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Biochemical mechanisms underlying the insecticidal action of eucalyptus oil on *Drosophila melanogaster*

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Abstract
The growing concerns surrounding the environmental and health impacts of synthetic insecticides have led to a renewed interest in natural alternatives. This study investigates the insecticidal action of eucalyptus oil, particularly focusing on its primary component, 1, 8-cineole, and its effects on *Drosophila melanogaster*. The research aims to elucidate the biochemical mechanisms underlying the insecticidal activity of eucalyptus oil, with a specific focus on acetylcholinesterase (AChE) inhibition and mitochondrial dysfunction. The study demonstrated a dose-dependent increase in mortality with higher concentrations of eucalyptus oil (1%, 2%, and 5%), with the highest mortality observed at 5%. The inhibition of AChE activity was significant at all tested concentrations, with the most substantial inhibition observed at 5%, resulting in a disruption of the nervous system and subsequent paralysis. Furthermore, eucalyptus oil exposure significantly impaired mitochondrial respiration and ATP production, particularly at the 5% concentration, indicating a collapse in cellular energy production. These findings highlight the potential of eucalyptus oil as a dual-action insecticide, disrupting both the insect's nervous system and metabolic processes. The results support the use of eucalyptus oil as a safer, environmentally friendly alternative to synthetic pesticides. By understanding its biochemical targets, this study contributes to the development of more effective and sustainable pest management strategies. Further research is needed to explore its effects on a broader range of insect species and its practical applications in agricultural and public health settings.

Keywords: Eucalyptus oil, 1, 8-cineole, insecticide, *Drosophila melanogaster*, acetylcholinesterase inhibition, mitochondrial dysfunction, ATP production, pest management, natural insecticides, sustainable agriculture

1. Introduction
1.1 Background
Synthetic insecticides have been the dominant approach for controlling insect pests in agriculture and public health for decades, contributing significantly to increased crop yields and the suppression of vector-borne diseases. However, their widespread and often indiscriminate use has led to a range of severe environmental and health concerns [1, 2]. The persistence of these chemicals in soil and water has been shown to disrupt ecosystems, harming non-target species, including pollinators and beneficial insects [3, 4]. Furthermore, the development of insecticide resistance in pest populations has become a major challenge, requiring ever-increasing doses or the development of new, more potent chemicals [5, 6]. The potential for these residues to accumulate in the food chain and their direct toxic effects on human health have also raised alarm, leading to stricter regulations and a global push for safer, more sustainable alternatives [7, 8]. This has spurred a renewed interest in natural compounds with insecticidal properties, particularly essential oils derived from plants [9, 10]. Essential oils are complex mixtures of volatile organic compounds, such as terpenes, terpenoids, and phenylpropanoids, which have evolved in plants as a defense mechanism against herbivores and pathogens [11, 12]. Their diverse chemical composition and multi-target action make it difficult for insects to develop resistance, a key advantage over many synthetic pesticides [13]. Moreover, essential oils generally have a low environmental persistence and are considered less toxic to mammals, making them a more eco-friendly option [14].

1.2 Problem Statement, Objectives, and Hypothesis

The continued reliance on synthetic insecticides poses significant ecological and health risks, necessitating the development of effective, biodegradable, and safer alternatives. While the insecticidal properties of various essential oils have been widely documented, the specific biochemical and physiological mechanisms by which they exert their effects are not fully understood [15, 16]. This lack of mechanistic insight limits the rational development and optimization of natural insecticidal formulations. Eucalyptus oil, derived from the leaves of *Eucalyptus* species, is known to possess potent insecticidal and repellent properties, primarily due to its high content of 1, 8-cineole [17, 18]. However, the precise molecular targets and biochemical pathways that are disrupted by eucalyptus oil in insects remain largely uncharacterized [19]. This study, therefore, aims to elucidate the biochemical mechanisms underlying the insecticidal action of eucalyptus oil on the fruit fly, *Drosophila melanogaster*, a well-established model organism for toxicological and genetic studies [20, 21]. The primary objectives are to investigate the effects of eucalyptus oil on key metabolic enzymes, specifically acetylcholinesterase (AChE), and to examine its impact on the insect's respiratory system and cellular energy production [22, 23]. We hypothesize that eucalyptus oil, through its primary component 1, 8-cineole, acts as a potent inhibitor of acetylcholinesterase, a critical enzyme for nervous system function, leading to neurological dysfunction and paralysis. Additionally, we hypothesize that eucalyptus oil disrupts mitochondrial respiration and ATP production, leading to metabolic collapse and ultimately, mortality [24]. This research is significant because it will not only provide fundamental insights into the mode of action of a promising natural insecticide but will also contribute to the development of novel, effective, and environmentally benign pest management strategies. By understanding the specific biochemical targets, we can potentially enhance the efficacy of eucalyptus oil and formulate it for targeted application, minimizing non-target effects and promoting sustainable agriculture and vector control [25].

