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# Bioactivity of essentials oils of *Hyptis suaveolens* (L.) on the reproductive parameters of the *Rhipicephalus (Boophilus) microplus*

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## ABSTRACT

The chemical composition of three essential oils of *Hyptis suaveolens* was performed by gas chromatography coupled with mass spectrometry and their bioactivity was deduced from a multi-dose immersion test in increasing progression.

The results show that the essential oils of *H. suaveolens* analysed are of two types: a sesquiterpene-dominant type (51.86%) very rich in β-caryophyllene (20.69%) and a monoterpene-dominant type (38.08%) which is distinguished from the first by a relatively high eucalyptol (1.8-Cineole) content (12.11%). Laboratory tests indicate a high toxicity of the β-caryophyllene chemotype on females of *R. (B.) microplus*. Only gorged females exposed to the two concentrations (1% and 2%) of this oil laid eggs with a laying delay of 168 hours. The egg laying inhibition rate recorded for these two concentrations is 65 and 98% respectively. The essential oil of *H. suaveolens* therefore represents a very interesting alternative to the use of synthetic antiparasitic.

**Keywords:** Essential oil, Hyptis Suaveolens, β-caryophyllene, R. (B.) microplus, oviposition.

## INTRODUCTION

Because of their ability to transmit diseases to domestic animals, ticks are ranked as the second most harmful arthropods in the world [1]. These diseases are considerably important to the health and productivity of the cattle herd. The development of dairy farming in Benin in recent years through the Support Project for the Milk and Meat Sector (PAFILAV) is therefore strongly compromised or at least slowed down by these diseases, especially in exotic pure-bred animals. Among the different species of ticks identified in Benin, the cattle tick *Rhipicephalus (Boophilus) microplus* whose development cycle is monoxenous, is a one-host cattle tick which has a tropical and subtropical distribution. This major ectoparasite has a very important economic impact on cattle husbandry throughout its area of distribution. The control of cattle ticks is usually done using conventional chemicals including synthetic pyrethroids (SP), organophosphates (OP), and amitraz (Am) with high acaricidal power [2]. Unfortunately, these products are persistent and have high toxicity to animal and human health and the environment. In addition, the selection of resistant individuals to the synthetic chemicals used is one of the problems to be solved.

Face of these difficulties, it would also be necessary to research and improve the use of natural acaricides which, while active, are biodegradable and well known by local communities [3]. Previous work has shown that some plants contain substances in their organs (root, bark, leaves) that have insecticidal, bactericidal, fungicidal and even acaricidal properties [4]. These broad-spectrum plants could be used as alternative acaricides [5].

*Hyptis suaveolens* (Lamiaceae) Poit, 1806 is herbaceous aromatic plant from tropical America. In Benin, *H. suaveolens* leaves traditionally were involved in several medicinal combinations in order to control certain diseases such as jaundice, hyperthermia, breast abscesses, hemorrhoids, anal oral candidiasis and generalized edema [6]. *H. suaveolens* of various originsgeographic, made the object of relevant scientific studies and many of these studies have reported can act as larvicide, insect growth regulators, and repellent and ovipositor attractant.

This work therefore aims to study the chemical composition of essential oils extracted from the leaves of *H. suaveolens* (L.) harvested in Benin's three main climatic zones and to evaluate their effects on the reproductive parameters of female *Rhipicephalus (Boophilus) microplus*. The objective is to try to answer the following two questions: Does geographical location have an influence on chemical compositions? Can chemical composition affect biological activity?

## MATERIALS AND METHODS

### Leaf harvesting

The leaves of *H. suaveolens* (L.), constitute the plant material. They were harvested early in the morning in their natural environments in Benin's main climatic zones during September and October 2017. The harvests were successively made in the communes of Sème-Podji, Dassa and Kérou respectively in the southern, central and northern climatic zones.

