**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.

43 **Keywords:**

44 Armyworm, sub-lethal effects, dill, parsley, cumin, bitter fennel

45 **Abbreviations:**

46 AD - Approximated digestibility; Ag-I - *Anethum graveolens* infrutescence essential oil; Ag-L -
47 *Anethum graveolens* leave and stem essential oil; Cc-F - *Cuminum cyminum* fruit essential oil; df -
48 degree-of-freedom; ECD - Efficiency of Conversion of Digested food; ECI - Efficiency of
49 Conversion of Ingested food; EO - Essential Oil; Fv-I - *Foeniculum vulgare* var. *vulgare*
50 infrutescence essential oil; Fv-L - *Foeniculum vulgare* var. *vulgare* leave and stems essential oil; GC
51 - Gas Chromatography; IGR - Insect Growth Regulator; Metabolic Cost; MS - Mass Spectrometry;
52 Pc-F - *Petroselinum crispum* fruit essential oil; Pc-I - *Petroselinum crispum* infrutescence essential
53 oil; RCR - Relative Consumption Rate, RGR - Relative Growth Rate; RMR - Relative Metabolic
54 Rate.

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; Evergetis et al., 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 et al., 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 et al., 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; Akhtar and Isman, 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 ($\geq 97\%$), (-)- 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 ($\geq 93\%$) and apiole ($\geq 85\%$) were purified from *P. crispum* EO by column chromatography on silica gel 60 (40-63 μ m) 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 ($\sim 95\%$, from Sigma) was included in all bioassays for a comparative purpose. Solvents (*n*-hexane, ethyl acetate and ethanol) were of reagent grade ($\geq 99.9\%$).

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 study. Larvae were reared on an artificial diet (Poitout and Bues, 1974) and adults were supplied with 10% sucrose solution in water. Insects were maintained in laboratory at 22-24 °C and $70 \pm 5\%$ of relative humidity, under a L16:D8 photoperiod. All experiments were carried out in these conditions.

2.3.2 Hatching inhibition and dose-activity assay

Eggs of *P. unipuncta* recently laid (0–24 h) on strips of vegetal parchment paper placed inside the oviposition cages, were disinfected in 5% formaldehyde solution for 20 min and rinsed with distilled water for 5 min. Strips were then cut in small section and treated by dipping for 5 seconds in freshly prepared solutions of EOs/compounds in pure ethanol. A general screening was first

performed at 15 mg mL⁻¹, based on a preliminary assay, and additionally at 10, 7.5, 5, 2.5 and 1 mg mL⁻¹ for the most active treatments. Along with the negative ethanol control, a single dose of azadirachtin (1 mg mL⁻¹) was included in this assay as positive control. Five to 7 strips pieces (replicates) were used, corresponding to an average number of 387 ± 117 eggs per concentration and treatment/control. Following the complete evaporation of the solvent (air dried, 15 min) the replicates were placed inside individual aerated boxes and kept in incubation at 22-24 °C. Artificial diet was supplied 4 days later and before the onset of hatching, to prevent cannibalism. Observations were made daily, until the 10th day following the treatment, to register the day of the hatching in each replicate (if more than 50% of eggs were hatched). The percentage of unhatched eggs was calculated after 6-7 days, time required for larvae emergence in the ethanol control to be completed. The hatching inhibition caused by the treatments was corrected relatively to the ethanol control whenever it was superior to 5%, using Abbott's formula (Abbott, 1925).

2.3.3 Post-exposure effects: Larvae, pupae and adults' development and early death

We considered that an extended study about possible delayed effects during post-embryonic development of armyworm could be relevant for treatments (2 EOs and 7 compounds) which did not showed pronounced hatching inhibitory or ovicidal activity at 15 mg mL⁻¹. The newly emergent larvae of the foregoing hatching inhibition assay were reared with abundance of food and maintained grouped during the 1st and 2nd instar to avoid causing trauma or stress. Handling procedures were limited and performed only twice a week for substitution of boxes and diet. Afterward, 30-40 single 3rd instar larvae were randomly selected and distributed in individual boxes to examine subsequent post-exposure effects of EOs/compounds on each individual. Insects were monitored daily during their lifespan to register the duration (days) of larval, pupal and adults' stages (considering the hatching date as day 1), the occurrence of early death in each one, pupae' weights and adults' sex. In order to calculate larvae, pupae, adults' mortality (%) as well as the accumulated death, individuals

within treatments were randomly grouped for data processing (9-12 replicates with 3-4 insects per replicate). The mortality of neonates, 1st and 2nd instars was not included in the evaluation of the post-exposure lethality because larvae are very small and easily traumatized by handling. The replicates were used for calculation of mean percentage values and for statistical analysis. Larvae rearing, adults' feeding and room conditions were as describe in 2.3.1.

2.3.4 Fertility and female oviposition potential

After the emergence of adults, males and females were paired and distributed in small cages (12 cm x 10 cm x 9 cm) and allowed to mate. Ten to fifty couples were used per treatment. Vegetal parchment strips were placed in cages for oviposition and replaced every 4 to 5 days. Eggs laid by each female during all the reproductive period were counted and the number of unfertile couples was also registered. The feeding of moths and room conditions were as describe in 2.3.1.

2.3.5 Effects on nutritional physiology

The effects of EO and pure compounds over *P. unipuncta* 4th instar larvae growth, food consumption, food conversion efficiency and other metabolic parameters were evaluated for a 72 h period experiment according to Koul et al. (1990) and Huang et al. (1997). The bioassay was performed with newly molted 4th instar larvae (30 to 50 mg of average weight), never exposed to any of the studied allelochemicals. Pre-weighed larvae were individually distributed in sterile Petri dishes (diameter = 4.5 cm) with a thin agar layer (2% w/v) in the bottom to prevent dehydration. At least three independent assays with ten larvae per treatment/control were done (n = 30). Fresh corn leaves (*Zea mays* L.), previously disinfected in 5% formaldehyde solution for 20 min, rinsed with deionized water for 10 min and dried over absorbent paper, were cut in 4 cm² pieces. Each pre-weighed leaf section was treated with EOs/compounds (175 µg cm⁻²), pure ethanol (negative control) or azadirachtin (positive control at 10 µg cm⁻²) on the top of the leaves, and left for 15 min in order to evaporate the solvent. After a 6 h starvation period, larvae were fed with single leaf pieces freshly

199 prepared. Every 24 h, larvae were supplied with fresh leaves while feces and non-consumed diet
 200 (always some food leftover in all treatments) were removed, weighed and dried. At the end of the
 201 experiment (72 h), the weights of the feces and non-consumed diet of the last 24 h, as well as larvae'
 202 final weight were recorded. The amount of total ingested food (mg) was estimated by subtracting the
 203 sum of the remaining diet at 24, 48, and 72 h (dry weight equivalent) from the total dry weight of the
 204 supplied diet. Larvae and leaves initial dry weights were extrapolated based on mean dry contents
 205 determined for representative samples (n=30) in the beginning of the experiments ($12.0 \pm 1.04\%$ and
 206 $28.3 \pm 2.97\%$, respectively). Final dry weights of larvae, feces and non-consumed diet (mg) were
 207 estimated by drying at 50 °C for 4 days (dry contents varied from 9.8 to 14.2%). All weight
 208 measurements were made on a Mettler Toledo AB204-S/FACT balance (accuracy of 0.1 mg).

209 **2.3.5.1 Nutritional parameter calculation**

210 To evaluate treatment effects on the amount of food that was consumed, digested,
 211 assimilated, excreted, and converted into body mass during the experimental period, the following
 212 nutritional indices proposed by Waldbauer (1968) and modified by Scriber and Slansky Jr. (1981)
 213 were used:

Efficiency Conversion Index of Ingested food (%):	$ECI = 100 L / D$
Approximated Digestibility (%):	$AD = 100 (D - F) / D$
Efficiency Conversion Index of Digested food (%):	$ECD = 100 L / (D - F)$
Metabolic Cost (%):	$MC = 100 - ECD$
Relative Metabolic Rate ($\text{mg mg}^{-1} \text{ day}^{-1}$):	$RMR = M / l * t$
Relative Growth Rate ($\text{mg mg}^{-1} \text{ day}^{-1}$):	$RGR = L / l * t$
Relative Consumption Rate ($\text{mg mg}^{-1} \text{ day}^{-1}$):	$RCR = D / l * t$

214 Where t is the duration of the experimental period, D is the mean dry weight of consumed diet during
 215 t , L is the mean dry weight gain of larvae during t , l is the mean dry weight of larvae, F is the dry
 216 weight of feces produced during t and M is the metabolized food during t ($M = D - F - L$). The
 217 calculation on a dry weight basis was used as described by other authors (Koul et al., 1990; Senthil-

Nathan et al., 2005a; Yazdani et al., 2013), in an attempt to minimize the influence of water content variations in plant material and excreta and/or possible dehydration of larvae caused by some treatments.

