

Larvicidal activity of ethanol extracts of *Azadirachta indica* (neem) against *Anopheles Arabiensis* and *Culex Quinquefasciatus* larvae, Gezira state, Sudan

Nosiba S Hamid¹, Mutaman A Kehail², Nasir A Ibrahim^{3*}, Eitimid H Abdel-Rahman⁴

¹ Department of Biology, Faculty of Science, Al immam Mohammed Bin Saud University, Saudi Arabia

² Department of Zoology, Faculty of Science, University of Gezira, Sudan

³ Biochemistry and Physiology Department, Faculty of Veterinary Medicine, Al-butana University, Sudan

⁴ Biology Department, Faculty of Science, University of Hail, Saudi Arabia

Abstract

Mosquitoes are among Sudan's most devastating malaria disease vector and some other endemic diseases in the world. The aim of this study was to evaluate the larvicidal activity of ethanol extract of flowers and leaves of *Azadirachta indica* (Neem) on *Anopheles arabiensis* and *Culex quinquefasciatus*. The selected natural products were collected from the City of Wad Medani, Gezira State, Sudan. The bioassay was assessed using the protocol of WHO. Various concentrations of each extract was tested on the active stage (3rd and early 4th instar) larvae of the two selected mosquito species. The percentage mortalities and their corresponding doses were used to calculate R^2 , LC_{50} and LC_{95} . The ethanol extract of *A. indica* leaves resulted in range of mean mortalities of 20-95% and 22-92%, respectively on *A. arabiensis* and *C. quinquefasciatus* larvae. Additionally, the LC_{50} 's was 77.62 and 52.48 ppm, while the LC_{95} 's was 219.46 ppm and 251.18 ppm, followed the same order of larvae. The ethanol extract of *A. indica* flowers showed a range of mean mortalities of 30 -97% and 32-97%, respectively on *A. arabiensis* and *C. quinquefasciatus* larvae. The LC_{50} 's was 11.22 ppm and 25.11 ppm and the LC_{95} 's was 41.68 ppm and 43.65 ppm, followed the same order of larvae. *A. indica* leaves and flower were found to have potential control measure for both *Anopheles* and *Culex* larvae, with slight degrees of toxicity. However, LC 's values and Log-Probity curve showed that, the flowers have higher larvicidal potentiality than the leaves against both larvae. Therefore, further studies should be carried out to determine the appropriate manner of application and examine the mode of action for these components.

Keywords: larvicidal, ethanol extract, *Azadirachta indica*, *anopheles Arabiensis*, *Culex quinquefasciatus*

Introduction

Azadirachta indica (Neem) is known as the Holy and Untouchable Tree, it has been used for medicinal properties in India for medicine assistants that cure all diseases for more than two millennia, and has been described as a village pharmacy since all parts of Neem tree are used as anthelmintic, antifungal, anti-diabetic, antibacterial, antiviral, contraceptive and sedative [1, 2]. Neem tree is used in many medicinal treatments such as skin diseases, healthy hair, improved liver function, blood detoxify, pest and disease control, fever reduction, dental treatments, cough, asthma, ulcers, piles, intestinal worms and urinary diseases [3]. There have been multiple global conferences and comprehensive scientific literature reporting on neems' potential medicinal and insecticidal properties [3, 4, 5]. Nevertheless, its value recognized by the United States National Academy of Science as a tree for solving global problems [6]. Furthermore, [7]. Documented its unique properties against insects' pests, agricultural and public health. It is grown in most tropical and subtropical areas of the world for shade, reforestation, and raw material processing for natural insecticides and medicines [8].

In general, insects from different orders have different behavioral patterns and behavioral responses when they are exposed to insecticide products [9]. And / or certain biological products such as Neem that have no environmental impacts [5]. As many reviews have been published on the behavioral and physiological properties of *A. indica* on insect life cycle effects, regarding development,

feeding behavior, growth, irregular and/or delayed molts, and mortality [10]. The effects were categorized in two ways: direct effects on cells and tissues and indirect effects exerted via the endocrine system [11]. As it describes the work carried out to date to identify the mode of action of azadirachtin from Neems at the cellular level. The differential effects between animal phyla and over non-target organisms are discussed and point to its potential success as a safe insecticide [12].

