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Larvicidal Effect of *Azadirachta indica* Extract on *Aedes aegypti* in Nnamdi Azikiwe University Environment, Awka South Local Government Area of Anambra State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CAI and JOO conceived the study and designed the study protocol. Authors JOO, CIN and OAO carried out sample collection and laboratory liaison. Authors UAU, CAI and CUU provided academic support and supervision. Authors CAI and IEO carried out the analysis and interpretation of data. Authors JOO, CIN, IEO and OAO drafted the manuscript. Authors UAU, CAI and MAI critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study assessed thelarvicidal ability of the ethanolic leaf extracts of Neem (Azadirachta indica)medicinal plants against *Aedes aegypti* mosquito larvae.
Study Design: The study was a random survey design:
Place and Duration of Study: The study was carried out in Nnamdi Azikiwe University environment, Awka South, Local Government Area, Anambra State, Nigeria between April and July, 2023.
Methodology: Respective concentrations of the plant extracts were added to twenty-five treatment trays each containing a larva (making up twenty-five larvae) and 15ml of its natural growth medium.A control, containing only larvae and natural growth medium was maintained. At regular intervals the mortality counts of larvae were monitored.
Results: It was found that the crude ethanolic neem leaf extract showed significant larvicidal effect at different concentrations.There is a strong correlation between mortalities observed in larvae and extract concentration, LC50=1.21ml, LC90=9.03ml.The statistical analysis revealed significant difference in the mortality response of *Aedes aegypti* larvae to different concentration of the leaf extract (P=0.000).
Conclusion: The neem plant, Azadirachta indica, ethanoic leave extract has larvicidal ability against *Aedes aegypti* mosquito larvae and can be safely used as a potent larvicidal agent.The active principles and optimum dosages, responsible for the larvicidal activity require further analysis to be isolated. This plant would be eco-friendly and may serve as suitable alternative to synthetic larvicides as they are relatively safe, inexpensive, and are readily available.

Keywords: Aedes aegypti; neem plant; Azadirachta indica; larvicides; larval concentration.

1. INTRODUCTION

"Aedes mosquitoes are pestiferous vectors responsible for the transmission of various dreadful diseases like yellow fever, causing millions of deaths annually" [1]. *Aedesaegypti i*s a vector of dengue fever infection, a serious public health problem in the world affecting both western and African countries [2]. "There are various methods for controlling *Aedes*mosquitoes including biological method but the use of chemical method is the most popular. Mosquito control focuses more on larval and pupal stage control than adult stage control" [3]. "One of the most common chemicals used is the larvicide temephos" [4]. "Chemical control is expensive, may be toxic to humans, its biodegradability is slow and mosquitoes can develop resistance against these chemicals" [3].

"Neem plant belongs to the genus *Azadirachta*of which *Azadirachta indica* (Family: Meliaceae) and its derived products have shown insecticidal property" [5]. "Azadirachtin, a biologically active compound in *A. indica* is an eco-friendly insecticide than synthetic insecticides that contribute in high cost and health effects" [6]. "*Azadirachta indica* is well known in Nigeria and itsneighboring countries as one of the most versatile medicinal plants having a wide spectrum of biological activity. Every part of the tree has beenused as traditional medicine for a household remedy against various human ailments" [7]. "The leaf extract of *Azadirachta indica* is highly toxic even at low doses which may eventually prove this plant to be a useful larvicide. The product of this plant can as well be utilized for preparing biocides or phytochemical from which the non-target organisms can be rescued from harmful vectors" [8]. "Nigerians still use medicinal plants to treat not only malaria but also several different illnesses and conditions. Some of these include;eczema, ringworm, acne, anti-inflammatory anti-hyperglycemic properties, and they are used to heal chronic wounds, diabetic foot, and gangrene. It is knownto remove toxins from the body. It also neutralizes free radicals and purifies the blood. It is used as an anticancer agent and it has hepato-renal protective activity and hypo-lipidemic effects" [7].

