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RESEARCH ARTICLE

In vitro evaluation of the acaricidal effect of three essential oils extracted from aromatic local plants on *Rhipicephalus (Boophilus) microplus* ticks in Benin.

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Abstract

Acaricidal activity, of essential oils extracted from the leaves of *Chenopodium ambrosioides*; *Ocimum canum* and *Citrus aurantium* in Benin, was tested *in vitro* on *Rhipicephalus (Boophilus) microplus* cattle tick using the test contact. The results showed that all these oils have a positive effect on *Rhipicephalus (Boophilus) microplus*. However, the effect of *Chenopodium ambrosioides* essential oil was stronger than that of the two other plants: 100% mortality at (0.25µl/cm^2) compare to 60% (0.25µl/cm^2) for *Ocimum canum* and 43% for (0.25µl/cm^2) *Citrus aurantium* respectively. The value of LD50 obtained was: 0.02µl/cm^2, 0.06µl/cm^2, and 0.75µl/ cm^2 for *Chenopodium ambrosioides*, *Ocimum canum* and *Citrus aurantium* essential oil respectively. Analysis of the variance of the daily cumulative mortality data in relation to the dose of essential oil used showed significant differences at 5% threshold. Thus, the use of *Chenopodium ambrosioides'* essential oil as an alternative control method of *Rhipicephalus (Boophilus) microplus* could be consider as for the development of cattle breeding in Benin.

INTRODUCTION

Ectoparasites infestation remains to be a crucial problem for livestock development in tropical countries **Bianchin *and al.* (2007)**. *Rhipicephalus (Boophilus) microplus* tick is one of the most dreaded parasites. Harm caused to animals include: weight loss, decrease of milk production, anemia, skin injury and in addition transmission of pathogen agents such as: *marginal Anaplasma*, *Babesia bovis* and *Babesia bigemina* **Hildebrandt *and al.* (2010)**. Commercial acaricides is currently the most used tick control tool. However, due to their extensive use, some species of ticks have developed resistance against main classes of available acaricides **Rodriguez-Vivas *and al*. (2007); Klafke *and al.* (2010); Rosado-Aguilar *and al.* (2010a)**. Moreover, these commercial acaricides are usually toxic for humans and the environment. In addition, these products are expensive, and their use is harmful for non-target species **(Sarda and al. (2007)**. Due to all these factors, synthetic acaricides are less regarded as an efficient tick control mean. Nowadays more study focus on developing novel control methods from plant extracts.

Indeed, the plant extracts contain mixtures of substances that may act synergistically, in different ways, what makes the development of resistance more difficult than in the case of classic acaricides **Chagas *and al.* (2011)**. For **Katoch *and al***., the efficiency of an ectoparasiticide can be improved by a judicious combination with another plant or an active ingredient that has additive properties **Katoch *and al.* (2006)**. Moreover, Africa in general and Benin in particular is full of important reserve of plants used in veterinary ethnomedicine that need to be promoted **Ogni *and al.* (2014)**. Thus, the present study aims to test the acaricidal effect of essential oils from *Chenopodium ambrosioides (L.); Ocimum canum (Sims)* and *Citrus aurantium* leaves on cattle tick *Rhipicephalus (Boophilus) microplus* in Benin.

1- Material and methods

1.1- Biological Material

The aerial part of *Chenopodium ambrosioides* (L.); *Ocimum canum* (Sims) and *Citrus aurantium* were essentially used in this study. These plants, after being identified and confirmed by the Benin National Herbarium, were respectively harvested in July 2014 in Dassa-Zoumé and Savalou (*Chenopodium ambrosioides, Ocimum canum*) and in August 2014 in Klouékanmè (*Citrus aurantium*). Plant treatment and essentials oil extraction was performed in the Applied Chemistry Research and Study Laboratory (LERCA) of the University of Abomey - Calavi.

1.2- Extraction of essential oils

The extraction of each of the essential oils was performed by steam distillation in a Clevenger device (1928) for about 5 hours. The various oils extracted were dehydrated using anhydrous sodium sulphate and stored in shaded glass bottles tightly sealed, covered with foil and stored at 4 ° C.

1.3- Collection and packaging of ticks

Male and female ticks of *Rhipicephalus (Boophilus) microplus*, were collected from the experimental farm of the department of animal production and health using forceps without breaking their rostrum and stored in perforated transparent bottles(1.5 to 2mm diameter).All the following test were conducted in the research unit of biotechnology in animal production and health. These ticks in a low state or null repletion had an average size of 4.7 mm and an average weight of 4.25mg.

