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Evaluation of the Insecticidal Activity of Essential Oil Extracted by Steam Distillation from Fresh Leaves of *Melaleuca leucadendron* on *Sitophilus zeamais*, a Pest of Maize Stocks

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ABSTRACT

Post-harvest losses due to insect pests represent a major challenge for cereal conservation in sub-Saharan Africa, particularly for maize storage. Conventional chemical insecticides, although effective, present risks of toxicity, environmental pollution, and the development of insect resistance. As a result, research has increasingly focused on natural, sustainable alternatives derived from aromatic plants. This study evaluates the insecticidal activity of essential oil extracted by steam distillation from fresh Melaleuca leucadendron leaves against Sitophilus zeamais, the main pest affecting maize stocks in Senegal. The extractions yielded an average of 0.75%, and GC-MS analysis revealed a composition dominated by sesquiterpenes, mainly γ-gurjunene (35.47%), nerolidol (17.47%), and farnesol (15.51%), indicating a high content of bioactive compounds. Maize grains were treated with different doses of oil (25 to 100 µL) and exposed to insects for 36 days, with adult mortality and the emergence of new generations being monitored and analyzed by ANOVA. The results show that mortality is highly dose- and time-dependent, with high doses causing total mortality on the first day, while lower doses induce a slower but longer-lasting residual effect. The emergence of new generations is significantly reduced, especially at high concentrations, and the efficacy is attributed mainly to sesquiterpenes, possibly enhanced by the synergistic effect of minor compounds. These observations suggest that M. leucadendron essential oil is a promising natural alternative to chemical insecticides for maize preservation, although further research is needed to optimize its formulation and stability and to assess its impact on grain quality.

Keywords:

Melaleuca leucadendro;

Sitophilus zeamais;

Insecticidal activity;

Essential oil;

Steam distillation

ABSTRAK

Kerugian pascapanen akibat hama serangga merupakan tantangan besar bagi konservasi sereal di Afrika sub-Sahara, terutama untuk penyimpanan jagung. Insektisida kimia konvensional, meskipun efektif, memiliki risiko toksisitas, pencemaran lingkungan, dan pengembangan resistensi serangga. Oleh karena itu, penelitian semakin difokuskan pada alternatif alami dan berkelanjutan yang berasal dari tanaman aromatik. Penelitian ini mengevaluasi aktivitas insektisida minyak atsiri yang diekstrak dengan distilasi uap dari daun Melaleuca leucadendron segar terhadap Sitophilus zeamais, hama utama tanaman jagung di Senegal. Ekstraksi menghasilkan rendemen rata-rata 0,75%, dan analisis GC-MS menunjukkan komposisi yang didominasi oleh seskuiterpen, terutama γ-gurjunene (35,47%), nerolidol (17,47%), dan farnesol (15,51%), yang menunjukkan kekayaan senyawa bioaktif. Biji jagung diberi perlakuan minyak dengan dosis berbeda (25–100 µL) dan dipapar serangga selama 36 hari. Mortalitas imago dan kemunculan generasi baru dipantau dan dianalisis dengan ANOVA. Hasil menunjukkan bahwa mortalitas sangat bergantung pada dosis dan waktu, dengan dosis tinggi mengakibatkan mortalitas total sejak hari pertama, sementara dosis yang lebih rendah menyebabkan efek residual yang lebih lambat namun bertahan lama. Kemunculan generasi baru berkurang secara signifikan, terutama pada konsentrasi tinggi, dan efikasinya terutama disebabkan oleh seskuiterpen, yang kemungkinan ditingkatkan oleh efek sinergis senyawa-senyawa minoritas. Pengamatan ini menunjukkan bahwa minyak atsiri M. leucadendron merupakan alternatif alami yang menjanjikan untuk insektisida kimia dalam konservasi jagung, meskipun penelitian lebih lanjut diperlukan untuk mengoptimalkan formulasi, stabilitas, dan menilai dampaknya terhadap kualitas biji-bijian.

