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INSECTICIDAL ACTIVITY AND CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM *ARTEMISIA JUDAICA* L. AGAINST *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE)

*Hany Kamal Abd-Elhady**

Department of Pesticides, Faculty of Agriculture, Menoufiya University, Shebin El-Kom 32511, Egypt

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Abstract: The insecticidal properties of essential oil derived via the hydro-distillation method from aerial parts of *Artemisia judaica* L. were tested against the cowpea weevil, *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae). The repellent activity assay of essential oil against *C. maculates* adults indicated that in concentrations of 63.7, 31.9, 15.9, 8.0 or 4.0 μg/cm^2, the oil reduced egg laying by 92.5, 86.0, 61.8, 42.7 and 12.5%, respectively. Also, the residual-film assay showed that after 72 hours of treatment, concentrations of 50 and 40% were highly effective against the *C. maculates* adults. The sub-lethal effects of essential oil were investigated on fecundity and F_1 progany by exposing adult females to treated seeds. Both LC_{25} and LC_{50} of essential oil significantly reduced F_1 progany production compared to the control. The chemical composition of the essential oil was analyzed by GC-MS and the resulting oil piperitone (32.4%), camphor (20.6%) and (*E*)-ethyl cinnnamate (8.2%) were found to contain the major constituents of the oil. This provided the insecticidal properties of the essential oil against cowpea weevil.

Key words: essential oil, *Artemisia judaica,* insecticidal activity, *Callosobruchus maculatus*, chemical composition, gas chromatography-mass spectrometry (GC/MS) analysis

INTRODUCTION

Artemisia judaica L. (family Asteraceae) is a perennial fragrant small shrub with pubescent leaves, which grows widely in Egypt (desert and coast) and in the Middle East (Sinai Peninsula, Jordan and Saudi Arabia). It is one of the common species of the genus *Artemisia* (Tackholm 1974). *A. judaica* is called ''Wormwood'' and it is used traditionally as a medicinal herb in Egypt. The plant has been used to treat gastro-intestinal disorders, poor eyesight, cardiovascular disease, skin disorders, and weak immune systems as well as to decrease the risk of atherosclerosis, cancer, and arthritis.

A number of volatile chemical constituents from the aerial parts of *A. judaica* have been identified. Phytochemical analysis shows it to be a rich source of flavonoids including apigenin, cirsimaritin, and various novel compounds (Liu *et al.* 2003, 2004). Oil from *A. judaica* may also have insecticidal properties.

The cowpea weevil, *Callosobruchus maculatus* (Fabricius) is a major pest of cowpea, *Vigna unguiculata* (L.) Walp. Cowpea, one of the main sources of protein for teaming populaces in poor tropical countries, is usually prone to attack by *C. maculatus*. The infestation usually commences from the field where the crops are grown and continues during storage. Synthetic chemical insecticides have proved very effective in the control of the beetle. However, the problems associated with chemical insecticides such as health hazards, insect resistance and cost, have led to continuous quest by food protectionists for alternatively safer, cheaper and more ecologically friendly methods of controlling the beetles (Ogunwolu and Idowu 1994). These natural substances have a relatively low mammalian toxicity (Isman 2000) and degrade rapidly in the environment (Rebenhorst 1996; Misra and Pavlostathis 1997).These properties make them attractive alternatives to synthetic insecticides for establishing new control practices with lower mammalian toxicity impact.

The purpose of this investigation was to evaluate the insecticidal properties of *A. judaica* oil against *C. maculates.* The identification of constituents of the essential oil was also determined by gas chromatography-mass spectrometry (GC/MS) analysis.

MATERIALS AND METHODS

1. Plant materials

The aerial parts of *A. judaica* were collected from the El-Arish Region of the Sinai Peninsula, Egypt in May, 2009 and shaded for 7–9 days at room temperature (25±2°C) until brittle. The plant material was identified by botanists in the Department of Agriculture Botany, Menoufiya University, Egypt. Air-dried aerial parts from the sample (200 g), were subjected to hydrodistillation in a Clevenger

*Corresponding address:
hany_elnanaay@yahoo.com

type apparatus for 3 h as described by Negahban *et al.* 2006. The oil was dried over anhydrous sodium sulphate and stored in a refrigerator at 5°C until required for the experiments.

