

Bioactivity, biochemical mechanisms, and olfactory effects of the essential oil from *Syzygium aromaticum* and its major compound eugenol on *Sitophilus zeamais* L. (Coleoptera: Curculionidae)

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Abstract

With the significant losses *Sitophilus zeamais* (Linnaeus, 1763) causes to corn from fields to storage and the harmful effects of excessive chemical insecticide use, this study aimed to assess the toxicity of *Syzygium aromaticum* essential oil and its main component, eugenol. It also looked into how they impact *S. zeamais* biochemical profile over time. The study analyzed chromatographic data for the oil and various parameters for the insects, including mortality rates, repellence, deterrence, adult emergence, and biochemical changes (proteins, lipids, sugars, and glycogen levels). Results showed that *S. aromaticum* essential oil had a significant lethal effect on *S. zeamais*, with an LC_{95} of 35.21 $\mu\text{L/L}$ and an LC_{50} of 13.7 $\mu\text{L/L}$, while eugenol's LC_{95} was 12.74 $\mu\text{L/L}$ and its LC_{50} was 7.33 $\mu\text{L/L}$ in contact toxicity tests. Both the oil and eugenol demonstrated repellent and fumigant effects, disrupting the insect's biology. In terms of nutritional impact, the substances affected the insects differently. The essential oil initially increased nutrient levels but then reduced them after 48 hours, while eugenol initially increased levels but eventually led to a decrease. In conclusion, both the essential oil and eugenol showed adverse effects on *S. zeamais*, altering crucial survival parameters. However, eugenol yielded more significant results and is readily available commercially, making it a promising candidate for developing new insecticidal products.

1. INTRODUCTION

Pests can wreak havoc on crops from the field to storage, causing considerable economic losses, sometimes up to 10% of the total annual production (Lorini 2015). *Sitophilus zeamais* (Linnaeus, 1763) (Coleoptera: Curculionidae), commonly known as the maize weevil, is a prime culprit in such infestations. With its penchant for lurking within grains, it not only leads to quantity and quality losses but also opens the door to contamination by other pathogens, posing risks to both human and animal health (Queiroz et al., 2020; Silva; De Farias, 2020).

Chemical liquid insecticides (preventive treatment), natural insecticide based on diatomaceous earth (preventive treatment), and fumigation with phosphine insecticide (curative treatment) are the three commonly involved methods in the control of *S. zeamais*. However, the continuous and indiscriminate use of these substances leads to the accumulation of toxic residues in food, environmental contamination, and the selection of resistant populations. Therefore, there is a need for the development of products with greater environmental safety (Ribeiro et al. 2020).

Exploration into novel biologically safe and effective molecules is evolving to address these voids, and in this pursuit, the examination of essential oils and their constituents has been burgeoning and garnering prominence in recent years. This interest stems from the rich composition of these substances and their abundance in nature, as well as their proven effectiveness in various fundamental parameters for insect survival, directly affecting their establishment in crops (Cruz et al., 2017; Silva et al., 2019a; Dutra et al., 2020).

The literature reports that among the oils studied so far, *Syzygium aromaticum* (Myrtales: Myrtaceae) stands out for its effectiveness against various stored grain pests. Gizawy et al. (2018) observed its highest efficiency through fumigation against *Sitophilus oryzae* (Coleoptera: Curculionidae), *Rhyzopertha dominicae* (Coleoptera: Bostrychidae), and *Tribolium castaneum* (Coleoptera: Tenebrionidae). Ramsha et al. (2019) and Mansour et al. (2021) observed repellent behavior against *T. castaneum* and *S. oryzae*, respectively. Soe et al. (2020) highlighted its residual effect on the mortality of *Callosobruchus maculatus* (Coleoptera: Bruchidae). However, the wide range of effects caused by essential oils and their compounds is not limited to the mortality and/or repellency of these organisms. Biological and physiological alterations result from the use of these substances, influencing their population dynamics and establishment as pests (Cruz et al., 2016; Dutra et al., 2019; Ebrahimifar et al., 2020).

Morphological changes in target organs, low assimilation of essential nutrients, and reduced defense capacity are some of the physiological alterations associated with the use of oils and their compounds. These alterations consequently affect the biological cycle and reproductive status, being more evident in holometabolous insects, where nutritional acquisition in immature stages is essential for offspring development (Cruz et al., 2017; Dutra et al., 2020; Guedes et al., 2020).

Given the above, this research aimed to study the effect of the essential oil of *Syzygium aromaticum* and its major compound, eugenol, on the maize weevil *Sitophilus zeamais*. It evaluated the following parameters: for the oil (chromatographic analysis); and for the insect pest (mortality, repellency, deterrence, adult emergence, and alterations in the biochemical profile, including proteins, lipids, sugars, and glycogen). The aim of this investigation was to enhance comprehension of the mechanisms of action of these substances in order to facilitate the development of more potent products.

2. MATERIAL AND METHODS

The research was conducted at the Alternative Pest Control Laboratory, situated at the Campus of Engineering and Agricultural Sciences (CECA) of the Federal University of Alagoas (UFAL) in Rio Largo, AL. Controlled environmental conditions were maintained, including a temperature of $25 \pm 2^{\circ}\text{C}$, relative humidity of $60 \pm 10\%$, and a 12-hour photoperiod. Additionally, experiments were carried out at the Insect Physiology Laboratory of the Department of Animal Morphology and Physiology (DMFA) at the Federal Rural University of Pernambuco (UFRPE).

Breeding *Sitophilus zeamais* Motschulsky 1855 (Coleoptera: Curculionidae). Corn grains of the BRS Catingueiro variety (variety code) were stored in a freezer at a temperature of 10°C for a minimum period of 10 days to eliminate any biological agents. Subsequently, these grains were placed in hermetically sealed glass jars and kept at room temperature for an additional 10 days to achieve hygroscopic equilibrium before being used for experiments and breeding. Later, these grains were placed in glass containers with a capacity of 2.5 liters, whose lids contained an opening covered with voile fabric for ventilation. Infestations with *S. zeamais* occurred biweekly, for which the glass jars were filled halfway with corn, and then the insects from the breeding were introduced, remaining in this environment for 10

days. They were then removed from the container, and the F1 generation was awaited, aiming to maintain the breeding and development of the experiments.

Obtaining the essential oil of *Syzygium aromaticum* (Myrtales: Myrtaceae) and its main compound, eugenol. The essential oil of *S. aromaticum* and eugenol were acquired from the specialized company Quinari® Casa das Essências – Ponta Grossa/PR (<https://www.quinari.com.br/>). According to the technical report from Quinari®, the essential oil is a 100% pure product, free from solvent and diluent, originating from Indonesia, obtained through steam distillation of leaves and flower buds. The major compound, eugenol, has a purity of 99.8%.

Chromatographic analysis with mass spectrometry (GC/MS) for chemical identification of *Syzygium aromaticum* essential oil. For the confirmation of the chemical analysis of the substances present in the clove essential oil, gas chromatography-mass spectrometry (GC/MS) was performed at the Natural Resources Research Laboratory (LPqRN) of the Federal University of Alagoas (UFAL). The samples were injected into the GC/MS model 2010 PLUS, Shimadzu, using a Nist-01 column (100% Dimethylpolysiloxane) with dimensions of 30 m length and 0.25 mm diameter. The method conditions were as follows: injector temperature set at 250°C in Split mode (with flow division), column starting at 60°C/3 min, followed by a gradient of 6°C/min until reaching 210°C, then increased by 15°C/min until reaching 300°C and held. Electron impact (EI) ionization was used with 70 eV (electron volts), with the ion source detector temperature set at 290°C. The interface temperature was 220°C, and the fragmentation values recorded ranged from a scan of 35 m/z to 500 m/z, with a total run time of 34 min. A mixture of linear hydrocarbons (C7H16-C30H32) was injected under the same experimental conditions as the samples, and the identification of the components was performed by comparing the obtained fragmentation spectra with the equipment database (Wiley 7 lib and NIST 08 lib) and through the use of the Kovats Retention Index, with each component calculated as previously described (Van Den Dool; Kratz, 1963; El-Sayed, 2011). The data were obtained and processed using the Shimadzu GC/MS Solution software.

