



Chemical Composition and Larvicidal Activity of Azadiractha Indica Seed Oil Against Dengue Vector Aedes Aegypti (L) (Diptera: Culicidae)

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ABSTRACT:

The purpose of this study is to evaluate the larvicidal activity of neem seed oil (*Azadiractha indica*) against the dengue vector, *Aedes aegypti* (Diptera: Culicidae). A number of diseases are mostly spread by mosquitoes which result a major threat. Many mosquito control strategies are adopted by which the population of mosquito is minimized. Excessive use of insecticides causes ill effects to exact mosquitoes. Since phytochemicals are suggested to control mosquitoes. They are bio-safe and eco-friendly the mosquitoes. Phytochemicals suggest bio-safe and eco-friendly options to control the disease. Eight main bioactive components were identified in the neem seed oil by GC-MS analysis, although at different amounts. Absorption spectra were used to determine the prominent peak's wave number, intensities, and vibrational assignments. Numerous functional groups were found, including alkane, carboxylic acid, alcohol, ester, phenol, and nitro compounds. Mortality was recorded 24 hours post-treatment with five different concentrations of neem seed oil (250, 200, 150, 100, and 50 ppm). A significant increase in larval mortality was observed with increased concentrations, reaching a median lethal concentration (LC₅₀) of 57.59 ppm and (LC₉₀) of 136.74 ppm. These findings imply that safe oil and its components have promising effects as larvicides for mosquito vector control. Therefore, this oil can be used as an effective controlling agent against *A. aegypti*.

1. Introduction

Mosquitoes (Family: Culicidae) are the most well-studied insects. Arthropods affect human health and welfare more than any other arthropod worldwide. It plays an important role in the transmission of many deadly diseases that seriously threat public health, such as filariasis, chikungunya, dengue fever, yellow fever, malaria and Zika [1-3]. Therefore, from a medical perspective, mosquitoes belonging to the genera *Aedes*, *Culex*, and *Anopheles* are important arthropods among the numerous bloodsucking insects [4-6]. Although there are more than 3,500 species in the world, they are responsible for nearly 10% of human diseases. Mosquito-borne diseases are the major cause of acute death in the human population. It affects About 700 million people are affected annually [7,8]. Particularly in countries with tropical and subtropical climates, this entails decreased labour and business productivity, costs, and the frequency of diagnosis, treatment, prevention, and management; however, vector-borne illnesses are widespread worldwide [9,10].

The small, dark-colored *Aedes aegypti* mosquito bears white lyre-shaped markings on its body and webbed legs. They like to bite inside and typically bite humans (CDC, 2015). These mosquitoes may lay their eggs in both natural and artificial water sources. They lay their eggs during the day in wide-open containers with organic matter (eg, decaying leaves, moss, etc.) and prefer dark containers located in the shade [11]. The main vector, *A. aegypti* large tracts of tropical and subtropical regions is prone to dengue fever, dengue hemorrhagic fever, and yellow fever [12-16]. Neem, (*Azadiractha indica*,) is a deciduous tree whose insect-repellent properties have long been known in northwestern India [17-19]. Research has shown that some components of neem trees have larvicidal properties against *Aedes* mosquitoes [20,21]. Allelochemicals in neem oil such as azadirachtin, nimbin and salanin interfere with the physiological processes of insects and inhibit their growth [22]. Neem-based insecticides are commonly used due to their multiple applications, such as repellent, anti-feeding activity and protection against non-target



organisms [23]. All mosquito species including *Aedes* [24], *Culex* [25], *Anopheles* [22] are well controlled by neem.

2. Materials and Methods

2.1. Study area and collection of oil extract

The present study was conducted from March 2022 to July 2022 at the P.G. and Research Department of Zoology, Raja Doraisingam Government Arts College, Sivagangai, Tamil Nadu, India. Neem seed oil was processed by a government-approved oil shop in Madurai, Tamilnadu, India (Fig. 1). GC-MS and FTIR analyses of bioactive compounds were performed in the Instrumentation Centre, ANJA College, Sivakasi, Viruthunagar, Tamil Nadu, India.



