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Bioactivity of some Apiaceae essential oils and their constituents against *Sitophilus zeamais* (Coleoptera: Curculionidae)

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Abstract

Sitophilus zeamais is a key pest of stored grains. Its control is made, usually, using synthetic insecticides, despite their negative impacts. Botanical insecticides with fumigant/repellent properties may offer an alternative solution. This work describes the effects of *Anethum graveolens*, *Petroselinum crispum*, *Foeniculum vulgare* and *Cuminum cyminum* essential oils (EOs) and (*S*)-carvone, cuminaldehyde, estragole and (+)-fenchone towards adults of *S. zeamais*. Acute toxicity was assessed by fumigation and topical application. Repellence was evaluated by an area preference bioassay and two-choice test, using maize grains. LC_{50} determined by fumigation ranged from 51.8 to 535.8 mg L^{-1} air, with (*S*)-carvone being the most active. LD_{50} values for topical applications varied from 23 to 128 µg per adult for (*S*)-carvone > cuminaldehyde > *A. graveolens* > *C. cyminum* > *P. crispum*. All EOs/standard compounds reduced significantly the percentage of insects attracted to maize grains (65–80%) in the two-choice repellence test, whereas in the area preference bioassay RD_{50} varied from 1.4 to 45.2 µg cm^{-2}, with cuminaldehyde, (*S*)-carvone and estragole being strongly repellents. *Petroselinum crispum* EO and cuminaldehyde affected the nutritional parameters relative growth rate, efficiency conversion index of ingested food and antifeeding effect, displaying antinutritional effects toward *S. zeamais*. In addition, *P. crispum* and *C. cyminum* EOs, as well as cuminaldehyde, showed the highest acetylcholinesterase inhibitory activity *in vitro* (IC_{50} = 185, 235 and 214.5 µg mL^{-1}, respectively). EOs/standard compounds exhibited acute toxicity, and some treatments showed antinutritional effects towards *S. zeamais*. Therefore, the tested plant products might be good candidates to be considered to prevent damages caused by this pest.

Introduction

Economic losses caused by storage pests are high, however, they strongly diverge with the type of crop, country, climatic region and duration of storage (Klys *et al.*, 2017). According to the same authors, in general, the global annual losses in the stored products due to insect activity are estimated at 10%. In addition to eating the grains, this pest is also a cause of food contamination by microorganisms (Magan *et al.*, 2003; Athanassiou *et al.*, 2017).

Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae), also known as the maize weevil, is a key pest of grains and grain products in different parts of the world, causing most of the losses in maize grains (Colares *et al.*, 2016; Ojo and Omoloye, 2016). Both the larval and adult stages of this insect devour the grains, causing postharvest significant damages. The control of these insects depends heavily on the use of synthetic insecticides, but their residues pose serious risks to the environment, animals and human, causing lethal effects on non-target organisms and pest resistance (Askar *et al.*, 2016; Colares *et al.*, 2016). To avoid such inconsistencies, the search for new alternatives for pest control is required. As a complementary approach or an alternative to synthetic pesticides, phytochemicals, namely essential oils (EOs) constituents are presently under consideration as ingredient of crop protection products, as well as in repellent formulations (Isman and Akhtar, 2007; Regnault-Roger *et al.*, 2012). Several studies have pointed out the value of Apiaceae plants and their potential application in the context of Integrated Pest Management and Integrated Vector Management (IVM) (Boulogne *et al.*, 2012; Evergetis *et al.*, 2012; 2013; Pavela and Vrchotová, 2013; Seo *et al.*, 2015). The supporting evidences of Apiaceae pesticidal activities against various types of damaging/noxious organisms, including stored-product insects are substantial (Chaubey, 2008; Ebadollahi, 2011, 2013; Ebadollahi *et al.*, 2012; Kim *et al.*, 2013).

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In view of their relatively high commercial relevance, we have been studying the pesticidal potential of some of the most important Apiaceae species: *Anethum graveolens* L. (dill), *Cuminum cyminum* L. (cumin), *Foeniculum vulgare* subsp. *vulgare* var. *vulgare* Mill. (bitter fennel) and *Petroselinum crispum* (Mill.) Nyman ex A.W. Hill (parsley) (Sousa *et al*., 2013, 2015*a*, 2015*b*, 2017). In the present work, EOs from these four plant species and some EO standard compounds were evaluated for their potential fumigant and contact toxicity, as well as repellent activity towards *S. zeamais* aiming to prevent damages caused by this pest on stored maize grain. Furthermore, their effects on nutritional physiology were evaluated through analysis of nutritional metrics, to assess to what extent EOs/standard compounds can disrupt insect feeding, metabolism and capacity of conversion of food into body mass.

Material and methods

Essential oils and chemical composition

A. graveolens (dill) plants were grown from commercial seeds while *F. vulgare* var. *vulgare* (bitter fennel) germplasm was obtained from a wild population. Voucher specimens of fruits and vegetative parts were deposited at the University of Porto (Portugal) herbarium (accession number PO1000MFF). Dill and bitter fennel green infrutescences (fruits in a pre-ripening phase) were collected from 5 and 14-months old plants, respectively. After 2 h of hydrodistillation in a Clevenger modified apparatus, the recovered EOs were dried with sodium sulphate and stored in brown sealed vials until use (−20° C). The EOs from *P. crispum* (parsley) and *C. cyminum* (cumin) fruits were purchased from Sigma-Aldrich, Co. Complete quantitative and qualitative profiles of dill, cumin, bitter fennel and parsley EOs herein tested were previously characterized (Sousa *et al*., 2017). The EO extracted from dill infrutescences was mainly constituted by (*S*)-carvone (66.4%), β-phellandrene + limonene (24.7%) and α-phellandrene (5.3%), while bitter fennel infrutescence EO contained estragole (64.9%), fenchone (15.8%) and β-phellandrene (5.5%). The EO from cumin fruits was rich in cuminaldehyde (39.4%), γ-terpinene (15.8%), β-pinene (12.4%), *p*-cymene (10.4%) and *p*-mentha-1,4-dien-7al (9.7%). Major compounds identified in parsley fruit EO were: myristicin (31.5%), apiole (15.9%), α-pinene (16.2%), β-pinene (13.6%) and 1-allyl-2,3,4,5-tetramethoxybenzene with carotol (8.6%).