Contact toxicity test with *Syzygium aromaticum* essential oil and its major compound, eugenol, on *Sitophilus zeamais* adults. In the glass containers with 40g of BRS Catingueiro maize seeds each, *Syzygium aromaticum* essential oil was added using an automatic pipette, which were then manually shaken for two minutes. Each 40g portion of maize was infested with 16 unsexed adults of *S. zeamais*, aged up to 15 days. After 48 hours of confinement, the mortality percentages were recorded. The same procedure was used for the major compound eugenol. Preliminary tests were conducted at different concentrations to find values close to the Upper Limit (UL) and Lower Limit (LL). Using the Bliss formula, concentrations for the definitive bioassay were defined, estimating the LC50 and LC99. The data were analyzed by Probit using the SAS Program (SAS Institute 2021) to estimate the lethal concentrations LC50 and LC95 for each treatment.

Repellency test of *Syzygium aromaticum* essential oil and its major compound, eugenol, on *Sitophilus zeamais* adults. The tests occurred in arenas composed of two glass containers connected to a central

container via two glass tubes. In one of the containers, 20 g of untreated corn grains were added, while in the other, the same amount of grains containing different concentrations of *Syzygium aromaticum* essential oil and its major compound eugenol, on filter papers in sachets, were added. In the central container, 16 non-sexed adults of *S. zeamais*, up to 15 days old, were released. The concentrations were based on those estimated in the contact test. After 48 hours, the insects in each container were quantified to evaluate repellency. The Repellency Index (RI) was calculated by the formula: $RI = 2G/(G + P)$, where G = % of insects attracted in the treatment and P = % of insects attracted in the control. RI values range from zero to two, where RI = 1 indicates similar repellency between the treatment and the control (neutral treatment), RI > 1 indicates lower repellency of the treatment compared to the control (attractive treatment), and RI < 1 corresponds to higher repellency of the treatment compared to the control (repellent treatment). The safety interval used to verify if the essential oil is repellent or not was obtained from the average RI (repellency index) and its respective standard deviation (SD), meaning that if the average RI is less than 1 - SD, the essential oil is repellent; if the average is greater than 1 + SD, the essential oil is attractive, and if the average is between 1 - SD and 1 + SD, the essential oil is considered neutral. This index is an adaptation of the formula mentioned by Lin et al. (1990) for consumption index.

Test for the emergence of *Sitophilus zeamais* adults treated with *Syzygium aromaticum* essential oil and its major compound, eugenol. After counting the eggs, the containers with all the material were kept in the laboratory to assess emergence. In this evaluation, the number of adults emerged in the treatment and control containers was counted separately until the end of emergence (five consecutive days without any further insect emergence).

Bioassay in Y-olfactometer of the essential oil of *Syzygium aromaticum* and its major compound, eugenol, on adults of *Sitophilus zeamais*. The behavioral response of *S. zeamais* adults to the oil of *S. aromaticum* and its major compound, eugenol, was assessed using a Y-olfactometer operated with a continuous airflow of 1.4 L/min, previously humidified and filtered with activated charcoal. For the odor source, a piece of filter paper (2 x 2 cm) impregnated with 10 µL of the treatment (oil or eugenol) or hexane (control) was used, with these being added to the base of each arm of the olfactometer. The insects were introduced into the base of the main tube of the olfactometer, and their behavior was observed for 10 min. The response was recorded when the beetle walked to the portion corresponding to 75% of the chosen arm. No response was recorded when it did not walk against the airflow and/or did not reach the considered portion during the 10 minutes observed. Twenty non-sexed adult insects were tested, with each individual considered as a repetition (20 repetitions/treatment). The odor source was changed for each tested individual. The experiments were conducted between 11:00 a.m. and 2:00 p.m. The data were subjected to statistical analysis using the Chi-square test.

Biochemical assays of the essential oil of *Syzygium aromaticum* and its major compound, eugenol, on *Sitophilus zeamais* adults. For the extraction and quantification of Total Soluble Proteins, Lipids, Total Sugar, and Glycogen, the lethal concentrations obtained in the toxicity bioassays by topical contact were used, applying the estimated lethal concentrations (LC25, 50, and 75). For eugenol, the concentrations were (LC25: 6.24 µL), (LC50: 7.33 µL), and (LC75: 8.6 µL), and for the *S. aromaticum* oil, (LC25: 9.3 µL),

(LC50: 13.7 μ L), and (LC75: 20.17 μ L). Extraction occurred at two time points, 24 and 48 hours, after the toxicity bioassays were set up. Each pool consisted of four adult *S. zeamais* insects, thus comprising one sample for each treatment. Ten samples per treatment were obtained, totaling 40 insects/treatment. Sample preparation (control and treatment) was carried out by macerating the insects in phosphate-buffered saline (PBS) (pH 7.4 and 0.1 M), in a ratio of four insects/5 mL of buffer. For each macerated group, 1.0 mL of the macerate (insect + buffer) was pipetted out using an automatic pipettor and stored in a labeled microtube (eppendorf). The entire procedure was performed at low temperature to prevent sample oxidation, and then the samples were separated. For this purpose, each sample was centrifuged for 3 minutes at 3000 rpm. After centrifugation, 100 μ L of each sample was separated for total soluble protein analysis, and 200 μ L was separated for lipid, total sugar, and glycogen analyses.

Extraction and Quantification of Total Soluble Proteins. Total soluble proteins were measured using the Bradford method (1976). For each sample, 100 μ L of macerate was transferred to glass centrifuge tubes. Bradford reagent (0.01% Coomassie Blue G-250; 8.5% phosphoric acid; 4.7% ethanol) was added to reach a final volume of 5 mL. The mixtures were homogenized using a vortex mixer and then left to rest for 2 minutes. Absorbance was measured with a UV-visible spectrophotometer (Biospectro SP-220) at a wavelength of 595 nm. The unit of measurement was μ g/mL. Results were analyzed using ANOVA, and means were compared using Tukey's test at a 5% significance level, utilizing the ProcGLM procedure in SAS (SAS 2001).

Extraction and Quantification of Lipids, Total Sugar, and Glycogen. The content of lipids, total sugar, and glycogen was evaluated using the Van Handel method (1985a, b). First, 200 μ L of the homogenized macerate were mixed with 200 μ L of sodium sulfate and 800 μ L of a chloroform-methanol solution (1:1) and then centrifuged at 3000 rpm for 3 minutes. The precipitate was used for glycogen analysis, while the supernatant was transferred to a graduated microtube, to which 600 μ L of distilled water was added, and centrifuged again at 3000 rpm for 3 minutes. Lipids were treated with a phosphoric acid/vanillin reagent, and total sugar and glycogen were treated with a sulfuric acid/anthrone reagent. Absorbance was measured using a UV-Visible spectrophotometer (190–1000 nm, Biospectro SP-220) at a wavelength of 625 nm. Results were recorded in μ g/mL. The data were subjected to ANOVA, and the means were compared using Tukey's test at a 5% probability level with the ProcGLM procedure of SAS (SAS 2001).

