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BIOACTIVITY OF EUCALYPTUS CV. "UROCAM" ESSENTIAL OIL ON THE CORN WEEVIL, *SITOPHILUS ZEAMAIS* (COLEOPTERA: CURCULIONIDAE)

Daniela Müller Lazzaretti [1]
Marco Aurélio Tramontin [2]
Paulo Roger Lopes Alves [3]
Veluma Ialú Molinari de Bastiani [4]
Jacir Dal Magro [5]
Lauri Lourenço Radünz [6]
Rafael Dal Bosco Ducatti [7]
Paulo Alfonso Floss [8]
Samuel Tadeu Tonin [9]
André Luiz Radünz [10*]

Resumo

O inseto *Sitophilus zeamais* é uma das mais importantes e severas pragas de grãos armazenados. Seu controle é realizado predominantemente através do uso de inseticidas sintéticos, realidade a ser repensada, visto as crescentes demandas por alimentos livres de agrotóxicos. Desta forma, buscou-se avaliar a bioatividade do óleo essencial de 'Eucalipto cv. Urocam' sobre *S. zeamais* com idade conhecida (10-30 dias). Para tanto foram pulverizados sobre grãos contaminados por *S. zeamais* as dosagens de 0,0; 1,0; 2,0; 3,0 e 5,0 L de óleo essencial por tonelada de grãos de milho. Os experimentos foram conduzidos com delineamento experimental totalmente casualizado e cinco

[1]Acadêmica no curso de Agronomia, Universidade Federal da Fronteira Sul, Câmpus Chapecó
[2]Professor Adjunto, Universidade Federal da Fronteira Sul, Câmpus Chapecó
[3]Professor Adjunto, Universidade Federal da Fronteira Sul, Câmpus Chapecó
[4]Doutoranda no Programa de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Santa Maria
[5]Professor no Programa de Pós-Graduação Stricto Sensu em Ciências Ambientais (UNOCHAPECÓ)
[6]Professor Associado, Universidade Federal do Rio Grande do Sul
[7]Mestrando do Programa de Pós-Graduação em Agronomia, Universidade Tecnologia Federal do Paraná – Câmpus Pato Branco
[8]Pesquisador na Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, Chapecó, SC
[9]Acadêmico no curso de Agronomia, Universidade Federal da Fronteira Sul, Câmpus Chapecó
[10]Professor Adjunto, Universidade Federal da Fronteira Sul, Câmpus Chapecó; *Corresponding author: andre.radunz@uffs.edu.br.

repetições. Avaliou-se a mortalidade e o índice de preferência do *S. zeamais*. Todos os dados foram submetidos ao teste F e quando interações significativas foram observadas, conduziu-se a análise de variância e testes de regressão (Software Assistat 7.7 Beta). O terpeno com maior presença no extrato de óleo essencial foi o Eucaliptol (74%). Pode-se inferir que o óleo essencial de 'Eucalipto cv. Urocam' apresenta potencial de utilização nas doses de 3,0 e 5,0 L t^{-1} de grão de milho, pois constatou-se elevada mortalidade dos insetos já nas primeiras horas de exposição, chegando a 100% nas primeiras 24 horas.

Palavras-chave: Biopesticida. Eucaliptol. Terpenos.

Abstract

Sitophilus zeamais is one of the more important and severe pests of stored grains. Its control is carried out predominantly by synthetic insecticides, a practice that must be rethought assuming the increasing demands for pesticide-free foods. This work aimed at evaluating the bioactivity of *Eucalyptus* cv. "Urocam" essential oil on 10- to 30-day-old *S. zeamais*. Essential oil from the leaves of *Eucalyptus* cv. "Urocam" was sprayed on corn kernels, infected by *S. zeamais*, at the rate dosage of 0.0; 1.0; 2.0; 3.0 and 5.0 L t^{-1}. A completely randomized experimental design with five replicates was used to conduct the experiment. All data were analyzed by the F test and whenever a significant interaction was found, analysis of variance and regression tests were performed (Assistat 7.7 Beta software). The terpene with the highest concentration in the *Eucalyptus* cv. "Urocam" essential oil was Eucalyptol (74%). Based on our results, it can be inferred that *Eucalyptus* cv. "Urocam" essential oil presents a good potential of use in the dosages of 3.0 and 5.0 L t^{-1} of corn kernels due to the high mortality of *S. zeamais* within the first hours after application, reaching 100% by the end of the first 24 hours.

