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Not just popular spices! Essential oils from *Cuminum cyminum* and *Pimpinella anisum* are toxic to insect pests and vectors without affecting non-target invertebrates

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A R T I C L E   I N F O

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A B S T R A C T

Each year the flavor and fragrance industry produces more than hundred thousand tons of essential oils. An important portion of this production might be directed to the fabrication of green pesticides relying on the well-documented efficacy of many essential oils against insect pests as well as vectors, besides their safety for human health and the environment. In this regard, two popular spices of economic importance, such as cumin (*Cuminum cyminum*) and anise (*Pimpinella anisum*), which are not only cheap, readily available, generally recognized as safe (GRAS), but also produces larger amounts of essential oils, are gaining interest for botanical insecticide development. Herein, we evaluated the efficacy of the essential oils obtained from seeds of these two spices on two agricultural pests, i.e., the peach-potato aphid *Myzus persicae* and the tobacco cutworm *Spodoptera littoralis*, and on two insect vectors, i.e., the common housefly *Musca domestica* and the lymphatic filariasis and Zika virus vector *Culex quinquefasciatus*. Furthermore, their safety on beneficial organisms such as the earthworm *Eisenia fetida* and the aphid predator *Harmonia axyridis* was assessed. The two essential oils, characterized by γ-terpinen-7-al (35.3%), cumin aldehyde (21.8%), and α-terpinen-7-al (15.4%), and (*E*)-anethole (93.0%), respectively, showed noteworthy effects against all pest targets, with anise more effective on larvae of *C. quinquefasciatus* (LC_{50} = 25.4 μl L^{-1}) and *S. littoralis* (LD_{50} = 57.3 μg $larva^{-1}$), whereas cumin was more active on adults of *M. persicae* (LC_{50} = 3.2 ml L^{-1}) and *M. domestica* (LD_{50} = 31.8 μg $adult^{1}$). Moreover, when compared at sub-lethal concentrations with the commercial insecticide α-cypermethrin, they were definitely devoid of toxic effects on *E. fetida* and *H. axyridis*. These results represent a milestone to manufacture and commercialize green formulations to be used in crop protection and to combat insect vectors of public importance.

1. Introduction

The Apiaceae family encompasses aromatic herbs endowed with and used as flavorings in food and beverages as well as in traditional medicines (Benelli et al., 2017a,b). In most cases, their aroma derives from mixtures of volatile components typically stored in internal secretory structures known as ducts and vittae from which they are extracted under the form of an essential oil (EO) (Maggi et al., 2015). In particular, the vittae are generally confined to the fruits, which are called schizocarps (including two single seeded elements known as mericarps) or commonly 'seeds'. These are used on an industrial level as spices due to the high EO content (Pavela et al., 2017). Main examples are given by anise (*Pimpinella anisum* L.), dill (*Anethum graveolens* L.), fennel (*Foeniculum vulgare* Mill.), cumin (*Cuminum cyminum* L.), caraway (*Carum carvi* L.) and coriander (*Coriandrum sativum* L.) (Singh et al., 2017). As reported by Lubbe and Verpoorte (2011), the worldwide production of these EOs is in the order of 1–50 t.

Given the well-documented use in the traditional medicine, their

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safety and the availability of the raw material from which they are obtained, the Apiaceae EOs represent an industrial source to be employed in different fields (Sayed-Ahmad et al., 2017). Among them, the agrochemical industry has recently regarded these EOs as potential weapons to manage arthropod pests and vectors (Yeom et al., 2012; Govindarajan and Benelli, 2016; Benelli, 2018a; Pavela et al., 2017; Chellappandian et al., 2018), thus being employable in Integrated Pest/Vector Management programs (IPM/IVM) (Senthil-Nathan, 2013; Benelli and Beier, 2017; Benelli et al., 2018a,b).

Among various Apiaceae species producing EOs with interesting potential in this field, we here focused on *C. cyminum* and *P. anisum*. Both species are annual herbs native to Egypt and widely cultivated in the Mediterranean area, Middle East, India and China (Gondaliya et al., 2018; Iannarelli et al., 2017). Their seeds enjoy a good reputation as flavorings of foodstuffs and liqueurs (Leung and Foster, 1996).

As an example, cumin is a spice owning a typical pungent and bitter taste, used to flavor meat and fish as well as an ingredient of curry and chili powder (Moghaddam and Pirbalouti, 2017). Its oil is used to flavor alcoholic drinks, desserts and as seasoning and ingredient of cosmetics and perfumeries (Singh et al., 2017). In the traditional medicine, cumin is a remedy against inflammations, toothaches, gastrointestinal and neurological disorders (Singh et al., 2017). Sweet tasting anise seeds are used to flavor coffee, bakery and confectionery products, and as ingredient of several liqueurs (Iannarelli et al., 2017). In the traditional medicine, the aniseeds infusion has been used as carminative, digestive, galactagogue and expectorant (Iannarelli et al., 2018). Both cumin and anise EOs enjoy the status of generally regarded as safe (GRAS) by the Food and Drug Administration (Sahana et al., 2011; Newberne et al., 1999) and this may favor their use as ingredients of botanical insecticides, particularly in organic farms and Integrated Pest Management programs (Pavela, 2016). Other hallmarks of these oils are their high yield (2–6 % for both) and relatively low price (60–70 €/kg for cumin, 7–9 €/kg for anise), along with the large-scale production of the raw material from which they are derived (around dozens of tons per year). This makes them largely utilizable on an industrial level (Lubbe and Verpoorte, 2011; Gondaliya et al., 2018).

