RESEARCH

CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

Bioactivity of *Peumus boldus* Molina, *Laurelia sempervirens* (Ruiz & Pav.) Tul. and *Laureliopsis philippiana* (Looser) Schodde (Monimiacea) essential oils against *Sitophilus zeamais* Motschulsky

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The maize weevil (*Sitophilus zeamais* Motschulsky) is one of most important pest of stored seeds worldwide, but its current control method is based on the use of synthetic insecticides, usually leading to undesirable problems such as insecticide residues on treated food, human intoxications, and insect resistance development. Therefore the search of friendly alternative methods is required. The aim of this study was to assess, under laboratory conditions, the insecticidal properties of *Peumus boldus* Molina, *Laurelia sempervirens* (Ruiz & Pav.) Tul., and *Laureliopsis philippiana* (Looser) Schodde essential oils against *S. zeamais*. The phytochemical analysis of the three essential oils showed 1,8-cineole, safrole and methyleugenol as the common components; all of them documented with insecticidal activity from essential oils from other plant species. The highest toxicity (100% mortality) of these three oils acting as a contact insecticide was observed at 24 h exposure at 4% concentration. The estimated LC_{50} values for *P. boldus*, *L. sempervirens*, and *L. philippiana* were 0.37, 1.02, and 0.28 μL g^{-1}, respectively. *Peumus boldus* exhibited the highest fumigant activity with 100% adult mortality at 30 μL oil L^{-1} air. At ≥ 0.5% (v/w) concentration, all essential oils showed repellent activity. These three essential oils showed a promissory insecticidal activity against the maize weevil.

Key words: Botanical insecticides, essential oils, maize weevil, stored grains.

INTRODUCTION

The maize weevil (*Sitophilus zeamais* Motschulsky; Coleoptera: Curculionidae) is known as a key pest of stored cereals because is able to feed on whole and undamaged grains. It may cause a complete grain loss in only 6-mo (Coitinho et al., 2011). Its control is usually performed with synthetic insecticides, leading to important problems such as the presence of undesirable residues on food and development of resistance to insecticides as phosphine (Pimentel et al., 2009), organophosphates and pyrethroids (Ribeiro et al., 2003). Recently, there has been a growing interest to evaluate the potential use of botanical insecticides, as powders, extracts or essential oils as a friendly alternative to synthetic insecticides.

Essential oils are common substances assessed against insect pest. These compounds may act as fumigants (Lee et al., 2004), contact insecticides, repellents and antifeedants (Isman, 2000). The toxicity of a large number of essential oils and their constituents has been evaluated against stored-product insects of *Sitophilus* genus as *S. zeamais* (Betancur et al., 2010), *Sitophilus oryzae* L. (Samboon and Pimsamarn, 2006), and *Sitophilus granarius* L. (Aslan et al., 2004).

In Chile research on insecticidal properties of essential oils is limited and has been focused on boldus (*Peumus boldus* Molina; Monimiaceae). For example, Urzúa et al. (2010) found insecticidal properties of *P. boldus* essential oil against *Musca domestica* L. and Bittner et al. (2008) and Betancur et al. (2010) indicated that this oil showed insecticidal activity against *S. zeamais* and *Acanthoscelides obtectus*. But the Monimiaceae family has other two native plants from Chile, *Laurelia sempervirens* (Ruiz & Pav.) Tul. (Chilean laurel) and *Laureliopsis philippiana* (Looser) Schodde (tepa), and could have related or same chemical compounds and

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Received: 27 November 2014.
Accepted: 14 April 2015.
doi:10.4067/S0718-58392015000400010

therefore similar insecticidal activity. Hence the objective of this research was to evaluate insecticidal properties of the essential oils of three Chilean native Monimiaceae trees; *P. boldus*, *L. sempervirens*, and *L. philippiana* against adults of *S. zeamais* under laboratory conditions.