Kata Kunci:

Melaleuca leucadendro;

Sitophilus zeamais;

Aktivitas insektisida;

Minyak atsiri;

Distilasi uap

INTRODUCTION

In many sub-Saharan African countries, cereals often suffer from low post-harvest yields and significant losses, primarily due to poor storage conditions and insect infestations. These losses not only reduce the quantity of available food but also compromise its nutritional quality and market value. The extent of these losses is closely linked to the edaphic and climatic characteristics of the region, where soils are often low in fertility, highly weathered, and sometimes affected by salinity and poor water retention. Recurrent droughts further exacerbate these conditions. Such abiotic stresses can strongly influence plant metabolism, particularly the synthesis of secondary metabolites, which play critical roles in plant defense and adaptation. For instance, Mirbakhsh & Sohrabi (2022) demonstrated that salinity stress significantly alters the physiological and biochemical responses of plants, affecting the production of compounds such as phenolics and flavonoids, which may influence the efficacy of plant-derived bioactive substances.

The most destructive post-harvest pests include *Sitophilus zeamais* Motschulsky, commonly known as the maize weevil, which attacks stored grains and causes substantial quantitative and qualitative losses (Cissokho et al., 2015; Regnault-Roger et al., 2012). To limit these damages, various strategies are employed, ranging from traditional methods such as ash application, use of plant-based repellents, and solar drying to modern approaches including synthetic insecticides and improved storage structures (Cissokho et al., 2015). However, the increasing resistance of insects to synthetic chemicals, along with concerns over toxic residues and environmental impacts, has driven research towards sustainable and eco-friendly alternatives (Isman, 2020; Soulama et al., 2015).

Among these alternatives, essential oils extracted from aromatic plants have shown promising insecticidal properties. Previous studies have demonstrated the effectiveness of oils from species such as *Cymbopogon nardus*, *Ocimum gratissimum*, and *Eucalyptus camaldulensis* against S. zeamais, reducing adult mortality, oviposition, and grain damage (Abdelgaleil et al., 2009; Diop et al., 2021). In particular, the essential oil of *Melaleuca leucadendron*, rich in 1,8-cineole, terpinen-4-ol, and α-terpineol, exerts its insecticidal activity through contact and inhalation, disrupting the physiological functions of the insects (Fall et al., 2017; Ndiaye et al., 2021). Recent studies indicate that this oil can induce high mortality in S. zeamais, reduce reproduction, and alter feeding behaviour, with efficacy increasing with concentration and exposure time (Ko et al., 2009; Selem et al., 2022).

Considering these findings, the present study aims to evaluate the contact insecticidal activity of essential oil extracted from Melaleuca leucadendron leaves against Sitophilus zeamais, the principal pest of stored maize in Senegal, providing a potentially effective and locally adapted alternative to conventional chemical insecticides.

MATERIALS AND METHODS

Plant material

Leaves were harvested in Hann Park, Dakar, Senegal (14°43’34” N and 17°26’02” W), washed with tap water to remove impurities, and lightly crushed to facilitate the passage of steam through the plant material.

Biological material and rearing

The individuals of *Sitophilus zeamais* used in this study were reared on dry maize grains (*Zea mays*), purchased locally at the Grand-Yoff market (Dakar). The initial strain came from the UCAD

Animal Biology Department. Rearing took place in 500-mL glass jars containing the maize kernels and a group of 50 to 60 adults. The humidity essential to their reproduction was maintained using moistened cotton. Mosquito netting was attached to the jars to allow ventilation while preventing the insects from escaping. The emergence of the first adults of the F1 generation was observed between days 17 and 21.

Extraction of essential oils

Melaleuca leucadendron essential oil was extracted by steam distillation from 150 g samples of fresh leaves. These were placed in a vertical column connected to a flask containing 1.5 L of distilled water, heated using a balloon heater. The vapors generated passed through the plant material, entraining the volatile compounds, which were then condensed using a cold-water condenser. The distillate obtained (a mixture of hydrolat and essential oil) was collected in an essencier, allowing separation by density difference. Extraction lasted 3 hours, and the oil obtained was stored in an amber glass bottle at 4°C until use.

Gas chromatography

GC-MS analysis of the essential oil was performed using an Agilent GC7890A gas chromatography system equipped with an automatic injector and a 5975C mass selective detector (Agilent Technologies). Compounds were separated on an HP-5MS capillary column (5% phenyl polysiloxane, 30.0 m × 0.25 mm i.d., 0.25 µm film thickness). The injection port temperature was set at 300 °C, and 1.0 µL of sample was injected in split mode (split ratio 30:1). Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. The oven temperature was programmed from 150°C (1 min hold) to 290°C at 10°C/min, then held at 290°C for 5 min. The detector transfer line, ion source, and quadrupole temperatures were 300 °C, 230 °C, and 150 °C, respectively. Mass spectrometry was performed in electron ionization (EI) mode at 70 eV with a mass range of 3-500 m/z. The compounds were identified by comparing the obtained spectra with the NIST08.L library using RTE Integrator software (Adams, 2009; El-Din et al., 2022; Sparkman et al., 2011).

