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Research Article

Menthol in pest control: Toxicidal activity on *Tenebrio molitor* (L., 1758) (Coleoptera: Tenebrionidae)

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Abstract. This study evaluated the insecticidal potential of menthol against *Tenebrio molitor* (L., 1758) (Coleoptera: Tenebrionidae), a significant pest of stored grains. Fourth and fifth instar larvae were exposed to menthol concentrations ranging from 0.1 to 9 g/L. Larval inactivity (24 and 48 hours) and mortality (72 hours) were analyzed. Higher concentrations (7, 8 and 9 g/L) showed greater efficacy, suggesting toxicity and anesthetic effects of menthol. These results indicate menthol as an environmentally sustainable alternative for pest management in stored grains.

Keywords: Integrated pest management, Natural pest management, Phytochemicals, Plant-derived biopesticides, Stored grain pests.

Introduction

Brazil produces approximately 288,136 million tons of grains per year (CONAB 2024). However, it is estimated that around 15% of this production is lost due to insect pests, fungi, and mycotoxins, with insects being the primary factor in grain losses during the storage period (Embrapa 2015; Lorini 2015). Among these insect pests, the beetle *Tenebrio molitor* (L., 1758) (Coleoptera: Tenebrionidae) stands out as a significant agricultural threat (Vigneron et al. 2019).

The main pest species of the family Tenebrionidae is commonly found in stored food warehouses, where both larvae and adults cause substantial damage to floury products, cereals, and stored grains (Costa Lima 1995; Fazolin et al. 2007). Its presence can lead to considerable economic losses, further emphasizing the importance of effective pest management strategies. Besides its detrimental impact, *T. molitor* serves as a valuable model in biological studies, particularly for evaluating the toxicity of plant extracts (Fazolin et al. 2007; Tavares et al. 2013; Menezes et al. 2014b), and is also utilized as a food source for predatory insects, bird feed, and even human consumption (Zamperlini et al. 1992; Martinson & Flaspohler 2003; Siemianowska et al. 2013).

Pesticides traditionally used for pest control are toxic, non-selective, and harmful to ecosystems, causing biodiversity loss (Kamlesh et al. 2024). Consequently, researchers have turned their attention to more sustainable pest control alternatives, such as biological methods (Gonçalves et al. 2003; Sousa et al. 2005) and chemical substances of plant origin (Céspedes et al. 2005; Fazolin et al. 2007; Pascutti et al. 2018). These plant-derived substances, originating from secondary metabolism, exhibit toxic effects by penetrating the insect's body through the cuticle and digestive tract (Afroz 2021), and cause interference in the respiratory and nervous systems (Viegas Junior 2003; Chaubey 2019; Souto et al. 2021).

Thus, botanical insecticides offer an environmentally friendly and cost-effective alternative to synthetic pesticides (Corrêa & Salgado 2011; Melo et al. 2011; Mishra et al. 2012). Essential oils have emerged as promising alternatives for controlling stored grain pests, offering a more sustainable approach compared to conventional pesticides (Mishra et al. 2012; Chaubey 2019; Leite et al. 2023). These naturally occurring phytochemicals, composed of complex mixtures of compounds, exhibit insecticidal, repellent, antifeedant, and developmental inhibitory activities against various coleopteran insects (Pérez et al. 2010; Chaubey 2019).

The plant families most used are Lamiaceae, Asteraceae, and Myrtaceae, which have shown efficacy in controlling insect pests (Pérez et al. 2010). Other notable examples include species from the Meliaceae and Annonaceae families, which have demonstrated insecticidal activity against common grain pests such as *Lasioderma serricorne* (Fabricius, 1792) (Coleoptera: Anobiidae) and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) (Leite et al. 2023; Lin et al. 2024). Extracts from *Melia azedarach* L. (Meliaceae) have shown significant insecticidal activity, reducing leaf consumption and increasing mortality in agricultural pests (Seffrin et al. 2008). Similarly, species from the Annonaceae family, such as *Annona muricata* L. and *Annona squamosa* L., have demonstrated insecticidal properties against various insect orders, including Coleoptera and Lepidoptera (Krinski et al. 2014; Durán-Ruiz et al. 2024).

