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Metodologia (Methodology)

Insecticidal activity and sublethal effects of essential oils on *Sitophilus zeamais* (Coleoptera: Curculionidae) and on *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae)

Atividade inseticida e efeitos subletais de óleos essenciais sobre Sitophilus zeamais *(Coleoptera: Curculionidae) e* Acanthoscelides obtectus *(Coleoptera: Chrysomelidae)*

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Abstract

The objective of this study was to evaluate the toxicity, the effects on the emergence and survival of the essential oils of *Cymbopogon nardus*, *Corymbia citriodora* and *Cymbopogon martini* and their mixtures, applied as fumigation, on *Sytophilus zeamais* and *Acanthoscelides obtectus*. The essential oil of *C. nardus* presented geraniol (38.93%) and citronellal (26.46%) as the main chemical compounds, *C. citriodora* presented citronelal (83.95%) and *C. martini* geraniol (85.67%). The essential oils and their mixtures showed mortality ranging from 3% to 16% on *S. zeamais*. However, on adults of *A. obtectus* they

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caused mortality ranging from 62.5% to 92.5%. The mixtures *C. martini* + *C. citriodora* and *C. martini* + *C. nardus* + *C. citriodora* caused 15% of emergence in adults of *S. zeamais*. The essential oil of *C. martini* decreased the survival of adults of *S. zeamais* in 20 days. The results showed that the essential oils and their mixtures caused toxicity and affected the emergence and survival of the adults of *S. zeamais* and *A. obtectus*. However, further studies are needed to improve essential oils to be used in the control of these stored grain pests.

Keywords: Fumigation, stored grain pests, toxicity, essential oil

Resumo

O objetivo do estudo foi avaliar a toxicidade, os efeitos sobre a emergência e sobrevivência dos óleos essenciais de *Cymbopogon nardus*, *Corymbia citriodora* e *Cymbopogon martini* e de suas misturas, aplicados em fumigação, sobre *Sitophilus zeamais* e *Acanthoscelides obtectus*. O óleo essencial de *C. nardus* apresentou geraniol (38,93%)e citronelal (26,46%) como principais componentes químico,, *C. citriodora* citronelal (83,95%) e *C. martini* geraniol (85,67%). Os óleos essenciais e suas misturas apresentaram mortalidade entre 3% e 16% sobre *S. zeamais*. No entanto, sobre adultos de *A. obtectus* exibiram mortalidade que variou de 62,5% a 92,5%. As misturas de óleos essenciais de *C. martini* + *C. citriodora* e *C. martini* + *C. nardus* + *C. citriodora* apresentaram 15% de emergência de adultos de *S. zeamais*. O óleo essencial de *C. martini* diminuiu a sobrevivência dos adultos de *S. zeamais* em 20 dias. Os resultados evidenciaram que os óleos essenciais e suas misturas causaram toxicidade e efeitos negativos sobre a emergência e sobrevivência de adultos de *S. zeamais* e *A. obtectus*. No entanto, mais estudos são necessários para o aprimoramento dos óleos essenciais, afim de que estes sejam empregados no controle destas pragas de grãos armazenados.

Palavras-chave: Fumigação, pragas de grãos armazenados, toxicidade, óleo essencial

Introduction

Brazil is currently one of the largest producers and exporters of maize (Farmnews, 2020), and one of the largest producers and consumers of beans in the world (Coêlho, 2019). Throughout the production cycle, many losses are generated by several factors, one of the main ones is the incidence of pests that affect these crops, which are responsible for about 10% of all national grain loss during the year (Lorini, 2015; Taddese et al., 2020). Among them, two insects can be cited as the more important: *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae) on maize, and *Acanthoscelides obtectus* Say, 1831 (Coleoptera: Bruchidae) on beans.