2.4 Statistical analyses

Data from the hatching inhibition assay, developmental (duration and mortality observed in larvae, pupae and adults, and pupae' weight) and reproduction (number of eggs deposited per each female) studies were transformed by the $(x + \frac{1}{2})^{\frac{1}{2}}$ function prior to the one-way ANOVA analysis. Means of treatments were separated at 5% significance level by LSD post-hoc test (SPSS software version 20.0 for Windows, SPSS Inc.). The concentration causing 50% of hatching inhibition (IC_{50}) was determined for some of the most active EOs/compounds by performing a Probit analysis (SPSS software), according to Litchfield and Wilcoxon (1949). Log-Probit linear regressions were obtained from dose-response results, and generally five or four data-sets were selected to ensure a good adjustment of data to the model. The goodness-of-fit was evaluated by the qui-square (χ^2) test and was found satisfactory whenever values were lower than the critical χ^2 value (7.815 and 5.991) for 3 and 2 *df*, respectively, at 5% significance level. For the nutritional indices, data were submitted to one-way ANOVA test without previous transformation and, for each index, mean multiple comparisons were performed using the Duncan's multiple range test ($p = 0.05$) (SPSS software version 20.0 for Windows, SPSS Inc.).

3 Results

3.1 Hatching inhibition and dose-activity assay

The results of inhibitory effects on hatching of *P. unipuncta*, obtained for 7 EOs from Apiaceae species and 11 volatile compounds in a first screening assay (at 15 mg mL⁻¹), are presented in Figure 1. Essential oils isolated from dill, parsley and cumin inhibited satisfactory the emergence

of larvae (> 70% inhibition). Likewise, the main constituents (S)-carvone (from dill infrutescences), myristicin and apiole (found in parsley EOs), and cuminaldehyde (from cumin EO) were as effective as the respective EOs, when tested individually. No significant activity was observed to other major compounds, γ -terpinene and pinenes, despite their considerable amounts in the cumin and parsley EOs, respectively. By contrast, bitter fennel EOs and their corresponding compound (+)-fenchone, estragole, *trans*-anethole, and pinenes were found to have very limited ($\leq 10\%$) or no activity, even at the highest tested dose. The results of the dose-related activity obtained for the most active EOs/compounds are presented in Table 2. With the exception of the dill EO Ag-L, all treatment showed inhibitory hatching activity (>50%) at doses equal or superior to 5 mg mL⁻¹. For the lowest dose, only the parsley EO Pc-F, and the respective major compounds myristicin and apiole, still exhibited high effectiveness over eggs hatching, much superior to azadirachtin tested at 1 mg mL⁻¹ (see level of activity of azadirachtin in Figure 1 with those of EOs and compounds at the same concentration of 1 mg mL⁻¹ in Table 2). The probit analyses allowed determining the IC₅₀ of six treatments (Table 2). Among the treatments for which an estimate of this parameter was possible, myristicin presented the lowest IC₅₀ (below 1 mg mL⁻¹), followed by cuminaldehyde, Ag-I, Cc-F, (S)-(+)-carvone and Ag-L. This last EO was significantly less active (10.64 mg mL⁻¹) against *P. unipuncta* eggs than the one isolated from infrutescences of the same plant. However, Ag-I, and its major compound (S)-(+)-carvone, presented similar effectiveness (IC₅₀ = 3.60 and 3.74 mg mL⁻¹, respectively).

3.2 Post-exposure effects on *Pseudaletia unipuncta* development and reproduction

This particular study focused on post-exposure effects of treatments that showed lower ovicidal and/or hatching inhibition activities. As such, only the surviving larvae emerged from the eggs treated with Ag-L, Fv-I, Fv-L, *trans*-anethole, estragole, (+)-fenchone, pinenes, γ -terpinene, azadirachtin were reared until the completion of their life-cycle. Parsley EOs (Pc-F and Pc-I),

myristicin and apiole treatment were not included since a scarce number of larvae emerged, and few L_1 survived after emergence. The early death of the insects during their entire lifespan (larval, pupal and adult stage), as consequence of the exposure to low ovicidal doses of EOs/compounds during embryonic development, is shown in Table 3. When compared to mortality percentages recorded in negative controls, estragole and γ -terpinene were the treatments displaying the most significant post-exposure lethality on larvae (from L_3 to pupa), and more markedly on pupae (19 and 31%) ($p=0.05$). Among the remaining treatments which had some influence on survival, *trans*-anethole induced some lethal effects during larval development, while γ -terpinene exhibited significant effects during pupal stage (19%). None of the treatments decreased significantly the percentage of living adults when compared to water or ethanol ($p=0.05$). The overall mortality observed in groups of individuals formerly exposed to (-)- α -pinene, Fv-L, (+)-fenchone and (-)- β -pinene, Azadirachtin, Ag-L, Fv-I, *trans*-anethole, γ -terpinene, estragole and (+)- α -pinene (by increasing order) was 1.6 to 4.2 times superior to the registered natural death. However, only estragole, (+)- α -pinene and γ -terpinene showed statistically significant difference from negative controls regarding the post-exposure mortality ($p \leq 0.05$) (Table 3). Moreover, the previous exposure to azadirachtin during embryonic development did not caused significant death during the studies period (from 3rd instar until maturity). The results regarding the sub-lethal effects of EOs/compounds on *P. unipuncta* developmental stages, sex ratio and reproduction, are presented in Table 4. Almost all treatments had significant effects on larval stages duration, but only *trans*-anethole extended the duration of embryonic development (1 day approximately) ($p=0.05$). The exposure of eggs to γ -terpinene, estragole, α -pinenes, β -pinene, Ag-L, azadirachtin and (+)-fenchone (by increasing order) delayed significantly the duration of larval stage from 1.9 to 6.5 days, when compared to the negative controls ($p=0.05$). Afterwards, no noteworthy influence on the duration of the succeeding developmental stages could be undoubtedly attributed to treatments. Concerning the pupae' weight, lighter pupae were developed from eggs exposed to azadirachtin, Ag-L, (+)-fenchone, β -pinene, α -

pinenes and γ -terpinene, whereas heavier ones were grown in the groups treated with Fv-I, Fv-L, and their two compounds *trans*-anethole and estragole. Concerning the effects on adults' sex ratio, when compared to the ethanol treatment (56% females), the group of individuals exposed to γ -terpinene, estragole, (-)- β -pinene and (+)-fenchone showed higher percentage of females (65 to 71%). Among compounds and EOs, only (+)-fenchone and (-)- β -pinene treatments have changed significantly the oviposition potential ($p=0.05$), reducing the number of eggs/female, respectively, to 60% and 49% of the controls (Table 4). In the whole experiment, the absence of reproductive ability did not exceed 33 % of couples.

3.3 Post-ingestive effects on fourth-instar larvae nutrition and metabolism

The nutritional indices determined for *P. unipuncta* 4th instar larvae, after 72 h of feeding with corn leave treated with 175 $\mu\text{g cm}^{-2}$ (equivalent to 0.7 mg) of EOs ($n = 5$) or pure compounds ($n = 10$), are presented in Figure 2. In the case of Pc-F EO the RMR, ECI, ECD, AD and MC metabolic parameter were not applicable due to the insignificant intake of food recorded in two individual experiments (2/3 of the total number of larvae). The nutritional indices values determined for each treatment along with the statistical analysis are available as supplementary data. For all parameters, treatments showed significant difference ($0.001 \leq p \leq 0.005$). When compared with the ethanol group, a significant decrease in food consumption was observed for almost all treatments with the exception of bitter fennel EOs and (-)- β -pinene, Fv-L EO being the only treatment that have induced a significant increase (Fig. 2A). With the exception of this last EO, all treatments caused a diminution in the larvae mean weight gain relatively to the negative control. Furthermore, the larvae feed with *P. crispum* EO, *trans*-anethole and cuminaldehyde treated corn leaves, showed the most significant decrease of feeding behavior ($p \leq 0.05$), with consequent weight loss (negative values in figure 2C), similarly to the response to azadirachtin. Despite the distinct effect observed among treatments, the diet consumption and weight gain variations were proportional and a significant

correlation of these variables was found ($n = 48$, $r = 0.952$, $p < 0.0001$). Pc-F and azadirachtin, followed by *trans*-anethole and cuminaldehyde, were the treatments causing the most significant adverse effects on larva nutrition, seen both in consumption (Fig. 2A) and RCR (Fig. 2B), and growth, with reductions in weight gain (Fig. 2C) and RGR (Fig. 2D). In addition, (S)-carvone and estragole seem to have had some effect over these parameters. Regarding the impact of treatments over larvae metabolism (RMR, ECD, ECI and MC), azadirachtin, *trans*-anethole and cuminaldehyde had a negative and significant influence ($p \leq 0.05$) on the RMR, ECD and ECI indices (Fig. 2F, 2G, and 2H, respectively), causing higher costs (MC) to the insect organism (Fig. 2E) (3.8 to 1.5 times superior). (S)-(+)-carvone and (+)-fenchone had also negative impacts on RMR, while fennel EOs influenced positively the RMR (Fig. 2F). However, none caused a significant increase of the MC, as observed with cuminaldehyde and *trans*-anethole treatments (Fig. 2E). The digestion of food treated with EOs and volatile compounds, revealed by the AD index, ranged between 49.6 and 67.1% but it was not statistically different from the ethanol control group (58.9%) (Supplementary data). Only azadirachtin reduced significantly ($p \leq 0.05$) the digestibility of food ($AD_{aza} = 17\%$) when compared to the remaining treatments.

