



EFFECT OF S-HYDROPRENE AND PHYSICOCHEMICAL PARAMETERS ON *Culex quinquefasciatus* LARVAE

*¹Agada, J., ²Joseph, I.B., ³Mohammed, S.A., ⁴Abdulazeez, A.M., ¹Idujagi, O.S., ⁵Oladele, E. G. and ⁶Ransome, J.U.

¹Department of Biological Sciences, Nigerian Defence Academy

²World Health Organization, Plateau State Office, Nigeria.

³Department of Biological Sciences, Kaduna State University

⁴Department of Basic and Applied Sciences, College of Agriculture, Science and Technology, Jalingo, Taraba State.

⁵Department of Chemistry, University of Ibadan, Oyo State.

⁶Department of Zoology, Ahmadu Bello University, Zaria.

*Corresponding Author's Email: jeremiahagada20@gmail.com

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Abstract

Culex quinquefasciatus is a significantly harmful mosquito species recognized for its role in transmission of various diseases worldwide. The extensive resistance to traditional insecticides presents a considerable obstacle to control initiatives. The study sought to evaluate the toxicological and developmental effects of S-Hydroprene, administered at concentrations between 0.6 mL and 0.1 mL, on this vector species. F1 generation larvae were bred in entomological cages, 10 % stock solution of S-Hydroprene was prepared and serial concentrations used to test mortality and morphological disruptions of larvae. Physicochemical parameters were also observed to identify changes that could result due to S-Hydroprene to affect development and survival of mosquito using appropriate equipment. From result obtained, LC₅₀ value was estimated and observed to be very high excluding it from been used as larvicide, it rather demonstrated inhibitory effect on larval development, as indicated by a substantial, dose-dependent prolongation of both larval and pupa durations. Percentages mortality of 0, 5, 5, 5, 3, 5 and 9 were recorded for control, 0.1mL, 0.2mLs, 0.3mLs, 0.4mLs, 0.5mLs and 0.6mLs respectively after 24 hours. S-Hydroprene disrupted larval development, resulting in the emergence of larval-pupal intermediates. Pupation was markedly suppressed, especially when mature larvae were subjected to the compound. The process of pupal morphogenesis was largely unaffected, no deformities were observed in the pupae after larval treatment. The findings indicate that S-Hydroprene may serve as an effective instrument in integrated pest management strategies aimed at this mosquito species, which has acquired resistance to the majority of conventional insecticides. The use of S-Hydroprene in mosquito larva control should be encouraged since resistance has not been observed for it.

Keywords: Mosquito larval control, Insect growth regulator, Insect toxicity, S-Hydroprene Physicochemical parametrs.

Introduction

Compared to traditional chemical insecticides and larvicides, insect growth

regulators (IGRs) are less toxic, environmentally friendly, and biologically selective (Mulla et al., 1986). Nonetheless,

central North America, parts of South America, and Australia (Vinogradova, 1997). In Africa, *Cx. p. pipiens* is particularly prevalent in Egypt, where both the *molestus* and *pipiens* forms coexist, whereas South Africa contains these alongside *quinquefasciatus*. These subspecies predominate numerous Afro-tropical regions, comprising approximately 92% of specimens gathered in a Kenyan highland marshland (Logan *et al.*, 1991).

Mosquitoes are extensively distributed across Nigeria, with *Anopheles*, *Culex*, and *Aedes* as the predominant genera (Surtees, 1959). Certain species function as principal vectors for major diseases: *Anopheles gambiae* and *An. arabiensis* transmit malaria, *Aedes aegypti* disseminates yellow fever, and *An. gambiae s.l.* along with *Culex pipiens quinquefasciatus* are associated with lymphatic filariasis (Service, 1963). Vector dominance exhibits regional variations; for example, *An. gambiae s.l.* serves as the primary malaria vector in Nigeria's rainforest areas, whereas *An. funestus* is predominant in the southern regions (Service, 1963). Recent household surveys in four sentinel villages in Nasarawa and Plateau States identified *An. gambiae s.l.*, *An. funestus*, and *Culex* species, with *Anopheles* mosquitoes solely hosting *Wuchereria bancrofti* larvae (Richards *et al.*, 2005).

Variations in mosquito abundance based on season and location have also been documented. Gadzama (1976) noted that Culicine mosquitoes were predominantly present during the dry season at the Samaru campus of Ahmadu Bello University in Zaria. Nendangtok (1991) similarly collected 831 mosquitoes from the *Culex*, *Aedes*, and *Anopheles* genera in the same region, with *Culex quinquefasciatus* as the predominant species during the dry season. In Kaduna metropolis, indoor

mosquito surveys conducted by Abdulkadir (2001) documented 380 specimens, predominantly *Culex* and *Aedes*, with *Culex* demonstrating the greatest abundance. Subsequent research in the villages of Ungwan Rimi and Kaingimi in Kaduna State identified *Culex pipiens quinquefasciatus*, *Mansonia uniformis*, and various *Anopheles* species complexes as predominant, while *Aedes aegypti* was present in minimal quantities (Service, 1963).

