Insecticidal activity of powder and essential oil of *Cryptocarya alba* (Molina) Looser against *Sitophilus zeamais* Motschulsky

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ABSTRACT

Cereals constitute a relevant part of human and domestic animal diet. Under storage conditions, one of the most significant problems of these crops is insect pests as the maize weevil (*Sitophilus zeamais* Motschulsky). This insect species is usually controlled by means of synthetic insecticides but problems as toxic residues and resistance has led to the search for more friendly control alternatives such as botanical insecticides. The aim of this research was to evaluate, under laboratory conditions, the insecticidal properties of the powder and the essential oil of peumo (*Cryptocarya alba* [Molina] Looser; Lauraceae) leaves against *S. zeamais*. The variables assessed were toxicity by contact and fumigant activity, adult emergence (F_1), repellent effect, and impact on wheat (*Triticum aestivum* L.) seed germination. A completely randomized design was used with five treatments and 10 replicates. The higher mortality levels were obtained at 80 g powder kg^{-1} grain and 40 mL essential oil kg^{-1} grain of *C. alba*; in both cases, the mortality of adult *S. zeamais* surpassed 80%. The emergence of adults *S. zeamais* (F_1) was reduced by 100% at 80 g powder kg^{-1} grain and 40 mL essential oil kg^{-1} grain. Germination of wheat seeds treated with *C. alba* powder and essential oil was not affected. Both, the powder and the oil treatments showed repellent effect, but not fumigant activity.

Key words: Bioinsecticide, maize weevil, peumo, stored grains.

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INTRODUCTION

Because of the grain damage on stored conditions, the maize weevil (*Sitophilus zeamais* Motschulsky) is a key pest of cereals worldwide. This insect species is usually controlled by means of synthetic insecticides but according to the Arthropod Pesticide Resistance Database (www.pesticideresistance.org) of Michigan State University, *S. zeamais* has 32 reports of resistance to nine different insecticides used against this pest on rice (*Oryza sativa* L.), maize (*Zea mays* L.), and sorghum (*Sorghum bicolor* L.) Hence, the use of botanical insecticides should be considered as an alternative.

The botanical insecticides have been used as powders, extracts, and essential oils for many years. These pesticides have shown contact, fumigant, antifeedant, and repellent activity against insects (Silva et al., 2003). The easiest way to use botanical insecticides against stored grain pest consist on drying the foliage and then mixing it with the grain. However, if fumigant effect is required, the essential oil is a better option.

The Chilean research about insecticidal activity of native plants derivates is very limited and focuses on a reduced number of species as boldo (*Peumus boldus* Molina; Monimiaceae) (Betancur et al., 2010), *Laurelia sempervirens* (Ruiz & Pav.) Tul. (Monimiaceae) (Torres et al., 2014), *Laureliopsis phillippiana* (Looser) Schodde (Monimiaceae) (Ortiz et al., 2012), and *Drimys winteri* J.R. Forst. & G. Forst. (Winteraceae) (Andrade et al., 2009). Hence, it is important to explore other Chilean plant species.

The peumo (*Cryptocarya alba* [Molina] Looser; Lauraceae), is a tree native from Chile that grows from southern Coquimbo to Los Lagos Region. It is a perennial tree with dense and dark foliage, reaching 15-20 m tall (Hoffmann, 1983). Its leaves contain 0.3% of essential oil, being the main components α-pinene, β-pinene, β-terpinene, cymol, terpinen-4-ol, and 1,8-cineole (Avello et al., 2012). Studies of essential oils extracted from other plants that contain terpinen-4-ol, 1,8-cineole or cymol as the main components, have shown insecticidal activity against insects pest of stored products as *S. zeamais* (Tapondjou et al., 2005; Kouninki et al., 2007), *Sitophilus granarius* L. (Coleoptera: Curculionidae) (Kordali et al., 2006) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) (Tapondjou et al., 2005), among others. The aim of this research was to assess, under laboratory conditions, insecticidal properties of the powder and the essential oil from *C. alba* foliage against *S. zeamais*.

