

Insecticidal effectiveness of seed and leaf oil extract of neem (*Azadirachta indica*) against larvae and adults of *Anopheles stephensi*

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ABSTRACT

This study was conducted to investigate the effect of neem (*Azadirachta indica*) seed and leaf oil extract on larvae and adults of *Anopheles stephensi* mosquitoes in the Biology Laboratory, Faculty of Education (Bayda), Omar Al-Mukhtar University, Libya. The *Anopheles* mosquito larvae 3rd larval instar was obtained from laboratory colony established in the insectary. Larvae were maintained at a temperature of $25 \pm 1^\circ\text{C}$, with a relative humidity of $80 \pm 5\%$, and a photoperiod of 12/ 12 hours light/ dark. For the larvicidal bioassay, six concentrations (50, 100, 150, 200, 250 and 300 ml/l) of seed oil and leaf extract of *A. indica*. A set of the control group using distilled water was included. The 100 % larval mortality was observed due to both *A. indica* seed oil and extract at concentration of 300 ml/l followed by 250 ml/l after exposure to 72 hours while the control treatment don't recorder any adults mortality percentage under treatment with neem oil and neem leaf extracts against *Anopheles stephensi* larvae. The results show that the mortality rate of *Anopheles stephensi* larvae increases with increasing concentration of both neem oil extract and neem leaf extract with increasing exposure period. The extracts of neem leaves of *Azadirachta indica* demonstrated strong larvicidal activity, with LC_{50} and LC_{90} values, with neem oil and neem leaf extracts.

Keywords: Neem (*Azadirachta indica*), *Anopheles stephensi* mosquitoes, larvicidal, larvicidal effects.

فعالية مستخلص زيت بذور وأوراق النيم (*Azadirachta indica*) كمبيد حشري ضد يرقات وبالغات *Anopheles stephensi*

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أجريت هذه الدراسة لدراسة تأثير مستخلص بذور وأوراق النيم (*Azadirachta indica*) على يرقات وبالغات بعوض *Anopheles stephensi* في مختبر الأحياء بكلية التربية (الببضاء)، جامعة عمر المختار، ليبيا. تم الحصول على الطور اليرقي الثالث ليرقات بعوض الأنوفيليس من مستعمرة معملية أنشئت في مختبر الأحياء. حُفظت اليرقات عند درجة حرارة 25 ± 1 درجة مئوية، ورطوبة نسبية $80 \pm 5\%$ ، وفترة ضوئية ١٢/١٢ ساعة ضوء/ظلام. لإجراء اختبار حيوي مبيد لليرقات، تم استخدام ستة تركيزات (50، 100، 150، 200، 250، 300 مل/لتر) من مستخلص زيت بذور وأوراق نبات *A. indica*. أدرجت معاملة الكنترول باستخدام الماء المقطر. لوحظت نسبة نفوق 100% لليرقات نتيجة لاستخدام كل من زيت بذور ومستخلص *Azadirachta indica* بتركيز 300 مل/لتر، يليه 250 مل/لتر بعد التعرض لمدة 72 ساعة، بينما لم تسجل معاملة الكنترول أي نسبة نفوق لليرقات البالغة بالمعاملة بزيت النيم ومستخلص أوراق النيم ضد يرقات *Anopheles stephensi*. تُظهر النتائج أن معدل نفوق يرقات *Anopheles stephensi* يزداد بزيادة تركيز كل من مستخلص زيت النيم ومستخلص أوراق النيم وزيادة فترة التعرض. أظهرت مستخلصات أوراق و زيت النيم نشاطاً قوياً في قتل اليرقات، بقيم LC_{50} و LC_{90} ، مع زيت النيم ومستخلصات أوراق النيم.

الكلمات المفتاحية: بعوض الأنوفيليس - زيت بذور ومستخلص النيم- اليرقات- الحشرات البالغة

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are major arthropod groups that existed over 180–220 million years ago (**Benelli and Duggan, 2018; Hillary and Ceasar, 2021**). Mosquitoes belong to two subfamilies: Anophelinae (Anopheles) and Culicinae (Aedes, Culex, and Mansonia), these two subfamilies pose a severe threat to humans and animals because of their widespread occurrence (**Gabrieli et al., 2014**).

According to **Govindarajan et al. (2016)**, mosquitoes continue to play a significant role in the transmission of the most deadly epidemic diseases, which threaten the lives of millions of people worldwide. Mosquito Numerous diseases are spread to humans by female mosquitoes, including malaria, which is one of the most severe diseases spread by female mosquitoes and kills over a million people annually and kills a kid every 30 seconds worldwide. Malaria is caused by the Plasmodium parasite. They also spread the nematode worms that cause elephantiasis, a disease that is not lethal but results in disability and social misery. As a source of permanent disability, it ranks second only to leprosy. Furthermore, they spread the virus that causes dengue fever, which is currently one of the most significant and pervasive viral diseases spread by mosquitoes. Indicators show that the disease is steadily increasing in both the number of endemic countries and the number of infection cases (**Govindarajan and Benelli, 2016**).

Because they are carriers for numerous harmful organisms that infect humans, mosquitoes pose a serious threat to public health, particularly in underdeveloped nations (**Medlock et al., 2015**). To name a few, they act as vectors for the spread of dengue fever, filariasis, malaria, and yellow fever (**Nazareth et al., 2016**). Africa accounted for 95% of the global malaria burden in 2020, with an estimated 241 million cases reported globally, according to the 2021 World Malaria Report. Additionally, of the 627 000 malaria deaths predicted worldwide in 2020, 96% were in the Africa Region, according to the World Health Organization (**WHO, 2021**). By using repellent, the human-vector contact transmission cycle can be interrupted (**Abiy et al., 2015**). Furthermore, utilizing bed nets and appropriate mosquito repellent on clothing has produced a lot of benefits, particularly in Africa, and protects the user (**Njumkeng et al., 2019**). Furthermore, one of the best methods for reducing mosquito bites outside is topical repellent (**Wilson et al., 2014**).

