

Essential oil of *Cinnamodendron dinisii* Schwanke for the control of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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ABSTRACT: The use of natural compounds is a less aggressive alternative for the control of insects in stored grains, in relation to synthetic chemical agents. Plants with insecticidal properties can be used as a source of these compounds to the direct application in pest control. In this work, the essential oil of *Cinnamodendron dinisii* was chemically characterized and tested regarding its insecticidal and repellent effect on the control of *Sitophilus zeamais* in stored grains. The essential oil was obtained by hydrodistillation and analyzed by gas chromatography–mass spectrometry (GC-MS). The insecticidal potential was evaluated through the maintenance of the insects during 24 hours in contact with several doses of the oil, in the absence of feed substrate. The Bioassays of repellency were conducted with lethal doses (LD₅, LD₂₅, LD₅₀ and LD₉₅) obtained from insecticidal bioassay. In order to compare the treatments, the preference index (PI) was used. The essential oil of *C. dinisii* had insecticidal activity against *S. zeamais*, causing a linear and crescent mortality with LD of 0.04, 0.17, 0.34 and 0.63 µL/cm², respectively. The repellency ranged from 55.4% to 85.2%, using the LD values previously mentioned. The DL₅ was neutral regarding repellence (PI index -0,09), but from DL₂₅ on, the PI index was between -0.1 and -1.0, indicating repellence activity.

Keywords: essential oil, insecticidal, repellence, *Sitophilus zeamais*.

RESUMO: Óleo essencial de *Cinnamodendron dinisii* Schwanke para controle de *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). O uso de compostos naturais é uma alternativa menos agressiva para o controle de insetos em grãos armazenados em relação aos agentes químicos sintéticos. Plantas com propriedades inseticidas podem ser usadas como fonte destes compostos para a aplicação direta no controle de pragas. No presente trabalho, o óleo essencial de *Cinnamodendron dinisii* foi caracterizado quimicamente e testado em relação ao seu efeito repelente inseticida no controle de *Sitophilus zeamais* em grãos armazenados. O óleo essencial foi obtido por hidrodestilação e analisado por cromatografia gasosa-espectrometria de massa (GC-MS). O potencial inseticida foi avaliado pela manutenção dos insetos durante 24 horas em contato com várias doses de óleo, na ausência de substrato alimentar. Os bioensaios de repelência foram realizados com as doses letais (DL₅, DL₂₅, DL₅₀ e DL₉₅) obtidas do bioensaio inseticida. Para comparar os tratamentos foi utilizado o índice de preferência (PI). O óleo essencial de *C. dinisii* apresentou atividade inseticida sobre *S. zeamais*, causando mortalidade linear e crescente com DL de 0,04, 0,17, 0,34 e 0,63 µL/cm², respectivamente. A repelência variou entre 55,4% até 85,2%, utilizando os valores de DL acima mencionados. A DL₅ mostrou-se neutra em relação à repelência (índice PI -0,09), mas a partir de DL₂₅ o índice PI foi entre -0,1 e -1,0, indicando atividade de repelência.

Palavras-chave: óleo essencial, inseticida, repelência, *Sitophilus zeamais*.

INTRODUCTION

Insects' attacks are related to significant economic losses in stored grains (Tibola et al., 2009). *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), popularly known as maize weevil, is

one of the main pests of stored grains in Brazil, which present elevated biotic potential, cross-infestation and that housing inside the whole grains (Pimentel et al., 2009). Besides, it has great reproductive

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potential and colonizes many hosts as wheat, corn, rice, barley, triticale and oats (Lorini, 2008).

Insecticidal plants are an alternative to the control of insects' in stored grains, and can be used to the synthesis of new products or even in the direct control of pests. Secondary metabolites or bioactive compounds present in some botany extracts are toxic to insects by affect its development during the grain colonization (Michaelraj & Sharma, 2006; Azmir et al., 2013). The bioactive compounds can be extracted from leaves, stems, flowers and fruits (Azmir et al., 2013). In these sense, natural insecticides based on essential oil represent an option for the protection of stored products (Isman, 2000).

