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Fumigant Effect of Tunisian Eucalyptus Essential Oils on Hidden *Callosobruchus maculatus* Individuals

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ABSTRACT

The objective of this work is to study the toxicity of essential oils of Tunisian origin on the hidden individuals of *C. maculatus* aged 12 and 18 days. The biological parameters studied are: the hatching rate of the eggs and their viability. Healthy seeds of cowpea are contaminated with adults of *C.maculatus* in Petri dishes. After 24 hours, we remove the weevils, and 4 to 5 days later, we sort the seeds bearing eggs and count 50 hatched eggs (2 to 3 eggs per seed). A number of seeds infested with 50 hatched eggs are placed in an oven until the 12th day. The doses used are: 6.5 µl / l; 12.5 µl / l; 25µl / l; 50 µl / l and 75 µl / l. For each dose, we have varied the duration of exposure: 24, 48, 72 and 96 hours. The same procedure is used for individuals aged 18 days. From the results obtained, we find that the number of adult individuals emerging from cowpea seeds decreases as the dose of essential oils increases. For *E. astringens* and *E. lehmanii*, the viability of *C. maculatus* is zero at 75 µl. No adult beetle has emerged after 48 hours of exposure and 72 hours for *E. maidenii* and *E. cinerea*. Concerning softwoods - regardless of the duration of exposure and the dose used, the viability of the young larvae varies between 50% and 75.5%.

KEYWORDS: Fumigation, *C.maculatus*, Essential Oils, Softwoods, Myrtaceae

INTRODUCTION

During seed storage, several generations of *C. maculatus* succeed one another and cause considerable losses of up to 90 and 100% (Sech *et al*., 1991; Ouedraogo and *al*., 1996) estimate the weight loss during storage at 800 g / kg of seed. In order to control the insect pests of the stored products, several methods are recommended. Preventive control is carried out prior to the installation of the pest (rigorous hygiene of means of transport, storage facilities, isolation of new crops from old ones in the warehouse and use of resistant packaging, plastic bag lined internally with cotton (Caswell, 1973 cited in Kellouche, 2004). Resistant cowpea varieties can improve the effectiveness of insecticides and reduce or eliminate treatments, while mitigating adverse effects (Kumar, 1991). According to Doumma *et al.,* (2001), varieties of *Vigna unguiculata* (063-84 and 044-84) reduce the infestation of *C. maculatus* by up to 80%, and physical means can be used to control stored grain pests. Mbata *et al.,* (1996) report 100% adult mortality after 24 to 100% exposure to carbon dioxide. 100% of the young larvae after 48 hours of exposure and 100% of the older larvae after 72 h of exposure have been reported by the same authors.

In contrast, few studies have been carried out on the effect of essential oils of Myrtaceae on pests of stored grains in general and on their larval stages, in particular.We can mention that of Keita *et al*., (2000) who evaluated the effect of *Tetraclinis occidentalis* on *C. maculatus* and Tapondjou *et al.* (2005) who studied the biological activity of the essential oil of *Cupressus sempervirens* and *E. saligna* on *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae).

In the present study, we attempt to evaluatefumigant toxicity of four species of genus *Euclyptus* essential oils namely *Eucalyptus lehmani*, *Eucalyptus astringens*, *Eucalyptus maideni* and *Eucalyptus cinerea* collected from the arboretum of Korbous (North Tunisia)and the Souinat arboretum against adults of one pest population (*Callosobruchus maculatus).*

MATERIAL AND METHODS

Material

Presentation of the host plant, Vigna unguiculata

The name *V. unguiculata* is taken from Latin Vigna = liana and unguiculus = nail, claw, because the top of the pod resembles a claw. It is grown as a dry and fresh vegetable. Most African cultivars belong to this group. The seeds used come from the local market.

Mass breeding of cowpea beetle

The insects used during all the tests, come from mass farms in a dark oven where temperature conditions of 30 ± 1°C and relative humidity of 70 ± 5% prevail. Individuals of *C. maculatus*, emerging from seeds not treated with essential oils, are introduced into glass jars containing healthy seeds of *V. unguiculata*. From the 30th to the 45th day, emerging individuals, less than 24 hours old, are used in the various tests. The cowpea seeds used as food for the beetle come from the local market.

