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INSECTICIDAL ACTIVITY OF CRUDE ESSENTIAL OILS OF FOUR AROMATIC PLANTS AGAINST *CALLOSOBRUCHUS MACULATUS* (COLEOPTERA: BRUCHIDAE)

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ABSTRACT: The contact toxicity, fumigant and oviposition deterrent activities of the essential oils from four plant species, geranium (Pelargonium graveolens), aniseed (Pimpinella anisum), German chamomile (Matricaria chamomilla) and Bitter orange bigarade (Citrus aurantium) were evaluated against Callosobruchus maculatus adults. Residue contact toxicity assayed by exposure of insect adults to thin film of oil in Petri dish showed that, at 24 h of treatment, P. graveolens oil was the most effective (LC50 = 3.5 mg/L) followed by P. anisum oil (LC50 = 4.9 mg/L). However, in the fumigation assay, after 24 h exposure, the LC50 values demonstrated that the most effective essential oils were P. graveolens (29.4 mg/L air) followed by P. anisum (50.0 mg/L air), and C. aurantium 79.38 mg/L air. The fumigant toxicity increased with increasing in exposure periods. The time needed for the essential oil to cause LT50 (median lethal time) was also estimated at the highest concentration used (2000 mg oil per kg cowpea seeds). Based on LT50 values, it was shown that C. aurantium oil was the most toxic material against insect LT50 = 10.24 hrs) followed by oils of P. anisum and M. chamomilla (LT50s were 19.5 h and 16.2 h, respectively). In addition, oviposition potency of C. maculatus was reduced significantly when insect adults were exposed to cowpea seeds mixed with sublethal concentrations of test oils. At the lowest concentration used (250 mg/kg), C. aurantium and Pimpinella anisum oils appeared to be the most effective in reducing oviposition rates compared to the control. With respect to the number of F1- progeny produced, the C. aurantium, P. anisum and M. chamomilla oils significantly reduced F1-progeny emergence compared to the control treatment. The chemical constituents of essential oils extracted from the four plant species used were also determined. The results suggested that these essential oils can be used as appropriate alternative to control of cowpea seed beetle.

Key words: Essential oils, contact toxicity, fumigant, oviposition deterrent, GC/MS analysis, cowpea seed beetle.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) (Walp.), is an important food legume for millions of people throughout the semi-arid regions of Africa, Asia, southern Europe, and North, Central, and South America (Singh *et al.,* 2003). The cowpea seed weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae), is the major pest of stored cowpea seed in the tropics and subtropics due to the favorable climatic conditions (Singh *et al.,* 1990; Dimetry *et al.,* 2007). The insect infests cowpeas in the field and the subsequent population buildup in storage can cause complete weight loss of stored cowpeas within six months if no prophylactic measures are put in place (Sanon *et al.,* 2005). Life history and

development of this insect on cowpeas have been described in early studies (Singh *et al.,* 1990; Edde and Amatobi, 2003). Control of this pest relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to users (Jembere *et al.,* 1995; Okonkwo and Okoye, 1996). Thus, repellents, fumigants, feeding deterrents and insecticides of natural origin are rational alternatives to synthetic insecticides. Herbal products are one potentially important source. Essential oils are secondary metabolism products in plants. These oils have strong aromatic components that give a plant its distinctive odor, favor, or scent (Koul *et al.,* 2008). A strong connection between medicinal and pesticidal plants was reported by several researchers (Yang and Tang, 1988). Recently, there has been a growing interest in research on the use of essential oils of aromatic plants for protection of stored products because of their complicated action mechanism to which insect pests find it difficult to develop resistance (Isman, 2008; Nerio *et al.,* 2009). Moreover, local availability, rapid degradation and low mammalian toxicity are a few advantages of the essential oils for the environment as cost-effective control agents (Isman & Machial, 2006; Liu *et al.,* 2007; Isman, 2008). Many aromatic plant species are indigenous to Egypt, however, the insecticidal activities of their essential oils have rarely been studied. The present research was therefore undertaken to investigate the bioactivity of the essential oils extracted from four plant species: Geranium, *Pelargonium graveolens*, Aniseed, *Pimpinella anisum*, German chamomile, *Matricaria chamomilla*, and Bitter orange bigarade, *Citrus aurantium*, grown in Egypt against adults of *C. maculatus.* Also, the active chemical constituents of essential oils of each tested plant species were analyzed.

MATERIALS AND METHODS

Insect Culture

The insects used in these experiments were obtained from a culture of *Callosobruchus maculatus* maintained in a glass jar containing seeds of cowpea in an incubator at 28 ± 2°C, 70 ± 5 % r.h. and under dark conditions. Parent adults were obtained from laboratory stock cultures maintained at the Department of Stored Product Pests, Plant Protection Research Institute, Sakha Agricultural Research Station. Fifty pairs of 1-2 day-old adults were introduced to a jar containing 100 g cowpea seeds for 24 h. After removing adults, the seeds containing eggs were maintained until the emergence of F1 adults. One-seven day old adults were used for all bioassays. All experiments were carried out under the same environmental conditions.

