

In-vitro Acaricidal efficacy evaluation trial of Ixodid ticks at Borana, Ethiopia.

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Abstract

The study was conducted from September 2011 to May 2012 in Borana pastoral community with the objectives of determining the efficacy of Amitraz 12.5% and Diazinon 60% Emulsion Concentrate (EC) against *Rhipicephalus pulchellus* and *Amblyoma gemma* using modified Adult Immersion Test (AIT) and to assess the pastoralist communities' perceptions on acaricide (usage, delivery and methods of tick control practice). Acaricide treatment is the only method for tick control in the community, where Amitraz was the most widely used followed by Diazinon. Manual spraying and hand dressing of each acaricide relying on frequent applications of often inadequate acaricide concentrations without alternation of acaricidal products was the common practice. The result of AIT on engorged female ticks revealed that, Amitraz 12.5% produced significantly higher ($P < 0.05$) level of oviposition inhibition (Percent control = 95.5%) in *A. gemma* and *Rh. pulchellus* than Diazinon 60% EC (Percent control = 80.9 %). Both acaricides however are relatively less efficient against *R. pulchellus* and *A. gemma*. Furthermore, Amitraz and Diazinon are the two mostly used acaricides by the community. Under laboratory condition, Amitraz seems to provide more oviposition inhibition effect against *Rh. pulchellus* and *A. gemma* than Diazinon 60% EC. Therefore, in vivo trial to assess the residual effect of these acaricides is suggested.

Key words: Acaricide, Cattle, Efficacy, Ethiopia, In-vitro evaluation, Ixodidae ticks

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Introduction

In Ethiopia, over the past decades ticks are mainly controlled by using a variety of acaricides; including organochlorines, organophosphates, carbamates, amidines or synthetic pyrethroids (Sileshi Mekonnen, 2001; Jobre *et al.*, 2001). However, with the most widespread, under or over concentration and frequent use of organochlorines and organophosphates compounds; ticks are likely to develop resistance in many countries (Furlong *et al.*, 2007); and in Ethiopia (Jobre *et al.*, 2001; Mekonnen *et al.*, 2004). Likewise, in Borana pastoral areas, where mostly use Amitraz 12.5% and Diazinon 60% EC, in various circumstances' animal health personnel's and livestock herders complained on failure of this two acaricides to kill ticks and toxicity associated with Diazinon usage (BZPADO, annual report, 2009/2010).

In view of the aforementioned facts in the inappropriate usage of acaricides, ticks are likely to develop resistance. Continuous studies on the responsible factors for the dynamics of tick population (Alanr, 2011) with the efficacy status of acaricides against the most abundant and important tick in particular area are necessary to carry out efficient tick control and/or tick burden reduction (Solomon Gebre, 2001). Therefore, the objectives of this study were to evaluate the efficacy of Amitraz 12.5% and Diazinon 60% EC against field population of engorged adult female *Rh. pulchilus* and *A. gemma* ticks in in-vitro condition using AIT (Adult Immersion Test) and to assess the perceptions of pastoralists' communities on the management, ways of acaricide usage and delivery; and methods of tick control practiced in the area.

Materials and Methods

Description of study area

The study was conducted from September 2011 to May 2012 at Yabello and Dire districts of Borana Pastoral area, of Ethiopia located at 570 and 665 kilometers far from the capital Addis Ababa respectively. The region has predominantly a semi-arid climate. The annual temperature varying between 21°C and 38°C with little seasonal variations and rainfall ranges from 350mm to 900mm, with considerable spatial and temporal variability in quantities and distribution (CARE, 2009). The region is characterized by bimodal rain with 60% occurring in the long rainy season (*Gana*) extending from mid-March to

May and erratic short rain season (*Hagayaa*) from mid-September through mid-November. The other two seasons are the cool dry (*Adoleessa*) extending from June to August and the major dry season (*Bonna*) from December to February (BZPADO, 2009/10).