Essential oils of *Mentha* species have been reported to exhibit toxic effects against insects (Khalfi et al. 2006; Samarasekera et al. 2008; Kumar et al. 2009), and menthol is a main component of this oil (Martins et al. 2000; Matos 2000). Although researchs have investigated the toxic effects of plant-derived monoterpenes, such as carvacrol, 1,8-cineole, and thymol, on *T. molitor* (Fazolin et al. 2007; Lima et al. 2011; Rodrigues et al. 2011), the impact of menthol on *T. molitor* remains unexplored. Considering that plant chemical compounds showed toxicity against bettle *T. molitor*, the menthol is promising for developing ecologically viable pest control of stored grains strategies. Thus, this study aims to evaluate the insecticidal potential of menthol (P.A.) as a contact surface treatment against immature stages of *T. molitor*.

Material and Methods

The experiment was conducted at the Invertebrate Zoology Laboratory of the University Center Uniacadêmica – Arnaldo Janssen Campus, Juiz de Fora, MG, Brazil. A total of 650 *T. molitor* larvae were used, sourced from a local supplier. The larvae were fed wheat bran (Zamperlini et al. 1992) and chayote slices [*Sechium edule* (Jacq.) Swartz (Cucurbitaceae)] to provide moisture (Fraenkel et al. 1950).

Menthol was weighed using an analytical scale and dissolved in distilled water heated to 60ºC combined with 1% DMSO (dimethyl sulfoxide) to prepare the following treatment concentrations: 0.1 g/L, 0.2 g/L, 0.4 g/L, 0.5 g/L, 0.7 g/L, 0.8 g/L, 0.9 g/L, 1 g/L, 3 g/L, 7 g/L, 8 g/L, and 9 g/L. The control group received distilled water with

1% DMSO. Each treatment concentrations was replicated five times. Subsequently, 5 mL of each treatment concentration was applied to 9.5 cm Petri dishes containing two layers of filter paper sterilized at 120°C for two hours. After the solutions had cooled, ten fourth- and fifth-instar larvae were placed in each dish, where they remained in direct contact with the contaminated surface for 48 hours. A physical stimulus was applied to the larvae using fine-tipped forceps after 24 and 48 hours of exposure to the menthol-contaminated surface. Larvae that did not respond to the stimulus were considered inactive. After 48 hours of direct exposure to the contaminated surface, the larvae were transferred to new Petri dishes containing dry filter paper without menthol or DMSO. The larvae were then maintained for an additional 24-hour period to confirm the lethality of the substance to *T. molitor*. The average temperature during the experiment was 22 ± 3°C, with a relative humidity of 70 ± 15%.

A generalized linear model (GLM) was used to evaluate the relationship between mortality (continuous variable) and menthol concentrations (categorical variable with twelve levels), employing a Poisson distribution family with a log link function. The differences between concentrations were compared using the Tukey post-hoc test. The adjusted model was validated using simulated residuals with the DHARMa package (Hartig 2022). The graphical analysis of standardized residuals did not reveal any evident patterns, suggesting a good model fit. The uniformity test showed that the residuals followed a uniform distribution (p = 0.95), indicating the adequacy of the model for the data. Additionally, the dispersion test (p = 0.19) found no evidence of overdispersion or underdispersion, confirming the variance assumption of the Poisson model. The analyses were performed using R software version 4.3.1 (R Core Team 2023), and the packages "multcomp" (Hothorn et al. 2018) and "stats" (R Core Team 2023). Graphs were generated using the "ggplot2" package (Wickham 2016).

Results and Discussion

The results showed that menthol concentrations have a significant impact on *T. molitor* mortality ($\chi^2_{12:}$ 87.03; $p < 0.001$) (Fig. 1). Additionally, significant differences were observed between the concentrations (Tab. 1). The differences in mortality rates among the concentrations suggest that menthol have a toxic effect on *T. molitor*.