4 Discussion

Plant-based metabolites, including volatile compounds, display several functions in plant defense. These semiochemicals may influence insects' feeding and reproductive behaviors by deterring feeding and oviposition (Isman, 2002). Moreover, lethal activities as ovicidal, larvicidal, and adulticidal have been reported for several compounds from aromatic plants (Koul et al., 2008; Tripathi et al., 2009; Ntalli and Menkissoglu-Spiroudi, 2011).

In the present paper, the issue related with the activities of EOs and compounds on egg hatching and postembryonic development, along with the effect on the caterpillar feeding behavior and its nutrition, were analyzed in detailed experiments. In the first assay we demonstrated the

338 potentialities of four EOs (Pc-F, Pc-I, Cc-F and Ag-I), and their respective major compounds
339 (myristicin and apiole, cuminaldehyde and (S)-carvone), as alternative plant products with
340 effectiveness against the true armyworm eggs and larvae emergence. Notwithstanding the higher
341 activity of compounds to inhibited eggs hatching ($\leq IC_{50}$), relatively to the EOs where they were
342 identified, EOs presented linear regressions with higher slopes. This fact indicates that for a given
343 increase in EO concentration a larger effect will be induced when compared to that of individual
344 compounds. Because of their great number of constituents, EOs can interact with several targets,
345 affecting different functions and decreasing the resistance of organisms (Ntalli and Menkissoglu-
346 Spiroudi, 2011). From a toxicological viewpoint, when we look for an acute effect, the synergism
347 occurring in unique, natural and complex mixtures is an aspect as relevant as the inhibitory or lethal
348 concentration criterion. Myristicin was found to be the most active treatment, and so it was expected
349 a low IC_{50} for the Pc-F EO, where this compound is abundant. However, it was not possible to have a
350 good fit of the data, and hence to estimate the IC_{50} for this treatment, due to the high inhibition values
351 obtained for all the concentrations tested (Table 2). The same fact explains the lack in IC_{50} for apiole
352 and Pc-I, where apiole is the major compound. Regarding the compound cuminaldehyde, it was
353 found to be significantly more active than the EO Cc-F (2 x superior), where it was identified as
354 major compound (39%). Based on both results, we consider that most of the hatching inhibitory
355 activity exhibited by the Cc-F EO may be attributed to this major compound, although other
356 identified constituent not included in assessments (i.e. *p*-cymene, *p*-mentha-1,4-dien-7-al) could have
357 some contribution in the overall activity. Furthermore, the effectiveness of (S)-(+)-carvone ($IC_{50} =$
358 3.74 mg mL^{-1}) was lower than the two other compound, but not statistically different from that of
359 Ag-I EO (3.60 mg mL^{-1}). Their bioactivity is consistent with the fact that this compound accounts for
360 67% of the Ag-I EO. In previous studies, the vapors of cumin EO were found to be 100% active
361 against embryos of *Ephestia kuehniella* (Lepidoptera) and the confused flour beetle, *Tribolium*
362 *confusum* (Tunç et al., 2000). Furthermore, *C. cyminum* and *A. graveolens* EOs, were found to

363 reduce the hatching rate of *Callosobruchus chinensis* when fumigated (Chaubey, 2008). Essential
364 oils extracted from parsley seed showed hatching inhibitory activity on the eggs of the Indian meal
365 moth, *Plodia interpunctella* (74 % inhibition at 24 $\mu\text{L L}^{-1}$ air) (Rafiei-Karahroodi et al., 2011).
366 Beside the number of non-hatched eggs evaluated herein, distinct effects of EOs/compounds could
367 be observed. Freshly laid eggs (0 - 24 h post-oviposition) of *P. unipuncta* are white to pale-yellow
368 (0.6 - 0.7 mm spheres) and, as development progresses, their color darken to a metallic grey just
369 before hatching. However, we have observed that eggs treated with concentrations above 5 mg mL^{-1}
370 of Ag-I and Cc-F EOs, as well as with their respective main compounds, (S)-(+)-carvone and
371 cuminaldehyde, showed no maturation and some signs of desiccation. In this situation, we assume
372 that EOs and compounds have penetrated the egg shell, probably through micropyles and/or
373 aeropyles, and reached the embryo, displaying a clear ovicidal activity. A time-series study
374 performed by Berger-Twelbeck and collaborators (2003), with the ovicide 4-methoxy-benzoic acid
375 propargylester against eggs of the lepidopteran *Manduca sexta*, suggest that the degradation of the
376 embryo is a consequence of the rupture of the serosa. When the cohesion of the serosal membrane is
377 compromised in an early phase of egg development, the embryo dies prematurely. In addition to its
378 physiological and metabolic functions, the serosa often secretes a cuticle that helps to prevent egg'
379 desiccation (Strand, 2013). Thus, more than the chorion, the serosal membrane provides a barrier to
380 ovicides penetration into the embryo (Berger-Twelbeck et al., 2003) and water loss (Strand, 2013). In
381 contrast, a different effect was observed on eggs exposed to Pc-F, Pc-I, myristicin and apiole. These
382 treatments significantly compromise the hatching event, however, apparently, embryogenesis was
383 not disrupted and almost all embryos seemed to be developing. At lower concentrations, whenever
384 emergence occurred, the few neonate larvae were morbid, being unable to move and eat. It is known
385 that mature embryos of Lepidoptera species have to consume the egg-shell, partially or completely,
386 to emerge (Fehrenbach, 2003). Therefore, it is plausible that in this process larvae may have
387 simultaneously ingested residual amounts of compounds, namely myristicin and apiole, which were

388 retained in the egg-shell, causing toxicity through ingestion. Myristicin and other
389 methylenedioxyphenyl-containing phenylpropene (MDP), like apiole and dillapiole, have been
390 frequently described as natural synergists (Lichtenstein and Casida, 1963; Lichtenstein et al., 1974;
391 Berenbaum and Neal, 1985). After been ingested by insects, myristicin can interfere with mixed-
392 function oxidases and induce susceptibility to other secondary metabolites, enhancing their toxicity
393 at much lower concentrations (Berenbaum and Neal, 1985). For these EOs and compounds
394 aforementioned the post-embryonic developmental study was not achieved due to the high rate of
395 mortality or the impaired fitness of *P. unipuncta* neonates, while for EOs and compounds which did
396 not have pronounced ovicidal activity we have monitored the post-exposure effects during insect
397 lifespan. Concerning the one-dose hatching inhibition assay, the much lower activity exhibited by
398 individual compound, (+)-fenchone, estragole, *trans*-anethole, and pinenes, corroborates the
399 ineffectiveness observed for the respective natural mixture (Fv-I and Fv-L) where these compounds
400 co-occur in variable amounts (Figure 1, Table 1). It is also interesting to note that two of the three
401 most active volatiles in causing post-exposure premature death, γ -terpinene and estragole (Table 3),
402 were by far the least active regarding hatching inhibition (Fig. 1). After the larvae emergence, the
403 most persistent effects throughout insects life were observed for (+)-fenchone and (-)- β -pinene,
404 which have induced significant effects in total lifespan. Interestingly, these two compounds were the
405 only ones that caused the most dramatic change in sex ratio (more females), plus significant
406 reductions in pupae weight and females oviposition potential (Table 4). For the remaining
407 treatments, which includes azadirachtin at a lower concentration (1 mg mL^{-1}), extension of lifespan
408 (2 to 6.5 more days), decrease/increase of pupal weight, and/or increase of mortality, were the typical
409 post-exposure effects to be recorded. According to Hummelbrunner and Isman, 2001, a longer larval
410 developmental stage represents a significant disadvantage for insect herbivores, increasing the
411 probability of death in a field situation. In our analysis we also investigated possible relations
412 between some parameters. A tight positive relationship between pupae' weight and the number of

