Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

The Effect of Azadirachta Indica (Neem) & Moringa Olifera Leaves on Control of Anopheles Mosquito Larvae

Najlaa Siddig Nasir[1], Yasir Ismail Adam[1], Emad A Abass[1], Mohamed Osman Elamin[2], Ali. M. Alshehri[2], Hatim. A. Natto[2], Hatim M. Badri[2], Wahaj A. Khan[2], Ahmed A. Osman[2], Sami Melebari[3]

[1]Department of Vector Control, Al-Zaiem Al-Azhri University, Sudan.
[2]Department of Environmental and Occupational Health, Faculty of Public Health & Informatics, Umm Al-Qura University, KSA, mobushara@uqu.edu.sa
[3]Department of Molecular Biology, MOH, KSA.

Control of Anopheles mosquitoes by use of chemical insecticides could have many disadvantages on the environment, human health, and non-target species. The search for herbal preparation and pure compounds that have no produce adverse effect on the non-target organisms, along with the scarce effect on the environmental characteristics, remains a top priority of research for scientists associated with the development of alternative vector control measures (1). We aimed to measure the effect of herbal extracts of moringa and neem in controlling Anopheles which are friendly to the environment and at the same time effective on controlling Anopheles larvae. An experimental study was carried out to assess the effect of Moringa olifera and Azadirachta indica (neem) leaves in control of Anopheles arabiensis mosquito larvae collected from rural areas in Sudan. Mosquitoes were collected from the 3rd and 4th instar larvae. Stock solutions of each plant were prepared with different dilutions of 40ml, 20ml, 10ml, and 5ml a control of larvae was prepared. Mortality was recorded after 24 hours. Showed that both plant extracts were toxic to mosquito larvae. The comparative toxicity was in favor of moringa olifera more than Azadirachta indica (neem). The mortality rate was recorded at (40 ml) concentration of Moringa olifera (93.3%) and Azadirachta indica (76.6%) 40ml. The two tested plants can be considered good larvicides. Triterpenoid is the active substance in neem leaves which is toxic to the Anopheles larvae. The study concluded that the aqueous extracts of Moringa olifera texture were observed more viscous than Azadirachta indica (neem), and this property may increase the mortality rate.

Keywords: Azadirachta Indica, Moringa olifera, Anopheles mosquito, Control, Larvae.

1. Introduction

Malaria is a major public health problem and is a cause of suffering and premature death in tropical and subtropical countries. In many endemic areas, it is becoming increasingly difficult to control because of the resistance of the parasite to anti-malarial drugs and the failure of

vector control measures. Malaria is endemic in 91 countries with about 40% of the world's population at risk. Each year there are 300-500 million clinical cases of malaria (90% of them in Africa) resulting in 1.5-2.7 million deaths, mostly children under 5 years [1, 2]. Epidemics are recurring in areas where transmission has been interrupted. Urban and peri-urban malaria are now substantial problems in certain areas of Africa and Asia. Increased population movement caused by war and socio-economic factors often culminates in malaria epidemics with high death rates. Three species of Anopheles are responsible for malaria transmission in Sudan, namely Anopheles Arabiensis, the most widespread. The other two species are Anopheles funestus and Anopheles gambiae [1, 3]. There are several methods of controlling mosquitoes but often a combination of methods will produce the best results. Natural methods are preferable before considering the use of pesticides. Control of mosquito larvae is generally more effective than controlling adult mosquitoes [4, 5]. Mosquitoes cannot be controlled easily or effectively without a proper understanding of their biology. It is essential to know, for instance, where mosquitoes breed and what factors favor their breeding, their flight range, and their biting and resting habits. measures taken to control mosquitoes, depending on particular circumstances, may be directed against the immature stages or the adult mosquitoes, or simply to prevent contact between man and the insect. The control of the aquatic stages of mosquitoes: This entails both deterring the breeding of mosquitoes and destroying eggs, larvae, and pupae that may be present in the breeding water. The measures of control may be chemical, biological, or mechanical [4, 6].