Mosquito-borne diseases pose a major threat to global health, affecting millions of people annually. The capacity of plant extracts to control mosquitoes has garnered attention as a viable environmentally friendly solution (**Ghosh et al., 2024**). Numerous fatal illnesses, including as chikungunya, dengue fever, malaria, and the Zika virus, are infamously spread by mosquitoes. These illnesses' high prevalence presents a serious threat to world health, which is why scientists are looking for more sustainable and alternative approaches to mosquito control. Utilizing plant extracts, which have been effective in deterring and eliminating disease-carrying mosquitoes, is one promising approach (**Ghosh et al., 2024**).

The use of synthetic insecticides against mosquitoes may lead to resistance development and potential health hazards in humans and the environment. Consequently, a paradigm needs to shift towards the alternative use of botanical insecticides that could strengthen an insecticide resistance management programme (**Muhammed et al., 2022**).

Synthetic insecticides have been used repeatedly to control mosquitoes, which has upset natural biological control systems and caused mosquito populations to rebound. Additionally, it has led to the emergence of resistance (**Liu et al., 2006**), unfavorable impacts on organisms that are not the intended target, and sparked environmental and human health concerns that prompt the search for substitute control methods (**Hayes and Laws 1991**). According to **Georgewill et al. (2010)**, plants are thought to be a rich source of bioactive compounds and could serve as a substitute supply of mosquito control agents.

To reduce the risk of contracting diseases spread by mosquitoes, it is possible to employ botanicals and their derivatives as insect repellent, especially against mosquitoes (**Shukla et**

al., 2018; De Sá *et al.*, 2019; Pandiyan *et al.*, 2019). Neem seed oil and other natural organic compounds derived from the *Azadirachta indica* neem tree have long been known to offer potential as mosquito repellents (Aremu *et al.*, 2009).

Alternative and environmentally friendly methods of controlling mosquitoes are now required due to the negative ecological effects of chemical pesticides and the growing problem of insecticide resistance. Examining the environmental and resistance problems related to chemical pesticides in detail is essential to improving knowledge of the topic. The various insect pest populations are managed using a variety of techniques. These techniques, which impact insect growth, development, and population, include chemical insecticides, biological control, synthetic insecticides, resistant types, and semi-chemicals (Ahmed *et al.*, 2018; Ahmed *et al.*, 2019).

Alternative, environmentally friendly mosquito control techniques are becoming more and more popular due to worries about the environment and chemical resistance in pesticides (Ahmed *et al.*, 2021; Iqbal *et al.*, 2021). According to Ahmed *et al.* (2021) and Iqbal *et al.* (2021), synthetic pesticides are now the most effective method of managing insect pests. In comparison to chemical pesticides, botanical insecticides are thought to be safer, more environmentally friendly, and biodegradable (Iqbal *et al.*, 2021; Ahmed *et al.*, 2021). Rich in bioactive substances such as flavonoids, terpenoids, phenols, and alkaloids, plant extracts have shown potential as natural insecticidal substitutes for preventing mosquito vectors (Iqbal *et al.*, 2021; Ahmed *et al.*, 2021).

The main ingredient that gives neem seeds their insect-repelling properties is azadirachtin, a complex tetra-terpenoid limonoid molecule. Furthermore, unlike synthetic DEET (N, N-diethyl-3-methylbenzamide), essential oil repellents are biodegradable, safe for the environment, and do not damage non-target organisms (Usharani *et al.*, 2019).

The neem tree, *Azadirachta indica*, is a versatile plant that has long been used in farming, traditional medicine, and even the textile industry. The neem tree produces a variety of compounds, including neem oil and neem extract, which have been demonstrated to possess insecticidal, antibacterial, and antifungal properties (Abd El-Salam *et al.*, 2024).

Of the about 99 physiologically active substances found in neem seeds, azadirachtin, nimbin, nimbidin, and nimbolides are the main molecules. Not only do these derived chemicals regulate insect growth and repel insects, but they also exhibit anti-feedancy, ovicidal activity, and fecundity suppression properties (Isman 2006). Antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer, and antifungal substances have been found in the seeds, bark, and leaves (Sudhir *et al.*, 2010). According to Patel *et al.* (2012), essential oils are complex, volatile, naturally occurring substances that have a strong smell and are produced by plants as secondary metabolites.

Neem oil has a bitter flavor, smells like sulfur or garlic, and is yellow to brown in color. It has been used to manage illnesses and pests for hundreds of years. It has a bitter taste since it is mostly made up of triglycerides and contains a lot of tri-terpenoid chemicals. According to Sudhir *et al.* (2010), the active constituents are Quercetin, Gedunin, Sodium Nimbin, Azadirachtin, Nimbin, Nimbidin, and Nimbidol.

The effectiveness of neem extracts in controlling mosquitoes has been impressive. By interfering with mosquitoes' physiological functions, the active ingredient azadirachtin impairs their capacity for eating and reproduction. Neem has been shown in studies to have larvicidal and pupicidal properties against a variety of mosquito species, including those that transmit dengue and malaria (Govindarajan *et al.*, 2016). The essential oil from neem showed good repellency against Anopheles mosquitoes, which spread malaria, according to a study by Maia and Moore (2011). Azadirachtin, a substance found in neem extracts, interferes with the mosquito's capacity to feed and procreate. Furthermore, compared to manufactured chemicals, neem extracts have a negligible environmental impact (Pramanik *et al.*, 2023).

Neem extract is especially useful in the textile sector since it can serve as a natural color fixative. This makes it a valuable component in the dyeing process since it can aid in enhancing the colorfastness of textiles. Furthermore, it has been discovered that neem extract possesses anti-bacterial qualities, which may aid in inhibiting the development of bacteria and fungi in textiles and prolonging their longevity. Neem extract is also being investigated for its potential to replace synthetic chemicals in the textile sector in a sustainable manner. Neem extract may provide a sustainable and natural way to dye and finish textiles as the market for non-toxic, environmentally friendly textile production methods expands (Abd El-Salam *et al.*, 2024).

In addition to the fact that mosquitoes did not develop resistance to chemical pesticides, some studies also demonstrated the benefits of employing medicinal plant extracts for mosquito control, stating that they are efficient and greatly lower the risks of negative environmental effects from their use. Many researchers worldwide are starting to examine some medicinal and herbal plants for mosquito control as a result of the recent surge in interest in the possible application of plant extracts as substitutes for synthetic pesticides (Choochote *et al.*, 2004).