Chemicals

Four high purity standard volatile compounds were included in all assays based on their relative abundance in the studied EOs (Sousa *et al*., 2017). The standards (*S*)-(+)-carvone (96%), cuminaldehyde (98%), estragole (98%) and (+)-fenchone (99.5%) were purchased from Sigma-Aldrich and Fluka (Aldrich chemical Co., St. Louis, MO, USA).

Ellman´s reagent (DTNB, 5,5′-dithionitrobenzoic acid; 99%), acetylthiocholine iodide (ATChI; ≥99%), berberine and the purified acetylcholinesterase (AChE, EC3.1.1.7) used for enzymatic assay were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

Bioassays

Insects

Adults of *S. zeamais* were obtained from a colony maintained for more than four generations in laboratory conditions in the Biology Department of Azores University (Ponta Delgada, S. Miguel, Azores archipelago, Portugal). Insects were reared with organic yellow corn grains (*Zea mays* local type) and maintained in plastic cages (35.7 cm × 23.5 cm × 13.4 cm) in the following conditions: 23 ± 1°C, 60% of relative humidity (RH), photoperiod of 14:10 (L: D) h. *Zea mays* grains were obtained from a biological production and acquired from local farmers with an average moisture content of 14 ± 0.5%. Unsexed adult weevils used in all the experiments were about 2 to 4 weeks old.

Fumigant toxicity

The fumigant insecticidal activity of the EOs and the standard compounds against *S. zeamais* adults were evaluated at different doses (25, 50, 75, 100, 156, 300, 525 and 600 mg L^{-1} air). Increasing amounts of EOs/standard compounds (from 1 to 24 mg) were pipetted onto a Whatman no. 1 filter paper disc (Ø 2 cm). Each paper disc was attached to the inner surface of a transparent plastic vial screw cap (total volume of 0.040 L) and a wire sieve was used inside the vial to prevent direct contact of the insects with the treated filter paper (fig. 1). Ten unsexed adult insects taken from the laboratory colony were placed with 1 g of yellow corn grains in a plastic vial (fig. 1). The caps were screwed tightly and hermetically sealed. Vials containing insects were turned upside down over the vials containing the impregnated filter paper disc, to allow saturation of the atmosphere with EO/standard compound vapours. The vials were kept in the dark, in an incubator at 23°C and 60 ± 5% RH. Five replicates were carried out for treatments and negative control groups. The number of dead insects was recorded daily by direct observation until 7 day after the start of treatment. Insects were considered dead if they did not respond to touch stimulation with a blunt needle.

Contact toxicity

The contact toxicity of EOs/standard compounds against *S. zeamais* adults was evaluated at different doses (0, 30, 50, 100 and 150 µg per insect). One microliter of the dilutions was topically applied to insects' pronotum using a micropipette. Controls were determined using distilled water. Both treated, and control insects were then transferred to a plastic Petri dish (10 insects/dish) containing corn grains and kept in incubators (23°C, 60 ± 5% RH). Insect mortality was observed daily until endpoint mortality was reached 7 days after treatment. The experiments were repeated in four times with ten insects per replicate.

Repellence bioassays

Area preference bioassays. The repellent effects of EOs/standard compounds on adult maize weevils were assessed as described by Cosimi *et al*. (2009) and Chaubey (2011). Petri dishes (Ø 9 cm) were used to confine *S. zeamais* during the experiment. Test emulsions of EOs/standard compounds at different concentrations (2.5, 7.5, 12.5 and 25 mg mL^{-1}) were prepared by diluting in ethanol and then into distilled water (1.5% v/v of ethanol in all the emulsions). Whatman filter paper (Ø 9 cm) was cut in equal halves. A total of 200 µL of the test emulsion was then uniformly applied to one half of the filter paper using micropipette to obtain the following doses per area unit: 16, 47, 78 and 156 µg cm^{-2}. The other half of the filter paper was moistened with 1.5% v/v of ethanol in distilled water, as a control treatment. Treated and untreated halves were placed together at the bottom of Petri dishes and fixed to their opposites. Twenty unsexed adults of *S. zeamais* were released at the centre of each Petri dishes then covered and

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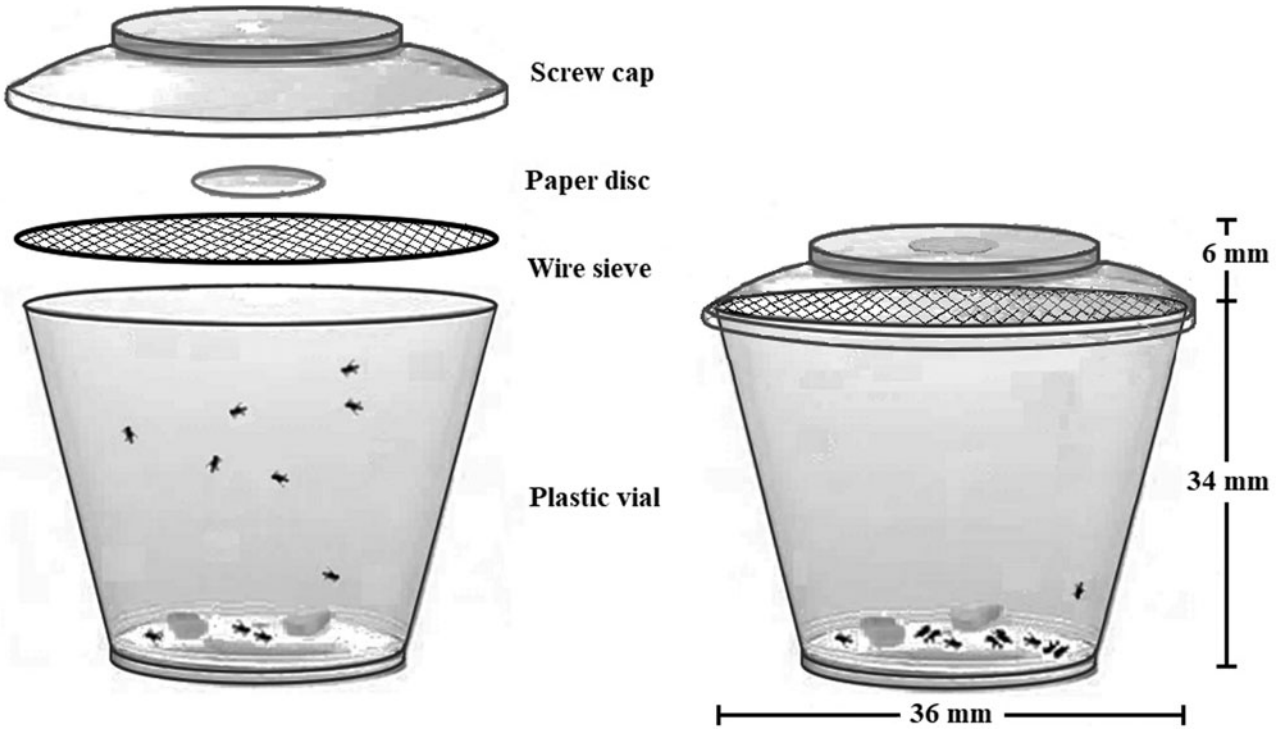