Sitophilus zeamais is a high destructive primary maize pest, as it presents cross infestation, that is, the ability to infest grains both in the field and in storage (Lorini et al., 2015; Chen et al., 2020). *Acanthoscelides obtectus* is also a high destructive bean pest primary since the larvae attack the grains opening galleries, causing their complete destruction, plus the qualitative losses such as the loss of nutritional value and the physiological quality of the seeds (de Campos et al., 2014; Taddese et al., 2020). When attacking grains in the field or in storage, these two insect pests, in addition to generating losses through destruction, can also create favorable conditions for weight loss and excess moisture and consequent proliferation of pathogenic fungi and bacteria (Ribeiro et al., 2015).

The primary method used to control stored grain pests is the application of chemical insecticides with a fumigant effect, such as phosphine and methyl bromide (Oliveira et al., 2018; Mahbub et al., 2020). However, despite the relative success, the inappropriate and frequent use of these products over the years can interfere in the populations of natural enemies, as promoting the development of pest resistant populations. Furthermore, they can cause poisoning in humans and animals through residues in food and persistence in the environment (Holtz et al., 2020).

In view of this problem, the search for more economically and ecologically viable and sustainable control methods is highly desired. That said, studies using essential oils extracted from plants has shown satisfactory results as an alternative pest control, as they are composed of secondary compounds with insecticidal activity; in addition, essential oils do not leave residues in food and are rapidly degraded in the environment (das Mercês et al., 2018). Ataíde et al. (2020a) obtained expressive mortality when used the essential oil of *Rosmarinus officinalis* L. Lamiaceae (Labiatae) on *A. obtectus;* Titouhi et al. (2017) evidenced that the essential oils of different plants of *Artemisia* (Asteraceae) were efficient to control *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae) and *Bruchus rufimanus* Boh. (Coleoptera, Bruchidae).

Hussein et al. (2017) confirmed that the essential oil of C*orymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnsonhas potential in alternative pest management, after testing the insecticidal effect of the oil on *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) and *Trialeurodes ricini* (Misra, 1924) (Hemiptera: Aleyrodidae). In a study, Ríos, Stashenko & Duque (2017) confirmed the larvicidal activity of several essential oils, including *Cymbopogon martini* (Roxb.) Wats (Poaceae) and *Cymbopogon nardus* (L.) Rendle f. *rectus* (Steud.) Roberty (Poaceae), against *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) larvae.

Therefore, the objective of this study was to evaluate toxicity and sublethal effects of essential oils of *Corymbia citriodora*, *Cymbopogon martini* and *Cymbopogon nardus* and their mixtures on *S. zeamais* and *A. obtectus*.

Material and methods

Biological material

The insects used in the bioassays were obtained from insect colony reared on the Laboratory of Entomology of the *Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário de Pragas e Doenças* (NUDEMAFI) at the *Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo* (CCAE-UFES) in Alegre, Espírito Santo, Brazil.

Obtaining the essential oils and preparation of oil mixtures

The essential oils were purchased in the company Phytoterápica® Ltda, located at Nova Cantareira Street, 2627, Tucuruvi, 02341-000-São Paulo- Brazil.

The mixtures of the essential oils of *C. citriodora*, *C. martini* and *C. nardus* were carried out according to Table 1 (Pavela, 2015).

Characterization of essential oils

The essential oils were analyzed by gas chromatography with a flame ionization detector (GC-FID) (Shimadzu GC-2010 Plus device) and by gas chromatography coupled to mass spectrometry (GC-MS) (Shimadzu device QP2010- Plus) following the adapted method of de Souza et al. (2017). The following chromatographic conditions were used in both analyses: fused silica capillary column (30 m x 0.25 mm) with Rtx®-5MS stationary phase (0.25 µm of film thickness); N2 (in GC/FID analysis) and He (in the GC-MS analysis) as carrier gas with a flow rate of 3.0 mL/min. The oven temperature followed a schedule in which it remained for 3 min at an initial temperature of 40 °C and then gradually increased by 3 °C/min until it reached 240 °C, remaining at this temperature for 5 min. The other chromatographic conditions were injector temperature of 250 °C; detector temperature of 280 °C; split ratio of 1:30. The GC/MS analysis was performed on equipment operating by electronic impact with 70 eV impact energy; scan speed 1,000; scan interval of 0.50 fragments/s and detected fragments from 29 to 400 m/z.