Study animals

The study was carried out on different cattle herds belonging to pastoralists of Yabello and Dire districts. From 36 cattle herds of six villages (three villages from each district), engorged adult females of *Rh. pulchellus* and *A. gemma* were collected for in-vitro efficacy evaluation of Amitraz 12.5% and Diazinon 60% EC. None of the cattle received acaricidal treatment one month before the start of tick collection for the experiments.

Study methodology and design

Two study districts and three PAs from each district were selected. All visible engorged female adult ticks were collected from 36 naturally infested cattle herds. A controlled experimental design was used to evaluate the efficacy and oviposition inhibition effect of Amitraz and Diazinon against *Rh. pulchellus* and *A. gemma* under laboratory condition. Pastoralists who use acaricides for tick control were approached to respond structured questionnaire, which has quantitative as well as qualitative questions, that helps to assess the perception of pastoralists towards the effect of ticks, delivery system of acaricide, as well as the different tick control options and methods of applications practiced in the area.

Collection and preparation of ticks

In each herd, all visible engorged adult female ticks were collected from 6 to 8 cattle. These ticks were placed individually in different plastic flasks prelabeled with time, date, place of collection and code number. Afterwards the ticks were transported to Yabello Regional Veterinary Laboratory within 24 hours of collection for the in-vitro acaricidal efficacy evaluation using modified adult immersion test (AIT). This laboratory protocol was first described by Drummond *et al.* (1973) and modified by FAO (2004) and the South Africa Bureau of Standards (SABS) in East London, South Africa. Ticks in the Test acaricides:

Amitraz 12.5% (Triatox; manufactured by Laboratorios microsules Uruguay S.A.) and Diazinon 60% EC (manufactured by Shandong Luxi Anim. Med. Share CO.LTD., China) were used for the evaluation experiments. These acaricides were purchased locally at veterinary pharmacy and stored at room temperature until use. Both acaricides were used at manufacturers recommended concentrations and guidelines on leaflets. Diazinon was used at concentration of 1:1000 while Amitraz 1.6:1000 diluted in distilled water. For all the preparations, the final volume was 1000mL.

In-Vitro acaricidal efficacy test using Adult Immersion Test (AIT)

The FAO modified protocol for the AIT and as suggested by Drummond *et al.* (1973) was used to conduct the experiment. For each acaricide, three replicates, containing 10 ticks were immersed in 20 ml of water or into 20 ml of each acaricide in a 100 ml plastic container. The ticks afterwards were held in the plastic containers before being removed and gently dried on absorbent paper. All treatment and control groups ticks were later stuck (ventral side up) with double-sided sticky tape in a Petri dish. The plates were then placed in larger, plastic boxes containing a moistened sponge for 7 days at temperature of 27⁰C with a photoperiod of 12:12 (L:D) h. Humidity was not measured but the sponge was freshly moistened every 24 hours. Seven days after immersion in acaricide solutions or distilled water, the number of ticks in each treatment for every experiment that produced eggs was counted and eggs produced by each group were weighed.

To estimate the efficacy of each acaricide, both groups (treated and control) were then tested using the egg laying test (ELT) method which involves the comparison of the egg mass of ticks treated with acaricide and the egg mass of untreated ticks and finally estimates the percentage control value, using the following formula:

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

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

Statistical data analysis: All collected data were entered into Microsoft Excel 2003 computer program then displayed by graphs and tables. Percent control (% C)

obtained with Egg Laying Test (ELT) for each acaricide was used to evaluate its effectiveness. Whereas, Independent samples t-test was used to examine mean Percent Control between acaricides. A P-value less than 0.05 at 95% confidence intervals was considered significant.

Results

Questionnaire survey: - Almost 80% of interviewed pastoralists, preferred to use Amitraz 12.5% while 17.1% of them were in favor of Diazinon 60% EC. In the study area, alteration of acaricide bases did not occur in systematic ways; when substitution occurred, the reason given was mainly disappointing result after treatment, low price and availability of acaricides on the local market. Moreover, almost all interviewed herders, responded that they started to use Amitraz 12.5% since 2-3 years, but they used Diazinon 60% EC since over a decade. At the time of this study, all respondents complained of diminishing efficacy of both acaricides and of intoxicated cattle treated with Diazinon 60% EC during the last one/two years in the area.

