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RESEARCH ARTICLE

Phytochemical composition of almond oil from *Melia azedarach* L. and its larvicidal, ovicidal, repellent and enzyme activities in *Culex pipiens* L.

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ABSTRACT

Melia azedarach L. (Meliaceae) is a botanical species with focal point of global research for its biological properties. The *Melia azedarach* tree is distinguished by its rapid growth, its adaptation to different temperate zones, as well as its insecticidal properties. All this made us think of exploiting it in biological control against different stages of mosquitoes. To this end, we aim, through the present work, to evaluate the effectiveness of *Melia azedarach* extracts against *Culex pipiens* mosquito. More specifically, our study focuses on determining the chemical composition of *Melia* almond oil, as well as the larvicidal, ovicidal and repellent activities on *Culex pipiens* L. mosquito as well as the activities of acetylcholinesterase (AChE) and glutathione-S-transferase (GST). Almond oil was extracted by a Soxhlet and subjected to gas chromatography–mass spectrometry (GC/MS). The yield was found to be 35.17%. The chemical composition revealed the presence of various phytoconstituents. A total of 7 compounds were identified, the main ones being 9,11-Octadecadienoic acid, methyl ester, (E,E)- (79.32%), 9-octadecenoic acid (Z)-, methyl ester (13.24%), hexadecanoic acid and methyl ester (3.69%). The larvicidal bioassays were performed according to the protocol recommended by the World Health Organization with concentrations varying from 20 to 80 mg/L depending on the exposure time (24, 48 and 72 hours). The almond oil exhibited remarkable larvicidal activity against fourth instar larvae and the lethal concentrations were determined (LC_{25}= 23.70 mg/L, LC_{50}=35.49 mg/L, LC_{90}=79.61 mg/L). The results also showed that the oil caused an ovicidal activity with a significant effect on egg hatch. The recorded hatching percentages were respectively 88.79% and 72.40% for the LC_{25} and LC_{50}, and this compared to the control series. Moreover, this oil exhibited significant repellency against adult mosquitoes. Furthermore, the enzymatic measurements performed on LC_{50} and LC_{90} treated larvae revealed a neurotoxic activity and a stimulation of the detoxification system as evidenced, respectively, by an inhibition of AChE and induction in GST activity. Overall, our data proved that *Melia azedarach* almond oil could be considered as a potent biorational alternative to synthetic insecticides for mosquito control.

Keywords: *Culex pipiens*; GC/MS; *Melia azedarach;* insecticidal activity; enzyme activities.

INTRODUCTION

Plant products have traditionally been used by human communities in many parts of the world against disease vectors and harmful insect species. The photochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositional attractants. They have deterrent activities observed by many researchers (Thanigaivel *et al*., 2017; Hafsi *et al*., 2022). The interest in new botanical compounds for biological control is based on their bioefficiency, their biodegradability, their respect for the environment, their physiological activity (Baranitharan *et al*., 2020; Kharoubi *et al*., 2020; Zeghib *et al*., 2020; Rechek *et al*., 2021) and usually their low mammalian toxicity (El-Sabrout *et al*., 2020). They provide a safer option for controlling various types of insects (Sukumar *et al*., 1991). It was listed and discussed 344 medicinal plant species that only showed important mosquitocidal activity.

Arthropod-borne diseases are predominant in more than a hundred countries worldwide (Benelli *et al*., 2016). Among arthropod vectors, mosquitoes are medically important insects and are considered as major public health pests. They transmit many dreadful and very serious to diseases humans and other warm-blooded vertebrates (Rehimi *et al*., 2011; Sarma *et al*., 2017). Therefore, mosquitoes spread a wide number of life-threatening diseases, such as malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile virus infection, and Zika virus (Benelli & Mehlhorn, 2016; Thanigaivel, 2017).

Culex pipiens L.1758 (Diptera: Culicidae) is an important species of mosquito under Culicidae family. It is the most widespread type of mosquito in the world and it is also a carrier of many diseases. In addition, it has been reported as the most abundant mosquito in north-eastern Algeria (Rehimi & Soltani, 2002; Bouabida *et al*., 2012; Arroussi *et al*., 2021; Hafsi *et al*., 2021). This species is more

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common in open and closed urban biotopes were the organic pollution is important.

Currently, mosquito control programs face a serious problem due to the repeated and indiscriminate use of synthetic pesticides which has led to resurgences in mosquito populations and the development of resistance (Jaoko *et al*., 2020). Moreover, it has caused adverse effects on non-target organisms as well as disturbances in natural systems. Therefore, the search for alternative control measures other than chemical insecticides continues intensively around the world. It has also led to a renewed interest in natural products which are a rich source of bioactive chemicals, and which are also promising sources for pest and mosquito control (Ntalli & Caboni, 2014; Ayinde *et al*., 2020). From these points, this great interest has focused on plant extracts as repellents and anti-mosquito agents.