Fig. 1: Collection of Oil extract

2.2. Gas chromatography mass spectroscopy

The Agilent chromatography GC (Model 7820A series) fitted with the VL-MSD detector (Model 5977E) was used to perform GC-MS analysis of the sample. For one minute, the temperature of the GC oven was set to 100°C. After that, it was increased to 270°C at a rate of 10°C per minute, and it stayed there for thirty minutes. Helium was used as the carrier gas, and it flowed at a steady 2 ml/min. A 1.0 µl sample injection was automatically performed into the column (DB-5) with the injector temperature set to 270- 270°C. The injection method employed was split-less. The substances were identified using a comparison of retention indices (RI), retention times (RT), WILEY mass spectra, NIST library data for the GC-MS instrument, and literature data.

2.3. Fourier Transform Infrared Spectrophotometer

The Fourier Transform Infrared Spectrophotometer (FTIR) is the most useful tool for identifying the types of chemical bonds, or functional groups that are present in compounds. The wavelength of light absorbed indicates the chemical bond, based on the annotated spectrum. An analysis of a molecule's infrared absorption spectra can reveal the chemical bonds. An extract of *A. indica* seed oil was used for FTIR analysis. 10 mg of the seed oil extract were encapsulated with 100 mg of KBr pellet to create translucent sample discs. The seed oil sample was put into an FTIR spectroscope (Shimadzu, 8400S) with a resolution of 4 cm⁻¹ and a scan range of 500 to 4000 cm⁻¹.

2.4. Mosquito Rearing

The colony of *A. aegypti* mosquitoes was reared in the insectary at the Raja Doraisingam Govt. Arts College, Sivagangai using the standard procedures described by Manh et al., [26, 27]. The insectary was maintained at 27 ± 3 °C, 70% – 80% relative humidity, and had a 12-hour light and 12-hour dark photoperiod. Adult mosquitoes were housed in breeding cages (30 cm x 30 cm x 30 cm) with a 10% sucrose solution, while larvae were kept in plastic trays with cat chow (Wiskcat). For the purpose of mosquito reproduction, the female mosquitoes were given the blood of live mice. The Raja Doraisingam Govt. Arts College, Sivagangai Guide for the Care and Use of Laboratory Animals was followed in the performance of these investigations.

2.5. Larvicidal activity

Larvicidal activity was studied using standard WHO methodology with a few minor adjustments [28]. The 200 ml of water in the 250 ml plastic cups with varying extract concentrations (50, 100, 150, 200, and 250 ppm). Larvae in their early fourth instar were added to each concentration. Various concentrations of extract (50, 100, 150, 200, and 250 ppm) were produced from the stock solution. 200 ml of water were added to 250 ml plastic containers containing early 20 fourth instar larvae for each concentration. Water was mixed with acetone to provide a control. After twenty-four hours, deaths were reported. Four replicates were kept at a time for every experiment. Abbott's Formula was used to adjust the observed percentage mortality [29].



$$\% \text{ of mortality} = \frac{\text{Number of larvae died}}{\text{Total number of larvae exposed}} \times 100$$

2.6. Statistical analysis

Mortality was recorded after 24 hours of exposure. Obtained values were subjected to logging probit regression analysis and to obtain LC₅₀ and LC₉₅ values with a 95 % confidence limit [30].

3. Results

The GC-MS characterization of seed oil extract of *A. indica* was analysed and presented in Table 1 and Fig. 2. Totally, twelve major chemical compounds were identified (Fig. 3), such as n-Hexadecanoic acid (0.46 %), cis-13-Octadecenoic acid, methyl ester (0.58 %), Oleic Acid (13.35 %), 9,17-Octadecadienal, (Z)- (2.79 %), 2-Methyl-7-phenylindole (0.54 %), Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (3.73 %), 1H-Indole, 5-methyl-2-phenyl- (1.82 %), Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl- (9.22 %), 1H-Pyrazol-3-amine, N,N-dimethyl-1-phenyl- (4.13 %), 1,1'-Binaphthalene, 5,5',6,6',7,7',8,8'-octahydro- (2.31 %), 1-Tributylsilyloxy-2-phenylethane (2.24%), 4H-Benzo[def]naphtho[2,3-] carbazole (7.92 %), 2-Ethylacridine, (1.99 %), Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- (3.30 %), Ergost-5-en-3-ol, (3.β)- (7.83 %), Supraene (3.14 %), Propanedioic acid, ethyl- diethyl ester (8.12%), 1,4-Bis (trimethylsilyl) benzene (1.41%), 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene (0.98%), beta-Sitosterol (9.29%), 6-Hydroxy-2-methylcyclohepta(b)pyridin-7-one (2.40 %), and Triphenyl phosphate (4.00 %) were present in the seed oil extract of *A. indica*.