Thus, the purpose of this study was to assess the insecticidal properties of neem (*Azadirachta indica*) seed and leaf oil extract against *Anopheles stephensi*.

MATERIALS AND METHODS

• Insect rearing

The third larval instar of *Anopheles* mosquitoes was gathered using the techniques outlined by Kauffman *et al.* (2017) from a laboratory colony set up in the insectary of the laboratory of economic entomology in the plant protection department, Faculty Education Omar Al-Mukhtar University, Al-Bayda. Under ideal conditions, the larvae were kept at $25 \pm 1^\circ\text{C}$, $80 \pm 5\%$ relative humidity, and a photoperiod of 12/12 light/dark hours.

• Collection of Anopheles Mosquito Larvae:

Larvae of the *Anopheles* mosquito, which were discovered in the stagnant water surrounding homes, were gathered using the Rathy (2015) method, which involves collecting larvae of the *Anopheles* genus for the study and using the Kent *et al.* (2005) method, which uses the larva morphology as the basis for selection and identification.

Larvicidal effects of seed oils: In a lab setting, against *Anopheles stephensi*. Different seed oil concentrations were investigated for their paricidal effects on *Anopheles stephensi* larvae.

• Statistical analysis:

Sigma Plot software Version 12.5 was used to do a one-way analysis of variance (ANOVA) on the acquired results. A P value of less than 0.05 indicated that the differences between the results were statistically significant. If necessary, all mortalities were adjusted using Abbott's (1925) formula. By dividing the number of dead and inactive larvae by the total number of larvae tested, the percentage of larval mortality was determined. Data analysis was conducted using IBM SPSS Statistics for Windows and Microsoft Excel.

RESULTS AND DISCUSSION

A) Insecticidal activity:

The insecticidal activity of neem oil (*Azadirachta indica*) and neem leaf extracts against *Anopheles stephensi* larvae in their third instar was demonstrated by the results presented in **Table (1) and Fig. (1)**. However, larvae's mean mortality increased progressively with the concentration of extract of neem oil. It was observed that after 72 hour of exposure to various concentrations of 50, 100, 150, 200, 250 and 300 mg/l) neem oil, the mean mortalities of *Anopheles stephensi* larvae were (23.33, 56.66, 71.66, 80.83, 99.58 and 100.00), respectively, while neem leaf extracts recorded the higher mean of mortality of *Anopheles stephensi* larvae after exposure to 72 hours under concentrations of 50, 100, 150, 200, 250 and 300 mg/l) were (21.66, 53.33, 68.33, 75.85, 98.61 and 100.00), respectively, When *Anopheles stephensi* larvae are treated with neem oil and leaf extracts, the control treatment does not record any adult mortality percentage. The findings indicate that when exposure duration and concentration of

neem leaf extract and neem oil extract rise, so does the mortality rate of *Anopheles stephensi* larvae.

The lethal toxicity doses of neem oil (*Azadirachta indica*) and neem leaf extracts against *Anopheles stephensi* larvae are shown in **Table (2) and Fig. (2)**. The extracts of neem leaves of *Azadirachta indica* demonstrated strong larvicidal activity, with an LC₅₀ value of 37.69 ppm and LC₉₀ value of 106.28 ml/l. In a similar vein, *Azadirachta indica* neem oil demonstrated good larvicidal activities with LC₅₀ and LC₉₀ values of 41.94 ppm and 97.02 ppm. With mortality rates that were dose-dependent and impacted by exposure duration, the results showed that the tested seed oil was effective against the target larvae. Of the seed oils, *A. indica* seed oil showed the best efficacy. Neem oil's ability to repel different mosquito species was evaluated by **Sharma, (1995)**. Plant-derived essential oils (EOs) are becoming more popular as a substitute for synthetic insecticides in the management of *Anopheles* larvae because of the spread of resistant strains of malaria vectors and the growing resistance to these pesticides (**Atenafu and Atnaf, 2025**).

In this regard, our results are in line with earlier studies that reported relatively high fatality rates in *Anopheles* species using extracts from a range of medicinal herbs (**Laojun and Chaiphongpachara, 2020**). For instance, research on *A. indica* and *O. sanctum* revealed that their bioactive phytochemicals, especially at higher concentrations, have strong larvicidal activity (**Pavela, 2015**). According to earlier studies, plant extracts had stronger larvicidal effects with extended exposure (**Ghosh et al., 2012**).

Despite their considerable larvicidal capability, synthetic larvicides like Temephos, which reliably achieve 100% mortality at the concentrations utilized, generally perform better than plant extracts. This disparity demonstrates how synthetic chemicals are more effective, yet plant-based insecticides are favored because of their safety for the environment (**Pavela, 2015; Abai et al., 2016**) and concerns over the development of resistance (**Abai et al., 2016**). The results clearly demonstrate a concentration response relationship, with higher concentrations of plant extracts associated with higher mortality rates.

It has been demonstrated that plant extracts with higher concentrations have superior larvicidal effects (**Oliveros-Díaz et al., 2022; Dey et al., 2023**). When **Dey et al. (2023)** studied extracts from *Annona muricata*, for instance, they discovered a similar pattern: higher concentrations were associated with higher mosquito larval mortality rates. This conclusion is in line with prior research that demonstrated longer exposure to plant extracts increased mosquito larval mortality rates. This is explained by the extracts' bioactive chemicals' cumulative effects, which may take some time to kill the larvae (**Daz and Rossini, 2012**).

For example, **Awosolu et al. (2018) and Chatterjee et al. (2023)** found that the LC₅₀ values for *A. indica* against *Aedes aegypti* ranged from 80 to 100 ppm, suggesting that *C. macrostachyus* may have a better larvicidal activity. **Hillary et al. (2024)** state that *C. macrostachyus*'s LC₅₀ against *Anopheles stephensi* is likewise lower than that of methanol extracts of *O. sanctum* (85 ppm). The potential of *C. macrostachyus* as an efficient all-natural pesticide is indicated by each of these findings. Depending on the plant species and extraction techniques used, these variances would suggest that the efficacy of plant extracts can vary considerably (**Njuabe et al., 2022**). According to a study (**Bagavan and Rahuman, 2011**), the presence of different phytochemicals in plant extracts may actually cause unequal biological reactions. Further evidence that variety affects *R. abyssinicus*'s efficiency may come from the species' larger confidence ranges. All *A. indica* extracts shown larvicidal action against *Anopheles* mosquito larvae, according to the experimental results of this investigation. This conclusion is consistent with a prior study conducted by **Ghosh et al. (2013)**. The extracts' larvicidal efficacy was incredibly diverse. Different phytochemical classes found in the plant may be the cause of the diverse biological actions of these plant extracts; these substances may work together or separately to provide larvicidal activity. Water, which has no effect on

mosquito larvae and exhibited an abnormal response, was utilized as the negative control. In line with a study by **Kajla et al. (2016)**, the data demonstrated that the larvicidal efficacy increased progressively as the dose was increased, reaching a peak of 100% mortality after 6 mg/ml of the extract.