MATERIALS AND METHODS

Essential oil extraction and analysis

Cryptocarya alba foliage was field-collected in December 2012 from Pinto county (36°42' S, 71°54' W, 286 m a.s.l.), Ñuble Province, Biobío Region, Chile, using the Vogel et al. (1997) criteria. The taxonomic identification of field-collected foliage was verified according to voucher CONC-CH 5488 and deposited in herbarium of Faculty of Agronomy of University of Concepcion at Chillan. Once field-collected, the leaves were dried out during 48 h in a stove (UNB 500, Memmert Gmbh, Schwabach, Germany) at 40 °C. After that, the foliage was grounded in an electric coffee grinder (A5052HF, Moulinex, Aleçon, France) to obtain a fine powder which was sieved throughout a 20 mesh (0.841 mm) (Dual Manufacturing, Chicago, Illinois, USA). According to Silva et al. (2003) this granulometry has the highest level of grain adherence. The essential oils were obtained by steam distillation during 4 h using distilled water in a Clevenger type apparatus (Kouninki et al., 2007). Subsequently, the oil was stored in amber-colored glass containers at 4.5 °C. Previous to the bioassays and analysis by gas chromatography (GC), the essential oil was treated with sodium sulfate to eliminate water traces.

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

Insects and grain

The insects used in those study were obtained from stock cultures maintained in the Laboratory of Entomology of University of Concepcion, Campus Chillan, and they were reared in 1 L glass flasks containing wheat at 30 ± 1 °C, 60 ± 5% RH and total darkness in a bioclimatic chamber (IPS 749, Memmert Gmbh, Schwabach, Germany). The sex was determined using the criteria proposed by Halstead (1963).

Wheat for insect rearing and bioassays was obtained from the fruit and vegetable market of city of Chillan. Only healthy grain it was washed with drinkable water and dried in a stove (UNB 500, Memmert Gmbh) at 25 °C for 12 h and then frozen at -4 ± 1 °C for 48 h prior to its use.

Bioassays of contact toxicity

The bioassay with powder was carried out using the methodology of Silva et al. (2005). The respective treatment was added into 250 mL glass jars containing 100 g of wheat grains. Then the container was hand shaken during 1 min. After that, 20 insect couples, 48 h-old were added. Untreated control consisted in a jar with 100 g wheat grains infested with 20 pairs of insects. After insect infestation, containers were transferred to a bioclimatic chamber (30 ± 1 °C, 60 ± 5% RH and total darkness). The percentage of mortality was assessed at 7 d after infestation (DAI) and was corrected by means of the Abbott's formula (Abbott, 1925), but if control exceeded 10% mortality, the whole respective replicate was discarded and repeated. An insect was considered dead when they failed to move after being prodded gently with a needle during 30 s. After recording the percent mortality, all insects were removed and the respective flasks returned to a bioclimatic chamber under previously described environmental conditions.

The contact toxicity bioassays with essential oil were carried out using the methodology of Obeng-Oferi and Reichmuth (1997). Solutions of 1 mL essential oil in acetone were applied to 400 mL glass flasks containing 200 g wheat grains. Then, the flasks were covered and hand shaken during 15 s to properly cover the grains with oil; then they uncovered and left for 2 h at room temperature (20 ± 5 °C) to allow the acetone to evaporate. After that, the flasks were infested with 20 insect couples, 48 h-old. The experimental units were stored in a bioclimatic chamber at 30 ± 1 °C, 60 ± 5% RH and total darkness. Mortality was assessed as previously described.

The evaluated treatments were 5.0, 10, 20, 40, and 80 g powder kg^{-1} grain and 2.5, 5.0, 10, 20, and 40 mL essential oil kg^{-1} grain. Every treatment had 10 replicates. To obtain the median lethal concentration (LC_{50}), data were subjected to Probit analysis using the PROC PROBIT procedure of Statistical Analysis System (SAS) software (SAS Institute, Cary, North Carolina, USA).

For both, the powder and the essential oil bioassays, after 36 (week 3), 50 (week 5) and 55 (week 7) DAI the emergence of the F_1 was assessed. The amount of F_1 adults were recorded and percent emergence was calculated, considering values of the untreated control as 100%.

The impact of the treatments on wheat germination was evaluated 55 DAI. To set up the experiment, 20 healthy wheat seeds per replicate were selected, then they were laid down on

a wet filter paper and kept under of 24 ± 2 °C and 60 ± 5% RH conditions in a bioclimatic chamber during 7 d. The grains used in bioassays were not certified, hence a relative germination considering untreated control as 100% was estimated.

