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## Toxicity of Diflubenzuron on Juveniles of African Brackish Water Shrimp from Lagoon Coastline and Mosquito Larvae from Breeding Places in a Tertiary Institution in Lagos, Nigeria

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**ABSTRACT:** Diflubenzuron (DFB) prevents chitin formation and cuticle deposition in insects. Its application can inadvertently enter into aquatic ecosystems, with potential adverse impacts on selected aquatic biota. Hence, the objective of this study was to evaluate the toxicity of diflubenzuron on juveniles of African brackish water shrimp from the Lagoon Coastline and mosquito larvae from breeding places in a tertiary institution in Lagos, Nigeria using appropriate standard techniques. The result of the lethal toxicity showed that  $LC_{50}$  toxicity factor of DFB on *Aedes and Culex* larvae were significantly higher (p < 0.05) for 24hrs compared to other time intervals. Exposure of the African brackish water shrimp to diflubenzuron had  $LC_5$ ,  $LC_{50}$ , and  $LC_{95}$  values of 0.115 mg/L, 8.510 mg/L, and 627.048 mg/L, respectively at 24hrs. Adult emergence inhibition of DFB on *Aedes and Culex* larvae showed highest adult emergence inhibition ( $\geq 90\%$ ) at 0.0001 mg/l after 12 days, while similar inhibition was achieved after 8 days for 0.005 mg/l. Diflubenzuron residue in water decreased with exposure time while that detected in *P. africanus* increased to a maximum of 42.13±3.7 ug/g and 301.03±13.7 ug/g for 100 ug/l and 1000 ug/l respectively after 14 days. The findings demonstrated that DFB is relatively stable in water, and its benign toxicity to non-target organisms makes it ideal for mosquito larval control.

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Palaemonetes africanus is a species of freshwater shrimp belonging to the Family Palaemonidae which is further divided into five sub-families namely Euryrhnchinae, Typhlocaridinae, Pontoniinae, Demoscaridinae Palaemoninae and (Marioghae, 1987). All the local species of the two genera (Palaemon and Palaemonetes) include maculatus, Palaemon Palaemon elegans and *Palaemonetes* africanus (Marioghae, 1987).

*Palaemonetes africanus* typically has a slender body with a semi-translucent exoskeleton. It displays unique patterns and a range of colors from brown to green. According to Ezemonye *et al.* (2007), shrimp are an essential part of the coastal food web because they serve as source of food for humans, fish, shellfish, birds, invertebrates, and small worms. The contamination of aquatic ecosystems by emerging micropollutants (EMs), such as pesticides and heavy

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metals, is increasingly rendering water unsuitable for human consumption (Kumar et al., 2023 and Rose et al., 2023). Pesticides, which are chemical mixtures designed to inhibit the growth of pests or other unwanted organisms (Mohamed and Paleologos, 2018), have become integral to modern agriculture. They are specifically formulated to target certain organisms, thereby enhancing crop yields. Globally, over 2 million tonnes of pesticides are used annually to manage pests, weeds, and insects (Syafrudin et al., 2021). Approximately 17.5% of this substantial volume of pesticides consists of fungicides, 29.5% of insecticides, 47.5% of herbicides, and 5.5% of other types of pesticides (Salem et al., 2014). Diflubenzuron is widely used as an insecticide and acaricide to control immature insect and acarian pests, such as rust mites and gypsy moths that harm crops like wheat, brassicaceous vegetables, cotton, fruit trees, tea plants, ornamental plants, and forest trees. Given its extensive applications, DFB can easily enter aquatic ecosystems through drainage, runoff, or drift, significantly impacting the aquatic biota (Huang et al., 2023). Diflubenzuron is widely used as an insecticide and acaricide to control larval insectile and acarian pests (like gypsy moths and rust mites) that harm cotton, wheat, brassicaceous vegetables, fruit trees, tea plants, ornamental plants, and forest trees. Given its numerous uses. DFB can easily enter aquatic systems through runoff, drainage, or drift and suppress the local biota (Huang et al., 2023). The prolonged stability of diflubenzuron in fishing grounds, with a half-life of up to 100 days, highlights the urgent need to investigate its toxicity to aquatic organisms (Han et al., 2022). Additionally, considering the critical role of shrimp in maintaining the ecological balance of inland water bodies, it is essential to assess DFB's impact on shrimp populations and evaluate the time needed for their recovery (Huang et al., 2023). Consequently, the objective of this paper was to evaluate the toxicity of diflubenzuron on juveniles of African brackish water shrimp from the Lagoon Coastline and mosquito larvae from breeding places in a tertiary institution in Lagos, Nigeria.

