LABORATORY PREPARATIONS OF LETHAL AND SUB-LETHAL BIO-ASSAYS CONCENTRATIONS OF DELTAMETHRIN AGAINST MALARIA MOSQUITO IN SULEJA, NIGERIA

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Laboratory Preparations of Lethal and Sub-lethal Bio-assays Concentrations of Deltamethrin Against Malaria Mosquito in Suleja, Nigeria

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Abstract— There is paucity of information concerning the resistance status of malaria mosquitoes (*Anopheles* mosquitoes) in Suleja. The present study therefore aims to prepare various CDC bio-assay concentrations of deltamethrin against *Anopheles* mosquito in Suleja metropolis, Nigeria. 25 samples of wild *Anopheles* mosquitoes were collected from Suleja and was exposed in replicate of four, to various concentrations of deltamethrin and then metabolic enzyme activities in exposed samples were examine following standard protocols. Data obtained was analysed using IBM-SPSS version 25.0 and results were expressed in 'Tables and Figures'. These were compared statistically among deltamethrin concentrations using Analysis of Variance at P=0.05 with the aid of Graph-Pad prism 8. 0.8µg/b, 0.6µg/b, 0.4µg/b, and 0.2µg/b of deltamethrin are sub-lethal concentrations of the insecticide that bring about 0% mortality insignificantly (P > 0.05) different from the mortality in the control experiment. As the mosquito population has developed resistance to recommended concentration of deltamethrin, the study recommends resistance monitoring exercise for effective vector control in Suleja.

Keywords___ Anopheles mosquito, CDC bottle, Suleja, Deltamethrin.

I. INTRODUCTION

The spread of malaria in Sub-Saharan Africa, particularly in Nigeria, has been attributed mostly to *Anopheles gambiae* (*s.l.*) mosquitoes [1, 2]. Malaria is the world's most important parasitic disease of public health importance [3]. Africa continues to have a startlingly high number of malaria cases, with 92% of recorded cases and over 93% of deaths from the disease, according to a recent World Health Organization (WHO) global malaria report [1]. In Africa, Nigeria is responsible for 25% of cases [3, 4].

Controlling mosquito vectors has been suggested as a key strategy to reduce the frequency and impact of malaria transmission in endemic areas of the world [5, 6]. This strategy seems to have run into a roadblock, though. This is due to mosquito vectors in Nigeria developing resistance to a variety of control methods, including chemical pesticides and insecticides [6, 7, 8]. The Centers for Disease Control (CDC) has promoted resistance monitoring in malaria endemic areas in order to confront the reality of mosquito resistance development [5, 9]. Monitoring resistance is essential for controlling mosquito vectors, especially when it comes to diseases like malaria. By allowing for prompt strategy modifications and guaranteeing that interventions continue to be effective, monitoring resistance in mosquito populations can greatly improve vector control programs [10, 11]. In particular, by routinely testing mosquito populations for resistance, authorities can identify early indicators of resistance and avoid the widespread failure of control operations, which can aid in mosquito vector management in a number of ways [11]. To the best of our knowledge, resistance status of Suleja's *Anopheles* mosquito species has not been documented. Therefore, the purpose of this study is to prepare different CDC bio-assay concentrations of deltamethrin, an insecticide recommended by the World Health Organization (WHO), against *Anopheles* mosquitoes in Suleja township, Nigeria.

II. METHODOLOGY

A. Collection Rearing of Mosquito

Anopheles larval collections were conducted in June 2024, resulting in 2,575 mosquito population among temporary rain puddles evenly distributed across all settlements in Suleja metropolis. This includes Suleja Township (latitude 9°10'50.12" N and longitude 7° 10' 45.80" E) and Gwazunu (Latitude: 9°08'13.18"N; Longitude: 7° 11' 46.79"E), Nigeria. Larval sampling was conducted and identified breeding sites were mapped using Garmin eTrex® GPS 10 personal navigators, following a standard protocol [12-15]. By carefully dipping white dippers at a 45° angle into designated breeding locations, skimming the water's surface, and then transferring the samples into collection bottles with labels, a consistent procedure was used to gather larval samples. These bottles were subsequently taken to the Suleja laboratory of the Centre for Tuberculosis and Other Neglected Tropical Diseases. Yeast was fed to the larvae samples every day while they were kept in regulated insectary settings (25–28 °C, ~70–80% humidity, and a 12-hour day/night cycle) [4]. A 10% glucose solution soaked in cotton wool was used to feed the emerging adult.

