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Essential Oils Composition and Toxicity Tested by Fumigation Against *Callosobruchus maculatus* (Coleoptera: Bruchidae) Pest of Stored Cowpea

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Composição e Toxicidade de Óleos Essenciais Testados por Fumigação Contra o *Callosobruchus maculatus* (Fabricius, 1775) Praga do Feijão-Caupi Armazenado

Resumo: A infestação de insetos durante o armazenamento do feijão-caupi (*Vigna unguiculata*) é um obstáculo econômico a agricultura. Neste contexto, foi proposto um teste em escala laboratorial, objetivando investigar o potencial fumigante dos óleos essenciais (OEs) de *Ocimum gratissium*, *O. basilicum*, *Cymbopogon nardus*, *C. citratus*, *Lippia alba*, *Mentha arvensis*, *Schinus terebinthifolius* e *Cordia verbenacea* no controle do ciclo de vida do caruncho do feijão-caupi (*Callosobruchus maculatus*). Assim, os OEs foram extraídos e analisados por CG/EM, depois foram usados para avaliar a mortalidade, a postura de ovos e emergência de novos adultos. Como resultado da análise dos OEs observou-se os compostos: linalol (32,8%) e eugenol (48,1%) para *O. basilicum*; eucaliptol (14,8%) e eugenol (74,5%) para *O. gratissimum*; acetato de mentol (10%) e mentol (73,3%) para *M. arvensis*; citronelal (50,3%) e nerol (10,8%) para *C. nardus*; citral (75,8 e 87,1%) respectivamente para *C. citratus* e *L. alba*; *α*-santaleno (35,8%) e *β*-sinensal (17,7%) para *C. verbenacea* e *α*-pineno (23,1%), *δ*-3-careno (32.1%) e limoneno (16.9%) para *S. terebinthifolius*. Nos testes biológicos, os OEs apresentaram efeitos tóxicos sobre o ciclo de vida do caruncho do feijão-caupi, inibindo acima de 80% a postura de ovos e mais que 98% a emergência de novos insetos, exceto o OE de *C. verbenacea*.

Palavras-chave: *Vigna unguiculata*; Caruncho do feijão-caupi; Atividade biológica; Óleo essencial; Cromatografia gasosa.

Abstract

Insect infestation on stored cowpea (*Vigna unguiculata*) is an economic obstacle to agriculture. In this context, a test in laboratorial scale was proposed aiming to investigate *Ocimum gratissimum*, *O. basilicum*, *Cymbopogon nardus*, *C. citratus*, *Lippia alba*, *Mentha arvensis*, *Schinus terebinthifolius* and *Cordia verbenacea* essential oils (EOs) fumigant potential on control of cowpea-weevil (*Callosobruchus maculatus*) life cycle. Thus, EOs were extracted and analyzed by GC/MS, furthermore, were used for evaluating mortality, oviposition and new adults emergence. As a result of EOs analyses linalool (32.8%) and eugenol (48.1%) in *O. basilicum*; eucalyptol (14.8%) and eugenol (74.5%) in *O. gratissimum*; menthyl acetate (10%) and menthol (73.3%) in *M. arvensis*; citronellal (50.3%) and nerol (10.8%) in *C. nardus*; citral (75.8 and 87.1%) in *C. citratus* and *L. alba* respectively; *α*-santalene (35.8%) and *β*-sinensal (17.7%) in *C. verbenacea* and *α*-pinene (23,1%), *δ*-3-carene (32.1%) and limonene (16.9%) in *S. terebinthifolius* were reported. In biological tests, EOs showed toxic effect on cowpea-weevil life cycle, inhibiting over 80 and 98% oviposition and new insects emergence, respectively, except for *C. verbenacea* EO.

Keywords: *Vigna unguiculata*; cowpea-weevil; biological activity; essential oils; gas chromatography.