3. RESULTS

Chromatographic analysis with mass spectrometry (GC/MS) for the chemical identification of *Syzygium aromaticum* essential oil. In the chromatographic analysis, five peaks were identified between 11 and 17 minutes of the chromatographic run (Fig. 1). The main component found in this oil was the phenylpropanoid eugenol, which accounted for about 85.96% of the total composition. This was followed by the sesquiterpenes β -caryophyllene, α -humulene, D-cadinene, and caryophyllene oxide (Table 1).

Table 1

Chemical composition of *Syzygium aromaticum* essential oil, identification based on GC-MS analysis and confirmation with standard compounds.

Nº	Name of compounds	RT	tRI	cRI	%
1	Eugenol	11.68	1325	1321	85.96
2	Trans β -caryolphyllene	13.39	1405	1399	11.44
3	α -Humulene	14.07	1431	1431	2.07
4	D- Cadinene	15.52	1501	1502	0.16
5	Caryophyllene oxide	16.40	1547	1547	0.37
RT = Retention time (minutes), tRI = tabulated retention index (Adams, 2007), cRI = calculated retention index, % = component percentage					

Contact toxicity test of *Syzygium aromaticum* essential oil and its major component, eugenol, on adult *Sitophilus zeamais*. In the contact toxicity test of *Syzygium aromaticum* essential oil on *Sitophilus zeamais*, Probit analysis revealed a 95% lethal concentration (LC₉₅) of 35.21 μ L/L and an LC₅₀ of 13.7 μ L/L. For the major component, eugenol, the LC₉₅ and LC₅₀ were 12.74 μ L/L and 7.33 μ L/L, respectively. The low chi-square value in the bioassays indicates the homogeneity of the test population (Table 2).

Table 2

Contact toxicity of *Syzygium aromaticum* essential oil and the major compound eugenol on *Sitophilus zeamais*

Product	N	Inclination $\pm$ (EP)	LC ₅₀ (95% IC)	LC ₉₉ (95%IC)	χ^2
<i>S. aromaticum</i> essential oil	400	4.013 $\pm$ 0.283	13.703 μ L (12.263–15.252)	35.212 μ L (30.476–2.024)	2.165
Eugenol	400	9.691 $\pm$ 0.810	7.33 μ L (7.10–7.60)	12.74 μ L (11.66–14.43)	6.29
N = Number of insects, EP = mean standard error, CI = confidence interval, LC = Lethal Concentration, χ^2 = Chi-square (significant at the 5% probability level).					

Repellency test of *Syzygium aromaticum* essential oil and its major component, eugenol, on adult *Sitophilus zeamais*. Adult *S. zeamais* exhibited a repellent behavior towards both the essential oil of *Syzygium aromaticum* and its major component, eugenol. It is important to note that lower concentrations of eugenol were required compared to the essential oil, due to its higher concentration (Fig. 2).

Emergence test of *Sitophilus zeamais* exposed to *Syzygium aromaticum* essential oil and its major component, eugenol. There was a decrease in the emergence rate of adult *S. zeamais* for treatments with both *Syzygium aromaticum* essential oil and its major component, eugenol, when compared to the control (Fig. 3).

Olfactory test using olfactometer with *Syzygium aromaticum* essential oil and its major component, eugenol. Adult *S. zeamais* showed a preference for the arm of the Y olfactometer containing only hexane (control) compared to the arm containing the essential oil or the major component, eugenol, confirming the repellent effect of the tested treatments (Fig. 4).

Biochemical Assays

For the 24-hour period at LCs₂₅, 50, and 75 of the major component eugenol:

There was a reduction in the quantification of total proteins in insects treated with LC₅₀ (53.638 ± 3.96) and LC₇₅ (58.142 ± 3.73) compared to the control (74.925 ± 4.93). However, insects from LC₂₅ (70.935 ± 3.36) did not differ from the control ($F = 6.29$; $P < 0.0001$). For lipids, there was a reduction in insects treated only with LC₂₅ (15.642 ± 0.94), while the others (LC₅₀ 30.142 ± 1.79 ; and LC₇₅ 29.685 ± 3.89) did not differ from the control (26.542 ± 1.40) ($F = 8.60$; $P = 0.0002$). In glycogen, all treatments reduced (LC₂₅ 16.212 ± 1.36 ; LC₅₀ 16.406 ± 0.80 ; and LC₇₅ 19.844 ± 0.73) when compared to the control (25.587 ± 1.16) ($F = 17.47$; $P < 0.0001$). For total sugar, insects treated with LC₂₅ (22.531 ± 1.06) showed a reduction, while those treated with LC₅₀ (39.481 ± 0.58) and LC₇₅ (71.225 ± 1.10) increased compared to the control (28.931 ± 0.96) ($\chi^2 = 35.5116$; $P < 0.0001$) (Fig. 5).

For the 48-hour period in LCs₂₅, 50 and 75 of the majority eugenol:

Insects treated with LC₂₅ (80.807 ± 1.73) did not differ from the control regarding proteins (79.807 ± 1.99). However, those treated with LC₅₀ (92.638 ± 1.38) and LC₇₅ (93.068 ± 1.31) increased compared to the other treatments ($F = 20.47$; $P < .0001$). There was an increase in lipid levels in insects treated with all three concentrations: LC₂₅ (49 ± 4.74), LC₅₀ (43 ± 3.48), and LC₇₅ (48.5 ± 3.70) compared to the control (35.371 ± 2.70) ($F = 2.89$; $P = 0.0488$). For glycogen, there was a reduction in insects treated with LC₂₅ (20.150 ± 1.81) and LC₇₅ (26.019 ± 1.72), while LC₅₀ (32.294 ± 2.01) did not differ from the control (39.377 ± 2.96) ($F = 14.23$; $P < .0001$). In total sugar, there was a reduction in insects treated with LC₂₅ (29.587 ± 0.88), LC₅₀ (44.950 ± 1.41) did not differ from the control (43 ± 4.31), and LC₇₅ (60.031 ± 1.25) increased ($F = 27.04$; $P < .0001$) (Fig. 6).

For the 24-hour period at LCs₂₅, 50, and 75 of *S. aromaticum* essential oil:

There was no statistical difference in total protein quantification between the control (74.926 ± 4.93) and insects treated with LC₂₅ (68.611 ± 3.28), LC₅₀ (84.258 ± 8.54), and LC₇₅ (96.065 ± 6.26) ($\chi^2 = 7.54$; $P = 0.0565$). There was an increase in lipid content in insects treated with LC₂₅ (68.611 ± 3.28), while those

from LC₅₀ (27.143 ± 2.58) did not differ from the control (26.542 ± 1.40), but only those treated with LC₇₅ (82.930 ± 5.67) showed an increase ($\chi^2 = 22.7715$; $P < .0001$). For glycogen, insects from LC₂₅ (10.33 ± 0.88) and LC₅₀ (8.552 ± 1.72) showed a reduction compared to the control (25.589 ± 1.16), while LC₇₅ (36.451 ± 4.82) increased ($F = 23.60$; $P < .0001$). In total sugar, all treatments LC₂₅ (89.914 ± 6.39), LC₅₀ (92.070 ± 10.91), and LC₇₅ (44.789 ± 1.70) increased compared to the control (28.931 ± 0.96) ($\chi^2 = 29.0929$; $P < .0001$).

