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Evaluation of *in Vitro* Effectiveness of Amitraz 12.5% and Diazinon 60% Against *Amblyomma variegatum* in Cattle in Sebeta Hawas District, Ethiopia

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Abstract: Ticks are ectoparasites that seriously affect public health and cause major production losses in the livestock industry. Most of the farmers use acaricides to treat or control ticks on their livestock; Amitraz and Diazinon were the most commonly used acaricides in the study site. The study was conducted from November 2021 to April 2022 in the SebetaHawas district. The present study evaluate the effectiveness of two commonly used acaricides (Amitraz 12.5% and Diazinon 60%)Emulsion Concentrate (EC) against *Amblyomma variegatum* collected from cattle. The effectiveness of these two regularly used acaricides on adult engorged females of A. variegatum was examined in an *in vitro* experimental design using the adult immersion test (AIT) method. The females were then observed for oviposition and mortality for 21 days. According to the analysis of the current investigation, the result of AIT on engorged female ticks revealed that Diazinon 60% produced a significantly higher ($P<0.05$) level of oviposition inhibition, or the mean efficacy was 89.92%, whereas the mean efficacy of Amitraz 12.5% was 70.21%.Diazinon (60%) inhibited oviposition more effectively against than Amitraz (12%). Therefore, further *in vivo* trials should be conducted to assess the effectiveness of these acaricides.

Key word: Acaricides · Amblyommavariegatum · In-Vitro Efficacy · Sebeta

INTRODUCTION

Ticks are without a doubt the most economically significant livestock ectoparasites on a global scale and over 20% of the exodid ticks also infest human [1]. Globally, there are more than 850 tick species within three families: soft ticks (Argasidae), hard ticks (Ixodidae) and Nuttalliellidae [2]. Hard ticks adopted a prolonged feeding duration, which facilitates microorganism transmission to livestock and they also fed a large amount of blood from their hosts, causing skin damage, irritation and discomfort, as well as tick paralysis [3]. Ticks are blood-sucking ectoparasites found worldwide, but they are more economically important in the tropical and subtropical zones. Ticks harm hosts both directly and indirectly. Direct harm results from blood loss, damage to hides and skins, serving as a route for secondary infection, the production of toxins and causing paralysis. Indirectly, ticks cause economic loss by contributing to reduced productivity of milk and meat, affecting fertility rates, anemia and increasing susceptibility to other diseases and death. It may also lead to hide and skin rejection at the tannery [4, 5]. Tick infestations pose a serious challenge to animals in both developed and developing countries. Both soft ticks (Argasidae) and hard ticks (Ixodidae) are very important vectors for the transmission of many bacterial, viral and protozoan pathogenic agents in humans, livestock and wild animals.

The ticks are the most important vectors of human and animal diseases caused by protozoa,rickettsiae, bacteria, viruses and some helminths. In Africa, 10 genera of ticks commonly infest domestic animals in 48 species: three are argasidae; seven are ixodidae [6]. In Ethiopia there are 8 genera of ticks which have major economically and veterinary importance these are Amblyomma, Aponoma, Ixodes, Haemaphysalis, Hyalomma and Rhipicephalus, Args and Orinthodorous, 44 species of ticks have been distributed throughout different agro-ecological zones of the nation [7] and [8]. Regardless of the enormous livestock population, the economic benefits of these animals remain insignificant. Economic losses from tick infestation and tick-borne diseases are directly related to farmers' socio-economic development [9, 10].

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Tick treatment relying on different application methods has been the main method of tick control in Africa. However, efficient and reliable control of ticks and tick-borne diseases (TBD) is still primarily based on the intensive use of acaricides, often without a local understanding of the factors responsible for tick distribution dynamics and the development of resistant tick strains, leading to numerous problems [11]. In Ethiopia, in recent decades, ticks have been primarily controlled using a variety of acaricides, although ticks are likely to develop resistance [12]. The objective of the current study was to evaluate the efficacy of the two most frequently used acaricides (Amitraz 12.5% and Diazinon 60%) for the control of cattle tick *A. variegatum* in Sebeta Hawas District, Ethiopia the study area.

