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Research paper

Efficacy of sea fennel (*Crithmum maritimum* L., Apiaceae) essential oils against *Culex quinquefasciatus* Say and *Spodoptera littoralis* (Boisd.)

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ABSTRACT

The effective management of insect pests and vectors still represents a major challenge in current entomology. *Culex quinquefasciatus* Say is a key vector of lymphatic filariasis, Rift Valley fever, West Nile, St. Louis encephalitis and Western equine encephalitis virus. Recently, the importance of the eco-friendly control of *C. quinquefasciatus* larval population using plant-borne biopesticides, including essential oils (EOs), has been stressed. *Spodoptera littoralis* (Boisd.) also known as tobacco cutworm, is one of the most destructive moth agricultural pests in tropic and sub-tropic regions. Despite the fact that sea fennel (*Crithmum maritimum* L., Apiaceae) is considered as a promising biosaline crop, its potential for commercial cultivation has not yet been exploited. Notably, it has been reported that in coastal marine environments insect pests stay away from sea fennel plants. This aromatic plant is endowed with secretory structures as ducts and vittae storing volatile compounds. In the present study, the chemical composition of sea fennel essential oils from three different EO accessions, namely France (Brittany), Central and South Italy (Marche and Sicily regions) have been analysed by gas chromatography-mass spectrometry (GC–MS), and their toxicity against larvae of *Cx. quinquefasciatus* and *S. littoralis* was evaluated. Selected pure compounds from the essential oils mentioned above were also tested. The essential oils showed notable variability in chemical composition, being dominated by dillapiole and γ-terpinene (French EO), limonene and γ-terpinene (central Italy EO) and thymol methyl ether and γ-terpinene (Sicilian EO). As found out, the significantly most efficient essential oils were obtained from the seeds and aerial parts of plants of the French region. For these EOs, LD_{50} values were estimated as 62.3 and 71.7 μg/larva for *S. littoralis*, respectively, and LC_{50} as 13.7 and 15.6 μl/L for *C. quinquefasciatus* larvae, respectively. γ-Terpinene and myristicin were the most effective on *C. quinquefasciatus* larvae showing LC_{50} values < 17 μl/L. Overall, these findings shed light on the insecticidal potential of *C. maritimum* EO against mosquitoes and agricultural pests, allowing to identify the most effective chemotype, and to stimulate cultivation of this species as a source of botanical insecticides.

1. Introduction

In the last ten years, the market of botanical insecticides and insect repellents has steadily increased due to the consumers' trust towards eco-friendly and reduced-risk natural products and to the awareness of agrochemical companies on the risk for human health derived from the use of synthetic insecticides (Benelli, 2015a; Pavela and Benelli, 2016a). As an example, it was estimated that the sales of biopesticides and green pesticides in the global pesticide market is expected to increase up to 20% by 2025 (Isman, 2015). Regarding the European Union, at present the European Food Safety Authority (EFSA) promoted the use of botanicals as low-risk active substances (LRASs) to be used for plant protection products through the EC Regulation No. 1107/2009. This should overcome the regulatory hurdles encountered by several botanicals to be used as insecticides (Chandler et al., 2011; Pavela, 2016; Pavela and Benelli, 2016b; Isman, 2017).

Among botanicals, plant essential oils (EOs) are recognized to have not only repellent effects on insects, but also selective contact,

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ingestion, and fumigant toxicity against insect and mite pests (Isman, 2000; Benelli, 2015b; Pavela, 2015a, 2015b; Govindarajan et al., 2016a, 2016b). The most important advantage of the use of EOs seems to be their relatively low mammalian toxicity, along with multiple mechanisms of action against insects (Isman, 2000; Pavela and Benelli, 2016b). For this reason, they result particularly suitable to be used not only as insecticides and crop protectants, but also for applications in schools, restaurants, hospitals and seniors' homes (Isman et al., 2011).