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Essential Oils Composition and Toxicity Tested by Fumigation Against *Callosobruchus maculatus* (Coleoptera: Bruchidae) Pest of Stored Cowpea

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1. Introduction

Bean (family: Fabaceae) are important vegetable protein sources and constituting basic diet of communities around the world, including Brazil (the largest consumer), however, global production sharing is lost during storage by weevil action (*Callosobruchus maculatus*), a cosmopolitan pest mainly infesting *Phaseolus* and *Vigna* genus grains, causing substantial losses to world grains production.[1-7]

The cowpea-weevil is an endemic pest in many regions of the world, including the Brazilian Northeast, causing mainly losses to cowpea production (*Vigna unguiculata*), ranging from 30 to 90% depending on producing region, grains total and seeds stored, due to insect life cycle that involving grains intake and larvae perforations, resulting on decrease of nutritional value, phytosanitary quality and seeds germination.[8-14]

The traditional method reducing economic damage caused by cowpea-weevil requiring fumigant pesticides uses, including magnesium phosphide (phosphine), designated by Brazilian Ministry of Agriculture, through the AGROFIT system, as well as, classified as extremely toxic and dangerous to environment.[15]

In Brazil, indiscriminate insecticides employment has promoted environmental impacts and human health damage, specially on rural worker who has more intensively been exposed to such products.[16-18]

On the other hand, plant natural products, such as essential oils, emerging as new molecules potential sources with insecticidal activity, becoming a potential alternative to pest storage control, in face to lower environmental and economical impact, in contrast to pesticide employment.[19-21]

Essential oils are mainly extracted by steam or hydrodistillation, it is a complex mixture of different substances classes, mainly terpenoids and phenylpropanoids, with diversified chemical structures produced by special metabolism[22-25] (Figure 1) and specially involved on species-specific ecological interactions,[26-28] for example inhibition of herbivory.[29,30]

The aim of this study was evaluating *Ocimum gratissimum* (L.), *O. basilicum* (L.), *Cymbopogon nardus* (L.) Rendle, *C. citratus* (DC), *Lippia alba* (Mill) NE Brown, *Mentha arvensis* (L.), *Cordia verbenacea* (DC) and *Schinus terebinthifolius* (Raddi) essential oils fumigation effects on cowpea-weevil life cycle, evaluation mortality, oviposition and new adults emergence.

Previous studies reporting *O. basilicum*,[31] *O. gratissimum*,[32] *Cymbopogon spp*[33-35] and *Lippia spp*[36-38]essential oils toxic effects against cowpea-weevil were reported on scientific literature. On the other hand, studies reporting *S. terebinthifolius* and *C. verbenacea* works essential oil effects were not demonstrated on cowpea-weevil. Biological activity in regarding to essential oil from those species against fungi, bacteria and insects were reported, as wel.[22,39-43]