## MATERIAL AND METHODS

*Study Area:* Lagos is a megacity in west Africa with a population of 17 million people (Olarinmoye *et al.*, 2020) and projected to reach 88.3 million by 2100 (Hoornweg and Pope, 2017). The city is bordered by the Lagos Lagoon, which spans approximately 50 km in length and 3 to 13 km in width, covering a total area of 6,354.7 km<sup>2</sup> (Badejo *et al.*, 2014). This lagoon is the fourth-largest lagoonal system in the Gulf of Guinea (Olarinmoye *et al.*, 2020) and the largest along the West African coast (Nkwoji *et al.*, 2023). It

serves as a nursery, breeding, spawning, and feeding ground for a variety of aquatic habitats, making it extremely significant ecologically (Elenwo and Akankali, 2015). *Aedes, Culex* larvae and juveniles of *Palaemonetes africanus* were collected differentially in University of Lagos Akoka Campus, at 3°24'07.6"E and Latitude 6°31'05.4"N.

#### Test Animal Collection:

*Mosquito larvae:* Mosquito breeding sites were identified within the University of Lagos Akoka campus, wherein three thousand  $1^{st} - 3^{rd}$  larval instars were collected with the aid of standard dippers, kept in containers, and transferred to the laboratory. These were then sorted into *Culex* and *Aedes* larvae via standard Morphological Keys (Jupp *et al.*,2002) and kept in different containers (2 litres volume) and kept inside mosquito cages to prevent/minimize escape upon adult emergence. The larvae were acclimatized for a maximum of 3 days prior to experimental procedure.

Palaemonetes africanus: A total of six hundred juveniles of Palaemonetes africanus (African brackish water shrimp) were collected off the coastline of University of Lagos lagoon front between 0530hrs and 0630hrs with the aid of sweep nets for two days in black containers and transported to the laboratory. They were sorted into three containers (20 litres volume) in batches of two hundred, with containers half-filled with brackish water with air pumps and covered with nettings to prevent/minimize escape/death. They were acclimatized for at least 7 days in this condition prior to experimental procedure.

*Test Compound Preparation:* Granules of Diflubenzuron (brand name Dimilin) were procured from the Applied Entomology Unit, Department of Zoology, University of Lagos. A total of ten granules (each weighing 1.05 mg) were dissolved in 1 litre of distilled water to make a stock solution. Thereafter, serial dilutions were made thereof, to getting the range-finding and definitive concentrations for test animal exposure.

*Experimental Procedure:* After series of rangefinding tests, the test animals were exposed to the following concentrations for the definitive tests for lethal toxicity. Only the mosquito larval populations were assessed for Adult Emergence Inhibition for the test compound, due to their relatively short developmental cycle. A total of ten *Palaemonetes africanus* juveniles were exposed to each concentration in triplicates, and Control. In the case of mosquito species, twenty  $2^{nd} - 3^{rd}$  instar larvae

were exposed to each concentration in triplicates, and Control.

#### Lethal toxicity:

*P. africanus* - 0.5, 1, 1.5, 2, 2.5, 3.5, 5 and control *Culex sp.* - 0.05, 0.1, 0.25, 0.5, 0.75 and control *Aedes sp.* - 0.05, 0.1, 0.25, 0.5, 0.75 and control *Adult Emergence Inhibition:* 

*Culex sp.* – 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05 and control

Aedes sp. - 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05 and control

Assessment of Test Compound Efficacy: Lethal toxicity (mortality) was assessed at 24 hrs intervals for 4 days, wherein an organism is considered death when it remains motionless on the water surface, and/or remain motionless when prodded with a forceps. Dead organisms are remained daily and cumulated over the exposure period. Pooled data are expressed as percentage mortality responses and express as LC<sub>5</sub>, LC<sub>50</sub>, and LC<sub>95</sub> respectively.

Adult Emergence Inhibition was assessed via the number of mosquito larval instars that pupated and emerged as adults upon exposure to varying concentrations of the test compound. Values were represented in percentiles.

