The Effectiveness of Seven Plant Essential Oils as Protectants of Cowpea Seeds against the Cowpea Beetle, *Callosobruchus maculatus* F.

Magdy I.E. Mohamed
Department of stared Product pests, plant protection Research institute, Agricultural Research Center, Sabahia, Alexandria, Egypt.

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ABSTRACT

The effect of seven plant essential oils isolated from Egyptian plants (*Achillea santolina, Artemisia judaica, Citrus reticulata, Schinus terebenthifolins, Mentha microphylla, Lantana camara, Majorana hortensis*), was evaluated against the adults of *allosobruchus maculatus* F. The tested essential oils showed potent contact toxicity against the adults of cowpea beetle, within 24h of exposure. The results showed that *M. microphylla* oil (LC_{50} =13.99 μg/cm^2) was the most toxic one, followed by *C. reticulata, L. camara, A. judaica,* while, *S. terebenthfolins* was the less effective oil. In the fumigant experiments, *M. microphylla, A. judaica* and *A. santolina* showed the highest activity against *C. maculatus* adults (LC_{50} = 0.41, 0.59 and 0.64 μl/L respectively, while the oils of *C. reticulata, L. camara, S. terebenthifolius* and *M. hortensis* had moderate activity (LC_{50} = 5.41, 5.99, 11.52 and 44.14 μl/L respectively). When mixed with cowpea seeds, the oil of *M. microphylla* caused complete morality (100%) of the adults *C. maculates* at 1 mg/g seeds, while at 5 mg/g, the oils of *A. santolina* and *A. judaica* gave the complete morality. No laid eggs and emerged adults were observed when the seeds treated with these oils. The results of this study indicate that the oils of *M. microphylla, A. santolina* and *A. judaica* could be used in the control *C. maculates*.

Key Words: Contact toxicity; fumigant toxicity; essential oils; Callosobruchus maculatus; stored cowpea.

INTRODUCTON

Essential oils are naturally occurring substances which are often responsible for a plants distinctive scent or taste. There are 17.500 aromatic species that occur in higher plants (Brumeton, 1999); however, the genera capable of producing the compounds that constitute essential oils are distributed in a limited number of families, such as Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zimgiberaceae and piperaceae. Plant essential oils in general have been recognized as an important natural resource of insecticides (Gbolade *et al.*, 2000). They have the potential to be ovicides, fumigants, insect growth regulators and insecticides against various insect species (Regnault-Roger *et al.*, 1993; Tsao *et al* 1995; Shaaye *et al.*, 1997). The essential oils major constituents, monoterpenes, are also of interest because of their toxicity to insects and other potent biological activities (Kubo *et al.*, 1994; Basilico and Basilico, 1999). Garcia *et al.* (2005) reported that the essential oil of *Baccharis saliciflia* (Asteraceae) had toxic and repellent effects against *T. castaneum*. As part of our continuous studies on the chemistry and insecticidal activities of natural products isolated from Egyptian plants, this study was aimed to evaluate the contact and fumigant toxicities of seven essential oils against the adults of *C. maculates*. In addition, the efficacy of essential oils for controlling the insect in stored cowpea seeds was also examined.

MATERIALS AND METHODS

1- Plant materials

Leaves of *Mentha microphylla* C. koch., fruits of *schinus terebenthifolius* Raddi and leaves of *Lantana camara* L. were collected from Faculty of Agriculture farm, Alexandria. The aerial parts *Artemisia judaica* L. and leaves of *Majoranan hortensis* Moench were collected from Sharm El-Sheikh, Sinai Peninsula. The aerial parts of Achillea *Santolina* L. were collected from Borg El-Arab City, Alexandria. The fruits *Citrus reticulata* Balance. were purchased from local markets in Alexandria City and the used part was peels. The plant materials were identified with assistance of the student's flora of Egypt book (Tackholm, 1974) and confirmed by prof. FathAllah Zaitoon of Plant Pathology Department, Faculty of Agriculture, Alexandria University.