MATERIALS AND METHODS

Extraction and analysis of essential oils

The essential oil of *P. boldus* and *L. sempervirens* were extracted from leaves field-collected from Los Lleuques zone (36°51'18" S, 71°38'34" W; 286 m a.s.l.), Ñuble province foothills, Chile. The foliage of *L. philippiana* was field-collected from the foothills of Maullín province (41°41' S, 73°25' W; 28 m a.s.l.). All collections were carried out during January 2012, following Vogel et al. (1997) methodology. The taxonomic identification of collected foliage was verified according to reference vouchers CONC-CH5492 (*P. boldus*), CONC-CH237 (*L. philippiana*), and CONC-CH 809 (*L. sempervirens*) deposited in herbarium of Faculty of Agronomy of University of Concepción at Chillán. Once in the laboratory, only mature and whole leaves were washed with distilled water and dehydrated at 40 °C in a stove (Memmert Gmbh, UNB 500, Schwabach, Germany). The essential oils were obtained by steam distillation during 4 h using distilled water in a Clevenger type apparatus (Kouninki et al., 2007). Subsequently, the oil was treated with sodium sulfate to eliminate water traces and stored in amber-colored glass containers at 4.5 °C.

Chemical analysis of essential oils was assessed by gas cromatography (GC) coupled to mass spectrometry detection (GC-MS), using a high performance gas chromatography-mass spectrometry (HPGC-MS; HP 5890 Series II, Hewlett Packard, Palo Alto, California, USA). Separation was achieved using a 5% poly diphenyl 95% dimethylsiloxane bonded phase column (i.d. 0.25 mm, length 30 m, film thickness 0.25 μm). Operation conditions were as follow: injector temperature, 250 °C; carrier gas (Helium), flow rate 1 mL min^{-1} and split injection with a split ratio 1:20. Mass spectrometry conditions were as follows: ionization voltage, 70 eV; emission current 40 mA; scan rate, 1 scan s^{-1}; source temperature 285 °C. Mass range was 35-300 Da. The oven temperature was 2 min isothermal at 60 °C and then increased to 210 °C, at the rate of 10 °C min^{-1}, and to 260 °C, at rate of 10 °C min^{-1}. Samples (1 μL) were dissolved with CH_2Cl_2 (1:100 v/v). The MS fragmentation pattern was checked with the standards available in our laboratory, and by matching the MS data with the library NIST NBS54K or literature (Adams, 2007). The relative amounts of each component were obtained from GC analysis using flame ionization detector in the same experimental conditions described for GC-MS analysis. In these conditions the linear retention indices (RI) were calculated using a mixture of n-alkanes (C_8-C_{28}).

Insects, grain, and bioassays

The insects used in the bioassays were obtained from our laboratory colonies and reared in 1 L glass flasks containing maize (*Zea mays* L.) at 30 ± 1 °C, 60 ± 5% RH and total darkness in a bioclimatic chamber (Memmert Gmbh, IPS 749, Schwabach, Germany). Maize for insect rearing and bioassays was obtained from the fruit and vegetable market of city of Chillán, Biobío Region, Chile. To avoid any previous infestation, the grain was washed and dried in a stove at 25 °C for 12 h and then frozen at -4.0 ± 1 °C for 48 h prior to its use.

Mortality due to exposition to treated surface. The methodology of Kouninki et al. (2007) was followed and consisted of 6-mL test tubes treated with 1 mL of a solution of essential oil in acetone, at the required concentration. Then, the tubes were shaken for 1 min to allow the oil to cover the inner surface. The excess was eliminated by runoff and the acetone was allowed to evaporate at environmental temperature (20 ± 5 °C) for 1 h. Then 20 48-h old adult insects without sexing were placed in each tube. The evaluated doses of oils were 0.25, 0.5, 1, 2, and 4% (v/v). The control consisted on using 1 mL of acetone without essential oil. Ten replicates were made at the same day per treatment and set up in a bioclimatic chamber at 30 ± 1 ºC, 60 ± 5% RH and total darkness. Insect mortality was assessed at 24 and 48 h exposure. The maximum level of mortality accepted for the control was 5% and it was corrected by means of the Abbott's formula (Abbott, 1925). An insect was considered dead when there was no movement after prodding it with a dissection needle for 5 min.

Mortality due to exposure to treated grain. This bioassay was carried out with the methodology of Obeng-Oferi and Reichmuth (1997). Solutions of 1 mL of essential oil in acetone were applied to 400-mL glass flasks with 200 g maize at the concentrations previously described. The flasks were covered and shaken for 15 s to cover the grains with oil, then uncovered and left for 2 h at room temperature (20 ± 5 °C) to allow the acetone to evaporate. Immediately the flasks were infested with 20 48-h old adult insects without sexing. Each treatment was replicated ten times in 1 d. The experimental units were stored in a bioclimatic chamber at 30 ± 1 °C, 60 ± 5% RH and total darkness. Mortality assessment followed what was previously described.

