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In vitro and *in vivo* screening of commonly used acaricides against Ixodid ticks in South Omo pastoral areas, South-Western Ethiopia

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A B S T R A C T

The study aimed to assess the *in vitro* and *in vivo* antitick efficacy of commonly used acaricides in order to suggest efficient acaricides to livestock owners was conducted in South Omo Zone, Southern Ethiopia. According to Drummond's method, the Adult Immersion Test (AIT) was applied to assess oviposition inhibition of engorged female ticks. Also, *in vivo* efficacy field trial of Deltamethrin 1% pour-on, Amitraz 12.5%, and Ivermectin 1% injection was conducted on a goat kept under a pastoral production system in the study area. A total of 528 fully engorged female *R. decoloratus* and *R. pulchellus* ticks were tested through the adult immersion test (AIT). A statistically significant (p<0.05) mean percent oviposition control was achieved by two in vitro tested acaricides. Amitraz induced 67.30±7.33 (min= 58.07%; max=74.02%) mean percent oviposition control while, Deltamethrin achieved 100±00 (min=100%; max=100%) on ticks from bovine. Similar results were obtained on ticks from caprine using Amitraz and Deltamethrin, and mean percent oviposition control was 82.90±2.7 (min=79.04%; max=85.01%) and 100±00 (min=100%; max=100%), respectively. A 61.45% and 73.15% oviposition control of *R. decoloratus* and *R. pulchellus* from bovine, and 81.77% and 84.03% oviposition control of *R. decoloratus* and *R. pulchellus* from caprine, respectively, were observed in *in vitro* studies. However, Deltamethrin was able to completely prevent oviposition in both bovine and caprine tick species. At the recommended field concentration, Amitraz, Ivermectin, and Deltamethrin, achieved 97.22%, 85.29%, and 99.03% effectiveness, respectively, in eliminating adult ticks from infested goats. We suggest both Amitraz and Deltamethrin were effective against Ixodid ticks infesting ruminants in the research area when used in the recommended dosages however; more confirmatory in vivo field data is required.

Keywords: Acaricide Efficacy, *In vitro* and *in vivo*, Bovine, Caprine, Tick, South Omo

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Introduction

In the tropics and subtropics, ticks are significant ectoparasites that parasitize farm animals, inflict significant financial losses, and spread zoonotic infections and tick-borne diseases (TBD) (Abbas *et al.*, 2014; Dantas-Torres *et al.*, 2012). Due to their significant negative impact, controlling tick infestation is the cornerstone in combating tick-borne diseases of veterinary and public health importance worldwide (Vayssier-Taussat *et al.*, 2015). Chemotherapy, which involves the direct application of acaricides to host animals, is the most popular approach for effectively controlling tick populations (Githaka *et al.*, 2022). However, acaricides are expensive and detrimental to the environment and non-targeted species (Moyo and Masika, 2009). Moreover, due to the misuse of chemical acaricides, there are numerous reports on the development of

acaricide-resistant species infesting domestic animals worldwide (Bishop *et al.*, 2023). This incidence of acaricide resistance development is more aggravated in African countries because tick control has been the responsibility of resource-poor farmers and pastoralists rather than the central government's veterinary departments (Bishop *et al.*, 2023).

In Ethiopia, ticks under the genus Ixodid have been widespread and are important parasites of livestock, causing significant economic losses to the livestock industry (Kaba, 2022; Yilma *et al.*, 2001). The only effective approach to combat tick infestation in Ethiopia is chemotherapy with acaricides. However, the widespread and improper use of accessible acaricides by uneducated pastoralists and farmers is troublesome regarding the emergence of acaricide resistance. Previous studies in Ethiopia indicated irregular spraying and other managerial constraints as anticipated causes that accelerate the emergence of acaricide resistance (Yilma *et al.*, 2001; Tesfaye and Abate, 2023).