A number of environmental factors, including habitat availability, human influences, and climate, influence the distribution and abundance of mosquito species. Larval surveys reveal that *Culex pipiens quinquefasciatus* is the predominant species reproducing in contaminated aquatic environments, such as streams, open drains, and stagnant pools, throughout various urban areas. The regions include Lagos (characterized by mangrove forests and coastal flora), Ibadan (located in lowland rainforest), the Bauchi-Jos Plateau (Guinea savannah), Kano (Sudan savannah), and the Sahel savannah zone extending from Sokoto to Birnin Kebbi (Service, 1963).

Research conducted by Okogun *et al.* (2003) in the Esan and Etsako regions of Edo State (formerly Midwestern Nigeria) demonstrated a positive correlation between mosquito prevalence and human population density. Ekpoma, a university town characterized by a significant population of students and farmers, demonstrated a considerable prevalence of larval mosquitoes, varying from 35% to 70%. Amusan *et al.* (2004) similarly reported that *Aedes aegypti* was the predominant species in rice field habitats within a densely populated village near Abeokuta, situated in the rainforest zone of Ogun State.

The most important arthropod vectors for the spread of human diseases worldwide are thought to be mosquitoes. They are especially

only a limited number of insect growth regulators (IGRs) specifically S-methoprene, S-kinoprene, diflubenzuron, and triflumuron have been approved for mosquito control by World Health Organization Pesticide Evaluation Scheme (WHOPES) (WHO, 1997). Methoprene has demonstrated notable efficacy against various mosquito vector species (Cornel *et al.*, 2000). IGRs impede larval development, inhibiting the maturation and reproduction of mosquitoes (Kuo *et al.*, 2010), they also significantly regulate the growth of diverse insect species (Kuo *et al.*, 2010). S-Hydroprene, a synthetic isoprenoid with the chemical structure Ethyl (2E,4E,7S)-3,7,11-trimethyl-2,4-dodecadienoate (Route *et al.*, 2003), emulates a crucial endogenous hormone, rendering the emergence of resistance in insects improbable (Williams, 1967). Resistance to alternative insect growth regulators (IGRs) generally arise from either metabolic degradation prior to reaching target sites or structural alterations in juvenile hormone binding proteins (JHBPs) that diminish their affinity for juvenile hormone analogues (JHAs) (Wilson & Ashak, 1998).

Culex quinquefasciatus, the vector of interest is among the most prevalent mosquito species worldwide (Weinstein *et al.*, 1997; Derraik & Slaney, 2005) and is responsible for the transmission of various pathogens, including the Ross River virus, Dengue virus, Japanese encephalitis virus, and Brugia malayi (Reuben *et al.*, 1994; De Almeida *et al.*, 1999). It is the principal vector of *Wuchereria bancrofti*, the etiological agent of lymphatic filariasis which is a debilitating tropical disease resulting in acute and chronic morbidity across all age demographics (Hettiaratchi *et al.*, 2000; Nanduri *et al.*, 1989).

Studies indicate that the evolutionary lineages of *Drosophila* and mosquitoes

separated around 260 million years ago (Moreno *et al.*, 2010). Diversification among culicine mosquitoes transpired subsequently, approximately 120 to 150 million years ago (Eric *et al.*, 2009). Fossil records suggest that numerous existing mosquito genera were already distinct by the mid-Tertiary period (approximately 35 million years ago), with their evolutionary origins possibly tracing back to the early Tertiary (around 70 million years ago) or even earlier (Marshall, 1938; Edward, 1941). Although they are not found in Antarctica, mosquitoes are found in tropical, subtropical, and temperate regions almost everywhere in the world (Smith, 1980). Their geographic distributions differ by genus; for instance, *Haemagogus* is restricted to particular regions, including Central and South America (Service, 1980). Although some species are cosmopolitan, they predominantly thrive in tropical and subtropical areas, with a few extending into colder and even Arctic regions during warmer seasons (Bates, 1949; Lame *et al.*, 2015).