For the 48-hour period at LCs 25, 50, and 75 of *S. aromaticum* essential oil:

For all treatments LC₂₅ (55.557 ± 5.50), LC₅₀ (53.320 ± 4.13), and LC₇₅ (60.870 ± 2.47), there was a reduction in total protein compared to the control (79.808 ± 1.99) ($F = 10.08$; $P < .0001$). Lipids followed the same pattern, decreasing in all treatments LC₂₅ (6.633 ± 0.18), LC₅₀ (5.605 ± 0.15), and LC₇₅ (4.840 ± 0.12) compared to the control (35.369 ± 2.70) ($\chi^2 = 22.7304$; $P < .0001$). In glycogen, there was a reduction in insects treated with the LC₂₅ (10.331 ± 0.88) and LC₅₀ (8.552 ± 1.72) and an increase in those treated with LC₇₅ (36.451 ± 4.82) ($F = 23.46$; $P < .0001$). Total sugar showed a reduction in insects from all three treatments LC₂₅ (35 ± 0.30), LC₅₀ (29.6 ± 0.32), and LC₇₅ (43.008 ± 0.33) compared to the control (48.008 ± 4.31) ($\chi^2 = 26.3805$; $P < .0001$) (Fig. 8).

Consistently with the ingested biomass, there was a phagoinhibitory effect of eugenol and clove essential oil on *S. zeamais*, indicating that the inhibitory effect depends on the tested concentration. The highest phagoinhibition index is achieved with LC₇₅ for both treatments when compared to the control and other concentrations (Fig. 9).

4. DISCUSSION

Factors such as cultivation location, harvest time, plant development stage, and climate and soil conditions can influence the chemical composition of an essential oil and, consequently, its insecticidal action (Lee et al. 2020). Generally, the essential oil of *Syzygium aromaticum* is predominantly composed of the phenylpropanoid eugenol, with minor components being various sesquiterpenes (Narayanan et al. 2013, Sebaaly et al. 2015, Costa Lima et al. 2021, Kume et al. 2021).

The biosynthesis of terpenes occurs through two distinct metabolic pathways: the methylerythritol phosphate (MEP) pathway and the mevalonic acid (MVA) pathway. These pathways produce the isoprene units, which are five-carbon compounds (Dewick 2009, Bernards 2010). Phenylpropanoids, on the other hand, are biosynthesized via the shikimic acid pathway and are characterized by a phenol group with a functional hydroxyl and an aromatic ring connected to a three-carbon side chain (Simões & Spitz 2004).

Regarding polarity, monoterpenes have lipophilic properties, while phenylpropanoids with non-polar substituent groups are hydrophobic. These properties are crucial for the toxicity of these compounds,

allowing for better penetration through the insect's exoskeleton due to polar affinity, which causes feeding deterrence. Thus, terpenes and phenylpropanoids achieve a close relationship between chemical structure and biological activity (Dell & McComb 1981, Rice & Coats 1994, Harmatha & Nawrot 2002, Kim et al. 2003).

As a result, these two classes of compounds have gained attention in the production of new insecticidal agents due to their high efficacy in controlling pest insects, attributed to their rich chemical composition. They can potentially induce changes in the metabolism and physiology of insects (Santos 2009, Santos et al. 2021).

The bioactivity of *S. aromaticum* essential oil has been tested on various stored grain pests. According to authors like Saad et al. (2018), Jairoce (2016), and Haddi et al. (2015), its effectiveness stems from the major compound eugenol. These findings align with the present research, where the CL99 of eugenol was three times more effective compared to the crude oil.

Another effect caused by these substances is their repellent action, which is highly valuable for developing insecticidal products for stored grain pests. By repelling these organisms, the degree of infestation during the storage period decreases. This effect is achieved through the action of allomones, which are chemical substances that benefit the emitter by protecting it from potential predators, particularly plant species against herbivorous insects (Pedotti-Striquer et al., 2006). The repellent effect of *S. aromaticum* oil was also observed by Trongtokit et al. (2005) when tested on mosquitoes of *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles dirus* (Diptera: Culicidae), thereby confirming the action of this substance.

Terpenes and phenylpropanoids also reduce the emergence of adults. This result occurs due to their antifeedant effect, which directly interferes with the development of insects (Tapondjou et al., 2005; Sayed et al., 2011; Ali et al., 2018; João et al., 2020). Similar to the results found in this research, a study conducted by Hag et al. (1999) observed that the essential oil of *S. aromaticum* inhibited the emergence of adults and larvae of *Culex pipiens* (Diptera: Culicidae) at a concentration of 200 ppm.

The proper acquisition of macronutrients like proteins, lipids, and carbohydrates can be altered due to the effects of terpenes and phenylpropanoids, either through an antifeedant effect or by altering the metabolism of these substances (Cruz et al., 2017). Considering that nutritional status is a key survival mechanism for insects, potential alterations can make the energy supply insufficient for essential activities like growth, defense, and reproduction, which are crucial for their establishment in the crop (Behmer, 2008; Gullan & Cranston, 2012).

Dutra et al. (2020) studied the effects of essential oils from *Piper corcovadensis*, *P. marginatum*, and *P. arboreum* on the nutritional parameters of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), a key pest of maize. They observed that at all tested doses (LC₃₀, 50, and 70), these oils affected the levels of proteins, lipids, carbohydrates, and glycogen. The authors attributed this effect to the chemical composition of the oils, namely terpenes and phenylpropanoids.

This phagoinhibitory effect was also observed in *S. zeamais* by Ferreira et al. (2018) through the action of the essential oil of *Eucalyptus torelliodora* (Myrtales: Myrtaceae). The authors attributed phagoinhibition to the terpenes present mainly in the essential oil, which, according to them, are the main phagoinhibitors of insects.

Omar et al. (2007) tested isolated terpenes from tropical species of *Rutales* and *Lansium domesticum* (Sapindales: Meliaceae) on *Sitophilus oryzae* (Coleoptera: Curculionidae), a stored grain pest from the same family as *S. zeamais*, and observed a significant phagoinhibitory effect. According to the authors, this effect is an important characteristic in screening new compounds with insecticidal potential. Guimarães et al. (2014) tested extracts from the fruit of *Capsicum baccatum* on *S. zeamais* and observed a decrease in insect weight due to the phagoinhibitory effect from a concentration of 20% of the extract, as well as a considerable repellent action. The phenylpropanoid Capsaicin was the substance present in the extract responsible for this effect.

Problems in the absorption and/or metabolism of nutrients can result in their redistribution among physiological processes, leading to changes in the quantity of these macromolecules. Depending on the xenobiotic, the behavioral pattern may vary (Cruz et al., 2017; Silva et al., 2019b). In the present study, an inverse pattern was observed, where insects treated with *S. aromaticum* oil generally showed a reduction in quantity in the first 24 hours followed by an increase at 48 hours. Conversely, the major compound, eugenol, initially increased, followed by a reduction.

Proteins play a role in various processes such as amino acid supply, morphogenesis, growth, egg production, cuticular sclerotization, neurotransmitter function, and reproduction (Kunkel & Nordin, 1985; Klowden, 2007; Salmela & Sundström, 2017; Huo et al., 2018).

Carbohydrates serve as the primary energy source for insects and can be converted into fats, contributing to the production of amino acids. They are also involved in cuticular development, gonad maturation, and pre-vitellogenic processes (Arrese et al., 2010). Among the reserve carbohydrates, we have trehalose and glycogen, both of which are converted into glucose and transported to areas of need when necessary (Klowden, 2007; Gullan & Cranston, 2012; Yamada et al., 2018).

Lipids provide energy for activities with high metabolic demands such as tolerating large temperature changes, metamorphosis, prolonged flight, egg production, and longevity. They also play a role in composing cellular membranes (Canavoso et al., 2001). Lipids are absorbed in the foregut and gastric ceca as fatty acids and diacylglycerols (Klowden, 2007). They are stored in the fat body, in cells called trophocytes (Gullan & Cranston, 2012), with over 90% of the stored lipids being triglycerides (Arrese & Soulages, 2010).

