

Research Article

Phytochemical Composition of Fruit and Leaf Terpenoid Extracts of *Melia azedarach* L. and Their Bioactivity Against *Culex pipiens* L. (Diptera: Culicidae)

Wail Ismael Hassan¹, Abbas Ali Zamani^{1*}, Yousif Dakheel Rashid² and Arezoo Jamshidi¹

¹Department of Plant Protection, College of Agriculture, Razi University, Kermanshah, Islamic Republic of Iran; ²Department of Biological Control, Al-Mussaib Technical College, Al Furat Al Asat Technical University, Iraq.

Abstract | *Culex pipiens* L. mosquitoes are among the most medically important vectors, transmitting pathogens such as West Nile virus, St. Louis encephalitis, and filarial worms. The development of resistance to conventional insecticides has intensified the search for eco-friendly alternatives, particularly botanical extracts with insecticidal properties. This study investigated the bioactivity of crude terpenoid extracts of *Melia azedarach* L. (Meliaceae) fruits and leaves against the immature stages of *C. pipiens* under laboratory conditions. Terpenoids were extracted using chloroform via a Soxhlet apparatus and tested at concentrations of 5, 7.5, and 10 mg/mL against eggs, larval instars (L1-L4) and pupae, with mortality rates recorded after 24-48 hours of exposure. Moreover, the repellent effects of both extract types on adult mosquitoes were evaluated using a chemotropometer device. Gas chromatography-mass spectrometry (GC-MS) analysis was performed to identify the bioactive constituents in the extracts. Results demonstrated concentration-dependent mortality across all developmental stages, with the highest efficacy observed at 10 mg/mL. Fruit extracts exhibited superior activity, causing 93.33% mortality of first instar larvae (L1) compared to 88.33% for leaf extracts at the same concentration. Egg mortality reached 86.25% by fruit extract, and 82.53% by leaf extract, while the pupae showed a lower susceptibility with 16.67% (by fruit extract) and 13.33% (by leaf extract) mortality. Adult repellency rates were 58% by fruit extract and 57% by leaf extract at the highest concentration. GC-MS analysis revealed 20 compounds in the fruit extracts, dominated by 9,12-octadecadienoic acid (20.28%) and n-hexadecanoic acid (19.20%), while the leaf extracts contained 32 compounds with 9,12-Octadecadienoic acid (44.23%) as the major component. These findings demonstrate that *M. azedarach* terpenoids, particularly from its fruits, possess significant larvicidal, ovicidal, and repellent properties, supporting their potential as sustainable alternatives for integrated mosquito control programs targeting *C. pipiens* populations. The differential efficacy between the plant parts and the developmental stages of mosquitoes provides valuable insights for optimizing botanical insecticide formulations.

Received | July 22 2025; **Accepted** | Aug 20, 2025; **Published** | December 08, 2025

***Correspondence** | Abbas Ali Zamani, College of Agriculture, Razi University, Iran. **Email:** azamani@razi.ac.ir

Citation | Hassan, W.I. A.A. Zamani, Y.D. Rashid and A. Jamshidi. 2025. Phytochemical composition of fruit and leaf terpenoid extracts of *Melia azedarach* L. and their bioactivity against *Culex pipiens* L. (Diptera: culicidae). Sarhad Journal of Agriculture, 41(5): 60-70.

DOI | <https://dx.doi.org/10.17582/journal.sja/2025/41.5.60.70>

Keywords | Mosquito control, *Culex pipiens*, Botanical insecticides, Larvicidal activity, Secondary metabolites, Vector management, *Melia azedarach*, Repellency effects.



Copyright: 2025 by the authors. Licensee ResearchersLinks Ltd, England, UK.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Introduction

The global burden of mosquito-borne diseases remains a significant public health challenge, with *Culex pipiens* L. complex mosquitoes serving as primary vectors for numerous pathogens, including West Nile virus, St. Louis encephalitis, and lymphatic filariasis (Ligsay *et al.*, 2021; Madhav *et al.*, 2024). These medically important insects have demonstrated remarkable adaptability to anthropogenic environments, thriving in urban settings where artificial water containers provide ideal breeding sites. The species' catholic feeding behavior, targeting both avian and mammalian hosts, including humans, enhances its vectorial capacity and complicates control efforts (Fonseca *et al.*, 2004; Shaikovich *et al.*, 2016).

The widespread use of synthetic insecticides against mosquitoes has led to significant operational challenges, including environmental contamination, the emergence of resistant mosquito populations, and various other ecological consequences (Abbasi *et al.*, 2022). Synthetic insecticides such as pyrethroids and organophosphates, once highly effective, now face reduced efficacy due to target-site mutations and enhanced metabolic detoxification in mosquitoes (Karunaratne *et al.*, 2018; Black IV and Snell, 2021). This resistance crisis is compounded by growing public concern over environmental contamination and non-target organism toxicity, particularly in aquatic ecosystems where mosquito larvae develop (Lange *et al.*, 2025). Furthermore, the withdrawal of certain insecticides from the market due to regulatory restrictions has left gaps in available control tools. These limitations have renewed scientific interest in plant-derived alternative pesticides that offer complex modes of action, potentially overcoming resistance while maintaining environmental safety. The development of botanical insecticides represents not just a return to traditional knowledge but an application of modern scientific approaches to harness nature's chemical diversity for sustainable vector control solutions (Souto *et al.*, 2021; Hillary *et al.*, 2024).

The Meliaceae family, particularly species such as *Azadirachta indica* A. Juss. and *Melia azedarach* L., has been extensively studied for its rich repertoire of bioactive compounds with insecticidal properties (Shilaluke and Moteetee, 2022). *M. azedarach*, commonly known as chinaberry or Persian lilac,

is a deciduous tree native to Asia but naturalized in tropical and subtropical regions worldwide. This species produces a diverse array of secondary metabolites, including limonoids, triterpenoids, and flavonoids, which have demonstrated growth-regulating, antifeedant, and toxic effects on various insect pests (Wang *et al.*, 2016; Tuan *et al.*, 2022). The tree's ecological adaptability and widespread distribution make it an attractive source for locally sustainable insecticide production in endemic regions.