### Extraction of essential oils and analysis of chemical constituents

The harvested leaves were transported to the laboratory where they were dried at a temperature of 17 °C away from sunlight. Samples were identified and certified under number AA6726/HNB at the Benin National Herbarium using the analytical flora of Akoègninou *et al.* [7]. The essential oils were extracted by simple distillation using a Clevenger hydrodistiller [8] at the Laboratory of Enzymology and Protein Biochemistry of the Institute of Applied Biomedical Sciences (ISBA). The dry leaves of *H. suaveolens* and distilled water were boiled for 2 to 3 hours, then the plant extracts condensed in a water cooler were recovered at the end of the process in a decanting tank.

The analysis of the chemical constituents of essential oils and the determination of their relative centesimal compositions were carried out in the LEXVA-ANALYTIC laboratory by gas chromatography (GC) equipped with a flame ionization detector (CG-FID) and by coupled gas chromatography and mass spectrometry (GC-SM) according to the following operating conditions (Table 1).

**Table 1:** Operating conditions

| | |
|---|---|
| Gas chromatograph: GC/MS 7890/5975C | - Apolar column: DB5 MS: 40 m 0.18 mm 0.18 µm;<br>- Temperature programming: 50 °C for 5min - 50 °C/min up to 300 °C<br>- Carrier gas: He: He: 1 ml/min;<br>- Sample: 4% in solution in acetone or hexane;<br>- Injection volume: 2 µl;<br>- Injector: 280 °C with 1/100 divider;<br>- Mass range: 33 to 550;<br>- The oil compounds are identified by a combined search for retention times (laboratory library) and mass spectra (NIST library 225 000 spectra) |
| Gas chromatograph: GC/FID 7890 | - Apolar column: DB5 MS: 40 m 0.18 mm 0.18 µm;<br>- Temperature programming: 50 °C for 5min - 50 °C/min up to 300 °C<br>- Carrier gas: He: He: 1 ml/min;<br>- Sample: 4% in solution in acetone or hexane;<br>- Injection volume: 2 µl ;<br>- Injector: 280 °C with 1/100 divider;<br>- Percentages (%) are calculated from the peak areas given by the GC/FID without the use of a correction factor. |

### Immersion test of female *R. (B.) microplus* gorged *R. (B.)* females

The slightly modified adult immersion test (AIT) of Drummond *et al.* [9] was used to evaluate the effect of essential oils on *R. (B.) microplus* gorged females.

Homogeneous batches of seven (07) *R. (B.) microplus females* were totally immersed for one minute in increasing concentrations of 1%, 2%, 3%, 3%, 4% and 5% initially prepared by diluting respective volumes of 0.25; 0.5 ; 0.75 ; 1 and 1.25 ml of the essential oil in distilled water with Tween 80 to 1% added as a non-toxic emulsifier for the mite.

Distilled water with Tween 80 added was used as a negative control, while the positive control was Alfacypermethrin acaricide molecule at the 1% concentration (recommended by the manufacturer). Three repetitions were made for each concentration and for each essential oil. The initial mass weight of all batches was determined using a precision balance.

After immersion, they were transferred to the boxes and covered with fine gaz fabric firmly attached to the edges with adhesive tape. This device has made it possible to keep them tamed and guarantee good ventilation until the eggs are laid.

The mass weight of eggs laid in each batch was determined eleven days after the start of oviposition in the negative control batch and parameters such as egg laying rate (TP) and egg laying inhibition rate (TIP) were determined according to the formula of Flores-Fernández *et al.* [10]:

**TP (%) = 100*(Mass weight of eggs laid / Initial engorged female weight)**

**TIP (%) =100*[(TP(negative control batch) – TP(treated batch))/ TP (negatif control batch)]**

Also, the laid eggs for both treated and untreated group were incubated at room temperature (28 ± 2°C) for 30 days to estimate percentage hatching. The hatching percentage of eggs was determined by visual estimation.

