

Lemongrass essential oil as a contact toxicant and repellent against the rice weevil, *Sitophilus oryzae*

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ABSTRACT

Stored-grain insects cause substantial quantitative and qualitative losses during storage, and the rice weevil, *Sitophilus oryzae* L., remains one of the most damaging pests of cereal commodities worldwide. Because reliance on synthetic grain protectants raises concerns about resistance, residue persistence, and environmental safety, plant-derived essential oils are increasingly being examined as low-risk alternatives. This study evaluated the contact toxicity and repellency of lemongrass essential oil against adult *S. oryzae* under laboratory conditions. Contact toxicity was assessed at 23.58, 31.44, 39.30, 47.16, and 55.02 $\mu\text{g}/\text{cm}^2$, and mortality was recorded after 24, 48, and 72 h. Repellency was assessed at 9.82, 19.65, 39.30, 78.60, and 157.95 $\mu\text{g}/\text{cm}^2$ over 5 h. Toxicity data were analyzed using a binomial generalized linear mixed model (GLMM) and time-specific probit models, whereas repellency data were analyzed using a binomial GLMM. Mortality increased with both dose and exposure time, rising from 0.00% at the lowest dose after 24 h to 76.67% at the highest dose after 48 and 72 h. The GLMM showed a strong positive dose effect on mortality, and the dose-response slope was significantly steeper at 48 and 72 h than at 24 h. Probit analysis showed that LD50 values declined from 49.86 $\mu\text{g}/\text{cm}^2$ at 24 h to 45.60 $\mu\text{g}/\text{cm}^2$ at 48 h and 45.27 $\mu\text{g}/\text{cm}^2$ at 72 h. Repellency was also dose-dependent, with the highest dose producing 60.00%-80.00% repellency over the 5 h observation period. These findings indicate that lemongrass essential oil possesses biologically relevant toxic and repellent activity against *S. oryzae* and may serve as a promising botanical component for stored-grain pest management, although formulation and storage-scale validation are still required.

Introduction

Stored grains are vulnerable to insect deterioration during post-harvest handling and storage, and *Sitophilus oryzae* L. is among the most destructive cosmopolitan pests of cereal commodities. Infestation reduces grain weight and quality and may accelerate contamination, heating, and secondary microbial spoilage. Continued dependence on synthetic protectants and fumigants has also heightened concerns

about residue issues, the evolution of resistance, and environmental safety (Campolo et al., 2018; Omar et al., 2024; Regnault-Roger et al., 2012).

Botanical insecticides, particularly essential oils, are receiving renewed attention because they are volatile, biodegradable, chemically diverse, and often active through multiple physiological and behavioral pathways. These properties make them attractive candidates for stored-product



protection, especially where reduced-risk or residue-conscious pest management is desirable (Ayllón-Gutiérrez et al., 2024; Isman, 2020; Regnault-Roger et al., 2012). At the same time, their practical use can be constrained by volatility, instability, and the need for formulation strategies that improve persistence and controlled release (Ayllón-Gutiérrez et al., 2024; Campolo et al., 2018).

Lemongrass, *Cymbopogon citratus*, is a well-known aromatic plant whose essential oil is dominated by citral-related compounds, particularly geranial and neral, together with other monoterpenoids that contribute to its biological activity (Majewska et al., 2019; Plata-Rueda et al., 2020). Previous work has shown that lemongrass essential oil and related terpenoid-rich oils can exert contact, fumigant, and repellent effects against stored-product coleopterans, including *Sitophilus* species (Kim et al., 2016; Plata-Rueda et al., 2020; Tawfeek et al., 2021). However, dose-specific evidence remains necessary for each target pest, assay system, and exposure interval before practical recommendations can be made.

The present study, therefore, investigated the contact toxicity and repellent effect of lemongrass essential oil against adult *S. oryzae* under laboratory conditions. The objectives were to quantify dose- and time-dependent mortality, estimate LD50 values through probit analysis, and determine whether repellency also increased with dose over short exposure periods.

Materials and methods

Insect culture and essential oil

Adults of *S. oryzae* were obtained from laboratory culture maintained on rice grain at room temperature (25 ± 5 °C). Lemongrass essential oil was used as the test material, and acetone served as the solvent for the contact bioassay. Lemongrass oil was selected because *C. citratus* essential oil is rich in bioactive terpenoids and aldehydes, which have documented pesticidal potential (Majewska et al., 2019; Plata-Rueda et al., 2020).

