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Evaluation of the Effects of S-Hydroprene and Various Leaf Extracts of *Azadirachta indica* (A.Juss 1830) on Larvae of *Aedes aegypti* (Linn., 1762)

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ABSTRACT

Aim: The study sort to evaluate the effect of S-Hydroprene and leaf extracts of *Azadirachta indica* on first and third instar larvae of *Aedes aegypti*. **Background**: One of the despised organisms that are significant to public health threat worldwide is Aedes aegypti. Biological control of the vectors of the organisms has become necessary in the midst of the associated health hazards with the use of juvenile hormone analogue. **Methods:** Serial concentrations (10 mg/L to 60 mg/L) of S-Hydroprene and methanol, n-hexane, chloroform and aqueous leave extracts of *Azadirachta indica* were tested on third instar larvae of *Aedes Aegypti* under laboratory conditions respectively. Survival, morphological deformation and mortality investigated vis-a-vis the effects of S-Hydroprene and the extracts. **Results**: n-hexane extract indicated higher morphological damage and mortality amongst the extracts of *Azadirachta indica* when compared with that of S-Hydroprene on *Ae. aegypti* third instar larvae at similar concentration. **Conclusion:** The leaf extracts of *Azadirachta indica* and S-Hydroprene caused morphological deformation of head and body of the mosquito. It also resulted in larval-pupal intermediate. The leaf extracts of *Az. indica* therefore could be considered and utilized in population management of noxious mosquito species to reduce their rate of disease transmission.

INTRODUCTION

Only a circumscribed number of Insect growth regulators (IGRs) such as S-methoprene, S-Kinoprene, diflubenzuron, triflumuron and a host of others have been approved for use in mosquito control by the World Health Organization (WHO) pesticide evaluation scheme (WHOPES) [1]. Among these IGRs, Methoprene has been reported to be effective against some mosquito vector species [2]. When mosquito larvae are exposed to IGRs, their life cycle is disrupted and they are prevented from reaching maturity or reproducing, they show very high level of regulatory activity against many insect species [3]. An example of IGR is S-Hydroprene which is an isoprenoid with the chemical name as Ethyl(2E,4E,7S)-3,7,11-trimethyl-2,4-dodecadienoate[4]. Insect resistances to IGRs such as Hydroprene have been considered to be unlikely because of their unique mode of action in structurally mimicking an essential hormone in the endogenous system [5]. 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) [6]. Mosquitoes are insects that are widely spread in the world [7]. *Aedes aegypti* which is among the thirteen genera of the family Culicidae also referred to as dangerous because of its significant public health threat worldwide [8]. This mosquito species has been known to be important vectors of Dengue viral encephalitis, Zika virus, Yellow fever, Elephantiasis and many other Arboviruses [9]. It was proposed that the term ''third generation pesticide'' be considered and applied to the potential use of the insect juvenile hormone (JH) as an insecticide, and suggested that it would not only be environmentally benignant but that the pest insects would also be unable to develop resistance [10]. Although, it took considerable number of years before the first commercial juvenile hormone analog made its debut [11]. From that time, many compounds that have shown to have adverse effect on the growth and development of insects have been synthesized and have been referred to collectively as 'insect growth regulators' [11]. Arthropods display a remarkable adaptability and diversity in colonizing very different and many ecological niches, between larval and adult stages of a given insect, there is several and distinct developmental and morphological changes that helps it to survive in different environments [12]. The development of this hormone as an insecticide was due to the idea that insects would be unable to develop resistance against JH if it was used as a control agent [5]. The Neem tree, (*Azadirachta indica* Linn.) is a member of mahogany family (*Meliaceae*) [13]. Individual plants can grow to a height of forty (40) to eighty (80) feet. It is probably indigenous to the Indian sub-continent,

but is now widespread in tropical and subtropical areas of Asia, Africa, Australia and South America, and the Pacific Islands. Neem is a traditional source of a wide variety of products including beauty aids, fertilizers, herbs, lumber, pesticides and numerous pharmaceuticals. They are all derived from different parts of the tree such as leaves, bark and the seeds [14]. Phytochemical components present in the neem plant that have been previously extracted include, alkaloids, steriods saponin, flavonoids tannins, glycoside cardiotonics, saponins, anthraquinones [15, 16]. Quercetin and sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties [17].

Study Area

This study was carried out in Ahmadu Bello University, Zaria which is in the Sabon Gari LGA, Kaduna State (latitude 11° 15′N to 11°3′N and longitude 7° 30′E to 7°45′E), an area that is characterized by a grassland ecosystem with trees being widely spaced so that the canopy does not close. The annual rainfall ranges between 1016 mm and 1524 mm with a relative humidity ranging between 60% and 80% [18].

