Original Article

Chemical Composition and Control Potential of *Melia azedarach* Extracts Against *Culex pipiens*

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Abstract

Background: *Culex pipiens* (Diptera: Culicidae) poses a persistent global health challenge. The overuse of synthetic insecticides has led to resistance and environmental damage, underscoring the need for sustainable alternatives. *Melia azedarach* (Meliaceae) represents a promising source of bioactive compounds. This study aimed to comprehensively evaluate the potential of alkaloid extracts from *M. azedarach* against all life stages of *Cx. pipiens* and to characterize their phytochemical composition.

Methods: Crude alkaloid extracts were prepared from both plant parts. Ovicidal, larvicidal, pupicidal and adult repellent effects, were assessed through laboratory bioassays. The chemical profile of the extracts was determined using Gas Chromatography-Mass Spectrometry (GC-MS).

Results: Bioassays demonstrated significant ovicidal activity, with 100% egg mortality at 1% (w/v) concentration. Larvicidal activity was also notable at 1% (w/v). The fruit extract caused 100% mortality across the first three larval instars, while the leaf extract caused 100% mortality in the first two instars and 96.6% in the third. In the fourth instar, larvae showed 93.33 and 91.67% mortality with the fruit and leaf extracts, respectively. The extracts exhibited significant repellent effects, with rates of 63.00% and 60.00% at 1% (w/v) and consistently negative equilibrium ratios. GC-MS analysis showed the fruit extract was rich in insecticidal fatty acid esters, while the leaf extract contained repellent terpenoids like Piperitenone Oxide. Linoleic acid was a major shared compound, potentially underpinning the broad-stage efficacy.

Conclusion: These findings support the use of *M. azedarach* extracts as a promising, locally accessible and environmentally responsible strategy for integrated *Cx. pipiens* mosquito management.

Keywords: Common house mosquito; Meliaceae; Mosquito control; Larvicidal activity; Plant extracts

Introduction

Culex pipiens (Diptera: Culicidae), a mosquito species with almost universal distribution, is a recognized vector of medical and veterinary importance, implicated in the transmission of pathogens that cause diseases affecting both human and animal populations (1–3). This mosquito's adaptability to a wide array of environments, ranging from densely populated urban centers to agricultural landscapes, enhances its ability to thrive in proximity to human settlements, thereby increasing the risk of pathogen transmission (4). Within Iraq, *Cx. pipiens* is a commonly encountered species, frequently found in residential areas and breeding in diverse water sources, including canals, stagnant pools and even domestic containers (5). While *Cx. pipiens* presence has been documented in Iraq (5), further investigations are warranted to fully understand its specific role in the local transmission dynamics of vector-borne diseases and to assess its potential impact on public health, considering factors such as insecticide resistance and changing environmental conditions (6). Such studies are crucial given the complex genetic structure and potential presence of morphologically similar sibling species within the *Cx. pipiens* assemblage in the region, as highlighted by molecular studies in neighboring Iran (7). The increasing global connectivity and changing climate patterns may further influence the distribution and vector competence of this species, emphasizing the need for continuous monitoring and research efforts to mitigate potential health risks (1).

The difficulties in managing *Cx. pipiens* populations are compounded by several factors, beginning with the escalating problem of insecticide resistance (8, 9), a phenomenon that significantly diminishes the effectiveness of conventional chemical interventions. This resistance arises from various mechanisms, including target-site mutations and enhanced metabolic detoxification, allowing mosquitoes to survive exposure to insecticides that would previously have been lethal (10). Furthermore, the widespread and often indiscriminate use of synthetic insecticides not only accelerates the selection for resistant mosquito strains but also poses substantial risks to non-target organisms, disrupting delicate ecological balances and leading to detrimental environmental consequences such as biodiversity loss and contamination of water sources (11, 12). Adding to the complexity, *Cx. pipiens* exhibits remarkable adaptability in its breeding habits, utilizing a wide range of aquatic habitats from highly polluted urban drainage systems to seemingly innocuous containers holding stagnant water around homes (5). This plasticity makes source reduction efforts, a cornerstone of integrated vector management, exceptionally challenging to implement effectively across diverse landscapes, demanding innovative and context-specific strategies to target and eliminate breeding sites. As a result, there is a pressing need for the development and implementation of alternative, environmentally benign and sustainable control approaches, with a particular emphasis on exploring the potential of plant-derived larvicides and other novel interventions that can provide effective and ecologically responsible solutions for managing *Cx. pipiens* populations (13–15). The success of these strategies hinges on a thorough understanding of local mosquito populations, their resistance profiles and the ecological factors that influence their abundance and distribution.

