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Nematicidal activity of essential oils: a review

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Abstract Plant parasitic nematodes are the most destructive group of plant pathogens worldwide and their control is extremely challenging. Plant Essential oils (EOs) and their constituents have a great potential in nematode control since they can be developed for use as nematicides themselves or can serve as model compounds for the development of derivatives with enhanced activity. This study reviews the plant EOs evaluated as potential nematicides and their toxic effects against pinewood nematode (*Bursaphelenchus xylophilus*) and root-knot nematodes (*Meloidogyne* spp.). Additionally, the nematicidal activity to *M. javanica* of several EOs from Spanish aromatic plants and their components is described.

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Abbreviations

EOs	Essentials oils
J2	Second-stage juveniles of *Meloidogyne* spp
GC-MS	Gas chromatography-mass spectrometry
GABA	Gamma-aminobutyric acid
AChE	Acetylcholinesterase

Introduction

Plant parasitic nematodes are the most destructive group of plant pathogens worldwide and their control is extremely challenging (Bird et al. 2009). They parasite a large variety of crops through worldwide and their impact on yield losses has been estimated to a billion of euros annually (Bleve-Zacheo et al. 2007). Plant parasitic nematodes attack theirs host by using a wide range of strategies. They can be ectoparasites, that feed on the outer plant tissues or endoparasitic that feed and live inside the plant tissues. Several important plant-parasitic nematodes are endoparasites. Sedentary endoparasites (cyst and root-knot nematodes), represent the most advanced and successful type of parasitism, they

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are biotrophic and induce profound changes in the roots of theirs host as they feed (Castagnone-Sereno et al. 2006). The root-knot nematodes, *Meloidogyne* spp, are one of the most economically damaging genera of plant-parasitic nematodes on horticultural and field crops. Migratory endoparasitic nematodes do not feed from a single site but move through the plant, causing extensive damage as they move and feed. Within this group should be noted the pine wood nematode, *Bursaphelenchus xylophilus*, that is the causal agent of pine wilt disease that causes serious economic losses in pine forests.

Synthetic nematicides have been used to protect moderate-to-high-value crops in intensive productions systems throughout most of the twentieth century. In the last decades, environmental and human health concerns have steadily reduced the availability of efficient commercial nematicides (Nyczepir and Thomas 2009; Sorribas and Ornat 2011). Therefore, less toxic pesticides need to be developed. Phytochemicals have a great potential in nematode control since they can be developed for use as nematicides themselves or can serve as model compounds for the development of derivatives with enhanced activity (Chitwood 2002).

Essential oils (EOs) are natural volatile substances found in a variety of plants. They are complex mixture of mainly terpenoids, particularity monoterpenes and sequiterpenes, and a variety of aromatic phenols, oxides, ethers, alcohols esters, aldehydes and ketones that determine the characteristic aroma and odor of the plant. Their chemical composition may vary considerably between aromatic plant species and varieties, and within the same variety from different geographic areas. In addition the effect of plant maturity at the time of oil extraction and the existence of chemotypic differences can also drastically affect their composition (Lahlou and Berrada 2003). Commercially, EOs are valuable natural products used in the cosmetic, food and pharmaceutical industries (Buchbauer 2000). Although aromatic plants and their essentials oils have been used since ancient times as antimicrobial and insecticidal agents, the interest in has been increased remarkably during the past decade. The presence of volatile monoterpenes on EOs provides an important defense strategy to the plant against insect pests and pathogenic organisms. These terpenoids also play a role in plant parasitic interactions, acting as signaling molecules (Batish et al. 2008). Thus, in the last few years much effort has been focused on the study of the nematicidal activity of plant EOs and their constituents as potential sources of commercial products for management of plan parasitic nematodes.

This study reviews the plant EOs evaluated as potential nematicides and their toxic effects against pinewood (*B. xylophilus*) and root-knot nematodes (*Meloidogyne* spp.). Additionally, the nematicidal activity to *M. javanica* of several EOs from experimentally cultivated Spanish aromatic plants and their components is described.

Essential oils with nematicidal effects

A large number of EOs extracted from different botanical families has been analyzed in vitro for nematicidal activity mainly against *B. xylophilus and Meloidogyne* spp (Table 1). Among EO-producing plants some families such Lamiaceae, Asteraceae, Myrtaceae, Rutaceae, Lauraceae and Poaceae have been widely studied. Especially the aromatic plants of the genera *Artemisia, Cympogon, Lavandula, Mentha, Oreganum, Ocimum, Rosmarinus, Thymus,* and aromatic trees of the genera *Citrus*, *Eucalyptus, Eugenia* and *Melaleuca* who have been traditionally used for protection of stored commodities, mainly in the Mediterranean region and in Southern Asia.

The majority of studies evaluating the nematicidal activity of EOs against *B. xylophilus* have been carried out in Korea, where the pine wilt disease, caused by the pinewood nematode, is the most serious problem in forests of the southern parts of the country. Thus, it has been demonstrated that EOs extracted from *Boswellia carterii, Cymbopogon citrates, Eugenia caryophyllata* (Park et al. 2005), *Cinnamomum zeylanicum, Coriandrum sativum, Litsea cubeba, Origanum vulgare, Pimenta dioica, Thymus vulgaris* (Kong et al. 2006), *Allium cepa, Paeonia moutan, Perilla frutescens, Schizonepeta tenuifolia* (Choi et al. 2007b, Choi et al. 2007c), *Trachyspermum ammi* (Park et al. 2007), *Brassica integrefolia, Pelargonium inquinans, Syzygium aromaticum* (Elbadri et al. 2008), *Coriandrum sativum, Liquidambar orientalis, Valeriana wallichii* (Kim et al. 2008) and *Gaultheria fragrantissima* and *Zanthoxylum alatum* (Kim et al. 2011) had significant activity to pinewood nematode under in vitro conditions. Moreover, the recently occurrence and increasing dispersion of *B. xylophilus* in Portugal promoted the study of the nematicidal activity of EOs from selected species of aromatic plants from the Iberian flora. Strong lethal effects to

Table 1 Plant EOs tested for nematicidal activity against root-knot nematodes (*Meloidogyne* spp.) and pinewood nematode (*B. xylophilus*)