The identification of the phytochemical compounds was confirmed based on the retention time, peak area, molecular formula and molecular weight. FTIR analysis of seed oil extract of *A. indica* was carried out and presented in Table 2 and Fig. 4. The compounds indicated show that the band at 3528.53, 3008.75, 2851.56, 2308.63, 2041.51, 1748.35, 1655.77, 1600.81, 1377.08, 1241.11, 1158.17, 1094.53, 871.76, 722.29 and 583.43 cm⁻¹. The broad band at 3528.53 cm⁻¹ O-H stretching in alcohol groups. The presence of peaks at 3008.75 cm⁻¹ and 2851.56 cm⁻¹ corresponding to the

carboxylic acid and C-H stretch of Alkane groups. The band at 2041.51 cm⁻¹ and 1748.35 cm⁻¹ to assign the N=C=S stretch Isothiocyanate and C=O stretch in Esters groups. The peaks at 1655.77 cm⁻¹ and 1600.81 cm⁻¹ corresponding to the C=N stretch Imine / Oxime and N-O stretch of Nitro compound groups. The peak at 1377.08 cm⁻¹, 1241.11 cm⁻¹ and 1158.17 cm⁻¹ in O=H bend Phenols, C-O stretch Alkyl aryl ether and C-O stretch Tertiary alcohol groups. The broad band at 1094.53 cm⁻¹ C-O stretch in Secondary alcohol groups. The peak at 871.76 cm⁻¹, 722.29 cm⁻¹ and 583.43 cm⁻¹ in C-H bend 1, 2, 4-trisubstituted, C=C bend alkenes and C-I stretch in Halo compounds.

LC₅₀= Lethal Concentration brings out 50% mortality and LC₉₀= Lethal Concentration brings out 90% mortality. LCL= Lower Confidence Limit, UCL= Upper Confidence Limit, χ²=Chi-square. Larvicidal activity of *A. indica* against fourth instar larvae of *A. aegypti* was estimated. The larval mortality of the fourth instar larvae of *A. aegypti* found to be increased with increasing concentrations of essential oil extract of *A. indica* LC₅₀ and LC₉₀ values of 57.59 ppm (36.5 – 73.7), and 136.74 ppm (108.0 – 204.6) was respectively (Table 3).

4. Discussion

The World Health Organization believes that every year, pesticides have an impact on the health of at least 3 million individuals globally. As a result, it appears that naturally occurring pesticides have a big influence on the development of future commercial pesticides, which are important for public health and environmental safety in addition to agricultural crop production [31]. Pesticides should not be harmful to non-target organisms and should be safe for the ecosystem. Eradicating mosquito larvae with larvicides is an essential step in avoiding illnesses carried by vectors. A desired and practical method of reducing mosquito populations at the community level is the use of larva-repellent plants. Plants produce compounds known as phytochemicals that are often hazardous to humans and mosquito larvae.