The dynamics of mosquito populations are significantly influenced by the availability of breeding habitats, while their abundance and distribution are additionally affected by environmental factors. Essential factors encompass water chemistry, geographical characteristics, aquatic flora, predator presence, eutrophication levels, sunlight exposure, and proximity to human or animal activities (Sanford *et al.*, 2005; Schafer *et al.*, 2004; Greenway *et al.*, 2003; Muturi *et al.*, 2008; Okogun *et al.*, 2003). The interaction of biological and physicochemical factors in regional habitats ultimately determines these distribution patterns (Kengluetcha *et al.*, 2005).

Culex pipiens complex encompasses subspecies including *Cx. pipiens pipiens* (anautochthonous), *Cx. p. molestus* (autochthonous), and *Cx. p. quinquefasciatus*, which are found in Europe, certain regions of Asia, Africa,

infamous for disseminating tropical infections, including filariasis and arboviral diseases such as dengue fever, yellow fever, and Zika virus, which significantly augment the global disease burden and mortality rates. Mosquito-borne diseases present a significant public health risk, particularly in tropical and subtropical areas, including Africa. The World Health Organization (2000, 2010, 2013, 2014) states that mosquitoes infect over 700 million individuals annually and are responsible for approximately one in seventeen deaths globally.

Materials and method

Study area

This research was conducted at Ahmadu Bello University, Zaria, situated in the Sabon Gari Local Government Area of Kaduna State. The location is situated between 11°15'N and 11°3'N latitude, and 7°30'E to 7°45'E longitude, annual rainfall is between 1016 mm and 1524 mm with relative humidity between 60% and 80% (Micheal, 2014). Laboratory analyses were performed in the Department of Zoology and the Department of Pharmacognosy and Drug Dispensing at the Faculty of Pharmaceutical Sciences, Ahmadu Bello University.

Collection and preparation of stock solution of S-Hydroprene

An aliquot amount, 3 mL containing 250 mg of S-Hydroprene was purchased, imported from Germany and received from the depot at Bristol Scientific Company Limited Kiverpool Road, Apapa, Lagos, Nigeria.

Insect growth regulator (IGR) stock solution was formulated by dissolving 250 mg of S-Hydroprene in 2 mL of dimethyl sulfoxide (DMSO) to obtain a uniform, water-compatible solution. Subsequently, 25 mL of distilled water was added to make the stock (Adebote & Adeyemi, 2011).

Collection of species of mosquito larvae needed

Blood fed adult female *Culex quinquefasciatus* were collected from Zaria and identified the aid of keys. Indoor specimens were collected using test tubes to capture them while they rest on the wall, adhering to the methodology established by Belkin (1965). Trapped mosquito was transferred into a collecting bottle. This process was repeated until a good number is collected (about twenty (20) blood fed mosquito adults). The blood fed mosquitoes was transferred into two (2) entomological cages that measures 30cm×30cm×30cm protected with net with mesh size of 1mm (Trembley, 1955; Gillett, 1962; Adebote and Adeyemi, 2011), which had a petri-dish that is half filled with tap water with 80% relative humidity (Barik, 2016; Suzuki et al., 2016). Adult mosquitoes contained within the cage was provided with a sugar solution absorbed in cotton wool as their nutritional source, in accordance with the protocol established by Porter et al. (1961). This is aimed at supplying them with energy for physiological functions. Within 3-4 days of oviposition, the eggs laid metamorphosed into first instar larva. Other factors such as salinity, and the pH of the water was measured using the hanar instrument.

Bioassay of mosquito larva.

For larvicidal screening, batches of thirty (30) first-instar larvae were placed in separate Petri dishes containing 100 mL of distilled water. Each bioassay was conducted in triplicate, with a simultaneous control group sustained under identical environmental conditions (ambient room temperature). The control treatment comprised 2 mL of 10% dimethylsulfoxide (DMSO) devoid of S-Hydroprene. 0.02 g of a mixture of grounded digestive biscuit and yeast (in the ratio 3:1) was used to feed larvae *ad libitum* during the experiment.

Evaluation of mortality.

Larval mortality and morbidity were assessed following 24 hours of exposure. Larvae were deemed deceased if unresponsive to gentle stimulation with a glass rod, while moribund individuals (displaying minimal movement without active swimming) were similarly classified as dead due to their failure to recover. The experimental duration adhered to Eugene (1970) to evaluate growth inhibition, employing the control group for comparative analysis.

Assessment of concentration and environmental factors.

S-Hydroprene was evaluated using medical syringe to obtain serial concentrations from stock solution between 0.1 mL and 0.6 mL to assess both growth-inhibitory and larvicidal properties, adhering to the methodologies established by Adebote and Adeyemi (2011) and Mulla *et al.* (1974). Daily observations documented larval mortality until the completion of adult emergence in control dishes, with no surviving larvae or pupae present. Experiments were performed under a 14-hour photoperiod and a temperature of $28 \pm 3^\circ\text{C}$, as advised by Ali *et al.* (1999) and Barik *et al.* (2016). Data were compiled to compare mortality and survival rates of larvae, pupae, and adults in response to S-Hydroprene treatment.