The increasing global burden of mosquito-borne diseases and the environmental and public health risks associated with synthetic insecticides have intensified the search for eco-friendly alternatives derived from plant metabolites. Conventional chemical insecticides, while effective, face challenges such as insecticide resistance in vector populations like *Cx. pipiens* (8, 9) and unintended ecological consequences, including harm to non-target organisms and contamination of aquatic ecosystems (11, 12). In response, phytochemicals, biologically active plant-derived compounds, have re-emerged as promising candidates for vector control due to their biodegradability, target specificity and reduced propensity to induce resistance (13, 14). Recent studies highlight the potential of plant extracts in mosquito control. Ragavendran et al. (16) reported significant mosquitocidal effects of *Sigesbeckia orientalis* (Asteraceae) on *Anopheles stephensi*, *Cx. quinquefasciatus* and *Aedes aegypti*, accompanied by histopathological damage to mosquito larvae and minimal non-target effects. Plants in the family Meliaceae, such as *Azadirachta indica* (neem) and *Melia azedarach*, are particularly notable for producing limonoids and alkaloids with demonstrated mosquitocidal activity (15, 16). Alkaloids, a class of nitrogenous organic compounds, are increasingly recognized for their insecticidal properties, as they disrupt physiological processes such as neurotoxicity, growth regulation and metabolic inhibition in arthropods. In *M. azedarach*, alkaloids derived from its fruits and leaves have shown significant bioactivity against insect pests, including larvicidal, pupicidal and oviposition-deterrent effects, as observed in studies targeting *An. stephensi* (17). These compounds interfere with molting hormone biosynthesis and induce morphological abnormalities in immature stages, offering a multi-modal mechanism of action that reduces the likelihood of resistance development. For instance, neem-based formulations disrupt larval development and oviposition behavior in mosquitoes while posing minimal environmental risks (15). Similarly, extracts of *M. azedarach* exhibit larvicidal, pupicidal and repellent properties against *An. stephensi*, suggesting their untapped potential for controlling other medically important vectors like *Cx. pipiens* (17).

Although the insecticidal properties of *M. azedarach* against mosquitoes are recognized, most research has utilized total crude extracts or essential oils. A targeted investigation into its alkaloidal constituents, a class of compounds with pronounced neurotoxic and growth-disrupting effects in insects, against *Cx. pipiens* remains limited. Furthermore, existing efficacy data often center on larval stages, leaving a knowledge gap regarding impacts on other critical phases of the mosquito life cycle. The potential for geographic variation in susceptibility among mosquito populations also underscores the need for local evaluation. To address these points, the present study focused on: (i) the preparation and chemical profiling via GC-MS of crude alkaloid isolates from *M. azedarach* fruits and leaves; (ii) a comprehensive assessment of their toxicity to eggs, all larval instars, pupae and adults of an Iraqi *Cx. pipiens* population and (iii) an evaluation of their behavioral influence on adult mosquitoes. This integrated approach, combining detailed chemistry with a full-life-cycle bioassay on a locally relevant vector population, offers new insights for developing locally adapted, plant-based control strategies.

Materials and Methods

Plant material collection

Fresh leaves and fruits of *M. azedarach* were collected in late May 2022 from the Babylon tourist resort in Iraq, where the plant grows abundantly along roadsides. Botanical identification was confirmed by Professor Dr Nida Mohammed of the Faculty of Science, University of Babylon. The collected plant parts were thoroughly rinsed with distilled water to remove soil and debris, then air-dried in the shade at 25–28 °C for 10–15 days to preserve thermolabile compounds. Subsequently, the dried material was finely ground into a homogeneous powder using a sterile electric grinder.

Collection of insect larvae, colony breeding, and insect identification

Samples of *Cx. pipiens* larvae were collected from a river swamp in Babylon City. The larvae were transferred to 1.8-liter containers filled with river water and poultry feed powder was added at a rate of 2 g per container to provide nutrition. These containers were placed inside mosquito breeding cages measuring 50× 50× 100 cm. To maintain optimal breeding conditions, the water level and larval population density were monitored closely and precautions were taken against overfeeding and fermentation of the breeding medium. The breeding medium was changed at the end of each larval meal and after adult emergence. Fresh fruits were provided as a source of sugars for adult males and females, while blood meals were offered using pigeons, which were prepared by removing feathers from the chest and abdomen and restricting them above the cage to allow females to feed at night. The mosquito colonies were maintained under controlled conditions: a temperature of 28±2 °C, relative humidity of 60±2%, and a photoperiod of 12 hours light: 12 hours darkness. Insect specimens from the colony were identified by Dr Malih Turkish AL-Husseini at the University of Kufa, Iraq.

Preparation of crude alkaloid extracts from *Melia azedarach* fruits and leaves

To prepare crude alkaloid extracts from the fruits and leaves of *M. azedarach*, a method adapted from Harborne (18) was employed. Ten grams of dried fruit or leaf powder was extracted with 200 mL of ethyl alcohol using a Soxhlet extractor over 24 hours at a temperature of 45 °C. The extracted material was then concentrated in a rotary evaporator. The concentrated extract was dissolved in 5 ml of ethyl alcohol, and 30 ml of 2% sulfuric acid was added to the alcohol extract. The presence of alkaloids was confirmed by a positive Mayer's test, indicated by a cloudy precipitate. To isolate the alkaloids, the acidic solution was adjusted to a pH of 9 by adding sufficient 10% ammonium hydroxide. The stock solution was transferred to a separating funnel, where 10 ml of chloroform was added, and the mixture was shaken and allowed to settle. This process was repeated three times, with the chloroform layer (containing the soluble alkaloid compounds) being collected each time, resulting in a combined volume of approximately 40 ml. The final chloroform solution was dried in an oven at 40–45 °C. The dried alkaloid extract was stored in a sealed glass container in the refrigerator until use. This extraction process was repeated multiple times to obtain a sufficient quantity of alkaloid compounds for further analysis (18, 19).

Stock solution preparation

To evaluate the biological activity of the crude alkaloid extracts, a stock solution was prepared. One gram of the dried extract was initially mixed with 2 ml of Tween 20 to enhance solubility, ensuring uniform dispersion of the alkaloids in the aqueous medium without affecting their activity (20). This mixture was then diluted with distilled water to a final volume of 100 ml, resulting in a stock solution with a concentration of 1% (w/v) (17). From this stock, working concentrations of 0.75% and 0.50% were prepared via serial dilution with distilled water. The control treatment consisted of 2 ml of Tween 20 diluted in 98 ml of distilled water, matching the solvent concentration used in the treatment groups (17).