2. GC/MS analysis

The oil compounds were isolated, identified and quantified on a Shimadzu GC-17A gas chromatograph (Shimadzu Corp., Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector (GC-MS QP-5050A). The GC-MS system was equipped with a TRACSIL Meta X5 column (Teknokroma S. Coop. C. Ltd., Barcelona, Spain; 30 m x 0.25 mm *i.d.*, 0.25 µm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min. at a split ratio of 1:10. The following temperature program was used: 40°C for 5 min.; rising at 3.0°C/min. to 200°C and held for 1 min.; rising at 15°C/min. to 280°C and held for 10 min. The injector and detector were held at 250 and 300°C, respectively. Diluted samples (1:10 pentane, v/v) of 0.2 µl of the extracts were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of *m/z* 45–450. The identification of individual compounds of essential oils was accomplished using two different analytical methods: (a) KI, Kovats indices in reference to *n*-alkanes (C_8–C_{32}) by NIST 2009; and (b) mass spectra (authentic chemicals and Wiley spectral library collection). Identification was considered to be tentative when it was based on mass spectral data only. The relative concentration of each compound in the essential oil was quantified according to the peak area integrated by the analysis program.

3. Insecticidal activity

3.1. Insects

The cowpea weevil insects used in this study were obtained from the Department of Stored-Product Pests, Plant Protection Research Institute, Agricultural Research Center, Dokki, Egypt. At this department, a standard culture has been maintained without exposure to any insecticides for several years on cowpea seeds (Su 1977) and maintained at 28±1°C, 70 ±5% relative humidity (RH) and 12 h:12 h lethal dose (LD). The cowpea seeds used for our experiments were previously sterilized by freezing at –20°C for one week to kill off any prior infestation. Then, the seeds were stored in sealed polyethylene bags at 5°C until required for the experiments (Giga and Smith 1983). The seed moisture content was approximately 13%, as described by Mian and Mulla 1982, which is consistent with that normally required for storage. At this moisture level, the growth of fungi and other micro-organisms is almost completely suppressed (Leahey and Curl 1982). For the toxicity tests, newly emerged adults (0 to 24 h old) were used.

3.2. Toxicity tests

3.2.1. Residual-film assay

The residue film technique was followed as described by Pangnakorn (2009) with some minor modifications for estimating the insecticidal activity of the tested oil against the cowpea weevil. An appropriate quantity of *A. judaica* oil was dissolved in acetone to obtain each test solution. One ml of oil solution was pipetted into each glass scintillation vial (25 ml; 5.5 cm x 3.0 cm diameter) and the vial was rolled for approximately 1 min. to ensure that all surfaces received treatment. Vials were air dried in a fume hood at 25°C for 24 h before use. The control vials were precoated with acetone only. A group of ten 0 to 24 h old adult female cowpea weevils was placed in the vial. For air flow, the vial was then covered with nylon cloth which was secured with adhesive tape. Five replications/treatments of the experiment were done and all were kept at 30±0.5°C. Insect mortality was recorded after 24, 48 and 72 h of treatment in separated experiments. Each experiment was replicated five times and the insecticidal activity of the oil was expressed as % mean mortality of adult insects.

3.2.2. Fumigation assay

The fumigant effects of the essential oil were evaluated against adults in sealed 3-l glass jars. An aliquot of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 or 6.0 ml of essential oil was deposited on Whatman filter papers (5 cm diameter) to generate concentrations (167 to 2,000 µl/l of air) of the oil without using any solvent. Fifty pairs of *C. maculates* adults were introduced into each jar containing 100 cowpea seeds. The number of dead adults per day was counted in three separated experiments; time was taken into consideration. Each concentration and control was replicated five times. Mortality was determined 24, 48 and 72 h after exposure under constant climatic conditions (25°C; 12 h: 12 h LD).

3.2.3. Repellency assay

The repellency assay was conducted according to the method described by Rezuanul *et al.* (2009) with some minor modifications. Twenty pairs of insects were enclosed in a plastic box (22 cm x 15 cm x 10 cm) with two glass Petri dishes (5 cm diameter) containing 40 cowpea seeds each. The dishes were positioned at opposite corners of the box. A 1,000 µl aliquot of test solution containing 63.7, 31.9, 15.9, 8.0 or 4.0 µg/cm^2 oil was applied to a filter paper disc (5 cm diameter) and placed on a Petri dish, while a filter paper disc without oil was placed on the opposite Petri dish as a control. After 48 h, the number of eggs laid on each cowpea was counted. The repellency capacity was quantified by comparing the number of eggs laid on cowpeas in the Petri dish with *A. judaica* oil against the number of eggs laid on cowpeas in the corresponding Petri dish without oil (the control).