MATERIALS AND METHODS

Description of the Study Area: This study was conducted in Sebeta, which is under Shegar sub-city, in the Oromia region of Ethiopia. The sub city is located 25 kilometers south-west of Addis Ababa city. The study area is subdivided into two agro-ecological zones, which cover highland (12%) and midland (88%) areas, respectively. The weather condition of the sub-city was altitude ranged between 1800 and 3385 (m.a.s.l.), The mean annual temperature of the study area ranged from 13.9°C to 25.4°C and the annual rainfall ranged between 860 to 1200 mm. According to The SSSCAO [13].

The major livestock species found in the study area were 6151 cattle, 1920 sheep, 1029 goats, 1571 horses, 893 mules, 2263 donkeys and 7527 poultry [13]. The farm was a mixed-crop livestock production system, where oxen are used for plowing to produce crops. Communal grazing land with a poor system of supplementary feeding throughout the year. Because the majority of the land in the sub-city was intensively farmed during the rainy season [13].

Study Population: Cattle found in and around Sebeta district were the target population. Animals were selected based on the criteria of their tick infestation level with engorged adult female ticks and those cattle that did not receive acaricide treatment one month before the trial [14]. From a total of 100 cattle herds, 50 cattle were selected for adult female engorged tick collection. Those kept under an extensive and semi-extensive production system in the study are.

Study Design: From November 2021 to May 2022, an experimental study design was carried out to look for acaricide resistance in cattle ticks in the study area. Similar to the above, a structured questionnaire was given to randomly chosen livestock owners with the main goal of obtaining some information regarding the cattle tick infestation, the source of the infestation, acaricide treatment history and economic losses caused by ticks. The following information was recorded: age, sex and breed of the animal.

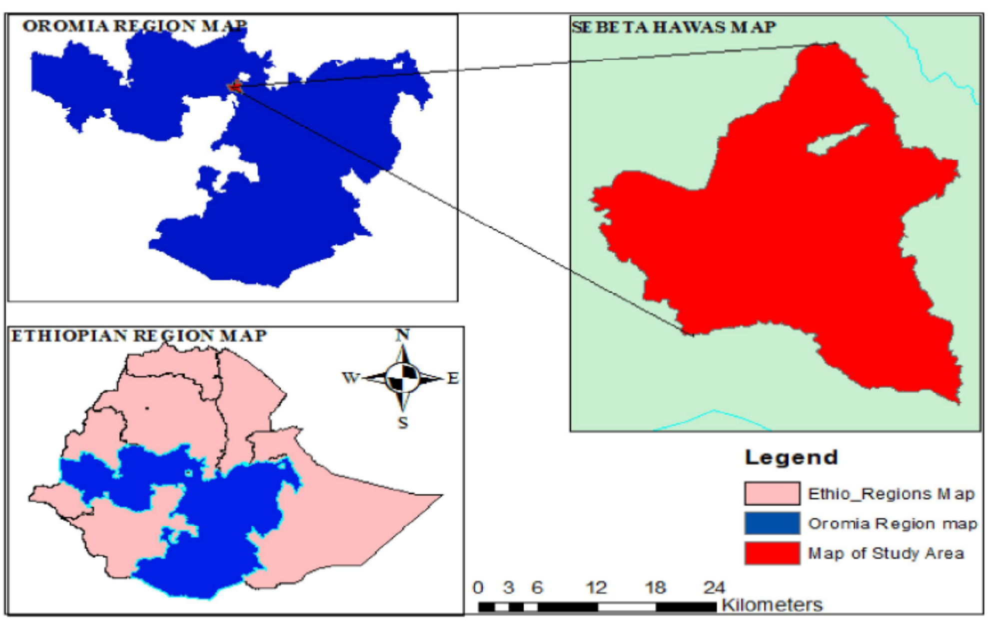

Fig. 1: Map of SebetaHawas District, Oromia special Zone, Oromia Regional State, Ethiopia.

