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Essential Oils of Umbelliferae (Apiaceae) Family Taxa as Emerging Potent Agents for Mosquito Control

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1. Introduction

Warm-humid areas around the globe constitute the cradle of humanity, providing their inhabitants the most favorable environments for living and agricultural production. In this "Garden of Eden", which spreads within the globe's temperate and tropical zones, is also thriving an annoying but dangerous daemon, the mosquito. This little devil constitutes the main vector of malaria and human encephalitis, both infectious diseases that account as major threats of public health (Becker et al., 2003). Recently, these threats have been spread to a broader geographical area, as a consequence of their vectors (*Aedes* sp., *Anopheles* sp. and *Culex* sp.) introduction into metropolitan areas of northern hemisphere, such as Chicago (Tedesco et al., 2010), New York (Peterson et al., 2006) and Paris (Delaunay et al., 2009). Since mosquito breeding habitats in both urban and rural areas are man-made (Imbahale et al., 2010), there are several restrictions limiting the efforts towards the development of an integrated vector management system. Todate, the history of evolutions of malaria vector interventions is directly connected with the mosquito control tools development, concerning either environmental modifications/manipulations or their chemical and/or biological control (Kilama, 2009).

In respect the chemical control, a significant milestone was the DichloroDiphenyl-Trichloroethane (DDT) synthesis by Zeidler in 1874. The DDT success was followed by the fast introduction of numerous chlorinated hydrocarbons, which were used in massive amounts for the control of mosquito-borne diseases (Ray, 2010). Despite their efficiency, the use of organochlorines had severe environmental impacts which were publicly (and dramatically) addressed by Carson (1962) in Silent Spring, initiating the development of insecticide resistant mosquito populations. These undesirable characteristics, in combination with concerns on public health risks, derived from the organochlorine residues detected in humans and animals, led to their ban in early 70's. Thus, they were replaced by less persistent chemicals, such as organophosphates, pyrethroids and avermectin derivates,

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substitutes that also display the major disadvantage of resistance development (Alves et al., 2010; Daaboub et al., 2008; Lima et al., 2011).

Recent research trend on mosquito chemical control mainly focuses on currently used compounds, aiming to enhance their potency and circumvent the problems connected with their application. In this respect, the so far developed pyrazole derivatives are quite efficient exhibiting however adverse environmental effects (Stevens et al., 2011), while the corresponding pyrroles display the desirable efficiency (Raghavendra et al., 2011) but adequate research on their environmental side effects is still underway. Amides, such as methazolamide and acetazolamide, were also evaluated as potent mosquito larvicides but were found to display significant bioaccumulation properties (Del Pilar Corena et al., 2006) which discourage their broad use. Finally, various novel pyrimido-quinolione molecules have been developed and assessed as highly toxic for other organisms (Rajanarendar et al., 2010). Ray (2010) recognized that the insecticide treated nets, in connection with the long lasting insecticidal nets, have resurrected the chemical control of malaria's mosquito vector. This may be rationalized considering that their targeted application resolves the problems connected with the environmental impacts of chemical control agents since limit their expansion, availability and environmental penetration.

Despite the numerous efforts and progress achieved, the efficacy of insecticidal nets in malaria prevention still constitutes a hot issue, since depends strongly upon a plethora of additional factors (Killeen & Smith, 2007). In particular, despite efforts (Pennetier et al., 2010) to overcome the recognized for longtime resistance development issues of insecticidal nets, todate these problems have not been resolved (Yadouleton et al., 2011). An additional drawback derives by the combined impact of herbicide application that promotes the cross-resistance to mosquito populations (Boyer et al., 2006; Riaz et al., 2009).

The corresponding biological control has dictated the development of novel-alternative mosquito control tools, including the sterile males technique (Patersson et al., 1968), the genetically modified mosquitoes (Gu et al., 2011; Lavialle-Defaix et al., 2011), the entomopathogenic fungi (Van Breukelen et al., 2011; Kanzok & Jacobs-Lorena, 2006) and bacteria. Among the tools developed the bacterial pathogens application is considered as the most prominent intervention, displaying species selective insecticidal ability (Hayes et al., 2011) which is considered as an efficient means for mosquito control without harmful impacts for the environment (Caquet et al., 2011). Major thresholds limiting the wider application of this technique are related with the induced pathogen introduction among the natural mosquito populations (Hancock et al., 2011) and the threats connected with bioaccumulation and resistance development (Tilquin et al., 2008). In general, the biological control tools are still under development, presenting todate a low degree of maturity for large-scale interventions.

Temephos was considered as one of the most potent-safe insecticides. Its recent exclusion from Annex I of the Directive 98/8/EC resulted in the discontinuation of its application in mosquito control programs by the European, emerging the development and use of new-safer insecticides. Thus, relative research directed towards the discovery-development of novel molecules, capable to control the mosquito populations without exhibiting the disadvantages of synthetic pesticides. In this respect, the plant originated natural compounds constitute a large deposit of such molecules, inherently allowing the retrieval of

various commercially successful molecules like pyrethrins. Todate, the search for novel, potent and safer pesticides from this deposit has already provided several candidates, either as pure compounds and/or their extracts. Specifically, various organic acids such as lactic and orthophosporic acids (Chakraborty et al., 2010), alkaloids (Talontsi et al., 2011) and plant proteins (Chowdhury et al., 2008) have been identified as efficient mosquito control agents. Furthermore, several plants were used as the maternal material to produce bio-products which were applied against mosquitoes with hopeful results (Shaalan et al., 2005; Sukumar et al., 1991). On the other hand, the plant derived Essential Oils (EOs) constitute a special category of natural products that exhibit the major advantage -for the mosquito control endeavor- of exhibiting an insect oriented mode of action with low penetrability to the ecosystems that does not affect larger animals. In addition, the natural diversity of their constituents addresses effectively the problem of resistance development (Isman, 2000).

