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Insecticide Activity of Essential Oils on the Development of Eggs and Adult of *Caryedon serratus* Olivier (Coleoptera: Chrysomelidae), Pest of Stored Groundnut

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Authors' contributions

This work was carried out with the collaboration of all authors. Author OI is the main instigator, he led the field work and laboratory analysis. Author NCR synthesized and analyzed the composition of essential oils used in our work. Authors DD and GW participated in the development of research protocols and analysis of the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

In Burkina Faso, the groundnut is attacked during storage by the weevil *Caryedon serratus* Olivier. The damage caused by this pest can reach 70% in the absence of protection methods.
To protect stored groundnut against *C. serratus*, essential oils extracted from four plants *Lippia multiflora* M, *Cymbopogon shoenanthus* (L.) Spreng, *Ocimum americanum* Sims and *Hyptis suaveolens* Poit obtained by hydro-distillation of leaves, were tested on adults and eggs of this pest.

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The application of essential oils in doses ranging from 10 to 25 µl induces in 24 hours a mortality ranging from 15 to 100% according to the type of essential oils. Thus the use of 10 µl of *Lippia multiflora* caused 100% mortality in 24 hours against 87.5% for *C. shoenanthus*, 27.5% for *Hyptis suaveolens* and 57.5% for *Ocimum americanum.*

The CL_{50} varied by 10.41 µl in 24 hours for *Hyptis suaveolens* oil, with *Lippia multiflora* it is 6.51 µl for only 5.17 µl for *Cymbopogon shoenanthus.*

The result show that the use essential oils against *C. serratus* in laboratory conditions has significantly reduced the eggs emitted by females and their lives.

Keywords: Groundnut; storage; Caryedon serratus; essential oil; Burkina Faso.

1. INTRODUCTION

Groundnut (*Arachis hypogea* L), is one of the major export crops of economic importance in Burkina Faso with more than 335,000 tons produced annually [1]. This crop plays an important role in the rural economy of Burkina Faso, because it constitutes both a food crop and a cash crop for producers [1]. On nutrition level, groundnut plays an important role for both urban and rural population. In fact, groundnut seeds, composed of approximately 50% of oil and 25% of protein, constitute with shea butter and cotton seed oil, the main source of lipids for the local populations of Burkina Faso. With a population growth estimated at 3% per year, there is a high demand for products derived from groundnut such as groundnut oil, butter cake etc). In spite of this prospect, groundnut is only available in quantity and quality during one short period of the year [1]. This situation is explained by the multiple constraints among which post-harvest during storage is the most crucial [2]. Indeed, stored groundnuts are attacked by several pests which often cause considerable losses [3-5]. Among these pests, the beetle *Caryedon serratus* (Olivier) bruchid from the family of Chrysomelidae appears to be the most dangerous for stored groundnut. It is the only pest that can attack unshelled groundnut during storage [6,5]. In Burkina Faso, the presence of this pest was announced by Robert [7] and Lafleur [8], according to the latter, *C. serratus* is the most harmful insect pest for stored groundnut. In the west of Burkina Faso where 25% groundnut production are cultivated, losses caused by *C. serratus* in stored groundnut reach 70% after six months of storage in absence of any treatment [9].

Losses caused by *C. serratus* on groundnut is at the basis of the development of protection methods of stored groundnut in several countries [10]. Among these methods is the use of insecticides or extracts from plant origin [11]. Many authors reported that a great number of plant species rich in essential oils have been used in stored cereals or legumes against crop pests [12-14]. The application of the essential oil from *Hyptis spigiera* Lam in laboratory for against cowpea weevil significantly reduced amount of eggs laid by the weevil was reported by Sanon et al. [15]. It is in this context that the work aimed to develop protection strategies groundnut by using essential oils. To achieve this aim, the insecticide activity of four essential oils extracted from *Lippia multiflora* M, *Cymbopogon shoenanthus* (L.) Spreng; *Ocimum americanum* sims and *Hyptis suaveolens* Poit on the development of the eggs and adults of *Caryedon serratus,* pest of groundnut during storage were determined.