Contact tests

The insecticidal activity of the essential oil was evaluated by direct contact on a food substrate. Sterile petri dishes 90 mm in diameter were used as the test medium. Each dish was carefully filled with 20 g of maize kernels. *Melaleuca leucadendron* essential oil was then applied directly to the kernels using a Pasteur pipette, in four separate volumes: 100 µL, 75 µL, 50 µL, and 25 µL. Each volume was tested in four independent replicates, for a total of 16 experimental units. After spraying, the dishes were left at room temperature for a few moments to allow the treatment to spread evenly. Next, 25 adult insects of the first generation (F1) of *Sitophilus zeamais*, aged between 0 and 2 days, were introduced into each box. The boxes were then stored at room temperature. Insect mortality was monitored daily for 36 days, with dead individuals removed every 24 hours using fine forceps. At the end of the test, the emergence of new generations was also noted.

Statistical analysis

The data collected were analyzed using an analysis of variance (ANOVA), based on the general linear model, in order to assess the effect of exposure time and doses applied on insect mortality and emergence. All the analyses were carried out using Jamovi software (version 2.7.2). To compare means between doses, a Tukey HSD post-hoc test was applied. Interpretation of the effect size (η^2) was based on the thresholds defined by Cohen (1988).

RESULTS AND DISCUSSION

Yield

Yields of essential oil from fresh *Melaleuca leucadendron* leaves varied between 0.48% and 0.89%, with an average of 0.75%. This fluctuation can be explained by various factors, including the mass of biomass processed, the physiological state of the leaves, and the harvesting period (October). Although the extraction method used, steam stripping over 3 hours, is commonly used, it may not have been able to recover all the volatile compounds present in the samples.

Chemical composition of the essential oil

Chemical analysis of the essential oil studied, carried out by gas chromatography coupled with GC/MS, identified several major and minor compounds (Table 1).

Monoterpenes and aromatic phenols represent a relatively small proportion, with α-terpinyl propionate at 2.42% and m-eugenol and isoeugenol at 0.52% and 0.55%, respectively.

Sesquiterpenes dominate the composition of the essential oil. The main constituents identified include β-caryophyllene (12.94%), γ-gurjunene (35.47%), nerolidol (17.47%), farnesol (15.51%), as well as α-gurjunene (1.11%), α-eudesmol (6.56%), isoaromadendrene epoxide (0.62%), δ-guaiene (0.56%), and β-elemene (0.31%). Among the sesquiterpenes, γ-gurjunene appears to be the predominant compound, accounting for more than a third of the total essential oil.

Among the other compounds identified, phytol (0.41%) and squalene (0.25%) complete the chemical profile of the essential oil. These results indicate that the oil analyzed is essentially characterized by a high content of sesquiterpenes, while monoterpenes and other compounds appear in low proportions.

Insecticidal activity of essential oils

Effects on adults

The ANOVA analysis (Table 2) carried out on the mortality data revealed that the ‘Doses’ factor had a significant, albeit modest, influence on mortality ($P < 0.001$; $\eta^2 = 0.007$). On the other hand, it was the duration of exposure that stood out as the main explanatory factor, accounting for 55% of the variance observed ($p < 0.001$; $\eta^2 = 0.550$). Furthermore, the interaction between dose and time was significant, suggesting that the impact of dose varies according to the duration of exposure.

Post-hoc tests (Table 3) show that doses D1 and D2 cause significantly higher mortality than D3 and D4. This result highlights a dose-dependent effect, where toxic efficacy increases with the concentration administered.

The curve representing the main effect of doses on mortality shows a progressive decrease in mortality from D1 to D4, indicating that the most concentrated doses are associated with increased mortality. In terms of the evolution of mortality over time, there is a very marked peak at the start, followed by a plateau where mortality rates stabilize at a relatively low level.

Finally, the interaction curve between doses and time shows that dose D1 leads to total mortality (100%) from day one, while dose D4 does not reach 30% even after 7 days.