According to 61.4% of respondents, the sources of acaricides for tick control in the study area are private pharmacies. Nevertheless 15.7% of them get from government veterinary clinics and 22.9 % from the local markets. None of the interviewed respondent had regular tick control schedule. Therefore, 97.1% of respondents treat their cattle for ticks whenever they observe high tick burden on their cattle, particularly on dairy cattle using most often knap sack hand sprayer and in few occasions hand dressing.

According to the view of most respondents concentration and dilution of acaricides was based on the extent of tick infestation and number of cattle population. None of the respondents had knowledge and awareness about the withdrawal period or expiration date before purchasing and application of acaricides.

In-vitro acaricide efficacy test: The mean percentage of oviposition inhibition of Amitraz 12.5% and Diazinon 60%EC against *A. gemma* and *Rh. pulchellus* were calculated by comparison of the mean egg mass laid by each tick species after treatment with the control group as presented in Table 1.

Amitraz 12.5% showed higher mean percentage of oviposition inhibition than

than Diazinon 60% EC. *A. gemma* treated with Amitraz laid no eggs, while few of *Rh. pulchellus* laid small batch of eggs with a mean weight of 0.033gm. In contrast, both tick species immersed in Diazinon 60% EC laid eggs with a mean weight of 0.037gm and 0.12gm by *A. gemma* and *Rh. pulchellus*, respectively. However, there was no statistically significant difference ($P>0.05$) between both acaricides regarding oviposition inhibition effect in both tick species (Table 2). Both tick species in the control group laid relatively large number of eggs with mean weight of 0.33gm and 0.373gm by *A. gemma* and *Rh. Pulchellus* respectively.

Table 1. Mean oviposition of engorged female *A. gemma* and *Rh. pulchellus* after immersion in Amitraz 12.5% and Diazinon 60% EC.

Treated ticks	Treatment	N	M ₁	S	M ₂	% C
<i>A. gemma</i>	Amitraz 12.5%	10	2.63	0	0.000	100.00
	Water control	10	2.45	8.67	0.333	0.000
<i>Rh. pulchellus</i>	Amitraz 12.5%	10	2.83	0.67	0.033	90.94
	Water control	10	2.75	9.67	0.373	0.00
<i>A. gemma</i>	Diazinon 60% EC	10	2.56	1.0	0.037	88.85
	Water control	10	2.45	8.67	0.333	0.000
<i>Rh. pulchellus</i>	Diazinon 60% EC	10	2.79	1.33	0.12	71.41
	Water control	10	2.75	9.67	0.373	0.00

N=Number of immersed female ticks; *M*₁=Engorgement weight (g); *S*=Number of ticks surviving after 7 days; *M*₂=Average egg mass per treatment group (g)

Table 2. T-test analysis of mean % of *A. gemma* and *Rh. pulchellus* oviposition inhibition between Amitraz 12.5% and Diazinon 60%EC.

Ticks	Acaricide	%C	N	Mean	SD	t-value	df	95% CI	S
<i>A. gemma</i>	Amitraz	100	3	100.0	0.0	2.438	4	-1.55-23.85	NS
	Diazinon	88.85	3	88.85	7.9				
<i>Rh. pulchellus</i>	Amitraz	90.94	3	90.94	11.3	2.26	4	-4.48-43.71	NS
	Diazinon	71.41	3	71.33	9.9				

%C=Percent control; *N*= Number of trials; *SD*=Standard Deviation; *NS*= Not significant

The overall mean % of oviposition inhibition effect of Amitraz 12.5% and Diazinon 60%EC was calculated by comparing the mean egg mass laid by both tick species treated with each acaricide versus with the mean egg mass of untreated control groups. Afterwards, the overall mean %C of each acaricide and their respective standard deviations as well as their minimum and maximum mean efficacy during the three replica of trial is presented in Table 3.

