RESEARCH

INSECTICIDAL PROPERTIES OF *Peumus boldus* Molina POWDER USED ALONE AND MIXED WITH LIME AGAINST *Sitophilus zeamais* Motschulsky (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

The insecticidal properties of boldus (*Peumus boldus* Molina) powder used alone and mixed with lime against adults of maize weevil (*Sitophilus zeamais* Motschulsky) were evaluated under laboratory conditions. Additionally, aeration effects (presence or absence) and temperature (room temperature vs. 3 ºC) on insecticidal properties were studied over time. A mortality rate of 100% was observed at 20 g kg^{-1} (w/w) of *P. boldus* powder when used alone and mixed with lime in proportions of 50:50, 60:40, and 80:20. The 50% lethal concentration (LC_{50}) for all treatments was < 5 g kg^{-1}, while LC_{90} was < 11 g kg^{-1}. Mixing corn grains with different insecticidal treatments did not affect maize germination. Temperature and aeration did not influence mortality of maize weevil adults. When the boldus powder was mixed with the infested maize 24 h before grinding, the toxicity to the parental adults and emergence of F1 adults with respect to the untreated control was 100 and 0%, respectively. The results were not satisfactory when boldus powder was stored during 30, 60, and 90 d and then mixed with the infested maize. Boldus foliage toxicity was high 24 h after grinding, but decreased significantly over time.

Key words: Boldus, maize weevil, stored corn.

INTRODUCTION

A main problem for farmers after cereal harvest is grain loss during storage. This problem is important for small producers in developing countries due to grain destruction by pests such as rodents, insects, fungi, and bacteria (Larrain, 1994). In Chile, about 60 insect species attack stored food. The main pests associated with stored grains are *Sitophilus zeamais* Molina, *Sitophilus oryzae* Linnaeus, and *Sitotroga cerealella* Olivier (Larraín, 1994). Pest control of stored grains is based mainly on the use of conventional insecticides and synthetic fumigants (Porca *et al.*, 2003). However, insecticide resistance, environmental contamination, and risk to human health are associated with the use of these toxics (Miranda *et al.*, 1994). Thus, there is a need for other less expensive and friendlier alternatives.

The problems caused by overusing synthetic insecticides have forced farmers and researchers to look for less dangerous alternatives (Henao, 1999; Mareggiani, 2001) such as those used for many years in developing countries (Paez *et al.*, 1990). Examples are the use of mineral powders and plant-derived substances with insecticidal properties (Miranda *et al.*, 1994; Tierto, 1994; Subramanyam and Hagstrum, 2000). These compounds kill on contact and many have shown antifeeding, repellent, and even attractant properties (Mbah and Okoronkwo, 2008; Parugrug and Roxas, 2008).

Boldus (*Peumus boldus* Molina) is a tree or shrub native to Chile with medicinal properties (Vogel *et al.*, 1999) which has shown toxic properties against *S. zeamais* in stored maize (*Zea mays* L.) (Silva *et al.*, 2003a; 2005), and might be used as a low risk alternative for pest control in subsistence agriculture (Silva *et al.*, 2003b). However, the use of boldus alone poses a logistic problem due to the difficulties associated in obtaining the necessary quantity of this plant. Other options include the use of mineral powders such as lime, calcium carbonate, diatomeus earth, and ash (Subramanyam and Roesli, 2000; Rahman *et al.,* 2003), or using boldus mixed with mineral powders. Thus, the aim of this study conducted under laboratory

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conditions was to evaluate the insecticidal properties of *P. boldus* powder used alone and mixed with lime against *S. zeamais*, and determine the effect of aeration and temperature on the length of the toxic activity.

MATERIALS AND METHODS

Plant material

P. boldus foliage was collected from the Andean foothills, 36°52' S, 71°22´ W and 1600 m.a.s.l., Bío-Bío Region, Chile. Once collected, leaves were dried during 48 h in a stove (Memmert Gmbh, UNB 500, Schwabach, Germany) at 40 ºC. After that, the foliage was ground in an electric coffee grinder (Moulinex ultra 505) to obtain a fine particulate powder, and sifted to a very fine powder with a 20 mesh (0.841 mm) sieve.

Maize cv. Laredo seeds were used in this study. Before use, grains were kept in a freezer at -4 ºC for 48 h in order to eliminate any external insect contamination that could affect the results.

Mineral powder

Lime was used as mineral powder because of its known insecticidal properties (Silva *et al*., 2004) and the wide availability of this compound in the market.

Insects

S. zeamais individuals were reared under controlled conditions in a bioclimatic chamber (Memmert Gmbh, INE 800, Schwabach, Germany), (25 ± 2 ºC and 16:8 L:D photoperiod) using maize cv. Laredo grains as food.