Given the above, the study of substances such as *S. aromaticum* oil and eugenol, which not only restrict their action to mortality but also have repellent and feeding-inhibitory effects, as well as altering the emergence of adults and the biochemical profile of these organisms, is crucial in the development of

new biotic and efficient insecticidal molecules that meet the principles of Integrated Pest Management (IPM).

5. CONCLUSIONS

The results demonstrated that both treatments, *Syzygium aromaticum* oil and its major compound eugenol, exhibit concentration-dependent adverse effects on survival, behavior, emergence, nutrition, as well as a significant repellent effect on *S. zeamais*. However, the most significant results were observed in insects treated with eugenol, making it more appealing for use due to its identified, pure, and commercially available nature, facilitating its availability and utilization in the biosynthesis of new products with effective insecticidal action.

Declarations

Ethical Approval Not applicable.

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AUTHOR CONTRIBUTION

This project was conceived collectively by all authors. Thiago Alves Pimenta was responsible for performing the experiments and writing the manuscript. Aleska Batista da Silva, Lílian Renata Alves Farias and Naely de Lima Silva contributed to the maintenance of the *Sitophilus zeamais* breeding, as well as to the assembly and evaluation of the experiments. Valéria Wanderley Teixeira and Álvaro Aguiar Coelho Teixeira provided the laboratory infrastructure and reagents necessary for performing the biochemical assays, quantification and extraction of total soluble proteins, lipids, total sugars and glycogen. Glaucilane Santos Cruz assisted and supervised the biochemical assays, in addition to contributing to the interpretation of the data obtained. Roseane Cristina Predes Trindade conceived and coordinated the project.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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Figures

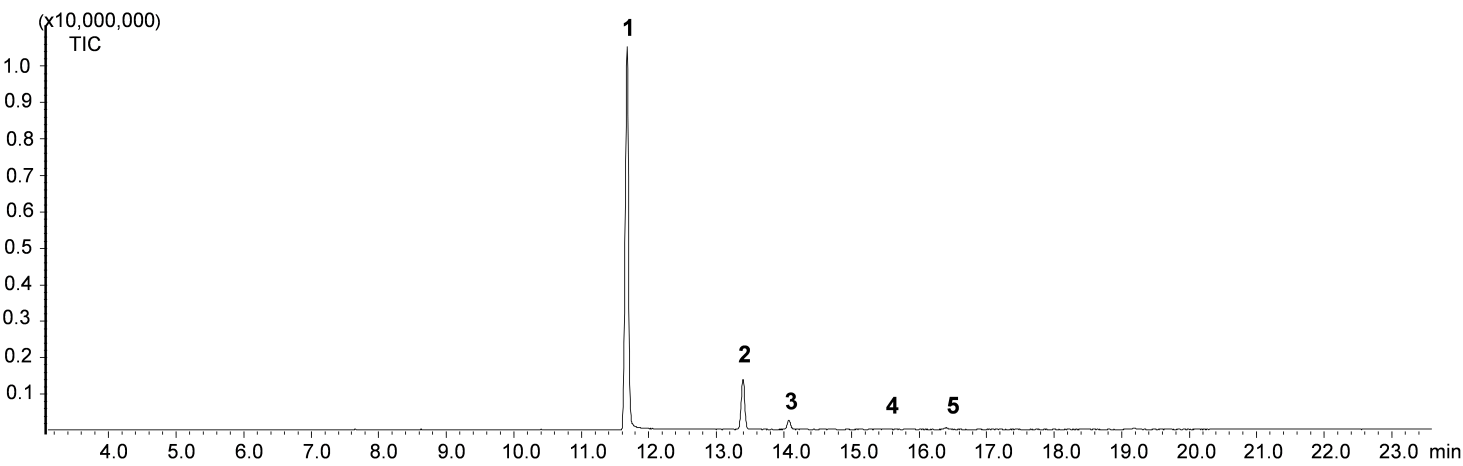


Figure 1

Chromatogram of *Syzygium aromaticum* essential oil, based on GC-MS analysis.

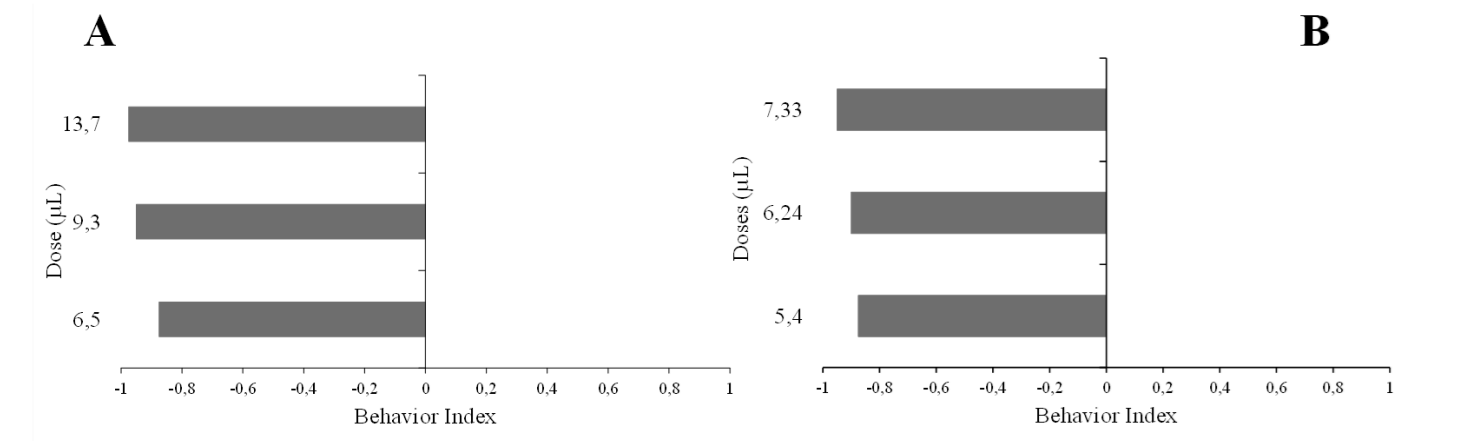


Figure 2

Repellent effect essential oil *Syzygium aromaticum* (A) and its major eugenol (B) on *Sitophilus zeamais*

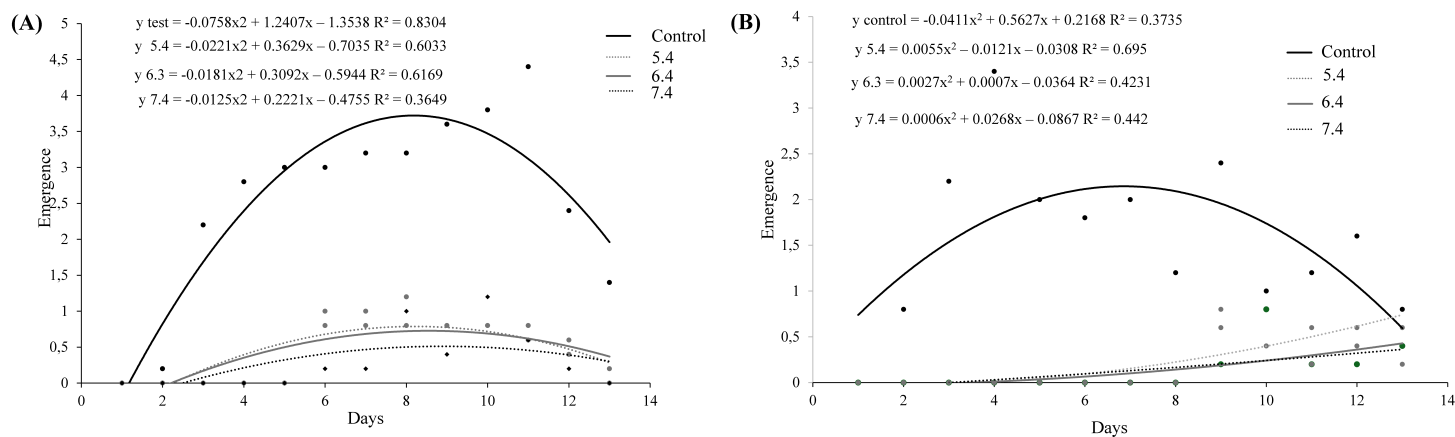


Figure 3

Emergence of *Sitophilus zeamais* subjected to treatments with the major compound eugenol (A) and the essential oil *Syzygium aromaticum* (B).