While previous research has established the general insecticidal potential of *M. azedarach* extracts, critical knowledge gaps remain regarding the comparative efficacy of different plant parts against mosquito developmental stages (Coria *et al.*, 2008; Selvaraj and Mosses, 2011; Popescu *et al.*, 2024; Ahmed, 2025). The terpenoid profile varies significantly between fruits and leaves, potentially leading to differential bioactivity against target organisms (Khaldi *et al.*, 2022; Song *et al.*, 2022). Furthermore, comprehensive studies examining the effects across the entire immature life stages *viz.* eggs, larvae, and pupae, while simultaneously evaluating adult repellency are lacking. Such holistic assessments are crucial for determining the practical utility of plant extracts in integrated vector management programs.

This study was designed to systematically evaluate the mosquito control potential of *M. azedarach* terpenoids through multiple approaches: (1) comparative extraction and chemical characterization of fruit versus leaf terpenoid fractions, (2) quantitative assessment of concentration-dependent mortality effects on eggs, all larval instars (L1-L4), and pupae of *C. pipiens*; (3) determination of repellent activity of *M. azedarach* extracts against adult mosquitoes using a controlled behavioral assay; and (4) the identification of major bioactive constituents through advanced chromatographic techniques. The findings provide critical data for optimizing plant-based formulations and contribute to the development of environmentally sustainable alternatives to conventional insecticides for mosquito control. Moreover, this multidimensional approach, examining the effects across all developmental stages while characterizing the active constituents, provides a robust foundation for future development of *M. azedarach*-based vector control products for combating insecticide-resistant mosquitoes.

Materials and Methods

Plant material collection and preparation

Fresh fruits and leaves of *M. azedarach* were collected from the Babylon tourist resort, Iraq (GPS: 32.5°N; 44.4°E) in May 2022. Plant samples were authenticated by Dr. Nida Mohammed (University of Babylon) and were deposited in the departmental herbarium. Plant materials were washed with distilled water, shade-dried (at 25±2°C for 7 days), and ground to a fine powder (40 mesh size) using an electric mill (Harborne, 1987).

Mosquito colony establishment

Larvae of *C. pipiens* were collected from the swampy river habitats in Babylon, Iraq (32.5°N; 44.4°E), and were transferred to 1.8 L containers filled with source water. Poultry feed powder (2 g per container) was provided as a nutrient source. Containers were housed in screened rearing cages (50 × 50 × 100 cm) under controlled conditions (at 28 ± 2°C, 60 ± 2% relative humidity, and 12:12 h light: dark cycle). Water quality was monitored daily to prevent fermentation, and the rearing medium was replaced after larval development or adult emergence. Adult mosquitoes were maintained on a 10% sucrose solution supplemented with fresh fruit. Female mosquitoes were blood-fed using restrained pigeons (with chest and abdomen feathers removed) placed atop cages overnight, following ethical guidelines. Mosquito species identification was confirmed morphologically by Dr. Malih Turkish Al-Husseini (University of Kufa).

Terpenoid extraction

To evaluate the biological activity of the terpenoid extracts, the following procedure, as described by Nathan *et al.* (2006), was adopted: For each 1.0 g of dry extract, 2 mL of Tween 20 (0.1% v/v) was added as an emulsifier to enhance the solubility without affecting active compounds. The mixture was then brought to a final volume of 100 mL with distilled water to prepare a 1.0% (10 mg/mL) stock solution. Serial dilutions of the stock solution were made to obtain different test concentrations of 0.75% (7.5 mg/mL) and 0.50% (5 mg/mL). Control treatments were prepared by adding 2 mL of Tween 20 to 98 mL of distilled water (0.1% v/v).

Bioassay procedures

Egg mortality test

Fresh egg rafts (230-290 eggs each) were collected

within 1-4 hours of oviposition and placed in plastic containers with 50 mL of test solutions (10%, 7.5%, or 5% extract concentrations) or control (0 mg/mL). Three replicates were performed per concentration. Hatching inhibition was assessed after 48 hours, with mortality calculated using Abbott's formula (Abbott, 1925).

Larval mortality test

Twenty larvae per instar (L1-L4) were exposed to 50 mL of test solutions in plastic containers, with 0.1 g poultry feed added per replicate (n=3). Mortality was recorded after 24 hours and adjusted using Abbott's correction (Abdulla and Aljanabi, 2020).

Pupal mortality test

The larval test protocol was followed for pupae, excluding food supplementation. Mortality was assessed after 24 hours with Abbott-adjusted calculations (Abdulla and Aljanabi, 2020).

Repellency bioassay

A custom chemotropometer (100 × 20 × 20 cm wooden box with 120 × 3 cm plastic tube) was used. Cotton rolls treated with extract solutions (50 mL distilled water + 1 mL Tween 20 + test concentration) were placed at one end, with controls (Tween 20 only) at the opposite end. Twenty adult mosquitoes were released at the center, and their distribution was recorded after 20 minutes. The apparatus was cleaned between trials (Hussein *et al.*, 2017; Mohammed *et al.*, 2021). Attraction and repellency ratios were calculated as:

$$AR (\%) = \frac{\text{Number of mosquitoes within 25 cm of the treated end}}{\text{Total number of mosquitoes introduced}} \times 100$$

$$RR (\%) = \frac{\text{Number of mosquitoes within 25 cm of the control end}}{\text{Total number of mosquitoes introduced}} \times 100$$

$$\text{Equilibrium Ratio} = AR - RR$$

A positive equilibrium ratio indicates a net attractant effect, while a negative value indicates a net repellent effect.

