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Insecticide activity of clove essential oil on bean weevil and maize weevil

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grain storage

ABSTRACT

Bean weevil and maize weevil can cause considerable damage to stored grains. These insects are mainly controlled with synthetic chemical insecticides, which may bring serious problems to human and environmental health. Therefore, this study aimed to evaluate the efficiency of the essential oil of clove [*Syzygium aromaticum* (L.) Merrill & Perry (Myrtaceae) (origin: Bahia, season Sep.2014-Feb.2015)] in the control of *S. zeamais* and *A. obtectus* under laboratory conditions. The essential oil was extracted through the classic hydrodistillation process and its chemical components were identified via gas chromatography. Oil efficiency was tested at the doses of 35, 17.9, 8.9, 3.6, 1.8, 0.4 and 0.2 μL g^{-1} (derived from a pilot study) for insect control and the LC_{50} was determined. The results showed that eugenol was the major compound. The essential oil caused mortality of 100% for both species 48 h after treatment with the concentrations of 17.9 and 35 μL g^{-1}. The LC_{50} for *A. obtectus* was 9.45 μL g^{-1}, against 10.15 μL g^{-1} for *S. zeamais*. The use of clove essential oil represents a promising alternative to be used under storage conditions for the integrated management of stored grains pests.

Palavras-chave:
estratégias de controle
infestação por insetos
armazenamento de grãos

Atividade inseticida do óleo essencial de cravo-da-índia sobre o caruncho-do-feijão e o gorgulho-do-milho

RESUMO

O caruncho-do-feijão e o gorgulho do milho podem causar grandes prejuízos aos grãos armazenados. Entre as alternativas de controle está a utilização de óleos essenciais de plantas com propriedades inseticidas; objetivou-se, assim, avaliar a eficiência do óleo essencial de cravo-da-índia no controle de *S. zeamais* e *A. obtectus* em condições de laboratório. O óleo essencial foi extraído por processo clássico de hidrodestilação e seus constituintes químicos foram identificados por cromatografia gasosa. A eficiência deste óleo foi testada nas doses 35; 17,9; 8,9; 3,6; 1,8; 0,4 e 0,2 μL g^{-1} (oriundas de um trabalho piloto) no controle dos insetos e determinada a CL_{50}. Os resultados mostraram que o eugenol foi o composto majoritário. O óleo essencial causou 100% de mortalidade para as duas espécies 48 h após o tratamento com as concentrações de 17,9 e 35 μL g^{-1}. A CL_{50} para *A. obtectus* foi 9,45 μL g^{-1} contra 10,15 μL g^{-1} do *S. zeamais*. A utilização do óleo essencial de cravo-da-índia representa uma alternativa promissora a ser usada em condições de armazenagem para o manejo integrado de pragas de grãos armazenados.

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Introduction

Many internal and external factors can compromise qualitative and quantitative characteristics of stored grains even after drying. Among these factors, pest insects stand out, which, besides attacking many crop development stages at the field, also damage the stored grains, thus being considered as cross-infestation pests (Scheepens et al., 2011). Among these pests, the bean weevil [*Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Chrysomelidae)] and the maize weevil [*Sitophilus zeamais* Motschulsky, 1885 (Coleoptera: Curculionidae)] stand out. They open galleries in the grains causing commercial depreciation, which is due to a series of characteristics of these insects, such as high biotic potential, capacity to attack grains both at the field and in deposits and capacity to survive at great depths inside the mass of grains (Faroni, 1992; Martins & Oliveira, 2009). The mean quantitative losses caused by pests in Brazil are estimated at approximately 10.0% of the total produced annually. This represents about 9.8 million tons per year, according to FAO and the Brazilian Ministry of Agriculture, Livestock and Supply (Lorini, 2005).

For the control of insects in stored grains, synthetic chemical products belonging to different toxicological classes are used. Despite the relative efficiency of these products, the intensive use can cause many problems, such as the occurrence of resistance in the insects, accumulation of residues in foods, damage to human health, environmental contamination, besides the increase in production costs (Campos et al., 2013). One alternative to the conventional control is the use of plants with insecticide properties, whose parts can be prepared and applied as powders, extracts and oils. These products have the advantages of low cost, easy acquisition and use, their application does not require qualified personnel and they do not have impacts on human health and the environment (Hernández & Vendramim, 1997; Mazzonetto & Vendramim, 2003).