3.2.4. Sub-lethal effects

Twenty g of cowpea seeds were put into 0.4-l glass jar (7 by 13 cm) and thoroughly mixed with 2 ml of oil diluted in acetone till the required concentration was achieved. To completely evapora™te the solvent, the treated samples were left overnight in open jars. Samples treated with acetone alone served as the controls. The dose-response data were subjected to probit analysis (Finney 1971) to determine the LC_{50} value for a 48-h exposure. Five males and five females (5♂ x 5♀) were intro-

duced into each jar (replicate) containing 20 g of cowpea treated seeds. Sub-lethal concentrations, LC_{25} and LC_{50} of *A. judaica* oil were used against *C. maculates* adults. The jars were covered with a piece of muslin secured with an elastic band and maintained in the laboratory under rearing conditions. The control and five replicates were used for each treatment. Adult females were allowed to lay eggs, and after 3 days the parent insects were removed and discarded. The insects in the different treatments were incubated under rearing conditions for oviposition. After 9 days of treatment, the mean number of eggs laid, the number of hatched eggs and percentage of emerged adults were noted.

4. Statistical analysis

The data was analyzed using analysis of variance (ANOVA), where significant differences between the treatments were observed. Mean values were significantly separated by using the least significant difference (LSD) test at 5% level (Sokal and Rohlf 1981). The computer software CoStat program, version 6.311, 2005 was used. The mortality percentages were corrected using Abbott's formula (Abbott 1925), and software to calculate probit analysis according to Finney (Finney 1971) was used.

RESULTS

1. Toxicity tests

1.1. Residual-film assay

The results showed the efficacy of residual contact toxicities of extracted essential oil from *A. judaica* on the cowpea weevil. Mortality counts were made at 24, 48 and 72 h after treatment. Data in table 1 show that after 72 h of treatment, concentrations of 50 and 40% were highly effective against the *C. maculates* adults,. The mortality percent seemed to be concentration dependant.

Table 1. Residual contact toxicities of extracted essential oil from *A. judaica* against the cowpea weevil *C. maculatus*

Treatment [%]	% mean mortality of adult insects[a]		
	24 h	48 h	72 h
10	0.0 c	1.0 d	4.0 e
20	0.0 c	7.0 c	15.0 d
30	1.0 c	9.0 c	28.0 c
40	4.0 b	15.0 b	41.0 b
50	12.0 a	26.0 a	62.0 a
LSD 0.05	1.800	2.001	2.540

[a]corrected mortality using the Abbott formula 1925 and % of mean mortality values followed by the same letter within each vertical column are not significantly different

1.2. Fumigation assay

The toxicity data of the tested essential oil were summarized on table 2. The data showed that LD_{50} values estimated after 48 and 72 h exposure were significantly less than that after a 24-h exposure.

Table 2. Fumigant toxicity test of extracted essential oil from *A. judaica* against adult cowpea weevil *C. maculatus*

Treatment	LC_{50} (confidence limits)[a] [µl/l of air]	Slope ±SE	Regression equation y = bx + a
24 hours	883 (794–980) a	2.2931 ±0.1714	y = 2.29x –1.755
48 hours	645 (578–715) b	2.4503 ±0.1695	y = 2.45x –1.885
72 hours	524 (376–676) b	2.4402 ±0.3115	y = 2.44x –1.635

[a]LC_{50} values followed by the same letter within each vertical column are not significantly different (95% CL do not overlap); SE – standard error

y – the dependent variable that the equation tries to predict;
x – the independent variable that is being used to predict y;
a – the y-intercept of the line

1.3. Repellency assay

A dose-dependent effect of the essential oil *A. judaica* on oviposition was observed. At concentrations of 63.7, 31.9, 15.9, 8.0 or 4.0 µg/cm^2, the oil reduced egg laying by 92.5, 86.0, 61.8, 42.7 and 12.5%, respectively when compared to the control.

