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Acaricidal activity of three plants extracts from the central region of Burkina Faso on adult *Rhipicephalus (Boophilus) microplus* cattle ticks

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ABSTRACT

Rhipicephalus (Boophilus) microplus is the most important and harmful tick species in the livestock sector in Burkina Faso today. This tick has developed resistance to most classes of synthetic acaricides. The objective of this work is to assess the efficiency of plant extracts on this tick to find an alternative. Thus, the acaricidal activity of hydroethanolic and hexanic extracts of *Ocimum americanum .L, Ocimum gratissimum .L* and *Laggera oloptera (DC) Adams* was evaluated on adults of this tick by the adult immersion method. The mortality, the inhibition of the laying, the hatching rate, and the effectiveness of each extract have been evaluated with concentrations at 100 mg/mL and at 200 mg/mL, respectively. The best mortality rate was 56%, with the hydroethanolic extract of *L. oloptera* at 200 mg / mL. The hydroethanolic extract of *O. gratissimum* was most effective with the best rates of egg inhibition and hatching rates at 200 mg / mL. The results of this study could be used for the development of new bio-acaricide formulations to fight against the tick R. (Boophilus) microplus effectively.

Keywords: Ticks, *O. gratissimum*, *O. americanum*, *Laggera oloptera*, phytochemical screening

INTRODUCTION

In West Africa, *Rhipicephalus (Boophilus) microplus* is the most harmful tick species in the livestock sector. It is a vector for the transmission of infectious agents to host animals and therefore contributes to decrease meat and milk production (Toure et al., 2012; Grisi et al., 2014). In Burkina Faso, the control of this tick is mainly based on the use of synthetic acaricides, mainly based on deltamethrin, flumetrine, and amitraz. However, the use of this means of control has led to the rapid development of resistance to the active ingredient (Adehan et al., 2016; Aca et al., 2017). Each time ticks survive an application of insecticide, they transmit genetic information to the following generations on the survival method faced with this product

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(Furlong et al., 2004). To adjust this problem of resistance to synthetic acaricides, research is increasingly focused on plant extracts. Thus, a study conducted by Hema (2018) in Burkina Faso, on the acaricidal properties of the hexanic extracts of *Ageratum conizoides*, *Cymbopogon giganteus*, *Laggera aurita*, and *Lippia multiflora* showed 100% larval mortality on the *R. (B.) microplus* tick.

Similarly, other studies have shown the effectiveness of plant extracts on this tick in Benin (Adehan et al., 2016), in Brazil (Silva et al., 2017; Politi et al., 2019) in India (Kumar et al., 2012) in New Caledonia (Lebouvier et al., 2013). To find new sources of natural acaricides, we were interested in three aromatic plants from Burkina Faso (*Ocimum gratissimum*, *Ocimum americanum*, and *Laggera oloptera*) whose biological activities have been reported in numerous works including their insecticidal properties (Nébié, 2006; Ouédraogo et al., 2016; Tia et al., 2019). The present study, therefore, aimed at assessing the acaricide efficacy of the extracts of these three plants on adults of the tick *R. (B.) Microplus* to find an alternative to the synthetic acaricides used in the field.

MATERIALS AND METHODS

Plant material

The plant material consisted of the aerial parts (stems, leaves, and flowers) of *Ocimum gratissimum*, *Ocimum americanum*, and *Laggera oloptera*. The plants were harvested in September 2017 in the central region of Burkina Faso (12 ° 25'17" North and 1 ° 29'14" West). Plant material was dried for two weeks in the laboratory of the Natural Substances Department of the Research Institute for Applied Sciences and Technologies (IRSAT) out of the sun. After drying, the plant material was pulverized using a mechanical grinder.

R. (B.) microplus tick strain

The tick strains used in our study were made up of adult females. They came from Kimini (10°06'24 "North and 4°46'50" West) and have been identified in the laboratory of the International Center for Research and Development on Livestock in the Subhumid zone (ICRDLS). These ticks were kept under control (27-28 °C, RH 70-80%, photoperiod 12h/12) in an oven. The eggs laid by these females were hatched for about four weeks to obtain larvae. These larvae were used to infest single oxen to get new adult females after 20-25 days. These were used to carry out acaricide tests.