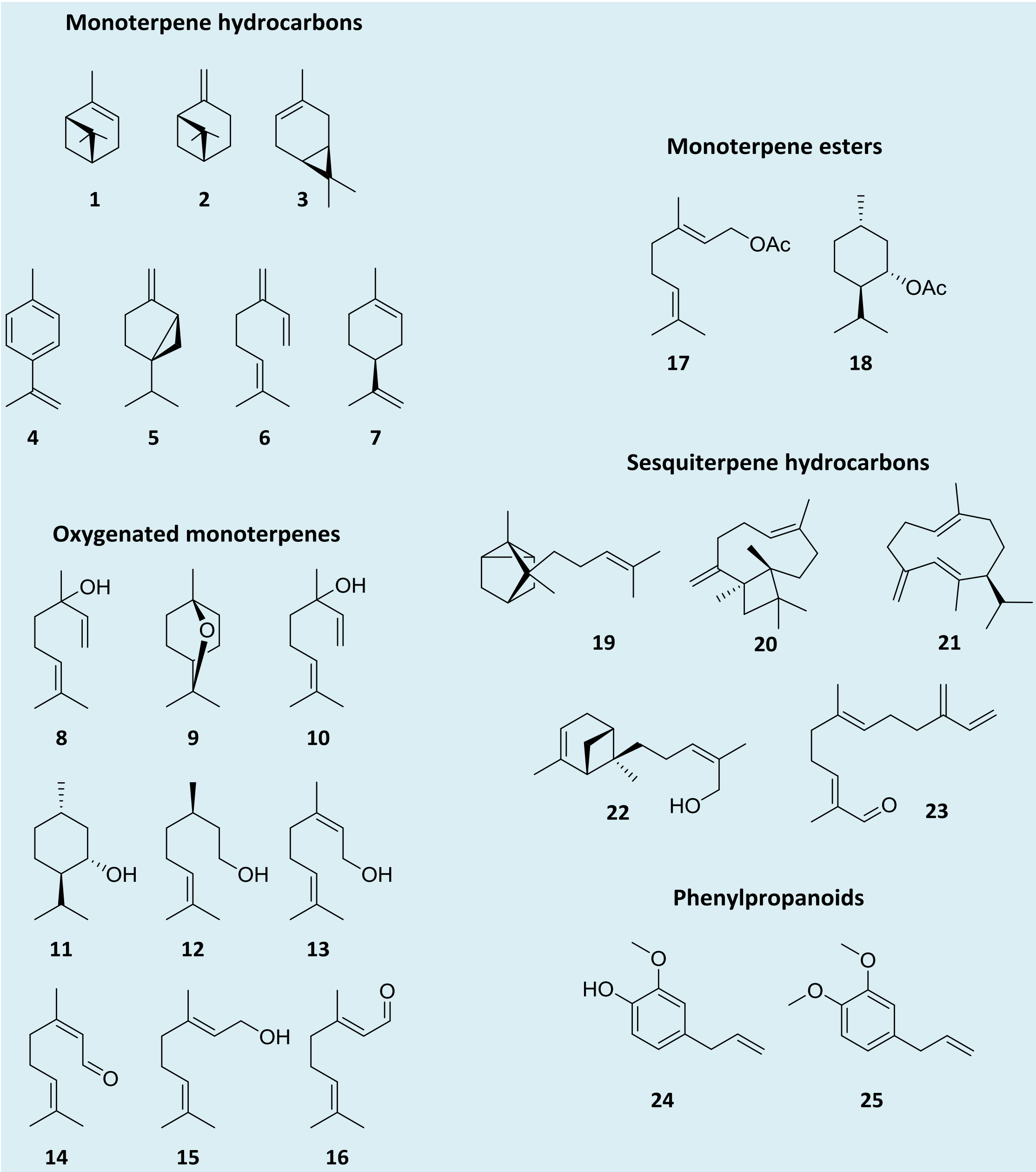

Figure 1. Some volatiles compound obtained from different aromatic plants. α-pinene **1**, β-pinene **2**, δ-3-carene **3**, ρ-cymene **4**, (−)-sabinene **5**, myrcene **6**, (−)-limonene **7**, linalool **8**, eucalyptol **9**, citronellal **10**, menthol **11**, citronellol **12**, nerol **13**, neral **14**, geraniol **15**, geranial **16** citronellyl acetate **17**, menthyl acetate **18**, α-santalene **19**, β-caryophyllene **20**, germacrene D **21**, (Z)-α-trans-bergamotol **22**, β-sinensal **23**, eugenol **24** and methyl eugenol **25**

2. Experimental

All procedures were performed at the Laboratory of Medicinal and Aromatic Plants, Department of Phytotechny, and Laboratory of Biochemistry and Molecular Biology of Arthropods, Department of Chemistry, UFRRJ.

2.1. General

The colony of C. maculatus used in this

work was initiated with animals originally supplied by Dr. J.H.R. Santos, Centro de Ciências Agrárias, Universidade Federal do Ceará, Fortaleza, Brazil. Stored cultures from this species have continuously been kept since 1978. Insects were reared on *V. unguiculata* seeds (commercial cultivar named Fradinho) in natural photoperiod at 29° (± 1°) and relative humidity of 65 ± 5%. *M. arvensis* (*RBR 32886*), *O. basilicum and S. terebinthifolius* (RBR 36405) were collected in Búzios (22°46'47.57"S, 41°54'6.62"W), and *C. verbenacea* (RBR 36381), *O. gratissimum* (RBR 36382), *C. citratus*, *C. nardus* and *L. alba* in Seropédica (22°45'39.65"S, 43°41'55.92"W), both in Rio de Janeiro, Brazil. Voucher specimen, from some plants, have been deposited on Biology Institute herbarium (UFRRJ) with the identification number, but other plants are still awaiting registration process.