2- Test insect

Culture of the cowpea weevil, *Callosobruchus maculatus* F. was maintained in our laboratory over 5 years without exposure to insecticides and reared on sterilized cowpea seeds. The seed moisture content was equilibrated at 13% insect rearing and all experimental procedures were carried out at 26 ± 1°C and 70 ± 5% RH and 2 12:12 light : dark photoperiod. Adults used for toxicity tests were 2-3 days post-emergence.

3. Isolation and analysis of essential oils

The plants materials were dried at room temperature (26±1°C) for five days. Essential oils were extracted by hydrodistillation in a Clevenger-type apparatus for two hours. The oils were dried over anhydrous sodium sulfate, and stored at 4°C. Essential oils were diluted (1/100 v/v) in diethyl ether and 1 µl was injected into a gas chromatography (TRACE GC 2000, THERMO) /mass spectrometry (SSQ 7000, FINNIGAN) (GC/MS) set-up as previously described (Mohamed and Abdelgaleil, 2008).

4- Contact toxicity assay

The insecticidal activity of the essential oils against the adults of *C. maculatus* was determined by direct contact application (Qi and Burkholder, 1981). A series of dilutions of oils were prepared using acetone as solvent. Aliquots of 1 ml of the dilutions were applied on the bottom of a glass Petri dish (9 cm diameter) to give a range of concentrations (2.5-500 µg/cm^2). After solvent evaporation for two minutes, 20 adults were introduced into each Petri dish. Control dishes with and without solvent were conducted. All treatments were replicated three times. The mortality percentages were recorded after 24 hours of treatment and LC_{50} values were calculated according to Finney (1971).

5- Fumigant assay

The toxicity of the oil vapours against the adults of *C. maculatus* was evaluated by using a modified fumigant toxicity assay as described by Huang *et al.,* (2000). One liter glass jars were used as fumigation chambers. Essential oils at volumes of 0.1, 0.2, 0.4, 0.8, 1, 2.5, 5, 10, 20, 40, 60, 80, and 100 µl were applied on filter pieces (2x3 cm) attached to the undersurface of screw caps of the glass jars. The caps were screwed tightly onto the jars containing 20 insects. Three replicates of each control and treatment were setup. Number of dead insects was recorded after 24 hours of treatment. The mortality percentages were calculated and LC_{50} values were determined as previously described.

6- Cowpea seed treatment and insect exposure

Stock solutions of the test plant essential oils were prepared in acetone. Fifty grams of cowpea seeds were placed in 300 ml glass jars. Cowpea seeds in glass jars were treated with 1 ml of the stock solutions of the test oils. Oil of *M. microphylla* was tested at application rates of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/g, while oils *A. santolina, A. judaica* and *L.camara* were tested at rates 0.5, 1.0, 2.0, 2.5 and 5.0 mg/g. The control jars were treated with acetone. All jars were shaken manually for approximately 2min to achieve equal distribution of the oils through the entire seed mass. The jars were left for 30 minutes for complete evaporation of the solvent. Each replicate was infested with 10 pairs of 2-3 days old *C. maculatus* adults immediately after oil treatment and jars were covered with muslin fastened by rubber bands. The jars were kept at 26 ± 1°C and 70±5 R.H. The adults mortality and number of laid eggs were examined after one week of treatment. Then 5 weeks after, the number of emerged adults of *C. maculatus* was counted and the adult emergence percentages were calculated. The following formula suggested by Mian and Mulla (1982) was used to determine the reduction percentage in the number of progeny % = (1-x/y) x 100, where x = the number of adults emerged in the treatment; y = the number of adults emerged in the control.

