Full Length Research Paper

**In-vitro efficacy evaluation of amitraz 0.025% and diazinon 0.06% against *Rhipicephalus pulchellus* and *Amblyomma gemma* in Borena pastoral area, Southern rangeland of Ethiopia**

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This study was conducted in Borena pastoral area, southern rangeland of Ethiopia to determine the in vitro efficacy of amitraz 0.025% and diazinon 0.06% against *Rhipicephalus pulchellus* and *Amblyomma gemma* using modified adult immersion. A total of 180 engorged adult female ticks (ninety ticks of each *A. gemma* and *R. pulchellus* species) were immersed in amitraz 0.025% or diazinon 0.06% at field recommended concentration (treated groups), or in distilled water (control groups) for 10 min and then incubated at 27 ± 1°C for 7 days. The oviposition response of each tick species in both groups was followed. The mean mass of eggs laid by each *A. gemma* and *R. pulchellus* in the treated group and those of untreated groups was compared to estimate the efficacy of each tested acaricide. Amitraz 0.025% has significantly higher (P < 0.05) in overall mean percent oviposition control (C% = 95.47) of *A. gemma* and *R. pulchellus* than diazinon 0.06% (C% = 80.9). At this recommended concentration, both amitraz 0.025% and diazinon 0.06% were less efficient in *R. pulchellus* oviposition control (C% = 90.94 for amitraz and C% = 71.41 for diazinon) than *A. gemma* oviposition control (C% = 100 for amitraz and C% = 88.85 for diazinon). The results of the study suggested that amitraz at field recommended concentration provides better efficient *R. pulchellus* and *A. gemma* oviposition inhibition than diazinon.

**Key words:** Amitraz, diazinon, Borena pastoral area, cattle, efficacy, in vitro testing, *Amblyomma*, *Rhipicephalus*.

**INTRODUCTION**

In tropical Africa, tick and tick borne diseases (TBDs) are economically very important diseases next to trypanosomosis (Belew and Mekonnen, 2011). Among 60 tick species found infesting both domestic and wild animal of Ethiopia, 30 species have been widespread and are important parasites of livestock (Solomon et al.,

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and causes significant economic losses to the livestock industry. The economic losses incurred from downgrading of hides and skins are enormous; its export yields foreign earnings of the country, second only to coffee (Sileshi et al., 2001).

*Rhipicephalus pulchellus* (*R. pulchellus*) and *Amblyomma gemma* (*A. gemma*) are the predominant species in arid and drier land (Walker et al., 2000). Similarly, their predominance is reported in the arid and drier land of Borena pastoral area (Solomon and Kaaya, 1998; Regassa, 2001; Solomon and Kaaya, 2001).

The currently available tools for tick control consists of acaricides relying on treatment with different application methods and/or formulations, tick resistant animals, tick vaccines, TBD vaccines and management interventions. The successful implementation of rational and sustainable tick control programme in grazing animals is dependent upon a sound knowledge of the ecology or epidemiology of the parasite as it interacts with the host in specific climatic, management and production environments (Alanr, 2011). Nevertheless, not all developing countries and those in transition may have such information available, due to a lack of human, economic and infrastructural resources (Food and Agriculture Organization (FAO), 2004).

In most situations, however, efficient and reliable control of ticks and TBDs are still based primarily on intensive use of acaricides, often without the local understanding of those responsible factors for tick distribution dynamics (Brito et al., 2011). Since acaricide introduction in South Africa around 1890, tick treatment relying on different application methods have been the main method of tick control in Africa, leading to numerous problems; environmental pollution, development of resistant tick strains and escalating costs (Alanr, 2011; Brito et al., 2011). To alleviate these problems, the most frequently used techniques to detect resistance in cattle tick are: the adult immersion test (AIT), larval packet test (LPT), and larval immersion test (LIT) (Castro-Janer et al., 2009).