Table 3. Overall mean percent oviposition control of Amitraz 12.5% and Diazinon 60%EC at field recommended concentration against adult female *A. gemma* and *Rh. pulchellus*

Acaricides	Min. Efficacy (%)	Max. Efficacy (%)	Mean efficacy (%±SD)
Amitraz 12.5%	78.38	100	95.47±8.663
Diazinon 60%EC	61.11	97.06	80.09±12.537

A mean %C for Amitraz 12.5% at recommended dose was found higher than for Diazinon 60%EC. The variance analysis showed that, regardless of the tick species acaricides had variable efficacy, according to the F-test value (ANOVA) at 5% significance (Table 4).

Table 4. Analysis of variance of the effects of Amitraz 12.5% and Diazinon 60% EC against oviposition response of *A. gemma* and *Rh. pulchellus*

	Sum of squares	df	Mean Square	F	Significance
Between groups	31530.515	2	15765.258		
Within groups	1161.072	15	77.405	203.673	0.000
Total	32691.587	17			

df= Degree of freedom; *F*= *F*-value

Discussion

The present questionnaire survey result revealed that the entire pastoralist respondents' livelihood depends on extensive livestock production system. Cattle rearing accounts for 61.68 % of livestock breeding and is practiced primarily for milk, gift of dowry and social prestige. Cattle population in the study area however, suffers from a number of constraints. 77.1% of the interviewed herders ranked tick infestation (locally called, “*Silmi*” or “*Dirandisa*”) as a second constraint, next to draught. Ticks affects livestock in general and cattle in particular by reducing milk production, growth and birth rate According to the respondents, tick infestation occurs throughout the year, but infestation was most prevalent during the long rainy season followed by the short rain season. Similar findings were recorded in Jimma Zone (Abebaw Gashaw 2004) and in Borena pastoral area (CARE, 2009; Teshale Sori, 2004).

Analysis of the questionnaire results, indicated that tick control in the area is entirely dependant on the use of acaricides, in which 80% of interviewed herders prefer Amitraz 12.5% and 17.1% Diazinon 60%EC, while 7.1% of them preferred to use Ivermectin. 84.24% of interviewed herders prefer to use knap sack hand spraying machine while 12.86% hand dressing.

The questionnaire survey results indicated dilution and application of acaricides most of the time accomplished by the livestock herders particularly during high tick infestations season. This observation was in agreement with the study made by Natała *et al.* (2005) in Ghana, who found that most farmers are who make unmeasured dilution and application of acaricides to treat ticks. The present study also revealed that there was no regular tick treatment program in the study area. Other work done in Ethiopia revealed that ticks on indigenous cattle are treated whenever the farmers bring their animals to the veterinary clinics either for tick control or for other complaints. There is no planned program of tick control except on dairy farms (Sileshi Mekonnen *et al.*, 2001).

Information on the status and magnitude of acaricide resistance is of paramount importance in deciding the appropriate tick and tick-borne disease control strategy in different localities in Ethiopia (Solomon Gebre *et al.*, 2004). However, the situation is compounded by the absence of an effective legislation for acaricide importation, marketing and monitoring. No one knows the origin of the acaricides available on local markets or monitors it. Similarly, the present

questionnaire survey indicated that private pharmacies, government veterinary clinics and local markets were the sources of acaricides in the study area as responded by 61.4%, 15.7% and 22.9% of interviewed herders, respectively. None of the respondents were aware on the proper usage and handling of acaricides and on the development of acaricides resistance by tick and its consequences for the livestock production. Not only the withdrawal periods of acaricides were not observed, but milking of cows and application of acaricides was often done simultaneously. Such procedures may create a serious human health risk and must be avoided.

