

## Evaluation of Amitraz and Diazinon against *Rhipicephalus decoloratus* and *Amblyomma variegatum* in Bako Agricultural Research Center

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### Abstract

Ticks are blood feeding ectoparasites that induce huge production losses in livestock industry and creating serious public health problems in the world. Although the use of chemicals is still the most effective method of tick control, uncontrolled applications may have accelerated the emergence of tick resistance to several active ingredients available. This study was conducted to assess the efficacy of commonly used acaricides (amitraz and diazinon) against *Rhipicephalus decoloratus* and *Amblyomma variegatum* collected from cattle by using Adult immersion test method. The *in-vitro* test employed an adult immersion technique. For laboratory experiment, adult ticks collected were exposed to Amitraz or Diazinon. The *in-vitro* assay showed no statistically significant tickicidal difference ( $p > 0.05$ ) between these compounds, although amitraz proved a relatively better efficacy. For both acaricides, doubled concentration was more effective in tick killing. In conclusion, comparing the efficacy of the two acaricides, amitraz is the preferable one. Furthermore, right application and choice of acaricides, avoidance of uncontrolled utilization of commercial insecticides and Strategies involving the early detection of resistance needs to be pursued in order to avoid any resistance against ticks in cattle.

**Keywords:** *Amblyomma varigaieum*; *Rhipicephalus decoloratus* ticks; Amitraz; Diazinon; Efficacy; *In-vitro* testing; Cattle

### Introduction

A wide range of internal and external parasitic diseases are found in domestic animals. Among external parasites, ticks are undoubtedly the most economically important ectoparasites of livestock on global scale [1]. The economic benefits of resolving questions about the epidemiology and control of tick-borne diseases in the vast cattle-producing areas of eastern and southern Africa, Latin America, Australia and the southern US motivated research by national and colonial governments in the affected countries plus efforts by international animal health companies to create and market products that provided a means for protecting cattle [2].

The infestation with ticks can cause vast losses in farm animal's production, due to tick borne diseases, tick paralysis and physical damage as well as to huge financial losses due to tick control [3]. The economic losses caused by the *Rhipicephalus microplus* tick are due to a reduction in meat and milk production, as well as a devaluation of leather due to skin lesions caused by high infestations and the possible installation of myiasis. This tick species is responsible for the transmission of Babesiosis (*Babesia bovis* and *B. bigemina*) and Anaplasmosis (*Anaplasma marginale*), hemoparasites that characterize the disease named Bovine Babesiosis and Anaplasmosis Complex. This tick also causes indirect economic harm due to the additional hours of work required, additional facility costs, acaricide acquisition and equipment used for its application [4].

The application of chemicals is still the most effective method of ticks' control. However, uncontrolled applications of commercial acaricides may have accelerated the emergence of tick resistance to several active ingredients available. Since acaricide introduction in 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 [5]. Likewise, in Ethiopia, over the past decade's ticks are mainly controlled by using variety of

acaricides; including organochlorines, organophosphates, carbamates, amidines or synthetic pyrethroids. 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 and in Ethiopia [6].

Repeated use of acaricides besides being the environmental hazard, it is exposed to be resisted by tick species through time, and this forces frequent application at high concentrations which is more critical to the environment. Tick acaricide resistance is reported in various parts of the countries where tick and tick borne diseases are of major problem. Since tick infestation is one of the major reported problems in the area, repeated use of acaricides is the only option in high tick seasons [6].

The most common method to control ticks is use of different types of chemical acaricides which are used in different method of applications such as dressing, spraying, systemic and dipping. The prolonged incorrect use of acaricides may cause resistance in ticks against acaricides. The resistance can be caused by numbers of mechanisms [7], and when resistant ticks survive, they pass this ability by genetic to their offspring, the higher reproductive rate of ticks that have heritable resistance factors well resulting to increase in proportion of population of tick that carry genes of acaricides resistance [8].

Ticks are of importance to veterinary medicine because they can be an annoyance, cause harm due to their blood feeding and they

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can transmit many pathologic organisms. Tick infestation and tick borne disease control is based mainly on the use of acaricides. Thus, the most widely used method for effective control of ticks is the direct application of acaricides to host animals. Tick acaricide resistance is reported in various parts of the countries where tick and tick borne diseases are of major problem. Since tick infestation and resistance to acaricides were one of the major problems in the area, repeated use of acaricides and inadequate application throughout an extended period may promote population selection of acaricide resistant of ticks in high tick seasons and increasing the resistance problem.

Therefore, the main Objective of this study was: To assess the efficacy of most frequently used acaricides for the control of ticks at Bako Agricultural Research Centre, Oromia Regional State, Ethiopia.

