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Activities of Apiaceae Essential Oils against Armyworm, *Pseudaletia unipuncta* (Lepidoptera: Noctuidae)

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ABSTRACT: Essential oils (EOs) from four Apiaceae species and 11 pure compounds were evaluated for their antifeedant, growth inhibitory, and insecticidal activities against *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) fourth-instar larvae. EOs from *Foeniculum vulgare* subsp. *vulgare* var. *vulgare*, *Anethum graveolens*, *Petroselinum crispum*, and *Cuminum cyminum* were characterized by gas-chromatography (GC) and mass spectrometry. Anti-insect activity varied according to plant specie/composition, type, and exposure period. EOs from *P. crispum* and *A. graveolens* fruits, *trans*-anethole and cuminaldehyde, exerted acute effects on larvae feeding and growth (FDI and GI > 70%). *A. graveolens*, *C. cyminum*, and *F. vulgare* EOs and some of their constituents were effective by fumigation (≥80%). Satisfactory contact toxicities (>70%) were observed for five compounds and all EOs, except *F. vulgare* EOs, when tested by the filter paper impregnation method. For the most active EOs/compounds, dose-dependent toxicity was determined and inverse relationships of LC_{50} with time were established.

KEYWORDS: *bitter fennel, dill, parsley, cumin, feeding deterrence, contact and fumigant toxicity*

INTRODUCTION

True armyworm, *Pseudaletia* (sin. *Mythimna*) *unipuncta* (Haworth) (Lepidoptera: Noctuidae), is a seasonal migrant and a polyphagous pest of grasslands and mixed pastures. It is one of the most important pests of agricultural crops in North America[1] and Europe.[2] In the Azores Islands (Portugal), high infestations by *P. unipuncta* during summer and early autumn[3] can cause considerable plant damage, corresponding to approximately 8% of the annual production and estimated at EUR 5 million.[4] The hazards of excessive pesticides use for insect pest control have increased the need for effective and biodegradable pesticides. In the last two decades, this growing awareness has motivated the research for safer and environmental friendly alternatives. Biological control with entomopathogenic nematodes[5] and application of bioactive natural compounds from plants with phagodeterrent/insecticide properties have been proposed for *P. unipuncta* control.[6,7] In the context of research for new biopesticides, extracts from plants have received special attention, and volatile constituents of essential oils (EOs) are among the candidates with highest potential.[8] The relative chemical simplicity and familiarization of the market for aromatic constituents in conjunction with the less toxic nature of these compounds are elements that contribute to the receptiveness to EOs applications. According to a literature survey on the biopesticidal potential of EOs from the year 2000 onward,[9] plants of Myrtaceae, Lamiaceae, Asteraceae, Apiaceae, and Rutaceae families are highly targeted for insecticidal activities against specific insect orders like lepidoptera, coleoptera, diptera, isoptera, and hemiptera. EOs have been explored for repellent, fumigant, larvicidal, and adulticidal activities against many species, and their potential has been the subject of several reviews.[8−12] In general, terpenoids are predominant constituents of plant EOs, but many other volatiles of distinct biosynthetic origin, such as aromatic and aliphatic constituents, may be detected in variable amounts.[13,14] Derived from phenylpropane, the aromatic compounds occur less frequently than the terpenes, and their occurrence is restricted to some botanical families, namely, Apiaceae, Lamiaceae, Myrtaceae, Rutaceae. Phenylpropane derivatives typically found in EOs are aldehydes (cinnamaldehyde), alcohols (cinnamic alcohol), phenols (chavicol, eugenol), methoxy derivatives (anethole, elemicine, estragole, methyl eugenole), and methylene dioxy compounds (apiole, myristicin, safrole).[15] The insecticidal activity of Apiaceae EOs has been evaluated against a number of arthropods, from stored product and agricultural pests to diseases vectors.[16−22] Moreover, synergistic properties and enhancement of synthetic insecticides activity or naturally occurring insecticides were attributed to methylenedioxyphenyl-containing phenylpropene constituents, like apiole and myristicin, frequently produced by

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Apiaceae species.[23−25] On the basis of the high bioactive potential of plants belonging to the Apiaceae family and their relatively safe utilization as herbs and spices, in traditional medicine and food, EOs extracted from *Foeniculum vulgare* Miller var. *vulgare*, *Anethum graveolens* L. (sin. A. sowa Roxb. ex Fleming), *Petroselinum crispum* (Mill.) Nym ex A.W. Hill (sin. *P. sativum* Hoffm or *P. hortensis* Auct), and *Cuminum cyminum* L., as well as 11 pure compounds, were assayed against *P. unipuncta* for an evaluation of their potential as natural control agents of this insect. The results of this research are here presented and discussed.

MATERIALS AND METHODS

Plant Material. Commercially available seed (packed by N.V. Somers, Co.) of *A. graveolens* (dill) and *P. crispum* (Italian giant parsley) were purchased for cultures establishment. *F. vulgare* spp. *vulgare* var. *vulgare* (bitter fennel) plants were grown from seed collected a year before from an identified and previously studied population growing wild in Braga, North of Portugal (41°36′04.21″ N; 8°26′50.25″ E). Plants were cultivated in an open field, from April to October 2010, under the same conditions without the use of fertilizers or pesticides. Fresh stems and leaves of fennel were collected before complete maturation of fruits, and green infrutescences of the three species were harvested for EO isolation. Additionally, mature and dried fruits collected from dill plants were kept for a posteriori EO isolation, 6 month later.

Essential Oils Extraction. EOs of the cultivated plants were isolated from preweighted samples (150−300 g) by a 2 h hydrodistillation in a Clevenger modified apparatus. Separately, representative samples were freeze-dried for extrapolation of respective biomass dry weights. Obtained EOs were dried over anhydrous sodium sulfate, and their volume was measured for yield calculation (mL of EO per 100 g of dried biomass). Pure EOs were stored in brown sealed vials at −20 °C until use. In addition, two commercialized EOs of *P. crispum* (parsley) and *C. cyminum* (cumin) fruits were purchase from Sigma-Aldrich (St. Louis, MO).

GC and GC−MS Analyses. A 10 μL volume of each EO was diluted in 100 μL of *n*-hexane prior to GC and GC−MS analysis. GC analysis were performed on a GC Focus (Thermo-Scientific) equipped with a TRX-5 capillary column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness) under the following conditions: hydrogen constant flow rate of 1.0 mL/min; splitless injector and flame ionization detector (FID) at 300 °C; oven program temperatures, from 60 to 285 °C, rising at 3 °C/min and kept at 285 °C for 15 min. Injections of samples were performed at least three times, with volumes of 0.3 or 0.4 μL. GC−MS analyses were carried on a Thermo Trace GC Ultra, with a TR-5 capillary column, coupled to a Thermo-Finnigan Q ion trap detector (ITD) (Thermo-Scientific) operating in EI mode at 70 eV. Helium was used as carrier gas at a constant flow of 1.5 mL/min and with a flux repartition of $^{1}/_{13}$. Injector and ion source temperature were 300 and 250 °C, respectively. The oven temperature program and injection conditions were as above-described for GC. Identification of compounds was performed on the basis of comparison of Kovats retention indices (KI) as well as on the MS fragmentation patterns. Some constituents were confirmed by comparison with available authentic standards. KI were calculated by means of coinjection with a series of *n*-alkanes (C_8−C_{34}) and compared with the respective KI reported in the literature and databases integrated in the GC−MS software (Excalibur 1.4, Thermo Electron Corp.). MS of compounds were compared with those found in the NIST mass spectra database (NIST, Wiley and TR libraries). Quantitative percentage data were obtained from GC FID analyses reports by peak area integration, without application of correction factors.