Bioassay for *Culex pipiens* mortality

The bioassay aimed to assess the impact of *M. azedarach* crude alkaloid extracts on the non-cumulative mortality of *Cx. pipiens* immature stages (eggs, larvae, pupae). Assays were performed in triplicate for each concentration and control.

Egg hatching inhibition test

Newly laid egg rafts (1–4 hours old), each containing 230–290 eggs, were collected from the breeding colony. Each raft was carefully transferred to a plastic container holding 50 mL of a test concentration (0.5, 0.75, or 1% w/v) or the control solution. After a 48-hour incubation period, the number of unhatched eggs was recorded to calculate the hatching inhibition rate. Egg mortality was then corrected using Abbott’s formula to account for any natural mortality observed in the control group (21).

Larval mortality bioassay

The larvicidal activity of *M. azedarach* extracts was evaluated against all_larval instars (1^{st} to 4^{th}). For each instar, twenty larvae were placed in individual plastic containers containing 50 mL of a test concentration or the control solution. To ensure adequate nutrition and prevent starvation-induced mortality, 0.1 g of finely ground larval food (a 1:1 mixture of yeast and fish food) was added to each container. Larval mortality was assessed after 24 hours of exposure. Three replicates were performed for each treatment and control group (21). Mortality rates were adjusted using Abbott’s formula to correct for natural mortality (21).

Pupal mortality bioassay

Ten pupae (newly formed within 24 hours) were transferred to plastic containers with 50 mL of the test concentrations or the control solution, following a similar protocol to the larval bioassay but without providing food (22). Mortality was recorded after 24 hours of exposure and data were corrected for control mortality using Abbott’s formula (21).

Chemotropism bioassay for adult mosquitoes

The attractant and repellent effects of the *M. azedarach* fruit and leaf alkaloid extracts on adult *Cx. pipiens* mosquitoes were evaluated using a chemotropism system modified from Hussein et al. (23). The apparatus consisted of a wooden box (100 cm L× 20 cm W× 20 cm H) housing a transparent plastic tube (120 cm long, 3 cm in diameter). A circular opening at the center of the tube facilitated the introduction of mosquitoes and was sealed with a removable plug afterward. A centimeter-graduated measuring tape was affixed along the tube's length to quantify mosquito movement. Both ends of the tube were sealed with cotton plugs to confine the mosquitoes and serve as surfaces for applying the test solutions.

For each treatment, one cotton plug (randomly selected per replicate) was saturated with 50 mL of a test solution (an effective concentration from the larval bioassays), while the plug at the opposite end received 50 mL of the control solution (distilled water with 2 % Tween 20). Twenty adult mosquitoes (3–5 days old, sugar-fed but starved for 4 hours before testing) were introduced into the center of the tube, after which the opening was immediately sealed. The mosquitoes were allowed to move freely within the tube for 20 minutes. The number of mosquitoes within a 25 cm zone of each end (treatment vs. control) was then recorded to determine the attractant and repellent effects. To align with the natural crepuscular activity of *Cx. pipiens*, all bioassays were conducted during dusk hours (18:00–20:00) (24).

The attractiveness ratio (AR) and repellency ratio (RR) were calculated as follows (25):

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

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

$$Equilibrium\ Ratio\ = AR - RR$$

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

To identify the chemical compounds present in the crude alkaloid extracts of *M. azedarach* fruits and leaves, gas chromatography-mass spectrometry (GC-MS) analysis was performed. A dried sample (1 g) of each extract type (fruit and leaf) was collected, labeled with a unique code. The samples were then sent to the Central Laboratory of Razi University, Kermanshah, for GC-MS analysis. The GC-MS analysis was conducted to determine the presence and identity of various chemical compounds within the alkaloid extracts, providing insights into the potential active components responsible for the observed biological activities.

Statistical analysis

To assess the impact of *M. azedarach* extracts on *Cx. pipiens*, the experiments followed a completely randomized design (CRD) within a factorial experimental framework. Mortality data were corrected for natural mortality using Abbott's formula (20). The Abbott's formula is as follows:

$$\%\ Corrected\ mortality = \frac{(Mortality\ in\ treatment - mortality\ in\ control}{100 - mortality\ in\ control} \times 100$$

To meet the assumptions of parametric testing, adjusted mortality percentages were arcsine transformed before analysis. Data were then subjected to analysis of variance (ANOVA), and significant differences between treatment means were determined using the Least Significant Difference (LSD) test at a significance level of $p \leq 0.05$. All statistical analyses were performed using SPSS 19 (IBM Corp., 2010).

Results

Eggs mortality

The impact of crude alkaloid extracts from *M. azedarach* fruits and leaves on *Cx. pipiens* egg mortality was significant (Table 1). The highest egg mortality rate (100%) was observed when eggs were treated with the highest concentration (1%) of both fruit and leaf extracts.

In contrast, the lowest mortality rates were recorded in the control groups, with only 0.053% and 0.048% mortality for the fruit and leaf extract controls, respectively. A clear dose-response relationship was evident, as increasing concentrations of both extracts led to higher egg mortality rates.

Statistical analysis revealed significant differences ($p< 0.05$) in egg mortality rates among the different concentrations of both fruit and leaf extracts. However, there were no significant differences between the fruit and leaf extracts at any given concentration, indicating that both extracts possess comparable ovicidal activity.