Insecticidal effect

The methodology proposed by Lagunes and Rodríguez (1989) was used to evaluate the insecticidal effect of the powders against *S. zeamais* adults. One hundred grams of maize were placed into 250 mL jars. Then, the respective treatment was added. The grain and toxic powder were manually mixed during 1 min to ensure that the grain was uniformly covered. The jar was then infested with 20 insect couples not older than 10 d. Sex was determined using the criteria proposed by Halstead (1963) who determined that the rostrum of the female is longer, thinner, and less ornamented than the male's. Once the jars were infested, they were transferred to the bioclimatic chamber (25 ± 2 ºC and 16:8 L:D photoperiod).

The evaluated treatments were *P. boldus* powder used alone (100:0), lime used alone (0:100), and a mixture of both in proportions of 20:80, 40:60, 50:50; 60:40, and 80:20 (w/w). In all cases, the treatments were evaluated at the 1, 10, and 20 g kg^{-1} (w/w) concentrations.

After 15 d of insect infestation, adults were removed from each jar and the mortality percentage was determined. The highest mortality in the untreated control was 10% whereas mortality in the toxic treatments was corrected with Abbott's formula (Abbott, 1925).

Estimation of lethal concentrations 50% (LC_{50}) and 90% (LC_{90})

Serial concentrations were evaluated to determine the range in which 0 and 100% mortality was observed. Then, from five to seven intermediate concentrations were added. A total of five replications were carried out on different days and each one included an untreated control.

Germination test

Maize grain germination was evaluated only in those treatments that caused at least 80% mortality. Ten seeds were randomly selected from each of the replications and germinated in Petri dishes on wet paper towel at 25 ºC. Seed germination was determined one week later.

Effect of the environmental factors on the insecticidal properties of *P. boldus* powder

To evaluate the environmental factors on the toxicity of boldus, two types of containers (500 mL capacity) were used: perforated (to evaluate aeration effect) and closed (to evaluate lack of aeration effect). Half of each type of container was kept at room temperature and half at 3 ºC in order to evaluate temperature effect. Boldus foliage was ground and stored during 24 h, 30, 60, and 90 d. Then, 100 g of corn grain was added to each container, mixed with boldus powder (10 g kg^{-1}), and infested as explained above. Adult mortality was determined 15 d after insect infestation and the emergence of F1 adults was evaluated after 55 d.

Experimental design

The toxic activity of boldus and lime used separately or as a mixture in different proportions was evaluated, as well as the germination test in a completely random design. Toxicity and germination tests consisted of 21 and 12 treatments, respectively. In both cases, five replications on different days were carried out. Each replication included an untreated control. The aeration and temperature effects on the residual toxicity of treatments were evaluated in a 2 x 2 factorial design with four treatments and five replications.

Statistical analysis

Before analysis, percentage mortality was transformed to the arcsine $\sqrt{x/100}$ and then subjected to an analysis of variance (ANOVA, $\alpha = 0.05$) with the SAS program (SAS Institute, 1998). Tukey´s multiple comparison test (α = 0.05) was used to determine treatment differences.

To determine the lethal concentrations that kill 50% (LC_{50}) and 90% (LC_{90}), data were subjected to the Probit analysis (Finney, 1971) using the Raymond Probit Analysis Program® software (Raymond, 1985).

RESULTS AND DISCUSSION

Mortality

As concentration increased, mortality also increased. With a 1 g kg^{-1} concentration, mortality tended to be higher as the proportion of lime in the mixture increased; the opposite was observed with 10 and 20 g kg^{-1} (Table 1). Mortality ranged from 8.2 to 100% (Table 1) in all treatments. The least effective treatments were those using a 1 g kg^{-1} concentration where mortality was < 50%. With 10 and 20 g kg^{-1}, mortality was > 50%, and with proportions of 60:40, 80:20, and 100:0, mortality of adult insects was 100% (Table 1). The high mortality percentages produced by *P. boldus* powder used alone agree with the results obtained by Paez *et al*. (1990), Silva *et al*. (2001; 2005) with 100%, 99.1%, and 99.3% insect mortality, respectively. The value obtained with lime was similar to that reported by Silva *et al*. (2004) with a 10 g kg^{-1} concentration and 100% mortality. The insecticidal effect of boldus is probably due to the alkaloids it contains. Alkaloids are known for their toxic properties, such as in nicotine (Lagunes and Villanueva, 1994), but boldine, the main boldus alkaloid, is present in very small concentrations (0.2 to 0.5%) (Vogel *et al.*, 1999). However, Perez *et al*. (2007) did not find any correlation between the alkaloid concentration and mortality of *S. zeamais*.

Table 1. Adult mortality of *Sitophilus zeamais* in maize treated with different proportions of *Peumus boldus* and lime.

