



The investigation of aqueous extract of neem bark and leaves against larvae mosquitoes in Khartoum state, Sudan

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Abstract

The present study aimed to investigate the aqueous extract of neems' bark and leaves against larvae mosquitoes in Khartoum State, Sudan. *A. indica* leaves and bark were collected from the Faculty of Public health and Environmental Health, University of Khartoum and identified and classify by Department of Botany Faculty of Science, University of Khartoum, the plant material washed by D.W water and alcohol then dried in shadow. Mosquito larvae (early 3rd and fourth instar) were collected from breeding sites pool of stagnant water from Gaba-alsunt-Khartoum state in a plastic bucket. Bioassay was applied according to WHO method in each test containing 20 leaves in 200 ml tap water, three replicates for each concentration of extract were done. after 24 hours the number of mortality was observed. The results were performed by calculating the death rate in each concentration The Porbit analysis was done using Excel 2016 to perform lethal concentrations for 50% of the mortality, LC₅₀ and LC₉₅ at 24h after treatment.at different concentrations. the results obtained from *A. indica* leaves and blend (bark + leave) water extracts were applied to mosquito larvae at concentrations, of 0.0125, 0.01, 0.005, 0.0005 and 0.00025 mg. The leaf extract was shown a range of mean mortality of between 45% and 95% after 24 hours and between 45%_and 98% after 48 hours. While water Extract *A.indica* blend (bark + leave) showed a range of mean mortality of between 50% and 90% after 24 hours and between 40%_and 95% after 48 hours. The probit analysis showed that the LLC50swas 0.0004 mg, 0.00039mg,0.00025 mg, and 0.00038mg after 24 hours for leaf and blend, respectively, while the LC95s were 0.0125 mg, 0.012mg, 0.0158 mg and 0.0125mg after 48 hours for leaf and blend, respectively.

Keywords: aqueous extract, neems' bark, leaves, mosquitoes, Khartoum State, Sudan

Introduction

Mosquitoes are a common insect that have related to transmitted many dangers disease to human and animals specially *ades aegypti* which transmitted serious viral diseases [6]. There are about 3500 species of mosquito in the world, most females fed on human and animal blood as meals that are important for their reproduction, this way of feeding is to mean the method of disease transmission 5). *Azedarach indica* (Neem) is a big tree reached in height about 20 m [1] can grow in tropical and subtropical regions with semi-arid to humid climates. Neem will typically experience mean temperatures of 25-35°C, There is, a considerable interest tree that prevents the spread of deserts and ameliorate desert environments [4] *Azedarach indica* contain active compounded belong to tetranortriterpenoid which consider as inhibitory growth to different stages of the insect [1]. Neem is effective against the mosquito in two ways, as a larvicide, and as a repellent. for control of malaria [2]. They stop feeding and die within 24 hours after treatment [3]. The control of mosquito indoor residual program it always focused to chemical control, however, in the last few years scientists became proposed to use biological control using plants as anti-insect, especially mosquitoes. Some study proven *A. indica* is more effective against mosquitoes [7]. The main active ingredient in neem is azedarach which is considered an insecticide and has a good impact on the environment, therefore, the use of neem as mosquitocidal became acceptable and applicable to fight mosquito and their stages [8]. The neem tree *Azadiracta*

indica extracts shave shown considerable lethal activity against insect pests of agricultural and public health importance [9]. Therefore, the present study aimed to investigation the aqueous extract of neems' bark and leaves against larvae mosquitoes in Khartoum State, Sudan.

Material and Methods

Plant Material

A. indica leaves and bark were collected from the Faculty of Public health and Environmental Health, University of Khartoum and identified and classify by the Department of Botany Faculty of Science, University of Khartoum, the plant material was washed with D.W water and alcohol and then dried in shadow.

