MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Toxicological and Histopathological Effects of Lemongrass Oils on *Oleander Aphids* (*Aphis nerii*)

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Received:8/6/2024
Accepted: 29/6/2024

Abstract: This study investigated the potential of lemongrass oil as a natural insecticide against *oleander aphids* using microscopic analysis of treated specimens. The findings revealed progressive damage to the aphids' internal tissues over time, with increasing severity at higher oil concentrations. This damage included tearing of the exoskeleton, compromising their defense mechanisms, shrunken salivary glands and dilated midgut, indicating impaired feeding and digestion, and even separation of organs, suggesting widespread cell death. These results strongly support the toxic effect of lemongrass oil on *oleander aphids* and its potential as a dose-dependent insecticide. Further research is recommended to optimize application methods, minimize environmental impact, and elucidate the underlying mechanisms of action, including potential effects on the aphid cytoskeleton.

keywords: *Oleander aphid*, Lemongrass oil, Insecticide, Natural product, Histological analysis, Tissue damage, Exoskeleton, Salivary gland, Midgut, Cell death, Dose-dependent.

1. Introduction

The presence of *oleander aphids* (*Aphis nerii*) signifies a substantial ecological and economic threat to cultivated *Nerium oleander* landscapes [1]. Beyond the detrimental aesthetic impact from sooty mold and discolored foliage, infestations significantly affect the economic viability of nurseries and landscapers [2, 3]. Reduced plant marketability, stunted growth, and increased disease susceptibility stemming from aphid presence translate to financial burdens [4]. Furthermore, the ecological significance of oleander in erosion control and pollinator attraction is jeopardized by widespread aphid populations, highlighting the urgency for establishing sustainable management strategies [5].

The widespread utilization of conventional insecticides for rapid pest control raises significant concerns regarding their long-term sustainability [6]. These chemical concoctions often exhibit non-target effects, harming beneficial insects like ladybugs and lacewings, which play crucial roles in maintaining ecosystem equilibrium [7]. Additionally, their persistence in soil and water poses a threat to wildlife and potentially even human health [8]. The development of resistance further complicates the issue, as continued usage selects for hardier aphid strains, rendering existing chemical options ineffective and necessitating the continuous development of novel formulations [9]. Lemongrass extract, with its inherent insecticidal properties, presents a potentially more sustainable alternative [10]. This natural product offers the potential to control aphid populations without disrupting the delicate ecological balance of an environment, paving the way for a more responsible and long-term approach to pest management [11].

Cymbopogon spp., commonly known as lemongrass, boasts a rich history of multifaceted contributions encompassing medicine, food, and even cultural significance [12, 13]. Within traditional medicine, particularly in Southeast Asia, its essential oils and teas have been utilized for their carminative properties, aiding in digestion and alleviating discomfort. Additionally, lemongrass exhibits antipyretic and analgesic effects, potentially reducing fever and aches without the drawbacks of conventional medications [14, 15]. Its historical use in combating skin infections, wounds, and even common colds suggests potential antimicrobial benefits [16, 17]. Furthermore, the calming aroma of lemongrass plays a valuable role in aromatherapy, believed to alleviate anxiety and promote relaxation, contributing to overall well-being [18].

Lemongrass (*Cymbopogon* spp.), despite its captivating fragrance and culinary uses [19], possesses potent insecticidal properties [20]. Its essential oil, rich in geraniol, citronellol, and limonene, acts as a multi-faceted defense against pests [21]. The pungent aroma disrupts insect behavior, while these compounds disrupt their nervous systems and exhibit insecticidal activity at higher concentrations [22]. Scientific evidence supports this diverse pest control potential. Studies demonstrate lemongrass extract's effectiveness against mosquitoes (offering a natural solution for disease control), aphids (presenting an eco-friendly alternative to chemical insecticides), and even termites (potentially safeguarding wooden structures) [23, 24]. Therefore, lemongrass extract emerges as a compelling option in the fight against harmful insects [25]. Its potent, scientifically confirmed insecticidal properties and natural advantages offer a promising alternative to traditional chemical methods, allowing it to serve as a gentle guardian of valued plants and the ecological balance.

