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Stage-specific toxicity of eucalyptus essential oil against the housefly (*Musca domestica L.*)

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Abstract
Housefly (*Musca domestica L.*) resistance to synthetic insecticides necessitates eco-friendly alternatives for effective vector control. This study evaluated the fumigant toxicity of Eucalyptus essential oil across four developmental stages (eggs, L3 larvae, pupae, adults) using standardized 24 h exposure bioassays at concentrations of 0-80 µL/L air. Dose-dependent mortality revealed marked stage-specific susceptibility, with L3 larvae exhibiting highest sensitivity (LD50 = 15.2 [13.8-16.9] µL/L), followed by adults (24.8 [22.1-28.3] µL/L), eggs (29.6 [26.4-33.2] µL/L), and pupae (54.7 [48.9-62.1] µL/L)—demonstrating 3.6-fold greater pupal tolerance. Complete mortality (100%) was achieved in larvae and eggs at 80 µL/L, positioning Eucalyptus oil as a potent botanical fumigant superior to neem oil benchmarks for mixed-age populations.

Keywords: *Musca domestica*, Eucalyptus oil, fumigant toxicity, stage-specific susceptibility, botanical insecticide

Introduction
Houseflies (*Musca domestica L.*) rank among the most significant mechanical vectors of over 100 human and animal pathogens, including *Salmonella*, *Shigella*, *E. coli*, and antibiotic-resistant bacteria, posing substantial public health risks in tropical regions like India where sanitation challenges exacerbate their proliferation. Global reliance on synthetic insecticides such as organophosphates and pyrethroids has led to widespread resistance, with field strains exhibiting up to 1000-fold tolerance and cross-resistance complicating conventional control. This resistance crisis underscores the urgent need for eco-friendly alternatives, particularly botanical essential oils that offer multi-site toxicity and reduced environmental persistence.
Eucalyptus essential oil, rich in 1,8-cineole (eucalyptol), has demonstrated potent insecticidal activity through fumigant, contact, and repellent modes, targeting octopaminergic systems absent in mammals. Previous studies confirm its efficacy against stored-product pests and mosquitoes, yet stage-specific toxicity against medically important flies remains underexplored. Notably, larval stages often exhibit heightened sensitivity due to thinner cuticles and active feeding, while pupae demonstrate resilience through hardened exoskeletons—a differential vulnerability exploitable for targeted integrated pest management (IPM).
The present study systematically evaluates Eucalyptus oil's dose-dependent toxicity across housefly developmental stages (eggs, L3 larvae, pupae, adults) using standardized fumigation bioassays. LD50 values, probit modeling, and two-way ANOVA elucidate stage-specific susceptibility patterns, providing baseline data for practical application in waste management facilities, livestock operations, and urban sanitation programs. These findings contribute essential toxicity benchmarks to support botanical IPM strategies amid escalating synthetic insecticide resistance.

Materials and methods
Insect culture
A laboratory culture of housefly, *Musca domestica* L., was maintained under controlled environmental conditions of approximately 27–32 °C, 70–80% relative humidity. Standardized rearing procedures were followed to obtain healthy and synchronized developmental stages for bioassay, with adults maintained on sugar- and milk-based diet and immature stages reared on a yeast-enriched moist medium.
Freshly laid eggs, third instar (L3) larvae, newly formed pupae, and newly emerged adults were collected separately from the stock colony for stage-specific toxicity assessment. Each developmental stage was handled carefully to avoid mechanical injury before treatment, and only healthy, active, and undamaged individuals were used in the experiments.

Test material
Eucalyptus oil was used as the test botanical insecticide. The required doses were prepared to obtain final concentrations of 0, 5, 10, 20, 30, 40, 50, and 80 µL/L air for fumigation bioassays. The control group received no eucalyptus oil and was maintained under identical experimental conditions.

Bioassay procedure
Stage-specific fumigation bioassays were carried out separately for eggs, L3 larvae, pupae, and adults. For each dose, a known number of individuals from each developmental stage was placed in airtight exposure containers of known volume, and the appropriate quantity of eucalyptus oil was introduced to achieve the desired concentration in µL/L air. This fumigation approach is suitable for essential oils because their volatile constituents disperse uniformly in enclosed space and exert toxic action through contact and respiratory exposure.
A separate untreated control was maintained for each stage. Mortality was recorded after 24 h of exposure. Individuals were considered dead when they showed no movement upon gentle probing. In the egg stage, failure to hatch after the observation period was considered mortality; in larvae and adults, absence of movement indicated death; and in