The Integrated Vector Management of mosquito species of public health relevance still represents a major challenge in current entomology and parasitology (Benelli and Mehlhorn, 2016; Benelli and Beier, 2017; Benelli et al., 2016a, 2016b; Ward and Benelli, 2017; WHO, 2016). In a number of Asian countries, *Culex quinquefasciatus* Say is a major vector of lymphatic filariasis, whereas in the African region, this mosquito can vector the Rift Valley fever virus. In the United States, *C. quinquefasciatus* transmits West Nile, St. Louis encephalitis and Western equine encephalitis virus (Turell et al., 2007; Vadivalagan et al., 2017). Recently, several researches stressed the importance of the eco-friendly control of *C. quinquefasciatus* larval population using plant-borne pesticides (Govindarajan and Benelli, 2016; Benelli, 2016a, 2016b).

Concerning agricultural insect pests, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), also known as tobacco cutworm, is one of the most destructive moth agricultural pests in tropic and sub-tropic regions. This species is extremely polyphagous, feeding on at least 87 species of economic importance, including maize, cotton, cereals, potatoes, vegetables and ornamental plants (Baldwin and Graves, 1991). Given that the pest may spread even to the temperate zone due to international transport of ornamental plants and vegetables, EPPO has listed *S. littoralis* as an A2 quarantine pest (OEPP/EPPO, 2015).

Among the botanical families used for the production of EOs, the Apiaceae family represents one of the most important either for the efficacy of its constituents on mosquito vectors and insect pests or for scalability for industrial production (Benelli et al., 2017a; Evergetis et al., 2013; Seo et al., 2015; Afshar et al., 2017). Monoterpene hydrocarbons, oxygenated monoterpenes and phenylpropanoids are the main chemical groups occurring in the Apiaceae EOs, having multiple mode and site of action in the insect nervous system and elsewhere (Isman et al., 2011).

Crithmum maritimum L. (Apiaceae), also known as sea fennel or rock samphire, is an aromatic halophyte, 20–50 cm tall, with pinnatisect, succulent, glabrous and glaucescent leaves, divided in linear-lanceolate leaflets; inflorescences are umbels with 10–32 rays bearing white-greenish flowers; fruits are ovoid schizocarps with 2 mericarps endowed with numerous vittae (Pignatti, 1982). It occurs especially in coastal areas of Europe and Mediterranean Sea, growing on cliffs, sands, stones and walls (Pignatti, 1982).

In Italy, it is locally known with several popular names as *finocchio marino*, *critmo, cretamo*, *spaccasassi*, *bacicci*, *basiggia*, *erba di San Pietro* and *salissia*. The same in France where it is known as *criste-marine*, *fenouil de mer* or *perce-pierre*. Because of its aromatic properties, the sea fennel is appreciated in cuisine, especially to make salads (Cornara et al., 2009). In particular, the leaves are consumed as fresh or steam cooked and as pickled vegetable (Bremness, 2004). In the Mediterranean regions, *C. maritimum* enjoys a good reputation also as a traditional remedy. As an example, in Mallorca (Spain), the pickled young aerial parts are eaten as antiscorbutic, digestive and diuretic (Carrió and Vallès, 2012). In the Amalfi coast (south Italy) the decoction of aerial parts has been used to treat whooping cough and pain (Savo et al., 2011). In Liguria (north Italy), the decoction of aerial parts and leaves were used against inflammations of urinary tracts and prostate, as liver detoxifying and for the relief of colics (Cornara et al., 2009). In central Italy, the juice of the plant is believed to be depurative, diuretic and carminative, whereas the infusion of the fruits is taken as stomachic, digestive and carminative (Tieri, 1984). In the veterinary medicine, the aerial parts are given to rabbits to integrate diet, to stimulate milk production and for healing several ailments (Cornara et al., 2009; Viegi et al., 2003).