Developing novel and effective insecticides is a major challenge nowadays, aimed to face the quick development of insecticide resistance, as well as to reduce pesticide toxicity for human health and the environment (Benelli, 2015a; Benelli and Mehlhorn, 2016). The potential of cumin and anise EOs as ingredients of botanical insecticides is supported by substantial scientific background. Indeed, the cumin EO exhibited strong toxicity and fumigant effects against *Anopheles gambiae* Giles (Deletre et al., 2015). It showed ovicidal activity against *Tribolium confusum* Jacquelin du Val and *Ephestia kuehniella* Zeller (Clark, 1998; Tunc et al., 2000) as well as ovicidal and insecticidal effects on *Sitophilus zeamais* (Motschulsky) (Chaubey, 2017). Contact toxicity and repellency were also reported against *Tribolium castaneum* Herbst and *Sitophilus oryzae* (L.) (Lashgari et al., 2014). Furthermore, *C. cyminum* EO exerted fumigant effects and contact toxicity on adults of *Blattella germanica* (L.) (Yeom et al., 2012). Encapsulation of *C. cyminum* EO with oil-loaded nanogels prolonged the efficacy against *Sitophilus granarius* and *T. confusum* (Ziaee et al., 2014). Cumin EO resulted toxic against the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) (Martinez-Velazquez et al., 2011).

Both anise EO and its main component (*E*)-anethole have been reported as potent larvicidal and adulticidal against mosquitoes. When tested against *Culex quinquefasciatus* Say 3^{rd} instar larvae, the anise EO showed an LC_{50} value of 25.9 ppm, which was consistent with that of its major component (*E*)-anethole (LC_{50} = 24.8 ppm) (Benelli et al., 2017a). Both anise EO and (*E*)-anethole were toxic to the West Nile vector *Culex pipiens* L. (Kimbaris et al., 2012). Noteworthy, aniseed EO was not toxic to non-target organisms such as *Daphnia magna* Straus at long exposure times and low concentrations (Pavela, 2014). Furthermore, anise EO and (*E*)-anethole exhibited fumigant effects against larvae of *Lycoriella ingenua* (Dufour) (Park et al., 2006) and contact toxicity against adults of *B. germanica* (Yeom et al., 2012) and *T. castaneum* (Nenaah and Ibrahim, 2011; see also Hashem et al., 2018). (*E*)-Anethole was found highly effective against the tephritid fruit flies *Bactrocera dorsalis* (Hendel), *Bactrocera cucurbitae* (Coquilett) and *Ceratitis capitata* (Wiedemann) (Chang et al., 2009).

With the aim to further explore the insecticidal spectrum of these two popular spices, we here evaluated the efficacy of their EOs against selected key agricultural pests, i.e., the green peach aphid *Myzus persicae* (Sulzer) (Rhyncota: Aphididae), and the tobacco cutworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), and insect vectors of high public importance, i.e., the lymphatic filariasis and Zika virus vector *Culex quinquefasciatus* Say (Diptera: Culicidae) (Benelli and Romano, 2017), and the house fly *Musca domestica* L. (Diptera: Muscidae) (Pavela, 2008; Davies et al., 2016). Moreover, the potential ecotoxicological consequences arising from the employ of *C. cyminum* and *P. anisum* EOs for insecticidal purposes were investigated testing both in toxicity tests on two terrestrial non-target invertebrates, i.e., the multicolored Asian lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) and the earthworm *Eisenia fetida* (Savigny) (Oligochaeta, Lumbricidae).

2. Materials and methods

2.1. Plant material

Schizocarps (seeds) of cumin were purchased in a local market of Damasco, Siria, in August 2017. Those of anise were collected from a field sited in Castignano, Ascoli Piceno, Italy (N 42°56′25″08; E 13°37′29″64, 475 m a.s.l.), in September 2015. In this case, a voucher specimen was collected, identified using available literature and deposited in the *Herbarium Universitatis Camerinensis* at the School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy, under the codex CAME 28168.

2.2. Hydrodistillation

Schizocarps of cumin (518 g) and anise (915 g) were crushed in a mortar to facilitate the release of the volatile oil, then immersed in a 10 L flask filled with 6 L of deionized water and subjected to hydrodistillation using a Clevenger-type apparatus according to the indications of European Pharmacopoeia (2005). They gave 3.1 and 2.0% (w/w) of pale and yellowish EO, respectively. Just after collection, the EOs were dehydrated using anhydrous sodium sulfate and stored in amber vials at 4 °C before chemical analysis and insecticidal assays.

2.3. GC–MS analysis

The qualitative and semi-quantitative analysis of the EOs from anise and cumin was performed by an Agilent 6890N coupled with a 5973N mass spectrometer. A HP-5 MS capillary column (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness; J and W Scientific, Folsom, CA) was used as a stationary phase with helium as the carrier gas flowing at 1 mL min^{-1}. The temperature programme consisted in a gradient from 60 °C (held 5 min) to 220 °C with an increase of 4 °C min^{-1}, then up to 280 °C at 11 °C min^{-1} (held 15 min). Mass spectra were acquired in electron impact mode with an electron energy of 70 eV; the acquisition operated at full scan in the range 29–400 mz^{-1}. Before injection, EOs were diluted (1:100) in *n*-hexane (Carlo Erba, Milan, Italy) and 2 μL were injected and analyzed three times. A mixture of *n*-alkanes C_8–C_{30} (Supelco, Bellefonte, CA) was run under the above operating conditions to calculate the temperature-programmed retention indices (RIs) using the formula of Van den Dool and Kratz (1963). The MSD ChemStation software (Agilent, Version G1701DA D.01.00) and the NIST Mass Spectral Search Program for the NIST/EPA/NIH EI and NIST Tandem Mass Spectral Library v. 2.3 were used to analyze data. The peak assignment was based on the