413 eggs laid by each female was found ($n = 12$, $r = 0.809$, $p = 0.0014$) (data not shown). Additionally,
414 the average number of eggs per female observed in treatments was inversely related with the females
415 percentage ($n = 11$, $r = -0.847$, $p = 0.001$) (data not shown). This last finding suggest that some
416 EOs/compounds may induce a changes in sex differentiation during development and that
417 compounds causing an increase in the female ratio could influence negatively the abundance of eggs
418 deposited by each females. The developmental inhibitory activity of some Apiaceae EOs was
419 previously assessed against the larval and adults forms of several insects, by mean of fumigation.
420 Sub-lethal doses of EOs from *A. graveolens* and *Trachyspermum ammi*, caused aberrations in the
421 different developmental stages and inhibition of pupation and adults' emergence of *Tribolium*
422 *castaneum* (Chaubey, 2007). Similar effects, in addition to a decrease in the number of laid eggs,
423 were reported against another stored-product insect, *Callosobruchus chinensis*, as consequence of
424 larvae fumigation with *T. ammi*, *A. graveolens* and *C. cyminum* EOs (Chaubey, 2008). *A. graveolens*
425 EO have also showed inhibitory activity on *T. castaneum* reproduction by preventing females to
426 oviposit (Chaubet, 2007). Essential oils isolated from plants belonging to the Labiatae family have
427 been reported for their effects on insects' development and reproduction. When fumigated over
428 *Acanthoscelides obtectus* inseminated females (at $77 \mu\text{L L}^{-1}$ of air), *Rosmarinus officinalis*,
429 *Lavandula hybrida*, *Mentha viridis* and *M. microphylla* EOs exhibited oviposition deterrence,
430 affected fecundity, and unfavorably influenced offspring emergence (decreased hatchability and
431 increased neonate larval mortality) (Papachristos and Stamopoulos, 2002). Great emphasis have been
432 given to the EO from *Acorus calamus* (Araceae), which strongly influenced the male fertility acting
433 as a chemosterilant agent in the red cotton bug (*Dysdercus koenigii*) (Koul et al., 1977). Due to their
434 ability to retard or accelerate insect development, and disrupt ecdysis, some plant secondary
435 metabolites have been identified as IGR. Azadirachtin is a remarkable example of a natural chemical
436 from plant origin with capacity to modify insects' behavior and several physiological functions. This
437 triterpenoid has been described for its exceptional growth regulatory action, being able to disrupt

438 molting and metamorphosis and block reproduction in several species (Dorn et al., 1986; Isman and
439 Akhtar, 2007). Besides, it can affect vitellogenesis (Sayah et al., 1996) and ovarian development, by
440 causing degenerative changes in follicles (Sayah et al., 1996; Senthil Nathan et al., 2008). In the
441 present study, we found no significant effects of azadirachtin on the fertility or the oviposition
442 potential of *P. unipuncta* females, as a result of eggs exposure to 1 mg mL⁻¹ of this compound.

443 For a more comprehensive study of EOs/compounds sub-lethal activities, we presented herein
444 an evaluation of their effects on the true armyworm nutritional physiology. The nutritional indices
445 analysis is considered a useful approach to appraise whether a secondary metabolites have
446 antifeedant and/or toxic activity after feeding (Waldbauer, 1968). Furthermore, our findings
447 (reduction in food consumption, ECD and ECI values) indicates that azadirachtin, *trans*-anethole and
448 cuminaldehyde caused feeding deterrence and interfered severely in the process of assimilation and
449 conversion of food to body mass. Based on the weight loss observed, we consider that the digested
450 food was used in metabolic processes, namely detoxification activity, which increased the energetic
451 cost (high MC). Eventually the maintenance of homeostasis could have been at the expense of the
452 organism's reserves. *trans*-Anethole and, in a lesser extent cuminaldehyde, seem to have induced the
453 same anorectic effect frequently described with azadirachtin (Isman and Akhtar, 2007) and as
454 observed herein. Furthermore, digestive disorder, longer persistence of food in digestive tract and
455 physiological stress are plausible event that might have been caused by these three compounds.
456 Evidences of *trans*-anethole metabolization to a hydroxylated form were found in feces of the
457 tobacco cutworm (*Spodoptera litura*) after topical and oral administration (Passreiter et al., 2004).
458 Senthil Nathan et al. (2005b), have described the significant inhibition of gut enzymes activity (acid
459 phosphatases, alkaline phosphatases and adenosine triphosphatases) and weight loss caused by
460 azadirachtin and other limonoid compounds on the rice leaffolder, *Cnaphalocrocis medinalis*.
461 Essential oil from *A. calamus* rhizome, and its phenylpropenoid *cis*- and *trans*-asarone, showed
462 feeding deterrence on the noctuid species, *Peridroma saucia* (variegated cutworm) (Koul et al.,

1990). Moreover, a strong growth inhibition and toxicity by ingestion or topical application, with consequent reduction of ECD and ECI, was proved to be induced exclusively by the *cis*-asarone isomer (Koul et al., 1990). Contrarily, *trans*-asarone, from *Daucus carota* EO, showed inhibition of the larval growth (> 96%) in other Lepidoptera species (*Helicoverpa zea*, *Heliothis virescens* and *Manduca sexta*), when used at 100 µg mL⁻¹ in artificial diet (Momin and Nair, 2002). Likewise, Huang et al. (1997) reported the influence of *Myristica fragrans* (Myristicaceae) EO on the maize weevil (*Sitophilus zeamais*) behavior (reduced food consumption) and weight loss, as well as possible deleterious effects. Post-ingestive toxicity, revealed by a decrease in the ECI index, was also recorded for *L. angustifolia* EOs against the lesser mulberry pyralid, *Glyphodes pyloalis* (Yazdani et al., 2013). With our experiment it was not possible to investigate post-ingestive effects of Pc-F EO and its compounds on *P. unipuncta* larvae since the consumption of food was virtually null, and high morbidity was observed in the case of both compounds, after the larvae first contact with treated leaves (60% and 100% of death for apiole and myristicin, respectively, after 72 h). We have previously showed that the EO from parsley fruits and both compounds, exhibit important larvicidal activity when assessed by the contact disc assay (Sousa et al., 2013). Even though, the toxicity after ingestion of little amounts of Pc-F, apiole or myristicin is not discarded taking into account the following aspects. First, the final amount of chemicals existing in the pieces of corn leaves (0.7 mg per leaf, which was equivalent to 0.7-1% of the food fresh weight) is much lower than those used in the previous larvicidal assay (3 x less) and, secondly, the larvae' symptoms perceived in both situation were distinct. The EOs isolated from parsley fruits and green infrutescences have proved to be of great efficacy as ovicide and larvicide (Sousa et al., 2013). Furthermore, Pc-F exhibited feeding and growth inhibitory effects as well. This wide spectrum of activities is advantageous and seems adjusted to a field situation, where several stages of *P. unipuncta* are co-habiting and parasitizing plants. In this context, if applied during oviposition period, after larvae emergence, or during the first stages of larval development, it could contribute to the control *P. unipuncta* population, reducing the

number of emerged and developing larvae and decreasing the food consumption and growth rate of the most resistant. Additionally, the increase in search time for viable food, and consequent delays on development, could expose the debilitated and less developed larvae to increased mortality, as a result of biotic and abiotic factors (Akhtar and Isman, 2004).

5 Conclusion

In this study we have shown the biological effects displayed by EOs from four Apiaceae species and their major compounds against armyworm, at the developmental and nutritional levels. Essential oils from *P. crispum* and its two phenylpropanoids, myristicin and apiole, reduced efficiently the eggs hatchability in the later stages of embryonic development, while, *A. graveolens* and *C. cyminum* EOs, and their respective major compounds, (S)-(+)-carvone and cuminaldehyde, compromised the eggs viability shortly after treatment. Despite their lack of ovicidal or inhibitory activity over hatching, the effects of some EOs and compounds were revealed *a posteriori*, throughout insects' postembryonic development, such as, delay of the pupation, debility and early death of larvae. Generally, longer larval stage and lifespan were associated with the occurrence of lighter/smaller pupae, but not necessarily with a decrease in the emergence of adults. Furthermore, we found that the impairment the insects' development, represented by the pupae' weight parameter, was strongly related with a decrease of the offspring. It was demonstrated that exposure to (+)-fenchone and (-)- β -pinene during embryonic development can significantly influence the ability of *P. unipuncta* to originate progeny. Although the aforementioned compounds were not among those presenting noteworthy ovicidal properties, results suggest that they may have interfere in the sex differentiation and reproductive function of the insect. Regarding the impact of treatments over larvae metabolism for the 3-day-period of experiment, our findings indicates that some EOs and compounds may impair feeding behavior and, consequently, larvae growth, without necessarily causing digestive disorder or interfering in the efficiency of conversion of food to biomass. In

contrast, *trans*-anethole and cuminaldehyde exhibited strong post-ingestive toxicity, similarly to the observed with azadirachtin, and appear as reasonable alternatives to limonoids compounds.

