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Toxicidade aguda, efeito subletal e alteração no comportamento de *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae) exposto aos componentes majoritários de óleos essenciais

Acute toxicity, sublethal effect and changes in the behavior of *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae) exposed to major components of essential oils

Toxicidad aguda, efecto subletal y cambio en el comportamiento de Lasioderma serricorne Fabricius (Coleoptera: Anobiidae) expuesto a componentes principales de aceites esenciales

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Resumo

O objetivo deste trabalho foi avaliar a toxicidade aguda, efeito subletal e alteração comportamental de *L. serricorne* expostos aos componentes majoritários de óleos essenciais, timol, cânfora, terpineol, canfeno, eucaliptol, limonene, βpineno e eugenol. Os adultos de *L. serricorne* de idade de 5 dias não sexados foram submetidos à toxicidade aguda por fumigação, utilizou câmaras de fumigação, recipientes de vidro com 200 ml de capacidade com 20 insetos, posteriormente foi feito a avaliação do efeito subletal, longevidade e a resposta comportamental. Os resultados mostram que os componentes majoritários e as misturas causaram toxicidade aguda, reduziu o tempo de sobrevivência e produção de proles e alterou a atividade comportamental de *L. serricorne*. Os componentes majoritários eucaliptol e terpineol apresentaram 65% de mortalidade, na qual as misturas terpineol+eucaliptol e cânfora+timol apresentaram 47 e 52% sobre os adultos de *L. serricorne*. A mistura entre terpineol+limonene permitiu apenas 4% de emergência de adultos de *L. serricorne*. A atividade comportamental foi alterada pela mistura eucaliptol+eugenol. Os resultados evidenciaram que os componentes majoritários e as misturas são promissores para elaboração e produção de novos inseticidas.

Palavras-Chave: Monoterpeno; Sesquiterpeno; Besouro do cigarro; Alimentos processados.

Abstract

The objective of this work was to evaluate the acute toxicity, sublethal effect and behavioral alteration of *L. serricorne* exposed to the major components of essential oils, thymol, camphor, terpineol, camphene, eucalyptol, limonene, βpinene and eugenol. The adults of *L. serricorne* , aged 5 days, not sexed, were submitted to acute toxicity by fumigation, used fumigation chambers, 200 ml glass containers with 20 insects, subsequently the sublethal effect, longevity and behavioral response. The results show that the major components and mixtures caused acute toxicity, reduced the survival time and production of offspring and altered the behavioral activity of *L. serricorne*. The major components eucalyptol and terpineol presented 65% mortality, in which the mixtures terpineol + eucalyptol and camphor + thymol presented 47 and 52% over adults of *L. serricorne*. The mixture between terpineol + limonene allowed only 4% ofadults to *L. serricorneemerge.* Behavioral activity was altered by the eucalyptol + eugenol mixture. The results showed that the major components and mixtures are promising for the preparation and production of new insecticides.

Keywords: Monoterpene; Sesquiterpene; Cigarette beetle; Processed foods.

Resumen

El objetivo deste trabajo fue evaluar la toxicidad aguda, el efecto subletal y la alteración del comportamiento de *L. serricorne* expuesto a los componentes principales de los aceites esenciales, timol, alcanfor, terpineol, canfeno, eucaliptol, limoneno, βpineno y eugenol. Los adultos de *L. serricorne* , de 5 días de edad, no sexados, fueron sometidos a toxicidad aguda por fumigación, utilizaron cámaras de fumigación, envases de vidrio de 200 ml con 20 insectos, posteriormente el efecto subletal, la longevidad y respuesta conductual Los resultados muestran que los principales componentes y mezclas causaron toxicidad aguda, redujeron el tiempo de supervivencia y la producción de descendencia y alteraron la actividad conductual de *L. serricorne*. Los componentes principales eucalyptol y terpineol presentaron un 65% de mortalidad, en el que las mezclas terpineol + eucalyptol y alcanfor + timol presentaron 47 y 52% sobre los adultos de *L. serricorne*. La mezcla entre terpineol + limoneno permitió que solo4% de los adultos de *emergiera elL. serricorne.* La actividad conductual fue alterada por la mezcla de eucaliptol + eugenol. Los resultados mostraron que los principales componentes y mezclas son prometedores para la preparación y producción de nuevos insecticidas.