Melia azedarach L. (Meliaceae) has several common names such as Chinaberry and Persian lilac. It is indigenous in India, and it has been introduced in The Unites States, Brazil, Argentina and Africa, because of its considerable climatic tolerance. It has been cultivated as an ornamental plant for unrecorded number of years and has adapted well (Baranitharan *et al*., 2020; Jaoko *et al*., 2020) and has been used as a good source of traditional medications. To date, many scientists around the world have focused their research on *M. azedarach* for its promising properties of interest in agriculture and medicine. Also, *M. azedarach* exhibits a range of biological activities. Extracts of its fruits, seeds and leaves have shown many properties (Lau *et al*., 2021) including pesticidal activity (Al-Rubae, 2009). The effectiveness of such extracts has been previously demonstrated against insects (Shadrach *et al*., 2018), as well as their antifeedant effects found in many insects (Pavela & Benelli, 2016; Bharanitharan *et al*., 2018). However, the oil extracted from this tree parts display several bioactivities against a wide range of insects and other organisms.

Considerable interest has been focused on the extracts from different parts of *Melia azedarach* such as leaves, seeds and seed kernels or seed almonds. It is for this purpose that the present work was carried out. Our objective is to study the chemical composition and to identify the major phytochemicals of almond extracts of *M. azedarach* through gas chromatography-mass spectroscopy (GC-MS). We are also looking to evaluate its larvicidal, and ovicidal activities, its repellency effect on the *Cx. pipiens* mosquito adults and lastly enzyme activities effects.

MATERIALS AND METHODS

Plants collection

Ripe fruits of *M. azedarach* were collected during March 2019 from the region Safsaf (36°53'43.91" North 7°44'01.51" East) in the city of Annaba-Algeria. The plant identification was kindly done by the botanist Dr. Tarek Hamel, and the voucher specimen (N° 273) was deposited in the herbarium of the Department of Botany, Faculty of Science, University of Badji Mokhtar Annaba, Algeria. The obtained samples passed through the following steps: The harvested fruits were sorted and then immersed in water for 48 hours to separate the fleshy pulp from the seeds. They were air-dried in the shade for 15 days and finally stored in a dry and ventilated place. A quantity of the seeds was carefully peeled one by one to separate the kernels from the cockles. Finally, it was ground with an electric grinder and then sifted to get a fine powder that was kept in dark bottles.

Oil extraction

The almond oil was extracted from *M. azedarach* according to the Association of Official Analytical Chemists (AOAC, 1975) method. A quantity of 30 g of powder is inserted into a cartridge and placed in a Soxhlet apparatus. A 500 ml flask is filled with 450 ml of hexane and placed in a suitable flask heater and connected to a cooling system. The solvent was removed using a rotary evaporator at a temperature of 58°C.

Mosquito rearing

The various instars of *Cx. pipiens* were obtained from a laboratory colony maintained in the Animal Biology Laboratory. The mosquito larvae were reared under laboratory conditions in plastic cups containing dechlorinated tap water until emergence. The larvae and adults were kept at temperature of 25±2°C, 70±5% relative humidity, and 14:10 light: dark photoperiod cycle (Rehimi & Soltani, 1999). Larvae were fed with fish food (Tetramine^{TM}). As soon as the pupae were transformed, they were placed in cages (20 x 20 x 20 cm^3) until emergence. The male mosquitoes were fed with the sugary solution of the dates suspended in the breeding cages. The taken quantity is according to the needs of each individual. On the other hand, after 3 days, the females began to take the blood meal of a pigeon and the produced eggs rafts were used for stock-rearing next generations. The newly exuviated fourth instar larvae are used in bioassays. The rearing water has been changed every four days.

Gas chromatography-mass spectrometry analysis

The fatty acid content was determined by gas chromatographic analysis of fatty acid methyl esters according to AFNOR (1998) standard T60-233. The obtained oils were subjected to the effect of 2N methanolic KOH solution. To 1g of oil, 10 mL of heptane and 0.5 mL of 2N methanolic KOH were added, after stirring for 20 seconds. It was left to stand for 24 h until the upper phase of the solution became clear. This fraction contains the ready fatty acid methyl esters (FAME) after dilution for injection to the GC-MS.

Chemical composition of *M. azedarach* almond oil was determined with gas chromatography–mass spectrometry (GC–MS), using Quadrupole mass spectrometer (Hewlett Packard Agilent 5973), operated at 70 eV, coupled directly to a Hewlett Packard Agilent 6890 plus gas chromatograph.

The chromatograph was equipped with a splitless injector (0.1 µl sample injected, carrier gas helium N 6.0, column flow 1 ml/min) and an HP-5MS capillary column (5% phenyl 95% dimethylpolysiloxane), 30 m – 0.25 mm, 0.25 µm film thickness). The injector temperature is 250°C and the oven temperature profile is as follows: 70°C for 5 min, 10°C/min to 130°C, isothermal for 2 min, 3°C/min to 220°C, isothermal for 4 min, 10°C/min to 280°C, isothermal for 15 min.

