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PEST MANAGEMENT

Pimenta pseudocaryophyllus Derivatives: Extraction Methods and Bioactivity Against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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Abstract
Plant-based insecticides can play an important role in integrated insect pest management (IPM), especially in protecting stored grains. The aim of this study was to evaluate the bioactivity of derivatives (powder, ethanolic extract, and essential oil (EO)) from the leaves of *Pimenta pseudocaryophyllus* (Myrtaceae), a Brazilian native species, against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), the main insect pest of stored corn. The powder and essential oil prepared from leaves showed a repellent effect. Moreover, the EO exhibited promising insecticidal activity through residual contact (LC_{50}=1522 mg kg^{-1}) and significantly decreased the F_1 progeny and the percentage of damaged grains. However, the essential oil obtained from *P. pseudocaryophyllus* leaves did not result in significant mortality of *S. zeamais* adults after 72 h of exposure by fumigation in concentrations up to 400 μL L^{-1} of air. Based on GC-MS analysis, 20 compounds were identified in the essential oil of *P. pseudocaryophyllus* leaves, being chavibetol (38.14%), methyl eugenol (11.35%), and terpinolene (9.17%) as the major constituents. Essential oil from *P. pseudocaryophyllus* leaves is an interesting source of compounds with grain-protectant properties and should be analyzed in future studies aiming to develop new bioinsecticides to use in the IPM of stored grains.

Introduction

The storage environment is characterized by a series of interactions among physical, chemical, and biological factors that may alter the quality of the stored products. One of the most important causes of qualitative and quantitative losses is the presence of insect pests and microorganisms (Neethirajan *et al* 2007). Many pest species are found in warehouses in tropical regions; for example, the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is frequently found in all economically important stored cereals (Kehinde & Angela 2004). Infestations by this insect pest can substantially reduce grain weight and increase grain moisture, thus providing favorable conditions for the growth of decomposing fungi (Lazzari & Lazzari 2009) and leading to even higher levels of post-harvest loss.

Integrated pest management (IPM) in stored grains is currently challenged by the growing frequency of populations that are resistant to available insecticides. Thus, the development of new management tools and low-risk insecticidal compounds is necessary (Moreau & Isman 2012). Compounds produced by plant secondary metabolism are

potential sources for developing low-risk insecticides, which differ from conventional synthetic insecticides in their low toxicity to mammals, rapid degradation, and local availability (Isman 2006, 2011, Regnault-Roger *et al* 2012).

The plant family Myrtaceae is one of the most important in the neotropics due to its high abundance (the highest within the order Myrtales) and species diversity (Paula *et al* 2010b). In addition, these plants secrete a large diversity of secondary metabolites, especially in the form of essential oils (EOs; mainly sesquiterpenes). Some of these metabolites exhibit important biological activities (Stefanello *et al* 2011), and the pharmacological and toxicological properties of various Myrtaceae species have received multidisciplinary interest. Recent studies have shown that many insect pest species are affected by essential oils from Myrtaceae plants. These essential oils can have several effects, including insecticidal action (knockdown effect), repellency, deterrence of feeding and oviposition, and growth inhibition (Ebadollahi 2013).

Pimenta pseudocaryophyllus is a native Brazilian species of the family Myrtaceae and is highly abundant in the Atlantic Forest and Cerrado biomes (Landrum & Kawasaki 1997). In addition to the known ethnopharmacological applications of various species of this genus (Paula *et al* 2008), previous studies have shown that compounds derived from *P. pseudocaryophyllus* have several biological effects, including potent antibacterial and antifungal activity (Lima *et al* 2006, Paula *et al* 2009, Custódio *et al* 2010). Furthermore, phytochemical studies revealed great chemical diversity in *P. pseudocaryophyllus* derivatives (Lima *et al* 2006, Paula *et al* 2008), indicating its potential as a source of a diversity of bioactive molecules, including those that could have insecticidal or insectistatic activity against insect pests such as insect pests of stored grains. Therefore, the primary objective of this study was to assess the bioactivity of derivatives (plant powder, ethanolic extract, and essential oil) obtained from *P. pseudocaryophyllus* leaves against the maize weevil. In addition, chemical analyses were performed to determine the composition of the active derivative(s).