Table (1): Larvicidal effect of *Azadirachta indica* seed oil and leaves extract

Conc. (mg/l)	Larval Mortality (%)					
	Neem seed oil			Neem leaves extracts		
	24h	48h	72h	24h	48h	72h
Control	0.00	0.00	0.00	0.00	0.00	0.00
50	10.00f	18.33f	23.33f	8.33f	16.66f	21.66f
100	26.66e	48.33e	56.66e	23.33e	46.00e	53.33e
150	46.66d	66.66d	71.66d	43.33d	63.33d	68.33d
200	60.00c	73.99c	80.83c	51.79c	70.30c	75.85c
250	90.42b	95.00b	99.58b	86.25b	91.39b	98.61b
300	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a

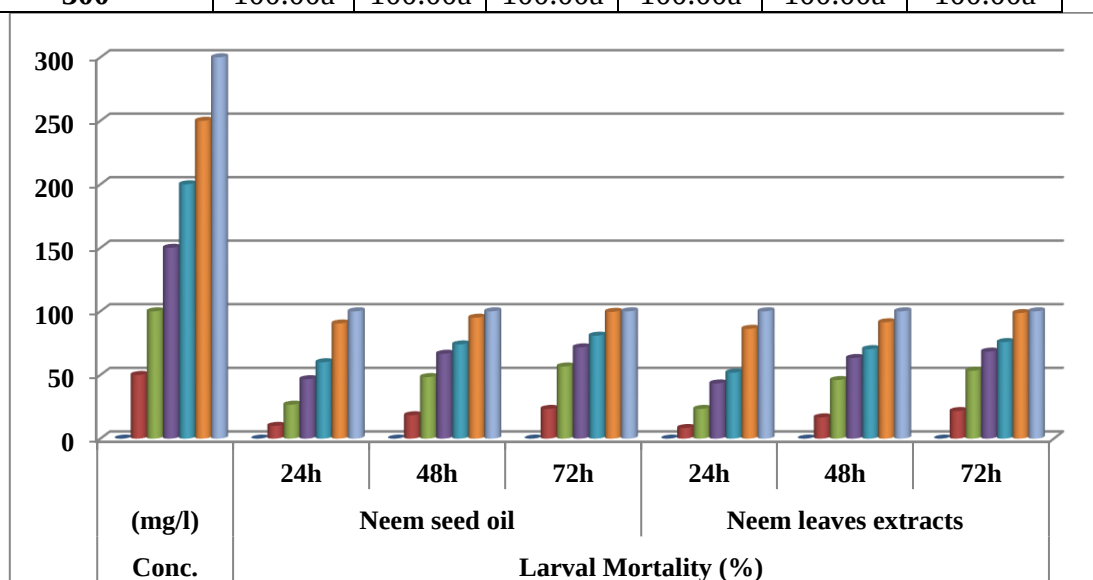


Fig. (1): Larvicidal effect of *Azadirachta indica* seed oil and leaves extract

Table (2): LC₅₀ and LC₉₀ (%) of leaf extracts of neem against the third instar larvae of *Anopheles stephensi*

Treatments	LC ₅₀	95% Confidence limit		LC ₉₀	95% Confidence limit		χ^2 (df = 5)
		LCL	UCL		LCL	UCL	
Neem seed oil	44. 52	41. 94	47. 20	97. 02	84. 78	107. 81	4.51
Neem leaves extracts	37. 69	36. 57	42.28	106. 28	95.72	120. 30	2.39

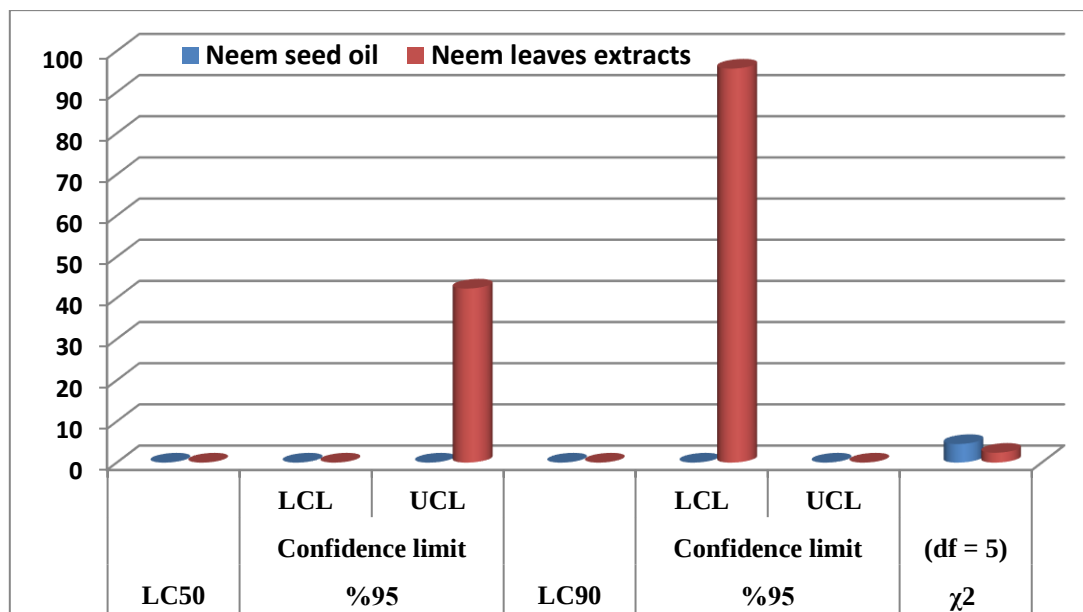


Fig. (2): LC₅₀ and LC₉₀ (%) of leaf extracts of neem against the third instar larvae of *Anopheles stephensi*