Figure 1. Diagram representing the experimental setup on the fumigant insecticidal activity.

kept in the dark. Six replicates were set for each concentration of EO/standard compound emulsion. Repellence evaluation was carried out 1 h after treatment, counting the number of insects present on both treated and untreated halves and expressing it as a percentage of repellence.

Two-choice. In the second assay, repellent activity of EOs/standard compounds against *S. zeamais* was performed following the method of França *et al.* (2012) with minor modifications, using a choice bioassay system, which consisted of two 70-millilitre plastic containers connected at their rims by a plastic tube (30 cm of length; 2 cm inner diameter). A circular hole was cut in the middle of the tube to facilitate the introduction of test insects into the bioassay system. Three pre-weighed corn grains were treated with a single dose of EOs/pure compound (1 μg g^{-1} diet) in glass bottles which were vigorously shaken to ensure proper mixing of corn grains with emulsions. One box contained, treated grains while the other box had untreated corn (negative control). Twenty unsexed adults were introduced into the plastic tube through the circular hole by means of a 0.5 cm diameter funnel. The number of insects present in the control box and the treated box was recorded after 1 and 3 h of treatment. The whole systems were thoroughly cleaned with ethanol and dried after each test to avoid any interference of other allelochemicals. The assay was repeated five times, for each EO/standard compound, using different cohorts of insects, which had not been previously exposed to any treatment.

Nutritional and antifeeding effects

The effects of EO/standard compounds over *S. zeamais* growth, food consumption and other metabolic parameters were evaluated for a 120-h period experiment. The bioassay was performed with adults (3–3.5 mg of average weight), never exposed to any of the studied allelochemicals. Groups of twenty pre-weighed adults were distributed in plastic containers (volume 110 mL). At least five independent assays with 20 adults per treatment were done ($n = 100$). After a 6-h starvation period, each group of unsexed adults was fed with three treated and pre-weighed corn grains. Grain treatments were prepared by vigorously shaking three corn grains into a glass vials containing 100 μL of EO/standard emulsion at a single concentration of 3% (w/v), or control solutions, to ensure proper mixing of corn grains with the liquid. Emulsions at the concentration of 3% (w/v) were obtained by a first dilution step of EOs/standard compounds in ethanol, followed by a gradual addition of distilled water up to the final volume (the final concentration of ethanol being 1.5% v/v). Two control groups one with water and another with ethanol (1.5% v/v) were included in the experiment. Corn grains were left for 10 min to evaporate the solvent and weighed before being placed into each container to feed insects. The weights of the non-consumed diet and insect alive, as well as eventual mortality were recorded after 120 h of assay under controlled conditions. All weight measurements were made on an analytical balance with an accuracy of 0.1 mg (Mettler Toledo AB204-S/FACT).

To estimate treatment effects on the food weight that was consumed, assimilated and converted into body mass in the 5 days of the experimental period the following parameters were evaluated: relative growth rate (mg mg^{-1} day^{-1}): $RGR = L/l \times t$; relative consumption rate (mg mg^{-1} day^{-1}): $RCR = D/l \times t$; efficiency conversion index of ingested food (%): $ECI = 100L/D$ and antifeeding effect (%), $AE = 100\ [(C-T)/C]$, where t is the duration of the experimental period, D the mean dry weight of consumed diet during t, L the mean dry weight gain of maize weevil adults during t, l the mean dry weight of maize weevil adults, C the mean consumption in the control (mg) and T the mean consumption in the treatment (Scriber and Stansky, 1981). The calculation on a dry weight basis was used as described by other authors (Koul

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Table 1. Acute toxicity of Apiaceae EOs and standard compounds against adults of *S. zeamais,* after 7 days of exposure

	Fumigant toxicity (mg L^{-1} air)			Contact toxicity (µg per adult)		
Treatments	LC_{50} (95% CL)[a,b]	Slope (±SEM)	H^c	LD_{50} (95% CL)[a,b]	Slope (±SEM)	H^c
Eos						
A. graveolens (infrutescences)	157.1 b (140.6–177.4)	3.03 ± 0.27	1.49	111.3 bc (94.2–139.1)	1.84 ± 0.27	1.96
C. cyminum (fruits)	229.4 c (200.7–265.1)	2.26 ± 0.27	0.89	120.4 bc (90.5–204.2)	1.09 ± 0.24	0.92
F. vulgare (infrutescences)	442.8 d (382.3–529.2)	2.10 ± 0.26	0.53	Mortality <28% at 150 µg per adult		
P. crispum (fruits)	535.8 d (438.0–713.1)	1.59 ± 0.21	0.44	128.2 c (112.0–154.3)	2.52 ± 0.33	1.05
Standard compounds						
(*S*)-Carvone	51.8 a (45.9–57.9)	2.67 ± 0.24	1.57	23.0 a (19.6–26.6)	1.78 ± 0.13	1.47
Cuminaldehyde	484.8 d (395.6–629.5)	1.51 ± 0.21	1.15	96.5 b (84.8–111.7)	2.35 ± 0.29	0.30
Estragole	501.2 d (397.9–688.4)	1.30 ± 0.17	1.30	Mortality <35% at 150 µg per adult		
(+)-Fenchone	424.8 d (336.1–586.7)	1.23 ± 0.28	1.01	Mortality <15% at 150 µg per adult		

[a]Estimated LC_{50} and 95% CL were determined by probit analysis based on dose-related adults' mortality.
[b]LC and LD values within the same column followed by the same letter are not significantly different based on non-overlapping of the 95% CL.
[c]*H*, Heterogeneity factor, χ^2:df.

et al., 1990; Senthil-Nathan *et al.*, 2005; Yazdani *et al.*, 2013), to minimize the influence of water variation, namely induced dehydration of insects possibly caused by some treatments.