South Omo is one of the pastoral zones in Ethiopia, which is inhabited by 1.75, 1.55, and 2.88 million cattle, sheep, and goats, respectively (CSA, 2021). Previous investigations in this zone indicated a high prevalence of tick infestation caused by *R. decoloratus* and *R. pulchellus* in domestic ruminants (Mebrahtu *et al.*, 2018). Another study conducted in this location showed a lack of awareness creation training, lack of acaricide dosage measurement and animal body weight estimation, and injection of acaricides by illiterate herdsmen as indicatives of poor acaricide usage practice (Tesfaye and Abate, 2023). These poor usage practices of limited acaricides lead to their efficacy deterioration. Therefore, acaricide chemicals that are already under extensive usage must be regularly checked to maintain the effectiveness of tick control and to mitigate the occurrence of acaricide resistance. However, no one study has been conducted in our study area that demonstrates the effectiveness of commonly used acaricides in conjunction with any of the aforementioned malpractices related to the use of acaricide chemicals. Hence, the main objective of this study was to evaluate the *in vitro* and *in vivo* effectiveness of commonly used acaricides on Ixodid ticks infesting cattle and goats in the study area.

Materials and Methods

Ethical approval

This study was approved and passed through a regional research review process held by the South Ethiopia Agricultural Research Institute (SEARI) (SEARI-07-008/2020). Humane handling of study animals was applied throughout the study period, and the study was continuously checked and reviewed by the institute and closely monitored by the principal investigator. All the animals that participated in this study were naturally tick-infested, and all the acaricide treatments conducted were according to the manufacturer's recommended dosage.

Study area description

This study was carried out in the Bena-Tsemay district of the South Omo zone, South-Western Ethiopia; which is 42 km far away from the zonal center, Jinka. The climatic condition of the Bena-Tsemay district is hot to warm and semiarid. The altitudinal range of the district varies from 500 m to 1800 m above sea level, with average daily temperature ranges between 15.6°C and 26.5°C. Generally, the Bena-Tsemay district is characterized by erratic rainfall, usually bimodal, occurring from September to December and March to May.

Study animals and their management system

The animals were local cattle types, and Woito-Guji goat breeds, reared under an extensive pastoral and agro-pastoral management system in the district. In the Bena-Tsemay district, mixed stocking of cattle and goats was more common, and they were fed dominantly on communal grazing pastures. A crash-type animal housing system is mainly constructed from locally available materials. The seasonal feed scarcity forces pastoral herds to travel long distances during dry periods. According to previous studies, ectoparasite infestations, especially of ticks (Mebrahtu *et al.*, 2018) were the main health threats that hindered animal production and productivity in the study area along with other diseases such as gastrointestinal helminthiasis (Tesfaye, 2021), trypanosomosis, Peste des Petits Ruminants (PPR) and Contagious caprine pleuropneumonia (CCPP).

Study design and acaricide preference

A randomized controlled trial was applied to assess the *in vitro* effectiveness of Deltamethrin (commercial emulsion concentration) and Amitraz 12.5% (Hebei Veyong Pharmaceutical Co., Ltd.) against Ixodid tick species collected from both bovine and caprine. Also, *in vivo* efficacy field trial of Deltamethrin 1% pour-on (SMASH™, Tagros chemicals India Pvt Ltd.), Amitraz 12.5% and Ivermectin 1% injection (Shenyang Sunvictor Pharmaceutical Co., Ltd. /China) was conducted on goat kept under pastoral production system in Bena-Tsemay district. According to our preliminary inventory, the above-mentioned acaricides were commonly used in the study areas, highly preferred by herdsmen and easily accessible in veterinary clinics of the study area. These acaricides were purchased from licensed importers, transported, stored and used according to the manufacturer's guidelines.

Study methodology

In vitro acaricide efficacy

Adult Immersion Test (AIT) was applied to assess oviposition inhibition of engorged female ticks as of Drummond *et al.* (1973). Seventy-two (72) adult engorged females of each tick species (*R. decoloratus* and *R. pulchellus)* from bovine and sixty (60) adult engorged females of each species (*R. decoloratus* and *R. pulchellus)* from caprine were separately collected. Ticks collected from bovines were randomly allocated into three treatment groups containing twenty four (24) engorged female ticks of each species separately. Ticks in Treatment-1 and Treatment-2 were subjected to test acaricides whereas ticks in treatment-3 were immersed in tap water and served as control. Similar treatment arrangement was applied for ticks collected from caprine except for the difference in the number of engorged female ticks in each treatment group (Treatment-1= 20, Treatment-2= 20 and Treatment-3= 20). A replicate of the above treatment arrangement for each acaricide treatment and control group was kept separately for each tick species collected from bovine and caprine during the study period. Therefore, a total of 528 engorged female ticks in a replicate per treatment per tick species were used during our in vitro experimentation.