2. Literature review

2.1 Umbelliferae (Apiaceae) family: A source of potent natural agrochemicals

Many EOs originated from diverse plant families have been considered and studied as potential sources of natural agrochemicals. In this respect, previous research results on Umbeliferae (Apiaceae) family plant materials revealed the significant acaricidal activities of butylidenepthalides isolated from *Angelica acutiloba* Kitagawa var. *sugiyame* Hikino (Kwon & Ahn, 2002) and the similar activity of the EO of *Foeniculum vulgare,* attributed to the presence of *p*-anisaldehyde and (+,−)-fenchone in the EO (Lee, 2004). These EOs were practically inactive in fumigant toxicity tests against *Lycoriella mali* though they are known to contain the active monoterpenes *a*-pinene and *β*-pinene (Choi et al., 2006), which are common constituents of many Umbelliferae EOs. Methanolic extracts of *Angelica dahurica*, *Cnidium officinale*, and *Foeniculum vulgare* were also tested against the Coleoptera *Lasioderma sericorne*, *Sitophilus oryzae* and *Callosobruchus chinensis* exhibiting a moderate activity only the second extract (Kim et al., 2003a; Kim et al., 2003b). Other EOs of this family screened as inactive against coleoptera were originated from the species *Anethum graveolens* L., *Apium graveolens* Houtt., *Coriandrum sativum* L., *Cuminum cyminum* L. and *Petroselinum sativum* L. (Regnault-Roger & Hamraoui, 1994; Papachristos & Stamopoulos, 2002). On the contrary, the EOs of *Pimpinella anisum* L. and *Cuminum cyminum* L. displayed excellent ovicidal and insecticidal activities against the *Tribolium confusum* du Val and the *Ephestia kuehniella* Zeller (Tunc et al., 2000). In addition, the aqueous extract of *Pimpinella anisum* exhibited good repellent effect against the adults of sweet potato whitefly *Bemisia tabaci* (Ateyyat et al., 2007).

These rather controversial results are not connected with the impressive activities that Umbelliferae EOs were found to exhibit against the Diptera, with the EO of *Ammi visnaga* displaying -among 19 EOs- the most potent ovicidal activity against *Mayetiola destructor* (Lamiri et al., 2001). In addition, tests against *Droshophila melanogaster* of furanocoumarins and pthalides isolated from *Angelica acutiloba* Kitagawa var. *sugiyame* Hikino revealed the hypothesis that the insecticidal properties of the plant extracts are connected with the acetylcholinesterase inhibition (Miyazawa et al., 2004). Finally, alkylpthalides originated from *Cnidium officinale* Makino were tested as extremely effective against *Droshophila melanogaster* (Tsukamoto et al., 2005).

2.2 Umbelliferae (Apiaceae) family: A strong focal point for mosquito control

Table 1 summarizes the test results against various mosquito species reported for all extracts and EOs derived from plants belonging to the Umbeliferae family. Same table also contains the test results of fourteen EOs, which appear herein for the first time. Results indicate that the organic phase of the *Cryoptaenia canadensis* extract is the most active against fourth instars of *Culex pipiens*, leading to the isolation –from the extract- of the acetylated very toxic (LC_{50} values lower than 10 mgl^{-1}) molecules of falcarinol and falcarindiol (Eckenbach et al., 1999). The larvicidal properties of hexane soluble fraction of *Apium graveolens* seeds -a plant with pleasant aroma- and three isolated compounds (sedanolide, senkyunolide-N, senkyunolide-J) against *Aedes aegypti* mosquitoes highlighted sedanolide as very active (100% mortality at 50 mgl^{-1}, Momin & Nair, 2001). As a consequence, a gel containing 5% of the *Apium graveolens* hexane extract was developed, providing full protection to volunteers from mosquito bites for two hours (Tuetun et al., 2009), while the ethanolic formulations from the same plant also provided protection against *Aedes aegypti*. Another formulation containing the aforementioned hexane extract and 5% vanillin showed strong repellent activities against different mosquito species (Tuetun et al., 2005, see also **Table 1** for details). The crude seed extract had no adverse effects on human volunteers skins when tested for several anti-mosquito properties (Choochote et al., 2004). This plant's EO exhibits potent larvicidal activity against two laboratory-reared mosquito species, the malaria vector *Anopheles dirus* and the vector of dengue *Aedes aegypti* (Pitasawat et al., 2007).

Species	Part used	Mosquito species	Bioactivity	Reference
Ammi visnaga	Seeds	*Culex quinquefasciatus*	Larvicidal	Pavela, 2008
Anethum graveolens	(not mentioned)	*Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus*	Larvicidal	Amer and Mehlhorn, 2006
Anethum graveolens	Leaves, twigs	*Aedes aegypti*	Larvicidal, effects on growth and development	Promsiri et al., 2006
Angelica archangelica	Fruits	*Culex quinquefasciatus*	Larvicidal	Pavela, 2009
Angelica sylvestris	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Apium graveolens	Seeds	*Aedes aegypti*	Larvicidal	Momin & Nair, 2001
Apium graveolens	Seeds	*Aedes aegypti*	Larvicidal, adulticidal, repellent	Choochote, 2004
Apium graveolens	Seeds	*Aedes aegypti, Aedes gardnerii, Aedes lineatopennis, Anopheles barbirostris, Armigeres subalbatus, Culex tritaeniorhynchus, Culex gelicus, Culex vishnui* group, *Mansonia uniformis*	Repellent	Tuetun et al., 2005

Species	Part used	Mosquito species	Bioactivity	Reference
Apium graveolens	Seeds	*Aedes aegypti, Anopheles ditrus*	Larvicidal	Pitasawat et al., 2007
Apium graveolens	Seeds	*Aedes, Anopheles, Armigeres, Culex, Mansonia*	Repellent	Tuetun et al., 2009
Athamanta densa	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Bupleurum fruticosum	Aerial parts	*Culex pipiens*	Larvicidal	Evergetis et al., 2009
Carum carvi	Fruits	*Aedes aegypti, Culex quinquefasciatus*	Larvicidal	Lee, 2006
Carum carvi	Seeds	*Aedes aegypti, Anopheles ditrus*	Larvicidal	Pitasawat et al., 2007
Carum ptroselinum	(not mentioned)	*Culex pipiens*	Larvicidal	Khater and Shalaby, 2008
Chaerophyllum heldreichii	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Conium divaricatum	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Conopodium capillifolium	Aerial parts	*Culex pipiens*	Larvicidal	Evergetis et al., 2009
Coriander sativum	Seeds	*Ochlerotatus caspius*	Larvicidal	Knio et al., 2008
Cryptotaenia canadensis	Fresh foliage, root, fruits	*Culex pipiens*	Larvicidal	Eckenbach et al., 1999
Daucus carota	Roots	*Aedes aegypti, Culex quinquefasciatus*	Larvicidal	Lee, 2006
Eleoselinum asclepium	Aerial parts	*Culex pipiens*	Larvicidal	Evergetis et al., 2009
Ferula assa-foetida	Stems	*Culex quinquefasciatus*	Larvicidal	Pavela, 2009
Ferula galbaniflua	(not mentioned)	*Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus*	Larvicidal	Amer and Mehlhorn, 2006
Ferula lancerottensis	Stems	*Culex quinquefasciatus*	Larvicidal	Pavela, 2008
Ferulago nodosa	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Foeniculum vulgare	Fruits	*Aedes aegypti*	Repellent	Kim et al., 2002
Foeniculum vulgare	(not mentioned)	*Aedes aegypti*	Larvicidal	Orozco & Lentz, 2005
Foeniculum vulgare	Flowers	*Culex pipiens*	Larvicidal, repellent	Trabousli et al., 2005
Foeniculum vulgare	Fruits	*Aedes aegypti, Anopheles ditrus*	Larvicidal	Pitasawat et al., 2007
Foeniculum vulgare	Stems, inflorencenc es, leaves	*Culex pipiens*	Larvicidal	Manolakou et al., 2009