2. MATERIALS AND METHODS

2.1 Groundnuts Varieties

The groundnut variety used for the study (HS 470 P) was obtained from ICRISAT.

2.2 Aromatic Plants, Extraction Methods and Chemical Analyze of Essential Oils

Essential oils which were subject of trial were extracted from several species of plant families. These plants were obtained from Bobo-Dioulasso located in the western part of Burkina Faso.

The plants used in this study are presented in Table 1.

The leaves of the plants were collected and sun dried in greenhouse for 96 hours before being sent to Research Laboratory on Natural Substances of IRSAT located in Ouagadougou for the extraction of essential oils.

Table 1. Insecticides plants used for the different tests

	Plants	Family
Essential oils	*Cymbopogon shoenanthus* L. Spreng	Poaceae
	Hyptis suaveolens Poit.	Lamiaceae
	Lippia multiflora Moldenke	Verbenaceae
	Ocimum americanum Sims.	Lamiaceae

2.3 Essential Oil Extraction

The oils were extracted by steam distillation using a Clevenger -type apparatus for 3 hours, using fresh or dry plant material as appropriate. Essential oils are collected by decantation, and then dried over anhydrous sodium sulfate. The calculation of the yields was made by the ratio of the volume of oil collected in milliliters on the weight in grams of plant material used.

2.4 Analysis by GC/MS

The essential oil were analyzed on a AGILENT gas chromatograph Model 7890, coupled to a AGILENT MS model 5975, equipped with a DB5 MS column (20 m X 0,18 mm, 0,18 µm), programming from 50℃ (3.2 min) to 300℃ at 8℃/mn, 5 min hold. Helium as carrier gas (1,0 ml/min); injection in split mode (1: 150) at 300℃. The MS working in electron impact mode at 70 eV; electron multiplier, 1800 V; ion source temperature, 230℃; mass spectra data were acquired in the scan mode in *m/z* range 33-550. Identification of the major compounds was achieved by comparison with retention times of purchased standards and after the Oils were put in small bottles and preserved in a refrigerator at 4℃ until when needed.

2.5 Test Insect

Adults of *C. serratus* of 48 hours old were obtained from TS 32 variety of groundnut. For each dose of essential oil tested, ten insects (5 females and 5 males) of *C. serratus* were used. In total, for each essential oil tested, 240 insects were used.

2.6 Effectiveness of Essential Oils on Lifespan of Adults *C. serratus*

To assess the effectiveness of essential oils extracted from plants, oils obtained from the four plants (*C. shoenanthus*, *H. suaveolens*, *O. americanum* and *L. multiflora*) were used at different doses. Into each one liter bottle, 20 groundnut pods were introduced, Thereafter, Five *C. serratus* couples of 48 hours old were also introduced. Seven doses (3 µL; 5 µL; 7 µL; 10 µL; 15 µL; 20 and 25 µL) each of the oil were measured using a pipette. The oils were then rubbed on Whatman filter (N°1) paper stuck inside the bottles with modeling clay; the bottles were then hermetically closed. The experiment was replicated four times in addition to the control experiment which did not receive any application. The insect mortality rate in groundnut treated groups was assessed 24 hours after the application of essential oil.

2.7 Assessment of the Efficacy of Essential Oils on Eggs of *C. serratus*

Careydon serratus of 48 hours old were insolated and introduced into pods of groundnut in a bottle for egg laying during 24 hours, the insects were then removed and eggs laid were counted and recorded using binocular. The pods with visible eggs were collected in one liter bottles at a rate of 20 eggs per bottle. Thereafter, extracted oils from *C. shoenanthus*, *H. suaveolens*; *O. americanum* and *L. multiflora* at 10 µl and 20 µl dose were tested. The fumigation of eggs was carried out 24 hourly, the pods were removed and put under observation while waiting for emergence of adults. The process of oils introduction was identical to what was described in the above. Each test with an essential oil consists of four repetitions in addition to the witness that is also repeated four times. At the conclusion of the experiment, the hatched and unhatched eggs rates were counted and recorded. The number of eggs laid by the females on groundnut pods was counted after 24 hours from the beginning of the treatment.