Acetylcholinesterase inhibition assay in vitro

The inhibitory effect of EOs and compounds on AChE activity was screened by *in vitro* assay using a purified AChE from electric eel (*Electrophorus electricus*) and following the method of Ellman *et al.* (1961). Ellman's colorimetric assay was adapted to 96-well microplates and performed at pH 8.0 in sodium phosphate buffer, using 0.25 U mL^{-1} of AChE and ATChI as a substrate (75 mM), in the presence of DTNB (3 mM) and different concentrations of EOs/standard compounds (Arruda *et al.*, 2012). The isoquinoline alkaloid, berberine, was used as a reference substance of plant origin (Jung *et al.*, 2009). The hydrolysis of the substrate was monitored by repeated spectrophotometric readings (absorbance at 415 nm) for different times of reaction (0, 150, 300 and 450 s) using a Bio-Rad Model 680 Microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). EOs/standard compounds' activity was assessed within a range of 6–7 concentrations (15.6, 31.3, 62.5, 125.0, 250.0, 500.0 and 1000.0 µg mL^{-1}) with four replicates per concentration. The percentage of inhibition was estimated based on the reaction rate (ΔAbs_{415nm} min^{-1}) obtained for each treatment/concentration and the control reaction without inhibitor: Inhibition (%) = 100−100($V_{sample}/V_{control}$). Assays were repeated 3 to 4 times to calculate mean inhibition (%).

Statistical analyses

Mean values calculated from dose-response data collected through the mortality, repellence and AChE inhibition assays were used to estimate, respectively, LC_{50} and LD_{50} values (the concentration required to kill 50% of the insects in the fumigation test and in topical application, respectively), RD_{50} values (the concentration required to repel 50% of the insects; area preference bioassays) and IC_{50} values (the concentration required to inhibit 50% of AChE activity). Each data set and independent group (EOs/standard compounds) was submitted to Probit analysis. Differences between the estimated LC_{50}, RD_{50} or IC_{50} values for EOs/standard compounds were considered significant based on the criterion of nonoverlap of the respective 95% confidence intervals (CI). The percentage of adults attracted to treated and untreated corn was analysed by a paired sample *t*-test (two-choice bioassays). For the nutritional indices, data were submitted to a one-way ANOVA test without previous transformation and, mean multiple comparisons were performed using the LSD test ($P = 0.05$). All analyses were performed using the statistical software SPSS 23.0 (IBM, 2015).

Results

Fumigant and contact toxicity

The fumigant toxicity of four EOs and four standard compounds against *S. zeamais* adults was dose-dependent, which allowed the estimation of lethal concentrations within acceptable confidence limits (CLs) through Probit analysis (table 1). In general, most of the data fitted well in the assumptions of this linear model. The LC_{50} values calculated for Apiaceae EOs/standard compounds ranged from 51.8 to 535.8 mg L^{-1}.

Based on LC values and the non-overlapping of CLs we have established two classes of toxicity with a probability of 95%. The first one, with LC_{50} values <200 mg L^{-1}, which includes the most effective treatments (*S*)-carvone (LC_{50} = 51.8 mg L^{-1}), and *A. graveolens* EO (LC_{50} = 157.1 mg L^{-1}), and the low toxicity class with LC_{50} > 200 mg L^{-1} comprising all the remaining EOs

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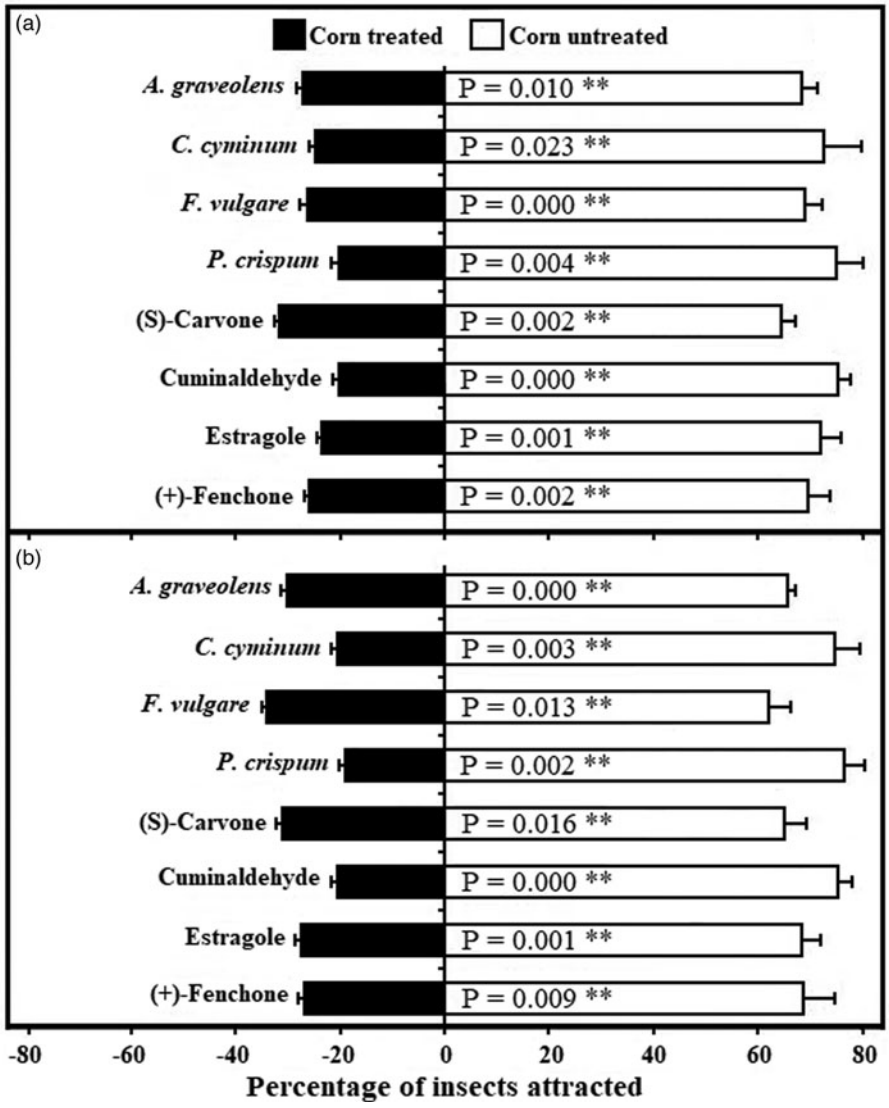

