Essential oil of *Peumus boldus* Molina against the nematode *Haemonchus contortus* (L3) and three stored cereal insect pests

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ABSTRACT

Alternative methods of control against different parasites affecting livestock and agriculture are currently being sought. The essential oil of *Peumus boldus* Molina has anthelmintic and insecticidal activity against the nematode *Haemonchus contortus* and stored cereal insect pests. The present study aimed to assess the in vitro activity of *P. boldus* essential oil against *H. contortus* (L3) unsheathed and *Sitophilus zeamais*, *Tribolium castaneum*, and *Plodia interpunctella*. The essential oil was obtained from leaves of *P. boldus* and its principal compounds identified by phytochemical analysis. Essential oil was tested at concentrations of 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 mg mL^{-1} on unsheathed L3 *H. contortus* larvae and in concentrations of 0, 35, 70, 100, 125, 150, 175 and 200 µL L^{-1} air on adult insects. The main constituents detected in the essential oil were 1,8-cineole, terpineol, terpinene-4-ol, γ-terpinene, *p*-cimene, methyl eugenol, safrole, (*E*)-germacrene D, β-tujene, and ascaridol. The nematode *H. contortus* and insects were exposed to the essential oil for 72 h. In a bioassay with *H. contortus* larvae, treatment with 2.0 mg mL^{-1} showed the highest toxicity with 99.2% mortality. Regarding insecticidal activity, *S. zeamais* was the most susceptible species with a mortality of 90% at 200 µL L^{-1} air. The present study suggests that the essential oil of *P. boldus* has potential anthelmintic and insecticidal activity.

Key words: Natural products, nematode, *Plodia interpunctella*, *Sitophilus zeamais*, stored cereal pests, *Tribolium castaneum*.

INTRODUCTION

The meat industry requires that animals and cereals they consume be maintained in a state of good health, but livestock and grains have parasites that affect their economy. In livestock, the nematode *Haemonchus contortus* (Rudolphi, 1803) Cobb (Strongylida: Trichostrongylidae) is a hematophagous parasite that causes histological damage to the abomasum, producing anemia; it can be fatal to young animals, causing significant economic losses worldwide (Pineda-Alegría et al., 2017). The main methods of combatting nematode infection are chemical treatments. However, frequent and excessive doses of anthelmintics have caused anthelmintic resistance. Interest has arisen in natural products of plant origin due to the undesirable side-effects of chemicals on non-target organisms, environmental hazards, and the development of resistance.

In the case of stored cereals, insects such as *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae) affect flour, maize, wheat, and rice, among others, generating economic losses. The loss of stored grains due to insect damage is estimated between 5% and 10% of worldwide production (Mesterházy et al., 2020), and its control is usually performed with synthetic insecticides, leading to critical problems such as the presence of undesirable residues on food and resistance development mainly to phosphine the main insecticide used in warehouse pest control (Agrafioti et al., 2019).

Essential oils from plants can be an alternative means of parasite and insect control. Against *H. contortus*, they have been assessed for effectiveness in treating animal diseases and as a nutraceutical alternative in ruminant nutrition. As for the anthelmintic activity of essential oils, it has been reported in previous studies with sheep that the essential oils of *Lippia sidoide* Cham., *Citrus ×sinensis* (L.) Osbeck, *Cymbopogon schoenanthus* (L.) Spreng., and *Eucalyptus staigeriana* F. Muell. ex F.M. Bailey. They reduce fecal egg counts load of *H. contortus* with an effect of 30%, 97%, 60%, and 99%, respectively (Squires et al., 2010; Macedo et al., 2010).

Many studies have demonstrated contact and fumigant toxicity of plant essential oils and their components to several species of stored product insects at different life stages. Essential oils of plants as *Eucalyptus* genus against *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and *T. castaneum* (Adak et al., 2020), *Mentha ×piperita* L. against *T. castaneum*, *Lasioderma sericoides* (Fabricius) (Coleoptera: Anobiidae) and *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae) (Pang et al., 2019), or *Chenopodium ambrosioides* L. against *S. zeamais* (Chu et al., 2011), among others.