Azadirachtin, a complex tetranortriterpenoid limonoid from the neem seeds, is the main component responsible for the toxic effects in different insect's type such as molen fly *Bactrocera cucubita* from Asia [10]. However, one of the few recorded field work that apply crude neem extracts on mosquito from Africa subtropical habitat as a potential larvicide that minimizes the accumulation of harmful residues on the Nabondo Plateau environment in western Kenya [13]. No published research done so far to define the mode of action of azadirachtin on local species of mosquitoes even they are among most devastating malaria disease vectors. Therefore, the present investigation aimed to test ethanol extract of neems' flowers and leaves on the *A. arabiensis* and *C. quinquefasciatus* larvae from Sudan.

Materials and Methods

Plant Material

The flowers and leaves of *A. indica* (Neem) were collected from Wad Medani City, Gezira State, that is located in the central part of Sudan, between the Blue Nile east 330 30'

and the White Nile west 320 40', within the dry climate area [14]. Fresh samples were collected randomly initially, plant taxonomy were reviewed for identification by members of the University of Gezira's Botany Department using available taxonomic reference [15]. and compared with old collections of the National Research Center for Medicinal and Aromatic Plants Institute. The plant specimens of *A. indica* were taken out and shade dried, and ground to fine powder.

Preparation of Ethanol Extracts

The ethanol extracts of the *A. indica* flowers and leaves were prepared with minor changes as defined by Mandal *et al.* [16]. The plant materials were washed 2-3 times with tap water and finally with distilled water and allowed to dry under shade at room temperature till the constant weight was obtained.

In short, 10 g of the powder was mixed with 100 ml of ethanol and left at room temperature for 48 hours, stirred manually and filtered. The weight of each of the dissolving fractions was used to calculate the original stock solution for each extract.

Collection and Rearing of Mosquito Larvae

Larvae of *A. arabiensis* and *C. quinquefasciatus* were collected from the breeding sites within the study area, and raised at 27±2°C and 70±5% relative humidity under laboratory conditions. The larvae were put in plastic and enamel trays containing tap water and housed at the Basic Sciences Laboratory, University of Gezira, Sudan. Larvae were fed on a diet of aquarium fish foods which contain the required nutrients.

Larval Bioassay

The larval bioassay tests were run following the protocol established by WHO [17]. In each test, 20 larvae of *A. arabiensis* and *C. quinquefasciatus* (the 3rd and early 4th instar) were put by means of dropper into small test cups each containing 250 ml tap water. A series of well calculated concentrations were added. Three replicates of each concentration for each extract were applied. Control, containing 20 larvae with tap water that is extraction-free. The percentage mortality of larvae toward each concentration was recorded after 24 hour. Also each test cup, the dead larvae were counted, put in glass slides to be photographed for monitoring the morphological changes (specially changes in color, status of digestive tract and the separation of some body parts). A digital microscope provided with camera was used for documentation of these observed changes.

Data and Statistical Analysis

Percentage larval mortalities corresponding to known concentrations after 24 hour were the row data used to run Probit analysis by using SPSS computer software programs version 19. The percentage mean mortality of larvae of *A. arabiensis* and *C. quinquefasciatus* were plotted with consistent log-concentrations to compare the larvicidal activity of all extracts and to calculate LC's values at specific P-value.