Table 1: GC-MS analysis of essential oil of *A. indica*

Sl. No	Retention Time	Area	Compounds	Formula	Molecular weight
1	11.72	0.46	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
2	12.97	0.58	cis-13-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296.48
3	13.43	13.35	Oleic Acid	C ₁₈ H ₃₄ O ₂	282.46
4	13.57	2.79	9,17-Octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264.44
5	16.43	0.54	2-Methyl-7-phenylindole	C ₁₅ H ₁₃ N	207.27
6	16.66	3.73	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	C ₁₆ H ₅₀ O ₇ Si ₈	579.248
7	16.70	1.82	1H-Indole, 5-methyl-2-phenyl-	C ₁₅ H ₁₃ N	207.27
8	16.75	9.22	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	C ₁₁ H ₁₂ NO ₂	207.23
9	17.07	4.13	1H-Pyrazol-3-amine, N,N-dimethyl-1-phenyl-	C ₁₁ H ₁₃ N ₃	187.24
10	17.29	2.31	1,1'-Binaphthalene, 5,5',6,6',7,7',8,8'-octahydro-	C ₂₀ H ₂₂ O ₂	294.40
11	17.34	2.24	Phenothiazine, 2-chloro-8-methoxy-	C ₁₃ H ₁₀ ClNOS	263.01
12	17.43	7.92	4H-Benzo[def]naphtho[2,3-] carbazole	C ₂₂ H ₁₃ N	291.10
13	17.59	1.99	2-Ethylacridine	C ₁₅ H ₁₃ N	207.27
14	17.89	3.30	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	C ₁₄ H ₄₄ O ₆ Si ₇	505.09
15	18.13	7.83	Ergost-5-en-3-ol, (3.beta.)-	C ₂₈ H ₄₈ O	400.68
16	19.22	3.14	Supraene	C ₃₀ H ₅₀	410.71
17	19.82	8.12	Propanedioic acid, ethyl-, diethyl ester	C ₉ H ₁₆ O ₄	188.22
18	19.92	1.41	1,4-Bis(trimethylsilyl)benzene	C ₁₂ H ₂₂ Si ₂	222.47
19	20.12	0.98	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	C ₁₇ H ₁₄ O ₄	282.29
20	20.58	9.29	beta.-Sitosterol	C ₂₉ H ₅₀ O	414.70
21	21.30	1.01	1,4-Bis(trimethylsilyl)benzene	C ₁₂ H ₂₂ Si ₂	222.47
22	21.48	2.40	6-Hydroxy-2-methylcyclohepta(b)pyridin-7-one	C ₁₁ H ₉ NO ₂	187.20
23	21.62	4.00	Triphenyl phosphate	C ₁₈ H ₁₅ O ₄ P	326.28

There are also several chemokines and growth inhibitors that have repellent or attractant qualities. In this investigation, *A. aegypti* larvae died due to neem chemicals. Compared to the original formulation, the neem oil formulation exhibited superior larvicidal action, but not after 12 to 15 days. Excellent mortality was exhibited by the formulation. The larvae were exposed because the emulsifier made it easier for the oil's active component to spread uniformly throughout the water.

For elevated levels of this material [32]. Our results on oil production are co-inside with those of earlier research [33,34] that utilized neem extracts at emulsified concentrations to kill mosquito larvae.

Additionally, according to our GC-MS analysis of eight major biological compounds, oleic acid (13.35%), β -sitosterol (9.29%), indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl (9.22%), propanedioic acid, diethyl ester (8.12%), 4H-Benzo[def]naphtho[2,3-]



carbazole (7.92%), and Ergost-5-en-3-ol (3.βeta.) (7.83%) are all naturally occurring fatty acids [35].

