INSECTICIDAL ACTIVITY OF *MELALEUCA BRACTEATA* (MYRTACEAE) ESSENTIAL OIL AGAINST THE EGYPTIAN COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The insecticidal activity of the essential oil from *Melaleuca bracteata*, a widely cultivated species in Egypt was investigated. The essential oil was extracted by steam distillation and its insecticidal activity against *S. littoralis* larvae was determined in a laboratory bioassay. The larvae of *S. littoralis* were highly susceptible to topical application of *M. bracteata* oil, with LC_{50} of 1.84 $\mu g/cm^2$. Chemical analysis of the *M. bracteata* oil by Gas chromatography coupled to mass spectroscopy (GC-MS) indicated that Methyleugenol is by far the major compound of the oil (87.6%) and contributes significantly to the insecticidal activity of the oil.

INTRODUCTION

In recent years, investigations aiming at the development of new agents for pest control have focused on the study of natural products (Addor, 1995). The discovery of active natural compounds that are more selective and less persistent will be beneficial for both the environment and agricultural product consumers, although natural products cannot automatically be assumed to be without risk. Of the studied natural products, essential oils of medicinal plants have been considered as potential sources of commercial pest control agents or as lead compounds (Isman, 1999). Many plant essential oils and phytochemicals are known to possess effective and selective insecticidal activity (Kim *et al.*, 2003).

Melaleuca (Myrtaceae) is a large genus includes more than 250 species. Some *Melaleuca* species are rich in essential oils and are used for medicinal purposes and cosmetics (Yatagai *et al.*, 1998). *Melaleuca bracteata* is widely cultivated species in Egypt as ornamental plant and has been reported to be extremely rich in essential oil (Aboutabl *et al.*, 1991). However, work on the anti-insect potential of essential oil of

M. bracteata is negligible. In order to find new ways for utilizing their oils and to investigate the toxicity of *M. bracteata* against insects, the oil was extracted and its insecticidal activities was studied on the cotton leafworm, *Spodoptera littoralis* as a model insect species. Also the chemical components of the oil was examined to identify the possible insect-active compounds.

MATERIALS AND METHODS

Collection of plant materials

Aerial parts (leaves and branches) of *M. bracteata* were purchased in local ornamental markets of Giza Governorate. Taxonomic identification of plants was performed by botanists of the Egyptian National Botanical Institute Dokki, Giza, Egypt, where voucher specimens are deposited.

Extraction of essential oils

Aerial fresh parts 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 (Ireland *et al.*, 2002).

Purification of methyleugenol

Crude essential oil was subjected to fractionation on silica gel according to Degen *et al.* (1999). One ml of oil 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 20-ml portions of 100% hexane, 5% diethyl ether in hexane, 100% diethyl ether, and 100% methanol. The diethyl ether fraction contained the pure methyleugenol (Degen *et al.*, 1999). Purity of compounds was checked by GC-MS (purity >99%).

GC-MS analysis

Identification of compounds in essential oil extracted from *M. bracteata* was carried out with a HP 5972A mass spectrometer (MS) coupled to HP-6890 gas-chromatography (GC) equipped with 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 software. 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 essential oil composition was computed from gas chromatography peak areas.

Contact Toxicity assay

To study the insecticidal properties of the tested compounds, the thin film procedure adopted by Pascual-Villalobos and Robledo (1998) 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 15, 30, 60, 120 and 240 µg active ingredient /ml acetone. One ml acetone solution at the selected concentration was spread in each dish using 1 ml pipette, with shaking of the dish to ensure the chemical distribution. This gave concentrations of 0.5, 1, 2, 4, and 8 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 the essential oil of *M. bracteata* exhibited concentration-dependant toxicity against larvae of *S. littoralis* with LC_{50} of 1.84 µg/cm^2 (Table 1). Previous studies showed that solvent extracts and essential oil of

certain Melaleuca species (*M. alternifolia*, *M. saligna*, M. *argentea*, *M. symphyocarpa* and *M. acacoides*) were toxic against *Varroa* mites (Sammataro *et al.*, 1998), European house-dust mite, *Dermatophagoides pteronyssinus* (Yatagai *et al.*, 1998) and the whitefly, *Trialeurodes vaporariorum*, (Choi *et al.*, 2003). On the other hand, Yatagai *et al.* (1998) mentioned that essential oil of *M. bracteata* has contact miticidal effect on *D. pteronyssinus* with LD_{99} of 1.3 1.84 μg/cm^2.

