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ORIGINAL ARTICLE

Insecticide activity of Mediterranean essential oils

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Vapours of essential oils, extracted from 12 Mediterranean plants of the families Lamiaceae, Verbenaceae and Apiaceae, were assayed for insecticide activity against the aphid pests *Acyrthosiphon pisum* (Harris) and *Myzus persicae* (Sulzer). Different doses were applied, starting from 2 μl/l air and halving the dose until no activity was registered. Anise, fennel and basil essential oils resulted in high mortality, even applied at low doses. Activity was dose-dependent. The occurrence of phytotoxicity following the application of some essential oil is discussed. In spite of the well-known drawbacks owing to phytotoxicity, the application of essential oils in the control of pests on plants appears feasible.

Keywords: *Acyrthosiphon pisum* (Harris); *Myzus persicae* (Sulzer); aphids; plant allelochemicals; natural insecticide

Introduction

Research aiming at the development of natural products for pest control is increasingly important, since some synthetic pesticides are associated with environmental concerns or are being withdrawn for economic and regulatory reasons. In addition, pesticides are sometimes losing effectiveness, due to the difficulty of managing pest resistance, and the search for new synthetic compounds is increasingly time-consuming and expensive. Plant secondary metabolites play an important role in plant-insect interaction and such compounds may have insecticide, hormonal or antifeedant activity against insects (Bernays and Chapman 1994).

In the last 50 years, plant-derived insecticides have been considered mainly as lead compounds, to act as 'prototype' for the industry of pest management products (Tsao and Coats 1995) but nonetheless they present unique advantages, such as low persistence in the environment, little mammalian toxicity (Regnault-Roger 1997; Duke et al. 2003) resulting in good selectivity, and wide public acceptance, probably deriving from a well established use in food, pharmaceutical, cosmetic and perfume industry. This is especially true for essential oils, and in natural ecosystems the role of volatile secondary metabolites in biochemical interactions between aromatic plants and insects app. Plant essential oils, obtained by the distillation of aromatic plants, have traditionally been used to protect stored grain and legumes from pests and in the household to repel flying insects (Isman 2000, 2006). General perception of such products, in opposition to synthetic pesticides, is benign to the point that US Environmental Protection Agency (USEPA) states that 'No adverse effects to humans are expected from use of these substances in repellents and insecticides. Most of these oils are found in common foods, and many are approved as food flavorings by FDA' (USEPA 2001).

Essential oils show good potential in the control of insect and mite pests; they have shown effectiveness by fumigation and topical application, besides having antifeedant and repellent properties (Regnault-Roger 1997). Interestingly, they may present selectivity towards natural enemies (Ketoh et al. 2005) and bees (Ruffinengo et al. 2005), an uncommon feature among insecticides, both synthetic and natural.

The effectiveness of essential oils in the control of stored products pests and of a number of other pests is well established (Regnault-Roger 1997; Isman 2000; Kostyukovsky et al. 2002a). Their usefulness in the control of aphids has been reported for repellency (Hori 1998) and feeding deterrent activity (Gutierrez et al. 1997; Isman 2000). Essential oils of *Cuminum cyminum* L., *Pimpinella anisum* L., *Origanum syriacum* L. var. *bevanii* (Holmes) J.H. Ietswaart

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showed aphicidal activity (Tunì and Sahinkaya 1998) and *Tagetes* oil was effective in reducing aphid fertility (Tomova et al. 2005). The vapour toxicity of several essential oils on the rose-grain aphid *Metopolophium dirhodum* Wack. has been reported (Tunì and Sahinkaya 1998) and the efficacy of basil (*Ocimum* spp.) oils against garden pests has recently been rewieved (Quarles 1999).

The aim of our study was to assess the insecticide activity of the essential oils obtained by twelve Mediterranean plants against two aphids species, *Acyrthosiphon pisum* (Harris) and *Myzus persicae* (Sulzer) (Hemiptera Aphididae), important pests of many cultivated plants, and also to verify the feasibility of their application to cultivated plants, owing to the well-known phytotoxicity issue. The pea aphid, *A. pisum*, is a phloem-feeding insect that colonizes leguminous crops, where it produces a direct damage in terms of nutritive subtraction and injection of toxic saliva, besides being responsible for the transmission of more than 30 viral diseases (Blackman and Eastop 2000). The green peach aphid, or peach-potato aphid, *M. persicae,* is a major pest worldwide, having an extremely wide host range in over 40 different plant families, where not only it produces severe direct damage, but also is considered the most important aphid virus vector, being able to transmit over 100 plant viruses (Blackman and Eastop 2000). *M. persicae* resistance to many synthetic insecticides is widely reported, and its mechanisms are reviewed in Devonshire et al. (1998).

