



Acaricidal Activity of Ethanolic and Volatile Extracts of The Leaves of Selected Plants Used in Veterinary Pharmacopeia on The Larvae of *Rhipicephalus Microplus* in Benin

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ABSTRACT

Key words:

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suaveolens,
Tephrosia
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gratissimum,
 Benin

A study was conducted on the effectiveness of ethanolic and volatile extracts of the leaves of four plants acclimatized in Benin on *Rhipicephalus microplus* tick, aiming to contribute to the research of acaricides, which are effective on these ticks and harmless to the environment. To this effect, the modified larvae immersion test was performed to determine the effectiveness of the extracts on the larvae of *Rhipicephalus microplus* aged 7 to 14 days. 5% concentration of the two types of extracts of *Lantana camara* L., *Hyptis suaveolens* (L.) Poit., *Tephrosia vogelii* Hook. f., and *Ocimum gratissimum* L. induced the *Rhipicephalus microplus* larval mortalities, extending from 32.5 to 88% with the ethanolic extracts on first time. Then again, essential oils extracted from these four plants led to 100% mortality from 1.25% of concentration. All the lethal doses at 50 and 90% of mortality of volatile extracts were statistically lower than those of the ethanolic extracts at 5% level of significance ($P < 0.05$). The extracts of *Ocimum gratissimum* L. and *Tephrosia vogelii* Hook. f. were the most active in vitro against *Rhipicephalus microplus* larvae. The results of the present study propose that, plant extracts constitute a promising option for the natural control against the safe strains of *Rhipicephalus microplus* in Benin and the world.

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1. INTRODUCTION

Cattle ticks constitute one of the primary dangers debilitating the health and the production of the world's farm animals due to their direct pathogenic effect on the parasitized animals (FAO, 2004; Rodriguez-Vivas et al., 2007; Usman et al., 2015). The habitual control method used against these ticks is the application of synthetic acaricides (mainly the organophosphorus, carbamates and pyrethroids). However, the intensive and abusive use of these chemicals for tick control in most of animal husbandries regardless of technical criteria, has quickened the occurrence of resistances within tick populations (Mendes et al., 2013).

Rhipicephalus microplus tick, introduced in Benin since 2004 following the importation of dairy cows of the Girolando breed from Brazil to Kpinnou rearing farm (De Clercq et al., 2012), currently represents one of the most resistant tick to acaricides in infested farms (Madder et al., 2012). To solve this, new alternative control methods are being explored among which is the use of plant extracts

having killing or repellent properties on ticks (Attia et al., 2011; Chagas et al., 2002; Fernandes et al., 2007). As a matter of fact, plant extracts are generally of low toxicity to mammals, they are water soluble, present minor secondary effects, and are less or not at all at the origin of resistances in tick populations (Chungsamarnyart et al., 1994).

Plants and their extracts are subject to increased attention as potential alternatives in animal treatment in recent years (El-Katcha, 2016). To date, about 55 plants species belonging to 26 families serve for the extraction of effective insecticidal substances against *R. microplus* (L. et al., 2011). *Ocimum gratissimum* (Lamiaceae), *Hyptis suaveolens* (L.) Poit. (Lamiaceae), *Lantana camara* L. (Bombacaceae), and *Tephrosia vogelii* (Leguminosae-Papilionoideae) are some of the plants which tested positive in microbiology in Benin, and by breeders against animal infections and infestations (Adjou and Mohamed, 2013; Adjou and Soumanou, 2013; Dossou-Gbete et al., 2006). It along these lines sounds imperative to evaluate the

effectiveness of the extracts of different parts of the previously stated plants against this new tick whose circulation is getting more extensive consistently (De Clercq et al., 2012).

Essential oils obtained by hydrodistillation from the leaves of these four plants, collected from different regions in Benin and in other studies, were analysed using Gas Chromatography (GC) and Gas Chromatography quadruple Mass Spectrometry (GC/MS). The major compounds in the order of importance of quantity obtained for *Ocimum gratissimum* were: thymol, α -terpinene, linalyl acetate, linalool, geranyl acetate, myrcene, and sabinene (Sessou et al., 2012; Yayi-Ladekan et al., 2011). Those of *Hyptis suaveolens* (L.) Poit. were: sabinene, α -terpinene, linalyl acetate, linalool, geranyl acetate, myrcene, and sabinene (Sessou et al., 2012; Yayi-Ladekan et al., 2011). Those of *Lantana camara* L. were: α -terpinene, α -pinene, camphene, octene-3-ol, sabinene, α -terpinene, octene-3-ol, phellandrene, δ -3-carene, α -terpinene, p-cymene, and 1,8-cineole (Houinsou et al., 2014), and finally, those of *Tephrosia vogelii* Hook. f. were: germacrene-B, elemol, caryophyllene, (E)-nerolidol, myrcene, and sabinene (Noudogbessi et al., 2012a).

Several African authors have evaluated the effect of these extracts that showed insecticidal and antifungal properties (Coelho et al., 2001; Houinsou et al., 2014; Lyndon et al., 1997; Noudogbessi et al., 2012a; Sessou et al., 2012; Sessou et al., 2013; Yayi-Ladekan et al., 2011). On the other hand, there are limited data reported on the acaricidal activity of the leaves of these plants against *R. microplus* in Benin (Dougnon et al., 2014; Ribeiro et al., 2010). In those ethanolic or volatile extracts previously studied, no active compounds against these ticks were not identified.

The objective of the present study is to assess in vitro the acaricidal effect of some extracts of the leaves of *Ocimum gratissimum* (Lamiaceae), *Hyptis suaveolens* (L.) Poit. (Lamiaceae), *Lantana camara* L. (Bombacaceae) and *Tephrosia vogelii* (Leguminosae-Papilionoideae) harvested in Benin for the control of *Rhipicephalus microplus* tick.

2. MATERIAL AND METHODS

2.1. Tick larvae production

Engorged females of *Rhipicephalus microplus* were collected on animals from the Kpinnou rearing Farm (N6.56828 E1.78623) in Athiémé, a Benin Municipality. These animals did not receive any acaricidal treatment no less than two months before the sampling. The sampled ticks were pasted on their back individually, and in lines, on a piece of

cardboard. This cardboard was put on a plastic plate of 20 cm x 25 cm with raised board to get the eggs laid by the ticks. Ticks were therefore left for egg laying period of 20 days, under an ambient temperature (at the laboratory of resistance tests of URBPSA/UAC). The harvested eggs were distributed in plastic tubes of about 650 cm³, at the rate of 0.5 gram of eggs per tube. Each of these tubes was then covered with a piece of white fine-knit material retained by the tube's lid adequately punctured to allow the eggs' ventilation. The eggs contained in the tubes were then placed in a steam room (Memmert steam room) at 27±1.5°C with 70-85% relative-humidity hatching conditions at the laboratory (Cen et al., 1998; Ibelli et al., 2012). Hatchings started one week after the laying, which lasted 20 days itself. The larvae used for the plant extracts effectiveness test were aged 14 to 21 days (Soberanes et al., 2002).