B) Adulticidal activities:

The Adulticidal activity of neem oil (*Azadirachta indica*) and neem leaf extracts against *Anopheles stephensi* adults in their third instar was demonstrated by the results presented in **Table (3) and Fig. (3)**. However, mean adults mortality percentage increased progressively with the concentration of extract of neem oil. It was observed that after 72 hour of exposure to various concentrations of 50, 100, 150, 200, 250 and 300 mg/l) neem oil, the mean mortalities of *Anopheles stephensi* adults were (11.67, 15.00, 18.33, 26.67, 55.00 and 84.00), respectively, while neem leaf extracts recorded the higher mean of mortality of *Anopheles stephensi* adults after exposure to 72 hours under concentrations of 50, 100, 150, 200, 250 and 300 mg/l) were (10.50, 13.50, 16.50, 24.00, 49.50 and 75.60), respectively, Neem oil and leaf extracts are used to treat *Anopheles stephensi* adults, while the control therapy does not record any adult mortality percentage. According to the findings, the mortality rate of adult *Anopheles stephensi* rises as exposure duration and concentration of neem leaf and oil extracts increase.

The lethal toxicity doses of neem oil (*Azadirachta indica*) and neem leaf extracts against *Anopheles stephensi* adult are shown in **Table (4) and Fig. (4)**. The extracts of neem leaves of *Azadirachta indica* demonstrated strong adulticidal activity, with an LC₅₀ value of 333.27 ppm and LC₉₀ value of 1031.74 ppm. In a similar vein, *Azadirachta indica* neem oil demonstrated good adulticidal activities with LC₅₀ and LC₉₀ values of 297.75 ppm and 762.63 ppm.

Depending on their action, plant extracts can be applied as repellents to prevent mosquito bites or as insecticides to kill adult or larval mosquitoes (**Sukumar et al., 1991**). Different phytochemical classes found in the plant may be the cause of the diverse biological actions of these plant extracts; these substances may work together or separately to provide larvicidal activity. Water, which has no effect on mosquito larvae and elicits a typical response, was employed as the negative control (**Chombo, 2021**). The outcome is consistent with research by **Okumu et al. (2007)** and **Ayinde et al. (2020)**, which claimed that neem oil formulation is a very successful larvicide for controlling anopheline mosquito vectors. Additionally, under field settings, *A. indica* oil has demonstrated strong larvicidal efficacy against many mosquito genera, such as *Aedes*, *Anopheles*, and *Culex* (**Mgbemena, 2010**).

Table (3): Adulticidal activities of *Azadirachta indica* seed oil and leaves extract

Conc. (mg/l)	Adult Mortality (%)					
	Neem seed oil			Neem leaves extracts		
	24h	48h	72h	24h	48h	72h
Control	0.00	0.00	0.00	0.00	0.00	0.00
50	5.33	7.76	11.67	4.80	6.98	10.50
100	8.20	8.67	15.00	7.38	7.80	13.50
150	13.33	16.67	18.33	12.00	15.00	16.50
200	18.33	21.67	26.67	16.50	19.50	24.00
250	23.33	48.33	55.00	21.00	43.50	49.50
300	61.55	65.00	84.00	55.40	58.50	75.60

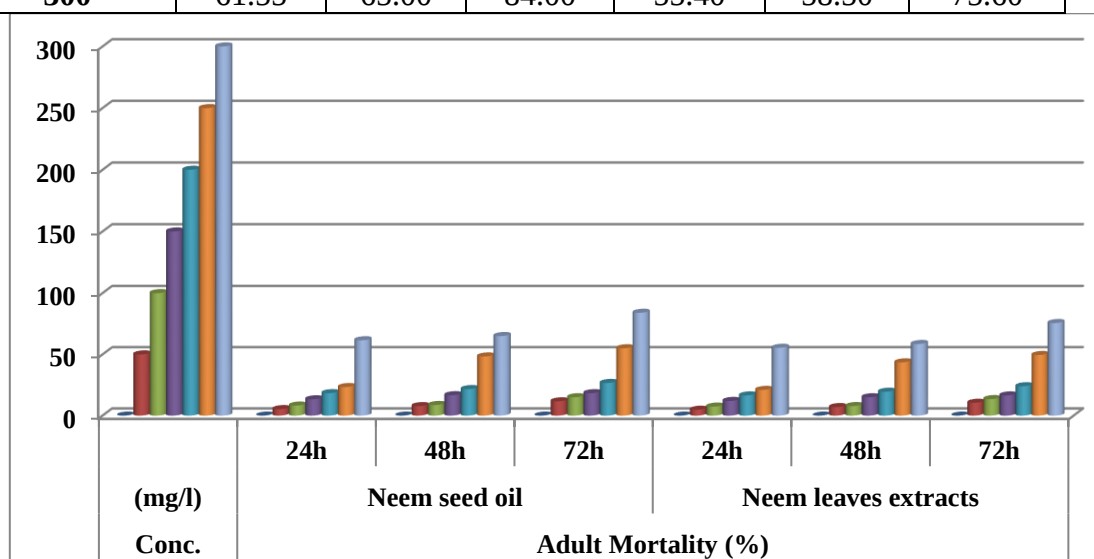


Fig. (3): Adulticidal activities of *Azadirachta indica* seed oil and leaves extract

Table (4): LC₅₀ and LC₉₀ (%) of leaf extracts of neem against the third instar larvae of *Anopheles stephensi*

Treatments	LC ₅₀	95% Confidence limit		LC ₉₀	95% Confidence limit		χ^2 (df = 5)
		LCL	UCL		LCL	UCL	
Neem seed oil	297. 75	254. 95	340. 56	762. 63	491. 13	1034. 13	0.88
Neem leaves extracts	333.27	268.92	397.63	1031.74	569.06	1494.43	0.75