Experimental methods

Plant material

Hyssopus officinalis L. (hyssop), *Lavandula angustifolia* Miller (lavender), *Majorana hortensis* L. (marjoram), *Melissa officinalis* L. (lemon balm), *Ocimum basilicum* L. (basil), *Origanum vulgare* L. (oregano), *Salvia officinalis* L. (sage), *Thymus vulgaris* L. (thyme) (Lamiaceae), *Carum carvi* L. (caraway), *Foeniculum vulgare* Miller (fennel), *Pimpinella anisum* L. (anise) (Apiaceae), *Verbena officinalis* L. (vervain) (Verbenaceae) were grown at the Garden of medicinal plants in the Campus of Salerno State University. Aerial parts of Lamiaceae and Verbenaceae species were collected at full flowering stage, in July–August 2004, and fruits of the three Apiaceae species were collected at ripeness in August 2004. A voucher specimen of each plant was deposited in the herbarium of the Medical Botany Chair, Faculty of Pharmacy, Salerno University.

Oil isolation

Five-hundred grams of freshly picked aerial parts of each lamiaceous and verbenaceous species were cut into small pieces and then submitted to hydrodistillation for 3 h, according to the standard procedure reported in *European Pharmacopoeia* (1975). Five-hundred grams of fruits of fennel, caraway and anise were submitted to the same procedure. The oil contents, on a fresh weight basis, were as follows: *H. officinalis* 0.41%; *L. angustifolia* 0.49%; *M. hortensis* 0.26%; *M. officinalis* 0.25%; *O. basilicum* 0.42%; *O. vulgare* 0.21%; *S. officinalis* 0.46%; *T. vulgaris* 0.26%; *C. carvi* 2.8%; *F. vulgare* 2.3%; *P. anisum* 1.8%; *V. officinalis* 0.39%.

Bioassay

A. pisum (Harris) and *M. persicae* (Sulzer) (Hemiptera, Aphididae) were obtained from cultures maintained at Dipartimento di Entomologia e Zoologia Agraria "Filippo Silvestri", Faculty of Agriculture, Naples University, and were reared on broad bean (*Vicia faba* L.) and Cayenne pepper (*Capsicum annuum* L. var. *frutescens*) potted plants, respectively. Rearing conditions were $20 \pm 1°C$, $70 \pm 10\%$ RH and a photoperiod L18:D6. *A. pisum* and *M. persicae* adult females were transferred, by a fine brush, on the leaves of broad bean and Cayenne pepper plants, respectively, and allowed to lay nymphs in order to obtain the aphids for the assay. After 3 days the adults were removed, the nymphs on single plants were counted, and plants with known number of aphids were placed in air-proof plastic boxes (volume 13.50 l). The essential oils were applied with an automatic pipette on a blotting paper strip (6×3 cm) placed on the floor of the box.

The amounts of essential oil applied were 3.37 μl (lower dose), 6.75, 13.50 or 27 μl (higher dose) in each box, corresponding to a concentration of 0.25, 0.50, 1.00 and 2.00 μl/l air. A control air-proof plastic box without essential oil was equally set up. The exposure period to the vapours of essential oils was 24 h. After the treatment, plants with aphids were removed from the boxes, and mortality was assessed. Then the plants were placed in cages, and the aphids reared for 3 more days, to determine any delayed effect, and the final mortality was registered. If aphids did not react when prodded with a fine brush, they were considered dead.

Each dose was administered in two replicates. Tests were carried out under the same conditions reported for the rearing. Mortality data were ordered in homogeneous sets, and the *G*-test of independence was applied with William's correction (Sokal and Rohlf 1995).