Compared to their synthetic counterparts, natural pesticides like lemongrass extract exhibit a temporarily disruptive effect on pest populations [26, 27]. Unlike conventional pesticides that accumulate in soil and water, lemongrass extract undergoes rapid biodegradation, minimizing long-term environmental residues [28]. This characteristic aligns with the concept of environmental stewardship, as it minimizes the introduction of persistent contaminants into the ecosystem. Additionally, lemongrass extract exhibits selective toxicity, primarily targeting targeted pests while minimizing harm to beneficial insects like pollinators [29]. This selectivity fosters ecological balance by preserving biodiversity and promoting healthy ecosystem function [30].

Conversely, conventional pesticides often disrupt trophic cascades within the food web, leading to unintended consequences for non-target organisms like earthworms [31]. By minimizing collateral effects, lemongrass extract contributes to a more inclusive and sustainable approach to pest management [32]. Furthermore, lemongrass extract primarily acts as a repellent through its strong scent, rather than directly killing pests [33]. This sublethal approach reduces the selection pressure for the development of resistance in pest populations, potentially offering a more sustainable long-term solution compared to conventional methods [34]. In conclusion, selecting natural pesticides like lemongrass extract can be considered an environmentally responsible choice for pest control, offering potential benefits in terms of reduced environmental impact, improved ecological balance, and decreased selection pressure for resistance [35].

This research aimed to explore the potential of lemongrass oil as a natural insecticide against *oleander aphids*. Histological examination was employed to assess the internal tissue damage caused by lemongrass oil at different concentrations and exposure times. By investigating the progressive damage and potential underlying mechanisms, the study sought to evaluate the overall insecticidal efficacy of lemongrass oil and its potential as a sustainable pest control solution.

2. Materials & Methods

2.1. Materials

2.1.1. Laboratory Equipment and Instruments:

The research utilized various instruments from international companies. A centrifuge (Eppendorf, Germany) was used for sample separation. Distillation and extraction processes employed a Clevenger apparatus (Gerhardt, Germany) and a distillator (Ika Dest, China), respectively. Maintaining consistent temperatures was achieved with an incubator (Nüve, Turkey) and an oven (Memmert, Germany), while a refrigerator (Philips, Netherlands) ensured proper sample storage.

Microscopic analysis was performed using a light microscope (Olympus, Japan). Sample preparation involved a vortex mixer (Quality Lab System, England) and a water bath (Memmert, Germany).

2.1.2. Chemicals and Biological Materials:

The research employed various chemicals from different sources. Analytical grade absolute ethanol (ROMIL pure chemistry, UK) was used for various purposes. Dimethyl sulfoxide (DMSO) from Santa Cruz Biotechnology (USA) served as a solvent. Deionized water, sourced locally in Iraq, was utilized throughout the study. Additionally, 10% formalin (Al-Nasr Chemical Industries Company, Egypt) was used for tissue fixation, while hematoxylin eosin (H&E) stain, also from Al-Nasr Chemical Industries Company (Egypt), was employed for histological analysis.

2.1.3. Source of plant material:

Lemongrass *Cymbopogon citratus* leaves were taken from a plant grown in the gardens of the University of Baghdad and dried in the laboratory of the College of Science. The leaves were washed with water and dried at room temperature, and ground using a grinder.

2.2. Methods

2.2.1. Study area: This study was conducted in Iraq's Baghdad University gardens, located in the Al-Jadria compass. The timeframe spanned from February to June 2023. Baghdad's central location within Iraq is reflected by its coordinates: 33°17′17″N, 44°23′35″E. Temperature and humidity data were sourced from an Iraqi Agrometeorological station.