Table 1. Chemical composition of essential oils

Peak	TR (min)	CAS NIST	Identified compounds	%	Qual	Chemical families
1	5,913	98-55-5	α-terpinyl propionate	2,42	89	**Monoterpenes**
3	6,550	501-19-9	m-eugenol	0,52	86	**Aromatic phenols**
4	6.810	1941-12-4	isoeugenol	0,55	91	
5	7,178	489-40-7	α-gurjunene	1,11	96	**Sesquiterpenes**
6	7,322	1000158-18-5	**β-caryophyllene**	**12,94**	93	
7	9,180	7212-44-4	**nerolidol**	**17,47**	96	
8	9,692 -	142-50-7	**farnesol**	**15,51**	87	
9	9,889				90	
10	10,383 -	22567-17-5	**γ-gurjunene**	**35,47**	95	
11	10,509					
12	10.598	473-16-5	**α-eudesmol**	**6,56**	90	
13	10,706	1000195-92-1	(E,E)-α- farnesene	0,31	47	
14	10,760	1000159-36-6	isoaromadendrene epoxide	0,62	64	
15	10,868	3691-11-0	δ-guajene	0,56	41	
16	11,020	62337-96-6	β-elemene	0,31	43	
18	13,157	150-86-7	Phytol	0,41	90	**other compounds**
20	18,408	111-02-4	squalene	0.25	92	