The efficiency of several vegetal species against *S. zeamais* and other grains-related pests has been demonstrated. Essential oils of *Elaeis guineensis* (palm oil) (Abulude et al., 2007), *Piper aduncum* and *Piper hispidinervum* (Piperaceae) (Estrela et al., 2006), *Vermonia amigdalya* (Compositae) (Asawalam & Hassanali, 2006), *Eucalyptus* spp (Myrtaceae) (Negahban & Moharramipour, 2007; Mossi et al., 2011), *Ocotea odorifera* (Lauraceae) (Mossi et al., 2014), were investigated to the control of *S. zeamais*. In all cases the insect was sensitive, at least in some degree, showing that *S. zeamais* can be controlled with bioactive compounds.

The use of natural products instead synthetic chemical pesticides is an alternative that can reduce the agriculture impact on the environment. The choice of native species as source of oil and/ or extracts employed in pest control could be a strategy to their sustainable use by local communities, and consequently contribute to their conservation. The *Cinnamodendron dinisii* Schwanke, popularly known as pimenteira, is a native tree of Atlantic Forest, encountered from southeast (Minas Gerais state) until the southern (Rio Grande do Sul state) of Brazil. Is a robust tree, with trunk clear and leaves with spice flavor. *C. dinisii* is abundant in Rio Grande do Sul state, inclusive in the neighborhood of agricultural regions (Souza & Lorenzi, 2012). The evaluation of the insecticide and repellent potential of essential oil of this species is relevant in order to support the development of molecules or products formulated for this purpose. In These sense, the objective of this work was characterize chemically the essential oil of *C. dinisii* and evaluate its insecticidal and repellent effect upon the control of *S. zeamais* in stored grains.

MATERIALS AND METHODS

Biological samples

Leaves of *Cinnamodendron dinisii* Schwanke (Canellaceae) was collected in Erechim, RS, Brazil (27°38'58.88"S, 52°16'12.52"W) in summer, always

in the morning. Exsiccates were identified and maintained at Padre Balduino Rambo Herbarium of URI – Erechim (HPBR n. 11.807). Following the collection, the vegetal samples were dried at 30-40°C until constant weight.

Extraction and characterization of essential oil

The essential oil of *C. dinisii* was extracted from aerial parts of the samples by hydrodistillation in Clevenger (Farmacopéia Brasileira, 1988). The oil was kept in glass recipient at -20° C until the subsequent assays. The chromatographic analyses of the essential oil was performed using a Shimadzu QP 5050A series gas chromatograph coupled to mass spectrometer (GC-MS), using a DB-5 fused silica capillary column (30m × 0.25 mm internal diameter × 0.25µm film thickness). Samples of 50.000 ppm dilluted in hexane were subjected to chromatography with flow of 1mL min⁻¹, 1.6 Kv detector and solvent cutting at 3.5 min. The temperature program used for the analysis was: initial temperature at 50°C, held for 3 min, and ramped at 5°C min⁻¹ to 130°C, then 20°C min⁻¹ to 250°C. Helium was used as a carrier gas at a flow rate of 1mL min⁻¹. The detector temperature was set to 250°C, and the injector temperature 240°C with injection port (split 1:20). The oil compounds were identified by comparison of mass spectra of each peak with those of authentic samples in a mass spectrum library (The Wiley Registry of Mass Spectral Data, 7th ed.), tables of Kovats indices (Adams, 2007) and references from literature.

Creation, development and maintenance of *S. zeamais*

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) were obtained in silos of corn storage. The insects were kept at 25°C (± 2°C) and 75% relative humidity in 1 liter glass bottle containing corn grains (Mossi et al., 2011). The corn used in the experiments was sterilized at -80°C (24 hours). For the assays, 20 adult insects were incubated in glass bottle with sterile corn during 15 days, until eggs outbreak. At this time, adult insects were discarded, using in the experiments, insects with 2 weeks of post-emergence (Mossi et al., 2014).

Evaluation of insecticidal activity

Essential oil of *C. dinisii* was applied in filter paper packed into circular plates, without feed substrate, on which were put glass beads to simulate grain presence. The tested doses were 115, 100, 85, 70, 55, 30, 15 e 5 µL/plate, corresponding respectively to 0.75, 0.65, 0.52, 0.45, 0.36, 0.19, 0.10 e 0.03 (µL cm⁻²). In each test were used 50

adult insect, not sexed. The plates were maintained in dark room, 20°C (± 2 C), and insect mortality was determined after 24 hour (Mossi et al., 2014). The experiments were repeated three independent times with 2 plates for each treatment. Were considered dead the insects who showed no reaction to the touch with metallic tweezers (Pinto Junior et al., 2010). The mortality curve was obtained plotting the oil dose applied against the number of death insects, which generate an equation used to calculate the lethal doses (LD₅, LD₂₅, LD₅₀ and LD₉₅). The statistical analyzes was performed by ANOVA plus Tukey test, with $p < 0.05$ considered significant.

