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(RESEARCH ARTICLE)



Larvicidal effect of S-hydroprene and leaf extracts of *Azadirachta indica* (A. Juss, 1830) on metamorphosis of *Culex quinquefasciatus* (Say, 1823)

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Abstract

Insect Growth Regulators (IGRs) are yet to be explored with the huge Nigerian plant biodiversity despite the bio-specificity and environmental friendly characteristics when compared to conventional chemicals. Safer, cheaper and reachable alternatives to the more popular insecticides that are hazardous to human health and the environment have become imperative. The study was designed to evaluate the effect of S-Hydroprene and various solvent leaf extracts of *Azadirachta indica* on the metamorphosis of *Culex quinquefasciatus* mosquitoes. Methanol, N-Hexane, Chloroform, Aqueous leaf extracts of *Azadirachta indica* and S-Hydroprene were tested on 1st and 3rd instar larvae of *Cx. Quinquefasciatus* under laboratory conditions for a consistent period of 6 - 15 days in which growth inhibition was monitored using the control to determine the rate of change for the concentrations 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L and 60 mg/L. S-Hydroprene had more deleterious effect on *Cx. quinquefasciatus* third instar larvae at 60 mg/L 2.8(9%) when compared with methanolic extract. N-Hexane extract killed more larvae at 60 mg/L 11.2(37%). Since extracts of *Az. indica* had less toxic effect, they allowed for the survival of *Cx. Quinquefasciatus* but extended the larval stages and prevented ecdysis. The first instar larval stage was maintained for sixteen (16) days which is at variance with the regular three to four days larval existence. Leaf extracts of *Az. Indica* and S-Hydroprene at concentration of 10 mg/L to 60 mg/L caused morphological deformations of the metamorphosis of *Culex quinquefasciatus*. The leaf extracts of *Az. indica* possess explorable larvicidal properties for control of larva and adult mosquitoes.

Keywords: *Azadirachta Indica*; *Culex quinquefasciatus*; Insect Growth Regulators; Larvicidal; Morphology; S-Hydroprene.

1. Introduction

Insect growth regulators (IGRs) are intrinsically non-toxic, biologically specific and environmentally friendly compared to other conventional chemical insecticides and larvicides [1]. Among these IGRs, Methoprene has been reported to be effective against some mosquito vector species [2]. Insect resistance to some IGRs other than Hydroprene may be due

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to either degradation of the artificially applied IGRs in the insect's body before reaching their target sites or the modification of the target sites resulting in reduced affinity of juvenile hormone binding proteins (JHBPs) to the juvenile hormone analogues (JHAs) [3]. Among all the species of mosquitoes known, *Culex quinquefasciatus* are the most widespread [4]. *Culex quinquefasciatus* are vectors of certain diseases and disease causing agents such as Ross River Virus, Alfuy, Almpiwari, Corriparta, Dengue, Sindbis and Japanese Encephalitis Virus [5, 6]. It is also the main vector of the filarial parasite *Wuchereria bancrofti* which causes the human lymphatic filariasis that lead to acute and chronic morbidity, affecting people of all ages and sexes throughout the tropical and sub-tropical areas of the world [7, 8]. *Azadirachta indica* A. juss (AI; Family: *Meliaceae*) is a popular medicinal plant originally grown in India [9] but is now being cultivated in almost every part of the world including Nigeria [10, 11] where it is popularly called "Dogonyaro". It is one of the most useful medicinal plants [12]. It is a large evergreen tree growing 10-11 m tall. The leaves are divided into numerous leaflets each resembling a full-grown leaf [13]. Every part of neem (*Azadirachta indica*) has been advocated to have medicinal properties [14]. Extracts from the plant offers a promising larvicidal against *Culex quinquefasciatus* and other species of mosquitoes, including growth inhibition, abnormal development, elongation of larval period and sometimes no pupation [15]. To evaluate the effects of extract of *Azadirachta indica* on *Culex quinquefasciatus* larvae.

