REPELLENT AND INSECTICIDAL ACTIVITIES OF VOLATILE CONSTITUENTS OF *ARISTOLOCHIA ELEGANS* MAST (ARISTOLOCHIACEAE) AGAINST *CORCYRA CEPHALONICA* (STAINTON) (LEPIDOPTERA: PYRALIDAE)

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Abstract

The toxic and repellent activities of the volatile constituents of *Aristolochia elegnas* Mast, were evaluated against storage pest *Corcyra cephalonica* (Stainton). The chemical composition of the hydrodistilled essential oil isolated from leaves of *A. elegnas* L. growing in Egypt was determined qualitatively and quantitatively using GC and GC–MS. Experimentally, a total yield of 0.38 % v/w of essential oil per 1kg fresh weight was gained which upon analysis yielded 40 compounds representing 98% of total essential oil. Bicyclogermacrene being the main component and the other major components identified were β-caryophyllene, limonene, germacrene D and isocaryophyllene. β-caryophyllene oxide and *E*-nerolidol were the prominent components of the oxygenated sesquiterpenes. The toxicity and repellency effect of the volatile constituents of *A. elegnas* was tested against *C. cephalonica* where, a significant inhibitory effect was reported. The high toxicity of the volatile constituents of *A. elegnas* may be due to the presence of dialkyl phthalates and limonene, while, high repellency may be due to the presence of β-caryrophyllene oxide and nerolidol Therefore, they could be suitable for using as insecticidal and repellent agents against stored products pests in the food industry.

INTRODUCTION

C. cephalonica (Stainton) is a serious pest of some important stored food such as rice, wheat, maize, sorghum, groundnut, cotton seeds, and cocoa beans (Cox *et al.*, 1981). During the past two decades, the genus Aristolochia (Aristolochiaceae) has attracted much interest and has been the subject of numerous chemical, pharmacological and insecticidal studies. Some *Aristolochia* species have been reported to possess insecticidal and repellent activities against stored products pests. Volatile oil of *A. elegans* caused high insecticidal and repellent activities and also, deformation rates against *C. cephalonica*, (El-Badawey, 2008). *Aristolochia argentina* (Griseb) showed a significant insecticidal activity against *Sitophilus oryzae* (L), a serious pest of stored wheat, rice and sorghum grains (Broussalis *et al.*, 1999).

Vila *et al.*, (1997) analyzed the volatile oils from roots, stems and leaves of *A. elegans*, bicyclogermacrene, caryophyllene and isocaryophyllene were the

predominant components of the oils from the leaves, whereas, *E*-nerolidol, was the main constituent of the oil from stems and roots.

Regarding the available literature there are no informations about the chemical composition of *A. elegans* cultivated in Egypt. The present study was a continuation of our previous research (El-Badawey, 2008), and mainly aimed to investigate of chemical composition of *A. elegans* oil and study of the insecticidal and repellent activities of volatile constituents in order to explore a novel natural insecticidal agent against *C. cephalonica* for the control of stored grain pests.

MATERIALS AND METHODS

1. Tested plants

A. elegans aerial parts were collected from Orman garden herbarium, Giza and Plant Protection Research Institute garden, Agricultural Research Center, Dokki, Giza. The identification of the plant was kindly done by Dr. Adel Okeal Director of El-Orman Garden, Giza, Egypt. A voucher herbarium specimen had been deposited in the Department of Pharmacognosy, Faculty of Pharmacy Al -Azhar University, Cairo, Egypt.

2. Stored grain insects

Insect rearing

Rearing of rice moth, *C. cephalonica* was carried out according to (Bernardi *et al*, 2000) with some modifications It was reared on artificial media composed of 50 % whole wheat flour , 45 % corn flour and 5 % yeast at a constant temperature of 30°C and 65 % RH in the dark.. This artificial diet produced adults after 22 days. Selected insects were obtained from the laboratory cultures at Department of Pest Physiology, Plant Protection Research Institute, Agricultural Research Center.

3. Preparation of the essential oil

The essential oil was prepared from the fresh *A. elegans* 1 kg (fresh weight) by hydrodistillation using a clevenger type apparatus. The distilled was extracted with ether after saturation with sodium chloride. The ether extract was dehydrated over anhydrous sodium sulfate. Solvent was removed under reduced pressure at 20 °C. The volatile constituents was packed in dark container and kept in refrigerator till analysis. Percentage yield was determined according to (Eight peak Index of Mass Spectra.1974).