### Statistical analysis

The data related to the immersion test were encoded in a database designed on Excel and analyzed with SAS 9.4 software (SAS, 2013. SAS® 9.4 Procedures Guide: Statistical Procedures, Second Edition. Cary, NC: SAS Institute Inc., 550p). A fixed effect linear has been adjusted to the initial mass, egg mass, egg laying rate and inhibition rate and includes the fixed effects of the chemical composition of the essential oils and doses (different concentrations). The interaction between chemical composition and dose was significant and was taken into account in the analysis model.

## RESULTS AND DISCUSSION

### Analysis of the chemical compounds of essential oils

The different essential oils obtained are pale yellow in color and have a very strong odor. Chromatographic analyses made it possible to identify the different compounds with the database (**Table 2**).

**Table 2:** The main compounds identified in essential oils of *H. suaveolens* according to climatic zones.

| Tr | Chemical Constituents | climatic zones of origin of the leaves of *H. suaveolens* | | |
|---|---|---|---|---|
| | | South | Center | North |
| | | % FID | | |
| *13.80* | Sabinene | 7.180 | 3.340 | 7.700 |
| *15.96* | Eucalyptol | 12.112 | 0.151 | 10.924 |
| *17.70* | Terpinolene | - | 6.698 | 2.528 |
| *17.73* | Linalool Trans-Oxide | 6.557 | - | - |
| *17.89* | Fenchone | 11.812 | 2.780 | 4.719 |
| *18.93* | Fenchol* | 0.894 | 1.127 | 11.810 |
| *27.75* | β-Caryophyllene | 10.338 | 20.691 | 12.457 |
| *29.63* | Bicyclogermacrène | 5.755 | 9.783 | 3.375 |
| *31.65* | Dehydro-Isolongifolene* | 2.465 | 8.432 | 2.354 |
| | *Monoterpenic hydrocarbons* | 38.087 | 19.236 | 27.37 |
| | *Oxygenated monoterpenes* | 3.567 | 3.555 | 15.162 |
| | *Monoterpenic oxydes* | 18.687 | 0. 456 | 11.405 |
| | *Sesquiterpenic hydrocarbons* | 27.98 | 51.865 | 29.191 |
| | *Oxygenated sesquiterpenic* | 2.048 | 8.7 | 4.01 |
| | *Terpenic ketones* | 0.977 | 0.256 | 0.289 |
| | *Alcohol* | 0.362 | 3.096 | 0.786 |
| | *Aldehydes* | 0.028 | 0.079 | 0.21 |
| | *Esters* | 0.557 | 0.689 | 0.31 |
| | *Diterpenes* | 3.028 | 2.363 | 5.742 |
| | TOTAL | 95.321 | 90,295 | 94,475 |

*Tr: Retention time; * Unidentified isomer; - Absent*

In this table, the essential oil obtained from the leaves of *H. suaveolens* harvested in the South is predominantly monoterpenic (38.08%) with a relatively high content of Eucalyptol (1.8-Cineole) (12.11%) followed by fenchone (11.81%). While, those extracted from the leaves harvested respectively in Central and Northern Benin are predominantly sesquiterpenic rich in β-caryophyllene (20.69 - 12.45%).

There is also analogy in the chemical composition of the essential oils of the leaves harvested in the southern and northern climatic zones. Indeed, when the content of β- Caryophyllene is low, Eucalyptol is relatively high and vice versa.

A comparison with the data in the literature confirms our results. Noudogbessi *et al*. [11] on seven samples of *H. suaveolens* collected from localities in Benin showed that they were mainly composed of 1,8-cineole (eucalyptol), sabinene, and β-caryophyllene. On the other hand, the essential oil analyzed by Kossouoh *et al.* [12] is rather rich in β-caryophyllene, trans- α-bergamotene, caryophyllene oxide and bicyclogermacene. These different profiles are also those obtained in Nigeria [13], Côte d'Ivoire [14] and Dakar [15].

