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Acaricidal Effect of Foam Soap Containing Essential Oil of Ocimum gratissimum Leaves on Rhipicephalus lunulatus in the Western Highland of Cameroon

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I. Introduction

Breeding of ruminants constitutes one of the main productions activities in many African regions in general and in particularly in Cameroon (Pamo et al., 2002). In Cameroon, small ruminants are used as source of income and for many other purposes almost every where in the country (Pamo et al., 2001; Pamo etal., 2004). Specifically, goats for breeders are easily mobilizable investment, having a very short development cycle (Lhoste et al., 1993). Following these considerations, it appears necessary to set interested in the breeding condition of goats in this zone where the demographic and hygienic conditions are not favorable for breeding activities.

Goat breeding is slowed down by various factors including nutrition; diseases and also ticks infestation which are the most important (Pamo et al., 2005; Tendonkeng et al., 2010). This Rhipicephalus lunulatus tick has been reported to be a common ectoparasite of goats in Cameroun and the surrounding countries (Pamo et al., 2005).

In fact, ticks in general and Rhipicephalus lunulatus are one of the main causes of mortality in farm animals (IEMVT, 1989). They are also responsible for secondary infections which could be bacterial, viral or protozoa related (Soulsby, 1982). Furthermore, each of the conventional methods of tick control is quite costly and environmentally unfriendly (Pamo et al., 2005). Attention is then shifted towards natural substances with therapeutic properties like essential oils extracted from some plants. Indeed, a substantial part of plants (leaves, fruits, flowers, stems and roots) contain antiseptic, anti-inflammatory, insecticidal, bactericidal healing substances (Kuiate, 1993). The acaricidal effect of essential oils of many plants has been documented in many studies (Pamo et al., 2002; Pamo et al., 2003; Pamo et al., 2005). An important example is Ocimum gratissimum whose leaves were shown to contain essential oils which make them to be sometimes irritating and toxic (Daget & Godron, 1995; Tapondjou et al., 2002; Pamo et al., 2002). Those previous studies then bring up the problem of conditioning theses essentials oils in better way for their efficient utilization for on farm ticks control. This study is therefore aimed at finding an efficient, cheap and easily applicable method of using essential oils to fight against ectoparasites in general and ticks in particular.

II. Materials and Methods

a) Extraction of O. gratissimum essential oil

Fresh leaves of O. gratissimum were harvested and sun dried for 3days. Extraction of essential oil was done by hydrodistillation (Kuiate, 1993). Two kilograms

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of dried leaves were soaked in 6 l of water and boiled for 10h in the modified Clavenger vendor. The evaporate was collected in an open mouth bottle and filtered

The yield of essential oil was calculated using the following formula:

$$\text{yield} = \frac{\text{weight of essential oil}}{\text{weight of O. gratissimum leaves}} \text{x100}$$

b) Collection and identification of ticks

Male and female R. lunulatus ticks, frequently found on ruminants in the highlands of west Cameroon, were collected by manual removal without breaking their rostrum. These ticks were fixed in ethyl acetate and identified as R. lunulatus according to Walker et al. (2002). To have an uninfested goat population, 10 West African dwarf goats were examined and all ticks removed. Another manual removal of ticks was done 30 days later. A total of 72 ticks were collected from the 10 goats. The average weight of engorging ticks was 0.5±0.1g and the average length was 6.5±0.4 mm. These two parameters (weight and length) were used in the tests.

c) Preparation of medicated soap

Palm oil liquid soap was used as vehicle for the essential oil. A volume of 900µl of essential oil was added to 450 g of liquid soap to obtain a concentration of 2µl/g base on which all doses were prepared. The solution was poured into the molds and allowed to solidify.

d) In vitro study

R. lunilatusticks were collected from various West African Dwarf goats and identified. A disc of N°1 Whatman filter paper measuring 62.63 cm^2 surface area were soaked with soap of various concentrations of the essential oil (0.00; 0.04; 0.06; 0.08; 0.10 µl/g) and placed in clean dry Petri dishes with four replicates each at room temperature (24°C, humidity 70%). Ten ticks randomly placed in each Petri dish and covered. The plates were examine each morning during 8 days and dead ticks, if any, were counted and removed. One soap sample without essential oil served as control. The mortality rate of the tick was calculated as described by Abott (1925), and lethal dose 50 (LD_{50}) was calculated according to Valette (1972).

$$Mc = \frac{M0 - Mt}{100 - Mt} X100$$

Where: Mc is the accrued and corrected death rate, M0 the death rate in Petri dishes treated and Mt the death rate in the control Petri dish (natural mortality).

e) In vivo study on the acaricide property of essential oil

The number of R. lunulatus ticks was counted at the preferential sites (ears, tail and head) (Tenekeu, 2002) on each of the 40 goats selected in this study. Soap with four concentration of essential oil selected through an anhydrous sodium sulphate column to eliminate the trace of water present in the essential oil. The oil was stored in the dark at room temperature. after in vitro test (0.00; 0.06; 0.08; 0.10 µl/g) was applied on batches of 10 goats. The preferential sites were examined for the number of live/dead ticks every 24h for 8 days. Three replicates of 10 goats/group/dose (120 goats) were carried out during on-farm trial. Ticks removed by engorgement were not taken into consideration during analysis.

f) Statistical analyses

The cumulative and corrected mortality percentages were submitted to analysis of variance (Mc Clave and Dietrich, 1979) and the differences between the treatments were analyzed by student's t- test.

