



**In Vitro Comparative Acaricidal Efficacy of  
Azadirachtin A and Amitraz on *Rhipicephalus*  
(*Boophilus*) *decoloratus* Larvae**

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**INTRODUCTION**

Ticks and tick borne diseases are among the most important constraints to livestock productivity in tropical regions of Africa (Uilenberg, 1982). They cause serious morbidity and mortality in susceptible exotic cattle and their crosses, limiting the genetic improvement of indigenous breeds. Tick infestation causes anaemia, secondary infections such as dermatophilosis, as well as direct loss in weight gain and milk production (Okello - Onen et al., 1994). Ticks also serve as vectors of viruses, rickettsiae, bacteria and protozoans (Jongejan and Uilenberg, 2004; Lord, 2008). In Africa, traditional control methods of ticks are based on the use of organophosphates, synthetic pyrethroids, amitraz and macrocyclic lactones (Pegram et al., 1989). However, these compounds have been partially successful due to their high cost, environmental pollution and development of resistance (Klafke et al., 2010; Fernández-Salas et al., 2012a).

Acaricide resistance is a major problem that hinders the control of the tropical cattle ticks in many parts of the world (Fernández-Salas et al., 2012b), where cattle production continues to suffer severe economic losses due to tick infestation (Barre´ et al., 2008). There is a worldwide tendency to reduce the use of

chemical acaricides because of the damage they cause to the environment and food chain (Ribeiro et al., 2011).

The use of plant products with insecticidal properties provides an alternative means with less environmental damage (Fernandes et al., 2007; Fernández-Salas et al., 2011) and public health effects, thus, there is an increasing interest in alternative anti-tick products and/or strategies based on the use of plant extracts. Natural bioactive compounds have been suggested as promising alternative for tick control (Ribeiro et al., 2007; Fernandes and Freitas, 2007; Ribeiro et al., 2011). They might offer additional advantages such as low toxicity to mammals and being more environment friendly (Batish et al., 2008, Rosado-Aguilar et al., 2010).

The neem (*Azadirachta indica* A. Juss, family: Meliaceae) is a very popular tree in most tropical and sub-tropical regions of the world. Medicinal properties resulting from antibacterial and antifungal activities have been attributed to the Neem tree (Choudhury, 2009), which contains at least 35 biologically active principles, among which Azadirachtin A, a tetranortriterpenoid, is one of the most prominent and active compound (Kraus, 2002, Morgan, 2004). Various preparations from the neem-tree have been used in pest control (Abdel-Shafy and

Zayed 2002, Hurbetus et al., 2003).

Thus this study was conducted to compare the efficacy of Neem Azal T/S 1% and Amitraz against *Rhipicephalus (Boophilus) decoloratus* larvae.

**KEYWORDS:** In vitro, Acaricidal efficacy, Amitraz, Neem, *Rhipicephalus decoloratus* larvae

## **MATERIAL and METHODS**

### **Neem Azal T/S®**

NeemAzal-T/S® was procured from Trifolio M GmbH as a 1% liquid preparation in a 2 litre container. Two fold serial dilutions were prepared with distilled water to obtain test concentrations of 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.0156 and 0.008%.

### **Amitraz (Tactic®)**

Amitraz (Tactic® 12.5% EC Intervet International B.V Boxmeer, The Netherlands) was obtained from the Small Animal Unit of the Veterinary Clinic, Federal College of Animal Health and Production Technology Vom, and was dissolved into distilled water to obtain 1.0, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156 and 0.008 % concentrations.

### **Collection of *Rhipicephalus (Boophilus) decoloratus***

Twenty five engorged female *R. (Boophilus) decoloratus* were collected from naturally infested cattle at Maiduguri cattle market, Borno State, Nigeria and were identified according to taxonomical keys of Walker et al. (2003).

