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Larvicidal Activity of *Ailanthus Altissima* and *Melia Azedarach* Against Malaria Vector (*Anopheles*)

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ABSTRACT

Plants are abundant in chemicals, drugs, and secondary metabolites that can serve as effective insecticides. Mosquitoes are the vectors of several deadly diseases, including malaria .Chemical insecticides have become less effective due to resistance in several mosquito species and negatively affect the environment. Plant-based alternatives have been highly recommended to overcome the hazards. The larvicidal activity of Ailanthus altissima and Melia azedarach leaf extract was evaluated against Anopheles stephensis. The extract was prepared using the maceration technique following the standard protocol of the World Health Organization. Late 3rd and early 4th instars (larvae) were exposed to seven concentrations of the plant extracts to calculate the LC50 and LC 90 values. Results showed that A. altissima extracts were most lethal at 950 mg/L (97%), and the minimum mortality was recorded at 150 mg/L (16%). While the LC50 of 312 mg/L and the LC90 of 1552 mg/L were recorded for A. altissima, on the other hand, M. azedarach shows maximum mortality at 950 mg/L (92%) and minimum mortality at 150 mg/L (14%), with LC50 values of 379 mg/L and the LC90 of 1786 mg/L. Both plant extracts were effective against Anopheles stephensis after 24 and 48 hours of exposure; however, the highest lethal effect of A. altissima was observed as compared to M. azedarach against the larvae of Anopheles. The findings of this study conclude that both plant extracts have larvicidal effects and could be used as eco-friendly alternatives to chemical insecticides.

Introduction

Mosquitoes belong to family Culicidae, they are largest group of insects, and more than 3,500 species of mosquitoes are recognized under 110 genera (Norris, 2004). Mosquitoes are found globally in tropical, subtropical, temperate region, inhabiting both aquatic and terrestrial habitats (Egbuche *et al.,* 2016). Mosquitoes are the primary vector for the spread of diseases, such as Japanese encephalitis, filariasis, schistosomiasis, yellow fever, dengue fever, and malaria (Kamaraj *et al.*, 2008). A study on

the systematic history of characteristics, distribution, and current epidemic status of viruses associated with mosquitoes in China. It was observed mosquitoes that mosquitoes consist of 41 genera and are categorized into 3500 species. According to epidemiology may carry a large number of viruses, making them one of the world's most significant disease vectors (Xia *et al.,* 2018).

Malaria is one of the main cause of illness in the country (Khan *et al.*, 2018). Malaria symptoms include fever and flu-like symptoms, such as chills, headache, pains in the muscles, fatigue, nausea, vomiting, and diarrhoea (Koussound and Ntoumi, 2016). Up to 63,000 death rate were reported due to 247 million cases worldwide. Of these, the WHO African area accounted for 94% of the recorded cases of malaria (WHO, 2022). In the recent years, there were 2.6 million recorded cases of malaria in 2021 and over 3.4 million suspected cases in 2022 from Pakistan (Nyasa *et al.*, 2021). A study done in the united states looked at how female *Anopheles* mosquitoes spread malaria. In 1880, Alphonse Laveran found malaria parasites in the blood of sick patients. It was also found that malaria parasites first grow in the liver before going into the blood (Cox,2010).

A. stephensi is an invasive species that was first recorded from the Horne of Africa in Djibouti (Seyfarth *et al.*, 2019). *Anopheles* mosquitoes have been identified in more than 500 species worldwide, and 70–80 of them are known to transmit malaria to people (Williams and Pinto, 2012). Ninety five million of Pakistan's 161 million citizens, or nearly 60% of the country's total population, reside in areas where malaria is endemic (WHO, 2013; Williams *et al.*, 2011). According to Zofou *et al.* (2014), controlling mosquitoes is still crucial for prevention infections in human and animals. The development and spread of resistance to synthetics chemical insecticides, primarily organophosphates, organchlorine, and Pyrethroid, has limited this progress (Aliyu, 2012; Karunamoorthi and Sabesan, 2013) . A study in Tehran tested plant-based repellents against *A.stephensis* mosquitoes. Plant-based repellents has been used traditionally for protection and can help develop naturalal alternatives to chemicals repellents (Asadollahiet al., 2019).