2. Material and methods

2.1. Study Area

Study was carried out in Ahmadu Bello University, Zaria, Nigeria (Figure 1). It lies between latitude 11° 15'N to 11°3'N and longitude 7° 30'E to 7°45'E. The area is characterized by a grassland ecosystem with trees being widely spaced so that the canopy does not close. The annual rainfall ranges between 1016mm and 1524mm with a relative humidity ranging between 60% to 80% [16].

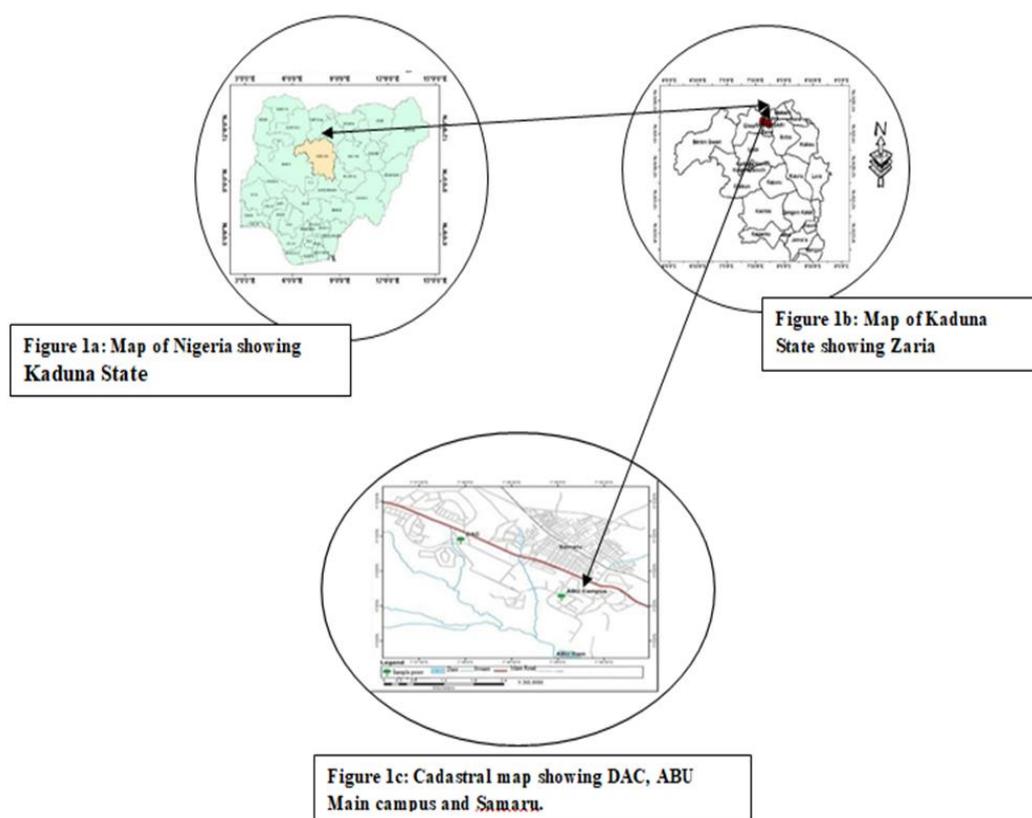


Figure 1 Cadastral map showing Nigeria, Kaduna State and Samaru campus

2.2. Collection and Identification of Plant material

Fresh leaves of *Azadirachta indica* were collected from the premises Ahmadu Bello University, Zaria and identified at the herbarium of the Department of Botany (voucher number 90104). The leaves were air-dried under the shade and pulverized using mortar and pestle.

2.3. Solvent Extraction of *Azadirachta indica* and Preparation of Stock Solutions

The plant material was extracted by weighing 500 g powder of *Azadirachta indica* each into four different glass containers with 1000 ml of methanol, distilled water, chloroform and N-Hexane respectively and allowed for 72 hours. The contents were filtered using muslin and cotton wool respectively and evaporated via the water bath at 60 °C – 65 °C.

Stock solution of the IGR was prepared by dissolving 250 mg of S-Hydroprene in 2 ml of dimethylsulphoxide (DMSO) to obtain a homogenous solution that is miscible with water. Twenty five milliliters of water was then added to obtain the stock. Same was carried out for the other solvent extracts without DMSO dilution as a result of its solubility in water according to [20]. Serial dilution for the stock solution was made afterwards.