Species	Part used	Mosquito species	Bioactivity	Reference
Foeniculum vulgare	Leaves	*Aedes albopictus*	Larvicidal	Conti et al., 2010
Heracleum sphondylium	Aerial parts	*Culex pipiens*	Larvicidal	Evergetis et al., 2009
Imperatoria ostruthium	Roots	*Culex quinquefasciatus*	Larvicidal	Pavela, 2009
Laserpitium pseudomeum	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Oenanthe pimpinelloides	Aerial parts	*Culex pipiens*	Larvicidal	Evergetis et al., 2009
Petroselimum crispum	Seeds	*Ochlerotatus caspius*	Larvicidal	Knio et al., 2008
Peucedanum neumayeri	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Peucedanum officinale	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Pimpinella anisum	Seeds	*Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus*	Larvicidal, adulticidal, ovicidal, oviposition-deterrent, repellent	Prajapati et al., 2005
Pimpinella anisum	Seeds	*Ochlerotatus caspius*	Larvicidal	Knio et al., 2008
Pimpinella peregrina	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Pimpinella rigidula	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Pimpinella tragium ssp tragium	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Scaligeria cretica	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Seseli montanum	Aerial parts	*Culex pipiens*	Larvicidal	Evergetis et al.,2009
Seseli pallasii	Stems	*Culex quinquefasciatus*	Larvicidal	Pavela, 2009
Seseli parnassicum	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Seseli tortuosum	Stems	*Culex quinquefasciatus*	Larvicidal	Pavela, 2008
Smyrnium rotundifolium	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Thamnosciadium junceum	Aerial parts	*Culex pipiens*	Larvicidal	(present study)
Trachyspermum ammi	Seeds	*Anopheles stephensi*	Larvicidal, oviposition-deterrent, vapor toxicity, repellent	Pandey et al., 2009

Table 1. Reported phytochemicals derived from plants belonging to Apiaceae family against various mosquito species.

Another EO found to possess potent larvicidal, oviposition-deterrent, vapor toxicity and repellent activities against *Aedes aegypti* was isolated from ajowan (*Tachyspermum ammi*, Pandey et al. 2009). *Anethum graveolens* extract exhibited larval toxicity with LC_{50} values from 27 to 20 mgl^{-1} (for 24 and 48 hours exposures respectively), while on growth survival and prolongation tests of the various instar larvae of *Aedes aegypti,* the second instar larvae was determined as the more susceptible. The lowest concentration of crude extracts of *Anethum graveolens* used (caused more than 50% larval mortality) was not toxic to guppy fish (*Poecilia reticulata*) at concentrations of 12.5 mgl^{-1} (Promsiri et al., 2006).

Among all EOs tested for mosquito control, the most potent was derived from *Foeniculum vulgare*, which caused the highest mortality against *Aedes albopictus* (Conti et al., 2010) and moderate against *Anopheles dirus* and *Aedes aegypti* (Pitasawat et al., 2007). Main component of this EO is methyl chavicol (more than 43%), while its methanolic extract (*trans*-anethole chemotype) was moderately active against *Aedes aegypti*, the yellow fever mosquito (Orozco & Lentz, 2005). The hexane fraction from its fruit-derived parts showed 99% repellency against *Aedes aegypti*, while the other fractions (chloroform, ethyl acetate and water: 37, 37 and 17% respectively) were practically inactive (Kim et al., 2002). Repellency and toxicity were also studied against *Culex pipiens* (Trabousli et al., 2005), indicating that the EO of *Foeniculum vulgare* was the most effective, while the repellency assays revealed protection time for almost one hour when applied at concentration of 3%.

Pimpinella anisum L. EO proved to possess equally potent larvicidal and ovicidal activities against *Anopheles stephensi*, *Aedes aegypti, Culex quinquefasciatus* and only larvicidal against *Ochlerotatus caspius* (Prajapati et al., 2005; Knio et al., 2008). Similar larvicidal activity results were also observed when the EOs of *Coriander sativum* and *Petroselinum crispum* were tested against *Ochlerotatus caspius* (Knio et al., 2008). The larvicidal tests of EOs of genus *Carum* were performed for *Carum carvi* against *Anopheles dirus* and *Aedes aegypti* and for *Carum petroselinum* against *Culex pipiens* (Lee, 2006; Pitasawat et al., 2007; Khater & Shalaby, 2008). The results were directly similar to those of the EO of *Daucus carota* (against *Anopheles dirus* and *Aedes aegypti*) proving their inability to cause 100% mortality at the lowest concentration (Lee, 2006).

Among the methanolic extracts of 118 Euroasiatic plants, tested for their larvicidal effects against *Culex quinquefasciatus,* the species *Ammi visnaga* and *Seseli pallasii* were determined as two of the most toxic materials tested, with LC_{50} values lower than 10 mgl^{-1} (Pavela, 2008, 2009). On the other hand, the extracts of *Angelica archangelica* and *Imperatoria ostruthium* exhibited LC_{50} values lower than 70 mgl^{-1}, while *Seseli tortuosum* and *Ferula lancerottensis* displayed moderate larvicidal activity (LC_{50} values around 430 mgl^{-1}). The only inactive Apiaceae plant tested was *Ferula assa-foetida* (LC_{50} value higher of 1000 mgl^{-1}), with the EO of *Ferula galbaniflua* exhibiting the weakest activity against *Culex quinquefasciatus* and *Anopheles stephensi* (mortality level less than 14% of dead larvae after 48 hours, Amer & Mehlhorn, 2006a). The same authors also reported that *Anopheles stephensi* was the most resistant to dill (*Anethum graveolens*), while the *Culex quinquefasciatus* the more sensitive. Dill was also evaluated for persistency to larvicidal effects under different conditions for 1 month after the preparation of its solutions. In all cases (open, closed, in light or in dark) the EO was active only when was used immediately after preparation (Amer & Mehlhorn, 2006b).

