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TOXICITY OF BOLDO *Peumus boldus* MOLINA FOR *Sitophilus zeamais* MOTSCHULSKY AND *Tribolium castaneum* HERBST

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The maize weevil (*Sitophilus zeamais* Motschulsky) and the red flour beetle (*Tribolium castaneum* Herbst) are two key pests of stored-grain products worldwide. The insecticidal activity of boldo (*Peumus boldus* Molina) powder, liquid ethanolic and hexanic extracts against *S. zeamais* and *T. castaneum* were evaluated under laboratory conditions. The evaluated variables were mortality, emergence of adult insects (F_1), and grain weight loss. The experimental design was completely randomized. The mortality in *S. zeamais* was 100% even at the lowest powder concentration (0.5% w/w), whereas emergence of F_1 adult insects was 0% and grain weight loss was ≤ 0.08%. For *T. castaneum,* only 8 and 16% w/w powder concentrations reached 100% mortality. The liquid ethanolic and hexanic extracts caused 100% mortality of *S. zeamais,* whereas only the ethanolic extract reached this value for *T. castaneum*. Therefore, the powder and the evaluated extracts of *P. boldus* were toxic for *S. zeamais* and *T. castaneum* and are promising against these and other stored-grain pests.

Key words: Botanical insecticides, stored grains, maize weevil, red flour beetle.

Stored grains are affected by several insect pests causing significant losses that may amount up to 50% of the harvest. In addition, pest damage to grain allows the entrance of pathogenic organisms such as fungus or bacteria (Regnault, 1997). The maize weevil (*Sitophilus zeamais* Motschulsky, Coleoptera: Curculionidae), rice weevil (*Sitophilus oryzae* Linnaeus, Coleoptera: Curculionidae), and grain moth (*Ephestia kuehniella* Zeller, Lepidoptera: Pyralidae) are three of the most important stored-grain pests in Chile (Larraín, 1994). Synthetic pesticides have been considered to be the most effective and easy to use tools against these insect pests (Huang and Subramanyam, 2005). However, their misuse has caused several problems such as the presence of high or illegal pesticide residues, human intoxication, and development of insect resistance (Roel and Vendramim, 2006).

The search for alternative methods includes using natural products that are both effective and environmentally friendly, such as botanical insecticides (Roel and Vendramim, 2006). These compounds have been traditionally used in developing countries against stored-grain pests such as *Sitophilus* weevils (De Oliveira *et al*., 2003). Many of the secondary metabolites of plants act as insecticides, ovicidal, ovipositional, and feeding deterrents, and growth retardants (Pugazhvendan *et al*., 2009). Currently, research about their use as grain protectants involves tropical plant species; therefore, the search for Chilean flora with promising activity is needed.

The perennial boldo tree *Peumus boldus* Molina (Monimiaceae) is native to Chile and in powder form has insecticidal activity against *S. zeamais* (Páez *et al*., 1990; Silva *et al*., 2003; 2005; 2006; Pérez *et al*., 2007), third instar larvae of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (Zapata *et al*., 2006), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Silva *et al*., 2010), as an extract against *Xanthogaleruca luteola* Müller (Coleoptera: Chrysomelidae) (Chiffelle *et al*., 2011), and as fungicide properties against *Penicillum* spp., *Fusarium* spp., *Aspergillus niger* Thieg., and *A. flavus* Link (Leite de Souza *et al*., 2005). The leaves of *P. boldus* contain a group of boldine alkaloids that have antioxidant, anti-inflammatory, and antipyretic properties (Vogel *et al*., 1999). However, the insecticidal properties of *P. boldus* extracts against *S. zeamais* and *T. castaneum* are not documented, so the aim of this research was to assess the insecticidal properties of *P. boldus* leaf powder and water, hexane, and ethanol extracts against *S. zeamais* and *T. castaneum* adults in the laboratory.

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MATERIALS AND METHODS

Study site, plant material, and insects

The study was carried out at the Laboratory of Entomology, Faculty of Agronomy, Universidad de Concepción in Chillán, Bío Bío Region, Chile.

Peumus boldus foliage was collected from trees in the park of the Universidad de Concepción, Chillán by following the criteria set out by Vogel *et al*. (1997).