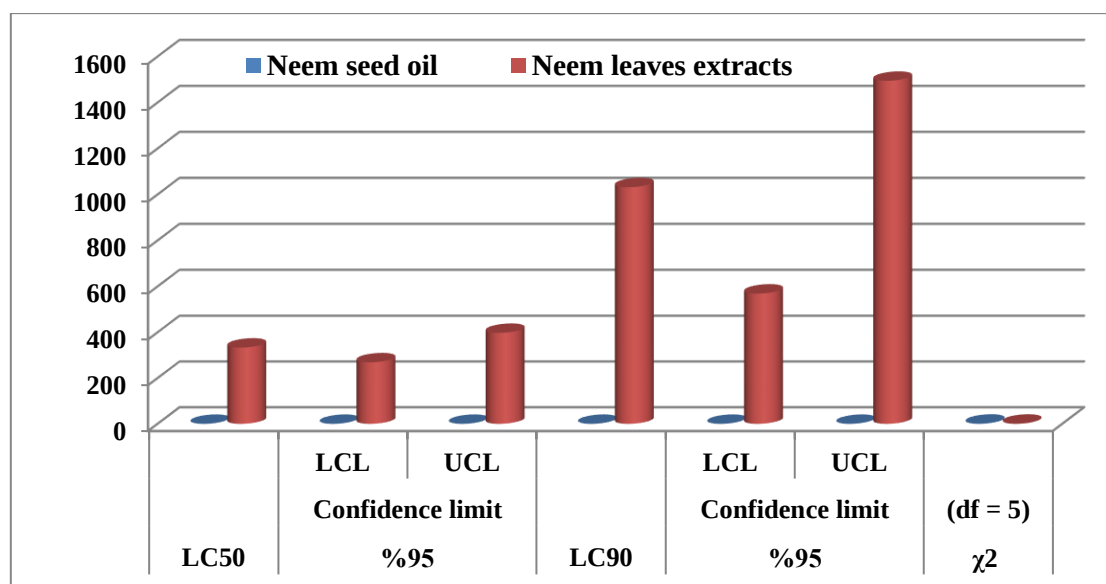


Fig. (4): LC₅₀ and LC₉₀ (%) of leaf extracts of neem against the third instar larvae of *Anopheles stephensi*

CONCLUSION

According to the results of this study, the neem plant, *Azadirachta indica*, has a larvicidal effect on mosquitoes. Because the plant's leaf extract is extremely poisonous, even at low concentrations, it may one day be used as a larvicide. To identify the active ingredients and ideal dosages that provide the larvicidal action, more research is necessary. This plant's product can also be used to make phytochemicals or biocides that can protect non-target organisms from dangerous vectors. This plant would be environmentally beneficial and could be a good substitute for synthetic larvicides, which are widely accessible, reasonably safe, and inexpensive in many parts of the world. This plant is environmentally beneficial and could be a good substitute for synthetic larvicides, which are widely accessible, safe, and reasonably priced worldwide.

REFERENCES

- Abai, M.R., Hanafi-Bojd, A.A. and Vatandoost, H. (2016). Laboratory evaluation of temephos against *Anopheles stephensi* and *Culex pipiens* larvae in Iran. J. Arthropod Borne Dis., 10: 510–518.
- Abd El-Salam, N. A., Shahin, A. R., Othman, H. A. and Hassabo, A. G. (2024). Neem tree extracts used in textile industries. Chem., 67: 159–169.
- Abiy, E., Gebre-Michael, T. and Balkew, M. (2015). Repellent efficacy of DEET, MyggA, neem (*Azadirachta indica*) oil and chinaberry (*Melia azedarach*) oil against *Anopheles arabiensis*, the principal malaria vector in Ethiopia. Malar J., 14: 1–6.
- Ahmed, N., Alam, M., Saeed, M., Ullah, H., Iqbal, T., Al-Mutairi, K.A. and Salman, M. (2021). Botanical insecticides are a non-toxic alternative to conventional pesticides in the control of insects and pests. In: Global Decline of Insects. IntechOpen.
- Ahmed, N., Chamila Darshanee, H.L., Fu, W.Y., Hu, X.S., Fan, Y. and Liu, T.X. (2018). Resistance of seven cabbage cultivars to green peach aphid (Hemiptera: Aphididae). J. Econ. Entomol., 111(2): 909–916.
- Ahmed, N., Darshanee, H.L.C., Khan, I.A., Zhang, Z.F. and Liu, T.X. (2019). Host selection behavior of the green peach aphid, *Myzus persicae*, in response to volatile organic compounds and nitrogen contents of cabbage cultivars. Front. Plant Sci., 10: 1–79.