Gas chromatography-mass spectrometry (GC-MS) analysis

Chemical characterization of the crude terpenoid extracts from *M. azedarach* fruits and leaves

was performed using gas chromatography-mass spectrometry (GC-MS). Dried samples (1 g each) were prepared in triplicate and coded before analysis. The analyses were conducted at the Central Laboratory of Razi University (Kermanshah, Iran). Separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness) with helium carrier gas (1.0 mL/min flow rate). Compound identification was performed by comparing mass spectra with the library and authentic standards (match threshold >85%), with relative abundances calculated from peak areas in total ion chromatograms.

Statistical analysis

The experimental design employed a completely randomized factorial arrangement to evaluate the treatment effects. Mortality data were adjusted for control group responses using Abbott's correction formula (Abbott, 1925) and were subjected to arcsine square-root transformation to meet the parametric assumptions. The transformed data were analyzed via two-way ANOVA (factors: concentration × plant part) using SPSS v19.0 (IBM Corp., Armonk, NY). The treatment means were compared using Fisher's Least Significant Difference (LSD) post-hoc test at a standard level of significance ($\alpha = 0.05$).

Results and Discussion

Ovicidal activity of *M. azedarach* extracts

The terpenoid extracts exhibited remarkable ovicidal properties, with fruit extracts showing slightly greater efficacy than leaf extracts, exhibiting 86.25% egg mortality at 10 mg/mL (1.0%) compared to 82.53% for leaf extracts (Table 1). The strong dose-response relationship in egg mortality suggests that terpenoids would interfere with critical developmental processes during embryogenesis through multiple potential mechanisms, including disruption of chitin synthase activity during chorion formation (Wink, 2015; Khaldi et al., 2022), inhibition of respiratory enzymes in developing embryos (Veni et al., 2017), and oxidative damage to embryonic membranes (Isman, 2020). While our results align with concentration-dependent ovicidal effects reported by Hussain et al. (2018a) for *Albizia lebbeck* extracts against *Culex quinquefasciatus*, the substantially higher mortality rates observed in our study (86.25% vs. 50.53% at comparable concentrations) demonstrate the superior efficacy of *M. azedarach* terpenoids as a potential ovicidal agent.

Table 1: Non-cumulative mortality of different immature stages of the common house mosquito *Culex pipiens* recorded at 24 h post-exposure to fruit and leaf terpenoid extracts of *Melia azedarach* under laboratory conditions.

Ex-tract	Concen-tration	Eggs	L1	L2	L3	L4	Pupa
Fruits	1%	86.25	93.33	88.33	78.33	48.33	16.67
	0.75%	79.35	81.67	76.67	66.67	38.33	11.67
	0.50%	69.13	58.33	53.33	38.33	28.33	0.00
	Control	4.33	0.0	0.0	0.0	0.0	0.0
Leaves	1%	82.53	88.33	83.33	73.33	43.33	13.33
	0.75%	75.76	76.67	71.67	56.67	36.67	8.33
	0.50%	65.04	56.67	51.67	36.67	25.00	0.00
	Control	4.89	0.0	0.0	0.0	0.0	0.0

Larvicidal efficacy of *M. azedarach* extracts

The terpenoid extracts demonstrated a significant larvicidal activity against *C. pipiens*, with pronounced concentration- and instar-dependent effects (Table 1). At the highest concentration tested (10 mg/mL), fruit extract induced 93.33% mortality in the first instar larvae (L1), while leaf extract showed slightly lower mortality (88.33%). Notably, susceptibility decreased progressively across larval stages, with fourth instar larvae (L4) exhibiting only 48.33 and 43.33% mortality for fruit and leaf extracts, respectively (Table 1). This pattern reflects the well-documented phenomenon of increased tolerance of xenobiotic compounds in later mosquito larval instars, likely due to an enhanced detoxification capability and thicker cuticular structures (Ochoa et al., 2018; Zhang et al., 2021). Statistical analysis revealed significant differences ($p < 0.05$) between all concentrations for both extract types, with fruit extract consistently outperforming leaf extract at 10 and 7.5 mg/mL concentrations. The observed stage-specific susceptibility aligns with previous findings for azadirachtin, where Alouani et al. (2013) reported LC_{50} values of 0.190 mg/L for first instar *C. pipiens* compared to 0.891 mg/L for second instars. A similar larval stage-specific response to botanical extracts is reported by Hassan et al. (2023). Mechanistic studies suggest that these effects may stem from terpenoid interference with midgut epithelial integrity, as demonstrated by Procópio et al. (2015), who observed cellular deformation and microvilli disruption in *Aedes aegypti* (L. in Hasselquist) larvae treated with *Schinus terebinthifolius* Raddi extracts. Our results compare favorably with other botanical larvicides,

including carvacrol ($LC_{50} = 5.5 \mu\text{g/mL}$ against *C. quinquefasciatus*; Ochoa *et al.*, 2018) and *Terminalia chebula* Retz. methanol extracts ($LC_{50} = 111.98 \text{ ppm}$; Veni *et al.*, 2017), suggesting *M. azedarach* terpenoids represent a potent alternative for mosquito control. The structure-activity relationships identified through molecular modeling further support the potential for rational design of enhanced terpenoid-based larvicides targeting the critical insect physiological processes (Ochoa *et al.*, 2018; De Almeida Teles *et al.*, 2024; Duarte *et al.*, 2024).

Table 2: Effect of different concentrations of melia azedarach fruit and leaf terpenoid extracts on the repellency and attraction behavior of the adults of common house mosquito culex pipiens under laboratory conditions.

