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Activities of Apiaceae essential oils and volatile compounds on hatchability, development, reproduction and nutrition of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae)

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ABSTRACT

The inhibitory activities of *Anethum graveolens*, *Cuminum cyminum*, *Foeniculum vulgare* and *Petroselinum crispum* Essential Oils (EOs), plus eleven volatile compounds, on embryonic development of a phytophagous pest, *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) were evaluated. Lethal and sub-lethal effects of some treatments during the postembryonic development were also monitored. The higher effectiveness to inhibit eggs hatching was achieved with *P. crispum* fruit EO and its two main compounds (apiole and myristicin). *F. vulgare* EOs, as well as the bioactive compound azadirachtin (1 mg mL^{-1}) and the volatiles *trans*-anethole, estragole, (+)-fenchone, α-pinenes, (−)-β-pinene and γ-terpinene (15 mg mL^{-1}), had negligible inhibitory effects on larvae emergence, although, some induced post-exposure mortality during larval phase. *A. graveolens* EO from leaves and stems, and six compounds, including azadirachtin, extended significantly the duration of larval development from 1.9 to 6.5 days. (+)-Fenchone and (−)-β-pinene reduced significantly the oviposition potential of females, by 46% and 33%, respectively.

Additionally, a quantitative nutritional approach was conducted in order to assess adverse effects of EOs and compounds on 4th instar larvae growth and metabolism. After 72 h of feeding with treated corn leaves (0.7 mg/leaf), *P. crispum* fruit EO (Pc-F) and azadirachtin (0.04 mg/leaf) caused significant adverse effects on larva nutrition (lower consumption and Relative Consumption Rate) and growth (weight loss and negative Relative Growth Rate), followed by *trans*-anethole and cuminaldehyde. As verified for the positive control azadirachtin, *trans*-anethole and cuminaldehyde had negative and significant influences on the Relative Metabolic Rate (RMR) and on the efficiency of conversion of food to biomass (measured as ECI and ECD), with consequent higher Metabolic Costs (MC) to the larvae.

P. crispum EO from fruits revealed great potential as a controlling agent of *P. unipuncta* in early life stages, acting as a hatching inhibitor, larvicide and phagodeterrent.

Abbreviations: AD, approximated digestibility; Ag-I, *Anethum graveolens* infrutescence essential oil; Ag-L, *Anethum graveolens* leave and stem essential oil; Cc-F, *Cuminum cyminum* fruit essential oil; df, degree-of-freedom; ECD, efficiency of conversion of digested food; ECI, efficiency of conversion of ingested food; EO, essential oil; Fv-I, *Foeniculum vulgare* var. *vulgare* infrutescence essential oil; Fv-L, *Foeniculum vulgare* var. *vulgare* leave and stems essential oil; GC, gas chromatography; IGR, insect growth regulator; MC, metabolic cost; MS, mass spectrometry; Pc-F, *Petroselinum crispum* fruit essential oil; Pc-I, *Petroselinum crispum* infrutescence essential oil; RCR, relative consumption rate; RGR, relative growth rate; RMR, relative metabolic rate.

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1. Introduction

Over the past three decades, the development of biorational insecticides has been the central goal of numerous investigations. Plant-based pesticides have become a popular alternative, among other complementary strategies integrating pest management programs. Volatile constituents of essential oils (EOs) represent one of the most reported classes of compounds from botanical origin with proved toxicity to a great variety of arthropods. Their potential applications as safer alternatives to conventional insecticides have been extensively discussed in several reviews (Regnault-Roger, 1997; Isman, 2000; Koul et al., 2008; Tripathi et al., 2009; Regnault-Roger et al., 2012). In contrast to conventional insecticides, EOs and their constituents are non-persistent in the environment, and no bioaccumulation or biomagnification has been reported to date (Regnault-Roger et al., 2012). From a phytochemical viewpoint, EOs are heterogeneous mixtures characterized not only by a great diversity (abundance of biosynthetically distinct compounds) but also by a high redundancy (several analogs of one class and enantiomers) (Bakkali et al., 2008; Regnault-Roger et al., 2012).