- Aremu, O. I., Femi-Oyewo, M. N. and Popoola, K. O. K. (2009).** Repellent action of neem (*Azadirachta indica*) seed oil cream against *Anopheles gambiae* Mosquitoes. Afri. Rev. Res. J., 3 (3): 12-22
- Aremu, O., Femi-Oyewo, M. and Popoola, K. (2009).** Repellent action of neem (*Azadirachta indica*) seed oil cream against *Anophele gambiae* Mosquitoes. Afri. Res Rev., 3: 12–22.
- Atenafu, G. and Atnaf, N. (2025).** Efficacy of seed oils from *Azadirachta indica* and *Schinus molle* and their combination against *Anopheles gambiae* s.l. J. Trop. Med., 1- 8.
- Awosolu, O., Adesina, F. and Iweagu, M. (2018).** Larvicidal effects of croton (*Codiaeum variegatum*) and neem (*Azadirachta indica*) aqueous extract against *Culex mosquito*. Int. J. Mosq. Res., 5: 15–8.
- Ayinde, A.A., Morakinyo, O.M. and Sridhar, M.K.C. (2020).** Repellency and larvicidal activities of *Azadirachta indica* seed oil on *Anopheles gambiae* in Nigeria. Heliyon., 6: 03920
- Bagavan, A. and Rahuman, A.A. (2011).** Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors. Asian Pac. J. Trop. Med., 4: 29–34.
- Benelli, G. (2020).** Plant-borne compounds and nanoparticles: challenges for medicine, parasitology and entomology. Environ. Sci. Poll. Res., 25: 10149–10150.
- Benelli, G. and Duggan, M.F. (2018).** Management of arthropod vector data – Social and ecological dynamics facing the One Health perspective. Acta Trop., 182: 80–91.
- Chatterjee, S., Bag, S., Biswal, D., Sarkar, P. D., Bandyopadhyay, R. and Sarkar, B. (2023).** Neem-based products as potential eco-friendly mosquito control agents over conventional eco-toxic chemical pesticides: A review. Acta Trop., 240: 106858.
- Choochote, W., Tuetun, B., Kanjanapothi, D., Rattanachanpichai, E., Chaithong, U., Chaiwong, P. and Pitasawat, B. (2004).** Potential of crude seed extract of celery, *Apium graveolens* L., against the mosquito *Aedes aegypti* (L.)(Diptera: Culicidae). J. Vector Ecol., 29 (2): 340–346.
- Daz, M. and Rossini, C. (2012).** Bioactive natural products from Sapindaceae: deterrent and toxic metabolites against insects. In: Perveen F(ed) Insecticidal pest engineering. IntechOpen.
- De Sá, E.L.R., Rodovalho, C.D.M. and De Sousa, N.P.R. (2019).** Evaluation of insecticide resistance in *Aedes aegypti* populations connected by roads and rivers: The case of tocanins state in Brazil. Mem Inst Oswaldo Cruz., 114.
- Dey, P., Mandal, S., Goyary, D. and Verma, A. (2023).** Larvicidal property and active compound profiling of *Annona squamosa* leaf extracts against two species of Diptera, *Aedes aegypti* and *Anopheles stephensi*. J. Vector Borne Dis., 60: 401–413.
- Gabrieli, P., Smidler, A. and Catteruccia, F. (2014).** Engineering the control of mosquito-borne infectious diseases. Genome Biology 15: 1–9.
- Georgewill O.A., Georgewill U.O. and Nwankwoala R.N.P. (2010).** Anti-inflammatory effects of *Moringa oleifera* lam extract in rats. Asian Pac J Trop Med. 3(2): 133-135.
- Ghosh, A., Chowdhury, N. and Chandra, G. (2013).** Plant extracts as potential mosquito larvicides. The Indian J. Med. Res., 135(5): 581–598.
- Ghosh, T., Saha, S., Dutta, A., Parida, S., Mondal, M. and Khalua, R. K. (2024).** Plant extracts in controlling disease vector mosquitoes. Int. J. Mosquito Res., 11(1): 89-91.
- Govindarajan, M. and Benelli, G. (2016).** a-Humulene and b-elemene from *Syzygium zeylanicum* (Myrtaceae) essential oil: highly effective and eco-friendly larvicides against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus* (Diptera: Culicidae). Parasitol. Res., 115 (7): 2771–2778.
- Govindarajan, M. and Benelli, G. (2017).** A facile one-pot synthesis of eco-friendly nanoparticles using *Carissa carandas*: ovicidal and larvicidal potential on malaria, dengue and filariasis mosquito vectors. J. Clust. Sci., 28: 15–36.

- Govindarajan, M., Rajeswary, M., Hoti, S.L. and Benelli, G. (2016).** Larvicidal potential of carvacrol and terpinen-4-ol from the essential oil of *Origanum vulgare* (Lamiaceae) against *Anopheles stephensi*, *Anopheles subpictus*, *Culex quinquefasciatus* and *Culex tritaeniorhynchus* (Diptera: Culicidae). Res. Vet. Sci., 104: 77–82.
- Hayes, J.B. and Laws, E.R. (1991).** Handbook of pesticide toxicology. San Diego: Academic Press.
- Hillary, V.E. and Ceasar, S.A. (2021)** Genome engineering in insects for the control of vector borne diseases. Prog. Mole. Biol. Transl. Sci., 179: 197–223.
- Hillary, V.E., Ceasar, S.A. and Ignacimuthu, S. (2024).** Efficacy of plant products in controlling disease vector mosquitoes: a review. Entomol Exp. Appl., 172: 195–214.
- Iqbal, T., Ahmed, N., Shahjeer, K., Ahmed, S., Al-Mutairi, K.A., Khater, H.F. and Ali, R. F. (2021).** Botanical insecticides and their potential as anti-insect/pests: are they successful against insects and pests? In: Global Decline of Insects.
- Isman, M. B. (2006).** Botanical insecticides, deterrent and repellents in modern agriculture and an increasingly regulated world. Ann. Rev. Entom., 51: 45-66
- Jacobson, M. (1990).** Review of neem research in the United States, In: Locke, J. C. and Lawson, R. H. (eds.) Proceedings of a workshop on neem's potential in pest management programs, USDA - Ars. Beltsville, MD. Ars., 86: 4 – 14.
- Kajla, M., Bhattacharya, K., Gupta, K., Banerjee, U., Kakani, P., Gupta, L. and Kumar, S. (2016).** Identification of the temperature induced larvicidal efficacy of agave angustifolia against *Aedes*, *Culex*, and *Anopheles* Larvae. Front. Publ. Health, 3: 286.
- Kauffman, E., Payne, A., Franke, M.A., Schmid, M.A., Harris, E. and Kramer, L.D. (2017).** Rearing of *Culex spp.* and *Aedes spp.* mosquitoes. Bio-protocol, 7(17): 2542-2542.
- Kent, S. and Chester, J. (2005).** Characteristics of Anophelines and Culicine. Dep. Health Human Serv., 1- 20
- Korunić, Z.K., Rozman, V., Liška, A. and Lucić, P. (2016).** A review of natural insecticides based on diatomaceous earths. Poljoprivreda, 22(1): 10–18.
- Laojun, S. and Chaiphongpachara, T. (2020).** Comparative study of larvicidal activity of commercial essential oils from aromatic rosemary, vanilla, and spearmint against the mosquito *Aedes aegypti*. Biodiver., 21: 2383–9.
- Li, M., Xia, Q., Lv, S., Tong, J., Wang, Z., Nie, Q. and Yang, J. (2022).** Enhanced CO₂ capture for photosynthetic lycopene production in engineered *Rhodospseudomonas palustris*, a purple nonsulfur bacterium. Green Chem., 24 (19): 7500–7518.
- Liu, N. (2015).** Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. Annu. Rev. Entomol., 60: 537–559.
- Liu, N., Xu, Q., Zhu, F. and Zhang, L. (2006).** Pyrethroid resistance in mosquitoes. Insect Sci., 13:159–166.
- Maia, M.F. and Moore, S.J. (2011).** Plant-based insect repellents: a review of their efficacy, development and testing. Malar. J., 10(1): 1–15.
- Medlock, J.M., Hansford, K.M. and Versteirt, V. (2015).** An entomological review of invasive mosquitoes in Europe. Bull. Entomol. Res., 105: 637–663.
- Mgbemena, I.C. (2010).** Comparative Evaluation of Larvicidal Potentials of Three Plant Extracts on *Aedes aegypti*. J. Amer. Sci., 6(10): 435-440.
- Muhammed, M., Dugassa, S., Belina, M., Zohdy, S., Irish, S. R. and Gebresilassie, A. (2022).** Insecticidal effects of some selected plant extracts against *Anopheles stephensi* (Culicidae: Diptera). Malaria J., 21(295): 1-10.
- Nazareth, T., Seixas, G. and Sousa, CA. (2016).** Climate Change and Mosquito-Borne Diseases. Clim Chang Manag; 109: 187–199.