Larvicidal activity

The larvicidal activity of *M. azedarach* almond oil has been evaluated according to the bioassay method recommended by the WHO (2005). A stock solution of the *M. azedarach* almond oil at 10% was prepared with ethanol. *Melia*'s almond oil was serially diluted in ethanol in order to perform the preliminary tests and select suitable concentrations for the various tests. Several primary tests were performed to determine the oil toxicity and obtain the desired concentrations in order to perform the toxicological tests. Subsequently, we have chosen a series of concentrations varying between 20 mg/L and 80 mg/L.

The test for each concentration was carried out using 25 newly exuviated fourth instar larvae of *Cx. pipiens* for treated and control series, the positive controls were exposed to ethanol (1 ml of ethanol) while the negative controls were exposed to dechlorinated water only. The larvae were placed in 100 ml containers of water. Four replicates were carried out for each concentration and control series. After treatment, the addition of food and water change was performed after 24 hours of exposure as well as recording of the observed mortality. The tests were performed under laboratory conditions.

The observed mortality percentages for the different series were determined and then corrected by the Abbott's formula (1925). No mortality was detected on negative and positive control tests.

Ovicidal activity

The ovicidal activity of *M. azeadrach* almond oil was tested with two sublethal and lethal concentrations determined by the larvicidal activity (LC_{25} = 23.70 mg/L, LC_{50} =35.49 mg/L). Both concentrations (LC_{25} and LC_{50}) were chosen to obtain the ovicidal effect. Prior to treatment, freshly laid eggs of *Cx. pipiens* were counted individually using a binocular magnifying glass. The eggs were then placed in 100 ml jars of water treated with both concentrations. Another group of eggs was incubated in dechlorinated water and served as a control group. Each test was conducted in five replicates. Newly hatched first instar larvae were counted under a binocular magnifying glass. Experiments were performed under laboratory conditions; the hatching rate was recorded after 24 h of treatment.

The percentage of hatching of eggs laid by females in the treated and control batches was calculated by the following formula:

$$\text{Percentage of hatching} = \frac{\text{Total number of larvae}}{\text{Total number of eggs}} \times 100$$

Repellent bioassays

The repellency assay was performed according to the methodology of Barnard *et al*. (2007). The used test method was that of the wire cage. In each cage, there were 25 females fasted for 72 hours, and the treatment consisted of a solution including different concentrations (23.70 mg/L, 35.49 mg/L and 79.61 mg/L) diluted in acetone. This solution was applied to an area of 25cm^2 of a volunteer forearm introduced into the cage for 30min. A second cage of mosquitoes was used as a control cage. Five repetitions were performed recording the number of bites. The repellency (*R*) was calculated using the following formula of Schreck (1977):

$$R\ (\%) = (C - T/C) \times 100\%$$

C: the number of mosquito bites on the control arm.
T: the number of bites in the treated arm.

Enzyme activities

This extract was applied to fourth-instar larvae at its lethal concentrations (LC_{50} and LC_{90}) and its effects on AChE and GST activities measured at different time points (0, 24, 48 and 72 h) after treatment were examined. In brief, the assay of Glutathione-S-Transferases (GSTs) was performed following the procedure of Habig *et al*. (1974), using 1-chloro-2,4- dinitrobenzene (CDNB) as an artificial substrate in the presence of a cofactor glutathione (GSH). After treatment the larvae were pooled in 1 ml phosphate buffered saline (0.1 M; pH= 6.5) in 4 replicates of 15 individuals. After centrifugation at 14,000 rpm for 30 minutes, the supernatant was used as the enzyme source.

The determination of acetylcholinesterase (AChE) was performed according to Ellman *et al*. (1961) method. Pooled fourth instar larvae (15 individuals per replicate, 4 replicates per series) were homogenized in this solution containing: 38.03 mg ethylene glycol tetraacetic acid (EGTA), 1 ml Triton X-100, 5.845 g NaCl and 80 ml Tris-buffer (10 mM, pH=7). After centrifugation (5000 rpm for 5 minutes), the assay for AChE activity on a 100 µl aliquot to which 100 µl DTNB was added (39.6 mg DTNB, 15 mg CO_3HNa (sodium bicarbonate), 10 mL Tris buffer (0.1 M, pH=7) as well as 1 mL Tris buffer (0.1 M, pH = 7). 100 µl acetylthiocholine substrate after 3-5 minutes (23.6 mg ASCh, 1 mL distilled water) was added. Measuring the absorbance at 412 nm for 20 min, each assay was performed in triplicate and results were expressed as mM/min/mg protein. The protein content was assessed according to Bradford (1976) using bovine serum albumin as a standard (BSA, Sigma).