7- Statistical analysis

The concentration – mortality data were subjected to probit analysis to obtain the LC_{50} values using the SPSS 12.0 software program (statistical package for Social Sciences USA). The values of LC_{50} were considered to be significantly different, if 95% confidence limits did not overlap. The mortality, laid eggs and emerged adults data were submitted to a one-way analysis of variance (AVOVA). Mean separations were performed by student-Newman-Keuls (SNK) (Cohort software Inc. 1985) test and differences at P = 0.05 were considered as significant.

RESULTS AND DISCUSSION

1- Contact toxicity of plant oils

The insecticidal activity of the seven oils was evaluated against the adults of *C. maculatus* using the residual film method. The values of LC_{50}, 95% condfidence limits, slopes and other parameters generated from regression lines are given in Table 1. All of the tested plant oils exhibited remarkable insecticidal activity. *M. microphylla* oil showed the strongest insecticidal activity with LC_{50} value of 13.99 µg/cm^2, followed by *C. reticulata, L. camara* and *A. judaica* as their LC_{50} values were 58.19, 83.04 and 97.82 µg/cm^2 respectively. On the other hand, the oils of *A. santolina* and *S. terebenthifolius* represented weak insecticidal activity. It has been reported that some plant oils such as cottonseed, soybean, maize and peanut had insecticidal activity against *C. maculates* (QI and Burkholder, 1981).

2- Fumigant toxicity of plant oils

Data of the fumigant toxicity of the tested essential oils against *C. maculatus* adults are given in Table 2. The essential oil of *M. microphylla* showed the strongest toxicity with LC_{50} value of 0.41µl/L. The toxicity of this oil was more 100 folds higher than oil of *M. hortensis*, more 20 fold higher than oil of *S. terebenthifolius* and more 10 fold higher than oils of *C. reticulate* and *L. camara*. On the other hand, the oils of *A. judaica* and *A. santolina* had toxicity close to toxicity oil of *M. microphylla*.The fumigant toxicity of some essential oils has been demonstrated against *C. maculatus*. For example, the essential oils of *Ageratum conyzoides, Citrus aurantifolia, Melaleuca quinquenervia, Carum copticum, Vitex pseudo-negundo, Artemisia scoperte, Ocimum basilicum* and *O. gratissimum* were found to possess fumigant toxicity against *C. maculatus* (Ke'ita *et al.*, 2001; Negahban *et al.*, 2006; sahaf and Moharramipour, 2008; Aboua *et al.*, 2010). The fumigant toxicity of these oils may be attributed to their active monoterpene constituents.

Table 1: Contact toxicity of the isolated essential oils against the adults of *Callosobruchus maculatus* (F.).

Oil	LC_{50}[a] ($\mu g/cm^2$)	95% Confidence limits (mg/cm^2) Lower	Upper	Slope ± S.E.[b]	Intercept ± S.E.[c]	$(\chi^2)^d$
Achillea santolina	100.34	90.75	108.83	4.18 ± 0.39	-3.33± 0.36	3.09
Artemisia judaica	97.82	91.38	103.64	6.46 ± 0.70	-5.13 ± 0.59	0.25
Citrus reticulata	58.19	26.89	81.62	1.30 ± 0.28	-0.74 ± 0.28	2.77
Schinus terebenthifolius	331.86	253.06	589.65	1.27 ± 0.27	-1.69 ± 0.29	1.28
Mentha microphylla	13.99	8.17	19.03	3.68 ± 0.30	0.19 ± 2.20	15.98
Lantana camara	83.04	73.45	90.90	4.68 ± 0.55	-3.39 ± 0.47	2.90
Majorana hortensis	115.44	98.77	130.88	2.32 ± 0.28	-2.01 ± 0.29	4.08

[a] The lethal concentration causing 50% mortality after 24 h.
[b] Slope of the concentration-mortality regression line ± standard error.
[c] Intercept of the regression line ± standard error.
[d] Chi square value

Table 2: Fumigant toxicity of the isolated essential oils against the adults of *Callosobruchus maculatus* (F.).