2.2. Essential Oils Composition and Content

Essential oils from fresh leaves were obtained by hydrodistillation in Clevenger apparatus for one hour, except for *S. terebinthifolius* fruits homogenized and distilled for three hours. Hydrodistilled (20 ml) were collected, partitioned with CH_2Cl_2 (4 × 5 ml), dried with anhydrous Na_2SO_4, concentrated in rotary evaporator and then with N_2 gas. The essential oil content (% *w*/*w*) was evaluated by the gravimetric methods and composition was analyzed by GC/FID and GC/MS.

2.3. GC/FID and GC/MS Analyses

GC analysis was carried out on a Hewlett-Packard 5890 II (Palo Alto, USA) apparatus equipped with flame ionization detection (FID) and a split/split less injector, on a 1:20 split ratio was used to separate and detect essential oil constituents. Substances were separated into the fused silica capillary column VF-5ms (30 m × 0.25 mm i.d., film thickness 0.25 µm, Agilent J&W). Oven temperature was programmed at 60° for 2 min, followed by heating at 5°/min to 110°, followed by heating at 3°/min to 150° and finally followed by heating at 15°/min until 290° and holding constant for 15 min. Injector temp., 220°; detector temp., 280°; carrier gas, He (1 ml/min). Injected volume was 1 µl on a 1:20 split ratio. Percentage of essential oil compounds was calculated from relative area of each peak analyzed by FID.

Essential oils were analyzed on a GC/MS QP-2010 Plus (Shimadzu, JPN). Carrier gas Flow (He), capillary column and temperature conditions for GC/MS analysis were the on same as described for GC/FID. Temperature injector, 220°; temperature interface, 250°. Mass spectrometer operating conditions were: ionization voltage, 70 eV; mass range, 40-400 *m/z* and 0.5 scan/s.

Constituents were identified by comparison of their mass spectra with those ones from authentic standards (Sigma-Aldrich, USA) or with those ones compiled on NIST libraries (2008). For numerous compounds, the identification was confirmed by comparison of their *RI*, what was obtained based on co-injection samples with C_8-C_{40} hydrocarbons mixture (Sigma-Aldrich, USA) based on Van Den Dool and Kratz,[44] and mass spectra with those ones reported by Adams.[45]

2.4. Biological Assay and Statistical Analysis

Falcon tube (50 ml) containing 30 grains of cowpea, 9 insects (6 male and 3 female) with 2 days old and healthy were used for biological assay. Aliquots with 10 µl of *O. basilicum* essential oil and 20 µl of other essential oils were transferred to paper discs filter, previously fixed to falcon tubes cap, providing concentration of 0.2 and 0.4 µl/cm^3. Same procedure was performed for control that did not receive essential oils. Test was carried out with five replicates per treatment. Then, tubes were closed and stored in chamber at 27° (± 1°). Mortality was recorded 48 h after essential oil application, then caps were removed and replaced by a fine mesh, oviposition was assessed after 72 h and new adults emergence after 27 days. Results obtained were subjected to variance analysis and means evaluated for significance test (with alpha = 0.05) using Sigmastat 2.03 (Systat Software Inc, USA) program. Results were converted to percentages relative to controls and showed in tables.

3. Results and Discussion

3.1. Essential oil Composition and Content

For evaluating effects from eight plant species on cowpea-weevil mortality, oviposition and new adults emergence *O. gratissimum*, *O. basilicum*, *C. nardus*, *C. citratus*, *L. alba*, *M. arvensis*, *S. terebinthifolius* and *C. verbenacea* essential oils were used. Contents were 1.0, 0.4, 0.6, 1.2, 0.5, 0.9, 0.3 and 1.3%, respectively and essential oils composition is present on Table 1.