Essential oils have multiple action mechanisms on the insects, such as acute toxicity, repellence, feeding reduction (deterrence), growth inhibition and limitations in development and reproduction (Coast, 1994). The essential oils of plant species belonging to the families of Asteraceae, Ranunculaceae, Myrtaceae, Brassicaceae, Apiaceae, Piperaceae, Lamiaceae, Lauraceae and Verbenaceae have shown repellence against insects of the order Coleoptera (Nerio et al., 2009). Clove [*Syzygium aromaticum* (L.) Merrill & Perry (Myrtaceae)] stands out among the plant species producing essential oils with insecticide potential for pest control (Ho et al., 1994; Paranhos et al., 2006; Correa, 2011; Afonso et al., 2012). The importance of the composition of clove essential oil must be highlighted and it varies according to the plant part where it is extracted from: sun-dried leaves, oven-dried leaves, peduncle and dried flower buds (Oliveira et al., 2009).

Although the previously mentioned species produce essential oils and their compositions have compounds with insecticide properties, little is known with respect to the effectiveness of these products in the control of pest insects of stored goods. Thus, this study aimed to evaluate the efficiency of clove essential oil in the control of *S. zeamais* and *A. obtectus,* under laboratory conditions.

Material and Methods

The experiment was conducted in the Laboratory of Insect Ecology, at the Federal University of Pelotas (UFPel) (Pelotas, RS) in 2014. The insects used in the experiment, *S. zeamais* and *A. obtectus*, were obtained from the insect rearing maintained by this laboratory. The experiment was set in a completely randomized design, with eight treatments and four replicates.

The specimens of *S. zeamais* and *A. obtectus* were maintained in laboratory in grains of maize (*Zea mays*) and bean (*Phaseolus vulgaris*), respectively stored in glass pots with capacity for 1 kg, wrapped with voile fabric and fixed with a rubber band. For the insect rearing, 20 unsexed adult insects were placed in recipients containing grains for 15 days. Then, the insects were removed and only the eggs were left. This procedure allowed obtaining insects with the same age for the tests.

Clove essential oil was extracted at the Laboratory of Lipidomics and Bioorganic (LLipidomicsBio) of the UFPel, through hydrodistillation using a Clevenger extraction apparatus, attached to a 2000-mL volumetric flask, and a heating mantle was used as the heat source. Clove flower buds were obtained from a local market specialized in spices in Pelotas-RS. The material was identified and taken to the LlipidomicsBio of the Center of Chemical, Pharmaceutical and Food Sciences, where the samples were ground, dried and the oil was extracted. The material came from the state of Bahia (season of Sept. 2013-Feb. 2014). 100 g of the sample (dried flower buds), ground in a knife mill, were weighed and 1500 mL of distilled water were added. Then, the temperature of the electric mantle was adjusted to 100 °C and, after 4 h of distillation, the essential oil was collected, centrifuged and stored in refrigeration to avoid probable losses of volatile constituents (Brasil, 2010).

Clove essential oil components were characterized through gas chromatography with flame ionization detector (GC/FID) and gas chromatography attached to mass spectrometry (GC/MS). The sample of clove essential oil was diluted in acetonitrile in the proportion of 1:9 (oil:acetonitrile) for later chromatographic injection.

Volatile molecules of the sample were identified through GC/MS (Model QP2010 SE) using an auto-injector Shimadzu® (Kyoto, Japan). A column of fused silica (Rtx-5MS®) with length of 30 cm, internal film width of 0.25 μm and diameter of 0.25 mm was used for the injection 1 μL of volume in the split mode (1:50). Ultrapure helium was used as the carrier gas at a flow rate of 1.22 mL min^{-1}. The temperature of the injector and the transfer line was 280 °C. The temperature ramp started at 50 °C, maintained for 1 min, and then increased at a rate of 10 °C min^{-1} until 280 °C, maintaining this value for 11 min. The total run time was 35 min. The mass spectrometer operated in the scan mode in a range of 35-700 m/z for the identification of the substances.