Concentration	Repellency rate (%)		Attractiveness rate (%)	
	Fruit extract	Leaf extract	Fruit extract	Leaf extract
1.0%	58	57	0.00	0.00
0.75%	48	45	0.00	0.00
0.50%	38	32	2.00	2.00

Pupicidal potential of *M. azedarach* extracts

The pupal stage of *C. pipiens* exhibited lower susceptibility to *M. azedarach* terpenoid extracts compared to the egg or larval stages, with fruit and leaf extract inducing only 16.67 and 13.33% mortality, respectively, at the highest concentration (10 mg/mL). While no pupal mortality was recorded at 5 mg/mL or in controls ($p < 0.05$), reflecting the protective advantages of the pupal stage, including its thick, sclerotized cuticle and metabolic dormancy that limit terpenoid penetration (Rajatileka *et al.*, 2011; Chatterjee *et al.*, 2023). Despite this inherent resistance, the concentration-dependent mortality ($R^2 = 0.94$ for fruit extract, $R^2=0.91$ for leaf extract) and the observed sublethal effects on emergence timing and adult morphology suggest that bioactive compounds can still disrupt the critical metamorphic processes, potentially through inhibition of chitin remodeling or hormonal interference (Ochoa *et al.*, 2018; Ali and Aljanabi, 2020). The superior efficacy of fruit extracts mirrors patterns seen in larval bioassays and aligns with findings for other botanical insecticides, including azadirachtin ($LC_{50} = 0.42\text{--}1.24 \text{ mg/L}$; Alouani *et al.*, 2009) and Albizia extracts (50% mortality at 0.75 mg/mL; Hussain *et al.*, 2018), though pupal LC_{50} values remain higher than for

larvae, emphasizing the importance of integrated approaches targeting multiple life stages for effective vector control.

Table 3: Terpenoid composition of melia azedarach fruit extract determined by gc-ms analysis.

Peak number	Compound name	Retention time/min	Area%
1	Hexanoic acid	16.044	7.23
2	1,1'-Biphenyl, 4-methyl-	38.876	0.87
3	Phenol, 2,4-bis (1,1-dimethylethyl)	39.682	2.37
4	Cetene	42.808	0.50
5	aR-Turmerone	45.740	1.98
6	1-Pentadecene	46.621	0.55
7	1-Octadecene	50.250	0.48
8	1,4-Benzenedicarboxylic acid	51.753	5.81
9	9,12-Octadecadienoic acid (Z,Z)-	60.103	4.30
10	Octadecenoic acid, methyl ester	61.103	1.55
11	1-Heptadecanecarboxylic acid	62.343	3.64

Repellent and attractant effects of *M. azedarach* extracts on adult *C. pipiens*

The chemotropometer assays revealed a significant repellent activity of *M. azedarach* terpenoid extracts against adult *C. pipiens*, with fruit extract showing slightly higher efficacy (58% repellency at 10 mg/mL) than leaf extract (57%) (Table 2). Concentration-dependent responses were evident across all treatments (38–58% for fruit extracts, 32–57% for leaf extracts), with statistical analysis confirming significant differences between the concentrations ($p < 0.05$) but no significant variation among plant parts at higher concentrations (@ 7.5 and 10 mg/mL). These findings align with Naimi *et al.* (2022), who reported 48.2% repellency for *M. azedarach* powders against *Tribolium castaneum*, (Herbst) and demonstrate superior performance to other botanicals such as *Trigonella foenum-graecum* L. (34.67%). Notably, attractant effects were minimal ($\leq 3\%$) and statistically insignificant ($p > 0.05$) across all tested concentrations (Table 3), suggesting the *M. azedarach* extracts' repellent properties dominate their behavioral impact (Carpinella *et al.*, 2006). This dual-action profile compares favorably with established repellents - while DEET and MyggA offer longer protection (6–8 hours; Abiy *et al.* 2015), *M. azedarach*