Statistical analysis

Larval mortalities were initially corrected by Formula of Abbott (1925) as follows:

$$\text{Corrected mortality (\%)} = \frac{\text{100–\% Mortality of control larvae}}{\text{\% Mortality of treated larvae–\% Martality of control larvae}} \times 100$$

The obtained results were statistically analyzed using a probit analysis (Finney, 1971) in order to determine the lethal concentrations and their 95% fiducial limits. The results were presented by the mean followed by the standard deviation (mean ± SD) for each test group.

The Prism software version 9.0.0 (Graph Pad Software Inc) was used to perform the analysis of variance (one-way and two-way ANOVA) on all experimental data. However, Tukey's test was used to test the difference between means such that P value that is less than 0.05 ($P<0.05$) was considered significantly different. The same statistical analysis procedure was used to calculate the correlation coefficient (R^2).

RESULTS

Chemical composition

The almond oil extracted from *M. azedarach* was analyzed by GC/MS. The oil content is 10.55 g from 30 g of *M. azedarach* almond powder, and the percentage of almond oil yield was 35.17%.

The GC-MS analysis of the studied sample has led to the determination of 7 compounds, representing 99.97% of the total content (Table 1). The Identification of constituents was done by comparing their mass spectral fragmentation patterns with those stored in the MS database (NIST, 2014). The vegetable oil profile is characterized by a high amount of 9,11-Octadecadienoic acid, methyl ester, (E,E)- (79.32%), followed by 9-Octadecenoic acid (Z)-, methyl ester (13.24%), Hexadecanoic acid, methyl ester (3.69%) as well as other compounds that were identified as minor components like Octadecanoic acid, methyl ester, 11-Eicosenoic acid, methyl ester, Eicosanoic acid, methyl ester, Docosanoic acid and methyl ester (3.45%).

Larvicidal activity

Toxicity tests are applied on newly exuviated fourth instar larvae (L_4) of *Cx. pipiens* with different concentrations of *M. azedarach* almond oil (20 to 80 mg/L). The percentage of mortality is recorded after 24, 48 and 72 hours of treatment. The obtained results are mentioned in Table 2. The results indicated that the larval mortality ranged from 18.99 to 76.83% after 24 hours of treatment, from 25.57 to 81.70% after 48 hours and from 28.41 to 84.94% after 72 hours, at concentrations ranging from 20 mg/L to 80 mg/L, respectively.

Table 1. Chemical composition of *Melia azedarach* almond oil with retention time (RT), and percentage concentration of constituents

N°	Retention time (RT)	Component name	Percentage (%)
1	34.70	Hexadecanoic acid, methyl ester	3.96
2	40.26	9,11-Octadecadienoic acid, methyl ester, (E,E)-	79.32
3	40.36	9-Octadecenoic acid (Z)-, methyl ester	13.24
4	40.91	Octadecanoic acid, methyl ester	2.92
5	46.20	11-Eicosenoic acid, methyl ester	0.33
6	47.18	Eicosanoic acid, methyl ester	0.16
7	51.59	Docosanoic acid, methyl ester	0.04
			Total: 99.97

Table 2. Larvicidal activity of *Melia azedarach* almond oil against the early fourth instar larvae of *Culex pipiens* after different exposure times (mean ± SD, n = 4 replicates each containing 25 larvae)

Time (hour)	Concentrations (mg/L)						
	20	30	40	50	60	70	80
24	18.99±2.2^{aA}	32.78±1.44^{bA}	43.08±1.32^{cA}	51.57±2.70^{dA}	57.25±2.9^{dA}	71.01±2.2^{eA}	76.83±2.8^{fA}
48	25.57±1.7^{aB}	36.06±1.39^{bA}	45.38±2.65^{cAB}	53.95±2.75^{dB}	59.01±4.0^{dA}	73.92±4.3^{eA}	81.70±5.6^{fA}
72	28.41±1.5^{aC}	38.44±1.36^{bA}	47.35±1.90^{cB}	55.55±0.00^{dB}	60.71±2.6^{dA}	76.83±2.8^{eA}	84.94±5.6^{fA}

For the same exposure time, means followed by different lowercase letter (a, b, c, d, e, f) are significantly different (p<0.05), whereas for the same concentration means followed by different capital letter (A, B, C) are significantly different (p<0.05).

