RESEARCH

INSECTICIDAL ACTIVITY OF *Peumus boldus* Molina ESSENTIAL OIL AGAINST *Sitophilus zeamais* Motschulsky

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ABSTRACT

In stored grains, the main agents diminishing production are insects, which can produce losses between 20% and 80% before harvest or under storage. The insecticidal properties of the essential oil of fresh leaves of *Peumus boldus* Molina against maize weevil (*Sitophilus zeamais* Motschulsky) adults were determined under laboratory conditions. The highest mortality (100%) was achieved at 4% concentration by contact with a treated glass surface. The same concentration in impregnated corn (*Zea mays* L.) grain, resulted in 98.7% mortality. Mortality by fumigant action at 6 h was 100% with 35 μL oil in 0.15 L (air volume). Concentrations 1, 2 and 4% of essential oil produced 0% F_1 adult emergence. At 10 d of residual effect, the 4% concentration reached 63.7% mortality. All treatments were repellent to adults of *S. zeamais* and corn grain germination was not affected by any treatment.

Key words: Maize weevil, boldus, stored grains, botanical insecticides.

INTRODUCTION

Cereal grains are staples in the diets of human beings and domestic animals. Consequently, their conservation is essential to have this basic food available on an ongoing basis (Huang and Subramanyam, 2005). Grains in storage are affected by several insect species that can destroy up to 50% of the harvest in 6 months. As well, storage allows the entrance of phytopathogenic organisms, such as fungus or bacteria (Regnault, 1997). Synthetic pesticides have been considered the most effective and accessible means to control insect pests of stored products (Huang and Subramanyam, 2005). However, their use can result in the presence of residues in the grains (Sanna *et al*., 2004).

The current trend is the search for and use of alternative methods to manage pests, which, in the economic context, are effective without presenting the risks associated with the use of conventional pesticides. One method consists of using plants with insecticidal properties that can be used as powder, extracts or oils (Mazzonetto and Vendramim, 2003). Botanic insecticides have been used traditionally in developing countries to control pests of stored grain, such as coleoptera of the genus *Sitophilus* (De Oliveira *et al*., 2003).

Aromatic plants have been used for both their medicinal and insecticidal properties. Their characteristic aroma derives from essential oils, many of which have proven fumigant and contact activity, as well as ovicide, anti-alimentary and repellent activity (Sanna *et al.,* 2004).

One of the native Chilean plants that has been evaluated as an insecticide in powdered form is boldo (*Peumus boldus* Molina) (Monimiaceae) (Silva *et al*., 2003a; 2003b; 2005a; 2005b; 2006), a tree that can reach a height of 20 m, with pruning it appears like a densely branched bush (Vogel *et al*., 2005). The leaves contain alkaloids, known together as boldine, which is attributed to having antioxidant, anti-inflammatory and antipyretic properties (Vogel *et al*., 1999). The leaves also have essential oils. Some 45 to 53% of the oil is composed of ascaridole, 1,8 cineole (30%), limonene, α-terpinol and terminen-4-ol (Vogel *et al*., 2005). The compound 1,8 cineole is also isolated from essential oil of plants such as *Eucalyptus globulus* Labill, *Gomortega keule* (Molina) I.M. Johnst., *Ocimum kilimandscharicum* Baker ex Gürke, *Ocimum kenyense* (Ayobangira), *Ocimum basilicum* L. and *Salvia officinalis* L., and is an effective fumigant for stored grain pest control (Bekele and Hassanali, 2001; Asawalam and Hassanali, 2006; Asawalam *et al*., 2008; Bittner *et al*.,

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2008). Consequently, the objective of this research was to evaluate the insecticidal properties of the essential oil of *P. boldus* against adults of *S. zeamais* under laboratory conditions.

MATERIALS AND METHODS

The study was carried out at the Insecticide Toxicology Laboratory of the Faculty of Agronomy of the Universidad de Concepción, Chillán Campus, Bío-Bío Region, Chile.

The insects used in the bioassays were obtained from colonies permanently maintained in the laboratory. They were reproduced in 1-L glass flasks containing maize (*Zea mays* L.) as a source of food. The insects were maintained in total darkness at a temperature of 30 ± 1 ºC.

Maize grains of with 14% moisture were used as an alimentary substrate. The maize was obtained from the fruit and vegetable market in Chillán. Only healthy grain was used to avoid any prior infestation that could affect the results of the bioassay. The grain was washed with potable water and frozen at a temperature of -4 ± 1 ºC for 48 h.