Nematode species evaluated	Plant source	Family plant	Part used	Country	Reference
Meloidogyne artiella	*Chrysanthemun coronarium**	Asteraceae	Flowers	Spain	Pérez et al. (2003)
Meloidogyne exigua	*Bixa Orellana**	Bixaceae		Brasil	Salgado et al. (2003)
	Cymbopogon nardus	Poaceae		Brasil	Salgado et al. (2003)
	*Melia azedarach**	Meliaceae		Brasil	Salgado et al. (2003)
	*Xylopia brasiliensis**	Annonaceae		Brasil	Salgado et al. (2003)
	*Eucaliptus camadulensis**	Myrtaceae		Brasil	Salgado et al. (2003)
	*Eucaliptus saligma**	Myrtaceae		Brasil	Salgado et al. (2003)
	*Eucaliptus urophylla**	Myrtaceae		Brasil	Salgado et al. (2003)
Meloidogyne incognita	*Cympogon flexuosus**	Poaceae	Foliage	India	Pandey et al. (2000), Sinha et al. (2006)
	*Eucaliptus citriodora**	Myrtaceae	Foliage	India	Pandey et al. (2000)
	*Eucaliptus hybrida**	Myrtaceae	Foliage	India	Pandey et al. (2000)
	*Mentha arvensis**	Lamiaceae	Foliage	India	Pandey et al. (2000)
				India	Sinha et al. (2006)
				India	Gupta et al. (2011)
	*Mentha piperita**	Lamiaceae			Pandey et al. (2000)
	*Mentha spicata**	Lamiaceae	Foliage	India	Pandey et al. (2000)
				India	Sinha et al. (2006)
	*Ocimum basilicum**	Lamiaceae	Foliage	India	Pandey et al. (2000)
				India	Sinha et al. (2006)
	*Pelargonium graveolensis**	Geraniaceae	Foliage	India	Pandey et al. (2000)
	Foeniculum vulgare	Umbeliferae	Flowers	Libano	Ibrahim et al. (2006)
	*Cympogon martinii**	Poaceae	Foliage	India	Pandey et al. (2000), Sinha et al. (2006)
	*Cympogon nardus**	Poaceae		India	Sinha et al. (2006)
	*Mentha citrata**	Lamiaceae		India	Sinha et al. (2006)
	*Tagetes minuta**	Asteraceae	Whole plant	India	Adekunle et al. (2007)
	*Pectis apodocephala**	Asteraceae	Aerial part	Brasil	Albuquerque et al. (2007)
	*Pectis oligocephala**	Asteraceae	Aerial part	Brasil	Albuquerque et al. (2007)
	*Eugenia caryophyllata**	Myrtaceae	Buds	Sigma	Meyer et al. (2008)
				India	Gupta et al. (2011)
	Croton regelianus	Euphorbiaceae	Leaves	Brasil	Torres et al. (2008)
	*Cymbopogon winterianus**	Poaceae		Brasil	Moreira et al. (2009)
	*Cymbopogon citratus**	Poaceae		Brasil	Moreira et al. (2009)
				India	Gupta et al. (2011)
	Eucalyptus terenticornis	Myrtaceae		Brasil	Moreira et al. (2009)
	*Lippia alba**	Verbenacea		Brasil	Moreira et al. (2009), Marino et al. (2012)
	*Lippia sidoides**	Verbenacea		Brasil	Moreira et al. (2009)
	Ocimum gratissimum	Lamiaceae		Brasil	Moreira et al. (2009)
	*Origanum vulgare**	Lamiaceae	Aerial part	Greece	Ntalli et al. (2011)
	*Origanum dictamnus**	Lamiaceae	Aerial part	Greece	Ntalli et al. (2011)
	*Mentha pulegium**	Lamiaceae	Aerial part	Greece	Ntalli et al. (2011)

Table 1 continued

Nematode species evaluated	Plant source	Family plant	Part used	Country	Reference
	*Melissa officinales**	Lamiáceas.	Aerial part	Greece	Ntalli et al. (2011)
	Chenopodium ambrosioides	Amaranthaceae	Aerial part	China	Chuan et al. (2011)
	*Carum capticum**	Apiaceae		India	Gupta et al. (2011)
	Cedrus deodara	Pinaceae		India	Gupta et al. (2011)
	*Eucaliptus globulas**	Myrtaceae		India	Gupta et al. (2011)
	*Kadsura heteroclite**	Schisandraceae	Stems	China	Li et al. (2011)
	Achillea millefolium	Asteraceae	Aerial part	Greece	Ntalli et al. (2011)
	*Eucaliptus meliodora**	Myrtaceae	Aerial part	Greece	Ntalli et al. (2011)
	*Foeniculum vulgare**	Apiaceae	Aerial part	Greece	Ntalli et al. (2011)
	Juglans regia	Juglandaceae	Aerial part	Greece	Ntalli et al. (2011)
	Laurus nobilis	Lauraceae	Aerial part	Greece	Ntalli et al. (2011)
	*Pimpinella anisum**	Apiaceae	Aerial part	Greece	Ntalli et al. (2011)
	*Pistacia terebinthus**	Anacardiaceae	Aerial part	Greece	Ntalli et al. (2011)
Meloidogyne javanica	*Achillea fragrantissima*	Asteraceae	Foliage	Israel	Oka et al. (2000)
	Artemisia arboresses	Asteraceae	Foliage	Israel	Oka et al. (2000)
	Artemisia dracunculus	Asteraceae	Foliage	Israel	Oka et al. (2000)
	*Artemisia judaica**	Asteraceae	Foliage	Israel	Oka et al. (2000)
	*Carum carvi**	Apiaceae	Foliage	Israel	Oka et al. (2000)
	*Coridothymus capitatus**	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	*Cymbopogon citratus**	Poaceae	Foliage	Israel	Oka et al. (2000)
	*Foeniculum vulgare**	Lamiaceae	Umbels	Israel	Oka et al. (2000)
	Laurus nobilis	Lauraceae	Foliage	Israel	Oka et al. (2000)
	Lavandula officialis	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	Mentha piperita	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	*Mentha rotundifolia**	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	*Mentha spicata**	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	Micromeria fruticosa	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	Myrtus communis	Myrtaceae	Foliage	Israel	Oka et al. (2000)
	Ocimum basilicum	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	Origanum dayi	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	Origanum syriacum	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	Origanum vulgare	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	Pelargonium graveolens	Geraniaceae	Foliage	Israel	Oka et al. (2000)
	Rosmarinus officinalis	Labiatae	Foliage	Israel	Oka et al. (2000)
	Salvia dominica	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	Salvia officinalis	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	Salvia triloba	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	Thymus vulgaris	Lamiaceae	Foliage	Israel	Oka et al. (2000)
	Cinnamomum verum	Lauraceae	Bark	Korea	Park et al. (2005)
	Leptospermun petersonii	Myrtaceae	Leaves	Korea	Park et al. (2005)
	*Haplophyllum tuberculatum**	Rutaceae	Aerial parts	Oman	Onifade et al. (2008)
	*Plectranthus cylindraceus**	Lamiaceae	Aerial parts	Oman	Onifade et al. (2008)
	Bacharis salicifolia	Asteracea	Aerial parts	Argentina	Sosa et al. (2012)
	Eupatorium arnotii	Asteracea	Aerial parts	Argentina	Sosa et al. (2012)

Table 1 continued

Nematode species evaluated	Plant source	Family plant	Part used	Country	Reference
	Eupatorium buniifolium	Asteracea	Aerial parts	Argentina	Sosa et al. (2012)
	Eupatorium inulaefolium	Asteracea	Aerial parts	Argentina	Sosa et al. (2012)
	*Eupatorium viscidum**	Asteracea	Aerial parts	Argentina	Sosa et al. (2012)
Bursaphelenchus xylophilus	*Agastache rugosa*	Lamiaceae	Whole plant	Korea	Park et al. (2005)
	Allium sativum	Liliaceae	Bulb	Korea	Park et al. (2005)
	Angelica dahurica	Apiaceae	Roots	Korea	Park et al. (2005)
			Roots	Korea	Choi et al. (2007b)
	Armoracia rusticana	Brassicaceae	Roots	Korea	Park et al. (2005)
	Asiarum sieboldi	Aristolochiaceae	Roots	Korea	Park et al. (2005)
	Atractylodes japonica	Asteraceae	Roots	Korea	Choi et al. (2007b)
	*Boswellia carterii**	Burceraceae	Resin	Korea	Park et al. (2005)
			Resin	Jin-Ah Korea	Kong et al. (2006)
			Resin	Korea	Choi et al. (2007b)
			Resin	Ethiopia	Park et al. (2007)
				Ethiopia	Kim et al. (2011)
	Cananga odorata	Annonaceae	Flower	Korea	Park et al. (2005)
	Carum carvi	Apiaceae	Seeds	Korea	Park et al. (2005)
			Seeds	Berje-USA	Kong et al. (2006)
				Egypt	Park et al. (2007)
	Chenopodium ambrosioides	Chenopodiaceae	Whole plant	Korea	Park et al. (2005)
			Whole plant	Korea	Choi et al. (2007b)
	Cinnamomum camphora	Lauraceae	Wood	Korea	Park et al. (2005)
	Cinnamomum verum	Lauraceae	Bark	Korea	Park et al. (2005)
	Citrus bergamia	Rutaceae	Peel	Korea	Park et al. (2005)
				Jin-Ah-Korea	Kong et al. (2006)
	Citrus limonum	Rutaceae	Peel	Korea	Park et al. (2005)
				Jin-Ah-Korea	Kong et al. (2006)
	Citrus paradise	Rutaceae	Peel	Jin-Ah Korea	Park et al. (2005), Kong et al. (2006)
	Citrus reticulata	Rutaceae	Rind	Italy/Brazil	Park et al. (2005)
			Leaves	Egypt	Kong et al. (2006), Kim et al. (2011)
	Citrus sinensis	Rutaceae	Peel	Korea	Park et al. (2005)
				Jin-Ah Korea	Kong et al. (2006)
	Cnidium officindle	Apiaceae	Roots	Korea	Park et al. (2005)
			Roots	Korea	Choi et al. (2007b)
	Cupressus sempervirens	Cupressaceae	Leaves	Korea	Park et al. (2005)
				Berje-USA	Kong et al. (2006)
	Curcuma longa	Zingiberaceae	Roots	Korea	Park et al. (2005)
				Korea	Choi et al. (2007b)
	*Cymbopogon citratus**	Poaceae	Whole plant	Korea	Park et al. (2005)
				Jin-Ah Korea	Kong et al. (2006)
				Portugal	Barbosa et al. (2010)