For the quantification, a GC/FID device (Model GC-2010, Shimadzu® - Kyoto, Japan) was used, with a HP1 column (30 m x 0.32 mm i.d. x 0.25 μm dimethylpolysiloxane). Hydrogen was used as the carrier gas at a flow rate of 1 mL min^{-1}. The temperature ramp started at 40 °C; then, it was gradually increased at a rate of 10 °C min^{-1} until reaching 300 °C, maintaining this temperature for 10 min. The temperature of the injector and the detector was 280 °C. The total time of analysis of close essential oil through GC/FID was 36 min.

The tests to evaluate the insecticide activity were carried out in Petri dishes (90 x 15 mm), by mixing 20 g of beans to the doses (defined in a pilot study) of 35, 17.9, 8.9, 3.6, 1.8, 0.4 and 0.2 $\mu L\ g^{-1}$ of clove essential oil diluted in Tween® (Polysorbate 20, hydrophilic tensioactive) at 0.2% for 2 min. The experiment had a control treatment, which consisted of only non-treated bean grains. Ten unsexed adults of *A. obtectus* were added to each plot, with ages between 15 and 20 days. The same procedure was performed for *S. zeamais* in 20 g of maize. The dishes were sealed with a transparent tape and maintained in a climatized B.O.D. (biochemical oxygen demand) chamber at 25 ± 3 °C, with RU (relative air humidity) of 70 ± 10% and photophase of 12 h. The evaluations of susceptibility were performed 24 and 48 h after the treatments. Insects that did no move for two minutes were considered as dead (Antunes et al., 2013).

The efficiency of control (EC %) was calculated through the equation of Abbott (1925):

$$EC = \frac{T - Tr}{T} \times 100$$

where:

T - number of insects alive in the control; and
Tr - number of insects alive in the treatment.

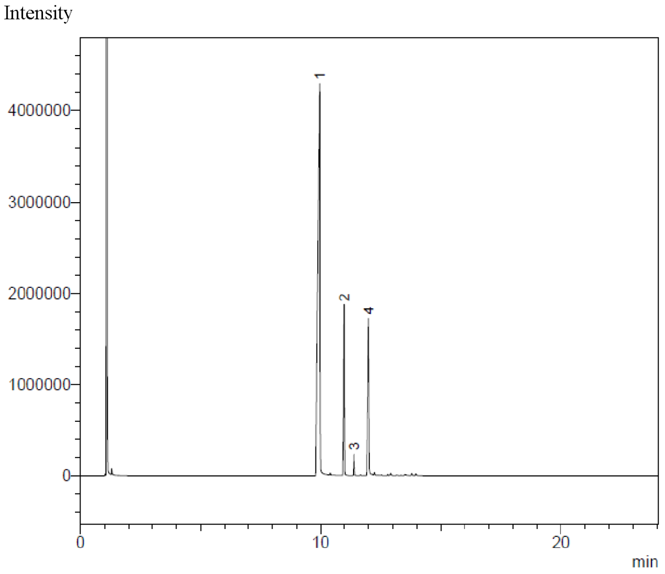
Mortality data were subjected to analysis of variance and the means transformed into $\sqrt{x + 0.5}$ were compared by Tukey test at 0.05 probability level ($p \leq 0.05$), using the program SAS® (SAS Institute Inc., 2000). The mean lethal concentration, sufficient to kill 50% of the population (LC_{50}), was also calculated through the correlation between the concentrations and cumulative mortalities of *A. obtectus* and *S. zeamais* 48 h after treatment. The LC_{50} was calculated using the statistical program GraphPad Prism Demo (version 5.0).

Results and Discussion

The results referring to the identification of components of the *S. aromaticum* essential oil are shown in Figure 1.

According to Table 1, the analysis of the essential oil of dried flower buds of *S. aromaticum* allowed the characterization of four components, with identification above 99%, among which eugenol was identified as the major component at the concentration of 62.72% (peak 1), then caryophyllene (18.46%), α-caryophyllene (2.84%) and eugenol acetate (15.97%). These values can vary depending on plant part and the condition in which the extraction is performed, such as sun-dried leaves, oven-dried leaves, peduncle and dried flower buds (Oliveira et al., 2009).