The essential oil was extracted from fresh leaves of *P. boldus* collected in the park of the Chillán Campus of the Universidad de Concepción. The leaves were washed with potable water to remove any possible detritus and the oil was obtained with steam distillation using distilled water, as suggested by Vogel *et al*. (1997). Subsequently, the oil was stored at a temperature of 4.5 °C in amber colored glass containers until they were used.

Bioassays

Mortality by contact with a surface of treated glass. The methodology of Kouninki *et al*. (2007) was used, with slight modifications that consisted of using 6-mL test tubes and applying 1 mL, instead of 40 mL and 3 mL, respectively, of a solution of essential oil in acetone (99% purity), at the required concentration. The tubes were agitated for 1 min for the oil to cover the interior surface. The excess was eliminated and the acetone was allowed to evaporate at ambient temperature for 1 h. Finally, 10 adult insects 48 h of age, without sexing, were placed in each tube. The evaluated doses of oil were 0.25, 0.5, 1, 2 and 4% and the control with 1 mL of acetone. Ten replicates were made per treatment. The treatments were kept in a bioclimatic chamber at a temperature of 30 ± 1 ºC. Insect mortality was assessed at 24; 48 and 72 h of exposure to the essential oil.

Mortality by contact with treated grain. This bioassay was carried out with the methodology of Obeng-Oferi and Reichmuth (1997). Solutions of 1 mL of essential oil of *P. boldus* in acetone were applied to 500-mL glass containers with 25 g of maize in concentrations of 0.25, 0.5, 1, 2 and 4%, plus a control with 1 mL of acetone. The flasks were covered and agitated for 15 s to uniformly cover the grains with oil. They were uncovered and left for 2 h at ambient temperature to evaporate acetone. The flasks were then infested with 20 adult insects 48 h of age, without sexing. Each treatment had ten replicates. The experimental units were stored in a bioclimatic chamber at a temperature of 30 ± 1 ºC and mortality was assessed at 24, 48 and 72 h exposure to the toxic.

Residuality

The methodology Obeng-Oferi and Reichmuth (1997) was used, with the difference that once the grain was mixed with the oil in the respective concentrations, it was stored in containers for 1, 5 and 10 d, respectively. With the elapse of these periods, each container (experimental unit) was infested with 20 adult insects 48 h of age, without sexing, and stored in a bioclimatic chamber at a temperature of 30 ± 1 ºC. For each of these storage durations, the concentrations of 0 (control); 0.25, 0.5, 1, 2 and 4% were assessed, with ten replicates for each treatment. Mortality was assessed at 24, 48 and 72 h after infestation.

Fumigant effect

This bioassay employed the methodology of Pires *et al*. (2006), which consisted of applying 0 (control), 5, 10, 15, 20, 25, 30 and 35 μL of essential oil on circular Whatman N°10 filter paper (Whatman, Maidstone, Kent, UK) 5.5 cm in diameter, which had been adhered to the covers of 150-m containers (air volume equivalent to 0.15 L), with 25 g of maize infested with ten adult insects, without sexing. The same procedure was used for the control with filter paper without treatment. There were ten replicates for each treatment. The experimental units were kept in a bioclimatic chamber at a temperature of 30 ± 1 ºC. Assessments of mortality were made at 6, 12 and 24 h of exposure. As the mortality rate in the control was lower than 5%, this was corrected with the Abbott formula (1925). An insect was considered dead when there was no movement after prodding it with a dissection needle.

Emergence of adults of F_1 compared to the control without treatment

Each experimental unit consisted of a 500-mL flask, 25 g of maize and 10 pairs of adult insects 24 h of age, which were allowed to freely reproduce for 21 d. Subsequently, the adult pairs were removed and the grain was mixed with essential oil of *P. boldus* diluted in acetone at concentrations of 0 (control); 0.125; 0.25; 0.5; 1; 2 and 4%, according to what was described by Obeng-Oferi *et al*. (1998). The control received only 1 mL of acetone. The flasks were kept in a breeding chamber at a temperature of 30 ± 1 ºC throughout the bioassay. Every treatment had 10 replicates. As a variable response, the percentage of emergence of adults of the F_1 generation was assessed weekly for 7 wk in comparison to the control without treatment. The morphology of the proboscis was used for sexual differentiation, that of the male being rougher and of a higher caliber than that of the female, according to what was described by Halstead (1963).