Chemicals. Eleven pure compounds were included in anti-insect activity assessments on the basis of their relative abundance in the studied EOs. *trans*-Anethole (*trans*-1-methoxy-4-(1-propenyl)benzene, 99% purity, MW = 148.2), (*S*)-(+)-carvone (96% purity, MW = 150.22), cuminaldehyde (4-isopropylbenzaldehyde, 98% purity, MW = 148.2), estragole (1-allyl-4-methoxybenzene, 98% purity, MW = 148.2), (+)-fenchone (99.5% purity, MW = 152.2), γ-terpinene (≥97% purity, MW = 136.23), (−)-β-pinene (99% purity, MW = 136.23), (−)-α-pinene, and (+)-α-pinene (98%, MW = 136.23) were purchased from Sigma-Aldrich and Fluka. The compounds myristicin (≥93%) and apiole (≥85%) were isolated from *P. crispum* EO by column chromatography. Several separations were performed on the silica gel column (18 cm of length, 22 mm of i.d., 25 g of Merck silica gel 60, 40−63 μm) using 0.4 g of parsley EO and a *n*-hexane−ethyl acetate mixture (93:7) as eluent. The composition of collected fractions was monitored by thin-layer chromatography using the same eluent and stationary phase (TLC silica gel 60_{F254} glass plates) and observation under UV at 254 nm. After solvent evaporation under nitrogen flux, the enriched fractions containing each compound were submitted to GC analysis for a purity appraisal, following the analytical conditions described in the previous section. For feeding deterrence/growth inhibition and paper disk assays, azadirachtin (∼95% purity, MW = 720.71, from Sigma) and lambda-cyhalothrin (Judo commercial formulation with 100 g L^{-1} of compound from Sapec Agro S.A) were used as positive controls, respectively. Solvents, *n*-hexane, ethyl acetate, and ethanol, were reagent grade (≥99.9%).

Bioassays. *Insects.* *P. unipuncta* fourth-instar larvae were obtained from an established laboratory colony maintained for more than six generations. Larvae were reared on an artificial diet[26] and adults were supplied with 10% honey solution in water. Insect colonies were maintained in laboratory at 23 ± 1 °C and 70 ± 5% relative humidity, under a L16:D8 photoperiod. Fourth-instar larvae of *P. unipuncta*, with the same age and weighing 30−50 mg, were used in this study. All experiments were carried out in an incubator under the same conditions described above.

Feeding Deterrence and Growth Inhibition. Antifeedant and growth inhibitory activities of EO and pure compounds against *P. unipuncta* were evaluated by the diet-no-choice method (adapted from ref 27) using corn (*Zea mays* L.) leaves as food. Three independent assays with 10 fourth-instar larvae per treatment (n = 30 individuals), including controls, were carried out on different days. Freshly collected leaves were previously disinfected in 5% formaldehyde solution for 20 min, rinsed with deionized water for 10 min, and dried over absorbent paper before cutting in 4 cm^2 equal pieces. Diet treatment consisted of a unique dosage by covering each preweighted leaf section with 17.5 μL of EOs/constituents (175 μg cm^{-2} or 0.7 mg/leaf from 40 mg mL^{-1} stock solutions in ethanol), pure ethanol (negative control), or azadirachtin (positive control at 10 μg cm^{-2} from a 2.3 mg mL^{-1} stock solution in ethanol), which was left for 15 min in order to evaporate the solvent before larvae feeding. Bioassays were conducted in sterile Petri dishes (ϕ = 4.5 cm) with a thin agar layer (2% w/v) covering the bottom, to prevent food and larvae dehydration. After a 6 h starvation period, larvae were preweighted, distributed individually in Petri dishes, and allowed to feed continuously on a treated piece of diet. Food replacements, with single pieces of leaves freshly prepared, were made every 24 h or whenever necessary. Larvae and diet (provided diet and/or nonconsumed diet) weights (mg) were recorded initially and after 48 and 72 h. After each assay, the final dry weight of larvae was estimated by drying at 50 °C for 4 days (dry contents varied from 9.8 to 14.2%). Larvae and leaves initial dry weights were extrapolated on the basis of mean dry contents determined for representative samples. Thirty individuals and pieces of corn leaves were used for this purpose, and mean dry weights contents were 12.0(±1.04)% and 28.3(±2.97)%, respectively.

Toxicity by Contact. The filter paper impregnation method was used to examine contact toxicity. In a first experiment, all EOs and pure compounds activities were screened at a single concentration (250 μg cm^{-2}, equivalent to 2 mg per filter paper). Bioassays were conducted in sterile Petri dishes (3.2 cm inner diameter and 1.0 cm high) containing one filter paper disk (8.0 cm^2) impregnated with 50 μL of EO/compound stock solutions (40 mg mL^{-1} of pure ethanol), ethanol (as solvent control), or positive control (lambda-cyhalothrin emulsion in distilled water at 40 mg mL^{-1}). The filter papers were air-dried for 15 min and a single fourth-instar larva was placed into each

Table 1. Apiaceae Plant Species and Plant Parts Used for Essential Oils Extraction and Characterization of Respective Essential Oils Used in Anti-Insect Activity Studies against *P. unipuncta* (armyworm)

scientific name	common name	origin	plant parts	yield (% ± SEM)[a]	detected compds[b]	ident (%)[c]
F. vulgare Mill. subsp. *vulgare* var. *vulgare*	bitter fennel	cultivated plants	fresh green infrutescences	3.27 ± 0.14 (n = 3)	15	99.4
			fresh stems with leaves	2.50 ± 0.35 (n = 6)	13	99.9
A. graveolens L.	dill	cultivated plants	fresh green infrutescences	3.30 ± 0.40 (n = 3)	10	98.6
			dried mature fruits	1.80 (n = 1)	11	98.7
P. crispum var. *neapolitanum* Danert	Italian parsley	cultivated plants	fresh green infrutescences	1.25 ± 0.12 (n = 3)	24	98.0
P. crispum (Mill.) Nyman ex A.W. Hill	parsley	France[d]	fruits		25	98.9
C. cyminum L.	cumin	Iran[d]	fruits		32	99.8

[a]Yields are expressed in mL of EO/100 g of dry biomass. Mean values were calculated for n hydrodistillations. [b]Number of compounds detected on GC-FID analysis with relative peak area ≥0.05%. [c]Identification percentages were calculated by sum of relative contents for identified compounds. [d]*P. crispum* and *C. cyminum* fruits EOs purchase from Sigma-Aldrich.