Bioassays of fumigant and repellent effect

The methodology to evaluate the fumigant effect of the *C. alba* powder was adapted from Tavares and Vendramim (2005). At the bottom of 200 mL plastic containers, a PVC tube of 5 cm long and 2.5 cm in diameter, containing the respective treatment, was stuck vertically with silicone. Then, the end of PVC tube was covered with a piece of fine organza fabric to prevent direct contact of insects with the powder, but allowing the release of volatile compounds into the environment. Then, the rest of the inner space between the container and the tube was filled with 20 g wheat grains, and infested with 20 unsexed insects. The untreated control consisted in plastic containers with 20 g wheat grains infested with 20 insects without powder in the tube. The mortality assessment by the fumigant effect was made 5 DAI. The treatments assessed were 5.0, 10, 20, 40, and 80 g powder kg^{-1} grain and each treatment consisted of 10 replicates.

To carry out the bioassay to evaluate the fumigant toxicity of essential oil, we used the methodology of Pires et al. (2006), which consisted of applying 0 (control), 15, 20, 25, 30, and 35 µL of essential oil on a circular Whatman nr 10 filter paper (5.5 cm diameter; Whatman, Maidstone, Kent, UK). Then, the treated paper was attached to undersurface of screw caps of a 500-mL glass container (air volume equivalent to 0.5 L) and 200 g wheat grain was added and infested with 10 adult insects, 48-h old without sexing. Mortality was assessed 5 DAI and 10 replicates were run for each treatment.

The methodology proposed by Mazzonetto and Vendramim (2003) was used by repellent effect. The experimental unit was a choice arena consisting in a central Petri dish with 20 adults, 48 h-old of *S. zeamais* without sexing. This Petri dish was connected to four dishes using tubes (10 cm long and 0.5 cm diameter), two dishes contained 20 g treated grain and the other two 20 g of untreated grain (Procopio et al., 2003). The choice arenas were kept in a bioclimatic chamber (30 ± 1 °C, 60 ± 5% RH and total darkness), for 24 h and the number of insects that moved to each treatment was recorded. Each treatment had 10 replicates and in each replicate, the treatments were randomly rotated to avoid the possible interference of external factors. The repellent index was calculated with these results according to Mazzonetto and Vendramim (2003), who classified the treatment as neutral if the index = 1, attracting if > 1 and repellent if < 1.

Experimental design and statistical analysis

The experimental design was a completely randomized design. The variable percentage was transformed to the $\sqrt{x}/100$ arcsine function prior to carry out ANOVA (α = 0.05) test with the SAS program to determine if at least one treatment was different from the rest. If so, a Tukey means comparison test was used ($p \leq 0.05$). Probit analysis responses were considered different when their respective fiducially limits did not overlap at a given mortality level (50%) (Robertson and Preisler, 1992).

RESULTS AND DISCUSSION

The principal constituents of the essential oil of *C. alba* were (E)-beta-bergamotene (15.6%), viridiflorol (8.5%), germacrene-D (7.65%), β-apo-13-carotenone (5.3%), linalool (4.4%), (-)-terpinen-4-ol (3.45%), 2-methyl-cyclopentane propanone (3.38%), α-farnesene (2.93%), β-himachelene (2.74%), 1,8-cineole (1.85%), β-cubebene (1.53), jasmoline 1 (1.47%), and safrole (1.1%) (Table 1). Our results do not agree with Niemeyer and Teillier (2007) and Avello et al. (2012), who mainly identified 1,8-cineole (21.4%) and 1-terpinen-4-ol (28.19%), respectively. We attribute these differences to the fact that there are differences in the field-collection date and on the geographical zone where the plant material was field-collected. Niemeyer and Teillier (2007) collected foliage on April (fall season) in Ocoa (32°54.957' S; 71°02.7080' W), Valparaiso Region, and Avello et al. (2012) and we collected foliage in Biobío Region but Avello et al. (2012) in November (spring season) in Nonguen Valley wood (36º49' S; 73º01' W) and we in December (summer season) in Pinto county (36°42' S; 71°54' W), Ñuble Province foothills.