Results

Table (1) show the larvicidal effect of ethanol extract of *A. indica* leaves at concentrations of (219.46, 175.56, 65.84,

43.89 and 21.95 ppm). The respective percentage mortalities were 95, 93, 36, 35, 20 on *A. arabiensis* and 92, 90, 55, 48, 22 on *C. quinquefasciatus*. The LC₅₀'s was 77.62 ppm for *A. arabiensis* and 52.48 ppm for *C. quinquefasciatus*, while the LC₉₅'s was 219.46 ppm and 251.18 ppm, followed the same order of larvae. Control mortality was 0. The used concentrations exhibit considerable potency on the tested larvae as was appeared in the Probit lines (Fig. 1).

Nevertheless, the larvicidal effect of ethanol extract of *A. indica* flower (Table, 2) showed a range of mean mortality of 30 -97% and 32-97% on *A. arabiensis* and *C. quinquefasciatus* larvae, at concentrations ranged between (9.19–45.93 ppm). The LC₅₀'s was 11.22 ppm for *A. arabiensis* and 25.11 ppm for *C. quinquefasciatus* larvae, while the LC₉₅'s was 41.68 ppm and 43.65 ppm, followed the same order of larvae. According to the obtained LC's values, it was clear that, the ethanol extract of *A. indica* flowers showed relatively higher larvicidal potentiality than leaves against both larvae, which can be easily noticed in (Figure 1).

The damage caused by the Ethanol extracts of *A. Indica* leaves and flower on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hours

After 24 hours of applying ethanol extracts of *A. indica* leaves and flower, a lethal effect and some morphological changes (in color, loss of the body hairs, swelling, decapitate and with disconnected head, Larvae damage,) were noticed (Plates, 1.a,b,c,d,e.). The change in the larval color submitted to *A. indica* leaves and flower was high in flower and leaves.

Table 1: The larvicidal effect of ethanol extract of *A. indica* leaves on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

Concentration (ppm)		<i>A. arabiensis</i>		<i>C. quinquefasciatus</i>	
Log	%Mortality	Probit	%Mortality	Probit	
219.46	2.341	95	6.64	92	6.41
175.56	2.244	93	6.48	90	6.28
65.84	1.818	36	4.64	55	5.13
43.89	1.642	35	6.14	48	4.95
21.95	1.341	20	4.16	22	4.23
Probit analysis					
R ²		0.92		0.99	
Slope		2.68		2.21	
x-coefficient		0.27		1.24	
LC ₅₀		77.62 ppm		52.48 ppm	
LC ₉₅		219.46 ppm		251.18 ppm	

Control mortality = 0

Table 2: The larvicidal effect of ethanol extract of *A. indica* flower on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

Concentration (ppm)		<i>A. arabiensis</i>		<i>C. quinquefasciatus</i>	
Log	% Mortality	Probit	% Mortality	Probit	
45.93	1.662	97	6.88	97	6.88
36.74	1.565	90	6.28	82	5.92
27.56	1.440	72	5.58	67	5.44
18.37	1.264	60	5.25	47	4.92
9.19	0.963	30	4.48	32	4.53
Probit analysis					
R ²		0.94		0.85	
Slope		3.26		3.06	
x-coefficient		1.19		1.30	
LC ₅₀		11.22 ppm		25.11 ppm	
LC ₉₅		41.68 ppm		43.65 ppm	

Control mortality = 0

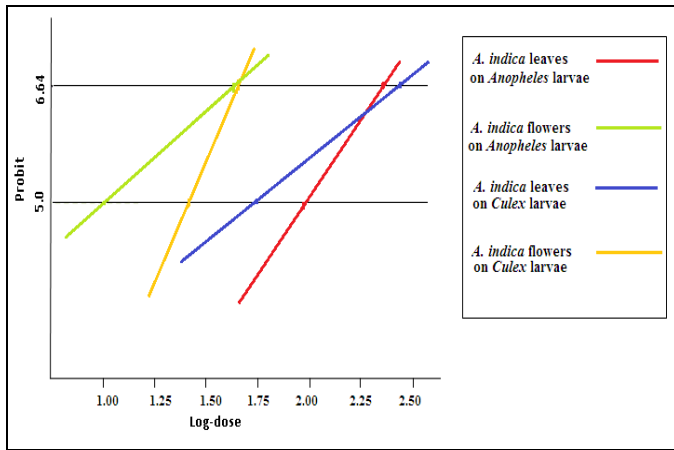


Fig 1: Log-Probability lines of *A. indica* leaves and flowers extracts on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