 $LC_5$  – The test compound concentration that can achieve at least 5% mortality of exposed test organism population

 $LC_{50}$  – The test compound concentration that can achieve at least 50% mortality of exposed test organism population

 $LC_{95}$  – The test compound concentration that can achieve at least 95% mortality of exposed test organism population

Percentage of inhibition of emergence (IE%): The adult emergence inhibition effect of Diflubenzuron was assessed against the larvae of Aedes and Culex in accordance with the WHO protocol (WHO, 2005) with slight modifications by (Panmei et al., 2019). A series of diflubenzuron concentrations (0.0001, 0.0005, 0.001, 0.005, 0.01and 0.05 mg/l, selected after preliminary trials) was taken from a Stock solution (10 mg/l). A total of 60 larvae of Aedes and *Culex* were exposed to a homogenous solution of 1 mL diflubenzuron and 199 mL dechlorinated water. Each concentration was run in three concurrent replicates. Control assay was run with 200 mL dechlorinated water. Larval growth/development was monitored at 24h interval post-exposure. After 8 days, adult emergence was assessed for all test concentrations and Control. Larval mortality was recorded daily and the percentage inhibition of adult

emergence (IE) was calculated using the following formula.

$$\% IE = 100 - (T \times 100 / C) T$$
 (1)

Where % IE = percentage adult emergence inhibition effect

T = percentage of adult emergence in the treatment,

C = percentage of adult emergence in the control treatment.

Chemical Analysis of Diflubenzuron in P. africanus:

Diflubenzuron Residue Extraction: Diflubenzuron residues in experimental water and in shrimps were performed using High Performance Liquid Chromatography (HPLC) procedure as described previously by Soltani and Morsli, (2003). At different exposure times (0, 3, 5, 7, and 28 days), 3 mL of experimental water sample and three shrimps were randomly sampled from Control and treated series and analyzed individually as follows. The harvested shrimps per treatment was rinsed using 3 mL of acetonitrile-water (50 - 50 by volume), then eviscerated and weighed. The remaining tissues were homogenized in 3 mL of acetonitrile-water (50 - 50) using a Sonifier cell disrupter B-30. After centrifugation (5000 g for 10 min) and evaporation of supernatant in Speed Vac (Varian), the various extracts from experimental water sample, and processed shrimp tissues were stored at -4°C for further analysis.

*High Performance Liquid Chromatography Analysis:* Single-residue methods employing HPLC-UV were used in depletion studies carried out in the mid-1990s for the quantification of diflubenzuron in shrimp tissues. The analytical method adopted herein (Thus et al., 1995) consisted of extraction of diflubenzuron from the whole digestion of shrimps (3 to 5 g) by solid-liquid extraction with acetonitrile (2 x 5 mL). The extract was evaporated to dryness at 50 °C, and dissolved in a solution of acetonitrile (1.5 mL), water (0.5 mL) and hexane (4 mL). The solution was vortexed, centrifuged and the hexane layer removed. An additional 4 mL of hexane, 1 mL of water and 4 mL of dichloromethane were added to the test tube. The mixture was vortexed, centrifuged and the dichloromethane layer separated. To the acetonitrile/water layer another 4 mL dichloromethane was added and the separation procedure repeated. The combined dichloromethane layers were mixed with sodium sulphate and the dried dichloromethane layer evaporated to dryness at 50 °C. The residue was dissolved in 4.0 mL of methylethylketone: petroleum ether, 2:25 v/v, with clean-up by solid phase extraction on a Florisil

cartridge (500 mg). The chromatographic separation was performed on a C18 column (Zorbax, 250 x 4.6 mm, 7.5  $\mu$ m particle size) at 35 °C, using acetonitrile: water, 1:1 v/v, as the mobile phase. Quantification was performed using a UV detector at 254 nm. The concentrations of diflubenzuron in the samples were calculated by comparing the peak height of the sample with the peak height of calibration solution

*Data Analysis:* Pooled data from the lethal toxicity (percentage mortality responses) were subjected to Probit Analysis (SPSS version 26) to extrapolate  $LC_5$ ,  $LC_{50}$ , and  $LC_{90}$  values on a daily basis. These values, with regression equation and degree of freedom were presented in tabular forms and graphics. Adult Emergence Inhibition was pooled and expressed in percentiles as graphics, using Microsoft Excel version 2016.

### **RESULTS AND DISCUSSION**

Lethal Effects of Diflubenzuron on Aedes and Culex Larval Populations: The toxicity of Aedes and Culex Larval Populations exposed to diflubenzuron showed that at 24hrs, the LC<sub>5</sub>, LC<sub>50</sub>, and LC<sub>95</sub> values were 0.115 mg/L, 8.510 mg/L, and 627.048 mg/L, respectively. A similar trend was observed for 48hrs, 72hrs and 96hrs. Toxicity factor of the LC<sub>50</sub> values of diflubenzuron on Aedes larvae showed significant different (p > 0.05) between 24hr value compared to the other time intervals, while no significant difference (p>0.05) was observed among 48hrs, 72 hrs. and 96 hrs. values. A similar trend was observed for Culex larval populations (Table 1).