Larvae Collection

Mosquito larvae (early 3rd and fourth instar) were collected from breeding sites pool of stagnant water from Sount forest -Khartoum state in a plastic bucket, it has water from the same breeding environment, and kept in the Laboratory at room temperature (27C⁰) after 24 hours was tested at the Medical Entomology Laboratory, Faculty of Public health and Environmental Health, University of Khartoum.

Extract preparation and bioassay

The leaves and bark of *A. indica* (Neem) were powdered and made aqueous solution containing 10g disliked in 100 ml of DW then flirtd after 24 hours. Bioassay was applied according to WHO (1996) method, in each test containing

20 leaves in 200 ml tap water, three replicates for each concentration of extract were done. after 24 hours the number of mortality was observed.

Statistical Analysis

The results were performed by calculating the death rate in each concentration using the following formula:

$$\text{Percentage of Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

The Probit analysis was done using Excel 2016 to perform lethal concentrations for 50% of the mortality, LC₅₀ and LC₉₅ at 24h after treatment at different concentrations, logarithms were added (+6) to convert the concentration logarithmic to a positive number. The death ratio was converted to Probit units using Finney table. The logarithmic concentrations were represented the X Probit units and. Y Points were represented and the toxicity line was drawn.

Results

Tables 1 and 2 show the results obtained from *A. indica* leaves and blend (bark + leave) water extracts were applied to mosquitoes larvae at concentrations, 0.0125, 0.01, 0.005, 0.0005 and 0.00025 mg. The leaves extract was showed a range of mean mortality of between 45% and 95% after 24

hours and between 45%_and 98% after 48 hours. While water Extract *A. indica* blend (bark + leave) was showed a range of mean mortality of between 50% and 90% after 24 hours and between 40%_and 95% after 48 hours. The probit analysis showed that, the LC₅₀'s was 0.0004 mg, 0.00039mg,0.00025 mg, and 0.00038mg after 24 hours for laves and blend, respectively, while the LC₉₅'s was 0.0125 mg, 0.012mg, 0.0158 mg and 0.0125mg after 48 hours for laves and blend, respectively (see depiction of “Log- Probit curve” in figure 1&2).

Table 1: The larvicidal effect water Extract *A. indica* leaves on Mosquitoes larvae

Conc. (mg)	Log- Conc	After 24hours		After 48hours	
		Mortality %	Probit	Mortality %	Probit
0.0125	1.9030-	95%	6.64	98%	7.05
0.01	2.0000-	85%	6.04	90%	6.28
0.005	2.3020-	75%	5.67	75%	5.67
0.0005	3.3010-	65%	5.39	70%	5.52
0.00025	3.6010-	45%	4.87	45%	4.87
R2		0.98		0.98	
slope		0.63		0.67	
x-coefficient		2.22		3.42	
LC ₅₀		0.0004mg		0.00039mg	
LC ₉₅		0.0125 mg		0.012mg	