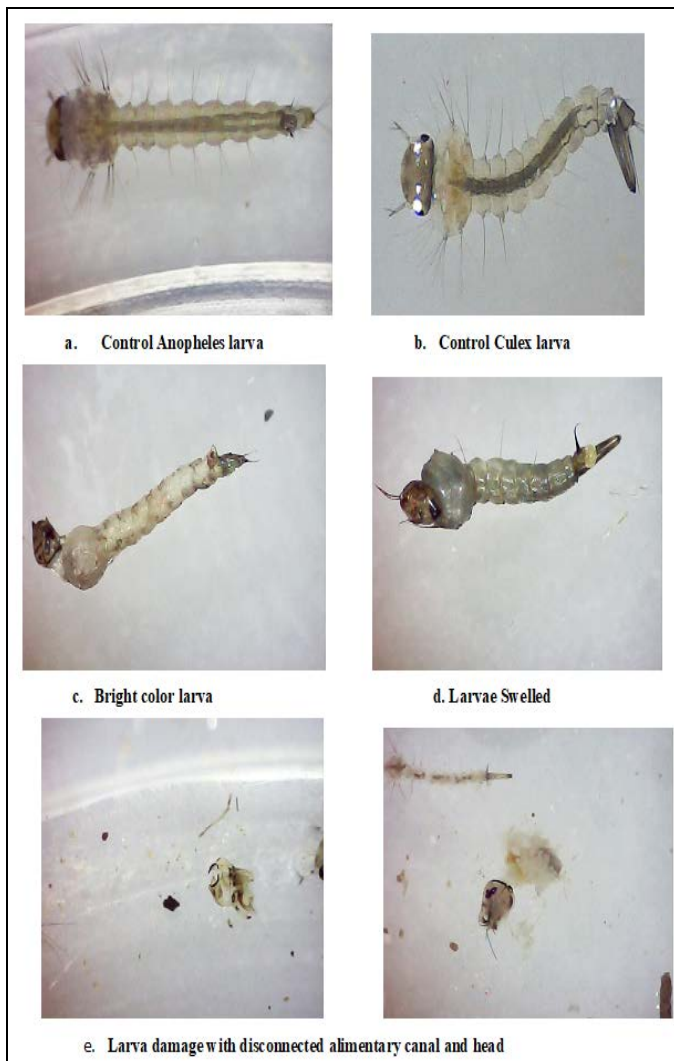


Fig 2: (a, b, c, d, e.) The damage caused by the Ethanol extract *A. Indica* flower on *A. arabiensis* and *C. quinquefasciatus* larvae after 24 hour

Discussions

In discovery of potent larvicides, crude aqueous extracts of the *A. indica* (Neem) were collected from the Wad Medani City, Central Sudan against larval stage of *A. arabiensis* and *C. quinquefasciatus*. The values of L_{50} and L_{95} , cleared that, the ethanol extract of local Neem flowers and leaves has a larvicidal activity against the tested larvae after 24 hours of