Presentation of aromatic plants used

We tested four species of plants: *Eucalyptus cinerea* (L'Her, 1789), *Eucalyptus maidenii* (Muell.,1890), *Eucalyptus lehmanii* (Muell, 1890), and *Eucalyptus astringens* (L'Her, 1789). The latter were extracted from their essential oil.

Methods

Essential oils extraction and chemical analysis

The Tunisian species (*E.lehmani*, *E. astringens*) come from Korbos (Korbos Arboretum). The latter is located in the northeast of Tunisia (Capbon), a region located in the bioclimatic stage with warm winters.

E. maidenii and *E. cinerea*come from the Souinat arboretum located concerning the species of Tunisian provenance, the extraction was carried out in the laboratory of ecology and sylvopastoral improvement at the INRGREF (Ariana, Tunis).

Essential oils were analyzed using an Agilent- Technologies 6890N Network GC system equipped with a flame ionization detector and HP- 5MS capillary column (30×0.25mm, film thickness0.25mm; Agilent-Technologies, Little Falls, CA, USA). The injector and detector temperatures were set 220°C and 290°C, respectively. The column temperature was programmed from 80°C to 220°C at a rate of 4°C / min, with the lower and upper temperatures being held for 3 and 10min, respectively. The flow rate of the carrier gaz (Helium) was 1.0ml / min. A sample of 1.0ml was injected, using split mode (split ratio, 1:100). The composition was reported as a relative percentage of the total area. The identification of the essential oil constituents was based on a comparison of their retention times to n-alkanes, compared to published data and spectra of authentic compounds were further identified and authenticated using their mass spectra compared to the Wiley version 7.0 library. Volatile compounds were ranged into groups (monoterpene, hydrocarbons, oxygenated monoterpenes, sesquterpenes oxygenated, sesquiterpene hydrocarbons, and other compounds.

Statistical analyzes

The results of our experiments were subjected to the variance analysis test according to several classification criteria. When the effect of the treatments is significant, the analysis is supplemented by the Newman and Keuls test at 5% (Stat box software, Dagnelie, 1998). The results of the various tests were also the subject of another statistical analysis with the Tukey test. This method makes it possible to compare the averages two to two, for the different doses of essential oils, with software R. If the adjusted probability is less than 0.05, the difference is significant; if the adjusted probability is greater than 0.05, the difference is considered insignificant (Millot, 2009).

Biopesticide tests

Healthy seeds of cowpea are contaminated with adults of *C. maculatus* in Petri dishes. After 24 hours, we remove the weevils. 4 to 5 days later, we sort seeds bearing eggs and count 50 eggs hatched (2 to 3 eggs / seed). A number of seeds infested with 50 hatched eggs are placed in an oven until the 12th day. These seeds are then introduced into a glass jar (one liter) in which we suspend a wire attached to the inner face of the lid. A piece of cotton is glued to the other end of the wire. We deposit a variable dose of essential oil (6.5 µl, 12.5 µl, 25 µl, 50 µl and 75 µl) on this cotton and we quickly close the lid of the jar. We have varied the duration of exposure: 24 h, 48 h, 72 h and 96 h for each dose. For each exposure time, each dose used and for the control, 4 replications are performed. These seeds, containing 12-day-old individuals, are then removed from the jars after exposure to the treatments and introduced into Petri dishes and placed in the oven. After 45 days, we count the emerging individuals in each Petri dish, for the different doses tested and the duration of exposure tested. The same procedure is used for individuals aged 18 days.

RESULTS AND DISCUSSION

Oil composition of *Eucalyptus*

Data of our study showed considerable similarity regarding chemical composition of the four essential oils. Analyses of the essential oils illustrated that monoterpenes are the major constituents in comparison to sesquiterpenes and diterpenes with respectively 97% for *E. lehmanii*, 95% for *E.cinerea*, 91% *E. maidenii* and 88 % for *E. astringens*. The major compounds were 1,8- cineole (56%, 55%, 71%, and 70%) and α pinene (25.08%, 25.55%, 14% and 7%)respectively for *E. lehmani*, *E. astringens*, *E. maidenii* and *E. cinerea* (Table 1 and 2).