Figure 2. Percentage of adults of *S. zeamais* attracted to untreated and treated corn grain with EO and standard compounds after (a) 1 h and (b) 3 h of experiment. **Statistically significant by the paired sample *t*-test.

(*F. vulgare*, *P. crispum* and *C. cyminum* EOs) and standard compounds (estragole, (+)-fenchone and cuminaldehyde).

The toxicity of Apiaceae EOs applied topically to the beetles is summarized in table 1. (*S*)-Carvone shows pronounced contact toxicity against *S. zeamais* (LD_{50} = 23 µg per adult) while *P. crispum* EO had a LD_{50} value of 128.2 µg per adult. The compound cuminaldehyde, and the EOs of *A. graveolens* and *C. cyminum* also revealed contact toxicity against *S. zeamais* (LD_{50} = 96.5, 111.3 and 120.4 µg per adult, respectively). Nevertheless, only (*S*)-carvone exhibited both strong fumigant and contact toxicity against the maize weevils.

Repellence

Results demonstrate that EOs and standard compounds have a good repellent activity against adults of *S. zeamais* when assessed by two methods (fig. 2 and table 2). In the area preference bioassays, cuminaldehyde, (*S*)-carvone and estragole are the most repellent (RD_{50} < 4.9 µg cm^{-2}) and significantly different from the others. Among the EOs tested, the EO extracted from *F. vulgare* infrutescences was the most repellent (RD_{50} = 24.0 µg cm^{-2}) (table 2). In the two-choice bioassays, at 1 h after the exposure, the most repellent was *P. crispum* fruit EO and cuminaldehyde, followed by *C. cyminum* fruit EO. After 3 hours of exposure, a similar pattern was noted, and no significant difference was observed in the results obtained between the two periods of observation (fig. 2).

Nutritional and antifeeding effects

The nutritional parameters determined for adults of *S. zeamais* when fed with corn grains treated with EOs/standard compounds (3% w/v emulsion), for a period of 120 h are presented in fig. 3. Mean values of RCR did not varied significantly, from 0.20 to 0.27 mg mg^{-1} day^{-1} ($F_{8,36}$ = 1.08, P = 0.397) and the LSD post hoc test indicated no statistical differences between treatments. Overall, the RCR values were much superior to the RGR mean values obtained (−0.028 to 0.012 mg mg^{-1} day^{-1}), which reflected on lower ECI values (−16.3 to 5.6%). With the exception of *P. crispum* fruit EO, none of the treatments showed significant decrease in the maize weevil RGR, when compared to the negative control. Concerning the ECI, only *P. crispum* EO and estragole presented a significant effect. *P. crispum* EO significantly impaired the ECI, while estragole seemed to have a promoting effect on this metabolic parameter. Moreover, the inhibition of

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Table 2. Repellency of Apiaceae EOs and standard compounds evaluated by the area preference bioassays against adults of *S. zeamais*, after 1 h of exposure

Treatments	RD_{50} (95% CL)[a,b]	Slope (±SEM)	Intercept (±SEM)	H^c
EOs				
A. graveolens (infrutescences)	37.9 d (26.4–50.6)	−1.00 ± 0.15	6.58 ± 0.27	0.00
C. cyminum (fruits)	45.2 d (35.6–55.4)	−1.27 ± 0.16	7.10 ± 0.28	1.82
F. vulgare (infrutescences)	24.0 c (16.3–31.8)	−1.26 ± 0.17	6.74 ± 0.29	2.42
P. crispum (fruits)	166.0 e (112.8–327.2)	−0.70 ± 0.13	6.55 ± 0.25	1.24
Standard compounds				
(*S*)-Carvone	3.6 a (0.1–10.4)	−0.37 ± 0.11	5.21 ± 0.19	0.06
Cuminaldehyde	1.4 a (0.1–4.3)	−0.61 ± 0.14	5.10 ± 0.23	2.09
Estragole	4.9 ab (0.1–14.7)	−0.33 ± 0.11	5.23 ± 0.19	0.08
(+)-Fenchone	28.4 b–d (10.6–44.7)	−0.58 ± 0.14	5.85 ± 0.27	0.13

Data are expressed as μg cm^{-2}.
[a]Estimated RD_{50} and respective 95% CL were determined by probit analysis based on adults' response to corn grains treated with increasing doses of EOs/standard compounds.
[b]RD values within the same column followed by the same letter are not significantly different based on non-overlapping of the 95% CL.
[c]*H*, Heterogeneity factor, χ^2:df.

S. zeamais adults' feeding behaviour (AE) with relation to the ethanol control group varied significantly, from −23.7 to 28.5% ($F_{7;30} = 2.35$, $P = 0.032$). Cuminaldehyde was found slightly stimulant (AE = −23.7 ± 10.3%), while *P. crispum* EO showed slightly deterrent effects on this insect (AE = 28.5 ± 10.9%). Furthermore, *P. crispum* EO and (*S*)-carvone exerted some acute toxicity (18 ± 3.0% and 6 ± 2.5% mortality, respectively). The observed percentages of mortality for other treatments were not significantly different from the negative control with ethanol.