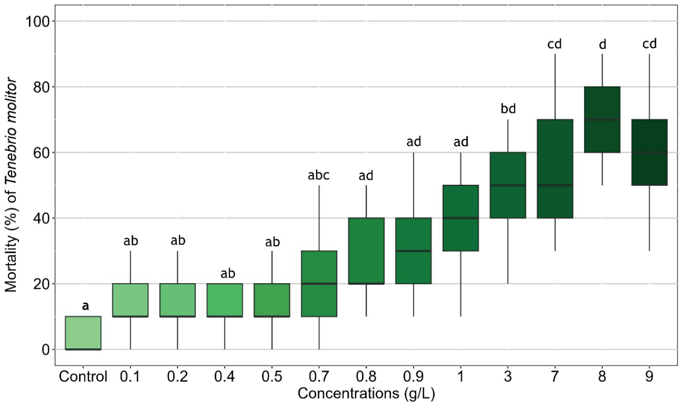

Figure 1. Mortality percentage of *T. molitor* at different concentrations of menthol (gradient colors from lowest to highest concentration). Box-and-whisker plots show the median (horizontal line), interquartile range (box), and the range from the upper and lower quartiles (whiskers). Lower-case letters indicate significant differences among concentrations, based on pairwise Tukey comparison.

Thus, this study revealed that menthol did not meet the criteria to be classified as an insecticidal substance by contact against *T. molitor* immatures, as its mortality rate was below 70%. According to Anvisa (2009), a substance must achieve mortality rates between 90 and 100% to be considered insecticidal. However, menthol showed moderate efficacy, with mortality rates ranging from 56 to 70% at concentrations of 7, 8, and 9 g/L, suggesting its potential use as a complementary tool in stored grain pest management. Its effect could contribute to reducing the number of adults and, consequently, limiting their proliferation (Coitinho et al. 2006; 2011). The insecticidal activity of *Mentha* sp. compounds against other stored grain pest beetles (Khalfi et al. 2006; Kumar et al. 2009; Mishra et al. 2012) differs from the results observed in the present study for menthol against *T. molitor*. This finding suggests that menthol may act synergistically with other substances found in the secondary metabolism of the *Mentha* sp. Similarly to our study, menthol also exhibited no insecticidal activity against *Aedes aegypti* (L., 1762) (Diptera: Culicidae) (Samarasekera et al. 2008).

Given that plant-derived substances may exhibit toxic activity towards insects, such as causing sudden death or triggering morphological (Tavares et al. 2013), physiological, and behavioral alterations (Grzesiuk et al. 2013; Pauliquevis et al. 2013), the present study observed several unsuccessful ecdyses followed by death. This suggests that menthol may induce some developmental alteration in the surviving insects. However, the immatures exhibited signs of being anesthetized when exposed to the substance during the 24 and 48-hour contact periods. Thus, this may have attenuated the toxic effect of menthol on the immatures of *T. molitor*.

The anesthetic effect was observed in 55.23% (n = 359) of the larvae after 24 hours, and a 6.46% increase (n = 42) in inactive larvae was observed after 48 hours, corresponding to a total of 401 inactive larvae (61.69%). After being transferred to Petri dishes and remaining for 24 hours without contact with menthol + 1% DMSO, it was observed that 31.69% (n = 206) of the larvae remained inactive and exhibited darkening of the exoskeleton, thus confirming the lethality of menthol on *T. molitor* (Fig. 2).

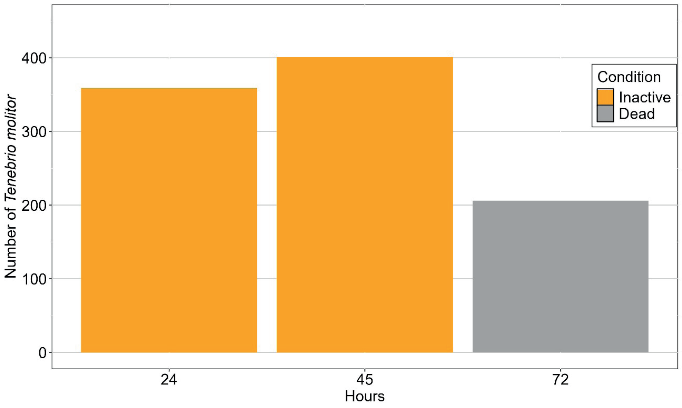

Figure 2. Number of *T. molitor* inactive (orange) and dead (grey) at different times after application.