Table 1: Concentration of terpenes in the various essential oils of Tunisian origin

	E. lehmanii	***E. astringens***	***E. cineria***	***E. maidenii***
Monoterpene hydrocarbons	12	11	20	12
(%)	97.86	88.47	95.41	91.98
Sesquiterpene hydrocarbons	1	5	8	6
(%)	0.18	3.78	3.26	5.39
Identified compound (%)	98.04	92.25	97.67	97.37
Other compound (%)	1.96	7.75	2.33	2.63

Table 2: Concentration (%) of the main compounds in the various essential oils tested.

	E. lehmanii	***E. astringens***	***E. maidenii***	***E. cinerea***
Monoterpene Hydrocarbons (%)	37.04	31.73	15.68	12.81
α Pinène	25.08	25.55	14.01	7
β Pinène	0.56	1.16	0.34	-
Camphène	0.26	0.10	0.19	0.10
4 Carène		-	0.19	12.68
β Myrcène	0.64	0.84	0.17	-
Terpinolène	0.35	0.17	-	0.11

O. Cymène	1.50	1.49	0.13	-
α Terpinène	8.65	0.32	0.65	-
β phéllandrène		2.10	-	-
Monoterpene oxygenated				
	60.82	56.74	76.21	76.12
Eucalyptol	56.90	55.40	71.93	70.89
Camphor D		-	0.25	
Fenchol	0.46	-	0.12	0.13
Terpinène 4 ol	0.32	0.30	0.24	0.66
Verbénol		-	0.12	-
Géraniol	0.19	-	-	-
Terpinéol	2.95	1.04	0.17	3.54
D sylvestrène	-	-		-
D carvone	-	-	0.11	-
Carvacrol	-	-	0.12	-
Bornéol	-	-	0.27	0.40
α pinène époxide	-	-	0.62	0.14
Isopinocarvéol	-	-	1.75	0.25
Trans carvéol	-	-	0.13	0.11
β Citral	-	-	0.38	-
Sesquiterpene hydrocarbons				
Caryophyllène	-		0.62	0.52
Aromadendrène	-	2.09	2.15	0.18
Varidiflorène	-	0.48	-	-
α Salinène	-	-	0.12	-
β Calarène	-		0.10	-
Gurjunene	-	0.48		0.11
β Cyclo citral	-	-	-	-
oxygenatedSesquiterpene	-	-		
Globulol	-	-	1.99	1.42
Spathulénol	0.18	0.30		0.36
Epiglobulol	-	0.43	0.34	-
Eudesmol	-	-	0.17	-
Selinénol	-	-	0.90	-
Nerolidol	-	-	-	0.37

Inhalation test on individuals aged 12 days

From the results obtained, we find that the number of adult individuals emerging from cowpea seeds decreases as the dose of essential oils increases. For *E. astringens* and *E. lehmanii*, the viability of *C. maculatus* is zero at 75 µl; no adult beetle has emerged after 48 h of exposure and from 72h and on for *E. maidenii* and *E. cinerea* (Table 3 and 4).

The results of the analysis of the variance for the viability parameter of 12-day-old individuals, after 24 hours of exposure to the various essential oils, reveal a very highly significant difference for the oil factor (p = 0.00), dose factor (p = 0.000) and the interaction of the two factors (p = 0.000).

For the 48-hour exposure period, a very significant difference is also observed for the dose factor, but no significant effect is noticed for the interaction of the two factors (P = 0.55). As for the 72h and 96h exposure times, the effect is very highly significant for the dose factor, the oil factor and the interaction of the two factors.

The results of the Tukey test has shown that the adjusted probability of the viability parameter of the young larvae of *C. maculatus* reveals a significant difference between the pairs of low and high doses (12.5 µl - 75 µl, 6.5 µl - 75 µl, 0 - 50 µl, 6.5 - 50 µl, 12.5 - 50) for the 48h, 72h and 96h exposure times, but no significant effect is observed for the duration of exposure, 24-hour exposure.

Tab1e 3: Viability rate (%) of *C. maculatus* after inhalation treatment of 12-day-old individuals with different essential oils for 24 hours.