Keywords: Biopesticide. Eucalyptol. Terpenes.

1. Introduction

The corn weevil, *Sitophilus zeamais* Motsch. 1855 (Coleoptera: Curculionidae), is one of the more important and severe pests of stored grains because in both stages, adult and larval, the insect feeds on grains and cause quantitative and qualitative losses to the stored products (OJO and OMOLOYE, 2016). In addition, *S. zeamais* is a "cross-infestation" insect (LORINI et al., 2015), which may contribute to its dispersion and increase its damages on stored products.

Currently, the use of synthetic insecticides is the most commonly used method for the control of *S. zeamais* (BOYER et al., 2012). However, despite the efficiency of these products, their indiscriminate use can lead, among other consequences, to the accumulation of toxic residues in food, environmental contamination, intoxication of the applicator, and to the selection of resistant populations (WEI et al., 2014).

Thus, the elaboration of alternative management methods to control *S. zeamais* becomes extremely necessary (MOREAU and ISMAN, 2012), especially when considering the ever-increasing search for pesticide-free foods, and the current discussions to find more sustainable productive models for agriculture.

For Lima et al. (2013), essential oils are still considered environmentally safer than synthetic pesticides due to the low molecular weight these compounds have, the low persistence in the environment and the lower toxicity to non-target organisms (MONTEFUSCOLI et al., 2014; FREIRES et al., 2015). The efficiency of essential oils on insect-pests has already been discussed by authors such as Albuquerque et al. (2013), Bacci et al. (2015), Campos et al. (2015) and Peixoto et al. (2015). These authors have shown the efficiency of essencial oils as for their insecticide action, repellency, feed deterrence, oviposition and inhibition of insect growth.

Eucalyptus cultivar "Urocam", a cross between *E. urophylla* × *E. camaldulensis*, is a widespread cultivar of *Eucalyptus* farmed in different regions of Brazil. As this cultivar is highly rich in essential oils, compounds toxic to insects and is farmed all over Brazil, it can be considered a good raw material for the extraction of specific products. Studies have shown that *Eucalyptus* presents great toxicity against several insects and microorganisms, acting as a repellent or feeding inhibitor (deterrence capacity) (BATISH et al., 2008; MACIEL et al., 2010; MOSSI et al., 2011).

Considering the economic importance, the damages caused by *S. zeamais* on stored products and the need of new methods and tools to enable a more sustainable management of this insect-pest, this work aimed at evaluating the bioactivity of *Eucalyptus* cv. "Urocam" essential oil on *S. zeamais*.

2. Material and Methods

The experiment was carried out at the Laboratory of Botany, Ecology and Entomology of the Universidade Federal da Fronteira Sul - Câmpus Chapecó. Colonies of *S. zeamais* were generated from specimens obtained/maintained in the Research Laboratory of EPAGRI (Agricultural Research and Rural Extension Company of Santa Catarina). The insects used in the bioassays were kept at controlled temperature (25 ± 2 °C), relative humidity (65 ± 5%) and a 12:12 (L:D) photoperiod. *Sitophilus zeamais* were kept in glass vials with a capacity of 2 liters, sealed with fine fabric (voile) in order to allow gas exchange with the environment and to avoid the escape of the insects. Corn kernels were used as substrate for the maintenance of *S. zeamais* (RIBEIRO, 2010).

