Repellent and Insecticide Activity of *Pelargonium* x *hortorum* against *Spodoptera littoralis* (Boisd.)

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Insecticide and repellent activity of an acetone extract and oil from fresh leaves of *Pelargonium* x *hortorum* (cv. Orangesonne) were evaluated against the 2nd and 4th instar larvae of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). The oil showed medium toxicity against the 2nd instar and low toxicity against the 4th instar larvae, while the extract showed high significant toxicity at all concentrations tested against the two instars. On the other hand, both oil and extract exhibited highly significant repellency against the two tested instars. Volatile constituents of the oil were also identified by GC-MS analysis.

Key words: Pelargonium x *hortorum*, *Spodoptera littoralis*, Oil

Introduction

Chemical pollution by pesticides has increased in a large scale due to their vast usage for controlling various pests and insects and to protect agricultural crops (Nathan, 2006). Consequently, an intensive effort has been made to find alternative methods of pest control. Botanical insecticides and microbial pesticides are highly effective, safe, and ecologically acceptable (Matthews, 1999). Botanical insecticides make existing integrated pest management programmes more effective and sustainable, while decreasing the reliance on synthetic insecticides (Zabel *et al*., 2002).

The importance of the plants of the genus *Pelargonium* in traditional medicine is well documented, and these form the basis of herbal medicines in areas of southern Africa (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996).

The genus *Pelargonium* comprises more than 250 species of perennial small shrubs which are limited in their geographical distribution. About 80% of these species are confined to the southern parts of Africa, while others occur in Australia, New Zealand, and in the Far East (Van der Walt and Vorster, 1983). There are few reports on the phytochemical and biological activities of *P.* x *hortorum* (syn. *P. zonale*). So the present work aimed to evaluate the repellent and insecticidal effects of an acetone extract and oil of *P.* x *hortorum* leaves on the cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) in the laboratory, and also to investigate the chemical composition of the *P.* x *hortorum* oil. The insecticidal activity and identification of *P.* x *hortorum* oil constituents appear to be the first reported in this field.

Material and Methods

Plant material

Fresh leaves of *P.* x *hortorum* (cv. Orangesonne) were collected at the gardens of the Faculty of Agriculture, Menoufia University, Shebin El-Koam, Egypt, in April 2008. Taxonomic identification of the plant was performed by botanists of the Egyptian National Botanical Institute, Dokki, Giza, Egypt according to Hay and Synge (1969).

Preparation of the crude acetone extract

Fresh leaves of *P.* x *hortorum* were ground to fine particles, and extraction was carried out according to the procedures of Warthen *et al*. (1984), with some modifications. In a 500-mL flask, 50 g of ground leaves were stirred for 3 h in 200 mL of acetone. After leaving the acetone solution

overnight, it was filtered over anhydrous sodium sulfate through Whatman No. 40 filter paper. The solid filtration residue was extracted again following the identical procedure, and the two filtrates were mixed. The solvent was removed using a rotary evaporator at (28 ± 2) °C, and a dark residue was obtained (2.93 g/50 g plant material). The acetone extract was used to prepare a stock solution. Series of concentrations (0.60, 1.25, 2.50, 5.00, and 10.00 g/100 mL) of *P.* x *hortorum* extract were made with acetone.

Extraction of the oil

Ground fresh leaves of *P.* x *hortorum* (600 g) were macerated in petroleum ether for 24 h with occasional stirring (three times). There after the mixture was filtered over anhydrous sodium sulfate through Whatman No. 40 filter paper. The resulting filtrate was evaporated under reduced pressure at (28 ± 2) °C with a recovery of oily material (2.64 g/600 g). After complete removal of the solvent, the oil was dissolved in petroleum ether to the desired concentrations for bioassay tests.