Larval mortality

The larvicidal activity of *M. azedarach* extracts against *Cx. pipiens* larvae was significant, with mortality rates varying depending on the extract concentration and larval instar (Table 1). The highest larval mortality was observed at the highest concentration (1%) for both fruit and leaf extracts, resulting in 100% mortality in the first two larval instars. The mortality of third instar larvae was 96.6% for the leaf extract, whereas it was 100% for the fruit extract. The fourth instar larvae exhibited lower mortality rates compared to the earlier instars, with 93.33% mortality observed for fruit extract and 91.67% for leaf extract at 1% concentration. At a concentration of 0.5%, the mortality rates for the fourth instar larvae were 51.67% and 45% for the fruit and leaf extracts, respectively, while no mortality was observed in the control group.

Statistical analysis indicated significant differences ($p\leq 0.05$) in mortality rates among the different concentrations of both fruit and leaf extracts. However, no significant differences were observed between the fruit and leaf extracts for the 4th larval stage at a concentration of 1%.

Pupal mortality

The *M. azedarach* extracts also exhibited pupicidal activity against *Cx. pipiens*, although the effect was less pronounced compared to the egg and larval stages (Table 1). The highest mortality rate in the pupal stage was observed at the highest concentration (1%), with mortality rates of 28.33 and 23.33% for the fruit and leaf extracts, respectively. The lowest mortality rates were observed at a concentration of 0.5%, with 11.67 and 6.67% mortality for the fruit and leaf extracts, respectively. No mortality was observed in the control group.

Attractant and repellent effects on adult mosquitoes

The crude alkaloid extracts from *M. azedarach* fruits and leaves exhibited a significant repellent effect on adult *Cx. pipiens* mosquitoes, as demonstrated using the chemotropometer (Table 2). The highest repellency rates were observed at a concentration of 1% (w/v), with 63.00 and 60.00% repulsion for fruit and leaf extracts, respectively. The lowest repellency rates were recorded at a concentration of 0.5% (w/v), with 45.00 and 43.00% repulsion for fruit and leaf extracts, respectively. Statistical analysis indicated significant differences ($p< 0.05$) between all concentrations for both fruit and leaf extracts. No significant differences were observed between fruit and leaf extracts at concentrations of 1% and 0.75% (w/v), nor at 0.5% (w/v). These results suggest that both fruit and leaf extracts are effective in repelling *Culex* mosquitoes and that the effect is concentration-dependent.

GC-MS analysis of *Melia azedarach* fruit and leaf alkaloid extracts

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted to identify the chemical compounds present in the alkaloid extracts of *M. azedarach* fruits and leaves. The analysis revealed the presence of 28 distinct chemical compounds in the fruit extract (Fig. 1), with 15 of these compounds appearing in a dominant form (Table 3). The three most abundant compounds identified in the fruit extract were Linoleic acid ethyl ester (62.121 min, 10.41%), (E)-9-Octadecenoic acid ethyl ester (62.315 min, 8.40%) and 9,12-Octadecadienoic acid (Z, Z)- (60.097 min, 5.04%). These compounds are known for their potential biological activities, including insecticidal and repellent properties.

In contrast, the leaf extract contained 16 chemical compounds (Fig. 2), with 11 of them appearing in a dominant form (Table 4). The most prominent compounds in the leaf extract were Cyclohexanone, 5-methyl-2-(1-met) (27.834 min, 13.75%), Piperitenone Oxide (33.430 min, 7.65%) and 2-Hexadecen-1-ol, 3,7,11,15-tetr (60.663 min, 7.87%).

Table 1. Non-cumulative mortality rates (mean% ± SE, n=3 biological replicates) of different life stages of *Culex pipiens* after 24-h exposure to various concentrations of *Melia azedarach* fruit and leaf alkaloid extracts under laboratory conditions (Babylon, Iraq, 2022)

Extract	Concentration	Eggs	L1	L2	L3	L4	Pupa
Fruits	1%	100±0.00^{ax*}	100±0.00^{ax}	100±0.00^{ax}	100±0.00^{ax}	93.33±1.73^{ax}	28.33±1.20^{ax}
	0.75%	82.25±0.45^{bx}	88.33±2.19^{bx}	83.33±1.76^{bx}	73.33±2.73^{bx}	58.33±3.28^{bx}	18.33±1.67^{bx}
	0.50%	75.49±0.41^{cx}	81.67±2.03^{bx}	78.33±4.41^{cx}	61.67±1.67^{cx}	51.67±2.40^{bx}	11.67±1.76^{bx}
	Control	0.05±0.01d^{x}	0.00±0.00c^{x}	0.00±0.00d^{x}	0.00±0.00d^{x}	0.00±0.00c^{x}	0.00±0.00c^{x}
Leaves	1%	100±0.00^{ax}	100±0.00^{ax}	100±0.00^{ax}	96.67±1.45^{ax}	91.67±2.03^{ax}	23.33±2.18^{ax}
	0.75%	80.45±0.44^{by}	83.33±2.73^{bx}	78.33±2.03^{bx}	68.33±1.20^{bx}	51.67±1.45^{by}	11.67±1.67^{by}
	0.50%	73.46±0.41^{cy}	78.33±1.67^{bx}	73.33±0.88^{bx}	61.67±1.76^{bx}	45.00±2.89^{bx}	6.67±1.45^{bx}
	Control	0.04±0.00^{dx}	0.00±0.00^{cx}	0.00±0.00^{cx}	0.00±0.00^{cx}	0.00±0.00^{cx}	0.00±0.00^{cx}

*Letters a, b, c, d in each column denote statistical comparisons (Tukey HSD after Anova; α=0.05) of mortality rates among different concentrations of the same extract (fruit or leaf). Letters x and y in each column represent statistical comparisons between fruit and leaf extracts at the same concentration using a t-test (α=0.05). Treatments with the same letter are not significantly different. For egg bioassay: one egg raft (230–290 eggs) per replicate, for larval bioassay: 20 larvae per instar per replicate and for pupal bioassay: ten pupae per replicate.