Plant products are gaining global attention as a biodegradable, affordable, and readily available alternatives to synthetics insecticides for controlling mosquitoes (Das *et al.,* 2010). Plants have been used for the management of insects for centuries in human communities. Secondary metabolites of plants play a vital role in the defence mechanism against insect attacks. Bio-active chemical in plants acts against insect species, acting as repellents, anti-feedants, moulting hormones, and attractants (Parabakan *et al.*, 2017).Plant extracts, such as saponin, steroids, isoflavonoids, essential oils, alkaloids, and tennis, are used as mosquito larvicides due to their non-targeted nature and lower environmental impact (Hemalatha *et al.*, 2015). Many medicinal plant are reported as mosquito codes and larvicides, but only a few are practically used for mosquito control (Afzal *et al.*, 2018). Medicinal plants parts, such as Nicotine, Rotenone, and Ryania, are used as insecticides against mosquitoes (Bekele *et al.*, 2018). Reserchers in Malaysia tested *Murraya koenigii* leaves for killing moasquito larvae. Methanol extract worked well and contained alkaloids, saponins, and flavonoids, with LC50 and LT50 values of 250 ppm and 450ppm after 36 hours.The study shows *M.Koenigi*i can be natural mosquito larvicide (Harith et al., 2018).

*Ailanthus altissim*a, called the "heaven of trees," is an ornamental plant in the *Simaroubacea* family. Although *A. altissima* is a short-lived tree, it may persist for over a century. They also reported that the phytoconstituents in *A. altissima* leaves contain antibacterial, herbicidal, insecticidal, and phytotoxic qualities (Rahman *et al.*, 2023). Most commonly occurring phenolic acids found in *A. altissima* leaves are Gallic acid, chlorogenic acid, p-coumaric acid, and sinapic acid. Flavonoids are also present in *A. altissima* leaves. It was shown that quercetin; isoquercetin, hyperoside, and apigenin are also present in the leaves of *altissima* (Boukhibar *et al.*, 2023). Leaves of *Ailanthus altissima* L. show potential as a natural herbicide and insecticide due to their secondary metabolites. The extracts strongly inhibited germination and growth of alfalfa (*Medicago sativa*), with greater effects observed in light than in darkness .At low concentration , some fractions even promoted plant growth .The extract also showed slight insecticidal activity against yellow fever mosquitoes larvae (*A.aegypti*) (Tsao et al., 2002).

Melia Azedarach is a member of the family Meliaceae. Other names for *Melia azedarach* include Chinese neem, bitter China berry, bitter China tree, and China tree. *Azedarach* and *Azadirachta* are the two species that make up the tiny genus *Melia.* Among these, the most well-known is the *M. azedarach* (*Meliaceae*) (Sharma *et al.*, 2013). This herb was discovered to be an antimalarial agent and was used to treat jaundice, ulcers, and the stomach (Duong *et al.*, 2018). Although it originated in Asia, it is currently found in regions of Southern Europe, North America, Africa, Northern Australia, and tropical South America. It is used as an antifungal, antiparasitic, and antimalarial drug in traditional Chinese medicine (Phua *et al.*, 2008). Many organic compounds, include terpenoids, flavonoids, steroids, acids, anthraquinones, alkaloids, saponins, and tannins were present in *M. azedarach* in phytochemical composition. Leaf of *M. azedarach* contain terpenoids and limonoids. Additionally, they include hexadecanoic or Palmitic acid (Sharma *et al.*, 2013). Many medicinal plant are reported as mosquito codes and larvicides, but only a few are practically used for mosquito control. In chennai, methanolic extracts of Azadirachta indica and Melia azedarach showed larvicidal activity showed lavicidal activity against *A.aegypt*i, *A.stephensi,* and *C.quinquefasiatus* at 100 ppm (Ravichandra et al., 2015).

WHO states that pyrethroid, sumithrin, organophosphate, and malathion are used to control mosquitoes. Pesticides destroy ecosystem and it is not good for people and animals that's why scientist use alternate and safe source for mosquito control (Ghosh *et al.*, 2012). Plant extract can be used for eco-friendly and bio-pesticides; it can also be used to overcome the environmental hazards of chemical insecticides. Medicinal plants have significant attention around the world because they provide a variety of safe, affordable preventive and curative treatments that can help achieve the goal of "health for all" (Afzal *et al.*, 2018). The objective of this to evaluate the larvicidal efficacy of *Ailanthus altissima* and *Melia azedarach.* Hence, this paper investigate the larvicidal activity of *Ailanthus altissima* and *Melia azedarach* against malaria vector (*anopheles)* mosquitoes larvae collected from the surroundings of Rawalakot in running water and were kindly provided by communicable disease control (CDC) Bagh AJK.