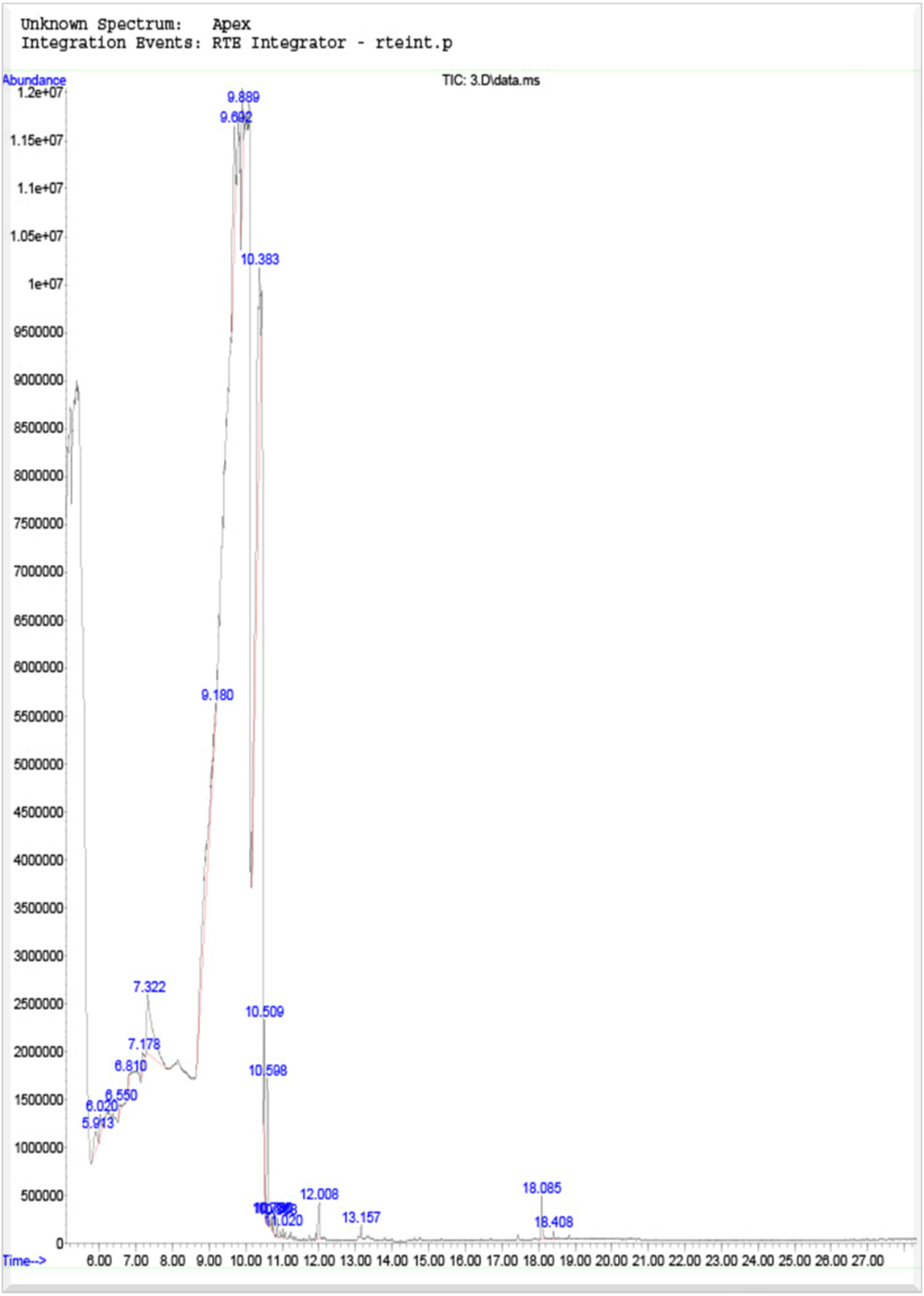

Figure 1. GC-MS (TIC) chromatogram of the essential oil

Table 2. Analysis of variance in mortality

	Sum of Squares	df	Mean Square	F	p	η^2
Overall model	3883.9	143	27.160	74.0	<0.001	
Doses	29.3	3	9.783	26.6	<0.001	0.007
Times	2223.5	35	63.529	173.0	<0.001	0.550
Doses*Times	1631.0	105	15.533	42.3	<0.001	0.403
Residuals	158.6	432	0.367			

Table 3. Post hoc comparisons – Doses

Comparison							
Doses		Doses	Mean Difference	SE	df	t	p_{tukey}
D1	-	D2	0.0627	0.0714	432	0.877	0.817
	-	D3	0.3576	0.0714	432	5.008	<0.001
	-	D4	0.5584	0.0715	432	7.813	<0.001
D2	-	D3	0.2950	0.0714	432	4.131	<0.001
	-	D4	0.4957	0.0715	432	6.936	<0.001
D3	-	D4	0.2008	0.0715	432	2.809	0.027