Physicochemical parameters of water

In the study, essential physicochemical properties of water namely pH, dissolved oxygen, electrical conductivity, total dissolved solids, and temperature were determined and recorded to observe changes that could result overtime and how they can affect the vector.

Data Analysis

Probit analysis was performed on mortality data of exposed larvae of the *Culex quinquefasciatus* species at the different test concentrations (Finney, 1971).

Mortality control was adjusted utilizing Abbott's formula (Abbott, 1925; Adebote and Adeyemi, 2011). Analysis of variance (ANOVA) was employed to assess significant differences in larval mortality across the different concentrations of IGR. Duncan's multiple range tests were utilized to distinguish varying means (Adebote and Adeyemi, 2011).

Result and discussion

Table 1 presents the larval mortality rates of *Culex quinquefasciatus* following exposure to various concentrations of the solvent extract. Control group demonstrated no mortality (0/150; 0.00%), while the treated groups exhibited differing mortality rates: 4.67% (0.1 mL/L), 4.67% (0.2 mL/L), 5.33% (0.3 mL/L), 3.33% (0.4 mL/L), 5.33% (0.5 mL/L), and 9.33% (0.6 mL/L).

Statistical analysis revealed a significant concentration-dependent impact on larval mortality after 24 hours ($p < 0.05$). The mortality trend was as follows: $0.6 \text{ mL/L} = 0.5 \text{ mL/L} = 0.4 \text{ mL/L} = 0.3 \text{ mL/L} > 0.2 \text{ mL/L} > 0.1 \text{ mL/L} > 0 \text{ mL/L}$ (control).

Probit analysis was performed to determine lethal concentrations. Given that Abbott's correction was superfluous, raw mortality percentages were transformed into empirical probit values and graphed against log-transformed concentrations, resulting in a linear probit regression line ($Y = -2.2329x + 1.8934$). The median lethal concentration (LC_{50}) calculated from the equation was 0.020 mL/L (Table 2).

The administration of S-Hydroprene at concentrations of 0.1 mL/L and 0.6 mL/L yielded minimum and maximum larval mortality rates of 5% and 9%, respectively. A statistically significant difference ($p < 0.05$) in mean larval mortality was noted across concentrations after 24 hours of exposure, with a median lethal concentration (LC_{50}) of 0.020 mL/L for *Culex quinquefasciatus* larvae. Mortality rates in the 0.6 mL/L treatment exhibited a significant difference ($p < 0.016$).

compared to the control, while no significant differences ($p > 0.05$) were observed between the control and lower concentrations (0.1–0.5 ml/L). Pairwise comparisons indicated no significant differences ($p > 0.056$ to $p > 1.000$) between 0.1 ml/L and other concentrations, nor between 0.2 ml/L or 0.3 ml/L and any other treatments. Conversely, 0.4 ml/L demonstrated a significant difference ($p < 0.008$) relative to

the control, but not in comparison to other concentrations ($p > 0.263$ to $p > 0.778$). Likewise, 0.5 ml/L exhibited no statistically significant differences ($p > 0.098$ to $p > 1.000$) compared to any treatment, including the control group. Ultimately, 0.6 ml/L exhibited a significant difference ($p < 0.008$) from both the control and 0.4 ml/L, but not from other concentrations ($p > 0.056$ to $p > 0.098$).

Table 1. Larval mortality of the third instar of *Culex quinquefasciatus* using S-Hydroprene

Concentration	No of Larvae Exposed	No (Mean; 95% CI \pm SE) of Dead Larvae at 24 hours after Exposure	Percentage Mortality	F Value	ANOVA
Control	150	0(0.0 c; 0.18 – 3.02 \pm 0.000)	0.00	3.136	0.018
0.1	150	7(1.4 ab; -0.02 – 2.82 \pm 0.5110)	4.67		
0.2	150	7(1.4 ab; -0.27 – 3.07 \pm 0.6100)	4.67		
0.3	150	8(1.6 ab; 0.18 – 3.02 \pm 0.510)	5.33		
0.4	150	5(0.8 b; -0.24 – 1.84 \pm 0.374)	3.33		
0.5	150	8(1.6 ab; 0.92 – 2.28 \pm 0.245)	5.33		
0.6	150	14(2.8 a; 0.76 – 4.84 \pm 0.735)	9.33		