Material and Methods

Collection of the plant material

Leaves of *P. pseudocaryophyllus* were collected on June 26, 2011 from specimens grown in the Caiçara de Pedrinhas neighborhood, Ilha Comprida municipality, state of São Paulo, Brazil (24°54′09.2″S, 47°47′10.8″W, 31 m asl). One voucher specimen, verified by Prof. Dr. Vicente Coffani Nunes (Universidade Estadual Paulista "Júlio de Mesquita Filho", Registro Campus), was deposited in the ESA herbarium (http://splink.cria.org.br/manager/detail?resource=ESA&setlang=pt) of the Departamento de Ciências Biológicas, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (ESALQ/USP), Piracicaba, SP, Brazil (record number 121119).

After collection, the leaves were separated from the remaining structures. Some of the leaves were used fresh to extract the essential oil, and the remaining leaves were dried to obtain the plant powder.

Preparation of the plant derivatives

*Plant powder***.** Leaves were dehydrated in a convection drying oven at 40°C for 48 h. Afterwards, the leaves were ground in a knife mill, and the resulting plant powder was stored in hermetically sealed glass receptacles and protected from light until use.

*Ethanolic extract***.** The crude extract was prepared by cold maceration in ethanol (99.5%) as a solvent, using a proportion of 1:5 plant powder/solvent (*w*/*v*). The resulting solutions were stirred for 10 min and left to rest for 72 h, after which the material was filtered through filter paper. The extraction process was then repeated with the material remaining on the filter using the same plant powder/solvent ratio. This procedure was repeated three times, resulting in a total of four filtrations. The solvent was removed from the remaining sample in a rotary evaporator at 50°C and −600 mmHg. The efficiency of the extraction process was determined after the complete evaporation of the solvent in an air-flow chamber.

*Essential oil***.** Fresh leaves were separated into 100-g samples, washed in running water, and cut into small pieces to optimize the extraction process by increasing the contact surface. The samples were then subjected to hydrodistillation in a Clevenger-type apparatus for 2 h at 110°C. The resulting mixture of water and oil (hydrolate) was decanted to separate the fractions and dried by adding anhydrous sodium sulfate (Na_2SO_4). The extraction yield was calculated from the fresh weight of the plant material (g of oil/g of fresh leaves). The essential oil was stored in a domestic freezer (~−10°C) until use.

Chemical analysis

The components of the essential oil were qualitatively and quantitatively analyzed in a 17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) coupled to a mass spectrometer (GC-MS) QP5000. The following conditions were used: J&W Scientific DB-5MS (5% phenyl, 95% methylpolysiloxane) fused silica capillary column with 30-m length×0.25-mm inner diameter×0.25-μm film thickness; 1.2 mL min^{-1} flow rate of helium as the carrier gas (99.99%); 0.5 μL injection volume; 1:20 split ratio; 250°C injector temperature; and 280°C detector

temperature. The oven temperature was programmed to 50°C for 1.5 min, followed by a rise of 4°C min^{-1} up to 200°C, then a rise of 10°C min^{-1} up to 250°C, and finally, a 5-min isotherm at 250°C. The mass spectrometer operated at 70 eV with rapid scanning at 0.5 scan s^{-1} for masses between 45 and 500 Da. The amount of each component was estimated from the normalized area (%) calculated using the areas of the GC-MS peaks sorted by elution order.

The volatile components were identified by comparing their mass spectra with spectra available in the literature (Adams 2007) and in the equipment's database (WILEY8, NIST05, NIST21, and NIST107) and by comparing the retention indices with those found in the literature. The retention indices (RI) were calculated from a homologous series of n-alkanes (C_9H_{20}–$C_{20}H_{42}$) injected under the same chromatographic conditions as the samples, using the equation proposed by Vand Den Dool & Kratz (1963).

Chavibetol was identified by mass spectrometry, retention index and confirmed by nuclear magnetic resonance (NMR ^1H and ^{13}C) of the essential oil in comparison to data published by Santos *et al* (2009). The NMR spectra (400 MHz for the frequency of ^1H and 100 MHz for ^{13}C) were acquired on a Bruker Avance spectrometer III 9.4 T using tubing Norell 5 mm ID, using deuterated chloroform ($CDCl_3$) as solvent.