The main secondary metabolite identified with insecticidal properties is the (*E*)-beta-bergamotene which is a sesquiterpene identified as a volatile compound released by plants in response to insect herbivory (Zhuang et al., 2012) and as a male pheromone of ectoparasitoid *Melittobia digitata* Dahms (Hymenoptera: Eulophidae) (Consoli et al., 2002). Huang et al. (2002) indicated that safrole has insecticidal activity against *S. zeamais* and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and according to Obeng-Oferi and Reichmuth (1997) this compound also affects *S. granarius*, *S. zemais*, *T. castaneum*, and *Prostephanus truncatus* (G.H. Horn) (Coleoptera: Bostrichidae). The other identified compounds are viridiflorol, germacrene-*d*, linalool, (-)-terpinen-4-ol and 1,8-cineole, which are components of the essential oils in other plant species, and have shown insecticidal activity against *S. zeamais* (Chu et al., 2012), *Sitophilus oryzae* L. (Coleoptera: Curculionidae) (Prieto et al., 2011), and *Cacopsylla chinensis* (Yang & Li, 1981) (Hemiptera: Psyllidae) (Liu et al., 2014), among others.

Contact toxicity

The highest mortality reached by the use of the *C. alba* powder was obtained with the treatment of 80 g powder kg^{-1} grain which caused 85% of mortality (Table 2), being

Table 1. Chemical composition of the essential oil from *Cryptocarya alba*.

Compound	RT†	Relative content	Identification††
	min	%	
Heptanal	4.73	0.18	MS, IK, S
Isoterpinolene	5.55	0.27	MS, IK, S
Sabinene	6.17	0.50	MS, IK, S
α-Felandrene	6.43	0.77	MS, IK, S
β-Terpinene	6.63	1.64	MS, IK, S
1,3,8-*p*-Menthatriene	6.76	0.15	MS, IK, S
1,5-Dimethyl-1,5-cyclooctadiene	6.83	0.43	MS, IK, S
1-8-Cineole	6.89	1.85	MS, IK, S
Isoterpinolene	7.79	0.45	MS, IK, S
Linalool	7.93	4.40	MS, IK, S
3-Isopentyl isovalerate	8.13	2.20	MS, IK, S
Unknown	8.18	0.26	MS, IK, S
cis-p-2-Menthen-1-ol	8.33	0.22	MS, IK, S
Pinocarveol	8.63	1.84	MS, IK, S
(3Z)-2,2,5,5-Tetra methyl hex-3-eno	8.86	0.58	MS, IK, S
(-)-Terpinen-4-ol	9.22	3.45	MS, IK, S
Terpineole	9.40	0.87	MS, IK, S
Linalyl formate	10.27	0.22	MS, IK, S
7-Octen-2-one	10.60	0.24	MS, IK, S
2-Nonanone	10.82	0.24	MS, IK, S
Safrole	10.87	1.01	MS, IK, S
α-Cubebene	11.69	0.73	MS, IK, S
β-Phenylethyl butyrate	12.25	0.32	MS, IK, S
(-)-Germacrene	12.27	0.44	MS, IK, S
E-β-Bergamotene	12. 81	15.60	MS, IK, S
(-)-Aristolene	12.95	0.28	MS, IK, S
β-Bisabolene	13.00	0.22	MS, IK, S
α-Caryophyllene	13.13	0.23	MS, IK, S
Isoledene	12.22	0.79	MS, IK, S
∂-Cadinene	13.43	0.18	MS, IK, S
Phenethyl isovalerate	13.44	0.76	MS, IK, S
Germacrene-*d*	13.47	7.65	MS, IK, S
α-Cadinene	13.49	0.16	MS, IK, S
α-Bulnesene	13.55	0.25	MS, IK, S
∂-Elemene	13.66	0.29	MS, IK, S
Bicyclosesquifelandrene	13.850	0.81	MS, IK, S
β-Cubebene	13.944	1.53	MS, IK, S
Cubinene	14.070	0.90	MS, IK, S
2-(1,3-Butadienyl) mesitylene	14.216	0.25	MS, IK, S
α-Farnesene	14.330	2.93	MS, IK, S
Unknown	14.540	0.52	MS, IK, S
1,3,3-Trimethyl-2-(3-methyl-2-methylene-but-3-enylidene)-1 -cyclohexanol	14.660	0.15	MS, IK, S
β-Apo-13-carotenone	14.710	5.30	MS, IK, S
Jasmoline I	14.866	1.47	MS, IK, S
3-Decine	15.060	0.25	MS, IK, S
β-Himachelene	15.220	2.74	MS, IK, S
β-Phenylethyl butyrate	15.280	0.17	MS, IK, S
3,9-Dodecadiine	15.340	0.28	MS, IK, S
α-Cubebene	15.410	0.84	MS, IK, S
2-Methyl-cyclopentane propanone	15.470	3.38	MS, IK, S
Veridiflorol	15.570	8.50	MS, IK, S
Dehydro-aromadendrene	16.105	0.45	MS, IK, S