2.2.2. Data collection:

The abundance of *Aphis nerii* on oleander plants (*Nerium oleander*, *Cascabela thevetia*, and *Pilea serpyllacea*) was monitored under standard horticultural practices, with no pesticide application during the study. Samples were collected at regular intervals (every 7 days) from the bottom, middle, and top sections of the plants, randomly selecting infested terminals within each zone. Three infested leaves were collected per sampling period, and the number of nymphs and adults was counted in the laboratory.

2.2.3. Studying Oleander Aphid Infestation Rates on Different Host Plants

The percentage of infestation of adults and nymphs on three plant hosts was studied through two experiments on the number density of plant leaves infected from the period from February 2023 to June 2023, as samples were taken to calculate the percentage of aphid infestations per week, through the presence of aphids on infected plant shoot apex, and the percentage was extracted percentage of infected plants according to the following equation (Eq. 1) [36]:

Percentage of Infected Shoot Apex (%) = (Number of Affected Shoot Apex / Total Number of Shoot Apex) x 100   Eq. (1)

Adult aphids were carefully collected and transported to the laboratory in ventilated paper bags for subsequent bioassay experiments.

2.2.4. Filed experiment conditions

Researchers evaluated the potential of lemongrass oil as an insecticide against adult oleander aphids on three heavily infested host plants: *Oleander Nerium*, *Cascabela thevetia*, and *Pilea serpyllacea*. The study compared three concentrations of lemongrass oil solution (5000, 10000, and 20000 ppm) applied at various time points (6, 12, and 24 hours) across two seasons (spring and summer). By analyzing the effectiveness of the oil against the aphids, the research aims to determine its potential as an eco-friendly alternative to conventional insecticides and assess its impact based on concentration, host plant, and seasonal variations.

2.2.5. Laboratory experiment conditions

Researchers evaluated the effectiveness of lemongrass essential oil (5000, 10000, and 20000 ppm) against adult oleander aphids in a controlled lab setting. Leaves from three different plants (*Nerium oleander*, *Pilea* *serpyllacea*, and *Cascabela thevetia*) were dipped in the oil solutions, air-dried, and placed in petri dishes with moistened filter paper. Ten adult aphids were then transferred onto each leaf disc. To compare effectiveness, some leaves were dipped in water only (control group). The researchers monitored and recorded aphid mortality at 6, 12, and 24 hours after treatment, while maintaining consistent temperature, humidity, and light conditions throughout the study. This experiment aimed to assess the potential of lemongrass oil as an insecticide for controlling oleander aphids on various host plants [37].

2.2.6. Extraction of essential oils from experiment plants:

One hundred grams of dried flower buds or leaves were combined with 500 milliliters of distilled water in a round-bottomed flask. This mixture was then subjected to hydrodistillation for 3 hours using a Clevenger apparatus. The heating temperature was maintained at 60°C throughout the process. The resulting mixture separated into two layers. The lower, water-based layer was discarded, while the upper layer containing the essential oils was collected. This upper layer yielded approximately 1 milliliter of essential oil, which was then stored in a sealed, labeled glass vial and refrigerated until further use [38].

2.2.7. Preparation of different concentration of plant extract:

To prepare different concentrations of the extracted oil for further experiments, the researchers used a dilution method. They started with a concentrated stock solution and diluted it with a solvent (DMSO) according to a specific formula: $C_1V_1 = C_2V_2$. This formula allows them to calculate the volume (V_2) required from the stock solution (C_1) to achieve a desired final concentration (C_2) in a specific final volume (V_2). This approach ensures they have various concentrations of the oil solution available for their research.

2.2.8. Histological Investigations:

To analyze potential tissue-level effects in oleander aphids treated with lemongrass and lavender, researchers used a modified version of Gad [39] method for histological processing. The aphids were first fixed and dehydrated, followed by clearing and infiltration with a solution to prepare them for sectioning. Individual specimens were then embedded on slides using celloidin and paraffin wax, before being sliced into thin sections and mounted for heat-fixing. The sections were subsequently stained with hematoxylin and eosin for visualization under a microscope, requiring deparaffinization, dehydration, and finally mounting in a suitable medium. This multi-step process allows for detailed microscopic examination of potential treatment-induced changes within the aphid tissues.