2.2. Fetching of the experimental plants

Lantana camara L., *Hyptis suaveolens* (L.) Poit., and *Ocimum gratissimum* were gotten from Abomey-Calavi Municipal in Benin (N6.63701 E2.32371), while *Tephrosia vogelii* Hook. f. was from Agbatou in Savalou Municipal (N7.83291 E1.69395). All these plants were certified by the National herbarium at the University of Abomey-Calavi (UAC) in Benin under the following identification numbers: AA6517/HNB, AA6518/HNB, AA6519/HNB and AA6520/HNB respectively.

The geographical positions of the places where these plants were harvested were recorded using a GPS, Garmin eTrex Venture. A part of each of the harvested plants was dried in open air, shielded from sunlight, then blended as a powder that served for the phytochemical screening and the preparation of the non-volatile extract.

2.3. Phytochemical Screening

The major families of secondary metabolites were searched in the plants according to the classical characterization methods. Tannins and Polyphenols were identified by the FeCl₃ and Stiasny were identified by bFlavonoides by Cyanidin reaction; Saponosides by Moss test; Quinones by Borntr identified by bTriterpenes and steroids by Lieberman-Burchard test; and finally, Alkaloids by Mayer and Dragendorf tests (Bruneton, 1999; Trease and Evans, 2002).

2.4. Preparation of the ethanolic extract

For each plant, 100g of powder previously obtained were diluted in 500ml of ethanolic (96°C) and agitated for 30 minutes, 4 times a day for 7 days

(Sessou, 2008). The extracted solution was submitted to an evaporation in a vacuum at a low temperature of 50°C in a Rotavapor® R-200 to evaporate the alcohol. The obtained solution was placed in a Memmert steam room at a temperature of 50°C to perfect the evaporation. The ethanolic extracts were made on account of the backing of the Laboratory of Study and Research in Applied Chemistry (LERCA) of the Polytechnic school of Abomey-Calavi (EPAC) in Benin. The alcohol utilized of every extraction of a plant is recycled safely to be used again in subsequent extractions of the same plant (Sessou, 2008). This possibility of alcohol recycling is an essential parameter in the profitability of this operation of ethanolic extraction of plants

2.5. Extraction of the essential oils

Hydrodistillation technique was used for the extraction of essential oils. It was done with 300g of fresh leaves in an extractor of Clevenger type at the Laboratory of Pharmacognosy and Essential Oils of the Faculty of Sciences and Techniques (FAST) of the University of Abomey-Calavi, Benin. These plants were previously spread under an ambient temperature of the laboratory from 25 to 28 an extractor of Clevenger type at the Laboratory of Pharmacognosy and after a 3 hours period. The technique consisted of a classical distillation in which the plant matter is put in water, and the whole solution is boiled. The water vapour loaded with volatile substances was condensed inside a cooler. Gases less dense than water were collected in flasks by simple settling at the surface of the water. These flasks of oil were secured with aluminium foil so as to protect them from any negative impact or any defilement from the light. The oil was then stored at 4d the light. The oil was then inside a cooler. Gases less dense than

2.6. Immersion test of the larvae

The immersion test was used to determine the effectiveness of the plant extracts on the larvae of *R. microplus* (Soberanes et al., 2002). Tween-20, an emulsifier, was diluted at 2% in distilled water to serve as control. This control solution was used to prepare a set of six solutions of concentrations, 0%; 0.15625% (1.5625 mg/mL); 0.625% (6.25 mg/mL); 1.25% (12.5 mg/mL); 2.5% (25 mg/mL); and 5.00% (50 mg/mL) for each of the two types of extracts of the four tested plants based on some effective concentrations used in previous studies (Rosado-Aguilar et al., 2010). Only the eggs in the tubes in which the hatching rates varied between 90% and 100% were used.

To perform this test, a tube containing tick larvae was placed on a plate of 20cm x 25cm half-filled with soapy water, to avoid them escaping. An amount of 0.70ml (Rosado-Aguilar et al., 2010) of each solution was introduced in petri dishes of 150mm diameter. About 100 to 200 larvae were delicately transferred with a N°4 brush in a closed clutch bag by a filter paper (Whatman Nm) of 8cm x 7cm folded in its middle and retained at two sides by clasps (Rosado-Aguilar et al., 2010). The third side of the clutch bag received its clasp after the introduction of the larvae. The envelopes containing the larvae were then immersed in the petri dishes' solutions for 10 minutes (Rosado-Aguilar et al., 2010). Four bags of larvae were elaborated by concentration of solutions. The envelopes were previously labelled with the date and the time of immersion, the origin of the tested larvae, the tested extract, the date and time at the end of the experiment. The packets of larvae were then placed in a steam room (Memmert) at the temperature of 27±1.5°C with 70 to 85% relative humidity for 24 hours (Rosado-Aguilar et al., 2010). At the end of the test, the envelopes were opened, and the containing larvae were counted using a gluing tip stick through a magnifying glass. The number of living and dead larvae was recorded. In order to stimulate the living and inactive larvae to move, air was delicately blown with the mouth into the opened packet. The larvae that remained inactive were considered dead. The larval mortalities were not corrected by the formula of Abott (Abott, 1925) recommended by the Food and Alimentation Organization (FAO, 2004), because, the mortalities recorded from the control groups were all greater or equal to 5%. To end up, the same larvae was immersed into a chemical acaricide, alphacypermethrin, at similar concentrations like those used for the four leave extracts of the tested plants, with the aim of making a comparison. The alphacypermethrin used was as a powder with 98% purity, and it was gotten from CSIRO (Commonwealth Scientific and Industrial Research Organization) through the CIRDES (International Centre of Breeding Research-Development in Sub-Humid Area) in Burkina Faso.

2.7. Statistical analyses

Regression Analyses of the doses' normality were performed with R software 2.15.3 version (R Core, 2013) using DRC package (version 2.3-96), specific to dose-responses curves models (Ritz and Streibig, 2005). Three functions: Model Four Log-logistic; Model Four, and Three of Weibull were tested in

order to choose the model that gives the lowest residual deviance.