MATERIALS AND METHODS

Collection, Identification and Solvent Extraction of *Azadirachta indica*

The leaf of *Azadirachta indica* was collected in the premises of Ahmadu Bello University, Zaria and identified at the herbarium of the Department of Botany (Voucher number 90104) after which it was air-dried under a shade, pulverized with pestle and mortar, and taken to the laboratory of the Pharmacognosy and Drug Development for extraction.

500 g powder of *Azadirachta indica* was weighed each into four different glass containers with 1000 ml of methanol, aqueous, chloroform and n-hexane respectively and allowed for 72 hours by way of maceration. The contents were filtered using muslin and cotton wool respectively and evaporated via the water bath at 60°C-65°C.

Preparation of Stock Solution of S-Hydroprene and the Leaf Extract of *Azadirachta indica*

Stock solution of the IGR was prepared by dissolving 250 mg of S-Hydroprene in 2 mls of dimethyl sulphoxide (DMSO) to obtain a homogenous solution that is miscible with water and 25 ml of water was 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 [19]. After this, serial dilution of *Azadirachta indica* was done.

Collection of Species of Mosquito Larvae and Identification of Adult Mosquitoes

Twenty (20) blood-fed adult female *Aedes aegypti* were collected with the use of a test tube to trap them while resting indoors on the walls of some rooms in the students' hostel in ABU main campus, transferred via entomological cages and bred according to [20] and [21] also considering salinity and the pH of the water as part of the determinant factors. Preserved female adult mosquitoes were observed under a microscope and identified by the use of published keys according to [22].

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

The larvae of the mosquitoes were fed with grinded digestive biscuits and baker's yeast in the ratio 3:1 throughout the experimental process continues until they began 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 the *Aedes aegypti* mosquitoes in batches of thirty (30). These were transferred into separate petri dish containing 100 ml of distilled water in replicate of five (5). Same replicates were made for the control but devoid of the neem extracts according to [19]. This experimental set-up was examined daily for any larval mortality and recorded. The experiment was terminated when both control and test samples no surviving larvae. Fourteen hour photoperiod and 28 ± 3°C temperature was maintained in the evaluation room during the tests according to [23]. The number of dead and moribund larvae was recorded after an exposure period of twenty four (24) hours according to [19]. The whole setup was allowed for a consistent period of 6 and 15 days in order to check for growth inhibition using the control to determine the rate of change at the following concentrations - (10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L and 60 mg/L).

Data Analysis

The number of dead larvae of *Aedes aegypti* in the various test concentrations used was 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 [19]. Analysis of variance (ANOVA) was used to test for significant differences in larval mortality amongst the various concentrations of solvent-based extracts while Duncan's multiple range tests was used 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 organisms at $P \leq 0.05$.

RESULTS AND DISCUSSION

Larval Mortality

Results indicate that larval mortality of the third instar larvae of *Aedes aegypti* was inconsistent with increase in concentration from 10 mg/L to 60 mg/L for all extracts of *Az. indica* using different media except in the case of aqueous extract which showed that all the mosquito larvae between 20 mg/L to 60 mg/L survived (Table 1). It was also observed that the result obtained from *Az. indica* third in star larvae using S-Hydroprene exhibited different pattern as mortality didn't increase with increase in concentration as compared with the data obtained using methanol extract with the highest being 2.6 (9%) and 0.8 (3%) mortality for S-Hydroprene (Table 1).

Table No. 1: Effect of some solvent extracts of *A. indica* and the IGR, S-Hydropreneon mortality of third instar larvae of *Aedes aegypti* after 24 hours