Effect of the derivatives on the adult survival, F_1 progeny, and damage caused by S. zeamais

All bioassays were carried out under controlled laboratory conditions (25±2°C; 60±10% RH; 14 h photophase) under a completely randomized experimental design.

The insecticidal activity of the plant derivatives (plant powder, ethanolic extract, and essential oil) was assessed using corn samples (10 g) placed in Petri dishes (6.1 cm in diameter and 2.1 cm in height) and separately treated with each derivative. The dishes were infested with 20 unsexed, 10–20-day-old adult weevils. The weevils originated from a population kept in the laboratory for approximately 20 generations with wheat grains used as breeding substrate. Adult survival was assessed on the 10th day of infestation. Individuals whose extremities were fully extended and that did not react to contact with a brush after 1 min of observation were considered dead. Ten replicates were used for each treatment (n=200).

The same sampling units used in the previous test were used to assess the effects of the plant derivatives on the F_1 progeny and on the damage sustained by the treated samples. For this purpose, the grains were treated with the respective derivatives and infested as before. After 10 days of infestation, the adults were removed and the sampling units were kept under the controlled conditions. The number of emerged adults was counted in each Petri dish 60 days after the initial infestation. The damage caused by *S. zeamais* feeding was also estimated at that time by counting the number of damaged or perforated grains in each sample based on a visual inspection of each grain. The grain weight loss (%) was then estimated using the equation proposed by Adams & Schulten (1976):

$$\mathrm{Wl}(\%) = (\mathrm{Ndg}/\mathrm{tNg})\,100C$$

where Wl = weight loss (%), Ndg = number of damaged grains, tNg = total number of grains, and C=0.125 if the corn is stored as loose grain or in cobs without husks and 0.222 if the corn is stored in cobs with husks.

The concentrations of each derivative in grams per kilogram (g of plant powder per kg of corn) were selected based on previous studies, as specified below for each derivative.

*Plant powder***.** To assess the bioactivity of the plant powder, corn samples (10 g) were treated with plant powder in concentrations of 10, 20, 40, and 80 g kg^{-1}. The control treatment did not receive any plant powder.

*Ethanolic extract***.** To assess the bioactivity of the ethanolic extract, corn samples (10 g) were sprayed with the extract at concentrations of 0.25, 0.5, 1, and 2 g kg^{-1} using a microatomizer coupled to a pneumatic pump, which was adjusted to supply a pressure of 0.5 kgf cm^{-2} through a spray volume of 30 L t^{-1}. These conditions were selected based on previous studies (Ribeiro *et al* 2013, 2014). The control samples were treated only with the solvent used to resuspend the extract [acetone/methanol (1:1, *v*/*v*)].

*Essential oil***.** To evaluate the insecticidal activity of the essential oil, corn samples (10 g) were treated with different concentrations of the essential oil (0.25, 0.5, 1, and 2 g kg^{-1}) solubilized in acetone using the same equipment and conditions earlier described. Similarly, the control samples were treated only with the solvent used to dissolve the essential oil (acetone).

Effects of the derivatives on the behavior of S. zeamais *adults*

The effects of the derivatives on the attractiveness of the corn to *S. zeamais* adults were assessed in two arenas composed of five Petri dishes (6.1 cm in diameter and 2.1 cm in height) mounted on a plastic base (30×30 cm). In each arena, one of the dishes was fixed to the center of the base and connected to the other dishes by plastic tubes of equal length.

Corn samples (10 g each) were treated with the respective derivatives in the same concentrations and using the same application procedures described above. The samples were placed in two Petri dishes located symmetrically on opposite sides of the arena, and grains treated only with the solvents used to solubilize the derivatives were placed in the other two Petri dishes (control). Afterwards, 50 unsexed, 10–20-day-old adults of *S. zeamais* were released in the central container. The number of insects found in each Petri dish

was counted after 24 h. Each treatment level consisted of 10 replicates (n=500).