Chemical measures: The use of larvicides, chemicals of various kinds which can be applied to the surface of the water of the breeding place to destroy the mosquito larvae, as well as their eggs and pupae. The larvicides in common use include oils, Paris green, and several insecticides such as Dichloro-Diphenyl-Trichloroethane (DDT), gammaxene, and dieldrin. The basic weakness of all larvicides is that they must be applied frequently. Many are not effective for more than a few days, and are generally employed where permanent methods of control are not feasible, or as temporary expedients until permanent measures can be installed [4, 7].

Herbal Plants control: Most plants contain compounds that may be used in preventing mosquitoes and other insects. These chemicals fall into several categories, including repellents, feeding deterrents, and toxins [8].

1- Neem (Azadirachtaindica):

Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Order: Sapindales

Family: Meliaceae

Genus: Azadirachta

Species: A. indica

Distribution: Azadirachtaindica is located in northern Australia, tropical Asia, Africa, Fiji,

Mauritius, Puerto Rico, the Caribbean, and many countries in South and Central America.

Habitat: Azadirachta indica can invade shrublands, open woodlands, grasslands, floodplains, riparian zones (banks of watercourses), coastal sites, and other distributed natural vegetation.

Description: Azadirachta indica is a fast-growing tree that can reach a height of 35-40 m. It is ever-green, but in severe drought, it may shed most or nearly all of its leaves. The branches are spread wide. The opposite, simple pinnate(once-divided) leaves are 20-40 cm long. Very young leaves are reddish to purplish. The shape of mature leaflets is more or less asymmetric and their margins are toothed (dentate).

The right and fragrant flowers arise from the junction of the stem and the petiole normally in more or less drooping flower clusters which are up to 25 cm long.

The fruit is a smooth olive-like drupe that varies in shape from elongate oval to nearly roundish, and when ripe is 1.4-2.8x1.0-1.5 cm. The fruit skin is thin and turns yellow when ripe. The better-sweet pulp is yellowish-white and very fibrous.

Uses of neem:

Neem extracts are frequently used in shampoo, toothpaste, soap, cosmetics, insect repellent, lotions and creams, and pet shampoo. Its high vitamin E content makes it effective in treating skin conditions such as eczema, psoriasis, acne, and skin allergies. Neem extract is effective in eliminating bacterial and fungal infections or parasites while its antiviral properties treat warts and cold sores. If applied in a paste, it soothes inflammation and reduces redness, moisturizes the skin keeps it smooth, and can lighten scars and pigmentation.

An extract called salannin from neem leaves is safer and more effective than synthetic insect repellents such as N, N-diethylm-toluamide (DEET). It repels mosquitoes, biting flies, sand fleas, and ticks. Adding neem oil to shampoo can also reduce scalp itching and dandruff. All parts of the neem plant are useful when ground into mulch and applied to soil. It can neutralize acidic soil, improve the water stagnant capacity, and improve the nutrient quality of the soil because the deep tap roots draw nutrients below the ground. Its rapid growth rate means that the neem tree is also sustainable as source of firewood [9].

1- Moringa oleifera:

Moringa oleifera is one of the world most useful trees. Almost every part of the tree can be used for food, medication, and industrial purposes.

Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Brassicales

Family: Moringaceae

Genus: Moringa

Species: Oleifera

Distribution: Moringa oliefera growth in any tropical and subtropical country with peculiar environmental features, namely, dry to moist tropical and subtropical climate. Moringa oleifra has found wide acceptance among various ethnicities in Nigeria, and its distribution is mainly in the Middle East, African, and Asian countries and is still spreading to other areas [10].

Habitat: This tree grows in a wide variety of soils, but it prefers well-drained, sandy, or loamy soils. It grows best in temperature within the range of 25-35 ○C.

Description: Moringa is a fast-growing, evergreen, deciduous tree of height 10-12 m. The leaves are bi-pinnate or more commonly tri-pinnate, up to 45 cm long. The flowers are fragrant and bisexual, surrounded by five unequal thinly veined yellowish-white penal. The fruits are pendulous, linear, and 20-50 in long.

Uses of moringa: It is used as a good food as it is highly nutritious. Moreover, it has medical uses as its flowers, leaves, and roots are used for the treatment of ascites, rheumatism, and venomous bites and as cardiac and circulatory stimulants in folk remedies. The oil is applied externally for skin disease. Leaf juice is used in hiccoughs, cooked leaves are given in influenza. The root bark is used as an antiviral, anti-inflammatory, and analgesic.