Finally, an interesting result was obtained during the study of several EOs using coupled gas chromatography-electroantennographic detection (GC-EAD), on the hypothesis that compounds can be detected by the antennae of the yellow fever mosquito, *Aedes aegypti*. Thus, cumin aldehyde and cumin alcohol the *Cuminum cynimum* EO components were identified as such molecules. It must be noted that for both components, their EO (cumin oil) was also EAD-active (Campbell et al., 2011)

2.3 Greek Umbelliferae (Apiaceae) plants extract activities against Culex pipiens mosquitoes

The larvicidal activity of the EO obtained from the stem of Greek *Foeniculum vulgare* was determined against *Culex pipiens* larvae, while methyl chavicol was determined as its main component (more than 32%). Although the LC_{50} value of methyl chavicol was more than 80 mgl^{-1}, the respective EO was determined as 2.1-fold more toxic (Manolakou et al., 2009). *Culex pipiens* larvae were also used to test the mosquito control properties of EO from various naturally growing plants throughout Greece, belonging to the following six different Apiaceae family taxa: *Heracleum sphondylium, Seseli montanum, Conopodium capillifolium, Bupleurum fruticosum, Oenanthe pimpinelloides, Eleoselinum asclepium.* All EOs tested displayed good larvicidal activities with LC_{50} values ranging from 40.26-96.96 mgl^{-1} (Evergetis et al., 2009)

As a continuation of our ongoing efforts to exploit the use of natural products for the development of environmentally friendly means for the mosquito population control, our interest was stimulated on the investigation of Umbeliferae (Apiaceae) plants EOs. In this context, we report herein the chemical composition and larvicidal activity results for 14 EOs originated from different taxon obtained during Greek Umbelliferae biodiversity studies (**Table 1**).

3. Materials and methods

3.1 Plant material

Fourteen different taxa of the Umbeliferae (Apiaceae) family, Apioideae subfamily belonging to seven tribes and twelve different genera have been collected during the present study. Representatives of the Apieae Tribe are *Pimpinella peregrina* L., and 5 Greek endemics, namely *Athamanta densa* Boiss. & Orph., *Pimpinella tragium* ssp *tragium* Vill., *Pimpinella rigidula* (Boiss. & Orph.) H. Wolf, *Seseli parnassicum* Boiss. & Heldr. and *Thamnosciadium junceum* (Sibth. & Sm.) Hartvig.; of Smyrnieae tribe *Scaligeria cretica* (Miller) Boiss. and *Smyrnium rotundifolium* Miller; of Angeliceae tribe *Angelica sylvestris* L.; of Scandiceae tribe the Greek endemic *Chaerophyllum heldreichii* Orph. Ex Boiss.; of Peucedaneae tribe *Ferulago nodosa* (L.) Boiss., *Peucedanum neumayeri* (Vis.) Reichenb, *Peucedanum officinale* L., and of Laserpitieae tribe the Greek endemic *Laserpitium pseudomeum* Orph., Heldr. & Sart. Ex Boiss (Pimenov & Leonov, 1993; Tutin et al., 1968).

Full collection details are provided in **Table 2.** A voucher specimen of each plant is deposited in the herbarium of the Agricultural University of Athens, Athens, Greece.

3.2 Essential oils isolation

The freshly collected plant materials (steams, leaves and flowers) were washed thoroughly, chopped off finely and subjected to steam distillation in a Clevenger-type apparatus, using the Microwave Accelerated Reaction System (MARS 5) at 1400 W for 40 min with 3 L of H_2O in order to obtain their EOs. The resulting oils were dried over anhydrous sodium sulphate and stored at 4 ^oC. The EO yield of each plant is included in **Table 3**.

Species	Abbreviation	Vegetative Stage	Date	Location
Angelica sylvestris L.	AS	Flowering	05.09.2004	Mt. Parnon, Peloponnisos, forest streams
Athamanta densa Boiss. & Orph. *	AD	Flowering	15.06.2005	Mt. Parnassos, Sterea Hellas, vertical cliffs
Chaerophyllum heldreichii Orph. Ex Boiss. *	CH	Flowering	25.07.2004	Mt. Parnon, Peloponnisos, forest clearings
Ferulago nodosa (L.) Boiss.	FN	Flowering	02.05.2005	Antikyra, Sterea Hellas, olive groves
Laserpitium pseudomeum Orph., Heldr. & Sart. Ex Boiss.*	LP	Flowering	15.07.2004	Mt. Oiti, Sterea Hellas, rocky slopes
Peucedanum neumayeri (Vis.) Reichenb	PN	Flowering	28.08.2004	Mt. Smolikas, Hepiros, forest clearings
Peucedanum officinale L.	PO	Flowering	15.07.2004	Mt. Oiti, Sterea Hellas, rocky slopes
Pimpinella tragium ssp tragium Vill. *	PT	Flowering	15.07.2004	Mt. Oiti, Sterea Hellas, rocky slopes
Pimpinella peregrina L.	PP	Flowering	14.05.2005	Iraklio, Is. Crete, olive groves
Pimpinella rigidula (Boiss. & Orph.) H. Wolf *	PR	Flowering	17.08.2004	Molai, Peloponnisos, roadside
Scaligeria cretica (Miller) Boiss.	SC	Flowering	22.05.2005	Vouliagmeni, Sterea Hellas, seaside
Seseli parnassicum Boiss. & Heldr. *	SP	Flowering	15.07.2004	Mt. Oiti, Sterea Hellas, forest clearings
Smyrnium rotundifolium Miller	SR	Flowering	02.05.2005	Distomo, Sterea Hellas, roadside
Thamnosciadium junceum (Sibth. & Sm.) Hartvig *	TJ	Flowering	25.07.2004	Mt. Parnnassos, Sterea Hellas, alpic ravine

*=Greek Endemic.

Table 2. Collection data.