The identification of the chemical components of the oils was performed by comparing their mass spectra with those available in the Willey7, NIST05, NIST 05s spectrotecs database (reference) with the co-injection of standards and by the Retention Indexes (IR). For the calculation of the, a mixture of linear n-alkanes (C7 to C40) was used as standard. The calculated IR for each compound was compared with values in the literature (Adams, 2007), being calculated using the Linear Temperature Programmed Retention Indexes equation.

Table 1. Proportion of essential oil mixtures 1:1 (v/v) used in the experiment of mortality, emergency and survivorship.

Mixtures	Proportions in the mixtures	Essential oils
1	1:1:1	*C. martini* + *C. Nardu s* + *C. citriodora*
2	1:1	*C. martini* + *C. nardus*
3	1:1	*C. martini* + *C. citriodora*
4	1:1	*C. nardus* + *C. citriodora*

Fumigation toxicity

Bioassays with *S. zeamais* and *A. obtectus* were conducted following the method adapted from Aslan et al. (2004), at a temperature of 25±2 °C, 70±10% RH and 12 hours of photophase. Ten adults (10 days old) of *S. zeamais* and 10 adults (5 days old) of *A. obtectus* were used. They were subjected to the fumigation effect of 20 µl of the essential oils and their mixtures. Each treatment was replicated 10 times, and the control had no product. After 72 hours, the mortality was assessed.

Sublethal effects

After 72 hours of exposure to essential oils and their mixtures, the alive adults of *S. zeamais* were sexed based on the characteristics of the head (Halstead, 1963) and adults of *A. obtectus* were sexed following the method of Quintela (2009). After sexing, six females and six males of both insects were placed in plastic pots (7 x 6 cm) (the lids of the plastic pots were perforated to allow air circulation) with 50 g of maize for *S. zeamais* and 50 g of beans for *A. obtectus*. For each treatment, five repetitions were performed. The plastic pots were placed in climatic chambers of the BOD type, at 25 ± 2 °C, 70 ± 10% RH and 12 hours of photophase, and left for five days for oviposition. After that, the adults were removed. Thirty days after the infestation, the number of adults that emerged was assessed every two days for a period of 10 days.

To assess the survivorship of the F_1 adult insects of *S. zeamais* and *A. obtectus,* the remainingadults from the last experiment were placed in plastic pots (7 x 6 cm), with perforated covers to allow air circulation and prevent escape of the insects, with 50 g of maize for *S. zeamais* and 50 g of beans for *A. obtectus*. The pots were placed in a BOD climate chamber at 25±2 °C, 70 ± 10% RH and 12 hours of photophase. Every three days, the number of alive and dead individuals was counted. This procedure was repeated until all insects died.

Data analysis

For the tests of acute toxicity by fumigation and sublethal effect, a completely randomized experimental design was used; an ANOVA was performed for data analysis followed by the Scott-Knott test for multiple comparison of the means ($p \leq 0.05$). Kaplan-Meier estimators (log-rank test) were used to analyze survival data. Adults of *S. zeamais* and *A. obtectus* that who did not survive until the end of the experiment were not included in the analysis.

Results

Characterization of essential oils

In the essential oil of *C. nardus*, 12 chemicals compounds were identified, the main compounds were citronellal (26.46%) and geraniol (38.93%). In the essential oil of *C. citriodora*, five chemicals compounds were identified, being citronelal (83.95%) the primary compound. In the essential oil of *C. martini*, six chemicals compounds were identified, with geraniol (85.67%) the most abundant.

Fumigation toxicity

For *S. zeamais*, the essential oil of *C. nardus* showed significantly higher mortality rate (15%) compared to other treatments; essential oils of *C. martini* and *C. citriodora* showed 5% and 3% mortality, respectively, which did not differ from control treatment. The mixtures between essential oils showed mortality rates similar to these last pure essential oils, except the mixture of *C. martini* + *C. nardus* + *C. citriodora* that caused a significantly mortality (16%) (Figure 1).