However, for the success of any tick management strategy, it is necessary to use a test that is practical, quick, economical and reliable to detect presence of resistance in target population (FAO, 2004). Enthusiasm for the development of standard protocols for modified AITs has been driven by the ease with which the test can be done, lack of any special equipment, and the fact that the test can be completed within only 7 days and this test mimicked the field condition better than the Shaw Larval Immersion Test (SLIT) (Jonsson et al., 2007).

Likewise, in Ethiopia, over the past decades ticks are mainly controlled by using variety of acaricides; including organochlorines, organophosphates, carbamates, amidines or synthetic pyrethroids (Sileshi, 2001; Yilma 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 Ethiopia (Adamu, 1996; Yilma et al., 2001; Sileshi et al., 2004). In Borena pastoral areas, where amitraz 0.025% and diazinon 0.06% were mostly used, in various circumstances, animal health personnel and livestock herders complained of failure of these two acaricides to kill ticks and toxicity associated with diazinon usage (Borena Zone Pastoral Area Development Office (BZPADO), 2009, 2010). Therefore, continuous studies on 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, 2001). The objectives of this study were to evaluate the efficacy of amitraz 0.025% and diazinon 0.06% against field population of engorged adult female *R. pulchilus* and *A. gemma* ticks under *in vitro* condition using AIT.

**MATERIALS AND METHODS**

**Characterizations of study area**

The study was conducted between March and April, 2012 at Borena pastoral area of Oromia regional state, Southern rangeland of Ethiopia, located at 565 km south of Addis Ababa, the capital city of Ethiopia. The region has predominantly a semi-arid climate, and physiographically, it is dominated by Savannah vegetation. Animal husbandry in the area is characterized by extensive livestock productions system and seasonal mobility. Cattles are the dominant livestock species (Cooperative for Assistance and Relief Everywhere (CARE), 2009). Cattle are heavily infested with *R. pulchellus* and *A. gemma*. Acaricide application is the main tick control methods in the area and their application seems to be regulated primarily by their availability. Cattle are treated for tick infestation whenever the farmers bring their animals to the veterinary clinics for other complaints; otherwise the pastoralists are treating by themselves. The organophosphates (diazinon 0.06% and amitraz 0.025%) are the two acaricides used by the pastoralist communities in the area to control ticks.

**Study methodology and procedures**

The fully engorged adult female ticks were collected from 17 different cattle herds at Borena pastoral areas, located roughly between 3 to 5° N and 37 to 42° E, covering an area of 120,000 km². The herds were selected on the history basis of communities’ complaints on acaricides failure. From each herd, 6 or 8 cattle, based on their infestation level with engorged adult female ticks, a total of 134 cattle, were selected. At each collecting site, cattle were restrained, and a maximum of five adult engorged female ticks of any species (as it is difficult to identify the tick species at collection site) were collected from the sampled cattle (Ducoenez, 2005). The ticks were placed in labeled plastic flasks with small holes and free of acaricide.

The choice of acaricides used was based on their commercial availability and patronage by farmers and veterinary clinic in Borena pastoral area. Thus, amitraz 0.025% was manufactured by Laboratorios Microsules Uruguay S.A. and diazinon 0.06% EC was...
manufactured by Shandong Luxi Animal Medicine Share Co. Ltd (China). The reconstitution of acaricides was done in cognizance to the manufactures recommended concentrations to be used on infested animals using distilled water. The indicated concentration for diazinon is 0.06% while that of amitraz is 0.025%. The formula, V1C1 = V2C2 was used to prepare the concentration of acaricides, where V1 and V2 are the volume of the acaricide to be drawn from the stock product and the final volume after reconstitution, respectively, C1 and C2 are the stock product concentration and the required final concentration after preparation, respectively. For all the preparations, the final volume was 1000 ml.