Conc. (mg/L)	No. per Exp.	N-Hexane Extract Mean Mortality ± SE	Aqueous Extract Mean Mortality ± SE	Chloroform Extract Mean Mortality ± SE	Methanol Extract Mean Mortality ± SE	S-Hydroprene Mean Mortality ± SE
Control	30	0.4 ± 0.25(1)	0.4 ± 0.25(1)	0.4 ± 0.25(1)	0.4 ± 0.25(1)	0.4 ± 0.25(1)
10	30	2.0 ± 0.32(7)^{de}	0.4 ± 0.25(1)^{a}	2.4 ± 0.25(8)^{c}	0.2 ± 0.20(1)^{b}	0.2 ± 0.20(1)^{a}
20	30	2.8 ± 0.37(9)^{cd}	0.0 ± 0.00(0)^{b}	2.4 ± 0.68(8)^{c}	0.4 ± 0.25(1)^{b}	0.4 ± 0.25(1)^{a}
30	30	4.0 ± 0.32(13)^{bc}	0.0 ± 0.00(0)^{b}	3.8 ±0.37(13)^{abc}	0.6 ± 0.40(2)^{b}	0.6 ± 0.25(2)^{a}
40	30	5.0 ± 0.32(17)^{b}	0.0 ± 0.00(0)^{b}	3.4 ± 0.75(11)^{bc}	1.2 ± 0.37(4)^{b}	0.4 ± 0.25(1)^{a}
50	30	11.0 ± 0.89(37)^{a}	0.0 ± 0.00(0)^{b}	5.2 ± 0.37(17)^{a}	1.2 ± 0.37(4)^{b}	0.8 ± 0.20(3)^{a}
60	30	10.4 ± 0.75(35)^{a}	0.0 ± 0.00(0)^{b}	4.6 ± 0.51(15)^{ab}	2.6 ± 0.75(9)^{a}	0.6 ± 0.25(2)^{a}
LC_{50}		1.109	-	1.135	1.019	1.691
F-Value		59.088	2.667	9.438	3.661	0.556
P-Value		0.000	0.036	0.000	0.008	0.762

Figures in parenthesis are in percentages. Superscripts with different letters are significantly different at $p \leq 0.05$.

Key: SE = Standard Error, Conc. = Concentration, No = Number

The result obtained by using n-hexane extract showed significantly higher mortalities of 11.0 (37%) at 50 mg/L compared to 0.8 (3%) mortality of S-Hydroprene at similar concentration. N-Hexane extract of *Az. indica* produced highest number of dead larvae 11.0 (37%) of *Ae. aegypti* while the least effect was by methanol extract 0.2 (1%) at 10 mg/L. When the effect of S-Hydroprene was compared with other extracts on the third instar larvae of *Ae.aegypti*, the highest mortality was 0.8 (3%) at 50 mg/L while the lowest was 0.2 (1%) at 10 mg/L obtained from methanol extract of *Az. Indica (Table 1).*

Larval Survival

The extracts of *Az. indica* on first in star larvae of *Ae. aegypti* had more survivors at 10 mg/L. This appeared to increase with increase in concentration for methanol and aqueous extracts but without change at 60 mg/L. In the case of S-Hydroprene, the effect was almost the same for all concentration where approximately single larvae 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. This however* suggests the toxic potency of chloroform in addition to the effect of *Az. indica* first in star larvae (Table 2).

Table No. 2: Effect of the solvent extracts of the leaf of *Az. indica* and the IGR S-Hydroprene on the survival of first instar larvae of *Ae. aegypti*

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

Figures in parenthesis are in percentages. Superscripts with different letters are significantly different at $p<0.05$.

Key: SE = Standard Error, Conc. = Concentration, No = Number

Mosquito survival in days

Treatment with extracts of *Az. indica* in 10 mg/L to 60 mg/L revealed survival of first instar larvae of *Ae. Aegypti* up to 9 days in chloroform and N-Hexane extracts and less in the other extracts. Those treated with S-Hydroprene lasted for 5 to 7 days without metamorphosing into pupae (Table 3).

Table No. 3: Mosquito larval survival in days following the application of S-Hydroprene and some solvent extracts of the leaf 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	5.20±0.37	5.20±0.37	6.60±0.25	5.20±0.37	5.20±0.37
10	6.40±0.81[a]	5.40±0.40[a]	8.40±0.60[a]	5.20±0.20[a]	6.80±0.20[a]
20	8.60±0.75[a]	5.40±0.40[a]	8.80±0.37[a]	5.40±0.00[a]	7.60±0.40[a]
30	9.00±0.71[a]	5.40±0.00[a]	9.20±0.37[a]	5.40±0.25[a]	7.00±0.45[a]
40	8.80±0.49[a]	5.60±0.00[a]	9.00±0.32[a]	5.20±0.20[a]	7.60±0.51[a]
50	8.40±0.75[a]	5.80±0.20[a]	8.00±0.45[a]	5.40±0.12[a]	7.40±0.25[a]
60	7.60±0.93[a]	6.60±0.40[a]	7.80±0.58[a]	5.80±0.20[a]	7.60±0.95[a]
F-Value	0.878	4.159	6.202	4.027	4.864
P-Value	0.524	0.004	0.000	0.005	0.002

Superscripts with different letters are significantly different at $p<0.05$

Key: SE = Standard Error, Conc. = Concentration, No = Number

Morphological Deformation Caused by IGR Extracts

The various leaf extract of *Azadirachta indica* and S-Hydroprene showed different effects on the body wall and chitinous exoskeleton of the organism. This includes deformation of larva-pupa intermediate, head and body decomposition. S-Hydroprene exerted highly significant morphological deformation on the organism when compared with aqueous extract of *Azadirachta indica* at all concentration (Figures 1-5). Larvae in the control experiments however showed no deformation (Figure 6).