Boldus (*Peumus boldus* Molina; Monimiaceae) is known worldwide for its therapeutic properties. According to pharmacopeias and treatises dealing with medicinal plants, boldo extracts have been used to treat headache, earache, rheumatism, "nervous weakness", dropsy, dyspepsia, menstrual pain, urinary tract inflammation and has also been claimed to be a sedative and mildly hypnotic. Its active components include alkaloids, essential oils, and flavonoids with boldine being the best-known alkaloid component (Benedetti-Ruiz and Barros-Asenjo, 2011). Specifically, leaves and bark contain its alkaloids known collectively as boldine, but boldo leaves contain between 0.4% and 0.5% of at least 17 different alkaloids belonging to the large benzylisoquinoline-derived family. Boldine is the major alkaloid as it accounts for 12%-19% of the total alkaloid content, which is considered to have antioxidant, anti-inflammatory, and antipyretic properties. Boldus as well have essential oils and some 45% to 53% of the oil is composed of ascaridole, 1,8-cineole, limonene, α-terpineol, and terpinen-4-ol (Pavela et al., 2019).

Thus, boldus essential oil is a candidate to assess its anthelmintic potential against the parasite *H. contortus*. According to Delgado-Díaz and Jara (2018), *P. boldus* has demonstrated anthelmintic activity against *Ascaris suum* Goeze. These authors assessed the efficacy of a hydroalcoholic extract of *P. boldus* leaves against eggs and larvae of *A. suum*, concluding that extract showed the same effectiveness as piperazine citrate, a drug used as an anthelmintic in some countries. Furthermore, Junkuszew et al. (2017) evaluated a formulation with essential oil of *P. boldus* against gastrointestinal parasites, obtained a reduction of the prevalence of *Eimeria* and *Capillaria* similar to a synthetic chemical agent containing albendazole. Finally, according to French (2018), *P. boldus* with *Achillea millefolium* L., *Dryopteris filix-mas* (L.) Schott, *Rosmarinus officinalis* (L.) Schleid., and *Salvia officinalis* L. are the top five plants with the highest number of anthelmintic compounds.

Regarding insecticidal activity, studies have shown that the powder, extracts and essential oil of *P. boldus* have contact and fumigant toxicity and insectistatic activity as repellent (Pizarro et al., 2014). Chiffelle et al. (2019) with an ethanolic extract inhibited feeding behavior of *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae) larvae. Pavela et al. (2019) indicated toxicity of *P. boldus* essential oil against *Musca domestica* L. (Diptera: Muscidae) and *Culex quinquefasciatus* Say (Diptera: Culicidae), Sombra et al. (2020) obtained ovicidal and pupicidal activity against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and Pizarro et al. (2014) found insecticidal activity against *S. zeamais*. Therefore, the study aimed to assess the in vitro insecticidal and nematicidal activity of *P. boldus* essential oil.

MATERIALS AND METHODS

Essential oil

The essential oil of *Peumus boldus* Molina was extracted from leaves collected in the field of the Los Lleuques zone (36°51'18"S, 71°38'34" W; 286 m a.s.l.), foothills of Ñuble Region, Chile. The collection was carried out during January 2019. The taxonomic identification of collected foliage was verified according to reference vouchers CONC-CH5492 deposited in the herbarium of the Faculty of Agronomy, University of Concepcion at Chillán. Once in the laboratory, only mature, 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 4 h of steam distillation using distilled water in a Clevenger-type apparatus. Subsequently, the oil was treated with sodium sulfate to eliminate residual water and stored in amber-colored glass containers at 4.5 °C. The chemical analysis of essential oil was carried out at the Laboratory of Pharmacognosy at the Faculty of Pharmacy, University of Concepcion, Concepción, Chile. The essential oil of *P. boldus* was assessed by

gas chromatography (GC) coupled to mass spectrometry (GC-MS), using a high-performance gas chromatographer-mass spectrometer (HPGC-MS; HP 5890 Series II, Hewlett Packard, Palo Alto, California, USA).

