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RESEARCH

Insecticidal activity of *Laurelia sempervirens* (Ruiz & Pav.) Tul. essential oil against *Sitophilus zeamais* Motschulsky

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The maize weevil *Sitophilus zeamais* Motschulsky is a worldwide key pest of stored products. Usually contact insecticides or fumigants are used against it, but problems as toxic residues, human intoxications, and resistance have triggered the search for alternative control methods as the use of essential oils. The objective of this research was to assess under laboratory conditions, the insecticidal properties of *Laurelia sempervirens* (Ruiz & Pav.) Tul. essential oil against *S. zeamais*. In contact toxicity bioassay assessed treatments were 0 (control), 1.25, 2.5, 5.0, 10, 20, and 40 mL essential oil kg^{-1} grain and 0 (control), 25, 50, 75, 100, 125, 150, and 175 μL essential oil L^{-1} air in fumigant toxicity tests. The highest toxicity by contact activity was reached by concentrations higher than 10 mL essential oil kg^{-1} grain (100% mortality). The same treatments totally inhibit F_1. The dose of 175 μL essential oil L^{-1} air showed a significant toxicity by fumigant activity causing 72.5% of dead insects. The other treatments did not surpass 5% mortality. In offspring effect (F_1) bioassay, all treatments had an insect emergence significantly lower than the control but concentrations equal or higher than 10 mL essential oil kg^{-1} grain prevented the emergence of F_1 during the 7 wk of bioassay. The residual effect of contact toxicity remained by 15 d. The treatments based on essential oil lead to a weight grain loss lower than control and germination was not affected. All assessed treatments showed repellent effect. The essential oil of *L. sempervirens* has promissory perspectives to maize weevil control.

Key words: Botanical insecticides, stored grains, maize weevil.

INTRODUCTION

The maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is considered a worldwide pest of stored products. The injury of this pest begins in the field and if in storage is not controlled, in 6-mo may cause complete grain destruction (Larraín, 1994). The larvae and adult feed on the endosperm and this damage allows the attack of secondary insect pests or fungi (Rees, 1996).

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Synthetic pesticides have been considered the most effective and accessible means to control these insect pests (Huang and Subramanyam, 2005). Usually the pest control of stored seeds is performed by means of the use of contact insecticides such as chlorpyriphos or malathion and the fumigants methyl bromide and phosphine (Pretheep-Kumar et al., 2010). However, their use has resulted in several problems such as the presence of pesticide residues, human intoxication and development of insect resistance (Roel and Vendramim, 2006). Hence a friendly alternative is required.

The botanical insecticides formulated as a powder, extracts and essential oils are alternatives to synthetic pesticides. The essential oils of aromatic plants are volatile, natural and complex compounds characterized by a strong odor and are constituted by secondary metabolites mainly of the terpenoids group (Bakkali et al., 2008). The insecticidal effect of essential oils is not fully elucidated but the symptoms of intoxicated insects suggest a neurotoxic effect (Tripathi et al., 2009). According to Isman (2000), studies with *Periplaneta americana* (Orthoptera: Blattidae) indicate that essential oils affect the octopamine receptor causing a breakdown of nervous system. Furthermore, Koul et al. (2008) explained that these compounds are safe for mammals. Many of pest control studies with essential oils have been focused in stored grain insect pests as *S. zeamais* (Asawalam and Hassanali, 2006; Betancur et al., 2010), *Sitophilus oryzae* L. (Coleoptera:Curculionidae) (Somboon and Pinsamarn, 2006), *Sitophilus granarius* L. (Aslan et al., 2004), *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) (Ko et al., 2009), *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) (Bittner et al., 2008), *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) (Emeasor et al., 2005) and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) (Obeng-Ofori et al., 1998) among others but the most effective essential oils are from plants not distributed in Chile.