The study of tick population dynamics and their survival in diverse natural habitats might be useful to initiate the formulation of integrated, suitable, efficient and economical tick control measures (Luciana *et al.*, 2011). However, in many countries current tick control relies exclusively on indiscriminate use of acaricides, often without knowledge on the factors responsible for the tick population dynamics. These leads to environmental pollution, development of resistant tick strains and escalating costs (Alan, 2011). Therefore, the FAO working Group on Parasite Resistance recommends the modified AIT, as proposed by Drummond (6) for the detection of acaricide resistance in the field (FAO, 2004). Despite the high variation of AIT results obtained for Synthetic Pyrethroids (SP), Organophosphates (OP) and Amitraz, AIT is a valuable tool for rapid and cheap detection of loss of susceptibility of acaricides to ticks (Jonsson *et al.*, 2007). In view of the aforesaid facts, the present study results may give some clue on factors responsible for acaricide efficacy failure under field condition.

Amitraz and Diazinon at recommended concentration exhibited 100% and 88.85% oviposition control of *A. gemma*. This indicated that all engorged female *A. gemma* treated with Amitraz were unable to lay eggs after 7 days of incubation. Although, Amitraz showed evidence of greatest effect on oviposition of *A. gemma*, the statistical comparison between Amitraz and Diazinon revealed no significant differences ($P>0.05$). The highest oviposition inhibition effect of Amitraz (100%) to *A. gemma* could be due to recent exposure acaricide to ticks in the study area.

A trial to assess the oviposition inhibition response of field collected engorged adult female *Rh. pulchellus* to Amitraz 12.5% and Diazinon 60%EC, revealed 90.94% and 71.41% effect respectively. No significant oviposition inhibition effect difference ($P>0.05$) was observed between the two acaricides.

However, Amitraz has a higher effect on oviposition of *Rh. pulchellus* than Diazinon when applied at field recommended dose. Such difference in efficacy between Amitraz and Diazinon might be associated with the higher sterilization effect of Amitraz than Diazinon at field recommended concentration. As an effective acaricide prevents the female ticks from laying eggs or the eggs do not hatch (Sileshi Mekonnen *et al.*, 2003). The efficacies of Amitraz 12.5% and Diazinon 60% EC varied from 78.38% to 100% and 61.11% to 97.06% respectively. The analysis of variance, revealed significant differences between the overall mean %C of the two acaricides, in which Amitraz 12.5% presented higher mean efficacy (%C=95.47%) than Diazinon 60% EC (%C=80.09%).

Several authors have studied the efficacy of Amitraz 12.5% against different tick species using AIT, with different susceptibility levels. In most of earlier conducted studies Amitraz 12.5% had shown high degree of acaricidal efficacy against ticks similar to the present finding. In this regard a closely comparable finding was reported by Sileshi Mekonnen (2003) at Sebeta, Ethiopia. In South Africa, Sileshi Mekonnen *et al.* (2002) also reported 100% efficacy of Amitraz 12.5% against ticks. Souza *et al.* (2003) in Southeast Brazil also obtained mean Amitraz efficacy of 95%. The minimum mean acaricidal efficacy of Amitraz in the present result was (78.38%) which agrees with the findings of Mendes *et al.* (2001). Contrary to the present study, Furlong *et al.* (2007) found mean acaricidal efficacy of Amitraz as 47.9%. In Northeastern Brazil low acaricidal effect of Amitraz (40.5% and 30.95%) was also reported by Santana (2000) and Campos and Oliveira (2005), respectively.

In the present study, the extent of oviposition by *Rh. pulchellus* and *A. gemma* oviposited was significantly greater when treated with Diazinon compared to treatment with Amitraz. This difference was most probably associated with prolonged exposure of these two acaricides to the most prevalent tick species in the area to Diazinon 60% EC. A majority (84.6%) of the interviewed persons declared that the use of Diazinon 60% EC had started more than 10 years ago in the area. Similarly in South Africa, Sileshi Mekonnen *et al.* (2002) observed relatively higher level of resistance to organophosphorous chemicals than to Amitraz 12.5% due to its utilization for over 10 years. Silva *et al.* (2000), in addition, strongly supports this finding and observed that sequential use of products from the same chemical group for long periods favored the development of resistance.