They were placed in plain universal bottles plugged loosely with cotton wool for aeration and transported to the Parasitology Laboratory National Veterinary Research Institute, Vom, Nigeria. Upon arrival, and incubated under laboratory conditions of 27±1.5°C temperature and 85% relative humidity (RH) to allow for egg laying (Ica et al., 2007). After egg laying the dead female

ticks were removed from the universal bottles, and the eggs were incubated under the same laboratory conditions. Larvae that emerged from the eggs were used for the Larval Packet Test 14-16 days post hatching.

### **Larval Packet Test**

The Larval Packet Test (LPT) was conducted as described by FAO (1971) with a few

modifications. Envelopes (6 x 5 cm<sup>2</sup>) were made by folding Whatman® No 4 filter papers, sticking the edges with liquid gum (Swangum®). The envelopes were allowed at room temperature in the laboratory for 24 hours for the liquid gum to dry and the odor to evaporate. Test solution was applied uniformly to each envelope using a pipette and placed on a perforated metal tray to allow excess fluid to drain.

Between 120-130 larvae of *R. (Boophilus) decoloratus* were introduced into treated envelopes using a fine brush. The open end of each filter paper envelope was sealed with a masking tape.

The envelopes of the control group were dipped into distilled water for one minute and kept on a separate tray. Three replicates were performed for each concentration. Each treatment group was kept separately in an incubator at 27±1.5°C and 85% of RH.

Larval mortality was recorded after 24 hours post exposure, when the envelopes were opened and inspected under a stereomicroscope (Carl Zeiss Micro imaging, GmbH, Germany). Only larvae that had the ability to walk were considered alive. Tests which produced 5% or more of mortality in the control group were repeated.

### **Statistical analysis**

The effect of larval mortality between treatments was analyzed using the Kruskal-Wallis test in Statgraphics statistical program (Statgraphics 15.2.06)

and doses of each acaricide tested were compared by MANOVA test.

Lethal concentrations LC<sub>50</sub> and LC<sub>99</sub> (concentration able to kill 50% and 99% of the larvae respectively) and slopes were calculated by interpolating the mortalities obtained for different concentrations using Probit analysis (LeOra, 2003). A value of  $p < 0.05$  was considered significant.

## RESULTS and DISCUSSION

Percentage mortality and concentration-mortality data of NeemAzal®-T/S and Amitraz on *R. (Boophilus) decoloratus* larvae are summarized in Tables I and II respectively. A dose dependent effect of both the compounds was evident on the larval mortality *In vitro*. There was no statistical difference between groups ( $p > 0.05$ ) at various concentrations. At the lowest concentration (0.008), 9.4% and 15.0% larval mortality was recorded while at 1% concentrations of NeemAzal®-TS and Amitraz, larval mortality was 91.4%

and 97.3% respectively ( $p < 0.05$ ) (Table I). The LC<sub>50</sub>, LC<sub>99</sub> and slopes for the three chemical tested are shown in Table II.

Neem tree extracts have been used to control crop and animal pests worldwide (Choudhury, 2009). Repellent and pesticidal properties have been demonstrated when different parts of the plant were used, and these qualities have been attributed to the different biologically active compounds (Kraus, 2002, Morgan, 2004). One of the most important is Azadirachtin A, which appears as a principal molecule in a new product Neem Azal®-T/S (Trifolio-M Lahnau GmbH, Germany) widely used for tick control in Nigeria. The toxicity of neem seed oil at concentrations of 20-100% against the larvae of *R. (Boophilus) decoloratus* in Nigeria has been documented with 100% mortality in 24-27 hours post exposure (Choudhury, 2009).