M. bracteata essential oil was subject to GC-MS analysis to identify the compound(s) responsible for its toxic effect on *S. littoralis*. Results of chromatographic analysis of *M. bracteata* oil are presented in Fig. 2 and Table 2. Eleven components were identified, most of them are common plant secondary metabolites and presented as minor constituents of the oil. However, the considered compound was methyleugenol (86% of *M. bracteata* crude oil constituents).

TABLE (I)

Results of probit analysis for toxicity of *M. bracteata* essential oil and purified methyleugenol on *S. littoralis* larvae

Probit analysis	Oil	Purified methyleugenol
LC_{50} (μg/cm^2)	1.84*	1.68*
Lower limit (μg/cm^2)	1.14	1.17
Upper limit (μg/cm^2)	2.79	2.66
Slope	3.47 ± 1.14	2.71 ± 0.83

* 95% confidence limit

Methyleugenol was reported to be very toxic against adult mite, *Tyrophagus putrescentiae* (Kim *et al.*, 2003), *Tribolium castaneum* and *Sitophilus zeamais* (Ho *et al.*, 1994). These reports, in addition to our observation in this study on treated *S. littoralis* larvae showed uncoordinated behavior, loss of glossiness of the cuticle and loss of body fluids before dying. Similar symptoms were reported for methyleugenol treated insects (Kim *et al.*, 2003) led us to suggested that methyleugenol is the insect-toxic component of *M. bracteata* oil. To test this suggestion, we isolated and purified methyleugenol from the oil using column chromatography (Figs 3&4) and its effects were tested on *S. littoralis*. Table 1 showed that pure methyleugenol was highly toxic against larvae of *S. littoralis* with LC_{50} of 1.68 μg/cm^2 (Table 1). There were no significant difference between LC_{50} of essential oil and the purified methyleugenol ($P > 0.05$, T-test). These results confirmed the suggestion that methyleugenol was the insect-active component of *M. bracteata* oil.

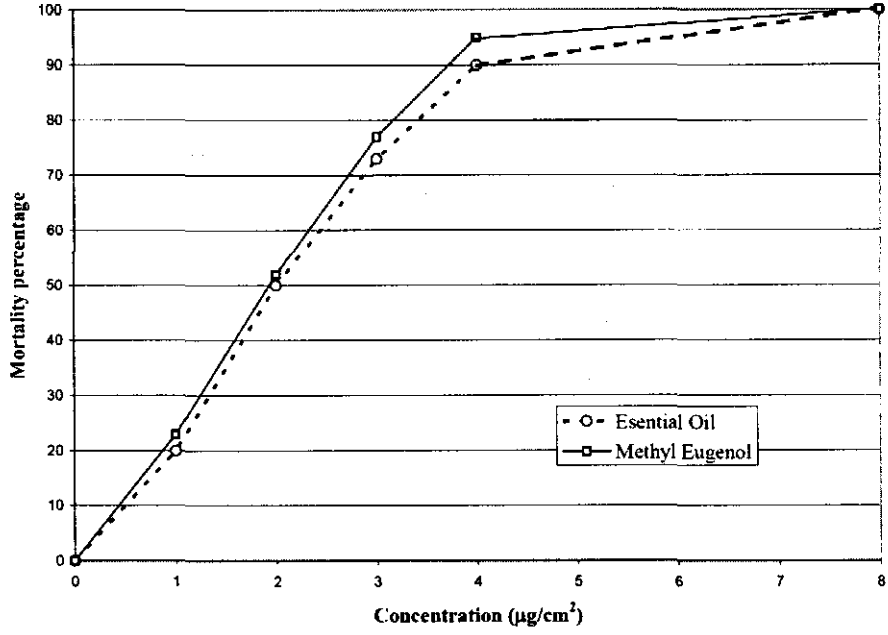

Figure 1. Contact toxicity of different concentration of M. *bracteata* essential oil and purified methyleugenol on *S. littoralis* larvae