The function of four log-logistical parameters with respective minimum and maximum values of 0 and 100 was used for the data's model using the DRM (Dose Response Mortality) command (residual deviance = 0.15). Then, the values of LC_{50} and LC_{90} and their Confidence Interval (CI) at 95% were estimated using ED command and delta option for the interval parameter. The models' lines were drawn in order to better assess the tendencies presented by each model. Besides, ANOVA and SNK (Student Newman Keuls) tests were performed on the mortalities obtained from each tested extracts in order to appreciate the significant differences that

could exist between the effects of the various extracts on the larvae and compare the averages two by two.

3. RESULTS

1. Extracts drawn

The yields of ethanolic extracts varied from 3.75% to 5.67%. They were similar for all the plants, but a little bit higher for *T. vogelii* (Table 1). Those of volatile extracts ranged between 0.04% and 0.32%. The yield of *O. gratissimum* in terms of essential oils is about ten times greater than the ones of each of the other plants.

Table 1: Extractions' yield of the ethanolic extracts and the studied essential oils.

Plant species	Yields	
	Ethanolic extracts (%)	Volatile extracts (%)
<i>Ocimumgratissimum</i>	4.50	0.32
<i>Lantana camara</i>	3.75	0.05
<i>Tephrosiavogelii</i>	5.67	0.07
<i>Hyptissuaveolens</i>	4.00	0.04

Yields followed by the same letters do not differ significantly ($p < 0.05$).

2. Major families of compounds present in the studied plants

The result of the phytochemical screening performed on the various leave powders obtained from the four tested plants revealed the presence of mucilage, catechic tannins, gallotannin, flavonoides, anthocyanes and leuco-anthocyanins, coumarins, reducing compounds, sterols, saponosides, and terpenes (Table 2). Mucilage was only present in the leaves of *L. camara*. The leaves of *O. gratissimum* are rich in coumarins and reducing compounds, which are completely absent in the three other plants. Gallic tannins, flavonoides, and anthocyanes are absent in *T. vogelii*. The phytochemical compositions of the four ethanolic extracts were very similar to the one of *T. vogelii*, which is partially different from the three others, for example, by the presence of saponosides. Catechic tannins, leuco-anthocyanins, reducing compounds, sterols, and terpenes were identified simultaneously in the ethanolic extracts.

3. Acaricidal effect of the tested ethanolic extracts and essential oils

Immersion of larvae revealed that LC_{50} and LC_{90} were reached at lower doses with essential oils, followed by, Alphacypermethrin, and after, by ethanolic extracts (Table 3). In addition, volatile extracts from *O. gratissimum*, *T. vogelii*, *H. suaveolens* (L.) Poit., and *L. camara* have the most significant effect on larvae of *R. microplus*, followed respectively by ethanolic extracts of *O. gratissimum*, *T. vogelii*, *Hyptis suaveolens*, and *Lantana camara* (Table 3). Utilizing ethanolic extracts, the complete dose-response ranges from 0 to 100 %, and has been reached for all given plants (Figure 1 & 2). Lethal concentrations at 50% mortality (LC_{50}) of essential oils were significantly lower than those of Alphacypermethrin. Nevertheless, the LC_{50} of volatile extracts were higher than the one of Alphacypermethrin. Results were similar between the lethal concentration with 90% (LC_{90}) of essential oils and ethanolic extracts of the leaves (Table 3).

Table 2: Phytochemical screening of the fresh plant leaves used

Secondary metabolites	<i>O. gratissimum</i>	<i>T. vogelii</i>	<i>H. suaveolens</i>	<i>L. camara</i>
Mucilage	-	-	-	+
Tannin	+	??	+	+
Catechic Tannin	+	+	+	+
Gallic Tannin	+/-	-	+	+
Flavonoides	+	-	+	+
Anthocyanes	+	-	+	+
Leuco-anthocyanes	+	+	+	+
Alkaloids	-	-	-	-
Saponosides	-	+	-	-
Coumarins	+	-	-	-
Reducing compounds	+	+/-	-	-
Free Anthraquinones	-	-	-	-
	o-heterosides		-	-
Combined anthraquinones	o-reduced geninheterosides		-	-
	c-hétérosides		-	-
Sterol and Terpenes			+	+

+: presence; -: Absence; +/-: Tracks

Table 3. Effect of the use of crude extracts of leaves in the control of *R. (B.) microplus*

Plant extracts	LC ₅₀		LC ₉₀	
	Estimate (%) [*]	CI (95%)	Estimate (%) [*]	CI (95%)
<i>Ocimum gratissimum</i> EO	0.32 ^c	(0.31-0.33)	0.51 ^c	(0.27- 0.75)
<i>Tephrosiavogelii</i> -EO	0.38 ^c	(0.37-0.39)	0.74 ^c	(0.71- 0.77)
<i>Hyptissuaveolens</i> -EO	0.51 ^d	(0.42-0.60)	0.68 ^c	(0.63- 0.73)
<i>Lantana camara</i> -EO	0.56 ^d	(0.50-0.62)	0.84 ^c	(0.60- 1.08)
Alphacypermethrin	1.18^c	(1.05 -1.31)	2.30^d	(1.68 -2.92)
<i>Ocimum gratissimum</i> -EE	1.77 ^{bc}	(1.63-1.91)	14.78 ^c	(09.46-20.08)
<i>Tephrosia vogelii</i> -EE	2.41 ^b	(2.33-2.49)	33.52 ^b	(28.30-38.74)
<i>Hyptis suaveolens</i> -EE	5.07 ^a	(4.75-5.4)	41.61 ^a	(20.33-62.89)
<i>Lantana camara</i> -EE	6.53 ^a	(6.30-6.76)	49.50 ^a	(38.81-60.19)

EO=Essential Oil; EE=Ethanollic Extract; CI=confidence intervals *Significant difference

The observations made at the level of Table 3 are in accordance with the appearance and position of the dose-response curves gotten at Figures 1 & 2. All of

the larvae of *R. microplus* died at less than 5% of alpha-cypermethrin concentration, while, it needed

more to get a 100% mortality of the larvae with the four tested ethanolic extracts (Figure 1).