## Materials and Methods

### Description of study area

The study was conducted at Bako Agricultural Research Center from November 2015 to March 2016. It is situated at 258 Km west of Addis Ababa, in Gobu Sayo District, East Wollega Zone of Oromia regional state, Western Ethiopia and some 4 km away from the main high way that gains Addis Ababa to Nekemte. It is located at Latitude of 090 06'N and Longitude of 370 09'E, and an Altitude of 1650 meters above sea level. The district has an average rain fall of 886.5 mm and an average temperature of 21.2°C. The area was generally concluded as "Woina Dega" even though some of it was kola. The main rainfall season was from May to September, dry season being from December to April and their humidity was 57.83% [9].

The total area of the Bako Tibe District is about 64,469 hectares of land with animal population of 141,393 Cattle, 12,880 Sheep, 14,641 Goats, 3,795 Horses, 8,668 Donkeys, 1,054 Mules, 97,709 Poultry, 5,237 Feline and 4,894 Canine [10]. The vegetation type of the area is characterized by common savannah vegetation's like mango tree, *stetrespermum kunthiamum* (botoro), Dokma (locally), pilio stigma thonningii (wanza) acashia absinica (grar), *Carissa idylis* (agamsa) and others. The area is reach with wild game animals in main river systems and in savannah. Some of these wild animals are Apes, pigs, antelopes, Columbus monkey, baboons and others. According to the settlers and people, there are bushbucks, hyenas and others [9].

### Study population

The study was conducted on different cattle breeds of Dairy Farm at Bako Agricultural Research Centre. It was carried out by collecting ticks from the animals 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.

### Tick collection

The Farm was selected on the history basis of complaints on acaricides failure. From the Farm, the engorged adult of *Rhipicephalus decoloratus* and *Amblyomma variegatum* were collected for *in-vitro* efficacy evaluation of Amitraz 12.5% and Diazinon 60% EC. At each collecting site, the entire body surfaces of the animals were examined thoroughly and adult ticks were collected from neck/dewlap, udder, perineum/anus and legs/belly in combination from the sampled cattle. The bottles were labeled by considering the predilection sites and sampled animals. All collected ticks were examined under stereomicroscope and identified to the Genus/species level using the taxonomic key described by [11].

## Study methodology and procedures

For laboratory experiment, the engorged adult ticks were collected from Dairy Farm at Bako Agricultural Research Centre. The *in vitro* acaricidal efficacy study was conducted on two dominant tick species infesting Cattle in the area. Accordingly, *Amblyomma variegatum* and *Rhipicephalus (R.) decoloratus* were collected and exposed to Diazinon and Amitraz according to Holdsworth et al. [12] immersion technique.

The recommended (1:1000 for diazinon, 1:625 for amitraz), double (2:1000 for diazinon, 2:625 for amitraz) and half doses were prepared. 1 ml of each liquor was added on Petri dish with a filter paper fit at its bottom. Then, the acaricide was evenly distributed and 10 ticks of equal size were placed on each Petri dish and it was closed. This was done separately for each species of tick. Distilled water was used as a control. The number of ticks alive or dead was counted after 24 hours of exposure. The experiment was repeated three times for precision and mean value was taken for the analysis.

## Data analysis

The Collected data were recorded, organized, edited and analyzed using statistical package for social sciences (SPSS) Version 20. The results generated from the investigation were expressed using descriptive statistics (mean  $\pm$  standard error of mean, percentage and graphs).

## Results

The present study revealed that a number of ticks died after exposure with acaricides in laboratory at recommended, half and double doses. There was no significant difference ( $p > 0.05$ ) between two acaricides on the killing effect at any of the three different concentrations tested against both species of ticks (Table 1). Although not statistically significant, amitraz seemed to be superior to diazinon as measured by antiparasitic efficacy (%) estimation against both *Amblyomma variegatum* and *R. decoloratus*. However, both compounds appeared to have a comparable and relatively better efficacy ( $> 90\%$  at recommended dose) against both *Amblyomma variegatum* and *R. decoloratus*. Both compounds produced maximum efficacy only at their double recommended dose. The mean number of ticks died after distilled water exposure was not more than one (Figures 1 and 2). For the *in vitro* experiments, antiparasitic efficacy (AE) of each treatment was calculated using the following equation [13]:

$$AE = [B - T/B] / B$$

Where AE is the antiparasitic efficacy, B is the mean number of surviving ticks in the control, and T is the mean number of surviving ticks in treatment (Table 1).

The sensitivity of *Amblyomma variegatum* to acaricides was compared based up on the result obtained. Even though, *Amblyomma variegatum* is sensitive to both acaricides at different concentration, it is relatively more sensitive to Amitraz as shown in Figure 1 above.