After measuring the weights of all engorged females, ticks in treatment groups (Treatment 1&2) were immersed in commercial acaricides of Amitraz 12.5% at recommended field concentration and prepared immersion concentration of deltamethrin from commercial emulsifiable concentrates as of FAO (2004). While ticks assigned in Treatment-3 (control group) were immersed in tap water. Tap water was used to reconstitute stock acaricide solution (Amitraz 12.5%) to the recommended field concentration. Thirty (30) minutes of immersion time was applied for this specific study. Immediately after discarding the immersion solution, ticks were cleaned and dried with absorbent paper and transferred to a petri dish with their ventral sides facing upwards for oviposition. Then, the ticks were incubated at temperature of 25^{0}C to 28^{0}C and 85-90% relative humidity for a period of oviposition (14 days) according to FAO (2004). The effect of each tested acaricide on the reproductive capacity of immersed female tick species was determined and then compared with the control groups. The egg masses laid by treated ticks were compared and the percentage control achieved by each in vitro tested acaricide was calculated according to FAO (2004) as follows:

$$\text{Percent control} = \frac{\text{MEC} - \text{MET}}{\text{MEC}} \text{ X } 100$$

Where,

MEC and MET are mass of eggs laid by control ticks and treated ticks, respectively.

In vivo acaricide efficacy

Before the commencement of an in vivo experimental trial, acaricide treatment history survey (to avoid previous treatment effect) and tick count were conducted on goats of selected herds. Ticks were counted on the preferred predilection sites, such as the ears, head, dewlap, back, abdomen, anus-vulva and tail, by the same person as per the procedure described by Bianchi *et al.* (2003). In this study, no particular consideration was given to the sex of the animal during selection i.e., both sexes were included based on the above-mentioned criterion only. Then, goats with a mean tick count of >20 and didn't receive acaricide treatment for at least one month were purposively selected and recruited for the trial. Each experimental goat was ear tagged and randomly assigned into four treatment groups, each containing 24-26 heads of goat. Goats in group-1 (24 goats), group-2 (24 goats) and group-3 (24 goats) were treated with field field-recommended concentration of Amitraz 12.5%, Ivermectin injection and deltamethrin 1% pour-on, respectively, while

goats in group-4 (26 goats) were left untreated as control. Each treatment group comprised animals from two study villages; i.e. each study village contributed 50% of the experimental animals to all assigned groups.

The test acaricides were prepared based on the manufacturer's recommendation. Amitraz 12.5% was prepared (1:625 for Amitraz) for hand spraying and sprayed manually by using a knapsack. Subcutaneous (SC) Ivermectin injection was undertaken based on the perfect body weight measurement of the animal and dosage prescription of the manufacturer (0.2 mg kg^{-1} or 0.02 ml kg^{-1}). Deltamethrin was applied along the backline from the base of the skull to the tail root and a few drops were placed on the ear based on the recommended dosage of 1 ml $5kg^{-1}$ body weight of animals. As the experimental protocol set for this specific study, goats in the control group were selected and tagged to separate from the treated group and were allowed to graze at separate grazing land.

The tickcidal effects of the acaricides were observed at regular intervals by tick counting and recording at Day 0 (at trial initiation day) and then at day 7, day 14 and day 21 after the trial on all acaricide treated and control group. Thus, the efficacy of each acaricide was estimated by comparing the tick loads on animals at the time of the treatment (pre-treatment count) with those obtained at day 7, day 14 and day 21 post-treatments and calculated by using the formula described by Marchiondo *et al.* (2013). Then, percentage tick control achieved by in vivo tested acaricide was calculated as follows:

$$\text{Percent control} = \frac{\text{Mc} - \text{Mt}}{\text{Mc}} * 100$$

Where,

M_C is the mean number of ticks on control group and M_t is the mean number of ticks on acaricide treated groups.

Data analysis

All collected data were entered into Microsoft Excel sheet 2010 and then transferred to SPSS Version 20. Descriptive statistics like mean and standard deviation was compared. Percent control (%C) of oviposition obtained for each acaricide was computed by comparing egg mass laid by acaricide treated group with the control group according to the modified FAO (2004) formulae mentioned above. An in vivo efficacy of each acaricide was estimated by comparing pre-treatment count tick loads with those obtained at Day 7, Day 14 and Day 21 post-treatment. Independent sample t-test was used to compare the mean tick burden between the treated and control groups. All the analysis results were thought statistically significant if $p \leq 0.05$ at 95% confidence intervals.

