Exposure to major components of essential oils and their mixtures cause mortality, sublethal effect and behavioral disturbance of *Sitophilus zeamais* (Motschulsky) (Coleoptera: curculionidae)

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Abstract

The *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), is an internal primary pest of stored grains of great economic importance. Few insecticides registered for fumigation control for *S. zeamais* exist, which makes it necessary to search for more control alternatives. Thus, the objective of this work was to assess acute toxicity, sublethal effect, survival and behavioral response of the major components of essential oils, Thymol, Camphora, Terpineol, Canpheno, Eucalyptol, Limonene, β Pinene and Eugenol; and the mixtures of these components on *S. zeamais*. The results showed that the mixtures of the major components caused acute toxicity, reduced survival time and offspring production, in addition to altering the behavioral activity of *S. zeamais*. The mixture Camphor + Canphene presented a synergistic effect, with LC₅₀ and LC₉₀ of 2.77 and 20.34 µl/ml, respectively. The mixtures of the major components proved to be promising products for the control of *S. zeamais*.

Keywords: Monoterpene, Sesquiterpene, Stored grain pest, Synergism

1. Introduction

The *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) is an internal primary pest of stored grains of great economic importance, because it can present cross infestation, in other words, infest grain in the field and also in the warehouse, where it penetrates into the mass of the grains. The *S. zeamais* has a high potential for reproduction, has many hosts, like wheat, corn, rice, barley and triticale [1,2].

The method used to control *S. zeamais* is the chemical, with fumigant synthetic insecticide (phosphine and methyl bromide) [3,4]. However, the inadequate application and intensive use of these insecticides, in grain storage locations over the years, have led to several problems, including the development of insect resistance [5,6]. Thus, the development of new insecticides that present a low risk of resistance development is necessary [7].

The majority components, which are substances of greater relative area that integrate the essential oils, have been considered a promising strategy for insect pest control, involving various modes of action, fumigation, repellency, contact and deterrent [8]. The essential oils, in general, have less toxicity in mammals, low persistence in the environment and decrease in insect resistance development [9,10,11].

In this study, the acute toxicity, sublethal effect and the behavioral response of major components Thymol, Camphora, Terpineol, Canpheno, Eucalyptol, Limonene, β pinene and Eugenol and their mixtures were evaluated on *S. zeamais*.

2. Material and Methods

2.1 Biological material

The *S. zeamais* was raised on corn, Zea mays L. Sp. Pl. 2: 971–972 (Poaceae), in an acclimatized room 25 ± 1 ºC, RH 65 ± 3% and photophase of 12 h in the Núcleo de Desenvolvimento Científico e Tecnológico em Manejo de Pragas e Doenças (NUDEMAFI) in the Centro de Ciências Agrárias e Engenharia da Universidade Federal do Espírito Santo (CCAE-UFES) in Alegre, Espírito Santo, Brazil.
Table 1: Proportions of mixtures of essential oils.

| Mixture | Mixing ratio | Majority components |
|---------|--------------|---------------------|
| 1       | 1:1:1        | Camphora + Campheno + Thymol |
| 2       | 1:1:1:1:1:1  | Limonene + Terpineol + β pinene + Eucalyptol + Eugenol |
| 3       | 1:1:1:1:1:1  | Camphora + Campheno + Thymol + β pinene + Eucalyptol + Eugenol |
| 4       | 1:1:1:1:1:1  | Limonene + Terpineol + β pinene + Eucalyptol + Eugenol |
| 5       | 1:1:1:1:1:1  | Limonene + Terpineol + β pinene + Eucalyptol + Eugenol |

2.4 Acute toxicity test by fumigation
The experiment was conducted at a temperature of 25 ± 1°C, relative humidity of 70 ± 10% and photophase of 12 h. The methodology adopted of Aslan et al. [9] was used to evaluate the fumigant effect. Glass containers, with 200 ml capacity, were used as fumigation chambers, where 20 S. zeamais adults, not sexual and 0-10 days old, were confined [12]. The majority components and their mixtures (Table 1), without any solubilization vehicle, were applied with automatic pipettor, 10µl in 18 cm² filter papers, attached to the bottom surface of the container lid. To avoid direct contact of the mixtures of the major components with the insects, the porous tissue (phyllum) between the cover was used where the filter paper was.