4. Chemical analysis of essential oil

4.1. Gas chromatographic-mass spectrometric (GC-MS) analysis

The prepared essential oil was subjected to GC / MS analysis using Shimadzu GC/ MS – QP 5050 A. Software Class 5000. Searched library: Wiley 229. LIB. Column: DB5, 30 m, 0.53 mm ID, 1.5 µm film. Carrier gas: Helium (flow rate 1ml / min.). Ionization mode: (70 ev). Temperature program: 40 ^{0}C (static for 2 min) then gradually increasing (160 ^{0}C at a rate of 2 ^{0}C/ min) up to 250 ^{0}C (static for 7.5 min). The detector temperature was 250 ^{0}C while the injector temperature was 250^{0}C.

4.2. Identification of the chemical constituents

Qualitative identification of the essential oil was achieved by library searched data base Willey 229 LIB as well as by comparing their retention indices and mass fragmentation patterns with those of the available references and with published data, (Egyptian Pharmacopia Genral Organization 1984.) and (Adams, 1989). The percentage composition of components of the volatile was determined by computerized peak area measurements.

5. Insecticidal activity

Toxicity study by contact treatment of insect (Thin Film Technique)

This technique was used according to (Pascual and Robledo. 1997). 1 ml of different concentrations (100 ,1000 , 10000, 100000 ppm) of total oil constituents in acetone was applied to filter paper disks (5 cm Diameter) and the solvent was allowed to evaporate at 25°C for 24h. For each concentration five replicates of ten larvae (last instar) in 9-cm petri dishes, in addition to a control (solvent only) were used, petri dishes were kept at 30°C in the dark. Mortality records were taken after 48 hours. Mortality was corrected according to (Abbott's formula, 1925). For the effective constituent LC_{50} (LC-P lines) values were calculated after 48 hours according to (Finney, 1971).

6. Repellency study by food preference method

Repellency was assessed according to the method (Liu *et al.*, 2006) with some modifications. Four glass jars (three glasses for oil treatments and one for control) was connected together at their rims by means of 30X10 cm nylon mesh tube. Circular hole (5 cm diameter) was cut at the middle of the mesh for introduction of the test insects. Twenty nonsexual adult insects were introduced into the nylon mesh tube through the circular hole.

Samples (75g) of wheat flour , corn flour , and yeast (50 : 45 : 5) were separately mixed with constituents of each oil in the glass jars at concentrations of 100 ,1000 , 10000,100000 ppm (constituent of oil/ sample grains) and kept at a constant temperature of 25°C in the dark for 24 h so as to allow the solvent (acetone)

to evaporate completely. An appropriate amount of acetone was used as control. For each treatment five replicates were carried out. After 3 h, the number of insects at each treated or control diet was counted and the repellency (%) was calculated by following formula :

$$\text{Repellency (\%)} = \frac{C - E}{T} \times 100$$

C is the insect numbers in the control jar, ***E*** is the insect numbers in oil treated jar and ***T*** is the number of total insects. *C*, *E* and *T* were the mean data of 5 replicates.

RESULTS AND DISCUSSION

1. Chemical analysis of constituents of oil

Phytochemical screening of *A. elegans* L. revealed the presence of terpenoid alkaloids and phenolic compounds. The essential oil of *A. elegans* leaves (50 g) was prepared by hydrodistillation. The obtained essential oil is yellow in color lighter than water and with aromatic odor with 0.8892 and 1.2831 of specific gravity and refractive index at 25 ^{0}C respectively. It yielded 0.38 % v/w of the essential oil, which is soluble in organic solvents such as petroleum ether, ether, chloroform, ethyl acetate and ethyl alcohol. The components of the essential oil were detected by TLC using n – hexane – ethyl acetate (97- 3), which showed several spots. GC of the essential oil revealed the presence of 40 peaks, all peaks were identified (Table 1). constituting 98% of total essential oil. They could be classified to monoterpene, sesquiterpenes, oxygenated sesquiterpenes hydrocarbons Cyclohexane dervatieves, Benzene derivatives and other compounds attaining 9.4 %, 51.7 %, 5.9 %, 5.1 %, 22.8 % and 3.1 % w/w respectively, for the first leaves sample (L_1) and 9.1 %, 50.8 %, 5.4 %, 5.2 %, 22.5 % and 3.0 % w/w respectively, for the second leaves sample (L_2).