Species	Part distilled	Weight of aerial parts (g)	Volume of oil (mL)
Angelica sylvestris L.	Aerial	920	0,5
Athamanta densa Boiss. & Orph. *	Aerial	450	0,5
Chaerophyllum heldreichii Orph. Ex Boiss. *	Aerial	530	0,7
Ferulago nodosa (L.) Boiss.	Aerial	400	0,7
Laserpitium pseudomeum Orph., Heldr. & Sart. Ex Boiss. *	Aerial	270	0,9
Peucedanum neumayeri (Vis.) Reichenb	Aerial	600	0,5
Peucedanum officinale L.	Aerial	*180*	*0,8*
Pimpinella tragium ssp tragium Vill. *	Aerial	650	1,5
Pimpinella peregrina L.	Aerial	500	0,4
Pimpinella rigidula (Boiss. & Orph.) H. Wolf *	Aerial	235	0,7
Scaligeria cretica (Miller) Boiss.	Aerial	200	0,5
Seseli parnassicum Boiss. & Heldr. *	Aerial	*200*	*0,5*
Smyrnium rotundifolium Miller	Aerial	530	0,9
Thamnosciadium junceum (Sibth. & Sm.) Hartvig *	Aerial	600	2,0

*=Greek Endemic.

Table 3. Essential oils yields.

3.3 Gas Chromatography-Mass Spectrometry (GC-MS) analyses

Gas Chromatography (GC). All GC analyses were carried out on a Agilent Technologies 7890A gas chromatograph, fitted with a HP 5MS 30m x 0.25mm x 0.25μm film thickness capillary column and FID. The column temperature was programmed from 60 to 280 °C at a initial rate of 3 °C/min. The injector and detector temperatures were programmed at 230 and 300 °C, respectively. Helium was used as the carrier gas at a flow rate 1 ml/min.

Gas Chromatography-Mass Spectrometry (GC-MS). The GCMS analyses were performed on the same instrument using the Agilent 5957C, VL MS Detector with Triple-Axis Detector system operating in EI mode (equipped with a HP 5MS 30m x 0.25mm x 0.25μm film thickness capillary column), using He (1 ml/min) as the carrier gas. The initial temperature of the column was 60 °C. The column was heated gradually to 280 °C with a 3 °C/min rate. The identification of the compounds was based on comparison of their retention indices (RI) (Van den Dool & Kratz, 1963), obtained using various n-alkanes (C9-C24). Also, their EI-mass spectra were compared with the NIST/NBS and Wiley library spectra and the literature (Adams, 1995; Massada, 1976). Additionally, the identity of the indicated phytochemicals was confirmed by comparison with available authentic samples.

3.4 Mosquito rearing

A colony of the species *Culex pipiens* biotype *molestus* is maintained for more than 25 years in the laboratory of Entomology of the Benaki Phytopathological Institute, Kifissia, Greece. Adult mosquitoes are kept in wooden framed cages (33x33x33 cm) with a 32x32 mesh at 25±2 ^{o}C, 80±2% relative humidity and photoperiod of 14:10 (L:D) h. Cotton wicks saturated with 10% sucrose solution are used as food source. Females lay eggs in round, plastic containers (10 cm

diameter x 5 cm depth) filled with 150 ml of tap water. Egg rafts are removed daily and placed in cylindrical enamel pans (with diameter of 35 cm and 10 cm deep), in order to hatch. Larvae are reared under the same conditions of temperature and light and are fed daily with baby fish food (TetraMin, Baby Fish Food) at a concentration of 0.25 gl^{-1} of water until pupation. Pupae are then collected and introduced into the adult rearing cages.

3.5 Larvicidal bioassays

Stock solutions of EOs tested were prepared in ethanol and maintained in a freezer as 1% mgl^{-1}solutions. They were dissolved in double distilled water to produce solutions of the tested materials in concentrations ranging from 5 to 150 mgl^{-1}. Prior to biological determinations the toxicity of each EO was evaluated (data not shown).

The larval mortality bioassays were carried out according to the test method for larval susceptibility, proposed by the World Health Organization (WHO, 1981). Twenty 3rd to 4th instar larvae of the species *Culex pipiens* biotype *molestus* were collected from the colony, placed in a glass beaker with 250 ml of aqueous suspension of the tested material at various concentrations and an emulsifier was added in the final test solution (less than 0.05%). Four replicates were made per each concentration and a control treatment with tap water and emulsifier was also included. Beakers with larvae were placed at 25±2 ^{o}C, 80±2% relative humidity and photoperiod of 14:10 h (L:D).

3.6 Data analysis

Larvicidal effect was recorded 48 h after treatment. Data obtained from each dose–larvicidal bioassay (total mortality, mgl^{-1} concentration in water) were subjected to probit analysis in which probit–transformed mortality was regressed against log_{10}–transformed dose; LC_{50}, LC_{90} values, and slopes were calculated (SPSS 11.0).

4. Results and discussion

4.1 Phytochemical analysis

Fourteen distinct Umbeliferae taxa (twelve genera) are studied herein, one of which is endemic to Greece (*Thamnosciadium* Hartvig). It must be noted that there are no literature reports and studies on the EOs and their chemical compostitions for the material obtained from the plants *Athamanta densa* Boiss. & Orph. (AD), *Chaerophyllum heldreichii* Orph. Ex Boiss. (CH), *Laserpitium pseudomeum* Orph., Heldr. & Sart. Ex Boiss. (LP), *Peucedanum neumayeri* (Vis.) Reichenb (PN), *Pimpinella tragium* ssp tragium Vill. (PT), *Pimpinella rigidula* (Boiss. & Orph.) H. Wolf (PR), *Scaligeria cretica* (Miller) Boiss. (SC), *Seseli parnassicum* Boiss. & Heldr. (SP) and *Smyrnium rotundifolium* Miller (SR). In addition, the discussion section on the related *taxa* EOs compositions includes ten (out of twelve) genera studied herein, since there are also no previous reports on the composition of EOs obtained from *Conium* L. and *Thamnosciadium* Hartvig genera.

In total seventy phytochemicals, representing 76.64 to 99.83 % of the respective EOs samples have been identified as their constituents using combined GC and GC/MS analyses and in certain occasions verified by NMR studies. The detailed qualitative and quantitative analytical data of the main constituents of steam volatiles (and their respective retention indices) are presented in **Table 4**.