Production of infecting larvae (L3) of *Haemonchus contortus*

The isolate used for *Haemonchus contortus* was "Hueytamalco", belonging to the Department of Helminthology, Centro Nacional de Investigación Disciplinaria (CENID)-Salud Animal e Inocuidad, Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), México. This strain was isolated from the feces of a sheep naturally infected by *H. contortus* in Hueytamalco, municipality of the State of Puebla, Mexico (Pineda-Alegría et al., 2017). To obtain infectious larvae of the nematode *H. contortus*, 3-mo-old sheep free of parasites were selected. The animals were obtained from the INIFAP institute, and this work complies with the ethical standards of the relevant Mexican guidelines regarding animal welfare and unnecessary animal suffering; these are well-established Good Management Practice policies at our institution. Sheep were infected orally with infectious larvae (L3) of *H. contortus* (at 350 L3 kg^{-1} body weight) and were used as egg-donor sheep (Liébano-Hernández et al., 2011). After 21 d, the McMaster technique was performed to confirm and determine the number of eggs per gram of feces. Once the presence of *H. contortus* was confirmed in the feces, the donor animal was placed in a metabolic cage. The feces of the donor animal were collected for coproculture, which was left to incubate for 7 d at 28 °C, then larvae (L3) were recovered using the Baermann technique. These larvae were stored refrigerated at 11 °C until use in bioassays. After the studies, sheep were dewormed and could continue with the planned cycle. These animals were not slaughtered. To eliminate *H. contortus* infecting larvae sheath with sodium hypochlorite, commercial sodium hypochlorite at 6% was used for ensheathment of infected larvae. Then, 10 mL 0.187% chlorine was prepared and larvae were exposed for 9 min. It was observed and quantified that more than 95% larvae no longer had the sheath. Subsequently, four washes were performed with distilled water, centrifuging for 1 min at 261.97 rad s^{-1} to eliminate sodium hypochlorite (González-Cortázar et al., 2020). Once the larvae were washed, the sediment formed in 50 mL Falcon tubes was recovered (Liébano-Hernández et al., 2011).

Insect rearing

The insecticidal activity was assessed against three biological models: *Tribolium castaneum*, *Sitophilus zeamais* and *Plodia interpunctella*. All insects were obtained from laboratory cultures maintained for the last 10 yr in a bioclimatic chamber (Memmert Gmbh, IPS 749, Schwabach, Germany) at 25-30 °C, 60% RH, and in total darkness. *Tribolium castaneum* adults were reared in 1-L glass containers with wheat flour with a 12%-13% moisture content mixed with yeast (10:1, w/w). *Sitophilus zeamais* adults were reared on whole maize at 12%-13% moisture content in 3-L glass containers, and the colony of *P. interpunctella* was reared on artificial diet of McGaughey and Beeman (1988) containing cornmeal (26%), whole wheat flour (23%), glycerol (16%) honey (14%), ground dog meal (10%), brewer's yeast (5%), rolled oats (4%) and wheat germ (2%) in 5-L glass containers.

Anthelmintic activity bioassays

Ensheathed *H. contortus* L3 larvae were exposed in vitro to the essential oil of *P. boldus*. The experiment was carried out in a multi-well plate (96 wells). First, 50 µL essential oil to be evaluated was diluted to different concentrations (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 mg mL^{-1}) in 4% methanol (MeOH), and 50 µL aqueous suspension of ensheathed L3 larvae of *H. contortus* were added, for a final volume of 100 µL and 200 larvae per well. A negative control (4% MeOH) and a positive control (ivermectin at 20 mg mL^{-1}) were used. The plates were packed with aluminum foil and incubated at 28 °C. Four replicates were made for each treatment. This methodology was used to evaluate different post-confrontation times (24, 48, and 72 h) (González-Cortázar et al., 2020). After the corresponding hours of exposure, mortality was observed under microscope (40X) by counting live and dead larvae. This quantification was done on slides (10 drops of 10 µL), observing the larvae, counting the degraded larvae and those that did not move when stimulated as dead. The live larvae were counted as those that moved even after incubation.

Insecticidal activity bioassays

The insecticidal activity of essential oil of *P. boldus* by inhalation toxicity was assessed against adults of *T. castaneum*, *S. zeamais*, and *P. interpunctella* using the method of Pizarro et al. (2014). A 2 cm diameter filter paper (Whatman N°1) was impregnated with essential oil in concentrations of 35, 70, 100, 125, 150, 175, and 200 µL L^{-1} air using a micropipette.

Each filter paper disk was then air-dried for 2 min and placed on the underside of the screw cap of a glass vial. In *T. castaneum* and *S. zeamais* bioassays, 20 adults were placed into 0.5-L glass vials before the cap was screwed tightly, and the lid was sealed with parafilm. In experiments with *P. interpunctella* adults, moths were placed individually in 0.15-L glass vials in groups of 20 vials per replicate. Each treatment had 20 replicates, and insect mortality was recorded 72 h after treatment. The maximum level of mortality accepted for the control was 5%, and the mortality value was corrected using Abbott's formula (Abbott, 1925). An insect was considered dead when it did not move after being prodded with a dissection needle for 5 min. All bioassays were carried out at 25 ± 1 °C, 60 ± 5% RH, and 16:8 h photophase.