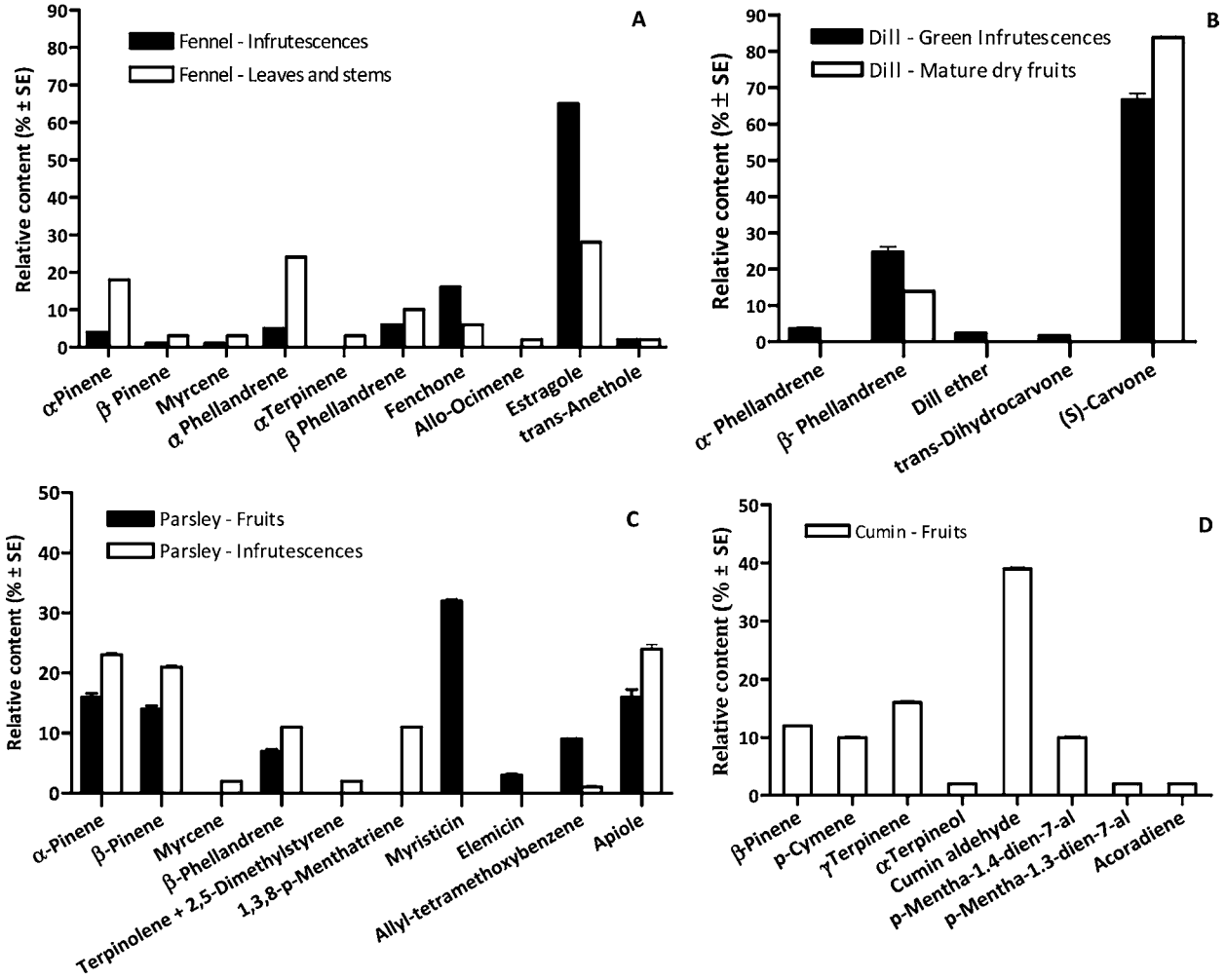

Figure 1. Composition of *F. vulgare* var. *vulgare* (bitter fennel), *A. graveolens* (dill), *P. crispum* (parsley), and *C. cyminum* (cumin) essential oils. (A) Volatile constituents identified in EOs of bitter fennel green infrutescences and fresh stems with leaves. (B) Volatile constituents identified in dill green infrutescence and mature dry fruit EOs. (C) Volatile constituents identified in parsley fruits and fresh green infrutescence EOs. (D) Volatile constituents identified in cumin fruit EO. Major constituents with relative contents above 1% are presented by order of elution in the TRX-5 GC capillary column. Identifications of EOs constituents were ≥98.0% (sum of detected compounds with relative contents ≥0.05%).

Petri dish, without food. About 12 h later, one piece of diet (corn leaves) was given to all living larvae. For each treatment three to six replicates of 10 larvae each were used. Mortality was assessed at 9, 24, and 48 h after treatment onset by observation of larvae with a stereoscopic microscope. Individuals were considered dead when no movement was detected after prodding with a dissecting pin. A dose–response evaluation was conducted afterward with the most active EOs/compounds following the same procedure. For each EO/compound a set of five to seven optimized doses were chosen among the following concentrations: 31, 63, 94, 125, 187, 250, 281, and 375 μg cm^{-2}. Assays were performed four times with 10 replicates per concentration including control groups with ethanol. Mortality was assessed after 24, 36, 48, and 72 h to determine lethal times (LTs) and evaluate lethal concentrations (LCs) variation over time.

Toxicity by Fumigation. The evaluation of the fumigant activity of EOs and pure volatile compounds was conducted using sealed plastic

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jars with a total inner volume of 0.043 dm^3 (4.5 cm of diameter and 2.7 cm high). For treatment, filter paper disks (ϕ = 3 cm) were impregnated with 50 μL of concentrated solution in ethanol containing 10.7 mg of EO or compound. Treated disks were disposed at the top of each jar, and the solvent was left to evaporate during 15 min. Ten fourth-instar larvae were placed inside each jar, and food was provided to prevent cannibalism. Contact of larvae with impregnated filter paper was avoided by disposing a thin net between. The tested concentration was chosen in an attempt to reproduce similar concentration of volatiles per volume of air (250 mg dm^{-3}) with those that probably occurred inside the 8.0 cm^3 Petri dishes used for contact toxicity screening (250 μg cm^{-2}). Three to six replicates per treatment and solvent control were done. Mortality was assessed at 12, 24, and 48 h by observation of larvae through the jar with a stereoscopic microscope. At 12 and 24 h, individuals were considered dead when no movement was detected, and in the end of experiment the mortality was confirmed by the normal procedure, prodding with a dissecting pin.

Statistical Analyses. The antifeedant effects were evaluated by the feeding deterrence index calculation, FDI (%) = $[(C - T)/C] \times 100$, where C is the weight of diet consumed in the control and T is the weight of diet consumed in the treatment.[28] Growth inhibition (GI) was estimated from the equation $[(C_L - T_L)/C_L] \times 100$, where C_L is the mean larval weight gain in the control and T_L is the mean larval weight gain in the treatment.[27] All calculations were performed on a dry weight basis. The data obtained for antifeedant, growth inhibition, and insecticidal activity of EOs/compounds were submitted to a one-way analysis of variance (one-way ANOVA) procedure. Prior to analysis, antifeedant and growth inhibition data were transformed by $\log_{10}(x + 1)$, while mortality rate in both fumigation and contact insecticidal assays were submitted to arcsine transformation. Means with significant variance and F-statistic were separated at 5% significance level by Duncan's test available in the IBM SPSS statistics package.[29] Dose−response data obtained by contact toxicity assay were submitted to probit analysis (IBM SPSS statistics) to calculate lethal concentrations (LC_{50} and LC_{90}) required to kill 50 and 90% of insects, at 24, 36, 48, and 72 h. The same procedure was used to determine lethal times (LT_{50} and LT_{90}), at a single concentration. Additionally, variations of LC_{50} over time were evaluated by submitting transformed data (LC_{50}^{-1}) to linear regression model, using Graph Pad Prism 4.[30]

RESULTS AND DISCUSSION

Essential Oils Composition. Essential oils yields obtained from cultivated plants of bitter fennel, dill, and parsley are summarized in Table 1. Qualitative and quantitative characterization of EOs was performed, and 98.0−99.8% of their constituents were identified. The highest EOs yields were obtained for *Foeniculum vulgare* var. *vulgare* and *A. graveolens* fresh plant material. The contents of the major EO compounds, representing more than 1%, are exhibited in Figure 1. The chemical compositions of EOs from *F. vulgare* var. vulgare infrutescences and leaves with stems were similar, although some differences of relative contents were found (Figure 1A) The EO extracted from umbels bearing green fruits was mainly constituted by estragole (65%) and fenchone (16%), with several monoterpenes hydrocarbons found in less amounts, representing 16.8%. A much higher content of monoterpene hydrocarbons was produced by young vegetative parts (leaves and stems) of bitter fennel plants (63%). The oxygenated monoterpene fenchone was considerably less abundant in vegetative parts (6%) than in infrutescences (16%), and in both samples, a low content of *trans*-anethole was determined (2%). Fennel natural populations with high estragole contents (50−65%) were already identified in Spain,[31,32] Portugal,[33] and Israel.[34] Estragole, *trans*-anethole, and fenchone proportions may vary considerably.[35] Based on several reports, the presence of fenchone in high amounts is a distinctive characteristic of bitter fennel.[34,36] With respect to *A. graveolens* EOs, very simple chemical profiles were found. No more than 11 constituents were detected for both EOs, and only two to five constituents were present above 1% (Figure 1B). Carvone was the major compound found in infrutescence and fruit EOs (67 and 84%, respectively), followed by β-phellandrene (25 and 14%, respectively). Dill ether (3,9-epoxy-1-*p*-menthene), a typical compound of this specie, and *trans*-dihydrocarvone were only detected in green infrutescence EO and in relatively low amounts. The composition of *A. graveolens* and its chemical variation throughout plant development have been well-described.[37,38] *S*-(+)-Carvone, phellandrenes, limonene, dill ether, and pinenes are the most characteristic compounds of EOs from this specie. The composition of dill EOs characterized herein are consistent with previous studies when we consider the harvesting time and the high-carvone profiles obtained. According to Callan and co-workers,[38] a significant increase of carvone is observed as the crop matures, and the levels of phellandrene, limonene, dill ether, and pinene, accumulated by dill plants during their development, decline. Thus, the aroma of dill EO from the leaves and stems of the plant before fruit formation is related to its phellandrene and dill ether contents, while, as we observed, carvone is abundantly accumulated in fruit-bearing umbels during maturation and after ripening. Relatively to *P. crispum* EOs, around 25 constituents were detected, although only seven or eight of these were found in substantial amounts (Figure 1C). Pinenes, β-phellandrene, allyltetramethoxybenzene, and apiole were found in both samples from different origins. However, some important chemical differences were recorded. Myristicin was the most representative phenylpropanoid of the parsley EO commercially available (32%), while it was totally absent in the infrutescence EO of cultivated plants. Myristicin is a phenylpropanoid frequently found in considerable amounts in parsley fruits and leaves.[39] Myristicin chemotypes or intermedium chemotypes, where this compound remains an important fraction of the EOs, were previously identified and related to the curly leaf or plain leaf parsley.[40,41] On the other hand, EO extracted from the green infrutescences of *neapolitanum* variety was mainly constituted by apiole (24%), a phenylpropene derivative generally found in roots of several Apiaceae species[23,24] and some parsley varieties,[41] namely, the root parsley, where it was detected in variable amounts (16−75%).[42] Therefore, the high apiole content found in *P. crispum* var. *neapolitanum* infrutescences is, to a certain extent, in agreement with a previous report for the giant Italian variety.[40] In the present study, phenylpropanoids constituted 59% of parsley fruit EO, whereas monoterpene hydrocarbons were the most important class of compounds in fresh infrutescences (57%). The predominance of these groups and the near absences of oxygen-containing monoterpenes or sesquiterpenes are not unusual for the specie and was already described for other plant parts.[41,42] The phytochemical analysis of *C. cyminum* fruit EO showed that 93% of the total content of this commercially available EO was represented by eight major compounds (Figure 1D), although 20 more minor ones (less than 1%) were detected and identified. The EO was composed by several monoterpene hydrocarbons (total of 42% of the EO) and two oxygenated monoterpenes, cuminaldehyde (39%) and *p*-mentha-1,4-dien-7-al (10%). Cumin seed EO and its respective chemical composition has been included in several studies.[43−45] Despite the high disparity among chemical descriptions,