Components	RI	PN	AS	TJ	SP	PO	CH	LP	PT	SC	PP	PR	FN	SR	AD	Identification
trans-2-hexanal	803												0.96		0.27	a, b
a-pinene	939	21.27	24.65	2.80		2.14	1.67	49.58	1.21	8.76			30.85	0.93	0.46	a, b, c
camphene	954	2.99	3.32			1.72							4.36			a, b, c
sabinene	975	2.76				1.15	71.76	24.73	4.30	13.74			1.96	0.93		a, b, c
β-pinene	979	2.66	1.33					8.51					1.79		8.86	a, b, c
myrcene	991	3.93	4.75	1.52		0.81	1.51	1.82					6.68	11.25	0.92	a, b, c
a-phellandrene	1003	2.53	2.35	3.83									1.33			a, b, c
a-terpinene	1017		3.58										0.32			a, b, c
p-cymene	1025	4.71						0.74								a, b, c
o-cymene	1026			0.91												a, b, c
limonene	1029	4.71		40.75		2.78			1.42	1.43					0.66	a, b, c
β-phellandrene	1030	12.76	42.96		1.42		10.86	6.73					10.20			a, b, c
cis-ocimene	1037	4.78		18.59									2.76		1.66	a, b, c
trans-ocimene	1050			0.82									1.01		5.16	a, b, c
γ-terpinene	1060	32.25					2.54	1.42		1.40			0.41			a, b, c
cis-sabinene hydrate	1070							2.53								a, b, c
terpinolene	1089			12.97												a, b, c
linalool	1097														0.50	a, b, c
trans-limonene oxide	1137			0.46												a, b
geijerene	1143								10.23							a, b
a-terpineol	1189						3.35	2.43		0.78						a, b, c
pregeijerene	1287								5.13							a, b
1-bornyl acetate	1289		3.84			81.13							0.52			a, b
2,3,4-trimethyl benzaldehyde	1359			2.16		4.68										a, b
2,3,6-trimethyl benzaldehyde	1371			0.62												a, b
isoledene	1376										0.69					a, b
a-copaene	1377									0.67			0.42		0.26	a, b
β-cubebene	1388													0.86		a, b
β-elemene	1391				10.85					0.50			0.51	2.09	0.33	a, b
aristolene	1407										19.92					a, b
calarene	1411										3.40				0.40	a, b
β-caryophyllene	1419		1.73		2.76				0.92	3.07			2.10			a, b, c
a-bergamontene	1435										62.15					a, b, c
γ-elemene	1437				3.22									0.72		a, b, c
β-humulene	1439				3.30					0.40						a, b, c
β-farnesene	1457									29.27		2.47			1.17	a, b, c
$C_{14}H_{30}O$ (m/z: 189, 147, 105, 91, 204)	1483														19.80	b
a-amorphene	1484											0.32				a, b
$C_{14}H_{28}O$ (m/z: 119, 91, 105, 145, 131)	1485								20.39							b
germacrene D	1487	2.55	4.42	0.89	13.02		2.53	0.71	0.87	28.37	0.85		6.42		1.21	a, b, c
β-selinene	1490				5.14						3.78	23.80			3.95	a, b, c
a-selinene	1498					0.95						3.53		5.28		a, b, c
a-zingiberene	1499											7.75				a, b
bicyclogermacrene	1500				6.25					3.51	1.21		4.04			a, b
a-farnesene	1506		1.84													a, b, c
β-bisabolene	1506				2.85						1.18	4.16			12.72	a, b, c
myristicin	1519											6.72			4.32	a, b, c
β-sesquiphellandrene	1523				30.39						1.04	1.80		0.92		a, b, c
δ-cadinene	1524	1.30	3.02							0.38						a, b, c
germacrene B	1561				10.64				19.28					2.13	0.98	a, b, c
spathulenol	1578				1.52					0.46						a, b, c
caryophyllene oxide	1583				1.02					0.61						a, b, c
β-elemenone	1601								1.72							a, b
isofuranogermacrene	1648													1.28		a, b
furanodiene	1649													11.81		a, b
a-bisabolol	1686		2.04													a, b
germacrone	1694								23.33					5.62		a, b
trans-isomyristicin	1721			10.14								7.74				a, b
trans-pseudoiso-eugenyl 2-methylbutyrate	1774											7.74				a, b
trans-epoxypseudoisoeugenyl 2-methylbutyrate	1783											26.72				a, b
furanoeremophil-1-one	1880													6.42	0.91	a, b
1*β*-acetoxyfuranoeudesm-4(15)-ene	1889													8.87		a, b
1*β*-acetoxyfuranoeudesm-3 ene	1911													20.72		a, b
$C_{12}H_{25}O_2N$ (m/z: 91, 55, 115, 129, 77)	1923														2.94	b
$C_{12}H_{25}O_2N$ (m/z: 91, 115, 55, 129, 77)	1943														8.58	b
$C_{13}H_{27}O_2N$ (m/z: 91, 115, 55, 129, 159)	2030														12.17	b
n-heneicosane	2100														0.59	a, b, c
tricosane	2300														0.32	a, b, c
pentacosane	2500														0.25	a, b, c
Total		99.20	99.83	96.46	92.38	95.36	94.22	99.20	88.80	93.35	94.22	92.75	76.64	79.83	89.39	

[a]Comparison of mass spectra with MS libraries and retention times
[b]Comparison of experimental RI with reported RI
[c]Comparison with authentic compounds
RI: Retention indices calculated against C_8 to C_{24} n-alkanes on the HP 5MS column.

Table 4. Chemical constituents of the essential oils tested.

The determined chemical composition of the EO from the aerial part of *Angelica sylvestris* L. (AS) is consistent with the literature reports for EOs obtained from its seeds (Bernard, 2001) and roots (Bernard & Clair, 1997), with α-pinene and β-phellandrene being the major components. Same compounds were reported as the prevailing phytochemicals in the EOs of *A. archangelica* L. *sensu lato* (Bernard, 2001; Nykanen et al., 1991; Bernard & Clair, 1997; Chalcat & Garry, 1997; Nivinskiene et al., 2005), while the EO of *A. glauca* is reported to contain β-phellandrene as major component and only small portions of α-pinene (Aghinotri et al., 2004; Kaul et al., 1996). Other *Angelica* L. taxa, such as *A. sinensis* (Dung et al., 1996; Kim et al., 2006), *A. gigas* (Kim et al., 2006), *A. acutiloba* (Kim et al., 2006), *A. heterocarpa* (Bernard, 2001; Bernard & Clair, 1997) and *A. tenuissima* (Ka et al., 2005) display a completely different, both qualitative and quantitative, EO composition profile.

In addition to *a*-pinene, which is the main constituent as previously reported by Demetzos et al. (2000), the studied EO of *Ferulago nodosa* (L) Boiss. (FN) was found to contain thirteen new components for the *taxon's* EO. More specifically, the molecules of *trans*-2-hexenal, myrcene, α-phellandrene, α-terpinene, β-phellandrene, *cis*-ocimene, *trans*-ocimene, γ-terpinene, bornyl acetate, β-elemene, β-caryophylene, germacrene D and bicyclogermacrene were also determined as constituents of this EO. With the exception of *trans*-2-hexenal all the abovementioned compounds have been assayed in the EOs of the following *Ferulago* W.D.J. Koch taxa; *F. asparagifolia* (Baser et al., 2001), *F. phialocarpa* (Masoudi et al., 2004b), *F. macrocolea* (Rustaiyan et al., 2005), *F. galbaniflua* (Rustaiyan et al., 2002a), and *F. thirkeana* (Baser et al., 2002).