The unhatched eggs determined by the presence of essential oils were calculated using the following formula [16]:

$$\text{Rate} = \frac{Nm - Ne}{Nm} \times 100$$

Nm: Number of laid eggs at the level of the control batch

Ne: Number of laid eggs at the level of treated batches with essential oils.

2.8 Evaluation of the Persistence of Essential Oils on the Lifespan of *C. serratus* Adults

To determine the effect of the persistence of essential oils, bottles containing groundnut pods were treated with essential oils (*C. shoenanthus*, *H. suaveolens*, *O. americanum* and *L. multiflora)* at a single dose of 15 µl. Essential oil was deposited on Whatman paper stuck inside the bottle. Bottles containing the pods were hermetically closed for 48 h, 72 h, 168 hours (7 days), 240 (10 days) and 360 hours (15 days). After each period, five newly emerged *C. serratus* were introduced into the treated bottles with four replications Parameters assessed included mortality of insects 24 h after introduction for each persistence period and the number of eggs laid by the females.

2.9 Statistical Data Analysis

Data collected were analysed using XLSTAT software version 6.1.9 and means were separated using Fisher Least Significant Difference (FLSD) at 5% level of probability. Mortality data were corrected using Abbott's formula [17]. Determination of CL_{50} was derived from the mortality caused by the different doses of essential oils. The data were analyzed by the WinDL32 software version 4.6 of CIRAD-CA from the Probit-log model of Finney [18].

3. RESULTS

3.1 Essentials Oils Composition

Analysis of essential oils that have been tested is presented in Tables 2a and 2b. At the Tables 2a, the chemical analyzes indicate that *Hyptis suaveolens* is mainly composed of Sabinene (36%), βcaryophylene (17%), β –pinene (5,5%) and Limonene (4.9%). For the oil extracted from *Ocimum americanum*, the same analyze indicate that this oil is mainly composed of Eucalyptol (41,42%), Camphre (10,83%), Alpha –pinene (9.63%) and Beta –pinene (5,69%).

In the Table 2b, the analyzes of the *C. shoenanthus* show that this oil consists mainly of piperitone (63.43%), of Elemol (9.75%) and Eremoligenol (4.94%). For *Lippia multiflora*, the major components were P-cymene (21.75%), Thymol (30%) and Acetate of Thymol (20.82%).

3.2 Effects of Essential Oils on Lifespan of *C. serratus*

The evaluation of essential oils extracted from four plants on the lifespan of adult *C. serratus* is shown on Table 3.

Table 2a. Main chemical constituents of the essential oils of *Hyptis suaveolens* and *Ocimum americanum* tested (in%)

Hyptis suaveolens		***Ocimum americanum***	
Compounds	**Proportions (%)**	**Compounds**	**Proportions (%)**
α -thujène	0, 9	Alpha -Thujène	0.183
α -pinène	**2,7**	Alpha –Pinène	**9.638**
α -terpinène	1.3	Camphène	3.662
sabinène	**36.0**	Béta-pinène	5.693
β-pinène	**5.5**	Myrcène	1.426
limonène	**4.9**	Limonène	**4.772**
βcaryophylene	**17,0**	Eucalyptol	**41.424**
Terpinolène	**7,3**	Para –Cymène	**1.305**
		Camphre	**10.837**
		α-Terpinéol	1.772
Oxyde de caryophyllène	1,9	Acétate de Bornyle	3.047
		α–Trans-Bergamotène	3.681
Alpha-cadinène	1,18	Béta caryophyllène	2.422
Bergamotol	1,6		