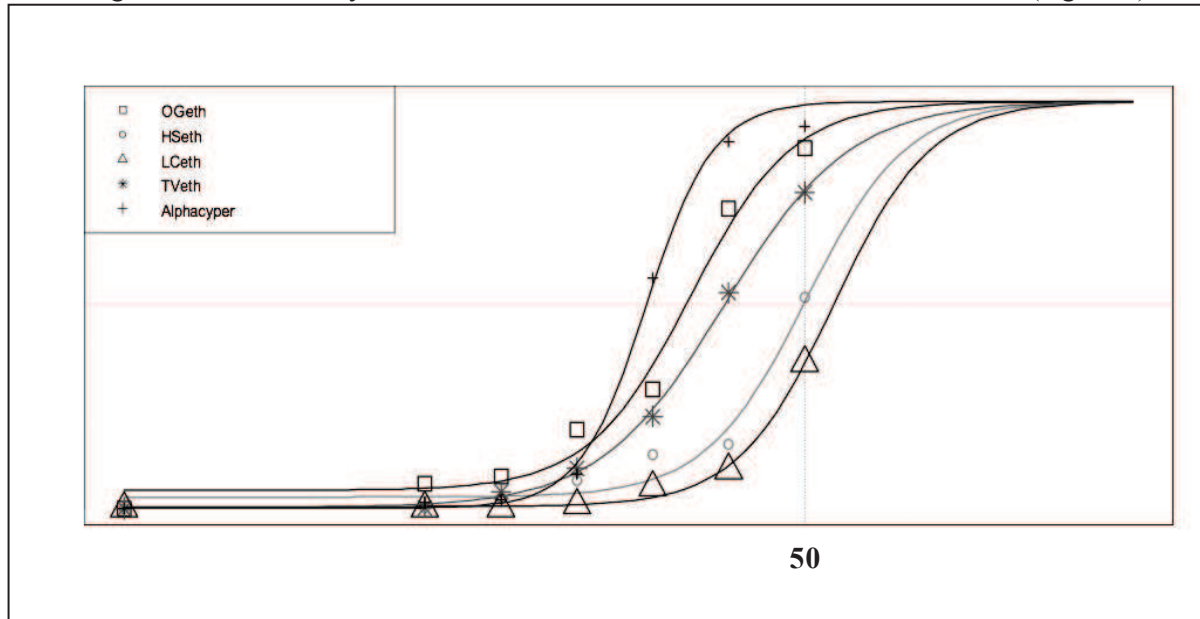


Figure 1: Dose-response curves of the leaves' crude ethanolic extracts

OGeth= crude ethanolic extracts of the leaves of *Ocimumgratissimum*; **HSeth**= crude ethanolic extracts of the leaves of *Hyptis suaveolens*, **LCeth**= crude ethanolic extracts of the leaves of *Lantana camara*; **TVeth**=crude ethanolic extracts of the leaves of *Tephrosia vogelii*; **Alphacyper**=Alphacypermethrin

The results obtained from the use of essential oils showed that, no mortality was noted at the dose control (0%). At about 5% concentration, the Alphacypermethrin like in the previous case provoked 100% mortality of the larvae. The tested essential oils had altogether a very high acaricidal activity on

the larvae of *R.microplus*, and gave 100% of mortality at less than 1% concentration (Figure 2). The analysis of this results show that, whatever the plant, among the tested ones, their essential oils have a higher acaricidal effect than their ethanolic extract.

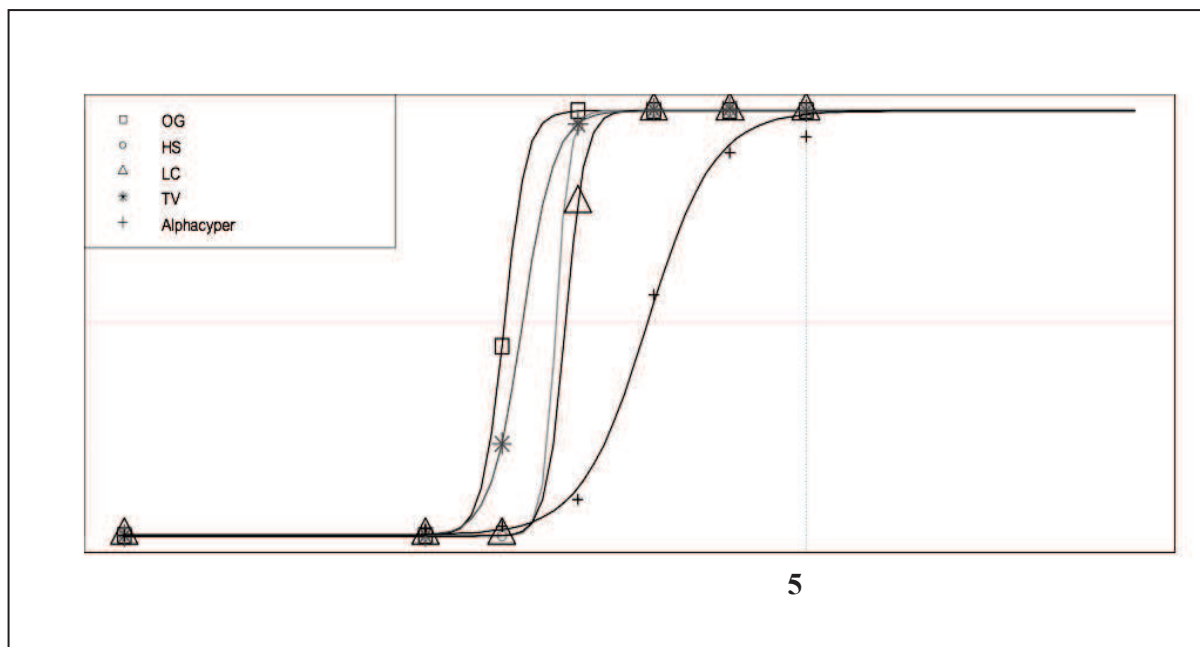


Figure 2: Dose-response curves of the essential oil extracts

OG= crude ethanolic extracts of the leaves of *Ocimumgratissimum*; **HS**= crude ethanolic extracts of the leaves of *Hyptissuaveolens*, **LC**=crude ethanolic extracts of the leaves of *Lantana camara*; **TV**=crude ethanolic extracts of the leaves of *Tephrosiavogelii*; **Alphacyper**=Alphacypermethrin