2.4. Collection of Adult Mosquitoes and Breeding of Larvae and Identification of Adult Mosquitoes

Twenty 20 blood-fed adult female *Culex quinquefasciatus* were collected by trapping them with the use of a test tube while resting on the walls of some rooms in the students' hostel in ABU main campus, transferred via entomological cages, identified by an entomologist and bred according to [21, 22] Preserved female adult mosquitoes were observed under a microscope and identified by the use of published keys according to [8].

2.5. Bioassay of Mosquito Larva Using S-Hydroprene and Leaf Extracts of *Azadirachta indica*

The established colonies maintained at optimum conditions of 25-27 °C and 70-80 % relative humidity in the Laboratory of Department of Zoological Sciences, ABU Zaria. The physicochemical parameters of the water – pH and dissolved oxygen were checked for breeding quality. The larvae of the mosquitoes were fed with grinded digestive biscuit throughout the experimental process continues until they to emerged as adult.

S-Hydroprene and the leaf extracts of *Azadirachta indica* were investigated based on concentration. Larval development and larvicidal efficacy were screened against the larval instars of *Culex quinquefasciatus*. For screening of larvicidal activity, the first larval instars of *Culex quinquefasciatus* were separated into a batch of thirty (30) and was transferred into separate petri dish containing 100ml of distilled water. This was again replicated into 5. The Same replication was made in the case of the control set-up but devoid of the neem extracts according to [20]. The whole setup was allowed for a consistent period of 6 - 15 days in which growth inhibition was monitored using the control to determine the rate of change for the concentrations 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L and 60 mg/L.

The number of dead larvae of *Culex quinquefasciatus* and *Aedes aegypti* in the various test concentrations used were subjected to probit analysis. Control mortality was corrected using Abbott's formula, $P = \frac{Po - Pc}{100 - Pc} \times 100$ (P = Abbott's corrected mortality, Po = percentage of observed mortality, Pc = percentage of control mortality) [20].

2.6. Statistical Analysis

Analysis of variance (ANOVA) was used to test for significant differences in larval mortality amongst the various concentrations of solvent- based extracts. Duncan's multiple range tests was employed in separating differing means. Student's T - Test was used to compare the efficacy of the extracts of *Azadirachta indica* and S-Hydroprene on the mosquito species. Values were considered statistically significant at $P \leq 0.05$.

3. Results and discussion

Larval mortality of the third instar of *Culex quinquefasciatus* increased as the concentration from 10 mg/L to 60 mg/L for all extracts of *Azadirachta indica* increased using different media.

That of S-Hydroprene exhibited similar pattern with the methanol. The results obtained with N-Hexane extract showed significantly ($0.000 P \leq 0.05$) -Table1.

Table 1 Effect of extracts of *A. indica* and S-Hydroprene on mortality of third instar larvae of *Culex quinquefasciatus* after 24 hours

Conc. (mg/L)	No. per Ex P.	N-Hexane Extract Mean Mortality \pm SE	Aqueous Extract Mean Mortality \pm SE	Chloroform Extract Mean Mortality \pm SE	Methanol Extract Mean Mortality \pm SE	S-Hydroprene Mean Mortality \pm SE
Control	30	1.0 \pm 0.32(3)	1.0 \pm 0.32(3)	1.0 \pm 0.32(3)	1.0 \pm 0.32(3)	1.0 \pm 0.32(3)
10	30	3.8 \pm 0.55(13) ^d	5.4 \pm 0.54(18) ^b	3.6 \pm 0.75(15) ^b	1.4 \pm 0.58(5) ^a	1.4 \pm 0.51(5) ^{a b}
20	30	5.4 \pm 0.75(18) ^{c d}	5.2 \pm 0.02(17) ^b	4.4 \pm 0.03(15) ^b	2.2 \pm 0.66(7) ^a	1.4 \pm 0.60(5) ^{a b}
30	30	6.8 \pm 0.97(23) ^{b c}	5.8 \pm 0.86(19) ^b	6.6 \pm 0.87(22) ^a	2.6 \pm 0.87(9) ^a	2.0 \pm 0.51(7) ^{a b}
40	30	8.6 \pm 0.75(29) ^{a b}	7.4 \pm 0.08(25) ^{a b}	7.0 \pm 0.32(23) ^a	2.0 \pm 0.75(7) ^a	1.4 \pm 0.37(5) ^c
50	30	9.0 \pm 0.25(30) ^{a b}	7.8 \pm 0.86(26) ^{a b}	7.8 \pm 0.86(26) ^a	3.2 \pm 0.15(7) ^a	2.2 \pm 0.25(7) ^{a b}
LC ₅₀		1.216	1.172	1.211	1.059	1.047
F-Value		14.453	8.095	10.956	1.212	1.674
P-Value		0.000	0.000	0.000	0.330	0.164