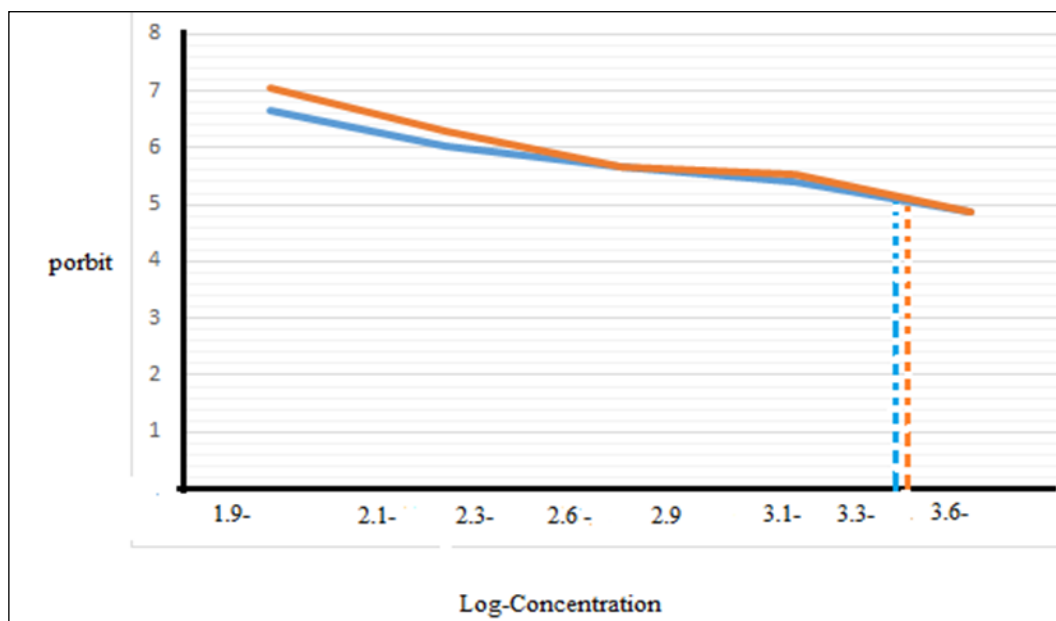


Fig 1: Log- Probit curve of the larvicidal effect water Extract *A. indica* leaves on Mosquitoes larvae

Table 2: The larvicidal effect water Extract *A. indica* blend (bark + leave) on Mosquitoes larvae

Conc. (mg)	Log- Conc	After 24hours		After 48hours	
		Mortality %	Probit	Mortality %	Probit
0.0125	1.9030-	90%	6.28	95%	6.64
0.01	2.0000-	85%	6.04	80%	5.84
0.005	2.3020-	75%	5.67	70%	5.52
0.0005	3.3010-	65%	5.39	60%	5.25
0.00025	3.6010-	50%	5.00	40%	4.75
R2	0.88			0.87	
slope	0.005			0.0039	
x-coefficient	4.72			0.025	
LC ₅₀		0.00025 mg		0.00038mg	
LC ₉₅		0.0158 mg		0.0125mg	