Wheat (*Triticum aestivum* L.) flour was employed to rear *T. castaneum*, whereas maize (*Zea mays* L.) grains with 14% moisture were used as food substrate for *S. zeamais*. To avoid any previous infestation, grains were washed and dried in an oven (Memmert Gmbh, UNB 500, Schwabach, Germany) at 25 ºC for 12 h and then frozen at -4 ± 1 ºC for 48 h prior to use.

Insects for the bioassays were provided by the Laboratory of Entomology of the Universidad de Concepción, Chillán. They were reared in 1-L glass flasks containing maize for *S. zeamais* or wheat flour for *T. castaneum* as food, respectively. Insects were maintained in total darkness at a temperature of 25 ± 1 ºC in a bioclimatic chamber (Memmert Gmbh, IPS 749, Schwabach, Germany).

Bioassays

Powder. Boldo leaves were washed and dried during 48 h at 40 ºC. Then, they were ground in an electric coffee grinder (Moulinex ultra 505) and passed through a 20 mesh (0.841 mm) (Dual Manufacturing, Chicago, Illinois, USA) sieve to produce fine powder.

For *S. zeamais*, 100 g of maize were placed in 250-mL jars (Silva *et al*., 2003) before adding the respective treatment and hand shaken 1 min to homogenously cover the grain prior to infestation with 20 insect couples not older than 10 d. Sex was determined with criteria proposed by Halstead (1963). After infestation, jars were transferred to a bioclimatic chamber (25 ± 1 ºC, 60% HR). The evaluated treatments were 0.5, 1.0 and 2.0% w/w. All insects were removed 15 d after infestation (DAI) and percentage of mortality was registered and corrected for statistical analysis by Abbott´s formula (Abbott, 1925). The maximum mortality level accepted for the untreated control was 10%. Insects were considered dead when they failed to move after being prodded gently with a needle for 30 s. Afterward, the percentage of adult emergence (F_1) was determined at 55 DAI. Grain weight loss was determined based on the difference between initial (100 g) and final weight.

For *T. castaneum*, 10 g of wheat flour were placed in 7-cm Petri dishes, mixing the flour with boldo foliage powder at 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0% w/w following the methodology by Chavez *et al*. (1992), and infesting each dish with 20 *T. castaneum* adults (unsexed 48-h-old). All dishes were located in a bioclimatic chamber at the same temperature as for *S. zeamais*. Mortality was recorded at 12 and 24 h and 3, 7, 14, and 21 DAI using the same criteria described above and corrected by Abbott´s formula (Abbott, 1925).

Extracts. The aqueous extract was obtained with the methodology of Prates *et al*. (2003) by placing 10 g boldo powder in a jar with 100 mL distilled water at boiling point, covering, filtering after 24 h through Whatman N°10 filter paper, and discarding the solid part. The extract was considered as the 100% stock compound. The ethanol and hexane extracts were obtained via Soxhlet extraction during 12 h (Kamaraj *et al*., 2008) and concentrated with a rotary evaporator (Fisatom, Sao Paulo, Brazil).

For *S. zeamais*, 6-mL test tubes with a 1 mL solution of each extract in distilled water and 0.2 mL of Tween® 20 surfactant (Kouninki *et al*., 2007) were employed at 0.25, 0.5, 1.0, and 2.0% v/v; an untreated control with 1 mL distilled water and the surfactant was covered with plastic film. Tubes were manually agitated for 1 min to properly cover the inner surface. The aqueous content was eliminated and test tubes were allowed to dry at room temperature for 1 h. Then, 10 unsexed 48-h-old adult insects were placed in each tube. Treatments were kept in a bioclimatic chamber. Mortality was recorded at 12, 24, 48, and 72 h. In this bioassay every extract had a reference control consisting of a tube with its inner surface covered with the respective solvent and infested with insects.

For *T. castaneum*, 10 g wheat flour were mixed in 7-cm Petri dishes with 2 mL of each extract solution at 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0% w/v and an untreated control with 1 mL distilled water; 2 mL Tween® 20 surfactant was added to each treatment (Jbilou *et al*., 2006). Each dish was infested with 20 unsexed 48-h-old *T. castaneum* adults. Mortality was recorded at 12, 24, 48, and 72 h after infestation and corrected by Abbott´s formula (Abbott, 1925). As in the *S. zeamais* bioassay, every extract had a reference control consisting of a Petri dish with wheat flour mixed only with the respective solvent and infested with insects.