Lethal Toxicity of Diflubenzuron on Palaemonetes africanus: The results from the bioassay showed that acute exposure to diflubenzuron on Palaemonetes africanus exhibited toxicity. Mortality was observed and noted across all the test concentrations though there were variations depending on exposure level. Palaemonetes africanus mortality increased with duration of exposure. Highest mortality was recorded at 96 hours and the second highest at 72 hours. The probit analysis revealed the concentration levels of diflubenzuron required to impact different percentages of the Palaemonetes africanus population at various time intervals. Palaemonetes *africanus* population had LC<sub>5</sub>, LC<sub>50</sub>, and LC<sub>95</sub> values of 0.115 mg/L, 8.510 mg/L, and 627.048 mg/L, respectively at 24hrs.A similar trend of LC<sub>5</sub>, LC<sub>50</sub>, and LC<sub>95</sub>was observed for Palaemonetes africanus population at 48 hrs., 72 hrs. and 96 hrs. (Table 2).

Adult Emergence Inhibition of Diflubenzuron on Aedes and Culex Larval Populations: Adult emergence percentage by Diflubenzuron during the 12-day period is shown in the Figures 1 and 2. In the control group, the efficacy in inhibiting adult emergence of both *Aedes* and *Culex* mosquitoes increase continuously and linearly from 8 days to 12 days. Highest adult emergence of 90% was observed at 0.0001 mg/l after 12 days, with no additional emergency observed at 8 days with concentration 0.005 mg/l, 0.01 mg/l, 0.05 mg/l. A similar trend of adult emergence was observed for mosquito larvae of *Culex* (Figure 2).

Diflubenzuron Residue Analysis: The amount of DFB residues detected in water and Palaemonetes africanus declined to the minimum  $(49.02 \pm 6.4 \text{ ug/g})$  and increase progressively to reach a maximum  $(42.13 \pm 3.7 \text{ ug/g})$  respectively at 100 ug/l concentration within 14day exposure (Table 3). A similar trend was observed for 1000 ug/l concentration (Table 3).



diflubenzuron

This study ascertained the toxicological effects of diflubenzuron (DFB) on the target organism (Aedes and Culex) and a non-target organism (Palaemonetes africanus) collected differentially from University of Lagos, Nigeria. The study also evaluates the Adult Emergence Inhibition of diflubenzuron on Aedes and Culex. The results revealed that DFB was found to be a potent insecticide for controlling mosquito populations. Mosquitoes are among the most significant insect vectors impacting the health of humans and animals worldwide (Torgby, 2019). Medically, they are the most important group of insects due to the numerous disease agents they transmit and the severe health issues these diseases cause. These include malaria, trypanosomiasis, and vellow fever.

Table 1. Relative to Alerty of diffuorization on Acaes and Calles failed and populations								
	Time	LC <sub>5</sub> (95% CL) mg/l	LC <sub>50</sub> (95% CL) mg/l	LC <sub>95</sub> (95% CL) mg/l	<b>Regression Equation</b>	Slope ± SE	DF	TF
	(hrs.)							
Aedes	24	0.051 (0.000245)	3.677 (2.140-98.412)	263.243 (26.911-52.82)	Y = 1.0X - 0.4	$-0.501 \pm .121$	4	5.3
	48	0.144 (0.034 - 0.280)	1.019 (0.713 - 1.292)	7.192 (4.443 - 19.808)	Y = 1.8X + 0.12	$-0.016 \pm 0.117$	4	1.5
	72	0.209 (0.090 - 0.332)	0.873 (0.659 - 1.062)	3.644 (2.717 - 6.071)	Y = 2.5X + 0.5	$0.157 \pm 0.119$	4	1.3
	96	0.157 (0.056 - 0.269)	0.694 (0.481 - 0.872)	3.064 (2.287 - 5.163)	Y = 2.25X + 0.7	$0.405 \pm 0.119$	4	1
Culex	24	0.013 (N/A)	8.011 (N/A)	5080.173 (N/A)	Y = 0.7X - 0.42	$-0.530 \pm 0.120$	4	9.4
	48	0.163 (0.043 - 0.306)	1.110 (0.805 - 1.396)	7.547 (4.666 - 20.432)	Y = 2X - 0.2	$-0.090 \pm 0.119$	4	1.3
	72	0.134 (0.033 - 0.260)	0.878 (0.594 - 1.121)	5.767 (3.747 - 3.747)	Y = 2X + 0.3	$0.114 \pm 0.117$	4	1.0
	96	0.175 (0.063 - 0.298)	0.855 (0.619 - 1.060)	4.175 (2.991-7.687)	Y = 2.5X + 0.5	$0.163 \pm 0.118$	4	1