In regarding to major compounds in plant essential oils and their percentages were noted: *Ocimum basilicum*, linalool (32.8%) and eugenol (48.1%); *O. gratissimum*, eucalyptol (14.8%) and eugenol (74.5%); *Mentha arvensis*, menthyl acetate (10%) and menthol (73.3%); *Cymbopogon nardus*, citronellal (50.3%) and nerol (10.8%); *C. citratus* and *Lippia alba*, citral composed by neral (32.5 and 36.6%) + geranial (43.3 and 50.5%); *Cordia verbenacea*, α-santalene (35.8%), (Z)-α-*trans*-bergamotene (12.9%) and β-sinensal (17.7%), *Schinus Terebinthifolius*, α and β-pinene isomers (23.1 and 11.5%), δ-3-carene (32.1%) and limonene (16.9%) (Table 1).

Table 1. *Essential Oils Composition Extracted by Hydrodistillation*

N.	Compound and class	KI	LRI	Percentage [a]							
				Ob	*Og*	*Ma*	*La*	*Cn*	*Cc*	*Cv*	*St*
1	*α*-pinene	939	935	-	-	-	-	-	-	2.2	23.1
2	sabinene	975	977	-	-	-	-	-	-	-	4.0
3	*β*-pinene	979	983	-	-	0.2	2.7	-	9.7	-	11.5
4	myrcene	990	996	-	-	0.2	-	-	-	-	3.9
5	*δ*-3-carene	1011	1015	-	-	-	1.3	-	-	-	32.1
6	*ρ*-cymene	1024	1031	-	-	-	-	-	-	-	7.1
7	limonene	1029	1033	-	-	0.5	-	0.79	-	-	16.9
8	eucalyptol	1031	1034	0.7	14.8	-	-	-	-	-	-
9	linalool	1096	1109	32.8	0.6	-	0.6	-	-	-	-
10	*cis*-verbenol	1141	1143	-	-	-	-	-	1.3	-	-
11	*trans*-verbenol	1144	1149	-	-	-	1.1	-	1.9	-	-
12	menthona	1152	1157	-	-	3.8	-	-	-	-	-
13	citronellal	1153	1159	-	-	-	-	50.3	-	-	-
14	isomenthone	1162	1165	-	-	2.6	-	-	-	-	-
15	neomenthol	1165	1169	-	-	1.7	-	-	-	-	-
16	menthol	1171	1175	-	-	73.3	-	-	-	-	-
17	4-terpineol	1177	1182	2.6	-	-	-	-	-	-	-
18	myrtenal	1195	1202	-	-	-	0.6	-	-	-	-
19	citronellol	1225	1229	-	-	-	-	6.8	-	-	-
20	nerol	1229	1231	-	-	-	-	10.8	-	-	-