2. Biological tests

- **Preparation of essential oil doses**

The concentrations used were determined according to an arithmetic progression after several preliminary tests for each of the active ingredients. Indeed, six doses of each essential oil were retained by diluting each time in 1 ml of solvent (acetone) successive volumes of 0; 1; 2; 4; 8 and 16 µl of each essential oil in test tubes. The contents of each tube was homogenized by stirring and each dose was uniformly spread on a filter paper washer of Whatman type No.1 of9 cm of diameter (63.6 cm^2) placed in a Petri dish of same diameter. After complete evaporation of the solvent, respective doses0.00; 0.016; 0.031; 0.062; 0.125 0.25µl / cm^2 were obtained on each filter paper. The dose of 0.00 represents the control dose made of solvent only.

- **Tests**

Tests were performed in vitro at 24ºC and 70% of humidity. They aim at evaluating the acaricidal effect of the three essential herbs putting them in direct contact **(Pomo *et al.*, (2003))** with *Rhipicephalus (Boophilus) microplus*. On the basis of the results of preliminary studies conducted on the viability of ticks in low repletion state separated from their host, we choose here to expose the ticks to different essential oils for 3days. Each treatment (dose) includes 3 repetitions and each repetition contained 10 active non sexed ticks put into petri dishes at various concentrations. The counting of dead ticks was done every 24 hours during the three days of exposure. The mortality rate in each box was calculated using Abbott's formula **(Abbott, 1925).**

$$Mc = \frac{Mo - Mt}{100 - Mt}$$

Mc = Corrected Mortality
Mo = Observed Mortality
Mt = Control Mortality

3. Statistical analysis of data

Data analysis was performed with the R Core Team (2014) software. For mortalities estimation and their standard deviations according to the doses and the number of days, we used the command "LSD. Test “of“agricolae " package after an analysis of variance by the "aov".

The average cumulative mortalities are obtained by the same approach. The probits are obtained with the "predict.glm" function after a function logistic regression of link "probit".
The lethal dose was determined by the function "dose.p" of "MASS" package and linear regression coefficients by the "lm" function of "Stats" package which also gives the R^2.

4- Results

4.1- Effect of essential oil of *Chenopodium ambrosioides* leaves on *Rhipicephalus (Boophilus) microplus*.

Table I showed the percentage of corrected mortality of *Rhipicephalus (Boophilus) microplus* subjected to different doses (µl/cm^2) of essential oil of *Chenopodium ambrosioides* according to the time. We found out that higher was the dose of essential oil tested, higher was the percentage of tick mortality over time. We reached 100% of ticks' mortality with the highest dose (0.25µl / cm^2) on the first day. The smallest dose of essential oil (0.016µl / cm^2) caused its highest ticks 'mortality percentage on day 3(57%).
The adjustment of the average cumulative mortalities percentages based on the doses of essential oil of the leaves over time allowed to get the following regression equation: $Y = 0.06X + 1.1$with $R^2 = 0.98$(Table IV).This result that is related to the R^2, a determination coefficient, indicates that 98% of the test effect variation can be explained by the regression line. Hence, it appears that most of the cumulative mortalities are due to the effect of the different doses.
The analysis of the variance of the daily cumulative mortality data according to the dose of essential oil showed a significant difference of their effect at 5% threshold.
On the first day, there was a significant difference between the mortality recorded in the treatment groups and the control group ($P>0.05$). Likewise, mortality recorded with the highest dose (0.25µl / cm^2) was significantly different compare with those recorded with doses 0.016; 0.031 and 0.062µl / cm^2 respectively ($P>0.05$). However, there is no significant difference between the mortality recorded with the highest dose and that recorded with dose0.125µl / cm^2.
On the second day, excepted the group of the lowest dose (0.016µl / cm^2), there was a significant difference between the mortality recorded in the 3 treatment groups and the control group ($P>0.05$). In the other hand, mortality observed in the group of the highest dose (0.25µl / cm^2) was significantly different from the one recorded in the group of doses 0.062µl/cm^2; 0.125µl/cm^{2}and 0,016µl/cm^2 respectively. Likewise both mortality recorded in the group of doses 0.062µl/cm^2 and 0.125µl/cm^{2}was significantly different from the one recorded in the group of lowest dose.
Trends observed on the third day were similar to that observed on the first day.

Table I: Effect of essential oil of *Chenopodium ambrosioides* leaves on *Rhipicephalus (Boophilus) microplus*.

	Exposure time (days)		
Doses (µl / cm^2)	**1**	**2**	**3**
0	**0±0^c**	**10±0^c**	**10±0^c**
0 .016	**47±12^b**	**47±12^{bc}**	**57±12^b**
0.031	**53±9^b**	**53±9^b**	**63±9^b**
0.062	**57±7^b**	**60±10^{ab}**	**63±13^b**
0.125	**70±25^{ab}**	**73±27^{ab}**	**80±20^{ab}**
0.25	**100±0^a**	**100±0a**	**100±0^a**

Column percentages with the same letter are not significantly different

4.2- Effect of essential oil of *Citrus aurantium* leaves on *Rhipicephalus (Boophilus) microplus*.