Table 2b. Main chemical constituents of the essential oils of *Cymbopogon shoenanthus* and *Lippia multiflora* (in%)

Cymbopogon shoenanthus		*Lippia multiflora*	
Compounds	**Proportions (%)**	**Compounds**	**Proportions (%)**
δ- 2 carène	5.36	α -thujène	1.22
Limonène	1.16	Myrcène	1.45
Pipéritone	**63.43**	p-Cymène	**21.71**
Elémol	**9. 75**	γ –terpinène	2.39
β Caryophyllène	1.61	Géranial	2.20
β eudesmol	2.25	Thymol	**30.00**
α eudesmol	2.57	Carvacrol	4.65
Eremoligenol	**4.94**	Acétate de thymol	**20.82**
		Acétate carvacrol	1.04

Table 3. Evolution of the mortality of adult *C. serratus* 24 hours after applying essential oils

	Mortality of *C. serratus* (en %)			
Oils concentration (en µl)	***Cymbopogon shoenanthus***	***Hyptis suaveolens***	***Lippia multiflora***	***Ocimum americanum***
3 µl	0 a	12.5 ± 2.5 a	15 ± 1.2 a	12.5 ± 9.57 a
5 µl	17.5 ± 5 a	12.5 ± 1.25 a	22.5 ± 1.7 a	17.5 ± 1.5 a
7 µl	40 ± 2.16 a	25 ± 1.29 a	22.5 ± 9.57 a	32.5 ± 2.5 a
10 µl	87.5 ± 5 a	27.5 ± 5 c	100 ± 0.0 a	57.5 ± 3.09 b
15 µl	100 a	42.5 ± 2 .21 c	100 ± 0.0 a	60 ± 2.4 b
20 µl	100 a	77.5 ± 9.57 b	100 ± 0.0 a	100 ± 0.0 a
25 µl	100 a	95 ± 10 b	100 ± 0.0 a	100 ± 0.0 a

Averages (± standard deviation) with the same letters on the same line do not differ significantly at the threshold of 5%

The result (Table 3) indicates that all the tested essential oils induced insecticide activity against adult *C. serratus* even at low doses. The lowest dose 3 µl (P< 0. 432; F= 1); 5 µl (P< 0.853; F= 0.22) and 7 µl (P< 0.405; F= 1.07) of essential oil did not shown any significant difference between the mortalities caused by essential oils. However, the mortality of adults of *C. serratus* induced by essential oils varies significantly (Pvalue) from the doses of 10 µl (P< 0.0001). Mortality of adults exposed to increasing essential oil doses varies according to type of essential oil. The oils from *C. shoenanthus* and *L. multiflora* were the most efficacious on groundnut beetles from 15 µl/l which cause 100% mortality, while for the other oils, it varies in the range of 42,5% (*H. suaveolens*) and 60% (*O. americanum*). However with *O. americanum,* total mortality is achieved with a dose of 20 µl, With *Hyptis suaveolens* oil, total mortality was observed with 20 µl.

3.3 Evaluation of Essential oils CL_{50} and CL_{90} on Adults of *C. serratus*

The effectiveness of essential oils used was assessed by determining the CL_{50} of each oil on adults of *C. serratus* (Table 4).

The results showed that CL_{50} differ according to essential oils. Oil extract from *L. multiflora* proved to be the more efficacious on groundnut adult beetles with CL_{50} 6.53 µl/l; it is followed by *C. shoenanthus* (7.10 µl/l) and *O. americanum* (7.89 µl/l). Essential oil of *H. suaveolens* gave the highest CL_{50} of 23.98 µl/l Values obtained CL_{90} showed that this parameter respected the same tendency as the one observed with the CL_{50}. In fact, contrary to CL_{50} the lowest CL_{90} was obtained with the oil extracted from *C. shoenanthus*. with 10.61 µl/l; it is followed by *L. multiflora* (11.69 µl/l).