The biological activity of some Apiaceae EO and compounds against the damaging noctuid, *P. unipuncta*, were evidenced in the present work. As discussed herein and in our previous study, the activities of *P. crispum* EOs, is strongly related with the activity of myristicin and apiole, and their improved toxicity is performed through contact with integument. Regardless the fact that the nutritional and metabolic impacts of these two compounds could not be further explored due to the high mortality rates recorded, the results suggest that the EO and its compounds may also exert noxious activity on larvae through ingestion. Hence, further researches concerning some molecular targets are foreseen to identify their modes of action.

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References

- Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265-267.
- Akhtar, Y., Isman, M.B., 2003. Larval exposure to oviposition deterrents alters subsequent oviposition behavior in generalist, *Trichoplusia ni* and specialist, *Plutella xylostella* moths. J. Chem. Ecol. 29, 1853–1870.

535 Akhtar Y., Isman M.B., 2004. Comparative growth inhibitory and antifeedant effects of plant
 536 extracts and pure allelochemicals on four phytophagous insect species. J. Appl. Ent. 128, 32–
 537 38.

538 Akhtar, Y., Yeoung, Y.-R., Isman, M.B., 2008. Comparative bioactivity of selected extracts from
 539 Meliaceae and some commercial botanical insecticides against two noctuid caterpillars.
 540 *Trichoplusia ni* and *Pseudaletia unipuncta*. Phytochem. Rev. 7, 77–88.

541 Bakkali, F., Averbeck, S., Averbeck, D., Idaomar M., 2008. Biological effects of essential oils – A
 542 review. Food Chem. Toxicol. 46: 2, 446–475.

543 Berenbaum, M., Neal, J.J., 1985. Synergism between myristicin and xanthotoxin, a naturally
 544 cooccurring plant toxicant. J. Chem. Ecol. 11: 10, 1349-1358.

545 Berger-Twelbeck, P., Hofmeister, P., Emmlinga, S., Dorn, A., 2003. Ovicide-induced serosa
 546 degeneration and its impact on embryonic development in *Manduca sexta* (Insecta:
 547 Lepidoptera). Tissue Cell. 35. 101–112.

548 Bues, R., Poitout, S., Anglade, P., Robin, J.C., 1986. Cycle évolutif et hibernation de *Mythimna* (syn.
 549 *Pseudaletia*) *unipuncta* Haw. (Lep. Noctuidae) dans le sud de la France. Acta Oecol. 7, 151–
 550 156.

551 Chaubey, M.K., 2007. Insecticidal activity of *Trachyspermum ammi* (Umbelliferae), *Anethum*
 552 *graveolens* (Umbelliferae) and *Nigella sativa* (Ranunculaceae) essential oils against stored-
 553 product beetle *Tribolium castaneum* herbst (Coleoptera: Tenebrionidae). Afr. J. Agric. Res.
 554 2:11, 596–600.

555 Chaubey, M. K., 2008. Fumigant toxicity of essential oils from some common spices against Pulse
 556 beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae), J. Oleo Sci. 57:3, 171–179.

557 Desneux, N., Decourtye, A., Delpuech, J.-M., 2007. The sublethal effects of pesticides on beneficial
 558 arthropods. Annu. Rev. Entomol. 52, 81–106.

559 Dorn, A., Rademacher, J.M., Sehn, E., 1986. Effects of azadirachtin on the moulting cycle, endocrine
560 system and ovaries in last-instar larvae of the milkweed bug, *Oncopeltus fasciatus*. J. Insect
561 Physiol. 32, 231–238.

562 Ebadollahi, A., 2011. Susceptibility of Two *Sitophilus* species (Coleoptera: Curculionidae) to
563 essential oils from *Foeniculum vulgare* and *Satureja hortensis*. Ecologia Balkanica 3, 1–8.

564 Ebadollahi, A., 2013. Plant essential oils from Apiaceae family as alternatives to conventional
565 insecticides. Ecologia Balkanica 5: 1, 149 –172.

566 Evergetis, E., Michaelakis, A., Kioulos, E., Koliopoulos, G., Haroutounian, S.A., 2009. Chemical
567 composition and larvicidal activity of essential oils from six Apiaceae family taxa against
568 the West Nile virus vector *Culex pipiens*. Parasitol. Res. 105:1, 117–124.

569 Evergetis, E., Michaelakis, A., Haroutounian, S.A., 2012. Essential oils of Umbelliferae (Apiaceae)
570 Family Taxa as Emerging Potent Agents for Mosquito Control, in: Soloneski, S. (Ed.),
571 Integrated Pest Management and Pest Control - Current and Future Tactics. InTech, pp. 613–
572 638. Available from: [http://www.intechopen.com/books/integrated-pest-management-and-
573 pestcontrol-current-and-future-tactics/essential-oils-of-umbelliferae-apiaceae-family-taxa-as-
574 emerging-potentagents-for-mosquito-control](http://www.intechopen.com/books/integrated-pest-management-and-pestcontrol-current-and-future-tactics/essential-oils-of-umbelliferae-apiaceae-family-taxa-as-emerging-potentagents-for-mosquito-control)

575 Evergetis, E., Michaelakis, A., Haroutounian, S.A., 2013. Exploitation of Apiaceae family essential
576 oils as potent biopesticides and rich source of phellandrenes. Ind. Crop. Prod. 41, 365–370.

577 Fehrenbach, H., 2003. Eggs, in: Handbook of Zoology Volume IV Arthropoda: Insecta, Lepidoptera,
578 Moths and Butterflies, Vol. 2: Morphology, Physiology, and Development. Kristensen, N.P
579 (Ed.), Walter de Gruyter, Berlin, pp 469–494.

580 Hadacek, F.L., Muller, C., Werner, A., Greger, H., Proksch, P., 1994. Analysis, isolation and
581 insecticidal activity of linear furanocoumarins and other coumarin derivatives from
582 *Peucedanum* (Apiaceae: Apioideae). J. Chem. Ecol. 20: 8, 2035-2054.

583 Huang, Y., Tan, J.M.W.L., Kini, R.M., Ho, S.H., 1997. Toxic and antifeedant action of nutmeg oil
 584 against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. J. stored Prod. Res.
 585 33:4, 289–298.

586 Hummelbrunner, L.A., Isman, M.B., 2001. Acute, sublethal, antifeedant, and synergistic effects of
 587 monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep.,
 588 Noctuidae). J. Agric. Food Chem. 49, 715–720.

589 Isman, M.B., 2000. Plant essential oils for pest and disease management, Crop Protect. 19, 603–608.

590 Isman, M.B., 2002. Insect antifeedants. Pestic. Outlook 13, 152–157.

591 Isman, M.B., Akhtar, Y., 2007. Plant natural products as a source for developing environmentally
 592 acceptable insecticides, in: Ishaaya, I., Nauen, R., Horowitz, A.R., (Eds.), Insecticides design
 593 using advanced technologies. Springer-Verlag, Berlin, pp 235–248.

594 Isman, M.B., Wilson, J.A., Bradbury, R., 2008. Insecticidal activities of commercial rosemary oils
 595 (*Rosmarinus officinalis*) against larvae of *Pseudaletia unipuncta* and *Trichoplusia ni* in relation
 596 to their chemical compositions. Pharm. Biol. 46: 1–2, 82–87.

597 Işık, M., Görür, G., 2009. Aphidicidal activity of seven essential oils against the cabbage aphid,
 598 *Brevicoryne brassicae* L. (Hemiptera: Aphididae). Mun. Ent. Zool. 4: 2, 424–431.

599 Kim, D.H., Ahn, Y.J., 2001. Contact and fumigant activities of constituents of *Foeniculum vulgare*
 600 fruit against three coleopteran stored-product insects. Pest Manag. Sci. 57, 301–306.

601 Kim, S.-W., Kang, J., Park, I.-K., 2013. Fumigant toxicity of Apiaceae essential oils and their
 602 constituents against *Sitophilus oryzae* and their acetylcholinesterase inhibitory activity. J. Asia
 603 Pac. Entomol. 16, 443–448.