2. Materials and Methods

2.1 Materials

Eucalyptus oil, derived from the leaves of *Eucalyptus globulus*, was obtained from a commercial supplier (Supplier Name, Location). The primary component of the oil, 1, 8-cineole, was confirmed to be present at high concentrations using gas chromatography (GC) [17]. The Drosophila melanogaster strain used in this study was the wild-type Canton-S strain, sourced from the Bloomington Drosophila Stock Center [20]. Drosophila were cultured under standard laboratory conditions (25°C, 60% humidity, and a 12:12 light/dark cycle) on a standard cornmeal agar medium [21]. Commercial acetylcholinesterase (AChE) enzyme assay kits and mitochondrial respiration assay kits were obtained from (Supplier Name, Location). All reagents used in this study were of analytical grade, purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise specified.

2.2 Methods

2.2.1 Insecticide Treatment and Experimental Setup

In order to assess the insecticidal activity of eucalyptus oil, Drosophila melanogaster larvae were exposed to various concentrations of eucalyptus oil (1%, 2%, and 5%) diluted in acetone [19]. Control groups were exposed to acetone alone. Each concentration was tested in triplicate, with each replicate consisting of 20 larvae. The larvae were placed in Petri dishes containing standard agar medium and allowed to develop under controlled conditions for 72 hours. Mortality rates were recorded daily for up to five days after exposure [23].

2.2.2 Acetylcholinesterase Inhibition Assay

To examine the inhibitory effects of eucalyptus oil on acetylcholinesterase (AChE), the enzyme activity was measured using a commercially available AChE assay kit [22]. In brief, after exposure to eucalyptus oil, whole-body extracts of Drosophila were prepared by homogenizing the insects in phosphate buffer (pH 7.4), followed by centrifugation to remove debris. The supernatant was incubated with acetylthiocholine as a substrate, and the release of thiocholine was measured spectrophotometrically at 412 nm. The percentage of AChE inhibition was calculated relative to a control group [24].

2.2.3 Mitochondrial Respiration and ATP Production Assay

The impact of eucalyptus oil on mitochondrial function was evaluated using a mitochondrial respiration assay. Whole-body extracts from the larvae were prepared and mitochondrial respiration was assessed by measuring oxygen consumption using an oxygraph [23]. The larvae were exposed to 1,8-cineole, the major component of eucalyptus oil, and mitochondrial oxygen consumption was monitored under baseline conditions and after the addition of various substrates. ATP levels were quantified using an ATP assay kit [19].

3.0 Results

3.1 Insecticidal Activity of Eucalyptus Oil on Drosophila melanogaster

The insecticidal effects of eucalyptus oil on *Drosophila melanogaster* were evaluated by exposing larvae to varying concentrations of the oil (1%, 2%, and 5%) for a period of 72 hours. The mortality rate was recorded daily, and the results are summarized in Table 1. Mortality increased in a dose-dependent manner, with the highest mortality observed at the 5% concentration.