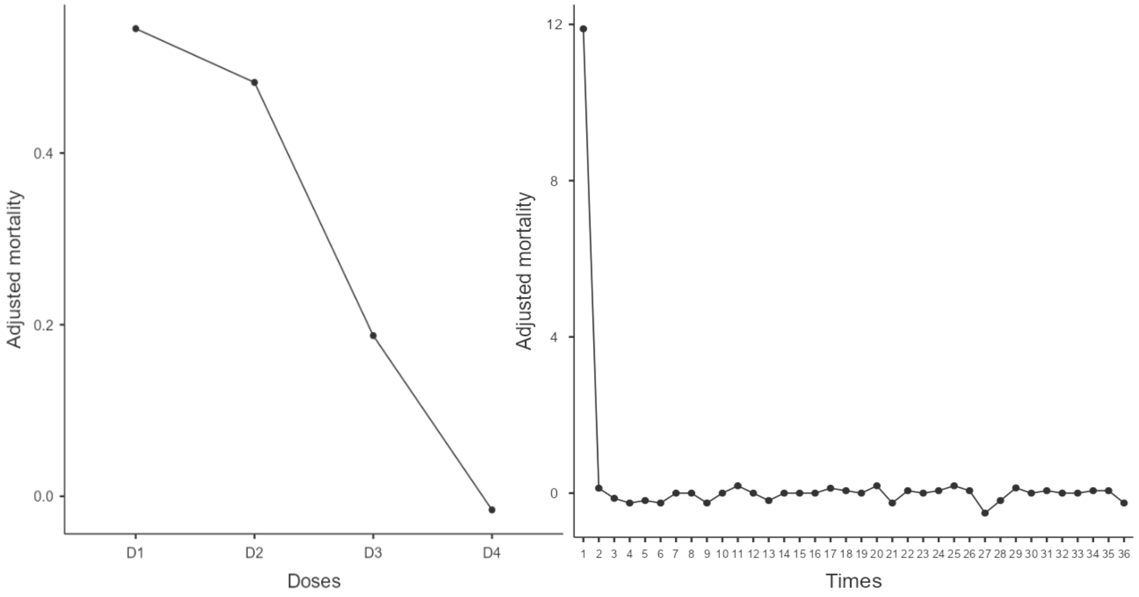

Figure 2. Main effect curves as a function of dose and time

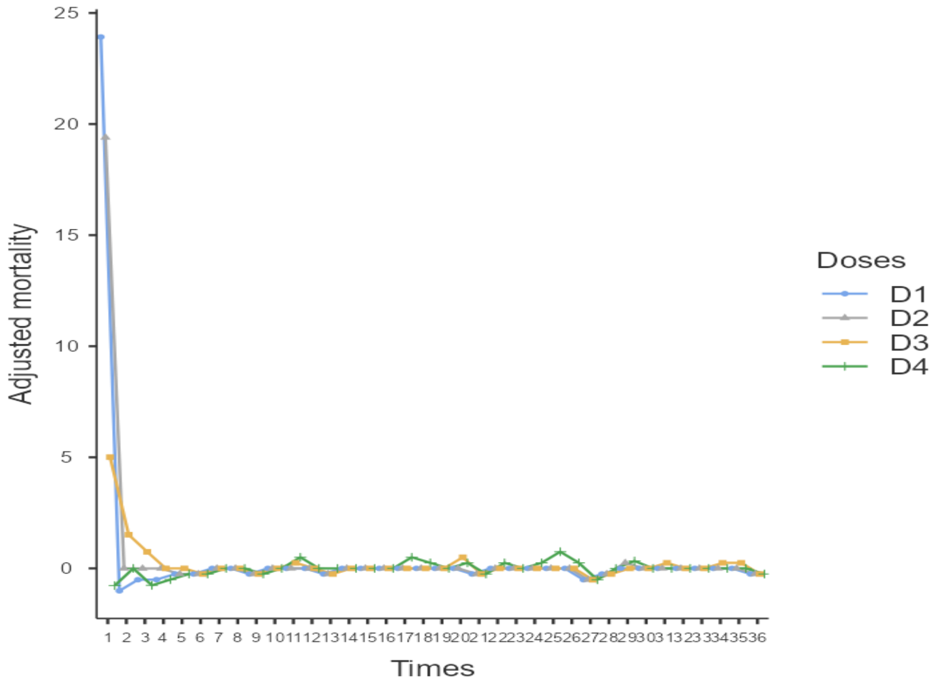

Figure 3: Dose*Time interaction curve for mortality

Finally, the results show that the oil used has a significant insecticidal effect on *Sitophilus*. This effectiveness depends on both the quantity applied and the exposure time. High doses caused rapid and significant mortality, while lower doses acted more slowly but remained effective over time. These observations suggest that this oil could represent a promising natural solution for controlling *Sitophilus* infestations, particularly in the context of protecting stored foodstuffs.

Effects on fecundity

The analysis of variance (ANOVA) performed on the corrected emergence data showed a significant effect of the dose factor ($p < 0.001$) and time ($p < 0.001$) on the results observed. The dose*time interaction factor, on the other hand, showed no significant effect ($p = 1.000$), suggesting that the effect of doses remained relatively stable over time. In terms of effect size (η^2), time explained around 26.4% of the total variance, making it the most influential factor. Dose explained around 6.2% of the variance, while the dose*time interaction explained only 4.7%, which remains relatively low.

Table 4. Analysis of variance for emergence

	Sum of Squares	df	Mean Square	F	p	η^2
Overall model	536.6	143	3.752	1.800	<0.001	
Doses	88.8	3	29.590	14.191	<0.001	0.062
Times	379.8	35	10.853	5.205	<0.001	0.264
Doses*Times	68.0	105	0.647	0.310	1.000	0.047
Residuals	900.8	432	2.085			

Analysis of the corrected emergence curve over time shows that the values tend to increase with each day, although the progression is irregular. At the beginning (between days 36 and 45), the results were very scattered and sometimes even negative. From day 50 onwards, emergence generally becomes positive and stabilizes at higher levels. This may indicate that the effect of the insecticide diminishes over time, perhaps due to degradation of the product or adaptation by the insects. The dose effect curve shows that the higher the dose, the lower the corrected emergence. For example, the highest dose (D1) gives an average value of around -1.15, while the lowest (D4) is around -0.50. This indicates that the treatment is more effective at higher doses, as it inhibits insect emergence to a greater extent.

The combined time and dose curve shows that all the doses follow a similar pattern over time, but the levels of emergence vary. D1 remains the most effective dose throughout the experiment, with the lowest values. D2 follows a similar trend, while D3 and D4 show higher emergences. This means that the time factor influences all the doses, but the concentration of the product remains a determining factor in effectiveness.