Table 2. Repellency and attraction responses of adult *Culex pipiens* to *Melia azedarach* fruit and leaf alkaloid extracts in a dual-choice olfactometer assay under laboratory conditions (Babylon, Iraq, 2022)

Concentration	Repellency rate (%)		Attractiveness rate (%)	
	Fruits	Leaves	Fruits	Leaves
1%	63.33±1.70^{ax*}	60.00±2.14^{ax}	0.00	0.00
0.75%	53.33±1.70^{bx}	51.67±1.87^{abx}	0.00	0.00
0.50%	45.00±2.24^{bx}	43.33±1.70^{bx}	1.67±1.70ax	3.33±1.44ax
Control	0.00	0.00	0.00	0.00

*Values are mean percentage ± standard error (SE) of three biological replicates (n=3), with 20 adult mosquitoes (3–5 days old) tested per replicate. Letters a, b, c, d in each column denote statistical comparisons (Tukey HSD after Anova; α=0.05) of repellency rates among different concentrations of the same extract (fruit or leaf). Letters x and y in each column represent statistical comparisons between fruit and leaf extracts at the same concentration using a t-test (α=0.05). Treatments with the same letter are not significantly different. The repellency rate measures the percentage of mosquitoes at the control end, using the control as the inherent reference.

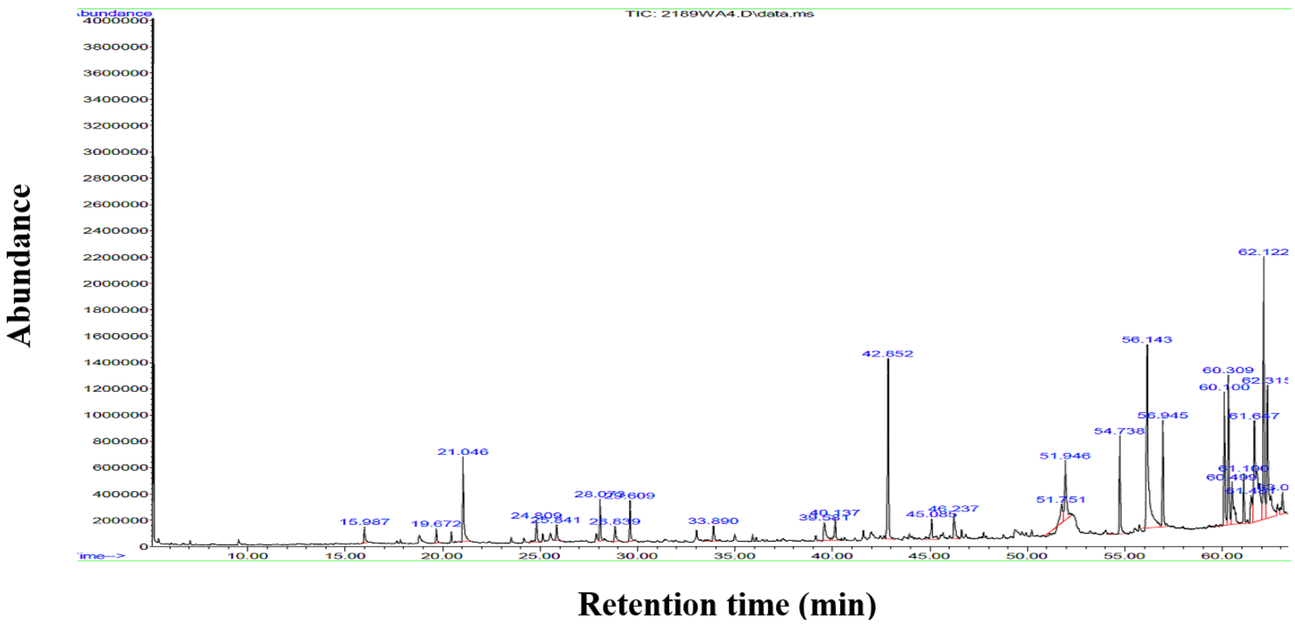

Fig. 1. GC-MS chromatograph of alkaloid extract from *Melia azedarach* fruits (analysis was performed at the Central Laboratory, Razi University, Kermanshah, Iran, 2022)