†Retention time.
††Compounds identified by comparison with mass spectra (MS) from database, Kovats retention indices (IK), and pure standards (S).

significantly higher in comparison with the other treatments ($p \leq 0.05$). The rest of treatments (5.0 to 40 g powder kg^{-1} grain) showed a level of mortality lower than 30%. The LC_{50} was 50 g powder kg^{-1} grain and according to this results, we can infer that the powder of *C. alba* foliage has the potential to control *S. zeamais* only at concentrations higher than 80 g powder kg^{-1} grain. Although, 80 g powder kg^{-1} grain showed a mortality greater than 80%, our results exhibit

Table 2. Mortality by contact effect of essential oil and powder of *Cryptocarya alba* against adults of *Sitophilus zeamais* and lethal concentration 50% (LC_{50}) and 95% (LC_{95}).

Treatments	Concentration	Mortality* (%)
Powder	5.0†	11.2b
	10.0	10.0b
	20.0	15.4b
	40.0	22.9b
	80.0	84.6a
	n†††	200
	b ± SE¶	1.70 ± 0.55
	LC§50	50
	(95% CL)&	44.2-56.7
	R^2	0.99
Essential oil	2.5††	15.5c
	5.0	13.0c
	10.0	23.0c
	20.0	59.5b
	40.0	94.0a
	n†	200
	b ± SE¶	1.33 ± 0.46
	LC§50	14.6
	(95% CL)&	12.8-16.2
	R^2	0.99

†g kg^{-1} grain; ††mL kg^{-1} grain; †††Total number of insects treated.
¶Probit adjustment slope (b) and standard error of slope (SE); §Lethal concentration: g powder kg^{-1} grain or mL essential oil kg^{-1} grain; &Confidence limits at 95%.
R^2: Coefficient of determination.
Values within a column with the same letter are nonsignificantly different according to Tukey's test ($p \leq 0.05$).

a lower toxicity than other vegetable powders assessed against *S. zeamais* as *D. winteri* (Andrade et al., 2009) and *P. boldus* (Silva et al., 2003), which have 100% mortality with concentrations similar or lower than 4.0% (40 g powder kg^{-1} grain). Moreover, a concentration of 8.0% should be considered impractical because of the high amount of required powder. For example, to protect 100 kg of cereal it will be required 8 kg of peumo powder; so we estimate that we required about 20 kg of *C. alba* foliage. However *C. alba* shows better results that *Eucalyptus globulus* Labill. (Myrtaceae) (Silva et al., 2003) and *Quillaja saponaria* Molina (Rosaceae) (Silva et al., 2005) that did not exceed 5% of dead insects.

In case of the essential oil, the treatment with the highest insect mortality was 80 mL essential oil kg^{-1} grain and caused 94% dead insects. This treatment had an estimated LC_{50} of 14.6 mL essential oil kg^{-1} grain (Table 2), being significantly more toxic ($p \leq 0.05$) than others assessed concentrations which did not surpass 60% mortality. Our results show a lower toxicity than essential oils from other Chilean native plants assessed against *S. zeamais*. Betancur et al. (2010), using *P. boldus* essential oil obtained mortality over 80% with concentrations equal or higher than 2.0% (20 mL essential oil kg^{-1} grain). In this case, the situation is similar to *C. alba* in the sense that the required oil concentration is impractically high. However, *C. alba* is more toxic than other essential oils evaluated against the same insect species as occurred with *Salvia hydrangea* DC. ex Benth (Hydrangeaceae) that at the concentration of 4.0% (40 mL essential oil kg^{-1} grain) led to 68.3% mortality (Kotan et

al., 2008), *Cupressus sempervirens* L. (Cupressaceae) and *Eucalyptus saligna* Sm. (Myrtaceae) that at 5.0% (50 mL essential oil kg^{-1} grain) of essential oil reached a 25% and 10% of dead insects, respectively (Tapondjou et al., 2005).