Palabras clave: Monoterpeno; Sesquiterpeno; Escarabajo del cigarrillo; Alimentos procesados.

1. Introduction

The *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae) (cigarette beetle) has since 1993 been widely distributed worldwide, especially in tropical and subtropical areas (Lorini et al., 2010). They cause significant economic damage to tobacco, cereals, processed foods and feed (Lorini et al., 2015). The adult cigarette beetle and the larvae dig galleries in the stored products, while the adults use the galleries only for laying and mating, while the larvae open galleries when feeding. This promotes quantitative losses, that is, weight loss and qualitative losses, among which, the presence of insects and the formation of galleries in stored products (Lorini et al., 2010).

Control of populations *L. serricorne* is done with applications of phosphine and pyretoids (White and Leesch, 1995; Phillip and Throne, 2010). However, the continued use of insecticides for decades has led to serious problems, such as developments in resistant insect populations increasing insecticide doses, contamination of non-target organisms, environmental pollution and rising storage costs (Rajendran and Narasimhan, 1994; Jovanovic et al., 2007; Phillip and Throne, 2010). The search for substances for insect control, which are environmentally friendly, becomes a necessity nowadays. Thus, essential oils and their major components have played a significant role in the development of insecticides (Bachrouch et al., 2010).

In recent years, research on essential oils extracted from plants, as well as studies such as the isolated or associated use of the major components present in essential oils, have been considered promising for insect pest control (Erdemir and Erler, 2017; Martins et al., 2017). They are typically characterized by having lower toxicity in mammals, low persistence in the environment and decreased development of insect resistance (Aslan et al., 2004; Santos et al., 2011; Oliveira et al., 2018).

The present study aimed to evaluate the acute toxicity, sublethal effect and the behavioral response of *L. serricorne* exposed to the major components thymol, camphor, terpineol, camphene, eucalyptol, limonene, βpinene and eugenol.

2. Material and Methods

The work carried out followed the laboratory research methodology of qualitative and quantitative nature, as proposed by Pereira et al. (2018). The work was developed during the year 2020, from january to april, in the laboratory at 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.

2.1. Biological Material

The adult *L. serricorne* were collected in cereals grains in Alegre-ES (Longitude; 41°31'59.54"O; Latitude; 20°45'47.14"S), and then were identified by the key proposed by Pereira and Almeida (2001). The breeding was developed in wheat germ, in an air-conditioned room at 34±1 ºC, relative humidity (RH) 70±10% and a photophase of 12 hour (Howe, 1957), at 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.

2.2. Obtaining the major components

The major components of the essential oils were purchased from the company Jacy Fragância Ltda, located at Limeira street, 281, São Fernando, Americana, 13454-214, São Paulo, Brazil.

2.3. Preparation of the mixtures of the major components

The mixtures of the major components, thymol, camphor, terpineol, camphene, eucalyptol, limonene, βpinene and eugenol were made in the proportion as shown in Table 1.

Table 1. Proportions of mixtures of major components (m/v).

Mixtures	Proportions of mixtures	major components
1	1:1:1	camphene +camphor+thymol
2	1:1:1:1:1	limonene+terpineol+βpinene+eucalyptol+eugenol
3	1:1	camphene +camphor
4	1:1	thymol +camphor
5	1:1	thymol +camphene
6	1:1	limonene+terpineol
7	1:1	limonene+βpinene
8	1:1	limonene+eucalyptol
9	1:1	limonene+eugenol
10	1:1	terpineol+βpinene
11	1:1	terpineol+eucalyptol
12	1:1	terpineol+eugenol
13	1:1	βpinene +eucalyptol
14	1:1	βpinene +eugenol
15	1:1	eucalyptol+eugenol

Source: Author himself.

The following mixtures of major components were tested:camphene +camphor+thymol, limonene+terpineol+βpinene+eucalyptol+eugenol, camphene +camphor, thymol +camphor, thymol +camphene, limonene+terpineol, limonene+βpinene, limonene+eucalyptol, limonene+eugenol, terpineol+βpinene, terpineol+eucalyptol, terpineol+eugenol, βpinene +eucalyptol, βpinene +eugenol, eucalyptol+eugenol. Acute toxicity testing by fumigation, sublethal effect, longevity analysis and behavioral response was performed.