Moringa supports a healthy cardiovascular system, promotes normal blood glucose levels, neutralizes free radicals that cause cancer, and supports the immune system, mental alertness, and bone strength. It has potential benefits for malnutrition, general weakness, lactating mothers, menopause, depression, and osteoporosis [11].

In this study, we aimed to measure the effect of herbal extracts of moringa and neem in control Anopheles.

2. Materials and Methods

This is an experimental study conducted to explore the effect of Azadirachta indica (neem) and Moringa oleifera leaves in control of Anopheles mosquito larvae.

Study area: Khartoum state, East-Nile locality-Elmahas Kutrang. Elmahas Kutrang is an agricultural region. Depended on groundwater for drinking, located near the Blue Nile River, and surrounded by three villages (Kutrang on the east side, Albanbonab on the west side, and Elmahas Tyba on north west side). On the south side, there are farms and pyres of animals on the extent of the river. On the north side, there was a large agricultural project (Dal Company).

Materials

Test tubes 2-Petri dishes3-Syringes 1-10 ml4-Plastic cups5-Dishes-spoons 6-Conical flask.7-Gauze.8-Bucket.9- Electric grinder 10-Plastic mesh.

Sample collection: 300 Anopheles arabiensis samples from 3rd-stage and 4th-stage larvae were collected by a spoon from larval habitats at Elmashas kutrang village-East Nile locality. Larvae were then placed in dishes and transmitted to the laboratory of the faculty of laboratory science Alzaem Alazhari University. Larvae were left overnight for 24 hours to get used to the environment of the lab before starting the experiments.

Experimental works: The experiment was performed according to WHO protocol for sensitivity tests [12]. 10 larvae were used for each concentration placed in five containers and 10 other larvae were used as a control group. The percentage mortality was calculated and then converted to probit units and log. Concentration was calculated and drawn to obtain a toxicity line and the LC50. readings were taken after 24 hours for each concentration.

Preparation of extracts: -15g,30g, and 45g of Neem and Moringa leaves were dried under the shed and then ground by an electric grinder into a powder. The powder was weighted by a sensitive balance scale, then 500ml of distilled water was added for each weight and left for 24h in the dark. 5ml,10ml,20ml, and 40ml concentrations were prepared from each extract for experiments to observe their effects on larvae. Concentrations were poured in plastic cups and distilled water was added to complete the volume to 200ml. 10 Larvae were picked by a dropper and placed in each concentration and experiments were repeated 3 times for each Neem and moringa extract.

Result analysis: Results were analyzed using probit regression analysis. A toxicity line was drawn and the LC50 was calculated.

3. Results

Table 1: Percentage mortality of Anopheles larvae exposed to different concentrations of Moringa leaves extracts for 24 hours. The mortality was obtained at a concentration (40 ml/l) (93.3%) followed by the concentration (20 ml/l) (66.6%) (Table 1).

Table 1. The percentage of Anopheles larvae exposed to moringa leaves extracts.

Concentration ml/l	No. of larvae exposed	No of dead larvae at each replicate			Total dead larvae	Mortality %
		Rep. 1	Rep. 2	Rep. 3		
40	30	8	10	10	28	93.3%
20	30	2	8	10	20	66.6%
10	30	4	8	6	18	60%
5	30	0	7	0	7	23%
Control	30	0	0	0	0	0

The mortality was obtained at concentration (40 ml/l) (76.6%) followed by the concentration (20 ml/l) (56.6%). Percentage mortality of Anopheles larvae exposed to different concentrations of Neem leaves extracts for 24 hours (Table 2).

Table 2. The percentage of mortality of Anopheles larvae exposed to Neem leaves extracts.

Concentration ml	No. of larvae exposed	No of dead larvae at each replicate			Total dead larvae	% mortality
		Rep. 1	Rep. 2	Rep. 3		
40	30	9	5	9	23	76.6%
20	30	6	2	9	17	56.6%
10	30	0	2	8	10	33.3%
5	30	0	2	7	9	30%
Control	30	0	0	0	0	0%

Probit analysis of two plants extracts showed that the highest mortality rate of moringa extract at concentration of 40 ml/l was 93.3% versus 76.6% in neem. While the lowest mortality rates were at concentration of 5ml/l yield 23% in moringa versus 30% in neem (Table 3).