Table 3. Toxicity of *Melia azedarach* almond oil applied on the fourth instar larvae of *Culex pipiens*: Determination of sublethal and lethal concentrations as well as their confidence intervals (95%)

Time (Hours)	LC_{25} (mg/L) 95% FL	LC_{50}(mg/L) 95% FL	LC_{90} (mg/L) 95% FL	Slope	R^2	Regression Curve
24	29.15 (24.83-33.30)	41.35 (37.70-44.99)	83.19 (70.97-101.50)	3.14	0.98	y = 14.44x+0.94
48	25.88 (20.24-31.21)	37.98 (33.16-42.70)	81.77 (66.25-108.50)	2.86	0.98	y = 13.33x+8.37
72	23.70 (17.91-29.10)	35.49 (30.46-40.38)	79.61 (63.55-108.00)	2.72	0.98	y = 12.81x+13.32

Table 4. Effect of *Melia azedarach* almond oil on egg hatch (%) against *Culex pipiens* (mean ± SD, n = 5)

Ovicidal activity	Control	LC_{25}	LC_{50}
Number of laid eggs	81.60±5.94^{a}	62.00±12.78^{b}	59,00±9.05^{b}
Number of hatched eggs	81.40±6.02^{a}	51.60±14.97^{b}	42.40±7.16^{b}
Egg hatchability (%)	98.65±1.38^{a}	82.33±10.50^{b}	71.81±3.70^{b}

For the same biological parameter, different letters (a, b) indicate a significant difference between treated series (p<0.05).

Table 5. The average percentage of the *Melia azedarach* almond oil repulsion against *Culex pipiens* mosquitos (mean ± SD, n = 5 replicates of 25 individuals each)

Percent (%) of repellency in time interval (min)	Concentrations (mg/L)		
	LC_{25}	LC_{50}	LC_{90}
5	79.04±7.19^{aA}	95.23±8.25^{aB}	100±0.00^{aB}
10	97.77±9.85^{bA}	100±0.00^{aA}	100±0.00^{aA}
20	96.80±2.97^{bA}	100±0.00^{aA}	100±0.00^{aA}
30	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}

For the same exposure time, means followed by different lowercase letters (a, b) are significantly different (p<0.05), whereas for the same concentration means followed by different capital letters (A,B) are significantly different (p<0.05).

Table 3 shows the values of the lethal concentrations LC_{50}, LC_{90} and the sublethal concentration LC_{25}. The results clearly showed that this oil has excellent larvicidal efficacy against *Cx. pipiens* larvae after 72 hours of exposure, with LC_{25}, LC_{50}, and LC_{90} values of 23.70 mg/L, 35.49 mg/L, and 79.61 mg/L, respectively.

Ovicidal activity

Table 4 summarizes the results obtained for ovicidal activity. These results showed that the treatment with *M. azedarach* almond oil on eggs caused a significant reduction in egg hatching with a concentration-response relationship, with a hatching percentage of 98.65% for the control series, 82.33% and 71.81% for the LC_{25} and LC_{50} concentrations, respectively. One-way ANOVA results showed a significant treatment effect ($F_{(2.12)}$ = 108.4; p<0.0001).

Repellency activity

The repellent activity of *M. azaderach* almond extract tested against adult females of *Cx. pipiens* showed a significant difference among the different concentrations (23.70, 35.49 and 79.61 mg/L) at different time intervals.

The result in Table 5 shows that the repellency effect of *Melia* oil was proportional to the exposure period (p < 0.05). The lowest repellency of adult females was obtained by applying the lowest concentration of *Melia* oil (23.70 mg/L). After only 5 min of exposure, the recorded mean was 79.04±7.19%, while the highest concentration (79.61 mg/L) caused a total repulsion (100%).

According to our experience results, the high concentration that was tested (79.61 mg/L) caused a 100% repellent effect from the first 5 minutes of the test, and the statistical analysis of the obtained data revealed a highly significant effect (p < 0.0001). So, almond oil from *M. azedarach* has been shown to be effective as one of the rapid repellants against *Cx. pipiens* females.

Enzyme activities

M. azedarach almond oil caused a significant induction on the specific activity of GST. Also, the comparison between the control series and the treated series after 24, 48 and 72 hours with LC_{50} and LC_{90} indicated that *M. azedarach* almond oil induced a significant increase in GST activity (Figure 1A).

The two-way ANOVA indicates a significant effect of time (p<0.0001), treatment (p<0.0001), and time-treatment interaction (p<0.0001).

On the other hand, the obtained results about AChE activity indicated a significant inhibition in the treated series compared to the controls from 0 hour (Figure 1B). Statistical analysis (ANOVA) revealed a very significant increase in time ($F_{(3,32)}$ = 236.4; p<0.0001), treatment ($F_{(2, 32)}$ = 104.7; p<0.0001) and time-treatment interaction ($F_{(6,32)}$ = 11.79; p<0.0001).