Key: SE = Standard Error, Conc. = Concentration, No = Number. Figures in parenthesis are in percentages. Superscripts with different letters are significantly different at $p \leq 0.05$

Approximately 9 (30%) of the first instar larvae of *Cx. quinquefasciatus* survived after sixteen (16) days with in both methanol and aqueous extracts of *A. indica* at 10 mg/L while only 2 (7%) of the organisms survived within same period of treatment with S-Hydroprene. However, increase in concentration of *A. indica* did not result in a corresponding increase in the number of survival for all solvent used for extraction (Table 2).

Table 2 Effect of the extracts of the leaf of *Az. indica* and S-Hydroprene on the survival of first instar larvae of *Culex quinquefasciatus*

Conc. (mg/L)	N-Hexane extract Mean Survival \pm SE	Aqueous extract Mean Survival \pm SE	Chloroform extract Mean Survival \pm SE	Methanol extract Mean Survival \pm SE	S-Hydroprene Mean Survival \pm SE
Control	24.00 \pm 0.63(80) ^a	24.00 \pm 0.63(80) ^a	24.00 \pm 0.63(80) ^a	24.00 \pm 0.63(80) ^a	24.00 \pm 0.63(80) ^a
10	2.40 \pm 0.51(8) ^b	8.80 \pm 0.14(29) ^{b c}	1.20 \pm 0.58(4) ^b	9.00 \pm 0.00(30) ^b	2.00 \pm 0.55(7) ^b
20	2.20 \pm 0.49(7) ^b	9.20 \pm 0.58(31) ^b	0.60 \pm 0.40(2) ^b	6.60 \pm 0.40(22) ^c	1.60 \pm 0.40(5) ^b
30	1.60 \pm 0.25(5) ^b	8.20 \pm 0.49(27) ^{b c}	0.60 \pm 0.40(2) ^b	6.40 \pm 0.68(21) ^c	1.40 \pm 0.60(5) ^b
40	1.80 \pm 0.20(6) ^b	7.80 \pm 0.49(26) ^{b c}	0.40 \pm 0.40(1) ^b	5.20 \pm 0.02(17) ^c	2.00 \pm 0.05(7) ^b
50	2.40 \pm 0.51(8) ^b	7.00 \pm 0.71(23) ^{c d}	1.80 \pm 0.66(6) ^b	6.00 \pm 0.70(20) ^c	1.60 \pm 0.68(5) ^b
60	1.20 \pm 0.20(4) ^b	6.60 \pm 0.40(22) ^d	0.40 \pm 0.25(1) ^b	6.40 \pm 0.98(21) ^c	1.60 \pm 0.25(5) ^b
F-Value	375.621	84.641	313.085	88.716	175.225
P-Value	0.000	0.000	0.000	0.000	0.000

Key: SE = Standard Error, Conc. = Concentration, No = Number
 Figures in parenthesis are in percentages. Superscripts with different letters are significantly different at $p < 0.05$

In the case of S-Hydroprene, the effect was almost the same for all concentrations where approximately one larva survived. The results obtained from methanol and aqueous extracts using various concentrations probably indicates the closeness of the potency of the active ingredients in methanol which is probably less toxic to the first instar larvae but the effect of the chloroform extract. of *Az. indica* definitely suggests the toxic potency of chloroform in addition to the effect of *Az. indica* to first instar larvae (Table 2).