Concentration	Treatment Boldus:lime (weight/weight)	Mortality[1]
g kg^{-1}		%
1	0:100	47.0fg
	20:80	43.8fgh
	40:60	20.6ij
	50:50	26.0ghi
	60:40	22.4hij
	80:20	15.2ij
	100:0	8.2j
10	0:100	66.0efi
	20:80	67.0efi
	40:60	93.8bc
	50:50	97.7abc
	60:40	100.0a
	80:20	100.0a
	100:0	100.0a
20	0:100	75.7ed
	20:80	90.4dc
	40:60	99.4ab
	50:50	100.0a
	60:40	100.0a
	80:20	100.0a
	100:0	100.0a

Treatments with the same letter in the columns are not statistically different according to Tukey test ($\alpha = 0.05$).
[1]Mortality corrected with Abbott's formula.

LC_{50} and LC_{90} values

Values for LC_{50} ranged from 0.41 to 4.27 g kg^{-1} and from 1.18 to 10.43 g kg^{-1} for LC_{90} (Table 2). The most toxic treatments at the LC_{90} level were the 60:40 and 80:20 proportions which required only 1.18 and 1.23 g kg^{-1} powder, respectively. These results confirm a positive synergistic effect of the mixture of *P. boldus* and lime. Slope values (Table 2) of the Dose-Probit Line (Ldp) (Figure 1) show that the greatest homogeneity in population response (slope 4.44) was achieved with the 50:50 treatment. Therefore, to achieve higher mortality, concentration must be increased less than in the other treatments. Ldp is a straight line indicating a unimodal response of *S. zeamais* to the treatments.

Germination test

No treatment was statistically different from the untreated control and values ranged from 86.7 to 100% (Table 3). Additionally, 100% germination was obtained with 40:60 and 10 g kg^{-1} (w/w), 50:50 and 60:40 with 20 g kg^{-1}, and 80:20 with 10 and 20 g kg^{-1}. *P. boldus* powder (100:0) at concentrations of 10 and 20 g kg^{-1} (w/w) achieved 96.7% and 93.3% germination, respectively. Consequently, the analysis of this parameter indicates that *P. boldus* powder used alone or mixed with lime does not significantly affect the percentage of seed germination.

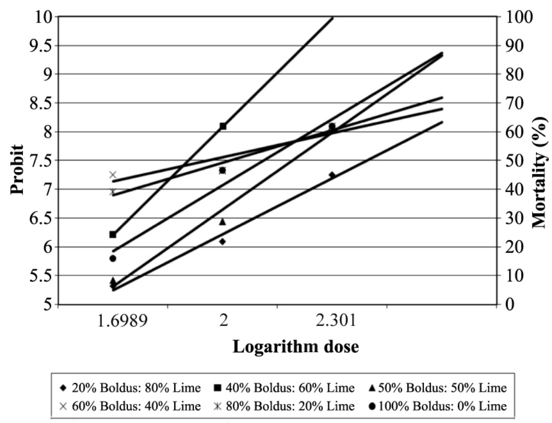

Figure 1. Dose-Probit Line (Ldp) for evaluated treatments to control *Sitophilus zeamais* in stored maize.

Table 2. Lethal concentrations of the most effective *Peumus boldus* and lime treatments on *Sitophilus zeamais* adults.

Boldus: Lime (Proportion weight:weight)	LC_{50}	LC_{90}	Equation Y = a + bx	Coefficient of determination (r^2)
	g kg^{-1}			
20:80	4.20	10.43	Y= 2.99 + 3.22X	0.99
40:60	3.26	7.39	Y= 3.16 + 3.58X	0.84
50:50	4.27	8.25	Y= 2.20 + 4.44X	0.98
60:40	0.14	1.18	Y= 6.17 + 1.38X	0.81
80:20	0.49	1.23	Y= 5.58 + 1.87X	0.96
100:0	2.86	6.30	Y= 3.26 + 3.81X	0.96

LC_{50}: Concentration that kills 50% of the treated individuals.
LC_{90}: Concentration that kills 90% of the treated individuals.

Table 3. Germination in maize seeds treated with different *Peumus boldus* and lime proportions.

Boldus: Lime (Proportion weight:weight)	Concentration	Germination
	g kg^{-1}	%
20:80	20	93.3a
40:60	10	100.0a
40:60	20	96.7a
50:50	10	93.3a
50:50	20	100.0a
60:40	10	96.7a
60:40	20	100.0a
80:20	10	100.0a
80:20	20	100.0a
100:0	10	96.7a
100:0	20	93.3a
Testigo	-	86.7a

Treatments with the same letter in the columns are not statistically different according to Tukey test ($\alpha = 0.05$).

