INSECTICIDAL ACTIVITY OF *ARTEMISIA HERBA-ALBA* (ASTERACEAE) ESSENTIAL OIL AGAINST THE EGYPTIAN COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Essential oil extracted from the leaves of *Artemisia herba-alba* by steam distillation was investigated for its toxicity against *S. littoralis* larvae. Results of the laboratory bioassay indicated that essential oil of *A. herba-alba* was toxic against *S. littoralis* larvae, with LC50 of 1300 ppm. Further fractionation of the oil by silica gel column chromatography resulted in separation of highly *S. littoralis* toxic fraction. The principal components of this fraction as detrmined by Gas chromatography coupled to mass spectroscopy (GC-MS) were Alpha-thujene, limonene, cis-ocimene, trans-beta-ocimene and davanone. It is suggested that both Alpha-thujene, and limonene could contribute significantly to the insecticidal activity of the oil.

INTRODUCTION

Plant secondary metabolites play an important role in plant–insect interactions and therefore such compounds may have insecticidal, hormonal or anti-feedant activity against insects. Medicinal plants can be screened for anti-insect activity and might lead to the identification of compounds active against insect pests. In this respect, much effort has been focused on plant essential oils as potential sources of commercial pest control agents or as lead compounds (Isman, 1999).

The genus *Artemisia* L. (family Asteraceae) comprises a variable number of plant species found throughout the northern half of the world (Marco and Barbera´, 1990). *Artemisia* spp. is rich in essential oils which have been used for centuries as an insect repellent, general pesticide, and as a spray to repel slugs and snails (Metspalu *et al., 2001*).

Artemisia herba-alba (Asso) is a dwarf shrub grows in the Middle East. It grows abundantly in Egyptian deserts such as Sinai (Segal, 1980 & Saleh *et al.*,

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1987) and west deserts (Ahmed *et al.*, 1990) where it is used extensively in folk medicine in helminthiasis, diabetes mellitus and other conditions such as jaundice (Migahid, 1978).

Although, the anti-insect potential of essential oil of various *Artemisia* species was extensively studied (Pascual-Villalobos and Robledo (1999) Kuusik *et al.*, 2000, and Metspalu *et al., 2001*), work on *A. herba-alba* essential oil is scarce. Soliman (2007) mentioned that essential oil of *A. herba-alba* is toxic to *Bemisia tabaci* (Gennadius), *Aphis gossypii* (Glover) and *Thrips tabaci* (Lindman). However, he did not determine the possible insect active compounds responsible for toxicity of the oil against these insect pests.

The objective of this study was to identify the possible insect-active compounds of *A. herba-alba*, using fractionation by column chromatography, GC-MS analysis and the cotton leafworm, *Spodoptera littoralis* as a model insect species.

MATERIAL AND METHODS

Collection of plant materials

Artemisia herba-alba was collected from Marsa-Matrouh, Egypt, in March 2005. Taxonomic identification of plants was performed by botanists of the Egyptian National Botanical Institute Dokki, Giza, Egypt.

Extraction of essential oils

Aerial fresh parts (leaves and branches) were cut into pieces and 150-200 g samples were prepared. The essential oils were extracted from samples by steam distillation using the method described by Duerbeck (1993). Following distillation for 4 h, pentane, 5–6 ml, was added to increase the volume of the oil obtained, which was then dried over anhydrous sodium sulphate and the pentane evaporated at room temperature.

Fractionation of essential oil

Crude essential oil was subjected to fractionation on silica gel column as follows: Oil (0.5 g) was mixed with 1 gm of silica gel then transferred onto the column (75 x 11 mm, silica gel 70-230 mesh, Merck Kieselgel 60), and eluted successively with 50-ml portions of 100% hexane, 100% chloroform, 100% ethyl acetate and 100% methanol. Solvent in each fraction was evaporated under reduced pressure. Residue was weight and stored at -20°C until used for toxicity assays and GC-MS analysis.

GC-MS analysis

Identification of compounds in essential oil extracted from *A. herba-alba* was carried out with a HP 5972A mass spectrometer (MS) coupled to HP-6890 gas-chromatography (GC) equipped with a HP-5MS capillary column (30 m x 0.32 ID. 0.25 µm film thickness). Electron impact (EI) mass spectra were obtained at 70 eV and the instrument scanning from 35 to 700 amu. Helium was used as the carrier gas at a flow rate of 1 ml/min. Injector temperature was 250°C; detector temperature was 280°C, and split was 20:1. Oven temperature was programmed from 35°C (5 min) to 80°C at 10°C/min and to 250°C at 4°C/min. Integration of peaks, drawing calibration table, and standard curve were performed using HP-Chemstation soft-ware. Identification of constituents present was based on computer matching against the library spectra (Wiley275L), built up using pure substances and components of known constituents. The percentage of composition was computed from gas chromatography peak areas.