The essential oil of *Laurelia sempervirens* has shown bactericidal (Montenegro et al., 2012), fungicidal (Bittner et al., 2009), and insecticidal (Bittner et al., 2008) activity. According to Niemeyer and Teillier (2007), Bittner et al. (2009), and Montenegro et al. (2012), the main chemical component of essential oil of *L. sempervirens* is safrole, which has exhibited toxic effect as a fumigant against *S. zeamais* and *T. castaneum* (Huang et al., 1999; 2002) but its activity as a contact insecticide against adult and immature insects, residual and repellent effect and effect on seed germination are not yet documented. Hence the aim of this research was to assess the bioactivity of essential oil of *L. sempervirens* against *S. zeamais*, under laboratory conditions.

MATERIALS AND METHODS

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

Extraction of essential oil

The essential oil was extracted from fresh leaves of *L. sempervirens* field-collected from Pinto county (36°42' S, 71°54' W; 286 m a.s.l.), province of Ñuble, Biobío Region, Chile. Leaves were washed with tap water to remove any possible detritus and the oil was obtained by steam distillation by 3 h using distilled water in a Clevenger apparatus, as suggested by Dongmo et al. (2012). Subsequently, the oil was dried out with anhydrous sodium sulfate and stored at 4.5 ± 1 °C in amber colored glass containers until they were used.

Insects and grain

The insects used in 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 30 ± 1 ºC, 60% RH in a bioclimatic chamber (Memmert Gmbh, IPS 749, Schwabach, Germany). The morphology of the proboscis was used for sexual differentiation, the one of the male being rougher and higher caliber in comparison to the female's proboscis, according to Halstead (1963). The maize (14% moisture) was obtained from the fruit and vegetable market in Chillan. Only healthy grain was used and with the aim to avoid any prior infestation that could affect the bioassay results, the grain was washed with drinkable water and frozen at -4 ± 1 ºC for 48 h.

Bioassays

Evaluation of contact toxicity was carried out with the methodology of Obeng-Ofori and Reichmuth (1997). Solutions of 1 mL essential oil of *L. sempervirens* diluted in acetone at concentrations equivalents to 1.25, 2.5, 5.0, 10, 20, and 40 mL essential oil kg^{-1} grain, plus a control treated with 1 mL of acetone were applied to 500-mL glass containing 200 g maize. Flasks were covered and shaken for 15 s to uniformly cover grains with oil. After that, they were uncovered and left for 2 h at room temperature to evaporate acetone. The flasks were then infested with 20 couples of insects 48 h old. Each treatment had 10 replicates. The experimental units were stored in a bioclimatic chamber at 30 ± 1 ºC, 60% RH and completely darkness. The insect mortality was assessed 15 d after infestation (DAI) and corrected by Abbott's equation (Abbott, 1925). Then data were subjected to Probit analysis (Finney, 1971) using the SAS PROC PROBIT procedure (SAS Institute, 1998) to estimate lethal concentration 50% (LC_{50}). After this evaluation, glass containers, without insects, were returned to bioclimatic chamber by an additional 40 days. Then 55 DAI, the adult insect emergence (F_1) was recorded considering control emergence as 100%. At the same time (55 DAI), the grain weight loss was recorded comparing the initial (200 g) with final weight. Based on preliminary observations, we assumed that the loss of humidity during the experiment equally affected all treatments.

The effect of essential oil of *L. sempervirens* on the germinate 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 apparent damage. 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 equivalents to 1.25, 2.5, 5.0, 10, 20, and 40 mL essential oil kg^{-1} grain were used as treatments. Every treatment had 10 replicates. The experimental units were kept at room temperature of 22 ± 5 ºC for 7 d. Subsequently, the relative percentage of germination was determined considering the control as 100%.