exposure and the high toxicity was exerted by the neem flowers extract

Neem, however, has been reported as an efficient larvicide against various animal phyla and species as reported by several authors [10, 12, 18, 19]. Due to its azadirachtin potential that inhibited the development of larvae as it has been to be highly effective in its activity as pesticides and/or medical properties [5, 20]. The results of this study showed that, mortality was based on the part of the plant used for extraction, the concentration and the species studied. This was in line with Boeke *et al.*, [20], and others as they reported that the neem tree provide many useful compounds which can be used as pesticides. However, the active compound in the neem tree and their concentrations vary with the parts of the plant used and with an effective dose concentration over various phyla and species of animals [20]. The previous author stated that “most dangerous neem-based products tend to be the non-aqueous extracts, with an average safe dose (ESD) of 0.002 and 12.5 mg/ kg bw/ day, as well as the unprocessed seed oil and the aqueous extracts (ESD 0.26 and 0.3 mg/ kg bw/day, 2 ml/kg bw/day, respectively) are less harmful, while most of the pure compounds show relatively low toxicity (ESD 15 mg/kg bw/day azadirachtin). One part per million (1 ppm) concentration of Azadirachtin is sufficient to produce almost 100% larval mortality [21]. In this research leave extract provide only 97% and 92% for, *A. arabiensis* and *C. quinquefasciatus* larvae, respectively. The neem oil formulation is a highly effective larvicide for anopheline mosquito vector control [22]. Neem oil had an LC_{50} value of 11 ppm after 8 days, which was nearly five times more toxic than the corn oil formulation. Adult emergence was inhibited by 50% at a concentration of 6 ppm. Significant reductions on growth indices and pupation, besides prolonged larval periods, were observed at neem oil concentrations above 8 ppm. The corn oil formulation, in contrast, produced no growth disruption within the tested range of concentrations [22]. The study was evaluating the damages caused by *Citrullus colocynthis* extracts on the larvae of *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes on the morphological bases. Some damage effects (morphological changes) were monitored on the dead larvae that subjected to the ethanol and acetone extracts, and it includes change in the larval color (to brighter color) in about 80% of dead larvae, disconnected heads in 25% of dead larvae, siphon lost in 30% of dead larvae [23]. Ethanol extract of *L. camara* leaves showed relatively higher larvicidal potentiality against *C. quinquefasciatus* larvae than *A. arabiensis* larvae. Also, leaves. In addition, some morphological changes were noticed on both species when they submitted to higher extracts of leaves and flower. Changes include larva and pupa damage with disconnected alimentary canal and head loss, as well as to failure to pupate on color pupa and morphogenesis termination of *A. arabiensis* and *C. quinquefasciatus* [24].

Conclusions

The obtained data showed that, the extracts of Neem leaves and flowers have been very effective as larvicides for both tested larvae. Also, flowers part exerted an excellent control work than leaves. Further studies should be carried out to determine the appropriate manner of preparation, application and evaluation of the mode of action of this extracts.

Conflict of interest

I (corresponding author) certify that there are NO have conflict of interest between authors in this work