Table 3. The comparative toxicity of water extracts of Moringa and Neem leaves.

Concentration ml/l	Moringa	Neem
40	93.3%	76.6%
20	66.6%	56.6%
10	6o%	33.3%
5	23.3%	30%
Control	0%	0%

Table 4. The concentration, log. Concentration, and probit unit of Anopheles larvae exposed to different concentrations of moringa leaves extracts.

Concentration ml	Log concentration	% mortality for different experiments			% (mean) mortality	Probit unit
		1	2	3		
40	1.6	80	100	100	93.3	6.84
20	1.3	20	80	100	66.6	5.41
10	1	40	80	60	60	5.25
5	0.7	0	70	0	23	4.26
Control	-	0	0	0	0	0

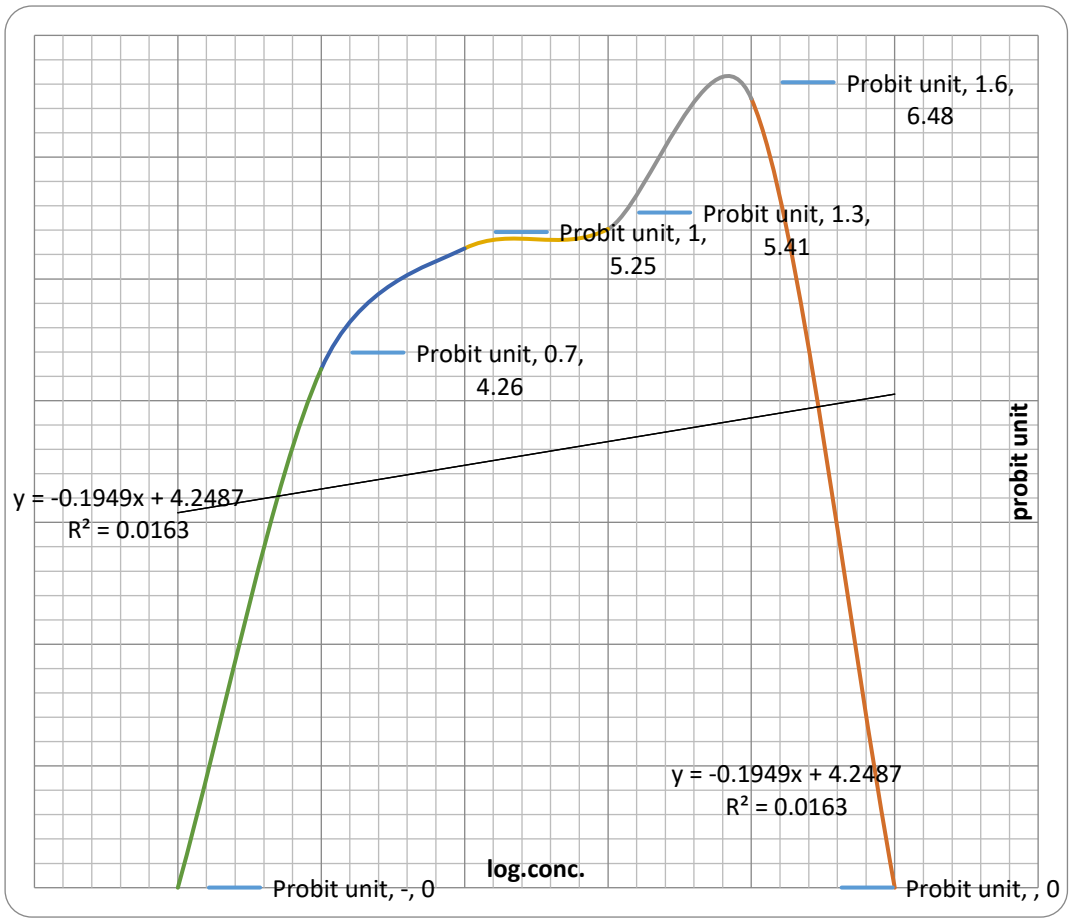

Fig. 1. The toxicity line of Moringa Olifera.