Essential oils (µl)	*E. cineria*	*E. maidenii*	*E. astringens*	*E. lehmanii*
0	48 ± 0.00	45 ± 0.00	37.50 ± 2.65	37.50 ± 2.65
6.5	44.50 ± 6.61	50 ± 0.00	39.74 ± 2.25	39 ± 3.56
12.5	47.75 ± 1.50	47.75 ± 1.50	38.75 ± 2.75	35.50 ± 4.12
25	49 ± 1.41	47.25 ± 1.50	47.25 ± 1.50	22.75 ± 2.88
50	39.75 ± 0.50	38.6 ± 7.90	6.50 ± 1.73	10.75 ± 3.20
75	35.75 ± 4.99	42.50 ± 5.00	1.25 ± 0.96	4.25 ± 1.50

Table 4: Viability rate (%) of *C. maculatus* after treatment of 12-day-old individuals with different essential oils for 48 hours

Essential oils (µl)	*E. cineria*	*E. maidenii*	*E. astringens*	*E. lehmanii*
0	50 ± 0.00	50 ± 00	37.50 ± 2.65	37.50 ± 2.65
6.5	50 ± 0.00	49.75 ± 0.50	36.50 ± 5.20	35.50 ± 3.00
12.5	47 ± 1.63	48.75 ± 1.26	31 ± 6.65	29 ± 5.35
25	44.75 ± 6.18	36 ± 14.44	18 ± 4.32	17.50 ± 3.79
50	28.75 ± 11.18	19 ± 8.08	4.50 ± 0.58	17.50 ± 3.79
75	49 ± 15.75	11.75 ± 4.50	0.00 ± 0.00	0.00 ± 0.00

Table 5: Viability rate (%) of *C. maculatus* after inhalation treatment of 12-day-old individuals with different essential oils for 72 hours

Essential oils (µl)	*E. cineria*	*E. maidenii*	*E. astringens*	*E. lehmanii*
0	50 ± 0.00	50 ± 0.00	37.50 ± 2.65	37.50 ± 2.65
6.5	49.25 ± 0.50	49.75 ± 0.50	31.75 ± 8.42	30.25 ± 4.50
12.5	39.25 ± 9.18	44.75 ± 2.13	21.75 ± 3.50	21.75 ± 3.50
25	36.75 ± 3.95	43 ± 6.78	13.25 ± 1.71	13 ± 2.58
50	7.25 ± 3.69	0.00 ± 0.00	2.50 ± 0.58	2.75 ± 2.22
75	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 6: Viability rate (%) of *C. maculatus* after treatment of 12-day-old individuals with different essential oils for 96 hours

Essential oils (µl)	*E. cineria*	*E. maidenii*	*E. astringens*	*E. lehmanii*
0	49 ± 0.00	49. ± 0.00	37.50 ± 2.65	37.50 ± 2.65
6.5	45.25 ± 5.41	49.50 ± 0.58	31 ± 8.29	27.75 ± 1.89
12.5	39.50 ± 9	43.50 ± 1.91	21.75 ± 1.71	20 ± 1.63
25	35.50 ± 7.90	42 ± 5.60	5.50 ± 23.42	6.25 ± 1.50
50	5.25 ± 5.91	3.50 ± 4.04	1.50 ± 1.29	1.25 ± 1.50
75	0.00 ± 0.00	0.00 ± 0.00	0 ± 0.00	0.00 ± 0.00

Inhalation test on individuals aged 18 days

The viability of individuals aged 18 days and exposed to different doses of essential oil during the various exposure periods decreases progressively for the four essential oils. As for the viability rate, it varies between 2 and 13% after 48h of exposure (Table 7 and 8).

After 72 hours of exposure to essential oils, the viability rate is less than 2%, and it vanishes after 96 hours of exposure (Table 9 and 10).

The results of the analysis of the variance have shown a very highly significant effect for the oil and dose factors and a significant difference for the interaction of the two factors in the individuals exposed to the different essential oils during 24h. As for the exposure periods of 48, 72 and 96 hours, the effect is very highly significant for dose, oil and interaction factors.

The results of the Tukey test have revealed a significant difference between couples of low and high doses (12.5 - 75 µl, 0 - 75 µl, 25 - 75 µl, 0 - 50 µl and 25 - 75 µl) whatever the duration of exposure to essential oils.