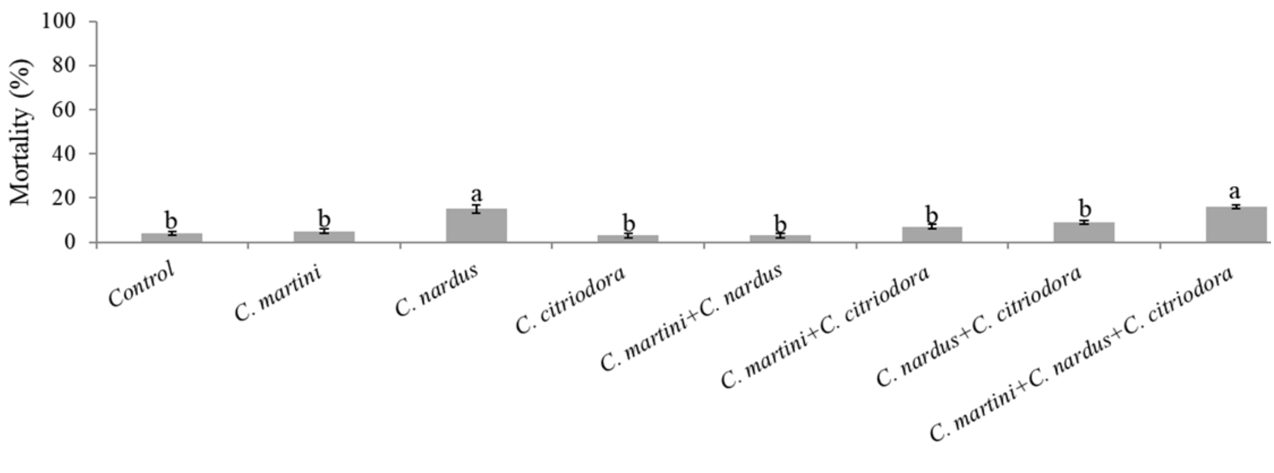

Figure 1. Mortality (%) of *S. zeamais* exposed to the essential oils of *Cymbopogon nardus, Corymbia citriodora* and *Cymbopogon martini and t*heir mixtures, under laboratory conditions. Means followed by the same letter are not significantly different by Scott-Knott test (p ≤ 0.05).

For *A. obtectus*, all essential oils presented significantly higher mortality rates compared to control treatment. *C. martini* was the essential oil that showed the highest mortality (92.5%), while the essential oils of *C. nardus* and *C. citriodora* showed a lower mortality rate (< 50%). All mixtures of essential oils presented significantly higher mortality rates compared to control treatment (ranging from ca. 30% to 78%) (Figure 2).