The different treatments were compared using a repellency index adapted from Lin *et al* (1990):

$$RI = 2G/(G+P)$$

where RI = repellency index, G = percent of insects on the grain treated with the derivative being tested, and P = percent of insects on the control grains. The RI and standard deviation were used to determine the classification interval (ClassI) for the treatment means using the following formula:

$$ClassI = 1 \pm t \times \left(SD/\sqrt{n}\right)$$

where t = tabulated t value ($_{n\text{-}1;\ \alpha:0.05}$), SD = standard deviation, and n = number of replicates. The derivative were considered neutral when the RI interval was within the ClassI being evaluated, repellent when the RI was below the lowest value obtained for the ClassI, and attractive when the RI was above the highest calculated ClassI value. In turn, the mean percentage repellency of each treatment was calculated from the following equation (Obeng-Ofori 1995):

$$PR(\%) = [(NC/NT)/(NC+NT)]100$$

where PR = mean percentage repellency; NC = total number of insects on the control grain, and NT = total number of insects on the grains treated with the derivative being tested.

Fumigant insecticidal activity of the essential oil

The fumigant insecticidal activity of the *P. pseudocaryophyllus* essential oil was assessed in 250-mL plastic containers used as fumigation chambers. Each chamber received 20 g of corn grains and was then infested with 30 adult weevils of undetermined sex and between 10 and 20 days of age. The essential oil was administered on rectangular patches of filter paper (5×2 cm), which were fixed in place on the lower part of each container's lid and isolated by a thin fabric (*voile*) to avoid direct contact of the weevils with the oil. Doses of 50, 100, 200, and 400 μL L^{-1} of air were tested. The control treatment contained no essential oil. The number of dead insects in each chamber was counted after 72 h of exposure. Six replicates of each concentration were used, with 30 weevils per replicate (n=180).

Response-concentration curves of the active derivatives

The results of the bioassays carried out with each derivative in the different methods of exposure were used to estimate the LC_{50} and LC_{90} (the concentrations required to kill 50% and 90% of the weevil population, respectively) for the active treatment. For this purpose, preliminary tests were performed with each derivative to determine the base concentrations that produced mortality rates of approximately 95% of the weevils and the concentrations that produced mortality rates similar to that observed in the control, according to the method described by Finney (1971). These tests were used to select the concentrations to be studied [six concentrations (range=0–4000 mg kg^{-1})]. Subsequently, the same bioassay procedures and experimental conditions earlier described were used.

Data analysis

Generalized linear models (Nelder & Wedderburn 1972) of the quasi-binomial type were used to analyze the proportions of mortality and damaged grains, whereas the quasi-Poisson model was used to analyze the number of emerged insects. The goodness-of-fit was assessed with a half-normal probability plot with a simulation envelope (Hinde & Demétrio 1998). When there were significant differences between treatments, multiple comparisons (Tukey's test, p<0.05) were carried out using the glht function in the multicomp package with adjusted p values for the treatments with qualitative levels, whereas nonlinear regressions were used for the treatments with quantitative levels. The possible relationships between the study variables were assessed using Spearman's nonparametric correlation analysis (p<0.05). All analyses were performed in the statistical software "R" version 2.15.1 (R Development Core Team 2012).

The lethal concentrations (LC_{50} and LC_{90}) were estimated using a binomial model with a complementary log-log link function (gompit model) via the PROBIT procedure in SAS software version 9.2 (SAS Institute 2011).

Results

None of the concentrations of the plant powder or ethanolic extract from *P. pseudocaryophyllus* leaves had a significant effect (p>0.05) on adult survival, F_1 progeny, or damage by *S. zeamais* (Table 1). However, the essential oil extracted from *P. pseudocaryophyllus* leaves caused significant adult mortality through residual contact in a concentration-dependent manner (Table 1). In addition to acute toxicity [LC_{50}=1.52 g kg^{-1} (95% CI=1.41–1.61), χ^2=5.68, df=4, n=1.200], the essential oil significantly reduced the F_1 progeny and therefore the damage caused by *S. zeamais* in the treated samples (Table 1). Adult mortality was negatively correlated with the F_1 progeny (r_s=−0.8438; p<0.0001) and with the percentage of damaged grains (r_s=−0.9052; p<0.0001), possibly indicating that acute toxicity is the main effect of the essential oil on *S. zeamais*; this effect was reflected in the other variables studied. In all cases, a second-degree polynomial model was fitted to the data to describe the effect of concentration on the studied variables (Fig 1). However, the

Table 1 Mean (± standard error) mortality rate at day 10, number of emerged insects (F_1 progeny), and damage after 60 days of infestation by *Sitophilus zeamais* in corn samples (10 g) treated with different concentrations of *Pimenta pseudocaryophyllus* derivatives.