The toxicity of the powder and the essential oil of *C. alba* against *S. zeamais*, is maybe due to the bioactivity compounds such as 1,8-cineole and terpineole. These compounds exist in other plant species with insecticidal activity. According to Calmasur et al. (2006), the essential oils of two species of the genus *Achillea* have 1,8-cineole and caused of 100% mortality against *S. granarius* and *T. confusum*. Furthermore, Clemente et al. (2007), using 1,8-cineole isolated from essential oil of *Lavandula spica* L. (Lamiaceae) documented 90% of mortality in *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Kotan et al. (2008) indicated that 1,8-cineole and terpineole have toxic effect against *S. granarius* and *T. confusum*.

The emergence of adult insects was observed from week 5 (50 DAI). The treatment of 80 g powder kg^{-1} grain showed an F_1 emergence of 0% (Table 3) exhibiting significant differences ($p \leq 0.05$) with all other treatments. At the week 7, the treatments of 5, 10, and 20 g powder kg^{-1} grain did not differ significantly from the untreated control, with 80.3%, 63.2%, and 70.4% of adult F_1 emergence, respectively. These treatments showed an F_1 emergence higher than the one observed with the 40 and 80 g powder kg^{-1} grain treatments which caused an emergence of F_1 adult *S. zeamais* of 10.5% and 0%, respectively. The F_1 values obtained with treatments of 40 and 80 g powder kg^{-1} grain show a higher emergence inhibition than other plants assessed as powder such as *E. globulus* (Silva et al., 2003) and *Chenopodium quinoa* Willd. (Chenopodiaceae) (Silva et al., 2005), which at concentrations of 2.0% (20 g powder kg^{-1} grain) showed 74% and 82% of *S. zeamais* emergence.

The essential oil showed a similar trend values because the emergence of insects started in the week 5, but in all evaluations, 20 and 40 mL essential oil kg^{-1} grain showed and F_1 lower than 1.0%. These treatments values were significantly lower than the observed in the rest of treatments and did not differ with those of the control with emergences between 46% and 56% (Table 4). Our results agree with those observed in the evaluation of other essential oils from Chilean native plants as *P. boldus* (Betancur et al., 2010) and *L. sempervirens* (Torres et al., 2014). In both cases, the insect emergence begins in the week 5 and the higher concentrations of essential oil showed a lower F_1. According to Papachristos and Stamopoulos (2002), the essential oils have ovicidal and larvicidal toxicity. However, in both bioassays (powder and essential oil), all wheat grains per replicate were checked we did not detected any dead larvae inside or outside grains, therefore an ovicidal effect is assumed.

All treatments with powder and essential oil of *C. alba* showed a germination percentage similar ($p > 0.05$) to the untreated control (Table 4). According these results, *C. alba* has advantages over other plant powder or essential oils that negatively affect germination. For example, according Pérez et al. (2007), *P. boldus* reduces maize germination. The higher germination values obtained (> 90%) in treatments of 20 and 40 mL essential oil kg^{-1} grain oil comply with the requirement to export seeds; thus increasing the possibility of using the more effective treatments to protect grain and seed.

Fumigant and repellent effect

All assessed treatment of powder or essential oil of *C. alba* showed 0% mortality. These results are maybe due to the lower concentration of 1,8-cineole (1.85%) and safrole (1.01%). According to Lee et al. (2004) 1,8-cineole is one of the most promissory monoterpenes with fumigant activity against insect pest control in stored products, and Huang et al. (2002) obtained a significant fumigant toxicity with safrol and isosafrol against *S. zeamais* and *T. castaneum*.

All treatments with powder and essential oil of *C. alba* showed a repellence index < 1 (Table 5), which according Mazzonetto and Vendramim (2003) classifies as repellent activity. Although the indexes with lower values (>repellency) were registered in treatments with higher concentrations of

Table 3. Emergence (F_1) of adults of *Sitophilus zeamais* to different concentrations of powder and essential oil of *Cryptocarya alba*.

Treatments	Concentration	Insect emergence (%)		
		Week 3	Week 5	Week 7
Powder	0.0†	0.0a	100.0d	100.0c
	5.0	0.0a	74.1d	80.3c
	10.0	0.0a	48.6c	63.2c
	20.0	0.0a	51.4c	70.4c
	40.0	0.0a	8.6b	10.5b
	80.0	0.0a	0.0a	0.0a
Essential oil	0.0††	0.0a	100.0b	100.0b
	2.5	0.0a	20.7b	46.1b
	5.0	0.0a	15.9b	55.7b
	10.0	0.0a	8.2ab	54.6b
	20.0	0.0a	0.0a	0.9a
	40.0	0.0a	0.0a	0.0a

†g kg^{-1} grain; ††mL kg^{-1} grain.
Emergence of control treatment was considered as 100%.
Values within a column with the same letter are not significantly different according to Tukey's test ($p \leq 0.05$).