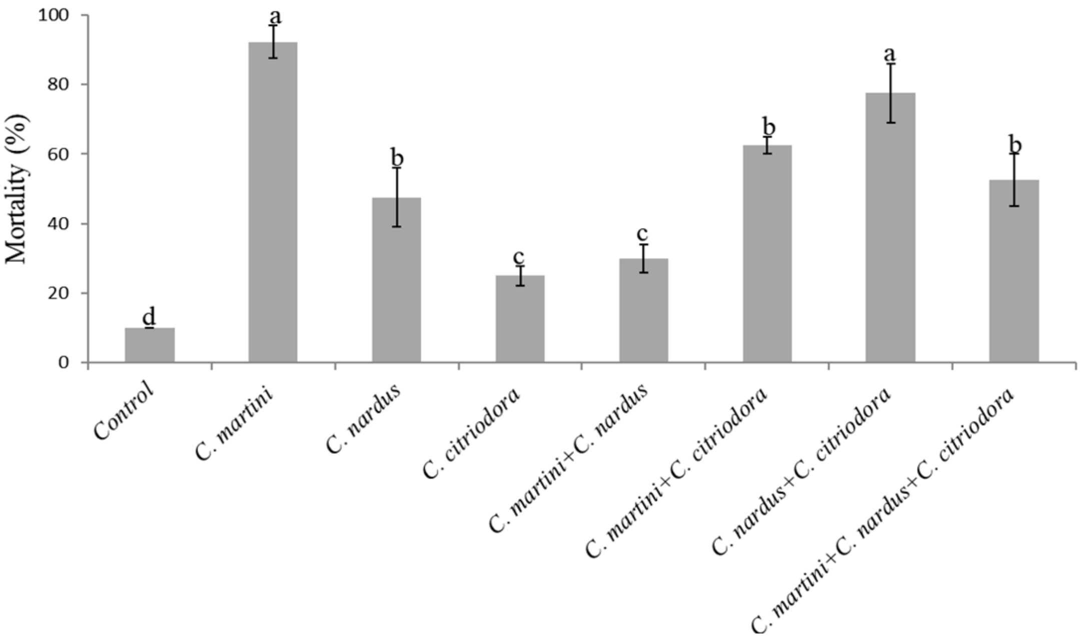

Figure 2. Mortality (%) of *A. obtectus* exposed to the essential oils of *Cymbopogon nardus, Corymbia citriodora* and *Cymbopogon martin* and their mixtures, under laboratory conditions. Means followed by the same letter are not significantly different by Scott-Knott test (p ≤ 0.05)

Sublethal Effects

In the emergency evaluation of *S. zeamais*, all essential oils (applied separately or in mixtures) showed a significant decrease in the number of adults emerged compared to the control treatment. The lowest rates were observed in the mixtures *C. martini* + *C. citriodora* (15%) and *C. martini* + *C. nardus* + *C. citriodora* (15%) (Figure 3).

Regarding the emergency *of A. obtectus*, there were no significant differences among the essential oils and their mixtures compared to the control treatment (Figure 4). In the evaluation of survivorship of *S. zeamais*, a significant difference was observed (log-rank test; $\chi 2 = 419.0$; df = 7; P < 0.001) compared to the control treatment. The essential oil of *C. martini* decreased the insect survival to 20 days (Figure 5). In *A. obtectus*, there was significant differences (log-rank test; $\chi 2 = 9.7$; df = 7; P < 0.001) in the adult survivorship. However, the essential oils of *C. citriodora* and mixtures *C. citriodora* + *C. nardus* and *C. nardus* + *C. martini* increased the adults' survival potential in relation to the control (Figure 6).