The most abundant compound of monoterpene is compound (2), (R_t 7.04) representing (7.8 % w/w) MS of compound 2 showed the molecular ion peak at M/Z 136 equivalent to $C_{10}H_{16}$, However other fragments for compound (2) 93, 79, 41, 67, 53, 105 and 121 prove the structure for compound (2) as lemonene.

Bicyclogermacrene, is the main component (20.2%), but other compounds were also found in relative amounts germacrene D (6.3%) , isocaryophyllene (5.6%), and α-humulene (1.6%) while, β-caryophyllene (15.5%) was also found to be one of the main components in the leaf oil of *A. elegans*. β-caryophyllene oxide (3.1%) and *E*-nerolidol (2.00 %) were the main components of oxygenated sesquiterpenes. These results are in agreement with (Vila *et al.*,1997). The essential oil of *A. elegans* are

characterized by the unusual presence of 1,2 di-*n*-butyl and 1,2 di-*n*-isooctyl phthalates (benzene derivatives) and all the samples analysed contained a significant amounts of these two components and these compounds were firstly isolated from *A. elegans*, however, these compounds does not seem to be widely distributed in the Aristolochiaceae. Other compounds were also found in relative amounts cyclohexane derivatives (1,3- dimethyl cyclohexane, 1,2- dimethyl cyclohexane 1,4- dimethyl cyclohexane 1,1,3- trimethyl cyclohexane 1,2,3- trimethyl cyclohexane, ethyl cyclohexane and 1-ethyl-3-methyl cyclohexane) and aliphatic hydrocarbons (Octane, 2,4 dimethyl heptane, *n*-Hexatriacontane, Hexadecene, n-Dotriacontane, Hexandioic acid dibutyl ester, Hexacosane and 9-Hexadecenoic acid).

2. Toxicity study

The essential oils of *A. elegans* at different concentrations were tested for their toxic activity against grains stored pest *C. cephalonica*. All treatments with essential oil caused significant mortality compared with control.

In fact, the evaluation of insecticidal activity of each constitute in the oil from leaves of *A. elegans*, was very hard, but some of these constituents were reported to have repellent and toxic effect against insects. The insecticidal constituents of many plants essential oils are monoterpenoids. The insecticidal activity of monoterpenes has been reported against stored products insects (Garcìa *et al.*, 2005). The compound limonene from *Citrus* spp showed insecticidal activities against *Rhyzopertha dominica* (F.), lesser grain borer, and *Tribolium castaneum* (Herbst), red flour beetle, which are important pests of stored grains (Prates, *et al.* 1998). Components (cymol and limonene) of essential oils of *Eucalyptus camaldulensis*, *Eucaltptus cameroni* and of the peal of *Citrus auranitum* have significant insecticidal action, being lethal to the stored products pests *R. dominica* and *T. castaneum*, limonene showed more effective control of *T. castaneum* than of *R. dominica* (Santos, *et al.* 1997).

The insecticidal activities of dialkyl phthalates have been demonstrated by several literatures, A study carried out by (Abdel-Aal, 1984) indicated that 1,2 di-*n*-butyl and 1-butyl-2- methyl propyl phthalates have generally toxic effect against *T. castaneum* and *Sitophilus granaries*, and also found that the toxicity of 1,2 di-*n*-butyl and 1-butyl-2- methyl propyl phthalates was 1.52 and 1.21, 0.30 times as toxic nicotine sulphate, malathion and pirimiphos-methyl respectively. (Streufert, 1980) reported that the acute toxicity of di-ethyl hexyl-1,2 benzene dicarboxylate against the midge larvae *Chironiums plumosus* was 1.8 mg/L and 7.2 mg/L for its degradation products. Also, (Ahmed, 1983) using the Devil' s apple *Datura ctramonium* (L.) that contains mainly 1,2-di-n-isooctyl phthalate against larvae of *Spodoptera littoralis* (Bosid.) with LC_{90} levels 0.34 and 1.5 mg/cm2, respectively. Phthalic acid esters

(PAEs) especially di-*n*-butyl phthalate (DBP) have become widespread in the environment and they have been found in sediments, waters, and soils (Chang, *et al* 2005). This confirmed our results (Table 2) and supports our conclusion, the high concentration of limonene, 1,2 di-*n*-butyl and 1,2 di-*n*-isooctyl phthalates at the present study was possibly the reason of the insecticidal effect of *A. elegans* oil against *C. cephalonica*.