Table 1 continued

Nematode species evaluated	Plant source	Family plant	Part used	Country	Reference
	Cymbopogon nardus	Poaceae	Whole plant	Korea	Park et al. (2005)
				Berje_USA	Kong et al. (2006)
	Eucalyptus citriodora	Myrtaceae	Leaves	Korea	Park et al. (2005)
				Jin-Ah Korea	Kong et al. (2006)
	Eucalyptus dives	Myrtaceae	Leaves	Korea	Park et al. (2005)
	Eucalyptus globulus	Myrtaceae	Leaves	Berje-USA	Kong et al. (2006)
	Eucalyptus smithii	Myrtaceae	Leaves	Korea	Park et al. (2005)
	Eucalyptus polybractea	Myrtaceae	Leaves	Korea	Park et al. (2005)
	Eucalyptus radiata	Myrtaceae	Leaves	Korea	Park et al. (2005)
	*Eugenia caryophyllata**	Myrtaceae	Lower bud	Korea	Park et al. (2005)
				Berje-USA	Kong et al. (2006)
	Evodia officinalis	Rutaceae	Fruits	Korea	Park et al. (2005)
			Fruits	Korea	Choi et al. (2007b)
	Lavandula officinalis	Lamiaceae	Flowering plant	Korea	Park et al. (2005)
	Leptospermum petersonii	Myrtaceae	Leaves	Korea	Park et al. (2005)
	Melaleuca dissitiflora	Myrtaceae	Leaves	Korea	Park et al. (2005)
	Melaleuca uncinata	Myrtaceae	Leaves	Korea	Park et al. (2005)
	Melaleuca linariifolia	Myrtaceae	Leaves	Korea	Park et al. (2005)
	Melaleuca quinquenervia	Myrtaceae	Leaves	Korea	Park et al. (2005)
	Mentha spicata	Lamiaceae	Whole plant	Korea	Park et al. (2005)
				Jin-Ah Korea	Kong et al. (2006)
	Nardostachys chinensis	Valerianaceae	Roots	Korea	Park et al. (2005)
			Roots	Korea	Choi et al. (2007b)
			Roots	China	Kim et al. (2008)
	Ocimum basilicum	Lamiaceae	Leaves	Korea	Park et al. (2005)
				Berje-USA	Kong et al. (2006)
	Pelargonium graveolens	Geraniaceae	Leaves	Korea	Park et al. (2005)
			Leaves	Berje-USA	Kong et al. (2006)
				Reunion	Park et al. (2007)
	Pimenta racemosa	Myrtacea	Leaves	Korea	Park et al. (2005)
				Jin-Ah Korea	Kong et al. (2006)
	Pimpinella anisum	Apiaceae	Flower bud	Korea	Park et al. (2005)
				Berje-USA	Kong et al. (2006)
					Elbadri et al. (2008)
	Piper nigrum	Piperaceae	Leaves	Korea	Park et al. (2005)
				Jin-Ah Korea	Kong et al. (2006)
	Zingiber officinale	Zingiberaceae	Rhizome	Korea	Park et al. (2005)
	Abies alba	Pinaceae		Jin-Ah Korea	Kong et al. (2006)
	Abies sibirica	Pinaceae		Berje-USA	Kong et al. (2006)
	Achillea millefolium	Asteraceae		Berje-USA	Kong et al. (2006)
	Acorus calamus	Acoraceae		Berje-USA	Kong et al. (2006)

Table 1 continued

Nematode species evaluated	Plant source	Family plant	Part used	Country	Reference
	Agothosma betulina	Rutaceae		Berje-USA	Kong et al. (2006)
	Amyris balsamifera	Rutaceae	Wood	Berje-USA	Kong et al. (2006)
				Caribbean	Park et al. (2007)
	Anethum graveolens	Apiaceae	Seeds	Berje-USA	Kong et al. (2006)
				Bulgaria	Park et al. (2007)
	Angelica archangelica	Apiaceae		Jin-Ah Korea	Kong et al. (2006)
	Aniba rosaeodora	Lauraceae		Berje-USA	Kong et al. (2006)
	Anthemis nobilis	Asteraceae	Blossoms	Jin-Ah Korea	Kong et al. (2006)
				France	Kim et al. (2008)
	Apium graveolens	Apiaceae	Flowering plant	Berje-USA	Kong et al. (2006)
				Morocco	Kim et al. (2008)
	Artemisia absinthium	Asteraceae	Aerial parts	Jin-Ah Korea	Kong et al. (2006)
				Portugal	Barbosa et al. (2010)
	Artemisia dracunculus	Asteraceae	Aerial parts	Berje-USA	Kong et al. (2006)
				Portugal	Barbosa et al. (2010)
	Artemisia pallens	Asteraceae	Leaves	Berje-USA	Kong et al. (2006)
				India	Park et al. (2007)
	Bulnesia sarmienti	Zygophyllaceae		Berje-USA	Kong et al. (2006)
	Cananga odorata	Annonaceae		Berje-USA	Kong et al. (2006)
	*Cinnamomum zeylanicum**	Lauraceae	Green leaves and bark	Berje-USA	Kong et al. (2006)
				Korea	Kong et al. (2006)
	Citrus aurantifolia	Rutaceae		Berje-USA	Kong et al. (2006)
	Citrus aurantium	Rutaceae		Berje-USA	Kong et al. (2006)
	Citrus reticulata	Rutaceae	Rind	Italy/Brazil	Kong et al. (2006)
			Leaves	Egypt	Kim et al. (2011)
	Commiphora myrrha	Burseraceae		Berje-USA	Kong et al. (2006)
	*Coriandrum sativum**	Apiaceae	Fruits	Berje-USA	Kong et al. (2006)
			Herb	Argentina	Park et al. (2007)
				Slovenia	Kim et al. (2008)
	Croton eluteria	Euphorbiaceae		Berje-USA	Kong et al. (2006)
	Cupressus funebris	Cupressaceae		Berje-USA	Kong et al. (2006)
	Cymbopogon martini	Poaceae		Berje-USA	Kong et al. (2006)
	Daucus carota	Apiaceae	Seeds	Berje-USA	Kong et al. (2006)
				France	Park et al. (2007)
	Ferula galbaniflua	Apiaceae		Berje-USA	Kong et al. (2006)
	Gaultheria procumbens	Ericaceae		Berje-USA	Kong et al. (2006)
	Helichrysum angustifolium	Asteraceae		Berje-USA	Kong et al. (2006)
	Hyssopus officinalis	Lamiaceae	Flowering plant	Berje-USA	Kong et al. (2006)
				France	Park et al. (2007)
	Juniperus communis	Cupresaceae		Berje-USA	Kong et al. (2006)
	Juniperus oxycedrus	Cupresaceae		Berje-USA	Kong et al. (2006)
	Juniperus virginiana	Cupresaceae		Berje-USA	Kong et al. (2006)