In the context of research for plant-based pesticides, plants belonging to the Apiaceae family have been identified as a valuable source of several bioactive compounds (Lichtenstein et al., 1974; Berenbaum and Neal, 1985; Hadacek et al., 1994; Momin and Nair, 2002; Evergetis et al., 2009, 2013; Pavela and Vrchotová, 2013). Activities of Apiaceae EOs as antifeedants, insecticides, insect repellents and growth regulators were recently reviewed (Ebadollahi, 2013 and references therein). *Foeniculum vulgare* Mill., *Anethum graveolens* L., *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill and *Cuminum cyminum* L., are four well-known aromatic plants abundantly produced in several countries and regularly used in culinary as herbs and condiments. The EOs extracted from these Apiaceae species have shown ovicidal, larvicidal, adulticidal, deterrent and/or repellent activities against several species of the Arthropoda taxa; from diseases vectors (Lee, 2006; Evergetis et al., 2012), agricultural pests (Işik and Görür, 2009) and household parasites (Seo et al., 2009; Yeom et al., 2012), to stored-product insects (Tunç et al., 2000; Kim and Ahn, 2001; Chaubey, 2008; Ebadollahi, 2011; Kim et al., 2013). The insecticidal activities of their EOs and/or respective major compounds against *Pseudaletia unipuncta*, one of the most important pests of agricultural crops in North America (McNeil et al., 2000) and Europe (Bues et al., 1986), were recently screened (Sousa et al., 2013). *Pseudaletia* (sin. *Mythimna*) *unipuncta*, Haworth 1809 (Lepidoptera: Noctuidae), also known as the true armyworm, is a seasonal and migrant noctuid which cause considerable plant damage in small grain crops (namely barley, oats, rice, rye and wheat), sugarcane, maize and forage grasses (Silva et al., 2003). A variety of extracts and allelochemicals from several botanical origins have been previously tested for their antifeedant and crop protection potential against *P. unipuncta* and other important phytophagous insects (Akhtar and Isman, 2004; Akhtar et al., 2008; Isman et al., 2008; Rosa et al., 2010). In general, the evaluation of plants extracts activities against many lepidopterous insects are centered on acute toxicity tests or feeding-deterrence assays, for estimation of the respective lethal and deterrent doses. Besides the direct induced mortality, sub-lethal effects of insecticides on arthropod physiology and behavior must be considered for a complete analysis of their impact (Desneux et al., 2007). In fact, compounds with lower toxicity may still confer protection to crops by reducing fitness of insect herbivores via inhibition of larval growth and/or by disruption of insects' development (Hummelbrunner and Isman, 2001), compromising the adults' emergence and progeny. In that way, several studies have examined the deleterious effects of plant extracts and botanicals on growth, development, lifespan, reproduction and/or nutrition of lepidopteran (Koul et al., 1990; Huang et al., 1997; Akhtar and Isman, 2003, 2004; Yazdani et al., 2013).

In the present work, EOs extracted from *F. vulgare* subsp. *vulgare* var. *vulgare* (bitter fennel), *A. graveolens* (dill), *P. crispum* (parsley) and *C. cyminum* (cumin) and some constituents were assessed for their hatching inhibitory activity against *P. unipuncta* eggs. The ensuing effects of the treatments, with lower toxicity to the embryos, were monitored through time by following mortality, as well as post-exposure sub-lethal effects over insects' development and reproduction. Furthermore, the post-ingestive effects of EOs/compounds on 4th instar larvae were evaluated in a short-time study by means of nutritional indices calculation, in order to evaluate in what extent EOs/compounds can disturb larvae nutrition, metabolism and capacity of conversion of food into body mass.

2. Materials and methods

2.1. Essential oils and chemical composition

Foeniculum vulgare subsp. *vulgare* var. *vulgare* (bitter fennel), *A. graveolens* (dill) and *P. crispum* var. *neapolitanum* (flat leaf parsley) cultures were established in an open-air field experimental trial. *F. vulgare* var. *vulgare* seeds were obtained from a wild population and the identification was based on morphochemical characteristics. Bitter fennel voucher specimens (fruits and plants) were deposited at the University of Porto (Portugal) herbarium under the accession number PO 1000MFF. Green vegetative parts (stems and leaves) and green infrutescences (fruits in a pre-ripening phase) were collected from bitter fennel plants, after 12 and 14 months of growth, respectively, and from 5-month-old dill plants. Green infrutescences bearing fully formed fruits were sampled from parsley plants after 8 months of development. All fresh samples were separately submitted to a 2 h hydrodistillation with 2–3 L of deionized water in a Clevenger modified apparatus, after which the EO were recovered, separated from the aqueous phase, dried with sodium sulfate, and stored in brown sealed vials at −20 °C until use. The EOs from fruits of *P. crispum* (parsley) and *C. cyminum* (cumin) were purchase from Sigma-Aldrich, Co.

The quantitative and qualitative characterizations of the eight EOs were performed as described previously (Sousa et al., 2013). The composition of the EOs used in this study is summarized in Table 1.

2.2. Chemicals

Eleven high purity standard volatile compounds were included in all assays based on their relative abundance in the studied EOs (Table 1). *trans*-Anethole (99%), (S)-(+)-carvone (96%), cuminaldehyde (98%), estragole (98%), (+)-fenchone (99.5%), γ-terpinene (≥ 97%), (−)-β-pinene (99%), (−)-α-pinene and (+)-α-pinene (98%) were purchased from Sigma-Aldrich and Fluka (Aldrich chemical Co., St. Louis, MO, USA). The compounds myristicin (≥93%) and apiole (≥85%) were purified from *P. crispum* EO by column chromatography on silica gel 60 (40–63 μm) with an *n*-hexane-ethyl acetate (93:7) eluent mixture and their relative amount in the enriched fractions determined by GC analysis. The compound azadirachtin (∼95%, from Sigma) was included in all bioassays for a comparative purpose. Solvents (*n*-hexane, ethyl acetate and ethanol) were of reagent grade (≥99.9%).

2.3. Bioassays

2.3.1. Insects

A *P. unipuncta* laboratory colony, maintained for more than six generations in the Biology Department of Azores University (Ponta Delgada, S. Miguel, Azores archipelago, Portugal) was used for this