TABLE (II)

Mean percent concentration and GC-retention time of all constituents identified by GC–MS analysis of crude essential oil of *M. bracteata*

No.	Compound	Retention time	Area (%)*
1	Alpha-pinene	4.96	0.92
2	Betal-pinene	5.59	1.25
3	Para-cymene	6.26	1.28
4	Limonene	6.32	0.89
5	1,8-Cineole	6.39	2.96
6	Terpinolene	7.21	1.12
7	Delta-3-carene	7.32	1.39
8	Alpha-terpineol	8.72	0.81
9	Methyleugenol	11.68	87.60
10	Germacrene-D	12.73	0.91
11	Cis-calmenene	13.19	0.87

- Relative intensity of *m/z*.

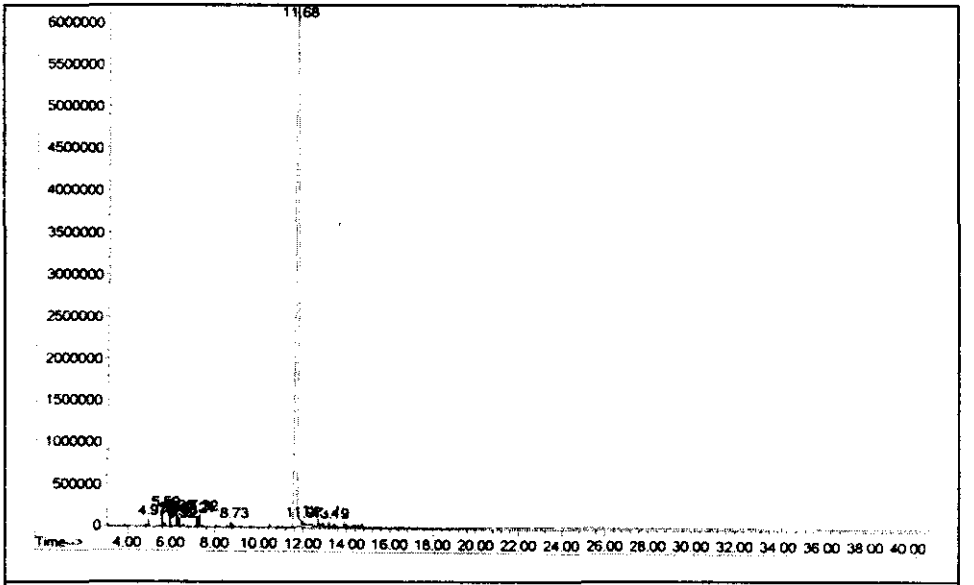

Figure 2. Gas chromatogram of compounds detected in crude essential oil of *M. bracteata* .