Table 1 continued

Nematode species evaluated	Plant source	Family plant	Part used	Country	Reference
	Lavandula angustifolia	Lamiaceae		Jin-Ah Korea	Kong et al. (2006)
	Lavandula intermedia	Lamiaceae		Berje-USA	Kong et al. (2006)
	Levisticum officinale	Apiaceae		Berje-USA	Kong et al. (2006)
	*Litsea cubeba**	Lauraceae	Fruits	Berje USA	Kong et al. (2006)
				Vietnam	Park et al. (2007)
	Melaleuca alternifolia	Myrtaceae		Berje-USA	Kong et al. (2006)
	Melaleuca viridiflora	Myrtaceae		Berje-USA	Kong et al. (2006)
	Melissa officinalis	Lamiaceae		Berje-USA	Kong et al. (2006)
	Mentha piperita	Lamiaceae		Berje-USA	Kong et al. (2006)
	Mentha pulegium	Lamiaceae	Aerial parts	Berje-USA	Kong et al. (2006)
				Portugal	Barbosa et al. (2010)
	Mentha spicata	Lamiaceae		Jin-Ah Korea	Kong et al. (2006)
	Myristica fragrans	Miristicaceae		Berje-USA	Kong et al. (2006)
	Myrtus communis	Myrtaceae	Aerial parts	Jin-Ah Korea	Kong et al. (2006)
				Portugal	Barbosa et al. (2010)
	*Origanum vulgare**	Lamiaceae	Aerial parts	Berje-USA	Kong et al. (2006)
				Portugal	Barbosa et al. (2010)
	Petroselinum crispum	Apiaceae		Berje-USA	Kong et al. (2006)
	*Pimenta dioica**	Myrtacea	Berries	Berje-USA	Kong et al. (2006)
				Jamaica	Park et al. (2007)
	Pimenta officinalis	Myrtacea		Berje-USA	Kong et al. (2006)
	Pimpinella anisum	Apiaceae		Berje-USA	Kong et al. (2006), Elbadri et al. (2008)
	Piper nigrum	Piperaceae		Jin-Ah Korea	Kong et al. (2006)
	Pogostemon patchouli	Lamiaceae	Whole plant	Jin-Ah Korea	Kong et al. (2006)
				Indonesia	Park et al. (2007)
	Rosa damascene	Rosaceae		Berje-USA	Kong et al. (2006)
	Rosmarinus officinalis	Labiatae		Berje-USA	Kong et al. (2006)
	Salvia lavendulaefolia	Lamiaceae		Jin-Ah Korea	Kong et al. (2006)
	Salvia officinalis	Lamiaceae		Jin-Ah Korea	Kong et al. (2006)
	Salvia sclarea	Lamiaceae		Jin-Ah Korea	Kong et al. (2006)
	Santalum album	Santalaceae		Jin-Ah Korea	Kong et al. (2006)
	Sassafras albidum	Lauraceae	Wood	Berje-USA	Kong et al. (2006)
				India	Kim et al. (2008)
	Satureja hortensis	Lamiaceae		Berje	Kong et al. (2006)
	Tagetes glandulifera	Asteraceae		Jin-Ah Korea	Kong et al. (2006)
	Thuja occidentalis	Cupressaceae		Jin-Ah Korea	Kong et al. (2006)
	Thymus capitatus	Lamiaceae		Berje USA	Kong et al. (2006)
	Thymus mastichina	Lamiaceae	Aerial parts	Berje-USA	Kong et al. (2006)
				Portugal	Barbosa et al. (2010)
	*Thymus vulgaris**	Lamiaceae		Berje USA	Kong et al. (2006), Elbadri et al. (2008)
	Valeriana officinalis	Valerianaceae	Roots	Berje-USA	Kong et al. (2006)
				China	Kim et al. (2011)

Table 1 continued

Nematode species evaluated	Plant source	Family plant	Part used	Country	Reference
	Vetiveria zizanoides	Poaceae		Jin-Ah Korea	Kong et al. (2006)
	Zingiber officinale	Zingiberaceae		Jin-Ah Korea	Kong et al. (2006)
	Acorus gramineus	Araceae	Roots	Korea	Choi et al. (2007b)
	Agastache rugosa	Labiatae	Whole plant	Korea	Choi et al. (2007b)
	Amomum cardamomum	Zingiberaceae	Fruit	Korea	Choi et al. (2007b)
	*Amomum globosum**	Zingiberaceae	Fruits	Korea	Choi et al. (2007b)
	Artemisia capillaries	Asteraceae	Whole plant	Korea	Choi et al. (2007b)
	Asiasarum heterotropoides	Aristolochiaceae	Root	Korea	Choi et al. (2007b)
	Atractylodes japonica	Asteraceae	Root	Korea	Choi et al. (2007b)
	Carpesium abrotanoides	Asteraceae	Fruits	Korea	Choi et al. (2007b)
	Curcuma zedoaria	Zingiberaceae	Root	Korea	Choi et al. (2007b)
	Cyperus rotundus	Cyperaceae	Root	Korea	Choi et al. (2007b)
	Forsythia koreana	Oleaceae	Fruits	Korea	Choi et al. (2007b)
	Juniperus chinensis	Cupressaceae	Wood	Korea	Choi et al. (2007b)
	Myristica fragrans	Myristicaceae	Fruits	Korea	Choi et al. (2007b)
	*Paeonia moutan**	Paeoniaceae	Roots	Korea	Choi et al. (2007b)
	*Perilla frutescens**	Labiatae	Leaves	Korea	Choi et al. (2007b)
	Poncirus trifoliata	Rutaceae	Fruits	Korea	Choi et al. (2007b)
	Santalum album	Santalaceae	Wood	Korea	Choi et al. (2007b)
	Saussurea lappa	Asteraceae	Roots	Korea	Choi et al. (2007b)
	Schizandra chinensis	Schizandraceae	Fruits	Korea	Choi et al. (2007b)
	*Schizonepeta tenuifolia**	Labiatae	Whole plant	Korea	Choi et al. (2007b)
	Styrax benzoin	Styraceae	Resin	Korea	Choi et al. (2007b)
	Zanthoxylum piperitum	Rutaceae	Fruits	Korea	Choi et al. (2007b)
	*Allium cepa**	Liliaceae	Bulb	Germany	Choi et al. (2007c)
	Cinnamonum cassia	Lauraceae	Leaves	Korea	Kong et al. (2006)
	Amyris balsamifera	Rutaceae	Wood	Caribbean	Park et al. (2007)
	Artemisia afra	Asteraceae	Flowering plant	South Africa	Park et al. (2007)
	Cananga odorata	Annonaceae	Blossoms	Indonesia	Park et al. (2007)
	Canarium luzonicum	Burseraceae	Resin	Phillipines	Park et al. (2007)
	Citrus clementina	Rutaceae	Rind	South Africa	Park et al. (2007)
	Copaifera reticulata	Fabaceae	Resin	Brazil	Park et al. (2007)
	Dipterocarpus turbinatus	Dipterocarpaceae	Resin	Indonesia	Park et al. (2007)
	Elettaria cardamomum	Zingiberaceae	Seeds	Equador	Park et al. (2007)
	Ferula galbaniflua	Apiaceae	Resin	Iran	Park et al. (2007)
	Fokienia hodgensii	Cupressaceae	Wood	Vietnam	Park et al. (2007)
	Larix europea	Pinaceae	Resin	Austria	Park et al. (2007)
	Lavandula hybrida	Lamiaceae	Flowering plant	France	Park et al. (2007)
	Melaleuca cajuputii	Myrtaceae	Leaves	Indonesia	Park et al. (2007)
	Myrocarpus fastigiatus	Fabaceae	Wood	Brazil	Park et al. (2007)
	*Trachyspermum ammi**	Apiaceae	Seeds	India	Park et al. (2007)
	*Brassica integrafolia**	Brassicaceae		Korea	Elbadri et al. (2008)