Table 7: Rate of viability of *C. maculatus* after treatment of 18-day-old individuals with different essential oils for 24hours

Essential oils (µl)	*E. cineria*	*E. maidenii*	*E. astringens*	*E. lehmanii*
0	50 ± 0.00	50 ± 0.00	50 ± 0.00	50 ± 0.00
6.5	50 ± 0.00	50 ± 0.00	41.50 ± 0.58	40.50 ± 1.95
12.5	45.50 ± 5.20	45.500 ± 5.20	38.75 ± 21.29	38.75 ± 3.40
25	43.25 ± 5.20	42.75 ± 7.27	34.75 ± 2.99	36.75 ± 3.20
50	39.50 ± 3.32	41.25 ± 2.99	27.75 ± 3.40	35 ± 5.94
75	31.25 ± 6.18	24.75 ± 8.46	24 ± 9.59	21.25 ± 6.95

Table 8: Viability of *C. maculatus* (%) after treatment of 18-day-old individuals with different essential oils for 48 hours

Essential oils (µl)	*E. cineria*	*E. maidenii*	*E. astringens*	*E. lehmanii*
0	50 ± 0.00	50 ± 0.00	50 ± 0.00	39.5 ± 1
6.5	47.25 ± 3.77	44.50 ± 3.70	37.5 ± 4.56	37.25 ± 4.86
12.5	44 ± 2.45	46 ± 3.56	36.75 ± 2.50	36.75 ± 2.50
25	45.25 ± 5.50	43.25 ± 6.35	32.50 ± 2.08	33.25 ± 3.95
50	16 ± 2.71	13.75 ± 3.30	12.25 ± 1.71	27.25 ± 3.50
75	3 ± 3.46	2.75 ± 0.50	13.50 ± 6.66	13.50 ± 6.66

Table 9: Viability rate (%) of *C. maculatus* after treatment of 18-day-old individuals with different essential oils, for 72h

Essential oils (µl)	*E. cineria*	*E. maidenii*	*E. astringens*	*E. lehmanii*
0	50 ± 0.00	50 ± 0.00	39.50 ± 1.91	39.50 ± 1.91
6.5	31 ± 2.83	40 ± 0.82	36.25 ± 3.6	34.50 ± 3.42
12.5	21.25 ± 3.93	47.52 ± 1.73	35.75 ± 2.87	36.75 ± 2.99
25	16.25 ± 11.81	44 ± 4.24	31.50 ± 3.42	31.50 ± 3.42
50	8.75 ± 10.11	10.25 ± 4.65	20.50 ± 2.52	23 ± 4.69
75	0.00 ± 0.00	5.75 ± 3.95	7.25 ± 3.20	7.00 ± 1.15

Table 10: Rate of viability of *C. maculatus* (%) after treatment of individuals aged 18 days with different essential oils, for 96 hours

Essential oils (µl)	*E. cineria*	*E. maidenii*	*E. astringens*	*E. lehmanii*
0	47 ± 0.00	47 ± 0.00	39.50 ± 1.91	39.50 ± 1.91
6.5	46.25 ± 3.50	45.50 ± 1.00	29.25 ± 4.99	30 ± 1.91
12.5	46.25 ± 2.87	44.75 ± 0.50	35.75 ± 2.87	32.75 ± 5.16
25	29.25 ± 12.20	16.75 ± 3.77	27.75 ± 5.80	27.75 ± 2.50
50	2.50 ± 3.79	2 ± 2.83	13.75 ± 1.71	11.50 ± 6.08
75	0.00 ± 0.00	0.00 ± 0.00	2 ± 0.82	2.50 ± 4.51

DISCUSSION

Results indicated that quantitative rather than qualitative variation in the composition of the essential oils was observed. Result in table 1 clearly demonstrated that both oils were rich in monoterpenoids compounds than sesquiterpenes with respectively for *E. lehmanii (*97%, 018%), *E. astringens* (88%, 3.78%), *E. maidenii* (91%, 5.39%) and *E. cinerea* (95%, 3.26%).

Our results are similar to those El Aissi (2011). Analyses of the essential oils illustrated that monoterpene are the major constituents in comparison to sesquiterpene with repectively for *E. lehmanii* (96.9% et 4.7%); *E. astringens* (81.7% et 15.6%), *E. maidenii* (86.5% et 12%) and *E. cinerea* (90.6% et 2.3%).

The predominant compound in most oils analysed was 1-8 Cinole for different species for the genus *Eucalyptus*: *E. lehmanii* (56.90%), *E. astringens* (55.40%), *E. maidenii* (71.93%) and *E. cinerea* (70, 89 %). El Aissi *et al*., (2011) reported that the eucalyptol is most importantcompounds for essential oils with repectively: *E. lehmanii* (56.6%), *E. astringens* (42.5%), *E. cineria* (70.4%), *E. maidinii* (57.8%). The second important compound is αpinène (7 and 25.5%).