2.4. Acute toxicity by fumigation

The experiment was carried out at a temperature of 34 ± 1 ºC, 70± 10% (RH) and a photophase of 12 h. In assessing the fumigant effect, the adapted methodology of Aslan et al., (2004) was adopted. Glass containers (volume 200 ml) were used as fumigation chambers, where 20 adult *L. serricorne* not sexed at 5 days old were confined. The major components and their mixtures (Table 1), without any solubilization vehicle, were applied with an automatic pipette, 10 µl in 18 cm filter papers, fixed on the bottom surface of the container lid. To avoid direct contact of the mixtures of the major components with the insects, fabric "*filó*" was used.

For each major component, their mixtures and control were performed five repetitions. After 72 hours of exposure to treatments, the number of adult *L. serricorne* dead was counted. To confirm the mortality of adults *L. serricorne,* they were touched with a fine bristle brush, the *L. serricorne* immobile were considered dead.

2.5. Sublethal effect

After 72 hours of exposure of adult *L. serricorne* to major components and their mixtures in acute toxicity tests, the *L. serricorne* survivors were sexed by the characteristics of the face (Jones, 1913). 20 g of wheat germ was infested with three males and six females, placed in a 150 ml plastic container. The lids of the plastic containers were punctured to allow gas exchange. The tests were carried out in climatic chambers type BOD, at 34±1 °C, 70±10% RH and 12 hours of photophase, left for 10 days for copulation. After that period, the adults were removed. Twenty-five days after the infestation, the quantity of adult insects emerged from the surviving parents of the acute toxicity tests were evaluated every two days for a period of 10 days.

2.6. Longevity analysis

The adult *L. serricorne* surviving the toxicity test were placed in plastic pots (7 x 6 cm) with perforated lids to allow gas exchange and prevent the escape of insects, with 10 g of wheat germ in each pot in a climatic chamber type BOD at 34 ± 1 ° C, 70 ± 10% RH and 12 hours of photophase. Every three days, the number of living and dead individuals was counted. This procedure was repeated until the death of the last insect.

2.7. Behavioral

The behavioral response bioassays were carried out in glass arenas (6 × 6 cm), in which half the majority components and their mixtures at 1% dissolved in acetone were sprayed with an airbrush. While the other goal was covered with a rigid pvc plastic sheet to avoid contamination of the other half. The other half used as a control was sprayed with acetone, following the same parameters as the previous spray. After the arenas were sprayed, they were left for 15 minutes to dry at a temperature of 25 ± 1 ºC, 50 ± 10% RH. The adult *L. serricorne* were acclimated for a period of 12 hours at a temperature of 25 ± 1 ºC, 50 ± 10% RH. After the periods of acclimatization and drying of the arenas, an adult *L. serricorne* was released in the center of the arena and maintained for 10 minutes. To avoid the flight of insects, the edges of the arenas were covered with double-sided tape. 30 insects were used for each treatment, each insect being one repetition. The walking activity in the arena was recorded using a Motorola 4K Full HD resolution digital video camera. Video Tox Trac software was used to track insects in the arena (Rodriguez et al., 2018). Through the Video Tox Trac software, the distance covered and the time spent without movement by insects in the treated and untreated area of the arena were counted. Insects that spent <1s in the half of the insecticide-treated arena were considered repelled, while those that spent <50% of the time on the insecticide-treated surface were considered irritated (Plata-Rueda et al., 2019).

2.8. Data analysis

For the acute toxicity tests by fumigation and sublethal effect, a completely randomized experimental design was used, with means compared by the Scott-Knott test ($p \leq 0.05$). Kaplan-Meier estimators (log-rank test) were used to analyze survival data.

Adult *L. serricorne* who did not survive until the end of the experiment were treated as censored data. The behavioral response data were submitted to unidirectional analysis of variance, with averages compared by the Scott-Knott test ($p \leq 0.05$). Acute toxicity, sublethal effect, survival analysis and behavioral response data were analyzed using the R statistical software (R Development Core Team program, 2010).