Contact toxicity bioassay

Contact toxicity was evaluated using a surface-film assay in 5-cm Petri dishes. One milliliter of each dilution was applied to the inner surface of a Petri dish, and the solvent was allowed to evaporate completely. Five doses were tested: 23.58, 31.44, 39.30, 47.16, and 55.02 $\mu\text{g}/\text{cm}^2$. Ten adult insects were released into each treated dish, and each dose was replicated three times. Mortality was recorded after 24, 48, and 72 h. The general approach is consistent with contemporary laboratory screening of essential oils against stored-product pests (Campolo et al., 2018; Demeter et al., 2021).

Repellency bioassay

Repellency was assessed in 9-cm Petri dishes using filter-paper arenas divided into treated and untreated halves. The treated half received the required dose of lemongrass essential oil, and the other half remained untreated. Five doses were tested: 9.82, 19.65, 39.30, 78.60, and 157.95 $\mu\text{g}/\text{cm}^2$. Ten adult insects were introduced at the center of each arena, and three replicate arenas were used per dose. The number of insects present on the untreated side was recorded hourly for 5 h, and the repellency percentage was calculated from the treated-versus-untreated distribution. This approach is suitable for short-term behavioral assessment of essential oil repellency in stored-product insects (Campolo et al., 2018; Plata-Rueda et al., 2020).

Statistical analysis

Observed mortality percentages were summarized by dose and exposure period. Contact-toxicity data were analyzed using a binomial GLMM with dose, exposure time, and their interaction as fixed effects and replicate unit as a random intercept. Time-specific probit models were then fitted to estimate LD50 values and 95% confidence limits for 24, 48, and 72 h. Repellency data were analyzed using a binomial GLMM with dose, hour, and their interaction as fixed effects and replicate unit as a random intercept. Statistical significance was evaluated at $p < 0.05$.

Results

Contact toxicity of lemongrass essential oil

Observed mortality increased progressively with dose and exposure period (Table 1; Figure 1). Mortality ranged from 0.00% at 23.58 $\mu\text{g}/\text{cm}^2$ after 24 h to 76.67% at 55.02 $\mu\text{g}/\text{cm}^2$ after both 48 and 72 h. At the two lowest doses, mortality remained low throughout the assay, whereas clear dose-dependent increases became evident at 39.30 $\mu\text{g}/\text{cm}^2$ and above.

Table 1. Mortality (%) of adult *Sitophilus oryzae* exposed to lemongrass essential oil under contact treatment.

Dose ($\mu\text{g}/\text{cm}^2$)	24 h	48 h	72 h
23.58	0.00	3.33	3.33
31.44	3.33	10.00	10.00
39.30	33.33	36.67	36.67
47.16	46.67	53.33	56.67
55.02	56.67	76.67	76.67

Table 2. Binomial GLMM results in contact toxicity of lemongrass essential oil against adult *Sitophilus oryzae*.

Term	Estimate	SE	OR	95% CI lower	95% CI upper	p
Intercept	-5.381	0.118	0.005	0.004	0.006	<0.001
48 h vs 24 h	-0.821	0.205	0.440	0.294	0.658	<0.001
72 h vs 24 h	-0.859	0.206	0.424	0.283	0.634	<0.001
Dose (per 10 $\mu\text{g}/\text{cm}^2$)	1.062	0.027	2.892	2.745	3.046	<0.001
Dose \times 48 h	0.294	0.047	1.342	1.225	1.471	<0.001
Dose \times 72 h	0.313	0.047	1.368	1.248	1.500	<0.001

Table 3. Probit-derived LD50 estimates of lemongrass essential oil against adult *Sitophilus oryzae*.

Exposure time (h)	LD50 ($\mu\text{g}/\text{cm}^2$)	Lower 95% CL	Upper 95% CL	Slope	χ^2
24.000	49.856	46.081	53.630	0.076	5.582
48.000	45.599	42.531	48.667	0.081	0.788
72.000	45.271	42.265	48.278	0.082	0.732

The binomial GLMM indicated a strong positive dose effect on mortality (Table 2). At 24 h, each 10 $\mu\text{g}/\text{cm}^2$ increase in dose increased the odds of death by 2.89-fold (OR = 2.89, 95% CI = 2.74–3.05, $p < 0.001$). The dose-response relationship became significantly steeper at 48 h (dose \times 48 h: OR = 1.34, 95% CI = 1.23–1.47, $p < 0.001$) and 72 h (dose \times 72 h: OR = 1.37, 95% CI = 1.25–1.50, $p < 0.001$), indicating stronger toxic action after longer exposure.