3. Results and Discussion

This research explored the potential of lemongrass oil as an insecticide against the *oleander aphid*. Histological examination of the treated aphids revealed progressive damage to their internal tissues over time, indicating a potentially toxic effect of lemongrass oil. These findings warrant further investigation to assess the full insecticidal potential of this natural product and its potential application as a sustainable pest control solution. Microscopic examination *oleander aphids* treated with lemongrass oil revealed progressive damage to their internal tissues over time, suggesting a toxic effect of the oil. Key findings include: tearing of the exoskeleton, potentially increasing vulnerability to infection and predation; shrunken salivary glands and dilated midgut, indicating impaired feeding and digestion; and separation of organs from the exoskeleton, suggesting cell death and tissue necrosis. The observed damage intensified with higher concentrations and longer exposure times, suggesting a dose- and time-dependent effect and the potential of lemongrass oil as a viable insecticide for *oleander aphid* control.

Examining the microscopic images reveals progressive damage to the oleander aphid's internal tissues over time, suggesting a potent toxic effect from lemongrass oil. At the initial 6-hour mark with the 5,000 ppm concentration (**Fig. 1a**), the aphid's structure appears normal, but potential stress is indicated by the presence of increased fat storage (FB) [40]. After 12 hours (**Fig. 1b**), the damage becomes evident, with shrunken salivary glands (SSG), a dilated midgut (DMG), and exoskeleton tearing (arrowheads) observed, suggesting disruption in feeding, digestion, and tissue integrity [41]. By 24 hours (**Fig. 1c**), the damage becomes severe, with complete separation between organs and the exoskeleton (asterisks) and exoskeleton ruptures (arrowhead), indicating widespread cell death and tissue necrosis, making survival unlikely [42]. These findings strongly support the toxic effect of lemongrass oil on oleander aphids and warrant further research to optimize concentration, application methods, and overall efficacy while minimizing environmental impact.

Microscopic analysis of oleander aphids exposed to a higher concentration of lemongrass oil (10,000 ppm) revealed similar but more severe tissue damage compared to the lower concentration (**Figs 2a-c**). This is evident in extensive exoskeleton tearing, heightened vulnerability to infection and predation, and pronounced damage to the salivary glands, midgut, and muscles, suggesting impaired feeding, digestion, and movement. The observed increase in fat body (FB) indicates a desperate attempt by the aphid to store energy and cope with the stress, but ultimately proves insufficient to combat the severe toxic effects of lemongrass oil. These findings highlight the dose-dependent impact of lemongrass oil *oleander aphids* and solidify its potential as a potent insecticide.

Microscopic examination of *oleander aphids* treated with a higher concentration of lemongrass oil (10,000 ppm) revealed progressively worsening tissue damage compared to the lower concentration, as evidenced in **Figures 2a-c**. Compared to the 6-hour exposure with 5,000 ppm (**Fig. 1a**), even the initial 6 hours at the higher concentration (**Fig. 2a**) showed more severe exoskeleton tearing and increased fat storage, suggesting a dose-dependent and rapid onset of damage [43]. This trend continued at 12 and 24 hours (**Figs. 2b** and **2c**), with progressive degeneration exceeding that observed at the lower concentration, including additional midgut damage, muscle degeneration, and widespread exoskeleton tears [44, 45]. Overall, these findings solidify the toxic effect of lemongrass oil on *oleander aphids*, demonstrating rapid and severe tissue damage at higher concentrations, supporting its potential as a potent insecticide.