Material And Methods

Collection of *Ailanthus altissima* and *Melia azedarach*:

The leaves of *Ailanthus altissima* and *Melia azedarach* were used to make extract for larvicidal activity. Leaves of *A. altissima* and *M. azedarach* were used for the preparation of plant extract these leaves are collected from Dothan Rawalakot District Poonch. The leaves of *A. altissima* and *M. azedarach* were taxonomically identified with the help of the flora of Pakistan and the plant list. The voucher specimen with accession number [UPR-BOT-1039 and UPR-BOT-1040] was submitted to the Herbarium, University of Poonch Rawalakot.

The leaves of *A. altissima* and *M. azedarach* were collected from field areas. To remove dust and dirt, the fresh leaves of these plants were initially washed three times in distilled water. The leaves were spaced evenly and dried for three weeks in a shaded place with daytime temperatures between 27 and 37 °C. During this time, they became crispy without losing their greenish colour.The dried leaves (700g) were grind in an electric chopper to get fine powder (Nahak and Sahu, 2010). The powder was kept in a glass jar until they use.

Plant Extraction

80 g of dried leaf powered was measured and dissolved in 800ml of ethanol then the sample were macerated for 72 hours at room temperature. The resulting material was filtered with Whatman filter paper No. 1. The remaining filtrate was used in a rotary evaporator under reduced pressure at 40 °C (Basra and Iftkhar, 2011).

Preparation of stock solution:

To create the stock solution, the crude extract was serially diluted to achieve the required concentration (Dey *et al.*, 2020). The 80 g of plant extract was dissolved in 800 mL of acetone to get the 80% stock solution. From this stock, solution different concentrations were prepared, ranging from 15, 30, 45, 60, 75, 80 and 95 % which were 150, 300, 450, 600, 750, 800 and 950 mg/L, respectively (Murugan *et al.*, 2012).



Fig 1.Prepartion of crude extracts of *A. altissima* and *M. azedarach* showing voucher / herbarium specimen ,dry leaves, ethanolic extract, filtration, and final crude extract.

Collection and rearing of mosquito larvae:

First and second instar larvae of *Anopheles* mosquitoes was collected from the surroundings of Rawalakot in running water and were kindly provided by communicable disease control (CDC) Bagh AJK. After collection, these alive larvae were transported to the laboratory of Entomology, Faculty of Agriculture, University of Poonch Rawalakot, for further processing. They were kept in the Growth Chamber at (27 ± 2 °C and 75–85% of relative humidity) with a 14:10 light-to-dark ratio. Artificial mosquitoes diet having powder of dog food biscuit and yeast powder with 3:1ratio was used (Kamaraj *et al.*, 2008; Veerakumar *et al.,* 2014).

Larvicidal bioassay

Twenty five larvae of late third and early fourth instars was placed in each plastic cup (4 treatments, 2 controls) with varying concentrations of plant extracts for the bioassay of each extract (Kumar *et al.*, 2023). Each experiment was conducted at a temperature of (30 ± 2 °C and relative a humidity of 70–75 %). Bioassay was carried out in paper cups having 249 mL of distilled water and 1mL extract of desired plant concentration and twenty five late 3rd and early 4th instar larvae was added in each cup. Six replicates of each concentration were used; two were the control while four were concentration treatment. One mL of acetone and two hundred and forty nine mL of distilled water was used in the control. After exposure for 24 and 48 hours and the number of dead larvae was counted. A larvae were considered dead when a larvae stopped moving after being probedin the cervical or syphon region with a needle (WHO, 2015)