Table 1 continued

Nematode species evaluated	Plant source	Family plant	Part used	Country	Reference
	*Pelargonium inquinans**	Geraniaceae		Korea	Elbadri et al. (2008)
	*Syzygium aromaticum**	Myrtaceae		Korea	Elbadri et al. (2008)
	Ammi visnaga	Apiaceae	Flowering plant	Morocco	Kim et al. (2008)
	Artemisia arborescens	Asteraceae	Flowering plant	Morocco	Kim et al. (2008)
	Chrysanthemum morifolium	Asteraceae	Flowering plant	China	Kim et al. (2008)
	Commiphora myrrha	Burseraceae	Resin	Somalia	Kim et al. (2008)
	Cyperus scariosus	Cyperaceae	Roots	India	Kim et al. (2008)
	Eriocephalus punctulatus	Asteraceae	Flowering plant	South Africa	Kim et al. (2008)
	Helichrysum angustifolium	Asteraceae	Blossoms	Croatia	Kim et al. (2008)
	Inula racemosa	Asteraceae	Roots	India	Kim et al. (2008)
	Leptospermum ericoides	Myrtaceae	Leaves	New Zealand	Kim et al. (2008)
	Lippia javanica	Verbenaceae	Flowering plant	Zimbabwe	Kim et al. (2008)
	*Liquidambar orientalis**	Altingiaceae	Resin	Turkey	Kim et al. (2008)
	Michelia alba	Magnoliaceae	Leaves	China	Kim et al. (2008)
	Miroxylon balsamum	Fabaceae	Resin	El Salvador	Kim et al. (2008)
	Nigella sativa	Ranunculaceae	Seeds	India	Kim et al. (2008)
	Ormensis multicaulis	Asteraceae	Blossoms	Morocco	Kim et al. (2008)
	Pastinaca sativa	Apiaceae	Whole plant	Croatia	Kim et al. (2008)
	Pogostemon patchouli	Lamiaceae	Whole plant	Indonesia	Kim et al. (2008)
	Salvia stenophylla	Lamiaceae	Leaves	South Africa	Kim et al. (2008)
	Styrax benzoin	Styracaceae	Resin	Indonesia	Kim et al. (2008)
	*Valeriana wallichii**	Valeraniaceae	Roots	India	Kim et al. (2008)
	Calamintha baetica	Lamiaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	*Chamaespartium tridentatum**	Fabaceae	Flowers	Portugal	Barbosa et al. (2010)
	Chamomilla recutita	Asteraceae	Flowers	Portugal	Barbosa et al. (2010)
	Cistus ladanifer	Cistaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	Crithmum maritimum	Apiaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	Cryptomeria japonica	Taxodiaceae	Leaves	Portugal	Barbosa et al. (2010)
	Foeniculum vulgare	Apiaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	Juniperus brevifolia	Cupressaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	Laurus azorica	Lauraceae	Aerial parts	Portugal	Barbosa et al. (2010)
	Laurus nobilis	Lauraceae	Leaves	Portugal	Barbosa et al. (2010)
	Lavandula dentata	Lamiaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	Lavandula luisieri	Lamiaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	Lavandula stoechas	Lamiaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	Lavandula viridis	Lamiaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	Myrtus communis	Myrtaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	Pittosporum undulatum	Pittosporaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	Salvia officinalis	Lamiaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	*Satureja montana**	Lamiaceae	Leaves	Portugal	Barbosa et al. (2010)
	*Thymbra capitata**	Lamiaceae	Aerial parts	Portugal	Barbosa et al. (2010)
	*Thymus caespititius**	Lamiaceae	Aerial parts	Portugal	Barbosa et al. (2010)

Table 1 continued

Nematode species evaluated	Plant source	Family plant	Part used	Country	Reference
	Thymus zygis	Lamiaceae	Aerial parts	Portugal	Barbosa et al. (2010)
				Spain	Kim et al. (2011)
	Artemisia dracunculus	Asteraceae	Plant	France	Kim et al. (2011)
	Abelmoschus seminis	Malvaceae	Seeds	India	Kim et al. (2011)
	Boswellia serrata	Burseraceae	Resin	India	Kim et al. (2011)
	Bulnesia sarmientoi	Zygophyllaceae	Wood	Brazil	Kim et al. (2011)
	Chamecyparia obutusa	Cupressaceae	Wood	Japan	Kim et al. (2011)
	Croton niveous	Euphorbiaceae	Bark	Latin America	Kim et al. (2011)
	Dipteryx odorata	Fabaceae	Seeds	Brazil	Kim et al. (2011)
	Ferula galbaniflua	Apiaceae	Resin	Iran	Kim et al. (2011)
	*Gaultheria fragrantissima**	Ericaceae	Leaves	Nepal	Kim et al. (2011)
	Hedychium spicatum	Zingiberaceae	Roots	India	Kim et al. (2011)
	Helichrysum bracteiferum	Asteraceae	Blossoms	South Africa	Kim et al. (2011)
	Hypericum perforatum	Asteraceae	Plant	France	Kim et al. (2011)
	Lippia citriodora	Verbenáceas	Flowering plant	Morocco	Kim et al. (2011)
	Myrtus communis	Myrtaceae	Flowering plant	Morocco	Kim et al. (2011)
	Michelia alba	Magnoliaceae	Blossoms	China	Kim et al. (2011)
	Nelumbo nucifera	Nymphaceas	Blossoms	India	Kim et al. (2011)
	Osmanthus fragrans	Oleaceae	Blossoms	China	Kim et al. (2011)
	Petroselinum sativum	Apiaceae	Herb	Hungary	Kim et al. (2011)
	Populus balsamifera	Salicaceae	Leaves	Canada	Kim et al. (2011)
	Tagetes minuta	Asteraceae	Blossoms	Egypt	Kim et al. (2011)
	Polianthes tuberose	Salicaceae	Blossoms	Brazil	Kim et al. (2011)
	Tasmannia lanceolata	Winteraceae	Fruits	New Zealand	Kim et al. (2011)
	Vitis vinifera	Vitaceae	Yeast	France	Kim et al. (2011)
	*Zanthoxylum alatum**	Rutaceae	Fruits	Nepal	Kim et al. (2011)

Active plant EOs are marked with asterisk

pinewood nematode were achieved with EOs from *Chamaespartium tridentatum*, *Origanum vulgare*, *Satureja montana*, *Thymbra capitata* and *Thymus caespititius* (Barbosa et al. 2010).