Plant Materials and Extraction Technique

The essential oils were extracted from four common ornamental plants that are grown in different areas of Egypt. Direct steam distillation technique was used for obtaining crude essential oils as described by Guenther (1977). In brief, test plant parts were put in a container equipped with condenser at which steam was passed through it carrying essential oils which condensed the vapor. Then, condensed vapor was received in receptor where may separate oil from water. Obtained oil was filtrated twice and maintained in refrigerator till experiment. Extraction time varied according to plant tissues used as follows: 1) Fresh herbs of Geranium, *Pelergonium graveoleft* (Geraniacae), were dried in laboratory condition without sun light for 48 h, cut in pieces, weighed and finally subjected to steam distillation for 2 h; 2) dry

flowers of German chamomile, *Matricaria chamomilla* (Chompositae) were subjected to distillation for 12 h; 3) dried seeds of Anise, *pimpinella anisum* (Umbelleferae) were used and subjected to distillation for 8 h; and 4) bitter orange fruits, Bigarade, *Citrus aurantium* (Rutaceae). The oils were separately isolated and dried on anhydrous sodium sulphate to remove water after extraction. Extracted oils were transferred to glass flasks that were filled to the top and kept at the temperature of 4 °C in a refrigerator for future study.

The constituents of essential oils for each test plant were analyzed by gas chromatography-mass spectrometry (GC/MS) using HP5890 system with a HP column (60 meter x 0.25 millimeter, 0.25 µm film thickness). Detector was flame ionization detector (FID). The mobile phase was nitrogen and hydrogen was the stationary phase. Initial temperature was 60 °C and maximum temperature was 250 °C. The injector temperature was 240 °C. Relative percentage amounts were calculated from peaks total area by apparatus software. The compounds were identified by matching the mass spectra data with those held in a computer library (Wiley 275.L). All steps of extraction and analysis procedure were carried out in the Analysis Laboratory of Hashem Brothers for Essential Oils and Aromatic Products (Kafr-ELsohby, Kalyoubeya, Egypt).

Contact Toxicity Assay

In this method, a serial dilution of each tested essential oil was prepared in acetone and one ml from each concentration was spread into a glass Petri-dishes (9 cm-in diameter) by moving the dishes gently in circle. The range of concentrations was chosen on the basis of a number of preliminary trials. The acetone was allowed to evaporate for 10 min prior to introduction of insects. Ten unsexed adults were transferred onto Petri dishes. Control dishes were treated with acetone only. Six concentrations were used for each oil, with three replicates for each concentration. Mortality percentages were recorded after 24, 48 and 72 h. All obtained results were corrected for natural mortality by using Abbott's formula (Abbott, 1925).

LT_{50} of Essential Oils

This experiment was designed to determine the time required for 50% mortality (LT50) when adult insects were exposed to a high concentration of each oil. A concentration of 2000 mg oil per kg of cowpea seeds was selected in this assay, based on preliminary trials designed by exposure insect adults to seeds mixed with different concentrations of each oil. A 1 ml aliquot of test oil diluted in acetone was applied on a glass jar (11.5 by 6 cm diameter) containing 20 g of cowpea seeds. The jars were shaken to mix the seeds with the tested oil. The acetone was allowed to evaporate for 10 min prior to introduction of insects. Ten unsexed adult insects (1-7- day old) were transferred onto jars. In control, cowpea seeds were mixed with solvent only. Jars were covered with muslin cloth and kept under laboratory condition. Mortality in adults was recorded every 6 hours. Three to five replicates were used for each bioassay. Percent of mortality was recorded and corrected by Abbot's formula (1925).

Fumigant Toxicity

The fumigant effect of essential oil against adults of *C. maculatus* was evaluated using an adopted technique described by Moravvej and Abbar (2008) and Taghizadeh-Saroukolai *et al.* (2010). Briefly, 6-cm diameter pieces of Whatman N° 1 filter paper were impregnated with 100 µl of an appropriate concentration of the essential oil. Then, the impregnated filter paper was attached to the bottom of the screw caps of a glass jar (170 ml). The solvent was allowed to evaporate for 1 min

before the cap was screwed tightly on the glass jar containing 10 unsexed insect adults (1- to 7-d-old). Three replicates were performed for each concentration. Control insects were exposed to filter paper treated with only acetone and kept under the same conditions. The insects in each treatment and control were incubated for three different intervals: 24, 48 and 72 h from the commencement of exposure. The insects had no contact with the impregnated filter paper and stayed at the bottom of the jars throughout the experiments. Mortality was recorded after 24 h from the commencement of exposure. Mortality data was corrected by using Abbott' formula (Abbott, 1925).

Oviposition Deterrence and Adult Emergence

To determine the effects of essential oils on oviposition deterrence and production of F_1-progeny, four concentrations were chosen for this bioassay: 250, 500, 1000 and 2000 mg/kg, based on preliminary trials. Briefly, seeds were cleaned and sterilized at 45 °C for 6 h in order to kill the eggs and developing larvae. For each tested concentration, 60 g of cowpea seeds were taken in a conical flask and mixed with each tested concentration, diluted in acetone, while seeds treated with only acetone used as control. After through mixing, the seeds were air dried and they were separated into three lots each 20 g seeds, stored in 400 ml-glass jar, and five sexed (5 pairs) of newly emerged adults were introduced into each jar. Three replicates were maintained for each concentration and controls. The jars were covered with muslin secured with elastic bands and kept under laboratory conditions. After 14 days, all insect adults were removed from each jar, and the number of eggs laid on both treated and untreated (control) seeds were recorded. Eggs were examined under binocular microscope and the number of eggs hatched was recorded. After the eggs were counted, the experimental set up was kept undisturbed till the emergence of F_1-adults from the treated and untreated seeds. The number of F1- adults emerged from the control seeds (C_n) and treated seeds (T_n) were recorded. The percentage reduction in F_1- adult emergence (PRA) was calculated (Ndomo and Ngamo, 2008), as: PRA = $[C_n - T_n]/C_n \times 100$.