"Neem extract has antiviral activity; almost every part of the tree has been in use since ancient times to treat several human ailments. The extract from bark, leaves, fruits, and root have been used to control leprosy, intestinal helminthiasis, and respiratorydisorders in children" [9]. "A leaf extract was found to lower raised levels of serum liver enzyme and paracetamol-induced liver necrosis. Hydro alcoholic leaf extract of *Azadirachta indica*

caused a dose-dependent hypotensive effect and oral administration of neem leaf extract showed an anxiolytic effect" [10]. "The antimalarial activities of neem seed and leaf extracts have been studied in vitro using malaria parasites. Components of the alcoholic extracts of leaves and seeds are effective in vitro against both chloroquine-resistant and sensitive strains of the malaria parasite. Recent investigations have shown that neem seed extract and its purified fractions inhibit the growth and development of asexual and sexual stages of drug-sensitive and resistant strains of the human malaria parasite,*P.falciparum*; Some reasons being that the medicinal herbs are readily available within the community,less and mild side effects if any, easy to prepare,and cheap. Some therapeutic drugs have severe effects on different people hence the choice to use herbs. Despite the ever-increasing reports with regards to the Larvicidal potentials of extracts of neem parts against known mosquito larvae, information on its growth inhibitory activity against mosquito larvae has been limited and scanty" [11].

"The incessant and haphazard use of conventional insecticides for the control of mosquito vectors has led to recent increase in the development of resistance and negative impacts on non-target organisms and the environment. Therefore, there is a need for development of biological effective mosquito control tools" [12,13]. "With Nigeria being one of the 10 countries with the highest burden of malaria in the year 2017, the development and adoption of alternative methods of integrated vector management remain the key" [12,14]. However, there is a dearth of information on the bio-insecticidal effects of the leaves of *Azadirachta indica* on *Aedes aegypti* in Nigeria.

Hence, this study determined theLarvicidal effect of leaf extracts of*Azadirachta indica* against *Ae.aegypti*in Nnamdi Azikiwe University environment,Awka South Local Government Area, Anambra State, Nigeria. Among the objectives were to rear mosquito eggs to larval stage and to evaluate the effect of ethanolic leaf extract of *Azadirachta indica* on *Ae. aegypti* larvae.

2. MATERIALS AND METHODS

2.1 Study Area

NnamdiAzikiwe University also called UNIZIKor NAU in short form is located along Onitsha-Enugu expressway, Awka, Anambra State, Nigeria between latitude 6^{o} 14' 15" and longitude 7^{o} 6' 56".Awka lies within the humid tropical rainforest belt of Nigeria characterized by trees, evergreen leaves, thick undergrowth, open vegetative lowland, interspersed with tall oil palm trees and deciduous trees. It experiences two distinct seasons brought about by the predominant winds that rule the area. The Southwestern monsoon winds and the Northeastern dry wind. The Southwestern monsoon winds from the Atlantic Ocean create heavy tropical rains that lasts for about seven months, from April to October while the Northeastern dry wind across the Sahara desert results to five months of dryness between November and March. In late December or beginning of January Awka experiences the harmattan that enters Nigeria usually in the form of dry and dusty wind characterized by gray haze limiting visibility and blocking the sun's rays. It has an annual rainfall of 1600 mm to 2000 mm on the average with amean annual temperature ranges between270C and350C.

2.2 Collection of Plant Materials

The leaves of *Azadirachta indica* was collected from various plantations around science village in Nnamdi Azikiwe University, Awka. Theleaves of *A. indica* wereair-dried in shade, before grinding into fine powder, using electric blender. The powdered sample was stored in a dark bottle with screw cap top.

2.3 Preparation of Ethanolic Extract of *A. indica*leafinto Powder

Ethanolic extracts of neem leafwas prepared using maceration method as performed by Dahchar et al. [15]. 100g of powder sample was soaked in 200 ml of ethanol (80% v/v). This was allowed to stand for 24 hours in a dark cupboard under room temperature. The content was shaken at a regular interval to ensure proper mixture. Thereafter, the mixture content was filtered through Whatman's Filter Paper. After the filtrate was obtained, the ethanol content of the mixture was removed using a water bath at 60 to 65^{0}C. The stock solution obtained was used for the preparation of different concentrations of the treatments used in the experiment.

2.4 Collection of Mosquito Larvae and Maintenance

Aedes mosquito eggs was purchased fromARBO-VIRUS in Enugu state, Nigeria and reared to larvae in the Parasitology and Entomology laboratory of NnamdiAzikwe University, Awka.