Contact Toxicity assay

To study the insecticidal properties of the crude oil and its fractions, the thin film procedure adopted by Pascual-Villalobos and Robledo (1999) was followed using 4th instar larvae of *S. littoralis*, as a model insect.

Bioassays were conducted in standard Petri dishes (actual measured area of ca. 30 cm^2). Tested extracts were diluted with acetone to give concentrations of 150, 300, 600, 1200 and 2400 µg active ingredient/ml acetone. One ml acetone solution at the selected concentrations was spread in each dish using one ml pipette, with shaking of the dish to ensure the chemical distribution. This gave concentrations of 5, 10, 20, 40, and 80 µg/cm^2 dish bottom area. Control dishes received only one ml pure acetone. Acetone was allowed to dry at room temperature for half hour. Actively feeding 4th instar larvae of *S. littoralis* were separated from laboratory mass rearing culture (Pest Physiology Dept., Plant Protection Research Institute) and 10 larvae were transferred to each dish. Five replicates (dishes) with ten larvae/replicate were prepared for each concentration as well as control treatment. After 24h at standard conditions (27±2°C and 70±5% RH) (Dahi, 2005), mortality of larvae was recorded.

Statistical analysis

All toxicity data were corrected for control mortalities according to Abbott's equation (Abbott, 1925). The LD_{50}, probit analysis and *t*-test were estimated using a software package "LD-Pline", Copyright of Ihab. M. Bakr, Plant Protection Research Institute, Egypt.

RESULTS AND DISCUSSION

Figure (1) shows that essential oil of *A. herba-alba* exhibited concentration-dependant toxicity against larvae of *S. littoralis* (Fig. 1) with LC50 of 26.8µg/cm2 (Table 1). Results of chromatographic analysis using GC indicated that crude essential oil of *A. herba-alba* contained many interfering compounds (Fig. 2) making it difficult to identify them by GC-MS analysis. Accordingly, essential oil was subjected to fractionation by column chromatography using solvents with different polarities to isolate the chemical compounds into broad groups based on their polarities. Table (1) and Fig. (1) Showed that the fraction eluted with chloroform had high contact toxicity against *S. littoralis* larvae with LC50 of 12.5 µg/cm2, while fractions eluted with hexane, ethyl acetate and methanol had no significant lethal effect on *S. littoralis* larvae. Thus, chloroform fraction was subjected to GC-MS analysis (Figure 3). Table (2) shows that chloroform fraction contained 11 main compounds. The oxygenated sesquiterpene "davanone" was the major component. Davanone is reported in several *Artmisia* species such as A. *pallens*, *A. rehan*, *A. douglasiana*, *A. herba alba*, *A. inculta*, *A. judaica*, *A. maritime* and *A. persica* among other species (Sadeghpour *et al.*, 2004). There were no reports on the toxicity of davanone against insects or animals. The only two compounds reported to have insecticidal effects and present in chloroform fraction of *A. herba-alba* oil are Alpha-thujene, and limonene. Limonene is well documented in the literature as an insecticide (Cook 1992; Ellis and Baxendale 1997; Ibrahim *et al.*, 2001 and Tripathi *et al.*, 2003). On the other hand, thujone is a neuorotoxic substance with high insecticidal properties (Chiasson *et al.*, 2001 and Metspalu *et al.*, 2001). Because of the above mentioned reports and the relatively high percentage of limonene and thujone in chloroform fraction, these two components are likely to be the main insecticidal ingredients against *S. littoralis* larvae with negligible activity attributable to the other constituents.

TABLE (I)

Results of probit analysis for toxicity of *A. herba-alba* essential oil and its fractions on *S. littoralis* larvae

Fractions	LC_{50} (µg/cm^2)	Lower limit	Higher limit
Crude oil	26.8	20.2	35.5
Hexane	-	-	-
chloroform	12.5	10.4	14.5
Ethyl acetate	-	-	-
methanol	-	-	-