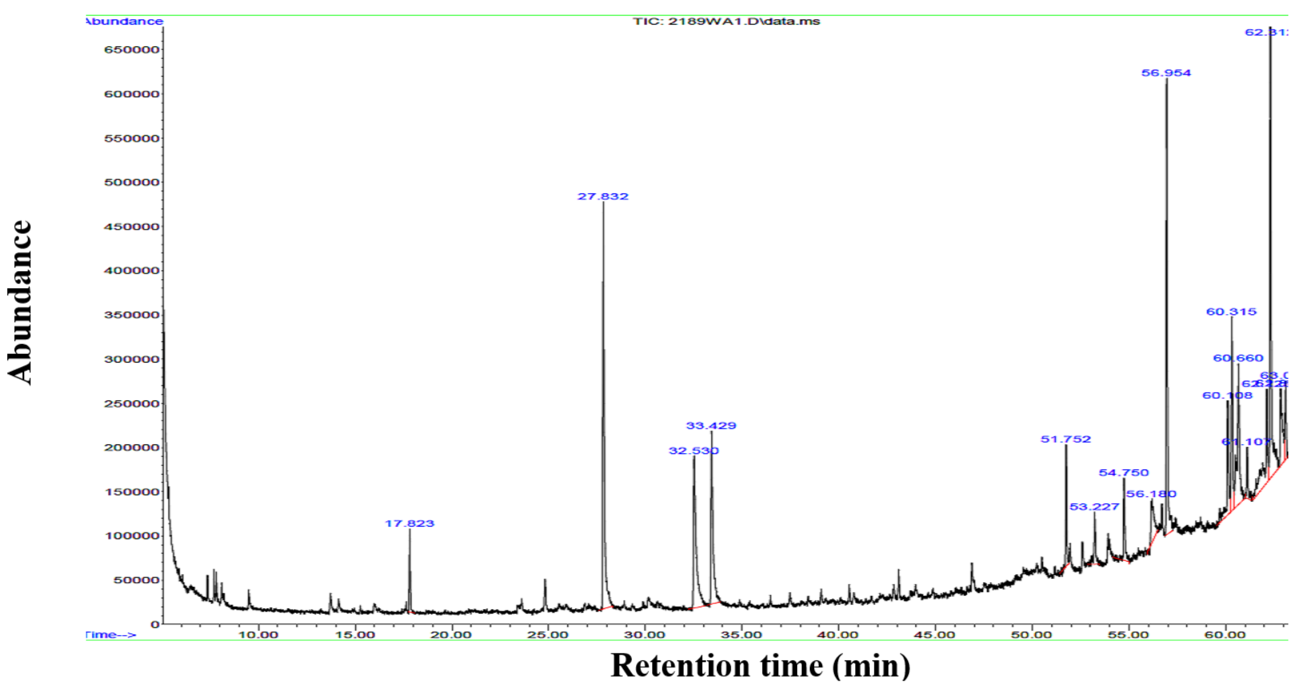

Fig. 2. GC-MS chromatograph of alkaloid extract from *Melia azedarach* leaves (analysis was performed at the Central Laboratory, Razi University, Kermanshah, Iran, 2022)

Table 3. Alkaloid composition of *Melia azedarach* fruit extract determined by GC-MS analysis (analysis was performed at the Central Laboratory, Razi University, Kermanshah, Iran, 2022)

Peak Number	Compound name	Retention time/min	Area%
1	Hexanoic acid, ethyl ester	15.987	0.69
2	Ethyl 2-(5-methyl-5-vinyltetrahy	19.673	0.57
3	Linalool L	21.045	3.70
4	Bicyclo[2.2.1]heptan-2-ol, 1,7,7	24.811	1.13
5	2-(4-methyl-3-cyclohexen-1-yl)-2	25.840	0.71
6	linalyl acetate	28.074	1.45
7	2,6-Octadien-1-ol, 3,7-dimethyl	33.893	0.91
8	Phenol, 4-ethyl-3-methyl	40.134	1.28
9	Bicyclo[4.4.0]dec-1-ene, 2-isopr	45.083	1.43
10	Bicyclo[7.2.0]undec-3-en-5-ol, 4	46.238	1.47
11	1,4-Benzenedicarboxylic acid, 1	51.753	0.56
12	2-Pentadecanone, 6,10,14-trimethyl	51.947	3.70
13	9,12-Octadecadienoic acid (Z,Z)-...	60.097	5.04
14	Linoleic acid ethyl ester	62.121	10.41
15	(E)-9-Octadecenoic acid ethyl ester	62.315	8.40

Table 4. Alkaloid composition of *Melia azedarach* leaves extract determined by GC-MS analysis (analysis was performed at the Central Laboratory, Razi University, Kermanshah, Iran, 2022)

Peak Number	Compound name	Retention time/min	Area %
1	Eucalyptol	17.821	1.73
2	Cyclohexanone, 5-methyl-2-(1-met	27.834	13.75
3	Piperitenone oxide	33.430	7.65
4	Neophytadiene	51.753	2.76
5	Hexadecanoic acid, methyl ester	54.748	2.61
6	9,12-Octadecadienoic acid (Z,Z)-...	60.109	4.28
7	2-Hexadecen-1-ol, 3,7,11,15-tetr	60.663	7.87
8	Heptadecanoic acid, 16-methyl	61.109	1.52
9	Ethyl (9Z,12Z)-9,12-octadecadien	62.126	5.90
10	Hexadecanamide	62.852	3.77
11	Octadecanoic acid, ethyl ester	63.098	2.40

Discussion

This study presents a comprehensive evaluation of the bioactivity and chemical composition of alkaloid extracts from *M. azedarach* fruits and leaves against *Cx. pipiens*, a prevalent mosquito vector in Iraq. Our results demonstrate that these locally sourced extracts exhibit significant and multifaceted insecticidal properties, affecting all developmental stages of the mosquito. Specifically, the extracts showed potent ovicidal and larvicidal activity, moderate pupicidal activity and a strong repellent effect on adults.

The complete inhibition of egg hatching at the highest concentration underscores the potent ovicidal activity of the *M. azedarach* alkaloid extracts. This effect may be attributed to the ability of alkaloid compounds present in the extracts to penetrate the eggshell, disrupt embryonic tissues and cause developmental abnormalities or death (26). This aligns with the findings of Veni et al. (27), who reported 100% egg mortality in *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* treated with methanolic extracts of *Terminalia chebula* Retz (Combretaceae). Similarly, Nathan et al. (17) found that methanolic extracts of *M. azeda-*

rach seeds and leaves significantly reduced egg hatchability in *An. stephensi*. These studies support the potential of *M. azedarach* extracts as a natural ovicidal agent for mosquito control.