The bioassay technique used was the modified AIT, a laboratory protocol first described by Drummond et al. (1973) and modified by FAO (2004). At the laboratory, from the total collected engorged adult female ticks, a sample comprising of 180 ticks (ninety ticks of each species) were used for each acaricide and species of tick as described. Both species were carefully washed on a sieve using a clean tap water and dried at room temperature on absorbent paper and each species divided into groups according to their size. Three groups of ten ticks of each species were randomly formed (Group I for amitraz, Group II for diazinon and Group III for control/water), and all ticks were pasted onto double sided adhesive strips on glass petridish, with their ventral sides facing upwards. This set up was then covered and incubated in a desiccator maintained at 72% relative humidity (RH) and temperature of 27°C. Three replicates were used for each acaricide and species of tick as described.

The efficacies of the acaricides were evaluated using the egg laying test (ELT). Thus, the overall mean efficacy was then estimated from all the three replica of trials. ELT method 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.

### Data management and analysis

All collected data were entered into Microsoft Excel 2007 computer program. All statistical analyses were performed using statistical package for social science (SPSS)-Version 19 for windows 2007. Percent control (C%) for each acaricide, obtained with ELT, was used to evaluate effectiveness. Independent sample t-test was used to observe the mean C% difference between the two acaricides. A P-value less than 0.05 at 95% confidence intervals was considered for significance.

### RESULTS

The oviposition response of *A. gemma* and *R. pulchellus*, after immersion in amitraz 0.025% and diazinon 0.06% in three replicates was determined and presented on (Table 1). In the trial, none of *A. gemma* treated with amitraz laid eggs, while few of the treated *R. pulchellus* laid small batch of eggs with mean weight of 0.033 g. In contrast, both *A. gemma* and *R. pulchellus* immersed in diazinon 0.06% laid eggs with mean weight of 0.037 and 0.12 g, respectively. However, there was no statistical significance variation (P > 0.05) between the two acaricides in the overall oviposition control of each tick species (Table 2). Both tick species in the control group laid relatively large batch of eggs with mean weight of 0.33 g by *A. gemma* and 0.373 g by *R. pulchellus*.

The overall mean C% of amitraz 0.025% and diazinon 0.06%, and their respective standard deviations as well as their minimum and maximum mean efficacy during the three replica of the trial is presented in Table 3. Therefore, although, amitraz showed evidence of greatest effect on oviposition on *A. gemma*, the statistical comparison between the overall mean C% of each acaricide revealed no significant differences (t = 2.438, df= 4, 95% confidence interval (CI) = -1.5498 to 23.8498, P > 0.05). Moreover, the overall mean C% analysis of the two acaricides on the inhibition of *R. pulchellus* oviposition also showed no statistical significant variation (t = 2.26, df = 4, 95% CI = -4.48 to 43.71, P > 0.05).

### Table 1. Mean oviposition of adult *A. gemma* and *R. pulchellus* after immersion in amitraz 0.025% and diazinon 0.06% EC at recommended concentration and 7 day incubation.

<table>
<thead>
<tr>
<th>Treated ticks</th>
<th>Treatment</th>
<th>N</th>
<th>M1</th>
<th>S</th>
<th>M2</th>
<th>C%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. gemma</em></td>
<td>Amitraz 0.025%</td>
<td>10</td>
<td>2.63</td>
<td>0</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td>Water control</td>
<td>10</td>
<td>2.45</td>
<td>8.67</td>
<td>0.333</td>
<td>0.00</td>
</tr>
<tr>
<td><em>R. pulchellus</em></td>
<td>Amitraz 0.025%</td>
<td>10</td>
<td>2.83</td>
<td>0.67</td>
<td>0.033</td>
<td>90.94</td>
</tr>
<tr>
<td></td>
<td>Water control</td>
<td>10</td>
<td>2.75</td>
<td>9.67</td>
<td>0.373</td>
<td>0.00</td>
</tr>
<tr>
<td><em>A. gemma</em></td>
<td>Diazinon 0.06% EC</td>
<td>10</td>
<td>2.56</td>
<td>1.0</td>
<td>0.037</td>
<td>88.85</td>
</tr>
<tr>
<td></td>
<td>Water control</td>
<td>10</td>
<td>2.45</td>
<td>8.67</td>
<td>0.333</td>
<td>0.00</td>
</tr>
<tr>
<td><em>R. pulchellus</em></td>
<td>Diazinon 0.06% EC</td>
<td>10</td>
<td>2.79</td>
<td>1.33</td>
<td>0.12</td>
<td>71.41</td>
</tr>
<tr>
<td></td>
<td>Water control</td>
<td>10</td>
<td>2.75</td>
<td>9.67</td>
<td>0.373</td>
<td>0.00</td>
</tr>
</tbody>
</table>