2.5 Larvicidal Bioassay

The Larvicidal Bioassay was carried out using WHO Standard test procedure [16] on second and third instar larvae i.e. L2 and L3, respectively. "25 healthy L2 and L3 instar larvae were introduced into the treatment petri-dish using a rubber pipette. The test wastreated in different concentration from 0.125; 0.250; 0.500; 0.750 and 1ml of the Azadirachtin. The control was set up where 25 instar larvae were introduced into petri-dish, and no treatment was added. Both the treatment and the control experiment were replicated four times.The larval mortality was observed for 24hrs, 48hrs and 72hrs. During the bioassay, the larvae were not fed" [17]. Mortality was regarded when there is no sign of any movement or even after mild touch with glass rod [18], and dead larvae were counted. If 30% mortality was recorded in the control, the experiment was discarded or the mortality was corrected using Abbott's formula, as follows:

> Corrected Mortality = Mortality in Test Bottle (%) - Mortality in Control Bottle (%) / 100%-Mortality in Control Bottle(%) * 100
>
> Or Corrected percentage of mortality = 1 - nin T after treatment / nin C after treatment * 100
>
> Percentage of Mortality = Number of dead larvae / Number of larvae introduced *100
>
> Where n = number of larvae or nymph, T = treated, C = control.

2.6 Data Analysis

The average larval mortality data obtained was subjected to Probitanalysis for calculating $LC_{50}$and LC_{90}(lethal concentrations) values, and their 95% confidence limits was estimated using a probit regression model to observe the relationship between percentage mortality of larvae and logarithmic concentration of the *A. indica*. Separate probit models were used for the extract. The analysis was carried out using the SPSS (statistical package for social science) version 20.0.

3. RESULTS

3.1 Effects of Various Concentration of Extract on *Aedes aegypti* Mosquito Larvae

Table 1 shows the mortality response to different concentration of after 72 hours. The result shows that the mortality increased with increase in concentration. The highest concentration of the 1ml recorded the highest mortality rate of 48.1%.While at 0.125% concentration, indicated a mortality rate of 9.72%. The tabled also shows that there is an increase in mortality with an increase in time. This shows that the mortality is concentration and time dependent. The statistical analysis revealed significant difference in the mortality response of *Aedes aegypti* larvae to different concentration of the leaf extract (P=0.000).

3.2 Determination of LC_{50} and $LC_{90}$for *A. indica* Leaf Extract

For *Aedes* Mosquito, the regression line y = 1.4657x + 3.4148 was obtained after plotting probit of mortality against log of concentration. LC_{50} value of 1.21ml was obtained from the equation as well as LC_{90} value of 9.03 ml. LC_{50} explains that it took a concentration of 1.21ml to kill 50% of the *Aedes* mosquito larvae. LC_{90} explain that the concentration of 9.03 ml of extractkilled 90% of the *Aedes* mosquito larvae.

3.3 Determination of LT_{50} and LT_{90} for*A. indica* Extract

Fig. 2 shows the result for 72 hours exposure time. Lethal time was plotted for extracts on a single line. This was done by plotting probit of mortality against log of time in hours. Each extracts regression line was obtained and it was used to obtain LT_{50} and LT_{90}.

The regression line ofY= 54.126x - 58.177 was obtained. It was then used to calculate LT_{50} and LT_{90}which were 14.70 hours and 15.50 hours respectively.

Table 1. The mortality effect of the different concentration and different time intervals of *A. indica* on *Ae. Aegypti*

Conc. (µl/ml)	24 hours	48 hours	72 hours	mean±se	Mortality (%)	Probit
1	8.5	12.3	15.3	12.03±1.97	48.1	4.9524
0.75	7	11.5	15.3	11.27±2.40	45.1	4.8769
0.5	3.75	7.8	11.3	7.62±2.18	16.61	4.0299
0.25	1.75	5.3	7.5	4.85±1.68	19.40	4.1367
0.125	0.5	1.8	5	1.34	9.72	3.5759
Control	0	0	0	0	0	
mean±se	4.3±1.52	7.74±1.95	10.88±2.06			
Mortality (%)	17.2	31.0	42.5			
Probit	4.0537	4.5041	4.8362			