4. DISCUSSION

I. Yield of ethanolic extracts and essential oils

The average yield of ethanolic extracts obtained from the plants' leaves ranged between 3.75% and 5.67%. Those of essential oils varied from 0.04% to 0.32%. For ethanolic extracts, the yield of *Tephrosia vogelii* Hook. f. was greater than the one of *Ocimum gratissimum* L., and that of *Hyptis suaveolens* (L.) Poit. was higher than the one of *Lantana camara* L., but with essential oils, it was the inverse situation. The yield of essential oils were five to ten times lower than those reported by other recent studies on the same plants species of the sampling area (Adjou et al., 2012; Houinsou et al., 2014; Noudogbessi et al., 2013; Noudogbessi et al., 2012b; R Core, 2013; Sessou et al., 2012; Yayi-Ladekan et al., 2011). This could be due to changes in harvesting season, sampling time in the day, the vegetative stage of the plant, and so many other environmental factors. The significant difference that the volatile extract of *Ocimum gratissimum* L. presented in terms of yield, compared to those of the three other plants shows that this plant contains more essential oils (Adjou et al., 2012).

i. Phytochemical composition of the ethanolic extracts

The variation of the chemical compositions of the extracts shows once again that the outcomes of plant extractions differ as per various parameters of which are the vegetative stage of the plant, and the period of the plants' harvesting (Richard, 1976; Yayi et al., 2004). The distinction between the phytochemical compositions of *T. vogelii* Hook. f. (*Leguminosae-Papilionoideae*) and those of the three other plants could be probably related to a Botanic family difference and soil related or environmental factors (Bachelier, 1978). This is the case of *O. gratissimum* L. and *H. suaveolens* (L.) Poit, which are all Lamiaceae, close to Bombacaceae to which *L. camara* L belongs.

ii. Acaricidal effects of the tested ethanolic extracts and essential oils

The exotic tick *R. microplus* became resistant to all chemical acaricides in Brazil (Domingues et al., 2002; Lovis, 2012; Santos et al., 2003) and in Benin (Madder et al., 2012). The same situation was described after the introduction of this tick through the importation of the dairy cows of Girolando breed from Brazil (Madder et al., 2012).

The biological effect of the leave extracts of four plants was used in the control of these ticks in the present study. The effectiveness of the ethanolic extracts of *Hyptis suaveolens* (L.) Poit., *Lantana*

camara L., *Tephrosia vogelii* Hook. f., *Ocimum gratissimum* L. and their essential oils were evaluated in this study by the mortality of the subjected larvae. The larval mortality obtained from this experiment was high and varied from 0.10% to 88.43% for the ethanolic extracts, and 21.63 to 100% for the volatile extracts, compared to alpha-cypermethrin with which the mortalities started from 0.15625% (Figure 1).

The lethal doses LD₅₀ and LD₉₀ of the ethanolic extracts on the larvae were all higher than the one of alpha-cypermethrin (Table 4). The maximum mortality at 5% concentration of the ethanolic extracts on the larvae were 35.79%, 51.87%, 77.66%, and 88.43% for *L. camara* L., *H. suaveolens* (L.) Poit, *T. vogelii* Hook. f. and *O. gratissimum* L. respectively. These results show that ethanolic extracts of the four plants have an important acaricidal activity on the larvae of *R. microplus*. The larvicidal potential of the ethanolic extracts on *R. microplus* could be explained by the chemical composition of the four plants. The phytochemical screening revealed the presence of catechictannins, saponosides, coumarins, reducing compounds, leuco-anthocyanins, and polyterpene-sterols in the leave extracts of the four plants. Coumarins are specific for *O. gratissimum* L., Saponosides for *T. vogelii* Hook. f., while reducing compounds are specific for the two others (*L. camara* L. and *H. suaveolens* (L.) Poit).

Most of these chemical compounds possess some biocides properties (Adjou and Soumanou, 2013). As a matter of fact, tannins showed an important acaricidal property on cattle tick *R. microplus* in the studies of (Fernandez et al., 2011). Saponosides also possess a preventive effect on microorganisms and/or parasites (Tava and Odoardi, 1996). Coumarins, also known as anticoagulants, are also repellents against flies and ticks (Emilie, 2011). The conjugated action of the reducing compounds and these other group of compounds could be at the origin of the larvicidal effect observed in vitro. The effect of the ethanolic extracts is first related to the compounds that all the four extracts possess in common (tannins, catechic tannins, leuco-anthocyanins). This activity could be reinforced in effectiveness order by the presence of saponosides, coumarins and reducing compounds. The activity of ethanolic extracts could also be due to Gallic tannins, compounds that are absent in the extracts of *T. vogelii*. This activity could be strengthened by the presence of compounds such as flavonoides and antocyanes, and probably minimized by the presence of mucilage. It is probable that, reducing compounds and coumarins contained in the extracts of *O. gratissimum*, and the

presence of reducing compounds and saponosides in the leaves of *T. vogelii* that made the ethanolic extracts of these two plants more effective.

Many other African authors assessed the effect of these extracts that showed insecticidal and antifungal properties (Coelho et al., 2001; Houinsou et al., 2014; Lyndon et al., 1997; Noudogbessi et al., 2012b; Sessou et al., 2012; Yayi-Ladekan et al., 2011). However, the acaricidal activity of the leaves of these plants against *R. microplus* was less reported. The tick mortality induced by various organ extracts of *Tephrosia vogelii* Hook.f. are known. (Kalume et al., 2012) observed the mortality rate of 95 and 100% while using concentrations of 10 and 20 mg/mL of the leaves of two different varieties of *Tephrosia vogelii* Hook.f. on *Rhipicephalus appendiculatus*. The same observations were made by (Matovu and Olila, 2007) while using the chloroform, methanolic, ether, and aqueous extracts of the stem and leaves of *Tephrosia vogelii* Hook.f. on the nymph and adult ticks in Uganda. (Gadzirayi et al., 2009) concluded from their various studies that concentrations of 50 to 100 g of *Tephrosia vogelii* Hook.f. leaves in 100 to 200 ml of water can be used by breeders for general tick control. (Sarda et al., 2007) evaluated the crude methanolic extract of *H. polyanthemum* on the larvae of *R. microplus* and recorded mortalities of 100%, 96.7%, 84.7% and 52.7%, respectively for the concentrations of 50, 25, 12.5 and 6.25 mg/mL.

In Benin, (Dougnon et al., 2014) reported that the ethanolic extract of the leaves of *Tephrosia vogelii* Hook.f. induced an in vivo mortality of 98.51% in *Amblyomma variegatum* tick. The same authors concluded that, the ethanolic extract of the *Tephrosia vogelii* Hook.f. leaves can be equally used as a larvicide. It was deduced that, the ethanolic extract which confirms the results obtained in the present study (Table 4). Results of the studies conducted by (Alitonou et al., 2004) from Benin on *Lantana camara* L. (Bombacaceae), demonstrated the volatile extracts of this plant as effective against tick infestations in general. The volatile extracts of the four plants brought about 100% of mortality from 0.625% of concentration for *O. gratissimum* L. and *T. vogelii*, 1.25% for *H. suaveolens* (L.) Poit. and *L. camara* L., contrary to the one of alpha-cypermethrin which was 5%.