Repellent effect

The methodology proposed by Mazzonetto and Vendramim (2003), with slight modifications, was used to assess the repellent effect of the essential oil of *P. boldus*. The experimental unit was a plastic Petri dish 5 cm in diameter containing 50 g of maize grains that had been impregnated with the respective concentrations of essential oil. The treatments were placed in a circle around a central Petri dish that contained 20 individuals of *S. zeamais* of 48 h of age, without sexing. The central Petri dish was connected to the others through tubes 10 cm long and 0.5 cm in diameter (Procopio *et al*., 2003). The evaluated treatments were 0 (control); 0.125, 0.25, 0.5, 1, 2 and 4% of oil in acetone. The experimental batch was maintained in a bioclimatic chamber for 24 h at a temperature of 30 ± 1 ºC. Subsequently, the number of insects present in each treatment was counted. Each treatment had 10 replicates and in each replicate the treatments were randomly rotated to avoid external factors from interfering. The repellent index is calculated with these results according to what was described by Mazzonetto and Vendramim (2003), in which the oil is classified as neutral if the index is equal to 1, attracting if it is higher than 1 and repellent if it is less than 1.

Germination test of treated grain

The effect of essential oil of boldo on the germinative power of the maize grains was assessed using the methodology described by Pérez *et al*. (2007). Groups of 30 seeds were randomly selected from seeds without any apparent damage. The seeds were mixed with oil in 150-mL flasks and placed separately in glass Petri dishes containing permanently moistened filter paper on the bottom. The following concentrations were used as treatments: 0 (control); 0.125, 0.25, 0.5, 1, 2 and 4% of oil. In total, 10 replicates were made. The experimental units were kept at a temperature of 30 ± 1 ºC for 7 d in a bioclimatic chamber. Subsequently, the percentage of germination was determined in comparison to the control.

Estimation of the equitoxic concentration

Lethal concentrations of 50% (CL_{50}) and 90% (CL_{90}) were estimated in the treatments of mortality by contact with a surface of treated glass, by contact with treated grain and by fumigation. In all cases, there was 24 h of exposure to the toxic and a Probit analysis (Finney, 1971) was used to estimate the equitoxic concentrations, using the PROC PROBIT procedure of the Statistical Analysis System program (SAS Institute, 1998).

Experimental design

The experimental design was completely random and percentage data were transformed to the arcsine function $\sqrt{x/100}$ for its ANOVA ($\alpha = 0.05$) with the Statistical Analysis System program (SAS Institute, 1998) to determine if any treatments differed from the others. In the case that there were differences, a Tukey means comparison test was employed with a significance of 95% ($p \leq 0.05$).

RESULTS AND DISCUSSION

Mortality by contact with a treated glass surface

In general, mortality increased with increased exposure time to the essential oil, which concurs with Bittner *et al*. (2008). The concentrations of 1, 2 and 4% exceeded 50% mortality at 24 h (Table 1) and the concentration of 4% caused 100% mortality. At 48 h, mortality at concentrations of 2 and 4% was 97.5 and 100%, respectively, without significant differences between them ($p > 0.05$) (Table 1). At 72 h, these same two concentrations provoked higher mortality. Based on this, the essential oil of *P. boldus* can be considered as effective as a contact insecticide at doses of 2 and 4%, given that at 48 h both showed an efficacy greater than 90%. These values are higher than those observed by Popovic *et al*. (2006), who reached mortality rates by contact of 26 and 28% with essential oil of *O. basilicum* and *S. officinalis*, respectively.

Mortality by contact with treated grain

Results close 100% were obtained at 24 h and with a concentration of 4% (Table 2), while at 48 and 72 h the concentration of 2% showed 85% mortality, without statistical difference with the concentration of 4% ($p > 0.05$). The direct proportionality between concentration and mortality concurs with what was obtained by Silva *et al*. (2003b; 2005a) and Pérez *et al*. (2007) using powder of *P. boldus* for control of *S. zeamais*. As well, Popovic *et al*. (2006) and Bittner *et al*. (2008) indicate that with all the essential oils used in the control of pests of stored grains, mortality increases as the concentration or exposure time increases. The mortality rate that was obtained by contact with treated grain was higher than that documented with essential oil of *O. grattissimum* and *V. amigdalina*, given that at a doses of 0.3% the mortality rate was lower than

Table 1. Mortality of *Sitophilus zeamais* adults exposed to a glass surface treated with different concentrations of essential oil of *Peumus boldus* under laboratory conditions.