In order to obtain 10- to 30-day-old insects, so as to perform the bioassays, 80 non-sexed adult specimens were placed in a container containing wheat grains so that they could lay their eggs. *Sitophilus zeamais* were allowed 15 days to lay the eggs, and then the adults were removed. The emergence of newly hatched *S. zeamais* insects occurred in a period of up to 35 days. Eggs were checked daily for the identification and removal of newly hatched insects (with a known age).

Leaves of *Eucalyptus* cv. "Urocam" were obtained from plants grown in the experimental area of EPAGRI, located in Guatambu – SC (27 ° 07 '55 "S and 52 ° 44' 04" W), southern Brazil. The extraction of the essential oil was carried out by the hydrodistillation method, in which 100 g of shaved leaves were placed in a volume of 500 ml of DI water. The distillation was held for a period of 90 min. After extraction, the oil was stored in bottles with screw cap and kept in a refrigerated environment (CAMPOS et al., 2014).

The identification of the chemical compounds present in the essential oil was obtained by gas chromatography on Agilent GC-MS (7890B) equipment coupled to a quadrupole mass spectrometer (5977A) (Agilent Technologies, Palo Alto, CA, USA). The operating conditions of the GC-MS system were as follows: Agilent 19091S capillary column, with the dimensions: 30m × 250mm × 0.25μm. The flow of the mobile phase (carrier gas: He) was adjusted to 1.2ml/min. The GC temperature program was 40 ºC (maintained for 4 minutes), with heating up to 200 ºC at a rate of 5 ºC min^{-1} (maintained for 1 minute). The temperature of the injector was 280 °C. For the analysis, the essential oil was dissolved in methanol at a concentration of 5 L ml^{-1}, the volume injected into the equipment was of 1 L. The chemical components present in the essential oil were identified by comparison with the equipment library (The National Institute of Standards and Technology, 2004). The relative amounts of each individual component were calculated using their respective peak areas in the chromatogram.

The bioassays, for the assessment of mortality and preference index, were conducted in a completely randomized experimental design, with five replicates. In all cases, corn kernels used in the experiments received no application of post-harvest products and the disinfestation of these kernels was carried out in an ultra-freezer with temperature of -80 °C, during a period of 24h.

In order to evaluate *S. zeamais* preference indexes for the *Eucalyptus* cv. “Urocam” essential oil, five arenas were built using plastic pots (10 cm in diameter and 4 cm in height). These five arenas were symmetrically interconnected to a central pot through plastic tubes with 0.5 cm in diameter. Fifty non-sexed 10- to 30-day-old adult insects were placed in the central pot. In the five arenas, symmetrically interconnected to this central pot, we added 10 g of corn kernels treated with the dosages of 0.0; 10.0; 20.0; 30.0 and 50.0 μL of essential oil, equivalent to 0.0; 1.0; 2.0; 3.0 and 5.0 L t^{-1} of corn kernels. In order to distribute the essential oil uniformly on the corn kernels, the homogenization was carried out in a glass container for 30 seconds. The arenas, interconnected to the central pot, were kept in a B.O.D (Biochemical Oxygen Demand) chamber, with controlled temperature (25 ± 2 °C) and 12:12 (L:D) photoperiod. The arenas were checked, for the preference index, after 0, 24, 48, 72, 96 and 120 h from the beginning of the assay.

The Preference Index (PI) was calculated through the following formula:

$$\mathrm{PI} = \frac{(\%\mathrm{IPT} - \%\mathrm{ipt})}{(\%\mathrm{IPT} + \%\mathrm{ipt})}$$

Where:
PI = Preference Index;
% IPT = % of insects in the treated pots;
% ipt = % of insects in the control pots.

Note: PI between -1.0 and -0.10 gives a repellent effect to the tested essential oil, while values between -0.10 and 0.10 give neutral effects and, values between 0.10 and 1.0 give attractive effects (PROCÓPIO et al., 2003).