Data Analysis

Median lethal concentration (LC50) and time needed for 50% mortality LT50) values with their confidence limits (95% CL) were calculated based on Finney' analysis (Finney, 1971) using Pc-Probit software program, and significant difference between LC50 values were estimated based on 95% CL overlapping. Analysis of variance (ANOVA) and Least Significant Difference (LSD) Test were employed using the Co-Stat software to compare means.

RESULTS

Chemical Constituents of Essential Oils

Results of the chemical analysis of essential oils extracted from the four plant species used are shown in Table 1. Extraction yields of 0.125, 1.5, 0.4 and 0.3 % (w/w) were obtained from Geranium, *Pelargonium graveolens*, Aniseed, *Pimpinella anisum*, German chamomile, *Matricaria chamomilla*, and Bitter orange bigarade, *Citrus aurantium*, respectively. The chemical analysis showed that sixteen major volatile compounds were identified in essential oil of *P. graveolens*, representing

Table (1): Chemical constituents of essential oils extracted from four plant species.

Main components	Composition %	Retention time (min)
Pelargonium graveolens		
Citranellol acetate	1.07	28.00
Phenylethyl tiglate	1.16	33.95
3,7-Dimethyl acetate	1.17	29.37
Rose oxide transe	1.27	13.04
Geraniol ester	1.46	38.51
Trans-Caryophyllene	1.51	26.95
Beta Bourbonene	1.87	25.44
Methylethyl	2.00	31.41
Guaniol	2.70	21.90
β–linalool	4.10	12.66
P-menthan-3-one trans	5.28	15.49
Cyclohexanone	5.82	15.50
Tetramethyl	6.25	35.44
Citronellyl formate	7.10	20.79
Geraniol	12.35	20.22
Citronellol	27.76	19.03
Matricaria chamomilla		
7,11-dimethyl-3-methylene	17.01	28.586
Germacrene-D	1.90	29.35
Germacrene-B	1.26	29.94
3,7,11-Trimethyle	1.14	30.45
5,8-Dimethylisoquinoline	1.11	30.64
Alpha-bisabolol	6.43	36.52
Bisabolol oxide B	7.43	35.28
Chamazulen	3.52	39.23
Bisabolol oxide A	40.54	40.52
Lend-in-dicycloether	6.32	44.87
Pimpinella anisum		
Transanisole	86.74	18.94
Estragol	4.08	25.68
Methyl chavicol	1.68	14.61
Citrus aurantium		
Limonene	88.65	16.54
Myrcene	2.00	20.35
beta-Linalool	1.00	19.63

88.58 % of the total oil. The oil contains a significant amount of citronellol (27.76%), geraniol (12.35%), citronellyl formate (7.1 %), *epi*-γ-eudesmol (6.06 %) , tetramethyl (6.25%), cyclohexanone (5.28%) (Table 1). Also, noticeable amounts of other constituents were present, linalool (4.1 %), guaniol (2.7%), methylethyl (2.00%). In *M. chamomilla* oil, ten major volatile components representing ca. 86.6 % of the total oil were detected (Table 1). The most abundant component was the terpenoid, α-bisabolo oxide A (40.54 %) followed by other terpenoids, 7,11-dimethyl-3-methylene (17.01 %), *bis*-abololoxide B (7.43%), α-bisabolo oxide B (6.43 %), lend-in-dicycloether (6.32%), and chamazulen (3.52%). In essential oil of *P. anisum*, the major constituent was *trans*-anisole with 86.74 %, then estragole with 4.08 %, and methyl-chavicol with 1.68 %. In essential oil of *C. aurantium*, the most abundant component was limonene with 88.65 % followed by myrcene (2%) and *β*-Linalool (1%) (Table 1).

Contact Toxicity

Based on LC50 data, it seems that at 24 h of treatment, *P. graveolens* oil was the most effective with LC50 of 3.5 mg/L (95% CL = 3.24 – 3.78 mg/L) followed by *P. anisum* oil, with 4.9 mg/L (Table 2). The slopes of concentration-response curves of oils were high (7.70 ± 0.55, 4.55 ± 0.35, respectively). In general, high values of the slopes of the concentration-response curves indicate that a small variation in concentration of the essential oil promotes large variations in mortality. Data also indicated that *C. aurantium* oil had the lowest effect on *C. maculatus* adults (LC_{50} = 15.0 mg/L) followed by *M. chamomilla* oil (LC50 = 12.0 mg/L). Similar trend was shown after 72 h of treatment.

