Insecticidal and repellent activity of the essential oil of *Cinnamomum camphora* var. *linaloolifera* Y. Fujita (Ho-Sho) and *Cinnamomum camphora* (L.) J Presl. var. *hosyo* (Hon-Sho) on *Sitophilus zeamais* Mots. (Coleoptera, Curculionedae)

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ABSTRACT: The aim of this work was to evaluate the insecticidal and repellency activity of the essential oil of *Cinnamomum camphora* var. *linaloolifera* Y. Fujita (Ho-Sho) and *Cinnamomum camphora* (L.) J Presl.var. *hosyo* (Hon-Sho), against the *Sitophilus zeamais* in maize grains. The essential oils were obtained by hydrodistillation and analyzed by GC-MS.The insecticidal activity was determined by the toxicity of different concentrations of essential oils during 24 hours of contact with the insects, in the absence of feed substrate. The Bioassays of repellency were conducted with lethal doses (LD_{50},LD_{25},and $LD_{12.5}$) obtained from insecticidal bioassay. In order to compare the treatments the preference index (PI) was employed. The analysis of the essential oils of *Cinnamomum camphora* leaves indicated 68% of camphor and 9% of linalool for the variation Hon-Sho and 95% of linalool to the variation Ho-Sho. The variation Ho-Sho presented greatest insecticidal activity than the variation Hon-Sho against the *Sitophilus zeamais*, with LD_{50} of 0.35 µL/cm^2, whereas in the variation Hon-Sho the ratewas 0.48 µL/cm^2. However, considering only the concentrations of linalool and camphor of Ho-Sho and Hon-Sho, the lethal doses' evaluation of these compounds were similar. The values of the preference index ranged from -0.3 to -0.8 for thevariation Ho-Sho and -0.2 to -0.7 for the variation Hon-Sho. The essential oils evaluated in this work showed repellent activity against *Sitophiluszeamais in vitro* and in trials performed in mini-silos.

Keywords: pest control; grains storage; maize.

RESUMO: Atividade inseticida e repelente do óleo essencial de *Cinnamomum camphora* var. *linaloolifera* Y. Fujita (Ho-Sho) e *Cinnamomum camphora* (L.) J Presl. var. *hosyo* (Hon-Sho) sobre *Sitophilus zeamais* Mots. (Coleoptera, Curculionedae). O objetivo deste trabalho foi avaliar a atividade inseticida e de repelência dos óleos essenciais de *Cinnamomum camphora* var. *linaloolifera* Y. Fujita (Ho-Sho) e *Cinnamomum camphora* (L.) J Presl. var. *hosyo* (Hon-Sho) contra *Sitophilus zeamais* em grãos de milho. Os óleos essenciais foram obtidos por hidrodestilação e analisados por CG-EM. A atividade inseticida foi determinada pela toxicidade de diferentes concentrações dos óleos essenciais durante 24 horas de contato com os insetos, na ausência de substrato alimentar. Os bioensaios de repelência foram realizados com as doses letais (DL_{50}, DL_{25}, e $DL_{12,5}$) obtidas do bioensaio inseticida. Para comparar os tratamentos foi utilizado o índice de preferência (PI). A análise de óleos essenciais de folhas de *Cinnamomum camphora* indicou a presença de 68% de cânfora e 9% de linalol na var. Hon-Sho e de 95% de linalol na var. Ho-Sho. A var. Ho-Sho apresentou maior toxicidade que var. Hon-Sho contra *Sitophilus zeamais*, com DL_{50} de 0,35 µL/cm^2, enquanto que na var. Hon-Sho foi de 0,48 µL/cm^2. No entanto, considerando apenas as concentrações de linalol e cânfora de Ho-Sho e Hon-Sho, a avaliação das doses letais destes compostos foram semelhantes. Os valores do índice de preferência variaram de -0,3 a -0,8 para a var. Ho-Sho e -0,2 para -0,7 para a var. Hon-Sho. Os óleos essenciais avaliados neste trabalho apresentaram atividade repelente contra *Sitophilus zeamais in vitro* e em experimentos realizados em mini-silos.

Palavras-chave: controle de pragas; armazenamento de grãos; milho.

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INTRODUCTION

In order to protect agricultural crops, high amounts of synthetic pesticides are used around the world. Nowadays, the maize weevil, *Sitophilus zeamais* – Motsch 1855, is the most relevant pest found during storage of maize grains (Mossi et al., 2011).