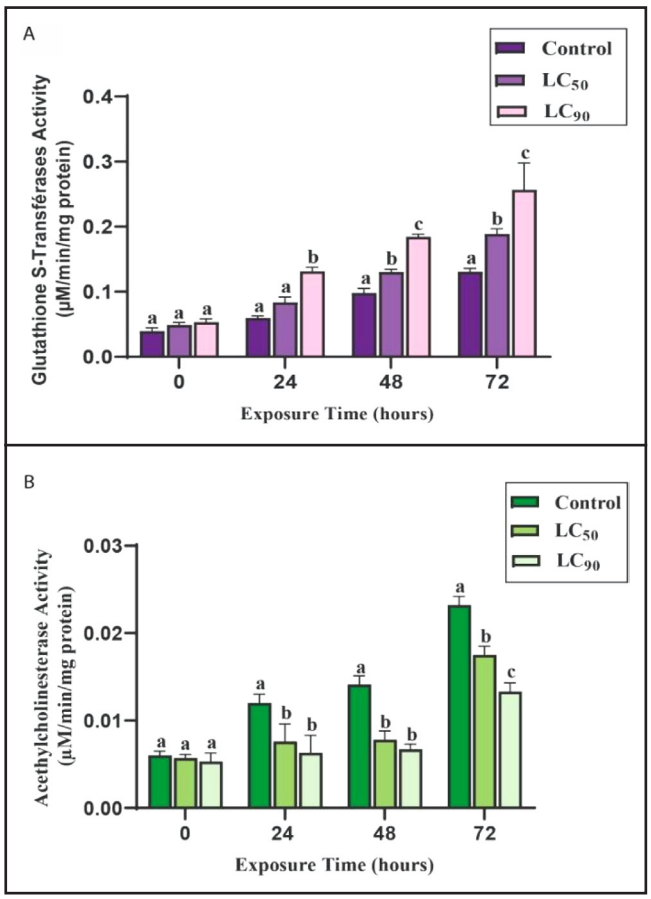

Figure 1. Effect of *Melia azedarach* almond oil extract on enzyme activities in fourth instar larvae of *Culex pipiens* (mean ± SD; n = 4).

A: GST activity (µM/mn/mg of protein)
B: AChE activity (µM/mn/mg of protein)

Different lowercase letters above the same exposure time indicate a significant difference ($p<0.05$).

DISCUSSION

As for the composition of Fatty Acid Methyl ester groups extracted from *M. azedarach* which the current study has proven it to contain many active substances, the results confirm a previous study by Hadjiakhoondi *et al*. (2006). Samples were prepared from 0.15 g of extracted fat, by saponification after esterification. The resulting fatty acid methyl esters were analyzed by GC/MS, on the hexanic extract of *M. azedarach* fruit, and its fatty acid methyl ester (FAME) was analyzed by gas chromatography coupled with mass spectrometry that detected Thirteen components, representing 86.84% of the total extract. The major constituents were methyl palmitate (18.8%), methyl linolenate (16.1%) and methyl linoleate (9.8%). In the works of Ranchitha *et al.* (2016), it was mentioned that the phytochemistry of *M. azedarach* ethanol extracts is related to the presence of triterpenoids and steroids, alkaloids and condensed tannins. These compounds are able to inhibit development or feeding of insects and also display ovicidal and insecticidal activity in insects. Additionally, many triterpenoids found in plants of the Meliaceae family are described as having insecticidal activity. GC-MS analysis results belonging to other works showed that the ethanol extract of *M. azedarach* fruit contains 27 compounds and is rich in many substances such as tannins, flavonoids and other phenolics compounds (Abdelslam *et al*., 2020).

In a previous study on *M. indica* seed oil from 42 samples from different parts of India, oleic, stearic, palmitic, linoleic, myristic, arachidic (eicosan) and behenic acids were detected. 90% and 99% of the total FAMEs were oleic acid (48.6-69.0%), palmitic acid (14.5-25.0%) and stearic acid (13.4-27.5%). Other fatty acids and methyl esters (myristic, arachidic, linoleic and behenic) were negligible or undetectable. Therefore, the large differences in the content of different fatty acids were evident (Kumar & Parmar, 1996). The analysis of compounds by gas chromatography and mass spectroscopy was performed in the methanolic extract of *M. azedarach* leaves by Al-Marzoqi *et al*. (2015), who showed the presence of 13 bioactive phytochemical compounds. This study presented a variety of compounds in *M. azedarach* including trichloromethanic acid, propanedioic acid, diethyl ester, 2-pyrrolidinyl-methylamine, butanedioic acid, diethyl ester, 2- piperidimethanamine, butanedioic acid, hydroxyl-, diethyl ester, 2,5-dimethylhexane-2,5- dihydroperoxide, dithiocarbamate, s-methyl-, n-(2-methyl-3-oxobutyl), triethyl citrate, y-sitosterol, ethyl 9,12,15-octadecatrienoate, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, and octadecane, 3-ethyl-5-(2-ethylbutyl). While study by Habib *et al*. (2017) with a gas chromatography- mass spectroscopy analysis of the extract of *M. azedarach* leaves in hexane. Different compounds were identified in this extract. Eight peaks of the non-polar plant extract were identified as 2-Undecanol, 4,6-decadienyl methyl ether, 13-docosenoic acid, 7,8-dihydrocarpesterol, glutaric acid, dimethyl ester, nonanoic acid, 1,2,3-propanetriyl ester, 1,3-glycerol dipalmitate 2-acetate, docosenoic acid and 1-methyl butyl ester.