21	neral	1238	1235	-	-	-	32.5	-	36.6	-	-
22	piperitone	1252	1256	-	-	2.3	-	-	-	-	-
23	geranial	1267	1267	-	-	-	43.3	-	50.5	-	-
24	menthyl acetate	1295	1297	-	-	10.0	-	-	-	-	-
25	citronellyl acetate	1352	1359	-	-	-	-	5.1	-	-	-
26	eugenol	1359	1363	48.1	74.5	-	-	1.3	-	-	-
27	geranyl acetate	1381	1389	-	-	-	1.3	5.8	-	-	-
28	methyl eugenol	1403	1403	1.3	-	-	-	-	-	-	-
29	*α*-cedrene	1411	1409	4.3	-	-	-	-	-	1.8	-
30	*α*-*cis*-berga48motene	1412	1420	-	-	-	-	-	-	1.0	-
31	*α*-santalene49	1417	1426	-	-	-	-	-	-	35.6	-
32	*β*- caryophyllene	1419	1429	-	2.6	0.6	7.8	-	-	-	-
33	*β*-gurgenene	1433	1444	1.9	-	-	-	-	-	-	-
34	*cis*-*β*-farnesene	1442	1453	-	-	-	-	-	-	8.8	-
35	*γ*-muurolene	1479	1482	-	-	-	-	2.0	-	-	-
36	germacrene D	1485	1483	-	1.6	1.1	2.4	-	-	-	-
37	*β*-selinene	1490	1491	-	3.5	-	-	1.4	-	-	-
38	*α*-selinene	1498	1512	-	1.2	-	-	-	-	-	-
39	*α*-farnesene	1505	1517	0.8	-	-	-	-	-	6.4	-
40	*cis*-*γ*-bisabolene	1515	1527	-	-	-	-	-	-	1.3	-
41	*δ*-cadinene	1522	1534	-	-	-	-	1.4	-	-	-
42	*cis*-nerolidol	1532	1546	-	-	-	-	-	-	1.0	-
43	elemol	1549	1559	-	-	-	-	5.7	-	-	0.9
44	germacren-4-ol	1575	1587	-	-	-	-	5.4	-	-	-
45	caryophyllene oxide	1583	1598	-	-	-	2.1	-	-	2.6	-
46	carotol	1594	1611	-	-	-	-	-	-	1.6	-
47	*epi*-*α*-cadinol	1640	1657	2.8	-	-	-	-	-	-	-
50	*α*-*trans*-bergamotol	1690	1695	-	-	-	-	-	-	12.9	-
51	*β*-sinensal	1699	1703	-	-	-	-	-	-	17.7	-
52	*α*-*trans*-bergamotol acetate	1794	1789	-	-	-	-	-	-	2.1	-
53	(*z*)-*epi*-*β*-santalol acetate	1805	1800	-	-	-	-	-	-	2.3	-
	Monoterpene hydrocarbons			-	-	0.9	4.0	0.8	9.7	2.2	98.6
	Oxygenated Monoterpenes			36.1	15.4	83.7	78.1	67.9	90.3	-	-
	Monoterpene esters			-	-	10	1.3	10.9	-	-	-
	Phenylpropanoids			49.4	74.5	-	-	1.3	-	-	-
	Sesquiterpene hydrocarbons			7.0	8.9	1.7	10.2	4.8	-	54.9	-
	Oxygenated Sesquiterpenes			2.8	-	-	2.1	11.1	-	35.8	0.9
	Sesquiterpene esters			-	-	-	-	-	-	4.4	-
	Total			95.3	98.8	96.3	95.7	96.8	100	97.3	99.5

[a] Percentage, based in compound area obtained by CG/FID analysis; Not detected (**–**), *Ocimum gratissimum* (**Og**), *O. basilicum* (**Ob**), *Cymbopogon nardus* (**Cn**), *C. citratus* (**Cc**), *Lippia alba* (**La**), *Mentha arvensis* (**Ma**), *Schinus terebinthifolius* (**St**) and *Cordia verbenacea* (**Cv**).

Essential oils extracted from *S. terebinthifolius* fruits and *C. citratus* showed monoterpene rich hydrocarbon profiles with low elution time in chromatographic column. However, *C. verbenacea* presented rich sesquiterpenes compositions. While *C. nardus*, *M. arvensis*, *L. alba* and *O. gratissimum* essential oils showed variable mono and sesquiterpene contents, including phenylpropanoids for *Ocimum* genus.

Essential oils chemical compositions (Table 1) are consistent to the results reported on literature,[46–53] but with small differences, consequences of environmental factors, nutrient availability, ecological interactions and genotypic differences, modulating the production and essential oil quality.[54] The wide factors range modulating essential oil quality might be the best explanation for information diversity on chemical profile for the same species.[55]

3.2. Biological Activity

Eight different essential oils extracted by hydrodistillation, each one with its chemical profile were used to compose biological assay treatments to cowpea-weevil insects, and results showed that *C. citratus* essential oil did not change on insect mortality, on the other hand, oviposition (96.1%) and the new adults emergence (98.5%) were strongly inhibited. Similar results in treatment with *S. terebinthifolius* essential oil causing low mortality (33.3%) were noted, however associated to higher oviposition inhibition (82.4%) and new adults emergence (96.9%).