Despite the increased presence of fat bodies in **Figure 2c**, suggesting a desperate attempt by the aphid to store energy and combat stress, the severe tissue damage ultimately proves insurmountable. Additionally, the distorted overall morphology of the aphid in the same figure hints at a potential impact of lemongrass oil on the cytoskeleton, a crucial structure for cell shape and function [46]. Taken together, these observations provide compelling evidence for the detrimental effects of lemongrass oil on *oleander aphid* tissues, opening avenues for further research to explore the underlying mechanisms of action and develop optimal control strategies using this natural product.

Examining the effects of the highest lemongrass oil concentration (20,000 ppm) in **Figures 3a-c** reveals even more severe tissue damage compared to the lower concentrations (5000 and 10000 ppm), further solidifying its potent insecticidal effect. This is evident in extensive exoskeleton tearing and ruptures, leaving the aphids virtually defenseless (**Figs. 3a-c**). Additionally, the observed damage to salivary glands, midgut, and muscles surpasses that seen at lower concentrations, suggesting a complete disruption of the aphid's ability to perform basic functions like feeding, digestion, and movement. Despite the increased presence of fat bodies indicating a desperate energy-storing attempt to combat the stress, this ultimately proven futile at this concentration. These findings strongly support the dose-dependent impact of lemongrass oil and its potential as a powerful and rapid-acting insecticide against *oleander aphids*.

Examining **Figure 3a** reveals severe damage to the soft tissues of oleander aphids exposed to the highest lemongrass oil concentration (20,000 ppm) even at the initial 6-hour mark. This is evident in torn exoskeleton (arrowhead), increased fat body storage (FB), and degenerated muscle tissue (DMU). This damage significantly surpasses that observed at the same time point with a lower concentration (10,000 ppm) in **Figure 2a**, highlighting the significant and rapid dose-dependent effect of lemongrass oil on the aphids [43].