Data analysis

In insecticidal and nematicidal bioassays, the experimental design was completely randomized. The mortality rate of ensheathed L3 larvae was calculated by using the formula described by Pineda-Alegría et al. (2017). In the case of anthelmintic activity, a Generalized Linear Model (GLM) was carried out obtaining average mortalities at 24, 48, and 72 h exposure of L3 of *H. contortus* with the respective evaluated concentrations. Statistical significance was determined by Tukey's *post hoc* test with significance set at $p < 0.05$. In insecticidal activity bioassays, the percent mortality was transformed into an arcsine function represented by $\sqrt{x/100}$ prior to carrying out ANOVA ($\alpha = 0.05$) to determine if at least one treatment was different from the rest. If so, a Tukey's means comparison test was used ($p \leq 0.05$). To obtain 50% (LC_{50}) and 95% (LC_{95}) lethal concentration of insects and 50% (EC_{50}) and 90% (EC_{90}) effective concentration of ensheathed larvae data were subjected to Probit analysis using PROC PROBIT procedure of Statistical Analysis System (SAS). All analyses were submitted with the SAS program (SAS Institute, Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Chemical analysis of essential oil

The foliage of *P. boldus* had a yield of 2.0% because 20 mL essential oil were obtained from 1 kg dry leaves. A total of 51.7% of components were identified (Table 1) and the remaining 48.3% were indicated as unknown by chromatography, but none exceeded 1.0% composition. The main components identified were ascaridol (24.37%), 1,8-cineole (14.85%), terpineol (2.14%), terpinene-4-ol (3.37%), γ-terpinene (3.94%) and safrole (1.45%) (Table 1).

Anthelmintic activity

Exposure of *H. contortus* larvae to the essential oil of *P. boldus* at 2 mg mL^{-1} resulted in 77.63% mortality after 24 h, 77.97% after 48 h, and 99.25% after 72 h (Table 2). In addition, *P. boldus* essential oil resulted in a mortality rate of 90% to 1.5 mg mL^{-1} at 72 h. The lowest EC_{50} was for *P. boldus* oil at 72 h (0.99 mg mL^{-1}). At 72 h exposure, EC_{99} was 1.91 mg mL^{-1} (Table 3).

The anthelmintic activity obtained from *P. boldus* agrees with a study against *Ascaris suum* (Delgado-Díaz and Jara, 2018). The activity of some of the constituents of *P. boldus* present in this study, such as ascaridol, 1,8-cineole, *p*-cimene, and methyl eugenol, among others, against *H. contortus* has been reported previously. Ascaridol was documented as an active molecule against *Ascaris* (Harder, 2002). This component is a bicyclic monoterpene that acts like many other terpenoids paralyzing and narcotizing the intestinal endoparasite, facilitating its expulsion by intestinal peristalsis of the host (Delgado-Díaz and Jara, 2018). The constituent 1,8-cineole, eugenol, *p*-cimene reported in *P. boldus* can also be detected in other plants reported to have anthelmintic activity. The species *Ocimum gratissimum* L. was assessed in vitro hatching tests against *H. contortus*, and among its main documented constituents were eugenol and 1,8-cineole. The essential oil of this plant was reported to have anti-hatching activity, but the most prominent activity was for eugenol with a 94% inhibition against *H. contortus* at a concentration of 0.25% (Pessoa et al., 2002). In the evaluation of the essential oil of *Artemisia lancea* Vaniot with 1,8-cineole as the principal component, the anti-hatching activity was 99% inhibition at 10 mg mL^{-1}, and the effective concentration to inhibit egg hatching was 1.82 mg mL^{-1}. When 1,8-cineole was assessed isolated presented an anti-hatching activity of CE_{50} 4.64 mg mL^{-1}. In addition, *Arisaema franchetianum* Engl. oil and *A. lobatum* Engl. with 1,8-cineole as more abundant chemical compounds in its essential oils showed an activity of 77% and 95% in larval development trials at 10 mg mL^{-1} (Zhu et al., 2013).

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

Compound	%
Ascaridol	24.37
1,8-Cineole	14.85
γ-Terpinene	3.94
Terpinene-4-ol	3.37
Terpineol	2.14
Safrole	1.45
Methyleugenol	0.46
(*E*)-Germacrene D	0.45
β-Tujene	0.32
p-Cimene	0.21
Isoterpinolene	0.10
Linalool	0.10
Total	51.70

Table 2. Mortality of *Haemonchus contortus* larvae after 24, 48, and 72 h treated with different concentrations of *Peumus boldus* essential oil.