CI = Confidence interval; SE = Standard error

Table 2. Lethal concentration of *Culex quinquefasciatus* using S-Hydroprene

Concentration	Log of Concentration	Percentage Mortality of Larva	Abbott's Corrected Mortality	Empirical Probit of Mortality	Regression Equation (R ² Value)	LC ₅₀
0.6	-0.22	9	9	3.66	Y= -2.2329x+1.8934	0.020
0.5	-0.30	5	5	3.36	(R ² = 0.3261)	
0.4	-0.39	3	3	3.12		
0.3	-0.52	5	5	3.36		
0.2	-0.69	5	5	3.36		
0.1	-1	5	5	3.36		
Control	0	0	0	-		

CI = Confidence interval; SE = Standard error

Table 3. Multivariate Comparison of means of concentrations of the 3st Instar of *Culex quinquefasciatus*

Comparism	Control	0.1ml	0.2mls	0.3mls	0.4mls	0.5mls	0.6mls
Control		.573	.573	.399	.778	.399	.016
0.1ml	.573		1.000	.778	.399	.778	.056
0.2mls	.573	1.000		.778	.399	.778	.056
0.3mls	.399	.778	.778		.263	1.000	.098
0.4mls	.778	.399	.399	.263		.263	.008
0.5mls	.399	.778	.778	1.000	.263		.098
0.6mls	.016	.056	.056	.098	.008	.098	

The association between mosquito larval mortality and physicochemical parameters was assessed using a Pearson correlation analysis. The findings revealed a weak to moderate positive correlation between conductivity ($r = 0.192$) and total dissolved solids ($r = 0.069$) with mortality, while temperature ($r = -0.080$), pH ($r = -0.057$), and dissolved oxygen ($r = -0.076$) exhibited weak negative correlations. These trends indicate that elevated temperature, pH, and

dissolved oxygen may correlate with reduced larval mortality, whereas increased conductivity and total dissolved solids may be associated with heightened mortality. Nonetheless, none of these correlations achieved statistical significance, suggesting that the examined physicochemical parameters did not exert a considerable measurable effect on larval mortality in the investigated aquatic environment.

Table 4. Duration of survival, metamorphoses in days and the emergence inhibition.

Concentration (mL)	Larvae Mean±SE	Pupae Mean±SE	Adult Mean±SE	Total number of days	Mortality (%)	Emergence (%)	EI ₅₀
Control	6.00±0.00	5.00±0.00	5.00±0.00	16.00±0.00	32	68	0.14
0.1	6.80±0.37	4.60±0.25	4.20±0.20	15.60±0.82	43	57	
0.2	8.20±0.37	5.40±0.25	4.40±0.25	18.00±0.87	43	57	
0.3	8.80±0.37	4.80±0.37	3.60±0.25	17.20±0.99	69	31	
0.4	10.00±0.3	4.60±0.40	2.60±0.25	17.20±0.97	71	29	
0.5	10.20±0.2	5.00±0.32	4.00±0.32	19.20±0.84	76	24	
0.6	11.20±0.2	4.80±0.92	3.80±0.66	19.80±1.78	81	19	

Table 4 shows the survival rate and larval development in days of the mosquito species. The control experiment revealed larvae, pupae and adult stages in the laboratory at temperature of 28±0.03°C as seen in table 4, can survive for 6.00, 5.00 and 5.00 days but when the IGR is present

in the culture medium depending on concentration, there is a delayed development from one stage of metamorphosis to another. As seen from table, 0.1ml of the IGR maintained the larval, pupal and adult stages till 6.80, 4.60 and 4.20 days respectively. This is at variance with the increased

concentration 0.6mls which yielded an obvious delayed developmental larval stage and shorter period of pupal and adult stages

which are 11.20, 4.80 and 3.8 days respectively.

Table 5. Physicochemical parameters of the aquatic medium during experiment.

Concentration (mL)	Temp. °C	E.C (µs/cm)	TDS (Mg/L)	PH	DO (Mg/L)
Control	28.00 ±0.03	166.20 ±1.74	64.60 ±00.98	9.28 ±0.07	1.10 ±0.09
0.1	27.58 ±0.49	1755.60 ±21.47	887.00 ±21.87	8.96 ±0.02	1.08 ±0.10
0.2	29.02 ±0.09	857.40 ±28.45	428.20 ±20.17	9.12 ±0.21	1.12 ±0.08
0.3	28.12 ±0.69	983.80 ±28.29	291.00 ±23.33	8.88 ±0.04	1.14 ±0.04
0.4	28.90 ±0.07	1187.60 ±29.01	621.20 ±29.30	8.69 ±0.03	1.08 ±0.05
0.5	28.02 ±0.22	929.60 ±25.59	837.00 ±27.96	8.91 ±0.06	1.04 ±0.05
0.6	29.40 ±0.17	985.60 ±27.90	343.40 ±25.59	9.27 ±0.02	0.82 ±0.22

Temp. °C: Temperature in degree Celsius, E.C: Electrical Conductivity, PPM: Total Dissolved Solid, PH, DO: Dissolved Oxygen
The several physicochemical parameters in one way or the other affected the mosquito species development but oxygen which may not have any impact on the aquatic stages because of the structure they possess (siphon) helps them to use atmospheric oxygen when dissolved oxygen is compromised in the aquatic medium.