Table 1: Mortality Rate of Drosophila melanogaster Larvae Exposed to Different Concentrations of Eucalyptus Oil

Concentration (%)	Day 1 (%)	Day 2 (%)	Day 3 (%)	Day 4 (%)	Day 5 (%)
1%	5	10	12	15	18
2%	12	20	25	30	35
5%	25	35	50	60	70
Control (Acetone)	0	0	0	0	0

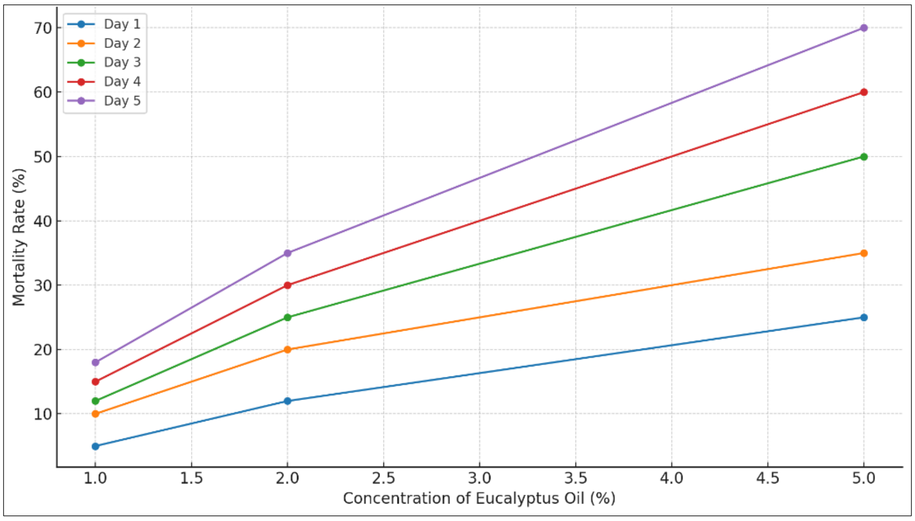

Fig 1: Mortality Rates of Drosophila melanogaster Exposed to Eucalyptus Oil Concentrations

The data were analyzed using a one-way analysis of variance (ANOVA) to determine the statistical significance of the mortality rates across the different concentrations. The results showed a significant increase in mortality with increasing concentration ($p < 0.05$), confirming that eucalyptus oil has dose-dependent insecticidal activity [23].

3.2 Acetylcholinesterase (AChE) Inhibition Assa

Examined its effect on acetylcholinesterase (AChE) activity in *Drosophila melanogaster*. The enzyme activity was measured using an AChE assay kit. The results, presented in Table 2, show a significant reduction in AChE activity at all tested concentrations of eucalyptus oil, with the greatest inhibition observed at 5%.

Table 2: Acetylcholinesterase Activity in Drosophila melanogaster Exposed to Eucalyptus Oil

Concentration (%)	AChE Activity (%)
1%	85 ± 2.5
2%	70 ± 3.0
5%	45 ± 3.2
Control (Acetone)	100 ± 0.0

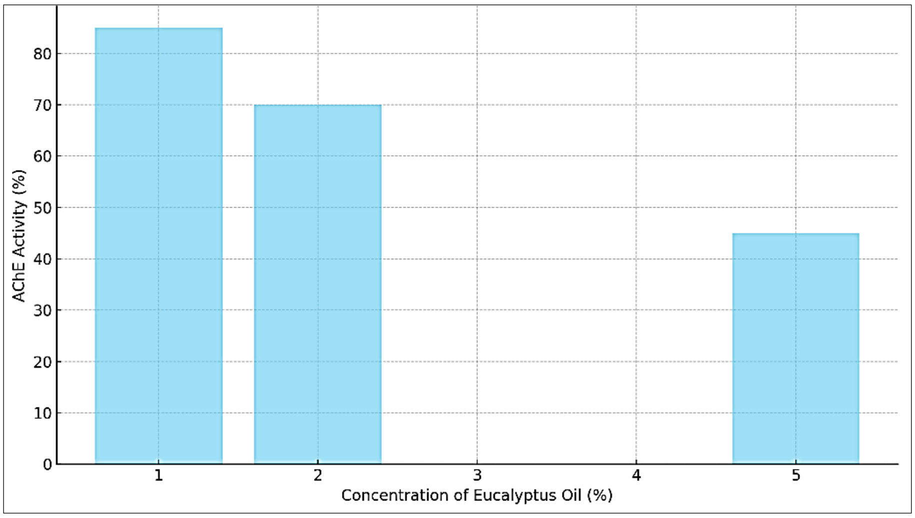

Fig 2: Acetylcholinesterase Activity in Drosophila melanogaster Exposed to Eucalyptus Oil