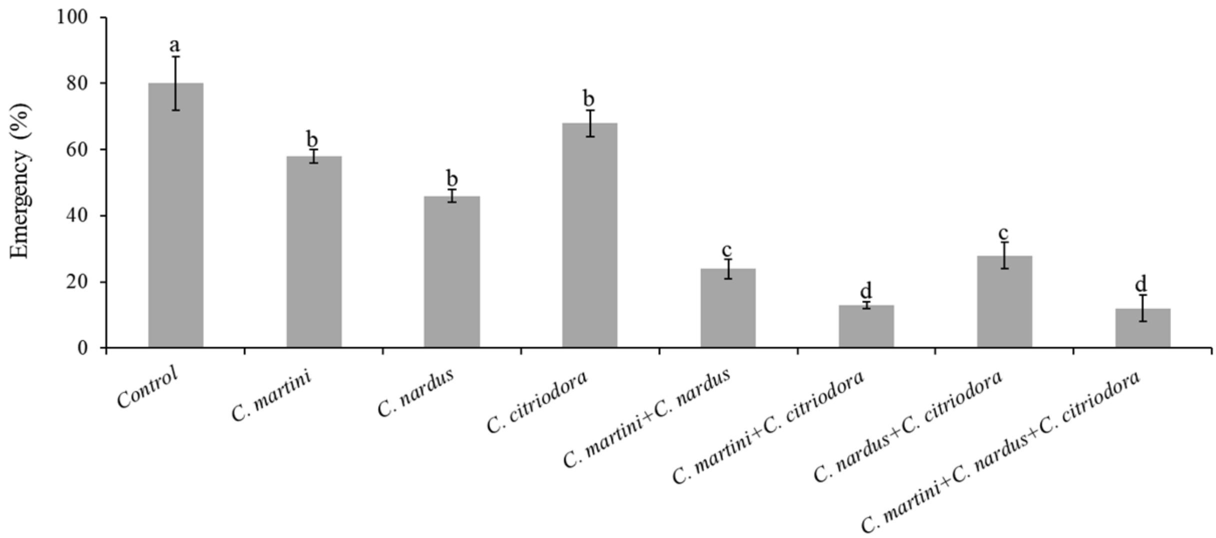

Figure 3. Emergency (%) of *S. zeamais* exposed to essential oils of *Cymbopogon nardus, Corymbia citriodora and Cymbopogon martin a*nd their mixtures, under laboratory conditions. Means followed by the same letter are not significantly different by Scott-Knott test (p ≤ 0.05).

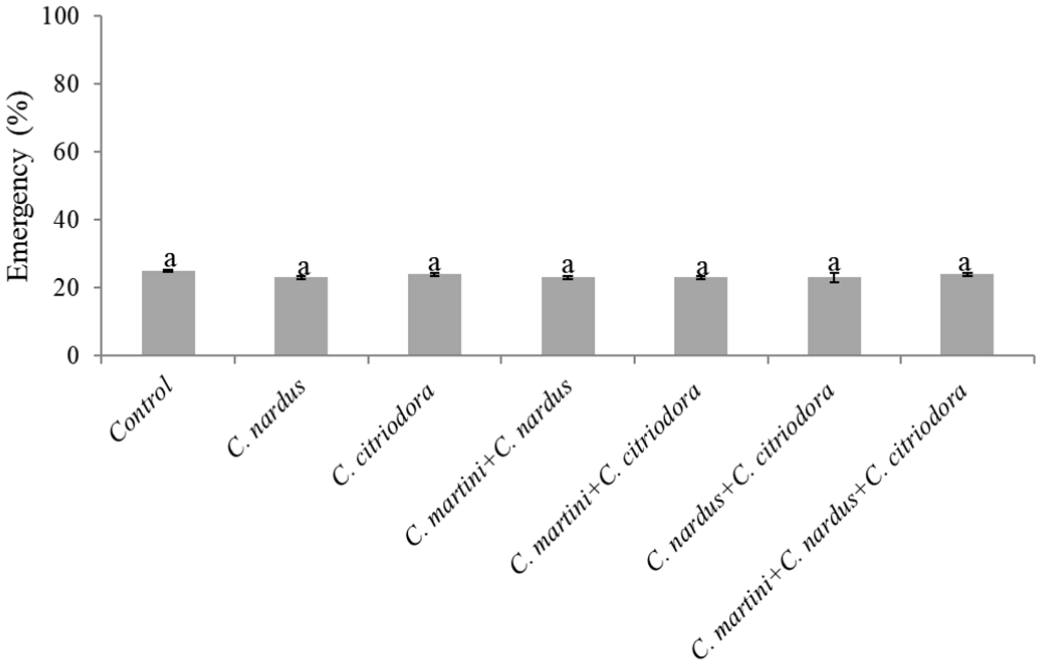

Figure 4. Emergency (%) of *A. obtectus* exposed to essential oils of *Cymbopogon nardus, Corymbia citriodora* and *Cymbopogon martin* and their mixtures, under laboratory conditions. Means followed by the same letter are not significantly different by Scott-Knott test (p ≤ 0.05).