LT_{50} Assay

Data in Table 3 show LT_{50} values of tested essential oils calculated at the highest concentration of 2000 mg oil/kg of cowpea seeds for *C. maculatus* adults exposed to oil-mixed seeds. Based on the overlap in 95% CL for LT_{50} values, *C. aurantium* oil seemed to be the most toxic material against insect adults compared to the other oils. The time needed for the *C. aurantium* oil to cause LT_{50} was 10.24 h (95% CL = 8.60 – 11.90 h), whereas the LT_{50} values for the essential oils of *P. anisum* and *M. chamomilla* were : 19.5 h (95% CL = 9.5- 31.2 h) and 16.2 h (95% CL = 13.6- 18.9 h), respectively. However, *P. graveolens* oil had the least activity (LT50 = 89.0 h, 95% CL= 57.9- 142.5 h). Similar trend was observed, at LT_{90} values, where the time needed for essential oils of *C. aurantium*, *M. chamomilla*, *P. anisum*, and *P. graveolens* to cause LT_{90}s for insect adults were: 40.1, 88.2, 267.5 and 293.7 h, respectively.

Fumigant Toxicity

Experiments were conducted to determine whether the insecticidal activity of tested essential oils against *C. maculatus* adults were attributable to fumigant action. Data in Table 4 indicated that, after 24 h exposure, there was significant difference in insecticidal activity between different oil-treatments, based on 95% CL overlapping. On the basis of LC_{50} values, after 24 h exposure, it was obvious that the most effective essential oils were *P. graveolens* (29.4 mg/L, 95% CL ranged from 21.8 to 29.69 mg/L) followed by *P. anisum* (50.0 mg/L, 95% CL ranged from 44.2 to 56.4 mg/L), and *C. aurantium* 79.38 mg/L, 95% CL ranged from 73.5 to 85.7 mg/L). However, *M. chamomilla* oil had the lowest fumigant activity (LC_{50}= 2,058 mg/L) after 24 h of fumigation, compared to other tested oils. Data also indicated that fumigant toxicity increased with increasing in exposure periods. After 72 h of exposure, LC_{50} values were decreased for essential oils of *P. graveolens* (20.0 mg/L), *P. anisum* (32.3 mg/L), and *C. aurantium* (58.8 mg/L).

Table (2): Insecticidal efficiency of essential oils from four plant species against *Callosobruchus maculatus* adults using contact toxicity assay.

Plant species	Hours after exposure	LC_{50} [a] (mg/L)	95% Confidence limits (mg/L)	Slope ± SE	χ^2
Pimpinella anisum	24	4.9bcd	4.29 - 5.58	4.55 ± 0.350	0.026
	48	3.7dfeg	3.10 - 4.40	4.00 ± 0.308	0.067
	72	2.5h	2.31 - 2.68	4.20 ± 0.312	0.108
Matricaria chamomilla	24	12.0a	10.34 - 13.92	3.90 ± 0.401	1.588
	48	5.8bc	5.00 - 6.72	4.00 ± 0.335	1.932
	72	4.3de	3.70 - 4.98	3.90 ± 0.401	0.021
Pelargonium graveoleft	24	3.5efg	3.24 - 3.78	7.70 ± 0.555	0.114
	48	3.2fg	2.93 - 3.48	6.25 ± 0.514	0.044
	72	2.9gh	2.66 - 3.16	6.70 ± 0.401	5.784
Citrus aurantium	24	15.0a	10.06 - 22.35	1.43 ± 0.121	1.186
	48	13.5a	10.60 - 24.40	1.43 ± 0.121	1.190
	72	8.5b	5.97 - 12.46	1.52 ± 0.125	1.051

[a] LC_{50} values in a column followed by the same letter(s) are not significantly different based on 95% CL overlapping.

Table (3): LT_{50} values of essential oils from four plant species against *Callosobruchus maculatus* adults fed on cowpea seeds treated with tested oils at 2000 mg/kg concentration.

Essential oils	Slope ± SE	LT_{50} [a] - hours (95% Confidence limits)	LT_{90} [a] - hours (95% Confidence limits)	χ^2
Pimpinella anisum	1.13 ± 0.12	19.5b (9.5 – 31.2)	267.5b (186.2 – 1424.5)	10.6
Matricaria chamomilla	1.74 ± 0.13	16.2b (13.6 – 18.9)	88.2c (72.5 – 112.5)	6.9
Pelargonium graveolens	2.5 ± 0.17	89.0a (57.9 –142.5)	293.7a (293.3 – 855.1)	79.3
Citrus aurantium	2.16 ± 0.16	10.24c (8.6 –11.9)	40.1d (34.1 – 48.8)	7.5

[a] LT_{50} and LT_{90} values in a column followed by the same letter(s) are not significantly different based on 95% CL overlapping.

Table (4): Fumigant toxicity of essential oils from four plant species against *Callosobruchus maculatus* adults exposed for 72 h at 30°C and 70% r.h.