In fumigant toxicity the bioassay was based on the methodology of Pires et al. (2006), which consisted of applying concentrations equivalents to 0 (control), 25, 50, 75, 100, 125, 150, and 175 μL essential oil L^{-1} air on circular (5.5 cm in diameter) Whatman nr 10 filter paper (Whatman, Maidstone, Kent, UK), which had been adhered to the covers of 200 mL containers (air volume equivalent to 0.2 L), with 25 g maize infested with 10 adult insects, without sexing. The same procedure was used for the control using an untreated filter paper. There were 10 replicates for each treatment. The experimental units were kept in a bioclimatic chamber at 30 ± 1 ºC, 60% RH and completely darkness. Assessments of mortality were made at 24 h exposure. As the mortality rate in the control was lower than 5%, this was corrected with the Abbott's formula (Abbott, 1925). An insect was considered dead when there was no movement after prodding it with a dissection needle. Finally, the LC_{50} was obtained with the same procedure described in contact toxicity bioassay.

The residual effect was assessed with the methodology of Obeng-Ofori et al. (1998). In 500-mL flasks, 200 g maize were mixed with essential oil of *L. sempervirens* in acetone at concentrations equivalents to 1.25, 2.5, 5.0, 10, 20, and 40 mL essential oil kg^{-1} grain, plus a control treated with 1 mL acetone. The flasks were covered and shaken 15 s to uniformly cover the grains with oil. Then, they were uncovered and left for 2 h at room temperature to allow the acetone to evaporate. After that, flasks were stored in a bioclimatic chamber by 1, 5, 10, and 15 d at 30 ± 1 ºC, 60% HR, and completely darkness. A total of 16 flasks per treatment were set up and on each date of evaluation, four of them were withdrawn from bioclimatic chamber and infested with 20 adult insects, without sexing. Immediately these flasks were returned to bioclimatic chamber and 15 DAI, mortality was assessed.

The bioassay of offspring effect (F_1) was carried out with the methodology of Obeng-Ofori et al. (1998). Each experimental unit consisted of a 500-mL flask, 200 g maize, and 20 couples of adult insects 24 h of age, which were allowed to freely reproduce for 21 d. After that, adult couples were removed and grain was mixed with essential oil of *L. sempervirens* diluted in acetone at concentrations equivalents to 1.25, 2.5, 5.0, 10, 20, and 40 mL essential oil kg^{-1} grain. The control received only 1 mL acetone. Every treatment had 10 replicates and the experimental units were stored in a bioclimatic chamber at 30 ± 1 ºC, 60% HR and completely darkness. The percentage of emergence of adults of the F_1 generation was assessed weekly for 7 wk in comparison to the control.

In repellent effect the methodology of Procopio et al. (2003) with slight modifications was used. The experimental unit was a choice arena consisting in a central plastic Petri dish (5 cm diameter) connected to another four dishes through tubes 10 cm long and 0.5 cm in diameter forming an "X". Two opposite dishes containing 20 g maize grains impregnated with the respective concentrations of essential oil, while other two dishes had maize grains treated only with acetone. In the central Petri dish 20 individuals of *S. zeamais* of 48 h of age without sexing were released. The evaluated treatments were 0 (control); 1.25, 2.5, 5.0, 10, 20, and 40 mL essential oil kg^{-1} grain. The experimental batch was maintained in a bioclimatic chamber for 24 h at 22 ± 5 ºC. Subsequently, the number of dead and alive insects in each dish was counted. Each treatment had 10 replicates. The repellent index was calculated according to 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.

Experimental design

The experimental design was completely random and percentage data were transformed to the arcsine $(x/100)^{1/2}$ for its ANOVA ($\alpha = 0.05$) prior to the analysis 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

In contact activity bioassay the highest mortality (100% dead insects) was reached by concentrations similar or higher than 10 mL essential oil kg^{-1} grain (Table 1) but without significant differences ($p > 0.05$) with 2.5 and 5.0 mL essential oil kg^{-1} grain that exhibited 80% and 85% of mortality respectively. The LC_{50} was 2.3 mL essential oil kg^{-1} grain with minimum and maximum values of 1.48 and 3.6 mL essential oil kg^{-1} grain, respectively (Table 1). These results are better than those obtained with the essential oils of *Peumus boldus* Molina, other tree of same botanical family where a concentration of 40 mL essential oil kg^{-1} grain obtain 80% dead insects (Betancur et al., 2010). The toxicity of our essential oil was higher than the one obtained against *Sitophilus* from other plants as *Ocimum basilicum* L. (Labiatae) and *Salvia officinalis* L. (Lamiaceae) (Popóvic et al., 2006), which need at least 20 mL essential oil kg^{-1} grain to obtain a similar mortality. Bittner et al. (2008) and Montenegro et al. (2012)

Table 1. Mortality by contact effect of essential oil of *Laurelia sempervirens* against adults of *Sitophilus zeamais*, lethal concentration 50% (LC_{50}) and emergence of adult insects (F_1).