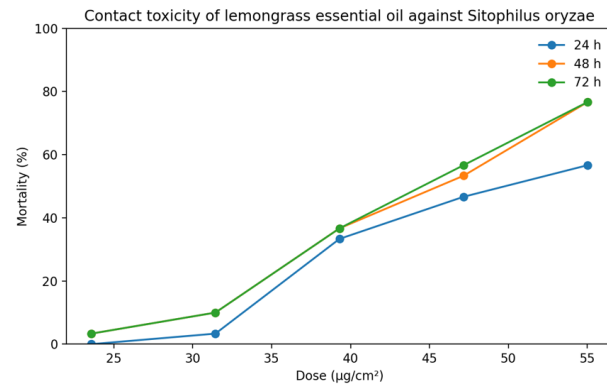


Fig. 1. Dose-dependent mortality (%) of adult *Sitophilus oryzae* at 24, 48, and 72 h after exposure to lemongrass essential oil. Editable Excel versions of Figures 1 and 2 are provided separately.

Table 4. Mean repellency (%) of lemongrass essential oil against adult *Sitophilus oryzae* at different observation times.

Dose ($\mu\text{g}/\text{cm}^2$)	1 h	2 h	3 h	4 h	5 h
9.82	33.33	26.67	33.33	13.33	26.67
19.65	33.33	33.33	26.67	46.67	46.67
39.30	46.67	60.00	46.67	40.00	40.00
78.60	66.67	53.33	46.67	40.00	66.67
157.95	80.00	73.33	60.00	66.67	66.67

Probit analysis supported the time-dependent increase in toxicity (Table 3). The LD50 declined from 49.86 $\mu\text{g}/\text{cm}^2$ at 24 h (95% CL = 46.08–53.63) to 45.60 $\mu\text{g}/\text{cm}^2$ at 48 h (95% CL = 42.53–48.67) and remained similar at 72 h (45.27 $\mu\text{g}/\text{cm}^2$, 95% CL = 42.26–48.28).

Repellent activity of lemongrass essential oil

Lemongrass essential oil also showed clear repellent activity against adult *S. oryzae*, with repellency generally increasing with dose (Table 4; Figure 2). Mean repellency at the lowest dose (9.82 $\mu\text{g}/\text{cm}^2$) ranged from 13.33% to 33.33% across the five hourly observations, whereas the highest dose (157.95 $\mu\text{g}/\text{cm}^2$) produced 60.00% to 80.00% repellency.

Table 5. Binomial GLMM results for repellency of lemongrass essential oil against adult *Sitophilus oryzae*.

Term	Estimate	SE	OR	95% CI lower	95% CI upper	p
Intercept	0.565	0.084	1.760	1.492	2.075	<0.001
2 h vs 1 h	0.058	0.191	1.060	0.729	1.540	0.761
3 h vs 1 h	0.057	0.182	1.059	0.741	1.512	0.755
4 h vs 1 h	-0.064	0.182	0.938	0.657	1.339	0.723
5 h vs 1 h	0.122	0.190	1.130	0.779	1.640	0.519
Dose (per 10 $\mu\text{g}/\text{cm}^2$)	0.113	0.012	1.120	1.093	1.147	<0.001
Dose \times 2 h	-0.024	0.028	0.977	0.924	1.033	0.406
Dose \times 3 h	-0.058	0.025	0.943	0.899	0.990	0.019
Dose \times 4 h	-0.042	0.025	0.959	0.913	1.008	0.103
Dose \times 5 h	-0.037	0.027	0.964	0.913	1.017	0.179

The binomial GLMM indicated that repellency was driven primarily by dose (Table 5). For each 10 $\mu\text{g}/\text{cm}^2$ increase in dose, the odds that an insect moved away from the treated half increased by 1.12-fold (OR = 1.12, 95% CI = 1.09–1.15, $p < 0.001$). Most hour effects were not significant, suggesting that repellency remained comparatively stable across the 5 h assay period. Only the dose \times 3 h interaction was negative and significant (OR = 0.94, 95% CI = 0.90–0.99, $p = 0.019$), indicating a modest attenuation of the dose effect at the third hour relative to the first hour.