Aromatic plants and their essential oils have been used since remote years in flavor and fragrances, as condiment or spice, in medicines, as antimicrobial/insecticidal agents, and to repel insect or protect stored products (Dorman & Deans, 2000; Bakkali et al., 2008). These essential oils represent effective alternatives to synthetic pesticides without producing adverse effects on the environment (Isman, 2000; Isman & Machial, 2006; Kordali et al., 2006). The repellence of insects with essential oils is an interesting, relatively new tool for the control of insects that mostly does not share the insecticides traditional problems have in relation to environmental and human toxicity (Isman, 2006).

Cinnamomum camphora, Lauraceae, is a Chinese medicinal plant widespread in China. The essential oils, compounds and their antimicrobial, antifungal insecticidal as well as repellent activity has been reported (Liu et al., 2001; Liu et al., 2006; Wang et al., 2005), but their effect in agricultural crops pests is not known.

In these work it was used var. Hon-Sho (*Cinnamomum camphora* (L.) J Presl. var. *hosyo*), which is high in camphor and Ho-Sho (*Cinnamomum camphora* var. linaloolifera Y. Fugita) which is high in linalool (Frizzo et al., 2000). These varieties are similar morphologic, however, the composition of essential oils is distinct, being considered physiologic varieties.

Essential oils from leaves of *Artemisia princeps* and seeds of *Cinnamomum camphora* displayed good repellent and insecticidal activities against storage pests *Sitophillus oryzae* and *Bruchus rugimanus*, which mixture was better (Liu et al., 2006).

Essential oils of *Cinnamomum camphora*, *Cymbopogon winterianus*, *Matricaria chamomilla*, *Mentha viridis*, *Prunus amygdalus*, *Rosmarinus officinalis* and *Simmondsia chinensis* were evaluated for their insecticidal and repellent properties against adults of *Rhyzopertha dominica*. Results indicated that adults of *R. dominica* were less susceptible to the different concentrations of tested essential oils as mortality was low (<10%), however, *C. camphora* was more effective as a repellent to *R. dominica* (Al-Jabr, 2008).

In this context, the aim of this work was to evaluate the insecticidal and repellency activity of essential oil of *Cinnamomum camphora* var. Ho-Sho and Hon-Sho, against the *Sitophilus zeamais* in maize grains.

MATERIAL AND METHODS

Plant materials

The essential oils of *Cinnamomum camphora*, var. Ho-Sho and Hon-Sho, were obtained from the Instituto de Biotecnologia da Universidade de Caxias do Sul (IB/UCS), Brazil and identified by Dr. Gabriel Pauletti. The dried leaves were hydro-distilled to extract the essential oil using a Clevenger-type apparatus for 4 h. The extraction conditions were established as 100 g of air-dried sample and 1:30 plant material/water volume ratio. Anhydrous sodium sulfate was used to remove the water after extraction. The extracted essential oil was stored in a refrigerator at 4 °C until analysis.

Characterization of the essential oils

The chromatographic analyses of the essential oils were performed using a Shimadzu QP 5050A series gas chromatograph (GC-MS; Osaka, Japan), using a DB-5 fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The temperature program used for the analysis was initial temperature at 50 °C, held for 3 min, and ramped at 4 °C/min to 300 °C. Helium was used as carrier gas at a flow rate of 1 mL/min. The detector temperature was set to 320 °C, and the injector temperature 280 °C with injection port (split 1:20). The identification of compounds was made by comparing the mass spectra obtained with those from the Wiley library and the retention time of analytical standards. The content of the major components of each extract is expressed as peak area percent.

Insects

Sitophilus zeamais was cultured in a controlled temperature and humidity chamber (25 ± 1 °C and 70–75 % relative air humidity (rh) in darkness. Parent adults were obtained from the laboratory stock cultures and kept at the EMBRAPA, Passo Fundo, RS, Brazil. The food media used were whole maize grains (Prates & Santos, 2000). The adults used in the experiment were 2–3 weeks post-emergence.

Contact toxicity on filter paper

The contact effect of essential oils against *Sitophilus zeamais* was evaluated on filter paper discs (Whatman N°1) (14 cm diameter, surface 153.9 cm^2), which were treated with different essential oil dosages of Ho-Sho and Hon-Sho varieties (*C. camphora*). The filter papers were placed in glass Petri dishes (14 cm diameter). An aliquot of 0µL (negative control), 10, 25, 50, 75, 100, 125, 150, 175 and 200 µL of each essential oil was applied to the filter paper discs corresponding to dosages of 0, 0.07, 0.16, 0.32, 0.49, 0.65, 0.81, 0.97, 1.14 and

1.3 µL/cm^2. An amount of 50 unsexed adults (3–7 days old) insect species (*Sitophilus zeamais*) was used separately into each dish and these were kept in darkness in the laboratory at 25 ± 1 °C and 70-75 % rh. The experiment was conducted in triplicate. Insect mortalities were analyzed after 24 hours. The percentage insect mortality was calculated using the Abbot correction formula for natural mortality in untreated controls (Abbot, 1925). The mortality rates were statistically evaluated by analysis of variance (ANOVA) followed by the Tukey test, using a confidence level of 95 % and by regression analysis.