This difference depends on ecotypes because the variation in chemical composition in plants is related to climatic factors, geographical origin, age of the plant but also ecological and pedological factors. Concentrations of these substances may also vary according to seasonality, circadian rhythm and plant development [16].

**Effect of essential oils on the reproductive parameters of engorged female of *R. (B.) microplus***

Compared to the negative control, the three essential oils of *H. suaveolens* caused a delay in oviposition in females of *R. (B.) microplus*, but this varies considerably depending on the composition of the essential oils (Table 3**)**. This delay is only 72 hours for females exposed to the dose of 3% of eucalyptol-rich essential oil. While, it is 144 hours for the dose of 2% of essential oils dominated by β-Caryophyllene. The same observations were made by Flores-Fernández *et al*. [10] with Lippia graveolens essential oil at a concentration of 1.25% resulted in a 24-hour delay in egg laying in females of *R. (B.) microplus*.

**Table 3:** Date of oviposition according to concentrations and essential oils and the climatic zone of origin.

| Climatic zone of the leaves of *H. suaveolens* | H.E concentration (%) | | Day corresponding to the beginning of egg laying | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ji | $J_1$ | $J_2$ | $J_3$ | $J_4$ | $J_5$ | $J_6$ | $J_7$ | $J_8$ | $J_9$ | $J_{10}$ | $J_{11}$ |
| South | $T_N$ | 0.00 | - | - | + | | | | | | | | | |
| | $C_1$ | 1 | - | - | - | + | | | | | | | | |
| | $C_2$ | 2 | - | - | - | - | + | | | | | | | |
| | $C_3$ | 3 | - | - | - | - | - | + | | | | | | |
| Center | $C_1$ | 1 | - | - | - | - | + | | | | | | | |
| | $C_2$ | 2 | - | - | - | - | - | - | - | + | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $C_1$ | 1 | - | - | - | + | | | | | |
| North | $C_2$ | 2 | - | - | - | - | - | - | - | - | + |
| | Alfacyper | 1 | - | - | + | | | | | | |

TN: negative control; Alfacyper (Alfacypermethrin solution); +: laying; -: no laying

This delay in laying is accompanied by a decrease in the number of eggs laid per female. The essential oils of *H. suaveolens,* whatever the chemical composition, have a significant effect on the fertility of female *R.* (*Boophilus*) *microplus* ticks. This effect ranged from a reduction in egg laying to total inhibition as the concentration of essential oils increases as shown in Table 4.

**Table 4:** Variation of the laying rate (TP) and the inhibition laying rate (TIP) according to the different dose and composition of essential oils.