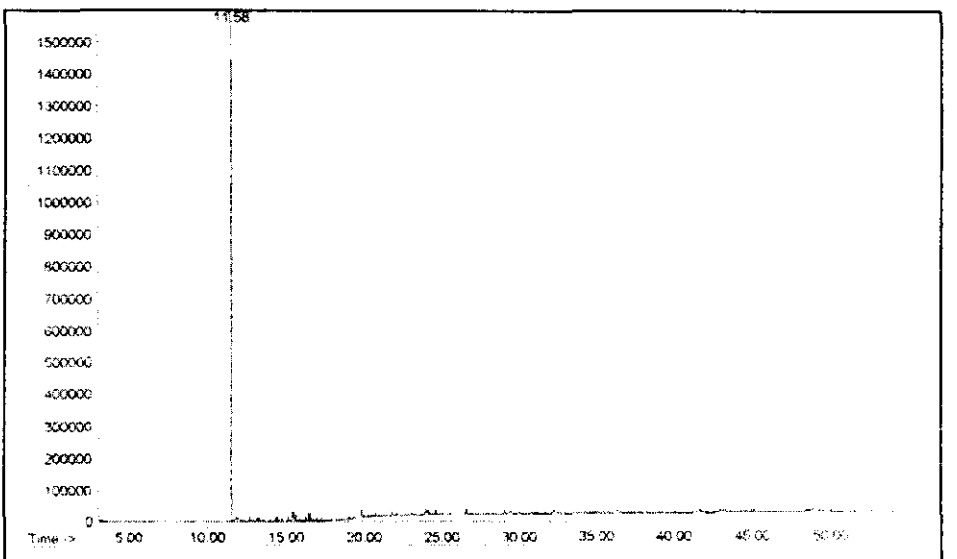

Figure 3. Gas chromatogram of methyleugenol purified from crude essential oil of M. *bracteata*.f

Melaleuca *bracteata*, is widely available plant species in Egypt with high yield of essential oil (Aboutbl *et al.*, 1991). There fore, it might present a promising agent for insect control alternative to chemical pesticides in Egypt and merit further

study as potential natural insecticide. However, although methyleugenol is a food flavoring agent and appears on the Flavor and Extract Manufacture's Generally Regarded as Safe (GRAS) list, there are several reports recorded adverse effects on yeasts, bacteria and rats (Kim *et al.*, 2003). Thus further research should be conduct on safety issues of this compound for human health before any attempts to use in any insecticidal formulation.

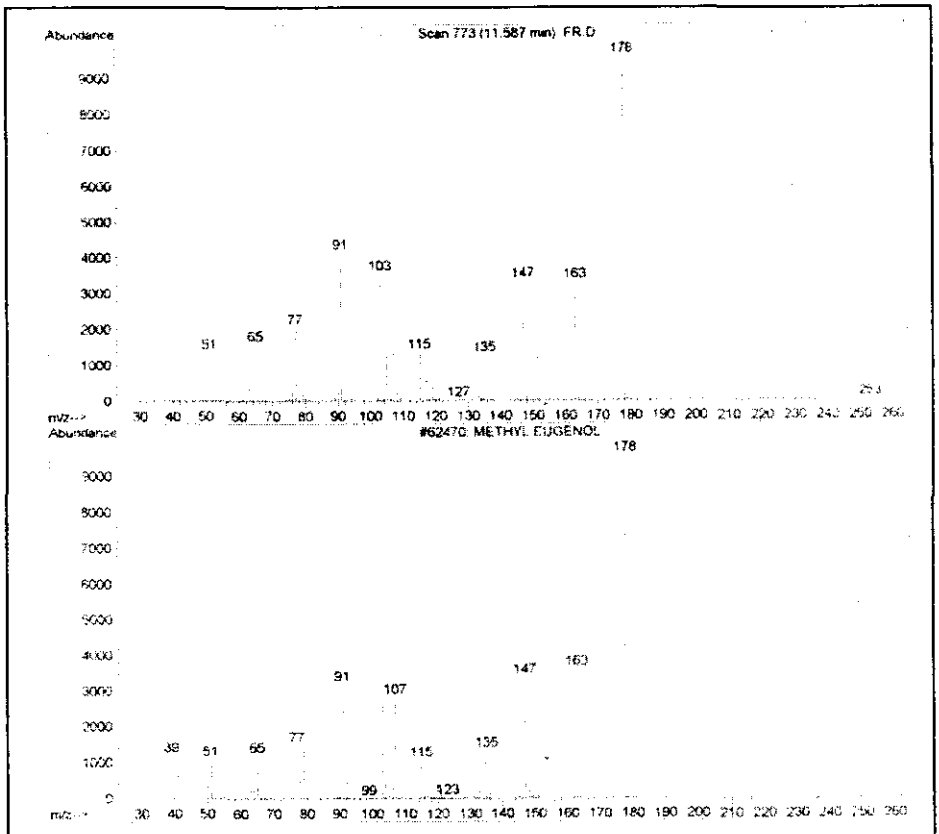

Figure 4. Mass spectrum of methyleugenol purified from crude essential oil of *M. bracteata* in comparison with authentic methyleugenol.

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