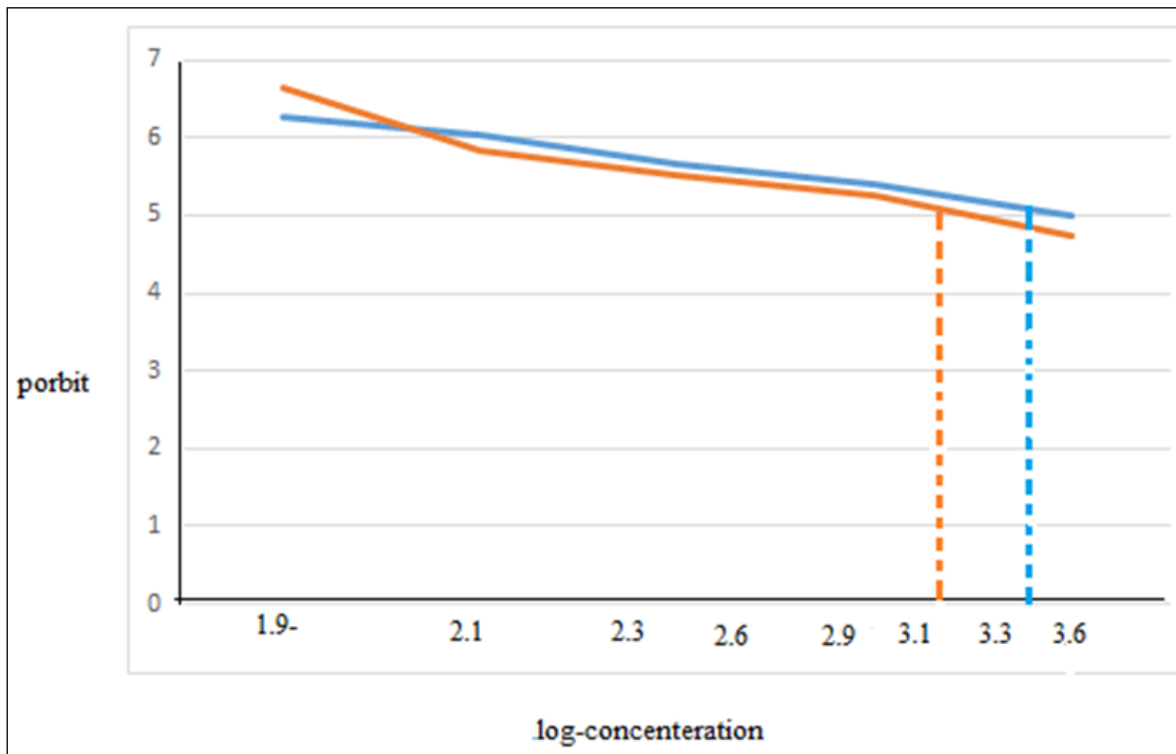


Fig 2: Log- Probit curve the larvicidal effect water Extract *A. indica* blend (bark + leave) on Mosquitoes larvae

Discussion

Mosquito control remains a powerful method to control mosquito-transmitted diseases, focusing on the early stages is very important for many reasons, such as because they are easy for handling, more sensitive to environmental factors, and more susceptible to synthetic and natural toxic products. Neem water extract was an effective larvicide, it was highly toxic to mosquito larvae and inhibited the development of pupae. The high rates of larval mortality observed at higher concentrations, within 48 hrs after exposure indicate the high toxicity of the product. *Azadirachta indica* (eco-friendly), use differently, insect repellent, Mixed dried neem leaves with stored grain or place them among warm clothes is useful to repel insects and mosquitoes [10], plant extracts are less expensive and highly efficacious for the control of mosquitoes [11]. The previous results support our findings obtained by a current study that found that the water extract of neem leaf was effective against mosquito larvae.

The results of this study showed high mortality, larval mortality increased from 45% at 0.00025mg to 95% at concentration 0.0125mg After 24hours, 45% at 0.00025mg to 98% at concentration 0.0125mg After 48 hours, Similarly a previous studies high mortality 94%, 80%, 97% [12, 13, 14], percentage larval mortality increasing with an increase in the concentration and the duration of exposure extract [15] LC50 value for the water Extract *A. indica* (leaves) was 0.0004mg for 24 hours, 0.00039mg for 48 hours, LC50 value for the water Extract (bark + leave) was 0.00025 mg for 24hours, 0.00038mg for 48. This compares with the LC50 previous studies showed LC50 Neem in 0.15% (0.36mg/ml, 0.69mg/ml) (3.02mg/ml) s [16], The LC50 value leaves (3.02mg/ml) [17] for 24hours, The LC50 bark (7250837.5ppm), The LC50 leaves (82.5 to 600ppm) for 48 hours [18], while the LC95 values of Ripe fruits ranged from (0.073 to 0.277) for 24 hours [19]. This compares with the LC95 in our study leaves (0.0125 mg) for 24 hours. (0.012mg) for 48 hours, LC90 bark + leave (0.0158 mg) for

24hours 0.0125mg for 48. The variation in LC50 and LC90 is due to mosquito species, and method of application. concentrations parts of the neem plant from which the products were extracted. The focus of this study is on neem leaf and bark. because the leaves are easier to harvest, especially at an earlier stage of tree development, and they are more able to regenerate. However, the leaves and bark have been found to be equally effective [20] Neem trees are found throughout Africa, and the manufacturing of these larvicides can be stimulated through local businesses [13]

Conclusion

According to our findings, the study concluded that neem-based larvicides may present an ideal option to increase efforts using simple and inexpensive application methods for controlling mosquitoes in their breeding sites.

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Conflict of interest

I (corresponding author) certify that there are NO any conflict of interest or competing financial interests between authors in this work

Ethics Statement

This work was approved by the Ethics Committee of the University of Khartoum. The data obtained from this work were analyzed according to guidelines of ethical standards of the Declaration of Helsinki.

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