Low mortality level was observed in cowpea-weevil treated with *C. citratus*, *C. verbenacea* and *S. terebinthifolius* essential oils, as well as, can be related to monoterpene esters lack and phenylpropanoids in essential oil composition, on the other hand, cowpea-weevil treated with *C. verbenacea* essential oil, with 94% sesquiterpene, showed low oviposition inhibition (33%) and new adults emergence (22.5%).

Table 2. *Biological Activity of Essential Oils*

Essential Oil	Concentration (µl/cm³)	Activity (%)[a]		
		Mortality	Inhibition of oviposition	Inhibition of emergence
Ocimum basilicum	0.2	88.3	83.5	100.0
Ocimum gratissimum	0.4	45.0	89.0	100.0
Mentha arvensis	0.4	58.8	88.0	100.0
Lippia alba	0.4	66.7	85.1	100.0
Cymbopongon nardus	0.4	71.7	99.1	100.0
Cymbopongon citratus	0.4	n.s.	96.1	98.5
Schinus terebinthifolius	0.4	33.3	82.4	96.9
Cordia verbenacea	0.4	25.9	33.0	22.5

[a] Mean values expressed as percentages relative to control (without essential oil); Results shown are different of control (t-Test; $\alpha = 0.05$); n.s. - not significant.

Possibly, higher *C. citratus* and *S. terebinthifolius* essential oil monoterpene percentages (100 and 98.6%, respectively) are responsible for high interference on cowpea-weevil life cycle, by effect of high oviposition inhibition (96.1 and 82.4%) and new adults emergence (98.5 and 96.9%).

C. nardus, *O. basilicum*, *O. gratissimum*, *L. alba* and *M. arvensis* Essential oils inhibited oviposition between 83-99% and 100% new adults emergence. However, it should be noted high *O. basilicum* essential oil effect against cowpea-weevil life cycle tested with half concentration (0.2 µl/cm^{-3}) in regarding to other treatments. It should also be explained that, unlike other treatments, O. basilicum essential oil was used in lesser concentration, in the reason of compound availability was also lesser, thus, requiring adjustment on assay.

Considering that essential oil has a complex chemical profile, designation of responsible component by biological activity is difficult. However, literature reported mechanisms action of several essential oil compounds on insects metabolism and physiology,[42] emphasizing that toxic action is due to synergism of different essential oil compounds.[21,22,42]

Other aspect observed is related to low molecular weight compounds percentages (with higher steam pressure) in essential oil and its higher toxicity, as showed on table 2. Similar observations were described to terpenes against *Aedes aegypti* and also concerning to monoterpene hydrocarbons with low molecular weight and compounds lipophilicity,[56–59] as well as, results evaluating several other essential oils effects by fumigation on cowpea-weevil and its toxic action.[31–38,40,41,60–62]

3.3. Considerations

It should be emphasized that this study, like others ones previously mentioned, point out to the possibility of essential oils employment as an alternative tool for storage pest control, in replacement of indiscriminate pesticide use, and its impact on the environment, health and the economy, especially, with regard to small farmers. However, this promising alternative requires more further studies in pilot scale, looking for developmental of new products and technologies related to agriculture.

It is also important mentioning that this work presents unpublished information about fumigant activity of two plant species against cowpea-weevil, *C. verbenacea* and *S. terebinthifolius* both native species from Brazilian Atlantic Forest. The first species showed low effect on cowpea-weevil life cycle, however, the second one presented 97% new adult emergency inhibition. *Schinus terebinthifolius* is an interesting plant for further studies development looking for new products for agriculture, mainly, considering specie availability and access, as well as, high dried fruit essential oil content.

4. Conclusions

It might be concluded that of *O. basilicum*, *O. gratissimum*, *M. arvensis*, *L. alba* and *C. nardus* essential oils have promoted cowpea-weevil life cycle interruption, after strongly inhibiting oviposition and new adults emergence, cause losses by grain perforations and phytosanitary quality, constituting to trading product depreciation. This study also demonstrated that *C. citratus* and *S. terebinthifolius* essential oils were very efficient for cowpea-weevil life cycle inhibiton. However, *C. verbenacea* essential oils did not efficiently work, showed inferior effects on mortality, oviposition and new adults emergence, as well.

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