The results of clove essential oil composition characterized by gas chromatography, in this study, indicated eugenol as the major component, which was also observed in the study of Cunha et al. (2012). The literature points to caryophyllene, α-caryophyllene and eugenol acetate as molecules with relative abundancy in this type of matrix. Other compounds are also present, but not in significant proportions (Santos et al., 2007).



1) Eugenol; 2) Caryophyllene; 3) α-caryophyllene; 4) Eugenol acetate

Figure 1. Chromatogram of the eugenol sample. Characterization of eugenol and derivatives of clove (*Syzygium aromaticum*) essential oil (dried flower buds obtained from the market in Pelotas-RS) performed through GC/FID

Table 1. Percentage of components in the essential oil of clove (*Syzygium aromaticum*) (dried flower buds) as a function of the retention time, performed through gas chromatography

Peaks	Names	Retention time (min)	Height	Area	Concentration (%)
1	Eugenol	9.96	4246585	25652213	62.72
2	Caryophyllene	10.98	1865255	4499842	18.46
3	α-caryophyllene	11.39	233282	510792	2.84
4	Eugenol acetate	11.99	1717661	6518306	15.97
Total			8062783	37181153	99.00

As observed in Table 2, there were no significant differences between the concentrations of 35 and 17.9 $\mu L\ g^{-1}$ of clove essential oil, with respect to its insecticide activity 24 h after treatment application. In other words, both were different compared with the other concentrations and the control, reaching mean cumulative mortalities for *A. obtectus* of 67.5 and 62.5%, respectively.

A. obtectus mortality 48 h after the treatments was higher at the concentrations of 17.9 and 35 $\mu L\ g^{-1}$ with cumulative values of 95 and 100%; at the third concentration (8.9 $\mu L\ g^{-1}$), the cumulative mortality 48 h after treatment was 45% and there was a significant difference in relation to the other treatments. The results show that *A. obtectus* mortality increases with the increment in the period of exposure to clove essential oil; thus, since there is a response to the increase in concentration, this is perceptible because the concentration of 0.2 $\mu L\ g^{-1}$ does not cause mortality when evaluated for the same period of exposure of the others (Table 2).

The cumulative mortality of *S. zeamais* was similar to that of *A. obtectus* (Table 2), reaching 67.5 and 62.5%, 24 h after treatment, and 100 and 82.5%, 48 h after treatment, for the concentrations of 35 and 17.9 $\mu L\ g^{-1}$, respectively. For both species, clove essential oil caused mortality of up to 100% at

Table 2. Cumulative mortality ± Standard error (SD) of *Acanthoscelides obtectus* and *Sitophilus zeamais*, as a function of concentrations of the essential oil of *Syzygium aromaticum* applied to bean and maize grains, 24 and 48 h after the treatment

Species	Concentration (μL g^{-1})	Mean cumulative mortality (%) ±SD	
		24 h	48 h
A. obtectus	35.0	67.5±0.4 a	100±0.0 a
	17.9	62.5±0.5 a	95.0±0.3 a
	8.9	32.5±0.4 b	45.0±0.4 b
	3.6	7.5±0.2 c	10.0±0.0 c
	1.8	5.0±0.3 c	7.0±0.3 c
	0.4	5.0±0.3 c	5.0±0.2 c
	0.2	0.0±0.0 c	0.0±0.0 c
	0.0	0.0±0.0 c	0.0±0.0 c
	CV (%)	17.32	23.09
S. zeamais	35.0	67.5±0.4 a	100±0.0 a
	17.9	62.5±0.4 a	82.5±0.2 a
	8.9	32.5±0.4 b	40.0±0.6 b
	3.6	5.0±0.3 c	7.5±0.2 c
	1.8	5.0±0.3 c	5.0±0.3 c
	0.4	2.5±0.1 c	2.5±0.3 c
	0.2	0.0 ±0.0 c	0.0±0.0 c
	0.0	0.0±0.0 c	0.0±0.0 c
	CV (%)	18.3	25.07

CV – Coefficient of variation; Means followed by the same letter in the columns do not differ significantly by Tukey test at 0.05 probability level

the maximum concentration (35 μL g^{-1}), 48 h after applying the product; however, there was no significant difference at the concentration of 17.9 μL g^{-1}.