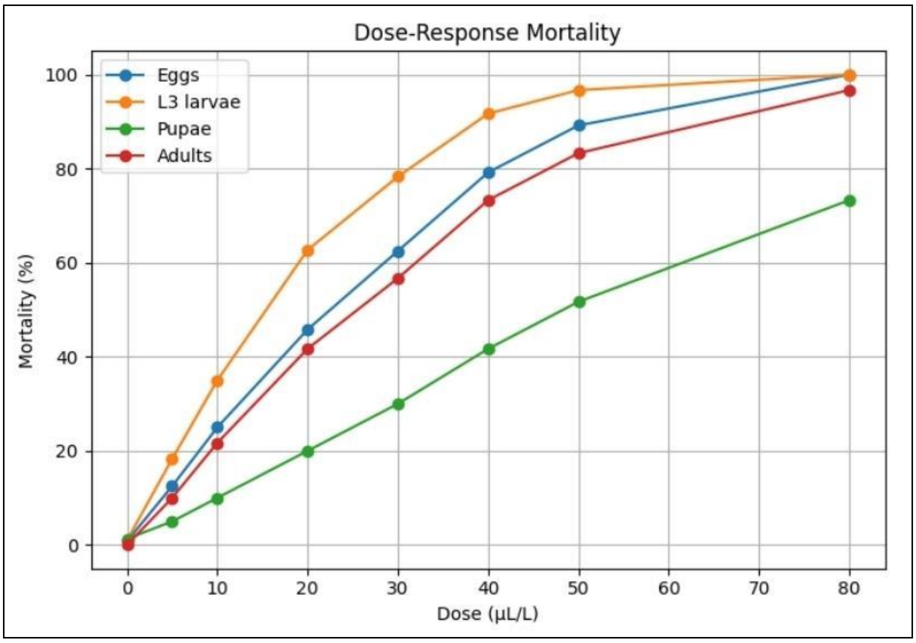
pupae, non-viability or failure to respond was taken as mortality.
The experiment followed a completely randomized design with multiple doses and four developmental stages. Each treatment was replicated, and mortality data were expressed as percent mean ± standard deviation (SD).
LD50 was calculated by using Miller and Tainter method (Miller and Tainter, 1944) [12]