Table 2. Effects of *F. vulgare* var. *vulgare*, *A. graveolens*, *P. crispum*, and *C. cyminum* Essential Oils and Pure Compounds on *P. unipuncta* Fourth-Instar Larvae Feeding on Fresh Corn Leaves Treated with 175 μg cm^{-2}, Assessed after 48 and 72 h of Treatment

treatments			mortality at 72 h (%)	relative mean consumed diet[a] (% ± SEM)[b]		feeding deterrence index (% ± SEM)[b]	
				48 h	72 h	48 h	72 h
controls		ethanol	0.0	43.7 ± 4.8 ghi	57.5 ± 5.3 e	–	–
		azadirachtin[c]	0.0	10.5 ± 1.5 bc	8.6 ± 1.1 ab	85.1 ± 1.1 g	93.3 ± 0.7 h
EOs	*F. vulgare*	infrutescences	0.0	33.6 ± 4.7 efg	56.4 ± 1.8 e	8.0 ± 1.7 b	11.5 ± 3.0 c
		stems and leaves	0.0	42.4 ± 1.1 ghi	65.4 ± 1.9 f	−26.2 ± 8.5 a	−20.3 ± 3.6 a
	A. graveolens	mature fruits	6.7	6.8 ± 2.6 abc	26.3 ± 8.6 c	84.7 ± 5.2 g	75.6 ± 6.4 g
		green infrutescences	3.3	28.7 ± 3.5 ef	39.9 ± 1.4 d	17.7 ± 6.5 bcd	31.5 ± 8.5 de
	P. crispum	fruits	32.4	0.1 ± 0.1 a	0.2 ± 0.2 a	99.7 ± 0.3 g	99.7 ± 0.3 h
	C. cyminum	fruits	3.3	22.5 ± 3.6 de	47.1 ± 3.8 de	44.6 ± 2.3 ef	32.9 ± 4.0 de
compds		*trans*-anethole	12.9	4.6 ± 0.7 ab	17.1 ± 3.9 bc	92.4 ± 1.7 g	84.6 ± 4.6 gh
		estragole	3.3	15.9 ± 1.8 cd	42.1 ± 4.8 de	59.5 ± 4.0 f	49.8 ± 5.8 f
		(+)-fenchone	0.0	48.6 ± 2.6 hi	52.9 ± 5.4 def	19.7 ± 2.8 bcd	25.5 ± 6.9 cd
		(*S*)-(+)-carvone	0.0	34.4 ± 1.1 efg	55.5 ± 6.3 def	46.3 ± 3.4 ef	45.9 ± 2.4 g
		myristicin	100[d]	–	–	–	–
		apiole	60.0[d]	–	–	–	–
		γ- terpinene	0.0	43.3 ± 5.1 ghi	53.8 ± 3.0 def	25.8 ± 1.8 cd	20.1 ± 4.9 c
		cuminaldehyde	12.9	8.4 ± 4.5 abc	22.3 ± 6.3 c	84.7 ± 4.4 g	74.0 ± 6.7 g
		(−)-α-Pinene	0.0	41.4 ± 7.9 fgh	53.3 ± 1.3 def	32.5 ± 3.7 de	29.8 ± 5.5 d
		(+)-α-pinene	0.0	47.9 ± 2.9 hi	54.1 ± 2.2 def	20.3 ± 4.1 bcd	21.0 ± 3.7 cd
		(−)-β-pinene	0.0	53.2 ± 6.3 i	66.6 ± 4.3 f	13.1 ± 6.2 bc	−6.0 ± 4.7 b

[a]Mean consumed diet per larvae (mg of dry leaf) relative to the initial mean dry weight of leaves. [b]Means values for three replicates with 10 fourth-instar larvae per replicate. Means (±SEM) in the same column followed by the same letters are not significantly different based on one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). [c]Positive control with 95% pure azadirachtin tested at 10 μg cm^{-2}. [d]High mortality was recorded in all experiments for the tested concentration.

Table 3. Effects of *F. vulgare* var. *vulgare*, *A. graveolens*, *P. crispum*, and *C. cyminum* Essential Oils and Pure Compounds (at 175 μg cm^{-2}) on Fourth-Instar Larvae of *P. unipuncta* Growth, Assessed after 48 and 72 h of Feeding on Treated Corn Leaves

treatments			relative mean weight gain[a] (% ± SEM)[b]		growth inhibition (% ± SEM)[b]	
			48 h	72 h	48 h	72 h
controls		ethanol	74.0 ± 6.3 fg	133.3 ± 6.6 h	–	–
		azadirachtin[c]	−7.7 ± 3.2 b	−9.7 ± 1.1[b]	112.2 ± 5.4 efg	127.4 ± 9.2 gh
EOs	*F. vulgare*	infrutescences	52.5 ± 6.7 e	81.0 ± 4.9 fg	34.0 ± 8.1 bc	43.2 ± 2.5 cd
		stems and leaves	84.7 ± 7.4 g	126.2 ± 4.1 h	−21.0 ± 6.7 a	−1.1 ± 2.3
	A. graveolens	mature fruits	7.2 ± 1.2 c	25.0 ± 4.3 c	91.2 ± 2.0 e	83.2 ± 3.2 f
		green infrutescences	53.5 ± 2.7 e	88.9 ± 3.5 fg	32.5 ± 7.2 bc	37.2 ± 6.5 cd
	P. crispum	fruits	−22.0 ± 2.5 a	−25.3 ± 1.2 a	136.3 ± 6.4 g	122.8 ± 1.4 h
	C. cyminum	fruits	31.1 ± 3.1 d	69.3 ± 0.7 e	49.6 ± 3.2 cd	38.9 ± 6.0 cd
compds		*trans*-anethole	−9.8 ± 4.2 b	−8.1 ± 5.5 b	132.2 ± 11.2 fg	117.4 ± 4.0 gh
		estragole	30.4 ± 4.2 d	53.2 ± 2.7 d	62.3 ± 1.5 d	62.6 ± 1.2 e
		(+)-fenchone	55.6 ± 4.8 ef	93.5 ± 6.0 g	23.6 ± 4.3 b	30.8 ± 3.0 c
		(*S*)-(+)-carvone	39.2 ± 2.0 de	74.3 ± 4.0 ef	46.4 ± 0.7 cd	46.8 ± 2.3 d
		myristicin[d]	–	–	–	–
		apiole[d]	–	–	–	–
		γ-terpinene	43.7 ± 7.2de	81.0 ± 4.2 efg	39.1 ± 6. 4 bc	38.0 ± 5.2 cd
		cuminaldehyde	−4.0 ± 1.0bc	−2.0 ± 1.0 b	106.4 ± 1.4 ef	101.7 ± 0.8 g
		(−)-α-pinene	40.9 ± 5. 4de	78.9 ± 8.8 efg	42.6 ± 4.8 c	39.8 ± 6.7 cd
		(+)-α-pinene	49.1 ± 7.4 e	88.7 ± 7.8 fg	40.0 ± 7.5 bc	35.3 ± 6.7 cd
		(−)-β-pinene	57.6 ± 7.0 ef	114.5 ± 6.7 h	32.8 ± 3.1 bc	12.2 ± 5.3 b