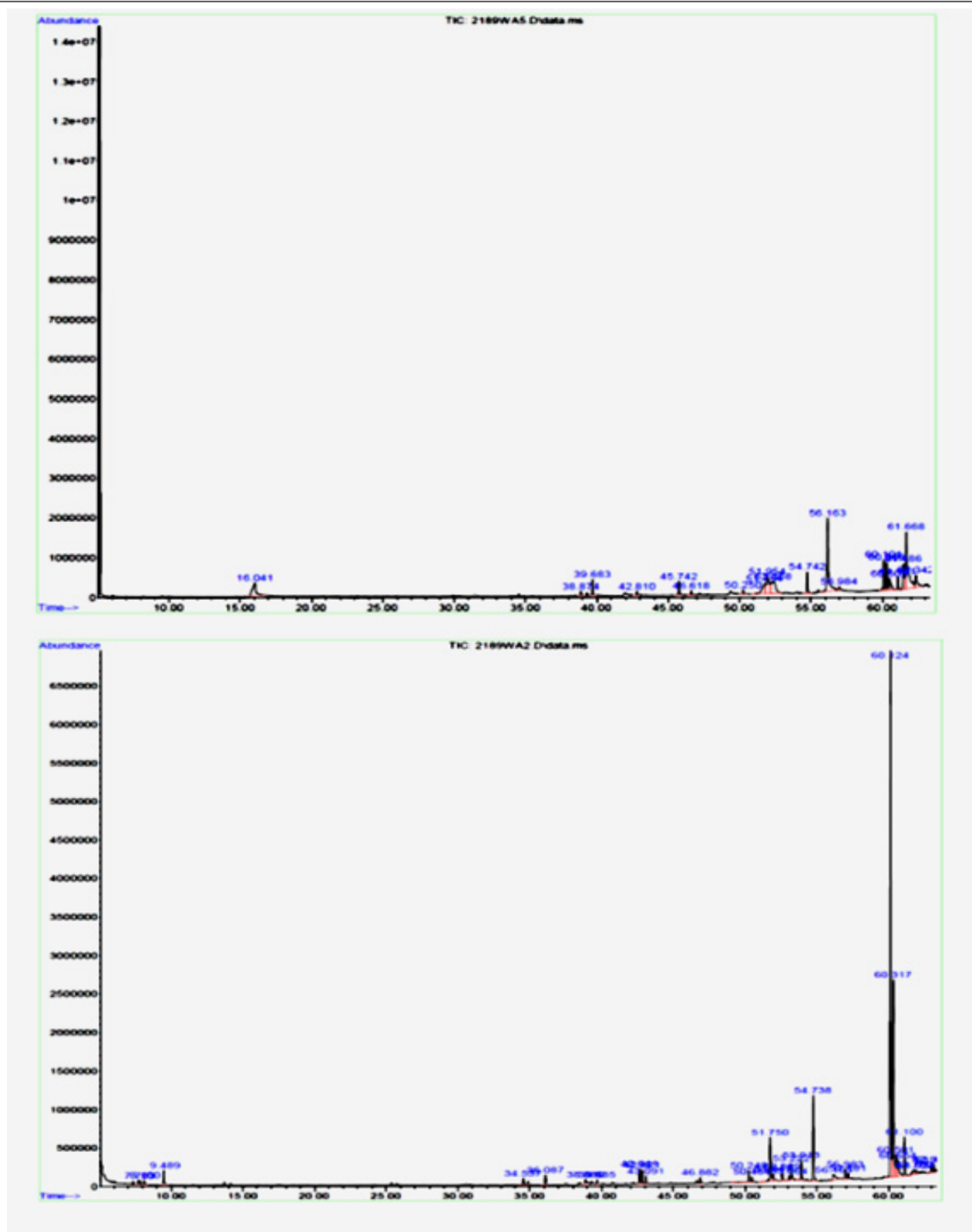


Figure 1: GC-MS chromatograph of terpenoid extract from *M. azedarach* leaves.

extracts show comparable efficacy to neem oil (70% repellency) and outperform citronellal (78% in vitro; Kim *et al.* 2005). The extracts' behavioral effects may stem from volatile terpenoids interfering with mosquito olfaction, as demonstrated by Nyasembe *et al.* (2012), who identified specific plant volatiles mediating *Anopheles* host-seeking. These results

support *M. azedarach* potential as an eco-friendly repellent (Carpinella *et al.*, 2006; Defagó *et al.*, 2009), though formulation improvements may be needed to match the duration of synthetic compounds while maintaining its advantage of negligible attractant properties that could compromise field efficacy.

Chemical characterization of *M. azedarach* extracts by GC-MS analysis

GC-MS analysis identified distinct phytochemical profiles in the fruit and leaf terpenoid extracts of *M. azedarach* (Figure 1), with fruit extracts containing 20 compounds dominated by hexanoic acid (7.23%), 1,4-benzenedicarboxylic acid (5.81%), and 9,12-octadecadienoic acid (Z,Z) (20.28%), while leaf extracts revealed 32 compounds including β -caryophyllene (14.2%), neophytadiene (3.70%), and phytol isomer (2.43%) as major constituents. The abundance of 9,12-octadecadienoic acid in fruits (20.28% of total terpenoids) and β -caryophyllene in leaves (14.2%) is particularly noteworthy, as these compounds have demonstrated insect growth-disrupting properties through interference with ecdysteroid activity (Morgan, 2009; Rasouli *et al.*, 2025) and larvicidal effects (Pavela, 2008), respectively. These chemical profiles align with previous findings by Jin *et al.* (2013) and Kharkwal *et al.* (2015), confirming the consistent presence of differential bioactive terpenoids and other phenolic compounds in *M. azedarach* fruits and leaves (M'rabet *et al.*, 2017; Lin *et al.*, 2022), while the observed variation in compound composition between plant parts (fruits vs. leaves) may explain their differential bioactivity against *C. pipiens* developmental stages, with fruit extracts generally showing superior efficacy that correlates with their higher content of known insecticidal compounds.

Table 4: Terpenoid composition of melia azedarach leaf extract determined by GC-MS analysis.

Peak number	Compound name	Retention time/min	Area %
1	3-Hexanone	7.705	0.16
2	3-Octanol	8.099	0.42
3	2-Pentanone, 4-hydroxy-4-methyl	9.488	1.05
4	1-Tetradecene	34.538	0.66
5	Caryophyllene	36.087	0.76
6	1,1'-Biphenyl, 4-methyl-	38.888	0.48
7	1H-Cycloprop[e]azulen-7-ol, deca...	42.643	1.18
8	1-Octadecene	50.250	1.28
9	Neophytadiene	51.747	3.70
10	Z-5-Nonadecene	53.062	0.37
11	3-Eicosene, (E)-	56.982	0.76
12	Phytol isomer	60.652	2.43
13	Methyl stearate	61.097	3.06

Conclusions and Recommendations

This study demonstrates that *M. azedarach* terpenoid extracts from its fruits and leaves exhibit a significant potential for controlling *C. pipiens* mosquitoes across multiple life stages. Fruit extracts showed a greater efficacy than leaf extracts, with 86.25% egg mortality, 93.33% first-instar larval mortality, and 58% adult repellency at the highest concentration (10 mg/mL). The pupal stage was more resistant, with only 16.67% mortality, likely due to its protective cuticle and metabolic dormancy. GC-MS analysis revealed distinct phytochemical profiles of both plant parts, with fruit extracts containing higher levels of bioactive compounds, including 9,12-octadecadienoic acid (20.28%), which may explain their stronger insecticidal effects. The extracts combined ovicidal, larvicidal, and repellent properties, without significant attractant effects, making them promising candidates for eco-friendly mosquito control. These findings further support the development of *M. azedarach* fruit-based formulations for targeted interventions against the immature stages of mosquitoes, potentially in combination with other vector control strategies. However, field trials are needed to evaluate their performance under natural conditions and to optimize the delivery systems for practical implications.

Acknowledgments

We are grateful to the Department of Plant Protection, Razi University, Iran, for supporting this project.

Novelty Statement

This *in vitro* study demonstrates the ovicidal, larvicidal, and repellency potential of crude terpenoid extracts of *M. azedarach* against *C. pipiens* mosquitoes. Moreover, fruit extracts exhibited a higher bioactivity than leaf extracts, and the variant profiles of the biochemical constituents of both types of extracts further corroborate this differential effect. Overall study findings advocate the development of *M. azedarach* fruit-based formulations combating immature stages of *C. pipiens* and other vector mosquitoes.

Author's Contribution

Wail Ismael Hassan and Abbas Ali Zamani: Conceived of the presented idea.

Yousif Dakheel Rashid: Developed the theory and performed the computations.