Treatment	Concentration	Mortality		
		24 h	48 h	72 h
	mg L^{-1}		%	
Metanol (4%)		0.00a	0.00a	0.00a
P. boldus essential oil	0.25	3.13ab	6.31b	3.04a
	0.50	5.75b	8.42b	15.11b
	0.75	12.10c	16.48c	28.31c
	1.00	13.90c	33.35d	38.46d
	1.25	16.60c	64.98e	78.97e
	1.50	36.70d	68.57e	90.75f
	1.75	69.22e	81.20f	99.32g
	2.00	77.63f	77.97f	99.25g
Ivermectin	20.00	100.00g	100.00g	100.00g
SE		1.58	1.62	1.72

Values with the same letter are not significantly different according to Tukey's test ($p < 0.05$).
SE: Standard error.

Table 3. Effective concentration 50% (EC$_{50}$) and 99% (EC$_{99}$) and 95% confidence intervals (CI) of different treatments of essential oil *Peumus boldus* against *Haemonchus contortus* L3.

Essential oil	Time	EC$_{50}$	CI	EC$_{99}$	CI
	h	mg mL^{-1}		mg mL^{-1}	
P. boldus	24	1.63c	1.59-1.67	3.06b	2.81-3.43
	48	1.20b	1.16-1.24	3.70b	3.38-4.13
	72	0.99a	0.94-1.02	2.03a	1.91-2.18

Values with the same letter in column are not significantly different according to Tukey's test ($P < 0.05$).

The *E. staigeriana* shares with boldo the components limonene, 1,8-cineole, and cimene, and an extract was assessed in a hatching test against *H. contortus* and a larval development test, obtaining 99% inhibition of hatching at 1 mg mL^{-1} and 98% inhibition of larval development at 8 mg mL^{-1}. The anthelmintic activity was attributed to the presence of limonene (Ribeiro et al., 2013). *Piper aduncum* L. plant has monoterpenes, sesquiterpenes, and 1,8-cineole as main chemical components, it was assessed in vitro in a hatching test against *H. contortus* and showed 89% inhibitory activity at 6 mg mL^{-1}, this biological activity was attributed to these compounds (Oliveira et al., 2014). It is worth mentioning that the previously reported molecules were found in essential oils of other plants exhibiting promising biopesticidal activity. Essential oils contain highly volatile secondary metabolites that reach a mass less than their molecular weight of 300 Da. This supports the hypothesis that some bioactive compound could cause this ovicidal effect with a sufficiently small size (less than 600 Da) to penetrate and kill the egg in the morula phase (Vargas-Magaña et al., 2014). In addition, the advantage of this size is that compounds can accumulate in effective concentrations in nematodes (D'Almeida et al., 2014).