[a]Mean weight gain of larvae (mg of dry larvae) relative to their initial mean dry weight. [b]Mean values for three replicates with 10 fourth-instar larvae per replicate. Means (±SEM) in the same column followed by the same letters are not significantly different based on one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). [c]Positive control with 95% pure azadirachtin tested at 10 μg cm^{-2}. [d]High mortality was recorded in all experiments for the tested concentration.

terpene hydrocarbons (β-pinene, *p*-cymene, γ-terpinene) and oxygen-containing monoterpenes, more precisely aldehydes (cuminaldehyde, *p*-mentha-1,3-dien-7-al, *p*-mentha-1,4-dien-7-al) and alcohols (cuminyl alcohol, α-terpineol, terpinene-4-ol), are the mostly reported constituents of cumin fruit EO. According to our results and a previous study,[43] other

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monoterpenoids, especially menthane-type monoterpenoids and some sesquiterpenoids (β-caryophyllene, β-farnesene), may be detected in lower contents.

Feeding Deterrence and Growth Inhibition. Bioactivities of six different EOs and nine pure compounds on *P. unipuncta* fourth-instar larvae feeding and growth after 48 and 72 h of experiment are presented in Tables 2 and 3, respectively. The FDI determined for EOs and compounds were significantly different [*F*-test = 46.31 (48 h), 46.42 (72 h); df = 47; $p < 0.001$] and varied from −26.2 to 99.7% and −20.3 to 99.7% after 48 and 72 h, respectively. Significant differences among treatments were also observed for GI results [*F*-test = 46.82 (48 h), 59.93 (72 h); df = 46; $p = 0.000$], as values ranged from −21.0 to 136.3% at 48 h and −1.1 to 127.4% at 72 h. Only *P. crispum* and *A. graveolens* fruit EOs and the pure constituents *trans*-anethole and cuminaldehyde showed effective feeding deterrence (FDI > 70%). Avoidance of food treated with *P. crispum* EO, *trans*-anethole, or cuminaldehyde by larvae was pronounced, especially in the first 48 h, which caused substantial weight loss (negative values in Table 3) and GI above 100%. Despite their higher concentration (175 μg cm^{-2}), the inhibitory effects of these treatments were statistically similar to those exhibited by the antifeedant compound azadirachtin at 10 μg cm^{-2} (17 times less concentrated). Additionally, low to moderate activity was attributed to *C. cyminum* fruit EO, (*S*)-(+)-carvone, and estragole (FDI ≤ 45% and GI ≤ 60%, after 48 h). Nonetheless, the low feeding deterrence activity of cumin fruit EOs described herein against *P. unipuncta* (45% at 175 μg cm^{-2}) was far greater than the activity recorded on *S. litura* (antifeedant index = 40% for 2 mg cm^{-2}).[46] Approximated feeding deterrence (50%) has been attributed to other EOs. For example, a DC_{50} value of 206 μg cm^{-2} was reported for clove oil against the same noctuid specie.[6] Relative to the remaining EOs/compounds, these were considered ineffective for feeding dissuasion and even stimulant, as was the case of the EO extracted from stems and leaves of *F. vulgare* var. vulgare and the compound (−)-β-pinene after 72 h (Table 2). For all treatments, excepting *P. crispum* fruit EO and the positive control, an increase in the consumption percentage was observed in the last 24 h, revealing some habituation to treated food. According to Usher and co-workers,[47] deprivation might allow acceptance of less preferred plants after repeated contacts and such behavioral flexibility would have adaptive value. Induction of detoxification enzymes and alteration of compounds toxicity are possible adaptive responses. Proportional increases of diet consumption and weight gain were recorded for the majority of treatments, and a significant correlation was found between the two variable at 48 h ($r = 0.887$, $p < 0.0001$) and 72 h ($r = 0.970$, $p < 0.0001$). However, the treatments with *trans*-anethole and fennel EOs did not fit into this trend, as larvae absolute weight gains were lower than those expected by the linear function after 72 h (absolute values not shown). These findings suggest that *trans*-anethole was not only phagorepellent but it may also have interfered in the process of assimilation and conversion of food to biomass. Postingestion effects, such as digestive disorders or physiologic stress, could be the primary cause for the larvae weight loss and death observed in 13% of individuals. Evidence of *trans*-anethole metabolization to a hydroxylated form was found in feces of the tobacco cutworm (*Spodoptera litura*) after topical and oral administration of the compound.[48] However, the impact on growth and larvae fitness was not referred to. An effective feeding deterrence of *trans*-anethole on fifth-instar larvae of *S. litura* was already reported and 50% deterrence (DC_{50}) was observed at a concentration of 103 μg cm^{-2}.[49] Regarding the results obtained for OEs and their major compounds tested individually, a few relations may be established. In the particular case of *A. graveolens*, the EO from green infrutescences was significantly less active than the EO from dry fruits. These findings suggest that the less significant deterrent effect might be a consequence of the lower (*S*)-(+)-carvone content in green infrutescences (64%), relative to fruit EO (84%), and/or the occurrence of antagonistic effects with other compounds (Figure 1B). However, when tested pure and at a slightly higher dosage, a significant decrease in the feeding deterrence of (*S*)-(+)-carvone was recorded, revealing the contribution of β-phellandrene, coexisting in the dill fruit EO in lower amount (14%), to enhance the antifeedant property of carvone in this natural mixture. For *C. cyminum* EO, we confirmed that its activity was closely related to the major constituent content, cuminaldehyde (39%), which was more active (GI and FDI values 2−2.6 times superior) when tested pure at a higher concentration (2.6 times). The results obtained for γ-terpinene and β-pinene showed that both compounds have negligible effects in this context when tested individually and at much higher dosage (respectively, 6.2 and 8 times more concentrated than in the OE). Nonetheless, the occurrence of synergism between cuminaldehyde and further compounds is not discarded. Fennel EOs showed irrelevant activities in this experiment, and the results obtained for both natural mixtures could not be related to any individual activity of the major compounds included in the study [*trans*-anethole, estragole, fenchone, (+)- and (−)-α-pinene, and (−)-β-pinene]. The stimulant effects observed with fennel stem and leaf EO is attributed to the specificity of the mixture presenting a considerable increase in the pinenes and phellandrenes proportion relative to (+)-fenchone and estragole, in comparison with the infrutescence EO. *P. crispum* EO was the most effective among the EOs, monoterpenes, and phenylpropane derivative compounds tested in the present work, as virtually no food intake was registered. Severe weight loss and, consequently, starvation was the major cause of the high mortality (32%). The phagorepellent property of this EO was evident (DC_{50} and DC_{90} should be inferior to 175 μg cm^{-2}) and certainly more related to the myristicin (32%) and apiole (16%) than pinenes contents (30%), even though some synergisms may occur. The strong toxicity observed for both tested phenylpropanoids (no consumption and 100% and 50% of death for myristicin and apiole, respectively, after 72 h) did not allow one to complete the evaluation of their effects at the unique dose tested, and further assays should be envisaged. As far as we know, less attention has been given to the anti-insect activities of EOs from this specie. We consider that the EO from parsley fruits has some potential, and a DC_{50} value close to the one estimated for rotenone on *P. unipuncta* third-instar larvae (62 μg cm^{-2})[6] is plausible. In the domain of antifeedant/deterrent biopesticides, a tremendous effectiveness has been attributed to *Meliaceae* species and their limonoid triterpenes compounds.[27,50,51] Feeding deterrent activity of *Azadirachta indica*, *Melia volkensii*, and *A. excelsa* extracts against *P. unipuncta* third-instar larvae were remarkable (DC_{50} = 0.6, 10.8, and 46.9 μg cm^{-2}, respectively).[6] The high effectiveness of the limonoid compound azadirachtin, against the same phytophagous specie, was confirmed in our study.

