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The acaricidal effect of the essential oil of *Ageratum houstonianum* Mill. flowers on ticks (*Rhipicephalus lunulatus*) in Cameroon

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Abstract

The acaricidal effect of the essential oil of the flowers of *Ageratum houstonianum* on *Rhipicephalus lunulatus* was tested in the Laboratory of Parasitology of the University of Dschang in the Western Highlands zone of Cameroon. Five doses of the essential oil (0.00, 0.016, 0.031, 0.062 and 0.125 µL/cm^2) in four replicates were used. Each replicate consisted of 10 ticks in a Petri dish with filter paper on the base of the dish uniformly impregnated with the product. The results of this study indicate that the essential oil of the flowers is toxic to *lunulatus* ticks. The mortality after six days of exposure in the control group was 10% while the tested doses had exterminated the ticks. The LD_{50} was 0.06653 µL/cm^2 at the end of the first day, indicating a potentially high efficiency of this product on this parasite.

Keywords: *Rhipicephalus lunulatus*, *Ageratum houstonianum*, acaricide, essential oil, flowers, LD_{50}, West African Dwarf goat
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Introduction

Ruminant husbandry constitutes one of the principal farming activities in most African countries in general, and in Cameroon in particular. Unfortunately, these animals depend essentially on natural pasture for their diet and are exposed to numerous constraints including disease, which affect growth (Lhoste *et al*., 1993; Pamo *et al*., 2003). Among these diseases, parasitic infestations are very common in the tropics where the environment provides favourable conditions for their development (Ukoli, 1984; Pamo *et al*., 2000). External parasites such as arthropods especially ticks feed on blood and transmit many diseases (IEMVT, 1989) which are complex to diagnose.

The control of ticks under commercial production systems is through the use of acaricides while small-holder farmers generally remove the ticks manually or use medicinal plants. Some tick populations have developed resistance to most acaricides (Barnett, 1961; Wharton, 1976; Hall, 1982; Ukoli, 1984). The large-scale use of synthetic insecticides has resulted in serious negative effects on man, animals and the vegetation (Knipling, 1952; Fivaz *et al*., 1992) with enormous consequences to the agricultural environment. The effects of the parasites on animal hinder the development of cost-effective animal production in environments where they occur and can severely affect profitability. Losses due to ticks in both tropical and temperate environments have been estimated to amount to several hundred million dollars per year (Soulsby, 1982). It is therefore necessary to look for alternative measures which are adaptable and less expensive, especially for the subsistence farmer with limited capital who constitutes the majority of animal rearers in Cameroon as well as in most of the sub-Saharan region.

The bark, fruit, roots and flowers of many plants contain therapeutic substances such as tannins and alkaloids (Lhoste *et al*., 1993). Essential oils have various biological activities including healing, antiseptic, anti-inflammatory, antipyretic, antispasmodic, insecticidal, bactericidal and fungicidal characteristics (Kuiate, 1993; Pamo *et al*., 2003). Herbal medicine is a cheap form of therapy and many plant products have been reported to be very effective (Lhoste *et al*., 1993). In view of this, a research programme at the University of Dschang, Cameroon, was initiated to study the possible acaricidal effects of some local plants.

Previous works carried out at the University of Dschang indicated that the essential oils of flowers of *Ageratum houstonianum* were toxic to insects of crops in storage such as *Tribolium confusum* and *Collosobruchus maculatus* (Pidjou, 1999). In addition, the bark of this plant is traditionally used to cure several diseases. The results which are reported here arose from an investigation into the effects of essential oils from *A. houstonianum* flowers on ticks (*Rhipicephalus lunulatus*) of West African Dwarf goats.

Materials and Methods

The work was carried out in Dschang which has an altitude of about 1420 m, 5°26 N and 10°26 E. The climate is typically equatorial with a long wet season (mid March -to mid November) and a short dry season (mid November to mid -March). The flowers of *A. houstonianum* were collected in the University of Dschang Campus and its surroundings. They were transported to the analytical chemistry laboratory for extraction.

The flowers of *A. houstonianum* were put into a 2 L water flask containing distilled water. Hydrodistillation was carried out for 10 hours using a modified Clevenger apparatus. The essential oil collected was dehydrated with anhydrous sodium tetrasulphate and the resulting clear yellowish product with a strong and persistent odour was preserved at room temperature in the dark. The principal constituents of the essential oil were identified by GC-MS using a HP 5890 II gaseous phase chromatograph coupled with a HP 5972 mass selective spectrophotometer and a DBwax capillary column (60 m x 0.25 mm). The furnace was regulated at temperatures from 60 ° to 220 °C at a speed of 5 °C per minute. Helium was the gas vector, the rate of flow was 0.9 mL per minute, the injection temperature 230 °C and that of the interphase 240 °C.

The different volatile components were identified by comparing their mass spectra and/or their retention rate to the standard reference of the Institut Für Getreide Verarbeitung Gmbh of Berlin, Germany. The quantification of each component was determined by integrating its peak on the spectrum of the gaseous phase chromatograph.

Ticks of the genus *Rhipicephalus*, which infest ruminants in the Western Highlands of Cameroon, were carefully collected from the goats in the University of Dschang Experimental Farm and the surroundings. The ticks collected were approximately 4.09 mm in length, flat and had not gorged. They were fixed with ethyl acetate and identification was carried out as described by Walker *et al*. (2002).

After several preliminary tests, doses were adopted for use and prepared by diluting in 1 mL of acetone 1, 2, 4 and 8 µL of the product. Each dose was uniformly spread with the aid of a micropipette on a round Whatman filter paper (63.62 cm^2). After complete evaporation of the solvent after 20 minutes, 0.016, 0.031, 0.062 and 0.125 $\mu L/cm^2$ doses were obtained on each filter paper. Filter papers impregnated with solvent (acetone) were used as control.