In addition, the differences in the efficacy of these two evaluated acaricides were most likely attributed from the widespread, frequency, irregular application, inadequate spraying, and improper mixing of acaricides and on the use of acaricides stored for a long time after dilution; which was the common feature of tick control situation in the study area. Such acaricides had loss efficacies and which may lead to failure to maintain the adequate lethal concentration. When animals are sprayed manually, a common acaricide application method in the study area, and proper treatment of predilection sites for certain tick species has to be taken into account. The use of an acaricide at incorrect concentration is one of the prime factors which affect the efficacy of an acaricide and causes of tick control failure (Natala *et al.*, 2005; Kirby, 2010; Alan, 2011). Similar investigation which agree with the present finding was observed in Ethiopia (Sileshi Mekonnen 2001; Yilma Jobre, *et al.*, 2001) and abroad the country (Bianchi *et al.*, 2003; Furlong *et al.*, 2007). Moreover, the pastoralists in the study area believed that they needed to increase the concentration of acaricides during the peak tick season to control the excessive tick burdens infesting their cattle or to decrease the concentration in order to apply acaricides to all of their cattle. This type of increased acaricide concentration would undoubtedly have lead to a higher selection pressure for tick resistance (Luciana *et al.*, 2011) as the high acaricide concentration would effectively kill all susceptible ticks leaving only a residue of highly resistant individuals in the population (Pegram *et al.*, 2000). Each successive application would be a selective process that would concentrate the genes responsible for the resistance and eventually the majority of the ticks in the population would be resistant to the acaricide being applied against them (Rosario-Cruz *et al.*, 2009).

During this study, almost all pastoralists complained of failure of acaricides to kill ticks after being treated and concluded that the acaricide was not working. Especially, this coincided with the period during which heavy tick burdens were present on cattle. In vitro laboratory tests, however, indicated that those acaricides that were mostly used for tick control in the area had relatively high acaricidal efficacy of Amitraz (95.47%) and Diazinon (80.09%). The result clearly showed that not the acaricide but the problem was dilution, preparation and application of acaricides according to major predilection sites of ticks.

Conclusion

The in vitro acaricidal efficacy trial result in the present study clearly indicated that most of engorged females of *Rh. pulchellus* and *A. gemma* ticks treated with Amitraz 12.5% laid significantly reduced eggs. Even those few eggs laid by both ticks were very small significantly reduced egg mass weight than those of the control groups. Furthermore both ticks immersed in Diazinon 60% EC induced less oviposition inhibition effect and laid eggs with higher egg mass weight than those ticks immersed in Amitraz.

Generally the acaricidal oviposition inhibition effect of Amitraz and Diazinon against engorged female ticks of *Rh. pulchellus* and *A. gemma* was 95.47% and 80.09% respectively. Tick control in both study areas are carried out mainly by using chemical acaricides, such as Amitraz and Diazinon. However, this method of tick control has various problems, particularly in the preparations (dilutions, dosage etc.) and application. Although there is no policy and regulation in the marketing, evaluation, method of preparation and application at the national level, effort to use acaricides at least following the manufacturer guidelines in the paramount important. Veterinary services in the area should do efficacy trials from time to time to detect possible acaricide resistance problems as well as to give basic awareness education to pastoralists on the use, preparation and application of chemical acaricides. Beside these aspects environmental protection and public health safety issues must be given the highest considerations.

Finally tick control in the area should be based on abundance and population dynamics of ticks rather than the opinion of users. In order to implement this, veterinary service and regional veterinary laboratory in the area must do basic researches on the ecology and biology of most prevalent tick species. Pastoralists must be given basic education (awareness creation) on the proper use and application of acaricide to avoid acaricide resistance problem and unnecessary effect on the environment, human, wild and domestic animals. To consolidate the findings of the present study it is highly recommended conducting further studies under laboratory and field conditions using other relevant methodologies, more other prevalent tick species and developmental stages.

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