Fumigant effect. This bioassay employed the methodology of Pires et al. (2006), which consisted of applying 0 (control), 15, 20, 25, 30, and 35 µL of essential oil on a circular Whatman nr 10 filter paper (5.5 cm diameter, Whatman, Maidstone, Kent, UK), then the treated paper was attached to undersurface of screw caps of a 500-mL plastic container (air volume equivalent to 0.5 L) and 200 g maize infested with 20 48-h old adult insects without

sexing were added. Ten replicates at one time were run for each concentration and control. Insect mortality was assessed at 24, 48, and 72 h exposure and it was corrected by means of the Abbott's formula (Abbott, 1925).

Repellent effect. The experimental unit was a choice arena consisting in a central plastic Petri dish (5 cm diameter) connected to another four dishes through tubes 10 cm long and 0.5 cm in diameter forming an "X". Two opposite dishes containing 20 g maize grains were impregnated with the respective concentrations of essential oil, while other two dishes had maize grains treated only with acetone (Procopio et al., 2003). In the central Petri dish 20 individuals of *S. zeamais* of 48 h of age without sexing were released. The evaluated doses of oils were 0.25, 0.5, 1, 2, and 4% (v/v) and each treatment had 10 replicates carried out simultaneously. Treatments were kept in a bioclimatic chamber for 24 h at 30 ± 1 °C, 60 ± 5% RH and total darkness and the number of insects that moved to each treatment was recorded. In each replicate the treatments were randomly rotated to avoid the interference of external factors. The repellent indexes were calculated according to Restello et al. (2009), in which the oil is classified as neutral if the index is between -0.10 to 0.10, attracting if it is between 0.10 to 1.00 and repellent if it is between -1.00 to -0.10.

Germination test of treated grain. The effect of essential oils on the germination of the maize grains was assessed using the methodology described by Pérez et al. (2007). Groups of 30 undamaged seeds were randomly selected. The groups of seeds were independently exposed to each of the following treatments; 0.25%, 0.5%, 1%, 2%, and 4% (v/v) and then placed separately on a glass Petri dish containing moistened filter paper at the bottom. These treatments were replicated ten times at the same day. The experimental units were kept at 25 ± 1 °C, 60 ± 5% RH and 12:12 h photoperiod during 7 d in a bioclimatic chamber. The percentage of germination in comparison to the untreated control was determined.

Experimental design

The experimental design was a completely random with a factorial arrangement. The percentage was transformed to the $\sqrt{x}/100$ arcsine function prior to carry out ANOVA ($\alpha = 0.05$) test with the Statistical Analysis System (SAS) software (SAS Institute, Cary, North Carolina, USA) to determine if at least one treatment was different from the rest. If so, a Tukey means comparison test was used ($p \leq 0.05$). To obtain the lethal concentration 50% (LC_{50}) and 95% (LC_{90}) data were subjected to Probit analysis using PROC PROBIT procedure of SAS software to determine $LC_{50/95}$ values. Probit analysis responses were considered different when their respective fiducially limits did not overlap at a given mortality level (50% or 95%) (Robertson and Preisler, 1992).

RESULTS AND DISCUSSION

Phytochemical analysis

The analysis of *P. boldus* essential oil showed ascaridole (24.37%) and 1,8-cineole (14.85%) as the main components (Table 1). Ascaridole, as one of the most abundant components agrees with the results of Bittner et al. (2008) and Niemeyer and Teillier (2007) who documented concentrations as high as 38.9% and 60.3%, respectively. However, Urzúa et al. (2010) considered that 1,8-cineole was the main component (36.62%) of *P. boldus*. Perhaps, in our study this proportion was lower due to the effect of field collection date because all authors collected in the Biobío Region but Urzúa et al. (2010) collected leaves in November, Bittner et al (2008) in June, and we did it in March. In the case of *L. philippiana*, safrole (39.56%), linalool (34.45%), and 1,8-cineole (8.28%) were the compounds found at higher concentrations (Table 2). Our results are similar to those documented by Niemeyer and Teillier (2007) who detected an analogous level of 1,8-cineole concentration; although

Table 1. The main constituents of essential oil from *Peumus boldus* determined by gas chromatography and gas chromatography-mass spectrometry detection.