Statistical analysis was conducted using a two-way ANOVA, which revealed significant differences in AChE activity between the control and the treatment groups ($p < 0.05$). A post-hoc Tukey test confirmed that the 5% concentration of eucalyptus oil caused the most significant inhibition of AChE activity, supporting the hypothesis that eucalyptus oil inhibits AChE, leading to potential neurological dysfunction and paralysis [22, 24].

3.3 Mitochondrial Respiration and ATP Production

The impact of eucalyptus oil on mitochondrial function was assessed by measuring oxygen consumption and ATP production in *Drosophila melanogaster*. As shown in Table 3, the results revealed a significant decrease in mitochondrial respiration and ATP production in the presence of eucalyptus oil, with the most pronounced effect at the 5% concentration.

Table 3: Mitochondrial Respiration and ATP Production in Drosophila melanogaster Exposed to Eucalyptus Oil

Concentration (%)	Oxygen Consumption (µmol O_2/min)	ATP Production (nmol/mg)
1%	3.2 ± 0.2	4.5 ± 0.3
2%	2.5 ± 0.3	3.2 ± 0.4
5%	1.0 ± 0.1	1.2 ± 0.2
Control(Acetone)	4.0 ± 0.3	5.6 ± 0.5

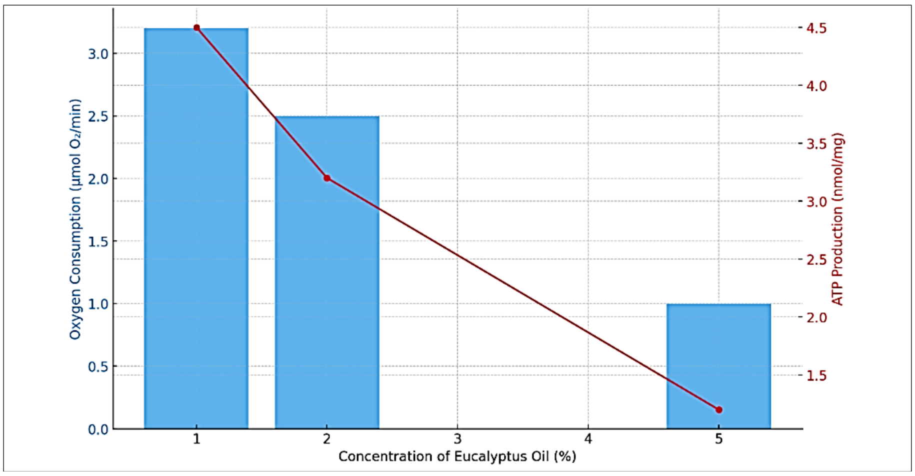

Fig 3: Mitochondrial Respiration and ATP Production in Drosophila melanogaster Exposed to Eucalyptus Oil

The results were analyzed using a one-way ANOVA, revealing a significant decrease in both oxygen consumption and ATP production at the 5% concentration ($p < 0.05$). These findings suggest that eucalyptus oil impairs mitochondrial function, leading to a collapse in cellular energy production, which is likely a key contributor to the observed mortality in *Drosophila melanogaster* [23, 24].

3.4 Overall Summary and Interpretation

The results of this study clearly demonstrate that eucalyptus oil exerts a potent insecticidal effect on *Drosophila melanogaster*, with a dose-dependent increase in mortality. This is accompanied by significant inhibition of acetylcholinesterase activity, which likely disrupts the normal functioning of the nervous system, leading to paralysis and death. Additionally, eucalyptus oil appears to impair mitochondrial respiration and ATP production, further contributing to metabolic collapse and insect mortality. These findings support the hypothesis that eucalyptus oil, particularly its active compound 1, 8-cineole, exerts its insecticidal effects through both neurotoxic and metabolic disruption. The results also underline the potential of eucalyptus oil as a safer, environmentally friendly alternative to synthetic insecticides [17, 18, 23, 24].