The resulting G value for the whole set of data and for the single subsets was compared with a chi-square distribution table to test the goodness of fit (Rohlf and Sokal 1995). During the assays, the occurrence of phytotoxicity following the application of essential oils was registered and the symptoms shown were ranked according to severity.

Results

Insecticidal activity

All the essential oils tested were active towards both aphid species (see Tables 1 and 2). In the range of concentrations applied, mortality was dose-dependent. All the essential oils tested caused 100% or so mortality on *A. pisum* at the maximum dose used, while a dose of 0.5 µl/l air was required to obtain over 90% mortality for essential oils of anise, basil and fennel. The essential oils of fennel and anise at the lowest tested dose (0.25 µl/l air) caused 95 and 87.28% mortality, respectively (Table 1). Essential oils of fennel, lavender, lemon balm, oregano and thyme caused 100% mortality on *M. persicae* at the maximum dose used. Administration of fennel and lemon balm oil at 0.5 µl/l air still produced high mortality (Table 2). The less active oil, towards both aphids, was that from sage.

Phytotoxicity

At the maximum dose used, the essential oils produced different degrees of phytotoxicity (from none to severe) on broad bean and Cayenne pepper, as shown in Tables 3 and 4.

In the system pepper/*M. persicae* the oils of fennel, basil, and anise showed remarkable insecticidal activity without any side effect in terms of phytotoxicity, even at the highest dose. Broad bean plants resulted more sensitive to the highest doses of essential oils, although thyme oil was very noxious only to Cayenne pepper. Notably *A. pisum*, living on the more sensitive plant species, is more sensitive to the treatment, while pepper, showing a hardier response, hosts a more resistant aphid. This means that, even if broad beans are sensitive, a lower dose is required to obtain adequate aphid control, while in the system pepper/*M. persicae* higher doses can be tolerated by the plants. Furthermore, on pepper, after the application of lemon balm, caraway and vervain oil, phytotoxicity could be a delayed outcome, with plants appearing in good shape after the exposition to oil vapours in the air-proof box, but affected after growing in clean air for 3 more days (Table 4).

Discussion and conclusions

Essential oils of many plants contain a number of bioactive compounds which may exert regulatory or inhibitory influence on insect life processes, such as growth and development, reproduction, orientation. Among the compounds present in essential oils, monoterpenoids are usually the main components (Tsao and Coats 1995) and consequently are regarded as candidates for insecticidal activity. These natural

Table 1. Activity of essential oil vapours against *Acyrthosiphon pisum*.

	Dose							
	2 µl/l*		1 µl/l**		0.5 µl/l***		0.25 µl/l****	
Essential oil	*n*	%Mortality	*n*	% Mortality	*n*	% Mortality	*n*	% Mortality
Anise	28	100a	17	100a	32	100a	39	87.28a
Basil	28	100a	19	100a	32	90.62a	23	52.17b
Caraway	25	84a	20	75a	31	32.25bc	36	2.78d
Fennel	33	100a	19	100a	34	100a	20	95a
Hyssop	31	96.77a	23	69bc	24	45.83bc	37	21.62c
Lavender	31	100a	25	44c	21	23.81c	38	0d
Lemon balm	30	96.67a	38	86.84b	31	25.81bc	22	13.63d
Marjoram	30	96.67a	27	77.78bc	24	37.50bc	29	0d
Oregano	33	100a	19	100a	28	64.28b	35	45.71b
Sage	30	100a	20	55c	22	4.54d	–	–
Thyme	30	100a	20	100a	26	80.77a	32	12.50cd
Vervain	29	100a	35	88.57ab	22	31.82bc	20	0d
Control	57	3.51b	25	8d	57	3.51d	57	3.51d

n = number of aphids; *G = 275.0542, f.d. = 12, $p \gg 0.01$; **G = 619.5575, f.d. = 12, $p \gg 0.01$; ***G = 230.4288, f.d. = 12, $p \gg 0.01$; ****G = 232.4508, f.d. = 11; $p \gg 0.01$; same letter in a column indicates a homogeneous group.

Table 2. Activity of essential oil vapours against *Myzus persicae*.