Table 1: Relative toxicity of diflubenzuron on Aedes and Culex larval populations

CL-95% Confidence limit, SE – Standard Error, DF – Degree of freedom, N/A – Not available. Toxicity Factor (TF) =  $LC_{50}$  of least pesticides concentration  $LC_{50}$  of more toxic pesticides tested against same species

**Table 2:** Relative Toxicity of diflubenzuron on Palaemonetes africanus

	Time (hr.)	LC <sub>5</sub> (95% CL) mg/l	LC <sub>50</sub> (95% CL) mg/l	LC <sub>95</sub> (95% CL) mg/l	<b>Regression Equation</b>	Slope ± SE	DF	TF
Palaemonetes africanus	24	0.114 (0.00 - 0.390)	8.972 (4.326-318.88)	707.384(61.569-51.33)	Y = 0.833X + -0.667	$-0.826 \pm 0.131$	5	9.73
	48	0.125(0.013 - 0.303)	2.346 (1.713 - 3.603)	15.926(15.926-15.377)	Y = 1.6667X + 0.333	$-0.479 \pm 0.121$	5	2.54
	72	0.094 (0.011234)	1.467 (1.003 - 1.972)	22.785(10.285-152.225)	Y = 1.5X + 0	$-0.230 \pm 0.117$	5	1.59
	96	0.125 (.033244)	.922 (0.624 - 1.184)	6.819 (4.491 - 14.999)	Y = 2X + 0.2	$0.067\pm0.117$	5	1

CL-95% Confidence limit, SE - Standard Error, DF - Degree of freedom, TF - Toxicity Factor

Toxicity Factor  $(TF) = LC_{50}$  of least pesticides concentration

 $LC_{50}$  of more toxic pesticides tested against same species

Exposure (Davs)	Concentration (ug/l)	Water residue (ug/l)	Water Residue	Fish residue (ug/g)	Fish Residue (%)
r · · · · · · · · · · · · · · · · · · ·			(%)		
0	0	0	0	0	0
	100	100	100	0	0
	1000	1000	100	0	0
3	0	0	0	0	0
	100	$76.41 \pm 10.2$	76.4	$11.07 \pm 4.3$	11.1
	1000	$842.10 \pm 32.4$	84.2	$87.12 \pm 11.4$	8.7
5	0	0	0	0	0
	100	$70.32 \pm 8.3$	70.3	$21.15 \pm 7.1$	21.2
	1000	$803.07 \pm 22.7$	80.3	$172.31 \pm 15.2$	17.2
7	0	0	0	0	0
	100	$65.07 \pm 5.1$	65	$27.02\pm5.1$	27
	1000	$782.32 \pm 11.4$	78.2	$219.17\pm10.8$	21.9
14	0	0	0	0	0
	100	$49.02\pm6.4$	49	$42.13\pm3.7$	42.1
	1000	$719.08 \pm 17.4$	71.9	$301.03 \pm 13.7$	30.1

Table 3: Diflubenzuron	residue analys	sis from ex	nerimental y	water and shrimn	samples
Lable 5. Diffuotization	residue analys	sis nom ca	permental v	water and sminp	sampics



Fig. 2: Adult emergence percentage of *Culex* exposed to diflubenzuron

A rising challenge in mosquito control globally is the increasing resistance to insecticides, which complicates efforts to manage mosquito populations in various regions (Belinato *et al.*, 2013). Medically, they are the most important group of insects due to the numerous disease agents they transmit and the severe health issues these diseases cause. These include malaria, trypanosomiasis, and yellow fever. A rising challenge in mosquito control globally is the increasing resistance to insecticides, which complicates efforts to manage mosquito populations in various regions (Belinato *et al.*, 2013).