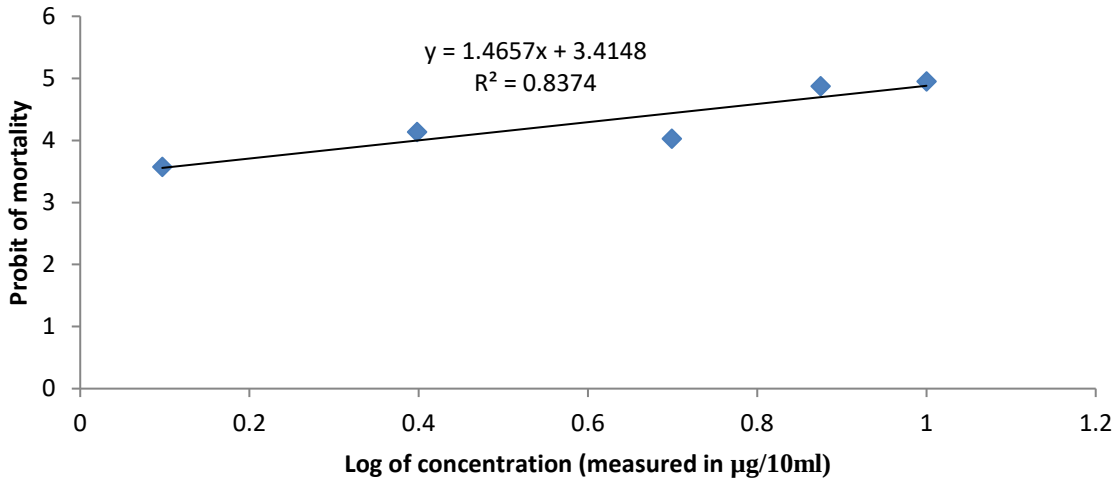

Fig. 1. Mortality responses of *Ae. aegypti* larvae after exposure to different concentrations of *A. indica* leaf extracts

LC_{50} and LC_{90} calculations for the extract.
Use the equation: y = 1.4657x + 3.4148
Probit LC_{50}
5.0 = 1.4657 log conc. + 3.4148
1.4657 log conc. = 5.0 – 3.4148
1.4657 log conc.= 1.5852
Log conc. = 1.5852/1.4657
Log conc.= 1.0815
Conc.= $\log^{-1}$ 1.0815
LC_{50} = 12.10
To convert to ml = 12.10/10
= 1.21ml
Probit LC_{90}
6.2816 = 1.4657 log conc. + 3.4148
1.4657 log conc.= 6.2816 – 3.4148
1.4657 log conc.= 2.8668
Log conc.= 2.8668/1.4657
Log conc.= 1.9559
Conc. = $\log^{-1}$ 1.9559
LC_{90} = 90.3
To convert to ml = 90.3/10
= 9.03ml

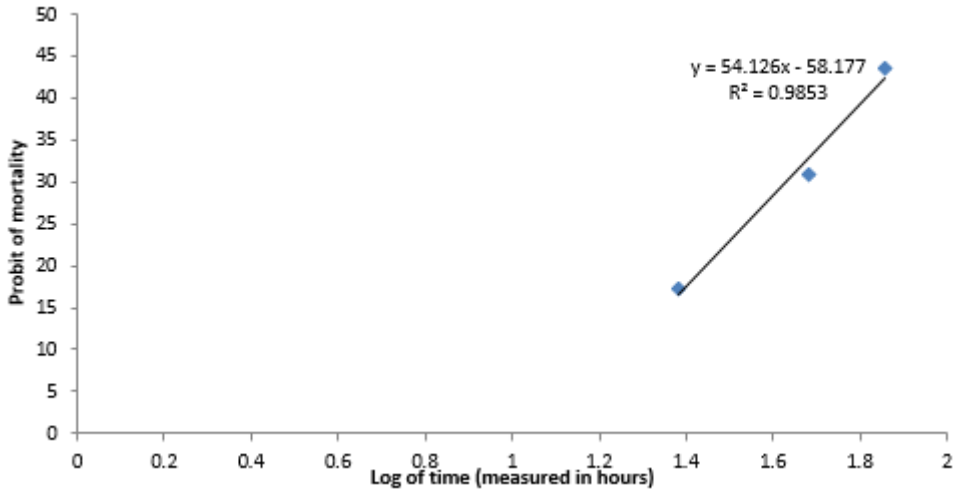

Fig. 2. Mortality responses of*Aedes aegypti* larvae after exposure to the extract at different time interval