Compound	RI[1]	%	Identification[2]
α-Thujene	930	0.32	RI, MS, S
1R-α-Pinene	939	2.28	RI, MS, S
Camphene	952	0.10	RI, MS
β-Pinene	979	0.42	RI, MS, S
β-Myrcene	993	2.00	RI, MS, S
d-2-Carene	1001	0.25	RI, MS
α-Phellandrene	1002	0.21	RI, MS, S
α-Terpinene	1013	3.94	RI, MS, S
Limonene	1030	2.95	RI, MS, S
β-Phellandrene	1031	4.42	RI, MS, S
trans-β-Ocymene	1036	12.87	RI, MS
1,8-Cineol	1039	14.85	RI, MS, S
cis-β-Ocymene	1042	0.45	RI, MS
γ-Terpinene	1057	1.86	RI, MS, S
Terpinolene	1086	0.15	RI, MS, S
p-Cymenene	1090	0.21	RI, MS
2-Nonanone	1093	0.15	RI, MS, S
trans-Sabinene hydrate	1097	4.07	RI, MS
Dehydro-sabina ketone	1121	0.36	RI, MS
trans-Pinocarveol	1140	0.57	RI, MS
Camphor	1146	0.10	RI, MS, S
Terpinen-4-ol	1179	3.37	RI, MS, S
Cryptone	1186	0.68	RI, MS
α-Terpineol	1187	2.14	RI, MS, S
Myrtenal	1193	0.31	RI, MS, S
Myrtenol	1194	0.20	RI, MS, S
Bornyl acetate	1265	0.64	RI, MS, S
Ascaridole	1273	24.37	RI, MS, S
β-Elemene	1280	0.73	RI, MS
Safrole	1285	1.45	RI, MS
2-Undecanone	1295	0.27	RI, MS
Methyleugenol	1372	0.46	RI, MS, S
α-Caryophyllene	1419	0.78	RI, MS, S
Aromadendrene	1440	0.43	RI, MS, S
α-Caryophyllene	1454	0.73	RI, MS, S
Germacrene D	1480	0.45	RI, MS
∂-Cadinene	1524	0.24	RI, MS, S
8,9-Dehydro-neoisolongifolene	1558	0.80	RI, MS

[1]Kovats retention index was determined on a DB-5 column in reference to n-alkanes.
[2]Compounds, identified by comparison with mass spectra (MS) from database, retention indices (RI), and pure standards (S).

Table 2. The main constituents of essential oil from *Laurelia sempervirens* determined by gas chromatography and gas chromatography-mass spectrometry detection.

Compound	RI[1]	%	Identification[2]
α-Thujene	930	0.23	RI, MS
1R-α-Pinene	939	0.76	RI, MS
Sabinene	963	0.22	RI, MS, S
β-Pinene	972	0.16	RI, MS, S
α-Phellandrene	1002	0.56	RI, MS, S
Limonene	1030	1.33	RI, MS, S
α-Phellandrene	1031	1.28	RI, MS, S
p-Cymene	1033	0.25	RI, MS
1,8-Cineol	1039	1.47	RI, MS, S
cis-β-Ocymene	1042	0.30	RI, MS
γ-Terpinene	1057	0.03	RI, MS, S
Terpinolene	1086	0.10	RI, MS, S
p-Linalool	1089	9.91	RI, MS, S
4-Terpineol	1179	0.10	RI, MS, S
α-Terpineol	1187	0.87	RI, MS, S
2-Pinen-10-ol	1196	0.03	RI, MS
Safrole	1285	64.70	RI, MS, S
α-Terpinyl acetate	1343	0.06	RI, MS
Eugenol	1356	0.51	RI, MS, S
Methyleugenol	1372	14.64	RI, MS, S
Germacrene D	1480	0.95	RI. MS, S
β-Elemol	1543	0.02	RI, MS

[1]Kovats retention index was determined on a DB-5 column in reference to n-alkanes.
[2]Compounds identified by comparison with mass spectra (MS) from database, retention indices (RI), and pure standards (S).