Preparation of plant extracts

The extraction protocol consisted of macerating twice successively for (03) hours, 250 g of powder from each plant in an ethanol/water mixture (80:20) for the hydro-ethanolic extracts, and in hexane for hexanic extracts. The residue obtained was extracted again for 16 hours before filtration. All filtrates obtained are combined and evaporated to dryness under reduced pressure using a rotary evaporator. Finally, the reduced hydro-ethanolic extract obtained was freeze-dried while the hexanic extract was placed in an oven to evaporate the extraction solvent.

Phytochemical screening

Each raw extract was subjected to phytochemical screening. Tannins, flavonoids, alkaloids, saponins, and triterpenes were searched for on the various extracts using standard procedures as described in the literature (Wagner and Bladt., 1996; Békro et al., 2007; Ramde-Tiendrebeogo et al., 2019; Nitiema et al., 2020).

Adult immersion test

Groups of 10 adult females of *Rhipicephalus (Boophilus) microplus*, randomly selected, were immersed for 5 min in Petri dishes (5.5 cm in diameter, 1.5 cm in height) containing 10.0 mL of ethanolic or hexanic extract. These extracts were prepared in a solution containing 2% of tween 20 at the concentrations of 100 mg/mL and 200 mg/mL (m/v). The 2% (v/v) tween 20 solution was used as a negative control. The ticks were then removed from the treatment solution, dried, and distributed individually in sterile Petri dishes. The boxes containing the treated ticks

were then incubated at 27-28 °C and 70-80% relative humidity. After two weeks of incubation, the tick death rate and the rate of inhibition of egg-laying were determined according to the equations of Sabatini et al. (2001):

- Mortality Rate (MR) = $\frac{\text{Number of dead ticks}}{\text{Number of ticks treated}} \times 100$
- Laying Index (LI) = weight of laid eggs (g)/weight of females (g)
- Ovulation Inhibition Rate (OIR) = $\frac{\text{LI (control)} - \text{IE (traited group)}}{\text{LI (control)}} \times 100$

The laid eggs were then collected in small boxes, weighed and incubated in the oven under the same conditions as before for about four weeks. At the end of the fourth week, the larval hatching rate was estimated by counting the larvae using a stereo-microscope. The efficacy of the extracts was calculated according to the equations proposed by Drummond et al. (1973):

- Hatching Rate(HR) = $\frac{\text{Number of eggs hatched}}{\text{Total number of eggs}} \times 100$
- Reproductive efficiency(RE) = $\frac{\text{weight of laid eggs (g)} \times \text{TE} \times 20{,}000}{\text{Weight of females (g)}}$
- Efficiency of Extracts(EE) = $\frac{\text{ER (control)} - \text{ER (traited group)}}{\text{ER (control)}} \times 100$

*: number of larvae obtained for 1.0 g of eggs (number obtained experimentally)

Statistical analysis

Statistical analysis was done by XLSTAT software. The data were processed and subjected to a report of variance (ANOVA). The differences between the means were determined by the Tukey test at a significance level of 5%. The lethal concentration to eliminate 50% (LC_{50}) of adult ticks for the extract that caused more than 50% mortality and its 95% confidence interval (CI) were determined by probit analysis.

RESULTS

Mortality rate and rate of inhibition of laying

The direct effect of the different extracts on adults of the tick *R. (B.) microplus* shows mortality rates ranging from zero (control group) to 56% (group treated at 200 mg/mL) for hydro-ethanolic extracts . The lethal concentration, which eliminates 50% (LC_{50}) of adult females, is obtained only with the hydro-ethanolic extract of *L. oloptera*, i.e., 197 mg/mL. For hexanic extracts, mortality rates range from zero (control group) to over 33% (group treated at 200 mg/mL).

Concerning the rates of inhibition of the laying, the values go from zero (control group) to more than 63% (group treated with 200 mg/mL) for hydro-ethanolic extracts. As for the hexanic extracts, they inhibit egg-laying from zero (control group) to more than 43% (group treated with 200 mg/mL). The results are reported in Tables 1 and 2.

The efficiency of the extracts on the hatching rate

Larval hatch rates vary from 100% (control group) to 24% (group treated at 200 mg/mL) and from 100% (control group) to 58%, respectively (group treated at 200 mg/mL) for hydro-alcoholic and hexanic extracts. Regarding the overall efficacy of the extracts, the values vary respectively from zero (control group) to 93% (group treated with 200 mg/mL) and from 0% (control group) to more than 65% (group treated with 200 mg/mL) for hydroalcoholic and hexane extracts (Tables 3 and 4).