| **climatic zone of leaves of H. *suaveolens*** | **Doses** | **Initial weight of engorged females (gr)** | **weight of eggs laid (gr)** | **Egg laying rate (%)** | **Inhibition rate (%)** |
|---|---|---|---|---|---|
| | 0,00 % | $1.05^{a} \pm 0.05$ | $0.37^{a} \pm 0.04$ | $35.24^{a} \pm 5.62$ | $0,00^{a} \pm 0,00$ |
| South | 1 % | $1.06^{a} \pm 0.03$ | $0.26^{ab} \pm 0.02$ | $23.77^{ab} \pm 0.25$ | $31.33^{ab} \pm 11.22$ |
| | 2 % | $1.02^{a} \pm 0.15$ | $0.16^{bc} \pm 0.03$ | $16.90^{b} \pm 04.14$ | $50.05^{b} \pm 18.48$ |
| | 3 % | $0.93^{a} \pm 0.09$ | $0.1^{bc} \pm 0.20$ | $12.90^{b} \pm 19.15$ | $68.83^{bc} \pm 46.26$ |
| | 4 % | $1.04^{a} \pm 0.08$ | $0.00^{c} \pm 0.00$ | $0.00^{c} \pm 0.00$ | $100.0^{c} \pm 0.00$ |
| | 5 % | $1.14^{a} \pm 0.06$ | $0.00^{c} \pm 0.00$ | $0.00^{c} \pm 0.00$ | $100.0^{c} \pm 0.00$ |
| Center | 1 % | $1.03^{a} \pm 0.11$ | $0.12^{bc} \pm 0.03$ | $12.31^{b} \pm 4.7$ | $65.78^{b} \pm 7.62$ |
| | 2 % | $0.85^{a} \pm 0.11$ | $0.003^{c} \pm 0.005$ | $0.46^{c} \pm 0.80$ | $98.88^{c} \pm 1.93$ |
| | 3 % | $0.88^{a} \pm 0.17$ | $0.00^{c} \pm 0.00$ | $0.00^{c} \pm 0.00$ | $100.0^{c} \pm 0.00$ |
| | 4 % | $0.82^{a} \pm 0.12$ | $0.00^{c} \pm 0.00$ | $0.00^{c} \pm 0.00$ | $100.0^{c} \pm 0.00$ |
| | 5 % | $0.68^{a} \pm 0.13$ | $0.00^{c} \pm 0.00$ | $0.00^{c} \pm 0.00$ | $100.0^{c} \pm 0.00$ |
| North | 1 % | $1.06^{a} \pm 0.03$ | $1.03^{c} \pm 1.78$ | $6.08^{c} \pm 10.54$ | $85.28^{c} \pm 2\ 5.48$ |
| | 2 % | $1.02^{a} \pm 0.15$ | $0.98^{c} \pm 1.70$ | $1.52^{c} \pm 2.64$ | $95.49^{c} \pm 7.79$ |
| | 3 % | $0.93^{a} \pm 0.09$ | $0.00^{c} \pm 0.00$ | $0.00^{c} \pm 0.00$ | $100.0^{c} \pm 0.00$ |
| | 4 % | $1.04^{a} \pm 0.08$ | $0.00^{c} \pm 0.00$ | $0.00^{c} \pm 0.00$ | $100.0^{c} \pm 0.00$ |
| | 5 % | $1.14^{a} \pm 0.06$ | $0.00^{c} \pm 0.00$ | $0.00^{c} \pm 0.00$ | $100.0^{c} \pm 0.00$ |
| | Alfacyper | $0.79^{a} \pm 0.30$ | $0.38^{a} \pm 0.19$ | $46.79^{d} \pm 9.27$ | $00.00^{d} \pm 00.00$ |
| **ANOVA** | | *NS (p= 0,145)* | ***(p<0,001)* | ****(p<0,0001)* | ****(p<0,0001)* |

Alfacyper: Alfacypermethrin; Mean ± Standard Error

But, unlike monoterpenically dominant essential oils, sesquiterpenically dominant essential oils (rich in β-Caryophyllene) appear to be more toxic to females of *R. (B.) microplus*. The egg laying inhibition rates recorded at the 2% concentration of these oils are 98.88 ± 1.93 and 95.49 ± 7.79, respectively, significantly different (p<0.0001) from 68.83 ± 46.26 for the 3% dose of monoterpenically dominant essential oil (Table 4). The three essential oils of *H. suaveolens* tested completely inhibit the fertility of female *R. (B.) microplus* from the 4% dose.

A comparison with the literature shows that terpene derivatives (sesquiterpene hydrocarbons) are mentioned as toxic. For example, the essential oil of *Piper nigrum*, which is predominant in sesquiterpene *β*-caryophyllene (26.2%), has caused a reduction in female *R. (B.) microplus* fertility [17]. The same effect was observed with *Piper marginatum* essential oil against Tetranychus urticae (Acari: Tetranychidae), where fertility was significantly reduced [18]. *β*-caryophyllene was one of the main compounds (16%) present in the essential oil of *P. marginatum,* such as the oils of *H. suaveolens* in this work. Also, the biological properties of caryophyllene have been confirmed by Zilda *et al*. [19] and Ribeiro *et al*. [20] in previous studies.