B. Insecticide Preparation And Dilutions

1ml from the original stock liquid solution of technical grade deltamethrin supplied by the Centre for Disease Control and Prevention (CDC) was diluted with 28.5ml (100%) acetone to give a standard concentration of 12.50µg/ml to be used to coat a 25ml capacity CDC bottle (12.50 µg/bottle) [5, 12].

Lower concentrations of deltamethrin were obtained by following the procedures described by [4] to obtain 1ml each of 15 µg/bottle, 5 µg/bottle, 1 µg/bottle, 0.9 µg/bottle, 0.8 µg/bottle, 0.6 µg/bottle, 0.4 µg/bottle, 0.2 µg/bottle and 0 µg/bottle (control, acetone only). This procedure is necessary to evaluate lethal and sub-lethal concentrations of the insecticide when mosquito is exposed to them. Summary of the dilutions is as shown in Table 1.

Table 1. Prepared Lower Concentrations of Deltamethrin

Serial number	Concentrations (µg/bottle)	Quantity of Stock Taken (ml)	Volume of Acetone (ml)
1	11.0	0.9800	0.0200
2	10.0	0.8000	0.2000
3	5.0	0.4000	0.6000
4	1.0	0.0800	0.9200
5	0.9	0.0720	0.9280
6	0.8	0.0640	0.9360
7	0.6	0.0480	0.9520
8	0.4	0.0320	0.9680
9	0.2	0.0160	0.9840
Control	0.0	-	1.0000

C. Mosquito Exposure to Deltamethrin Insecticide

Four replicates of the aforementioned concoction of concentrations were given to 2-3 days olds female *Anopheles* mosquitoes in order to identify the lethal and sub-lethal ones. According to. [4], this is accomplished by applying each concentration to a dried-clean, clearly labeled 25 ml CDC vial, after which 25 adult female mosquito samples were added to each of the four replicates, including the control. Over the course of a day, the mortality rate of exposed mosquitoes was tracked.

The number of adult mosquitoes (samples size) used for this experiment was determined based on CDC-Global Manual for Bottle Bioassay [5]. 25 adult mosquito samples in 4 replicates for each concentration.

- For each concentration = 100 mosquito samples.
- For 9 lower concentrations in this experiment: 10 × 100 = 1000
- Control experiment (without insecticide) = 100
- Total Sample = 1000 + 100 = 1100 adult mosquito sample size
- All exposed mosquito sample were kept for possible biochemical analysis

D. Biochemical Assay for Measurement of Detoxification Enzymes in Adult Mosquitoes Exposed to a Selected Lethal and a Sub-Lethal Concentrations of Deltamethrin

Thirty non-blood female adult mosquitoes were selected each from (a) population exposed to standard deltamethrin concentration and (b) population exposed to 0.2 µg/CDC bottle deltamethrin concentration and analysed for detoxifying enzyme activities. Control of these experiments was made of 30 non-blood female adult mosquitoes not exposed to the insecticide. Assays of monooxygenase (P450), Glutathione-S-Transferase (GST), and esterase, the major detoxification enzymes, were carried out using procedures described by WHO [6]. All of the assays were three-fold duplicates.

E. Statistical Analysis

Using IBM-SPSS version 25.0, data on mosquito mortality was analyzed and presented in Tables and Figures. Using Graph-Pad Prism 8, they were statistically compared across deltamethrin concentrations using Analysis of Variance at P=0.05.

III. Results

Centre for Disease Control and Prevention (CDC) recommended concentration of Deltamethrin (12.5µg/b) could not bring about 100% mortality of adult *Anopheles* mosquitoes after 30 minutes of exposure as shown in Table 1. This was only achieved after 35 minutes. Mosquito mortality rate decreases with decrease in concentration of deltamethrin insecticide (Figure 1). For instance, the mortality of mosquitoes decreases across 30 minutes mark as the concentrations of the insecticides decreases. Also, 26.00 (26 %) mean mosquito mortality was recorded under 15.0µg/b after 30 minutes, however, only significantly ($P < 0.05$) lower mean mortality of 7.00 (7 %) was recorded for the same mosquito population exposed to 10.0µg/b under the same condition. Also, 21.5µg/b would result in 50% mosquito mortality, that is LC50, after 30 minutes whereas the feat could only be achieved by 5.0 µg/b after 1hour exposure. 90% mosquito mortality, that is LC90 was achieved by 15.0µg/b at 40 minutes of mosquito exposure.