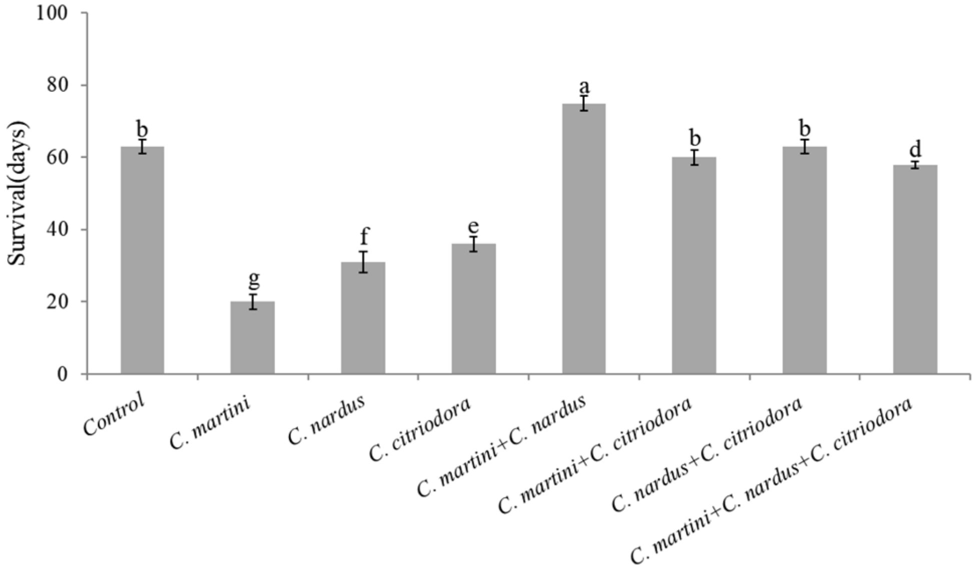

Figure 5. Survivorship (%) of *S. zeamais* exposed to essential oils of *Cymbopogon nardus, Corymbia citriodora* and *Cymbopogon martin* and their mixtures, under laboratory conditions. Means followed by the same letter are not significantly different by Scott-Knott test (p ≤ 0.05).

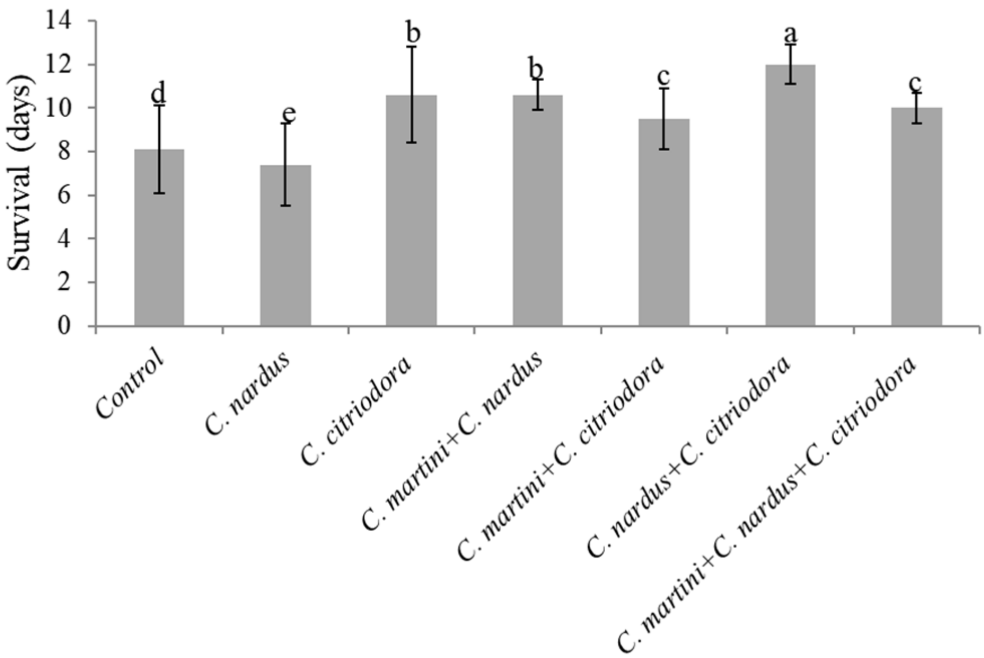

Figure 6. Survivorship (%) of *A. obtectus* exposed to essential oils of *Cymbopogon nardus, Corymbia citriodora* and *Cymbopogon martin* and their mixtures, under laboratory conditions. Means followed by the same letter are not significantly different by Scott-Knott test (p ≤ 0.05).