Figure 1: Decomposing head of *Aedes aegypti* first instar larvae

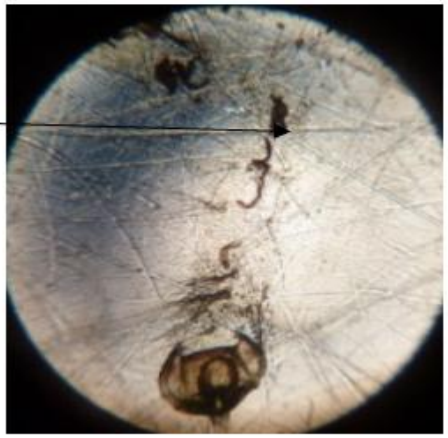

Figure 2: Decomposing abdomen of *Aedes aegypti* first instar larvae

Figure No. 3: Larva-pupa intermediate of *Aedes egypti* first instar larvae

Figure No. 4: Decomposing body parts of *Aedes egypti* first instar larvae

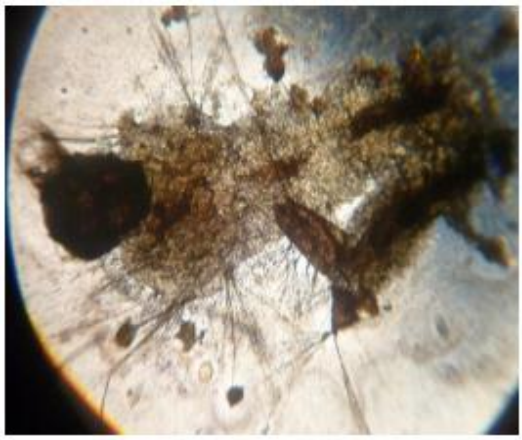

Figure 5: Decomposing abdomen of *Aedes aegypti* first instar larvae

Figure 6: Control

Figure 1 - 6: Morphological effect of *Azadirachta indica* (10mg/L to 60mg/L) and S-Hydroprene (10mg/L to 60mg/L) on experimental and control groups of first instar larvae of *Aedes aegypti*

Moreover, the third instar larvae of *Ae. aegypti* were not easily susceptible to the solvent extracts because increase in concentration of the extracts did not result in high mortality. This is probably because the organisms were less sensitive to the extracts.

Only n-hexane extract indicated higher mortality when compared with the effect of S-Hydroprene on *Ae. aegypti* third instar larvae at similar concentration.

The observed inhibitory effects of S-Hydroprene on third instar larvae of *Ae. aegypti* is similar to the work of [24] who reported S-Hydroprene to prolong the larval stages of mosquito larvae and [25] who worked on juvenile hormone esterase.

The apparent increase in the survival of methanol and aqueous *Az. indica* treated *Ae. Aegypti* (first instar larvae) is probably due to the mild toxic effect of methanol on the test organisms while the S-Hydroprene had uniform effect on the survival of same for all concentrations probably due to its mild effect.

The increased survival of the methanol and aqueous extracts treated *Ae. Aegpti* first instar larvae was probably because the methanol solvent had some mild toxic effect on the test organisms while the S-Hydroprene had uniform effect on the survival of first instar larvae for all concentrations. This is probably because S-Hydroprene allows the survival of the test organism with mild effect at each concentration.

The morphological deformation observed on the test organisms after twenty-four hours and subsequent days, thus disrupting the life cycle of *Ae. aegypti* confirms the work reported by [26].

CONCLUSION

S-Hydroprene and the leaf extracts of *Az. indica* have some biologically active components which is larvicidal but not sufficient to yield up to fifty percent mortality of *Aedes aegypti* larvae between 10 mg/L and 60 mg/L.n-hexane *Az. indica* leaf extract effected the highest mortality (37%) at 60 mg/L, this may be detrimental when economic consideration for use comes into play. The leaf of *Azadirachta indica* could be used as a growth inhibitor or regulator. Further studies to identify specific active ingredients causing larval delay and morphological deformation and identification other plants with IGR potentials are recommended.

CONFLICT OF INTEREST

Authors hereby declare no conflict of interest.

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