	Mortality ± DE		
Concentrations	24 h	48 h	72 h
%		%	
0.25	0.00 ± 0.0d	5.00 ± 0.98c	15.00 ± 7.5d
0.5	12.50 ± 5.6dc	35.00 ± 7.2bc	57.50 ± 8.8c
1	52.50 ± 8.5bc	67.50 ± 8.9ba	72.50 ± 9.2bc
2	77.50 ± 11.7ba	97.50 ± 13.4a	97.50 ± 10.9ba
4	100.00 ± 0.0a	100.00 ± 0.0a	100.00 ± 0.0a

Values followed by the same letters in the column do not differ according to the Tukey test ($p \leq 0.05$); DE: standard deviation.

Table 2. Mortality of *Sitophilus zeamais* adults exposed to maize grain treated with different concentrations of essential oil of *Peumus boldus* under laboratory conditions.

	Mortality ± DE		
Concentrations	24 h	48 h	72 h
%		%	
0.25	0.00 ± 0.0d	1.25 ± 0.18b	2.50 ± 1.2b
0.5	0.00 ± 0.0d	1.25 ± 0.21b	3.75 ± 2.0b
1	7.50 ± 0.2c	8.75 ± 2.2b	13.75 ± 2.4b
2	82.50 ± 6.7b	85.00 ± 7.9a	87.50 ± 9.8a
4	98.75 ± 8.9a	98.75 ± 11.2a	98.75 ± 12.4a

Values followed by the same letters in the column do not differ according to the Tukey test ($p \leq 0.05$); DE: standard deviation.

30% at 48 h and 82% at 7 d of exposure (Asawalam and Hassanali, 2006; Asawalam *et al.*, 2008).

Residuality

As the time increases between impregnation of the grain and infestation with adults of *S. zeamais*, mortality decreases in all the treatments and when the exposure time to treated grain increases, the mortality of adults of *S. zeamais* also increases (Table 3). During the first day of exposure, the concentration of 4% reached a mortality of 97.5%, and this value maintained the same statistical significance at 48 and 72 h ($p \leq 0.05$). At 10 d, mortality was less than 64% with the highest concentration. These results could be due to the loss in the concentration of the active compounds are vulnerable to light and temperature. The lost insecticidal power over time concurs with the research of Silva *et al.* (2005a), who concluded that the residual effect of *P. boldus* does not exceed 30 d.

Fumigant effect

It was observed in the fumigant action bioassay that 35 μL of oil in a volume of 0.15 L has a rapid toxic effect, producing 100% mortality in 6 h (Table 4). At 12 h of exposure, the doses of 15, 20, 25, 30, and 35 μL in 0.15 L^{-1} also showed 100% mortality (Table 4). At 24 h, the treatments higher than 20 μL of oil in 0.15 L caused 100% mortality. According to Vogel *et al.* (2005), the essential oil of *P. boldus* contains 1,8 cineole. This compound is also present in the essential oil of *Rosmarinus officinalis* L., *Eucalyptus blakelyi* Maiden and *Melaleuca fulgens* R. Br., which have demonstrated fumigant action against the mite *Tetranychus urticae* Koch (Miresmailli *et al.,* 2006) and insects like *Sitophilus oryzae* L., *Tribolium castaneum* Herbst. and *Rizopertha dominica* F. (Lee *et al.*, 2003). The results obtained are superior to those observed with other essential oils of Chilean plants that contain 1,8 cineole, such as *G. keule* and *Laurelia sempervirens* (Ruiz & Pav.) Tul., which provoke 100% mortality at 72 h (Bittner *et al.*, 2008). This level of mortality has been reached in other countries at 48 h with the essential oils of *Achillea biebersteinii* Afan and *A. wilhelmsii*, and at 96 h with oil of *Pistacia* spp. (Aslan *et al.*, 2004). As well, Bekele and Hassanali (2001) indicate that there is a synergism among the components of the essential oils of plants such as *O. kilimandscharicum* and *O. kenyense*, so that their use to control as an oil would be more effective than their compound in isolated form, given that among the other benefits are reducing the risk of resistance.

Table 3. Mortality over time of *Sitophilus zeamais* adults exposed to different concentrations of essential oil of *Peumus boldus* under laboratory conditions.