GC-MS of the oil

Identification of volatile compounds in the oil extracted from *P.* x *hortorum* leaves was carried out with an HP 5972A (Hewlett-Packard, Palo Alto, CA, USA) mass spectrometer coupled to an HP 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5MS capillary column (30 m x 0.32 mm ID, 0.25 μm film thickness). Electron impact (EI) mass spectra were obtained at 70 eV and 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 10:1. The oven temperature was programmed from 35 °C (5 min) to 80 °C at 10 °C/min and to 250 °C at 4 °C/min. Identification of the compounds present was based on computer matching against the library spectra (Wiley275 L), built up using pure substances and known compounds.

Strain of cotton leafworm S. littoralis

The *S. littoralis* strain was obtained from the Faculty of Agriculture, Cairo University, Giza, Egypt, and was reared in the laboratory of the Physiology Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, as described by El-Defrawi *et al.* (1964), under constant laboratory conditions at (25 ± 1) °C, (70 ± 5)% relative humidity, and a photoperiod of 16 h:8 h light:dark. Adults were fed with a 15% solution of honey. Filter paper was provided as an oviposition substrate, and it was replaced periodically.

Toxicity assay

The leaf-dipping technique, similar to that described by Tabashink *et al.* (1987), was used to determine the toxicity of the acetone extract and oil of *P.* x *hortorum* leaves against the 2nd and 4th instar larvae using concentrations of 0.60, 1.25, 2.50, 5.00, and 10.00 g/100 mL. Eight castor leaves were dipped for 5 s in each concentration, and then the treated leaves were left for natural air-drying and were distributed in four jars (2 leaves/jar). Ten of 2nd and 4th instar larvae were allowed to feed on treated leaves for 48 h, then, larvae were fed on untreated leaves for 24 h. Four replicates of ten larvae were fed on acetone-treated leaves for 72 h and another four replicates of 10 larvae were fed on petroleum ether-treated leaves for 72 h to serve as control. Larval mortality was recorded after 72 h. Mortality was calculated using the Abbott formula (Abbott, 1925) and subjected to Probit analysis according to Finney (1971).

Repellency assay

Repellency was assessed according to the area preference method of Obeng Ofori *et al.* (1998), with some modifications. Concentrations of 1.25, 2.50, 5.00, and 10.0 g/100 mL of acetone extract or oil were applied to one half of filter paper discs with a pipette, and the solvent (acetone for acetone extract or petroleum ether for the oil) on the other half as control. After the solvent was completely volatilized, each filter paper was placed in a culture dish of 9 cm diameter, and thirty larvae of *S. littoralis* were placed in the centre of the paper, covered with perforated lids lined with 4-mm wire mesh and banned with a rubber band. Three replications of each treatment were performed. After 24 h the numbers of larvae present on the treated (T) and the control (C) discs were counted. Percentage repellency (PR) values were computed using the formula: $PR = [(C - T)/(C + T)] \cdot 100$.

Statistical analysis

PR data were analysed using analysis of variance after arcsine transformation. Negative *PR* values were treated as zero.

Significance was calculated by ANOVA and Duncan's multiple range tests (ANOVA of arcsine square root transformed percentages). Differences between the treatments were determined by Tukey's multiple range test ($P < 0.05$) (Snedecor and Cochran, 1989).

Results and Discussion

Chemical analysis of constituents of P. x hortorum leaf oil

GC-MS analysis (Table I) showed that *P.* x *hortorum* leaf oil is composed of citronellol (4.7%), α-humulene (2.3%), citronellyl propionate (6.1%), β-bisabolene (1.8%), citronellyl butyrate (2.0%), γ-selinene (8.1%), 2,6,10,14-tetramethylpentadecan (6.1%), neophytadiene (9.3%), 1,2-benzenedicarboxylic acid bis(2-methylpropyl)-ester (4.2%), di-*n*-butyl phthalate (3.2%), heptadecane (7.5%), oleic acid methyl ester (5.1%), phytol (8.3%), di-isooctyl adipate (6.3%), pentacosane (6.1%), di(2-ethylhexyl) phthalate (7.5%), α-tocopherol (1.1%), squalene (2.3%), and cyclooctacosan (8.0%). The chemical constituents of *P.* x *hortorum* oil have been identified for the first time in the present work.