Experimental design and statistical analysis

Treatments had 10 replicates and bioassays were replicated three times on separate days with a completely randomized experimental design. The response variables were transformed to arcsine $\sqrt{x}/100$ prior to ANOVA ($\alpha = 0.05$) analysis with the SAS program (SAS Institute, 1990). Statistical differences were determined through Tukey tests ($P \leq 0.05$).

RESULTS AND DISCUSSION

Effect of powder on *S. zeamais*

All powder treatments caused 100% mortality (Table 1). At 1.0 and 2.0% concentrations mortality was similar to the results obtained by Páez *et al*. (1990), Silva *et al*. (2003; 2005; 2006), and Cruzat *et al*. (2009). However, at

Table 1. Mortality, adult insect emergence (F_1), and grain weight loss in maize treated with *Peumus boldus* powder at concentrations of 0.5, 1.0, and 2.0% (w/w) to control *Sitophilus zeamais* under laboratory conditions.

Concentration	Mortality[1]	Emergence[1] (F_1)	Weight loss[1]
%	% w/w		
0.5	100 ± 0.0a	0.0 ± 0.0b	0.12 ± 0.03b
1.0	100 ± 0.0a	0.0 ± 0.0b	0.09 ± 0.001bc
2.0	100 ± 0.0a	0.0 ± 0.0b	0.04 ± 0.001c
Control[2]	0.0 ± 0.0b	100 ± 0.0a	4.91 ± 0.2a

[1]Values within a column with the same letter are not significantly different according to Tukey test (P ≤ 0.05).
[2]Mortality in control was corrected by Abbott´s formula (Abbott, 1925).

0.5%, we observed 100% mortality as compared to ≤ 30% reported by these researchers. According to Pérez *et al*. (2007), differences in toxic potency are probably related to the field-collection date of plant material, and there was no emergence of F_1 adults. In all treatments with powder, grain weight loss was < 0.5%, which is significantly lower (P ≤ 0.05) than the untreated control (Table 1).

Effect of powder on *T. castaneum*

The insecticidal effect of *P. boldus* powder against *T. castaneum* adults increased over time (Table 2). However, this species is less affected than *S. zeamais* for which concentrations of 8.0 and 16.0% lead to 100% mortality at 14 DAI. The reduced sensibility of *T. castaneum* to vegetable powders agrees with data found by Denloye *et al*. (2010), who obtained higher mortality with extracts (LC_{50} = 0.04 g L^{-1}) than with powders (LC_{50} = 250 g kg^{-1}) using *Chenopodium ambrosioides* L. (Chenopodiaceae). *Peumus boldus* powder is more potent against *T. castaneum* than other vegetable powders derived from plants such as *Khaya senegalensis* (Bamaiyi and Bolanta, 2006) or *Punica granatum,* and *Murraya koenigii* (Gandhi *et al*., 2010). Furthermore, plant powder gives a greenish color to the treated flour, thus affecting its quality for human consumption. This is why some researchers suggest not mixing powder with flour (Kordan *et al*., 2003; Husain and Hasan, 2008; Pugazhvendan *et al*., 2009).