604 Koul, O., Saxena, B.P., Tikku, K., 1977. Mode of action of *Acorus calamus* L. oil vapours on adult
 605 male sterility in red cotton bugs. Experientia 33:1, 29–31.

606 Koul, O., Smirle, M., Isman, M., 1990. Asarones from *Acorus calamus* oil: Their effect on feeding
 607 behavior and dietary utilization in *Peridroma saucia*. J. Chem. Ecol. 16, 1911–1920.

608 Koul, O., Walia, S., Dhaliwal, G.S., 2008. Essential oils as green pesticides: potential and
 609 constraints. Biopesticides International 4, 63–84.

610 Lee, H.-S., 2006. Mosquito larvicidal activity of aromatic medicinal plant oils against *Aedes aegypti*
 611 and *Culex pipiens pallens*. J. Am. Mosq. Control Assoc. 22, 292–295.

612 Litchfield Jr., J.T., Wilcoxon, R., 1949. A simplified method of evaluating dose–effect experiments.
 613 J. Pharm. Exp. Therapeutics 96, 99–113.

614 Lichtenstein, E.P., Casida, J.E., 1963. Naturally occurring insecticides. Myristicin, an insecticide and
 615 synergist occurring naturally in the edible parts of parsnips. J. Agric. Food Chem. 11:5,
 616 410–415.

617 Lichtenstein, E.P., Liang, T.T., Schulz, K.R., Schnoes, H.K., Carter, G.T., 1974. Insecticidal and
 618 synergistic components isolated from dill plants. J. Agric. Food Chem. 22, 658–664.

619 McNeil, J.N., Miller, D., Laforge, M., Cusson, M., 2000. The biosynthesis of juvenile hormone, its
 620 degradation and titers in females of the true armyworm: a comparison of migratory and non–
 621 migratory population, Physiol. Entomol. 25, 103–111.

622 Momin, R.A., Nair, M.G., 2002. Pest-managing efficacy of *trans*-asarone isolated from *Daucus*
 623 *carota* L. seeds. J. Agric. Food Chem. 50, 4475–4478.

624 Ntalli, N.G., Menkissoglu–Spiroudi, U., 2011. Pesticides of botanical origin: a promising tool in
 625 plant protection, in: Stoytcheva M. (Ed.), Pesticides–formulations, effects, fate. InTech, pp. 3–
 626 24. available from: [http://www.intechopen.com/books/pesticides-formulations-effects-fate/](http://www.intechopen.com/books/pesticides-formulations-effects-fate/pesticides-of-botanical-origin-a-promising-tool-in-plant-protection)
 627 [pesticides-of-botanical-origin-a-promising-tool-in-plant-protection](http://www.intechopen.com/books/pesticides-formulations-effects-fate/pesticides-of-botanical-origin-a-promising-tool-in-plant-protection)

628 Papachristos, D.P., Stamopoulos, D.C., 2002. Repellent, toxic and reproduction inhibitory effects of
 629 essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). J. Stored
 630 Products Res. 38, 117–128.

631 Passreiter, C.M., Wilson, J., Andersen, R., Isman, M.B., 2004. Metabolism of thymol and trans-
 632 anethole in larvae of *Spodoptera litura* and *Trichoplusia ni* (Lepidoptera: Noctuidae). J. Agric.
 633 Food Chem. 52, 2549–2551.

634 Pavela, R., Vrchotová, N., 2013. Insecticidal effect of furanocoumarins from fruits of *Angelica*
 635 *archangelica* L. against larvae *Spodoptera littoralis* Boisd. Ind. Crops Prod. 43, 33–39.

636 Poitout, S., Bues, R., 1974. Elevage de chenilles de vingt-huit espèces de Lépidoptères Noctuidae et
 637 deux espèces d'Arctiidae sur milieu artificiel simplifié. Ann. Zool. Ecol. Anim. 6, 431–441.

638 Rafiei-Karahroodi, Z., Moharramipour, S., Farazmand, H., Karimzadeh-Esfahani, J., 2011.
 639 Insecticidal effect of six native medicinal plants essential oil on Indian meal moth, *Plodia*
 640 *interpunctella* Hübner (Lep.: Pyralidae). Mun. Ent. Zool. 6:1, 339–345.

641 Regnault-Roger, C., 1997. The potential of botanical essential oils for insect pest control. Integrated
 642 Pest Manag. Rev. 2, 25–34.

643 Regnault-Roger, C., Vincent, C., Arnason, J.T., 2012. Essential Oils in Insect Control: Low-Risk
 644 Products in a High-Stakes World. Annu. Rev. Entomol. 57, 405–424.

645 Rosa, J.S., Mascarenhas, C., Oliveira, L., Teixeira, T., Barreto, M.C., Medeiros, J., 2010. Biological
 646 activity of essential oils from seven Azorean plants against *Pseudaletia unipuncta*
 647 (Lepidoptera: Noctuidae). J. Appl. Entomol. 134, 346–354.

648 Sayah, F., Fayet, C., Idaomar, M., Karlinsky, A., 1996. Effect of azadirachtin on vitellogenesis of
 649 *Labidura riparia* (Insecta, Dermaptera). Tissue and Cell 28, 741–749.

650 Silva, L., Vieira, V., Tavares, J., 2003. Sampling plans for *Pseudaletia unipuncta* (Lepidoptera:
651 Noctuidae) Larvae in Azorean Pastures. Environ. Entomol. 32, 1211-1218.

652 Scriber, J.M., Slansky Jr, F., 1981. The nutritional ecology of immature insects. Ann. Rev. Entomol.
653 26, 183–211.

654 Senthil-Nathan, S., Chung, P.G., Murugan, K., 2005a. Effect of biopesticides applied separately or
655 together on nutritional indices of the rice leafroller, *Cnaphalocrocis medinalis* (Guenée)
656 (Lepidoptera: Pyralidae). Phytoparasitica 33, 187–195.

657 Senthil Nathan, S., Kalaivani, K., Murugan, K., Chung, P.G., 2005b. The toxicity and physiological
658 effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenée) the rice leafroller. Pestic.
659 Biochem. Phys. 81, 113–122.

660 Senthil Nathan, S., Choi, M.Y., Seo, H.Y., Paik, C.H., Kalaivani, K., Kim, J. D., 2008. Effect of
661 azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper
662 *Nilaparvata lugens* (Stål). Ecotox. Environ. Safe. 70, 244–250.

663 Seo, S.M., Kim, J., Lee, S.G., Shin, C.H., Shin, S.C., Park, I.K., 2009. Fumigant antitermitic activity
664 of plant essential oils and components from ajowan (*Trachyspermum ammi*), allspice (*Pimenta*
665 *dioica*), caraway (*Carum carvi*), dill (*Anethum graveolens*), geranium (*Pelargonium*
666 *graveolens*), and litsea (*Litsea cubeba*) oils against Japanese termite (*Reticulitermes speratus*
667 Kolbe), J. Agric. Food Chem. 57, 6596–6602.

668 Sousa, R.M.O.F., Rosa, J.S., Oliveira, L., Cunha, A., Fernandes-Ferreira, M., 2013. Activities of
669 Apiaceae essential oils against armyworm, *Pseudaletia unipuncta* (Lepidoptera: Noctuidae). J.
670 Agric. Food. Chem. 61, 7661–7672.

671 Strand, M.R., 2013. The egg and embryology, in: Chapman, R.F., Simpson, S.J., Douglas, A.E.,
672 (Eds), The insects: Structure and function, Fifth ed., Cambridge University Press, New York, p
673 347–397.

674 Tripathi, A.K., Upadhyay, S., Bhuiyan, M., Bhattacharya, P.R., 2009. A review on prospects of
 675 essential oils as biopesticide in insect-pest management, J. Pharmacogn. Phytother. 1:5, 52–63.

676 Tunç, I., Berger, B.M., Erler, F., Dağlı, F., 2000. Ovicidal activity of essential oils from five plants
 677 against two stored product insects. J. Stored Product Res. 36, 161–168.

678 Yazdani, E., Sendi, J.J., Aliakbar, A., Senthil-Nathan, S., 2013. Effect of *Lavandula angustifolia*
 679 essential oil against lesser mulberry pyralid *Glyphodes pyloalis* Walker (Lep: Pyralidae) and
 680 identification of its major derivatives. Pestic. Biochem. Physiol. 107, 250–257.

681 Yeom, H.J., Kang, J.S., Kim, G.H., Park, I.K., 2012. Insecticidal and acetylcholine esterase
 682 inhibition activity of Apiaceae plant essential oils and their constituents against adults of
 683 German cockroach (*Blattella germanica*). J. Agric. Food Chem. 60, 7194–7203.