This study examined the larvicidal activity of *M. azedarach* almond oil against *Cx. pipiens* mosquitoes. The obtained results revealed a variable sensitivity of *Cx. pipiens* larvae resulting in low to very high mortality rates when switching from one concentration to another. Compared to other results, the *M. azedarach* plant extract and plant parts exhibit a wide range of insecticidal activity against different species of insects. This result is entirely consistent with the explanations given in the study by Ilahi *et al*. (2012), which showed the larvicidal activities of different parts of *M. azedarach* against *Culex quinquefasciatus*. There was a continuous increase in mortality of 3rd and 4th instar larvae with increasing concentration of extracts. Ranchitha *et al*. (2016) proved that acetone leaf extract of *M. azedarach* possess the larvicidal activity against the dengue vector *A. aegypti* with various concentrations (300-700 ppm) for 24 hours. The observed LC_{50} and LC_{90} values of *M. azedarach* acetone extract were respectively 587.832, and 805.308 ppm, for the fourth instar larvae. The phytochemicals which are present in this extract may individually or synergistically possess the larvicidal activity against the developmental stages.

Melia azedarach insecticidal activity was comparable with the well known lipopesticide azadirachtin found in Neem tree. For this reason, Aydine *et al*. (2020) found that Neem oil obtained by hexane extraction was lethal to third and fourth instar *Anopheles gambiae* larvae. All concentrations of emulsified Neem oil above 1000 ppm (0.5-10%), caused 100% mortality against the third and the fourth instar larvae within 24 hours. Effectiveness results of *M. azedarach* insecticidal extracts coincide with those obtained by other authors, such as Manzano *et al*. (2020) who evaluated the larvicidal effect of Neem extract on *Aedes aegypti* larvae. The best larvicidal activity (93% mortality) was obtained at 72 hours of 50 mg/L^{-1} extract. However, Nour *et al*. (2012) reported the larvicidal activity of extracts from different parts of *Azadirachta indica* (leaf, stem, and root) against *Aedes aegypti* larvae where they indicated that the leaves produce the greatest activity. The lethal concentrations, namely LC_{50} and LC_{90}, were respectively 3.85 g/L and 7.35 g/L after 24 hours of exposure. As well, they were respectively 3.57 g/L and 7.68 g/L after 72 hours of treatment with *M. azedarach* oil.

Another similar study by Al-Mehmadiand Al-Khalaf (2010) showed that the effect of *M. azedarach* extract on 3rd instar larvae of *Culex quinquefasciatus* increases with increasing concentration, confirming the result that the LC_{50} after 24h was 1.035 and 0.75

mg/L after 48h. A regression analysis showed a significant correlation between the concentration of the plant extract and the larval mortality. According to the work of Kharoubi *et al*. (2021) which were carried out under the same experimental conditions and using the same biological material, *Mentha x piperita* essential oils at concentration series of 100, 150, 200, 300, 400 and 500 ppm applied directly to fourth instar *Cx. pipiens* larvae resulted in mortality rates of up to 95.91% after 72 hours of treatment. Undoubtedly, plant derived toxicants are valuable sources of potential insecticides. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs (Ranchitha *et al*., 2016).

The present study shows that *M. azedarach* almond oil has significant ovicidal activity against *Cx. pipiens* mosquitoes. These results are consistent with those of Kamaraj *et al*. (2010) showing that aqueous and hydro-alcoholic extracts of *M. azedarach* leaves and seeds caused 100% inhibition of egg hatch. which was inhibited at 12.5 mg/mL. In other research works as in Malarvanan *et al*. (2009), petroleum ether, hexane, chloroform, acetone and water from *Melia dubia*, *Cipadessa baccifera*, *Clause nadentata* and *Dodonaea angustifolia* are reported to have ovicidal activity against cotton bollworms. According to Maria Ruth *et al*. (2019), in the dose range of 10 to 80 ppm, the ovicidal activity of the extracts of white flowers and fruits on the eggs of *Aedes aegypti* showed that the ovicidal activity at hatching decreased from 83 to 31.50%. On the other hand, the results of Alouani *et al*. (2019) revealed a low ovicidal effect of the *Azadirachtin* on the eggs of *Cx. pipiens* under laboratory conditions. Finally, Kharoubi *et al*. (2021), according to their experiments which took place under the same conditions as ours, they indicated that the essential oil of *M. x piperita* has ovicidal properties on *Cx. pipiens* with an egg hatch percentage of 53.67% and 8.13% at LC_{25} and LC_{50}, respectively.