Yousif Dakheel Rashid and Arezoo Jamshidi: Verified the analytical methods. **Wail Ismael Hassan Arezoo Jamshidi and Abbas Ali Zamani:** Data collection, analysis, and writing

Generative AI or AI assisted technology statement

Authors declare that no generative AI and AI-assisted technologies were used for this manuscript.

Conflict of Interest

The authors have declared that no conflict of interest exists.

References

- Abbasi, E., M. Vahedi, M. Bagheri, S. Gholizadeh, H. Alipour and M.D. Moemenbellah-Fard. 2022. Monitoring of synthetic insecticides resistance and mechanisms among malaria vector mosquitoes in Iran: A systematic review. *Heliyon.*, 8(1): <https://doi.org/10.1016/j.heliyon.2022.e08830>
- Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18(2): 265-267. <https://doi.org/10.1093/jee/18.2.265a>
- Ali, A.N. and M.M. Aljanabi. 2020. Toxicity of aqueous and organic solvent extracts of *Melia azedarach* (L.) leaves in the larvae and pupae of *Culex pipiens*. *Biopest. Int.*, 16(1).
- Abiy, E., T. Gebre-Michael, M. Balkew and G. Medhin. 2015. Repellent efficacy of DEET, MyggA, neem (*Azadirachta indica*) oil and chinaberry (*Melia azedarach*) oil against *Anopheles arabiensis*, the principal malaria vector in Ethiopia. *Malar. J.*, 14: 1-6. <https://doi.org/10.1186/s12936-015-0705-4>
- Ahmed, S. 2025. Evaluation of concentration and time duration of *Parthenium hysterophorus* and *Melia azedarach* in field conditions for mosquito control. *ApexMed.*, 1(1): 35-45. <https://doi.org/10.63056/ACAD.001.05>
- Alouani, A., N. Rehim and N. Soltani. 2009. Larvicidal activity of a neem tree extract (azadirachtin) against mosquito larvae in the Republic of Algeria. 2(1): 15-22.
- Alouani, A., N. Rehim and N. Soltani. 2013. Bioefficacy of azadirachtin in controlling *Culex pipiens pipiens* (Diptera: Culicidae). *Jordan J. Biol. Sci.*, 6(3): 217-222. <https://doi.org/10.12816/0001536>
- Black, W.C. IV, T.K. Snell, K. Saavedra-Rodriguez, R.C. Kading and C.L. Campbell. 2021. From global to local new insights into features of pyrethroid detoxification in vector mosquitoes. *Insect.*, 12(4): 276. <https://doi.org/10.3390/insects12040276>
- Brugueras, S., B. Fernández-Martínez, J. Martínez-de la Puente, J. Figuerola, T.M. Porro, C. Rius, A. Larrauri and D. Gómez-Barroso. 2020. Environmental drivers, climate change and emergent diseases transmitted by mosquitoes and their vectors in southern Europe: A systematic review. *Environ. Res.*, 191: 110038. <https://doi.org/10.1016/J.ENVRES.2020.110038>
- Carpinella, M.C., M.T. Defagó, G. Valladares and S.M. Palacios. 2006. Role of *Melia azedarach* L. (Meliaceae) for the control of insects and acari: Present status and future prospects. *Adv. Phytomed. Adv.*, 3: 81-123. [https://doi.org/10.1016/S1572-557X\(06\)03005-4](https://doi.org/10.1016/S1572-557X(06)03005-4)
- Chatterjee, S., S. Bag, D. Biswal, D. Sarkar, R. Bandyopadhyay, B. Sarkar, A. Mandal and T. Kanti. 2023. Neem-based products as potential eco-friendly mosquito control agents over conventional eco-toxic chemical pesticides a review. *Acta Trop.*, 240: 106858. <https://doi.org/10.1016/j.actatropica.2023.106858>
- Coria, C., W. Almiron, G. Valladares, C. Carpinella, F. Ludueña, M. Defago and S. Palacios. 2008. Larvicide and oviposition deterrent effects of fruit and leaf extracts from *Melia azedarach* L. on *Aedes aegypti* (L.) (Diptera: Culicidae). *Bioresour. Technol.*, 99(8): 3066-3070. <https://doi.org/10.1016/j.biortech.2007.06.012>
- De Almeida Teles, A.C., B.O. Dos Santos, E.C. Santana, A.O. Durço, L.S.R. Conceicao, D. Roman-Campos and M.R.V. Dos Santos. 2024. Larvicidal activity of terpenes and their derivatives against *Aedes aegypti* – a systematic review and meta-analysis. *Environ. Sci. Pollut. Res.*, 1-16. <https://doi.org/10.1007/s11356-024-35479-w>
- Defagó, M.T., A. Mangeaud, V. Benesovsky, C. Trillo, C. Carpinella, S. Palacios and G. Valladares. 2009. *Melia azedarach* extracts – a potential tool for insect pest management. *Phytopharmacol. Ther. Values V. Studium Press LLC. Houston. Texas. USA:* 17-33.