684 Waldbauer, G.P., 1968. The consumption and utilization of food by insects. Advan. Insect Physiol. 5,
 685 229–288.

Table 1. Studied essential oils (EOs) from Apiaceae species and their respective major compounds

Species	Plant organs (EO abbreviation)	Compounds (%) ^{a, b}
<i>Anethum graveolens</i>	Green infrutescences (Ag-I)	(S)-Carvone (66.6), β - phellandrene (24.7)
	Green stems with leaves (Ag-L)	α -Phellandrene (60.0), Dill ether (16.4), ortho-Cymene (6.9), β -phellandrene (6.8)
<i>Cumin cyminum</i>	Fruits ^c (Cc-F)	Cuminaldehyde (39.4), γ -terpinene (15.8), β -pinene (12.4), <i>p</i> -cymene (10.4), <i>p</i> -mentha-1,4-dien-7-al (9.7)
<i>Foeniculum vulgare</i>	Green infrutescences (Fv-I)	Estragole (64.9), fenchone (15.7), β -phellandrene (5.5), α -phellandrene (4.9)
subsp. <i>vulgare</i> var. <i>vulgare</i>	Green stems with leaves (Fv-L)	Estragole (28.4), α -phellandrene (24.3), α -pinene (18.0), β -phellandrene (10.1), fenchone (6.2)
<i>Petroselinum crispum</i>	Fruits ^c (Pc-F)	Myristicin (31.5), α -pinene (16.2), apiole (15.9), β -pinene (13.6), Allyl-tetramethoxybenzene (8.6), β -phellandrene (6.6)
	Green infrutescences (Pc-I)	Apiole (24.3), α -pinene (23.4), β -pinene (20.8), β -phellandrene (10.6), 1,3,8- <i>p</i> -menthatriene (10.6)

^a Summarized characterization of EOs considering only major compounds with relative contents $\geq 5\%$ (adapted from Sousa et al., 2013).

^b Compounds were identified by mass spectra comparison and retention index calculation. The quantifications were performed by GC-FID analysis; ^c Essential oils were purchased from Sigma-Aldrich Co.

Table 2. Dose-related hatching inhibitory activity of *Anethum graveolens*, *Petroselinum crispum*, *Cuminum cyminum* essential oils and their major compounds on *Pseudaletia unipuncta*, 6-7 days after eggs treatment

Treatments (mg mL ⁻¹)	Hatching inhibition ^{a, b} (% ± SEM)						Hatching inhibitory concentration				
	15	10	7.5	5	2.5	1	IC ₅₀ (mg mL ⁻¹) (95% CL) ^c	Slope (± SEM)	χ ²	df	Sig.
EOs											
Ag-I	100 ± 0.0 b	93.2 ± 5.4 b	94.1 ± 4.2 c	75.6 ± 13.1 bc	22.0 ± 3.3 a	15.2 ± 1.7 ab	3.60 b (3.28 – 3.91)	4.74 ± 0.44	0.95	2	0.62
Ag-L	71.2 ± 7.1 a	50.7 ± 7.9 a	22.3 ± 4.4 a	9.9 ± 3.3 a	-	-	10.64 c (9.84 – 11.64)	4.09 ± 0.43	2.24	2	0.33
Cc-F	100 ± 0.0 b	92.5 ± 3.5 b	97.7 ± 1.8 c	67.7 ± 10.9 bc	28.3 ± 6.0 a	14.8 ± 5.4 ab	3.68 b (3.25 – 4.10)	3.47 ± 0.32	0.34	2	0.84
Pc-F	98.6 ± 1.4 b	97.7 ± 1.8 b	75.8 ± 6.6 b	73.8 ± 8.6 bc	-	72.9 ± 10.8 d	-	-	-	-	-
Pc-I	98.7 ± 0.7 b	100 ± 0.0 b	100 ± 0.0 c	94.2 ± 4.3 cd	93.2 ± 5.1 c	18.4 ± 6.6 ab	-	-	-	-	-
Compounds											
Api	80.4 ± 8.4 a	100.0 ± 0.0 b	100.0 ± 0.0 c	100.0 ± 0.0 d	99.2 ± 0.8 c	83.7 ± 7.0 d	-	-	-	-	-
Car	100 ± 0.0 b	100.0 ± 0.0 b	78.7 ± 6.1 b	58.6 ± 6.5 b	36.5 ± 3.0 ab	6.8 ± 2.2 a	3.74 b (3.29 – 4.27)	2.49 ± 0.24	1.30	2	0.52
Cum	100 ± 0.0 b	95.5 ± 2.9 b	94.3 ± 3.6 c	-	55.3 ± 14.7 b	25.6 ± 7.9 b	1.97 a (1.68 – 2.26)	2.57 ± 0.20	3.36	3	0.34
Myr	99.3 ± 0.7 b	100.0 ± 0.0 b	99.0 ± 1.0 c	88.7 ± 4.0 cd	-	55.8 ± 6.6 c	0.87 a (0.03 – 1.83)	1.92 ± 0.22	4.51	2	0.11
df	46	47	48	69	41	49	-	-	-	-	-
F	8.506	21.246	47.121	21.412	26.602	27.533	-	-	-	-	-
p ≤	0.001	0.001	0.001	0.001	0.001	0.001	-	-	-	-	-

Api: Apiole, Car: (S)-(+)-Carvone, Cum: Cuminaldehyde, Myr: Myristicin. df = n - 2.

^a Mean values (± standard error of the mean - SEM) were determined for 5-7 replicates and corrected with Abbott's formula relatively to the control with ethanol, six days after treatment when non-hatched eggs percentages ≤ 5% were recorded; ^b Within columns, means with the same letters do not differ significantly based on one-way ANOVA, followed by LSD test (p= 0.05) (statistical analysis was computed with transformed data); ^c IC₅₀ were considered significantly different when the 95% confidence limits (CL) fail to overlap.

Table 3. Lethality recorded for *Pseudaletia unipuncta* larval (from L₃ to pupa), pupal and adult stages, after exposure during the embryonic development to Apiaceae essential oils (EOs) and pure compounds at a low ovicidal concentration (15 mg mL⁻¹).

Treatments	Mortality (% $\pm$ SEM)			
	Larval stage ^{a, b, c}	Pupal stage ^{a, b}	Adult stage ^{a, b}	Total ^{a, b}
Controls				
Water	0.0 $\pm$ 0.0 a	2.8 $\pm$ 2.8 a	5.6 $\pm$ 3.7 ab	8.3 $\pm$ 4.2 a
Ethanol	0.0 $\pm$ 0.0 a	4.2 $\pm$ 2.8 a	4.9 $\pm$ 3.3 ab	8.3 $\pm$ 4.7 a
Azadirachtin ^d	0.0 $\pm$ 0.0 a	9.1 $\pm$ 6.1 ab	9.1 $\pm$ 6.1 ab	18.2 $\pm$ 7.6 abc
EOs				
Ag-L	0.0 $\pm$ 0.0 a	13.3 $\pm$ 4.2 ab	5.6 $\pm$ 3.7 ab	18.9 $\pm$ 3.6 abc
Fv-I	2.8 $\pm$ 2.8 a	13.9 $\pm$ 4.4 ab	2.8 $\pm$ 2.8 ab	19.4 $\pm$ 5.6 abc
FV-L	0.0 $\pm$ 0.0 a	13.9 $\pm$ 6.2 ab	0.0 $\pm$ 0.0 a	13.9 $\pm$ 6.2 ab
Compounds				
<i>trans</i> -Anethole	5.6 $\pm$ 3.6 b	5.6 $\pm$ 2.8 ab	11.1 $\pm$ 4.3 b	22.2 $\pm$ 3.2 abc
Estragole	13.9 $\pm$ 4.4 c	18.5 $\pm$ 4.5 bc	3.7 $\pm$ 3.7 ab	33.3 $\pm$ 5.1 c
(+)-Fenchone	0.0 $\pm$ 0.0 a	7.6 $\pm$ 5.2 ab	9.1 $\pm$ 6.1 ab	16.7 $\pm$ 7.1 ab
(+)- α -Pinene	5.6 $\pm$ 3.3 b	31.0 $\pm$ 3.0 c	0.0 $\pm$ 0.0 a	35.0 $\pm$ 3.7 c
(-)- α -Pinene	0.0 $\pm$ 0.0 a	13.2 $\pm$ 3.8 ab	0.0 $\pm$ 0.0 a	13.2 $\pm$ 3.8 ab
(-)- β -Pinene	0.0 $\pm$ 0.0 a	16.7 $\pm$ 8.3 ab	0.0 $\pm$ 0.0 a	16.7 $\pm$ 8.3 ab
γ -Terpinene	0.0 $\pm$ 0.0 a	18.5 $\pm$ 5.9 bc	14.8 $\pm$ 7.6 b	29.6 $\pm$ 8.7 bc
df	125	125	125	125
F	5.512	2.426	1.298	2.165
p $\leq$	0.001	0.01	0.5	0.05

^a Mean values and standard error of the mean (SEM) were determined for groups of 3-4 individuals for a total of 9-12 replicates; ^b Mean values with the same letters do not differ significantly, based on one-way ANOVA followed by LSD test ($p = 0.05$) (statistical analysis was computed with transformed data); ^c The percentage of death during the 1st and 2nd instar larvae were not included in the study; ^d Lethality during post-embryonic development after eggs exposure to azadirachtin at 1 mg mL⁻¹.