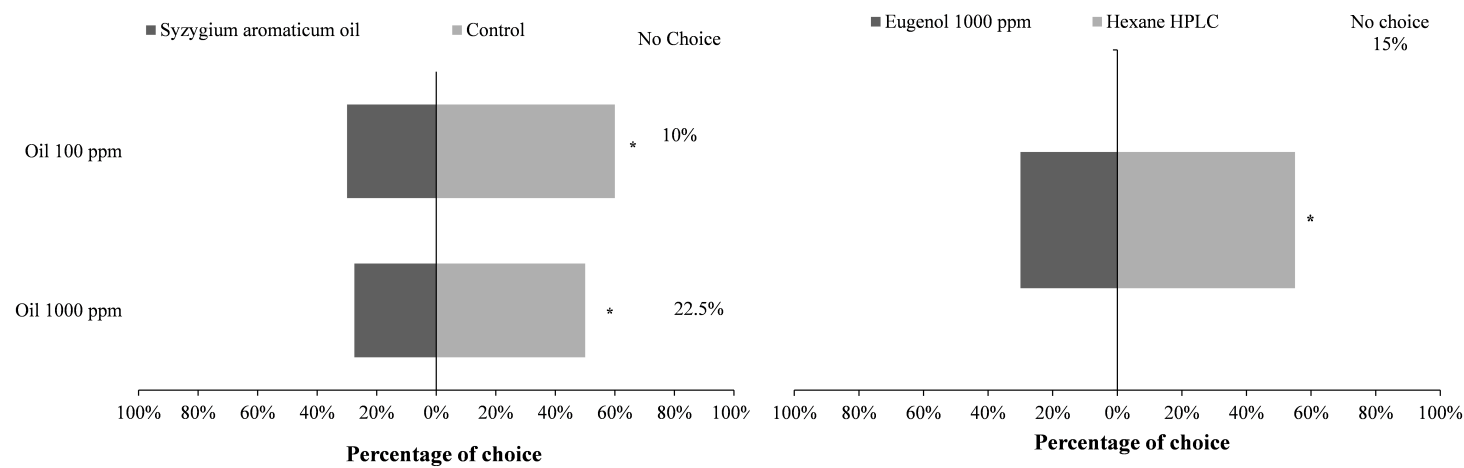


Figure 4

Behavioral response of *Sitophilus zeamais* to *Syzygium aromaticum* oil at 100 ppm (n=40) and 1000 ppm (n=40) in a double-choice olfactometer. (*) indicates a significant difference between treatments using the Chi-square test 100 ppm (GL: 1, X2: 5.7166; P= 0.0168); 1000ppm (GL: 1, X2: 4.3074; P= 0.0379). Behavioral response to eugenol compound diluted at 1000 ppm in a dual-choice olfactometer. (*) indicates a significant difference between treatments using the Chi-square test (GL=1; X2=4.4234; P= 0.0354).

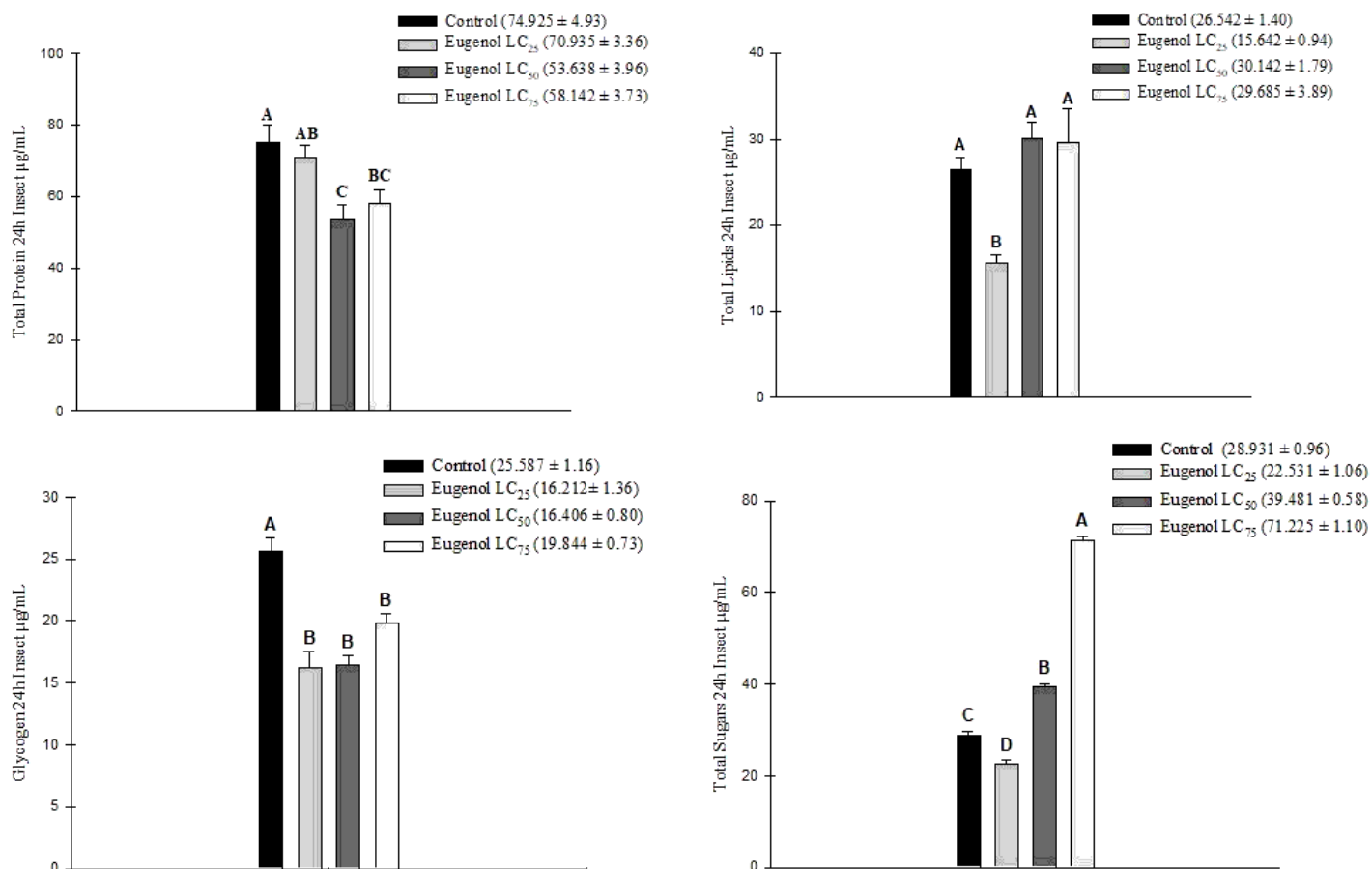


Figure 5

Amount of proteins (A), lipids (B), glycogen (C) and total sugar (D) (means $\pm$ standard error) of *Sitophilus zeamais* subjected to the topical contact toxicity test at lethal concentrations (25-50-75) of the major compound eugenol for 24 hours. Bars followed by different letters differ significantly by Tukey's test ($P < 0.05$)