3. Results

3.1. Acute toxicity by fumigation

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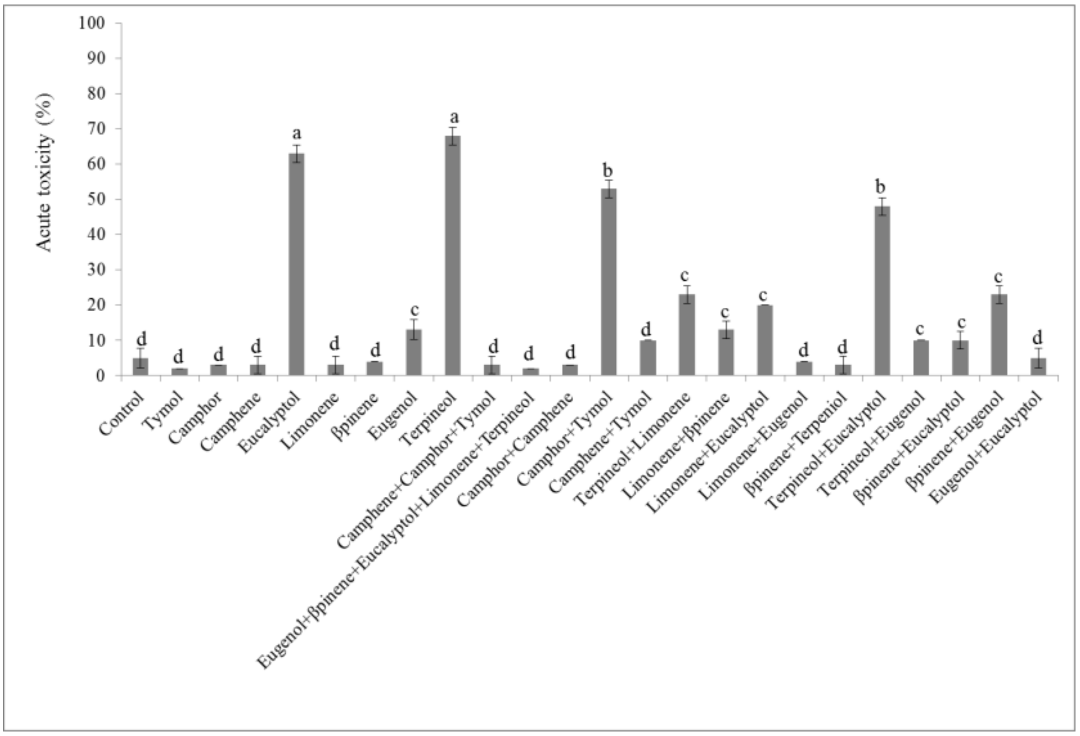
The acute toxicity of the major components and their mixtures against adult *L. serricorne* was shown in Figure1.

Figure 1. Percentage of dead adult *L. serricorne* at a temperature of 34 ± 1 ºC, 70 ± 10% RH and a photophase of 12 h, by major components and their mixtures. Equal letters do not differ statistically from each other by the test Scott-Knott, at 5% probability.



Source: Author himself.

Among the major components evaluated, eucalyptol and terpineol showed 65% mortality ($F_{23, 72}$= 102.70; P < 0.001) at the highest dose applied (10µl / ml). The other components (thymol, camphor, camphene, limonene, eugenol and βpinene) exhibited between 10 and 25% of mortality ($F_{23, 72}$= 102.70; P < 0.001). Regarding the mixtures (Fig.1), in which terpineol+eucalyptol and camphor+thymol presented 47 and 52% ($F_{23, 72}$= 102.70; P < 0.001) mortality, while the others provided between 10 and 25% mortality ($F_{23, 72}$= 102.70; P < 0.001).

3.2. Sublethal effect

The sublethal effect of the major components and their mixtures against *L. serricorne* adults is found in Figure 2.