Combinations of these compounds with other essential oil components like β-caryophyllene, α-humulene, germacrene D and β- pinene, generally enhanced activity (Setzera *et al.*, 2007). In addition, caryophyllene oxide, significantly enhances the activity of oil (Angela *et al.*, 2007).

The medium lethal concentrations (LC_{50}) could be calculated for the first and the second sample leaves of oil extract, the results showed that essential oil of (L_1) was higher larvicidal activity (the least LC_{50}) than essential oil of (L_2) against *C. cephalonica* at all concentrations. The medium lethal concentrations (LC_{50}) for the two essential oil leaves were 10.05 and 33.58 ppm.

3. Repellency action

The presence of oxygenated sesquiterpenes caryophyllene oxide and nerolidol with slightly high concentration in essential oil of *A. elegans* may be the reason for the repellent effect of *A. elegans* oil against *C. cephalonica*, this conclusion was confirmed by (Maurice, *et al* 2004) found that caryophyllene oxide and a sesquiterpene alcohol exhibited relatively high repellency against *Anopheles gambiae* and (Lwande, *et al* 1999), stated that, nerolidol from the most repellent components against *Rhipicephalus appendiculatus*. The highest repellency value (90%) recorded at the highest concentration (100000 ppm) and L_1 is more repellent than L_2 with all concentrations (Tabe 3).

Therefore, these compounds or their mixtures might contribute to the whole repellent and insecticidal activity of *A. elegans* oil against the stored grains products. However, the volatile constitutes of *A. elegans* oil were rarely described as a kind of insecticidal or repellent ingredients. From the previous results, essential oil of *A. elegans* and their constituents exhibit acute toxic and repellent effects against *C. cephalonica* grains stored pest..

Table 1. Chemical composition of the volatile oil from leaves of *A. elegans*.

No.	Components	Rt (min)	Ratio (%)	
			(L_1)	(L2)
	Monoterpene hydrocarbons		**9.40**	**9.10**
1-	β-Pinene	3.93	1.60	1.90
2-	Limonene	7.04	7.80	7.20
	Sequiterpens hydrocarbons		**51.70**	**50.80**
3-	Isocaryophyllene	10.97	5.60	5.60
4-	β-Elemene	11.03	1.50	1.50
5-	β-Caryophyllene	11.35	15.50	15.10
6-	Aromadendrene	11.98	0.40	0.60
7-	α-Humulene	12.00	1.60	1.30
8-	Germacrene B	12.93	0.30	0.30
9-	Germacrene D	13.37	6.30	6.00
10-	Bicyclogermacrene	14.79	20.20	19.90
11-	Valencene	14.89	0.20	0.30
12-	β- Farnesene	15.12	0.10	0.20
	Oxygenated sesquiterpenes		**5.90**	**5.40**
13-	Z-Nerolidol	14.01	0.20	0.20
14-	E-Nerolidol	14.27	2.00	2.30
15-	Caryophyllenol	14.72	0.10	0.10
16-	β-Caryophyllene oxide	15.03	3.10	2.50
17-	Viridithenol	17.93	0.20	0.10
18-	Spathanlenol	18.33	0.30	0.20
	Cyclohexane dervatieves		**5.10**	**5.20**
19-	1,3- dimethyl cyclohexane	3.14	1.50	1.30
20-	1.2- dimethyl cyclohexane	3.21	1.00	1.00
21-	1,4- dimethyl cyclohexane	3.39	0.50	0.70
22-	1,1,3- trimethyl cyclohexane	3.73	0.60	0.70
23-	Ethyl cyclohexane	3.70	0.40	0.40
24-	1,2,3- trimethyl cyclohexane	3.91	0.80	0.90
25-	1-Ethyl-3-methyl cyclohexane	4.61	0.30	0.20
	Benzene derivatives		**22.80**	**22.50**
26-	Ethylbenzene	4.02	1.50	1.50
27-	P- Xylene	4.12	0.80	0.70
28-	m-Xylene	4.44	1.10	1.30
29-	1-ethyl-3-methylbenzene	4.61	0.50	0.50
30-	1,3,5 trimethylbenzene	5.84	0.20	0.20
31-	1,2- Di-butyl-phthalate	21.94	10.30	9.30
32-	1,2- Di-isooctyl- phthalate	48.05	8.40	9.00
	Other compounds		**3.10**	**3.00**
33-	Octane	3.28	0.70	0.60
34-	2,4 dimethyl heptane	3.51	0.60	0.60
35-	*n*-Hexatriacontane	13.26	0.20	0.20
36-	Hexadecene	3.84	0.40	0.30
37-	n-Dotriacontane	46.93	0.50	0.50
38-	Hexandioic acid dibutyl ester	36.56	0.20	0.20
39-	Hexacsane	44.38	0.40	0.30
40-	9-Hexadecenoic acid	62.62	0.10	0.30
	Total identified		**98.00**	**96.00**