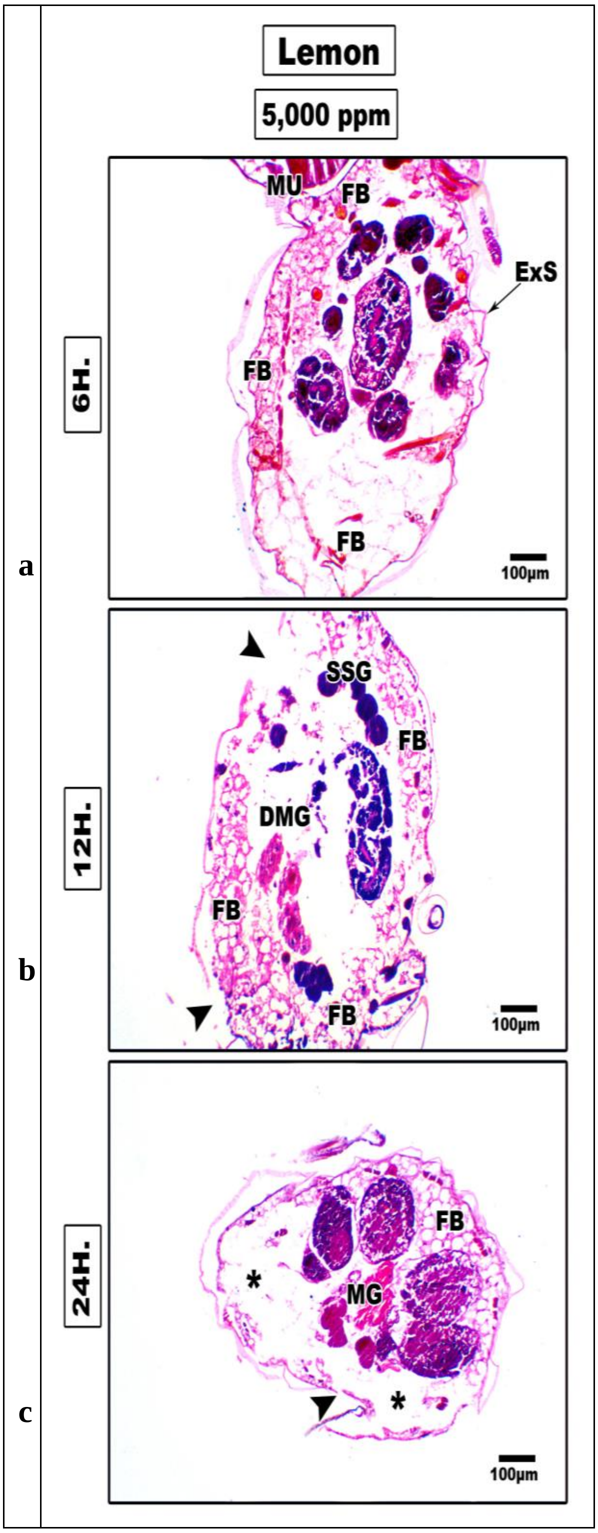

Figure 1. A photomicrograph of a paraffin section of *oleander aphid*'s soft tissues containing 3 periods with 3 conc. which showing: (a) 6 hours: 5.000 ppm, normal muscle (MU) fat body (FB) and Exoskeleton (ExS). (b) 12 hours: 5.000 ppm shrunken salivary gland (SSG), dilated midgut (DMG), fat body still found (FB) and tearing of exoskeleton (arrow heads). (c) 24 hours: 5.000 ppm noticed a separation (asterisks) in between exoskeleton and internal organs. Fat bodies (FB) are present, exoskeleton rupture in one place (arrow head), midgut (MG).