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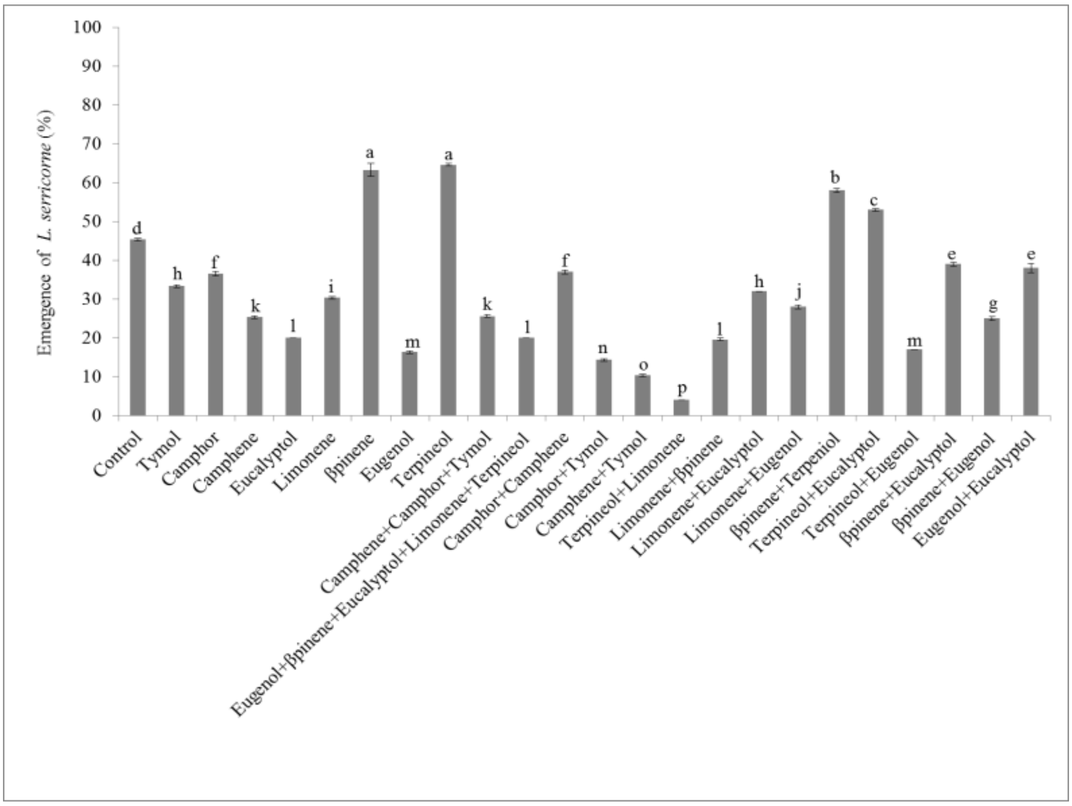
Figure 2. Emergency percentage of *L. serricorne* at a temperature of 34 ± 1 ºC, 70 ± 10% RH and a photophase of 12 h, by major components and their mixtures. Equal letters do not differ statistically from each other by the Scott-Knott test, at 5% probability.



Source: Author himself

The mixture of terpineol + limonene showed a lower emergence (4%) of L. serricorne ($F_{23, 48}$= 852.18; $P < 0.001$), followed by the mixture of camphene+thymol that exhibited 10% ($F_{23, 48}$= 852.18; $P < 0.001$). The other 13 mixtures provided emergence of L. serricorne between 30 and 50% ($F_{23, 48}$= 852.18; $P < 0.001$). The major components evaluated separately which allowed the highest emergence percentage were: eugenol and eucalyptol with 16 and 20% ($F_{23.48}$= 852.18; $P <0.001$) respectively. Insects exposed to the components βpinene and terpineol (both with 64%) exhibited an emergence superior to the control($F_{23, 48}$= 852.18; $P < 0.001$).

3.3. Survival

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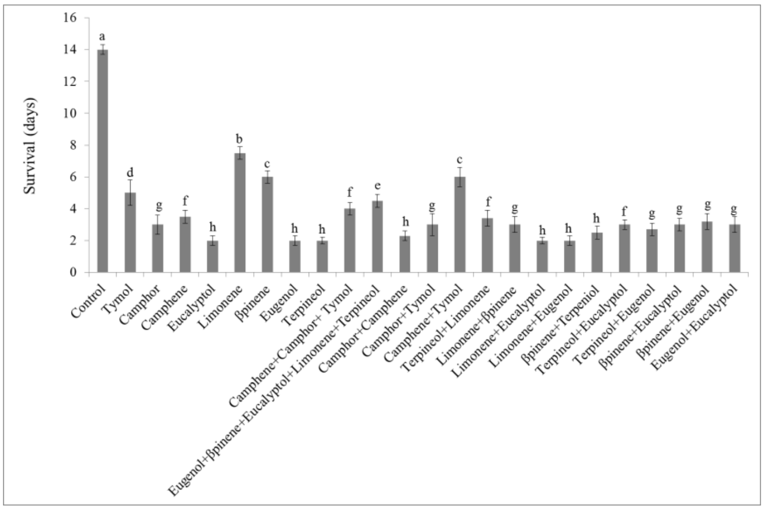
Analysis of adult *L. serricorne* exposed to major components and their mixtures indicated significant differences between treatments (log-rank test; $\chi2 = 296$; df = 23; $P < 0.001$) in Figure 3.