Results

The mean in vitro oviposition inhibition response of *R. decoloratus* was 61.45% when treated with Amitraz 12.5% at the recommended field concentration. Meanwhile, mean oviposition inhibition of 73.15% was observed when R. pulchellus was immersed in Amitraz at 12.5% at the recommended field concentration and incubated. An oviposition inhibition response of 100% was achieved on both (*R. decoloratus* and *R. pulchellus*) tick species collected from bovine after incubation on deltamethrin (Table 1).

Amitraz 12.5% induced 81.76% mean in vitro oviposition inhibition response of *R. decoloratus* at recommended field concentration. Meanwhile, mean oviposition inhibition of 84.03% was observed when R. pulchellus was immersed in Amitraz at 12.5% at the recommended field concentration. However, complete (100%) mean oviposition inhibition was achieved by deltamethrin on both tick species sampled from caprine (Table 1).

Table 1. In vitro efficacy of tested acaricide on adult *R. decoloratus* and *R. pulchellus* collected from Bovine and Caprine.

Animal species	Trial	Tick species	Acaricides	N	Engorged female weight (g)	Egg mass (g)	% control
Bovine	Trial-A	*R. decoloratus*	Amitraz 12.5%	24	4.95	0.5807	58.07%
			Deltamethrin	24	5.06	0.00	100.00%
			Control	24	5.50	1.385	
		R. pulchellus	Amitraz 12.5%	24	24.38	0.690	74.02%
			Deltamethrin	24	24.10	0.00	100.00%
			Control	24	23.72	2.656	
	Trial-B	*R. decoloratus*	Amitraz 12.5%	24	4.66	0.469	64.84%
			Deltamethrin	24	5.00	0.00	100.00%

			Control	24	4.83	1.334	
		R. pulchellus	Amitraz 12.5%	24	24.02	0.641	72.28%
			Deltamethrin	24	23.56	0.00	100.00%
			Control	24	23.99	2.312	
Caprine	Trial-A	*R. decoloratus*	Amitraz 12.5%	20	4.22	0.249	79.04
			Deltamethrin	20	4.36	0.00	100.00
			Control	20	4.32	1.188	
		R. pulchellus	Amitraz 12.5%	20	20.08	0.209	83.05
			Deltamethrin	20	19.29	0.00	100.00
			Control	20	19.85	1.233	
	Trial-B	*R. decoloratus*	Amitraz 12.5%	20	4.37	0.189	84.51
			Deltamethrin	20	3.99	0.00	100.00
			Control	20	4.03	1.22	
		R. pulchellus	Amitraz 12.5%	20	18.92	0.148	85.01
			Deltamethrin	20	19.35	0.00	100.00
			Control	20	19.47	0.993	

There was a statistically significant (p<0.05) difference in overall mean oviposition inhibition response among tested acaricides on tick species collected from bovine and caprine. Deltamethrin (100%) resulted in a significantly higher overall mean oviposition inhibition response over Amitraz 12.5% (67.30%) at recommended field concentration on ticks sampled from bovine. Similarly, the efficacy of deltamethrin was significantly higher than Amitraz at 12.5%, with their respective mean oviposition inhibition response of 82.90% and 100% for tick species collected from caprine (Table 2).

Table 2. Overall mean percent oviposition control of tested acaricide against adult engorged female *R. decoloratus* and *R. pulchellus*.

Animal sampled	Acaricide	Oviposition control	Mean difference	SD	p - value	95% CI	
						Lower	Upper
Bovine	Amitraz 12.5%	67.30	-32.69	7.33			
	Deltamethrin	100.00	-32.69	0.00	0.003*	-44.36	-21.03
Caprine	Amitraz 12.5%	82.90	-17.09	2.705			
	Deltamethrin	100.00	-17.09	0.00	0.001*	-21.40	-12.79

Our in vitro oviposition inhibition test on engorged female *R. decoloratus* collected from bovine indicated 61.46% control of oviposition using Amitraz 12.5% at recommended field concentration. Whereas, oviposition inhibition response of 73.15% was achieved using the same acaricide on *R. pulchellus* engorged females sampled from bovine (Table 3). Similarly, amitraz induced 81.77% and 84.03% mean percentage oviposition inhibition, respectively of *R. decoloratus and R. pulchellus* detached from caprine. All female ticks from both bovine and caprine showed complete (100%) oviposition inhibition after immersing in deltamethrin (Table 3) and all the ticks died (movement cessation) after 24 hours of immersion. This study indicated that there was statistically significant (p<0.05) difference in mean oviposition inhibition of Amitraz 12.5% on the same tick species from different animals (Table 3).