Table I: Effects of acaricides on mortality of *Rhipicephalus. decoloratus* larvae 24 hours post larval packet test

NeemAzal T/S <sup>®</sup>				Amitraz			
D (%)	MM	ML	PM	D (%)	MM	ML	PM
1	117	11	91.4a	1	110	3	97.3a
0.5	98	26	79.0a	0.5	100	10	91.2a
0.25	87	43	67.1a	0.25	54	51	51.4a
0.125	50	56	47.2a	0.125	71	49	59.2a
0.0625	21	98	17.6a	0.0625	45	94	32.4a
0.0312	18	96	16.1a	0.0312	25	105	19.2a
0.0156	28	92	23.3a	0.0156	30	96	23.8a
0.008	11	106	9.4a	0.008	17	96	15.0b
C	5	109	4.4a	C	3	109	2.5a

D- Dose; MM- mean mortality; ML- Mean live; PM- Percent mortality; C-control group

Different letters between lines indicate significant statistical difference.

Table II: Comparison of Lethal Concentrations ( $L_{50}$  and  $L_{99}$ ) of Neem Azal T/S<sup>®</sup> and Amitraz tested against *Rhipicephalus decoloratus* larvae 24 hours post larval packet test.

Acaricide	N	$\chi$ (df)	Slope (std error)	$LC_{50}$ (95% confidence level)	$LC_{99}$ (95% confidence level)
NeemAzal <sup>®</sup>	132	106.85 (22)	1.320 (0.044)	0.130 (0.105-0.163)	7.522 (4.141-6.725)
Amitraz	115	144.83 (22)	1.290 (0.045)	0.096 (0.075-0.125)	6.099 (2.991-16.890)

n: sample;  $\chi$ :Chi square; (df): degree freedom; std error: standard error; LC: lethal concentration.

For this, the objectives of the present study were to validate the dose of NeemAzal<sup>®</sup>-T/S 1% in its use against *R. (Boophilus) decoloratus* larvae from Nigeria and to compare its mortality results with those obtained with a chemical acaricide Amitraz used in tick control in Nigeria.

Reports of bioactive compounds from plant extracts with larvicidal activity against *Hyalomma anatolicum excavatum*, *Rhipicephalus sanguineus* and *Rhipicephalus microplus* have been documented (Abdel-Shafy and Zayed 2002; Fernandes et al., 2007; Fernandez-Salas et al., 2011; Ribeiro et al., 2011).

In vitro laboratory comparative bioassays from this study revealed similarity in mortality of *R. (Boophilus) decoloratus* larvae between Amitraz and Neem Azal<sup>®</sup>-TS, a new prospective bioactive product for tick control in Nigeria.

A dose dependent effect of both the compounds was evident on the mortality of *R. (Boophilus) decoloratus* In vitro. Although Amitraz appeared to be more potent than Neem Azal<sup>®</sup>-TS, the difference was not statistically significant except at the lowest concentration of 0.008%. The  $LC_{99}$  of Amitraz and Neem Azal<sup>®</sup>-TS were similar in this study which indicates the potential for the use of Neem Azal<sup>®</sup>-TS for tick control.

A 100% mortality of *H. anatolicum excavatum* larvae was reported with Neem

Azal<sup>®</sup>-TS at 12.8% concentration in 48 hours, and 72-100% at 1.6 % in 24-72 hours (Abdel-Shafy and Zayed, 2002). It appears a longer contact time between Neem Azal<sup>®</sup>-TS and the tick is needed to cause high mortality, a situation not quite feasible during the rainy season in Nigeria where animals graze on open fields exposed to rain which may wash off the chemical.

Neem Azal<sup>®</sup>-TS seemed to be less potent, but have good acaricidal effect against *R. (Boophilus) decoloratus* larvae at high concentrations. The biological activities of Neem Azal<sup>®</sup>-TS have been related to medicinal properties of Azadiractin A, a seed extract that acts against ectoparasites (Hurbetus et al., 2003). What is not known however is whether the ticks can develop resistance to low concentrations of Neem Azal<sup>®</sup>-TS preparations, a problem with most chemical acaricides.

In conclusion, the result of this study shows that a natural plant product Neem Azal<sup>®</sup>-TS has a comparable efficacy against *R. (Boophilus) decoloratus* larvae compared to Amitraz.

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