For each major component and their mixtures, five repetitions were performed. After 192 hours of exposure to treatments, the number of S. zeamais adults killed was counted. To confirm the mortality of adult S. zeamais, these were touched with a fine bristle brush. The immovable S. zeamais were considered dead.

2.5 Estimation of lethal concentration
In the previous test, 10µl of the major components and their mixtures were used to check the toxicity on adult S. zeamais. The majority components and their mixtures, which obtained a minimum mortality of 80%, were submitted to the lethal concentration estimate, in the following concentrations (µL/mL):0 (control); 1; 4; 5; 6; 8; 10. The lethal concentrations LC₅₀ and LC₉₀ were estimated using Probit regression [13].

2.6 Sublethal Effect Test
After 192 hours of exposure of adult S. zeamais to major components and their mixtures, the survivors of S. zeamais were sexed by the characteristics of the face [14]. 50 g of corn was infested with three males and six females, in a 150 ml plastic container with the screened lid. Posteriorly, the containers were placed in BOD, at 25 ± 1 °C, 70 ± 10% RH and 12 hours of photophase, left for 10 days for copulation and oviposition [15]. After this period, the adults were removed. 35 days after infestation, the number of adults emerged were assessed every two days, for a period of 10 days. The emerged adults were discarded after counting.

2.7 Survival Analysis
The adult S. zeamais surviving the toxicity test were placed in plastic pots (7 x 6 cm) with perforated lids, for air intake, with 30 g of corn in each pot. Every five days, the corn was changed and also the number of living and dead individuals were counted in each treatment. This procedure was repeated until the death of the last insect.

2.8 Behavioral responses
The adult S. zeamais was placed in a glass arena (15 x 15 cm) lined with filter paper. The inner edges of the arena were covered with EVA fabric to prevent the escape of insects. The behavioral response bioassays were carried out in arenas. At half of each arena, the major components and their mixtures, 1% dissolved in acetone, were sprayed with the aid of an airbrush. The other half was used as a control treated only with acetone. After the treatment of the arenas, they were left to dry in the shade for 15 minutes. An adult S. zeamais was released in the center of the treated arena and kept in the arena for 10 minutes. 30 insects were used for each treatment, resulting in 30 repetitions, in a completely randomized design. For each insect, the walking activity inside the arena was recorded using a Motorola Full HD 4K digital video camera. A Tox Trac video tracking system was used to analyze the videos, measure the distance that insects have traveled and the time spent resting in each half of the arena [16]. The insects that passed <1 s on the half of the arena treated with insecticide were considered repelled, while those who have passed <50% of the time on the insecticide-treated surface were considered irritated [17].

2.9 Data analysis
Para os ensaios de toxicidade aguda por fumigação e efeito subletal, the completely randomized experimental design was used, with means compared by the Scott-Knott test (p≤0.05). Para análise dos dados de sobrevivência, estimators of Kaplan-Meier (log-rank test) was used. The adult S. zeamais that survived until the end of the experiment were treated as censored data. Behavioral response data was submitted to unidirectional analysis of variance, with means compared by the Scott-Knott test (p≤0.05). The data of acute toxicity, sublethal effect, survival analysis and behavioral response were analyzed using the R Development Core Team program [18].