Oil	LC_{50}[a] ($\mu l/L$)	95% Confidence limits ($\mu l/L$) Lower	Upper	Slope ± S.E.[b]	Intercept ± S.E.[c]	$(\chi^2)^d$
Achillea santolina	0.64	0.62	0.65	17.65 ± 2.16	3.39 ± 0.41	0.00
Artemisia judaica	0.59	0.56	0.62	12.21 ± 2.16	2.73 ± 0.41	1.21
Citrus reticulata	5.41	3.03	7.85	2.10 ± 0.21	- 1.54 ± 0.18	8.89
Schinus terebenthifolius	11.52	9.61	13.38	1.98 ± 0.28	- 2.10 ± 0.33	3.28
Mentha microphylla	0.41	0.36	0.45	4.36 ± 0.69	- 1.66 ± 0.22	1.95
Lantana camara	5.99	4.49	7.71	2.63 ± 0.23	- 2.05 ± 0.20	5.95
Majorana hortensis	44.14	28.80	324.14	1.10 ± 0.38	- 1.81 ± 0.50	0.14

[a] The lethal concentration causing 50% mortality after 24 h.
[b] Slope of the concentration-mortality regression line ± standard error.
[c] Intercept of the regression line ± standard error.
[d] Chi square value.

3- Effect of essential oils on mortality of *C. maculatus* in treated cowpea seeds,

The results of contact and fumigant toxicities demonstrated that essential oil of *M. microphylla*, *A. santolina*, *A. judaica*, and *L. camara* were the most effective against *C. maculatus*. Therefore, these four essential oils were tested for their potential to control the insect in stored cowpea seeds. The mortality of *C. maculatus* adults in treated cowpea seeds with different application rates after one week is presented in Tables 3 and 4. The results showed that there were significant differences between essential oils and among the application rates of each oil on the percentages of adult mortality. Oil of *M. microphylla* caused the height mortality at the application rates of 1 mg/g followed by the oil of *A. santolina*, and *A. judaica* which caused complete mortality at rate of 5 mg/g. At the same rate and time, the oil of *L.camara* caused 70.0% mortality. The results to oils *M. microphylla* and *A. judaica* are in good agreement with our previous study on the activity of the tested essential oils against *S. oryzae* and *T. castanum* (Mohamed *et al.*, 2009)

4- Effect of essential oils on laid eggs and adults emergence of *C. maculatus* in treated cowpea seeds

The results of the reduction of laid eggs, progeny production and adult emergence of *C. maculatus* after 5 weeks of seed treatment are presented in Table 3 and 4. In general, all of the tested essential oils at the tested application rates showed significant reduction in number of laid eggs and emerged adults compared with control. The potency of essential oils was in order of *M. microphylla*, *A. judaica*, *A. santolina* and *L. camara*. No adults emerged in the treatment of *M. microphylla* at the rate of 1.0 mg/g. Similarly, oils of *A. judaica* and *A. santolina* completely inhibited the emergence of the adults at rate 5 mg/g. The oil of *L. camara* caused low mortality percentages but caused high reduction percentages of 93.8 at rate of 5.0 mg/g. The strong insecticidal activity of *M. microphylla* oil observed in this study agreed with the results of Abdelgaleil, *et al.* (2010).

Progeny reduction in the treated seeds is perhaps more important than parental mortality, because a seed protectant should protect the seed for along storage period (Athanassiou *et al.*, 2005). In our work, progeny reduction of *C. maculatus* was significantly high on cowpea seed treated with the tested essential oils, which suggests that long-term protection for the treated cowpea seeds. There were few reported studies on the efficacy of essential for control *C. maculatus* in stored seeds (Ke'ita *et al.*, 2001; Ketoh *et al.*, 2005; Raja and William, 2008).