Table 3. Effectiveness of chemicals between ticks collected from cattle and ticks collected from goats.

Tick species	Acaricide type	Ticks sampled from	Mean efficacy	SD	p- value	95% CI	
						Lower	Upper
R. decoloratus	Amitraz	Bovine	61.45	4.78	0.043*	-39.04	-1.59
		Caprine	81.77	3.87			
R. pulchellus	Amitraz	Bovine	73.15	1.23	0.014*	-16.52	-5.24
		Caprine	84.03	1.39			
R. decoloratus	Deltamethrin	Bovine	100	00	NA	NA	NA
		Caprine	100	00			
R. pulchellus	Deltamethrin	Bovine	100	00	NA	NA	NA
		Caprine	100	00			

*SD= Standard Deviation, *= Statistically Significant, NA= Not Analyzed (due to zero SD)*

Treatment with Amitraz 12.5% on caprine at recommended field concentration resulted in 97.23% and 97.21% overall reduction in tick count, respectively, in Luka and Diziaman study sites (Table 4). Similarly, an overall mean tick count reduction of 85.60% and 84.98% using Ivermectin and 99.45% and 98.61% by using deltamethrin pour-on was achieved, respectively, in both study sites (Table 4). Ivermectin achieved a maximum tick count reduction of 87.55% and 89.64% in Luka and Diziaman villages at day-21. Likewise, the maximum tick count reduction of 99.75% and 99.44%, respectively, in Luka and Diziaman villages was attained by deltamethrin pour-on on the seventh day of post-acaricide application (Table 4).

Table 4. Mean tick count on goats treated with acaricides and percentage control/efficacy achieved.

Mean tick count ± SEM (% Efficacy)						
Study villages	Acaricide used	Day 0	Day 7	Day 14	Day 21	Overall % efficacy
	Amitraz 12.5%	38.28±2.77	2.56± 0.23 (96.05%)	1.52± 0.28 (98.21%)	2.84± 0.39 (97.18%)	97.23%
Luka	Ivermectin	33.65±2.24	12.27± 0.69 (80.31%)	10.31± 0.64 (87.34%)	12.04± 0.83 (87.55%)	85.60%
	Deltamethrin 1%	50.46±3.25	0.15± 0.09 (99.75%)	0.46± 0.17 (99.43%)	0.69± 0.17 (99.28%)	99.45%
	Control	48.00±4.21	62.31±4.91	81.42±5.52	96.69±5.35	
	Amitraz 12.5%	40.04±1.77	1.36± 0.24 (97.29%)	1.12± 0.23 (98.02%)	2.44± 0.25 (96.49%)	97.21%
Diziaman	Ivermectin	38.92±1.81	12.68± 0.48 (74.80%)	6.60± 0.41 (88.32%)	7.20± 0.43 (89.64%)	84.98%
	Deltamethrin 1%	58.35±5.82	0.27± 0.11 (99.44%)	0.92± 0.26 (98.30 %)	1.15± 0.24 (98.27%)	98.61%
	Control	42.81±3.75	48.35±4.64	54.34±4.68	66.81±3.28	

SEM= Standard Error of Mean

An increased number (50%) of ticks on the control group (Table 5) might be due to the high challenge of tick infestation in the study area. Overall efficacy of Amitraz and deltamethrin on day 21st post treatment indicating prolonged protection of these two acaricides beyond 21st days at recommended field concentration. However, Ivermectin ed a decline in efficacy below the FAO recommended cut-point in both study locations.

Discussion

Our in vitro acaricidal efficacy trial revealed variation in mean percentage oviposition control achieved by Amitraz at field recommended concentration on tick species collected from bovine and caprine. However, complete (100%) and non-significant mean percentage oviposition inhibition control was recorded by Deltamethrin on gravid female *R. decoloratus* and *R. pulchellus* from bovine and caprine. Mean oviposition inhibition (61.45%) achieved by Amitraz on *R. decoloratus* collected from bovine was much lower than earlier reports of 98.27% (Gashaw *et al.*, 2018) and 89.08% (Asha and Eshetu, 2015) on the same tick species from cattle respectively from Sebeta Awash district and selected areas of Wolaita and Dawuro zones.