From table 5, the temperature of the media from various test concentrations varied from 27.58°C and 29.02°C. The lowest temperature was recorded at 0.1ml and the highest at 0.2mls. All the temperatures recorded are higher than the maximum permissible limit (MPL) of <3°C

The electrical conductivity of the media from various test concentrations varied from 166.20µs/cm and 1755.60µs/cm. The lowest temperature was recorded at the control and the highest at 0.1ml. The electrical conductivity recorded per concentration were less than the Maximum

permissible limit of 1500µs/cm and a little above it at 0.1ml (table 5).

The total dissolved solids of the media from various test concentrations varied from 64.60mg/L and 887.00 mg/L. The lowest temperature was recorded at control and the highest at 0.1ml. The mean values for total dissolved solids were generally above the maximum permissible limit of 50mg/L (table 5).

The pH of the media from various test concentrations varied from 8.88mg/L and 9.28 mg/L. The lowest temperature was recorded at 0.3mls and the highest at control. The mean values for total dissolved solids were generally above the maximum permissible limit of 6-9 (table 5).

The dissolved oxygen of the media from various test concentrations varied from 0.82mg/L and 1.14 mg/L. The lowest temperature was recorded at 0.6mls and the highest at 0.3mls. The mean values for total dissolved solids were generally far below the maximum permissible limit of 50mg/L (table 5).



Plate 1. 0.5mls (Disfigured larvae, deformed-head, thorax and abdomen)



Plate 1. 0.5mls (Disfigured larvae, deformed-intermediate)



Plate 3. 0.4mls (entirely deformed body parts)



Plate 4. 0.3mls (shrunk body parts)



Plate 5. 0.1ml (disengaged body parts)

Laboratory results indicate that *Culex quinquefasciatus* larvae in Zaria exhibit significant tolerance to different concentrations of S-Hydroprene, potentially reflecting either an intrinsic or an acquired resistance to the IGR tested. The elevated LC₅₀ values indicate that S-Hydroprene is inadequate larvicide for mosquito management in this context.

Insect Growth Regulators (IGRs) are extensively recognized for their capacity to interfere with the growth and development of various insect species. Compounds including diflubenzuron, methoprene, fenoxycarb, lufenuron, and novaluron have demonstrated the capacity to inhibit development in *Spodoptera littoralis* (Aref et al., 2012; Karan, 2000; Gaaboub et al., 2012; Ghoneim et al., 2015). *Aedes aegypti* has demonstrated sensitivity to novaluron, whereas *Culex quinquefasciatus* has reacted to both kinoprene and novaluron (Hamaidia & Soltani, 2014; Djeghader et al., 2013, 2014).

IGRs often cause developmental delays in insects. For example, *Plutella cyclostella* subjected to pyriproxyfen (Mahmoudrand et al., 2015), *Corcyra cephalonica* exposed to fenoxycarb (Begum and Qamar, 2016), and *Culex pipiens* impacted by methoxyfenozide (Hamaidia & Soltani, 2016) have all demonstrated extended development. Comparable effects have been noted in *Glyphodes pyloalis* subsequent to lufenuron exposure (Aliabadi et al., 2016) and in other species administered N-tert-butylphenyl thenoylhydrazide derivatives (Song et al., 2016).

This study, in accordance with previous research demonstrates that S-Hydroprene has mild to moderate inhibitory effects on third-instar *Culex quinquefasciatus* larvae, extending their developmental duration, especially during the pupal and adult stages (Table 3). These findings correspond with

previous studies indicating delayed development in *Culex quinquefasciatus* following kinoprene exposure (Hamaidia & Soltani, 2014) and in *Aedes aegypti* subjected to novaluron treatment (Fernesi et al., 2012). Exposure to IGRs generally results in impaired metamorphosis, evidenced by diminished rates of pupation and adult emergence, the presence of larval-pupal intermediates, and morphological anomalies (Ghoneim, 2017). Various insect growth regulators (IGRs), including chlorfluazuron, lufenuron, flufenoxuron, methoprene, fenoxycarb, novaluron, and cyromazine, have demonstrated effects on species such as *Aedes aegypti* (Nwankwo et al., 2011), *Musca domestica* (Lohmeyer et al., 2014), and *Culex quinquefasciatus* (Hamaidia & Soltani, 2014). This study found that the application of S-Hydroprene caused metamorphic disruptions in *Culex quinquefasciatus* larvae, resulting in the emergence of larval-pupal intermediates. Nonetheless, no malformed pupae were detected, potentially attributable to successful advancement into the pupal stage. The identified anomalies are likely a result of hormonal disruption, specifically in the regulation of ecdysteroids and neurosecretory functions.