- Duarte, J.L., S. Duchon, L.D. Di Filippo, M. Chorilli and V. Corbel. 2024. Larvicidal properties of terpenoid-based nanoemulsions against the dengue vector *Aedes aegypti* L. and their potential toxicity against non-target organism. PLoS One., 19(2): e0293124. <https://doi.org/10.1371/journal.pone.0293124>
- Fonseca, D.M., N. Keyghobadi, C.A. Malcolm, C. Mehmet, F. Schaffner, M. Mogi and R.C. Wilkerson. 2004. Emerging vectors in the *Culex pipiens* complex. Sci., 303(5663): 1535-1538. <https://doi.org/10.1126/science.1094247>
- Harborne, J.B. 1987. Phytochemical methods (guide to modern methods of analyzing plants). Transl. K. Padmawinata and I. Soediro. ITB., Bandung.
- Harborne, J.B. 1987. Metode fitokimia penuntun cara modern menganalisis tumbuhan. http://perpustakaan.stifar.ac.id/index.php?p=show_detail&id=448&keywords=harborne
- Hassan, S.A. and B.A.M. Al-Chalabi. 2023. Effects of puncture vine plant (*Tribulus terrestris* L.) extract on all larval stages of mosquito, *Culex pipiens* (Diptera: Culicidae). Sci. J. Univ. Zakho., 11(1): 50-53. <https://doi.org/10.25271/sjuoz.2023.11.1.981>
- Hillary, V.E., S.A. Ceasar and S. Ignacimuthu. 2024. Efficacy of plant products in controlling disease vector mosquitoes a review. Entomol. Exp. Appl., 172(3): 195-214. <https://doi.org/10.1111/eea.13401>
- Hussain, S.F., S.K. Jebur and F.S. Al-Zubaidi. 2018. The effects of extracted terpenoids of *Albizia lebbek* on the biological performance of mosquito, *Culex quinquefasciatus* (Diptera: Culicidae). https://hdl.handle.net/10520/ejc-ento_v29_n1_a26
- Hussein, H.S., M.Z.M. Salem and A.M. Soliman. 2017. Repellent, attractive and insecticidal effects of essential oils from *Schinus terebinthifolius* fruits and *Corymbia citriodora* leaves on two whitefly species, *Bemisia tabaci* and *Trialeurodes ricini*. Sci. Hortic., 216: 111-119. <https://doi.org/10.1016/j.scienta.2017.01.004>
- Isman, M.B. 2020. Botanical insecticides in the twenty-first century fulfilling their promise? Annu. Rev. Entomol., 65(1): 233-249. <https://doi.org/10.1146/annurev-ento-011019-025010>
- Jin, Q., C. Lee, J.W. Lee, M. Lee, M.K. Lee and B.Y. Hwang. 2013. A new apotirucallane-type triterpenoid from the fruit of *Melia azedarach*. Nat. Prod. Sci., 19(4): 342-343. <https://doi.org/10.5555/20143079593>
- Karunaratne, S.P., W.P.P. De Silva, T.C. Weeraratne and S.N. Surendran. 2018. Insecticide resistance in mosquitoes – development, mechanisms and monitoring. Ceylon J. Sci., 47(4). <https://doi.org/10.4038/cjs.v47i4.7547>
- Khaldi, R., N. Rehim, R. Kharoubi and N. Soltani. 2022. Phytochemical composition of almond oil from *Melia azedarach* L. and its larvicidal, ovicidal, repellent and enzyme activities in *Culex pipiens* L. 531-538. <https://doi.org/10.47665/tb.39.4.008>
- Kharkwal, G.C., C. Pande, G. Tewari, A. Panwar and V. Pande. 2015. Volatile terpenoid composition and antimicrobial activity of flowers of *Melia azedarach* Linn. from north west Himalayas, India. J. Ind. Chem. Soc., 92(January): 141-145.
- Kim, J., C. Kang, J. Lee, Y. Kim, H. Han and H.K. Yun. 2005. Evaluation of repellency effect of two natural aroma mosquito repellent compounds, citronella and citronellal. Entomol. Res., 35(2): 117-120. <https://doi.org/10.1111/j.1748-5967.2005.tb00146.x>
- Lange, S.K., C.R. Connelly, Z. Tai, N. Foley, J. De Leon Rivera, S. Lozano and R.J. Nett. 2025. A scoping review to determine if adverse human health effects are associated with use of pyrethroids for mosquito control. J. Med. Entomol., tjaf012. <https://doi.org/10.1093/jme/tjaf012>
- Ligsay, A., O. Telle and R. Paul. 2021. Challenges to mitigating the urban health burden of mosquito-borne diseases in the face of climate change. Int. J. Environ. Res. Pub. Health., 18(9): 5035. <https://doi.org/10.3390/ijerph18095035>
- Lin, M., X. Bi, L. Zhou and J. Huang. 2022. Insecticidal triterpenes in Meliaceae: plant species, molecules, and activities: Part II (*Cipadessa*, *Melia*). Int. J. Mol. Sci., 23(10): 5329. <https://doi.org/10.3390/ijms23105329>
- M'rabet, Y., N. Rokbeni, S. Cluzet, A. Boulila, T. Richard, S. Krisa and K. Hosni. 2017. Profiling of phenolic compounds and antioxidant activity of *Melia azedarach* L. leaves and fruits at two stages of maturity. Ind. Crop. Prod., 107: 232-243. <https://doi.org/10.1016/j.indcrop.2017.05.048>
- Madhav, M., K.R. Blasdel, B. Trewin, P.N.