The insecticide activity of clove oil was confirmed by Paranhos et al. (2006) in a study testing the effect of clove essential oil on *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae). These authors observed high insect mortality and decrease of oviposition by females when treated with this oil. This effect can be due to the characteristics of the chemical compounds present in the composition of this oil, especially eugenol, one of the main compounds in clove essential oil (Huang et al., 2000).

The fumigation and contact effects of essential oils and their chemical constituents are still little studied with respect to the immature stages of *S. zeamais* and there are more studies with other pests. Rajendran & Sriranjini (2008), in a study with *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) and *A. obtectus*, Fabricius (1975) observed that the active stages (adults and non-diapausing larvae) are more susceptible than the sedentary stages (eggs and pupae), due to the differences in respiratory rates.

Immediately after treatment application on the populations of *A. obtectus* and *S. zeamais* on the Petri dish, there was an intense agitation of the insects, which moved randomly around the recipient. After 5 min, the insects tried to escape through the surface of the dish. Four hours later, almost all the insects were motionless and apparently dead and, after 48 h of exposure, a mortality of 100% was observed for the highest concentrations in both populations. This behavior was probably favored by the effects of fumigation and contact of clove essential oil. According to El-Nahal et al. (1989), the period of exposure to the essential oil is more important than the applied dose; however, according to the results obtained under the conditions of this study, the period of exposure follows the applied dose in terms of efficiency (Table 2).

Rahman & Schmidt (1999), in study with *Acorus calamus* (L.) (Acoraceae) essential oil in the form of vapor, observed that the highest mortality of *Callosobruchus phaseoli* (Gyllenhal, 1833) (Coleoptera: Chrysomelidae) was related to the increase in the period of exposure to the oil. Campos et al. (2013) evaluated the repellent and insecticide activity of the essential oil of *Baccharis articulata* (Lam.) Pers. on the bean weevil (*A. obtectus*) and observed that the dose of 52 μL caused insect mortality of 90% with exposure time of 32 h. Smaniotto et al. (2010) observed the bioactivity of *Cabralea canjerana* (Vell.) Mart. (Meliaceae) on *A. obtectus* and observed efficiency of 100% for the raw extract (concentration of 1%), followed by the hexane extraction (concentration of 1%), with efficiency of 84.2%. The fractions of ethyl acetate, chloroform and essential oil showed the lowest efficiencies in the control of this insect.

Some essential oils cause mortality, repellence, growth effects and reductions in oviposition and emergence. In another study, Savaris et al. (2014) observed efficiency of 100% of essential oil of *Cunila angustifolia* Benth (Lamiales: Lamiaceae) in the control of *S. zeamais*, and the major component was pulegone with participation of 56.1% in the composition of its oil. This was also observed in a study conducted by Savaris et al. (2012), in which all the doses of *C. angustifolia* essential oil showed efficiency of 100% for the mortality of *A. obtectus* after 24 h; the other treatments showed low efficiency, compared with the effect of this essential oil.

In this experiment, 100% mortality was observed at the concentration of 35 μL g^{-1} of clove essential oil. A similar study was performed by Prado et al. (2013), testing the insecticide effect of the essential oil of *C. angustifolia* in the control of *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera: Tenebrionidae), besides an efficiency of 100% for the mortality of larvae and adults, at the concentrations of 5 and 10%.

Afonso et al. (2012) confirmed the effects of methanol and hexane extracts of *S. aromaticum* on the repellence of maize weevil in rice grains, and eugenol had effect on the adults. In another study, Ho et al. (1994) reduced significantly the emergence of this insect in rice grains treated with hexane and methanol clove extracts at the dose of 1 mL 100 g^{-1} of rice.