Rt : Retention time

L_1= The first leaves sample L_2= The second leaves sample

Table 2. Toxic effect and LC50 of larvae caused by contact application of volatile oil constituents of A. elegans against C. cephalonica.

Test component	Corrected mortality (%)	
Conc. (ppm)	First leaves sample (L_1)	Second leaves sample (L_2)
100	64.0	59.0
1000	73.0	65.0
10,000	85.0	77.0
100,000	93.0	89.0
LC_{50} (ppm)	10.05	33.58

Table 3. Repellency (%) of essential oil constituents against stored grain rice moth, C.. cephalonica at different concentrations

Test component	Repellency (%)	
Conc. (ppm)	First leaves sample (L_1)	Second leaves sample (L_2)
100	37.8	33.7
1000	56.6	40.8
10,000	79.4	73.0
100,000	90.4	87.9

CONCLUSION

The essential oil from *A. elegans* could be considered as a new alterative clue for natural insecticide of plant origin in control of storage pest *C. cephalonica* and 1,2 di-*n*-butyl and 1,2 di-*n*-isooctyl phthalates, limonene, β-caryophyllene oxide and *E*-nerolidol were not only the main constitutes of essential oil from the leaves of *A. elegans* but also the main insecticidal and repellent activities against *C. cephalonica*.

It is particularly important to avoid the use of plastics because phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Great care must be experienced to prevent such contamination and exhaustive cleanup of reagents, solvents, water and glassware may be required to eliminate background phthalate contamination.

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الإنشطة الطاردة والإبادية للمكونات الطيارة لنبات الإرستولوشيا إليجانس ضد فراشة الأرز

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يهدف هذا البحث إلي دراسة التأثير السمي والطارد لمكونات الزيت العطري لنبات الإرستولوشيا إليجانس علي اليرقات والحشرات الكاملة لحشرة فراشة الأرز والتى تعتبر من آفات الحبوب المخزونة الهامة. قد أوضحت الدراسة أن مستخلص الزيت العطري لنبات الإرستولوشيا إليجانس كان ذا تأثير سمي عالي مع جميع التركيزات المستخدمة (١٠، ١٠٠، ١٠٠٠، ١٠٠٠٠، ١٠٠٠٠ جزء من مليون). وقد يرجع ذلك إلي وجود مادة الليمونين ومشتقات البنزين أو إستترات حمض الفاثاليك (ثنائي البيوتيل – وثنائي الأيزوأوكتيل فيثاليت) ، أظهرت الدراسة أيضاً أن خلاصة الزيت العطري للنبات المختار لها تأثيرطارد مع جميع التركيزات المستخدمة بسبب وجود مادتي أوكسيد بيتا-كاريوفيللين و النيروليدول. أثبتت الدراسة أن نسبة الموت تزيد كلما زاد التركيز. وبناءاً علي هذه النتائج نقترح إستخدام الزيت العطري لنبات الإرستولوشيا إليجانس في المكافحة المتكاملة لآفة الحبوب المخزونة فراشة الأرز كمادة سامة وطاردة للحشرات .