Evaluation of repellent activity

The Bioassays of repellency were conducted

$$PI = \frac{\% \text{ of insects in test plates} - \% \text{ of insects in control plates}}{\% \text{ of insects in test plates} + \% \text{ of insects in control plates}}$$

Where PI values means:

- 1.00 to -0.10, repellent activity
- 0.10 to +0.10, neutral repellence
- +0.10 to +1.0, attract activity

RESULTS AND DISCUSSION

Extraction and characterization of *C. dinisii* essential oil

The yield of *C. dinisii* essential oil was 0.62% after 2 hour of extraction, which was higher than yield of 0.17% obtained by Torres et al. (2010) with *Capsicodendron dinisii* (synonymy of *C. dinisii*).

Based on chromatography analyses were identified 23 major compounds in the essential oil of *C. dinisii*, with predominance of bicyclogermacrene (26.19%), spathulenol (24.21%), terpinyl acetate (16.34%) and alpha terpineol (7.34%) (Table 1).

Chemical compounds were grouped into 4 classes: monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes. A fifty class formed by acetates (ester derived from monoterpene alcohols) was separated from oxygenated monoterpenes due its high concentration. As observed in Table 1, the majority fraction is composed by sesquiterpenes (37.92%) and oxygenated sesquiterpenes (27.28%) which corresponds to 65.20% of all identified compounds.

No data were found in the literature about insecticidal activity of the major compounds present in the essential oil analyzed in these work. However, it was described that α -terpinolene (Pietro et al., 2010), α -terpineol (Lima et al., 2009) and germacrene D and germacrene B (Bamontri & Mazoochi, 2009) have insecticidal properties and are present in the essential oil analyzed in this work, but in lower percentages, except the alpha terpineol which

in an arena with a central plate (14 cm diameter, 2 cm height, 153.9 cm², without feed substrate) interconnected to four plates disposed diagonally, with 20 g of corn each (Mossi et al., 2014), being that in two opposite plates were tested the lethal doses (LD₅, LD₂₅, LD₅₀ e LD₉₅) and the two other plates were used as control (without oil). Adult insects were starved of feed for 3 hours before the experiment (Tavares & Vendramim, 2005) and then put in the central plate (20 insects). After 24 hours, the number of insects that migrated to each peripherally plate was determined. The repellency assay was repeated three independent times with 2 plates for each treatment and the average percentage were used to calculate the preference index (PI) (Procópio et al., 2003):

showed percentage of 7.34%. Thus, the insecticidal action of *C. dinisii* oil (described below) is probably related to synergistic effect of several compounds instead action of major compounds acting isolated.

Insecticidal activity

The mortality of *S. zeamais* after 24 hours- exposure to different dosage of *C. dinisii* oil is shown in (Figure 1). This result demonstrate an insecticidal action with a linear correlation between oil dosage and insect mortality ($R^2 0.9503$). Based on the equation generated by mortality curve, LD₅₀ was established in 51.69 $\mu\text{L}/\text{plate}$ (0.34 $\mu\text{L}/\text{cm}^2$). The LD₉₅, LD₂₅, LD₅ were calculated in 97.04 $\mu\text{L}/\text{plate}$ (0.63 $\mu\text{L}/\text{cm}^2$), 26.50 $\mu\text{L}/\text{plate}$ (0.17 $\mu\text{L}/\text{cm}^2$) and 6.35 $\mu\text{L}/\text{plate}$ (0.04 $\mu\text{L}/\text{cm}^2$), respectively. These values of LD were subsequently used in the repellence assay.

Comparison between mortality produced by several concentrations of the essential oil is present in (Table 2). The doses of 100 and 115 $\mu\text{L}/\text{plate}$ resulted in 97.33% and 100% of lethality, respectively, not differing statistically each other, but differing in relation to the lower doses. In other words, 100 $\mu\text{L}/\text{plate}$ (0.65 $\mu\text{L}/\text{cm}^2$) is a dose sufficiently efficient to the control of *S. zeamais* in experimental conditions and could be used in mini silos and repellence assays.