Results
Stage-Specific Mortality Induced by Eucalyptus Oil. Eucalyptus oil triggered dose-dependent mortality in all developmental stages of the housefly (*Musca domestica*), with 24 h percent mortality increasing sharply from 0.0–1.3% at the control dose (0 µL/L) to kill (73.3–100%) at 80 µL/L. Larvae (L3) proved most susceptible, achieving 96.7 ± 1.9% mortality at 50 µL/L and 100% at 80 µL/L, followed closely by adults (83.3 ± 2.9% at 50 µL/L; 96.7 ± 1.9% at 80 µL/L). Eggs showed intermediate sensitivity, reaching 89.2 ± 2.5% at 50 µL/L and 100% at 80 µL/L, while pupae were most tolerant, with only 51.7 ± 3.3% mortality at 50 µL/L and 73.3 ± 3.5% at the highest dose. The bioassay data demonstrated a clear and progressive dose-dependent toxic action of eucalyptus oil against all developmental stages of *Musca domestica* after 24 h of fumigant exposure. (Table 01)
Mortality escalated progressively across doses: low at 5 µL/L (5.0–18.3%), moderate at 20 µL/L (20.0–62.7%), and high at 40–80 µL/L (>73%). Mortality in the untreated control remained negligible throughout the study, indicating that the recorded lethality in the treated groups was attributable to eucalyptus oil rather than to handling or natural causes. The response pattern also showed marked variation among developmental stages, confirming that susceptibility to the fumigant differed substantially across eggs, third instar larvae, pupae, and adults. A stage-wise examination of mortality values showed that the L3 larval stage was the most susceptible developmental form. Larvae exhibited measurable mortality even at the lowest tested concentration and their mortality rose rapidly with each increase in dose. At 5 µL/L air, larval mortality already reached 18.3 ± 2.1%, which increased to 35.0 ± 2.4% at 10 µL/L air and 62.7 ± 3.1% at 20 µL/L air. Beyond this level, the increase remained steep, with mortality values of 78.3 ± 2.9%, 91.7 ± 2.5%, 96.7 ± 1.9%, and finally 100.0 ± 0.0% at 30, 40, 50, and 80 µL/L air, respectively. This pattern indicates that larval physiology was highly vulnerable to the volatile toxic constituents of eucalyptus oil and that comparatively low concentrations were sufficient to induce major lethality in this stage.
Adult flies ranked as the second most susceptible stage. Mortality in adults rose from 10.0 ± 1.8% at 5 µL/L air to 21.7 ± 2.1% at 10 µL/L air, then increased further to 41.7 ± 3.1% at 20 µL/L air and 56.7 ± 3.1% at 30 µL/L air. At the higher dose range, adults recorded 73.3 ± 2.9%, 83.3 ± 2.9%, and 96.7 ± 1.9% mortality at 40, 50, and 80 µL/L air, respectively. Thus, adult flies were strongly affected by eucalyptus oil fumigation, although they required somewhat higher concentrations than larvae to achieve comparable mortality.
Eggs displayed an intermediate level of susceptibility. The egg stage registered 12.5 ± 2.4% mortality at 5 µL/L air, followed by 25.0 ± 2.4% at 10 µL/L air and 45.8 ± 3.1% at 20 µL/L air. Mortality increased to 62.5 ± 3.5%, 79.2 ± 3.3%, and 89.2 ± 2.5% at 30, 40, and 50 µL/L air, and complete inhibition of hatching or full mortality was achieved at 80 µL/L air. The egg stage therefore appeared less sensitive than larvae and adults at low to intermediate doses, yet still responded strongly at the upper end of the tested concentration range.
Pupae were the least susceptible stage throughout the experiment. Their mortality remained distinctly lower than that of the other developmental stages at every comparable dose. At 5 µL/L air, pupal mortality was only 5.0 ± 1.2%, rising to 10.0 ± 1.8% at 10 µL/L air, 20.0 ± 2.4% at 20 µL/L air, and 30.0 ± 2.9% at 30 µL/L air. Even at 40 and 50 µL/L air, mortality reached only 41.7 ± 3.1% and 51.7 ± 3.3%, respectively, while the highest tested dose of 80 µL/L air produced 73.3 ± 3.5% mortality. These observations clearly indicate that the pupal stage had the greatest tolerance to eucalyptus oil fumigation, most likely because of its more protected developmental structure.
Taken together, the raw mortality data established the following order of susceptibility after 24 h exposure: L3 larvae > adults > eggs > pupae. The data therefore shows that eucalyptus oil exerted potent fumigant toxicity against all stages of housefly development, but the extent of toxicity depended strongly on the developmental stage exposed as shown in graph 01.
Using Miller and Tainter methods, the LD50 values were calculated as 15.4 µL/L air for L3 larvae, 25.5 µL/L air for adults, 22.5 µL/L air for eggs, and 48.3 µL/L air for pupae. These values confirmed that larvae required the lowest dose to achieve 50% mortality and were therefore the most sensitive developmental stage, whereas pupae required the highest dose and were the least sensitive. The LD50 calculation supports the conclusion that eucalyptus oil acts as a potent fumigant against housefly developmental stages, although susceptibility varies substantially with stage. (Table 02)

Table 1: Effect of Eucalyptus oil on stage-specific mortality of housefly (24 h mortality; mean ± SD %)

Dose (µL/L)	Eggs (mean ± SD %)	L3 larvae (mean ± SD %)	Pupae (mean ± SD %)	Adults (mean ± SD %)
0	0.8 ± 0.5 (0.2)	1.0 ± 0.0 (0.0)	1.3 ± 0.6 (0.3)	0.0 ± 0.0 (0.0)
5	12.5 ± 2.4 (1.2) *	18.3 ± 2.1 (1.2) *	5.0 ± 1.2 (0.6) *	10.0 ± 1.8 (0.9) *
10	25.0 ± 2.4 (1.2) ***	35.0 ± 2.4 (1.2) ***	10.0 ± 1.8 (0.9) ***	21.7 ± 2.1 (1.1) ***
20	45.8 ± 3.1 (1.6) ***	62.7 ± 3.1 (1.6) ***	20.0 ± 2.4 (1.2) ***	41.7 ± 3.1 (1.6) ***
30	62.5 ± 3.5 (1.7) ***	78.3 ± 2.9 (1.4) ***	30.0 ± 2.9 (1.4) ***	56.7 ± 3.1 (1.6) ***
40	79.2 ± 3.3 (1.7) ***	91.7 ± 2.5 (1.2) ***	41.7 ± 3.1 (1.6) ***	73.3 ± 2.9 (1.4) ***
50	89.2 ± 2.5 (1.2) ***	96.7 ± 1.9 (1.0) ***	51.7 ± 3.3 (1.7) ***	83.3 ± 2.9 (1.4) ***
80	100.0 ± 0.0 (0.0) ***	100.0 ± 0.0 (0.0) ***	73.3 ± 3.5 (1.7) ***	96.7 ± 1.9 (1.0) ***

Asterisks indicate significance vs. control (*[$p < 0.05$]; ***[$p < 0.001$]); values in parentheses are arcsine-transformed.