However, these results are still contrary to the work of Dedome *et al*. [21] with essential oils extracted from *Monanthotaxis parvifolia* (Oliv.) and *Xylopia parviflora* rich in caryophyllene oxide inhibited egg laying in *microplus R. (B.)* females at only 62.33 and 49.82% respectively at a dose of 10% [21a, 21b]. The effect of the oils therefore seems to be related to the main chemical groups identified in them.

The bioactive compounds of essential oils are known to have disruptive effects on the basic metabolic, biochemical, physiological and behavioural functions of ticks. The sesquiterpenic components present in the essential oils of *H. suaveolens* therefore disrupt ovarian development in female ticks. They suppress and reduce the rate of ovarian development and thus reduce the number of eggs emitted by females. Eggs are also altered by the active components of essential oils during ovarian development, hindering hatching ability after oviposition [23, 24]. According to Touré [25], the biological activity of an essential oil is related to its chemical composition, the functional groups of the majority compounds and the possible synergistic effects between the minority components. Thus, the nature of the chemical structures that compose it, but also their proportions, play a decisive role.

In addition, the commercial solution of alfacypermethrin showed no in vitro activity on egg laying and fertility in *R. (B.) microplus* female. This suggests a resistance of *R. (B.) microplus* to Alfacypermethrin as described by many authors [26, 27, 28].

Visual examination of the incubated eggs revealed that all eggs laid by ticks treated with essential oils of *H. suaveolens* did not hatch, while those laid by untreated ticks did. The lethality of essential oils on eggs laid was total for all concentrations tested. The essential oils of *H. suaveolens* therefore interfere with the development of eggs by blocking hatching rates. According to Don Pedro [29], the ovicidal activity of essential oils is due to the direct toxicity of their compounds that inhibit the metabolic activity of eggs. This indicates a high ovicidal potential of the essential oil of *H. suaveolens.*

## CONCLUSION

This study shows that geographical origin has a quantitative and/or qualitative influence on the chemical composition of essential oils of the same plant species and this variation is related to biological activity.

Indeed, the essential oils of *H. suaveolens* rich in sesquiterpenic compounds, mainly β-caryophyllene, have shown very interesting activity on the laying capacity and fertility of the engorged female *R. (B.) microplus*. The minimum concentration of 2% of these oils caused a delay of more than a week in gorged females with an inhibition percentage of nearly 99%. This essence can therefore be used as an active raw material in the formulation of bio-acaricides. Our work is continuing in this direction in order to find an appropriate formulation applicable in farms to effectively control ticks, especially the invasive tick *R. (B.) microplus*.