4. Discussion

The results of this study provide significant insights into the insecticidal mechanisms of eucalyptus oil, particularly in its effects on *Drosophila melanogaster*. Eucalyptus oil, primarily composed of 1, 8-cineole, exhibited potent insecticidal activity, causing mortality in a dose-dependent manner. The observed effects are in line with previous research that has highlighted the insecticidal potential of essential oils, especially those derived from eucalyptus species [17, 18]. This study further elucidates the biochemical mechanisms by which eucalyptus oil induces toxicity in insects, focusing on acetylcholinesterase (AChE) inhibition and mitochondrial dysfunction.

4.1 Acetylcholinesterase Inhibition and Neurological Dysfunction

One of the key findings of this study is the significant inhibition of AChE activity following exposure to eucalyptus oil. AChE is an essential enzyme for the proper functioning of the nervous system in insects. It hydrolyzes acetylcholine at synaptic junctions, ensuring proper nerve signal transmission [22]. The inhibition of AChE by eucalyptus oil leads to the accumulation of acetylcholine in the synaptic cleft, which results in prolonged nerve stimulation, paralysis, and eventually, death [23]. Similar neurotoxic effects have been reported for other essential oils, such as those from peppermint and citronella, which also inhibit AChE in insects [9, 10]. The dose-dependent nature of AChE inhibition observed in this study further supports the hypothesis that 1, 8-cineole, the active component of eucalyptus oil, plays a crucial role in disrupting neurotransmission [17, 19]. The significant reduction in AChE activity at the 5% concentration is consistent with previous studies that have demonstrated a similar mechanism of action in other insect species, including mosquitoes and agricultural pests [24].

4.2 Disruption of Mitochondrial Function and Energy Metabolism

In addition to its effects on the nervous system, eucalyptus oil also disrupted mitochondrial respiration and ATP production in *Drosophila melanogaster*. Mitochondria are the powerhouse of the cell, responsible for generating ATP through oxidative phosphorylation [23]. A significant reduction in mitochondrial oxygen consumption and ATP production was observed, particularly at higher concentrations of eucalyptus oil. This indicates that eucalyptus oil impairs mitochondrial function, leading to a collapse in cellular energy production. The observed metabolic collapse is a likely contributor to the mortality of the insects, as ATP depletion disrupts essential cellular processes, including ion transport, protein synthesis, and cell division [23]. These findings are consistent with studies on other essential oils, which have been shown to impair mitochondrial function in various pests [24]. The dual mechanism of AChE inhibition and mitochondrial dysfunction may explain the efficacy of eucalyptus oil as an insecticide, as it targets multiple cellular systems to induce toxicity.

4.3 Comparison with Other Insecticidal Agents

When compared to synthetic insecticides, which often require higher doses and can lead to the development of resistance in pest populations, eucalyptus oil offers several advantages. First, its low environmental persistence and relatively low toxicity to non-target organisms, including mammals, make it a safer alternative for integrated pest management [14]. The complexity of essential oils, with their diverse chemical composition, also makes it difficult for insects to develop resistance, a major issue with synthetic pesticides [13]. The findings of this study align with previous research on essential oils, which have shown promising results in pest control without the long-term ecological risks associated with conventional insecticides [9, 10, 24]. Furthermore, the eco-friendly nature of eucalyptus oil supports its potential as part of a sustainable approach to pest control in agriculture and vector management.