Likewise, the current in vitro mean percentage efficacy of Amitraz on *R. pulchellus* (73.15%) was far below 90.94% percentage oviposition control achieved in Borana pastoral areas (Eshetu *et al.*, 2013) but almost comparable to 77.44% mean percentage oviposition inhibition on *R. microplus* in Sao Paulo state (Mendes *et al.*, 2001). Amitraz also produced high mean oviposition response inhibition on other tick species, such as *Amblyomma variegatum* (94.51%), *R. microplus* (95%) and *Amblyomma gemma* (100%), as reported by Asha and Eshetu (2015), Eshetu *et al.* (2013) and De Souza *et al.* (2003), respectively. However, amitraz's in vitro test on globally known acaricide-resistant tick species, *R. microplus* has shown surprisingly lower efficacies in different countries, including 14.2% and 56.3% in Colombia (Lopez-Arias *et al.*, 2014), 30.95% in Brazil (Campos Junior and de Oliveira, 2005) and 48.4% in Brazilian Southwestern Amazon (Brito *et al.*, 2011). The difference in efficacy of the same acaricide on ticks collected from the same area could be due to mixing infestations from other locations, as pastoral herds moved frequently to distant (wildlife-domestic animal junctions) to search for water and feed during the dry season and returning with an infestation of ticks with different acaricide exposure histories.

This study confirmed that amitraz was more effective against the tick species *R. decoloratus* (81.77%) and *R. pulchellus* (84.03%) from caprine than it was against the same tick species from bovine. However, earlier studies in Ethiopia (Marchiondo *et al.*, 2013; Gashaw *et al.*, 2018; Asha and Eshetu, 2015); indicated better in vitro amitraz efficacies on bovine ticks, although these investigations didn't make comparison of efficacy on ticks from different animals. Similarly, Ravindran and his colleagues from India confirmed that amitraz has 100% in vitro activity and completely blocks eclosion on *R. annulatus* (Ravindran *et al.*, 2018). Generally speaking, the diminished amitraz in vitro efficiency on the most common tick species in our study area may indicate the potential development of acaricidal resistance. *R. decoloratus* and *R. pulchellus* infestations of a variety of domestic ruminants may enhance their exposure to this acaricide at levels that are either below or over the advised field concentration. This repeated exposure to levels above or below a sufficient acaricide concentration could lead to the development of resistant strains. Additionally, the lowered in vitro efficacy of amitraz in this particular study might be linked with previous study's speculations, which anticipated that this acaricide can take several days to kill ticks and some surviving ticks can complete engorgement and lay viable eggs (Burridge *et al.*, 2003). Moreover, the methodology used (AIT) had its own drawback in predicting the full-field efficacy of this acaricide. However, it can certainly diagnose the emergence of acaricide resistance, as stated by FAO (2004) and WAAVP (Holdsworth *et al.*, 2006). The total (100%) in vitro oviposition suppression by deltamethrin on both tick species may be related to the occasional use of this acaricide in the region due to its high market price. According to the herdsmen of the study area, deltamethrin was very rarely used in the area for the control of tsetse fly instead of tick which indicates its minimum exposure to tick species in this region. This result concurs with the report from Egypt by Arafa *et al.* (2019), who found complete (100%) oviposition inhibition induction by deltamethrin. Similarly, Mekonnen *et al.* (2004) revealed that almost all ticks treated with related synthetic pyrethroids, such as cypermethrin, could not lay eggs. However, lower deltamethrin in vitro efficacy of 93.54% (Asha and Eshetu, 2015) and 67.10% (Brito *et al.*, 2011) were reported from selected areas of Wolaita and Dawuro zones and Brazilian Southwestern Amazon, respectively.