Plant species	Exposure Period (hrs)	LC_{50}[a] (mg/L)	95% Confidence limits (mg/L)	Slope ± SE	χ^2
Pimpinella anisum	24	50.0g	44.23 – 56.48	4.55 ± 0.359	0.074
	48	50.0g	44.23 – 56.48	4.55 ± 0.359	0.074
	72	32.34h	29.10 – 35.90	5.26 ± 0.431	0.042
Matricaria chamomilla	24	2058.0a	1607.8 – 2634.2	2.33 ± 0.268	0.645
	48	1234.8b	964.7 – 1580.5	2.33 ± 0.186	0.993
	72	493.30c	408.2 – 597.6	3.03 ± 0.232	0.035
Pelargonium graveolens	24	29.4i	21.8 – 29.7	1.89 ± 0.139	0.861
	48	20.0j	15.7 – 25.4	2.44 ± 0.192	1.45
	72	20.0j	15.7 – 25.4	2.44 ± 0.192	1.45
Citrus aurantium	24	79.38d	73.5 – 85.7	7.14 ± 0.561	0.134
	48	70.5e	64.7 – 77.0	6.67 ± 0.525	0.058
	72	58.8f	53.9 – 64.0	6.67 ± 0.537	0.234

[a]LC_{50} values in a column followed by the same letter(s) are not significantly different based on 95% CL overlapping.

Oviposition Deterrence and Adult Emergence

The inhibition rates in the production of the first generation (F_1) of the *C. maculatus* adults in grains treated with various essential oil concentrations are presented in Table 5. The results show that the deterrent activity of tested essential oils in reducing the potency of insect females in egg laying varied with different plant species and concentrations. *Citrus aurantium* and *P. grave*olens completely deterred oviposition at the highest concentration used (2000 mg/kg). Numbers of eggs laid decreased with increasing concentrations for all essential oils used. For example, treatment with *M. chamomilla* oil resulted in reducing oviposition rate with an average of 124.3, 99.0, 25.3 and 24.3 eggs at 250, 500, 1000 and 2000 mg/kg, respectively, compared to 268.7 eggs for the control treatment. Data also show that percentages of egg hatching decreased with increasing concentrations for all essential oils tested. At concentration of 1000 mg/kg, egg hatch percentages for treatments of *M. chamomilla, P. graveolens, C. aurantium, and P. anisum* oils were 4.7, 42.7, 76.9, and 81.5 %, respectively, compared to 87.2 % in the control. With respect to the number of F1- progeny produced, it was clear that adult emergence significantly decreased with increasing oil

Table (5): Means of fecundity rate and F_1-progeny production in *Callosobrochus maculatus* fed on cowpea seeds treated with tested oil at different concentrations compared to control treatment.

Oil Concentration (mg/Kg)	Mean no. of eggs laid	Mean no. of hatch	% Hatch	Mean no. of emerged adults	% Reduction[a] in F_1-progeny
Pimpinella anisum					
Control	268.7±13.7a	234.3±18.0a	87.2	79.7± 5.1a	-
250	56.0 ± 3.8f	47.7± 3.24f	85.2	43.3± 2.4bcd	45.67
500	32.0 ± 2.2fg	26.6± 2.0fg	83.3	24.5± 1.9cdef	69.26
1000	14.0 ± 0.7g	11.4± 0.9gh	81.5	10.0± 0.7fghi	87.45
2000	10.0 ± 0.7g	4.10± 0.2h	40.7	3.1± 0.2ghi	96.11
Matricaria chamomilla					
Control	268.7±13.7a	234.3± 18.0a	87.2	79.7± 5.1a	-
250	124.3±9.9bcd	97.3± 7.0d	78.3	22.3± 1.6defg	72.02
500	99.0 ±7.2def	46.3± 4.2e	49.8	18.3± 1.4efgh	77.03
1000	25.3 ± 2.1fg	11.3± 0.7gh	44.7	1.0± 0.08ih	98.75
2000	24.3 ± 1.8fg	9.3± 0.7gh	38.3	0.0± 0.0i	100
Pelargonium graveolens					
Control	268.7±13.7a	234.3±18.0a	87.2	79.7± 5.1a	-
250	179.0± 12.7b	154.0±12.6b	86	75.5± 6.2a	5.27
500	158.5±11.3bc	128.9±7.5c	81.3	51.5± 3.7b	35.38
1000	112.5±6.8cde	48.0±3.8e	42.7	47.5± 2.8bc	40.40
2000	0.0 ± 0.0h	--	--	--	--
Citrus aurantium					
Control	268.7±13.7a	234.3±18.0a	87.2	79.7± 5.1a	-
250	64.5± 4.5ef	51.0±5.59e	79.7	37.5± 3.3bcde	52.95
500	33.5±2.55fg	27.0±2.21f	80.5	25.2± 1.8cdef	68.38
1000	32.5±1.85fg	25.0±1.95fg	76.92	15.5± 1.2efgh	80.55
2000	0.0 ± 0.0h	--	--	--	--

Means followed by the same letter(s) in each column are not significantly different (P=0.05; LSD test).

concentrations (Table 5). For example, the numbers of F1-progeny of *C. maculatus* produced from *M. chamomilla* oil-treatment were 22.3, 18.3, 1.0 and 0.0 at concentrations of 250, 500, 1000, and 2000 mg/kg, respectively, compared to 79.7 adults in the control. At the lowest concentration used, 250 mg/kg, *M. chamomilla* oil was the most effective causing 77.02% reduction in F_1-progeny production followed by *C. aurantium* (52.9%) and *P. anisum* (45.67%).