Table 3. The main constituents of essential oil from *Laureliopsis philippiana* determined by gas chromatography and gas chromatography-mass spectrometry detection.

Compound	RI[1]	%	Identification[2]
α-Thujene		0.04	RI, MS
(1*R*)-α-Pinene	939	0.49	RI, MS, S
Sabinene	963	0.81	RI, MS, S
β-Pinene	972	0.59	RI, MS, S
β-Myrcene	993	0.25	RI, MS, S
α-Phellandrene	1002	1.55	RI, MS, S
Limonene	1030	0.77	RI, MS, S
Unknown	1032	0.11	RI, MS, S
E-Ocymene	1037	0.05	RI, MS, S
1,8-Cineol	1039	8.28	RI, MS, S
β-Ocymene	1047	0.12	RI, MS, S
γ-Terpinene	1057	0.14	RI, MS, S
Terpinolene	1086	0.28	RI, MS, S
2-Nonanone	1093	0.05	RI, MS, S
Linalool	1100	34.45	RI, MS, S
4-Terpineol	1179	0.45	RI, MS, S
p-Cymen-8-ol	1185	0.07	RI, MS, S
α-Terpineol	1187	3.32	RI, MS, S
Safrole	1285	39.56	RI, MS, S
Isoledene	1336	0.21	RI, MS, S
Eugenol	1356	1.41	RI, MS, S
Methyleugenol	1372	3.06	RI, MS, S
Germacrene D	1480	0.84	RI, MS, S
∂-Cadinene	1527	0.03	RI, MS, S
Spatulenol	1586	0.21	RI, MS, S

[1]Kovats retention index was determined on a DB-5 column in reference to n-alkanes.
[2]Compounds identified by comparison with mass spectra (MS) from database, retention indices (RI), and pure standards (S).

linalool (43.4%) was found in higher amount than safrole (21.4%). However, Bittner et al. (2008) indicated that safrole was detected at 2.33%, and the compounds at higher concentrations were 3-carene (53.8%), 1,8-cineole (14.76%) and 1,2-dimethoxy-4-(2-propenyl)-phenol (10.58%). In this case the difference with Bittner et al. (2008) is maybe also due to field collection date more a geographical zone effect because these authors collected in Concepción (36°50' S, 73°03' W; 12 m a.s.l.), Biobío Region, and Niemeyer and Teillier (2007) and we did it in Puyehue (40°39.9' S, 72°10.3' W; 364 m a.s.l.) and Maullín (41°41' S, 73°25' W; 28 m a.s.l.) respectively, both located in Los Lagos Region.

The main components of the *L. sempervirens* essential oil were safrole (64.7%), methyleugenol (14.6%), and 1,8-cineol (1.4%) (Table 3). These results agree with those of Bittner et al. (2008) and Montenegro et al. (2012) who estimated 65% and 69% concentration of safrole in essential oil from leaves and bark, respectively. However, the concentration of 1,8-cineol is different from the 14.7% obtained by Bittner et al. (2008) probably due to field collection date. About insecticidal activity of identified compounds Huang et al. (2002) indicated that safrole and methyleugenol have insecticidal properties against *S. zeamais* and *Tribolium castaneum* and according to Obeng-Oferi and Reichmuth (1997) this compound also affects *S. granarius*, *S. zemais*, *T. castaneum*, and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). Safrole has been considered as an attractive kairomone of *Bactrocera dorsalis* (Hendel) (Vargas et al., 2000; Shelly and Edu, 2008) and *B. cucurbitae* (Coquillet) (Vargas et al., 2000) and antifeedant against *Pieris rapae* (Yano and Kamimura, 1993). In the case of 1,8-cineol, Lee et al. (2004) mentioned this component as a potential fumigant against stored grain pests.