III. Results and Discussion

a) In vitro study

The yield of the oil extraction was 0.62 %. This yield was higher than that obtain by Pamo et al. (2003) which was 0.50 %. This difference can be explained by many factors including the distillation method and the period during which the plant was harvested.

All the three concentrations tested in the study showed some acaricidal effect on R. lunulatus. Efficiency increased as the concentration of essential oil increased and with the duration of exposure. The highest dose (0.10 µl/g) killed all ticks by day six of exposure while, the lowest dose (0.04 µl/g) caused 80 % of mortality within the eighth day of the trial. By the end of the study, control dose killed only 30% of ticks (Figure 1). This study established that the essential oil obtained from O. gratissimum, and incorporated in soap as carrier was toxic to ticks, and toxicity was directly proportional to the concentration of the essential oil in the soap. The toxicity of these soaps containing essential oil can be mainly attributed to the predominance of Phenolics and Terpenoics compounds present in the essential oil. The main compound of this essential oil is Thymol. This compound is a highly selective chemical substance attacking specific aspects of the endocrine system of insect, thus inducing a toxic effect (Ojimelukwe and Alder, 1999). The high toxicity of this essential oil suggest, that Thymol, known for his insecticidal and acaricidal effect (Tapondjou et al., 2003; Tapondjou et al., 2005; Ndomo et al., 2009) react in synergy with other monoterpens like y-terpinen, p-cymen, terpenoids like β-caryophyllen, and eugenol which insecticidal activities have been documented (Tchoubougnang, 1996; Obeng-Ofory et al., 1997; Tapondjou et al., 2005; Ndomo et al., 2009). Action of phenolics compound such as linalool and y-terpinol is also important. These substances are recognized as important insecticides, fungicides and bactericides (Hassanali et al., 1990; Obeng-ofory et al., 1997). This reality was confirmed by Pamo et al. (2003)

whodemonstrated the acaricidal effect of crude essential oil fromO. gratissimum leaves. Finding from these studies showed that these essential oils which were mainly rich in monoterpen in general and in thymol in particular can induce not only toxic effect but also metabolic disorder in R. lunulatus that may impact their development and reproductive process. The high mortality record in the control group can be explained by other soap components such as soda.

The regression equation derived by comparing the average cumulative mortalities with the concentration of essential oil ($Y=656.25X+11.455$; $R^2=0.9213$; Figure2) revealed that 97.96% of the correlation with mortality doses could be assigned to the concentration of the essential oil.

The adjustment of the average cumulative mortality percentages with doses in time led to the regression equation: $Y= 656.25X +11.455$ ($R^2=0.9213$).

Following the transformation of the mortality percentages to probits at the end of the second day of exposure, the regression line $Y=5.1015X+10.19$ ($R^2= 0.9621$) showed that after 2 days of exposure, the LD_{50} was 0.061μl/g. These confirm the degree of toxicity of soap containing essential oil of O. gratissimum on R. lunilatus.

b) In vivo study

The cumulative mortality of ticks was significantly higher in all treatment groups than the control by third day after treatment. This continued to increase with time in each group. By the end of study, the mortality was almost more than three time higher in group 2 and 3 and four time in group 4 as compared to control (Figure 3). Accordingly, soap containing essential oil at the rate of 0.10μl/g would be effective in completely eliminating the R. lunilatus ticks on goat within a week.

The regression equation ($373.63X+5.473$, $R^2= 0.999$) on mortality versus concentration of essential oil suggests that increasing concentration of essential oil in the soap had increasing effect on ticks.

Similarly, the regression line ($Y= 3.0771X+6.5929$, $R^2= 0.9868$) suggest that by the second day of the treatment, the LD50 was 0.066μl/g. There was no appeared side-effect of the medicated soap application on behaviors and/or health of goats. The in vivo mortalities were relatively lower as compared to those achieved in vitro with LD_{50} of 0.061μl/g. This could be due to the fact that on-farm, the application of medicated soap was carried out on ticks that were allowed to continue feeding. In the laboratory the ticks were not fed, and thus were under stress. This could have weakened them with regard to synergetic activity of the ingredients of the medicated soap.