The LD₅₀ obtained in this work (0.34 $\mu\text{L}/\text{cm}^2$) was similar to the values described with essential oil of other vegetal species that have good insecticidal activity upon *S. zeamais*, as *Schinus molle* that

TABLE 1. Compounds and their respective percentage in essential oil of *C. dinisii*.

Compound	Kovats Index	Peak area (%)
Terpinolene	1064	1.03
Camphor	1139	0.73
Alpha Terpineol	1148	7.34
Linalyl Acetate	1261	0.46
Isopulegyl Acetate	1275	2.72
Gama Elemene	1340	0.35
Alpha Cubebene	1345	0.16
6-Metil-5-hepten-6-one	1348	0.95
Terpinyl Acetate	1352	16.34
Alpha Copaene	1377	1.69
Geranyl Acetate	1382	1.25
Beta Elemene	1393	0.48
Iso Caryofyllene	1438	2.72
Alpha Humulene	1467	0.83
Beta caryophyllene	1470	0.34
Germacrene D	1487	1.14
Bicyclogermacrene	1517	26.19
Germacrene B	1562	0.49
Isoledene	1569	3.53
Caryophyllene Oxide	1573	0.75
Spathulenol	1619	24.21
Viridiflorol	1754	0.27
Drimenol	1764	2.05
Groups		
Monoterpenes		1.03
Oxygenated Monoterpenes		9.02
Monoterpene Esters		20.77
Sesquiterpenes		37.92
Oxygenated Sesquiterpenes		27.28
Total		96.02%

Kovats index (Adams, 2007).

showed LD₅₀ of 0.25 µL/ cm² (Fernandes & Favero, 2014); *Piper aduncum* and *Piper hispidinervum*, with LD₅₀ of 0.51 µL/ cm² and 2.85 µL/ cm², respectively (Estrela et al., 2006); *Eucalyptus saligna* with LD₅₀ of 0.36 µL/ cm² and *Cupressus sempervirens* LD₅₀ of 0.84 µL/ cm² (Tapondjou et al., 2005). These data indicate that several plants have potential to the control of *S. zeamais*, and then the choice of the best specie should take into account factors related to sustainable management, as regional availability and ecological importance of the species.

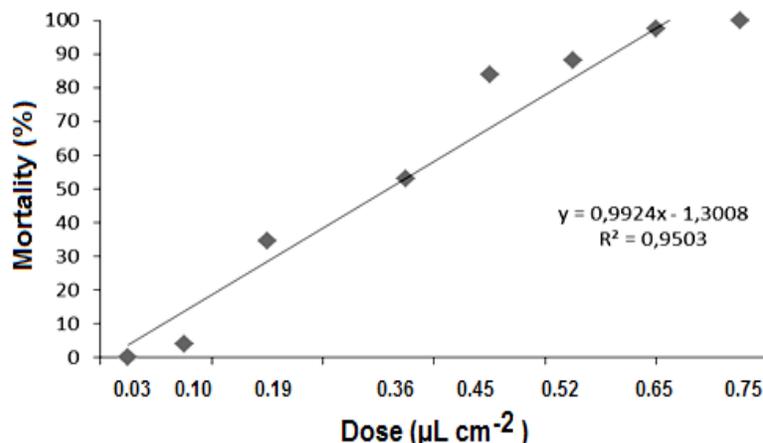
Repellence activity

The repellency assays of *C. dinisii* essential oil upon *S. zeamais*, were performed using LD₅, LD₂₅, LD₅₀ and LD₉₅ aforementioned, the data are

summarized in (Table 3).

Doses between 0.17 µL/cm² and 0.63 µL/ cm², show no statistical differences each other, presenting a good repellent effect of *C. dinisii* essential oil (around 72.9 and 85.2%). Similar data were obtained by Ootani et al. (2011) for *Cymbopogon nardus* and *Corymbia citriodora*. Oils of these two species had repellence between 86.6% and 98.8% upon *S. zeamais*, in concentrations range from 0.66 until 1.32 µL/ cm².