The EO of *Peucedanum officinale* L. (PO) is dominated by bornyl acetate, which was previously found only in *P. scoparium* (Masoudi et al., 2004a). It is also characterized by the presence of 2,3,4-trimethyl benzaldehyde, which has not been previously reported as constitutent of *Peucedanum* L. EOs. In addition, the EO tested was found to contain five molecules, namely *a*-pinene, sabinene, myrcene, limonene and β-selinene, never reported in a EO of *P. officinale* (Jaimand et al., 2006). These five phytochemichals are abundant in the general profile of *Peucedanum* L. EOs, as reported for *P. scoparium* (Masoudi et al., 2004a), *P. zenkeri* (Menut et al., 1995), *P.vertcillare* (Fraternale et al., 2000), *P. petiolare* (Rustaiyan et al., 2001) and *P. cervariifolium* (Bazgir et al., 2005).

The EOs of *Pimpinella* L. have been thoroughly studied, mainly because the application of their several taxa as culinary herbs and/or spices. Though the EOs of fourteen (14) *taxa* were studied, only one (Tabanca et al., 2005) refers to PP (*Pimpinella peregrina* L.) and none to PT (*Pimpinella tragium* ssp tragium VIII) and PR (*Pimpinella rigidulla* Boiss. & Orph. H. Wolf). The main constituent of EO of PO is *a*-bergamontenene, reported so far only for *P. anagodendron* (Velasco-Negueruela et al. 2005) and *P. anisum* (Santos et al., 1998). Two additional components determined herein, β-bisabolene and β-sesquiphellandrene, have not been reported in previous studies for PP but are well documented for *P. anagodendron* (Velasco-Negueruela et al. 2005), *P. junoniae* (Velasco-Negueruela et al. 2003), *P. anisum* (Santos et al., 1998), *P. anisetum* (Baser et al., 1999; Tepe et al., 2006) and *P. tragioides* (Askari & Sefidcon, 2007). New entries, for this genera EO components list, are isoledene, aristolene, calarene and β-selinene which were also assayed in the EO of PP. On the contrary, the EO of PR is characterized by the complete absence of monoterpenes, advocating previous record of β-selinene and introducing *a*-amorphene, *a*-selinene and *trans*-isomyristicin as components of the *Pimpinella* L. EOs. Finally, the EO of PT has only two differences as

compared to the genus EO components, an unidentified component and *β*-elemenone. In general, its composition is in accordance with the phytochemical profiles reported for the EOs of *P. aromatica* (Baser et al., 1996), *P. serbica* (Ivanic et al., 1983), *P. flabellifolia* (Tepe et al., 2006), *P. aurea* (Tabanca et al., 2005; Assadian et al., 2005), *P. acuminata* (Melkani et al., 2006), *P. barbata* (Fakhari & Sonboli, 2006), *P. rupicola* (Velasco-Negueruela et al. 2005), *P. corymbosa* and *P. puberula* (Tabanca et al., 2005).

Major components of the EO of *Scaligeia cretica* (Miller) Boiss (SC) are *a*-pinene, *β*-farnesene and germacrene D, which have also been detected in previous studies on the EOs of *Scaligeria* DC. In this respect, the EO of *S. lazica* contains *β*-farnesene as major and *a*-pinene, germacrene D as minor components (Baser et al., 1993). On the contrary, the EO of *S. tripartite* contains *β*-farnesene and germacrene D as minor compounds, while *a*-pinene is absent (Tabanca et al., 2007). Compounds assayed herein and never reported before in the EOs of *Scaligeria* DC are *a*-terpineol, *β*-elemene and *β*-humulene.

The EOs of *Laserpitium pseudomeum* Orph. Heldr. & Sart Ex Poiss. (LP) contains *a*-pinene, *β*-pinene, sabinene and *β*-phellandrene as major components, all well known constitutents of the EOs of *Laserpitium* L. Previous literature reports indicated that the EOs of *L. latifolium* contains *a*-pinene and *β*-pinene as major components (Borg-Karlson et al., 1994), the *L. petrophilum a*-pinene and sabinene (Baser et al., 1997), while the molecule of *β*-phellandrene is present in traces in both EOs. On the contrary, the phytochemical profile of the EO of *L. siler* is completely different containing mainly limonene and perillaldehyde (Chizzola et al., 1999)

The EO composition of *Smyrnium* L. has also been scarcely investigated, since only three *taxa's* EOs, namely *S. perfoliatum* (Molleken et al., 1998a; Tirillini et al., 1996; Tirillini & Tosi, 1992), *S. cordifolium* (Amiri et al., 2006) and *S. olusatrum* (Molleken et al., 1998b), have been studied todate. The studied of EO of *Smyrnium rotundifolium* Miller (SR) contains 7 major components, with the molecule of *a*-selinene reported for the first time as EO component of *Smyrnium* L.. Other compounds present in large quantitites are furanodiene (reported as major constitutent in *S. olusatrum*), myrcene, furanoeremophil-1-one, 1*β*-acetoxyfuranoeudesm-4(15)-ene, 1*β*-acetoxyfurano eudesm-3-ene (detected in *S. olusatrum* and *S. perfoliatum,* Molleken et al., 1998) and germacrone (present in *S. cordifolium*).

The phytochemical profile of *Chaerophyllum* L. EOs was studied previously for *C. macropodum* (Baser et al., 2006), *C. crinitum* (Baser et al., 2006; Nematollahi et al., 2005), *C. macrospermum* (Sefidcon & Abdoli, 2005; Rustaiyan et al., 2002b, Mamedova, 1994), *C. bulbosum sensu lato* (Mamedova & Akhmedova, 1991; Kokkalou & Stefanou, 1989), *C. aksekiense* (Baser et al., 2000b), *C. coloratum* (Vajs et al., 1995), *C. azoricum* (Pedro et al., 1999) and *C. prescotii* (Letchamo et al., 2005). The more significant differentiation among the literature results and the assayed herein EO of *Chaerophyllum heldreichi* Orph. Ex Boiss (CH) comprises the identification for first time of *a*-terpineol as main component of EO of *Chaerophyllum* L..