Residuality	Concentration	Mortality ± DE		
		24 h	**48 h**	**72 h**
Days		%		
1	0.25	0.94 ± 0.1d	2.81 ± 1.6c	2.50 ± 1.1c
	0.5	1.88 ± 0.9d	2.81 ± 1.7c	12.50 ± 2.7c
	1	10.00 ± 1.4c	30.00 ± 4.6b	55.00 ± 7.9b
	2	71.25 ± 6.2b	90.00 ± 7.7a	92.50 ± 11.9a
	4	97.50 ± 13.4a	97.50 ± 17.1a	97.50 ± 10.0a
5	0.25	0.00 ± 0.0c	1.25 ± 0.3c	2.19 ± 1.9d
	0.5	1.25 ± 0.7cb	5.00 ± 1.6c	12.50 ± 3.4dc
	1	7.50 ± 1.5cb	15.00 ± 5.5cb	27.50 ± 5.8c
	2	8.75 ± 2.6b	32.50 ± 9.2b	68.75 ± 12.7b
	4	77.50 ± 7.7a	92.50 ± 12.3a	93.75 ± 11.7a
10	0.25	0.00 ± 0.0b	0.00 ± 0.0b	0.00 ± 0.0c
	0.5	0.00 ± 0.0b	0.00 ± 0.0b	2.50 ± 1.8bc
	1	0.00 ± 0.0b	0.00 ± 0.0b	11.25 ± 8.1bc
	2	0.00 ± 0.0b	7.50 ± 2.2a	38.75 ± 16.6ba
	4	12.50 ± 6.4a	35.00 ± 11.1a	63.75 ± 19.9a

Values followed by the same letters in the column do not differ according to the Tukey test ($p \leq 0.05$); DE: standard deviation.

Emergence of adults of F_1 in comparison to the control

In all evaluated treatments, adult insects were observed to emerge beginning at week 5 of the infestation. The lowest level of emergence of F_1, compared to the control without treatment was observed in the concentrations of 1, 2 and 4%, with values of 0% until week 7 (Table 5). However, these three concentrations only showed significant differences from the other treatments ($p \leq 0.05$) in week 7. In week 5 all concentrations were statistically equals ($p > 0.05$), while in week 6, 0.125% differed from the other treatments ($p \leq 0.05$). According to Paranagama *et al*. (2003), the majority of essential oils produce a significant inhibition of oviposture and emergence of F_1 of coleoptera associated with stored grain, which concurs with the results of the current research.

Repellent effect

All the treatments evaluated had a repellent effect. The value of the index decreased as the concentration increased (Table 6). Consequently, the essential oil of *P. boldus* can

Table 4. Mortality of *Sitophilus zeamais* adults by the fumigant action of *Peumus boldus* essential oil under laboratory conditions.

Treatment	Mortality ± DE		
	6 h	**12 h**	**24 h**
μL 0.15 L^{-1}	%		
5	0.00 ± 0.0d	0.00 ± 0.0d	2.50 ± 0.9b
10	0.00 ± 0.0d	17.50 ± 4.3b	35.00 ± 3.7b
15	45.00 ± 6.2c	82.50 ± 9.2b	97.50 ± 14.8b
20	80.00 ± 8.9b	100.00 ± 0.0a	100.00 ± 0.0a
25	87.50 ± 9.9b	100.00 ± 0.0a	100.00 ± 0.0a
30	90.00 ± 11.2ba	100.00 ± 0.0a	100.00 ± 0.0a
35	100.00 ± 0.0a	100.00 ± 0.0a	100.00 ± 0.0a

Values followed by the same letters in the column do not differ according to the Tukey test ($p \leq 0.05$); DE: standard deviation.

Table 5. F_1 emergence of *Sitophilus zeamais* adults exposed to different concentrations of *Peumus boldus* essential oil under laboratory conditions.

Concentration (%)	Emergence of adult insects[1] ± DE			
	Week 1 to 4	Week 5	Week 6	Week 7
	%			
0.125	0.00 ± 0.0a	9.52 ± 1.1a	38.10 ± 5.6a	83.14 ± 19.8a
0.25	0.00 ± 0.0a	14.29 ± 5.4a	30.95 ± 7.2ba	55.81 ± 7.6ba
0.5	0.00 ± 0.0a	0.00 ± 0.0a	7.14 ± ba	34.88 ± 14.8b
1	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0b	0.00 ± 0.0c
2	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0b	0.00 ± 0.0c
4	0.00 ± 0.0a	0.00 ± 0.0a	0.00 ± 0.0b	0.00 ± 0.0c