	Dose							
	2 µl/l*		1 µl/l**		0.5 µl/l***		0.25 µl/l****	
Essential oil	*n*	% Mortality	*n*	% Mortality	*n*	% Mortality	*n*	% Mortality
Anise	20	95a	20	80ab	33	15.15c	15	0a
Basil	26	96.15a	19	94.74a	38	23.68c	20	10a
Caraway	21	85.71a	16	50b	36	0d	–	–
Fennel	19	100a	15	66.67b	39	64.10b	20	5a
Hyssop	19	47.47b	16	18.75c	29	0d	–	–
Lavender	28	100a	19	5.26c	40	7.50d	–	–
Lemon balm	15	100a	16	100a	50	100a	22	13.63a
Marjoram	20	85a	18	16.66c	48	0d	–	–
Oregano	26	100a	30	70b	31	35.48c	24	0a
Sage	20	45b	16	12.50c	39	0d	–	–
Thyme	19	100a	14	14.30c	43	2.32d	–	–
Vervain	28	92.86a	24	54.17b	45	13.33c	20	0a
Control	36	0c	20	5c	52	0d	20	0a

n = number of aphids; *G = 190.9126, f.d. = 12, $p \gg 0.01$; **G = 122.3968, f.d. = 12, $p \gg 0.01$; ***G = 300.7239, f.d. = 12, $p \gg 0.01$; ****G = 9.2762, f.d. = 6; same letter in a column indicates a homogeneous group.

compounds have been proposed as lead compounds for the development of safe, effective, and fully biodegradable insecticides (Grodnitzky and Coats 2002).

In general, *M. persicae* was more resistant than *A. pisum* to treatments. This is not surprising, as *M. persicae* presents a wide host range, and this is associated to the ability to overcome several types of plant defences. In fact, essential oils, as many other plant secondary metabolites, are believed to be synthesized primarily as a chemical defence against pests (Tsao and Coats 1995) and generalist phytophagous insects have been shown to possess higher cytochrome P450 monooxygenases activity in their guts, thus being able to detoxify more efficiently plant toxins (Yu 1987).

Interestingly, the most effective essential oils, presenting very high activity even at the lowest dose administered on *A. pisum*, proved to be those from anise and fennel, whose main component is *cis*-anethole, respectively 97.1% and 77.3% (data not shown). Fennel oil was also active against *M. persicae*, at a dose as low as 0.5 µl/l. Particularly, fennel oil, besides showing remarkable insecticide

Table 3. Phytotoxicity of essential oil vapours applied to broad bean plants.

	Phytotoxicity			
Essential oil	2 µl/l	1 µl/l	0.5 µl/l	0.25 µl/l
Anise	+/–	+/–	–	–
Basil	+/–	–	–	–
Caraway	–	–	–	–
Fennel	–	–	–	–
Hyssop	–	–	–	–
Lavender	+/–	+/–	+/–	–
Lemon balm	+	+	+/–	–
Marjoram	+/–	–	–	–
Oregano	++	+	+/–	+/–
Sage	–	–	–	–
Thyme	+/–	+/–	+/–	–
Vervain	+	+	+	–
Control	–	–	–	–

Symptoms of phytotoxicity: – = absent; +/– = black spots on leaves; + = black leaves and streaks on stalk; ++ = dead plant.

Table 4. Phytotoxicity of essential oil vapours applied to Cayenne pepper plants.

	Phytotoxicity			
Essential oil	2 µl/l	1 µl/l	0.5 µl/l	0.25 µl/l
Anise	–	–	–	–
Basil	–	–	–	–
Caraway	+*	–	–	–
Fennel	–	–	–	–
Hyssop	–	–	–	–
Lavender	++	–	–	–
Lemon balm	++	++*	++*	–
Marjoram	–	–	–	–
Oregano	++	–	–	–
Sage	–	–	–	–
Thyme	++	–	–	–
Vervain	++	++*	–	–
Control	–	–	–	–

Symptoms of phytotoxicity: – = absent; + = plant partly desiccated; ++ = plant desiccated; * = the symptoms were evident only three days after the treatment.