* 95% confidence limit

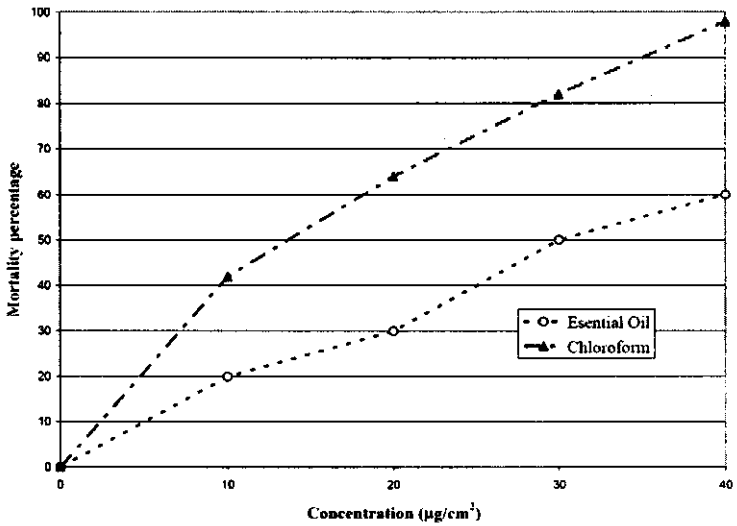

Figure 1. Contact toxicity of different concentration of *A. herba-alba* essential oil and chloroform fraction on *S. littoralis* larvae

TABLE (II)

Mean percent concentration and GC-retention time of all constituents identified by GC–MS analysis of crude essential oil of *Artemisia herba-alba*

No.	Compound	Retention time	Area (%)*
1	Beta phellandrene	8.61	2.25
2	Alpha-Thujene	8.79	5.2
3	Myrcene	9.40	2.72
4	Limonene	9.47	7.71
5	Cis-Ocimene	9.51	9.36
6	Tans-Ocimene	9.69	11.55
7	Allo-Ocimene	9.91	3.62
8	Beta-caryophyllene	10.2	1.13
9	Alpha-Humulene	10.4	1.29
10	Curcumene	14.51	3.10
11	Davanone	16.22	51.45

- Relative intensity of *m/z*.

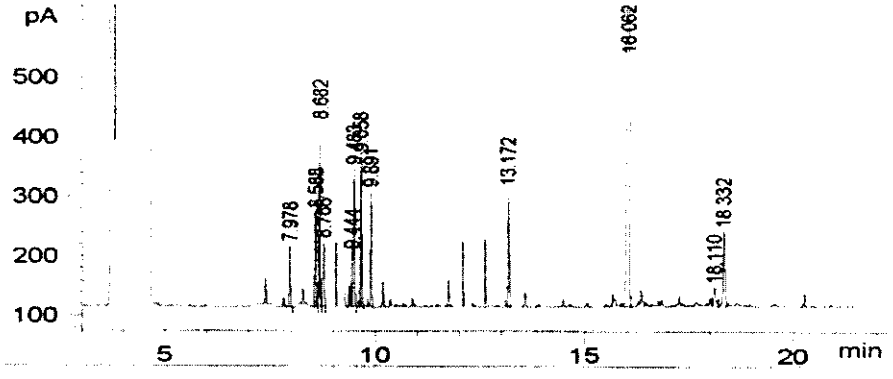

Figure 2. Gas chromatogram of compounds detected in crude essential oil of *A. herba-alba*.

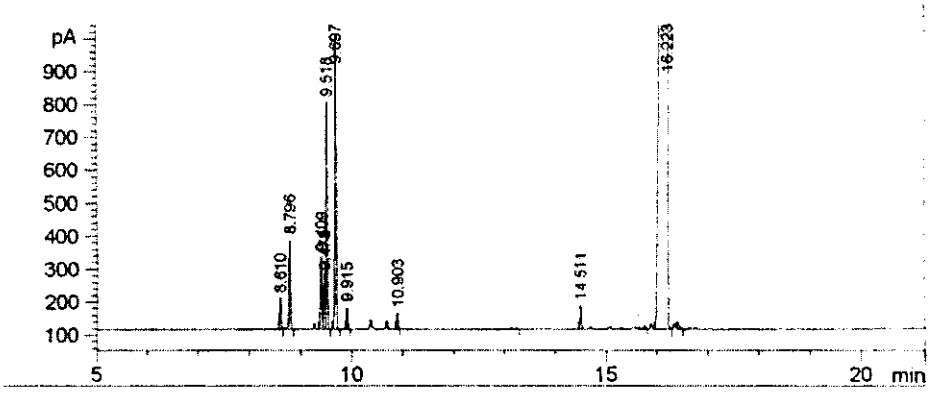

Figure 3. Gas chromatogram of chloroform fraction of *A. herba-alba* essential oil.

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