[1]Emergence of the control is considered as 100%.
Values followed by the same letters in the column do not differ according to the Tukey test ($p \leq 0.05$); DE: standard deviation.

be considered a repellent for adults of *S. zeamais* and the concentration has a direct relationship with the degree of repellence, which concurs with what was documented by Nuñez (2005) for powder of *P. boldus*. Similar results were obtained with other plants where the major component is 1,8 cineole, such as *Vernonia amygdalina* (Lam) and *O. grattisimum* L., whose oils, according to Asawalam and Hassanali (2006) and Asawalam *et al*. (2008) are highly repellent against *S. zeamais*.

Germination test of treated grain

The percentage of germination of the grain varied from 85.19 to 100%, without statistical differences among the treatments ($p > 0.05$) (Table 7). These data concur with those of Silva *et al*. (2006), but contradicts those of Pérez *et al*. (2007), who affirmed that the powder of *P. boldus* significantly affect maize germination at concentrations higher than 1%. This difference could be because the compound or compounds that affect germination are found in the powder and not in the essential oil of *P. boldus*.

Table 6. Repellence index of essential oil of *Peumus boldus* against *Sitophilus zeamais* adults under laboratory conditions.

Concentration (%)	Repellence index (IR) ± DE
0.125	0.79 ± 0.013
0.25	0.68 ± 0.072
0.5	0.63 ± 0.032
1	0.45 ± 0.018
2	0.13 ± 0.008
4	0.16 ± 0.004

IR > 1 attracting oil; = 1 neutral oil; < 1 repellant oil.
DE: standard deviation.

Table 7. Maize grain germination exposed to different concentrations of *Peumus boldus* essential oil.

Concentration (%)	Germination ± DE (%)
Testigo	100.00 ± 0.0a
0.125	100.00 ± 0.0a
0.25	85.19 ± 14.1a
0.5	86.67 ± 12.3a
1	96.30 ± 3.5a
2	100.00 ± 0.0a
4	88.89 ± 11.1a

Values followed by the same letters in the column do not differ according to the Tukey test ($p \leq 0.05$); DE: standard deviation.

Estimation of equitoxic concentration

The values of CL_{50} for the mortality bioassays by contact with treated grain, by contact with a surface of treated glass, and by fumigant action were 1.54, 1.06 and 10.26 μL oil 0.15 L^{-1}, respectively. The CL_{90} values for these bioassays were 2.34, 2.36 and 14.28 μL oil 0.15 L^{-1}, respectively (Table 8). There were confidence limits at both the levels of CL_{50} and CL_{90}, consequently there were no statistical differences between the bioassays of exposure to treated grain and to a surface of treated glass. However, these values were higher than those of the bioassay evaluating mortality by fumigant action (Table 8), which implies less toxicity of *P. boldus* by this method.

Table 8. Toxicity of essential oil of *Peumus boldus* against *Sitophilus zeamais* adults exposed to maize grain treated or a treated glass surface.

Bioassay	n[1]	CL	Concentration	Confidence limits	b ± ES[2]
Mortality by contact with treated grain	500	50	1.54[3]	1.30 - 1.83	7.06 ± 0.61
		90	2.34[3]	1.95 - 3.36	
Mortality by contact with the surface of treated glass	1000	50	1.06[3]	0.75 - 1.49	3.66 ± 0.45
		90	2.36[3]	1.63 - 5.20	
Mortality by fumigant action	700	50	10.26[4]	7.53 - 12.13	1.9 ± 0.48
		90	14.28[4]	12.08 - 23.35	

[1]Number of treated insects.
[2]Slope (b) Probit adjustment and standard error of the slope (ES).
[3]mL oil L^{-1}.
[4]μL oil 0.15 L^{-1}.

CONCLUSIONS

The essential oil of *P. boldus* is toxic for adults of *S. zeamais*, whether by exposure to the surface of treated glass, exposure to treated grain, or by fumigant action. It also significantly reduces the emergence of F_1 in comparison to the control and has a repellent effect. The residual effect is significant for 5 d and does not affect germination of the maize grain.