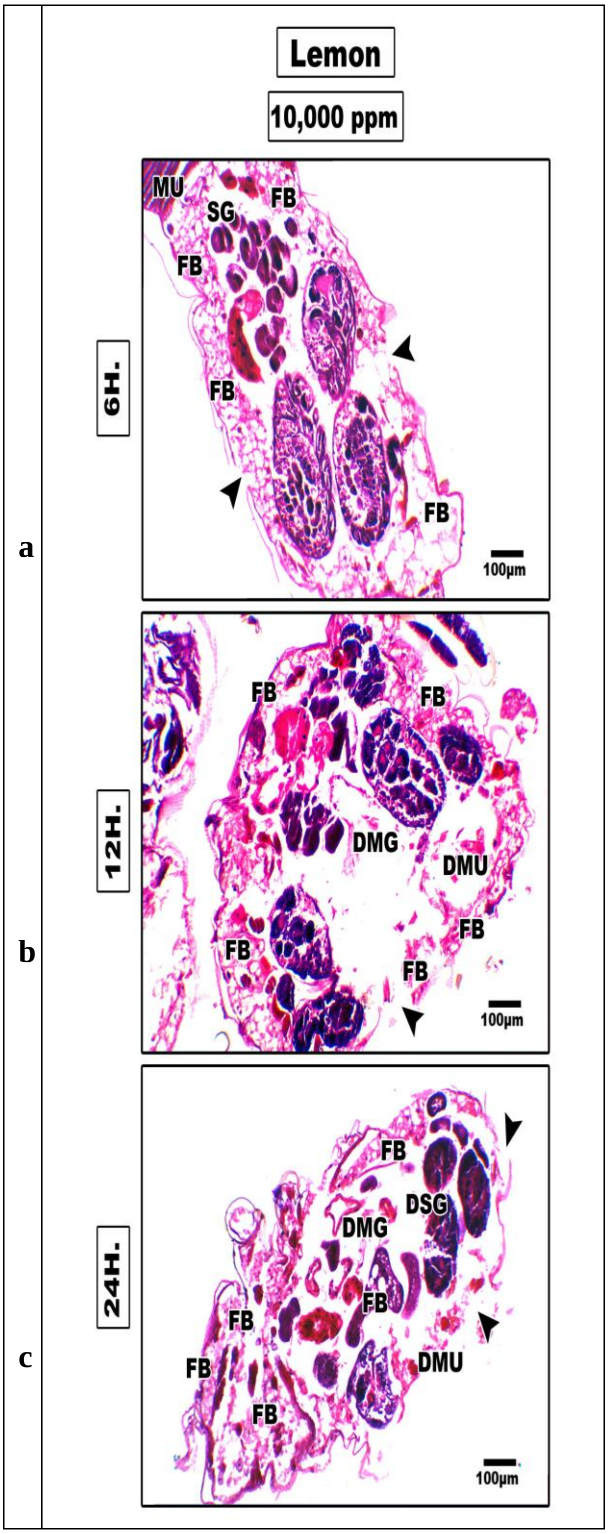

Fig 2. (a) 6 hours: 10.000 ppm, salivary glands (SG), tearing exoskeleton (arrow heads) appears in two areas with more fat bodies (FB) than previous tissue. (b) 12 hours:10.000 ppm increasing fat body area (FB), damage midgut (DMG) and tearing of exoskeleton (arrow head), degenerated muscle (DMU). (c) 24 hours:10.000 ppm numerous fat bodies area (FB) appear, degenerated salivary gland (DSG),

damage midgut (DMG) with tearing of exoskeleton (arrow heads). Degenerated muscle (DMU).

