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Life expectancy and lethal effect of *Culex quinquefasciatus* larvae due to S-Hydroprene and leaf extracts of *Azadirachta indica*

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Abstract
Culex quinquefasciatus is a dorminant mosquito species in Nigeria and a major cause of several diseases. The study seeks to determine the effects of S-Hydroprene and leaf extracts of *Azadirachta indica* on life expectancy of third instar larvae of *Culex quinquefasciatus*. Concentrations (ranging from 10 mg/L to 60 mg/L) were tested on the larvae for mortality, survival and life expectancy of the medically important *Cx. quinquefasciatus* mosquito species under laboratory conditions of temperature (25° - 27), relative humidity (70% – 80%) and photoperiod (14hrs). Chloroform extract killed more larvae of *Cx. quinquefasciatus* at 60 mg/L (70.67%) among leaf extracts while methanol killed less (32%). S-Hydroprene showed more deleterious effect at 60 mg/L (82%). Since extracts of *Az. indica* had less toxic effect, they allowed for the survival of *Cx. quinquefasciatus* and extended the larval period. S-Hydroprene and the leaf extracts of *Azadirachta indica* are capable of increasing life expectancy of *Culex quinquefasciatus* larvae at certain concentrations until they all died out or a few adults emerge. To control *Culex quinquefasciatus* therefore, extracts of *Azadirachta indica* can be used to reduce the number of adult emergence in our environment and in turn reduce their rate of disease transmission.

Keywords: Mortality, life expectancy, *Azadirachta indica*, *Culex quinquefasciastus*, insect growth regulators, larvicidal, S-Hydroprene

1. Introduction
Mosquitoes are associated with human activities and are a major vector of tropical diseases which include malaria, yellow fever and elephantiasis and other arbovirals leading to acute and chronic morbidity [1]. They cause nuisances and affecting humans of all ages and sexes throughout tropical and sub-tropical areas of the world [2, 3]. *Culex quinquefasciatus* is distributed worldwide [1]. Because insect growth regulators (IGRs) are non-toxic, biologically specific and environmentally friendly compared to other conventional chemical insecticides and larvicides [4], they can be very useful in the control of mosquito species. Insect resistance to some IGRs other than Hydroprene may be due 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) [5]. *Azadirachta indica* also known as Neem, is a popular medicinal plant originally grown in India [6] but is now being cultivated in almost every part of the world including Nigeria [7]. It is one of the most useful medicinal plants [8] where every part has been reported to have medicinal properties [9]. Extracts from the plant offers a promising larvicidal effect against *Culex quinquefasciastus* and other species of mosquitoes, including growth inhibition resulting in the elongation of larval periods [10].
This research is aimed at evaluating the effect of S-Hydroprene and leaf extracts of *Azadirachta indica* on the life expectancy of *Culex quinquefasciastus* larvae.

2. Materials and Methods
2.1 Collection, Identification and stock preparation of Leaf of *Azadirachta indica* and S-Hydroprene
Fresh leaves of *Azadirachta indica* were collected in Ahmadu Bello University, Zaria and identified at the herbarium and extracted after the method of [11] using methanol, chloroform,

N-Hexane and aqueous solvents.

S-Hydroprene was purchased and received from Bristol Company. 10% Stock solution of S-Hydroprene and leaf extracts of *Azadirachta indica* were prepared using 25 ml distilled water. 2 ml of dimethylsulphoxide (DMSO) was first used to obtain a homogenous solution that will be miscible with water each of S-Hydroprene and the leaf extracts with exception of aqueous solvent extract of *Azadirachta indica* according to [12]. Serial concentrations were used for the bioassay.

2.2 Collection and Identification of Adult Mosquitoes

Blood fed female adult *Culex quinquefasciastus* were collected in Ahmadu Bello University, Zaria Postgraduate student hostel using a test tube and identified according to the key of [3].

2.3 Larval Breeding

Engorged female mosquitos were kept in entomological cages and fed with 10% sugar solution soaked in cotton wool to as a source of energy for physiological function. 200 ml of distilled water was placed in a petri dish to allow for oviposition. Once female mosquitos were ready, they oviposit and the eggs hatch into larvae. Larvae were fed using grounded biscuit and yeast in the ratio three to one respectively until third instar larvae.

2.4 Bioassay of *Culex quinquefasciatus* Larva with S-Hydroprene and Leaf Extracts of Azadirachta indica

The optimum conditions of temperature (25-27 °C) and 70-80% relative humidity were maintained in the Laboratory for optimum breeding requirement. The larvae of the mosquitoes were fed with ground digestive biscuit and baker's yeast throughout the experimental process until all larval died or pupae resulted.

S-Hydroprene and the leaf extracts of *Azadirahcta indica* were investigated base on concentration from 10 mg/L to 60 mg/L. For screening of larvicidal activity, the third larval instars of *Culex quinquefasciastus* were separated into batches of one hundred and fifty (150) and was transferred into petri dishes per concentration containing 500 ml of distilled water. The same replication was made control experiment but devoid of neem extracts and S-Hydroprene according to [12]. The number of dead larvae of *Culex quinquefasciatus* in the various test concentrations was recorded and life expectancy calculated and presented according to [13].

3. Results and Discussion

Life table presented reveals the effect of S-Hydroprene and various solvent extracts of the leaf of *Azadirachta indica.*

From the table, 150 mosquito larvae were used for each concentration in the experiment. After a 24 hours of applying S-Hydroprene and the leaf extracts of *Azadirachta indica*, it was observed that the number that survived reduced with increase in concentration from 10 mg/L to 60 mg/L.