Discussion

The present study demonstrates that lemongrass essential oil exerts both contact toxic and repellent effects against adult *S. oryzae*. In the toxicity bioassay, mortality increased with both dose and exposure duration, and the significant

dose \times time interaction indicated that the toxic effect intensified after 48 and 72 h. This pattern is consistent with the general view that essential oils can act as dose-dependent neuroactive toxicants whose effects accumulate or intensify with exposure duration (Isman, 2020; Regnault-Roger et al., 2012).

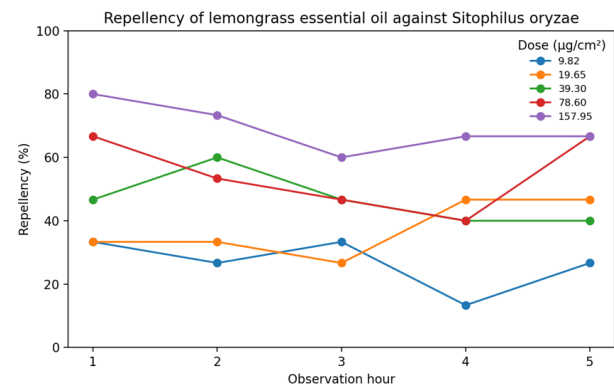


Fig. 2. Mean repellency (%) of adult *Sitophilus oryzae* across 5 h of exposure to lemongrass essential oil at different doses. Editable Excel versions of Figures 1 and 2 are provided separately.

The reduction in LD50 from 24 h to 48 h, followed by stabilization at 72 h, indicates that biologically active constituents of lemongrass oil remain effective over the observation window. Lemongrass essential oil is typically rich in citral-related aldehydes such as geranial and neral, compounds widely associated with pesticidal action in aromatic oils (Majewska et al., 2019; Plata-Rueda et al., 2020). Similar lemongrass-based toxicity has been reported for *S. granarius* and other stored-product beetles, supporting the view that Cymbopogon-derived volatiles can function as broad-spectrum stored-product protectants (Demeter et al., 2021; Plata-Rueda et al., 2020; Tawfeek et al., 2021).

Repellency in the present study was also clearly dose-related, with the highest dose maintaining strong repellency throughout the 5 h assay. This is important from a practical perspective because repellency may reduce landing, feeding, movement, and colonization even when complete mortality is not achieved. Comparable behavioral responses have been documented for



essential oils tested against *Sitophilus* species, suggesting that olfactory disruption and avoidance behavior contribute materially to pest suppression (Campolo et al., 2018; Kim et al., 2016; Regnault-Roger et al., 2012).

The limited contribution of observation hours in the repellency GLMM suggests that short-term repellency was relatively stable once a sufficiently strong dose was applied. That stability is encouraging for screening purposes, although it should not be interpreted as evidence of long residual activity under real storage conditions. Essential oils are volatile and susceptible to oxidation, so persistence under warehouse or farm storage conditions is often far lower than under controlled laboratory bioassays (Ayllón-Gutiérrez et al., 2024; Campolo et al., 2018).

Taken together, the results place lemongrass essential oil among the more promising botanical candidates for stored-product pest management. However, the work remains a laboratory-scale proof of concept. Before practical recommendations, further studies should evaluate formulation, persistence on grain, effect on grain odor or acceptability, compatibility with integrated pest management, and performance in semi-field or storage-bin systems. Such translational work is widely recognized as the major next step in the development of plant-derived insecticides (Ayllón-Gutiérrez et al., 2024; Isman, 2020).

Conclusion

Lemongrass essential oil showed significant contact toxicity and repellency against adult *Sitophilus oryzae* under laboratory conditions. Mortality increased with both dose and exposure period, whereas repellency was driven mainly by dose and remained strong at the highest concentration during the 5 h assay. These findings support the potential of lemongrass essential oil as a botanical component for stored-grain pest management. Nevertheless, realistic storage-scale validation, formulation development, and persistence studies are still

needed before operational use can be recommended.

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Conflict of interest

The authors declare they have no conflicts of interest.

Author contributions

IM conceptualized and supervised the study. He also contributed to data curation and statistical interpretation. PM conducted the laboratory work, organized the primary dataset, and drafted the first version of the manuscript. MN contributed to literature synthesis and manuscript revision. SHAM contributed to critical review and refinement of the manuscript. All authors read and approved the final version.

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