The larvicidal effect of the volatile extracts of *O. gratissimum* L. and that of *T. vogelii* Hook.f. are similar but different from those of *H. suaveolens* (L.) Poit. and *L. camara* L., which are both identical as well. The ethanolic extract of *H. suaveolens* (L.) Poit. and *L. camara* L. acts similarly on the larvae of *R. microplus*, but differently from those of *O.*

gratissimum L., and *T. vogelii* Hook.f. that are identical. Below are four effective groups in the order of the most to the least effective groups of all the eight extracts:

- i. essential oils of *O. gratissimum* L. and *T. vogelii* Hook.f.
- ii. essential oils of *H. suaveolens* (L.) Poit. and *L. camara* L.
- iii. ethanolic extracts of *O. gratissimum* and *T. vogelii* Hook.f.
- iv. ethanolic extracts of *H. suaveolens* (L.) Poit. and *L. camara* L.

Essential oils possess higher antimicrobial properties than other types of extracts, including ethanolic extracts (Burt, 2004). It was also demonstrated that essential oils which have high presence of esters are particularly active (Alitonou et al., 2004). They are followed by: aromatic hydrocarbons, oxides, phenols, aldehydes, ketones, and alcohols (Alitonou et al., 2004). The volatile extracts of *O. gratissimum* L., *T. vogelii* Hook.f., *H. suaveolens* (L.) Poit., and *L. camara* L. were more effective on the larvae of *R. microplus* than their ethanolic extracts, with a significant difference at 5% level of confidence. These volatile extracts contain a number of natural chemical compounds playing various biological roles, including repelling, anti-appetence or toxicity on the ticks. This is probably thanks to thymol, α -terpinene, p-cymene, α -thujene, myrcene, sabinene in one hand and germacrene-B, elemol and caryophyllene on the other hand. The high activity of essential oils of *O. gratissimum* could be related to its high thymol concentration, which is a phenolic compound (Prajapati, 2012). An important characteristic of thymol is its hydrophobicity which allows it to integrate the lipids of the membrane of fungal cells, disrupting thus their structures, and making them more permeable to ions escaping and all other cell contents (Sessou et al., 2012). It is probably the presence of these compounds in the volatile extracts of the leaves of *O. gratissimum* L. and *T. vogelii* Hook.f. that made them more effective. *O. gratissimum* as other tested extracts, are aromatic and medicinal plants of whose uses are numerous in traditional therapy, and in human and animal consumption (Dossoukpevi et al., 2013). For high consumption rates, it is necessary to develop some strategies that will immensely boost production, and as well the preservation of phylogenetic resources, so as to be free from being set in a trap of competition against harvest intended for human consumption. The improving result of works of these plants by the in vitro test of their united-nodal explants are promoted (Dossoukpevi et al., 2013).

The *O.gratissimum* is a plant greatly consumed in human feeding in Benin and of which essential oil is greatly produced and marketed. Although, the result of the four vegetables extracts contents (Sessou et al., 2012). It is probably the presence of these compounds in the vareless, *Lantana* remains a toxic herb for chewing animals. The livestock poisoning by *Lantana Camara* was reported in India, Indonesia, United states of America, Australia, and some parts of Africa. The chewing animals (buffalo, sheep, goats) and non-chewing ones like horse, rabbit, pig, and female rat are sensitive to hepatotoxic action of the toxin from different species of *Lantana sharm*.

5. CONCLUSION

The assessment of the effectiveness of ethanolic and volatile extracts of *Lantana camara* L., *Hyptissuaveolens* (L.) Poit., *Tephrosiavogelii* Hook.f. and *Ocimumgratissimum* L. stimulated significant mortality in the exotic tick larvae of *Rhipicephalus microplus*. The control of *R. microplus* using plant extracts seems to be one of the means to look into in seeking solutions to fight against ticks in general, and especially *Rhipicephalus microplus*. Of importance is that, some additional tests are much necessary before a real working of these results in the field. Among which are: toxicity tests of which that invivo of plants extracts about engorged female, and those invitro of the same extracts on artificial infestations of *Rhipicephalus microplus* in station on the one hand, and on natural infestations of ticks in a real zone on the other hand. Necessary efforts should be put in place for the isolation of the active substances of these plants in order to make them easily available to breeders and technicians'; new viable molecules on ticks by and large, and of utmost importance, the *Rhipicephalus microplus*. Today, a huge significant quantity of extracts of some plants, of which that of *O. gratissimum*, is produced and marketed in Benin without any harmful effects on the harvests production for human feeding. For this purpose, the same technologies used can be put into use for the production.