Concentration (g kg^{-1})	Mortality (%)[a]	No. emerged insects[b]	% damaged grains[a]	Sample weight loss	
				Total (%)[c]	Relative[d]
Plant powder					
80	1.5±0.76	41.7±3.48	70.8±3.99	8.9	84.7
40	2.5±1.34	40.2±3.75	73.2±2.82	9.1	87.4
20	3.0±1.52	49.7±3.05	78.3±3.18	9.8	93.4
10	3.0±1.52	44.7±4.87	78.1±2.50	9.8	93.3
Control	0.5±0.50	52.3±3.01	83.7±3.02	10.4	100.0
F	1.0624 [ns]	1.893 [ns]	2.4981 [ns]	–	–
p value	0.3862	0.1282	0.05586	–	–
Ethanolic extract					
2	1.0±0.66	51.4±2.70	91.4±2.18	11.4	104.7
1	0.0±0.00	50.7±2.71	92.4±1.16	11.5	105.8
0.5	0.5±0.50	43.4±2.11	86.8±2.75	10.9	99.6
0.25	1.0±0.66	45.2±2.21	88.5±1.64	11.0	101.2
Control	0.0±0.00	43.6±3.46	87.4±2.65	10.9	100.0
F	2.4838[ns]	2.0756[ns]	1.3836[ns]	–	–
p value	0.05696	0.0998	0.2548	–	–
Essential oil					
2	60.0±6.47	16.5±3.28	36.2±6.40	4.5	41.6
1	14.5±2.63	41.1±2.18	75.7±2.83	9.5	87.1
0.5	3.0±1.33	50.8±3.32	81.3±2.26	10.2	93.5
0.25	1.0±0.66	46.7±2.71	85.3±2.20	10.7	98.1
Control	2.0±1.52	51.9±3.80	87.0±2.98	10.9	100.0
F	44.522	21.552	28.07	–	–
p value	<0.0001	<0.0001	<0.0001	–	–

[a] Significant differences between treatments (GLM with quasi-binomial distribution, $p<0.05$).

[b] Significant differences between treatments (GLM with quasi-Poisson distribution, $p<0.05$).

[c] Calculated by means of the Adams and Schulten's (1976) formula.

[d] Calculated based on the relative comparison between the treatment and the respective control.

[ns] Non-significant.

essential oil obtained from *P. pseudocaryophyllus* leaves did not result in significant mortality of *S. zeamais* adults after 72 h of exposure by fumigation in concentrations up to 400 µL L^{-1} of air (Table 2).

The powder and essential oil extracted from *P. pseudocaryophyllus* leaves had a repellent effect on *S. zeamais* adults (Table 3), with larger concentrations of each derivative resulting in greater repellency.

The extraction yield of the essential oil using the hydrodistillation was 0.81% (g of oil/g of fresh leaves). The GC-MS identified 20 constituents in the essential oil obtained from *P. pseudocaryophyllus* leaves. The major compounds were the phenylpropanoids chavibetol (38.14%) and methyl eugenol (11.35%) and the terpenoid terpinolene (9.17%) (Table 4, Fig 2). However, two other components of essential oil (0.89% of their relative composition) were not identified by means of this technique.

Discussion

This study reports for the first time the biological activity of *P. pseudocaryophyllus* against an insect pest species of stored grains. Our results show that the activity of derivatives from this species against *S. zeamais* may vary with the extraction method (and the consequent modifications of the chemical profile of the constituents found in the derivative), the type of exposure (contamination) of the target insect to the product, and the concentration used.