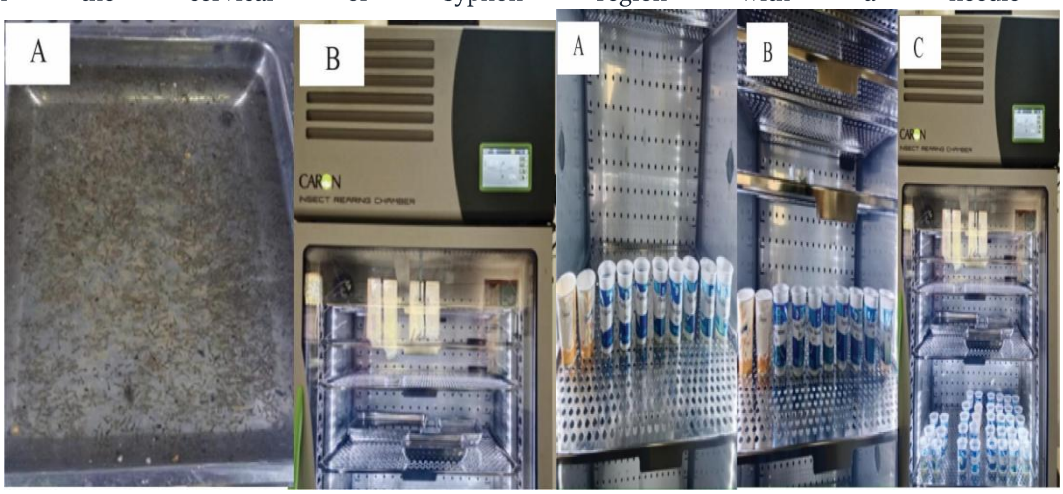


Fig 2. Collected wild samples of Anopheles larvae placed in rearing chamber and tested with different concentrations of Crude extract of *M. azedarach* and *A. altissima* for larvicidal activity.

Abbot's formula (Chauhan *et al.*, 2016) was used to correct the mortality in control, if the mortality ranges between 5-20 % and if exceeded 20 % than trail were repeated.

The following formula was used to calculate percentage mortality.

$$\text{Percentage mortality} = \frac{\text{No. of dead larvae}}{\text{No. of larvae introduced}} \times 100$$

While the mortality in control was corrected by using the Abbot's formula

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

DATA ANALYSIS:

Microcall Origin software was used to create the graphical display of the data after they had been analyzed using Statistical 8.1. Using the SPSS software version 21, Probit analysis was used to determine the LC_{50} and the LC_{90}.

Results

In the present study, the plant, common name, family, habit, leaf are used for bioassay test was given in (Table1). The results showed that larvicidal activity of ethanolic extracts of two medicinal plants species screened against *A. stephensi* larvae. The larvicidal activity of plant extracts of *A.altissima* shows a strong effect as compare to *M. azedarach* on the larvae of mosquitoes after 24 and 48 hours of exposure at 950ml; however, the highest death rate was observed at 95 mL (97%), and 150 mL (16%)

had the lowest mortality rate after 24 h. After 48 hours, the maximum death rate of *A, stephensi* larvae was observed at 950 mL, in which 100% of the larvae were killed, and the minimum death rate was observed at 150 mL (48%) of the *A. stephensi* larvae.The maximum number of mortalities of *M. azedarach* was observed at 950 mL (92%) after 24 hours, and the least mortality was observed at 150 mL (14%) after 24 hours. After 48 hours, the highest mortality percentage was recorded at 950 mL (99%), and the lowest mortality was observed at 150 mL (28%) in (Table 2, Fig 3 and 4).

Table.1 Detail of medicinal plant screened for larvicidal activity

Sr.No	Botanical name	Local name	Family	Habit	Part use
1.	*M. azedarach*	Darekh	Meliaceae	Tree	leaf
2.	*A. altissima*	Darva	Simaroubaceae	Tree	leaf

Table 2. Total mortality of plant extracts of *Melia azedarach* and *Ailanthus altissima against A.stephensi* larvae

Concentration %/mg/L	Larvae Expose	Percent Larval Mortality (± SE) *Ailanthus altissima* 24hrs	*Ailanthus altissima* 48hrs	*Melia azedarach* 24hrs	*Melia azedarach* 48hrs
15/150	100	16	48	14	28
30/300	100	49	55	39	50
45/450	100	58	80	41	66
60/600	100	68	80	58	80
75/750	100	78	100	69	87
80/800	100	93	99	90	100
95/950	100	97	100	92	99
Control	100	00	00	00	00

Fig 3. Percentage mortality of *A. stephensi* larvae against different concentrations of *A.altissima* plant extracts after 24 and 48hours