Graph 1: Represents the dose-response mortality of housefly (*Musca domestica*)

Table 2: Miller and Tainter interpolation data used for LD50 estimation

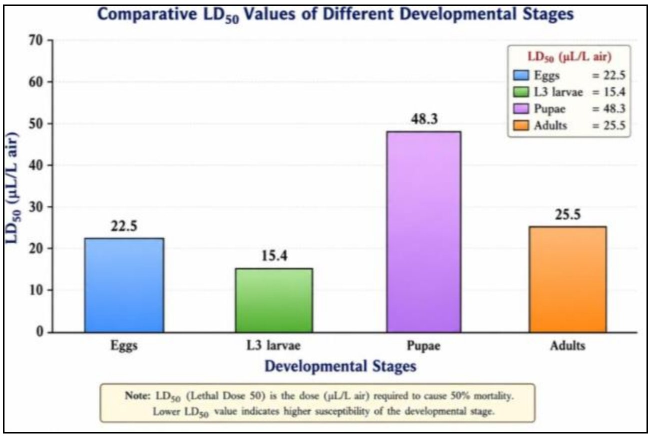
Developmental Stages	Lower dose (µL/L air)	Mortality at lower dose (%)	Higher dose (µL/L air)	Mortality at higher dose (%)	Position of 50% mortality	Estimated LD50 (µL/L air)
Eggs	20	45.8	30	62.5	(50 - 45.8) / (62.5 - 45.8) = 0.2515	22.5
L3 larvae	10	35.0	20	62.7	(50 - 35.0) / (62.7 - 35.0) = 0.5415	15.4
Pupae	40	41.7	50	51.7	(50 - 41.7) / (51.7 - 41.7) = 0.8300	48.3
Adults	20	41.7	30	56.7	(50 - 41.7) / (56.7 - 41.7) = 0.5533	25.5

LD50 Calculation by the miller and tainter method

The median lethal dose (LD50) was calculated and written here by applying the Miller and Tainter method. In this method, percentage mortality is first corrected conceptually for extreme values, then converted into probit units, and the corresponding logarithm of dose is used to estimate the point at which probit 5, representing 50% mortality, intersects the dose–response line. For practical presentation, the 50% lethal point may be obtained by interpolation between the two tested doses that bracket 50% mortality for each developmental stage. This is fully consistent with the Miller and Tainter logic, because the probit line is defined from the relationship between transformed mortality and log dose. Here eggs crossed the 50% level between 20 and 30 µL/L air, larvae between 10 and 20 µL/L air, pupae between 40 and 50 µL/L air and adults between 20 and 30 µL/L air. This already anticipates the biological ranking observed in the raw results, with larvae requiring the lowest fumigant dose to kill half the exposed population and pupae requiring the highest. (Table: 03)

Table 3: LD50 values calculated by using Miller and Tainter method

Developmental stage	LD50 (µL/L air)
Eggs	22.5
L3 larvae	15.4
Pupae	48.3
Adults	25.5



Graph 2: Represents the comparative LD50 values of different developmental stages

The LD50 calculations above are based on the observed 24 h**.** The LD50 values were estimated from the observed mortality data using the Miller and Tainter method while retaining the original dose unit, µL/L air. The estimated LD50 values were 15.4 µL/L air for L3 larvae, 25.5 µL/L air for adults, 22.5 µL/L air for eggs, and 48.3 µL/L air for pupae. These values confirmed that larvae required the lowest dose to achieve 50% mortality and were therefore the most sensitive developmental stage, whereas pupae required the highest dose and were the least sensitive**.** Graph 02, represents the comparative LD50 values of different developmental stages of housefly, *Musca domestica*.

The present study demonstrates that eucalyptus oil has substantial fumigant toxicity against Musca domestica, producing a clear dose-dependent increase in mortality across all tested developmental stages. At the same time, the response was strongly stage-specific, with L3 larvae showing the greatest susceptibility, followed by adults and eggs, while pupae remained the most tolerant stage. This pattern indicates that the insecticidal efficacy of eucalyptus oil is shaped not only by dose but also by the biological and structural characteristics of the developmental stage exposed. The high sensitivity of L3 larvae is toxicologically plausible because larvae are physiologically active, comparatively less protected by hardened external structures, and continuously exposed to the surrounding medium in which volatile compounds disperse. In contrast, pupae are enclosed within a more protective outer covering and are metabolically less active, which may limit penetration and reduce immediate toxic impact. The lower LD50 observed in larvae and the markedly higher LD50 observed in pupae therefore reflect a biologically meaningful difference in developmental vulnerability rather than a simple statistical variation.