LT_{50} and LT_{90} calculations for the extract.
Use the equation: y = 54.126x - 58.177
Probit LT_{50}
5.0 = 54.126 log conc. – 58.177
54.126 log conc. = 5.0 + 58.177
54.126 log conc.= 63.177
Log conc = 63.177/54.126
Log conc.= 1.1672
Conc. = $\log^{-1}$ 1.1672
LT_{50} = 14.70hrs
Probit LT_{90}
6.2816 = 54.126 log conc. – 58.177
54.126 log conc.= 6.2816 + 58.177
Log conc.= 64.4586/54.126
Log conc.= 1.1909
Conc.= $\log^{-1}$ 1.1909
LT_{90} = 15.50hrs

4. DISCUSSION

"The extract of the neem tree, *Azadirachtin*, was tested in the current study. It has been proven that crude or partially-purified plant extracts are less expensive and highly effective for the control of *Aedes* mosquitoes than the purified extracts" [1].

"The experimental results obtained from this study revealed that all *A. indica* extracts showed larvicidal activity against *Aedes* mosquito larvae" [19]. "The larvicidal activity among extracts was extremely broad. The various biological activities of these plant extracts may be due to various phytochemical classes, existing in the plant; these compounds may jointly or independently contribute to producing larvicidal activity. The negative control used was water which has no effect on the mosquito larvae and showed a normal response" [1].

"The data showed that larvicidal effect rose gradually with an increase in dose until it reached a peak of 48.1% mortality after 1ml of the extract", [20] and [21]. Beyond this dose the larvicidal effect observed was constant until the highest concentration of 20mg/ml which is in total agreement with the findings of Chombo [10]. The LC_{50} value for the ethanolic extract of *Azadirachtin indica* was 1.21ml, while the LC_{90} value for the ethanolic extract of *Azadirachtin indica* was 9.03 ml for 72h.

The most important physiological effect of *A.indica*on insects is the growth regulatory effect. It is because of this property that the family *Meliaceae* has emerged as a potent source of insecticides. Exposure of mosquito larvae to sub-lethal doses of neem leaves extract in the laboratory prolonged larval development, reduced pupal weight, and oviposition as was also noticed by Murugan et al. [22]; Su and Mulla [23].This study when fully developed and applied will contribute to a great decrease in the use of synthetic insecticides, and consequently increase the opportunity for the application of botanical pesticides in controlling the various medicinally important pests naturally, since these botanical pesticides are usually active against a limited number of species including specifictarget insects, less expensive and easily biodegradable to non-toxic products.

5. CONCLUSION

From the findings of this work, the neem plant, *Azadirachta indica* has a larvicidal efficacy on the mosquito, *Ae. aegypti*. Thehigh toxicity of the planteven at low doses provesit can bea useful larvicide.To isolate the active principles and optimum dosages responsible for the larvicidal activity, further analysis is needed.This plant would be friendlyto the eco-system and can be a suitable alternative to synthetic larvicides as they are comparatively inexpensive, safe and are readily available in many parts of the world.

ETHICAL APPROVAL

A letter of approval was collected from the Head of Department, Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria and was taken to the chief Technologists of the department to allow us use the laboratory. The letter was also taken to the national ARBO virus Institute Enugu, Nigeria when purchasing the eggs of the mosquito.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chombo T. Larvicidal potential of crude methanolic leaf extract of Azadirachta Indica On Mosquito Larvae. Student's Journal of Health Research Africa. 2021;2 (3):11.
2. Champakaew D, Junkum A, Chaithong U. *Angelica sinensis* (Umbelliferae) with proven repellent properties against *Aedesaegypti*, the primary dengue fever vector in Thailand. Parasitology Research. 2015;114:2187-2198.
3. Becker T, Loch G, Beyer M. FOXO-dependent regulation of innate immune homeostasis. Nature. 2010;463:369-373.
4. Tiwary M, Naik SN, Tewary DK, Mittal PK, Yadev S. Chemical composition and larvicidal activities of the essential oil of *Zanthoxylumarmatum* DC (Rutaceae) against three mosquito vectors. Journal of Vector Borne Disease. 2007;44:198-204.
5. Obomanu FG, Ogbalu OK, Gabriel UU, Fekarurhobo GK, Adediran BI. Larvicidal properties of *Lepidagathisalopecuroide s*and *Azadirachta indica* on *Anopheles gambiae* and *Culex quinquefasciatus.* African Journal of Biotechnology. 2006;5 (9):761-765.
6. Mondali NK, Mojumdar A, Chatterje SK, Banerjee A, Datta JK, Gupta S. Antifungal activities and chemical characterization of Neem leaf extracts on the growth of some selected fungal species *in vitro* culture medium. Journal of Applied Science and Environmental Management. 2009;13(1): 49-53.
7. Kumar VS, Navaratnam V. Neem (Azadirachta indica): Prehistory to contemporary medicinal uses to humankind. Asian Pacific Journal of Tropical Biomedicine. 2013;3(7):505–514.
8. Remy KM, Logaswamy S. Larvicidal efficacy of leave extracts of two botanicals against the mosquito vector *Aedes aegypti* (Diptera: Culicidae). Indian Journal of Natural Products and Resources. 2010;1 (2):208-212.
9. Gunasekaran K, Vijayakumar T, Kalyanasundram M. Larvicidal and emergence inhibitory activities of NeemAzal T/S 1.2 per cent EC against vectors of malaria, filariasis & dengue. Indian Journal of Medical Research. 2009;130(2):112-114.
10. Chombo T. Larvicidal potential of crude methanolic leaf extract of azadirachta