Sample Size Determination: The Adult Immersion Test (AIT), which was the methodology given by [14], was used to calculate the sample size. Thus, for this test, 90 *Amblyomma variegatum* engorged female ticks were specifically collected from cattle under extensive and semi-extensive management systems at the study areas. These ticks had no signs of injury or color changes. A total of 90 ticks were divided into three groups at random: Group I for Amitraz, Group II for Diazinon and Group III for control and water. Each group contained 30 ticks (10 for the primary assay and 20 for the replica trial).

In terms of the questionnaire survey, 100 owners were interviewed at household level totally in four villages in the study areas using the formula provided by Arsham [15].

$$N = \frac{0.25}{SE^2}$$

where:

N = sample size

SE = Standard error assuming the standard error of 5% at a precision level of 0.05 and the confidence interval of 95%.

Sampling Methods: The study site is located in the Oromia region, in the Sebeta district. Purposive sampling was carried out in four villages known as Jawe, Tafki, Café Here and Dima. These villages were selected based on the burden of cattle tick infestation and the failure rate of acaricidal drugs [16]. From each village, 25 cattle of all ages were selected with the following inclusion criteria: they did not receive acaricide treatment one month prior to the trial and had a high tick infestation burden. They were selected for sampling and well-engorged female ticks were collected from each host.

Ticks Collection and Transportation: The engorged adult female ticks were carefully removed from the cattle to preserve morphological traits while the entire surface of the cattle was meticulously checked. Ticks were taken from the neck, back, udder, inguinal region, anus and vulva, tail and legs, among other body parts. The sampling of tick specimen depends on the host type and skin cover (fur, feather and reptile skin), the time available for inspection (sedated, alert or dead animals), tick size (larvae, in particular, may be difficult to see), the infestation rate and the experience of the investigator Petney *et al*. [17, 18]. The ticks were removed off each host and put in separate, small boxes with a few tiny holes for air circulation. A green leaf or piece of grass was then put on the bottom of the box to keep the ticks moist or prevent desiccation during transportation. The samples were then labeled with the host, location and date of collection. The samples were then delivered to the Animal Health Institute's (AHI) acarology laboratory.Upon arrival at the lab with the tick samples, the ticks were cleaned with distilled water and allowed to dry on fresh white paper. According to Walker *et al.* [19] and Guglielmone *et al.* [20], the ticks were next identified at the species level based on morphological traits using a stereomicroscope. In addition, the efficacy of acaricide was assessed in vitro. The trials used the acaricidesAmitraz 12.5%, produced by Shandong Luxi Animal Laboratorios China and Diazinon 60%, produced by Adami Tulu Pesticide Processing, S.Co., Ethiopia. Additional commonly used acaricide treatments were acquired from a veterinary pharmacy in the study area. Acaricides were then kept at room temperature until they were used.

***In-vitro* Acaricides Study Resistance:** The adult immersion test (AIT) and a questionnaire survey were used to assess community perceptions of acaricide (source of drug, reduced productivity, use and tick control methods). For each acaricide, three replicates containing adult, 10 engorged female Amblyommavariegatum ticks were immersed in 25 ml of the treatment solution and 25 ml of water for the control group in a 100 ml plastic container for 30 seconds with gentle agitation and the acaricide was poured off through the sieve and the ticks were placed in petri dishes on top of the Whatman filter paper until dry. All ticks from each group were coded, then deposited into a Petri dish and sealed with tape. All Petri dishes containing ticks from the treated and control groups were kept at room temperature for 24 hours. Ticks were transferred into an incubator at 28°C and 75-85 percent relative humidity. These ticks were observed for oviposition and death for a total of 21 days. The weight of the eggs laid by the treated ticks was recorded in comparison with the control ticks after 21 days. The egg was incubated in the same conditions and the percentage of hatching eggs will be calculated. The index fecundity of egg laying and percentage inhibition of oviposition, percentage control and percentage resistance were calculated using the following FAO [14] formula:

Control percentage =
Reproductive Index (IF) =
Percentage inhibition of oviposition (IO %) =
Percentage resistance = 100-control

Whereas MEC=mass of egg control, MET = mass of egg treatment, IFCG = Index fecundity of control group, IFTG = index fecundity of treatment group FAO [14].