The nematicidal effects of EOs from spices and medicinal plants on root-knot nematodes have been widely reported. Several studies described the high mortality that EOs of *Cymbopogon* grasses (*Cymbopogon martini motia*, *C. flexuosus* and *C. winterianus*) caused to juveniles (J2) of root-knot nematodes (*Meloidogyne incognita* and *M. javanica*) (Sangwan et al. 1985; Saxena et al. 1987). Oka et al. (2000) evaluated the nematicidal activity of 25 plant spices and aromatic plants against *M. javanica*. They showed that EOs from *Artemisia judaica, Carum carvi, Corridothymus capitatus, Cybopogon citratus, Foeniculum vulgare, Mentha rotundifolia, Mentha spicata* caused J2 immobilization and egg hatching inhibition at 1,000 µl/l. High toxicity of *Cymbopogon* (*C. citratus, C. flexuous, C. martinii, C. nardus* and *C.winterianus*), *Mentha* (*M. arvensis, M. citrata, M. piperita, M. pulegium and M. spicata*), *Perlargonium graveolens* and *Ocimun basilicum* EOs to *M. incognita* J2 at different concentrations has been reported (Leela et al. 1992; Pandey et al. 2000; Sinha et al. 2006; Moreira et al. 2009; Ntalli et al. 2010). Pérez et al. (2003) demonstrated that *Chrysantemum coronarium* oil (at 2, 4, 8, and 16 µl/ml), significantly reduced egg hatching, J2 survival and reproduction rate of *M. artiella.* EOs from *Eucalyptus* spp. also have nematicidal activity (Batish et al. 2008). Specifically, *E. citriodora* and *E. hybrida*, are toxic to *M. incognita* J2 (Saxena et al. 1987) even at low concentrations (Pandey et al. 2000), *E. camadulensis*, *E. saligma* and *E. urophylla* caused the mortality of *M. exigua* J2 (Salgado et al. 2003) and *E. globulus* and *E. meliodora* acted on *M. incognita* (Gupta et al. 2011; Ntalli et al. 2011). High mortality of *M. exigua* J2 was induced by *Bixa orellana*, *Melia azedarach* and *Xylopia brasiliensis* oils (Salgado et al. 2003). EOs from aerial parts of *Pectis oligocephala* and *P. apodocephala* exhibited mortality effects against *M. incognita* J2 (Albuquerque et al. 2007). A soy lecithin/detergent formulation of clove (*Eugenia caryophyllata*) oil induced egg and J2 mortality of *M. incognita* (Meyer et al. 2008) and the application of clove oil reduced *M. hapla* gall numbers of on carrots (Douda et al. 2010). *Tagetes* spp. are known for their ability to suppress plant-parasitic nematodes in the field (Krueger et al. 2007) and a further in vitro study has revealed that different oil concentrations (1–4 %) from *T. minuta* have strong toxicity to eggs and juveniles of *M. incognita* (Adekunle et al. 2007). The potential of *Haplophyllum tuberculatum* and *Plectranthus cylindraceus* oils to control root-knot nematodes have been investigated. Although both oils were toxic to *M. javanica* (12.5 µg/ml), the greatest juvenile toxicity and egg hatching inhibition effects were obtained by 1:1 mixtures of the two oils (Onifade et al. 2008). EOs from *Lippia* spp. (*L. sidoides and L. alba*) induced *M. incognita* J2 mortality (Moreira et al. 2009; Marino et al. 2012). Ntalli et al. (2010) have shown high nematicidal activity of *Origanum vulgare* and *O. dictamnus* EOs against *M. incognita*, with 1.55 and 1.72 µl/ml EC_{50} respectively. Moreover, *O. majorana* essential oil has been tested for *M. hapla* management on carrot (Douda et al. 2010). Recently, nematicidal effects against *M. incognita* of EOs from *Chenopodium ambrosioides*, *Foenicum vulgare, Kadsura heteroclita, Pistacia terebinthus, Pimpinella anisum* have been demonstrated (Chuan et al. 2011; Li et al. 2011; Ntalli et al. 2011), while Sosa et al. (2012) have shown strong toxic effects of *Eupatorium viscidum* essential oil to *M. javanica* juveniles.

Results and discussion

Nematicidal EOs from Spanish cultivated Lamiaceae

The nematicidal effects of the selected EOs from experimentally cultivated Spanish aromatic plants are shown in Tables 2, 3 and 4. Most of the tested oils, *M. arvensis*, *M. rotundifolia, M. spicata, Satureja montana, Thymus mastichina*, *T. vulgaris* and *T. zygis,* induced 100 % mortality of J2 *M. javanica* at 1 mg/ml after 12, 48 and 72 h exposure. *S. montana* oil was the most effective with the lowest LC_{50} (0.041 mg/ml) and LC_{90} (0.087 mg/ml). The rest of LC_{50} and LC_{90} values ranged from 0.300 to 0.204 and 0.456–0.320 mg/ml, respectively.

Egg hatching tests are more accurate than counting immobile juveniles in a particular population (Oka et al. 2000). In soil, root knot nematode eggs are aggregated within egg masses surrounded by a gelatinous matrix which serve as a protective barrier against soil-borne antagonists (Kok et al. 2001; Orion et al. 2001). Therefore, the egg hatch inhibition activity tested on egg masses is an indication of the extract/compound ability to penetrate the gelatinous matrix and act on nematode eggs. The egg hatchability test (Table 4) indicated that *Mentha arvensis*, *M. spicata, S. montana, T. vulgaris* and *T. zygis* EOs strongly suppressed *M. javanica* egg hatching after 5 days of incubation. The percentage reduction ranged from 86 to 99 %. After their exposure to the EOs, the hatch rate of eggs masses immersed in water increased over time. At 27 days, *M. spicata*, *M. rotundifolia*, *S. montana,* and *T. vulgaris* oils inhibited over 50 % egg mass hatching, with a relative suppression rate of 61, 58, 52 and 59 % respectively. These results

Table 2 Effects of 11 plant essentials oils (1 µg/µl) on mortality of second stage juveniles (J2) of *Meloidogyne javanica*

Botanical name	J2 mortality (%)[a] after		
	24 h	48 h	72 h
Hyssopus officinalis	0	0	0
Lippia alba	0	0	10.6
Mentha arvensis	100	100	100
M. longifolia	0	0	0
M. piperita	0	0	4.5
M. rotundifolia	100	100	100
M. spicata	100	100	100
Satureja montana	100	100	100
Thymus mastichina	100	100	100
T. vulgaris	100	100	100
T. zygis	100	100	100

[a] Corrected according to Scheider–Orelli's formula. Values are means of four replicates

Table 3 LC_{50} and LC_{90} values of selected EOs against second stage juveniles (J2) of *Meloidogyne javanica*

Botanical name	LC_{50} mg/ml[a] (95 % CL[b])	LC_{90} mg/ml[a] (95 % CL[b])
Mentha arvensis	0.291 (0.284–0.298)	0.409 (0.396–0.425)
M. rotundifolia	0.204 (0.196–0.213)	0.320 (0.308–0.334)
M. spicata	0.293 (0.286–0.300)	0.403 (0.392–0.418)
Satureja montana	0.041 (0.037–0.044)	0.087 (0.083–0.094)
Thymus mastichina	0.300 (0.289–0.313)	0.456 (0.430–0.489)
T. vulgaris	0.224 (0.218–0.230)	0.337 (0.327–0.348)
T. zygis	0.226 (0.220–0.233)	0.333 (0.323–0.344)

[a] Mortality was observed 72 h after treatment. Five concentrations were used to obtain LC_{50} and LC_{90}, and four replicates were used for each treatment

[b] CL denotes confidence limit

Table 4 Effects of active essentials oils (1 µg/µl) on hatching of eggs in eggs masses of *M. javanica* in time

Botanical name	Relative hatch suppression rate (%)[a] in time[b]					
	0	2	7	13	20	27
Mentha arvensis	86	67	35	42	37	36
M. rotundifolia,	41	73	67	71	67	58
M. spicata	94	95	70	69	63	61
Satureja montana	98	85	57	59	53	52
Thymus mastichina	0	30	6	11	10	10
T. vulgaris	96	76	69	62	60	59
T. zygis	99	90	51	38	35	34

[a] Each value represents the hatch inhibition in the respective treatment corrected according to the control (Scheider–Orelli's formula). Values are means of four replicates

[b] Time 0: after 5 days of immersion in test solutions; time 2: 2 days of immersion in water after time 0; time 7:7 days of immersion in water after time 0; time 13: 13 days of immersion in water after time 0; time 20: 20 days of immersion in water after time 0; time 27: 27 days of immersion in water after time 0

indicate that the effects of EOs tested are stronger on J2 mortality than on egg mass hatching.

The chemical compositions of the EOs evaluated are shown in Table 5. A total of 13 compounds were identified. In *Mentha arvensis* and *M. spicata* oils, the most abundant compound was carvone, followed by 1,8-cineole and menthol. The major components of *M. rotundifolia* oil were piperitone oxide and two unidentified compounds. Thymol was found as the main compound in *T. vulgaris* and *T. zygis* oil, followed by p-cymene, γ-terpinene and carvacrol. Carvacrol was the major constituent of *S. montana* oil. The most abundant compound in *T. mastichina* oil was 1,8-cineole, followed by α-terpineol, linalool and β-pinene.