X=3.48 ml

Table 5. Concentration, log. concentration and probit unit of Anopheles larvae exposed to different concentrations of neem leave extracts.

Concentration ml	Log concentration	% mortality for different experiments			% (mean) mortality	Probit unit
		1	2	3		
40	1.6	90	50	90	76.6	5.71
20	1.3	60	20	90	56.6	5.15
10	1	0	20	80	33.3	4.56
5	0.7	0	20	70	30	4.48
Control	-	0	0	0	0	0

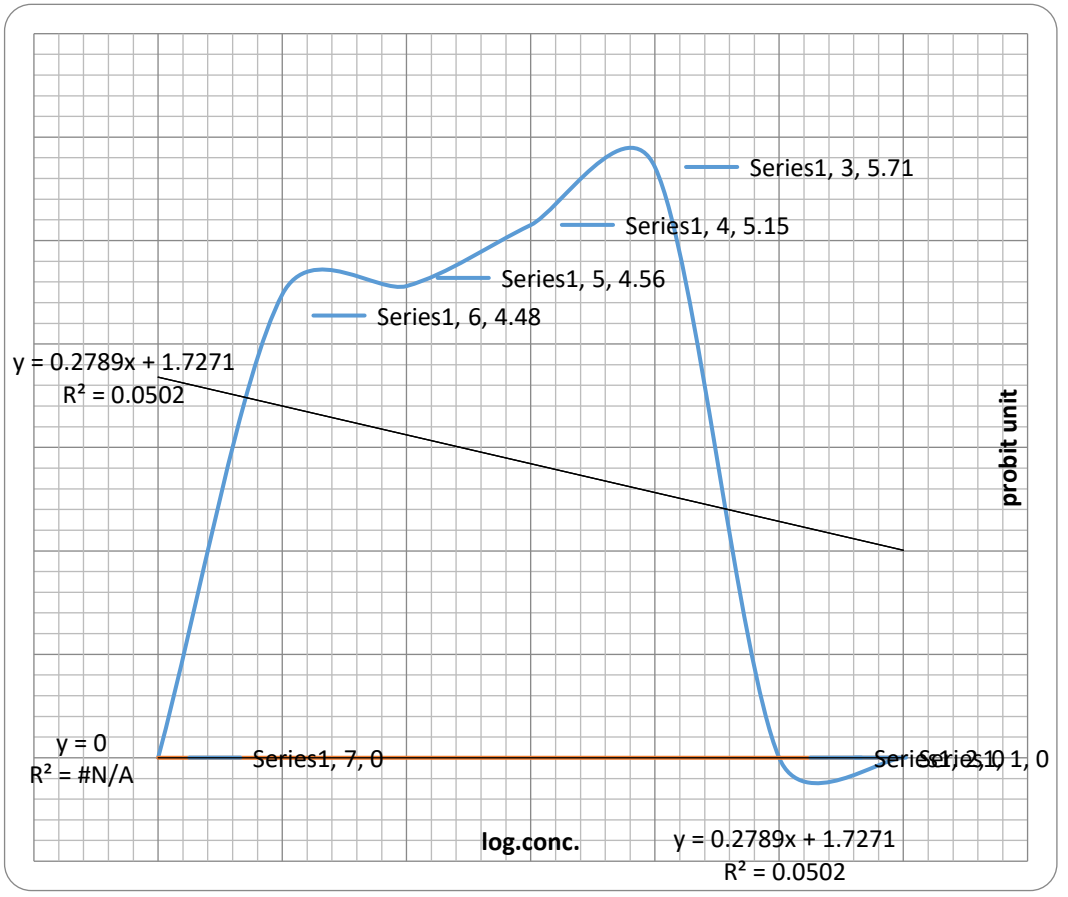

Fig. 2. the toxicity line of Neem.