RESUMEN

Actividad insecticida del aceite esencial de *Peumus boldus* Molina sobre *Sitophilus zeamais* Motschulsky. Los principales agentes que disminuyen la producción en los granos almacenados son los insectos, antes de la cosecha y en el almacenamiento pueden causar pérdidas de 20 a 80%. Se evaluaron las propiedades insecticidas del aceite esencial de hojas frescas de *Peumus boldus* Molina para el control de adultos de gorgojo del maíz (*Sitophilus zeamais* Motschulsky) en laboratorio. La mayor mortalidad (100%) por contacto con una superficie de vidrio tratada se obtuvo con la concentración de 4%. Esta misma concentración produjo 98,7% de mortalidad en exposición a grano de maíz (*Zea mays* L.) tratado. El efecto fumigante a las 6 h de exposición fue 100% con 35 μL de aceite en 0,15 L (volumen de aire). Con las concentraciones de 1, 2 y 4% de aceite esencial, el porcentaje de emergencia de la F_1 fue 0%. A los 10 d de efecto residual se alcanzó 63,7% de mortalidad con la concentración de 4%. Todos los tratamientos fueron repelentes para adultos de *S. zeamais* y ningún tratamiento afectó la germinación de los granos.

Palabras clave: gorgojo del maíz, boldo, granos almacenados, insecticidas vegetales.

LITERATURE CITED

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18:265-267.

Asawalam, E.F., S.O. Emosairue, and A. Hassanali. 2008. Essential oil of *Ocimum grattissimum* (Labiatae) as *Sitophilus zeamais* (Coleoptera: Curculionidae) protectant. African Journal of Biotechnology 7:3771-3776.

Asawalam, E.F., and A. Hassanali. 2006. Constituents of the essential oil of *Vernonia amygdalina* as maize weevil protectant. Tropical and Subtropical Agroecosystems 6:95-102.

Aslan, I., H. Özbek, Ş. Kordali, Ö. Çalmaşur, and A. Çakir. 2004. Toxicity of essential oil vapors obtained from *Pistacia* spp. to the granary weevil, *Sitophilus zeamais* (L.) (Coleoptera: Curculionidae). Journal of Plant Diseases and Protection 111:400-407.

Bekele, J., and A. Hassanali. 2001. Blend effects in the toxicity of the essential oil constituents of *Ocimum kilimandscharicum* and *Ocimun kenyense* (Labiataea) on two post-harvest insect pests. Phytochemistry 57:385-391.

Bittner, M., M.E. Casanueva, C. Arbert, M. Aguilera, V. Hernández, and J. Becerra. 2008. Effects of essential oils from five plants species against the granary weevil *Sitophilus zeamais* and *Acanthoscelides obtectus* (Coleoptera). Journal of the Chilean Chemical Society 53:1455-1459.

De Oliveira, S., J. Vendramim, J. Ribeiro, e J. Barbosa. 2003. Bioatividade de diversos pós de origem vegetal em relaçao a *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae). Ciência e Agrotecnologia 27:1231-1236.

Finney, D. 1971. Probit analysis. 333 p. Cambridge University Press, Cambridge, UK.

Halstead, D.G.H. 1963. External sex differences in stored-products Coleoptera. Bulletin of Entomological Research 54:119-134.

Huang, F., and B. Subramanyam. 2005. Management of five stored-product insects in wheat with pirimiphos-methyl and pirimiphos-methyl plus synergized pyrethrins. Pest Management Science 61:356-362.

Kouninki, H., T. Hance, F.A. Noudjou, G. Lognay, F. Malaisse, M.B. Ngassoum, *et al*. 2007. Toxicity of some terpenoids of essential oils of *Xylopia aethiopica* from Cameroon against *Sitophilus zeamais* Motschulsky. Journal of Applied Entomology 131:269-274.

Lee, B.H., P. Annis, F. Tumaalii, and S. Lee. 2003. The potential of 1,8-cineole as a fumigant for stored wheat. p. 230-234. *In* Proceedings of the Australian Postharvest Technical Conference, Canberra. 25-27 June 2003. Commonwealth Scientific and Industrial Research Organisation (CSIRO), Canberra, Australia.

Mazzonetto, F., e J. Vendramim. 2003. Efeito de pós de origem vegetal sobre *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) em Feijao armazenado. Neotropical Entomology 32:145-149.

Miresmailli, S., R. Bradbury, and M. Isman. 2006. Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. Pest Management Science 62:366-371.

Nuñez, P. 2005. Control de *Sitophilus zeamais* Motschulsky con polvos de C*henopodium ambrosioides* L. y *Peumus boldus* Mol., solos y en mezcla con carbonato de calcio. 32 p. Tesis Ingeniero Agrónomo. Universidad de Concepción, Facultad de Agronomía, Chillán, Chile.