Phytochemical screening of plant extracts

The results obtained during phytochemical screening (Table 5) mainly show the presence of tannins, coumarins, alkaloids, and flavonoids in hydro-ethanolic extracts. For hexanic extracts, the main chemical groups found are terpenes and sterols.

Table 1: Mortality rate as a function of extract concentration.

EXTRACTS		Mortality rate (%)		
		Control	100 mg/mL	200 mg/mL
Hydro-ethanolic	OA	0	0	0
extracts	OG	0	13.33 ± 0.97$^{(b)}$	30.00 ±0.66 $^{(a)}$
	LO	0 $^{(C)}$	36.6 ± 0.76 $^{(a)}$	56.00 ± 1.14 $^{(a)}$
Hexanic extracts	OA	0	0	0
	OG	0	0	0
	LO	0 $^{(C)}$	16.66 ± 8.94 $^{(b)}$	33.33± 7.14 $^{(a)}$

Identical letters in the same column indicate identical statistical groups. The different letters show different statistical groups; ***OA****: Ocimum americanum,* ***OG****: Ocimum gratissimum;* ***LO****: Laggera oloptera.*

Table 2: Ovulation Inhibition Rate (OIR) as a function of the concentration of extract.

EXTRACTS		Ovulation Inhibition Rate (%)		
		Control	100 mg/mL	200 mg/mL
Hydro-ethanolic	OA	0 $^{(C)}$	24.29 ± 4.93 $^{(a)}$	36.76 ± 5.16 $^{(b)}$
Extracts	OG	0 $^{(C)}$	50.21 ± 5.06 $^{(a)}$	63.32 ± 10.21 $^{(a)}$
	LO	0 $^{(C)}$	46.57 ± 7.66 $^{(a)}$	60.88 ± 11.96 $^{(a)}$
Hexanic	OA	0 $^{(C)}$	22.36 ± 7.01$^{(b)}$	36.09 ±1.44 $^{(a)}$
extracts	OG	0 $^{(C)}$	20.90 ± 2.25 $^{(a)}$	23.87 ± 3.85 $^{(a)}$
	LO	0 $^{(C)}$	26.32 ± 7.58 $^{(b)}$	43.17 ± 1.77 $^{(a)}$

Identical letters in the same column indicate identical statistical groups. The different letters show different statistical groups; ***OA****: Ocimum americanum,* ***OG****: Ocimum gratissimum;* ***LO:*** *Laggera oloptera.*

Table 3: Egg hatching rate as a function of extract concentration.

EXTRACTS		Egg hatching rate (%)		
		Control	100 mg/mL	200 mg/mL
Hydro-ethanolic	OA	Témoin	94.27 ± 0.25$^{(a)}$	90.17 ± 2.26$^{(a)}$
Extracts	OG	100 $^{(a)}$	72 ± 8.71$^{(d)}$	24 ± 11.13$^{(d)}$
	LO	100 $^{(a)}$	94 ± 2$^{(a)}$	91.3 ± 7.02$^{(a)}$
Hexanic	OA	100 $^{(a)}$	98.67 ± 1.15$^{(a)}$	92.67 ± 4.16$^{(a)}$
Extracts	OG	100 $^{(a)}$	76 ± 8 $^{(b)}$	63.33 ± 5.03 $^{(c)}$
	LO	100 $^{(a)}$	74 ± 12.16$^{(b)}$	58.66 ± 5.03$^{(c)}$

Identical letters in the same column indicate identical statistical groups. The different letters show different statistical groups; ***OA****: Ocimum americanum,* ***OG****: Ocimum gratissimum;* ***LO****: Laggera oloptera.*

Table 4: Efficiency of extracts as a function of concentration.