Assessment of lethal doses and repellency bioassay

The different 50% lethal doses (LD_{50}) were calculated using probit analysis (Finney, 1971). The values of LD_{50} calculated for each var. Ho-Sho e Hon-Sho (*C. camphora*), were evaluated in triplicate in the repellence bioassay.

The repellence action of the essential oils was carried out in triplicate, using a system containing five Petri plates (diameter=14 cm), codified as A, B, C, D and E. In plates A and B, 10 g of maize and the essential oils were added with dose of LD_{50}, half of LD_{50} and quarter LD_{50}. In plates C and D (blanks) only the substrate was added and in plate codified as E (central), 50 adults of *Sitophilus zeamais* were delivered and after 24 hours the number of insects were counted. Each dish was kept in darkness at 25 ± 1 °C and 70–75% rh. Aiming to compare the different treatments, the Preference Index (PI) was established, as cited by Procópio et al. (2003), defined as:

$$PI = \frac{\text{\% of insects of test plants - \% of insects of blank experiment}}{\text{\% of insects of test plants + \% of insects of blank experiment}}$$

Where:

PI of -1.00 to - 0.10, test plant repellent;

PI of -0.10 to +0.10, test plant neutral;

PI of+0.10 to +1.00, test plant attractant.

To simulate the repellent effect of essential oils in field conditions, repellency bioassays were also performed in minisilos. In this experiment, the Petry plates were replaced by silos with 6 cm diameter x 4.5cm height (95.4 cm^3) containing 50 g of grain in each container (proportional dimensions to real silos).The different doses (0.12, 0.25, 0.37, 0.50, 0.75,1.00, 1.50 and 3.00 µL/cm^3) were applied directly to the grain at two opposite silos. The other procedures were kept identical to the previous experiment.

RESULTS AND DISCUSSION

Chemical constituents of the essential oils

The main compounds of essential oil from Hon-Sho and Ho-Sho leaves were characterized by more than 94% of monoterpenes. Hon-Sho leaves oil were composed by 68% of camphor and 9% of linalool. The Ho-Sho leaf essential oil was almost exclusively formed by linalool (95%), with no other constituent representing more than 1%. These essential oils are similar to the most common compositions described in the literature (Fujita et al., 1974; Dung et al., 1993), where linalool content ranged 66 to 91%.

Filter paper contact toxicity

The essential oil of the *Cinnamomum camphora* var. Ho-Sho was more efficient than var. Hon-Sho against *Sitophilus zeamais* (Table 1).

The significantly higher percentage of mortality (p<0.05) after 24 hours of exposure, were obtained with concentrations up 0.81 and 1.14 µL/cm^2, respectively.

The linear regression equation of *S. zeamais* mortality was Y = 74.35X + 18,68 (R^2 = 0.903) for Ho-Sho essential oil and Y = 78.40X + 8.523 (R^2 = 0.954) for Hon-Sho essential oil, indicating a dose-dependent response in mortality rates of *S. zeamais*.

The results obtained in this work are interesting when compared with some authors as Tapondjou et al. (2005). These authors Showed that 1.56 µL/cm^2 of the essential oil of *Eucaliptus saligna* was necessary to obtain 100% of mortality of *S. zeamais* in the fourth day of exposure and 0.78 µL/cm^2 of the essential oil of *Cupressus sempervirens* to obtain 100% of mortality in the fifth day.

Lethal doses assessment

The probit analysis showed LD_{50}=0.35 ± 0.02 µL/cm^2 for Ho-Sho and LD_{50}=0.48 ± 0.02 µL/cm^2 for Hon-Sho, indicating that Ho-Sho essential oil was more toxic than Hon-Sho essential oil.

However, it could be seen that, considering only the linalool and camphor concentrations, the Lethal doses assessment (LD_{50}) are quite similar. Ho-Sho essential oil have LD_{50}=0.35 µL/cm^2 with 95% of linalool which corresponds to 0.33 µL/cm^2 of linalool and the Hon-Sho have LD_{50}=0.48 µL/cm^2, with 68% of camphor which corresponds to 0.326 µL/cm^2 of camphor, for lethal dose (LD_{50}).