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## REFERENCES

1. Parola P, Raoult D. Ticks and tick-borne bacterial disease in humans: an emerging infection threat. Clinical Infectious Diseases. 2001; 32:897-928.
2. Wood TG, Pearce MJ. Termites in Africa: the environnemental impact of control measures and damage to crops, tree, rangeland and rural buildings. Sociobiology. 1991; 19(1):221-234.
3. Njan-Nloga AM, Saotoing P, Tchouankeu JC, Messi J. Journal of Entomology. 2007; 4(6):444-450.
4. Brahim O, Saadia M, Fouad M, Saadia, Agron. Soc. Environ., 2006; 10(2):67-71.
5. Pamo ET, Amvam ZPH, Tendonkeng F, Kana JR, Fongang MD, Tapondjou LA. Livestock Research Rural Development, 2004; 16(9).
6. Adjanohoun EJ, Adjakidjè V, Ahyi MRA, Ake Assi L, Akoegninou A, d'Almeida J *et al*., Médecine traditionnelle et Pharmacopée, Contribution aux études ethnobotaniques et floristiques en République Populaire du Bénin, *ACCT*, Paris, France, 1989.
7. Akoègninou WJ, van der Burg LJG, Van der Maesen, Flore analytique du Bénin. Backhuys Publishers, 2006, 1043.
8. Clevenger JF. Apparatus for the determination of volatile oil. Journal of American Pharmaceutics Association. 1928; 17:336-341.
9. Drummond RO, Ernst SE, Trevino JL, Gladney WJ, Graham OH, Journal of Economic Entomology 1973; 66:130-133.
10. Flores-Fernández JM, Padilla-Camberos E, Castillo Herrera GA, Martínez-Velázquez M. Tropical Biomedicine. 2016; 33(2):290-294.
11. Noudogbessi JP, Agbangnan P, Yehouenou B, Adjalian E, Nonviho G, Akibou OM *et al*. International Journal. Medecinal Aromatique. Plants, ISSN 2249 – 4340, 2013; 2(3):191-199.
12. Kossouoh C, Moudachirou M, Adjakidje V, Chalchat JC, Figueredo G. A Comparative study of the chimical composition of the leaves and fruits deriving the essential oil of *Hyptis suaveolens* (L.) Poit. From Benin, 2010; 22:507-509
13. Eshilokun AO, Kasali AA, Giwa-Ajeniya AO, Flavour Fragr J 2005; 20:528-530.
14. Tonzibo ZF, Florence AB, Bédi G, Chalchat JC. European Journal of Scientific Research. ISSN 1450-216X 2009; 38(4):565-571.
15. Ngom S, Diop M, Mbengue M, Faye F, Kornprobst JM. et A. Samb, Afrique Science. 2014; 10(4):109-117.
16. Gobbo-Neto L, Lopes NP, Quimica Nova. 2007; 30(2):374-381.
17. Vinturelle R, Mattos C, Meloni J, Nogueira J, Nunes MJ, Vaz JS, *et al*. Biochemistry Research International, 2017, Article ID 5342947, 9p https://doi.org/10.1155/2017/5342947.
18. Ribeiro N, Camara C, Ramos C. Chilean Journal of Agricultural Research. 2016; 76(1):71-76.
19. Zilda CG, Izabel GD, Maria VCL, Ana CL, Ana MCH, Claudia MR *et al*. Diogenes, Experimental Parasitology. 2011; 129:175-178.
20. Ribeiro VLS, Avancini C, Gonçalves K, Toigo E, Von Poser G. Veterinary Parasitology. 2008; 151:351-354.
21. Dedome SLO, Alitonou GA, Sessou P, Tchobo FP, Yovo M, Yessinou E *et al*. Sohounhloue, American Journal of Applied Chemistry. 2017a; 5(3):53-56.
22. Dedome SLO, Alitonou GAP, Sessou FP, Tchobo M, Yovo E, Yessinou S *et al*. Sohounhloue, IJPCBS 2017b, 7(2), 149-154.
23. H.F. Khater, Ecosmart Biorational Insecticides: Alternative Insect Control Strategies. Insecticides - Advances in Integrated Pest Management. 2012, 17-61.
24. Shyma K, Gupta J, Ghosh S, Patel K, Singh Veer. Parasitology Research. 2014; 16(9).
25. D. Toure Etudes chimique et biologique des huiles essentielles de quatre plantes aromatiques medicinales de côte d'ivoire. Thèse de doctorat Chimie organique. Université Felix Houphoeut Boigny, Côte d'Ivoire. 2015, 153.
26. Yessinou RE, Akpo Y, Sidick A, Adoligbe C, Youssao AKI, Akogbeto M *et al*. Ticks and Tick-borne Diseases. 2018; 9:665-671.
27. Mendes MC, Duarte FC, Martins JR, Klafke GM, Fiorini LC, Barros ATM. Rev. Bras. Parasitol. Veter. 2013; 22:379-384.
28. Fernandez-Salas RI. Rodrıguez-Vivas, A.M. Alonso-Dıaz, J. Parasitol. 2012; 98(5):1010-1014.
29. Don Pedro KN. Pestic. Sci. 1989; 26:107-116.

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