In vivo experimental trial on caprine indicated significant variation ($P<0.05$) between ivermectin and each of amitraz and deltamethrin in removing adult tick; however, it was insignificant between Amitraz and Deltamethrin in both villages. The highest adult tick removal was achieved by Deltamethrin, followed by Amitraz and Ivermectin. Deltamethrin attained 99.45% and 98.61% adult tick removal in Luka and Diziaman village. Likewise, amitraz achieved 97.23% and 97.21% adult tick removal and Ivermectin resulted in 85.60% and 84.98% adult tick removal, respectively in Luka and Diziaman villages. However, each acaricide a non-significant difference in their efficacy in the study villages, which might be attributed to similar acaricide preference and usage practice by pastoralists of the study villages. According to our in vivo trial, deltamethrin and amitraz were in the range of internationally and nationally recommended cut-off points for acaricide efficacy as of FAO (2004). Ivermectin, however, fell short of the threshold for acaricide efficacy, which may be related to the pastoral community's abuse of the drug. The South Omo zone pastoral community's preference for an acaricide typically depends on its cost and accessibility (Tesfaye, 2021). Ivermectin's affordability, accessibility, and careless application by uneducated pastoralists may have resulted in a reduction in its effectiveness. Contrary to ivermectin, other acaricide options (amitraz and deltamethrin) were less frequently used by pastoral communities due to their prohibitive price, which may have led to their better field efficacy in our study. In addition to the frequency of acaricide usage, the efficacy of acaricide may deteriorate on its storage condition as most of the frequently used acaricides in our study location were handled by uneducated herdsmen and stored for a long time in the herdsmen's house for future use.

This study's in vivo tickicidal efficacy of deltamethrin was in agreement with the results of Mekonnen (2014), who reported 97.3-100% efficacy. Similarly, Asha and Eshetu (2015) reported closely comparable deltamethrin efficacy of 97.15% and 97.81% in the Dawuro zone and dairy farm around Wolaita, respectively. Moreover, slightly lower in vivo efficacy (94.62%) from Sodo Zuria (Asha and Eshetu 2015) and higher (100%) from Egypt (El-Bahy *et al.*, 2015) and (100%) from Pakistan (Malik *et al.*, 2021) was recorded. In contrast, the lowest in vivo deltamethrin efficacy of 21.6% was reported on cattle from Egypt (Arafa *et al.*, 2021).

According to this investigation, amitraz achieved 97.23% and 97.21% adult tick removal, respectively, in Luka and Diziaman villages at 21 days post-treatment. This acaricide was found to be an efficacious chemical next to deltamethrin on caprine at the recommended field concentration. In line with our findings, Asha and Eshetu (2015) reported 99.67% and 99.88% efficacy of amitraz from the Dawuro zone and Soddo Zuria, respectively, which is comparable to our finding within 21 days of post-treatment. However, better efficacy of 100% tick removal within 3 days post-treatment with a further 21 days of protection from re-infestation was reported from other parts of Ethiopia (Mekonnen, 2001). Additionally, compared to our finding, amitraz also induced superior efficacy of 100% with 28 days of post-treatment protection and 100% efficacy with 50 days of post-treatment protection in India (Kapoor and Sharma, 2014).

Ivermectin achieved 85.60% and 84.98%, respectively in Luka and Diziaman, which was below an international and national recommended acaricide efficacy threshold according to FAO (2004). According to information obtained from herdsmen of the study villages, ivermectin was the most frequently used acaricide due to its lower market price as compared to deltamethrin and amitraz. Therefore, the lower efficacy of this acaricide might be associated with extensive and misuse of the drug by illiterate herdsmen due to a lack of technical understanding of the functioning of acaricide. The efficacy report of 87.67% from Sodo Zuria (Asha and Eshetu, 2015) was closely comparable to the current report. However, the same author confirmed higher efficacy of ivermectin in the Dawuro zone (97.76%) and dairy farm around Wolaita (97.02%). Also, complete (100%) adult tick removal by ivermectin was recorded in other countries like Egypt (Arafa *et al.*, 2019) and Pakistan (El-Bahy *et al.*, 2015) at 7 and 5 days post-treatment, respectively.

Conclusion

This study confirmed that amitraz, deltamethrin and ivermectin had shown varying efficacy in vitro and in vivo. Through an in vitro efficacy study, amitraz showed inferior efficacy of inhibiting the oviposition of gravid female ticks compared to deltamethrin, which in turn completely (100%) ceased oviposition of engorged female ticks of both species from bovine and caprine. Our in vivo investigation confirmed amitraz and deltamethrin as effective on caprine at the recommended field concentration. However, ivermectin efficacy was below the international and national recommended acaricide efficacy threshold. Therefore, we recommend that awareness be created for herders on the issue of measuring the dosage, acaricide dilution, and frequency of its usage on animals to curb the deterioration of available acaricides. Finally, due to the difficulty in tracing a sufficient number of tick-infested animals, this study used a small number of engorged female ticks with minimal replications for adult immersion tests (AIT). Therefore, we suggest that further in vitro investigation be conducted to validate the efficacy of commonly used acaricides. In addition, in vivo data is to be validated by different researchers.

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