The study indicated that the sensitivity of *R. decoloratus* to both acaricides was compared based up on the result obtained. *R. decoloratus* is moderately resistant to Diazinon when compared with amitraz as shown in Figure 2 above.

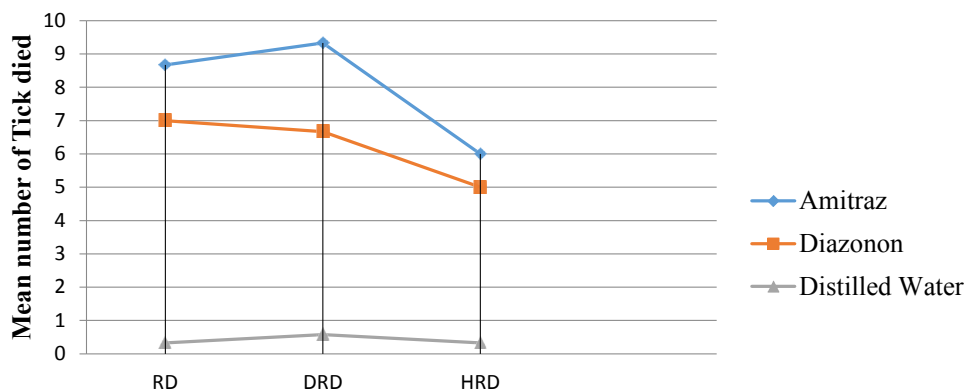
## Discussion

The present study revealed that a number of ticks died after exposure with acaricides in laboratory at recommended, half and double doses. Even though, there was no significant difference ( $p > 0.05$ )

Treated tick	Treatment	NE	MNS	MND	AE (%)
<i>Amblyomma variegatum</i>	Amitraz RD	10	1.33 ± 0.577	8.67 ± 0.577	98.52
	Amitraz HRD	10	4.00 ± 1.000	6.00 ± 1.000	95.56
	Amitraz DRD	10	0.67 ± 0.577	9.33 ± 0.577	99.25
	Diazinon RD	10	3.00 ± 1.000	7.00 ± 1.000	96.67
	Diazinon HRD	10	5.00 ± 1.000	5.00 ± 1.000	94.45
	Diazinon DRD	10	3.33 ± 0.577	6.67 ± 0.577	96.31
	Distilled Water	10	9.50 ± 0.548	0.50 ± 0.548	0
<i>R. decoloratus</i>	Amitraz RD	10	3.00 ± 1.000	7.00 ± 1.000	96.89
	Amitraz HRD	10	5.00 ± 1.000	5.00 ± 1.000	94.82
	Amitraz DRD	10	1.67 ± 0.577	8.33 ± 0.577	98.27
	Diazinon RD	10	5.00 ± 1.000	5.00 ± 1.000	94.82
	Diazinon HRD	10	6.00 ± 1.000	4.00 ± 1.000	93.79
	Diazinon DRD	10	3.33 ± 0.577	6.67 ± 0.577	96.55
	Distilled Water	10	9.83 ± 0.408	0.17 ± 0.408	0

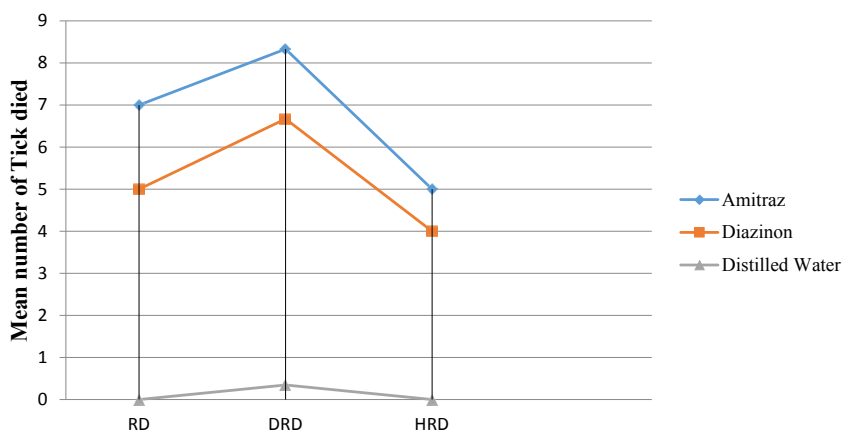
Values are mean ± SD; RD=Recommended Dose; DRD=Double Recommended Dose; HRD=Half Recommended Dose; NE=Number of Ticks Exposed; MNS=Mean Number of Ticks Survived; MND=Mean Number of Ticks Died; AE=Antiparasitic Efficacy

**Table 1:** *In vitro* ticks killing effect of Diazinon and Amitraz at recommended, Half and double doses of 24 hours post exposure of *Amblyomma variegatum* and *R. decoloratus*.