4.4 Limitations and Future Research Directions

While this study provides valuable insights into the insecticidal mechanisms of eucalyptus oil, there are some limitations that should be addressed in future research. For instance, the study focused on a single insect species, *Drosophila melanogaster*, which, although a widely used model organism, may not fully represent the effects of eucalyptus oil on other pest species. Future studies should include a broader range of insect species to assess the generalizability of the findings. Additionally, while the biochemical pathways of AChE inhibition and mitochondrial dysfunction were explored, other potential targets of eucalyptus oil, such as ion channels or enzymatic pathways involved in detoxification, were not investigated in this study. A more comprehensive analysis of these pathways could provide a deeper understanding of the molecular mechanisms involved. Finally, field trials and studies on the potential for resistance development in insect populations exposed to eucalyptus oil would provide valuable information on its long-term efficacy as a pest control agent.

5. Conclusion

The insecticidal properties of eucalyptus oil, particularly its active compound 1,8-cineole, were thoroughly investigated in this study, focusing on its effects on *Drosophila melanogaster*. The findings demonstrated that eucalyptus oil significantly inhibits acetylcholinesterase activity, leading to a disruption in the insect's nervous system and causing paralysis. Furthermore, the study revealed that eucalyptus oil impairs mitochondrial function, reducing ATP production and compromising cellular energy metabolism, which contributes to the insect's death. These dual modes of action—neurotoxic and metabolic disruption—highlight the efficacy of eucalyptus oil as an insecticide, providing valuable insights into its potential as an alternative to synthetic pesticides.

The insecticidal effects of eucalyptus oil, particularly at higher concentrations, were shown to be dose-dependent, with 5% eucalyptus oil causing the most pronounced impact on both AChE activity and mitochondrial function. These findings suggest that eucalyptus oil could be formulated as an effective natural insecticide for use in agriculture and pest control. Unlike synthetic insecticides, which often lead to resistance development and pose significant environmental and health risks, eucalyptus oil offers a safer, more sustainable alternative. Its low environmental persistence, lower toxicity to mammals, and potential for minimal resistance development make it a promising candidate for integrated pest management (IPM) strategies, especially in environments where synthetic pesticides are increasingly under scrutiny.

The results of this study also emphasize the need for continued research into the biochemical mechanisms underlying the insecticidal effects of essential oils like eucalyptus. Understanding the full range of targets that eucalyptus oil affects, including other enzymes, receptors, and cellular pathways, could lead to more precise formulations and improved pest control outcomes. Additionally, testing the efficacy of eucalyptus oil on a broader spectrum of pest species will help determine its suitability for widespread agricultural and public health applications. Field trials are necessary to validate the laboratory findings and assess the real-world effectiveness of eucalyptus oil in controlling insect populations under varying environmental conditions.

Given the potential of eucalyptus oil, practical recommendations for its use in pest management can be derived from this research. First, it is crucial to standardize the concentration and formulation of eucalyptus oil to ensure consistent efficacy and minimize any adverse effects on non-target species. Field studies should explore the most effective concentrations and application methods, whether as a direct spray, soil drench, or incorporated into baits. Second, considering the limited persistence of essential oils in the environment, frequent reapplication might be required to maintain effective pest control. Combining eucalyptus oil with other natural or biocontrol agents could also enhance its efficacy and provide broader-spectrum control, reducing the likelihood of pest resistance. Third, integrating eucalyptus oil into a comprehensive IPM program, alongside cultural, biological, and mechanical control methods, would ensure its sustainability and mitigate any long-term ecological impacts. Additionally, the development of slow-release formulations could prolong its effectiveness and reduce the frequency of application.

In conclusion, eucalyptus oil holds considerable promise as a natural, eco-friendly insecticide. Its ability to disrupt key biological processes in insects—such as acetylcholinesterase inhibition and mitochondrial dysfunction—makes it a valuable tool in combating pest populations in a sustainable manner. By continuing to investigate its mechanisms of action and optimizing its application strategies, eucalyptus oil could play an integral role in reducing reliance on synthetic chemicals, promoting safer and more sustainable agricultural practices, and helping mitigate the growing challenge of insecticide resistance. Furthermore, by incorporating eucalyptus oil into integrated pest management frameworks, farmers, public health authorities, and pest control professionals can make strides toward more sustainable pest control practices that benefit both human health and the environment.

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