Acetylcholinesterase inhibition assay

Concerning the results obtained by the *in vitro* assay with purified AChE, all the EOs and the standard compounds showed a dose-dependent inhibitory activity (table 3). With basis on this preliminary study, we found evidences that EOs from parsley and cumin fruits ($IC_{50} = 185$ and 235 μg mL^{-1}, respectively) have a more significant inhibitory action over the hydrolytic activity of the AChE, when compared to dill and bitter fennel infrutescence EOs. Moreover, cuminaldehyde and (*S*)-carvone, previously identified as major volatile constituents of cumin fruit EO (39%) and dill infrutescence EOs (66%), respectively (Sousa *et al.*, 2017), showed inhibitory effects comparable to their corresponding EOs. Cuminaldehyde and (*S*)-carvone were also the most effective among the four tested standard compounds (214.5 and 368.1 μg mL^{-1}, respectively). When comparing all results, bitter fennel EO and both its major compounds, estragole and fenchone, showed the lowest anticholinesterase activity *in vitro* ($IC_{50} = 465.4$, 605.7 and 726.5 μg mL^{-1}, respectively).

Discussion

Due to their high volatility, EOs and their constituents, present a strong fumigant action by penetrating the insect body via the respiratory system. Such exposure through the gaseous phase is acutely toxic to insects and became a relevant handling strategy to control an insect damaging stored-product (Coats *et al.*, 1991; Kim *et al.*, 2003; Lee *et al.*, 2003; Liu *et al.*, 2011; Ebadollahi, 2013; Massango *et al.*, 2016) and repellence (Bedini *et al.*, 2016; Lee *et al.*, 2017). Some EOs also exhibit antifeedant properties (Benzi *et al.*, 2009).

In general, contact and fumigant insecticidal activities of plant EOs and monoterpenes against stored product pests have been successfully reported (Tripathi *et al.*, 2000; Lee *et al.*, 2003; Abdelgaleil *et al.*, 2009; Wang *et al.*, 2009; Yang *et al.*, 2011; Kordali *et al.*, 2012). In the present study, although most of the EOs and compounds were toxic to S. zeamais, their toxicity varied with the type of bioassay. In contact toxicity assays, dill infrutescence EO, cumin fruit EO, (*S*)-carvone and cuminaldehyde showed the highest toxicity towards S. zeamais. In fumigant toxicity assays, dill infrutescence EO and (*S*)-carvone were found significantly more effective as fumigants, than other treatments. The relatively high biological activity of the dill and cumin EOs is probably related to the high concentration of (*S*)-carvone (66%) and cuminaldehyde (39%), respectively (Sousa *et al.*, 2015*a*). In general, the toxicity displayed by several plant species EOs against stored pests has been related to their major components, mostly monoterpenes (López *et al.*, 2011; Kumar *et al.*, 2012). For example, cuminaldehyde exhibited strong contact and fumigant toxicities against *Blattella germanica* (Yeom *et al.*, 2012), and *S. oryzae* (Chaubey, 2011; Kim *et al.*, 2013). Another major compound, (*S*)-carvone also possessed insecticidal activity against *S. oryzae*, *Tribolium castaneum, Rhyzopertha dominica*, *Cryptolestes pusillus* and *Callosobruchus chinensis* (Abdelgaleil *et al.*, 2009; Fang *et al.*, 2010; López *et al.*, 2010; Kim *et al.*, 2013). Likewise, Yildirim *et al.* (2013) found that the oxygenated monoterpenes carvone, dihydrocarvone, menthone, terpinen-4-ol, 1,8-cineole, fenchone, linalool and limonene oxide have some insecticidal potential towards *S. zeamais* adults.

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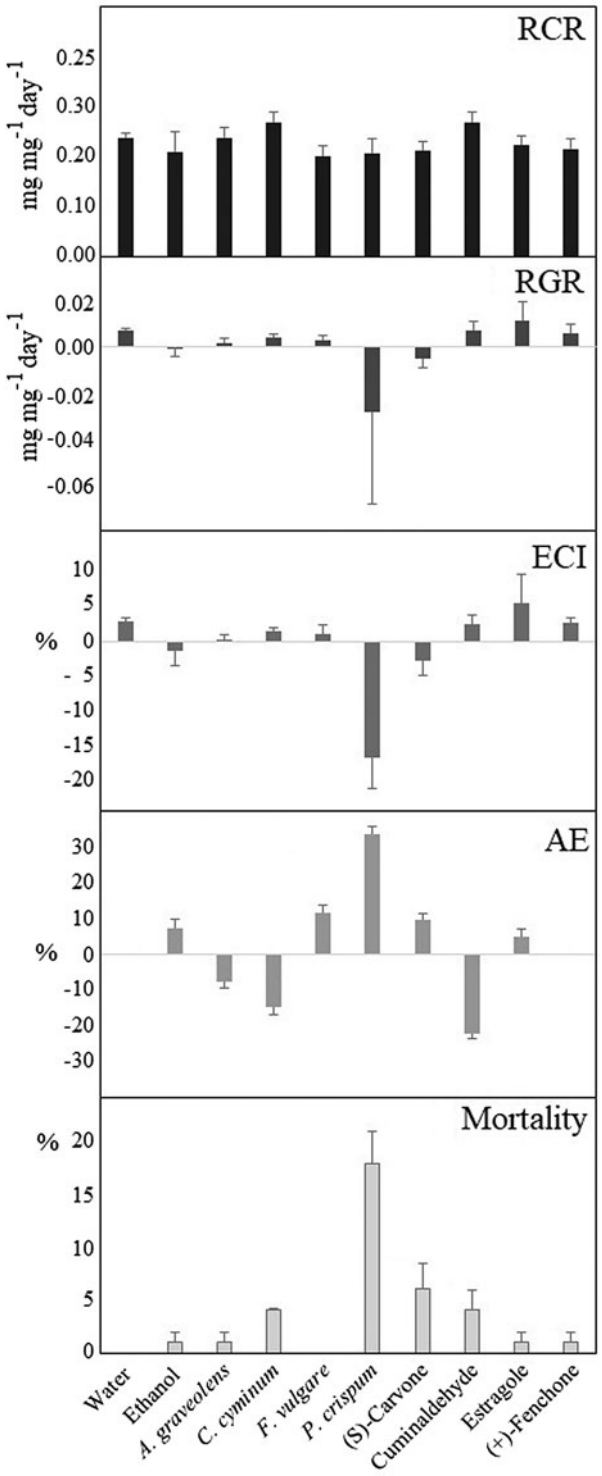

Figure 3. Estimated nutritional indices and feeding deterrent activity of Apiaceae EOs and standard compounds on *adults* of *S. zeamais*, after 120 h of treatment.