In addition, in other studies (Nerio et al., 2009; Correa, 2011) showing the potential of use of clove essential oil, whether by contact, repellence or fumigation, the authors observed that clove essential oils can become an alternative to the conventional insecticides for the mortality of populations of *A. obtectus* and *S. zeamais*. However, for the application of the essential oil, it is necessary to develop technologies that allow greater permanence of the compounds close to the mass of grains, increasing control efficiency and grain preservation.

The results show that, after 48 h, the lethal concentrations sufficient to kill 50% of the individuals of *A. obtectus* and *S. zeamais* were equal to 9.45 and 10.15 μL g^{-1}, respectively. The obtained LC_{50} results confirmed the hypothesis that there are differences in the response to the composition of the clove essential oil used in this study between the different species.

According to Table 3, the LC_{50} to control the population of *A. obtectus* (9.45 μL g^{-1}) was lower compared with the LC_{50} to control *S. zeamais* (10.15 μL g^{-1}), demonstrating that *A. obtectus* is more susceptible to clove essential oil than *S. zeamais*. The

Table 3. Lethal concentration of clove essential oil necessary to kill 50% of adults of *Acanthoscelides obtectus* and *Sitophilus zeamais* applied to bean and maize grains, and its slope 48 h after the treatment

Species	Concentration (μL g^{-1})	Cumulative mortality (%) 48 h	Lethal concentration (LC$_{50}$) (95% CI) after 48 h	Slope (95% CI)
A. obtectus	35.0	100	9.45 (8.75-10.5) (μL g^{-1})	4.05 (2.2-5.9)
	17.9	95.0		
	8.9	45.0		
	3.6	10.0		
	1.8	7.5		
	0.4	5.0		
	0.2	0.0		
S. zeamais	35.0	100	10.15 (6.65-15.4) (μL g^{-1})	2.5 (1.6-3.4)
	17.9	82.5		
	8.9	40.0		
	3.6	7.5		
	1.8	5.0		
	0.4	2.5		
	0.2	0.0		

CI – Confidence interval

lower susceptibility of *S. zeamais* can be associated with the resistance observed in maize weevil populations to insecticides, which has been pointed as one of the factors responsible for the occurrence of failures in the control of this pest (Ribeiro et al., 2003; Fragoso et al., 2007).

Estrela et al. (2006) observed that oils of *Piper aduncum* (Linnaeus, 1753) (Piperaceae) and *Piper hispidinervum* C.D.C. (Piperaceae) were also toxic to adults of *S. zeamais*, showing LC$_{50}$ of 0.56 and 1.32 μL 20 g^{-1} of maize grains, respectively. Negahban & Moharramipour (2007) observed that essential oils of *Eucalyptus intertexta* R.T. Baker (Myrtaceae), *Eucalyptus sargentii* Maiden and *Eucalyptus camaldulensis* Dehnh have fumigation effect in adults of *Sitophilus oryzae* (*Linnaeus*, 1763) (Coleoptera: Curculionidae), with LC$_{50}$ of 6.93, 12.91 and 12.06 μL L^{-1} of air, respectively, after 24 h of exposure. In the present study, the concentrations of 17.9 and 35 μL g^{-1} of clove essential oil were not significantly different with respect to the mortality of both insect populations; thus, the concentration of 17.9 μL g^{-1} can be considered as the best one for the control of both populations.

In this context, the results obtained in the present study show the possibility of use of essential oils, such as clove oil, as alternatives for the management of *S. zeamais* and *A. obtectus* in the storage of maize and bean grains, especially for organic and family agriculture products, because the release of this product is easier to be obtained compared with synthetic insecticides.

Other eugenol sources, such as clove basil (*Ocimum gratissimum* L.) can be used; for being a bushy plant with fast growth, it can be used for the industrial extraction of eugenol, reaching contents of up to 67% (Cortez et al., 1998).

CONCLUSIONS

1. Eugenol is the compound responsible for the insecticide action of clove essential oil, applied for the control of *S. zeamais* and *A. obtectus*.

2. Clove essential oil is an efficient alternative method for the control of *S. zeamais* and *A. obtectus*.

3. The acute lethal concentrations of clove essential oil to kill 50% of the insect population are 9.45 μL g^{-1} for *A. obtectus* and 10.15 μL g^{-1} for *S. zeamais*.

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