Table 4. Evaluation of essential oils CL_{50} and CL_{90} on adults of *C. serratus*

	C. shoenanthus	***H. suavolens***	***L. multiflora***	***O. americanum***
CL_{50} (µl/l)	7.10 ± 0.01	23.98 ± 0.27	6.53 ± 0.08	7.89 ± 0.074
χ^2	2.46	0.40	44.23	10.45
CL_{90} (µl/l)	10.61 ± 0.02	218.20 ± 1.37	11.69 ± 0.13	17.71 ± 0.12

3.4 Effects of Oils on Egg Laying and Incubation of Eggs

3.4.1 Effect of essential oils on egg laying by *C. serratus*

The egg laying ability of *C. serratus* females subjected to treatments with essential oils is presented on Table 5. All the essential oils tested, prevent the oviposition by females except *L. multiflora* with doses ranging from 3 µl to 25 µl the result showed that average number of eggs laid by the females decreases gradually with increase in the oil doses. However from 10 µl, no laying was observed in the different batches.

3.4.2 Effect of essential oils on the development of the eggs of *C. serratus*

The results on the ovicide activity of essential oils are presented on Table 6. All essential oils used have effects on egg development and The number of hatched eggs varied significantly with essential oils applied (10 µl $P < 0.0001$; $F = 54.07$; 20 µl $P < 0.0001$; $F = 83.43$).

The average hatched eggs rate at the level of the witness was 73.75±4.78%; when the eggs are under essential oils treatment at 10 µl. the hatched eggs rate observed varied between; 23.75±14.93% (for *C. shoenanthus*) to 31.25±14.36 (for *H. suavoelens)*. When we use 20 µl, the reduction in the hatched eggs rate was observed, the hatch rate was 18.75±10.30% for *O. americanum*; 12.5±9.57% for *C. shoenanthus.* 8.75±4.78% for *L. multiflora* and 18.75±9.46% for *H. suavoelens*.

3.5 Effect of the Persistence of Essential Oils on Lifespan of *C. serratus*

At 48 hours after application of treatments, the results shows that the death rate was significantly higher compared with the witness ($F= 26.08$; $P<0.0001$). The essential oils extracted from *C. shoenanthus. L. multiflora* and *O. americanum* induced total mortality to *C. serratus*. Consequently exposure time of insects with *H. suaveolens* oil recorded was 95% mortality.