3. Results
3.1 Acute toxicity of major components and mixtures
The efficacy of the major components in terms of lethal effect against S. zeamais adults is shown in Figure 1. Of the eight major components tested, one (Eucalyptol) caused acute toxicity of 60% (F2, 90=29.90; P< 0.001) in the highest
applied dose of 10µl/ml. However, the other seven
components (Thymol, Camphora, Campheno, Limonene,
Eugenol, Terpineol and β pinene) caused mortality among 30
and 45% (F_{22}, 92=29.90; P<0.001). 15 mixtures were
evaluated (Fig. 1). The (Camphora+ Campheno) mixture
presented a synergistic effect, with a LC₅₀ and LC₉₀ of 2.77
µl/ml air and 20.34 µl/ml air, respectively (Table 2), while 11
mixtures showed an antagonistic effect and three mixtures
were stable, showing no antagonistic and synergistic effect.

Table 2: Lethal concentration of the (Camphora+ Campheno) mixture on S. zeamais adult females.

| Treatment                  | LC₅₀ (µl/ml air) IC 95% | LC₉₀ (µl/ml air) IC 95% | Slope±SE | Chi Square (χ²) | p-Value |
|----------------------------|-------------------------|-------------------------|----------|----------------|---------|
| Camphor + Camphene         | 2.77 (2.16±3.34)        | 20.34 (14.57±34.28)     | 1.90 ± 0.23 | 2.84          | 0.589   |

A Straight slope.
B Chi Square.
C p-Value.

3.2 Sublethal effect of major components and mixtures
For the sublethal effect, presented in Fig. 2, the majority
components Eugenol, β pinene and Eucalyptol feature
between 16 to 20% (F_{22}, 92=6.91; P<0.001) of emergency of
S. zeamais adults, followed by Campheno with 23% (F_{22},
92=6.91; P<0.001); Thymol, Camphora and Terpineol 36 to
40% (F_{22}, 92=6.91; P<0.001) and Limonene 46% (F_{22}, 92=6.91;
P<0.001). The sublethal effect of the 14 mixtures shown in Figure 2,
seven mixtures showed synergism (Campheno + Camphora +
Thymol; Thymol + Camphora; Thymol + Campheno; Limonene + β
pinene; Limonene + Eugenol; Terpineol + β pinene; Terpineol +
Eucalyptol), with an emergency rate of S. zeamais adults between
3 to 8% (F_{22}, 92=6.91; P<0.001). However, seven mixtures remained stable, showed no
antagonism and synergism.
### 3.3 Survival Analysis
The survival analysis of *S. zeamais* adults exposed to major components and their mixtures indicated significant differences between treatments (log-rank test; $\chi^2 = 130; df = 22; P < 0.001$). The Limonene+ β pinene mixture provided shorter average survival time (11%), followed by the Camphora+Thymol mixture (20%) Fig. 3.

![Fig 3: Survival analysis of the majority compounds and mixtures on *S. zeamais*.

### 3.4 Behavioral responses
The behavioral responses of adult *S. zeamais* released in semi-treated arenas are shown in Fig.4 and Fig.5. The distance covered was greater in the majority component Thymol (382 cm) ($F_{23, 696} = 57.66; P < 0.001$) and Terpineol (272 cm) ($F_{23, 696} = 57.66; P < 0.001$). The shortest distance traveled was in the mixtures β pinene+Eucalyptol and Terpineol+Limonene (15 cm) ($F_{23, 696} = 57.66; P < 0.001$) (Fig.4). The rest times were longer in the major components Thymol (12 s) ($F_{23, 691} = 30.79; P < 0.001$), followed by Terpineol (10.88 s) ($F_{23, 691} = 30.79; P < 0.001$), Eucalyptol (10.74 s) ($F_{23, 691} = 30.79; P < 0.001$) and β pinene (10.54 s) ($F_{23, 691} = 30.79; P < 0.001$) and mixtures Camphora+Thymol (8.58 s) ($F_{23, 691} = 30.79; P < 0.001$) compared to the control. However, the mixture β pinene+Eucalyptol (4.66 s) ($F_{23, 691} = 30.79; P < 0.001$) and Terpeniol+Limonene (2 s) ($F_{23, 691} = 30.79; P < 0.001$) showed less rest time than the control Fig.5.