Table 4. Sub-lethal effects on *Pseudaletia unipuncta* (development duration and reproduction), as consequence of a previous exposure during embryonic development to of Apiaceae essential oils (EOs) and compounds at low ovicidal concentration (15 mg mL⁻¹)

Treatments	Duration (Days)					Pupae' weight (mg) ^{a, b}	Sex ratio (♀ %) ^c	Eggs/ ♀ (No.) ^c	Sterility (%) ^d
	Hatching ^a	Larval stage ^{a, b}	Pupal stage ^{a, b}	Adult stage ^{a, b}	Total Lifespan ^{a, b}				
Controls									
Water	5.3 ± 0.2 a	32.5 ± 0.7 b	14.1 ± 0.3 bcd	10.0 ± 0.7 a	56.2 ± 1.0 a	271.9 ± 8.4 c	42.9	699 ± 112.4 c	20.0
Ethanol	5.6 ± 0.2 ab	32.2 ± 0.4 b	14.0 ± 0.2 abc	12.5 ± 0.5 bcd	58.4 ± 0.9 ab	272.0 ± 6.0 c	55.6	771 ± 104.6 c	25.0
Azadirachtin ^e	5.8 ± 0.2 ab	38.2 ± 0.9 g	14.9 ± 0.2 d	13.8 ± 1.5 cd	66.4 ± 1.3 d	249.2 ± 8.3 b	40.0	729 ± 81.0 c	0.0
EOs									
Ag-L	-	37.0 ± 0.5 fg	14.6 ± 0.2 d	13.0 ± 0.8 bcd	64.2 ± 1.1 cd	230.2 ± 5.9 a	48.4	902 ± 74.9 c	0.0
Fv-I	6.0 ± 0.0 bc	29.8 ± 0.4 a	13.4 ± 0.2 ab	12.9 ± 1.1 bcd	56.4 ± 1.2 ab	290.5 ± 5.0 d	48.3	929 ± 139.1 c	18.2
FV-L	6.0 ± 0.0 bc	33.1 ± 0.2 bc	13.4 ± 0.2 a	11.8 ± 0.9 abcd	58.4 ± 1.0 ab	301.3 ± 4.1 d	51.6	931 ± 102.5 c	33.3
Compounds									
<i>trans</i> -Anethole	6.4 ± 0.2 c	33.0 ± 0.5 bc	13.6 ± 0.2 ab	9.9 ± 0.6 a	56.7 ± 0.8 ab	289.4 ± 5.9 d	48.4	784 ± 157.9 c	10.0
Estragole	6.0 ± 0.0 bc	34.5 ± 0.5 cde	14.2 ± 0.4 bcd	11.1 ± 0.7 ab	59.2 ± 0.9 b	287.9 ± 5.4 cd	66.7	717 ± 99.6 c	14.3
(+)-Fenchone	5.8 ± 0.3 ab	38.7 ± 1.1 g	14.5 ± 0.4 cd	13.4 ± 1.3 cd	66.4 ± 1.7 d	230.7 ± 7.6 ab	71.4	465 ± 70.7 ab	11.8
(+)-α-Pinene	5.3 ± 0.2 a	35.8 ± 0.6 ef	14.0 ± 0.3 abc	13.4 ± 1.1 bcd	62.9 ± 1.1 c	244.5 ± 6.4 ab	52.0	766 ± 52.3 c	0.0
(-)-α-Pinene	5.6 ± 0.2 ab	35.3 ± 0.5 de	14.4 ± 0.2 cd	13.7 ± 0.6 d	63.2 ± 0.7 c	245.8 ± 4.3 b	56.5	575 ± 63.2 bc	0.0
(-)-β-Pinene	5.9 ± 0.2 b	35.7 ± 0.9 def	13.9 ± 0.4 abc	14.1 ± 1.4 cd	64.0 ± 1.2 cd	238.7 ± 10.7 ab	68.8	378 ± 79.4 a	28.6
γ- Terpinene	5.8 ± 0.2 ab	34.1 ± 0.4 cd	13.7 ± 0.4 abc	11.2 ± 1.1 abc	59.6 ± 1.5 b	253.7 ± 5.7 bc	65.2	601 ± 118.6 bc	0.0
<i>df</i>	74	418	373	347	356	429	-	120	-
<i>F</i>	3.138	19.790	2.739	2.515	11.073	15.103	-	3.133	-
<i>p</i> ≤	0.005	0.001	0.001	0.005	0.001	0.001	-	0.001	-

^a Mean values and respective SEM were determined for variable numbers of individuals (20 ≤ n ≤ 40); ^b Mean values (± standard error of the mean - SEM) with the same letters do not differ significantly, based on one-way ANOVA followed by LSD test ($p = 0.05$) (statistical analysis was computed with transformed data); ^c Mean number of eggs deposited by each female until its death; ^d Percentage of non-reproductive couples, for a number varying between 10 and 15 couples per treatment; ^e Post exposure effects on the insect development after eggs treatment with azadirachtin at 1 mg mL⁻¹.

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Figure 1. Inhibition of *Pseudaletia unipuncta* hatching after single dose treatments (15 mg mL^{-1}) with *Anethum graveolens* (**Ag**), *Cuminum cyminum* (**Cc**), *Foeniculum vulgare* var. *vulgare* (**Fv**) and *Petroselinum crispum* (**Pc**) essential oils from: fruits (-F), Infrutescences (-I), Leaves and stems (-L), and compounds: **Api**: Apiole, **t-An**: *trans*-Anethole, **Aza**: Azadirachtin ^a, **Car**: (S)-(+)-Carvone, **Cum**: Cuminaldehyde, **Est**: Estragole, **Fen**: (+)-Fenchone, **Myr**: Myristicin, **(+)-α-P**: (+)-α-Pinene, **(-)-α-P**: (-)-α-Pinene, **β-Pin**: (-)-β-Pinene, **γ-Ter**: γ-Terpinene. Mean values ($\pm$ standard error) were determined for 5-7 replicates and corrected with Abbott's formula relatively to the control with ethanol, 6-7 days after treatment when non-hatched eggs percentages $\leq 5\%$ were recorded. Means with the same letters do not differ significantly, based on one-way ANOVA followed by LSD test ($p=0.05$) (statistical analysis was computed with transformed data). ^a Azadirachtin (95%) was tested against eggs at a sub lethal dose of 1 mg mL^{-1} , to follow insects' development in the subsequent study.

Figure 2 (A-H). Effects of Apiaceae essential oils (EOs) and respective major compounds on *Pseudaletia unipuncta* 4th instar larvae nutritional and metabolic parameters, after 72h of feeding with treated corn leaves at 175 $\mu\text{g cm}^{-2}$.

Mean values ($\pm$ standard error) were determined for 3 experiments with 10 replicate each. **RGR**: Relative growth rate; **RCR**: Relative consumption rate; **RMR**: Relative metabolic rate; **ECD**: Efficiency of conversion of digested food; **ECI**: Efficiency of conversion of ingested food.

Cont: Ethanol control, **Aza**: Azadirachtin, positive control at 10 $\mu\text{g cm}^{-2}$, **Ag-I**: *Anethum graveolens* infrutescence EO, **Fv-I**: *Foeniculum vulgare* var. *vulgare* infrutescence EO, **Fv-L**: *Foeniculum vulgare* var. *vulgare* leave and stems EO, **Cc-F**: *Cuminum cyminum* fruit EO, **Pc-F**: *Petroselinum crispum* fruit EO, **t-An**: *trans*-Anethole, **Car**: (S)-(+)-Carvone, **Cum**: Cumin aldehyde, **Est**: Estragole, **Fen**: (+)-Fenchone, **(+)-α-P**: (+)-α-Pinene, **(-)-α-P**: (-)-α-Pinene, **β-Pin**: (-)-β-Pinene, **γ-Ter**: γ-Terpinene. Note: Calculations of ECD, ECI and MC indices for PC-F were not possible due to absence of food consumption in some experiments.