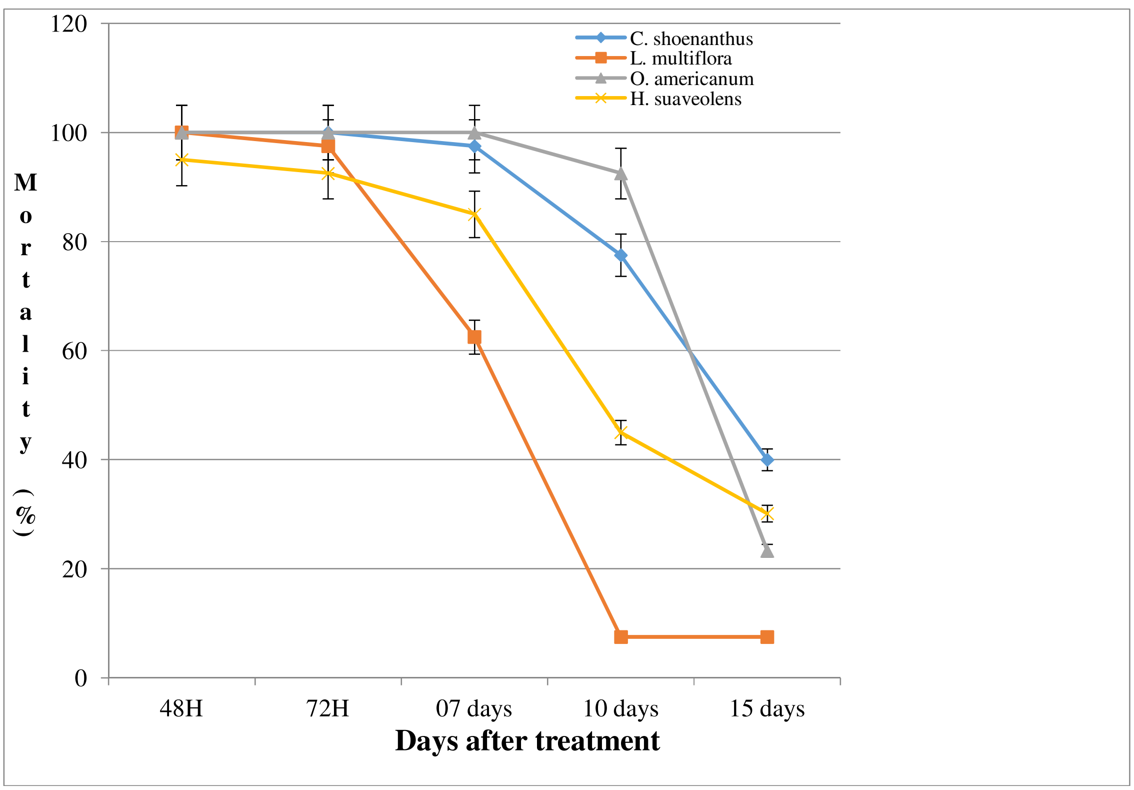

Fig. 1. Death rate evolution of *C. serratus* according to the persistence of essential oils

Table 5. Evolution of the average number of eggs laid by females of *C. serratus* according to the concentrations of essential oils

HE	Average number of eggs laid according to the concentrations (µl)							
	0 µl/l	**3 µl/l**	**5 µl/l**	**7 µl/l**	**10 µl/l**	**15 µl/l**	**20 µl/l**	**25 µl/l**
C. shoenanthus	-	0.0 a A	0. 0 a A	0 ± 0 a A	0 ± 0	0 ± 0	0 ± 0	0 ± 0
L. multiflora	-	48 ±17.49 b A	26.25 ±14.77 b B	14.25 ± 8.9 b B	0± 0	0 ± 0	0 ± 0	0 ± 0
O. americanum	-	0.0 a A	0.0 aA	0.0 a A	0± 0	0 ± 0	0± 0	0 ± 0
H. suaveolens	-	0.0 a A	0.0 a A	0.0 a A	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Témoin	34.7 ± 20.07	-	-	-	0 ± 0	0 ± 0	0 ± 0	0 ± 0

The averages (± standard deviation) with the same alphabetical lower-case letters on the same column do not differ significantly at the threshold of 5%. The averages with the same capital letters on the same line do not differ significantly at the threshold of 5%

Table 6. Ovicide activity of four essential oils on fresh eggs of *Caryedon serratus* after 24 hours exposure

Essential oil	10 µl	20 µl
Ocimum americanum	28.75±6.29	18.75±10.30
Cymbopogon shoenanthus	23.75±14.93	12.5±9.57
Lippia multiflora	28.75±9.46	8.75±4.78
Hyptis suaveolens	31.25±14.26	18.75±9.26
Witness	73.75±4.78	

Similarly at 72 hours after application of treatments oils extracted from *C. shoenanthus* and *O. americanum* recorded 100% mortality. With the other treatments *H. suaveolens* and *L. multiflora,* the mortality recorded was 92.50 and 97.50% respectively.

At 168 hours (7 days) after application results indicated that the application of extracts of *O. americanum* recorded 100% mortality while oils from *C. shoenanthus* and *H. suaveolens* recorded 97.50 and 62.50% respectively.