Resistance to the primary neurotoxic insecticide classes has become widespread among various populations of insect disease vectors. Diflubenzuron, a benzoylurea derivative and chitin synthesis inhibitor, is a key compound in the arthropod cuticle (Lechekhab and Soltani, 2018). When leached by rainfall, it contaminates aquatic ecosystems, including sediments where all stages of the crustacean life cycle occur (Lechekhab and Soltani, 2018). Diflubenzuron impacts mosquito larval development at all larval instars and other stages (Lau et al., 2018). The resulting of this finding showed that diflubenzuron causes lethal effects to Aedes and Culex larvae at DFB concentrations of 0.5 to 5 mg/l. This research is comparable to that of Gartenstein et al. (2006), whose findings indicated that DFB causes lethal effects to Artemia larvae at DFB concentrations of 0.13 to 1  $\mu$ gL<sup>-1</sup>. Lethal toxicity refers to the process by which toxic substances enter the body and disrupt the normal functioning of the target organ (Yuniari et al., 2016).

It is typically assessed through trials designed to evaluate the relative toxicity of a chemical to aquatic organisms over a specific, limited period of time. According to Libralato *et al.* (2016) and Yuniari *et al.* (2016), common toxicity tests include the LC50 - 96hour test, which measures the concentration of toxic substances that can cause the death of 50% of the population or test organisms within 96 hours. The results of this study showed that diflubenzuron exhibited significant toxicity to *Palaemonetes africanus.* These findings are consistent with previous research by Huang *et al.* (2023), who tested diflubenzuron at doses of 0, 0.74, 2.222, 6.667, 20, and 60 µg/L for shrimp (*Neocaridina palmata*).

Insect growth regulators (IGRs) are recognized as environmentally friendly pest control agents and more selective insecticides (Fansiri et al., 2022). They disrupt the normal functions of target insect pests by interfering with their growth and developmental processes, thereby reducing survival rates and impairing reproduction (Deep et al., 2018). The primary modes of action of IGRs include destroying insect eggs, disrupting developmental stages, preventing adult eclosion, and causing reproductive incapacity. According to Fansiri et al. (2022), the efficacy of IGRs in inhibiting adult emergence varies depending on their specific modes of action. In this study, diflubenzuron, a chitin synthesis inhibitor (CSI), effectively inhibited the adult emergence of Aedes and Culex mosquitoes. The results of this study are consistent with those of Fansiri *et al.* (2022) who observed that the  $EI_{50}$  and EI<sub>95</sub> of Ae. aegypti adult emergence ranged from 0.240 to 2.412 ppb and 0.444 to 4.040 ppb, respectively. This was also in tandem with Lau et al. (2018) who also noted that diflubenzuron has showed complete inhibition for more than 4 weeks up to 4 months. Despite diflubenzuron's (DFB) poor water solubility, water can serve as a significant exposure pathway for non-target organisms, particularly when DFB is used as a mosquito larvicide (Zaidi et al., 2013). Laboratory pesticide residue testing is employed to identify and quantify pesticide residues in food and environmental samples. DFB residues from runoff and discharge tend to persist in the environment, adversely impacting the viability of natural crustacean populations (Gartenstein et al., 2006). This persistence is attributed to DFB's relatively slow degradation in brackish estuarine waters (Langford et al., 2014). According to Zaidi et al. (2013), high-performance liquid chromatography (HPLC) is the most effective method for analyzing benzoylphenylurea compounds like DFB due to its high sensitivity. Rapid extraction procedures combined with HPLC analysis facilitate the precise

determination of individual residue levels (Soltani et al., 1983). Quantitative analysis of DFB in surface water and Palaemonetes africanus using HPLC revealed а significant reduction in DFB concentrations in water, accompanied by a progressive accumulation in the fish. The observed reduction in water concentrations may result from degradation or factors such as pH, salinity, and water temperature, which influence DFB's toxicity and persistence (Zaidi et al., 2013). These findings align with Zaidi et al. (2013), who studied DFB degradation in freshwater and its bioconcentration in Gambusia affinis using HPLC methods.

*Conclusion:* Pesticides were to improve human life by boosting agricultural output and reducing transmissible illnesses. However, their disadvantages had outnumbered their advantages, creating serious environmental problems; hence the need for substitute pest management techniques. This research showed that DFB exhibited notable toxicity on target organisms (*Aedes* and *Culex* larval populations), as well as juveniles of non-target species (*Palaemonetes africanus*). Nevertheless, further research is essential to comprehensively evaluate the efficacy of DFB in different mosquito species, its effect on non-target organisms molting processes and the main metabolites generated during its breakdown.

*Declaration of Conflict of Interest*: The authors declare no conflict of interest.

*Data Availability Statement:* Data are available upon request from the first author or corresponding author or any of the other authors.

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