N-Hexane and chloroform extracts of *Az. Indica* treatments on the first instar larvae of *Cx. quinquefasciatus* survived for fifteen (15) to sixteen (16) days without metamorphosing into pupae in concentrations which range from 10 mg/L to 60 mg/L (Table 3).

Table 3 Mosquito larval survival in days following the application of S-Hydroprene and extracts of *Az. Indica*

Conc. (mg/L)	N-Hexane extract Mean Days ± SE	Aqueous extract Mean Days ± SE	Chloroform extract Mean Days ± SE	Methanol extract Mean Days ±SE	S-Hydroprene Mean Days ± SE
Control	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00
10	13.80±0.37 ^b	10.40±0.58 ^{bc}	15.20±0.20 ^a	12.40±0.25 ^a	12.00±1.09 ^a
20	15.60±0.25 ^a	11.00±0.40 ^c	15.40±0.25 ^a	12.00±0.32 ^a	13.80±0.74 ^a
30	15.80±0.20 ^a	11.60±0.51 ^{abc}	15.00±0.45 ^a	12.40±0.25 ^a	12.80±1.56 ^a
40	15.80±0.20 ^a	11.80±0.58 ^{abc}	15.00±0.00 ^a	12.20±0.20 ^a	13.60±0.75 ^a
50	15.60±0.40 ^a	12.40±0.45 ^{ab}	15.00±0.00 ^a	11.80±0.37 ^a	12.00±0.45 ^a
60	15.80±0.6 ^a	12.60±0.51 ^a	14.80±0.20 ^a	12.20±0.37 ^a	12.80±0.74 ^a
F-Value	67.048	12.181	147.608	32.741	6.110
P-Value	0.000	0.000	0.000	0.000	0.000

Key: SE = Standard Error, Conc. = Concentration, No = Number Superscripts with different letters are significantly different at p<0.05

First instar larvae of *Cx. Quinquefasciatus* treated with S- Hydroprene survived from 12 to 14 days across 10 mg/L to 60 mg/L ranges of concentration.

The effect of treatment with Leaf extracts of *Az. Indica* and S-Hydroprene on the morphology of the organisms was in form of abnormalities on the body wall and chitinous exoskeleton (Figures 2, 3 and 4). These effects were not observed in the control group (Figure 5).



Figure 2 Decomposition Head and Abdomen of *C. quinquefasciatus*



Figure 3 Decomposing body parts of *C. quinquefasciatus*



Figure 4 Larva-pupa intermediate of
Culex quinquefasciatus



Figure 5 Control

Figure 2 – 5: Morphological effect of *Azadirachta indica* (10 mg/L to 60 mg/L) and S-Hydroprene (10 mg/L to 60 mg/L) on experimental and control groups of first instar larvae of *Culex quinquefasciatus*

All extracts of *Az. Indica* produced varying degrees of mortality on the test larvae. N-Hexane extract was observed to kill more mosquito larvae than the other extracts of *Az. indica* and S-Hydroprene at same concentration. It also however killed more mosquito larvae than S-Hydroprene at the same concentration. This is probably because N-Hexane solvent extracted more active ingredients from *Az. indica* than the other solvents. This is similar to the work of [23] that used methanol extract of *Az. indica* against third and fourth instar larvae of *C. quinquefasciatus* in India and obtained total kill of the test specimen. The observed effect of N-Hexane extract of neem on larvae of *C. quinquefasciatus* is probably because of the failure of larvae to metamorphose into the pupae stage due to the inhibition of chitin formation or due to their inability to shed their exo-cuticle and inadequate intake of air to split the old cuticle. This concurs with the observation made by [24], [25]

4. Conclusion

Both leaf extracts of *Az. Indica* and S-Hydroprene at concentration of 10 mg/L to 60 mg/L caused morphological deformations of larval-pupal intermediate and damage of head and body on *Culex quinquefasciatus*. N-Hexane extract gave the highest effect with mortality rate of 37 % (60 mg/L). Furthermore, S-Hydroprene and the leaf extracts of *Az. indica* possess explorable larvicidal properties for control of larva and adult mosquitoes. This may open another vista to compare the current larvicides and this often mention third generation larvicides.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors hereby declare no conflict of interest.

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