At 168 hours after application of treatments, oil extracted from *O. americanum* significantly reduced mortality of adults of *C. serratus* by 92.50% followed by *C. shoenanthus* oil (77.50%). However there were not significantly different from each other (F=26.085; P<0.0001). Similar trend was observed with *L. multiflora* and *H. suaveolens* Application of treatments to the groundnut pods at 360 hours (15 days) before the introduction of insects showed 50% reduction in mortality irrespective of the type of treatment applied. Thus the most potent oil was *C. shoenanthus* (40% mortality); followed by *H. suaveolens* (30.14% mortality). *L. multiflora* 7.50% and O*. americanum* 23.33% respectively.

4. DISCUSSION

All the tested essential oils showed insecticidal activity against adult *C. serratus*. However mortality varied according to the type of essential oils applied in relation with the chemical compositions of these different ones [19]. This difference between mortality is due to the variation of the chemical composition of essential oils used [19]. These observations indicated that oil of *Lippia multiflora* is composed of Thymol, P-cimene and Thymol acetate while Hyptis *suaveolens* is composed of sabinene and β-caryophyllene which may be the reasons for their insecticidal activities [20]. Similarly *Cymbopogon shoenanthus* is composed mainly of Piperitone [20]. Similar results were reported by the authors who have worked on the cowpea beetle *Callosobruchus maculatus* by using essential oils of *Hyptis suaveolens* [21,22]. The chemical composition of oils may also play a crucial role in their biological effectiveness which is determined by the presence of the chemical grouping in the oils [23]. Nebie [20] and Ilboudo et al. [22] reported that essential oils contain oxygenated monoterpenic compounds with alcohol or ketone functions are the functional groups of the compounds as for the oil extracted from. Neuciro et al. [24] shows that oil extracted from *Hyptis suaveolens* showed that it is composed of hydrocarbon monoterpene at low rates making them less active. Essential oils extracted from *C. shoenanthus*, *L. multiflora* and *O. americanum* have proved lethal concentrations 50 (CL_{50}) on adult beetle *C. serratus* thus testifying their efficacy. The egg laying ability of female *C. serratus* in the presence of essential oils was disturbed and even inhibited. The reduction of females' lifespan equally affected reduction of the number of laid eggs. This inhibition of the egg laying may be attributed to the physiological disturbance caused by essential oils on the females and in addition, it was reported that monoterpenes inhibits the oviposition of females [25]. Also the egg laying by females of Bruchidae (*C. maculatus* and *C. subinnotatus* PIC) is inhibited by the presence of the essential oil vapors [26]. Essential oils vapors extracted from aromatic plants may cause physiological dysfunction which disturbs the normal functioning of insect ovarioles [27]. This situation may block the sphincters that are likely to push eggs towards the genital opening for their emission [25].

The insecticidal activity of essential oils in this research work confirms the result of Don Pedro [28] who reported the toxicity of their chemical compounds by penetrating the respiratory tube thereby affecting the metabolic activity. The results on the ovicide activity of essential oils indicates that essential oils from *L. multiflora* and *C. shoenanthus* are the most efficacious in preventing eggs from hatching. These results are similar to the work of the author who pointed out that the most efficacious oils on eggs are those extracted from the citral type plants such as *L. multiflora* whose vapors dissolve the ovular contents of eggs [21].

The evaluation of the persistence of essential oils indicated that the effectiveness of oils decreases with the duration of treatment. Similar results were reported on the cowpea beetle *C. maculatus* [21].

5. CONCLUSION

The treatments of stored groundnut with essential oils have shown that all tested oils have insecticidal effects which vary according to plant species. All essential oils used present an insecticidal activity with a persistence that may reach 10 days. Beyond 14 days, the insecticide effectiveness of essential oils decreases significantly. Therefore the use of essential oils can be an effective way to be explored for a better protection of stored food products; since this method has the advantage of being both eco-friendly and protecting consumers unlike some synthetic chemical products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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