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7. REFERENCES

- Abbott, W. 1925. A Method of Computing the Effectiveness of an Insecticide. J. Econ. Entomol. 18: 265-267.
- Adjou, E., Dahouenon-Ahoussi, E., Degnon, R., Soumanou, M., Sohounhloué, D. 2012. Bioefficacy of Essential Oil of *Lantana Camara* from Benin Against the Growth of Fungi and Aflatoxin Production. J Rec. Adv Agri 1: 112-121.
- Adjou, E.S., Mohamed, MS. 2013. Efficacité Des Extraits De Plantes Dans La Lutte Contre Les Moisissures Toxinogènes Isolées De L'Arachide En Post-Récolte Au Bénin. J. Appl. Biosci. 70: 5555-5566.
- Adjou, E.S., Soumanou, M.M. 2013. Efficacité des extraits de plantes dans la lutte contre les moisissures toxinogènes isolées de l'arachide en post-récolte au Bénin. J. Appl. Biosci. 70: 5555-5566.
- Alitonou, G., Avlessi, F., Bokossa, I., Ahoussi, E., Dangou, J., Sohounhloué, D. 2004. Composition Chimique Et Activités Biologiques De L'Huile Essentielle De *Lantana Camara* Linn. C. R Chimie 7 : 1101-1105.
- Attia, S., Grissa K., Ghrabi-Gammar Z., Catherine Mailleux, A., Lognay, G., Hance, T., Le Goff, G. 2011. Contrôle De Tetranychus urticae Par Les Extraits De Plantes En Vergers d'Agrumes. Faunistic Entomology 63 : 229-235.
- Bruneton, J. Pharmacognosie, Phytochimie, Plantes médicinales. Lavoisier Technique & Documentation., Paris, France ; 1999.
- Burt, S. 2004. Essential Oils: Their Antibacterial Properties and Potential Applications in Foods – a review. Int. J. Food Microbiol. 94: 223-253.
- Cen, A., Rodríguez-Vivas, R., Wagner, G. 1998. Studies on the Effect on Infection by Babesia Sp. on Oviposition of Boophilus Microplus Engorged Females Naturally Infected in the Mexican Tropics. Vet. Parasitol. 78: 253-257.
- Chagas, A., Prates, H., Leite, R., Furlong, J. 2002. Açõlarvicida De Derivados arilsulfonílicos Da (+)-Cânfora e da (+)-Isopinocanfona, Em *Rhipicephalus (Boophilus) Microplus*. Arq. Bras. Med. Vet. Zootec. 54: 462-467.
- Chungsamarnyart, N., Ratanakreetakul, C., Jansawan, W. 1994. Acaricidal Activity of the Combination of Plant Crude Extracts to Tropical Cattle Ticks. Kasetsart Journal: Natural Sci. 28: 649-660.
- Coelho, B., Young, M., Giesbrecht, A., Roque, N., Bolzani, V. 2001. Antifungal Polysulphides From *Petiveria Alliacea* L. Phytochem. 57: 743-747.
- De Clercq, E.M., Vanwambeke, S.O., Sungirai, M., Adehan, S., Lokossou, R., Madder, M. 2012. Geographic distribution of the invasive cattle tick *Rhipicephalus microplus*, a country-wide survey in Benin. Exp. App. Acarol. 58: 441-452.

- Domingues, L., Bello, A., Cunha, A., Brasil, B., Barros, A., Leite, R., Silaghi, C., Pfister, K., Passos, L. 2002. Survey of Pyrethroid and organophosphate resistance in Brazilian field populations of *Rhipicephalus (Boophilus) Microplus*: detection of C190A mutation in domain II of the para-type Sodium channel Gene. Vet. Parasitol. 189: 327 – 332.
- Dossou-Gbete, G., Salifou, S., Aboh, A., Dossa, S. 2006. Evaluation Participative de l'importance des Tiques et méthodes endogènes de lutte au nord Bénin: Perspectives et valorisation. RASPA. 4: 61-68.
- Dougnon, T., Farougou, S., Kpodékon, T., Hounmanou, G. 2014. Comparative study of the ethanolic extract effect of *Tephrosia vogelii* Leaves and Alfapor® (Alpha-Cyperméthrine) of the Tick *Amblyomma variegatum* on Borgou cattle. Int. J. Biosci. 5: 1-7.
- El-Katcha, M.I., Soltan, M.A., Essi, MS. 2016. Effect of Garlic Extract Supplementation on Growth Performance, Nutrient Digestibility and Some Blood Serum Biochemical Changes of Fattening Lambs. AJVS. 48: 124-133.
- Emilie, V. Réactivité des polyéthers ionophores et des coumarines: vers des systèmes moléculaires efficaces pour la santé animale. In. de l'Université de Lyon, France.; 2011.
- FAO. 2004. Resistance management and integrated parasite control in ruminants: guidelines, pp. 25-77. Module1. Ticks: Acaricide Resistance: Diagnosis, Management and Prevention. FAO, Rome, Italy.
- Fernandes, F., Leles, R., Silva, I., Freitas, E. 2007. Etude de l'activité de sapindussaponari (Sapindaceae) sur les larves de la tique brune du chien *Rhipicephalus Sanguineus* (Latreille 1806) (Acari : Ixodidae). Arq. Bras. Med. Vet. Zootec. 59: 145-149 .
- Fernandez, B.G., Gaspar, P., Brás-Pereira, C., Jezowska, B., Rebelo, S.R., Janody, F. 2011. Actin-Capping Protein and the Hippo pathway regulate F-actin and tissue growth in *Drosophila*. Development. 138: 2337-2346.
- Gadzirayi, C., Mutandwa, E., Mwale, M., Chindundu, T. 2009. Utilization of *Tephrosia Vogelii* in Controlling Ticks in Dairy Cows by Small-Scale Commercial Farmers in Zimbabwe. Afr. J. Biotechnol. 8: 4134-4136.
- Houinsou, F.R., Euloge, S.A., Danhouenon, E.A., Dominique, S.C, Soumanou, M.M. 2014. Bioactivity of Essential Oil from Fresh Leaves of *Lantana Camara* against Fungi Isolated from Stored Cowpea in Southern Benin. Int. J. Biosci. 5: 365-372.
- Ibelli, A., Giglioti, R., Regitano, L., Alencar, M., Chagas, A., Paço, A., Oliveira, H., Duarte, J., Oliveira, M. 2012. Resistance of Cattle of Various Genetic Groups to the Tick *Rhipicephalus Microplus* and the Relationship with Coat Traits. Vet. Parasitol. 186: 425-430.
- Kalume, M., Losson, B., Angenot, L., Tits, M., Wauters, J., Frédérick, M., Saegerman, C. 2012. Rotenoid Content and *in Vitro* Acaricidal Activity of *Tephrosia Vogelii* Leaf Extract on the Tick *Rhipicephalus Appendiculatus*. Vet. Parasitol. 190: 204-209.
- Borges, L.M., Sousa, L.A., Barbosa Cda, S. 2011. Perspectives for the use of plant extracts to control the cattle tick *Rhipicephalus (Boophilus) microplus*. Rev. Bras. Parasitol. Vet. 20: 89-96.
- Lovis, L. 2012. Evaluation of acaricide resistance in the cattle tick, *Rhipicephalus (Boophilus) microplus*, using a new in vitro test and molecular tools. Thèse de doctorat: Université de Neuchâtel. pp. 1-204.
- Lyndon, J., Lawrence, A., Earle, V. 1997. An Insecticidal and Acaricidal Polysulfide Metabolite from the Roots of *Petiveria Alliacea*. Pest. Sci. 50: 228-232.
- Madder, M., Adehan, S., De Deken, R., Adehan, R., Lokossou, R. 2012. New foci of *Rhipicephalus microplus* in West Africa. Exp. Appl. Acarol. 56: 385-390.
- Matovu, H., Olila, D. 2007. Acaricidal Activity of *Tephrosia vogelii* Extracts on Nymph and Adult Ticks. Int. J. Trop. Med. 2: 83-88.
- Mendes, E.C., Marcia, C.M., Mario, E.S. 2013. Diagnosis of amitraz resistance in Brazilian populations of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) with larval immersion test. Exp. Appl. Acarol. 61: 369.
- Noudogbessi, J-P., Pascal, A., Boniface, Y., Elvis, A., Guévara, N., Mokimou, A., Valentin, W., Gilles, F., Jean-Claude, C., Dominique, S. 2013. Physico-chemical properties of *Hyptis Suaveolens* essential oil. Int. J. Med. Arom. Plants. 3: 191-199.
- Noudogbessi, J., Sessou, P., Wotto, V., Figueredo, G., Chalard, P., Chalchat, J., Dansou, K., Sohounhloué, D. 2012a. Chemical Compositions and Preventive Activity of Essential Oils Extracted From the Leaves of Two Varieties of *Tephrosia (Leguminosae-Papilionoideae)* Collected in Benin on *Callosobruchus Maculatus* (Fabricius). Asian Journal of Research in Chemistry. 5: 1431-1436.
- Noudogbessi, J., Sessou, P., Wotto, V., Figueredo, G., Chalard, P., Chalchat, J., Dansou, K., Sohounhloué, D. 2012b. Chemical Compositions and Preventive Activity of Essential Oils Extracted from the Leaves of Two Varieties of *Tephrosia (Leguminosae-Papilionoideae)* Collected in Benin on *Callosobruchus Maculatus* (Fabricius). Asian J. Res. in Chemistry. 5: 1431-1436.
- Prajapati, S. 2012. Biomonitoring and speciation of road dust for heavy metals using *Calotropis procera* and *Delbergia sissoo*. Environmental Skeptics and Critics. 1: 61-64.
- R Development Core Team. 2013. R: A Language and Environment for Statistical Computing. Vienna, Austria: The R Foundation for Statistical Computing. ISBN: 3-900051-07-0. Available online at <http://www.R-project.org>.
- Ribeiro, V., Dos Santos, J., Bordignon, S., Apel, M., Henriques, A., Von Poser, G. 2010. Acaricidal Properties of the Essential Oil from *Hesperozygis Ringens* (Lamiaceae) on the Cattle Tick *Boophilus microplus*. Bioresour. Technol. 101: 2506-2509.
- Richard, H. Quelques Épices Et Aromates Et Leurs Huiles Essentielles ; 1976
- Rodriguez-Vivas, R.I., Rivas, A.L., Chowell, G., Fragoso, S.H., Rosario, C.R., Garcia, Z., Smith, S.D., Williams, J.J., Schwager, S.J. 2007. Spatial distribution of acaricide profiles (*Boophilus microplus* strains susceptible or