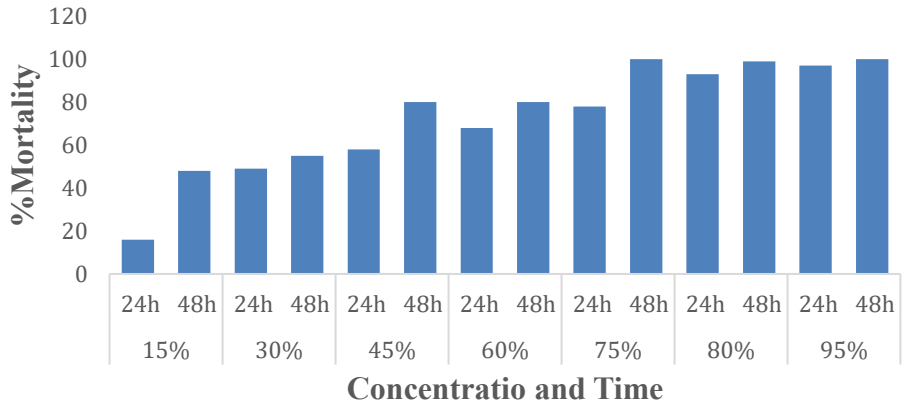
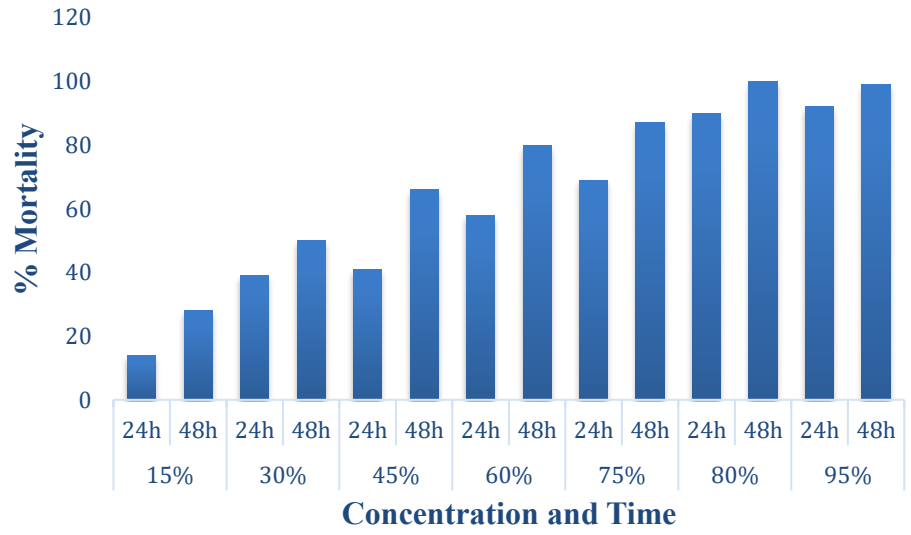


Fig4. Percentage mortality of *A. stephensi* larvae against different concentrations of *M. azedarach* plant extracts after 24 and 48hours



In *A. altissima,* the highest concentration of 950 mL caused 97 ± 2.51% larval mortality, while the lowest concentration of 150 mL caused a larval mortality rate of 16 ± 1.63%. At the highest concentration of 950 mL, larval mortality caused 93 ± 2.30%, while at the lowest concentration of 150 mL, larval mortality was 14 ± 2.58% of *M. azedarach.* The ethanolic extracts from *A. altissima* had a value of LC_{50} and LC_{90} of (LC_{50}: 312 ppm) and (LC_{90}: 1.552 ppm), followed by the ethanolic extracts of *M. azedarach* (LC50: 379 ppm) and (LC_{90}: 1.786 ppm). The mean and (SE) values for *A. altissima* and *M. azedarach* are 2.881 ± 0.215 and 2.982 ± 0.219 respectively.The value of P for *A. altissima* and *M. azedarach* is ≤ 0.05 in (Table 3).

Table 3. Log Probit analysis of concentrations of *Ailanthus altissima* and *Melia azedarach*

Plants	N1	LC_{50}[2] mg/L C.L3)	LC_{90} mg/L C.L)	Mean ± SE	r^2 (df)	P-Value
Ailanthus altissima	1700	312 (234.496 - 379.816)	1.552 (1117.210 - 2800.789)	2.881 ± 0.215	13.433(5)	≤ 0.05
Melia azedarach	1700	379 (236.590 - 503.504)	1.786 (1098.161 - 2579.728)	2.982 ± 0.219	35.445(5)	≤ 0.05