The percentages of corrected mortality of *Rhipicephalus (Boophilus) microplus* subjected to different doses (µl / cm^2) of essential oil of *Citrus aurantium* over the time are reported in Table II. Although ticks mortality increased by day 3for all treatment, there was already a remarkable increased of tick's mortality percentage with doses (0.016; 0.031 and 0.062) of essential oil during the first two days. The highest mortality (43%) of ticks was obtained with the highest dose (0.25µl / cm^2) on day 3. The highest mortality caused (23%) by the lowest dose (0.016µl / cm^2) was obtained on day 3.
Analysis of the average cumulative mortalities percentages according to the essential oil doses extracted from the leaves of *Citrus aurantium* over time (Table V) allowed us to obtain the following regression equation: $Y = 0.5X +0.06$ where$R^2 = 0.99$. This result related to the determination coefficient R^2indicates that 99% of the test effect

variation can be explained by the regression line. Hence, It appears that most of the cumulative mortalities is only due to the effects of the different doses.

The results of the analysis of variance of daily cumulated mortality data according to the doses showed some significant differences at 5% threshold. At the end of the first day, no significant difference was noted between the mortalities of the control group and the different treatment groups. On the second day, mortality (10 ± 0) caused by the control dose was not significantly different from the one caused by following treatment doses including: 0.016; 0.031 and 0.062µl / cm^2. Furthermore, mortality recorded in the lowest dose treatment was significantly different compare to mortality induced by the higher doses (0.125; 0.25µl / cm^2).

On the third day, significant difference was observed between the mortality recorded with the control dose and those recorded with all the treatments groups. Likewise the mortality induced by the lowest dose (0.016µl / cm^2) was significantly different from the mortality caused by the higher doses 0.125 and 0.25µl / cm^2 respectively.

Table II: Effect of essential oil from the leaves of *Citrus aurantium* on *Rhipicephalus (Boophilus) microplus*.

	Exposure time (days)		
Doses (µl / cm^2)	**1**	**2**	**3**
0	10±0^a	10±0^b	10±0^d
0 .016	3±3^a	13±3^b	23±3^c
0.031	7±7^a	17±7^{ab}	30±6^{bc}
0.062	10±0^a	20±0^{ab}	33±3^{abc}
0.125	13±3^a	30±6^a	40±6^{ab}
0.25	13±3^a	30±6^a	43±3^a

Column percentages with the same letter are not significantly different

4.3- Effect of essential oil extracted from the leaves of *Ocimum canum* on *Rhipicephalus (Boophilus) microplus.*

Table III shows the percentage of corrected mortality of *Rhipicephalus (Boophilus) microplus* subjected to different doses (µl/cm^2) of essential oil of *Ocimum canum* according to the time. Overall, ticks' mortality percentage increased with higher doses, however significance difference was observed only between the highest dose (0.25 µl/cm^2) and the other $^($0 .016, 0.031, 0.062 and 0.125µl/cm^2.In another hand, on day 2 and day 3 although there was an increase of the mortality rate with higher doses, no significance difference was observed.

The analysis of the average cumulative mortalities percentages of different dose of essential oil of *Ocimum canum* leaves over time (Table VI) allowed us to obtain the following regression equation: Y = 0.1X+0.7With R^2 = 1. This result related to the R^2 coefficient of determination indicated that 100% of the test effect variation can be explained by the regression line. Therefore it appeared that 100% of the cumulative mortalities are only due to the effects of various doses.

In regards to the analysis of variance of daily cumulative mortality data depending on the dose of this oil, there was a significantly different effect at the 5% threshold.

On day 1, mortality (0 ± 0) in the control group was significantly (P>0.05) different from those observed in all the treated groups. Similarly, the mortality recorded with the highest dose (0.25µl / cm^2) was significantly (P>0.05) different from that recorded with doses (0.016; 0.031; 0.062 and 0.25µl / cm^2).

On day 2, mortality observed in the control group (0 ± 0) was significantly different from the mortality from treated groups. Furthermore, there is no significant difference between the mortality caused by the highest dose and the one caused by other doses (0.016; 0.031; 0.062µl / cm^2). The same trend was observed on day 3.

Table III: Effect of essential oil of the leaves of *Ocimum canum* on *Rhipicephalus (Boophilus) microplus*.