Extracts

***Sitophilus zeamais*.** The hexanic extract was the most toxic after 24 h exposure in all evaluated concentrations and caused 100% mortality (Table 3). The aqueous and ethanolic extracts reached 100% mortality at 72 h. Considering the polarity of used solvents (water is polar, ethanol has intermediate polarity, and hexane is non-polar), we inferred that the insecticidal properties of *P. boldus* extracts are due to polar and non-polar molecules because all extracts reached 100% mortality. It is probable that insecticidal properties of boldo extracts are due to a group of molecules with joint action similar to neem (*Azadirachta indica* J. [Meliaceae]) (Schmutterer, 1990). The highest toxicity of hexanic extract as compared to ethanolic extract agrees with Hincapie *et al*. (2008), who evaluated *Annona muricata* L. (Annonaceae) extracts at a 5% concentration and obtained 100% *S. zeamais* mortality with the hexanic extract, while the ethanolic extract under the same conditions cause no mortality (0%). We estimated that *P. boldus* has a higher insecticidal potency than other plant extracts such as *Brassica napus* (L.) (Brassicaceae) (Salem *et al*., 2007), *Clerodendrum inerme* L. (Verbenaceae), *Withania somnifera* L. (Solanaceae), *Gliricidia sepia* L. (Fabaceae), *Cassia tora* L. (Caesalpiniaceae), and *Eupatorium odoratum* L. (Asteraceae) (Yankanchi and Gadache, 2010). In these cases, at least a 5% concentration was needed to reach 80% mortality. Using *Jatropa curcas* L. (Euphorbiaceae) extracts, a 20% concentration was required to reach a 70 to 90% mortality (Asmanizar and Idris, 2012).

***Tribolium castaneum*.** Plant powder extracts also gave a greenish color to the treated flour. The aqueous extracts caused no mortality (0.0%) in all the evaluated concentrations. The hexanic extract killed 6.7% of treated individuals at 16.0% concentration (Table 4). The highest mortality (100%) was achieved by the ethanolic extract during 12 h exposure at concentrations ≥ 2%. It seems that *P. boldus* is more toxic than other plant extracts such as *Manilkara zapota* (L.) (Sapotaceae) (Osman *et al*., 2011), *Chrysanthemum* spp. (Asteraceae) (Haouas *et al*., 2008), *Hyptis spicigera* Lam. (Labiatae) (Othira *et al*., 2009), *Peganum harmala* L. (Nitrariaceae) (Jbilou and Sayah, 2008), and *Mantis alcaduriaei* (Spach) Briq. & Cavill (Asteraceae) (Boussaada *et al*., 2008) in which only larval

Table 2. Mortality of *Tribolium castaneum* adults in flour wheat mixed with *Peumus boldus* powder at concentrations of 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0% (w/w) under laboratory conditions.

	Mortality[1]					
Concentration	12 h	24 h	3 d	7 d	14 d	21 d
%	% w/w					
Control[2] (0.0)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.5	0.0 ± 0.0b	3.5 ± 0.27a	7.0 ± 0.22c	11.1 ± 0.2d	13.7 ± 0.2e	14.5 ± 0.25e
1.0	0.0 ± 0.0b	3.5 ± 0.22a	10.5 ± 0.55c	22.2 ± 1.25d	41.1 ± 5.5d	41.6 ± 5.5d
2.0	1.7 ± 0.22b	5.2 ± 0.32a	10.5 ± 0.90c	46.2 ± 2.75c	66.6 ± 6.66c	64.5 ± 6.00c
4.0	5.2 ± 0.56a	8.7 ± 0.44a	26.3 ± 1.75b	75.9 ± 2.90b	80.3 ± 5.5b	79.1 ± 2.50b
8.0	5.2 ± 0.56a	10.5 ± 0.55a	26.3 ± 2.00b	98.1 ± 5.5a	100 ± 0.0a	100 ± 0.0a
16.0	5.2 ± 0.56a	12.2 ± 0.60a	64.9 ± 5.55a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a

[1]Values within a column with the same letter are not significantly different according to Tukey test (P ≤ 0.05).
[2]Mortality in control was corrected by Abbott´s formula (Abbott, 1925).

Table 3. Mortality of *Sitophilus zeamais* adults treated with *Peumus boldus* extracts in water, hexane, and ethanol at concentrations of 0.5, 1.0, and 2.0% (w/v) under laboratory conditions.