Toxicity tests

Data presented in Table II revealed that the acetone extract and the oil had significant effects on the larvae of *S. littoralis*. The acetone extract was more effective than the oil against the 2nd and 4th instar larvae at all concentrations tested. The highest toxicity rates for the acetone extract of *P.* x *hortorum* were recorded with 70% and 50% mortality of the 2nd and 4th instars, respectively, at the highest concentration of 10.00 g/100 mL, while the oil caused 45% mortality of the 2nd instar at the same concentration. The LC_{50} values of the acetone extract were 2.47 and 8.02 g/100 mL with the 2nd and 4th instar larvae, respectively, while the value for the oil was 10.93 g/100 mL with the 2nd instar and there was low toxicity with the 4th instar.

Many plant oils show a broad spectrum of activities against pest insects and plant pathogenic fungi ranging from insecticidal, antifeedant, re-

Table I. GC-MS analysis of *P.* x *hortorum* leaf oil.

Compound	Rt [min]	*m/z*	Relative content (%)
Citronellol	12.235	156, 141, 127, 101, 85, 71, 55	4.7
α-Humulene	12.409	205, 189, 170, 147, 121, 93, 77, 55	2.3
Citronellyl propionate	12.636	212, 188, 169, 141, 113, 101, 85, 57	6.1
β-Bisabolene	12.951	204, 189, 161, 119, 93, 69, 85, 53	1.8
Citronellyl butyrate	14.246	226, 211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57	2.0
γ-Selinene	15.073	204, 161, 137, 121, 105, 81, 57	8.1
2,6,10,14 Tetramethylpentadecan	15.94	268, 183, 155, 113, 85, 57	6.1
Neophytadiene	18.158	244, 226, 207, 179, 123, 95, 68, 57	9.3
1,2-Benzenedicarboxylic acid bis(2-methylpropyl) ester	18.642	279, 223, 205, 167, 135, 104, 76, 57	4.2
Di-*n*-butyl phthalate	20.194	278, 223, 205, 169, 149, 135, 121, 104, 57	3.2
Heptadecane	20.779	212, 197, 183, 169, 141,113, 85, 57	7.5
Oleic acid methyl ester	22.328	296, 253, 169, 141, 113, 85, 57	5.1
Phytol	22.512	298, 278, 196, 138, 95, 71, 55	8.3
Di-isooctyl adipate	26.573	371, 313, 259, 241, 191, 147, 129, 112, 83, 57	6.3
Pentacosane	28.471	352, 281, 267, 253, 239, 225, 197, 183, 169, 155, 141, 127, 85, 71, 57	6.1
Di(2-ethylhexyl) phthalate	29.139	390, 279, 167, 149, 132, 113, 83, 5	7.5
α-Tocopherol	34.258	337, 308, 276, 250, 125, 83, 55	1.1
Squalene	36.079	410, 367, 341, 299, 257, 231, 177, 161, 137, 121, 95, 69, 53	2.3
Cyclooctacosan	38.9	392, 364, 336, 307, 281, 264, 224, 207, 181, 153, 125, 111, 97, 83, 57	8.0

Table II. Toxic effect and LC_{50} value of the acetone extract and oil of *P.* x *hortorum* leaves against 2nd and 4th instar larvae of *S. littoralis*.

Treatment	Corrected mortality (%) ± SD			
	Acetone extract		Oil	
	2nd instar	4th instar	2nd instar	4th instar
Control	0.00	0.00	0.00	0.00
0.60 g/100 mL	32.50 ± 0.17[e]	10.00 ± 0.15[e]	7.50 ± 0.26[e]	0.00 ± 0.00[c]
1.25 g/100 mL	40.00 ± 0.00[d]	15.00 ± 0.50[d]	22.50 ± 0.00[d]	0.00 ± 0.00[c]
2.50 g/100 mL	45.00 ± 0.10[c]	32.00 ± 0.11[c]	32.50 ± 0.25[c]	0.00 ± 0.00[c]
5.00 g/100 mL	60.00 ± 0.10[b]	45.00 ± 0.23[b]	37.50 ± 0.11[b]	5.00 ± 0.40[b]
10.00 g/100 mL	70.00 ± 0.50[a]	50.00 ± 0.27[a]	45.00 ± 0.20[a]	7.50 ± 0.20[a]
LC_{50} (g/100 mL)	2.47	8.02	10.93	62.64
F value	3487.50***	3497.50***	3168.75***	1704.55***
LSD	0.81	0.94	0.82	0.27