X=38.04 ml

4. Discussion

The use of herbal products is one of the reasonable methods for mosquito control [13]. In this study, we revealed that Moringa olivera and Azadirachta indica water extracts are toxic when applied to Anopheles larvae at (40ml/L) concentrations where recorded mortality of (93.3%) for Moringa and (76.6%) for neem at 40ml/L concentration. A similar study had been

conducted in Sudan and revealed that the larvicidal activity of moringa and neem seeds water extracts are both effective in control of Anopheles larvae which agrees with the findings of this study [13]. In another study conducted by Pradu et al. showed that Moringa olifera seed extracts are effective against the control of mosquitoes Anopheles stephensi larvae, and their findings agreed with the findings of this study [14]. A study conducted by Abdel Ouaheb et al. in Algeria (2009) revealed that neem tree extract is effective in control of Culex pipiens stage 4 larvae which also agrees with the findings of this study as neem is effective in the control of different Culicidae (Culex, Anopheles) [15]. In India Dua et al. observed that neem oil formulation was found effective in the control of mosquitos' larvae Anopheles, Culex, and Aedes which agrees with the findings of this study [16]. In Brazil, Ferreira et al. (2009) observed that water extract of moringa olifera has a toxic effect on the third larval stage of Aedes aegypti even at low concentrations which agrees with the findings of this study since third-stage larvae of Anopheles recorded toxic effects with neem extracts [17, 18]. In India Tandon and Sirohi (2010) observed that the effect of neem aqueous extract on Culex larvae was 99% mortality which agrees with the findings of this study [19, 20]. In Hawaii, United States, a study conducted by Ohia et al. (2013) revealed that moringa seeds aqueous extract has an effect on Anopheles gambiae larvae which agrees with the findings of this study where moringa olivera has a toxic effect on Anopheles arabiensis larvae [21, 22].

5. Recommendations

From the results obtained in this study, we recommend the usage of Neem and Moringa extracts as larvicides for Anopheles mosquitoes. Furthermore, we encourage the extraction of the active substance of Moringa and neem and test their sensitivity to other vector controls. Further studies on neem leaves and moringa leaves are required to explore their potent effects on vector control along with the suitable dose by accuracy.

6. Conclusion

The percentage mortalities of Anopheles mosquitoes against Azadirachta indica (Neem) leaves and Moringa olifera leaves was highest at a concentration of 40 ml/L for both neem and moringa and was recorded at 93.3% and 76.6%. The lethal concentration (LC50) of Moringa olifera capable of killing 50% of Anopheles mosquitoes was 3.48 ml. The lethal concentration (LC50) of Azadirachta indica capable of killing 50% of Anopheles mosquitoes was 38.04 ml. The aqueous extracts of Moringa olifera texture were observed more viscous than Azadirachta indica (neem), and this property may increase the mortality rate.

Conflict of interest statement: Authors declares no conflict of interest in this study

Funding information: No funds received for this study

References

1. M. A. El Sharif Gasim, "The mosquitoes," Practical and Medical Entomology, vol. 1, p. 16-27, 2010.
2. Dainius R, Audra J, Kornelijus M, Zygimantas P, Deividas C, Povilas D. Healing of Post-

Extraction Alveolar Defects by Primary and Secondary Intention: A Systematic Literature Review. Ann Dent Spec. 2024;12(2):47-54. https://doi.org/10.51847/KyxflfwkM5