Extract	Concen-tration	Mortality[1] 12 h	24 h	48 h	72 h
		% w/w			
Water	Control[2]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.5	0.0 ± 0.0b	0.0 ± 0.0b	46.7 ± 3.3a	100 ± 0.0a
	1.0	0.0 ± 0.0b	0.0 ± 0.0b	46.7 ± 3.5a	100 ± 0.0a
	2.0	13.3 ± 1.1a	13.3 ± 1.1a	66.7 ± 4.0a	100 ± 0.0a
Ethanol	Control[2]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.5	0.0 ± 0.0b	0.0 ± 0.0b	46.7 ± 3.0a	100 ± 0.0a
	1.0	0.0 ± 0.0b	0.0 ± 0.0b	46.7 ± 3.0a	100 ± 0.0a
	2.0	13.33 ± 1.1a	13.33 ± 1.1a	66.7 ± 5.5a	100 ± 0.0a
Hexane	Control[2]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.5	0.0 ± 0.0b	100 ± 0.0a	-	-
	1.0	0.0 ± 0.0b	100 ± 0.0a	-	-
	2.0	26.7 ± 2.25a	100 ± 0.0a	-	-

[1]Values within a column with the same letter are not significantly different according to Tukey test ($P \leq 0.05$).
[2]Mortality in control was corrected by Abbott´s formula (Abbott, 1925).

Table 4. Mortality of *Tribolium castaneum* adults treated with wheat flour mixed with *Peumus boldus* extracts in water[1], hexane, and ethanol at concentrations of 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0% (w/v) under laboratory conditions.

Extract	Concen-tration	Mortality[2] 12 h	24 h	48 h	72 h
		% w/w			
	0.0[3]	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b
Ethanol	0.5	22.8 ± 3.3c	31.5 ± 3.2c	33.3 ± 3.1c	33.3 ± 3.0c
	1.0	47.3 ± 3.5b	59.6 ± 3.0b	68.4 ± 2.6b	73.6 ± 2.2b
	2.0	98.2 ± 0.01a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	4.0	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	8.0	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	16.0	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	0.0[3]	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b
Hexane	0.5	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b
	1.0	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b
	2.0	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b
	4.0	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b
	8.0	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b
	16.0	5.0 ± 1.0a	6.7 ± 1.2a	6.7 ± 1.2a	6.7 ± 1.2a

[1]The aqueous extracts caused no mortality (0.0%) in all the evaluated concentrations.
[2]Values within a column with the same letter are not significantly different according to Tukey test $P \leq 0.05$).
[3]Mortality in control was corrected by Abbott´s formula (Abbott, 1925).

mortality was documented. Some researchers suggest that plant extracts are powerful repellents but not promising contact insecticides against *T. castaneum* (Kanvil *et al*., 2006; Saidana *et al*., 2007).

CONCLUSIONS

The foliage powder of *Peumus boldus* is more effective against *Sitophilus zeamais* as compared to *Tribolium castaneum* under laboratory conditions. The hexanic and ethanolic extracts are more effective against *S. zeamais* and *T. castaneum* than the aqueous extract. These results must be field-validated in order to evaluate their usefulness as a chemical tool against these insect pest species.

Toxicidad del boldo, *Peumus boldus* Molina, sobre *Sitophilus zeamais* Motschulsky y *Tribolium castaneum* Herbst. El gorgojo del maíz (*Sitophilus zeamais* Motschulsky) y el gorgojo castaño de la harina (*Tribolium castaneum* Herbst) son plagas primarias de productos almacenados a nivel mundial. Se evaluó en laboratorio la actividad insecticida de polvo y extractos líquidos etanólicos y hexánicos del boldo (*Peumus boldus* Molina) sobre *S. zeamais* y *T. castaneum*. Las variables evaluadas fueron mortalidad y emergencia de insectos adultos (F_1) y pérdida de peso de los granos con un diseño experimental completamente al azar. La mortalidad en *S. zeamais* fue 100%, incluso con la concentración menor (0,5% p/p) mientras que la emergencia de insectos adultos y la pérdida de peso de granos de maíz fue ≤ 0,08%. Para *T. castaneum* sólo las concentraciones de 8 y 16% p/p de polvo causaron una mortalidad de 100%. Los extractos en agua, etanol, y hexano tuvieron un efecto insecticida de 100% en *S. zeamais*, mientras que en *T. castaneum* sólo el extracto en etanol alcanzó este valor. Por lo tanto, el polvo y los extractos evaluados de *P. boldus* presentan actividad insecticida contra *S. zeamais* y *T. castaneum* y son promisorios para utilizarse contra éstas y otras plagas de granos almacenados.

Palabras clave: insecticidas vegetales, granos almacenados, gorgojo del maíz, gorgojo castaño de la harina.

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