Mortality tests were conducted in B.O.D. chambers, with controlled temperature (25 ± 2 °C) and 12:12 (L:D) photoperiod using circular Petri dishes, in which the essential oil was applied with the aid of an automatic pipettor (Labmate Pro and Research Plus) and homogenized with 10 g of corn kernels at the dosages of 0.0; 10.0; 20.0; 30.0 and 50.0 μL of essential oil, equivalent to 0.0; 1.0; 2.0; 3.0 and 5.0 L t^{-1} of corn kernels. In this experiment, 20 non-sexed 10- to 30-day-old adult insects were used in each Petri dish and the counts were performed after 0, 24, 48, 72, 96 and 120 h from the beginning of the assay, evaluating the number of dead insects per dish.

Due to thanatosis (characteristic presented by the insects), the insect that did not show any movement during three minutes of observation was considered dead (ANTUNES et al., 2011).

The data were analyzed by the F test and whenever we found a significant interaction, we conducted an analysis of variance (ANOVA) and regression tests, which were performed by the Assistat 7.7 Beta software (SILVA and AZEVEDO, 2009).

3. Results and Discussion

The GC-MS results for the essential oil extracted from the leaves of *Eucalyptus* cv. “Urocam” are shown in Table 1. The 5 major compounds correspond to 97.6% of the total components identified in the essential oil.

Table 1. Major compounds found in the essential oil extracted from *Eucalyptus* cv. "Urocam" leaves.

Compounds	Concentration (%)
α-Pinene	5,63
Limonene	6,57
Eucalyptol	74,93
α-Terpineol	6,41
Terpinyl acetate	4,25
Total (%)	**97,59**

The composition found in the essential oil extracted from *Eucalyptus* cv. "Urocam" in terms of major chemical compounds, is similar to other cultivars and/or species reported in the literature (LUCIA et al., 2012).

Eucalyptus is an important source of essential oils with wide applications in different areas of science (Hyldgaard et al., 2012). The chemical composition of eucalyptus essential oils can vary widely for different species, as well as the respective biological activity (Batish et al., 2008). The main compounds found are as follows: Hydroxyacetaldehyde, eugenol, 4-vinyl syringol, levoglucosan, 4-vinylguaiacol, 2-hydroxy-3-methyl-2-cyclopenten-1-one, α-pinene, α-felandrene, eucalyptol, p- cymene, limonene, eucamalol, citronellal, among others (Barbosa et al., 2016; Batish et al., 2008; Cheng et al., 2009). Other species of Eucalyptus (*E. nitens* and *E. globulus*) have been shown to be effective in controlling *Sitophilus zeamais*. The chemical composition of these plants is similar to the plant of this study, with eucalyptol as its main component, in addition to α-pinene and terpenyl acetate (González-Guiñez et al., 2016).

According to the F test, there is a significant interaction between the dosage and the time of exposure of *Eucalyptus* cv. "Urocam" essential oil on *S. zeamais* mortality, indicating there is a dependence between these variables.

The interaction between dosage and time of exposure to *Eucalyptus* cv. "Urocam" essential oil, showed that the use of 1 L t^{-1} of corn kernels, regardless of the time evaluated, showed no significant mortality (Figure 1). Therefore, this concentration does not represent a viable form to control *S. zeamais*. When analyzing the dosage of 2 L t^{-1}, we found an increase in insect mortality due to the longer time of exposure to the essential oil (Figure 1). However, the mortality of *S. zeamais* was lower than 80%, even when evaluated at 120 hours of exposure. According to Pinto Júnior et al. (1997), insecticides must have a minimum effectiveness of 80% to be considered effective.

When analyzing the dosages of 3.0 and 5.0 L t^{-1} of corn kernels, it is possible to observe a high mortality of *S. zeamais* within the first hours of exposure to the essential oil, reaching 100% by the end of the first 24 hours (Figure 1). Considering the results obtained in the present experiment, it is possible to affirm that it is viable, regarding insect mortality, to use *Eucalyptus* cv. "Urocam" essential oil in order to control *S. zeamais* infestations.