indica on mosquito larvae. Student's Journal of Health Research, Africa. 2021;2(3):11.
Available:https://doi.org/10.51168/sjhrafrica.v2i3.16

11. Dua VK, Pandey AC, Raghavendra K, Gupta A, Sharma T, Dash AP. Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. Malaria Journal. 2009;8(1):124.
12. Ayinde AA, Morakinyo OM, Sridhar MKC. Repellency and larvicidal activities of *Azadirachta indica* seed oil on *Anopheles gambiae* in Nigeria. Heliyon. 2020;6(5).
DOI: 10.1016/j.heliyon.2020.e03920
13. Govindarajan M, Rajeswary M, Arivoli S, Tennyson S, Benelli G. Larvicidal and repellent potential of Zingibernimmonii (J. Graham) Dalzell (Zingiberaceae) essential oil: an eco-friendly tool against malaria, dengue, and lymphatic filariasis mosquito vectors? Parasitology Research. 2016;115: 1807–1816.
14. World Health Organization. World malaria report. World Health Organization, Geneva; 2018.
15. Dahchar Z, Bendali-Saoudi F, Soltani N. Larvicidal activity of some plant extracts against two mosquito species *Culex pipiens*and *Culisetalongiareolata*. Journal of Entomology and Zoology Studies. 2016; 4(4):346-350.
16. World Health Organization. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. 2013;18-20.
17. Kumar D, Chawla R, Dhamodaram P, Balakrishnan N. Larvicidal activity of *Cassia occidentalis* (Linn.) against the larvae of bancroftian filariasis vector mosquito *Culex quinquefasciatus*. Journal of Parasitology Research. 2014.
Available:http://dx.doi.org/10.1155/2014/236838
18. Ali EOM, Shakil NA, Rana VS, Sarkar DJ, Majumder S, Kaushik P, Singh BB, Kumar J. Antifungal activity of nano emulsions ofNeem and citronella oils against phytopathogenic fungi, *Rhizoctonia solani*and Sclerotium rolfsii. Industrial Crops and Products. 2017;108:379-387.
19. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. The Indian Journal of Medical Research. 2013;135(5):581-598.
20. Kajla M, Bhattacharya K, Gupta K, Banerjee U, Kakani P, Gupta L, Kumar S. Identi1cation of the Temperature Induced Larvicidal EZcacy of Agave angustifolia against Aedes, Culex, and Anopheles Larvae. Frontiers in Public Health. 2016; 3:286.
21. Lama PS, Olayinka MD, Ezekiel A, Tongjura JDC, Ombugadu RJ, Akyengo O. Effect of Azadirachta indica (Neem) seed extract on larvae and adult Anopheles mosquitoes in Nasarawa Local Government Area, Nasarawa State, Nigeria. International Journal of Applied Biology. 2022;6(2):179-185.
22. Murugan K, Babu R, Jeyabalan D, Senthil Kumar N, Sivaramakrishnan S. Antipupational effect of neem oil and neem seed kernel extract against mosquito larvae of Anopheles stephensi (Liston). Journal of Entomology Research. 1996;20: 137-139.
23. Su T, Mulla MR. Oviposition bioassay responses of Culex tarsalis and Culex quinquefasciatus to neem products containing azadirachtin. Entomologia Experimentalis et Applicata. 1999;91:337-345.

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