Toxicity Evaluations. The toxicity of seven EOs and nine compounds evaluated by fumigation and disk contact assay

Table 4. Toxicity of *F. vulgare* var. *vulgare*, *A. graveolens*, *P. crispum*, and *C. cyminum* Essential Oils and Pure Compounds against *P. unipuncta* Fourth-Instar Larvae Evaluated by Fumigation (250 μg cm^{-3}) and Assessed after 12, 24, and 48 h of Treatment

treatments				mortality (% ± SEM)[a]		
			n[b]	12 h	24 h	48 h
control		ethanol	300	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
EOs	*F. vulgare*	infrutescences	40	40.0 ± 5.8 b	97.5 ± 2.5 g	100.0 ± 0.0 e
		stems and leaves	50	6.0 ± 4.0 a	58.0 ± 5.8 cd	96.0 ± 2.4 de
	A. graveolens	mature fruits	30	96.7 ± 3.3 d	100.0 ± 0.0 g	100.0 ± 0.0 e
		green infrutescences	60	65.8 ± 9.4 c	90.0 ± 6.3 fg	100.0 ± 0.0 e
	P. crispum	fruits	40	5.0 ± 2.9 a	5.0 ± 2.9 ab	5.0 ± 2.9 a
		green infrutescences[c]	40	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	C. cyminum	fruits	50	14.0 ± 4.0 ab	58.0 ± 8.0 cd	80.0 ± 8.4 bc
compds		*trans*- anethole	40	0.0 ± 0.0 a	34.3 ± 3.3 bc	68.9 ± 8.2 b
		estragole	40	0.0 ± 0.0 a	72.5 ± 2.5 de	97.5 ± 2.5 de
		(+)- fenchone	40	2.5 ± 2.5 a	12.5 ± 6.3 ab	22.5 ± 7.0 a
		(*S*)-(+)- carvone	40	95.0 ± 2.9 d	100.0 ± 0.0 g	100.0 ± 0.0 e
		myristicin[d]	–	–	–	–
		apiole[d]	–	–	–	–
		γ-terpinene	40	82.5 ± 4.8 c	92.5 ± 3.9 fg	100.0 ± 0.0 e
		cuminaldehyde	40	28.0 ± 9.2 ab	80.0 ± 7.1 ef	88.0 ± 4.9 cd
		(−)-α-pinene	40	0.0 ± 0.0[a]	0.0 ± 0.0 a	0.0 ± 0.0 a
		(+)-α-pinene	40	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
		(−)-β-pinene	40	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a

[a]Means (±SEM) of mortality in the same column followed by the same letters are not significantly different based on one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). [b]Number of tested insects. [c]EO extracted from the *P. crispum* var. *neapolitanum* Danert. [d]These compounds were not included in the fumigation assessment, as they are less volatile at room temperature.

Table 5. Toxicity of *F. vulgare* var. *vulgare*, *A. graveolens*, *P. crispum*, and *C. cyminum* Essential Oils and Pure Compounds against *P. unipuncta* Fourth-Instar Larvae Evaluated by Contact Disk Assay at 250 μg cm^{-2} and Assessed after 9, 24, and 48 h of Treatment

treatments				Mortality (% ± SEM)[a]		
			n[b]	9 h	24 h	48 h
controls		ethanol	60	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
		lambda-cyhalothrin[c]	40	0.0 ± 0.0 a	12.5 ± 4.8 ab	62.5 ± 6.3 b
EOs	*F. vulgare*	infrutescences	30	3.7 ± 3.3 ab	7.4 ± 3.3 a	7.4 ± 3.3 a
		stems and leaves	30	3.6 ± 3.3 ab	7.1 ± 3.3 a	7.1 ± 3.3 a
	A. graveolens	mature fruits	40	83.3 ± 3.3 f	100.0 ± 0.0 g	100.0 ± 0.0 f
		green infrutescences	60	50.0 ± 7.1 e	88.3 ± 5.4 ef	95.0 ± 3.4 f
	P. crispum	fruits	60	10.0 ± 7.7 ab	60.0 ± 6.3 cd	93.3 ± 3.3 ef
		green infrutescences[d]	40	0.0 ± 0.0 a	40.0 ± 9.1 bc	67.5 ± 4.8 bc
	C. cyminum	fruits	40	32.5 ± 7.5 cd	80.0 ± 3.2 de	92.0 ± 2.0 ef
compds		*trans*-anethole	60	20.0 ± 10.0 bc	73.3 ± 8.0 de	95.0 ± 2.2 f
		estragole	40	2.5 ± 2.5 ab	5.0 ± 5.0 a	15.0 ± 6.4 a
		(+)-fenchone	40	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
		(*S*)-(+)-carvone	40	37.5 ± 4.8 cde	97.5 ± 2.5 fg	97.5 ± 2.5 f
		myristicin	50	2.0 ± 2.0 ab	72.0 ± 4.9 d	100.0 ± 0.0 f
		apiole	40	0.0 ± 0.0 a	20.0 ± 7.1 ab	72.5 ± 8.5 cd
		γ-terpinene	40	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
		cuminaldehyde	50	44.0 ± 9.3 de	75.0 ± 9.9 de	80.0 ± 8.2 de
		(−)-α-pinene	40	0.0 ± 0.0 a	0.0 ± 0.0 a	0,0 ± 0.0 a
		(+)-α-pinene	40	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
		(−)-β-pinene	40	2.5 ± 2.5 ab	2.5 ± 2.5 a	2.5 ± 2.5 a

[a]Means (±SEM) of mortality in the same column followed by the same letters are not significantly different based on one-way ANOVA followed by Duncan's Multiple range test ($p < 0.05$). [b]Number of tested insects. [c]Pyrethroid insecticide. [d]EO extracted from the *P. crispum* var. *neapolitanum* Danert.

with *P. unipuncta* larvae are described in Tables 4 and 5, respectively. Differences recorded among treatments by fumigation [*F*-test =27.55 (12 h), 31.59 (24 h), 52.57 (48 h); df = 62; $p < 0.001$] and filter paper impregnation [*F*-test =16.72 (9 h), 22.87 (24 h), 62.57 (48 h); df = 84; $p < 0.001$] were significant. In general, some activity was observed in fumigant and contact toxicity assays performed at the unique screening dosages of 250 μg cm^{-3} and 250 μg cm^{-2}, respectively. *A. graveolens* EOs exhibit larvicidal properties in both assays, causing mortalities above 50% in the first exposure periods (9

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Table 6. LC_{50} and LC_{90} ($\mu g\ cm^{-2}$) Estimated by the Disk Assay Method for the Most Active Essential Oils and Constituents against Fourth-Instar Larvae of *P. unipuncta* after 24 h of Treatment

treatments			n^a	$LC_{50}{}^{b,c}$ (95% CL)	$LC_{90}{}^{b,c}$ (95% CL)	Slope^{b,c} (±SEM)	$H^{b,d}$
EOs	*A. graveolens*	green infrutescences	200	107.7 a (95.0−121.3)	343.6 b (268.5−509.3)	2.5 ± 0.32 a	2.60
	P. crispum	fruits	250	156.0 b (137.4−181.6)	641.4 b (451.6−1159.1)	2.1 ± 0.28 a	0.68
	C. cyminum	fruits	200	196.8 c (181.9−215.7)	404.1 b (344.0−509.8)	4.1 ± 0.42 b	1.38
compds		*trans*- anethole	200	178.7 bc (161.4−202.6)	504.7 b (396.2−726.0)	2.8 ± 0.31 ab	1.89
		(*S*)-(+)-carvone	200	108.6 a (102.4−113.9)	160.0 a (150.8−153.0)	7.6 ± 0.75 c	1.46
		myristicin	200	157.5 b (143.1−175.8)	443.5 b (355.4−615.6)	2.9 ± 0.30 ab	0.73
		cuminaldehyde	280	151.4 b (133.2−176.3)	661.6 b (482.6−1052.3)	2.0 ± 0.21 a	0.51