Concentration	Mortality*	Emergence (F_1)*
mL essential oil kg^{-1} grain	%	%
0.00	0.0a	100.0a
1.25	5.0a	93.5a
2.5	80.0b	8.3b
5.0	85.0b	9.3b
10	100.0b	0.0b
20	100.0b	0.0b
40	100.0b	0.0b
CV, %	20.9	27.6
n†	100	
B ± SE¶	5.42 ± 0.43	
LC_{50}§	2.3	
(95% CL)&	1.48-3.60	
R^2	0.92	

*Values within a column with the same letter are not significantly different according to Tukey ($p \leq 0.05$).
CV: Coefficient of variation; R^2: coefficient of determination.
†Total number of insects treated.
¶Probit adjustment slope (b) and standard error of slope (SE).
§Lethal concentration = mL essential oil kg^{-1} grain.
&Confidence limits at 95%.

identified safrole as the main essential oil from foliage and bark of *L. sempervirens*, which according to Huang et al. (2002) has biological activity as contact insecticide against *S. zemais* and *T. castaneum*.

In insect adult emergence treatments, between 2.5 and 40 mL essential oil kg^{-1} grain are significantly similar and showed an emergence lower than 10% (Table 1). These results agree with Sabbour (2013), who documented that in grains treated with oil of *Jatropha curcas* L. (Euphorbiaceae) there were lower emergence of *S. oryzae* and *Ephestia küehniella* Zeller (Lepidoptera: Pyralidae) in comparison with untreated grains. Furthermore, Ngamo et al. (2007) indicated that sublethal doses of essential oil significantly reduced the amount of grain damage since the rate of oviposition was reduced.

Treatments over 0.25 mL essential oil kg^{-1} grain, the grain weight loss was significantly lower in comparison to the control (Table 2). The only treatment without significant difference with the control was 0.125 mL essential oil kg^{-1} grain that caused 12.3% of grain weight loss. Similar trend was observed with the powder of *P. boldus* (Pizarro et al., 2013) and tepa (*Laureliopsis philippiana* [Looser] Shodde; Monimiaceae) (Ortiz et al., 2012).

Germination of maize was not affected by the essential oil of *L. sempervirens*, since all treatments showed germination rates higher than 90%. No significant differences between all treatments and control were observed (p > 0.05). The international germination threshold required by seed exportation is 90% (González, 1995), so the essential oil of *L. sempervirens* could be used to protect maize used as seed. These results are similar to those obtained by researches using other essential oils from the Monimiaceae family (Betancur et al., 2010) as well as with the use of *P. boldus* powder (Pizarro et al., 2013) and *L. philippiana* leaf powder (Ortiz et al., 2012), since in all these cases seed germination was not affected.