Why *T. molitor* individuals enter an anesthetic state in contact with menthol is not clear. However, this phenomenon is likely due to menthol's interaction with the insect nervous system, particularly through its modulation of ion channels associated with sensory and pain perception, such as those in the transient receptor potential (TRP) family. Menthol's known activity on TRP melastatin 8 (TRPM8) and gamma-aminobutyric acid subtype A (GABAa) receptors, both linked to sedative, anxiolytic, anesthetic, and anticonvulsant effects (Hall et al. 2004), supports this hypothesis.

In *T. molitor*, the inactivity observed may also be related to TRP neuroreceptors, as seen in the modulation of thermoregulatory behavior by capsaicin and capsazepine (Olszewska & Tegowska 2011). Menthol's lipophilic nature allows it to penetrate the insects cuticle and cellular membranes, further disrupting physiological processes and neural signaling, potentially inducing paralysis or anesthetic effects. This interplay highlights the need for further investigation into the specific mechanisms by which menthol affects *T. molitor*.

In contact with the menthol + 1% DMSO contaminated surface, the immatures of *T. molitor* exhibited escape behavior as they attempted to leave the Petri dish. This behavior is similar to that displayed by *Ripicephalus microplus* (Canestrini, 1987) (Acari: Ixodidae) (Novelino et al. 2007). This suggests that menthol may act as a repellent for insect pests of stored grains, similarly to essential oils from plants of the *Mentha* genus (Knaak & Fiuza 2010; Magalhães et al. 2015).

Table 1. Comparison of mortality in *T. molitor* larvae by different menthol concentrations. Values indicate the estimated effect on mortality (β ± SD), the z-value, and the significance probability (p-value) (with significant p-values in bold).

Concentrations	β ± SD	z-value	p-value
0.1 – Control	1.25 ± 0.80	1.56	0.931
0.2 – Control	1.25 ± 0.80	1.56	0.931
0.4 – Control	1.10 ± 0.82	1.35	0.978
0.5 – Control	1.25 ± 0.80	1.56	0.931
0.7 – Control	1.71 ±0.77	2.22	0.544
0.8 – Control	1.95 ± 0.76	2.57	0.297
0.9 – Control	2.08 ± 0.75	2.77	0.192
1.0 – Control	2.25 ± 0.74	3.03	0.100
3.0 – Control	2.49 ± 0.74	3.38	**0.035**
7.0 – Control	2.64 ± 0.73	3.61	**0.016**
8.0 – Control	2.86 ± 0.73	3.94	**<0.01**
9.0 – Control	2.71 ± 0.73	3.71	**0.011**
0.2 - 0.1	0.00 ± 0.53	0.00	1.000
0.4 - 0.1	-0.15 ± 0.56	-0.28	1.000
0.5 - 0.1	0.00 ± 0.53	0.00	1.000
0.7 - 0.1	0.45 ± 0.48	0.94	0.999
0.8 - 0.1	0.69 ± 0.46	1.50	0.949
0.9 - 0.1	0.83 ± 0.45	1.82	0.816
1.0 - 0.1	1.00 ± 0.44	2.26	0.515
3.0 - 0.1	1.23 ± 0.43	2.87	0.153
7.0 - 0.1	1.39 ± 0.42	3.28	**0.048**
8.0 - 0.1	1.61 ± 0.41	3.89	**<0.01**
9.0 - 0.1	1.46 ± 0.42	3.47	**0.027**
0.4 - 0.2	-0.15 ± 0.56	-0.28	1.000
0.5 - 0.2	0.00 ± 0.53	0.00	1.000
0.7 - 0.2	0.45 ± 0.48	0.94	0.999
0.8 - 0.2	0.69 ± 0.46	1.50	0.949
0.9 - 0.2	0.83 ± 0.45	1.82	0.817
1.0 - 0.2	1.00 ± 0.44	2.26	0.515
3.0 - 0.2	1.23 ± 0.43	2.87	0.152
7.0 - 0.2	1.39 ± 0.42	3.28	**0.048**
8.0 - 0.2	1.61 ± 0.41	3.89	**<0.01**
9.0 - 0.2	1.46 ± 0.42	3.47	**0.027**
0.5 - 0.4	0.15 ± 0.56	0.28	1.000
0.7 - 0.4	0.61 ± 0.51	1.19	0.992
0.8 - 0.4	0.85 ± 0.49	1.74	0.863
0.9 - 0.4	0.98 ± 0.48	2.05	0.671
1.0 - 0.4	1.15 ± 0.47	2.46	0.368
3.0 - 0.4	1.39 ± 0.46	3.04	0.098
7.0 - 0.4	1.54 ± 0.45	3.42	**0.031**
8.0 - 0.4	1.76 ± 0.44	3.99	**<0.01**
9.0 - 0.4	1.61 ± 0.45	3.60	**0.017**
0.7 - 0.5	0.45 ± 0.48	0.94	0.999
0.8 - 0.5	0.69 ± 0.46	1.50	0.949
0.9 - 0.5	0.83 ± 0.45	1.82	0.817
1.0 - 0.5	1.00 ± 0.44	2.26	0.514
3.0 - 0.5	1.23 ± 0.43	2.87	0.153
7.0 - 0.5	1.39 ± 0.42	3.28	**0.048**
8.0 - 0.5	1.61 ± 0.41	3.89	**<0.01**
9.0 - 0.5	1.46 ± 0.42	3.47	0.026