The EO of *Seseli parnassicum* Boiss. & Heldr. (SP) was found to contain three new compound entries, *β*-humulene, *β*-selinene and *β*-sesquiphellandrene, as compared with the EO of the Seseli L. *taxa* (also including the synonymous *Lomatopodium* Fisch. et C.A. Mey *taxa*). The remaining components are in accordance with the EO content of same *taxa* plants, such as *S. montanum* (Evergetis et al., 2009), *S. campestre* and *S. peucedanoides* (Baser et al. 2000a;

Bulatovic et al. 2006) and in *S. buchtormence*. These compounds were also present in the EOs of *S. resinosum* and *S. tortuosum*, obtained from the fruits and not the herbal part of the plants (Dogan et al. 2006). The *L. khorassanicum* and *L. staurophyllum* EOs were assayed to contain mostly aliphatic terpenes, while the corresponding cyclic terpenes were present in smaller amounts compared to EOs of Seseli L. (Sedghat et al. 2003; Sefidkon et al. 1997).

Finally, the investigated EO of *Athamanta densa* Boiss. & Orph., contains as major constituents myristicin and various unidentified alkaloids, which account for almost 24 % of its weight. The literature reports of EOs of *Athamanta* L. indicate that they mainly contain either myristicin, such as the EOs of *A. sicula* (Camarda & Di Stefano, 2003), *A. turbith sensu lato* (Tomic et al., 2009), *A. macedonica* (Verykokidou et al., 1995) and *A. haynaldi* (Zivanovic et al., 1994), or apiole as in *A. sicula* (Camarda & Di Stefano, 2008).

4.2 Larvicidal assays

The investigated EOs were evaluated ─for the first time─ in respect to their larvicidal activities against 3rd- 4th instar larvae of *Culex pipiens*. The relative results expressed as the respective LC_{50} and LC_{90} values are included in **Table 5**. Among the EOs tested only two were rather inactive (AS and PP, displaying LC_{50} values above 150 mgl^{-1}), while the EOs of SC and SP were moderately active displaying LC_{50} values above 100 mgl^{-1} (111.99 and 122.54 mgl^{-1} respectively).

Essential Oils tested	LC_{50} (95% CL)[a]	LC_{90} (95% CL)[a]	Slope (±SE)
Athamanta densa	10.15 (9.49-10.73)	15.75 (14.52-17.76)	6.72±0.80
Pimpinella tragium ssp tragium	40.13 (32.43-45.95)	71.10 (61.51-91.00)	5.15±0.52[b]
Pimpinella rigidula	40.31 (34.75-43.64)	60.41 (55.66-70.57)	7.29±1.44[b]
Thamnosciadium junceum	44.17 (41.52-46.62)	64.42 (59.94-71.28)	7.82±0.86[b]
Peucedanum neumayeri	47.40 (40.25-54.15)	81.47 (68.63-113.57)	5.44±0.53[b]
Chaerophyllum heldreichii	53.61 (50.29-56.55)	75.96 (71.53-82.15)	8.46±0.87
Laserpitium pseudomeum	56.73 (53.50-59.60)	79.59 (75.18-85.71)	8.46±0.86
Ferulago nodosa	67.39 (64.17-70.41)	95.59 (89.90-103.94)	8.43±0.84
Smyrnium rotundifolium	80.32 (76.88-84.16)	105.30 (98.33-116.61)	10.89±1.29
Peucedanum officinale	86.46 (82.27-90.30)	125.05 (117.23-136.95)	7.99±0.84
Scaligeria cretica	111.99 (107.86-115.47)	133.83 (128.35-143.21)	6.58±0.73[b]
Seseli parnassicum	122.54 (115.54-141.06)	167.15 (143.83-268.76)	6.30±0.68
Angelica sylvestris	>150		
Pimpinella peregrina	>150		

[a] LC values are expressed in mgl^{-1} and they are considered significantly different when 95% CL fail to overlap.
[b] Since goodness–of–fit test is significant ($P<0.05$), a heterogeneity factor is used in the calculation of confidence limits (CL)

Table 5. LC_{50} and LC_{90} values for the tested essential oils against larvae of *Culex pipiens* biotype *molestus*.

The EO derived from the endemic in Greece plant *Athamanta densa* was determined as the most active since displayed the highest toxicity against mosquito larvae, with LC_{50} value 10.15 mgl^{-1}. The EO tested contains a series of compounds which were not found in the other EOs tested, such as bisabolene and the unidentified compounds $C_{14}H_{30}O$, $C_{12}H_{25}O_2N$ and $C_{13}H_{27}O_2N$, which have to study more thoroughly in order to determine their activities. The remaining EOs (PR, TJ, PT, PN, CH, LP, FN, SR and PO) displayed LC_{50} values ranging from 40.31 to 86.46 mgl^{-1}. No significant relationship between toxicity and phytochemical content was detected.

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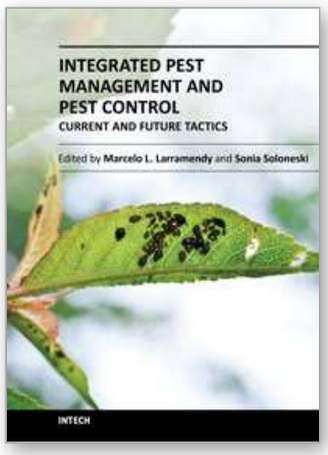

Integrated Pest Management and Pest Control - Current and Future Tactics

Edited by Dr. Sonia Soloneski

ISBN 978-953-51-0050-8
Hard cover, 668 pages
Publisher InTech
Published online 24, February, 2012
Published in print edition February, 2012

Integrated Pest Management is an effective and environmentally sensitive approach that relies on a combination of common-sense practices. Its programs use current and comprehensive information on the life cycles of pests and their interactions with the environment. This information, in combination with available pest control methods, is used to manage pest damage by the most economical means and with the least possible hazard to people, property, and the environment.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Epameinondas Evergetis, Antonios Michaelakis and Serkos A. Haroutounian (2012). Essential Oils of Umbelliferae (Apiaceae) Family Taxa as Emerging Potent Agents for Mosquito Control, Integrated Pest Management and Pest Control - Current and Future Tactics, Dr. Sonia Soloneski (Ed.), ISBN: 978-953-51-0050-8, InTech, Available from: http://www.intechopen.com/books/integrated-pest-management-and-pest-control-current-and-future-tactics/essential-oils-of-umbelliferae-apiaceae-family-taxa-as-emerging-potent-agents-for-mosquito-control

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