[a]Number of tested insects excluding controls. [b]Values were determined by probit analysis based on mortalities recorded by contact toxicity assays performed at different optimized concentrations (five or six concentrations between 31 and 375 $\mu g\ cm^{-2}$). [c]LC_{50} and LC_{90} values and 95% confidence limits (CL) are expressed in $\mu g\ cm^{-2}$ of essential oil or compound required to kill insects. LC values and slopes within a column followed by the same letter are not significantly different based on nonoverlapping of 95% CL. [d]*H*, heterogeneity factor (χ^2/degree of freedom)

and 12 h). Similar results were observed for *C. cyminum* EOs, although its activity was stronger through contact (93%), and *F. vulgare* var. vulgare and *P. crispum* EOs gave ambiguous results when comparing the two assays. Fumigant effectiveness was registered with fennel EOs (100 and 96%, after 48 h treatments), while negligible toxicity was recorded in the disk contact assay. On the contrary, *P. crispum* fruit and green infrutescence EOs did not show significant effects when larvae were exposed to its vapors, despite the evident contact toxicities of these EOs evaluated by paper impregnation (94 and 68% mortality at 48 h). With respect to the tested compounds, estragole, γ-terpinene, and (+)-fenchone were more active by fumigation than by contact with larvae body. The toxicity of *trans*-anethole though contact was higher (96%) relatively to its isomer estragole (15%), despite its lower fumigation effectiveness (69%). Similar observations were described by Bhardwaj[52] against the tobacco armyworm. Cuminaldehyde larvicidal activity was approximately the same in both assays (88 and 80% of mortality after 48 h of fumigation and contact with impregnated paper, respectively). The results obtained with both toxicity assays demonstrated that some EOs and pure compounds might have broad activity and probably several modes of action (dill EOs, cumin EO, carvone, cuminaldehyde, and *trans*-anethole), while others can be more active by a specific way of entrance/contact (bitter fennel and parsley EOs, estragole, and γ-terpinene). *A. graveolens* EOs have been widely recognized for their insecticidal properties namely against *Aedes aegypti* larvae,[24,53] the Japanese termite (*Reticulitermes speratus*),[22] and the coleopterans *Acanthoscelides obtectus*,[54] *Callosobruchus chinensis*,[19] and *Callosobruchus maculatus*.[55] Likewise, a strong activity of *C. cyminum* EOs on insects and other arthropods has been reported. Cumin EO was effective as fumigant against the pulse beetle (*C. chinensis*), *Lycoriella ingenua* larvae, *A. obtectus*, and the cotton aphid (*Aphis gossypii*),[19,45,54,56] and as contact larvicidal agent against *S. litura*.[46] In some of these studies, and as we observed, the toxicity of dill and cumin EOs were closely related with high contents of carvone and cuminaldehyde, respectively. Carvone and cuminaldehyde were 100% and 98% active by fumigation at 5×10^{-3} mg mL^{-1} air against *L. ingenua*.[45] Cumin and dill EOs, characterized by 43% of cuminaldehyde and 35% of carvone, respectively, were among the most fumigant EOs against *A. obtectus*.[54] Bitter fennel EOs and its major constituent, estragole, were much more effective without skin contact. A plausible hypothesis is that the delivery of these oils to target tissues may occur through a vapor phase, preferentially through penetration via the respiratory system. There are several evidence of the fumigant potential of *F. vulgare* EOs and/or its major constituents against stored product coleopterans,[17] aphid,[21] mosquitoes species,[18,57] and lepidopteran.[58] On the basis of the differences observed on the fumigation screening for bitter fennel EOs and their major compounds [fruit EO ≥ stem and leave OE ≥ estragole > *trans*-anethole > (+)-fenchone ≫ α- and β-pinenes], we consider that the fumigation activity of both OEs is certainly related with the estragole content. Nevertheless, synergist/antagonist activities between major and minor constituents are probable. It has been demonstrated that some ketone monoterpenes, namely (+)-fenchone, were more effective as fumigants than alcohols[58] and that phenol, alcohol, aldehyde, and ketone compounds are generally more toxic than hydrocarbons.[22,45] Kim and Ahn[17] observed a higher activity of estragole and (+)-fenchone against the two pests *Sitophilus oryzae* and *C. chinensis* relative to *trans*-anethole. In our study, (+)-fenchone was found to be less active as fumigant relative to both phenols, the aldehyde, and the monoterpenoid hydrocarbon tested. Regarding parsley fruit EOs, chemical profiles had an important role in the bioactivities reported herein. A low fumigant activity of fruit EO was already reported by Regnault-Roger and co-workers[54] for a EO constituted by 43% of apiole and 10% of thymol (LC_{50} > 100 mg dm^{-3}). The absence of toxicity through the fumigation method can be explained, to some extent, by the predictable reduced concentrations of phenylpropane derivatives in the vapor phase at room temperature, as a consequence of their high boiling points (276.5 and 294 °C for myristicin and apiole, respectively) and low vapor pressures (0.008 and 0.003 mm/Hg for myristicin and apiole, respectively). Thus, on the basis of observed results, toxicity exerted at the tissue level via penetration through the insect cuticle or digestive apparatus (during grooming) is very plausible. In fact, some insecticidal potential has been attributed

Table 7. LT_{50} and LT_{90} (h) for the Most Active Essential Oils and Four Pure Compounds Estimated by the Disk Assay Method against Fourth-Instar Larvae of *P. unipuncta*, at 125 μg cm^{-2}

treatments			n^a	mortality at 72 h (%)	$LT_{50}{}^{b,c}$ (95% CL)	$LT_{90}{}^{b,c}$ (95% CL)	slope^{b,c} (±SEM)	$H^{b,d}$
EOs	*A. graveolens*	green infrutescences	190	85.0	18.1 a (14.5−21.3)	83.2 b (65.7−119.4)	1.9 ± 0.23 a	1.35
	P. crispum	fruits	160	78.0	29.7 b (23.5−34.5)	118.0 bc (87.1−216.3)	2.1 ± 0.38 a	0.35
	C. cyminum	fruits	160	40.0	–[e]	–[e]	–	–
compds		*trans*-anethole	160	70.0	40.1 c (34.8−46.0)	151.4 bc (107.4−294.3)	2.2 ± 0.38 a	1.43
		(*S*)-(+)-carvone	200	95.0	13.4 a (10.5−16.0)	48.4 a (41.1−60.7)	2.3 ± 0.26 a	2.57
		myristicin	160	97.5	25.2 b (22.4−27.5)	45.8 a (41.9−51.8)	4.9 ± 0.57 b	1.56
		cuminaldehyde	160	62.5	44.0 c (37.1−53.5)	238.2 c (155.6−494.7)	1.8 ± 0.25 a	0.40

[a]Number of tested insects excluding controls. [b]Values were determined by probit analysis based on mortalities recorded on the contact toxicity assay performed over time (24, 36, 48, and 72 h) at 125 μg cm^{-2}. [c]LT_{50} and LT_{90} values and 95% confidence limits (CL) are expressed in hours of treatment required to kill insects. LT values and slopes within a column followed by the same letter are not significantly different based on nonoverlapping of 95% CL. [d]*H*, heterogeneity factor (χ^2/degree of freedom). [e]Not determined for the chosen concentration of 125 μg cm^{-2}. Plotting of Probit versus $\log_{10}$(concentration) for linear regression was not possible due to low mortalities recorded over time.