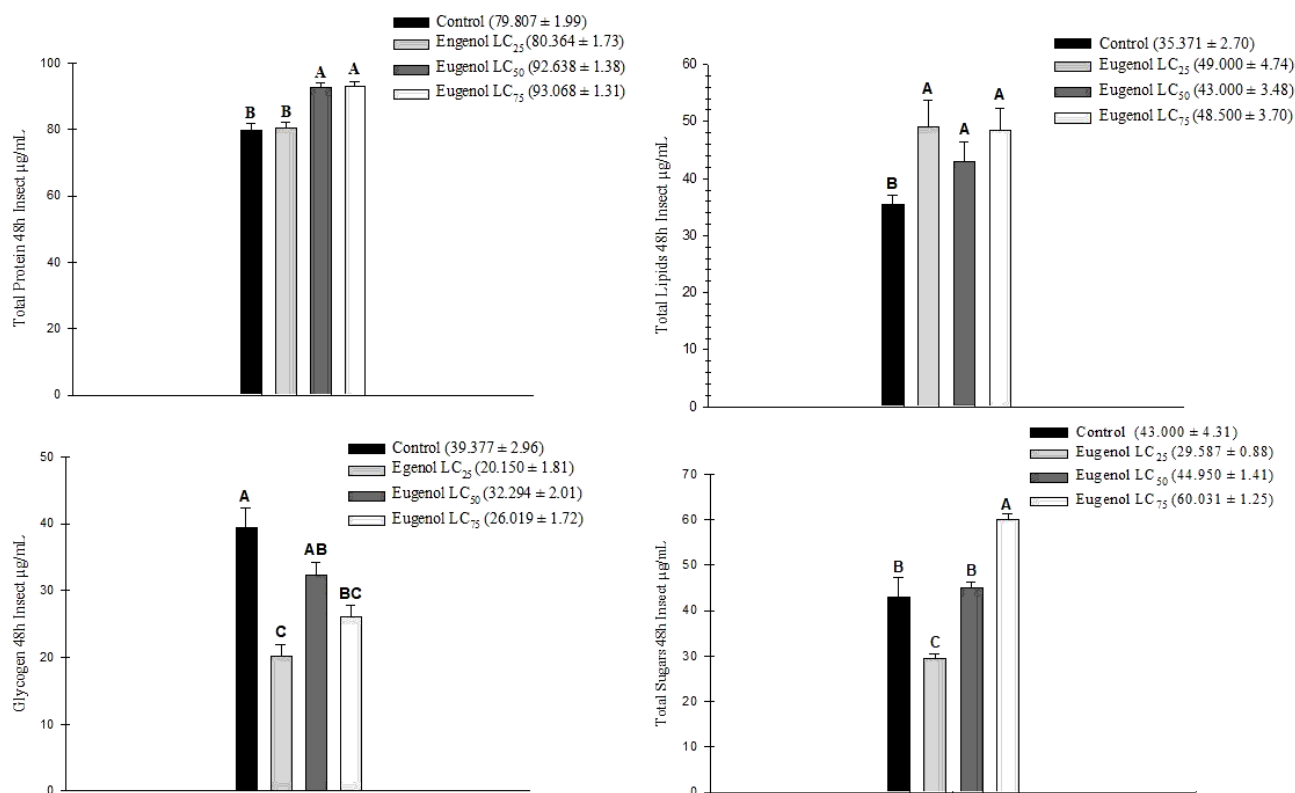


Figure 6

Amount of proteins (A), lipids (B), glycogen (C) and total sugar (D) (means $\pm$ standard error) of *Sitophilus zeamais* subjected to the topical contact toxicity test at lethal concentrations (₂₅₋₅₀₋₇₅) of the major compound eugenol for 48 hours. Bars followed by different letters differ significantly by Tukey's test ($P < 0.05$)

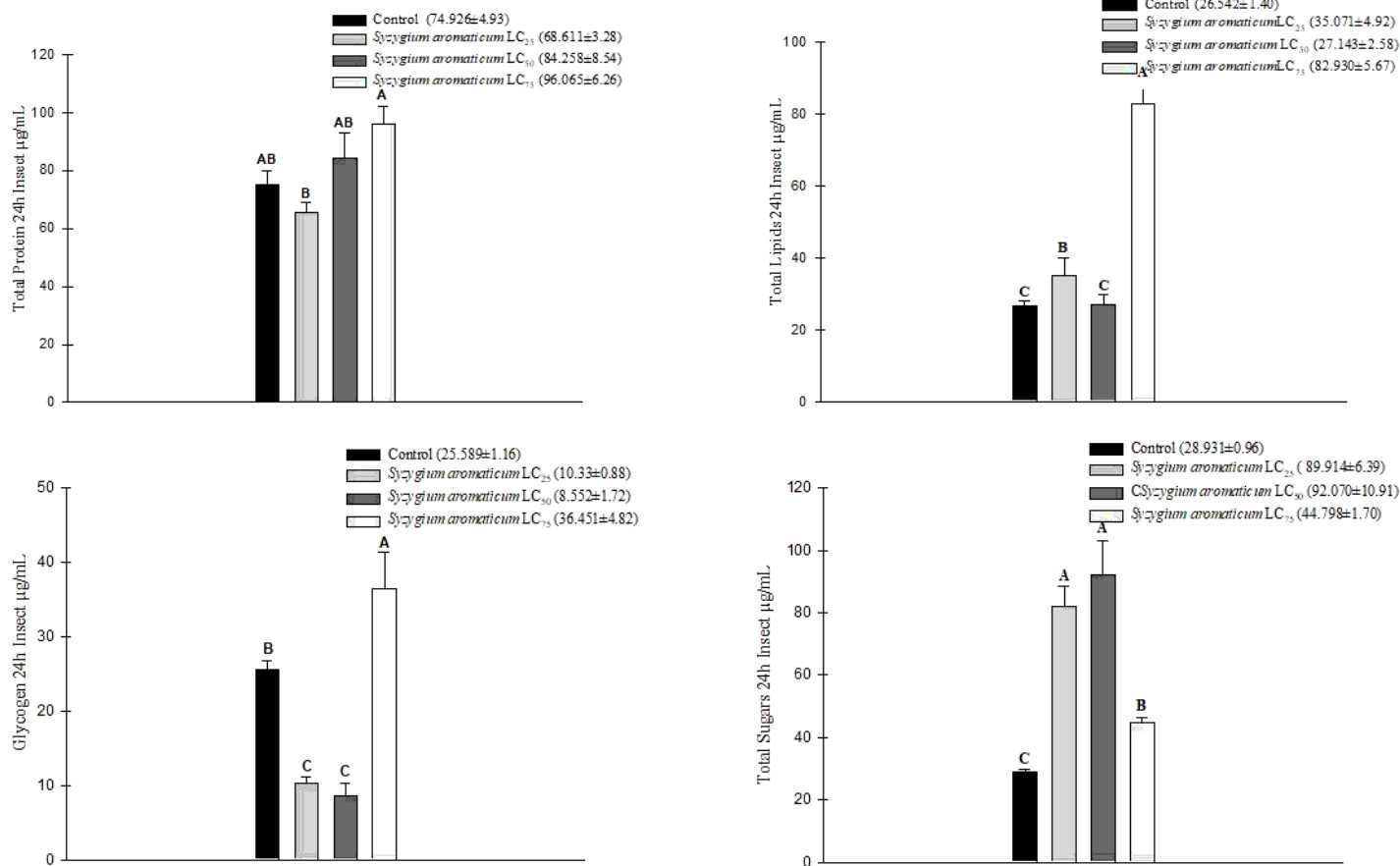


Figure 7

Amount of proteins (A), lipids (B), glycogen (C) and total sugar (D) (means $\pm$ standard error) of *Sitophilus zeamais* subjected to the topical contact toxicity test at lethal concentrations (25-50-75) of *Syzygium aromaticum* oil for 24 hours. Bars followed by different letters differ significantly by Tukey's test ($P < 0.05$)