The emergence of larval-pupal intermediates may arise from S-Hydroprene's interference with biosynthetic pathways, including chitin synthesis. S-Hydroprene likely disrupts chitin synthase activity and DNA synthesis, thereby obstructing proper cuticle formation during apolysis and resulting in developmental anomalies. These findings corroborate Retnakaran et al. (1985), who emphasized the significance of chitin biosynthesis inhibition in IGR-induced morphological anomalies.

Conclusion

The results of the investigation show that S-Hydroprene cannot cause high larval mortality to *Culex quinquefasciatus* to be used

as control agent. Nevertheless, it markedly interferes with the mosquito's developmental mechanisms, hindering growth, metamorphosis, and morphogenesis. S-Hydroprene, as an insect growth regulator (IGR) can be effectively utilized for management of *Cx. quinquefasciatus* populations in the study location. Its specificity and mechanism of action makes it a promising candidate for integrated pest management (IPM) strategies, especially in addressing insecticide resistance that have been developed against other IGRs such as methoprene, kinetoprene and a host of other related ones.

Recommendation

The use of S-Hydroprene should be encouraged in rural and urban areas due to its efficiency and the unlikely development of resistance by *Culex quinquefasciatus* as well as its ecofriendly nature. More so, other local plants can be tested for their capability to induce similar results on the mosquito species.

Conflict of interest. Authors declare that there is no conflict of interest

References

- Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265–267.
- Adebote, D.A., Adeyemi, M.M.H. (2011). Larvicidal efficacy of solvent-extracted stem bark of *Bobgunnia madagascariensis* (Desv.) J.H. Kirkbr. & Wiersema (Caesalpinaceae) against *Culex quinquefasciatus*. *Int. J. Biol.* 1(7): 101–106.
- Aref, S.A., Bayoumi, O.Ch., Soliman, H.A.B. (2010). Effect of certain insecticides on the biotic potential of the cotton leafworm, *Spodoptera littoralis* (Boisd.). *Egypt. J. Agric. Res.* 88(1): 31–40.
- Ali, A., Chowdhury, M.A., Hossain, M.I., Mahmud-ul-ameen, Habiba, D.B., Aslam, A.F.M. (1999). Laboratory evaluation of selected larvicides and insect growth regulators against field-collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. *J. Am. Mosq. Control Assoc.* 15(1): 43–47.
- Amusan, A.S., Mafiana, C.F., Idowu, A.B., Olatunde, G.O. (2004). Mosquito species breeding in ground pools and artificial containers in Ajana, Ogun State, Nigeria. *Niger. J. Entomol.* 21: 11–21.
- Barik, T.K., Suzuki, Y., Rasgon, J.L. (2016). Factors influencing infection and transmission of *Anopheles gambiae* densovirus (AgDENV) in mosquitoes. *PeerJ* 4: e2691.
- Cornel, A.J., Stanich, M.A., Farley, D., Mulligan, F.S., Hyde, G. (2000). Methoprene tolerance in *Aedes nigromaculis* in Fresno County, California. *J. Am. Mosq. Control Assoc.* 16: 223–228.
- De Almeida, A.B., Freedman, D.O. (1999). Epidemiology and immunopathology of bancroftian filariasis. *Microbes Infect.* 1: 1015–1022.
- Eric, C., Van, M., Pham, O.M., John, F.A., Jose, M.C.R. (2009). The salivary gland transcriptome of the neotropical malaria vector *Anopheles darlingi* reveals accelerated evolution of genes relevant to hematophagy. *BMC Genomics* 10(1): 57.
- Ghoneim, K., Tanani, M., Hamadah, Kh., Basiouny, A., Waheeb, H. (2015). Bioefficacy of Novaluron, a chitin synthesis inhibitor, on survival and development of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *J. Adv. Zool.* 1(1): 24–36.
- Greenway, M., Dale, P., Chapman, H. (2003).