In the (Table 4), are shown the preference indexes (PI) of *S. zeamais* in relation to the essential oil dosage. The lower PI was obtained with 0.63 µL/ cm² (LD₉₅), pointing to the better repellent effect upon the insects. The concentration of 0.04 µL/ cm² (LD₅) was neutral in relation to the repellency and the

FIGURE 1. Mortality curve of *S. zeamais* treated with *C. dinisii* essential oil.**TABLE 2.** Insectidal action of *C. dinisii* essential oil upon *S. zeamais*.

Doses	Mortality (%) [*]
0.03 µL/cm ² (5 µL/plate)	0.0 ^f ± 0.0
0.10 µL/cm ² (15 µL/plate)	4.0 ^e ± 2.0
0.19 µL/cm ² (30 µL/plate)	34.7 ^d ± 5.0
0.36 µL/cm ² (55 µL/plate)	54.0 ^c ± 4.0
0.45 µL/cm ² (70 µL/plate)	84.0 ^b ± 4.0
0.52 µL/cm ² (80 µL/plate)	88.0 ^b ± 2.0
0.65 µL/cm ² (100 µL/plate)	97.3 ^a ± 1.1
0.75 µL/cm ² (115 µL/plate)	100.0 ^a ± 0.0

* The data are presented as media ± SD (standard derivation). Different letters indicate statistical differences at $p < 0.05$ (as analyzed by ANOVA plus Tukey test).

TABLE 3. Repellent activity of *C. dinisii* essential oil upon *S. zeamais*.

Dose	Repellency (%)
0.04 µL/cm ² (LD ₅)	55.4 ^b ± 9.3
0.17 µL/cm ² (LD ₂₅)	72.9 ^{a,b} ± 4.6
0.34 µL/cm ² (LD ₅₀)	78.3 ^a ± 9.8
0.63 µL/cm ² (LD ₉₅)	85.2 ^a ± 6.0

* The data are presented as media ± SD (standard derivation). Different letters indicate statistical differences at $p < 0.05$ (as analyzed by ANOVA plus Tukey test).

doses of 0.17 µL/cm² (LD₂₅) and 0.34 µL/cm² (LD₅₀) was intermediate effect of repellency.

The data shown a linear correlation between oil dosage and PI, i.e., as high was the dose of *C. dinisii* lower will be the PI of the insects, increasing the repellency.

There are no studies in the literature about *C. dinisii* effect upon *S. zeamais*, but the results obtained in the present work are similar to those

TABLE 4. Preference index (PI) of *S. zeamais* after treatment with different doses of *C. dinisii* essential oil.

Dose	Preference index (PI) [*]
0.04 µL/cm ² (LD ₅)	-0.09
0.17 µL/cm ² (LD ₂₅)	-0.37
0.34 µL/cm ² (LD ₅₀)	-0.48
0.63 µL/cm ² (LD ₉₅)	-0.62
Linear correlation between oil dosage and PI	$y = -0.8203x - 0.1501$ (R ² = 0,8626)

* PI values means: -1.00 to -0.10, repellent activity; -0.10 to +0.10, neutral repellence; +0.10 to +1.0, attract activity.

encountered with several species described as repellents against the refereed insect. Procópio et al. (2003) investigating the PI of six vegetal species upon *S. zeamais*, verified the better repellence with *Eucalyptus citriodora* (PI -0.81). Mossi et al. (2011), using LD₅₀ between 0.08 and 0.79 µL/cm², observed PI values from -0.50 until -0.69 for several *Eucalyptus* spp. Reichert et al. (2013) demonstrated that *Baccharis dracunculifolia* had PI ranging from -0.67 and -0.93 para *S. zeamais*, when tested in the concentration of 0.06 to 0.65 µL/cm².

Considering the possibility that insects can acquire resistance against actives principles from different origins, the study of essential oils can be an alternative to the seek new bioproducts used directly on insect control or even, new molecular models to the production of pesticides less aggressive to the environment. The essential oils and their byproducts have been considered a good choice to the insect's control, since they have quickly degradation, causing minor effects upon beneficial species of insects (Tripathi et al., 2009). Besides, the application of native species as source of oils and/ or extracts employed in favor of the agriculture

could be a strategy to their sustainable use by local communities, and consequently contribute to their conservation. In this study, the application of *C. dinisii* in the combat of *S. zeamais* showed promising results, which and could be explored to the adequate management of this tree in fragments of Atlantic Forest.

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