Table 8. Linear Variation of the Inverse LC_{50} with Time of Exposure Assessed for the 3-Day Period of Treatment

		SEM			
treatments	equations[a]	slope	*y*-intercept	*p*	*r*
A. graveolens EO	$LC_{50}{}^{-1} = 2.7 \times 10^{-4}t + 2.3 \times 10^{-3}$	2.3×10^{-5}	1.1×10^{-3}	0.007	0.992
P. crispum EO	$LC_{50}{}^{-1} = 1.4 \times 10^{-4}t + 4.1 \times 10^{-3}$	3.8×10^{-6}	1.8×10^{-3}	0.061[b]	0.939
C. cyminum EO	$LC_{50}{}^{-1} = 0.49 \times 10^{-4}t + 4.1 \times 10^{-3}$	4.5×10^{-6}	0.22×10^{-3}	0.008	0.992
trans-anethole	$LC_{50}{}^{-1} = 1.3 \times 10^{-4}t + 2.6 \times 10^{-3}$	2.4×10^{-5}	1.1×10^{-3}	0.031	0.969
(*S*)-(+)-carvone	$LC_{50}{}^{-1} = 2.3 \times 10^{-4}t + 6.3 \times 10^{-3}$	1.6×10^{-5}	7.9×10^{-4}	0.005	0.995
myristicin	–[c]	–	–	–	–
cuminaldehyde	$LC_{50}{}^{-1} = 0.62 \times 10^{-4}t + 5.2 \times 10^{-3}$	5.6×10^{-6}	0.27×10^{-3}	0.008	0.992

[a]$LC_{50}{}^{-1}$ (μg^{-1} cm^2) was correlated with time of exposure, *t* (h). [b]Slope was not significantly different from zero ($p > 0.05$). [c]The variation of myristicin $LC_{50}{}^{-1}$ for the studied period does not fit into the linear regression model.

to phenylpropane derivative compounds, but their activity is greatly influenced by the variations in the isomeric forms and/or functionalities attached to either the aryl ring or the alkyl side chain.[22] An EO fraction, rich in dillapiole, extracted from *Anethum sowa* had ovicidal activity but no fumigant toxicity against *C. maculatus*.[55] Lichtenstein and collaborators[24] reported that apiole, dillapiole, and myristicin were much more toxic to fruit flies than D-carvone and that apiole, having two methoxy groups, appeared to be more toxic than myristicin, which has just one. Conversely, in the present study, the contact toxicity of myristicin was significantly superior to the apiole insecticidal activity recorded at 24 and 48 h and (*S*)-(+)-carvone was much more effective than both phenylpropenes, in the first 24 h of exposure.

Determination of LCs and Toxicity over Time of Exposure. Paper impregnation was the chosen method to probe toxicity caused by contact exposure of *P. unipuncta* fourth-instar larvae to EOs and pure compounds. Lethal parameters (LCs and LTs for 50 and 90% of mortality) were determined for treatments that showed higher effectiveness in the single-dose screening assay: dill green infrutescence, parsley and cumin fruit EOs, and constituents *trans*-anethole, (*S*)-(+)-carvone, myristicin, and cuminaldehyde. After 24 h of treatment, LC_{50} of EOs were considered significantly different and (*S*)-(+)-carvone LC_{50} value was found to be statistically distinct from that of other compounds (Table 6). Results also indicate that for the evaluation of insecticidal properties the LC_{50} parameter has a better discrimination power, since less significant differences were detected among treatments based on LC_{90}. Thus, for a 24 h evaluation, *A. graveolens* EO and (*S*)-carvone were considered the most active (108 and 109 μg cm^{-2}) and *C. cyminum* was the least effective of all treatments (197 μg cm^{-2}). On the basis of accumulated mortalities recorded over time of exposure, we were able to compare the treatments toxicity for an intermediate single-dose, with larvicidal effects above 50% (Table 7). The 50% killing performance achieved with *A. graveolens* green infrutescence EO, and its major compound, was significantly higher than *P. crispum* fruit EO and myristicin, *trans*-anethole, or cuminaldehyde. After 13 and 18 h of treatment at a medium concentration, 50% of individuals were killed by (*S*)-(+)-carvone and dill EO, respectively, while more than twice that time was needed for *trans*-anethole and cuminaldehyde. Linear regressions between the inverse of OE/constituents activities (LCs^{-1}) and time of exposure (h) were established, except for myristicin, and they are presented in Table 8. All slopes were significantly different from zero with the exception of that of *P. crispum* ($p = 0.062$, $r = 0.94$). The comparison of the different slopes gives useful information about the potency of treatments. A higher effectiveness and toxicity were recorded for EOs or constituents with highest slopes, as was the case for *A. graveolens* infrutescence EO and its major compound, (*S*)-

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(+)-carvone, followed by *P. crispum* EO and *trans*-anethole. Concentrations needed to obtain 50% of activity reduced substantially over time, including for myristicin (concentrations were 2.4−2.0 smaller between 24 and 72 h). Lower decreases of LCs_{50} were observed for *C. cyminum* and its principal constituent cuminaldehyde (1.48 and 1.45 less, respectively). Furthermore, similar patterns of variation (approximated slopes) were found for dill and cumin EOs and their major compounds (*S*)-(+)-carvone and cuminaldehyde, respectively, evidencing the direct relation existing between these compounds and the performances of EOs where these are found in abundance. Regardless of the fact that some retarded/cumulative effects may have occurred in individuals exposed to a certain dose during the specific studied period, we consider that this evaluation gives a strong indication of the performance and insecticidal potency of tested EOs/compounds. Time-series approaches help to differentiate, among candidates with considerable activity, which have acute/faster activity, for a reduce amount of toxicant, from others. Effectiveness for short time exposure may contribute to reduce costs and environmental impact. From toxicological, economic, and environmental viewpoints, these are desirable attributes for novel biopesticides, especially for rare or fewer abundant natural compounds from botanical species.

The present work revealed the anti-insect potential of EOs/compounds from four well-known Apiaceae species on a major agricultural pest. Anti-insect activity varied according to plant specie/composition and type of exposure. Additionally, results indicated that the same mixture/compound may have different fates and consequences in one specie and that several or distinct mechanisms may be involved in fumigant and contact toxicant action. Sublethal toxicity through feeding might have been the cause of growth and consumption inhibitions observed for some treatments. In the field context, a reduction in food consumption, by debilitated and less developed larvae, might help to mitigate some of the damage caused by this pest. Also, according to Akhtar and Isman, growth inhibition and feeding deterrence can increase the time of developmental stages and the search for viable food, exposing herbivores to increased mortality as a result of biotic and abiotic factors.[59]

Fumigant activities were demonstrated in this study for dill and fennel EOs, estragole, (*S*)-(+)-carvone, and γ-terpinene, as well as a promising contact toxicity of parsley EO and *trans*-anethole. Insecticidal activities of dill and cumin EOs and their major compounds (*S*)-(+)-carvone and cuminaldehyde were recorded in all evaluations performed against *P. unipuncta* caterpillar, suggesting that these may have multiple toxic effects. The acute/strong toxicity of EO from dill infrutescences was noteworthy and directly related with the extremely high content of (*S*)-(+)-carvone (67 and 84%), a compound with recognized insecticidal activity. Cuminaldehyde, the major compound of cumin oil (39%), was responsible, to some extent, for the EO anti-insect properties, but it did not entirely justify the results (additive effects or synergisms with other constituents). The promising activities recorded for parsley EOs seems to be related with specific activities of phenylpropenes. In general, the individual evaluation proposed for all major compounds of the studied OE was not sufficient to explain the observed properties of respective EOs. However, it is important to emphasize that a more complete evaluation of several constituents individually may not necessarily lead to a better understanding of the toxicity of such complex mixtures (the whole is probably not the sum of its parts). As an alternative, an efficient approach have been proposed by Jiang et al. using artificial blends with omitted components to discriminate the role of each compounds on the overall toxicity of a reproduced EO.[60]

A. graveolens, *C. cyminum*, and *P. crispum* EOs tested and characterized herein were effective against *P. unipuncta*. The application of these EOs as deterrents and/or insecticides for the control of this lepidopteran could be advantageous considering the great productivity of these annual/biannual species and the high availability, low cost, and satisfactory efficiency of their EOs.

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Notes

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