Discussion

The essential oil of *C. nardus* presented geraniol and citronellal as main chemical compounds. Samarasekera et al. (2006) found the same primary compounds in this essential oils. In the research of Tu et al. (2018), hedycaryol was found as the main compound. This difference may be related to the place or the time the plant was collected (Isman, 2006). The essential oil of *C. citriodora* presented citronellal as the primary chemical compound, similarly, as found by Insuan and Chahomchuen (2020) and Gusmão et al. (2013). The essential oil of *C. martini* exhibited geraniol as primary chemical compound, as reported by Devil et al. (2020).

Essential oils are secondary metabolites comprising different bioactive compounds and have been getting importance as alternative to synthetic insecticides (Sarma et al., 2019). The main compounds found in the essential oils of the present study are oxygenated monoterpenes (Abifarin et al., 2020), which act causing toxic interference in the biochemical and physiological functions of herbivorous insects, when inhaled, ingested or absorbed by the integument. They can also act as repellents (War et al., 2012). The low mortality in *S. zeamais* by action of the essential oils can be explained due to the resistance developed by the insect as a defense mechanism, by metabolization or detoxification, allowing to modify the toxic substances in a sufficient rate to prevent the action at the target site, or even it even metabolizes it to quickly eliminate it from the body (Lorini, 1999).

The mixtures of essential oils showed mortality on *A. obtectus*. However, there was no significant difference between the mixtures and the essential oil of *C. martini*. Thus, it is inferred that the binary mixtures between some essential oils did not enhance the insecticidal activity (Pavela, 2015). In *S. zeamais,* it was observed that, like pure oils, mixtures between essential oils were not effective in controlling them, which can be explained by the non-potentiation of binary combinations due to antagonistic effects between two or more compounds (de Lima et al., 2019), or by the defense mechanism of the insect mentioned above. Another factor that may explain the non-potentiation of mortality is that insects reduce their respiratory rates when exposed to essential oil terpenes. Thus, they can detach themselves from the compounds found in lesser quantity and be less exposed to the action of the more toxic compounds (Plata-Rueda et al., 2018).

For sublethal effects, several studies showed that the action of major compounds in essential oils can have negative effects on the physiology of insects via neurotoxic actions (Izakmehri et al., 2013; Pavela et al., 2020; Ahmadpour et al., 2021), decreasing the survivorship capacity and the likelihood of emergence of adults (Ataíde et al., 2020b). For adult emergence of *S. zeamais*, there were significant differences in the number of adults, and the lower emergence rates were obtained in the mixtures of essential oils, thus may be said that the mixtures of essential oils potentiated the ovicidal activity on *S zeamais*. However, for *A. obtectus,* the oils and their mixtures were not effective. The ovicidal activity of the essential oils can occur due to complications caused by neurotoxic actions in the physiology of insects, making them infertile or making eggs unviable (Correa et al., 2015; Dar et al., 2017).

Observing the survivorship of *S. zeamais*, mixtures of essential oils provided the highest rates, confirming the effectiveness of synergism in interfering with the detoxification systems present in the insect's physiology (de Araújo, 2014). However, in *A. obtectus*, weobserved that instead of low survival, the insects from the treatments survived more than those from the control. The causes of the event are not known for certain, but it appears that some of the compounds present in the essential oils have positive effects on the physiology of insects, allowing them to survive longer than the control. Kouloussis et al. (2017) found similar results when testing orange, *Citrus sinensis* (L.) Osbeck (Rutaceae), essential oil on the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae); adult insects with food restriction in contact with the oil showed greater survivorship than the control insects.

Conclusion

The essential oils of *Corymbia citriodora*, *Cymbopogon martini* and *Cymbopogon nardus* and their mixtures are toxic and decreased the emergence and survival in adults of *S. zeamais*. However, essential oils and their mixtures caused mortality in adults of *A. obtectus*, but had no effect on emergence and survival. Therefore, further studies are needed to improve essential oils to be used to control these stored grain pests.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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