In previous phytochemical investigations, the EO composition of *C. maritimum* was proven to be highly sensitive to geographic origin of the samples and other factors, revealing to be characterized by different chemotypes, namely aromatic monoterpenes-type (thymol methyl ether/carvacrol methyl ether), monoterpene hydrocarbons-type (α-pinene/limonene, γ-terpinene/sabinene, sabinene/γ-terpinene/limonene, γ-terpinene/limonene, β-phellandrene/(*Z*)-β-ocimene/*p*-cymene, γ-terpinene/sabinene/β-phellandrene), phenylpropanoids-type (dillapiole, dillapiole/methylchavicole) and their intermediate forms (Katsouri et al., 2011; Glamoclija et al., 2009; Tsoukatou et al., 2001; Senatore et al., 2000; Coiffard et al., 1993; Özcan et al., 2001; Baser et al., 2000; Polatoglu et al., 2016; Barroso et al., 1992; Pateira et al., 1999; Jallali et al., 2014).

The sea fennel EO proved to be effective as antifungal (Glamoclija et al., 2009) and antibacterial agent (Flamini et al., 1999; Senatore et al., 2000).

Despite the fact that sea fennel is considered as a promising biosaline crop, its potential for commercial cultivation has not yet been exploited (Renna et al., 2017). It is worth to mention that sea fennel seems to be promising as a source of botanical insecticides since some insects are repelled by this plant. In this respect, the sea fennel EO proved to be effective against Nylander (*Pheidole pallidula*) ants (Tsoukatou et al., 2001) as well as against stored product pests, especially *Sitophilus* oryzae L. and *Oryzaephilus surinamensis* L. (Coleoptera) (Polatoglu et al., 2016). On the other hand, no data are available on the insecticidal activity of sea fennel EO against the mosquito vector *C. quinquefasciatus* and the moth pest *S. littoralis*. Therefore, in the present work we evaluated the toxicity of different accessions of sea fennel EOs showing different chemical composition against *C quinquefasciatus* and *S. littoralis* larvae. Thus, our goal was to identify the volatile mixture produced by sea fennel most effective against these two insects. Selected pure compounds from the essential oils mentioned above have been also tested. Lastly, the inhibitory activity of essential oils on acetyl cholinesterase (AChE) activity was evaluated on both pests. Results of this study are going to give credit to an overlooked Mediterranean crop and to improve its cultivation as a source of botanical insecticides.

2. Materials and methods

2.1. Plant material

Plant material, including aerial parts, leaves, flowers and seeds (See Table 1), was collected from sea fennel populations growing along sea cliffs in Le Conquet (Finistere, Brittany, France, N 48°20′49.31", O 4°46′12.04"", 5 m asl), Senigallia (Marche, central Italy, N 43°44′30", E 13°11′05", 3 m asl) and Sicily (Italy, site 1: Salinelle, Palermo, N 38°01′48", E 13°18′40", 6 m asl; site 2: Acqua Calda, Lipari, N 38°31′11", E 14°56′00", 2 m asl) in September 2016, August 2016, and October 2015 and April 2016, respectively. Botanical identification was performed by K. Cianfaglione, F. Maggi and E. Schimmenti using available literature (Pignatti, 1982), and voucher specimens were deposited in the Herbarium of Géoarchitecture, UFR Sciences & Techniques, Université de Bretagne Occidentale, Brest, France, *Herbarium Universitatis Camerinensis*, School of Biosciences and Veterinary Medicine, University of Camerino, Italy, and in Department STEBICEF, University of Palermo, Palermo, Italy, under the codes BRECK9, CAME 27833, and PAL 16/22aMB and PAL 16/22bMB, respectively. After collection, the plant material was dried in a ventilated dark room at ~ 25 °C for one week before undergoing hydrodistillation.

2.2. Isolation of essential oils

Dry aerial parts and seeds of French sample (264–707 g), flowering aerial parts of central-Italy sample (720 g) and leaves and flowers of