DISCUSSION

Essential oils (EOs) are generally products of rather complex compositions used contemporaneously in aromatherapy, and for centuries as aromatic medicinal plant species in traditional systems of medicine. Aromatic formulas are used for the treatment of a variety of illnesses, including those that affect the CNS (Almeida *et al.,* 2004). Volatile compounds presenting sedative or stimulatory properties have been identified in EOs from aromatic medicinal species spread across different families and genera. The majority of these substances have small structures with less than 12 carbons and present low polarity chemical functions, being therefore quite volatile. Since most natural EOs are formed by complex mixtures, their bioactivity(ies) are obviously dependent on the contribution of their various components. In the current study, we evaluated the insecticidal efficiency of the crude essential oils extracted from four plant species commonly grown in Egypt were tested by different techniques against adults of *Callosobruchus maculatus.* Several studies demonstrated that the essential oils obtained via steam distillation of those four aromatic plants are often used as fragrances in the perfume industry and more recently for aromatherapy and as herbal medicines. For example, studies carried out by Džamić *et al.* (2014) on *P. graveolens* oils, Ouedrhiri *et al.* (2015) on *Citrus aurantium* oils, Sharafzadeh and Alizadeh (2011) on *Matricaria chamomilla* oils, and Shojaii and Fard (2012) on *Pimpinella anisum* oils. However, little data are not available on the insecticidal activity of such EOs against the stored-product insects. Earlier attempts to explore the toxicity of essential oils against the pulse beetle, *Callosobruchus chinensis* have been made and proved that essential oils affect insects by antifeedant, repellent, oviposition inhibitory, ovicidal and progeny production inhibitory activities by disrupting metabolic pathways (Chaubey, 2011; Chaubey, 2013).

In the present study, the LT50 data showed that *Citrus aurantium* oil seemed to be the most toxic material against *C. maculatus* adults, compared to the other oils used (LT50 = 10.24 h). In agreement with our findings, a study carried out by Moravvej and Abbar (2008) indicated that the oils extracted from the fruit peels of four different species of citrus including *Citrus aurantium* had high fumigant activity against *C. maculatus* adults; the mortality increased with concentration and exposure time from 3 to 24 h after treatment. Their results suggest that citrus peel oils can be used as potential control measure against cowpea beetles. For fumigation, our data demonstrated that after 24 h exposure, it was obvious that the most effective essential oils were *P. graveolens* followed by *P. anisum* and *C. aurantium*. Data also indicated that fumigant toxicity increased with increasing in exposure periods. Recently, Chaubey (2014) reported that *Allium sativum* essential oil significantly repelled the pulse beetle, *C. chinensis*, adults at a very low concentration as the oviposition capacity decreased in choice oviposition assay. This volatile oil caused fumigant and contact toxicity in bruchid adults in a concentration-dependent manner. In addition, *A. sativum* essential oil reduced egg laying capacity in *C. chinensis* adults in oviposition inhibition

assay performed either by fumigation or contact method. Also, *A. sativum* essential oil reduced hatching rate in *C. chinensis* eggs when fumigated (Chaubey, 2014). In oviposition deterrence and F1-progeny production assay, our results showed that the deterrent activity of tested essential oils reduced the potency of *C. maculatus* females in egg laying and varied with different plant species and concentrations. *Citrus aurantium* and *Pelargonium grave*olens completely deterred oviposition at the highest concentration used (2000 mg/kg). Numbers of eggs laid decreased with increasing concentrations for all essential oils used.

At the lowest concentration used, 250 mg/kg, *M. chamomilla* oil was the most effective causing 77.02% reduction in F1-progeny production followed by *C. aurantium* (52.9%) and *P. anisum* (45.67%). Since adult emergence is based on the proportion of hatched eggs that develop into adults inside the seeds, the results suggest that essential oil vapours cross the seed coat and therefore, interfere with the larvae development (Braga *et al.,* 2007). In fumigant toxicity assay, *Anethum sowa* and *Artemissia annua* essential oils have been reported to show ovicidal and oviposition-deterrence in *C. maculatus* (Tripathi *et al.,* 2001). Also, Elhag (2000) revealed the oviposition inhibition activity of several essential oils against *C. maculatus*. The exposure of the cowpea seeds to the vapour of *tri*-decanone is very effective to control their infestation by *C. maculatus* since adult emergence was reduced as compared to untreated seeds (Braga *et al.,* 2007). The number of eggs laid and fecundity were reduced when *C. maculatus* was exposed to fumigation with garlic essential oils (Douiri, 2013). In general, higher the concentration of essential oil, the higher the reduction in adult emergence. The reduction in adult emergence could either be due to the reduction in egg hatching rate or death of larva. Don-Perdo (1996) reported that tridecanone, a component of essential oil, exhibits fumigant toxicity and its efficacy in protecting the cowpea seeds against *C. maculatus* which is mainly due to its ovicidal activity. Mode of action of essential oil constituents has not been known yet, although, it may be due to the suffocation and inhibition of various biosynthetic processes of insect (Don-Perdo, 1996). Toxicity of menthol, methonene, limonene, α-pipene, β-pipene and linalool against *S. oryzae* is proved to its effect on acetylcholines-terase (AChE) enzyme activity (Lee *et al.,* 2001). Findings of the present study indicate that essential oils can be a promising tool in insect pest management. However, before its application, it must be kept in mind that essential oil should be toxic to target insects and but not toxic to non-target organisms such as other beneficial insects and other animals such as fish, birds and humans (Chaubey, 2014). There are several other factors that must be considered during the evaluation of insecticides like risk associated to users, mode of exposure, degradation in the environment and chronic toxicity to be used effective for control of stored-product insect populations (Chaubey, 2014).