- Njuabe, M.T., Fru, C.T., Desire, S. and Awono-Ambene, H. P. (2022).** Larvicidal Activity of *Momordica foetida* (Cucurbitaceae), *Gnidia glauca* (Thymelaeaceae) and *Vepris soyauxii* (Rutaceae) extracts on *Anopheles gambiae* mosquitoes and their acute toxicity on rats. J. Environ. Sci. Pub. Health, 6: 31–45.
- Njumkeng, C., Apinjoh, T.O. and Anchang-Kimbi, J.K. (2019).** Coverage and usage of insecticide treated nets (ITNs) within households: Associated factors and effect on the prevalence of *Malaria parasitemia* in the Mount Cameroon area. BMC Public Health, 19: 1216.
- Okumu, F.O., Knols, B.G.J. and Ulrike F. U. (2007).** Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. Malaria J., 6:63
- Oliveros-Díaz, A.F., Pájaro-González, Y., Cabrera-Barraza, J., Hill, C., Quiñones, F. W. and Olivero-Verbel, J. (2022).** Larvicidal activity of plant extracts from Colombian North Coast against *Aedes aegypti* L. mosquito larvae. Arab J. Chem., 15: 104365.
- Pandiyan, G.N., Mathew, N. and Munusamy, S. (2019).** Larvicidal activity of selected essential oil in synergized combinations against *Aedes aegypti*. Ecotoxicol. Environ. Saf., 174: 549–556.
- Patel, E. K., Gupta, A. and Oswal, R. J. (2012).** A review on: mosquito repellent methods. Int. J. Pharmac., Chem. Biol. Sci., 2(3): 310-317
- Pavela R. (2015).** Essential oils for the development of eco-friendly mosquito larvicides: a review. Indian Crops Prod., 76:174–87.
- Pavela, R. and Benelli, G. (2016).** Essential oils as eco-friendly biopesticides? Challenges and constraints. Trends Plant Sci., 21 (11): 1000–1007.
- Pramanik P, Tewari S, Mukherjee P, Banka R, Sen S. (2023).** Indian traditional plants: Medicinal properties and Human health. J. Surv. Fisher. Sci., 10 (1): 6170-6179.
- Qualls, W.A. and Xue, R.D. (2009).** Field evaluation of three botanical repellents against *Psorophora ferox*, *Aedes atlanticus*, and *Aedes mitchellae*. J. Am. Mosq. Control Assoc., 25 (3): 379–381.
- Rahuman, A.A., Bagavan, A., Kamaraj, C., Saravanan, E., Zahir, A.A. and Elango, G. (2009).** Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol. Res., 104 (6): 1365–1372.
- Rathy, M. C., Sajith, U. and Harilal, C. C. (2015).** Larvicidal efficacy of medicinal plant extract against the vector mosquito *Aedes Albopictus*. Int. J. Mosquito Res., 2(2): 80-82.
- The Role of Agricultural Extension in Using Digital Transformation to Address Salinity Problems and Decreasing Water Resource Efficiency in the Al-Jabal Al-Akhdar Region - Libya. (2026). *Al-Farooq Journal of Sciences*, 2(2), 140-154. <https://doi.org/10.65405/6mdyya78>
- Sharma, S. K., Dua, V. K. and Sharma, V. P. (1995).** Field studies on the mosquito repellent action of neem oil. Southeast Asian J. Trop. Med. Pub. Health, 26(1): 180-192.
- Sharma, V. P., Ansari, M. A. and Razdan, R. K. (1993).** Mosquito repellent action of neem (*Azadirachta indica*) oil. J. Amer. Mosquito Cont. Association, 9(3): 359-366
- Shukla, D.K., Wijayapala, S. and Vankar P.S. (2018).** Effective mosquito repellent from plant based formulation. Popul Dyn Aedes Mosq larvae from peridomestic water bodies, 5: 19–24.
- Sudhir, K. P., Debasis, M. Goutam, G. and Chandra S. P. (2010).** Biological action and medicinal properties of various constituent of *Azadirachta indica* (Meliaceae)” an Overview, Ann. Biol. Res., 1(3): 24-34.
- Sukumar, K., Perich, M.J. and Boobar, L.R. (1991).** Botanical derivatives in mosquito control: a review. J. Am. Mosq. Cont. Assoc., 7: 210–37.

- Usharani, K., Dhananjay, N. and Manjunatha, R. (2019).** Neem as an organic plant protectant in agriculture. 8: 4176 4184.
- WHO, World Malaria Report (2021).**
Evaluation of the antifungal efficacy of *Salvia officinalis* extract against some clinical oral candidiasis isolates: a comparative study with standard antifungals. (2026). مجلة الفاروق للعلوم, 2(1), 1242-1252. <https://doi.org/10.65405/95gkdw08>
- Wilson, A.L., Chen-Hussey, V. and Logan, J.G. (2014).** Are topical insect repellents effective against malaria in endemic populations? A systematic review and meta-analysis. Malar J., 13: 446.
- Zhang, J., Shen, Q., Ma, Y., Liu, L., Jia, W., Chen, L. and Xie, J. (2022).** Calcium homeostasis in Parkinson's disease: from pathology to treatment. Neurosci. Bull., 38 (10): 1267–1270.