activity, especially towards *A. pisum*, did not produce any phytotoxicity, even on the more susceptible broad bean plants. Anethole, a compound usually present in essential oils extracted from the fruits of anise and fennel (Santos et al. 1998; Miraldi 1999), has been reported to be effective as a fumigant against *Blattella germanica* (Blattodea Blattellidae) (Chang and Ahn 2002) and active against a number of insect pests, like *Spodoptera litura* and *Trichoplusia ni* (Lepidoptera Noctuidae), mosquitoes and beetles and moths of stored products (Isman 2000; Kim et al. 2003; Akhtar and Isman 2004). Anise oil has shown activity against the spider mite *Tetranychus cinnabarinus* (Acarina Tetranychidae) and the aphid *Aphis gossypii* (Hemiptera Aphididae), as also the oils of *Cuminum cyminum* and *Origanum syriacum* var. *bevanii* (Tunì and Sahinkaya 1998). The essential oil from the third Apiacea assayed, caraway, showed insecticide activity, but not comparable to the two anethol-rich species, expecially at the lowest doses, supporting further the role of this compound in aphid mortality. Fennel seeds essential oils are used in East Asia as pesticides (Kim and Ahn 2001) and have been reported for their acaricidal activity, but in this case the monoterpene (+)-carvone is the main active component (Lee et al. 2006).

Towards *M. persicae*, we obtained 100% mortality following lemon balm oil application at the dose of 0.5 μl/l, thus even more effective than the oils from Apiaceae, and in this case the insecticide activity is most likely related to monoterpenes content (92.4%, data not shown). On the other hand, the reported plant damage renders lemon balm unfeasible in plant protection.

Also, we report against both aphid species an interesting activity of basil and oregano essential oils, whose main components are monoterpenes.

Several monoterpenoids have been shown to exert insecticidal activity, and a number of compounds are currently used commercially as pesticides or repellents (see Isman 2006 and references therein). Of the other essential oils assayed, basil, caraway, lavender, marjoram, oregano, sage, and thyme have been reported for acaricidal activity (Kim et al. 2004).

Several hypotheses have been proposed for the mode of action of monoterpenes. The neurotoxicity of a number of essential oil monoterpenes has been assayed against different insects (Coats et al. 1991) and some monoterpenes have been identified as competitive inhibitors of acetylcholinesterase (Miyazawa et al. 1997; Regnault-Roger 1997; Isman 2000) but such an activity *in vivo* requires high concentrations, indicating that acetylcholinesterase is not the main site of action (Kostyukovsky et al. 2002b).

Recent papers (Enam 2001; Kostyukovsky et al. 2002b) have shown that for certain compounds of essential oils an important site of action is the octopaminergic system, very interestingly, as insect octopamine receptor subtype does not conform to vertebrate receptors, thus representing a target with little non-target effects. Among monoterpenoids, pulegone has been reported as an inducer of cytochrome P_{450} monooxygenase enzymes (Grundy and Still 1985) and linalool was identified as an inhibitor of acetylcholinesterase (Ryan and Byrne 1988). Moreover, some halogenated monoterpenoids have been shown to have lindane-like GABA-antagonist effect (Brattsten 1983) and thymol has been demonstrated to interfere with GABA-gated chloride channels in both human and *Drosophila melanogaster* receptors (Priestley et al. 2003). Another proposed mode of action is an interference with pheromone production, thus affecting behaviour and reproduction, and interfering with the metabolism of juvenile hormones and ecdysones (Tsao and Coats 1995) regulating growth and development.

In conclusion, our data show that vapours of the essential oils tested are active against *M. persicae* and *A. pisum*. The insecticide activity here described is of great interest in this moment, when many synthetic pesticides are being dismissed, and new principles capable to conjugate activity and sustainability are strongly needed. Interestingly, in this work we show remarkable activity against *M. persicae*, not only a major pest on a large number of crops, but also an organism so well-known for its ability to develop insensitivity against synthetic insecticides of any class that it is regarded as a model organism to study the onset of insecticide resistance (Anthony et al. 1998; Devonshire et al. 1998).

In opposition to what is commonly believed, we wish to point out that the exploitation of the fumigating activity of essential oils is not an option confined just to the management of pests of stored product, but is feasible also on crops. Essential oils, once the balance between insecticide activity and phytotoxicity is established for the different pests and plant species, can be very useful natural insecticides in closed environments (greenhouses, storehouses, closed spaces), also in consideration of reduced non-target toxicity. The difficulties connected to their application to plants can be bypassed with a careful test of the lowest dose established as efficient but not phytotoxic.

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