Repellency

Based on the Preference Index (Table 2), the essential oils tested in this work were efficient against the adults of *Sitophilus zeamais*. The values of the Preference Index of essential oils ranged from -0.3 to -0.8 for var. Ho-Sho and -0.2 to -0.7 for var. Hon-Sho. According Procópio et al. (2003) an oil

TABLE 1. Percentage of mortality of *Sitophilus zeamais* in stored maize grains after 24 hours of exposure under different doses of essential oils of Ho-Sho and Hon-Sho.

Concentration (μL/cm²)	% of mortality after 24 h of exposure	
	Ho-Sho	Hon-Sho
control	$0^{f} \pm 0$	$0^{g} \pm 0$
0.07	$20.7^{e} \pm 3.1$	$5.3^{g} \pm 2.5$
0.16	$42.3^{d} \pm 3.2$	$21.0^{f} \pm 4.4$
0.32	$47.3^{cd} \pm 4.9$	$40.0^{e} \pm 4.6$
0.49	$53.0^{c} \pm 3.0$	$53.3^{d} \pm 4.7$
0.65	$70.0^{b} \pm 2.6$	$69.3^{c} \pm 4.7$
0.81	$96.3^{a} \pm 2.5$	$80.7^{b} \pm 3.2$
0.97	$96.7^{a} \pm 3.0$	$85.0^{b} \pm 3.5$
1.14	$100^{a} \pm 2.0$	$96.0^{a} \pm 2.6$
1.30	$100^{a} \pm 1.6$	$98.0^{a} \pm 2.0$

* Means followed by different letters in column are significantly different by Tukey test (p<0.05).

TABLE 2. Efficiency of the treatments of repellency activity with different doses of essential oil of *Cinnamomum camphora* Nees & Eberm, var. Ho-Sho and Hon-Sho against *Sitophilus zeamais* in stored maize grains.

Concentration	Ho-Sho		Hon-Sho	
	(μL/cm²)	PI*	(μL/cm²)	PI
LD_{50}	0.35	-0.8	0.48	-0.7
LD_{25}	0.18	-0.5	0.24	-0.5
$LD_{12.5}$	0.09	-0.3	0.12	-0.2

*Preference Index (PI)

should have preference index lower than -0.10 to consider repellency activity.

Procópio et al. (2003) evaluated the repellency effect of fragments of leaves of six vegetables species against adults of *Sitophilus zeamais* and verified, based on the PI, that two species caused repellency, the leaves of *Eucalyptus citriodora* (Myrtaceae) (PI = -0.81) and *Capsicum frutescens* (Solanaceae) (PI= -0,17).

The highly toxic and repellent effects of main constituents of these oils, camphor and linalool, have been demonstrated by other researchers (Chen et al., 2013; Ajayi et al., 2014). Linalool was shown to exhibit fumigant toxicity against *S. zeamais* (Wang et al. 2011; Liu et al., 2013; Yildirim et al., 2013) and the *C. camphora*, with camphor as major component, showed repellent activity (Liu et al., 2006) and high insecticidal activity against *S. oryzae* (Hamed et al., 2012).

When assessing the repellency performed in silos, was observed effect at doses above 0.75 and 1.00 μL/cm³ for Ho-Sho and Hon-Sho, respectively (Table 3).

The lower doses were shown to be neutral but not attractive. Extrapolating the results, would be needed a minimum of 1.43 and 1.91L/ton of Ho-Sho and Hon-Sho essential oil, respectively for repelling *Sitophilus zeamais* in field conditions.

TABLE 3. Efficiency of the treatments of repellency activity with different doses of essential oil of *Cinnamomum camphora* Nees & Eberm, var. Ho-Sho and Hon-Sho against *Sitophilus zeamais* in maize storage silo.

Concentration (μL/cm³)	Ho-Sho	Hon-Sho
	PI*	PI
0 (control)	0.5	0.7
0.12	0.4	0.3
0.25	0.1	0.2
0.37	0.1	0.1
0.50	-0.1	0.2
0.75	-0.3	-0.1
1.00	-0.5	-0.3
1.50	-0.7	-0.5
3.00	-0.7	-0.5

*Preference Index (PI)

The comparison between the composition of the different essential oils evaluated in this work,

showed that both Ho-Sho, rich in linalool, as Hon-Sho, rich in camphor, have insecticidal and repellent activity against *Sitophilus zeamais*.

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