	Exposure time (days)		
Doses (µl / cm^2)	**1**	**2**	**3**
0	0±0^c	0±0^b	10±0^b
0 .016	23±9^b	43±12^a	47±15^a
0.031	23±3^b	47±12^a	50±10^a
0.062	27±7^b	47±12^a	53±9^a
0.125	33±7^b	57±9^a	57±9^a
0.25	50±10^a	60±10^a	60±10^a

Column percentages with the same letter are not significantly different

Table IV: **Percentages of average cumulative mortalities of *Rhipicephalus (Boophilus) microplus* depending on essential oil doses of *Chenopodium ambrosioides* leaves.**

Doses (µl / cm^2)	Average cumulative mortalities	Standard deviation
0 .016	50	±6
0.031	57	±5
0.062	60	±5
0.125	74	±12
0.25	100	±0

Table V: **Percentages of average cumulative mortalities of *Rhipicephalus (Boophilus) microplus* depending on essential oil doses from *Citrus aurantium* leaves.**

Doses (ml / cm)	Average cumulative mortalities	Standard deviation
0 .016	13	±3
0.031	18	±5
0.062	21	±4
0.125	28	±5
0.25	29	±5

Table VI: **Percentages of average cumulative mortalities of *Rhipicephalus (Boophilus) microplus* depending on essential oil doses from *Ocimum canum* leaves**

Doses (µL/cm^2)	Average cumulative mortalities	Standard deviation
0 .016	38	±7
0.031	40	±6
0.062	42	±6
0.125	49	±6
0.25	57	±6

Table VII: **Logarithm of essential oil doses from *Chenopodium ambrosioides* leaves and mortality percentages probits of *Rhipicephalus (Boophilus) microplus* after two days of exposure.**

Dose	Log (Dose)	Mortality (%)	Probit (Y)
0 .016	-1.8	51	0.02
0.031	-1.5	64	0.4
0.062	-1.2	76	0.7
0.125	-0.9	85	1
0.25	-0.6	92	1.4

Table VIII: **Logarithm of essential oil doses from *Citrus aurantium* leaves and mortality percentages probits of *Rhipicephalus (Boophilus) microplus* after two days of exposure.**

Dose	Log (Dose)	Mortality (%)	Probit (Y)
0 .016	-1.8	23	-0.74
0.031	-1.5	27	-0.61
0.062	-1.2	32	-0.48
0.125	-0.9	37	-0.34
0.25	-0.6	42	-0.21

Table IX: Logarithm of essential oil doses from *Ocimum canum* leaves and mortality percentages probits of *Rhipicephalus (Boophilus) microplus* after two days of exposure.

Dose	Log (Dose)	Mortality (%)	Probit (Y)
0 .016	-1.8	42.1	-0.2
0.031	-1.5	46	-0.1
0.062	-1.2	51	0.02
0.125	-0.9	55	0.13
0.25	-0.6	60	0.24

Discussion

The essential oil extracted from the leaves of *Chenopodium ambrosioides*; *Ocimum canum* and *Citrus aurantium* have an *in vitro* acaricidal effect on *Rhipicephalus (Boophilus) microplus* adult cattle ticks in state of low repletion as showed by the result of the contact test. These results suggest that essential oil extracted from plants could be used for the fight against *Rhipicephalus (Boophilus) microplus*. This statement was supported by several works. For instance, **Bisen *and al.* (2009)** conducted an *in vitro* test on the efficacy of Neem (*Azadirachta indica*) and the Karanj (*Pongamia pinnata*) seeds oils on *Rhipicephalus (Boophilus) microplus* and found that Karanj seed oil has higher efficiency (70%). Various species of Cymbopogon were also tested on *R. (B.) microplus*. The essential oil of *Cymbopogon winterianus* Jowitt was tested against larvae and engorged females. Here, full inhibition of eggs hatching was observed at a concentration of 7% and that of eggs-laying at a concentration of 10%. All larvae died at concentrations between 6 and 7% **Martins (2006).**In our study, *Chenopodium ambrosioides* (L.) appear to be the most efficient plant. The acaridial effect of these essential oils against *Rhipicephalus (Boophilus) microplus* may be due to their component which are known to have diverse virtue including insecticide, antihelmintic, larvicide, antiparasitic, antimicrobial, and bactericidal one **El Idrissi *and al*.(2014); Okombe. Embeya (2013); Eveline Solon Barreira Cavalcanti *and al*. (2004); Akantetou *and al*. (2011); Mawussi (2009)**. Indeed, at the highest dose used, the essential oil of *Chenopodium ambrosioides* showed 100% of mortality (0.25µl / cm^2) compare to 60% (0.25µl / cm^2) for *Ocimum canum* (Sims) and 43% (0,25µl / cm2) for *Citrus aurantium.* Thus, *Chenopodium ambrosioides* (L.) is the most effective essential oil in our study based on the classification scale of acaricidal effectiveness depending on the corrected mortality **Chungsamarnyart *and al*. (1991).**The essential oil of *C.ambrosioides* can be recommended to replace synthetic acaricides for the control of *Rhipicephalus (Boophilus) microplus*. This is can help to reduce the negative impact of synthetic acaricides such as wastes, resistance and environmental pollution.

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