IV. Conclusion

The essential oil of O. gratissimum is toxic to R. lunilatus ticks, both in vitro and in vivo. The toxic effect of the medicated soap containing the essential oil on ticks increased as the concentration of essential oil increased and persisted during the entire period of the study. A low LD50 (0.061μl/g for in vitro and 0.066μl/g for in vivo application) of the essential oil suggests that it bar potential or acaricidal agent for R. lunilatus ticks. Further studies on the chemical nature of the components of essential oil as well as the separation of the different fractions would improve our Knowledge and understanding. Identification of the active ingredient in the essential oil would improve quality and efficiency of the product. The effects of this essential oil on other tick species need to be studied. Likewise, the effect of repeated application of the medicated soap on adults ticks and their others life stage needs some evaluation.

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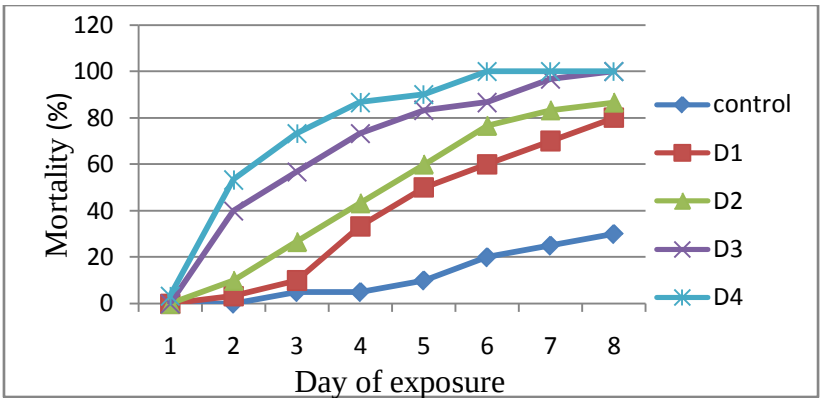

Figure 1 : Cumulative ticks mortality (%) following in vitro treatment with soap containing different doses of O. gratissimum leaves essential oil

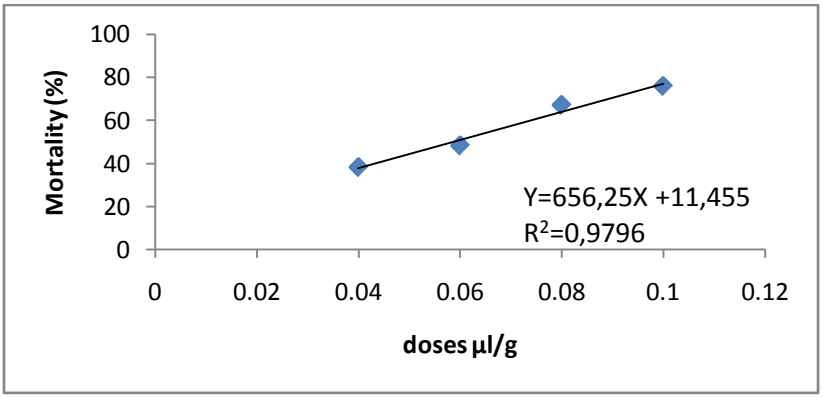

Figure 2 : Regression line of the cumulative mortality of R. lunulatus and concentration of essential oil

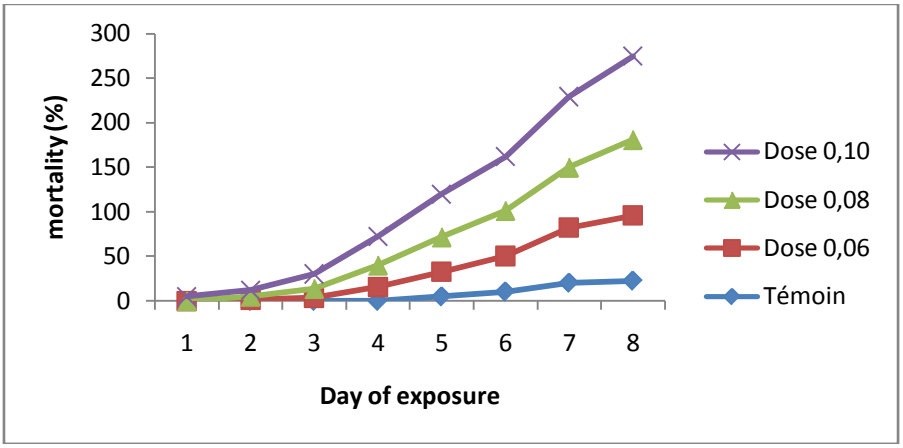

Figure 3 : Cumulative ticks mortality (%) following in vivo treatment with soap containing different doses of O. gratissimum leaves essential oil