3. Vinichenko MA, Zavalishina SY, Kartashev VP, Makurina ON. Physiological Features of the Cardiovascular System in Hypertensive Men Under Conditions of Regular Physical Activity. J Biochem Technol. 2023;14(1):57-63. https://doi.org/10.51847/9a5O0KSRNz
4. Centers for Disease Control and Prevention (CDC). "Prevention and control of west Nile virus", 2015. https://www.cdc.gov/westnile virus.com
5. Gandhi DA, Memon A, Kheur S. Liquid Biopsy to the Rescue of Oral Cancer Diagnostics- A Narrative Review. Clin Cancer Investig J. 2023;12(6):14-8. https://doi.org/10.51847/zwbQR2I4Gl
6. Beibalaeva SN, Magomedova AT, Kuramagomedova AG, Gadzhimagomedov MM, Belyaeva VA, Rahimova AN, et al. Characteristics of Biologically Active Properties and Therapeutic Use of Ulomoides Dermestoides Beetles. Entomol Appl Sci Lett. 2023;10(1):83-8. https://doi.org/10.51847/VScQR2GaF2
7. Singar FAW. Characterization of Defatted Cake Prepared from Egyptian Olive's Fruit (Wateeken Cultivar) and Its Biological Activity. World J Environ Biosci. 2024;13(2):31-5. https://doi.org/10.51847/R7K4g1FOdt
8. J. Banupriya, and V. Maheshwari, "Effects of mosquito repellent finishes by herbal method on textiles," International Journal of Pharmacy & Life Sciences, vol. 4, no. 11, 2013.
9. B. Albert, "The Neem tree Azaidirachta indica," village volunteers.org. 2010. https://www. villagevolunteers.org.
10. A. Leone, A. Spada, A. Battezzati, A. Schiraldi, J. Aristil, and S. Bertoli, "Moringa oleifera seeds and oil: Characteristics and uses for human health," International Journal of Molecular Sciences, vol. 17, no. 12, p. 2141, 2016.
11. E. Chukwuebuka, "Moringa Oleifera "the mother's best friend," International Journal of Nutrition and Food Sciences, vol. 4, no. 6, p. 624-630, 2015.
12. World Health Organization, "Guidelines for laboratory and field testing of mosquito larvicides (No. WHO/CDS/WHOPES/GCDPP/2005.13)," World Health Organization, 2005.
13. A. A. M. Zaroug, "The effect of Azadirachta indica(neem) seeds, Moringa olifera seeds and Caltropisprocera(usher) leaves in control of Anopheles arabiensis mosquito larvae," M.S.C. thesis. AL zaiem Al azhari University, 2015.
14. K. Prabhu, K. Murugan, A. Nareshkumar, N. Ramasubramanian, and S. Bragadeeswaran, "Larvicidal and repellent potential of Moringa oleifera against malarial vector, Anopheles stephensi Liston (Insecta: Diptera: Culicidae)," Asian Pacific Journal of Tropical Biomedicine, vol. 1, no. 2, p. 124-129, 2011.
15. A. Alouani, N. Rehimi, and N. Soltani, "Larvicidal activity of a neem tree extract (Azadirachtin) against mosquito larvae in the Republic of Algeria," Jordan Journal of Biological Sciences, vol. 2, no. 1, p. 15-22, 2009.
16. V. K. Dua, A. C. Pandey, K. Raghavendra, A. Gupta, T. Sharma, and A. P. Dash, "Larvicidal activity of neem oil (Azadirachta indica) formulation against mosquitoes," Malaria Journal, vol. 8, p. 124, 2009.
17. P. M. Ferreira, A. F. Carvalho, D. F. Farias, N. G. Cariolano, V. M. Melo, M. G. Queiroz, A. M. Martins, and J. G. Machado-Neto, "Larvicidal activity of the water extract of Moringa oleifera seeds against Aedes aegypti and its toxicity upon laboratory animals," Anais da Academia Brasileira de Ciencias, vol. 81, no. 2, p. 207-216, 2009.
18. Alanazi HMN. Virtual Reality in Leisure: Its Impact on Social Interaction and Physical Activity Among Saudi University Students. J Organ Behav Res. 2023;8(2):261-80. https://doi.org/10.51847/8OnKjAVQFX
19. P. Tandon, and A. Sirohi, "Assessment of larvicidal properties of aqueous extracts of four plants against Culex quinquefasciatus larvae," Jordan Journal of Biological Sciences, vol. 3,

no. 1, p. 1-6, 2010.
20. Elmeged LSMA, Alshehri KM. Protective and Histological Effects of Kumquat (Citrus japonica) Extract in Rats Injected with Carbon Tetrachloride (CCl4). Pharmacophore. 2024;15(2):43-53. https://doi.org/10.51847/Wo07IlXueK
21. C. M. D. Ohia, G. R. E. E. Ana, and O. M. Bolaji, "Larvicidal Activity of aqeous extract of moringa oleifera seeds on anopheles gambiae and its effects on poecilia reticulate," Agrosearch, vol. 13, no. 3, p. 176-185, 2013.
22. Drobotova AN, Filippova VV, Ovechko OY, Leshchenko YY, Belova PS, Tabukhova AZ. Definition, Diagnosis, Tactics for Postpartum Bleeding: A Literary Review. Arch Pharm Pract. 2024;15(1):127-30. https://doi.org/10.51847/HaHb7qs9mn