Nematicidal activity of oils from *Mentha* species has been widely studied. High activity has been recorded in vitro for oils of *M. rotundifolia* and *M. spicata* against *M. javanica* (Oka et al. 2000) and for *M. arvensis* and *M spicata* oils to *M. incognita* (Pandey et al. 2000; Sinha et al. 2006). Nonetheless this is the first report of the nematicidal effects of *M. arvensis* oil to *M. javanica.* Among *Thymus* species, only *T. vulgaris* oil has been tested against root-knot nematodes by Oka et al. (2000), but only a moderate effect on J2 immobilization and egg hatching inhibition of *M. javanica* at 1,000 µl/l was reported. This divergence with the present findigs may be explained by intraspecific variability in plant chemical composition (Koul et al. 2008). *T. vulgaris* is native to the Mediterranean region. It grows wild in almost all the countries bordering Mediterranean area, Asia and Central Europe and it is extensively cultivated in Spain, Germany, France, England and various other neighboring countries, so there are a

Table 5 Main components of evaluated essential oils

Botanical name	Main components
Hyssopus officinalis	1,8-Cineole (53 %), β-pinene (12 %), pinocamphone (6 %)
Lippia alba	Linalool (77 %), 1,8-cineole (5 %)
Mentha arvensis	Carvone (75 %), 1,8-cineole (14 %), menthol (1 %)
M. longifolia	Menthone (49 %), menthol (24 %)
M. piperita	Menthone (41 %), menthol (31 %)
M. rotundifolia	Piperitone oxide (25 %), M^+(166), 67, 138, 41 (25 %), M^+(166), 67, 138, 41 (25 %)
M. spicata	Carvone (79 %), 1,8-cineole (12 %), menthol (2 %)
Satureja montana	Carvacrol (76 %), p-cymene (2 %)
Thymus mastichina	1,8-Cineole (79 %), α-terpineol (6 %), linalool (3 %), β-pinene (2 %)
T. vulgaris	Thymol (49 %), p-cymene (29 %) γ-terpinene (7 %), carvacrol (4 %)
T. zygis	Thymol (74 %), p-cymene (9 %) γ-terpinene (7 %), carvacrol (4 %)

large number of ecotypic variations and chemotypic races or populations. The nematicidal activity of *T. vulgaris* oil could significantly increase its commercial value since the global production of oil is 20–30 tons per year and in Spain is the largest producer (Joy et al. 2001).

This study demonstrates for the first time the nematicida activity of *S. montana, T. zygys* and *T. mastichina* EOs against root-knot nematodes. Oils from *T. zygys* and *S. montana* have shown nematicidal effects against *B. xylophylus* (Kong et al. 2006; Barbosa et al. 2010) but no nematicidal activity was found of *T. mastichina* essential oil to the pinewood nematode (Kong et al. 2006; Barbosa et al. 2010). Both *Thymus* species*, T. mastichina* and *T. zygys,* are Iberian endemic plants. The nematicidal effects of their oils are of interest for future integrated pest management programmes (Koul et al. 2008) and to promote their cultivation in the Mediterranean region.

Nematicidal activity of EO major components

A series of 13 mono and sesquiterpenes (Fig. 1) and seven paired mixtures were selected to be tested on *M. javanica* based on the EOs composition (Tables 6 and 7). The highest mortality effects (100 %) on *M. javanica* J2 (0.5 mg/ml, 72 h incubation) were induced by carvacrol, geraniol and thymol, followed by citronellol. These compounds were also very effective at egg mass hatching inhibition and their values of relative suppression rate ranged from 71 % (geraniol) to 91 % (carvacrol). These results agreed with previous findings where carvacrol, geraniol and thymol showed high nematicidal activity against *M. javanica* (Oka 2001), *M. incognita* (Al-Banna et al. 2003; Ntalli et al. 2010), and *B. xylophilus* (Choi et al. 2007a; Kong et al. 2007; Park et al. 2007) while 1,8-cineole, menthol, menthone and pinene were not effective against *M. javanica* (Al-Banna et al. 2003).

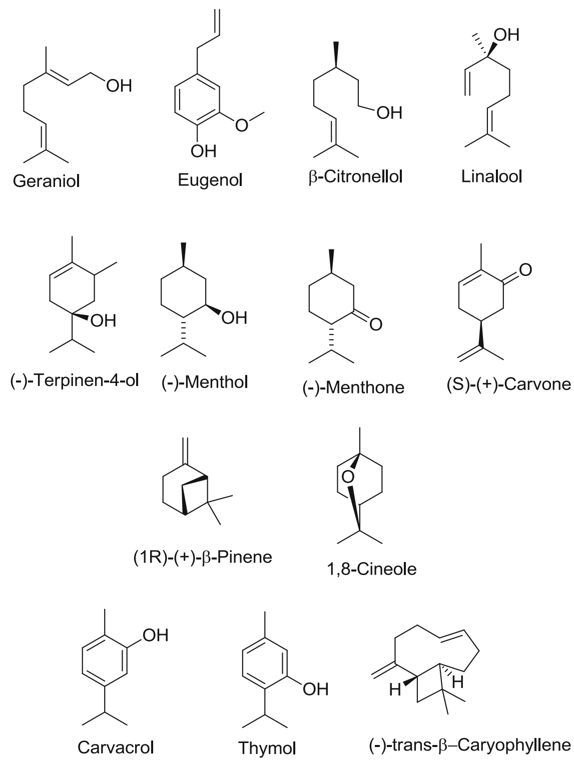

Fig. 1 Chemical structure of the terpenoids tested against *Meloidogyne javanica*

Table 6 Effects of 13 compounds and seven paired mixtures (0.5 μg/μl) on mortality of second stage juveniles (J2) of *Meloidogyne javanica*

Compound/s	J2 mortality (%)[a] at 72 h
Carvacrol	100
Carvone	3.06
β-Caryophyllene	1.86
1,8-cineole	0
Citronellol	83.1
Eugenol	13.1
Geraniol	100
Linalool	0
Menthol	38.6
Menthone	0
β-Pinene	0
Terpineol	0
Thymol	100
Carvone/cineole (80-20)	100
Carvone/cineole (50-50)	1.53
Carvone/menthol (95-5)	97.3
Carvone/menthol (50-50)	5.94
Cineole/β pineno (95-5)	3.6
Cineole/terpineol (90-10)	2
Menthol/cineole (25:75)	6

[a] Corrected according to Scheider–Orelli's formula. Values are means of four replicates

Interestingly, Echeverrigaray et al. (2010) indicated that among acyclic alcohols, those with the hydroxyl group at C1 (geraniol and citronellol) were more effective against *M. incognita* than linalool, with the hydroxy group at C3 (Fig. 1). Similarly to our results, geraniol exhibited higher nematicidal activity to *M. incognita* than citronellol, indicating that the position of the functional group and the double bond can also affect the activity of the monoterpenoid (Park et al. 2007).

Among the binary terpene mixtures, only carvone/cineole (0.4/0.1 mg/ml) and carvone/menthol (0.475/0.025 mg/ml) were active, causing 100 and 97 % J2 mortality and 87 and 85 % final egg mass hatch suppression respectively. It is noteworthy that each component alone (carvone, 1,8-cineole and menthol) and the same component mixtures at different concentrations showed no nematicidal activity, suggesting a synergistic interaction of these compounds at certain concentrations.

The nematicidal activity of *S. montana*, *T. vulgaris* and *T. zygis* EOs could be explained by their content in active carvacrol (88 %) and thymol (49 and 74 %) respectively. The nematicidal effects of *M. arvensis* and *M. spicata* EOs could be attributed to synergistic interaction of carvone with 1,8-cineole and menthol. The activity of *M. rotundifolia* EO could be due to other components such as isomers of 1,2-epoxymenthylacetate as suggested by Oka et al. (2000) since piperitone lacked nematicidal effects. On the other hand, the nematicidal activity of *T. mastichina* EO did not correlate with that of its major components alone (1,8-cineole, terpineol, linalool and β-pinene) or in binary mixtures of 1,8-cineole with other compounds. Therefore, the contribution of each component to the activity of an EO follows a complicated pattern of interactions (Ntalli et al. 2010) including synergistic and/or antagonistic interactions that contribute to the overall toxicity of the oil (Lahlou 2004).