The concentration-dependent larvicidal activity observed in this study reveals important differences in potency between the fruit and leaf alkaloid extracts. The fruit extract caused 100% mortality across the first three larval instars, while the leaf extract caused 100% mortality in the first two instars and 96.6% in the third at 1% (w/v). This suggests that while both extracts are effective larvicides, the leaf extract's efficacy may decrease more rapidly than the fruit extract's at lower concentrations. The results also showed that the effect of extract concentration was more influential on larval mortality rate than the effect of extract type.

These findings are consistent with other studies demonstrating the larvicidal potential of *M. azedarach* extracts. Ranchitha et al. (28) found that acetone leaf extracts of *M. azedarach* were effective against *Ae. aegypti* larvae, with the fourth instar larvae showing more resistance than the first, second and third instars. Preliminary phytochemical analysis of the acetone extract revealed the presence of various compounds, including alkaloids, phenols, saponins, flavonoids and tannins, which may contribute to the observed larvicidal activity. Similarly, Rudayni et al. (29) reported that ethanolic extracts of plants caused morphological abnormalities and mortality in *An. arabiensis* and *Cx. quinquefasciatus* larvae. Baz et al. (30) evaluated the larvicidal, ovicidal, and growth-inhibiting effects of extracts from five medicinal plants against *Cx. pipiens*. Their study showed significant bioactivity and identified diverse chemical compounds, supporting the potential of plant-based agents for mosquito control. Ghazawy et al. (31) investigated the insecticidal potential of *Asafetida* plant extract against *Cx. pipiens* and reported significant antioxidant, antimicrobial and odor-retardant properties. Their results showed substantial larval mortality with LC_{50} values indicating strong bioactivity, suggesting *Asafetida* as a promising plant-based agent for mosquito control.

The observed larvicidal effects may be attributed to various mechanisms of action, including the disruption of larval development, interference with feeding and induction of morphological abnormalities. Procópio et al. (32) found that *Schinus terebinthifolius* (Anacardiaceae) leaf extract caused intense disorder of the larval midgut epithelium in *Ae. aegypti* larvae, leading to cell deformation, hypertrophy and disruption of microvilli, which ultimately affected larval survival.

The findings of this study, along with those of others, suggest that *M. azedarach* extracts could be a valuable tool for controlling mosquito populations at the larval stage, providing an environmentally friendly alternative to synthetic insecticides.

The pupal stage of *Cx. pipiens* demonstrated notable resilience to the alkaloid extracts, showing significantly lower mortality compared to eggs and larvae across all tested concentrations. These results indicate that *Culex* pupae possess a higher tolerance to the effects of *M. azedarach* extracts compared to eggs and larvae, particularly at lower concentrations. The pupicidal effect was concentration-dependent, with higher concentrations leading to greater mortality.

Similar findings have been reported in other studies. Koomson (33) found that mortality in *Anopheles* pupae treated with *Alchornea cordifolia* (Euphorbiaceae) leaf extract was directly proportional to the extract concentration. Abdulla and Aljanabi (22) also reported pupicidal activity of *M. azedarach* extracts against *Cx. pipiens*, with higher concentrations of both cold and hot water extracts, resulting in increased pupal mortality.

The lower pupicidal activity compared to larvicidal and ovicidal effects may be due to the protective cuticle of the pupa and its reduced feeding activity. The results clearly show that eggs and the first three larval instars are more vulnerable, as the highest mortality rate (100%) was achieved at 1% (w/v) concentration. In contrast, pupae treated with the 0.5% (w/v) concentration had the lowest mortality rates of 11.67% and 6.67% for fruit and leaf extracts, respectively.

Beyond lethal effects on immature stages, the alkaloid extracts exhibited a pronounced behavioral effect on adult *Cx. pipiens*, functioning primarily as a spatial repellent. In our olfactometer assay, both fruit and leaf extracts significantly reduced mosquito orientation towards treated zones, with repellency rates reaching up to 63.33% and 60.00% at 1% (w/v) concentration, respectively. This repellent property is consistent with reports of *M. azedarach* efficacy against other insect pests. Naimi et al. (34) found that *M. azedarach* powder had a high repellency rate against *Tribolium castaneum* (Coleoptera: Tenebrionidae). Ngo et al. (35) concluded that organic mosquito coils made from Neem and Papaya leaves were more effective in repelling mosquitoes compared to inorganic coils. These findings support the potential of *M. azedarach* extracts as a natural repellent for mosquito control.

In contrast to the repellent effect, the *M. azedarach* extracts showed minimal attractant properties toward adult *Cx. pipiens* mosquitoes (Table 2). The highest attraction rates were observed at a concentration of 0.5% (w/v), with only 2.00% and 3.00% attraction for fruit and leaf extracts, respectively. No attraction was observed at concentrations of 1% and 0.75% (w/v). Statistical analysis showed slightly significant differences in the percentage of expulsion of adult *Cx. pipiens* mosquitoes between the concentrations of 0.75% and 0.5% (w/v) for the leaf extract, while no significant differences were observed among all concentrations of the fruit extract. Additionally, no significant differences were observed between fruit and leaf extracts at all concentrations.