The results corroborate with those obtained by Restello et al. (2009), when evaluating the insecticide activity of *Tagetes patula* on *S. zeamais* at concentrations of 0.5, 10.0, 20.0, 30.0 and 50.0 μL per Petri dish. They have reported that in the higher concentrations of essential oil (30.0 and 50.0 μL), the mortality of *S. zeamais* was of 100%, gradually reducing to the lower concentrations.

For *Azadirachta indica* essential oil it was established that the concentration of 16% in an exposure time of 120 hours could already be considered efficient for the control of *S. zeamais*, despite its efficacy of only 75% (BARANEK, 2008). Thus, the mortality of *S.*

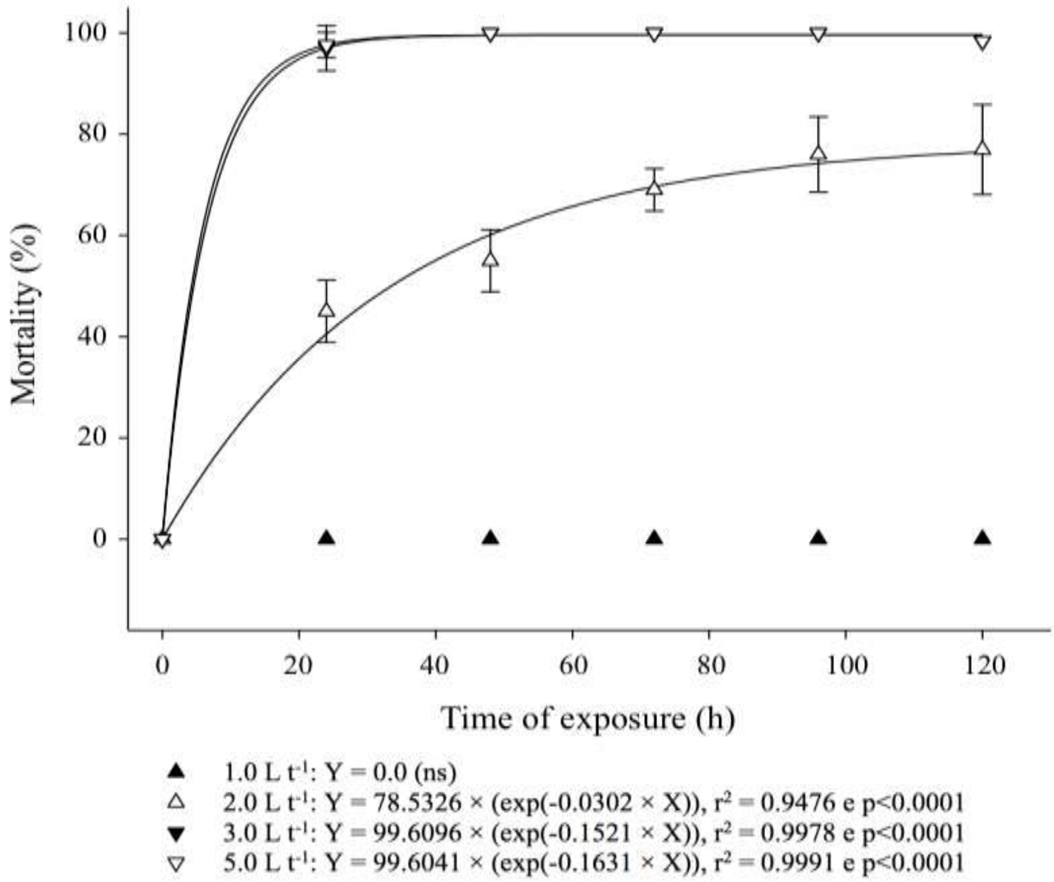

Figure 1. Mortality of *Sitophilus zeamais* as a function of the *Eucalyptus* cv. "Urocam" essential oil dosage and the time of exposure, 2018.

zeamais presented by the use of *Eucalyptus* cv. "Urocam" essential oil in its highest concentration (50 µL) for an exposure time of 120 h can be considered highly efficient.