Figure 3. Survival analysis of *L. serricorne* at a temperature of 34 ± 1ºC, 70 ± 10% RH and a photophase of 12 h, of the major components and their mixtures. Equal letters do not differ statistically from each other by the Scott-Knott test, at 5% probability.



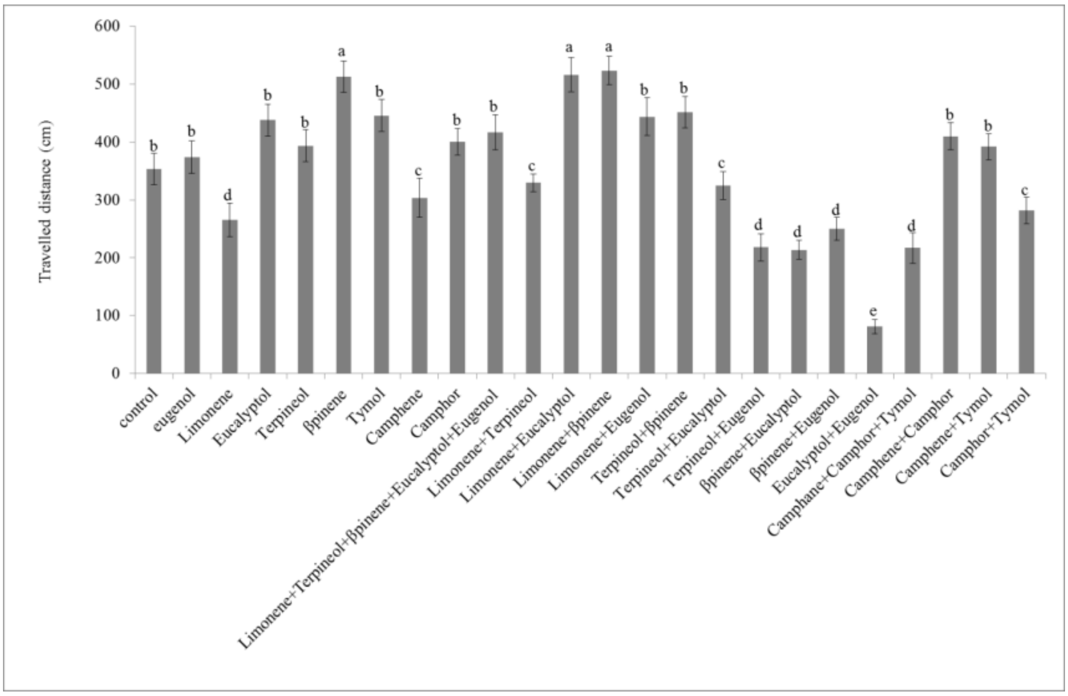
Source: Author himself

The major components eucalyptol, eugenol and terpineol provided a shorter average survival time of 2 days, followed by mixtures βpinene+terpeniol, limonene+eugenol, limonene+βpinene and camphor+camphene.

3.4. Behavioral responses

The behavioral responses of adult *L. serricorne* are expressed in the Figure 4 and Figure 5.

Figure 4. Behavioral response of *L. serricorne* in distance covered in arenas (cm) at a temperature of 25 ± 1 ºC, 50 ± 10% RH, of the major compounds and mixtures. Equal letters do not differ statistically from each other by the Scott-Knott test, at 5% probability.