**Different Concentrations of ectoparasiticides to which *Amblyomma variegatum* was exposed**

**Figure 1:** Relative comparison of *in vitro* killing effect of amitraz and diazinon at recommended, double and half concentrations against *Amblyomma variegatum*. RD=Recommended Dose; DRD=Double Recommended Dose; HRD=Half Recommended Dose.



**Different Concentrations of ectoparasiticides to which *Rhipicephalus decoloratus* was exposed**

**Figure 2:** Relative comparison of *in vitro* killing effect of amitraz and diazinon at double, recommended and half concentrations against *R. decoloratus*. RD=Recommended Dose; DRD=Double Recommended Dose; HRD=Half Recommended Dose.

between two acaricides on the killing effect at any of the three different concentrations tested against both species of ticks, amitraz seemed to be effective than diazinon as measured by antiparasitic efficacy estimation against both *Amblyomma variegatum* and *R. decoloratus*. However, both compounds appeared to have a comparable and relatively better efficacy (>90% at recommended dose) against both *Amblyomma variegatum* and *R. decoloratus* and produced maximum efficacy only at their double recommended dose.

The present result showed that Amitraz (98.52) is more effective than Diazinon (96.67) at a recommended dose to *Amblyomma variegatum*. Present study agrees with Eshetu et al. [14] who reported that Amitraz at recommended concentration provides better efficient oviposition inhibition than Diazinon on *Amblyomma* and other ticks. However, the present study disagrees with the report of Furlong et al. [15] who found mean efficacy of 47.9% for amitraz, Santana [16] who reported a low efficacy of amitraz 40.5%, Campos and Oliveira [17] of 30.95% and Camillo et al. [18] also observed low efficacy of Amitraz in some tick populations, in Northeast region of Brazil. This difference might be associated with the method of application of acaricides.

The present finding shows that, Amitraz is the most preferable than Diazinon to control *R. decoloratus* ticks with the antiparasitic efficacy of 96.89% whereas that of diazinon is 94.82%. This finding was nearly similar with the finding of Dinka et al. [19] who worked at Borana and reported 100% efficacy of Amitraz and Souza et al. [20], in Southeast Brazil obtained mean Amitraz efficacy of 95%. The result also in consistent with the report of different authors from different areas at different time reported that the effectiveness of Amitraz over Diazinon [21]. Current study reveals that the sensitivity of *Amblyomma variegatum* to acaricides was compared based up on the result obtained. Even though, *Amblyomma variegatum* is sensitive to both acaricides at recommended and double dose, it is relatively more sensitive to Amitraz, but with no great variation on number of tick died due to the application of both drugs. Both compounds seemed to have a comparable and relatively better *in-vitro* efficacy (>90% at recommended dose) against *Amblyomma variegatum* with Antiparasitic Efficacy 98.52 of Amitraz and 96.67 Diazinon Recommended dose, after 24 hours post exposure. This finding is consistent with the report of similar result indicated by Turkson and Botchey [22] at Ghana, who reported that field strain of *Amblyomma variegatum* is resistant to organophosphates like Diazinon.

Generally, the species of ticks used in the study were compared based on their susceptibility to the acaricides used. Even though, both *Amblyomma variegatum* and *Rhipicephalus decoloratus* were sensitive to both acaricides at different concentration; they were relatively more sensitive to Amitraz than Diazinon The present study illustrated that the superiority of Amitraz over Diazinon in each concentration against both *Amblyomma variegatum* and *R. decoloratus*. The result also revealed that *Rhipicephalus* was relatively resistant to both acaricides than *Amblyomma*. This resistance might be due to regular use of limited acaricide in the area since there is high infestation of ticks. The observation of the result was after 24 hours, which could be resulted by making *Rhipicephalus* was seemed resistant to the drug.

## Conclusion and Recommendations

The present research work demonstrated that amitraz has relatively conserved its tickicidal efficacy *in vitro*, on both tested tick species than diazinon. A clue of tick insusceptibility to diazinon was noted suggesting a need to consider correct application and choice of acaricides in order

to avoid any resistance against ticks. The differences in the efficacy of the two evaluated acaricides (Amitraz and Diazinon) were most likely attributed from one of widespread, frequency, irregular application, inadequate spraying, and improper mixing of acaricides and on the use of acaricides stored for a long time after dilution.

Therefore, the following some basic management were forwarded as the recommendation to reduce the chances of developing acaricides resistance:

- Avoidance of uncontrolled utilization of commercial insecticides and dependence on limited type of acaricides and appropriate and good application of acaricides as prescribed by manufacturer.
- Use of appropriate dose of acaricides during treatment.
- Strategies involving the early detection of resistance and the use of integrated tick control are recommended.

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## Competing Interest

We declare that we have no any personal interest that inappropriately influences writing this article.

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