Based on the results obtained on the effect of essential oils of Tunisian origin on hidden individuals, post-embryonic viability rates decrease as the dose and duration of exposure increase. On the other hand, individuals aged 12 days are more sensitive than older larvae. Thus, no individual emerged from the seeds treated for 96 h with the oils of the different species of eucalyptus, at a dose of 75µl/l. The essential oils of the *Myrtaceae* are more effective than those of the conifers. We can link this efficiency to their volatility index. In fact, the essential oils of eucalyptus evaporate rapidly and have a faster effect than coniferous ones (their volatility index is low, and evaporate slowly). The essential oils of conifers evaporate more slowly than those of *Eucalyptus* type.

In 18-day-old individuals, exposed to different doses and treatment times, there was emergence of adults irrespective of the essential oil used. Similar results were obtained by Regnault Roger *et al*., (2008), who used the seeds of *V. unguiculata* containing stage 2 and stage 4 larvae exposed to the essential oil of *O. basilicum*, at a dose of 5 µl/l. The mortality rates obtained are 95% in L_2 and at the rate of 12% for L_4. We believe that the viability of the various stages of development of *C. maculatus* (eggs and individuals aged 12 and 18 days) has been affected by the duration of exposure to terpene compounds of essential oils. Indeed, when they are exposed longer, this rate decreases. Furthermore, Regnault Roger *et al.,* (2008) showed that monoterpenes in the majority of essential oils develop early and late ovicidal and larvicidal activities and anti-nutritional activity against hopper larvae in the cotyledons of cowpea seeds. This low sensitivity of hidden larvae of *C. maculatus* to essential oils may be due to the seed coats which protect them and which slow down the penetration of volatile compounds. Little work has been done on the activity of essential oils with respect to the hidden stages of stored insect pests. According to Regnault Roger *et al*., (2008), the physicochemical constituents of the seed would inhibit the penetration of the compounds present in the atmosphere of the flask, and the larvae would only be exposed to low concentrations of insecticide substance inside the galleries. According to the same authors, terpenes penetrate less well into seeds than sulfur compounds. This low penetration may be one of the causes of the reduced mortality rate of larvae. Similarly, Kellouche (2004) has found that larvae of *C. maculatus* in cowpea seeds are not affected by eugenol (the main compound of clove essential oil) even at high doses (80 µl/l).This author first assumed that this lack of mortality may be due either to insensitivity of larvae or to protection by seeds. But with other complementary tests on the larvae extracted from the seeds of *V. unguiculata* and treated with this substance at a lower dose (8 µl/l), the results have revealed a 100% larval mortality. Thus the hidden larvae are inaccessible to the terpenic compounds, because of the protection provided by the different envelopes of the seed. In the previous tests, by contact and inhalation, total adult mortality was observed, respectively, from doses of 25 µl/50 g and 25 µl/l. On the other hand, the effect of essential oils on hatching and the viability of eggs and individuals aged 12 and 18 days varies greatly depending on the duration of exposure and the dose used. Thus, the viability rate of these stages becomes zero only from 96h of exposure at a dose of 75 µl/l, in the

Eucalyptus type. We believe that the dose of essential oil used is not sufficient to significantly affect hidden eggs and larvae. Regnault Roger *et al*., (2008) also report that the toxicity of vapors of essential oils to adults of *C. maculatus* is greater in the absence of cowpea seeds than in their presence. The proportion of essential oil absorbed is, however, insufficient to be toxic to the larvae which grow inside the seeds. We believe that the effectiveness of *Eucalyptus* essential oils is linked to the action of the major compound, which is 1-8 cineola.For Obeng Ofori (1997), this terpene causes the mortality of eggs and young larvae in *Sitophilus granarius*, *Sitophilus zeamais* and *T. castaneum* at a dose of 3µl/kg after 3 hours of exposure. Similarly, Ketoh and *al*. (1998) has reported the ovicidal and larvicidal activity of *E. Citriodora* with respect to *C. maculatus*, at a dose of 33 µl/l. Osekre *et al*., (2002) has obtained similar results with palm oil whose application (1 ml /10 seeds) on eggs deposited on 10 seeds or directly on the larvae of *C. maculatus* growing within the seeds of cowpea has caused 100% mortality.

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