Source: Author himself

The travelled distance was greater in the major component βpinene 512 cm (F23, 696= 14.46; P < 0.001), followed by the mixtures limonene+eucalyptol and limonene+βpinene that presented the distance covered between 515 to 520 cm (F23, 696= 14.46; P <0.001). However, the shortest distance covered was in the eucalyptol + eugenol 81 cm mixture (F23, 696= 14.46; P < 0.001).

Figure 5. Behavioral response of *L. serricorne* of time (s) without movement in arenas at a temperature of 25 ± 1 ºC, 50 ± 10% RH, of the major compounds and mixtures. Equal letters do not differ statistically from each other by the Scott-Knott test, at 5% probability.

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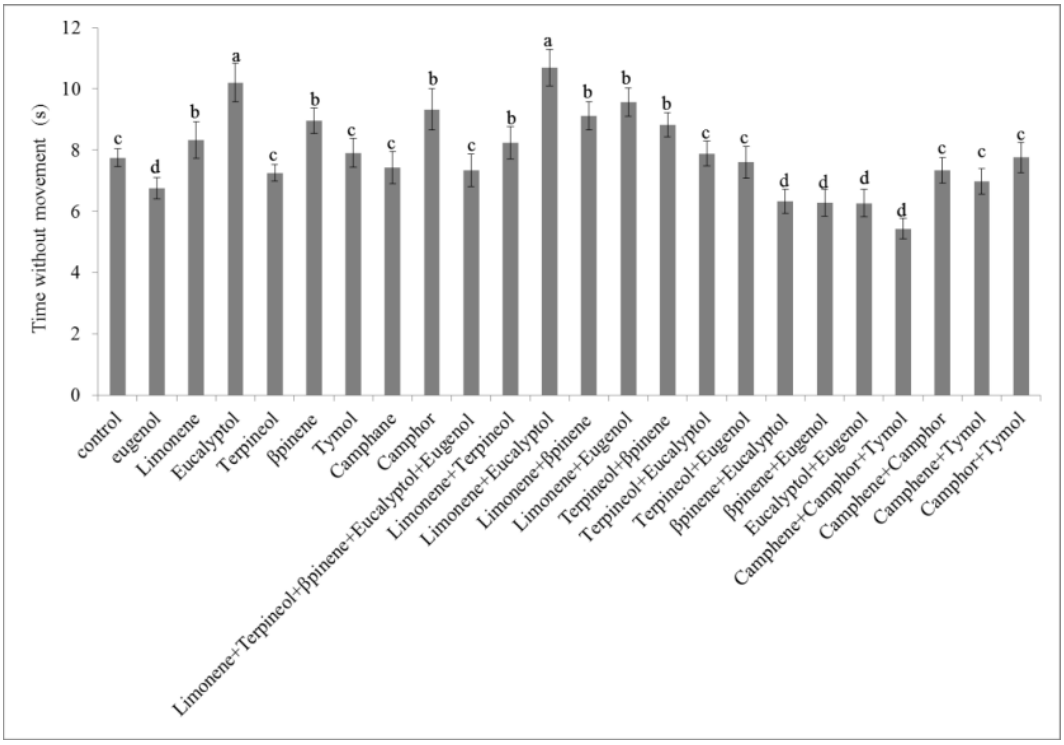

Source: Author himself.

For the time that the insects were without movement, the major components eucalyptol, limonene, βpinene and camphor showed higher values, between 8 and 10 seconds ($F23.696 = 6.74$; $P < 0.001$), followed by the mixtures limonene+eucalyptol, limonene+terpineiol, limonene+βpinene, limonene+eugenol and terpineiol+βpinene. However, the major component eugenol and the mixtures βpinene+eucalyptol, βpinene+eugenol, eucalyptol+eugenol, camphene+camphor+thymol presented the shortest time (6 seconds) without movement ($F_{23, 696} = 6.74$; $P < 0.001$).