Insecticidal activity

The insecticidal activity obtained by fumigation with essential oil of *P. boldus* was higher against *S. zeamais*, with a mortality of 90% at concentrations equal to or greater than 125 μL L^{-1} air, and there was nonsignificant difference (p > 0.05) between treatments (Table 4). In *P. interpunctella*, toxicity did not reach 30% of dead insects, and in *T. castaneum*, the maximum assessed concentration showed a mortality of 30% (Table 4). The highest toxicity of *P. boldus* essential oil against *S. zeamais* was observed more clearly when comparing CL$_{50}$ because the values were 2.67, 2.70, and 1.60 μL essential oil L^{-1} air for *T. castaneum*, *P. interpunctella*, and *S. zeamais*, respectively (Table 5). Results with *S. zeamais* agree with Pizarro et al. (2014), who obtained 100% mortality with 20 μL essential oil L^{-1} air. The observed toxicity was mainly due to the action of ascaridole and 1,8-cineole present in the essential oil at 24.37% and 14.85%, respectively. Chu et al. (2011) obtained the strongest fumigant activity against *S. zeamais* adults with (*Z*)-ascaridole with a LC$_{50}$ value of 0.84 mg L^{-1} air, followed by isoascaridole with an LC$_{50}$ value of 2.45 mg L^{-1} air. Zhu et al. (2012) in bioassays with *Blattella germanica* L. (Blattodea: Blattidae) male adults obtained a LC$_{50}$ of 0.55 mg L^{-1} air for ascaridole and 2.07 mg L^{-1} air in isoascaridole with maximum mortality of 100%. Concerning the insecticidal mode of action, de Castro et al. (2016) in larvae of *Culex quinquefasciatus* treated with essential oil of *P. boldus* with 89.5% ascaridole found morphological changes in the midgut, with cells possessing a cytoplasm that contained small vacuole-like structures, as well as a nucleus with decondensed chromatin and a cell apex with a short brush border. The fat body cells showed larger protein granules, which were acidophilic relative to the larvae of the control group. In the case of 1,8-cineole, according to Liska et al. (2015), this monoterpenoid has a promising potential for use as a fumigant, inducing excellent results in the control of stored products insects. These authors evaluated 1,8-cineole against the pupal stage of *T. castaneum*, obtaining a 30% of mortality but the serial effect of metamorphosis interference showing deforming adults, mainly in male pupae. These deformities were less or more expressed on elytra developed adults while 1,8-cineole concentration increase. In other insects, 1,8-cineole showed 100% mortality of the red imported fire ant (*Solenopsis invicta* Buren; Hymenoptera: Formicidae) workers, even at the lowest concentration of 20 μL L^{-1} after 24 h exposure (Xie et al., 2019). Kumar et al. (2012) assessed the insecticidal effect of 1,8-cineole by fumigation against *M. domestica* obtained for larvae a CL$_{50}$ of 2.4 μL L^{-1} and 100% mortality of pupae. According to Isman (2020), toxic and behavioral effects attributed to mono and sesquiterpenes as 1,8-cineole are a consequence of neurotoxicity or interaction with one or more receptors in the arthropod nervous system. Jankowska et al. (2018) indicated that in insects, essential oils cause paralysis followed by death, and the inhibition of acetylcholinesterase is one of the most investigated mechanisms of action. Studies conducted by Xie et al. (2019) found that 1,8-cineole significantly inhibited the acetylcholinesterase activity in the red imported fire ant workers. But Tak and Isman (2015) indicated that 1,8-cineole could stimulate the response of pheromone-sensitive sensilla of American cockroaches (*Periplaneta americana* L.; Blattodea: Blattidae), receptor cells modulated by octopamine, a neurotransmitter present only in arthropods.

This is the first report of boldo against *H. contortus*, although the main constituents detected in its essential oil has shown potential anthelmintic activity against *H. contortus* forming part of essential oils of other plants. The present study suggests that *P. boldus* essential oil has potential anthelmintic and insecticidal activity against *H. contortus* and *S. zeamais*, respectively, but further studies may explore in vivo conditions and possible mechanisms of action, and considering high volatility of some compounds, the next step should be to formulate the essential oil as an emulsion or encapsulation to increase its residuality.

Table 4. Insecticidal activity by fumigant effect of *Peumus boldus* essential oil against *Tribolium castaneum*, *Sitophilus zeamais* and *Plodia interpunctella*.

	Mortality		
Treatment	*Tribolium castaneum*	*Plodia interpunctella*	*Sitophilus zeamais*
μL essential oil L^{-1} air	%	%	%
35	3.7d	1.3d	55.5c
70	4.3d	5.7cd	63.2bc
100	5.4cd	8.8c	70.3b
125	10.5bcd	12.4bc	90.3a
150	13.1bc	16.3ab	93.9a
175	18.5b	19.9a	99.3a
200	30.0a	21.9a	100.0a

Values within a column with the same letter are not significantly different according to Tukey's test (p > 0.05).

Table 5. Lethal concentration 50% (CL$_{50}$) and 95% (CL$_{95}$) by fumigant effect of essential oil of *Peumus boldus* against *Tribolium castaneum*, *Plodia interpunctella* and *Sitophilus zeamais*.

Essential oil	N[1]	CL$_{50}$ (Lower-Upper)[2]	CL$_{95}$ (Lower-Upper)[2]	Slope ± SE	χ^2	Df
		——— µL essential oil L^{-1} air ———				
T. castaneum	1400	2.67 (2.4-4.05)	3.52 (2.9-6.9)	1.9 ± 0.53	0.0003	5
P. interpunctella	1400	2.70 (2.53-3.04)	3.57 (3.17-4.41)	1.9 ± 0.35	0.0001	5
S. zeamais	1400	1.60 (0.97-1.78)	2.30 (2.07-2.88)	2.5 ± 0.62	0.0009	5

[1]Total number of insects tested in 10 replicates per treatment.
[2]Confidence limits at 95% of probability.
SE: Standard error; χ^2: chi-square; Df: degrees of freedom.

CONCLUSIONS

The essential oil of boldo has anthelmintic and insecticidal activity against unsheathed larvae of *Haemonchus contortus* and adult of *Sitophilus zeamais*, respectively.

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