Table 7 Effects of active compounds and paired mixtures (0.5 μg/μl) on hatching of eggs in eggs masses of *M. javanica* in time

Compound	Relative hatch suppression rate (%)[a] in time[b]				
	0	3	9	16	23
Carvacrol	98	96	94	92	91
Citronellol	82	79	77	79	80
Geraniol	21	70	63	68	71
Thymol	99	93	86	84	84
Carvone/cineole (80/20)	98	94	97	86	87
Carvone/menthol (95/5)	93	95	85	84	85

[a] Each value represents the hatch inhibition rate in the respective treatment corrected according to the control (Scheider–Orelli's formula). Values are means of four replicates

[b] Time 0: after 5 days of immersion in test solutions; time 3: 3 days of immersion in water after time 0; time 9:9 days of immersion in water after time 0; time 16: 16 days of immersion in water after time 0; time 23: 23 days of immersion in water after time 0

The mode of action of EOs and their constituents is of practical importance for nematode control because it may give useful information on the most appropriate formulation and delivery means. Oka et al. (2000) have reported a strong relationship between nematicidal and insecticidal activity and suggested the involvement of EOs components in interrupting the nematode nervous system. Some EOs have been reported to interfere with the neuromodulator octopamine (Kostyukosky et al. 2000) or GABA-gated chloride channels of insect pests (Priestley et al. 2003) and Lee et al. (2001) showed evidence that EOs of *Mentha* species inhibited AChE activity. In addition, EOs may disrupt the cell membrane of the nematode and change its permeability (Oka et al. 2000). Lambert et al. (2001) and Bakkali et al. (2008) reported that EOs damaged bacteria and yeast membrane integrity, affecting pH cellular homeostasis and equilibrium of inorganic ions. The nematicidal activity of carvacrol and thymol, might be mediated through the tyramine receptor, as the two compounds were able to trigger a signaling cascade that lead to nematode mortality by interacting with a receptor like SER-2 (Lei et al. 2010). Likewise, it has been reported that the activity of geraniol and citronellol are essentially due to membrane and ion channel perturbations modifying membrane-bound protein activity and the intracellular signaling pathways (Warber 1998; Tsuchiya 2001; Kaur et al. 2011).

Conclusions

Essential oils contain functional nematicide compounds which could be used for the control of pinewood (*B. xylophilus*) and root-knot nematodes (*Meloidogyne* spp.). Experimentally cultivated Spanish aromatic plants EOs have nematicide properties, but, direct in vitro tests must be complemented by in vivo soil based experiments to confirm the efficacy of this aromatic plant EOs.

This review demonstrates the significant progress undertaken in the last decade to investigate the nematicidal activity of plant EOs and these constituents. Although commercial nematicides based on plant EOs have not yet appeared in the market place there is a clear need to develop novel products based on essential oil formulations. These formulations should target plant parasitic nematodes affecting high-value crops and substitute existing commercial nematicides coming off the market due to changes in the Plant Protection Products Directive (91/414/EEC). The main advantages of using essential oil-based pesticides are their low mammalian toxicity and environmental persistence. Their short residual half lives on plants also enhance their compatibility with biological control agents (Isman et al. 2011). Moreover, the potential disadvantages as limited persistence and phytotoxicity could be mitigated by the application of microencapsulation techniques EOs when formulated, to protect them from degradation, evaporation and to provide a controlled release. In this sense, exhaustive studies have been recently performed in order to obtain stable capsules of EOs using biodegradable polymers as carrier materials and high effectiveness of microencapsulation has been achieved by high-pressure technology application (supercritical fluids processes) (Martín et al. 2010; Varona et al. 2009; Varona et al. 2010). At present, nematicide EOs based products may be developed, with formulations that provide efficacy at low concentrations, to minimize risk to the environment. This will enhance user safety and is likely to provide more durable pest control due to the complexity of chemical composition and naturally occurring variability.

Materials and methods

Plant materials

A series of aromatic plant species were selected for their experimental cultivation based on their medicinal and/or condiment value (Burillo and García-Vallejo 2003). Among the species cultivated the following were selected to be tested for their nematicidal effects: *Hyssopus officinalis, Lippia alba*, *Mentha arvensis*, *M. longifolia*, *M. piperita, M. rotundifolia*, *M. spicata, Satureja montana, Thymus mastichina*, *T. vulgaris* and *T. zygis.*

Essential oil and extract preparation

Flowers and leaves were manually separated from the twigs and then hydrodistilled separately for 2 h in a Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia.

Essential oil analysis

Flower and leaf EOs were analyzed by GC-MS using an Agilent 6890 N gas chromatograph (Agilent Technologies, Palo Alto, California, USA) coupled to an Agilent 5973 N mass detector (electron ionization, 70 eV) (Agilent Technologies, Palo Alto, California, USA) and equipped with a 25 m × 0.2 mm id HP-1 (methylpolyisiloxane, 0.20 µm film thickness) and a 30 m × 0.25 mm id Carbowax (polyethylene glycol, 0.25 µm film thickness) capillary columns (Hewlett-Packard). Working conditions were as follows: injector temperature, 260 °C; temperature of the transfer line connected to the mass spectrometer, 280 °C; column temperature 70–90 °C, 5 °C min^{-1}. El mass spectra and retention data were used to assess the identity of compounds by comparing them with those of standards or found in the Wiley Mass Spectral Database (Wiley, 2001). Quantitative data were obtained from the TIC peak areas without the use of response factors.

Chemicals

Analytical standards of 1,8-cineole (99 %), (+)-carvone (98,5 %) and carvacrol (97 %) were purchased from Fluka (Buchs Switzerland). Eugenol (99 %), geraniol (98 %), β-caryophyllene (80 %), β-citronellol (99 %), linalool (99 %), (−)-menthone (90 %) menthol (99 %) β-pinene (99 %) α-terpineol (97 %) and thymol (99.5 %) were purchased from Sigma-Aldrich.

Nematodes

M. javanica population was maintained on *Lycopersicon esculentum* plants (var. Marmande) in pot cultures at 25 ± 1 °C, > 70 % relative humidity. Egg masses of *M. javanica* were handpicked from infected tomato roots. Second-stage juveniles (J2) were obtained from hatched eggs by incubating handpicked egg masses in a water suspension at 25 °C for 24 h.

Bioassays

Effect on J2

EOs and their components were suspended in distilled water containing DMSO with 0.5 % Tween 20. Test solutions (5 µl) were filled into a cell of a 96-cell plate (BD Falcon, San Jose, CA, USA) containing 90–150 nematodes in 95 µl of test solution. Thus, the total volume of the solution in a cell was 100 µl. The concentration of EOs and each components tested in a cell was 1 and 0.5 mg ml^{-1}, respectively. As a control, four wells were treated with the water/DMSO/Tween 20 in the same volume as the tests. All treatments were replicated four times. The plates were covered to prevent evaporation and were maintained in the dark at 25 °C. After 72 h, the dead J2 were counted under a binocular microscope (20×). The J2 were considered dead when they did not move on probing with a fine needle (Cayrol et al. 1989). In addition, the most nematicidal essential oils, which exhibited over 99 % mortality rates, were further screened to asses J2 mortality after 24 and 48 h using the immersion bioassay and for the determination of LC_{50} and LC_{90} values after 72 h.

Effect on egg mass hatching

Three (for EOs) or two (for the components) sterilized healthy egg masses of nearly uniform size were transferred to a 96-well plate (BD Falcon, San Jose, CA, USA) containing different treatments solutions described above with nematicidal activity.

Egg masses placed in sterilized distilled water served as controls. Four replicates of each treatment and control were included. The plates were covered to prevent evaporation and incubated in the dark at 25 °C. After 5 days the hatched J2 were counted, test solutions were removed and wells with egg mass were washed and filled with sterilized distilled water. The egg hatch was monitored over 4 weeks, until hatching was complete in the control treatment, and then relative hatching percentages (compared with the controls) were recorded.

Statistical analysis

The data of nematicidal activity are presented as percent dead J2 corrected according to Scheider—Orelli's formula. Effective lethal doses (LC_{50} and LC_{90}) were calculated by Probit analysis. Five concentrations of selected EOs were used to obtain the LC_{50} and LC_{90} and four replicates were used in each concentration.

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