Effect of environmental factors on insecticidal properties of *P. boldus* powder

Mortality. There were no statistical differences with respect to aeration (presence or absence) or temperature (room temperature or 3 ºC). However, the time that the boldus powder remained in storage (24 h, 30 d, 60 d, and 90 d) greatly affected treatment toxicity. Mortality was > 97% in all treatments after 24 h, whereas at 30, 60, and 90 d, mortality was < 53% (Table 4) which is not considered promising according to criteria set by Lagunes and Rodríguez (1989). Our results agreed with those obtained by Paez *et al*. (1990) and Silva *et al*. (2003b; 2005) who claimed a mortality of 100%, 99.1%, and 99.3%, respectively after 24 h. Mortality with boldus powder at 30, 60, and 90 d storage was similar to that found by Silva *et al*. (2005), who obtained percentages close to 2% for the same periods.

Emergence of F1 adults

There were no aeration (presence or absence) or temperature (room temperature or 3 ºC) effects on the emergence of F1 adults with respect to the untreated control (Tukey, $\alpha = 0.05$) (Table 5). Mortality was greatly influenced by boldus powder storage time (24 h, 30, 60, and 90 d) before insect infestation (Table 5). There was no emergence of F1 adults after 24 h. In the rest of the treatments, the emergence of F1 adults compared to the untreated control did not reach satisfactory results according to criteria set by Lagunes and Rodríguez (1989). The result obtained after 24 h was probably due to the 100% mortality achieved in parent adults and to the fact that the parent females died before laying eggs.

CONCLUSIONS

Mortality of adults was positively correlated with treatment concentration. At 1 g kg^{-1} (w/w), toxicity increased as the proportions of boldus in the mixture increased, but the opposite was observed at 10 and 20 g kg^{-1} (w/w). In all cases, the evaluated treatments did not affect maize germination. There were no aeration (presence or absence) or temperature (room temperature

Table 4. Mortality of *Sitophilus zeamais* adults treated with *Peumus boldus* powder with 1 g kg^{-1} concentration and exposed to different environmental conditions.

	Mortality (%)			
Treatment	**24 h**	**30 d**	**60 d**	**90 d**
Aeration				
Room temperature	97a	13a	13a	32a
3 ºC	98a	25a	10a	48a
No aeration				
Room temperature	100a	20a	17a	33a
3 ºC	100a	37a	18a	53a

Values with the same letter and inside the same column are not statistically different according to Tukey test ($\alpha = 0.05$).

Table 5. Emergence of F1 *Sitophilus zeamais* adults treated with *Peumus boldus* powder with 1 g kg^{-1} concentration and subjected to different environmental conditions.

	Emergence of F1 adults with respect to the untreated control (%)			
Treatment	**24 h**	**30 d**	**60 d**	**90 d**
Aeration				
Room temperature	0a	83a	90a	61ab
3 ºC	0a	53a	94a	47ab
No aeration				
Room temperature	0a	66a	85a	99ab
3 ºC	0a	48a	88a	45ab

Values with the same letter and inside the same column are not statistically different according to Tukey test ($\alpha = 0.05$).

or 3 ºC) effects on treatment toxicity. Mortality and emergence of F1 adults compared to the untreated control was highly influenced by the time the boldus powder was stored before insect infestation. Once the foliage of boldus is ground, its toxic properties remain satisfactory during a short time (24 h).

RESUMEN

Propiedades insecticidas del polvo de *Peumus boldus* Molina: solo y en mezcla con cal contra *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Se evaluaron las propiedades insecticidas del polvo de boldo (*Peumus boldus* Molina) solo y en mezcla con cal, bajo condiciones de laboratorio. Adicionalmente, se evaluó el efecto de la aeración (presencia vs. ausencia) y de la temperatura (temperatura ambiente vs. 3 ºC) sobre la mortalidad y emergencia de adultos de la F1. La concentración de 20 g kg^{-1} (p/p) del polvo de boldo ya sea solo o en combinación con cal en las proporciones de 50:50, 60:40 y 80:20 mostraron 100% de mortalidad. La concentración letal 50% (CL_{50}), en todos los tratamientos fue menor a 5 g kg^{-1} (p/p) mientras que la CL_{90} no superó 11 g kg^{-1} (p/p). La mezcla del polvo con los granos de maíz tanto solo como en mezcla con cal no afectó la germinación. La temperatura y la aeración no afectaron la mortalidad de los adultos parentales ni la emergencia de adultos de la F1. Cuando se mezcló el maíz con el polvo de boldo molido 24 h antes de la infestación con adultos, la mortalidad de los adultos parentales y la emergencia de adultos de la F1 fue de 100 y de 0%, respectivamente. Los resultados no fueron satisfactorios cuando el polvo de boldo almacenado durante 30, 60 y 90 d fue mezclado con el maíz infestado. La toxicidad del follaje de boldo es alta 24 h después de pulverizarse; si el tiempo es mayor, la toxicidad declina significativamente.

Palabras clave: boldo, gorgojo del maíz, maíz almacenado.

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