Seed germination results of most effective essential oils, *M. microphylla*, at rate (1 mg/gm), *A. Judaica* and *A. santolina* at rate (5 mg/g) revealed that these oils reduced the germination percentage of the treated seeds compared with control. *A. judaica* oil caused the highest reduction in seed germination followed by oil of *A. santolina* with germination percentages of 46.7 and 51.6, respectively, while oil of *M. microphylla* had the greatest effect on seed germination (61.7%) at rate application of 1 mg/g. The presence of naturally occur insecticidal components has been known for centuries. However few of essential oils are commercially used in management of stored-product insects. Problems associated with the use of synthetic insecticides, such as the development of resistance, persistence of residues in stored products, and damage to the environment and human health (Lorini and Galley, 1999, Zettler and Arthur, 2000) have generated interest in naturally occurring compounds. Some of active natural products with interesting insecticidal potential such as the tested essential oils are isolated from edible plants; therefore they are considered being safer to human and environment than other chemicals. The results presented in this study suggest that some essential oils such as *M. microphylla*, *A. judaica* and *A. santolina* could be efficient protectants against *C. maculatus* in stored cowpea seeds and also could be used in integrated pest management program of this insect.

Table 3: Effect of *Mentha microphylla* oil applied to cowpea seeds on mortality, laid eggs and adult emergence of *Callosobruchus maculatus* (F.).

Application rate (mg/g)	Mortality (% ± SE) after 1 week	Mean No. of eggs (± SE) after 1week	Emerged adults after 5 weeks		
			(Mean No. of adults (± SE)	Emergence (%)	Reduction (%)
control	10.0 ± 0.0d	785 ± 60.77a	471.7 ± 22.36a	60.1	0.0
0.01	13.33 ± 3.33d	769 ± 40.19a	462 ± 25.54a	60.1	2.1
0.05	16.67 ± 1.66d	723 ± 12.17a	409 ± 16.38a	56.6	13.3
0.1	30.8 ± 3.94c	535 ± 24.46b	230 ± 21.80b	43.0	51.2
0.5	61.6 ± 3.34b	387 ± 9.46c	90.0 ± 6.66c	23.3	80.9
1.0	100.0 ± 0.0a	0.0 ± 0.0d	0.0 ± 0.0c	0.0	100.0

Data are expressed as means ± S.E. from experiments with three replicate.
Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

Table 4. Effect of isolated oils applied to cowpea seeds on mortality, laid eggs and adult emergence of *Callosobruchus maculatus* (F.).

Application rate (mg/g)	Mortality (% ± SE) after 1week	Mean No. of eggs (± SE) after 1week	Emerged adults after 5 weeks		
			Mean No. of adults (± SE)	Emergence (%)	Reducti on (%)
control	10.0 ± 0.0h	785 ± 60.77a	471.7 ± 22.36a	60.1	0.0
		***Achillea santolina* oil**			
0.5	15.0 ± 0.0gh	773 ± 9.46a	469 ± 16.83ab	60.0	1.6
1.0	20.0 ± 0.0fg	710 ± 18.19a	422.4 ± 16.19b	59.5	10.5
2.0	54.0 ± 2.36d	386 ± 41.19b	129 ± 14.75e	33.4	72.6
2.5	73.0 ± 4.72bc	205 ± 10.28f	51 ± 3.05fg	24.9	89.1
5.0	100.0 ± 0.0a	0.0 ± 0.0g	0.0 ± 0.0h	0.0	100.0
		***Artemisia judaica* oil**			
0.5	11.7 ± 1.66gh	472 ± 9.72bc	279 ± 7.0c	59.1	40.8
1.0	18.3 ± 1.66fg	421 ± 12.17cd	202 ± 10.41d	47.9	53.3
2.0	23.7 ± 1.85f	360 ± 11.85d	126 ± 9.30e	35.0	73.2
2.5	78.0 ± 2.25b	145 ± 7.02f	21 ± 1.52 gh	14.5	82.8
5.0	100.0 ± 0.0a	0.0 ± 0.0g	0.0 ± 0.0h	0.0	100
		***Lantana camara* oil**			
0.5	11.7 ± 1.66gh	739 ± 15.11a	442 ± 13.88ab	59.8	6.2
1.0	13.3 ± 3.33gh	735 ± 17.02a	436 ± 9.54ab	59.3	7.5
2.0	35.0 ± 1.73e	504 ± 12.90b	291 ± 15.54c	57.7	38.2
2.5	58.0 ± 1.52d	283 ± 7.57e	73 ± 7.0f	25.8	84.5
5.0	70.0 ± 2.89c	195 ± 7.94f	29 ± 2.6gh	14.8	93.8