N= Total number

[2]LC= Lethal concentration

[3]CL= Confidence limit/

Discussion

In the current study, crude extracts of both *A. altissima* and *M. azedarach* showed larvicidal activity against *A. stephensi,* but *A. altissima was* most effective, with a lower LC_{50} and a higher mortality rate. The mortality of *A. altissima* with ethanolic extract against *A. stephensi* larvae was effective, with an LC_{50} value of 312 mg/L and an LC_{90} value of 1.552 mg/L. The death rates of *M. azedarach* with ethanolic extract against *A. stephensi* larvae were observed with LC_{50} and LC_{90} values of 379 and 1.786 mg/L, respectively. In this study, the larvicidal activity of *A. altissima* crude was found to be the highest at the lowest tested concentration. Crude extract showed larvicidal effects against late 3rd and 4th instar larvae of *A. stephensi*, respectively. The crude extract of *A. altissima* had a significant larvicidal effect at a dose of 750 mg/mL. The maximum larval mortality was

observed at 150 to 950 mg/L within 24 hours, with LC_{50} and LC_{90} values of 312 and 1.552 mg/L, respectively. Previous studies from Pakistan are similar to our findings. A previous study reported the chemical composition, larvicidal activity, and repellent activities of *Mentha longifolia*, *Salsola imbricata*, *Erigeron bonariensis*, *E. canadensis*, *Ailanthus altissima*, and *Zanthoxylum armatum* plants against *Aedes aegypti* mosquitoes. The essential oils of these plants showed 99.0%, 96.8%, 40.2%, 41.7%, 29.1%, and 13.2% repellency against *mosquitoes* at a tested concentration of 33.3 µg/cm2, respectively. Essential oils of *M. longifolia*, *S. imbricata*, *E. bonariensis*, *E. canadensis*, and *A. altissima* have the most common components, such as piperitone oxide (45.5%), carvone (39.9%), matricaria ester (43.1%), and eugenol (24.4%) (Abbas *et al.*, 2023). Another previous study similar to our finding: the insecticidal activities against *Sitophilus zeamaise* were demonstrated by *A. altissima* (Haroon *et al.*, 2018). More over, similar to *A. altissima,* indigenous plant extracts were observed for mosquitocidal activity against different stages of Culex quinquefasciatus.The LC_{50} and LC_{90} values for late 3rd instar larvae and pupae after 24 hours of exposure range from 93–1856.7 ppm, 424–763.5 ppm, and 3667.3–17427.9 ppm, respectively, as reported from Pakistan by Hussain *et al.* (2021). Crude extract of *M. azedarach* show larvicidal effects against late 3rd and early 4^{th} instar larvae of *A. stephensi*. The death rates of larvae were observed at 150 to 950 mg/L within 24 hours, with LC_{50} and LC_{90} values of 379 and 1.786 mg/L, respectively. Similar to current findings, the larvicidal activity of *M. azedarach* was also reported by Ojo (2022). *M. azedarach* leaf was used against *Anopheles gambiae* larvae with an LC_{50} of 1.09 mg/mL. Another previous study is similar to the current finding: the significant larvicidal activity of *M. azedarach* extracts was reported by Coria *et al.* (2008) against *Aedes aegypti*. The *M. azedarach* leaf exhibited potent larvicidal properties. All larvae were killed before pupation, and larval morality was significantly greater from the earliest larval instar ($F = 278.100$, $df = 6, 28$). Another finding was similar to our finding against *Culex quinquefasciatus* from Saudi Arabia, and their result showed that the histopathological effects of *M. azedarach*, a plant extract used as a bioinsecticide against *Cx. quinquefasciatus* larvae, have been reported. The findings acquired from this study may help to clarify the mode of action of this plant extract (Al-Mehmadi *et al.*, 2010). This study concludes that *A. altissima* leaf extract is a powerful larvicide against vector mosquito species and that the extract's harmful potential varies depending on the larval stage, exposure duration, and extract concentration, with a lower value of (LC_{50} 312 mg/L) as compared to the highest (LC_{50} 379 mg/L) value of *M.* azedarach. Plant extract is a cheap, safe, and simple natural product that may be used to reduce mosquito populations.

Conclusion

This study concludes that the leaves of *A. altissima* show effective larvicidal activity against *Anopheles* larvae as compared to *M. azedarach*. *A. altissima* leaf extract shows a strong larvicidal effect; the extract's harmful potential varies depending on the larval stage, exposure duration, and extract concentration, with a lower value of (LC_{50} 312 mg/L) as compared to the highest (LC_{50} 379 mg/L) value of *M.* azedarach. Plant extract is a cheap, safe, and simple natural product that may be used to reduce mosquito populations.

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