The variables analyzed in our bioassays enabled us to assess the potential activity of the essential oil extracted from

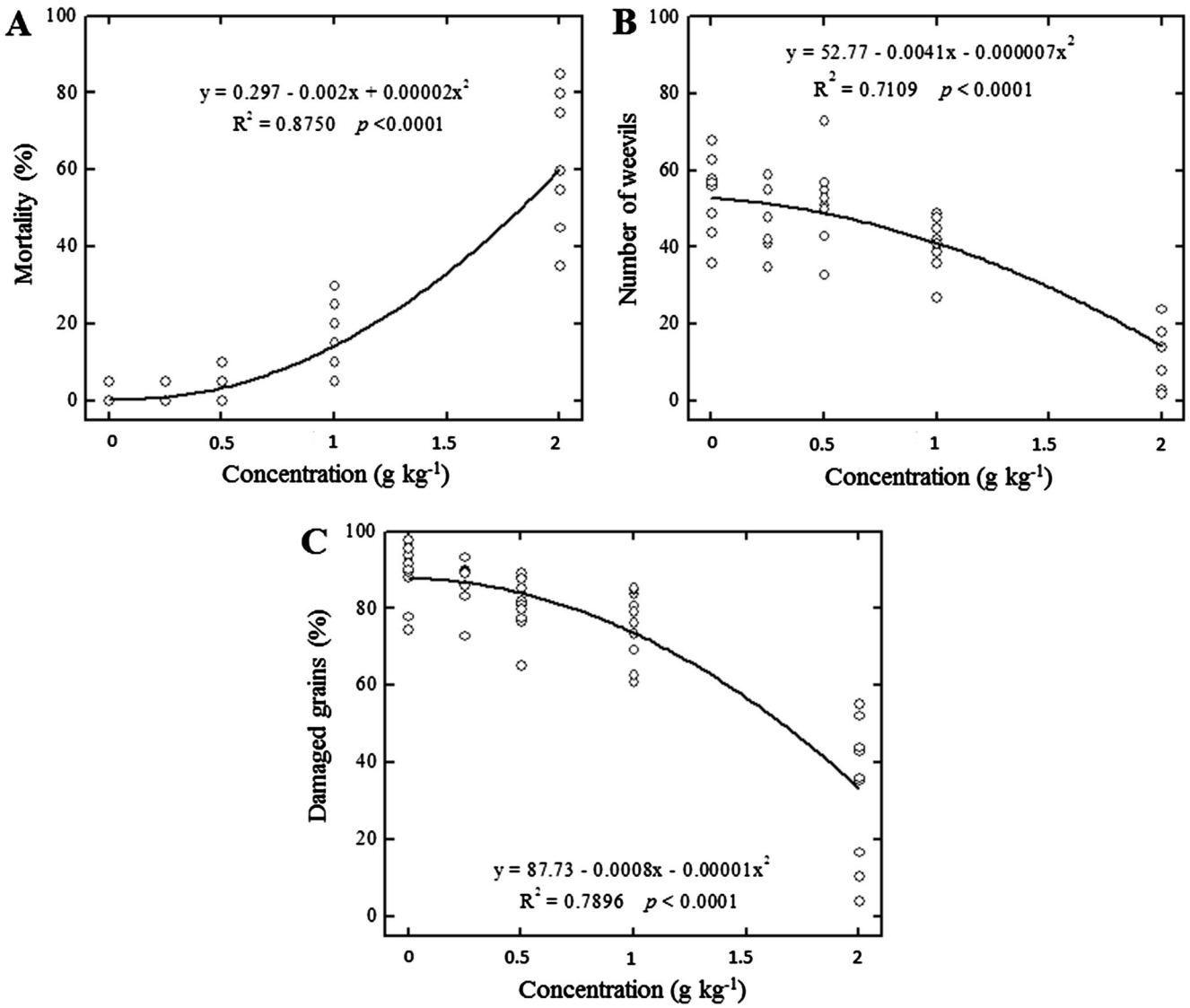


Fig 1 Bioactivity of different concentrations of the essential oil extracted from *Pimenta pseudocaryophyllus* leaves against *Sitophilus zeamais*. **a** Adult mortality by day 10. **b** Number of emerged insects (F_1 progeny). and **c** Damage sustained by the corn samples after 60 days of infestation, estimated as the proportion of damaged grains.