In this study, bitter fennel and parsley EOs showed low fumigant toxicity to *S. zeamais*. However, Ebadollahi (2011) previously demonstrated that *F. vulgare* (seed) EO display fumigant activity against adults of *S. oryzae* and *S. granarius* (the wheat weevil), while Maroufpoor *et al.* (2016) observed that *P. crispum* EO shows significant fumigant toxicity against tree important stored product pests, *Callosobruchus maculatus* (Coleoptera: Bruchidae), *Plodia interpunctella* (Lepidoptera: Pyralidae) and *Ephestia kuehniella* (Lepidoptera: Pyralidae). Such differences of results point to the fact that EO isolated from different plant parts or origin may exhibit distinct fumigant toxicity, which can be attributed not only to their mechanism of action over different targeted arthropods, but also to variable qualitative and quantitative composition, differences in volatile component physical properties (distinct vapour pressure and boiling point), and their equilibrium in the gaseous phase.

The literature survey indicated that EOs obtained from Apiaceae family are active as repellents for Coleoptera and insects of other orders. Based on the comparison of estimated RD_{50} (table 2) cuminaldehyde, (*S*)-carvone and estragole, followed by bitter fennel EO, exhibit strongest repellent activity against *S. zeamais*. On the other hand, it was not possible to establish a clear relationship between EOs biological activities and their main constituents. This could be due to the natural occurring blend of constituents, or due to the presence of other minor compounds, as suggested by other authors (Bertoli *et al.*, 2012; Ebadollahi, 2013).

Concerning the antinutritional effects towards *S. zeamais*, the studied parameters indicate that for their most parts EOs/compounds have little impact on the efficiency of the metabolic process and growth of adults, and little or no antifeedant activity at the tested dose. Maize weevils presented similar consumption rates for the 120 h-period of experiment (no alternative food offered) independently of the treatment. Significant effects on RGR, and ECI during the time of the assay were only obtained for parsley EO. This EO significantly decreased the RGR and ECI parameters and caused morbidity, which suggests toxicity through ingestion. In our previous investigation, the same parsley fruit EO caused significant anti-nutritional effects (feeding and growth inhibition with weight loss) to the caterpillar *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) (Sousa *et al.*, 2015*a*). Evidences of EOs activities on the maize weevil feeding behaviour and nutrition, with significant reduction of the nutritional indices and moderate antifeedant effects, have been reported for several plant species, namely nutmeg (*Myristica fragrans*, Myristicaceae) (Huang *et al.*, 1997), *Evodia rutaecarpa* (Rutaceae) (Liu and Ho, 1999), cardamom (*Eletaria cardamomum,* Zingiberaceae) (Huang *et al.*, 2000) and red ginger (*Alpinia purpurata*, Zingiberaceae) (de Lira *et al.*, 2015). However, investigations concerning Apiaceae EOs are scarce. Possible negative effects of EOs/compounds toward other stored-grain pest feeding behaviour, nutrition and metabolism were also studied, as the EOs from leaves and fruits of pepper tree (*Schinus molle*), and from leaves of *Tagetes terniflora*, *Cymbopogon citratus* and *Elyonurus muticus* on *S. oryzae* (Benzi *et al.*, 2009; Stefanazzi *et al.*, 2011) and from eugenol, isoeugenol, methyleugenol and EOs from leaves of *Tagetes terniflora*, *Cymbopogon citratus* and *Elyonurus muticus* on *Tribolium castaneum* (Huang *et al.*, 2002; Stefanazzi *et al.*, 2011). In the present study, we conclude that the tested dose of EOs/compounds might not be effective to protect stored maize grain from *S. zeamais*, since AE were negligible 5 days after the application of emulsions. However, these results might be attributable to the relatively low dose tested when compared to those used in similar studies. For example, EOs or their compounds exhibited potential feeding deterrence when applied at a concentration of 14,400 ppm (Huang *et al.*, 2000), 13.2 mg g^{-1} of diet (Huang *et al.*, 2002) and 37.5 µL g^{-1} of diet (de Lira *et al.*, 2015). Also, we found that values obtained in the control for the nutritional indices RCR and ECI differed considerably from values described in the literature for *S. zeamais* probably because

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Table 3. *In vitro* inhibitory effect of Apiaceae EOs and standard compounds on AChE activity

Treatments	IC_{50} (95% CL)[a,b]	Slope (±SEM)	Intercept (±SEM)	χ^{2c}
Eos				
A. graveolens (infrutescences)	370.5 bc (288.2–523.6)	1.35 ± 0.16	1.54 ± 0.35	1.94
C. cyminum (fruits)	234.9 ab (184.9–316.9)	1.17 ± 0.12	2.23 ± 0.25	1.90
F. vulgare (infrutescences)	465.4 c (331.0–759.3)	1.04 ± 0.12	2.22 ± 0.26	0.85
P. crispum (fruits)	185.1 a (143.3–253.5)	1.00 ± 0.11	2.72 ± 0.23	2.61
Standard compounds				
(*S*)-Carvone	368.1 ab (245.7–667.7)	0.83 ± 0.11	2.87 ± 0.23	0.80
Cuminaldehyde	214.5 a (176.6–269.7)	1.44 ± 0.13	1.64 ± 0.27	2.13
Estragole	605.7 c (400.0–1221.7)	0.97 ± 0.15	2.30 ± 0.34	1.78
(+)-Fenchone	726.6 bc (515.7–1187.4)	0.92 ± 0.11	2.36 ± 0.27	0.85
Berberin[d]	1.9 (1.5–2.4)	1.23 ± 0.11	4.66 ± 0.06	0.05