For the preference tests, all the results were converted to Preference Indexes (PI) of *S. zeamais* on *Eucalyptus* cv. "Urocam" essential oil at different concentrations. Therefore, it was possible to verify that for *Eucalyptus* cv. "Urocam" essential oil, except for time 0 h, all tested treatments showed repellency to *S. zeamais* regardless of the time of exposure and the essential oil dosages (Table 2). This finding shows a high potential of use for *Eucalyptus* cv. "Urocam" essential oil in the management of *S. zeamais* and that this repellency might be associated with the presence of eucalyptol as the major compound of *Eucalyptus* cv. "Urocam" essential oil.

When working with the use of essential oil, the repellent action is one of the more important properties for the control of stored grain insect-pests, because the infestation tends to be smaller when the oil has a greater repellency, which contributes to the reduction of egg laying and the number of hatched eggs (COITINHO et al., 2006).

Also, repellency is understood as a reaction that occurs in the sensory system of the insect, which is activated when the insect is submitted to undesirable substances. The reaction of the insects to these substances is due to the chemoreceptors they have, which are located all over their body and are responsible for analyzing the conditions of the surrounding environment, orientating the insects to opposite directions if the conditions are not favorable (ALMEIDA and SILVA, 2013).

Table 2. Preference indexes (PI) for *Sitophilus zeamais* as a function of *Eucalyptus* cv. "Urocam" essential oil dosages at different times of exposure. [1]Classification, where N = Neutral, R = Repellent and A = Attractive.

Dosage (µL)	Time (h)	PI	Classification
10	0	0	N[1]
10	24	-0.95	R
10	48	-0,92	R
10	72	-0,94	R
10	96	-0,90	R
10	120	-0,90	R
20	0	0	N
20	24	-0,95	R
20	48	-0,97	R
20	72	-0,94	R
20	96	-0,96	R
20	120	-0,95	R
30	0	0	N
30	24	-0,78	R
30	48	-0,84	R
30	72	-0,79	R
30	96	-0,85	R
30	120	-0,84	R
50	0	0	N
50	24	-0,69	R
50	48	-0,83	R
50	72	-0,78	R
50	96	-0,81	R
50	120	-0,79	R

Similar results to those found in this work were found in the evaluation of *Corymbia citriodora* and *Cymbopogon nardus* essential oils for the control of *S. zeamais*. These oils provided a repellent effect at concentrations of 0.660; 0.881; 1.101 and 1.321 µL cm^{-2}, corresponding to a repellency percentage from 86.6% to 98.8% (OOTANI et al., 2011).

Likewise, other *Eucalyptus* species also presented a repellent effect to the corn weevil. *Eucalyptus citriodora* and *E. globulus*, in dosages of 50 µL in 20 g of corn kernels, presented repellent effects with percentages of repellency of over 87% for *E. citriodora* and 71.1% for *S. zeamais* adults (COITINHO et al., 2006).

Finally, it can be verified that the major chemical compounds identified in *Eucalyptus* cv. "Urocam" essential oil are present in several essential oils with proven insecticide activity (MACIEL et al., 2010; WANG et al., 2011), which may help explain the positive results observed on the control of *S. zeamais* in this work.

3.1 Conclusions

It can be inferred that *Eucalyptus* cv. "Urocam" essential oil presents potential of use in the dosages of 3.0 and 5.0 L t^{-1} of corn kernels, because it was verified a high mortality of the insects within the first hours of exposure, reaching 100% by the end of the first 24 hours. For insect repellency, regardless of the exposure time and the used dosages, a significant effect was observed. Eucalyptol was the major compound found in *Eucalyptus* cv. "Urocam" essential oil, corresponding to almost 74% of the total identified compounds.

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