REFERENCES

Abbott, W.S. (1925). A method for computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

Braga, Y.F.B., T.B. Graneiro, E.A. Freire, H.L. Lopes, J.N.S. Bezerra and Mano (2007). Insecticidal activity of 2-tridecanone against the cowpea weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae). Anais da Academia Brasileira de Ciências, 79(1):35-39.

Chaubey, M. K. (2011). Fumigant toxicity of essential oils against rice weevil

Sitophilus oryzae L. (Coleoptera: Curculionidae). J. Biol. Sci., 11: 411-416.

Chaubey, M. K. (2013). Biological activities of *Zingiber officinale* (Zingiberaceae) and *Piper cubeba* (Piperaceae) essential oils against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Pak. J. Biol. Sci.,16 (11): 517-523.

Chaubey, M. K. (2014). Biological activities of *Allium sativum* oil against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Herba Polonica, 60 (2):41-55.

Dimetry, N.Z., S. El-Gengaihi, and A.M.E. Abd El-Salam (2007). Protection of stored cowpea from *Callosobruchus maculatus* (F.) attack by some plant extracts formulations in different storage sacks, Herba Polonica,53: 71–84.

Don-Perdo, K.M. (1996). Investigation of single and joint fumigant insecticidal action of citrus peel oil components. Pest Sci 1996; 46:79-84

Edde, P.A. and C.I. Amatobi (2003). Seed coat has no value in protecting cowpea seed against attack by *Callosobruchus maculatus* (F.), Journal of Stored Products Research, 39 (1): pp. 1–10.

Elhag, E.A. (2000). Deterrent effects of some botanical products on oviposition of the cowpea bruchid *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Int. J. Pest Manag., 46:109-113

Finney, D. L. (1971). Probit Analysis. Cambridge University Press. Cambridge.

Guenther E. (1977). The essential oils. Vol. 4 New York: Van Nostrand; Individual essential oils of the plant family Myrtaceae.

Isman, M. B. (2008). Botanical insecticides: for richer, for poorer. Pest Management Science, 64: 8-11.

Isman, M. B. and C.M. Machial (2006). Pesticides based on essential oils: from traditional practice to commercialization, 29-44 pp. *In*: Naturally Occuring Bioactive Compounds. (Eds. Rai, M. and Carpinella, M.C.), Elsevier, Amsterdam.

Jembere, B., D. Obeng-Ofori, A. Hassanali, G.N.N. Nyamasyo (1995). Products derived from the leaves of *Ocimum kilimanndscharicum* (Labiatae) as post-harvest grain protectants against the infestation of three major stored product insect pests. Bulletin of Entomological Research, 85: 361-367.

Koul, O., S. Walia, and G.S. Dhaliwal (2008). Essential oils as green pesticides: potential and constraints. Biopesticides International, 4: 63-84.

Lee, B.H., W.S. Choi, S.E. Lee and B.S. Park (2001). Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). Crop Protection, 20: 317-320.

Liu, Z. L., S.H. Goh and S.H. Ho (2007). Screening of Chinese medicinal herbs for bioactivity against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). Journal of Stored Product Research, 43: 290-296.

Moravvej, G. and S. Abbar (2008). Fumigant toxicity of citrus oils against cowpea seed beetle *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Pakistan Journal of Biological Sciences, 11: 48-54.

Ndomo, A. F. and L.T. Ngamo (2008). Insecticidal effects of the powdery formulation based on clay and essential oil from the leaves of *Clausena anisata* (Willd.) J.D. Hook ex. Benth. (Rutaceae) against *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). J. Pest. Sci., 81: 227-234.

Nerio, L. S., J. Olivero-Verbel and E.E. Stashenko (2009). Repellent activity of essential oils from seven aromatic plants grown in Colombia against *Sitophilus zeamais* Motschulsky (Coleoptera). Journal of Stored Products Research, 45: 212-214.

Okonkwo, E.U. and W.I. Okoye (1996). The efficacy of four seed powders and the essential oils as protectants of cow-pea and maize grain against infestation by *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Nigeria. International Journal of Pest Management, 42: 143-146.

Sanon, A., L.C.B. Dabir´e, A. P. Ou´edraogo and J. Huignard (2005). Field occurrence of bruchid pests of cowpea and associated parasitoids in a sub humid zone of Burkina Faso: importance on the infestation of two cowpea varieties at harvest, Journal of Plant Pathology, 4 (1): 14–20.

Sharafzadeh, S. and O. Alizadeh (2011).German and Roman Chamomile. Journal of Applied Pharmaceutical Science, 1(10): 1-5.

Shojaii, A. and M. A. Fard (2012). Review of pharmacological properties and chemical constituents of *Pimpinella anisum.* International Scholarly Research Network, 2012: 1-8.

Singh, B. B., H. A. Ajeigbe, S. A. Tarawali, S. Fernandez-Rivera, and M. Abubakar (2003). Improving the production and utilization of cowpea as food and fodder, Field Crops Research, 84 (1-2): 169–177..