Data Analysis: Raw data collected from questionnaires and laboratory results was coded into appropriate variables and entered into a Microsoft Excel spreadsheet. Acaricide resistance was determined by comparing all tick eggs treated with acaricides in the previous 21 days to all tick eggs in the control group. All statistical analysis was performed using statistical packages [21]. The percent control (%c) obtained with the egg-laying test (ELT) for each acaricide was used to evaluate its effectiveness. An independent sample t-test was used to compare the mean percent control of acaricides. All significant values were set at P 0.05 and a 95% confidence level.

RESULTS

Questionnaire Result

Socio-Demographic Characteristics: More than half (57%) of the 100 respondents were female. Regarding age group, 35 (35%) of the study participants were between 31 and 40 years old. With regard to educational status, 86 (86%) of participants did not have formal education.

General Perception of Respondents on Ticks Infestation on Cattle: According to the questionarysurvey, most respondents were able to describe an infestation of ticks, which are locally known as "Silmii" Afan Oromo. About 100% of respondents were aware that a tick infestation was occurring. According to the survey, 51% and 49% of farmers, respectively, reported that the frequency of tick infestation was increasing and that there had been no change in its occurrence in the study area.

In-vitro Acaricide Resistances Test: In the present study, two end-use products, amitraz (12.5%) and diazinon (60%), were tested for acaricide resistance to A. variegatum. Comparing the mean number of eggs laid with the control group, the mean percent inhibition of the oviposition of Amitraz (12.5%) and Diazinon (60%) EC against A. variegatum was calculated. Diazinon (60%) had a higher average incidence of oviposition inhibition than Amitraz (12.5%). A.variegatum treated with Amitraz 12.5% laid more eggs, with an average weight of 1.336g, while A.variegatum treated with Diazinon 60% laid fewer eggs than Amitraz 12.5%, with an average weight of 0.447g.

Table 1: Socio-demographic information of the study participants in SebetaHawasDistrict

Variables	Village	Frequency	Percent
Pas	Jawe	23	23
	C/Hora	24	24
	Tafki	24	24
	Dima	29	29
Sex	Male	43	43
	Female	57	57
Age	18-20	32	32
	31-40	35	35
	41-60	25	25
	Above 60	8	8
Educational status	No formal Education	86	86
	Elementary	9	9
	High school(9-12)	4	4
	College	1	1
	Degree and Above	0	0

However, there was a statistically significant (P<0.05) difference between the two acaricides with respect to the inhibiting effect of oviposition in A. variegatum tick species in the study area. A.Variegatum ticks in the control group laid large numbers of eggs, weighing an average of 4.462g. The response of *A. variegatum* collected from Sebeta Hawas district to recommended dose concentrations of Amitraz 12.5% and diazinon 60% in terms of egg mass weight, index fecundity (IF) and percentage inhibition of ovulation (%IO) is presented in (Table 2).

The difference in inhibition of oviposition(%IO) effect between the two acaricides was statistically significant in the triplettrials tested by recommended dose; Diazinon at 60% seemed to be higher than Amitraz at 12.5% efficacy against *A. variegatum*. However, both acaricides appeared to have similar efficacy (at 90% of the recommended dose) against A. variegatum. Both acaricides produced maximum efficacy at their recommended doses.

DISCUSSION

Ticks are considered to be most important to the health of domestic animals in African countries, including Ethiopia and are very important vectors for the transmission of many bacterial, viral and protozoan pathogens in humans and livestock. The present study found that bovine tick infestation was the main constraint

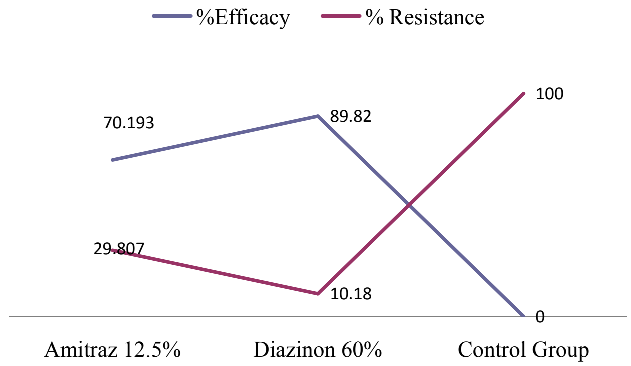

Fig. 2: Percentage of resistance and percentage of efficacy amitraz 12.5% and diazinon60% to cattle ticks' at SebetaHawas district.