The negative equilibrium ratios consistently obtained across all tested concentrations indicated a predominant repellent effect of *M. azedarach* extracts, resulting in a reduction in the tendency of *Cx. pipiens* mosquitoes to approach treated areas. These findings align with the observed repellency rates and further suggest that adult *Cx. pipiens* mosquitoes neither prefer nor are attracted to the crude alkaloid extract of the *M. azedarach* plant, even under conditions of confinement and at low concentrations.

The lack of attraction to *M. azedarach* extracts contrasts with the attraction of mosquitoes to certain plant hosts for sugar feeding, which is essential for their nutrition and survival. Nyasembe et al. (36) found that *An. gambiae* mosquitoes were attracted to certain plant species based on their sugar content and volatile compounds. The current results suggest that *M. azedarach* extracts do not contain compounds or sugars that attract *Cx. pipiens* mosquitoes.

The distinct chemical profiles revealed by GC-MS analysis provide a plausible chemical basis for the differential and multi-stage bioactivities observed for the fruit and leaf alkaloid extracts. These compounds may contribute to the observed biological activities of the leaf extract.

The identification of these compounds suggests that both fruit and leaf extracts of *M. azedarach* are complex mixtures of various chemical constituents. Similar findings have been reported in other studies, such as Shilaluke and Moteetee (37) and Kamali et al. (38), which have also identified a range of compounds in *M. azedarach* extracts. Omowanle et al. (39) noted that neem oil, derived from a related species (*A. indica*), contains limonoids like azadirachtin A, nimbin and salannin, among other active ingredients. These compounds are known for their insecticidal properties, suggesting that the compounds identified in this study may contribute to the observed biological activity of the *M. azedarach* extracts.

In this study, GC-MS analysis was employed to characterize the chemical composition of crude alkaloid extracts from *M. azedarach* fruits and leaves at a fixed extraction concentration. The chemical profiles obtained represent the relative abundance of compounds within these crude extracts. However, it should be noted that the relative proportions of individual compounds remain constant across different bioassay concentrations tested, which involve dilution of the whole extract rather than alteration of its chemical profile. Therefore, the dose-dependent mortality observed in the bioassays reflects the combined effects of the entire mixture of compounds present, rather than the influence of a single isolated component. While correlation analysis between compound concentration and mortality is a valuable approach, it requires quantitative measurements of individual compounds at varying concentrations, ideally through testing of purified compounds or fractionated extract components. Future investigations aiming to isolate and test individual bioactive constituents at multiple dosages will be essential to establish direct quantitative relationships between specific compounds and insecticidal effects. Meanwhile, the current compositional data, together with previously reported biological activities of the major identified compounds, provide useful insights into the potential contributors to the observed toxic effects.

This study represents several key advances in the search for plant-based mosquito control agents. The principal highlights of our work include: (1) the demonstration of significant multi-stage bioactivity of *M. azedarach* alkaloid extracts, exhibiting potent ovicidal, larvicidal, pupicidal and adult repellent effects against *Cx. pipiens*; (2) the revelation, via GC-MS analysis, of distinct chemical profiles between fruit and leaf extracts, with fatty acid esters dominating the fruits and cyclic ketones prevailing in the leaves, providing a chemical rationale for their observed bioactivities; and (3) the validation of a sustainable approach by utilizing locally abundant and readily available plant parts (fruits and leaves), enhancing the practical potential for developing an integrated and eco-friendly vector management strategy.

While this study provides valuable insights into the potential of *M. azedarach* extracts, some limitations should be acknowledged. First, the bioassays were conducted under controlled laboratory conditions, which may not fully reflect the efficacy of the extracts in complex field environments. Second, the chemical analysis identified the major compounds, but the specific modes of action and potential synergistic effects among these compounds remain to be elucidated. Third, the bioassay design, optimized for a broad life-stage screening, utilized three set concentrations. Consequently, it did not permit the determination of precise median lethal concentrations (LC_{50} and LC_{90}) via probit analysis, which requires a full concentration-gradient experiment. Finally, the potential effects of the extracts on non-target organisms were not evaluated, which is a crucial consideration for future environmental risk assessments before practical application.

Conclusion

This study provides compelling evidence for the multifaceted potential of *M. azedarach* fruit and leaf alkaloid extracts as environmentally responsible agents for *Cx. pipiens* mosquito control. The extracts demonstrated significant bioactivity across multiple life stages, from ovicidal and larvicidal effects to pupicidal activity and adult repellency. The chemical profiling of the extracts via GC-MS revealed a complex composition of bioactive compounds, which likely contribute to the observed effects. These findings support the further exploration and development of *M. azedarach*-based biopesticides as a sustainable and locally accessible approach for integrated mosquito management, reducing the reliance on synthetic insecticides and mitigating their associated environmental impacts. This study offers key advancements over previous research by demonstrating the multi-stage efficacy of *M. azedarach* extracts from egg to adult stages, linking these bioactivities to detailed comparative phytochemical profiles and highlighting the practical potential of using locally abundant plant parts for sustainable mosquito control.

In summary, based on our findings, it is recommended to conduct field trials of *M. azedarach* extracts to confirm laboratory results under natural conditions. Integration of these plant-based extracts into sustainable mosquito control programs may reduce the dependency on chemical insecticides. Future research should also explore long-term environmental impacts and assess the potential for resistance development.

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Ethical consideration

This laboratory study was conducted on an insect species (*Cx. pipiens*). This article does not contain any studies with animals performed by any of the authors. However, all efforts were made to minimize suffering and maintain the mosquitoes in optimal conditions throughout the study.

Conflict of Interest Statement

The authors declare there is no conflict of interest.

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