Singh, S.R, L.E.N. Jackai, J.H.R. Dos Santo, and C.B. Adalla (1990). "Insect pest of Cowpea," *In:* Insect Pest of Food Legumes, S.R. Singh, Ed., pp. 43–80, John Wiley & Sons, Chichester, UK.

Taghizadeh-Saroukolai, A., S. Moharramipour and M.H. Meshkatalsadat (2010). Insecticidal properties of *Thymus persicus* essential oil against *Tribolium castaneum* and *Sitophilus oryzae*. Journal of Pest Science, 83: 3-8.

Tripathi, A. K., V. Prajapati, S.P.S. Khanuja and S. Kumar (2001). Toxicity, feeding deterrence and effect of activity of 1,8-cineole from *Artemisia annua* on progeny production of *Tribolium castaneum* (Coleoptera: Tenebrionidae). J. Econ. Entomol., 94: 979-983

Yang, R.Z. and C.S. Tang (1988). Plants used for pest control in China: a literature review. Economic Botany, 42: 376-406.

النشاط الابادي للزيوت الخام الأساسية المستخلصه من أربعة نباتات عطرية ضد خنفساء اللوبيا

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الملخص العربي

في هذا البحث تم استخلاص الزيوت الأساسية (الطيارة) من أربعة نباتات عطرية شائعة تزرع في جمهورية مصر العربية، لتقييم فعاليتها كمبيدات مباشرة بالملامسه، أو كسموم غازية (مدخنات)، أو كمثبطات لوضع البيض،

علي حشرة خنفساء اللوبيا *Callosobruchus maculatus*. والنباتات الأربعة هي: العتر *Pelargonium graveolens* ، اليانسون *Pimpinella anisum* ، شيح البابونج *Matricaria chamomilla* ، النارنج *Citrus aurantium*. وقد أظهرت نتائج ٢٤-ساعة للتقييم الحيوي للزيوت عن طريق تعريض الحشرات بالتلامس المباشر لمتبقي سطح رقيق من الزيت علي قاع طبق بتري زجاجي ، فعالية زيت العتر وزيت اليانسون حيث كانت قيم التركيز النصفي القاتل ٣.٥ ، ٤.٩ مجم/لتر، علي الترتيب. وأظهرت نتائج التقييم الحيوي بتعريض الحشرات للغازات المتطايرة عن الزيت دون تلامس الحشرة له Fumigation ، فعالية زيوت العتر، اليانسون، النارنج ، حيث كانت قيم LC_{50} المسجله بعد ٢٤ ساعة من التعرض هي: ٢٩.٤، ٥٠.٠، ٧٩.٣٨ مجم/لتر هواء، علي الترتيب. وقد لوحظ أن التأثير السام للزيوت كمدخنات يزداد بطول فترة التعرض. وفي اختبار آخر لتقدير الزمن المنقضي لقتل ٥٠% من الحشرات المعرضة لتركيز ما من الزيوت المختبرة LT_{50} ، أظهرت النتائج أن زيت النارنج كان أسرع الزيوت تأثيراً علي الحشرة عندما أختبر علي تركيز ٢٠٠٠ مجم زيت/ لكل كجم بذور لوبيا، مسجلاً LT_{50} = ١٠.٢٤ ساعة من بداية تعرض الحشرة للزيت، يليه زيوت اليانسون، شيح البابونج بقيم LT50s = ١٩.٥ ، ١٦.٢ ساعة، علي الترتيب. وأجري اختبار آخر لتقييم التأثير المثبط للزيوت علي معدلات وضع البيض في الحشرات المعرضة لتركيزات تحت مميته من كل زيت مختبر. وقد أوضحت النتائج فعالية زيوت النارنج، اليانسون ، عند اختبارها علي تركيز منخفض (٢٥٠ مجم/كجم بذور لوبيا)، حيث كان متوسط عدد البيض في المعاملات ٦٤.٥ ، ٥٦.٠ بيضه، علي الترتيب ، مقارنة بمتوسط ٢٦٨.٧ بيضه في حالة الكونترول المقارن. بالاضافة إلي ذلك، فقد سجلت النتائج أيضاً، انخفاضاً معنوي اًفي أعداد حشرات الجيل الأول الناتج F1-progeny في المعاملات بزيوت النارنج، شيح البابونج، واليانسون ، عند أقل تركيز مستخدم (٢٥٠ مجم/كجم) وذلك بنسب ٧٢.٠٢ ، ٥٢.٩٥ ، ٤٥.٦٧% ، علي الترتيب ، عن مثيلتها في الكونترول. وأيضاً في هذه الدراسه، تم تحليل كيماوي لتقدير المكونات الرئيسية لكل زيت، باستخدام طرق الفصل الكروماتوجرافي، والتعريف باستخدام جهاز كروماتوجرافيا الغاز GC/MS. ويستخلص من نتائج هذه الدراسة البحثيه، إمكانية استخدام الزيوت النباتية الأساسية، كبدائل للمبيدات التقليدية المخلقة ضمن برامج المكافحة المتكامله، في مكافحة حشرات المخازن، في حال ثبوت أمانها علي الثدييات والكائنات الحية غير المستهدفة.