1.4. Sub-lethal effects

Data in table 3 indicated that adult females exposed to seeds treated with LC_{25} or LC_{50} of *A. judaica* essential oil were significantly affected by the treatments. Egg hatch percentages were significantly decreased by the LC_{25}-and LC_{50}-treatment when compared to the control (72.48%). Also, the F_1 progeny production was significantly reduced in both LC_{25}, LC_{50} treatments, with 38.52 and 17.65% emergence, compared to 77.78% emergence in the control.

Table 3. Surface film treatment of cowpea seeds with sub-lethal concentration of essential oil from *A. judaica* on the biology of *C. maculatus*

Treatment	Mean number of eggs laid (±SD) (% reduction)[a]	Mean number of eggs hatched (±SD) (% hatch)	Mean number of emerged adults (±SD) (% emergence)[b]
LC_{50}	234 (±41) a [21.48]	68 (±11) c [29.06]	12 (±3) c [17.65]
LC_{25}	265 (±33) a [11.07]	109 (±16) b [41.13]	42 (±6) b [38.53]
The control	298 (±29) a	216 (±24) a [72.48]	168 (±23) a [77.78]

Means followed by the same letter(s) within each vertical column are not significantly different at p = 0.05

[a][(Number of eggs laid in the control – number of eggs laid in treatment)/number of eggs laid in the control] x 100

[b](Number of emerged adults/number of hatched eggs) x 100

2. GC/MS analysis

The hydrodistillation of the dried aerial parts of *A. judaica* L. gave light yellowish oil with a yield of 0.7% (w/w). Forty-seven components were identified in the oil, representing 94.8% of the total composition (Table 4). The

major components of the essential oil were piperitone (32.4%), camphor (20.6%), (*E*)-ethyl cinnamate (8.2%) and terpinene-4-ol (4.6%). The essential oil of *A. judaica* L. was rich in monoterpenoids and ester of cinnamic acid.

Table 4. Chemical composition of the essential oil isolated by hydrodistillation from aerial parts of *A. judaica* analyzed by GC-MS

Number	RI[a]	Compound[b]	Peak area [%][c]
1	923	α-pinene	0.3
2	937	camphene	0.3
3	956	β-pinene	0.2
4	971	myrcene	0.3
5	989	α-phellandrene	1.2
6	1012	α-terpinene	0.3
7	1021	1,8-cineole	0.4
8	1037	artemisia ketone	1.4
9	1062	artemisia alcohol	0.3
10	1073	terpinolene	0.2
11	1079	fenchone	0.6
12	1089	linalol	0.3
13	1097	β -thujone	0.6
14	1105	chrysanthenone	3.9
15	1114	camphor	20.6
16	1131	*iso*-borneol	0.2
17	1141	terpinene-4-ol	4.6
18	1152	lavandulol	0.5
19	1164	borneol	2.2
20	1179	α-terpineol	0.3
21	1196	verbenone	0.3
22	1202	carveol	0.4
23	1230	piperitone	32.4
24	1240	geraniol	0.8
25	1248	perilla aldehyde	0.2
26	1257	geranial	0.5
27	1265	bornyl acetate	3.0
28	1296	carvacrol	0.2
29	1334	citronellyl acetate	0.7
30	1355	(*E*)-ethyl cinnamate	8.2
31	1374	α-ylangene	0.2
32	1385	β-elemene	0.2
33	1414	α-cedrene	0.3
34	1429	β -caryophyllene	0.3
35	1435	(*E*)-β -farnesene	0.3
36	1442	*allo*-aromadendrene	0.4
37	1481	valencene	1.3
38	1487	β -bisabolene	0.2
39	1498	γ -cadinene	0.7
40	1541	spathulenol	0.3
41	1548	caryophyllene oxide	1.1
42	1560	davanone	0.3
43	1597	1-*epi*-cubenol	1.4
44	1610	humulene oxide II	1.2
45	1616	T-cadinol	0.4
46	1622	β -eudesmol	0.2
47	1649	cadalene	0.6
		total	94.8