Each treatment dose consisted of four replicates, each made up of 10 non-sexed ticks selected randomly and introduced into a Petri dish. The toxic effect of the essential oil on the ticks was evaluated in the laboratory at 24 °C and 70% relative humidity. Daily mortality rates for eight days were calculated using the Abbott formula (Abbott, 1952).

$$M_c = \frac{M_o - M_t}{100 - M_t} \times 100$$

M_c = Corrected mortality rate
M_o = Mortality rate in treated dishes
M_t = Mortality rate in control dish (natural mortality)

Analysis of variance (McClave & Dietrich II, 1979) was carried out on the data and differences between treatments, when significant, were separated using the Duncan Multiple Range test. The logarithmic regression of the doses and the probit were used to determine the LD_{50} of the product.

Results and Discussion

The extraction yield of the essential oil was 0.2% and the principal chemical components are presented in Table I. Precocen I (48.01%) and Precocen II (36.55%) are the major substances in the oil. Figure 1 summarises the corrected cumulative mortality of *R. lunulatus* for the different doses during the experimental period. The mortality increased with increased doses and also in the course of time (day), reaching a maximum of 100% on the fifth day with the group on the highest dose (0.125 $\mu L/cm^2$) and sixth day with the group with the lowest dose (0.016 $\mu L/cm^2$).

Table 1 Principal chemical constituents of the essential oil in *Ageratum houstonianum*

Name of components	Percentage (%)
Demethoxy ageratochromene (Precocen I)	48.01
Ageratochromene (Precocen II)	36.55
β-caryophyllen	8.37
Germacrene D	2.34
Acetate of bornyl	2.29
β-cubebene	1.22
β-farnesene	0.66

The essential oil of *A. houstonianum* flowers was toxic to *R. lunulatus*. On the fifth day of exposure, the highest doses had killed all the ticks, producing a significantly ($P < 0.05$) different result between the group with the highest dose (0.125 µL/cm^2) and the other treatment group. There was no significant ($P > 0.05$) difference in the mortality observed between the groups on 0.016 µL/cm^2 and 0.031 µL/cm^2 doses. The same result was recorded between the mortality recorded with doses 0.031 and 0.061 µL/cm^2. Irrespective of the dose used, the mortality observed among the treatment groups on the fifth day was significantly ($P < 0.01$) higher than that of the control group.

The transformation in the profit of mortality rates after the first day of exposure was determined and the regression of the data obtained based on the logarithm of the doses gave the following equation:

$Y = 1.1641x + 6.3701$ ($R^2 = 0.9752$) and the LD_{50} calculated was 0.06653 µL/cm^2.

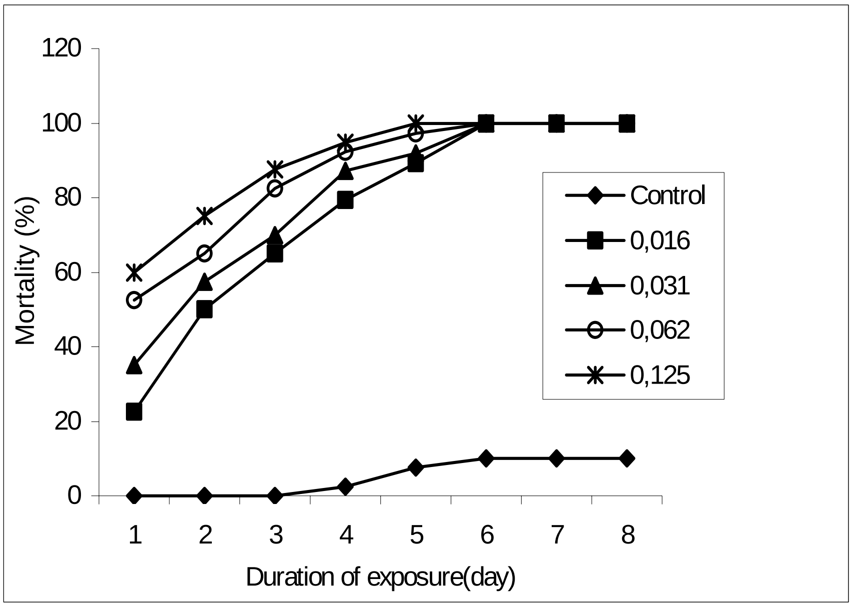

Figure 1 Effect of essential oil (µL/cm^2) of *Ageratum houstonianum* on *Rhipicephalus lunulatus*

According to the chemical analysis (Table I) Precocens I and II are the most important components of the essential oil. The toxicity of the oil to *R. lunulatus* may be attributed to these two substances or to a synergistic interaction between these components and the other constituents of the essential oil. These results are in agreement with those of Pamo *et al.* (2003) who worked with the leaves of the same plant. Bowers *et al.* (1976) noticed that adult insects coming into contact with the Precocens became sterile while there is early metamorphosis among the immature insects and immediate death of the premature adults. It is possible that these compounds have similar actions on the ticks. The metabolism of insects activates the Precocens leading to production of epoxyde which reacts with the proteins of "corpora allata", destroying them. The

Precocens are highly specific chemical substances, which attack certain areas of the insect endocrine system causing not only toxic effects but also disturbing the development process and reproduction.

Conclusion

It appears that the essential oil from *A. houstonianum* flowers has a toxic effect on ticks of the genus *Rhipicephalus*. The mortality rate increases with dose and time. Irrespective of the treatment there was 100% mortality after six day of exposure. The LD_{50} calculated was 0.06653 µL/cm^2 on the first day of exposure.

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