to be continued...

Table 1. Continued...

Concentrations	β ± SD	z-value	p-value
0.8 - 0.7	0.24 ± 0.40	0.60	1.000
0.9 - 0.7	0.37 ± 0.39	0.96	0.999
1.0 - 0.7	0.55 ± 0.38	1.44	0.962
3.0 - 0.7	0.78 ± 0.36	2.14	0.601
7.0 - 0.7	0.93 ± 0.36	2.63	0.266
8.0 - 0.7	1.16 ± 0.35	3.35	0.039
9.0 - 0.7	1.00 ± 0.35	2.85	0.162
0.9 - 0.8	0.13 ± 0.37	0.37	1.000
1.0 - 0.8	0.31 ± 0.35	0.87	1.000
3.0 - 0.8	0.54 ± 0.34	1.60	0.918
7.0 - 0.8	0.69 ± 0.33	2.12	0.620
8.0 - 0.8	0.92 ± 0.32	2.90	0.142
9.0 - 0.8	0.76 ± 0.32	2.36	0.443
1.0 - 0.9	0.17 ± 0.34	0.51	1.000
3.0 - 0.9	0.41 ± 0.32	1.26	0.988
7.0 - 0.9	0.56 ± 0.31	1.79	0.838
8.0 - 0.9	0.78 ± 0.30	2.59	0.286
9.0 - 0.9	0.63 ± 0.31	2.03	0.683
3.0 - 1.0	0.23 ± 0.31	0.76	1.000
7.0 - 1.0	0.39 ± 0.30	1.31	0.983
8.0 - 1.0	0.61 ± 0.29	2.14	0.600
9.0 - 1.0	0.46 ± 0.29	1.56	0.933
7.0 - 3.0	0.15 ±0.28	0.55	1.000
8.0 - 3.0	0.38 ± 0.27	1.42	0.966
9.0 - 3.0	0.22 ±0.27	0.82	1.000
8.0 - 7.0	0.22 ± 0.25	0.88	1.000
9.0 - 7.0	0.07 ± 0.26	0.26	1.000
9.0 - 8.0	-0.15 ± 0.25	-0.62	1.000

Conclusion

This research provides insight into the toxic potential of menthol on *T. molitor* larvae at different tested concentrations. The concentrations of 7, 8, and 9 g/L caused higher mortality rates in the individuals, suggesting their potential use in controlling the proliferation of this pest insect. Additionally, menthol demonstrated anesthetic and repellent effects on the immatures, which makes it potentially useful for the development of less aggressive pesticides.

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Authors' Contributions

A.M.O.T.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, and Writing – review & editing. P.A.F.: Conceptualization, Investigation, Methodology, Supervision, and Writing – review & editing.

Conflict of Interest Statement

The authors affirm that they have no conflicts of interest, whether financial, professional, or personal, that could have influenced the research or its outcomes.

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