Table 2 Fumigant insecticidal activity of the essential oil extracted from *Pimenta pseudocaryophyllus* leaves against *Sitophilus zeamais* adults after 72 h of exposure.

Concentration (μL L^{-1} of air)	Mortality (%)
400	1.1±1.11
200	1.1±0.70
100	2.8±2.18
50	0.5±0.55
Control (Acetone)	1.1±0.70
F	0.5523^{ns}
p value	0.6991

^{ns} Non-significant difference (GLM with a quasi-binomial distribution, $p>0.05$).

P. pseudocaryophyllus leaves as a grain protector. The phenylpropanoid chavibetol, which is the major constituent of the oil (38.14% of its total composition) is reported as the major component of the essential oil from *P. pseudocaryophyllus* leaves (Santos *et al* 2009, Marques *et al* 2010, Barata *et al* 2011). However, edaphoclimatic and seasonal differences among the regions where the specimens used in the different studies were cultivated may explain the differences in the proportions of the major constituents and in the presence or absence of minor constituents (Paula *et al* 2010a, Barata *et al* 2011). Still, the occurrence of intraspecific chemical variation (chemotypes) has been reported in this Myrtaceae species (Paula *et al* 2011) and should be considered and analyzed in future studies aiming to develop new bioinsecticides from this species.

Table 3 Repellency of *Sitophilus zeamais* adults by corn samples (10 g) treated with different concentrations of *Pimenta pseudocaryophyllus* derivatives.

Concentration (g kg^{-1})	RI (Means±SD)	ClassI	CL	PR
Plant powder				
80	0.4±0.22	1±0.16	R	62.9
40	0.4±0.34	1±0.24	R	61.5
20	0.5±0.26	1±0.18	R	46.4
10	0.5±0.31	1±0.22	R	42.0
Ethanolic extract				
2	1.0±0.22	1±0.15	N	5.4
1	1.1±0.27	1±0.19	N	15.7
0.5	1.1±0.14	1±0.11	N	−16.4
0.25	1.1±0.21	1±0.15	N	5.6
Essential oil				
2	0.3±0.10	1±0.12	R	69.3
1	0.4±0.14	1±0.18	R	64.5
0.5	0.5±0.20	1±0.25	R	50.4
0.25	0.8±0.11	1±0.14	R	15.4

RI repellency index; *ClassI* classification interval; *CL* classification (*N* neutral, *A* attractive, *R* repellent); *PR* percentage repellency.

In general, terpenes are neurotoxic to insects by affecting the acetylcholinesterase activity or the octopamine receptors (Isman 2000). Based on a *Periplaneta americana* Linnaeus cell culture and *Drosophila melanogaster* Meigen brains, Enan (2001) showed that eugenol [$C_{10}H_{12}O_2$ (isomer of chavibetol)] acts on insects by mimicking the activity of octopamine and increasing the levels of intracellular calcium. Terpenic compounds may reach their target sites by entering the organism (insect) through its airways, thus rapidly interfering with the insect physiology (Rajendran & Sriranjini 2008), although we did not observe it in our fumigation bioassay. Although some of the compounds found in this essential oil have been shown to have fumigant insecticidal activity against various pest species of stored grains (Mondal & Khalequzzaman 2010, Coitinho *et al* 2011, Liu *et al* 2013), the low concentrations of the active constituents in the crude essential oil or possible changes in the physical-chemical properties (especially the volatility) of the fumigant insecticidal compounds when used in blends may explain these results. Similarly, Cox *et al* (2001) demonstrated that the non-oxygenated terpenes found in the essential oil of *Melaleuca alternifolia* (Myrtaceae) reduce the antimicrobial activity of terpinen-4-ol (the main active component of this oil) by reducing its aqueous solubility, possibly through an increase in the carbon-chain length of the total composition.

In addition to the insecticidal and repellent activity of the essential oil, the *P. pseudocaryophyllus* leaf powder also had a significant repellent effect on adult *S. zeamais*. This effect was most likely due to the presence of more polar and less

Table 4 Chemical composition of the essential oil of *Pimenta pseudocaryophyllus* (Myrtaceae).