Table 4. Germination of wheat mixed with different concentrations of powder and essential oil of *Cryptocarya alba*.

Treatments	Concentration	Germination (%)
Powder	0.0†	100.0a
	5.0	82.1a
	10.0	77.9a
	20.0	86.3a
	40.0	86.7a
	80.0	88.3a
Essential oil	0.0††	100.0a
	2.5	95.0a
	5.0	93.5a
	10.0	86.5a
	20.0	90.0a
	40.0	96.0a

†g powder kg^{-1} grain, †† mL essential oil kg^{-1} grain.
Values within a column with the same letter are not significantly different (Tukey, $p \leq 0.05$).

Table 5. Repellence index of powder and essential oil of *Cryptocarya alba* against adults of *Sitophilus zeamais*.

Treatments	Concentration	Repellence index (IR)	Effect
Powder	5.0†	0.98	Repellent
	10.0	0.92	Repellent
	20.0	0.92	Repellent
	40.0	0.91	Repellent
	80.0	0.82	Repellent
Essential oil	2.5††	0.28	Repellent
	5.0	0.19	Repellent
	10.0	0.27	Repellent
	20.0	0.21	Repellent
	40.0	0.19	Repellent

†g powder kg^{-1} grain, ††mL essential oil kg^{-1} grain, IR: neutral (IR = 1), repellent (IR < 1), attractive (IR > 1).

powder or essential oil, the repellent effect is detectable. According to Conti et al. (2010) this effect is directly related with monoterpenes concentration. Thus if the monoterpenes concentration is lower, usually the most abundant components in essential oils, the lower will be the repellency.

CONCLUSIONS

The powder and essential oil of foliage of *Cryptocarya alba* under laboratory conditions have contact insecticidal activity and repellence effect against adults of *Sitophilus zeamais* without affecting wheat germination. The essential oil *C. alba* may have potential to be developed as a new natural contact insecticide to control insects of stored products.

REFERENCES

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

Andrade, Y., G. Silva, N. Zapata, M. Tapia, J. Rodríguez, y A. Lagunes. 2009. Toxicidad de polvos de canelo (*Drimys winteri* J.R. et G. Forster) contra *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) en laboratorio. Agro-Ciencia 25(1):33-40.

Avello, M., C. López, C. Gatica, E. Bustos, A. Brieva, E. Pastene, et al. 2012. Efectos antimicrobianos de extractos de plantas chilenas de las familias Lauraceae y Atherospermataceae. Revista Cubana de Plantas Medicinales 17(1):73-83.

Betancur, J., G. Silva, J.C. Rodríguez, S. Fischer, and N. Zapata. 2010. Insecticidal activity of *Peumus boldus* Molina essential oil against *Sitophilus zeamais* Motschulsky. Chilean Journal of Agricultural Research 70:399-407.

Calmasur, O., S. Kordali, O. Kaya, and I. Aslan. 2006. Toxicity of essential oils of vapours obtained from Achillea spp. to *Sitophilus granaries* (L.) and *Tribolium confusum* (Jacquelin du Val). Journal of Plant Diseases and Protection 113:37-41.

Clemente, S., G. Mareggiani, A. Broussalis, y G. Ferraro. 2007. Actividad insecticida de 1,8-cineol sobre mosca de los frutos *Ceratitis capitata* Wied. (Diptera: Tephritidae). Dominguezia 23:29-34.

Consoli, F.L., H.J. Williams, S.B. Vinson, R.W. Matthews, and M. Cooperband. 2002. *trans*-Bergamotenes-male pheromone of the ectoparasitoid *Melittobia digitata*. Journal of Chemical Ecology 28:1675-1689.

Conti, B., A. Canale, P. Cioni, and G. Flamini. 2010. Repellence of essential oils from tropical and Mediterranean Lamiaceae against *Sitophilus zeamais*. Bulletin of Insectology 63:197-202.

Chu, S., Z. Liu, S. Du, and Z. Deng. 2012. Chemical composition and insecticidal activity against *Sitophilus zeamais* of the essential oils derived from *Artemisia giraldii* and *Artemisia subdigitata*. Molecules 17:7255-7265.