Values in a column followed by the same letter are not significantly different (ANOVA, Duncan's multiple range test, $P < 0.05$).
SD, standard deviation.
***, highly significant effect.

pellent, oviposition-deterrent, growth-regulatory, and antivector activities (Koul *et al*., 2008). Pavela (2005) reported that twenty essential oils applied by fumigation were highly toxic to the 3rd instar of *S. littoralis* larvae. Some plants belonging to the genus *Pelargonium* had toxic effects against insects as described by many investigators. Kabera *et al.* (2011) showed that essential oils of *P. graveolens* and *Cymbopogon citratus* had a significant insecticidal activity against maize weevil (*Sitophilus zeamais*) with a maximum mortality rate of 100%. Gopalan and Madhusudhan (1968) stated that *P. graveolens* was active against *Spodoptera litura* (F.). Gopalan *et al*. (1987) found that at 420 μg/nymph, *P. graveolens*, *Vetiveria zizanioides*, and *Ocimum basilicum* caused 90, 85, and 80% mortality of the 5th instar nymphs of the pyrrhocorid *Dysdercus cingulatus*, respectively. *P. graveolens* had antifeedant properties against slugs (Warrell, 1991). It was concluded that plant essential oils (geranium oil, spikenard oil, muskmelon oil, and patchouli oil) are promising for development as potential botanical pesticides (Wang *et al*., 2000). The leaf extract of *P. citrosum* protected against mosquito bites and killed mosquitoes directly when it was placed in an enclosed area as described by Yu *et al*. (2004). The extracts of *P. hortorum* limited Colorado potato beetle feeding and development (Lamparski and Wawrzyniak, 2005). The essential oil of *P. citrosum* was found to be the best for killing larvae of *Aedes aegypti* (Zaridah *et al*., 2006). Eight oils (*P. roseum*, *Origanum vulgare*, *O. compactum*, *Mentha pulegium*, *O. basilicum*, *O. majorana*, *Thymus vulgaris*, and *P. graveolens*) were lethal in doses ranging from 10 to 20 μg/fly against the house fly, *Musca domestica* (L.) (Pavela, 2008).

The chemical analysis of *P.* x *hortorum* oil identified three phthalate derivatives [1,2-benzenedicarboxylic acid bis(2-methylpropyl) ester, di-*n*-butyl phthalate, and di(2-ethylhexyl) phthalate], which form about (14.9%) of the oil constituents. Phthalate esters are one of the most produced chemical groups in the world and are used mainly as plasticizers (Thuren, 1986). Of the phthalates, di(2-ethylhexyl) phthalate reduces the reproduction in *Daphnia magna* (Sanders *et al*., 1973). Di(2-ethylhexyl) phthalate is toxic only at high levels (>10 mg/L) (Peakall, 1975; Streufert *et al*., 1980). The toxicity of di-*n*-butyl phthalate to fish has been found to be relatively low (Mayer and Sanders, 1973). However, phthalic acid esters were found to accumulate in invertebrates to a degree similar to that found with the same species of invertebrates exposed to organochlorine insecticides (Johnson *et al*., 1971). The oil of *P.* x *hortorum* contains also about 5.1% of oleic acid methyl ester. It was reported that fatty acid methyl esters have toxic effects against insects. The potency of plant fatty acids was reported by Abdallah *et al.* (2009) against *Aphis craccivora*, and Messina and Renwick (1983) and Abdallah *et al*. (1986) against weevil species. Tare and Sharma (1991) compared the larvicidal properties of different fatty acid