4. Discussion

The major components of essential oil can cause insect mortality, as demonstrated in the present study, where in the evaluated economically viable dosage it promoted up to 60% of mortality in adult *L. serricorne*, among which, eucalyptol and terpineol and the mixtures terpineol+eucalyptol and camphor+thymol were the most promising. These components are monoterpenes that play a lipophilic role, which can bind to the trachiolar fluid, inhibiting enzymes or deactivating proteins in cells (Pavela, 2015). Chopa and Descamps (2012) and Kiran and Prakash (2015) suggest that the acute toxicity of grain pests stored by essential oils and major compounds is associated with biochemical, physiological responses and morphological differences (cuticle thickness), so they present contraction and muscle paralysis, a toxic effect on the nervous system. Zhao et al., 2013 and Prowse et al., 2006 reported that essential oils and major components had neurotoxic effects on enzymes from *Delia radium* L., *Muscado mestica* L., *Cacopsylla chinensis* and *Diaphorinacitri kuwayama*, both insects exhibited leg hyperextension, hyperactivity and immobilization.

Enzymes (SOD = Superoxide dismutase, CAT = Catalase, GSH = Glutathione and GSSG = Glutathione disulfide) are present in all aerobic organisms, responsible for the antioxidant system that provides the primary defense against oxidative stress that alters the normal physiological processes of insects (FontagneÂ-Dicharry et al., 2014). The compounds present in essential oils can alter the production of these enzymes, changing the biological process, insect life span and percentage of insects that will emerge (Aslanturk et al., 2011). Another important enzyme, acetylcholinesterase (AChE) which is an excitatory neurotransmitter, which can be reduced or even blocked by the components of essential oils, so it can kill insects by preventing the passage of the nervous impulse, and can also generate other physiological complications, decrease survival time and change insect emergence rate (Qin et al., 2010).

Regarding the sublethal effects, the effect on reducing longevity and emergency can be highlighted. Studies have shown that the major components of essential oils have neurotoxic action, which can trigger effects on the physiology of insects, reducing the ability to survive and the percentage of emergence. However, essential oils and their major components are reported as antagonists of various neurotransmitters and neuromodulators in insects such as acetylcholine, GABA (gamma-aminobutyric acid), octopamine and tyramine (Enan, 2005; Park et al. 2001; Tong and Coats, 2012). Affecting multiple biological activities in insects, reproduction, longevity, pheromone release (Haddi et al. 2015).

The behavioral response of *L. serricorne* showed that the major components and mixtures showed promising results for controlling this pest. Insect repellency actions are related to the detection of substances by the olfactory sensillae present in the antennae mainly as an insect response, triggering an escape behavior (Oliveira et al., 2018). (Missbach et al., 2014). These changes in insect behavior occur as a result of the action of toxic compounds on the nervous system, which stimulate or reduce the mobility of insects (Plata-Rueda et al., 2019). The opposite effect can also occur, the substance can attract insects. Oliveira et al. (2018) demonstrated that ρ-cymene attracted adult *Sitophilus zeamais* (Motschulsky)

(Coleoptera: Curculionidae). The present work showed that thymol, βpinene, limonene + eucalyptol and limonene + βpinene also attracted adult *L. serricorne*. However, in the same work by Oliveira et al. (2018), the major component thymol acted as an adult repellent for *S. zeamais*.

Behavioral responses can be influenced by the mode of action and the extent of the effects of lethal and sublethal doses (Hoy et al., 1998; Liu and Trumble, 2004). In insects, many factors can affect behavior, including quality of the resource fragment, type of habitat, specific interactions, encounters with edges of the fragment and presence of toxins, among others (Hoy et al., 1998; Bowler and Benton, 2005). Toxins can also lead insects to avoid or spend less time in areas with high concentrations (Hoy et al., 1998). These effects can be caused by the mechanisms and actions of essential oils or their major components, in which the physiological actions have been shown to be related to the disturbing capacity of the neurotransmitter GABA, as well as to the inhibition of the enzyme acetylcholinesterase in the insect's nervous system (Correa et al. al., 2015).

Essential oils, which are products that contain several major components, can cause mortality from fumigation, contact and ingestion, in addition to causing repellency. Therefore, its major components may have specific functions in the nervous system, altering the functions of some enzymes, thus causing changes in the physiology of insects.

5. Final Considerations

The results showed that the major components and their mixtures caused mortality, reduced survival time and progeny production, in addition to altering the behavioral activity of adult *L. serricorne*.

Therefore, these products can be used to prepare new insecticides, helping to control this insect in food storage units.

Need to conduct more detailed research on the effect of these major components on the physiology of insects, in order to better understand the mechanism of action of the major components that caused mortality.

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