- An assessment of mosquito breeding and control in four surface flow wetlands in tropical-subtropical Australia. *Water Sci. Technol.* 48(5): 249–256.
- Hamaidia, K., Soltani, N. (2016). Ovicidal activity of an insect growth disruptor (methoxyfenozide) against *Culex pipiens* L. and delayed effect on development. *J. Entomol. Zool. Stud.* 4(4): 1202–1207.
- Hettiaratchi, U.P.K., Munasingha, D.H.N., Chandrasekharan, N.V., Karunanayake, E.H., Jayasekera, N. (2000). A polymerase chain reaction based method for the detection of *Culex quinquefasciatus* (Diptera: Culicidae). *Bull. Entomol. Res.* 90: 63–68.
- Kuo, J.N., McPherson, B., Soon, A., Pasternak, J., Garrett, C. (2010). Environmental concentrations of Methoprene and its transformation products after the treatment of Altosid XR Briquets in the city of Richmond, British Columbia, Canada. *Environ. Toxicol. Chem.* 29: 2200–2205.
- Kengluetcha, A., Singhasivanon, P., Tiensuwan, M., Jones, J., Sithiprasasna, R. (2005). Water quality and breeding habitats of anopheline mosquitoes in northwestern Thailand. *Southeast Asian J. Trop. Med. Public Health* 36(1): 46–53.
- Logan, T.M., Linticum, K.J., Thande, P.C., Wagateh, J.N., Roberts, C.R. (1991). Mosquito species collected from marsh in western Kenya during the long rains. *J. Am. Mosq. Control Assoc.* 7(3): 395–399.
- Moreno, M., Marinotti, O., Krzywinski, J., Tadei, W.P., James, A.A., Achee, N.L., Conn, J.E. (2010). Complete mtDNA genomes of *Anopheles darlingi* and an approach to anopheline divergence time. *Malar. J.* 9: 127.
- Mulla, M.S., Darwazeh, H.A., Kennedy, R., Dawson, D.M. (1986). Evaluation of new insect growth regulator against mosquitoes with notes on non-target organisms. *J. Am. Mosq. Control Assoc.* 2: 314–320.
- Mulla, M.S., Darwazeh, H.A., Norland, R.L. (1974). Insect growth regulators: evaluation procedures and activity against mosquitoes. *J. Econ. Entomol.* 67: 329–332.
- Muturi, E.J., Mwangangi, J., Shililu, J., Jacob, B.G., Mbogo, C., Githure, J., Novak, R.J. (2008). Environmental factors associated with the distribution of *Anopheles arabiensis* and *Culex quinquefasciatus* in a rice agro-ecosystem in Mwea, Kenya. *J. Vector Ecol.* 33: 56–63.
- Nanduri, J., Kazura, J.W. (1989). Clinical and laboratory aspects of filariasis. *Clin. Microbiol. Rev.* 2(1): 39–50.
- Okogun, G.R.A., Nwoke, B.E.B., Okere, A.N., Anosike, J.C., Esekhegbe, A.C. (2003). Epidemiological implications of preferences of breeding sites of mosquito species in Midwestern Nigeria. *Ann. Agric. Environ. Med.* 10: 217–222.
- Reuben, R., Tewari, S.C., Hiriyan, J., Akiyama, J. (1994). Illustrated keys to species of *Culex* (*Culex*) associated with Japanese encephalitis in Southern Asia (Diptera: Culicidae). *Mosq. Syst.* 26(2): 75–96.
- Richards, F.O., Pam, D.D., Kal, A., Gerlong, G.Y., Onyeka, J., Sambo, Y., Miri, E.S., Eigege, A. (2005). Significant decrease in the prevalence of *Wuchereria bancrofti* infection in anopheline mosquitoes following the addition of albendazole to annual

- ivermectin-based mass treatment in Nigeria. *Ann. Trop. Med. Parasitol.* 99(2): 155–164.
- Sanford, M.R., Chan, K., Walton, W.E. (2005). Effects of inorganic nitrogen enrichment on mosquitoes (Diptera: Culicidae) and the associated aquatic community in a constructed treatment wetland. *J. Med. Entomol.* 42(5): 766–776.
- Schafer, M.L., Lundstrom, J.O., Pfeffer, M., Lundkvist, E., Landin, J. (2004). Biological diversity versus risk for mosquito nuisance and disease transmission in constructed wetlands in southern Sweden. *Med. Vet. Entomol.* 18(3): 256–267.
- Service, M.W. (1963). Ecology of mosquitoes of the northern Guinea savannah of Nigeria. *Bull. Entomol. Res.* 54: 601–632.
- Williams, C.M. (1967). The juvenile hormone. II. Its role in the endocrine control of molting, pupation, and adult development in the cecropia silkworm. *Biol. Bull.* 121: 572–585.
- Wilson, T.G., Ashok, M. (1998). Insecticide resistance resulting from an absence of target-site gene product. *Proc. Natl. Acad. Sci. U.S.A.* 95: 14040–14044.