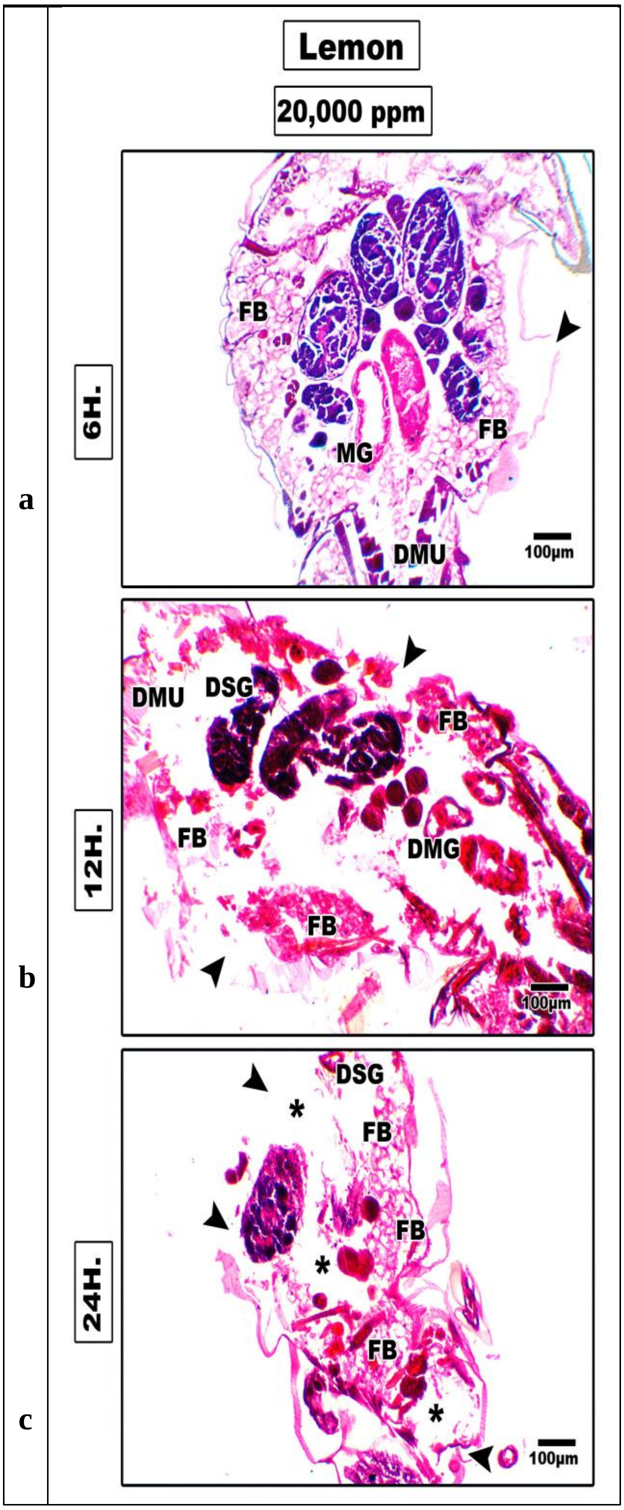

Figure 3. (a) 6 hours: 20.000 ppm, midgut (MG), tearing exoskeleton in one area (arrow head), increasing fat bodies (FB) in between (ExS) and internal organs. Degeneration muscle (DMU) is noticed. (b) 12 hours: 20.000 ppm tearing and rupture exoskeleton (arrow heads) degenerated muscles are noticed (DMU) with damage of salivary glands (DSG). Dilated midgut (DMG) is opserved with a lot of fat bodies (FB). (c) 24 hours: 20.000 ppm degenerated and rupture exoskeleton (arrow heads) in most of places with wide separation (asterisks). Increasing fat bodies in many areas (FB) and damage in salivary glands are noticed (DSG). (H&E, x100).

Figure 3b showcases the extensive damage caused by the highest lemongrass oil concentration (20,000 ppm) at the 12-hour mark. Compared to the lower concentration at the same time point (**Fig. 2b**), the damage is significantly more severe, as evidenced by widespread tearing and ruptures of the exoskeleton (multiple arrowheads), degenerated muscles (DMU), salivary gland damage (DSG), and a dilated midgut (DMG) containing numerous fat bodies (FB). This dramatic increase in tissue damage highlights the potent and dose-dependent impact of lemongrass oil, with the higher concentration accelerating the toxic effects to a much greater extent [47].

Microscopic examinations revealed progressively worsening tissue damage in oleander aphids treated with increasing concentrations of lemongrass oil, as evidenced in **Figures 3a-c**. Compared to lower concentrations, the highest concentration (20,000 ppm) caused significantly more severe damage at each time point, evident in extensive exoskeleton tearing and ruptures, widespread muscle degeneration, and damage to various vital organs (**Figs. 3a-c**). The presence of abundant fat bodies throughout various concentrations suggests the aphids' desperate attempt to combat stress, ultimately proving futile at higher concentrations. Notably, even the lowest concentration (5,000 ppm) caused noticeable damage over time, making it a potentially viable and less drastic option for oleander aphid control [48]. Overall, these findings demonstrate the dose-dependent toxic effect of lemongrass oil on oleander aphids, supporting its potential as a potent and potentially sustainable insecticidal solution.

While the presence of increasingly abundant fat bodies across all concentrations (**Figures 3a-c**) indicates the aphids' stressed attempts to store energy, ultimately, this proves insufficient to combat the severe tissue damage caused by lemongrass oil. Furthermore, the drastically distorted overall morphology of the aphid in **Figure 3c** suggests a potentially significant impact of the oil on the cytoskeleton, a crucial structure vital for cell shape and function. These observations combined solidify the potent toxic effect of lemongrass oil on oleander aphids, even at lower concentrations, and warrant further investigation into the underlying mechanisms and optimal application strategies for this potential green insecticide.

4. Conclusion

In conclusion, this study employed histological analysis to explore the potential of lemongrass oil as an insecticide against *oleander aphids*. The observed progressive damage to the aphids' internal tissues over time, increasing in severity with higher concentrations, strongly suggests a potent toxic effect of lemongrass oil. This damage manifested as exoskeleton tearing, impaired feeding and digestion, and even cell death. These findings highlight the potential of lemongrass oil as a dose-dependent insecticide and a promising green alternative for *oleander aphid* control. Further research is warranted to optimize application methods, minimize environmental impact, and elucidate the underlying mechanisms of lemongrass oil's toxicity, including its potential effects on the aphid cytoskeleton.

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