These findings are important in the broader context of housefly management. Musca domestica is a medically and economically important pest whose control remains difficult because breeding sites are often abundant, population turnover is rapid, and adults can recolonize treated areas quickly. In addition, long-term dependence on conventional insecticides has created resistance problems that reduce the reliability of many existing chemical-control programs, increasing the need for alternative and complementary agents. The present results support that direction by showing that eucalyptus oil has measurable fumigant activity against multiple life stages of the housefly. The study also agrees with the wider literature on botanical insecticides and essential oils. Plant-derived products have attracted growing attention because they are often biodegradable, chemically diverse, and capable of exerting insecticidal, repellent, and growth-modifying effects through more than one physiological mechanism. Essential oils in particular have been described as reduced-risk pest-management tools with insecticidal and fumigant activity against a broad range of insects, although commercial development has progressed more slowly than laboratory research would suggest. The present findings are therefore consistent with the established view that botanical volatiles can contribute meaningfully to pest-control systems where reduced environmental persistence is advantageous.

Evidence from previous eucalyptus-oil studies further supports the interpretation of the present data. Vapors from eucalyptus essential oils and their major components have shown toxicity to other muscid flies, including *Haematobia irritans,* indicating that the insecticidal potential of eucalyptus volatiles extends beyond a single target species. Eucalyptus oils have also demonstrated fumigant potential in other insect systems, reinforcing the broader relevance of the present results. The current study adds to this evidence by showing clear stage-specific toxicity in *M. domestica*, which is especially important for practical control planning.

A major strength of the present results is the consistency of the dose-response relationship. Mortality increased progressively from low to high concentrations in all developmental stages, and the statistical analysis demonstrated significant effects of dose, stage, and dose × stage interaction. This indicates that eucalyptus-oil efficacy cannot be interpreted independently of population stage structure. A concentration that is highly effective against larvae may not produce equivalent suppression of pupae under the same exposure conditions, which is a critical consideration when translating laboratory findings into operational use.

From an applied perspective, the results suggest that eucalyptus oil may be particularly useful when directed toward larval populations developing in manure, decomposing organic matter, refuse, and other breeding substrates. Because larvae showed the lowest LD50 and the highest susceptibility, treatment at this stage could interrupt population development before adult emergence and thereby reduce subsequent fly abundance more effectively. This makes eucalyptus oil especially promising as a stage-targeted botanical fumigant within integrated housefly-management systems.

Since pupae were the most tolerant stage, eucalyptus-oil fumigation alone may not provide complete suppression in mixed-age infestations where large pupal populations are present. Surviving pupae could continue development and contribute to reinfestation after the fumigant effect declines. For this reason, eucalyptus oil should be regarded as a promising component of control, but not necessarily as a fully sufficient stand-alone intervention under field conditions.

This stage-selective performance supports the need for integration with other control measures. Sanitation remains one of the most critical elements of successful housefly management because it reduces attractive odors, eliminates breeding media, and limits the establishment of immature stages. Source reduction, moisture management, exclusion, and other compatible control tactics can complement eucalyptus-oil fumigation and improve overall suppression. The present findings therefore fit most appropriately within an integrated pest-management framework in which eucalyptus oil is used strategically rather than in isolation

Conclusion

Eucalyptus essential oil demonstrated potent, stage-selective fumigant toxicity against Musca domestica under laboratory conditions, producing dose-dependent mortality across eggs, L3 larvae, pupae, and adults while preserving the original dose unit (µL/L air). Larvae were the most susceptible stage (lowest LD50), whereas pupae exhibited the greatest tolerance (highest LD50), indicating that developmental stage strongly modulates fumigant sensitivity. These results suggest that eucalyptus oil can serve as an effective botanical alternative to synthetic insecticides for housefly management, particularly when treatments are timed to target larval populations or when higher doses are applied to control mixed-age infestations. However, because pupae showed partial tolerance at the tested concentrations, integrated control programs should combine eucalyptus-based fumigation with complementary measures (mechanical sanitation, larval habitat reduction, or targeted contact treatments) to achieve comprehensive suppression. Field validation and formulation optimization are recommended to confirm operational efficacy, minimize non-target impacts, and translate laboratory LD50 benchmarks into practical application rates for waste-management and livestock settings.The present study indicates that eucalyptus oil has real potential as a botanical fumigant for housefly control, but its best utility will likely be achieved when it is deployed strategically within an integrated pest-management framework rather than used as a stand-alone intervention. The strongest practical benefit appears to lie in suppression of susceptible larval populations, while the greater tolerance of pupae highlights the need for combined and stage-aware management strategies.

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