In fumigant toxicity evaluations the highest mortality was 72.5% in treatment of 175 μL essential oil L^{-1} air (Table 3). This treatment was significantly more potent than other treatments which did not exceed 5% dead insects. The LC_{50} was 177 μL essential oil L^{-1} air with a minimum and a maximum value of 170.5 and 184 μL essential oil L^{-1} air, respectively (Table 4). The lower fumigant activity of *L. sempervirens* essential oil was documented by Bittner et al. (2008), who found that 8 μL L^{-1} air caused 20% *S. zeamais* mortality. The insecticidal activity of essential oil of *L. sempervirens* could be attributed to the presence of safrole (Bittner et al., 2009; Montenegro et al., 2012), which according to Huang et al. (1999) has shown fumigant toxicity against *S. zeamais* and *T. castaneum*. Although others species such as *Cuminum cyminum* L. (Apiaceae) (Chaubey, 2011), *Piper nigrum* L. (Chaubey, 2011) in concentrations of 60 μL essential oil L^{-1} air and *Piper hispidinervum* (C. DC.), *Piper marginatum* Jacq. (Coitinho et al., 2011) (Piperaceae), *Schinus terebinthifolia* Raddi (Anacardiaceae), and *Melaleuca leucandendra* (L.) L. (Myrtaceae) (Coitinho et al., 2011) with concentrations between 2.8 μL 40 g^{-1} essential oil showed higher toxicity. Perhaps because of the temperature, the essential oil of *L. sempervirens* did not reach the 90% of dead. Laznik et al. (2012) assessed the effect of five temperatures on insecticidal effect of four essential oils against *S. granarius* obtained better results at 40 ºC.

In residual effect concentrations of essential oil similar or higher than 1 mL essential oil kg^{-1} grain, sustains its

Table 2. Germination and weight loss of maize treated with *Laurelia sempervirens* essential oil to *Sitophilus zeamais* control.

Concentration	Grain germination*	Grain weight loss*
mL essential oil kg^{-1} grain	%	%
0.00	100.0a	13.2a
1.25	91.7a	12.3a
2.5	95.0a	1.7b
5.0	100.0a	1.4b
10	90.0a	0.1b
20	93.3a	0.1b
40	95.0a	0.1b
CV, %	8.75	45.7

*Values within a column with the same letter are not significantly different according to Tukey ($p \leq 0.05$).
CV: Coefficient of variation.

Table 3. Toxicity by fumigant effect of essential oil of *Laurelia sempervirens* against adults of *Sitophilus zeamais*.

Concentration	Mortality*
μL essential oil L^{-1} air	%
25	0.0a
50	0.0a
75	0.8a
100	0.8a
125	2.5a
150	4.2a
175	72.5c
CV, %	13.6
n†	60
B ± SE¶	10.7 ± 0.43
LC_{50}§	177
(95% CL)&	170.5-184.0
R^2	0.73

*Values within a column with the same letter are not significantly different according to Tukey ($p \leq 0.05$).
CV: Coefficient of variation; R^2: coefficient of determination.
†Total number of insects treated.
¶Probit adjustment slope (b) and standard error of slope (SE).
§Lethal concentration = μL essential oil L^{-1} grain.
&Confidence limits at 95%.

Table 4. Residual effect as contact insecticide of essential oil of *Laurelia sempervirens* against *Sitophilus zemais* adults.

	Mortality (%)			
Concentration	1 d	5 d	10 d	15 d
mL essential oil kg^{-1} grain				
1.25	8.8a	8.7a	8.2a	8.2a
2.5	100.0b	100.0b	100.0b	78.3b
5.0	100.0b	100.0b	100.0b	85.0b
10	100.0b	100.0b	100.0b	100.0b
20	100.0b	100.0b	100.0b	100.0b
40	100.0b	100.0b	100.0b	100.0b
CV, %	40.9	41.0	41.3	35.87

*Values within a column with the same letter are not significantly different according to Tukey ($p \leq 0.05$).
CV: Coefficient of variation.

insecticidal activity during 15 d (Table 4). Although concentrations of 0.25 and 0.5 mL essential oil kg^{-1} grain with a mortality of 78.3% and 85%, respectively, did not show significant differences ($p > 0.05$) with 1, 2, and 4 mL essential oil kg^{-1} grain of essential oil of *L. sempervirens*. Our results agree with those of Coitinho et al. (2010) that working with the essential oils of *P. hispidinervum*, *P. marginatum*, *M. leucadendra*, *S. terebinthifolia* and *Eugenia uniflora* L. (Myrtaceae) concluded that the contact insecticidal persistence does not reach 30 d.