Table 2: Recommended DD dependent response of amitraz 12.5% and diazinon 60% against*A.variegatum* collected from SebetaHawas district.

TN	TP	NEFT	EFW	EMPTG	IF	%IO	% cont
1	Amitraz (12.5%)	10	8.144	1.295	0.159	66.17	70.17
	Diazinon (60%)	10	12.527	0.542	0.043	90.85	87.51
	Control (water)	10	9.219	4.341	0.47	100	0
2	Amitraz (12.5%)	10	8.025	1.102	0.137	71.03	71.67
	Diazinon (60%)	10	9.79	0.403	0,041	91.33	89.64
	Control (water)	10	8.22	3.89	0.473	100	0
3	Amitraz (12.5%)	10	11.025	1.612	0.146	69.5	68.74
	Diazinon (60%)	10	9.875	0.396	0.04	91.7	92.32
	Control (water)	10	10.736	5.156	0.48	100	0

NB: TN=Trial number, NEFT=Number of immersed female ticks, EFW=Engorgement Female weight (gram),EMPTG=egg mass per treatment group (gm), (IF)=index of fecundity(gram), (IO) = inhibition oviposition

Table 3: Mean oviposition of *A.variegatum*after adult immersion test inamitraz 12.5% and diazinon 60% at recommended DD after 21 day incubation.

TT	TP	TN	Av.wit(g)	AEM(g)	IF(Aver. EM	% cont.	RI %
A.variegatum	Amitraz 12.5%	30	9.064g	1.336g	0.146g	70.193	29.807
	Diazinon 60%	30	10.730g	0.447g	0.041g	89.82	10.18

TT=Treated ticks, TP=Treatment product, TN=Trial Number, Av.wit =average weight of immersed ticks, AEM =Average Egg Mass= (gram), IF Index of fertility, (% cont.) percentage control, RI= reproductive index.

Table 4: T-test analysis of mean percent *A. variegatum* oviposition control between Amitraz12.5% and Diazinon 60% EC at recommended DD.

Treated ticks	Acaricides	N	Mean	St.Dev	t-Value	df	P-Value
A.variegatum	Amitraz 12.5%	3	70.21	1.47	-12.054	4	.000
	Diazinon 60%	3	89.92	2.41			

Table 5: Overall mean percent oviposition control of Amitraz 12.5% and Diazinon 60% EC at field recommended DD and their standard deviation against adult female *A.variegatum*.

Acaricides	Minimum efficacy	Maximum efficacy	Mean efficacy ± SD
Amitraz 12.5%	68.74	71.67	70.21±1.47
Diazinon 60%	87.51	92.32	89.92±2.41

affecting health and productivity in the study area. This was confirmed by the results of the questionnaire survey, which found that tick infestation was identified as a significant external pest in cattle by 100 respondents. Similar findings have been reported by Abdella *et al.* [22] in southwestern Ethiopia, where an infestation of cattle ticks has been a major impediment to animal productivity.

Even though the level of precision depends on the experience of livestock keepers, most farmers in the present study were aware of the clinical signs suggestive of cattle tick infestation, which were used as the basis for treatment. The most frequent signs of tick infestation were swelling around the udder, lameness, emaciation and the formation of a wound. Similarly, studies conducted in tick-infested areas of Brazil by Faza *et al*. [23].

In the present study, higher numbers of respondents were well aware of the seasonality of tick infestations.

This indicates that tick infestations peak during the summer and rainy seasons, whereas a few respondents believed the tick infestation lasted all year [24]. This was in line with the findings of Nejash [25], from Ethiopia. This could be attributed to the favorable temperature and moisture conditions for tick development and hatching.