Obeng-Ofori, D., and Ch. Reichmuth. 1997. Bioactivity of eugenol, a major component of essential oil of *Ocimum suave* (Wild.) against four species of stored-product Coleoptera. International Journal of Pest Management 43:89-94.

Obeng-Ofori, D., Ch. Reichmuth, A. Bekeles, and A. Hassanali. 1998. Toxicity and protectant potential of camphor, a major component of essential oil of *Ocimum kilimandscharicum*, against four stored product beetles. International Journal of Pest Management 44:203-209.

Paranagama, P., Ch. Adhikari, K. Abeywickrama, and P. Bandara. 2003. Deterrent effects of some Sri Lankan essential oils on oviposition and progeny production of the cowpea bruchid, *Callosobruchus maculatus* F.) (Coleoptera: Bruchidae). Journal of Food Agriculture & Environment 1:254-257.

Pérez, F., G. Silva, M. Tapia, y R. Hepp. 2007. Variación anual de las propiedades insecticidas de *Peumus boldus* sobre *Sitophilus zeamais*. Pesquisa Agropecuaria Brasileira 42:633-639.

Pires, J., J. De Morais, e S. De Bortoli. 2006. Toxicidade de óleos essenciais de *Eucalyptus* spp. sobre *Callosobruchus maculatus* (Fabr., 1775) (Coleoptera: Bruchidae). Revista de Biologia e Ciéncia da Terra 6:96-103.

Popovic, Z., M. Kostic, S. Popovic, and S. Skoric. 2006. Bioactivities of essential oils from basil and sage to *Sitophilus oryzae* L. Biotechnology & Biotechnological Equipment 20:36-40.

Procopio, S., J. Vendramin, J. Ribeiro, e J. Santos. 2003. Bioatividade de diversos pós de origen vegetal em relaçao a *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae). Ciencia e Agrotecnologia 27:1231-1236.

Regnault, R.C. 1997. The potential of botanical essential oils for insect pest control. Integrated Pest Management Review 2:25-34.

Sanna, G., E. Bazzoni, and M. Moretti. 2004. Microencapsulated essential oils active against Indian meal moth. Boletín de Sanidad Vegetal Plagas 30:125-132.

SAS Institute. 1998. Language guide for personal computer release. 6.03 ed. 1028 p. SAS Institute, Cary, North Carolina, USA.

Silva, G., R. Kiger, R. Hepp, y M. Tapia. 2005b. Control de *Sitophilus zeamais* con polvos vegetales de tres especies del género *Chenopodium*. Pesquisa Agropecuaria Brasileira 40:953-960.

Silva, G., A. Lagunes, y J. Rodríguez. 2003a. Control de *Sitophilus zeamais* (Coleoptera: Curculionidae) con polvos vegetales solos y en mezcla con carbonato de calcio en maíz almacenado. Ciencia e Investigación Agraria 30:153-160.

Silva, G., O. Orrego, R. Hepp, y M. Tapia. 2005a. Búsqueda de plantas con propiedades insecticidas para el control de *Sitophilus zeamais* en maíz almacenado. Pesquisa Agropecuaria Brasileira 40:11-17.

Silva, G., D. Pizarro, P. Casals, y M. Berti. 2003b. Evaluación de plantas medicinales en polvo para el control de *Sitophilus zeamais* Motschulsky en maíz almacenado. Revista Brasileira Agrociencia 9:383-388.

Silva, G., M. Tapia, R. Hepp, G. Bustos, y F. Osses. 2006. Evaluación de boldo (*Peumus boldus* Molina) y cal para el control de *Sitophilus zeamais* Motschulsky. Agrociencia 40:219-228.

Vogel, H., I. Razmilic, y U. Doll. 1997. Contenido de aceite esencial y alcaloides en diferentes poblaciones de boldo (*Peumus boldus* Mol.). Ciencia e Investigación Agraria 24:1-6.

Vogel, H., I. Razmilic, P. Muñoz, U. Doll, and J. San Martín. 1999. Studies of genetic variation of essential oil and alkaloid content in Boldo (*Peumus boldus*). Planta Medica 65:90-91.

Vogel, H., I. Razmilic, J. San Martín, U. Doll, y B. González. 2005. Plantas medicinales chilenas. 192 p. Editorial Universidad de Talca, Talca, Chile.