EXTRACTS		Efficiency of extracts (%)		
		Control	100 mg/mL	200 mg/mL
Hydro-ethanolic Extracts	OA	0 $^{(b)}$	67.15 ± 5.21 $^{(a)}$	79.62 ± 8.96 $^{(a)}$
	OG	0 $^{(b)}$	53.28 ± 3.43 $^{(b)}$	93.90 ± 3.74 $^{(a)}$
	LO	0 $^{(b)}$	46.57 ± 7.66 $^{(b)}$	62.58 ± 14.87$^{(a)}$
Hexanic Extracts	OA	0 $^{(b)}$	38.79 ± 7.07 $^{(b)}$	65.92 ± 8.12 $^{(a)}$
	OG	0 $^{(b)}$	26.29 ±13.65$^{(b)}$	59.00 ± 30.13 $^{(a)}$
	LO	0 $^{(b)}$	22.79 ± 4.19$^{(b)}$	41.27 ± 5.22$^{(a)}$

Identical letters in the same column indicate identical statistical groups. The different letters show different statistical groups; ***OA****: Ocimum americanum,* ***OG****: Ocimum gratissimum;* ***LO****: Laggera olpotera.*

Table 5: Results of phytochemical assays.

Extraits	Secondary metabolites						
	Tannins	alkaloids	Flavonoids	Saponins	Sterols and terpenes	Quinones	Coumarins
OA $_{HA}$	+	+	+	-	-	-	+
OG $_{HA}$	+	+	+	-	-	-	+
LO$_{HA}$	+	+	+	-	+	-	+
OA$_{Hex}$	-	-	-	-	-	-	-
OG $_{Hex}$	-	-	-	-	+	-	-
LO$_{Hex}$	-	-	-	-	+	-	-

OA:*Ocimum americanum****; OG:*** *Ocimum gratissimum****; LA:*** *Laggera olopera****; HA:*** *Hydro-alcoholic****, Hex: Hexanic; sign +:*** *presence of compound****; sign - :*** *Absence of compound****.***

DISCUSSION

The parameters measured during this study were: the rate of tick death, the percentage of inhibition of egg-laying, the rate of hatching, and the efficiency of the extracts.

The results showed cases of mortality, especially with the hydro-ethanolic extracts of *O. gratissimum* and *L. oloptera*. Indeed, *O. gratissimum* and *L. oloptera* respectively caused maximum mortality rates of 30% and 56% at the concentration of 200 mg/mL with an LC_{50} of 197 mg/mL for *L. oloptera*. These values are low compared to those obtained with the methanolic extract of *Atropa belladonna*, which showed a mortality rate of 100% at 200 mg/mL and an LC_{50} of 68.75 mg/mL (Godara et al., 2014). Similarly, a mortality rate of up to 100% was obtained with the hydro-ethanolic extract of *Murraya koenigii* at 100 mg/mL with an LC_{50} of 29.7 mg/mL (Singh et al., 2015). Finally, *Piper nigrum* fruit extracts have caused mortalities ranging from 12.5 to 95.8% and those from *Piper longum* from 29.2 to 87.5% at the same concentration. This result shows that the effect of the extracts is more linked to the plant species than to the nature of the extraction solvent.

The rate of inhibition of egg-laying varied from zero for the controls to 60.88% and 63.32%, respectively, for *O. gratissimum* and *L. oloptera* at 200 mg/mL. It shows that the quantities of eggs laid in the ticks treated decreased significantly ($P < 0.05$) compared to the controls. These results corroborate with those of Godara et al. (2014) who also found a 44.2% reduction in egg-laying with the methanolic extract of *Atropa belladonna* at 200 mg/mL. Similarly, another study showed more significant inhibitions of egg-laying of 96.9% and 89.3% at 200 mg/mL, respectively, with the ethanolic extract of *Piper nigrum* and *Piper longum* (Godara et al., 2018).

Relative to hatching rates for laid eggs, *O. gratissimum* had a maximum value of 24% and *L. oloptera*, 91.3% at the concentration of 200 mg/mL. These results are in agreement with those of Godara et al. (2014), who achieved 100% inhibition of egg hatching with the methanolic extract of *Atropa belladonna* at 100 mg/mL. Likewise, Parveen et al. (2014) showed 100% inhibition with the ethanolic extract of *Artemisia absinthium* at only 50 mg/mL.

Finally, the maximum efficiency values obtained by *O. gratissimum* and by *L. oloptera* are 93.90% and 62.58% at the concentration of 200 mg/mL, respectively. Politi et al. (2012) obtained a lower maximum efficiency value (42.45%) with the ethanolic extract of the aerial parts of *Tagetes patula* at 200 mg/mL. Similarly, Broglio-Micheletti et al. (2009) obtained efficiency values of 18.35% with the ethanolic extract of leaves of *Cymbopogon citratus* and 59.24% for the ethanolic extract of flowers of *Syzygium malaccensis* at the same concentration. However, Conceição et al. (2017) achieved an efficiency of up to 90% with the ethanolic extract of *Ocotea aciphylla* at only 50 mg/mL.