Regarding the repellent effect, under laboratory conditions and according to the used protocol and which is mentioned in the reference by Donald *et al.* (2007), our results reveal the presence of a very significant repellent effect of *M. azedarach* almond oil against *Cx. pipiens* adults. These results are similar to the work of Dadé *et al*. (2019), where it is shown that the extracts *M. azedarach* fruits are effective against the nymphs of *Triatoma infestans* in terms of repulsive activities. Tandon & Sirohi (2009) stated that 5% ethanolic *M. azedarach* extract repelled a minimum of 30% beetles of *Raphidopalpa foveicollis* Lucas in one hour and a maximum of 65% beetles in 48 hours whereas 10% extract repelled a maximum of 76% beetles in 48 hours. *M. azedarach* oil at 2% provided 95.13% protection for 7 h 20 min, while 5% oil provided 96.20% protection for 8 h 20 min against *Phlebotomus orientalis* (vector of visceral leishmaniasis) (Kebede *et al*., 2010). Plants of the Meliaceae family, including Neem, *Azadirachta indica*, *Carapa procera*, *Melia azedarach*, *Khaya senegalensis,* and *Trichilia emetica*, which contain limonoids, have insecticidal and repellent effects on insects (Pavela & Benelli, 2016). Neem provided a protection of 98.2% for 8 hours against *Anopheles darlingi*. Although Neem has low toxicity to the skin, it can cause skin irritation, such as dermatitis when used undiluted. To this fact, the US Environmental Protection Agency (US EPA) has not approved its use as a topical insect repellent (Reutemann & Ehrlich, 2008; Maia & Moore, 2011). Neem is widely advertised as a natural alternative to DEET, and it has been tested for repellency against a wide range of arthropods of medical and veterinary importance. MiteStop®, based on a Neem seed extract, had a considerable repellent effect on bloodsucking mosquitoes, tabanids, ceratopogonids, simuliids, as well as licking flies (Al-Quraishy *et al*., 2012). Several field studies in India have shown that Neem preparations have a very high efficacy, which is in contrast with the results of other researchers' studies on moderate insect repellency. However, these comparative results may be due to different methods and solvents used to carry insect repellents. According to the number of works carried out on this subject, it is seen that the field of plant-based repellents is moving forward. This comes down to consumers who demand means of protection from arthropod bites that are safe, pleasant to use and environmentally sustainable because usually, repellents do not kill mosquitoes, they simply reduce human-vector contact.

In fact, some plant oils cause neurotoxic effects in insects by interacting with acetylcholinesterase (AChE) (Pavela & Benelli, 2016). Our results assert that the plant extract used in our experiments had an inhibitory effect on the AChE enzyme.

A similar study by Shahat *et al*. (2020) reported that extracts of *Origanum syriacum*, *Pergularia tomentosa*, *Senna italica*, and *Otostegia fruticose* significantly decreased AChE activity of 3rd instar larvae of *Cx. pipiens*. The work of Dris *et al*. (2017) confirms our results on the same mosquito species but with an essential oil extracted from the plant of *Ocimum basilicum* which inhibited AChE activity in fourth instar larvae of *Cx. pipiens*.

Our results are in agreement with the work of Hafsi *et al*. (2022) who used ethanolic extract of *Tecoma stans* leaf against the fourth instar larvae of *Cx. pipiens* which showed an induction of GST activity. Similar results have been presented by Kharoubi *et al*. (2020) who showed an increase in GST activity after treatment of *Cx. pipiens* larvae using the essential oil of *M. rotundifolian*. Other works are in agreement with our results, using the essential oil of *Thymus vulgaris*, where they found an increase in GST activity after treatment of *Cx. pipiens* larvae (Bouguerra *et al*., 2018).

CONCLUSION

Plants are still a source of biochemicals used in many fields. Currently, natural products have received considerable attention as potential bioactive agents for the management of insect disease vectors.

The main objectives of this research were to assess the mosquito-repellent properties of a plant locally known as *M. azedarach*, to determine the main chemical components, and to identify the effects of these biopesticides and biomarkers.

The analysis by GC/MS of almond oil extracted from *M. azedarach* indicated the presence of many chemical compounds including 9,11-Octadecadienoic acid, methyl ester, (E,E)-, as the main compounds of this oil. In this research, the *M. azedarach* almond oil has been shown to be effective as a larvicide and ovicide against *Cx. pipiens*, as well as having a repellent activity against adults. Therefore, it caused a neurotoxic activity as evidenced by the inhibitor effect on AChE. Furthermore, it induced activation of the detoxification system through a significant increase in GST activity on the treated larvae. So, *M. azedarach* almond oil can be considered as a potential biopesticide to control mosquito populations.

In addition, this research provides scientific support for the use of plant extracts in control programs against different species of mosquitoes as well as other harmful insects.

The present study highlights a specific mosquitocides product of botanical origin, biodegradable, and environmentally safe.

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Conflict of interest

The authors declare that they have no conflict of interest.

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