![Fig 4: Behavioral response in traveled distance (cm) of the majority compounds and mixtures on *S. zeamais*.](image-url)
4. Discussion
The lethal effect of the major components can be added with the preparation of mixtures as was observed with (Camphora+Campheno), that showed acute toxicity on S. zeamais adults (CL50 2.10 µl/ml), concentration lower than phosphine (PH3) used on Trogoderma granarium Everts, 1899 (Coleoptera: Dermestidae) and Rhyzopertha dominica (F.) (Coleoptera, Bostrichidae) [19,20]. The high acute toxicity presented by the (Camphora+ Campheno) mixture can be related to the synergism of Camphora with Campheno, because they belong to different groups, oxygenated monoterpenes and bicyclic monoterpene. Interaction of major components of different group was studied by Tak and Isman that showed the interaction between monoterpene component α-terpineol (+) vanillin phenolic on Tetranychus urticae Koch (Acari: Tetranychidae) [21].

Although it is difficult to define precisely, the effectiveness of individual major components on insects occurs due to the complexity of the effects on them, causing acute toxicity, repellency and sublethal effect [10]. For sublethal effect, the mixes (Campheno+Camphora+Thymol; Thymol+Camphora; Thymol+Campheno; Limonene+ β pinene; Limonene+Eugenol; Terpineol+Terpineol; β pineno+Eucalyptol; β pineno+Eugenol) developed antagonistic effect. This antagonistic effect may be due to the competition between substances, because both substances can compete for the same receptor system, preventing access and combination of substances to receptors, blocking its effects [24].

The major components and their mixtures interfered in the survival of adult S. zeamais. However, some essential oils and major components may have slow action by fumigation, ingestion or topical, similar to the synthetic insecticide cyantraniliprol [25, 26]. According Lopez and Pascual-Villalobos, the AChE of different insects can be inhibited by essential oils and their compounds [27]. The decrease in the activity of AChE causes a direct change in insect behavior, like flight, copulation and oviposition, as well as changes in many other insect biological processes, in addition to indirectly affecting lipid remobilization in oxidative metabolism of ovogenesis [28, 29]. Santos et al., evidenced that the essential oil of Piper aduncum L. (Piperaceae) had a significant impact on the survival of the caterpillars of Helicoverpa armigera Hubner (Lepidoptera: Noctuidae) [30]. The behavioral response test indicated that the mixtures β pinene+Eucalyptol and Terpineol+Limonene and the major components Thymol and Terpineol had substantial effects on S. zeamais. The mixtures β pinene+Eucalyptol and Terpineol+Limonene were repellents for S.zeamais, while Thymol and Terpineol had attractive effects. These effects may be related to the detection of substances by the olfactory sensilla of insects, responsible for triggering escape behavior. Thus, when S. zeamais recognize the substances, the locomotion activities as an escape strategy increase [31]. These changes in the walking patterns of insects occur as a result of the action of toxic compounds on the nervous system that stimulate or reduce the mobility of insects [17]. However, the result presented by Thymol in the present work contradicts the result presented by Oliveira et al., where Thymol acted as insect repellent [8]. This difference, in the results presented above, may be related to the part of the plant where the essential oil was extracted. Silva et al. and Tripathi et al. showed that different parts of plants can have different major components and different effects on the biological material [32,33].
5. Conclusion
Our results show that the major components and their mixtures caused mortality, reduced the survival time and progeny production, in addition to altering the activity of walking of S. zeamais. Thus, the majority components, Thymol, Terpineol and Eucalyptol and the mixtures Campheno + Camphora, Campheno + Thymol, Thymol + Camphora, Thymol + Campheno, Limonene + β pinene, Limonene + Eugenol, Terpineol + β pinene, Terpineol + Eucalyptol, β pinene + Eucalyptol and Terpineol + Limonene exhibit lethal and sublethal effects on S. zeamais and can be used as an alternative to other synthetic insecticides, helping to decrease the development of insecticide resistance to insecticides.

6. Declaration of Competing Interest
All authors of this manuscript declare that have no conflict of interest.

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