N = Number of immersed female ticks; M1 = engorgement weight (g); S = number of tick survived after 7 days incubation; M2 = average egg mass per treatment group (g).
Table 2. T-test analysis of mean percent A. gemma and R. pulchellus oviposition control between amitraz 0.025% and diazinon 0.06% EC at recommended concentration.

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

C% = Percent control; N = number of trails; SD = standard deviation; NS = not significant; df = difference.

Table 3. Overall mean percent oviposition control of amitraz 0.025% and diazinon 0.06% EC at field recommended concentration against adult female A. gemma and R. pulchellus

<table>
<thead>
<tr>
<th>Acaricide</th>
<th>Min. efficacy (%)</th>
<th>Max. efficacy (%)</th>
<th>Mean efficacy (±SD%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitraz 0.025%</td>
<td>78.38</td>
<td>100</td>
<td>95.47±8.663</td>
</tr>
<tr>
<td>Diazinon 0.06% EC</td>
<td>61.11</td>
<td>97.06</td>
<td>80.09±12.537</td>
</tr>
</tbody>
</table>

Min = minimum, Max = maximum.

DISCUSSION

Several authors have studied the efficacy of amitraz on different tick populations using AIT. The results have shown different susceptibility levels. In most of the studies, amitraz revealed high degree of tickicidal efficacy that was agreed with the present finding. Similarly, a closely comparable finding, C% of 98 to 100, was reported by Sileshi (2001) at Sebeta, Ethiopia. In South Africa, Sileshi et al. (2002) also reported 100% efficacy for amitraz at the same dilution rate. Moreover, Souza et al. (2003) in Southeast Brazil obtained mean amitraz efficacy of above 95%. The minimum mean efficacy of amitraz observed in the present study (78.38%) is in accordance with that of Mendes et al. (2001) finding and he found an average efficacy of 77.44%.

In contrast, Furlong et al. (2007) found mean efficacy of 47.9% for amitraz. Camillo et al. (2009) also observed the presence of resistance with a low efficacy of Amitraz in some tick populations of RioGrande do Sul State, Brazil. In Northeast region of Brazil, a low efficacy of amitraz, with a control of 40.5 and 30.95% was reported by Santana (2000) and Campos and Oliveira (2005), respectively. In our case, we can assume that the tick populations were susceptible to amitraz.

The efficacy variation between the two presently tested acaricides might be associated with the high sterilization effect of amitraz compared to diazinon when applied at field recommended concentration (Sileshi et al., 2003). The oviposition response variation of each tick species is also most probably associated with prior exposure of these tick species to diazinon, as its usage began over the past 10 years in the study area. Sileshi et al. (2003) in South Africa observed relatively higher level of resistance to diazinon than amitraz.

The use of an acaricide at incorrect concentration is also one of the prime factors which affect the efficacy of an acaricide and causes of tick control failure (Natala et al., 2005; Kirby, 2010; Alanr, 2011). It has been observed during the study period that the pastoralists in the 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. This type of increased acaricide concentration can lead to a higher selection pressure for tick resistance (Brito et al., 2011; Pegram et al., 2000). In the present study, the two acaricides have conserved their efficacy on both tested tick species. However, a right tick control management needs to be pursued in order to avoid any resistance.

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