- resistant to acaricides) in southeastern Mexico. Vet. Parasitol. 146: 158-169.
- Rosado-Aguilar, J., Aguilar-Caballero, A., Rodriguez-Vivas, R., Borges-Argaez, R., Garcia-Vazquez, Z., Mendez-Gonzalez, M. 2010. Acaricidal activity of extracts from *Petiveria alliacea* (Phytolaccaceae) against the cattle tick, *Rhipicephalus (Boophilus) microplus* (Acari:Ixodidae). Vet. Parasitol. 168: 299-303
- Santos, T., Klafke, G., Pappen, F., Nizoli, L., Biegelmeyer, P., Farias, N. 2003. Comparison of Three Larval Bioassays to Evaluate Susceptibility of *Rhipicephalus (Boophilus) Microplus* to Amitraz. Rev. Bras. Parasitol. Vet. 22: 495-501.
- Sarda, R.V.L., Toigo, E., Bordignon, A.L., Goncalves, K., Von Poser, G. 2007. Acaricidal properties of extracts from the aerial parts of *Hypericum polyanthemum* on the cattle tick *Boophilus microplus*. Vet. Parasitol. 147: 199-203.
- Sessou, P. 2008. Efficacité des extraits de plantes sur les bactéries *Salmonella oakland* et *Salmonella legon*. Mémoire de DIT, Université d'Abomey-Calavi, Bénin. pp. 1-70.
- Sessou, P., Farougou, S., Alitonou, G., DjenontinTindo, S., Yèhouénou, B., Azokpota, P., Youssao, I., Sohounhloué, D. 2012. Chemical Composition and Antifungal Activity of Essential Oil of Fresh Leaves of *Ocimum Gratissimum* from Benin Against Six Mycotoxigenic Fungi Isolated from Traditional Cheese WagashiJ. Soc. Ouest-Afr. Chim. 35: 41-48.
- Soberanes, N., Santamaría, V., Fragoso, H. 2002. First Case Reported of Amitraz Resistance in the Cattle Tick *Boophilus Microplus* in Mexico.Tec. Pecu. Mex. 40: 81-92.
- Tava, A., Odoardi, M. 1996. Saponins from *Medicago* SPP.: Chemical Characterization and Biological Activity Against Insects in: Waller G, Yamasaki K (ed.) Saponins used in food and agriculture, 405: 97-109.
- Trease, G.E., Evans, W.C. 2002. Pharmacognosy, in 15th Ed. Saunders Publishers: London; pp. 42-393.
- Usman, I.S., Mani, A.U., Mohammed, I.D. 2015. Indigenous Foot and Mouth Disease Control Methods among Nomadic Cattle Fulanis in Adamawa State, Nigeria. Alex. J. Vet. Sci. 45: 71-78.
- Yayi, E. 1998. Contribution à l'étude des huiles essentielles de plantes aromatiques du Bénin: cas de *Ocimum basilicum*, *Ocimum canum* et *Ocimum gratissimum* dans la perspective de leur production, in, Thèse de doctorat en chimie organique des substances naturelles, Université Nationale du Bénin: pp. 1-182.
- Yayi, E., Joachin, D., Léon Akanni, A., Mansour, M., Jean-Claude, C. 2004. *Ocimum. Gratissimum* L., Siège De Variations Chimiques Complexes Au Cours Du Développement. C. R. Chimie.7 : 1013-1018.
- Yayi-Ladekan, E., Dossou, S., Fernand, G., Bénédicta, G-K., Joachim, G., Claude, J., Mansourou, M., Georges, C., Joëlle, Q-L. 2011. Variation Diurne De La Composition Chimique Et Influence Sur Les Propriétés Antimicrobiennes De L'Huile Essentielle De *Ocimum Canum* Sims Cultivé Au Bénin. Int. J. Biol. Chem. Sci. 5: 1462-1475.