Data are expressed as means ± S.E. from experiments with three replicate.
Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

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الملخص العربى

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مجدى إبراهيم الدسوقى

قسم أفات الحبوب المخزونة – معهد بحوث وقاية النبات – مركز البحوث الزراعية – الصبحية – الإسكندرية – مصر

تم تقييم تأثير سبع من الزيوت النباتية المعزولة من النباتات المصرية التالية *Achillea santolina, Mintha microphylla, Schinus terbenthifolius, Citrus reticulata, Artemisia judaica, Majorana hortensis, Lantana camara* ضد خنفساء اللوبيا فى بذور اللوبيا . تم التقييم بطريقة الملامسة باستخدام اطباق بترى وسلسلة من تركيزات الزيوت المعزولة ضد الحشرة الكاملة لخنفساء اللوبيا واخذ نسبه الموت بعد ٢٤ ساعة من المعاملة وكان زيت *M. microphylla* اكفأ الزيوت حيث أعطى قيمة LC_{50} تساوى ١٣,٩٩ ميكروجرام/سم٢ يليه باقى الزيوت وأقلهم كفاءة زيت *S. terbenthifolius* حيث أعطى قيمة LC_{50} تساوى ٣٣,٨٦ ميكروجرام/سم٢ بعد ٢٤ ساعة من المعاملة . وتم التقييم ايضا بطريقة التدخين وكان أيضا زيت *Microphylla M.* الأعلى كفاءة حيث اعطى قيمة LC_{50} ١٤,٠ تساوى ميكروليتر/ لتر يليه باقى الزيوت وكان زيت *L. camara* هو الاقل كفاءة بقيمة LC_{50} تساوى ١٤,٤٤ ميكروليتر/لتر بعد ٢٤ ساعة من المعاملة . وعند خلط حبوب اللوبيا بسلسلة من تركيزات هذه الزيوت فى وجود الحشرة الكاملة لخنفساء اللوبيا كان زيت *M. microphylla* هو الأقوى فعند تركيز ١ مجم/جم بذور لوبيا سبب موت كامل للحشرة بعد اسبوع من المعاملة. كذلك زيوت *A. santolina* و *A. judaica* أعطيا نسبة موت ١٠٠% ولكن عند تركيز ٥مجم /جم بذور ، أما زيت *L. camara* اعطى نسبة موت متوسطة (٧٠%) عند نفس التركيز . كذلك تم خفض نسبة وضع البيض كذلك الحشرة الكاملة الناتجة بعد خمسة اسابيع من المعاملة حتى وصلت النتائج الى ١٠٠% موت للحشرة الكاملة وعدم وضع بيض وكذا عدم خروج حشرات كاملة وذلك بالنسبة لزيت *M. microphylla* عند تركيز ١ مجم/جم . أما زيت *A. santolina* و *A. judaica* أعطيا نفس النتيجة ولكن عند تركيز ٥ مجم/جم. أما زيت *L. camara* كان الأقل فعالية فى هذه الدراسة. يمكن الإقتراح بإن تلك الزيوت ذات الكفاءة العالية فى موت الحشرة الكاملة ومنع وضع البيض وكذا منع خروج الافراد الكاملة ممكن ان يكون لها دور مهم كواقيات لبذور اللوبيا ضد خنفساء اللوبيا.