- Paradkar and A.J. López-Denman. 2024. *Culex*-transmitted diseases: mechanisms, impact and future control strategies using *Wolbachia*. *Virus.*, 16(7): 1134. <https://doi.org/10.3390/v16071134>
- Mohammed, N.A.G., A.A.H. Hassan and M.O. Mohammed. 2021. The effect of pyrrolidinium bromide salt in the life of the southern cowpea beetle *Callosobruchus maculatus* (Fab) (Coleoptera: Chrysomelidae). *IOP Conf. Ser. Earth Environ. Sci.*, 910(1). <https://doi.org/10.1088/1755-1315/910/1/012140>
- Morgan, E.D. 2009. Azadirachtin, a scientific gold mine. *Bioorg. Med. Chem.*, 17(12): 4096-4105. <https://doi.org/10.1016/j.bmc.2008.11.081>
- Naimi, I., M. Zefzoufi, H. Bouamama and T.B. M'hamed. 2022. Chemical composition and repellent effects of powders and essential oils of *Artemisia absinthium*, *Melia azedarach*, *Trigonella foenum-graecum* and *Peganum harmala* on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Ind. Crops Prod.*, 182: 114817. <https://doi.org/10.1016/j.indcrop.2022.114817>
- Nathan, S.S., G. Savitha, D.K. George, A. Narmadha, L. Suganya and P.G. Chung. 2006. Efficacy of *Melia azedarach* L. extract on the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Bioresour. Technol.*, 97(11): 1316-1323. <https://doi.org/10.1016/j.biortech.2005.05.019>
- Nyasembe, V.O., P.E. Teal, W.R. Mukabana, J.H. Tumlinson and B. Torto. 2012. Behavioural response of the malaria vector *Anopheles gambiae* to host plant volatiles and synthetic blends. *Parasit. Vect.*, 5(1): 234. <https://doi.org/10.1186/1756-3305-5-234>
- Ochoa, S.A., J.C. Basurto, L.M.R. Valdez, L.E.S. Torres and B.N. Torres. 2018. In vitro and in silico studies of terpenes, terpenoids and related compounds with larvicidal and pupacidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Chem. Cent. J.*, 12: 1-21. <https://doi.org/10.1186/s13065-018-0425-2>
- Pavela, R. 2008. Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). *Parasitol. Res.*, 102: 555-559.
- Popescu, V.S., L. Zhang, G. Papa, C. Giuliani, G. Ribaud, G. Abate, D. Bulgari, E. Mac Sweeney, M. Pucci, M. Bottoni, F. Milani, D. Zizioli, I. Negri, A. Gianoncelli, E. Gobbi, D. Uberti, L. Lucini, M. Memo, G. Fico, G. Peron and A. Mastinu. 2024. Ecotoxicological evaluation of an aqueous phytoextract of *Melia azedarach* L. *Sci. Total Environ.*, 950: 175314. <https://doi.org/10.1016/j.scitotenv.2024.175314>
- Procópio, T.F., K.M. Fernandes, E.V. Pontual, R.M. Ximenes, A.R.C. De Oliveira, C.D.S. Souza, A.M.M. De Albuquerque Melo, D.M.D.A.F. Navarro, P.M.G. Paiva, G.F. Martins and T.H. Napoleão. 2015. *Schinus terebinthifolius* leaf extract causes midgut damage, interfering with survival and development of *Aedes aegypti* larvae. *PLoS ONE.*, 10(5): e0126612. <https://doi.org/10.1371/journal.pone.0126612>
- Rajatileka, S., J. Burhani and H. Ranson. 2011. Mosquito age and susceptibility to insecticides. *Trans.R.Soc.Trop.Med.Hyg.*, 105(5): 247-253. <https://doi.org/10.1016/j.trstmh.2011.01.009>
- Rasouli, F., H. Abbasipour and A. Rezazadeh. 2025. Phytochemical properties and insecticidal activity of hexane extract of *Melia azedarach* fruit against *Tuta absoluta* (Meyrick). *Arch. Phytopathol. Plant Prot.*, 1-14. <https://doi.org/10.1080/03235408.2025.2451996>
- Selvaraj, M. and M. Mosses. 2011. Efficacy of *Melia azedarach* on the larvae of three mosquito species *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Eur. Mosq. Bull.*, 29(1): 116-121.
- Shaikevich, E.V., E.B. Vinogradova, A. Bouattour and A.P.G. de Almeida. 2016. Genetic diversity of *Culex pipiens* mosquitoes in distinct populations from Europe: contribution of *Cx. quinquefasciatus* in Mediterranean populations. *Parasit. Vector.*, 9: 1-16. <https://doi.org/10.1186/s13071-016-1333-8>
- Shilaluke, K.C. and A.N. Moteetee. 2022. Insecticidal activities and GC-MS analysis of the selected family members of Meliaceae used traditionally as insecticides. *Plant.*, 11(22): 3046. <https://doi.org/10.3390/plants11223046>
- Song, M., G. Chan, L.G. Lin, D. Li, K. Zhang, X.Q. Zhang and Q.W. Zhang. 2022. Triterpenoids from the fruits of *Melia azedarach* L. and their cytotoxic activities. *Phytochem.*, 201: 113280. <https://doi.org/10.1016/j.phytochem.2022.113280>
- Souto, A.L., M. Sylvestre, E.D. Tölke, J.F. Tavares, J.M. Barbosa-Filho and G. Cebrián-Torrejón. 2021. Plant-derived pesticides as an

- alternative to pest management and sustainable agricultural production: prospects, applications and challenges. *Molecul.*, 26(16): 4835. <https://doi.org/10.3390/molecules26164835>
- Tanvir, M., M.A. Riaz, M.Z. Majeed, M.I. Zafar, M. Tariq and M.B. Tayyab. 2022. Comparative efficacy of selected biorational insecticides against larvae of southern house mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae). *Pak. J. Zool.*, 54(5): 2229.
- Tuan, N.N., P.C. Kuo, V.D. Hoang, V.T. Hien, L.D. Quang, T.T. Hieu and T.D. Thang. 2022. The limonoids and other constituents from the fruits of *Melia azedarach* and their biological activity. *Rec. Nat. Prod.*, 16: 307-315. <http://doi.org/10.25135/rnp.277.21.06.2097>
- Veni, T., T. Pushpanathan and J. Mohanraj. 2017. Larvicidal and ovicidal activity of *Terminalia chebula* Retz. (Family: Combretaceae) medicinal plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *J. Parasitol. Dis.*, 41(3): 693-702. <https://doi.org/10.1007/s12639-016-0869-z>
- Wang, Y., X. Chen, J. Wang, H. Xun, J. Sun and F. Tang. 2016. Comparative analysis of the terpenoid biosynthesis pathway in *Azadirachta indica* and *Melia azedarach* by RNA-seq. *Springerpl.*, 5(1): 819. <https://doi.org/10.1186/s40064-016-2460-6>
- Wink, M. 2015. Modes of action of herbal medicines and plant secondary metabolites. *Medicin.*, 2(3): 251-286. <https://doi.org/10.3390/medicines2030251>
- Zhang, C., Q. Shi, T. Li, P. Cheng, X. Guo, X. Song and M. Gong. 2021. Comparative proteomics reveals mechanisms that underlie insecticide resistance in *Culex pipiens pallens* Coquillett. *PLoS Negl. Trop. Dis.*, 15(3): e0009237. <https://doi.org/10.1371/journal.pntd.0009237>