In conclusion, essential oils are effective against insects, especially in high doses. The effect of time is visible, but the dose plays an essential role in limiting emergence.

Discussion

Chemical analysis of the essential oil, obtained by steam distillation from fresh *Melaleuca leucadendron* leaves, shows a chemical profile dominated by sesquiterpenes, with a particularly high proportion of γ-gurjunene (~35.5%), followed by nerolidol (17.5%), farnesol (15.5%), and β-caryophyllene (12.9%), accompanied by other compounds such as α-eudesmol, α-gurjunene, α-terpinyl propionate, phytol, and squalene. These results are consistent with the widely documented chemical variability in *M. leucadendron*. For example, some oils from leaves grown in India are particularly rich in (E)-nerolidol: up to 76.6-90.9% of oxygenated sesquiterpenes, with β-caryophyllene (1.5-4.5%) (Padalia et al., 2015). Other publications mention a chemotype dominated by (E)-nerolidol (85.7%) (Joshi et al., 2021). Conversely, certain oils, particularly those from Egypt, Indonesia, and Senegal, have a high 1,8-cineole content (44.8-64.3%) (Diallo et al., 2022; Tran et al., 2024). There are therefore clearly distinct chemotypes depending on geographical origin, extraction methods, and the condition of the raw material.

Melaleuca leucadendron essential oil exhibits strong insecticidal activity against *Sitophilus zeamaïs*, mainly due to its high sesquiterpene content, with γ-gurjunene, nerolidol, farnesol, and β-caryophyllene as the main contributors. These compounds cause rapid mortality at high doses and ensure a prolonged effect at low concentrations. Monoterpenes and other minor constituents, although present in low proportions, can potentiate this action through synergistic effects. Thus, the effectiveness of this essential oil results from the interaction between its major and minor components, confirming its potential as a natural alternative for the preservation of stored foodstuffs. The observed activity can be mainly attributed to oxygenated sesquiterpenes such as nerolidol and farnesol, whose contact insecticidal effect has been associated with acetylcholinesterase inhibition, as shown in the case of Anopheles (in vitro and in silico) (Rants'o et al., 2023). Furthermore, β-caryophyllene and γ-gurjunene, although less studied individually, could enhance overall efficacy through synergistic effects, particularly by improving the cuticular penetration of the compounds (Tak & Isman, 2017).

Previous studies reported in the literature corroborate these results. Among these, Fall et al. (2017) studied the insecticidal effect of fumigation with *Melaleuca leucadendron* essential oils on *Sitophilus*, highlighting an efficacy dependent on exposure time with an average mortality rate of over 60% after six days. In addition, an LC50 of 0.031 mL/L of air was observed after eight days. Similarly, Ziane & Bouredji (2024) showed that *Eucalyptus globulus* (Myrtaceae) essential oil also has insecticidal activity through fumigation against *Tribolium castaneum*, which varies depending on the dose and exposure time. They observed mortality rates ranging from 14 to 32% at a dose of 5 µL/mL and from 96 to 100% at 25 µL/mL between 24 and 72 hours, respectively. In addition, they reported a LC50 of 12.36 µL/mL at 24 hours, which decreased to 8.38 µL/mL after 72 hours, in addition to a strong repellent effect after 72 hours for all doses tested.

CONCLUSION

The study showed that essential oil extracted from fresh *Melaleuca leucadendron* leaves has significant insecticidal activity against Sitophilus zeamais, the main pest affecting maize stocks. Its chemical composition, dominated by sesquiterpenes such as γ-gurjunene, nerolidol, and farnesol, largely explains its observed effectiveness. The results highlight a clear relationship between dose, exposure time, and insect mortality: high concentrations cause rapid and total mortality, while lower concentrations ensure a prolonged residual effect. In addition, the emergence of new generations is significantly reduced, confirming the potential of this oil as a post-harvest protection tool.

These observations position *M. leucadendron* essential oil as a natural and more environmentally safe alternative to chemical insecticides, thus contributing to the sustainable management of stored commodities. However, before large-scale application, further investigation is needed to optimize its formulation, improve its stability during storage, and assess its potential effects on the organoleptic and nutritional quality of the grains. Such an approach would further enhance the value of this local plant resource and offer producers an accessible and environmentally friendly solution for corn preservation.

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