[a] RI, retention index on a TRACSIL Meta X5 column

[b] compounds are listed in order of their elution from a TRACSIL Meta X5 column

[c] compound percentage

DISCUSSION

The use of natural products can be considered as an important alternative for the control of stored product pests. The results from this study indicated that the essential oil of *A. judaica* exhibited effective toxicity to *C. maculatus* in all tests (fumigation, repellency, surface film, egg hatch and adult emergence). Essential oils can affect insects in several ways: they may disrupt major metabolic pathways and cause rapid death, act as contact insecticides (Saxena *et al.* 1992), fumigants (Shaaya *et al.* 1997), repellents (Plarre *et al.* 1997), and deterrents or can modify oviposition. The essential oil had a good fumigation effect on adult insects. According to the results, we observed similar trends to the surface film toxicity of *A. judaica* essential oil. Similar observations about other plant extracts have also been made. For example, Wang *et al.* (2006) showed that *Artemisia vulgaris,* strongly repelled *T. castaneum. Azadirachtin* has also been demonstrated to be repellent to three stored product insects (Xie *et al.* 1995). Plant extracts may also accelerate development or interfere with the life-cycle of insects in other ways (Bell *et al.* 1990). Results of the sub-lethal effects indicated that *A. judaica* oil proved to be an effective biocontrol agent. Similar results were obtained with the essential oils of *Tagetes minuta* L., *Hyptis suaveolens* Poit., *Ocimum canum* L., *Ocimum basilicum* and *Piper guineense* Schum (Keita *et al.* 2002). There have been no previously reported studies concerning the activity of *A. judaica* used as a fumigant on insect pests. The fumigant activity of some essential oils from the *Artemisia* species have been evaluated against a number of stored product insects. Fumigant toxicity of essential oils has been reported from *A. annua* against *S. oryzae* (Aggarwal *et al.* 2001), and *A. aucheri* oil showed fumigant properties on stored insect (Shakarami *et al.* 2004). Fumigant properties of *A. sieberi* oil on *S. oryzae* and *T. castaneum* were also shown (Negahban *et al.* 2006, 2007). In this study, the mortality of *C. maculatus* tested both by contact and by fumigation varied with the dose of the essential oil. High mortality rates and inhibition of F_1 progeny production were recorded by contact with seeds treated with essential oil for *C. maculatus*. The vapours of the essential oil exhibit a strong toxic action against the adults of *C. maculatus.* It has also been established, that the essential oil generally remains more toxic and its effect is persistent.

The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids (Regnault-Roger and Hamraoui 1995; Ahn *et al.* 1998). Mono-terpenoids are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions (Lee *et al.* 2002). Due to their high volatility, they are fumigant and gaseous and might be of importance for stored-product insects (Ahn *et al.* 1998). Therefore, insecticidal activity of *A. judaica* may be related to these components. The toxicity exhibited by essential oils and their constituent monoterpenes marks them as potentia1 alternative compounds to currently used fumigants (Huang *et al.* 2000). The monoterpene camphor might show a broad insecticidal activity against stored insects according to a more

detailed study by Dunkel and Sears (1998). They demonstrated the potent toxic effects of camphor from *A. tridentata* Nutt against *T. castaneum.* Therefore, toxicity of the *A. judaica* could be attributed to higher concentrations of camphor.

The chemical composition of the essential oil from *A. judaica* could be changed according to geographical distribution and it might be an effective factor affecting its insecticidal activity. The principal components of *A. judaica* essential oil are piperitone and *trans*-ethyl cinnamate, which have been found to play a key role in controlling *Spodoptera littoralis* (Abdelgaleil *et al.* 2008). In general, the cytotoxic activity of essential oils is mostly due to the presence of phenols, aldehydes and alcohols (Sacchetti *et al.* 2005). It is also possible that various minor components may be involved in some type of synergism with other active components (Yu *et al.* 2004). The results of the present study suggest the possible use of *A. judaica* essential oil in research for selecting new natural biocontrol components. because *A. judaica* oil has potential insecticidal activity. However, further investigations on the insecticidal mode of action of the oil, its effect on non-target organisms, and field evaluation are needed.

CONCLUSION

Extracted volatile oils from the aerial parts of *A. judaica*, using the steam distillation process, were found to have an insecticidal effect against *C. maculatus*. A concentrated extraction of 50% was shown to have the highest mortality according to the residue film test. However, the effectiveness decreased when the extraction was diluted. In this study, we were able to show the essential oil of *A. judaica* to be an active fumigant, at low concentrations. The efficacy of *A. judaica* oil described here might have more fumigant toxicity than that of related species. All these results indicate that essential oil of *A. judaica* is a source of boilogically active vapours. In addition to fumigant activities, the essential oil showed a strong repellent effect on *C. maculatus.* Therefore, aerial parts of *A. judaica* show a potential to be developed into biopesticide for controlling the cowpea weevil insects.

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