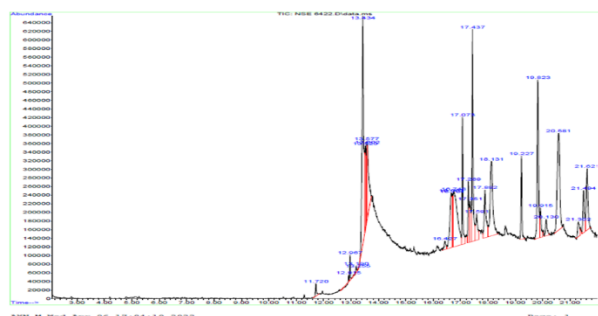


Fig. 2: GC-MS analysis of seed oil extract of *A. indica*

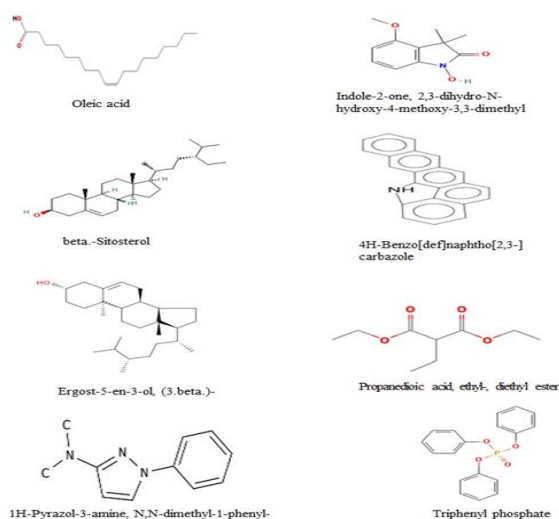


Fig. 3: GC-MS analysis of major bioactive compounds in neem seed oil extract

Table 2: Functional groups of the components of seed oil extract of by FTIR

Sl.No	Absorption (Cm ⁻¹)	Functional Groups	Compounds	Intensity
1	583.43	C-I stretching	Halo compound	Strong
2	722.29	C=C bending	Alkene	Strong
3	871.76	C-H bending	1, 2, 4-trisubstituted	Strong
4	1094.53	C-O stretching	Secondary alcohol	Strong
5	1158.17	C-O stretching	Tertiary alcohol	Strong
6	1241.11	C-O stretching	Alkyl aryl ether	Strong
7	1377.08	O-H bending	Phenol	Medium
8	1600.81	N-O stretching	Nitro compound	Strong
10	1655.77	C=N stretching	Imine / oxime	Medium
11	1748.35	C=O stretching	Esters	Strong
12	2041.51	N=C=S stretching	Isothiocyanate	Strong
13	2851.56	C-H stretching	Alkane	Medium
14	3008.75	O-H stretching	Carboxylic acid	Strong
15	3528.53	O-H stretching	Alcohol	Strong

Although commercial samples may be yellowish in the maximum proportion of 13.35%, the oil is colourless and odourless. According to Pravin Kumar et al., [36], "oleic" refers to or is generated from olive oil, which is mostly made up of oleic acid. The most significant sterol in the oil was beta-sitosterol, which is known to have a role in lowering cholesterol. According to Saeidnia et al., [37], this sterol is also crucial in lowering heart disease, rheumatoid arthritis, male pattern baldness, and prostate edema. According to Ogunles et al. [38], a number of fatty acids have antibacterial and antifungal properties, including oleic acid and n-hexadecanoic acid. In this study *A. indica* seed oil recorded LC₅₀ and LC₉₀ values of 57.5ppm and 136.7 ppm against the larvae of *A. aegypti*, respectively. According to Suganya et al., [39] concentration increased with increased mortality in *S. indicum*. Increased concentrations resulted in increased mortality of larvae, and research confirmed that the higher the concentration level, the higher the level of toxicity [40]. Furthermore, Sugumar et al. [41] found a positive correlation between larvicidal mortality and exposure time and concentration. In this experiment, the extract concentration rose from 1000 ppm to 5000 ppm, resulting in an approximately five-fold increase in the mortality rate between the initial and final concentrations. Furthermore, a number of studies discovered a positive relationship between time factor and larval mortality [42-45].

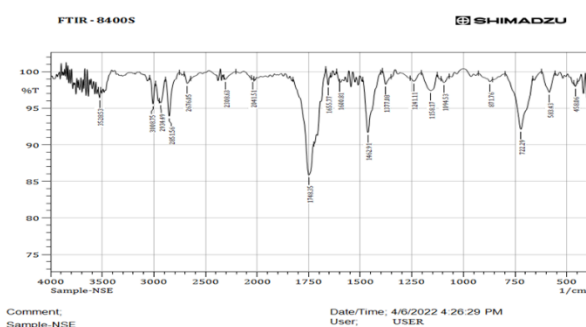


Fig. 4: Functional groups of the components of seed oil extract of by FTIR

Table 3: Larvicidal activity of *A. indica* seed oil against *A. aegypti* larvae after 24 h exposure

Concentration (ppm)	% Mortality	LC ₅₀	LC ₉₀	LC ₅₀ L-UC	LC ₉₀ L-UC	χ^2
Control	0					
50	45					
100	75					
				36.	108.	
150	90	57.	136.	5-73.	0-204.	0.8
				7	6	52
200	100					
250	100					

LC₅₀= Lethal Concentration brings out 50% mortality and LC₉₀= Lethal concentration brings out 90% mortality. LCL= Lower Confidence Limit, UCL= Upper Confidence Limit, χ^2 =Chi-square

Conclusion

Neem seed oil formulation effectively suppressed mosquito larvae at different nesting places in natural field settings. Neem seed oil contains compounds that are safer for the environment, less harmful than traditional pesticides, and can be used to manage diseases spread by vectors. Neem seed oil also prevents pests from developing resistance.

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