[a]Estimated IC_{50} and 95% CL were determined by probit analysis based on mean values of inhibition. Values are expressed in μg mL^{-1} of EOs/standard compounds required to inhibit 50% of the enzymatic activity.
[b]LC values within the same column followed by the same letter are not significantly different based on non-overlapping of the 95% CL.
[c]χ^2 values were determined for 4 degrees of freedom.
[d]Berberin was used as the positive control.

of a different diet and longer duration of the experiment. Camaroti *et al.* (2018) and de Lira *et al.* (2015) reported lower RCR but superior ECI values when assays were performed with wheat flour disks for 7 days. For the RGR index described for S. *zeamais*, values tend to be very low varying from 0.042 mg mg^{-1} day^{-1} after 3 days (Liu and Ho, 1999) to approx. 0.01 mg mg^{-1} day^{-1} for a 7-days assessment (Camaroti *et al.*, 2018). In the present work, The RGR was nearly null for the negative control groups which indicate that, despite the higher consumption rate of maize, adults did not growth during such short period of time. In general, in most studies performed with a flour disk diet, the efficiency of conversion of ingested food into body mass by adults of *S. zeamais* is not very high (4.2, 11, 17 and 22.4%) (Huang *et al.*, 1997, 2000, 2002; Liu and Ho, 1999, respectively).

Similar to several synthetic pesticides (e.g. carbamates and organophosphates), the toxic properties exhibited by EOs and their constituents have been frequently related to a neurotoxic mode of action achieved, in part, via the octopaminergic system and/or through an inhibitory action on the cholinergic synapses, where AChE regulates nerve impulse transmissions by rapidly breaking downs acetylcholine (ACh) into choline and acetate (Coats *et al.*, 1991; Kostyukovsky *et al.*, 2002; Tripathi *et al.*, 2009; Rattan, 2010).

EO constituents have been reported for their potential AChE inhibitory effects (Ingkanian *et al.*, 2003; López and Pascual-Villalobos, 2010; Aazza *et al.*, 2011; Arruda *et al.*, 2012; Yeom *et al.*, 2012; Orhan *et al.*, 2013; Seo *et al.*, 2015), and the possible correlation between enzyme inhibition *in vitro* and acute *in vivo* toxicity in insects was recently examined by Isman and Tak (2017). In our study, parsley EO and cuminaldehyde, followed by the cumin fruit EO, exhibited the most significant inhibitory effect over AChE *in vitro* activity (lowest IC_{50} value). However, in accordance with the categories recently described by Santos *et al.* (2018) (high potency, $IC_{50} < 20$ μg mL^{-1}; moderate potency, $20 < IC_{50} < 200$ μg mL^{-1} and low potency, $200 < IC_{50} < 1000$ μg mL^{-1}); only parsley EO could be considered of moderate potency. The EO from *A. graveolens* infrutescences presented a low inhibitory action over AChE activity, which may be attributable to the joined action of (*S*)-carvone with other major components, such as the cyclic monoterpene hydrocarbons α- and β-phellandrene (not tested). Previous reports established that α- and β-phellandrene, and (*S*)-carvone, might act as a non-competitive inhibitor of AChE (Bonesi *et al.*, 2010; Jankowska *et al.*, 2018). The lowest toxicity of estragole and (+)-fenchone (highest IC_{50} values) may be a possible explanation for the low inhibitory action of bitter fennel EO over AChE. The weak anticholinesterase activity of *F. vulgare* EO on purified AChE from electric eel was previously reported by Aazza *et al.* (2011), although the authors estimated a much higher IC_{50} value (2.5-fold superior). Contrariwise, López *et al.* (2010) identified fenchone as a competitive inhibitor of AChE with an IC_{50} of 0.4 mM, which is approximately 10 times inferior to the value we determined in this work (726.6 μg $mL^{-1} \approx 4.77$ mM). Putting into perspective, the potency of the assessed EOs and their constituents as AChE inhibitors might not be so relevant, since these were 97 to 383 times less active than berberine (based on IC_{50} values). In general, the most notable AChE inhibitors naturally occurring in plant showed effects at a much lower order of magnitude (below 15 μM) (Santos *et al.*, 2018). Despite the inhibitory actions herein recorded and evidences of acute effects in the contact toxicity assay for some treatments, the

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present findings do not permit to establish any correlation of the *in vitro* AChE inhibitory activity with the acute toxicity results obtained *in vivo* against *S. zeamais*. Therefore, conclusions about a possible mode of action are limited. According to Isman and Tak (2017), the degree of inhibition that has been observed in several studies might be too low to consider AChE inhibition as the major mode of action of EOs and monoterpenoids exhibiting insecticidal properties.

Conclusion

In this study, we have shown the lethal and sublethal effects of EOs from four common Apiaceae species and some of their constituents against *S. zeamais*, with regard to their toxicity, repellent activity and impact on insect feeding, metabolism and growth. *A. graveolens* infrutescence EO and the respective major compound, (*S*)-carvone, demonstrated the highest fumigant and contact toxicity, while cuminaldehyde, (*S*)-carvone and estragole followed by EO from *F. vulgare* exhibited high repellent activity. Moreover, *P. crispum* EO and cuminaldehyde were found to influence maize weevil's nutrition, but only *P. crispum* fruit EO showed significant negative impacts. Concerning the possible inhibitory effects of EOs/compounds on AChE *in vitro* activity, the findings suggest that *P. crispum* and *C. cyminum* EOs, as well as cuminaldehyde, were more effective ($IC_{50} = 185$, 234.9 and 214.5 μg mL^{-1}, respectively) than the remaining tested EOs and volatile compounds, but all were relatively weak AChE inhibitors relatively to other naturally occurring plant products described in the literature. In addition, the present work gives evidences of the potential use of dill and cumin EOs, and their major oxygen-containing monoterpenes (S)-carvone and cuminaldehyde, respectively, as possible natural fumigants for the control of *S. zeamais*.

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Conflict of interest. The authors declare that there are no conflicts of interest.

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