The current response showed that acaricides and ivermectin were commonly used drugs for the treatment and control of cattle tick infestations. The drugs used to treat tick infestations were acaricides (64%) and ivermectin (36%). This study supported the findings of Brito *et al*. [11], who stated that most farmers prefer acaricides and ivermectin to other drugs.

Almost all the respondents treat their cattle against tick infestation and they believe that the treatment charges are expensive, with an overall average purchase price of 20-30 ETB per bovine per treatment According to the questionnaire response, the tick infestation reduces the milk and meat production of cattle and impacts agricultural activities. Almost all of the respondents answered that the impact of tick infestation mostly lies in crop production through the availability and cost of cattle that provide traction power [25].

Amitraz (12.5%) and Diazinon (60%) demonstrated different efficacies in this study. The percentage control of Amitraz (12.5%) and Diazinon (60%) varied, resulting in 70.19% and 89.82%, respectively. The analysis of variance revealed significant differences between the resistance percentages of the two acaricides, in which Amitraz (12.5%) presented a higher resistance percentage (29.89%) than Diazinon (60%) (10.18%).

Presently, the detection of A. *variegatum* tick resistance by the AIT proposed by FAO [10] and Drummond *et al*. [26], for evaluating the susceptibility to amitraz 12.5% and diazinon 60% in cattle A. variegatum ticks in the study area is underway. Despite the variation in AIT results obtained for 12.5% and diazinon 60%, the finding was agreed upon [27]. According to 42%, 40% and 18% of the interviewed herders, private pharmacies, government veterinary clinics, private clinics and other shops were the sources of acaricides in the study area.

However, in many countries, current tick control relies exclusively on the indiscriminate use of acaricides, often without knowledge of the factors responsible for tick population dynamics and how these factors contribute to developing resistant tick strains and escalating costs [28]. Therefore, the FAO Working Group on Parasite Resistance recommends the modified AIT, as proposed by Drummond *et al.* [26], for the detection of acaricide resistance in the field. The current study's findings may provide some insight into the factors that contribute to acaricide efficacy failures in the field.

Amitraz at 12.5% revealed a low percentage of efficacy in the current study, which was consistent with the findings of Furlong *et al.* [30], who discovered a mean efficacy of 47.9% for amitraz Camilo *et al.* [29], also observed the presence of resistance and low efficacy of Amitraz in some tick populations Rao *et al.* [31] and lower efficacy was reported by Mekonnen *et al.* [32], who showed an average efficacy of 77.4%.

In the current study, *A. variegatum* oviposition was greater when treated with Amitraz 12.5% than when treated with Diazinon 60%. This difference was most probably associated with prolonged exposure of these two acaricides to the most prevalent tick species in the study area. Similarly, Mendes *et al.* [33] discovered a relatively higher level of resistance to organophosphorus chemicals and Amitraz 12.5% due to its long-term use in South Africa. Silva *et al.* [34], in addition, strongly support this finding and observe that the sequential use of products from the same chemical group for long periods favors the development of resistance.

The *A. variegutum* used in this study on acaricide susceptibility was not more sensitive to either acaricide at recommended dose concentrations but was relatively more sensitive to Diazinon at 60% than Amitraz at 12.5%.The result also revealed that A. variegatum was relatively resistant to Diazinon (60%) compared to Amitraz (12%). This resistance might be due to the use of acaricides bought from other shops or because animal owners spray acaricides on their animals. The results showed that after 21 days, it was developing resistance to both acaricides, particularly Amitraz 12.5%.

CONCLUSSIONS AND RECOMMENDATIONS

- Limit the use of acaricides purchased from an unknown drug vendor or another store.
- Farmersmust be trained in order for acaricides to be used correctly.

Further tests using other detection methods involving larval and in vivo resistance trials at the field level should be conducted to assess both acaricides.

Policymakers and veterinary regulatory bodies, especially in tick-endemic and epidemic areas, essential to be aware with tick management strategies that could reduce dependency on acaricide.

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