In offspring effect (F_1) treatments with essential oil of *L. sempervirens* caused a reduction in the emergence of the next generation (F_1) of *S. zemais*. The F_1 emergence in all treatments was significantly lower than the one observed in the control (Table 5). The highest reduction of F_1 adult emergence (< 11%) was observed in treatments from 5 to 40 mL of essential oil kg^{-1} grain. The F_1 adult insect emergence was observed beyond week 5. As the concentration increased, the F_1 emergence decreased as described by Pizarro et al. (2013), who concluded that assessed treatments had toxic effect against immature stages of *S. zeamais*.

All evaluated treatments showed a repellent index lower than 1, and according to Mazzonetto and Vendramim (2003) these values are classified as repellents (Table 6). Using the essential oil of *P. boldus*, Betancur et al. (2010) at the same oil concentrations we used, obtained indexes between 0.79 and 0.16 indicating lower repellent activity than the one observed with the essential oil of *L. sempervirens*. According to Paranagama et al. (2004) this trend of increasing repellence with increasing dose of essential oil is common.

Table 5. Effect, under laboratory conditions, of essential oil of *Laurelia sempervirens* on *Sitophilus zemais* offspring (F_1).

	Emergence (F_1) (%)			
Concentration	Weeks 1, 2, 3, and 4	Week 5	Week 5	Week 5
mL essential oil kg^{-1} grain				
0.00	0.0a	100.0a	100.0a	100.0a
1.25	0.0a	4.8b	38.7b	56.7b
2.5	0.0a	4.9b	12.6c	43.6b
5.0	0.0a	0.0b	9.7cd	10.7c
10	0.0a	0.0b	0.0d	0.0c
20	0.0a	0.0b	0.0d	0.0c
40	0.0a	0.0b	0.0d	0.0c
CV, %	0.0	74.8	56.7	47.2

*Values within a column with the same letter are not significantly different according to Tukey ($p \leq 0.05$).
CV: Coefficient of variation.

Table 6. Repellence index of different concentrations of *Laurelia sempervirens* essential oil against *Sitophilus zeamais*.

Concentration	Repellence index* (IR)
mL essential oil kg^{-1} grain	
1.25	0.46
2.5	0.30
5.0	0.32
10	0.08
20	0.06
40	0.02

*IR = 1 Neutral (N), IR < 1 Repellent (R), IR > 1 Attracting (A).

CONCLUSIONS

The essential oil of *Laurelia sempervirens* has biological activity as a contact insecticide and repellent activity against *Sitophilus zeamais*, without affecting maize grain germination.

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

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

Bakkali, F., S. Averbeck, D. Averbeck, and M. Idoamar. 2008. Biological effects of essential oil-A review. Food and Chemical Toxicology 46:446-475.

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.

Bittner, M., M. Aguilera, V. Hernández, C. Arbert, J. Becerra, and M.E. Casanueva. 2009. Fungistatic Activity of essential oils extracted from *Peumus boldus* Mol., *Laureliopsis philippiana* (Looser) Schodde and *Laurelia sempervirens* (Ruiz & Pav.) Tul. (Chilean Monimiaceae). Chilean Journal of Agricultural Research 69:30-37.

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.

Chaubey, M.K. 2011. Fumigant toxicity of essential oils against rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Journal of Biological Sciences 11:411-416.

Coitinho, R.L., J.V. De Oliveira, M.G. Gondim, e C.A. Da Camara. 2010. Persistência de óleos essenciais em milho armazenado, submetido á infestaçao de gorgulho do milho. Ciência Rural 40:1492-1496.

Coitinho, R.L., J.V. De Oliveira, M.G. Gondim, e C.A. Da Camara. 2011. Toxicidade por fumigação e ingestão de óleos essenciais para *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera Curculionidae). Ciência e Agrotecnología 35:172-178.

Dongmo, H., H. Womeni, G. Piombo, N. Barouh, and L.A. Tapondjou. 2012. Bioefficacy of essential and vegetable oils of *Zanthoxylum xanthoxyloides* seeds against *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Journal of Food Protection 73:547-555.