At the end of assay, it was observed that mortality increased with increase in concentration 10 mg/L to 60 mg/L when treated with S-Hydroprene and the leaf extracts of Chloroform, Methanol and Aqueous while N-Hexane did not result in corresponding increase with increase in concentration. The least mortality (32%) was observed on the application of methanol solvent extract of *Azadirachta indica* at 10 mg/L and the highest (70%) due to chloroform at 60 mg/L. When the effect of S-Hydroprene is compared with that of the leaf extracts of *Azadirachta indica*, it is worthy of note that S-Hydroprene at 10 mg/L effected 52% mortality which compares with that of 20 mg/L and 40 mg/L of N-Hexane and aqueous extracts respectively while at 60 mg/L, S-Hydroprene yielded the highest (82%) which is comparable to that of chloroform (70.67%) at 60 mg/L.

Table 1: Life table expectancy of *Culex quinquefasciatus* treated with S-Hydroprene and the leaf extracts of *Azadirachta indica*

Treatment	Concentration (mg/L)	Age (X)	Lx	Lx	Dx	100qx	Sx	Tx	ex (days)
S-Hydroprene	Control	3rd Instar	150	146	22	14.67	85.33	146	1.95
	10		150	141	78	52.00	48.00	750	10.00
	20		150	131	104	69.33	30.67	609	8.12
	30		150	126	106	70.67	29.33	478	6.37
	40		150	122	106	70.67	29.33	352	4.69
	50		150	116	114	76.00	24.00	230	3.07
	60		150	114	124	82.67	17.33	114	1.52
N-Hexane	10		150	140	74	49.33	50.67	724	9.65
	20		150	136	67	44.67	55.33	584	7.79
	30		150	130	74	49.33	50.67	448	5.97
	40		150	125	66	44.00	56.00	318	4.24
	50		150	95	94	62.67	37.33	193	2.57
	60		150	98	94	62.67	37.33	98	1.31
Chloroform	10		150	138	60	40.00	60.00	791	10.55
	20		150	138	77	51.33	48.67	653	8.71
	30		150	133	92	61.33	38.67	515	6.87
	40		150	131	96	64.00	36.00	382	5.09
	50		150	124	97	64.67	35.33	251	5.35
	60		150	127	106	70.67	29.33	127	1.68
Methanol	10		150	145	35	23.33	76.67	858	11.44
	20		150	145	42	28.00	72.00	713	9.51
	30		150	143	46	30.67	69.33	568	7.57
	40		150	144	48	32.00	68.00	425	5.67
	50		150	144	57	38.00	62.00	281	3.75
	60		150	137	69	46.00	54.00	137	1.83
Aqueous	10		150	148	58	38.67	61.33	885	11.80
	20		150	148	59	39.33	60.67	737	9.83
	30		150	148	70	46.67	53.33	589	7.85

	40		150	147	77	51.33	48.67	441	5.88
	50		150	147	84	56.00	44.00	294	3.92
	60		150	147	83	55.33	44.67	147	1.96

x = developmental stage, Ix = number entering stage, Lx = number alive between stage x and x+1, dx = number that died in stage x, 100qx = percent apparent mortality, Sx = survival rate within stage, Tx = total number of age x units beyond the age, ex = life expectancy

Consequently, extracts that yielded low mortality allowed for high number of larval survival. This is observed in larvae treated with methanol extract of the leaf of *Azadirachta indica* where the percentage of survival from 10 mg/L to 60 mg/L is higher compare to the values obtained from S-Hydroprene and the remaining leaf extracts.

Life expectancy of the larvae of *Culex quinquefasciatus* reduced with increase in concentration when treated with S-Hydroprene and the leaf extracts of *Azadirachta indica*. On overall, the highest life expectancy (11.80 days) was observed at 10 mg/L of the aqueous extract while the lowest (1.31 days) was observed for N-Hexane extract at 60 mg/L. Comparing life expectancy of S-Hydroprene and the leaf extracts of *Azadirachta indica*, N-Hexane extract compares very closely with S-Hydroprene from 10 mg/L to 60 mg/L.

In the study, larval mortality may be due to the inhibition of larvae to molt, inability of larvae to feed and the toxic active components (which is larvicidal) of S-Hydroprene and the leaf extracts of *Azadirachta indica*. Extracts that yielded the least mortality depicts less toxicity which in turn allowed for larval survival and extended period. This is in agreement with the work of [14] and [15] showing that extracts of *Azadirachta indica* can cause death of larvae of *Culex quinquefasciatus* in large number only when large quantity or higher concentration is used in the assay. This therefore signifies that the quantity that was used can effect less mortality disqualifying from the list of good larvicides. Larvae survived longer than expected in a natural setting because S-Hydroprene and leaf extracts of *Azadirachta indica* possess certain active substances that mimic juvenile hormone of the larvae thereby altering growth process which either led to death or increased the larval period. This is in agreement with the work of [16] who showed various concentration of extract from the leaf can delay metamorphoses by extending larval period. S-Hydroprene and the leaf extracts of *Azadirachta indica* may have altered two possible protein targets (tubulin and hsp60) which may be the cause of the observed inhibitory effects. This was described by [17] and [18] showing that such interference alters spindle formation and in turn negatively affects mitosis.

It is worthy of note therefore that S-Hydroprene and the leaf extracts of *Azadirachta indica* possess similar properties that can delay the emergence of adult *Culex quinquefasciatus* which in turn will reduce vector potential for disease transmission and nuisance caused.

4. Conclusion

S-Hydroprene and the leaf extracts of *Azadirachta indica* are capable of increasing the life expectancy of *Culex quinquefasciatus* larvae at certain concentrations until all death results or a few larvae metamorphose into pupae. To control *Culex quinquefasciatus* therefore, extracts of *Azadirachta indica* can be used to reduce the number of adult emergence in our environment.

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