References

1. Maithani A, Parcha V, Pant G, Dhulia I, Kumar D. *Azadirachta indica* (neem) leaf: A review. *Journal of Pharmacy Research*. 2011; 4(6):1824-1827.
2. Hashmat I, Azad H, Ahmed A. Neem (*Azadirachta indica* A. Juss) - A nature's drugstore: an overview. *Int. Res. J. Biol. Sci.* 2012; 1(6):76-79.
3. Sujarwo W, Keim AP, Caneva G, Toniolo C, Nicoletti M. Ethnobotanical uses of neem (*Azadirachta indica* A. Juss.; Meliaceae) leaves in Bali (Indonesia) and the Indian subcontinent in relation with historical background and phytochemical properties. *J. Ethnopharmacology*. 2016; 189:186-193.
4. Reed E, Majumdar SK. Differential cytotoxic effects of azadirachtin on *Spodoptera frugiperda* and mouse cultured cells. *Entomologia Experimentalis et Applicata*. 1998; 89(3):215-221.
5. Tembe-Fokkungan EA, Charles F, Kaba N, Donatien G, Michael A, Bonaventure N *et al.* The Potential Pharmacological and Medicinal Properties of (*Azadirachta indica* A. Juss) in the Drug Development of Phyto-medicine. *Journal of Complementary and Alternative Medical Research*. 2019; 7(1):1-18.
6. National Research Council. *Neem: a tree for solving global problems*. The Minerva Group, Inc, 2002, 144.
7. Ahmed S, Grainge M. Potential of the neem tree (*Azadirachta indica*) for pest control and rural development. *Economic botany*. 1986; 40:201-209. <https://doi.org/10.1007/BF02859144>
8. Umar MR. Trees Mensuration in Forestry and Wildlife Nursery Plantation University of Maiduguri Campus, Borno State, Nigeria. *Asian Journal of Research in Agriculture and Forestry*. 2020; 6(2):8-20.
9. Zibae A, Malagoli D. The potential immune alterations in insect pests and pollinators after insecticide exposure in agroecosystem. *Invertebrate Survival Journal*. 2020; 17:99-107.
10. Khan FZA, Sagheer M, Hasan M, Saeed S, Ali K, Gul HT *et al.* Toxicological and repellent potential of some plant extracts against stored product insect pest, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). *International Journal of Biosciences*. 2013; 3(9):280-286.
11. Sayah F, Idaomar M, Soranzo L, Karlinsky A. Endocrine and neuroendocrine effects of azadirachtin in adult females of the earwig *Labiduraria parva*. *Tissue and Cell*. 1998; 30(1):86-94.
12. Jennifer AM, Alasdair JN. Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. *Anais da Sociedade Entomológica do Brasil*. 2000; 29(4):615-632.
13. Imbahale SS, Mukabana WR. Efficacy of neem chippings for mosquito larval control under field conditions. *BMC Ecology*. 2015; 15(1):8.
14. Van der Kevie W. Climatic zones in the Sudan. *Soil Survey Administration*, 1976.
15. Harrison MN, Jackson JK. Ecological classification of the vegetation of the Sudan, *Forests Bulletin, Sudan*. 1958 2:45.
16. Mandal S, Mandal MD, Pal NK. Antibacterial potential of *Azadirachta indica* seed and *Bacopa monniera* leaf extracts against multidrug resistant *Salmonella enterica* serovar *typhi* isolates. *Archives of Medical Science*. 2007; 3(1):14.
17. WHO: Pesticides and their Application for the Control of Vectors and Pests of Public Health Importance. World Health Organization 2006; WHO Pesticides Evaluation Scheme, WHO/CDS/WHOPES/GCDPP /2006.1.
18. Reed E, Majumdar SK. Differential cytotoxic effects of azadirachtin on *Spodoptera frugiperda* and mouse cultured cells. *Entomologia Experimentalis et Applicata*. 1998; 89(3):215-221.
19. Zhou Y, Zhang PW, Chen XT, Liu BJ, Cheng DM, Zhang ZX *et al.* Integrated LC-MS and GC-MS-based untargeted metabolomics studies of the effect of Azadirachtin on *Bactrocera dorsalis* larvae. *Scientific Reports*. 2020; 10(1):1-11.
20. Boeke SJ, Boersma MG, Alink GM, van Loon JJ, van Huis A, Dicke M *et al.* (Safety evaluation of neem (*Azadirachta indica*) derived pesticides. *Journal of Ethnopharmacology*. 2004; 94(1):25-41.
21. Schreck CE. Protection from blood-feeding arthropods. *Wilderness Medicine: Management of Wilderness and Environmental Emergencies*. 3d ed. St. Louis: Mosby, 1995, 813-30.
22. Okumu FO, Knols BG, Fillinger U. Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. *Malar J*. 2007; 6:63. Doi: 10.1186/1475-2875-6-63.
23. Nosiba S Hamid, Mutaman A Kahil, Nasir A Ibrahim. Damages caused by *Citrullus Colocynthis* extracts on larvae of *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes. *International Journal of Biology Research*. 2020; 5(3):5-7.
24. Ahmed Ali Alghamdi, Nosiba SH Basher. Efficacy of leaves and flowers ethanol extracts of the invasive species *Lantana camara* Linn as a Mosquito larvicidal. *International Journal of Mosquito Research*. 2020; 7(5):43-47.