Emeasor, K.C., R.O. Ogbuji, and S.O. Emosairue. 2005. Insecticidal activity of some seed powders against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) on stored cowpea. Journal of Plant Diseases and Protection 112:80-87.

Finney, D. 1971. Probit analysis. 272 p. Cambridge University Press, Cambridge, London, UK.

González, U. 1995. El maíz y su conservación. 399 p. Trillas, México DF, México.

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

Huang, Y., H. Ho, H. Lee, and Y. 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.

Huang, Y., H. Ho, and K. Manjunatha. 1999. Bioactivity of safrole and isosafrole on *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). Journal of Economic Entomology 92:676-683.

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

Isman, M.B. 2000. Plant essential oil for pest and disease management. Crop Protection 19:603-608.

Ko, K., W. Juntarajumnong, and A. Chandrapatya. 2009. Repellency, fumigant and contact toxicities of *Litsea cubeba* (Lour.) Persoon against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). Kaestsart Journal (Natural Sciences) 43:56-63.

Koul, O., S. Walia, and G.S. Dhaliwal. 2008. Essential oils as green pesticides: Potential and constraints. Biopesticides International 4:63-84.

Larraín, P. 1994. Manejo integrado de plagas en granos almacenados. IPA La Platina 81:10-16.

Laznik, Z., M. Vidrih, and S. Trdan. 2012. Efficacy of four essential oils against *Sitophilus granarius* (L.) adults after short-term exposure. African Journal of Agricultural Research 7:3175-3181.

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.

Montenegro, I., A. Madrid, L. Zaror, R. Martínez, E. Werner, H. Carrasco-Altamirano, et al. 2012. Antimicrobial activity of ethyl acetate extract and essential oil from bark of *Laurelia sempervirens* against multiresistant bacteria. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 11:306-315.

Ngamo, T.L.S., A. Goudoum, M.B. Ngassoum, P.M. Mapongmetsem, G. Lognay, F. Malaisse, et al. 2007. Chronic toxicity of essential oils of 13 local aromatic plants towards *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). African Journal of Agricultural Research 2:164-167.

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-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.

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

Paranagama, P.A., K.H.T. Abeysekera, L. Nugaliyadde, and K.P. Abeywickrama. 2004. Repellency and toxicity of four essential oils to *Sitophilus oryzae* L. (Coleoptera:Curculionidae). Journal of the National Science Foundation Sri Lanka 32:127-138.

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.

Pizarro, D., G. Silva, M. Tapia, J.C. Rodríguez, A. Urbina, A. Lagunes, et al. 2013. Actividad insecticida del polvo de *Peumus boldus* Molina (Monimiceae) contra *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 12:420-430.

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

Pretheep-Kumar, P., S. Mohan, and P. Balasubramanian. 2010. Insecticide resistance stored product insects. 64 p. Lambert Academics Publishing, Saarbrücken, Germany.

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.

Rees, P. 1996. Coleoptera. p. 1-40. In Subramanyam, B., and D. Hagstrum (eds.) Integrated management of insects in stored products. Marcel Dekker, New York, USA.

Roel, A., e J.D. Vendramim. 2006. Efeito residual do extrato acetato de etila de *Trichilia pallida* Swartz (Meliaceae) para lagartas de diferentes idades de *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae). Ciencia Rural 36:1049-1054.

Sabbour, M.M. 2013. Bioactivity of natural essential oils against *Sitophilus oryzae* and Ephestia *küeniella*. Scientia Agriculturae 1:15-20.

Somboon, S., and S. Pimsamarn. 2006. Biological activity of *Cleome* spp. extracts against the rice weevil, *Sitophilus oryzae* L. Agricultural Science Journal 37:232-235.

SAS Institute. 1998. Language guide for personal computers, release 6.03 edition. SAS Institute, Cary, North Carolina, USA.

Tripathi, A.K., S. Upadhyay, M. Bhuiyan, and P.R. Bhattacharya. 2009. A review on prospects of essential oils as biopesticide in insect-pest management. Journal of Pharmacognosy and Phytotherapy 5:52-63.