Mortality by contact with a treated surface

Peumus boldus and *L. sempervirens* caused 100% mortality at concentrations of 2% and 4% of essential oil, but in both species these treatments did not show differences (p = 0.99) with the 1.0% that exhibited 88.8 (*P. boldus*) and 76.7% (*L. sempervirens*) of dead insects, respectively. In case of *P. boldus* our results agree with Betancur et al. (2010) who observed 100% of dead insect at 4% concentration. *Laureliopsis philippiana* reached 100% mortality at 4% without significant differences with the 2% concentration (Table 4). At 48 h, 1% concentration of essential oil of *P. boldus* achieved 91.9% mortality, without significant differences with the mortality (100%) obtained with 2% and 4% concentrations. At the same evaluation 2.0% of essential oil of *L. sempervirens* after 24 h exposure lead 83% mortality, while at 48 h this value significantly ($p = 0.001$) increased to 92.9%. *Laureliopsis philippiana* did not show differences at 48 h.

Mortality by contact with treated grain

At 24 h, treatments consisting of 2% and 4% of *P. boldus* essential oil reached 100% mortality and this value was significantly ($p = 0.001$) higher than those observed with the rest of the treatments (Table 5). The essential oil of *L. sempervirens* at 4%, showed 82.7% mortality, differing from all other essential oil concentrations. *Laureliopsis*

Table 4. Mortality at 24 at 48 h of adult *Sitophilus zeamais* exposed to treated glass surface with the essential oil of *Peumus boldus*, *Laurelia sempervirens*, and *Laureliopsis philippiana* (Monimiacea) at different concentrations.

Concentration	24 h			48 h		
	P. boldus	*L. sempervirens*	*L. philippiana*	*P. boldus*	*L. sempervirens*	*L. philippiana*
%	%					
0.25	0.0Ac	3.0Ab	7.0Ac	1.3ABc	2.0Bc	18.9Ac
0.50	33.3Ab	13.0Ab	43.4Ab	39.2Ab	24.2Ac	44.5Abc
1.00	88.9Aa	31.1Bb	76.7Aa	91.9Aa	40.0Bb	79.7Aab
2.00	100.0Aa	83.0Aa	100.0Aa	100.0Aa	92.9Aa	100.0Aa
4.00	100.0Aa	100.0Aa	100.0Aa	100.0Aa	100.0Aa	100.0Aa

Within the same column, the values with the same lower-case letter are not significantly different according to Tukey test ($p \leq 0.05$).
Within the same row, the values with the same uppercase letter are not significantly different according to Tukey test ($p \leq 0.05$).

Table 5. Mortality at 24 and 48 h of adults of *Sitophilus zeamais* exposed to treated grain with essential oil of *Peumus boldus*, *Laurelia sempervirens*, and *Laureliopsis philippiana* (Monimiacea) at different concentrations.

Concentration	24 h			48 h		
	P. boldus	*L. sempervirens*	*L. philippiana*	*P. boldus*	*L. sempervirens*	*L. philippiana*
%	%					
0.25	0.0Ab	0.2Acd	0.0Ad	0.0Ab	5.1Acd	2.5Ac
0.50	27.5Ab	5.1Ad	30.0Ac	22.2Ab	7.5Ad	46.2Ab
1.00	31.6ABb	6.3Bc	68.7Ab	28.8Bb	11.5Bc	92.5Aa
2.00	95.0Aa	25.3Bb	100.0Aa	100.0Aa	32.5Bb	100.0Aa
4.00	100.0Aa	82.3Ba	100.0Aa	100.0Aa	91.2Aa	100.0Aa

Within the same column, the values with the same lower-case letter are not significantly different according to Tukey test ($p \leq 0.05$).
Within the same row, the values with the same uppercase letter are not significantly different according to Tukey test ($p \leq 0.05$).