Compound	RI exp. [a]	RI lit.[b]	Percent (%)
NI	–	–	0.64
α-pinene	934	932	2.59
Camphene	941	946	2.19
β-pinene	979	974	0.90
Myrcene	990	988	1.31
α-felandrene	1006	1002	5.94
δ-3-carene	1008	1008	2.51
α-terpinene	1016	1014	1.16
p-cymene	1024	1020	8.21
Limonene	1028	1024	1.31
β-felandrene	1030	1025	0.95
1,8-cineole	1032	1026	3.93
β-(E)-ocimene	1045	1044	2.80
γ-terpinene	1057	1054	3.79
Terpinolene	1085	1086	9.17
Linalool	1099	1095	0.17
NI	–	–	0.25
Terpinen-4-ol	1180	1174	1.73
p-cymen-8-ol	1186	1179	0.48
Eugenol	1350	1356	0.48
Chavibetol	1367	1372[c]	38.14
Methyl eugenol	1398	1403	11.35
Total			99.11

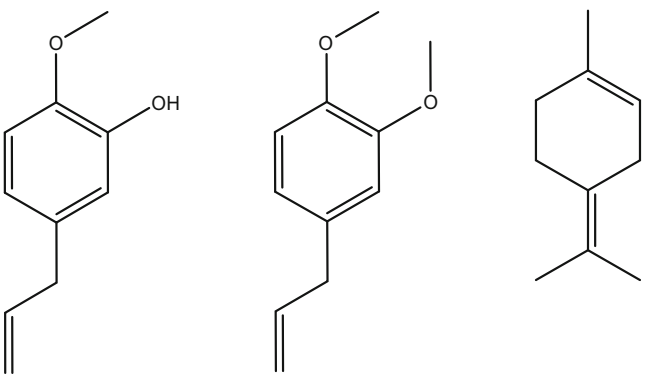
[a] Retention index calculated using the equation of Vand Den Dool & Kratz (1963).

[b] Retention index obtained from the literature (Adams 2007) based on the equation of Vand Den Dool & Kratz (1963).

[c] Retention index according to Santos *et al* (2009).

[NI] Compound not identified.

[–] Not determined.



(A) (B) (C)

Fig 2 Chemical structures of the major compounds found in the essential oil of *Pimenta pseudocaryophyllus*.**a** Chavibetol ($C_{10}H_{12}O_2$). **b** Methyl eugenol ($C_{11}H_{14}O_2$). **c** Terpinolene ($C_{10}H_{16}$).

volatile terpenic compounds in the plant powder, which were not lost when the leaves were dried due to the exposure to a slow drying in a low-temperature process. Although the action of chavibetol has not been studied yet, the effects of other components of the essential oil of *P. pseudocaryophyllus* on the behavior of various pest species of stored grains have been previously reported (Obeng-Ofori & Reichmuth 1997, Ogendo *et al* 2008, Zapata & Smagghe 2010, Ukeh & Urnoetok 2011, Suthisut *et al* 2011). In these studies, the compounds were tested in isolation or as major constituents of the essential oils of different aromatic species, and the attractiveness level varied with the concentrations and proportions of the oil constituents. In light of these findings, layers of *P. pseudocaryophyllus* leaves might be used for grain protection using the technique of grain enveloping, especially in the storage units of smallholders in the Vale do Ribeira (southern of the state São Paulo, Brazil), where *P. pseudocaryophyllus* is abundant. This grain protection technique is already used with inert powders and involves placing layers of plant material below and above the grain mass, thus preventing insect pests from entering the grain through the effect of the plant material on host-plant selection behavior (repellency).

Based on these results, we conclude that *P. pseudocaryophyllus* is an interesting source of active compounds with insecticide activity against stored-product pest. However, more studies are required for the development of a botanical insecticide based on *P. pseudocaryophyllus* essential oil, especially devoted to the isolation and identification of the main active compound(s) and to the understanding of the role of each constituent in their overall biological activity. Moreover, complementary studies are also necessary to determine the activity-structure relationships (configurations and functional groups) of these terpenic compounds and to modulate their biological properties to facilitate the development of more efficient and inexpensive artificial blends to use in the IPM of stored grains.

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