As for the hexanic extracts, the best results were obtained by *L. oloptera* at 200 mg/mL. Indeed, cases of mortality have been noted only with this extract, with a maximum value of 33.33%. This value is lower than that obtained by Castro et al. (2014), who have shown mortality of more than 95% at 150 mg/mL with *Acmella oleracea* hexanic extract. The maximum rate of inhibition of spawning recorded was 43.17%. This rate is lower than that obtained by Santos et al. (2018), which is 100% at 200 mg/mL with the hexanic extract of *Digitaria insularis* leaves. Similarly, other studies show that the hexanic extracts of *Ageratum Conizoides* (Hema et al., 2018) and the leaves of *Neoglaziovia variegata* (Dantas et al., 2015) caused inhibition of egg-laying of 60% and 94.1% respectively at 200 mg/mL. As for hatching rates, the maximum value was 58.66%. However, *Lippia multiflora* hexanic extract has shown an inhibition rate below this value, which is 51% (Hema et al., 2018). The leaves of *Neoglaziovia variegata* also showed a 99.8% higher hatching rate (Dantas et al., 2015). Finally, the overall efficiency obtained was 41.27% at 200 mg/mL. This value is lower than that obtained by Dantas et al. (2015), which was 99.8% with the hexanic extract of the leaves of *Neoglaziovia variegata*.

The phytochemical screening performed on the extracts of the three plants essentially reveals the presence of flavonoids, alkaloids, and tannins for hydro-alcoholic extracts and sterols and terpenes for hexanic extracts. In addition, numerous studies have shown that the acaricidal activity of plant extracts is linked to its composition as secondary metabolites. Indeed, the acaricidal activity of the ethanolic extract of *Tagetes gracilis* has been attributed to the presence of flavonoids (Abdala et al., 2003). Likewise, the flavonoids could be responsible for the acaricidal activity of the ethanolic extract of *Tagetes maxima* (Parejo et al., 2004). Godara et al. (2014) also reported that the alkaloids are implicated in the acaricide activity observed by the ethanolic extract of *Atropa belladonna*. Coumarins are known for their repulsive action against flies and ticks (Emilie, 2011). Finally, it has been shown that tannins are responsible for the acaricidal activity observed with extracts of Acacia pennatula, Piscidia piscipula, Leucaena leucocephala and *Lysiloma latisiliquum* (Fernandes-Salas et al., 2011). The acaricide activity followed by the different extracts could therefore be linked to the presence of these chemical constituents. This activity was more pronounced with hydro-alcoholic extracts than hexanic ones. This could be explained by the fact that it is much more linked to polar than nonpolar compounds.

Some extracts did not cause direct mortality on the *R. (B.) Microplus* tick despite the presence of secondary metabolites. According to Furlong (2000), this would probably be explained by the fact that ticks generally develop different survival mechanisms in the face of acaricides: such as reducing the penetration rate of the product by modifying the external coat, changes in metabolism, storage and excretion of acaricides and changes in the site of action of acaricides.

Conclusion

In this study, we evaluated the acaricidal activity of the hydro-alcoholic and hexanic extracts of *O. americanum*, *O. gratissimum*, and *L. oloptera* on adults of the tick *R. (B.) microplus*. The hydro-alcoholic extracts were the most active, and among these, that of *O. gratissimum* and *L. oloptera* were the most remarkable. The acaricide activity thus observed is mainly due to the presence of secondary metabolites such as tannins, alkaloids and flavonoids. The results of this study could, therefore, be used to develop new bio-acaricide formulations effective for the control of the *R. (B.) microplus* tick in Burkina Faso. However, it would be interesting to assess the toxicity of the two essential oils beforehand.

COMPETING INTERESTS

The authors declare that there is no competing interest.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all authors. Authors AC and MK designed the study and wrote the protocols. Authors AC, MK, AB, AT, and SZ conducted experimentation and statistical analysis. Author AC wrote the first draft of the manuscript. Authors RB, MK, and VB interpreted the results and reviewed the manuscript. Authors JCO, MKONE, and RN managed the study and designed the journal. All authors read and approved the final manuscript.

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