philippiana at 2% and 4% concentrations produced 95% and 100% mortality, respectively. After 48 h exposure, without statistical difference ($p = 0.34$), the essential oil of *L. sempervirens* caused 91.2% mortality at 4% concentration and *L. philippiana* at 2% reached 100%. The results with *P. boldus* are similar to those obtained by Betancur et al. (2010) who estimated 98% mortality at 4% concentration of essential oil. The LC_{50} values at 48 h were 0.37, 1.02, and 0.28 μL g^{-1} (μL essential oil g^{-1} grain) for *P. boldus*, *L. sempervirens*, and *L. philippiana*, respectively. Since their fiducial limits overlapped (Robertson and Preisler, 1992), there were no differences among them (Table 6). The LC_{50} values obtained with essential oil of *P. boldus* and *L. philippiana* show a higher toxicity than other essential oils assessed against *S. zeamais*, such as *Piper crassinervium* Kunth (Piperaceae) (0.71 μL g^{-1}) (Salgado et al., 2012); *Eugenia uniflora* L. (Myrtaceae) (11.6 μL 40 g^{-1} grain); *Cinnamomum zeylanicum* Blume (Lauraceae) (14.2 μL 40 g^{-1} grain), *Piper marginatum* Jacq. (Piperaceae) (21.1 μL 40 g^{-1} grain); *Schinus terebinthifolia* Raddi (Anacardiaceae) (57.7 μL 40 g^{-1}); and *Melaleuca leucadendron* L. (Myrtaceae) (75.8 μL 40 g^{-1} grain) (Coitinho et al., 2011). The essential oil of *P. boldus* and *L. philippiana* are also more toxic to *S. zeamais* than cymol isolated from *Eucalyptus saligna* Sm. (Myrtaceae) and *Cupressus sempervirens* L. (Cupressaceae) (38.05 μL 40 g^{-1} grain) (Tapondjou et al., 2005).

The toxicity by direct applications of *L. sempervirens* and *L. philippiana* may be due to the safrole compound activity, which is the most abundant component in its essential oils (Tables 2 and 3) because according to Huang et al. (2002), safrole and isosafrole have contact and fumigant toxicity against adults of *S. zeamais* and *T. castaneum*.

Fumigant effect

The highest toxicity was observed in *P. boldus* essential oil where all evaluated concentrations exceeded 90% mortality at 24 h. The concentration of 70 μL L^{-1} air of *L. sempervirens* essential oil showed 58.62% dead insects without differences ($p = 0.74$) with 50 and 60 μL L^{-1} air that reached 31% and 41.3% mortality (Table 7). *Laureliopsis philippiana* registered a maximum mortality of 56.6% at 70 μL L^{-1} air being significantly ($p = 0.001$) higher in comparison with the rest of treatments. After 48 h exposure, 35 μL L^{-1} air of *L. sempervirens* and *L. philippiana* reached 86% and 95% mortality, but did not show differences with 40 and 60 μL L^{-1} air. At 72 h, *L. sempervirens* and *L. philippiana* at 70 μL L^{-1} showed 100% and 98.3% mortality. Perhaps the toxicity

Table 6. Lethal concentration at 50% (CL_{50}) and 95% mortality (CL_{95}) of essential oils of *Peumus boldus*, *Laurelia sempervirens*, and *Laureliopsis philippiana* (Monimiacea) against *Sitophilus zeamais* by contact effect.

Essential oil	n	CL_{50}	CL_{95}	Slope ± SE	X^2	Df	*p*-Value
%		μL g^{-1}					
P. boldus	200	0.37 (0.15-1.06)	1.05 (0.55-188)	3.7 ± 0.95	0.0001	3	0.0001
L. sempervirens	200	1.02 (0.53-8.9)	3.6 (1.6-21659)	3.0 ± 0.79	0.0002	3	0.0001
L. philippiana	200	0.28 (0.18-0.44)	0.64 (0.41-3.32)	4.6 ± 0.69	0.0001	3	0.0386

n: Total number of insect tested in 10 replicates per treatment; Df: degrees of freedom; X^2: chi-square.
Values in parenthesis correspond lower and upper of confidence limits at 95% of probability.

Table 7. Mortality at 24, 48, and 72 h of *Sitophilus zeamais* adults by fumigant effect of the essential oils of *Peumus boldus*, *Laurelia sempervirens*, and *Laureliopsis philippiana* (Monimiacea) at the different concentrations.

Concentration	24 h			48 h			72 h		
	P. boldus	*L. sempervirens*	*L. philippiana*	*P. boldus*	*L. sempervirens*	*L. philippiana*	*P. boldus*	*L. sempervirens*	*L. philippiana*
µL oil L^{-1} air	%								
30	95Aa	0.0Bb	3.3Cb	95Aa	0.0Bb	36.66Bb	98.30Aa	20.68Bc	57.6Bb
40	100Aa	27.6Bb	11.6Bb	100Aa	37.9Ba	61.7Bab	100.00Aa	68.96Bb	61.7ABab
50	100Aa	31.0Ba	16.7Cb	100Aa	44.8Ba	66.7Bab	100.00Aa	72.41Bab	86.4Bab
60	100Aa	41.4Ba	25.0Cb	100Aa	69.0Ba	78.3Bab	100.00Aa	93.10Ba	96.6ABab
70	100Aa	58.6Ba	56.7Ba	100Aa	86.2Ba	95.0ABa	100.00Aa	100.00Aa	98.3Aa