constituents against *Aedes aegypti* and found that oleic acid was the most effective one. Deshpande *et al.* (1974) reported oleic acid as insecticidal component of *Nigella sativa* (Ranunculaceae), which was found to be toxic to the pulse beetle, *Callosobruchus chinensis*. Barakat *et al*. (2004) reported that the ethanol and hexane crude extracts of *Cassia fistula* (L.) reduced pupation, egg production, and hatchability, and increased sterility; the dominant constituents were the fatty acids linoleic, hexadecanoic, and octadecanoic acid, respectively. In conclusion, the medium insecticidal activity of *P.* x *hortorum* oil may be due to the presence of organic phthalates and oleic acid methyl ester.

Repellency bioassay

The repellency rates of *P.* x *hortorum* oil and acetone extract against *S. littoralis* are shown in Table III. The repellent rates of the oil were higher than those of the extract at all concentrations tested with the 4th instar larvae, while the repellency rates were approximately the same with the 2nd instar larvae. On the other hand, a higher repellency rate was recorded with the 4th instar compared with the 2nd instar larvae at all concentrations tested. Generally, repellency increased with the increase of concentration. The highest repellency (74.44%) of oil was recorded at the highest concentration tested (10.00 g/100 mL) with 4th instar larvae; also, the highest repellency rate (60%) for the extract was recorded with the same larvae and at the same concentration. There are many pervious investigations on the repellent activity of plants of the genus *Pelargonium*. Wyrostkiewicz (1987) reported that the extracts of *Pelargonium* were highly effective in repelling adults and larvae of the potato pest *Leptinotarsa decemlineata*. A leaf extract of *P. citrosum* was effective in repelling mosquitoes (Yu *et al*., 2004). Clove oil (50%) combined with geranium oil (50%) or with thyme oil (50%) prevented biting by *Anopheles albimanus* for 1.25 – 2.5 h (Barnard, 1999). Choice and no-choice tests showed that all but *Pelargonium* oil had a repellent action. Furthermore, eucalyptus strongly reduced fecundity, decreased egg hatchability, and increased neonate larval mortality (Stamopoulos, 1991).

Some of the *P.* x *hortorum* oil constituents have been reported to have repellent action on insects such as citronellol, phthalates, and phytol. The monoterpenes, α-pinene, limonene, terpinolene, citronellol, citronellal, and camphor which are common constituents of some oils, have been reported to possess high repellent properties against various insects (Perttunen, 1957; Moore, 1974). Phthalic acid esters and di-*n*-butyl phthalate are used as insect repellents (Farm Chemicals, 1971). Odalo *et al*. (2005) stated that one of the most potent repellents against *Anopheles gambiae* was phytol.

In conclusion, *P.* x *hortorum* leaf oil could be considered as new repellent reagent in pest control, while the acetone extract could be used as new insecticide in *S. littoralis* control. Purification of the active ingredient from *P.* x *hortorum* leaves is in progress.

Table III. Repellency of the acetone extract and oil of *P.* x *hortorum* leaves against 2nd and 4th instar larvae of *S. littoralis*.

Treatment	Repellency (%) ± SD			
	Acetone extract		Oil	
	2nd instar	4th instar	2nd instar	4th instar
1.25 g/100 mL	22.22 ± 0.32	32.22 ± 0.017	21.11 ± 0.09	46.66 ± 0.09
2.50 g/100 mL	36.66 ± 0.01	46.66 ± 0.03	37.77 ± 0.01	50.00 ± 0.00
5.00 g/100 mL	45.55 ± 0.10	50.00 ± 0.43	43.33 ± 0.05	62.22 ± 0.03
10.00 g/100 mL	55.55 ±0.08	60.00 ± 0.10	53.33 ± 0.04	74.44 ± 0.02
F value	16525.86***	9414.97***	16886.28***	9922.00***
LSD	0.36	0.39	0.33	0.41

Values in the columns are all significantly different from each other (ANOVA, Duncan's multiple range test, $P < 0.05$).
SD, standard deviation.
***, highly significant effect.

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