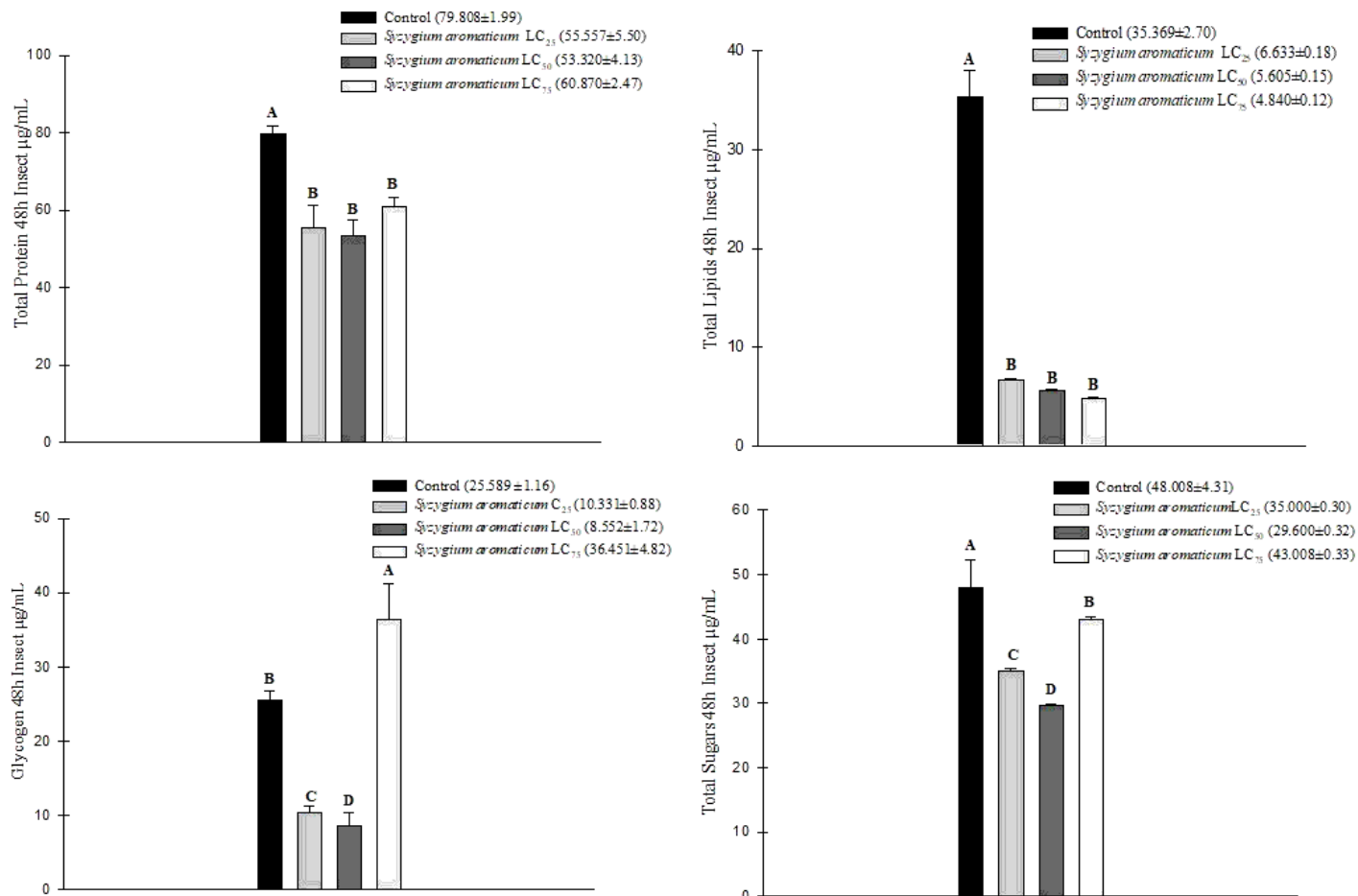


Figure 8

Amount of proteins (A), lipids (B), glycogen (C) and total sugar (D) (means $\pm$ standard error) of *Sitophilus zeamais* subjected to the topical contact toxicity test at lethal concentrations (LC₂₅-LC₇₅) of *Syzygium aromaticum* oil for 48 hours. Bars followed by different letters differ significantly by Tukey's test (P < 0.05).

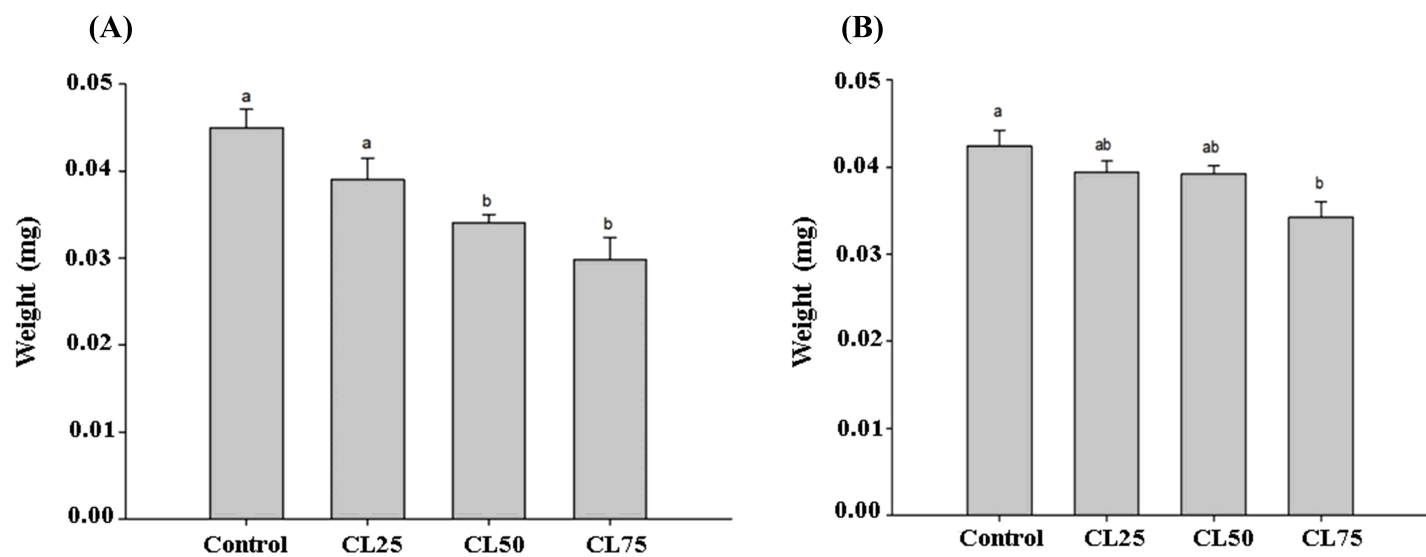


Figure 9

Phagoinhibitory effect of the major compound eugenol (A) and *Syzygium aromaticum* oil (B) on *Sitophilus zeamais*.