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

Hoffmann, A. 1983. El árbol urbano en Chile. Fundación Claudio Gay, Santiago, Chile.

Huang, Y., H. Ho, H. Lee, and H. Yap. 2002. Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). Journal of Stored Product Research 38:403-412.

Kordali, S., I. Aslan, O. Calmasur, and A. Cakir. 2006. Toxicity of essential oils isolated from three *Artemisia* species and some of their major components to granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Industrial Crops and Products 23:162-170.

Kotan, R., S. Kordali, A. Cadir, M. Kesdek, Y. Kaya, and H. Kilic. 2008. Antimicrobial and insecticidal activities of essential oil isolated from Turkish *Salvia hydrangea* DC ex Benth. Biochemical Systematic and Ecology 36:360-368.

Kouninki, H., T. Hance, F.A. Noudjou, G. Lognay, F. Malaisse, M.B. Ngassoum, et al. 2007. Toxicity of some terpenoids of essential oil of *Xylopia aethiopica* from Cameroon against *Sitophilus zeamais* Motschulsky. Journal of Applied Entomology 131(4):269-274.

Lee, B.H., P.C. Annis, F. Tumaalii, and W. Choi. 2004. Fumigant toxicity of essential oils from the Myrtaceae family and 1-8-cineole against 3 major stored-grain insects. Journal of Stored Products Research 40:553-564.

Liu, X.C., Q.Z. Liu, W.P. Shi, and Z.L. Liu. 2014. Evaluation of insecticidal activity of the essential oil of *Eucalyptus robusta* Smith leaves and its constituent compound against overwintering *Cacopsylla chinensis* (Yang et Li) (Hemiptera: Psyllidae). Journal of Entomology and Zoology Studies 2:27-31.

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.

Niemeyer, H., y S. Teillier. 2007. Aromas de la flora nativa de Chile. Universidad de Chile-Fundación para la Innovación Agraria (FIA), Santiago, Chile.

Obeng-Oferi, D., and C.H. 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.

Ortiz, A., G. Silva, A. Urbina, and N. Zapata. 2012. Bioactivity of tepa (*Laureliopsis philippiana* (Looser) Shodde) powder to *Sitophilus zeamais* Motschulsky control in laboratory. Chilean Journal of Agricultural Research 72:68-73.

Papachristos, D.P., and D.C. Stamopoulos. 2002. Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Journal of Stored Product Research 38:117-128.

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.

Prieto, J.A., O.J. Patiño, W.A. Delgado, J.P. Moreno, and L.E. Cuca. 2011. Chemical composition, insecticidal, and antifungal activities of fruit essential oil of three Colombian *Zanthoxylum* species. Chilean Journal of Agricultural Research 71:73-82.

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.

Robertson, J.L., and H.K. Preisler. 1992. Pesticide bioassays with arthropods. 224 p. CRC Press, Boca Raton, Florida, USA.

Silva, G., R. Kiger, R. Hepp, y M. Tapia. 2005. Control de *Sitophilus zeamais* Motschulsky con polvos vegetales de tres especies del género *Chenopodium*. Pesquisa Agropecuária Brasileira 40(10):953-960.

Silva, G., D. Pizarro, P. Casals, y M. Berti. 2003. Evaluación de plantas medicinales en polvo para el control de *Sitophilus zeamais* Motschulsky en maíz almacenado. Revista Brasileira de Agrociência 9(4):383-388.

Tapondjou, A.L., C. Adler, D.A. Fontem, H. Bouda, and C. Reichmuth. 2005. Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. Journal of Stored Products Research 41:91-102.

Tavares, M., e J. Vendramim. 2005. Bioatividade da erva-de-santa-maria, *Chenopodium ambrosioides* L., sobre *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae). Neotropical Entomology 34:319-323.

Torres, C., G. Silva, M. Tapia, J.C. Rodríguez, I. Figueroa, A. Lagunes, et al. 2014. Insecticidal activity of *Laurelia sempervirens* (Ruiz & Pav.) Tul. essential oil against *Sitophilus zeamais* Motschulsky. Chilean Journal of Agricultural Research 74:421-426.

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.

Zhuang, X., T.G. Köllner, N. Zhao, G. Li, Y. Jiang, L. Zhu, et al. 2012. Dynamic evolution of herbivore-induced sesquiterpene biosynthesis in sorghum and related grass crops. The Plant Journal 69:70-80.