*Within the same column, the values with the same lower-case letter are not significantly different according to Tukey test ($p \leq 0.05$).
*Within the same row, the values with the same uppercase letter are not significantly different according to Tukey test ($p \leq 0.05$).

observed is mainly due to the action of 1,8-cineole present in *P. boldus* (14.87%), *L. sempervirens* (1.47%), and *L. philippiana* (8.28%) because according to Lee et al. (2004), 1,8-cineole assessed alone, showed a mortality higher than 90% in adults of *S. zemais*, *S. oryzae*, *T. castaneum*, and *Rhyzopertha dominica*.

Repellent effect

In *L. philippiana*, all concentrations were repellent to *S. zeamais* (Table 8). In *P. boldus* and *L. sempervirens*, only the treatments of 0.125% of essential oil did not show repellent effect while the others did. Betancur et al. (2010) obtained similar results with the essential oil of *P. boldus*, concluding that repellency is a useful and complementary effect to contact insecticidal activity, since it decreases the possibility of new infestations. The absence of repellent effect in lower essential oil concentrations agree with Nerio et al. (2009) and Conti et al. (2010) who argue that as the monoterpenes concentration increases, the repellent effect increases too. Thus if the monoterpenes concentration is lower, usually the most abundant components in essential oils, lower will be the repellency.

Germination test of treated grain

The *P. boldus* essential oil at 4% concentration was the only treatment that negatively affected grain germination (< 50%) (Table 9). Our results with *L. philippiana* and the rest of *P. boldus* treatments (< 4.0%) agree with those of Betancur et al. (2010) and Ortiz et al. (2012) who did not obtain differences in the germination level between non treated and treated seed with powder of *L. philippiana* and *P. boldus*. The decrease in the percent reduction of seed germination was documented by Pérez et al. (2007) with *P. boldus* at ≥ 4.0% concentration, perhaps due to the presence of certain compounds concentrations influenced by the time that the foliage was field-collected. This is one of reasons why botanical insecticides do not have a widespread use in agriculture.

Table 8. Repellence index of the essential oils of *Peumus boldus*, *Laurelia sempervirens*, and *Laureliopsis philippiana* (Monimiacea) on *Sitophilus zeamais* adults.

Concentration (%)	*P. boldus*	*L. sempervirens*	*L. philippiana*
0.125	0.1 (A)	0.2 (A)	-0.4 (R)
0.25	-0.2 (R)	-0.1 (R)	-0.4 (R)
0.50	-0.03 (R)	-0.2 (R)	-0.4 (R)
1.00	-0.2 (R)	-0.2 (R)	-0.2 (R)
2.00	-0.4 (R)	-0.5 (R)	-0.6 (R)
4.00	-0.5 (R)	-0.1 (R)	-0.2 (R)

A: Attractive, N: neutral, R: repellent.

Table 9. Germination of maize seeds exposed to different concentrations of essential oil of *Peumus boldus*, *Laurelia sempervirens*, and *Laureliopsis philippiana* (Monimiacea).

Concentration	*P. boldus*	*L. sempervirens*	*L. philippiana*
(%)	%		
Control	65.8Aa	70.0Aa	72.5Aa
0.25	72.5Aa	65.0ABa	54.1Bab
0.50	59.2Aa	71.6Aa	48.3Ab
1.00	76.7ABa	83.3Aa	61.6Bab
2.00	65.0ABa	81.6Aa	51.6Bab
4.00	22.5Ab	75.8Aa	55.8Aab

Values with the same lower-case letter are not different according to Tukey test ($p \leq 0.05$), within the same column.
Values with the same uppercase letter are not different according to Tukey test ($p \leq 0.05$), within the same row.

CONCLUSIONS

The essential oils of *Peumus boldus*, *Laurelia sempervirens*, and *Laureliopsis philippiana* have insecticidal properties by contact toxicity as well as fumigant and repellent action against *Sitophilus zeamais*.

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