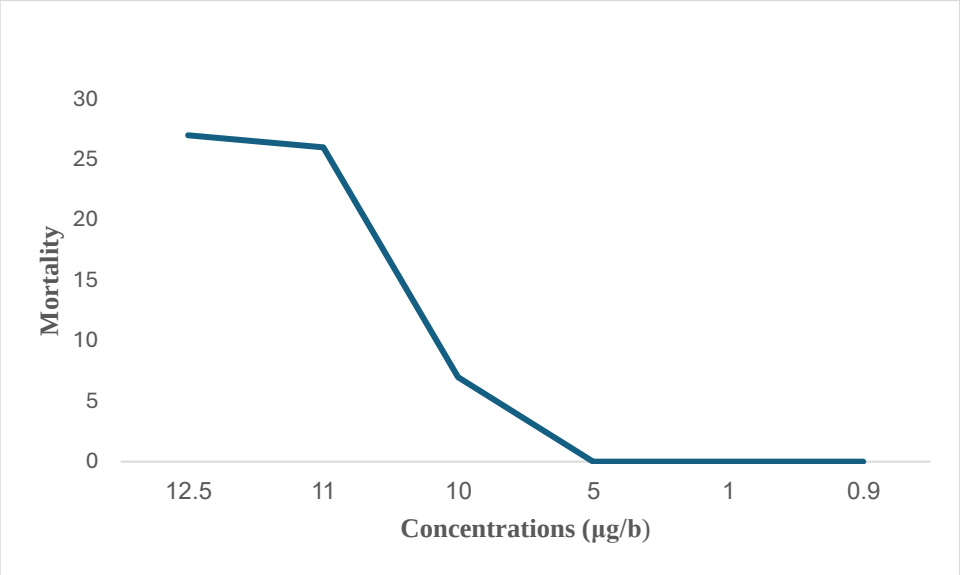

Fig. 1. Mortality Rates of Adults *Anopheles* Mosquitoes after 30 minutes Exposure to Standard, Lethal and Sub-Lethal Concentrations of Deltamethrin

Table 2. Mean Mortality (± SD) and Mortality Rate (%) of Deltamethrin Lethal Concentrations Against *Anopheles* Mosquito in Suleja

Time (min)	12.5µg/b	11.0µg/b	10.0µg/b	5.0 µg/b	1.0µg/b	0.9µg/b
0	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)
15	15.00±1.89^{b}(15)	10.00±0.58^{a}(10)	7.00±1.51^{a}(7)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)
30	27.00±0.50^{b}(27)	26.00±0.50^{b}(26)	7.00±1.51^{a}(7)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)
35	100.00±00^{c}(100)	33.00±1.71^{b}(33)	10.00±0.58^{a}(10)	1.00±0.50^{a}(1)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)
40	100.00±00^{c}(100)	90.80±2.22^{c}(90.8)	26.00±0.50^{b}(26)	1.00±0.50^{a}(1)	0.00±0.00^{a}(0)	0.00±0.00^{a}(0)
45	100.00±00^{c}(100)	100.00±00^{c}(100)	26.00±0.50b(26)	4.00±0.83a(4)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)
60	100.00±00c(100)	100.00±00c(100)	100.00±00^{c}(100)	33.00±1.71^{b}(33)	15.00±1.89^{b}(15)	0.00±0.00^{a}(0)
24 hours	100.00±00^{c}(100)	100.00±00^{c}(100)	100.00±00^{c}(100)	100.00±00^{c}(100)	15.00±1.89^{b}(15)	1.00±0.50^{a}(1)

Superscripts with different alphabets along the row are significantly different at P<0.05; n= 100; µg/b= Microgram per 25ml CDC bottle.

Insignificantly different (P > 0.05) mean mortality (0.00±0.00) and percentage mortality (0 %) was recorded even after 24 hours that adult *Anopheles* mosquitoes have been exposed to 0.8µg/b, 0.6µg/b, 0.4µg/b, 0.2µg/b and 0.0µg/b (control) concentrations of deltamethrin as shown in Table 3 below.

Table 3. Mean Mortality (± SD) and Mortality Rate (%) of Deltamethrin Sub-Lethal Concentrations Against *Anopheles* Mosquito in Suleja

Time (min)	0.8µg/b	0.6µg/b	0.4µg/b	0.2 µg/b	0.0 µg/b (Control)
0	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)
15	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)
30	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)
35	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)
40	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)
45	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)
60	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)
24 hours	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)	0.00±0.00$_{a}$(0)

Subscripts with different alphabets along the row are significantly different at P<0.05; n= 100; µg/b= Microgram per 25ml CDC bottle

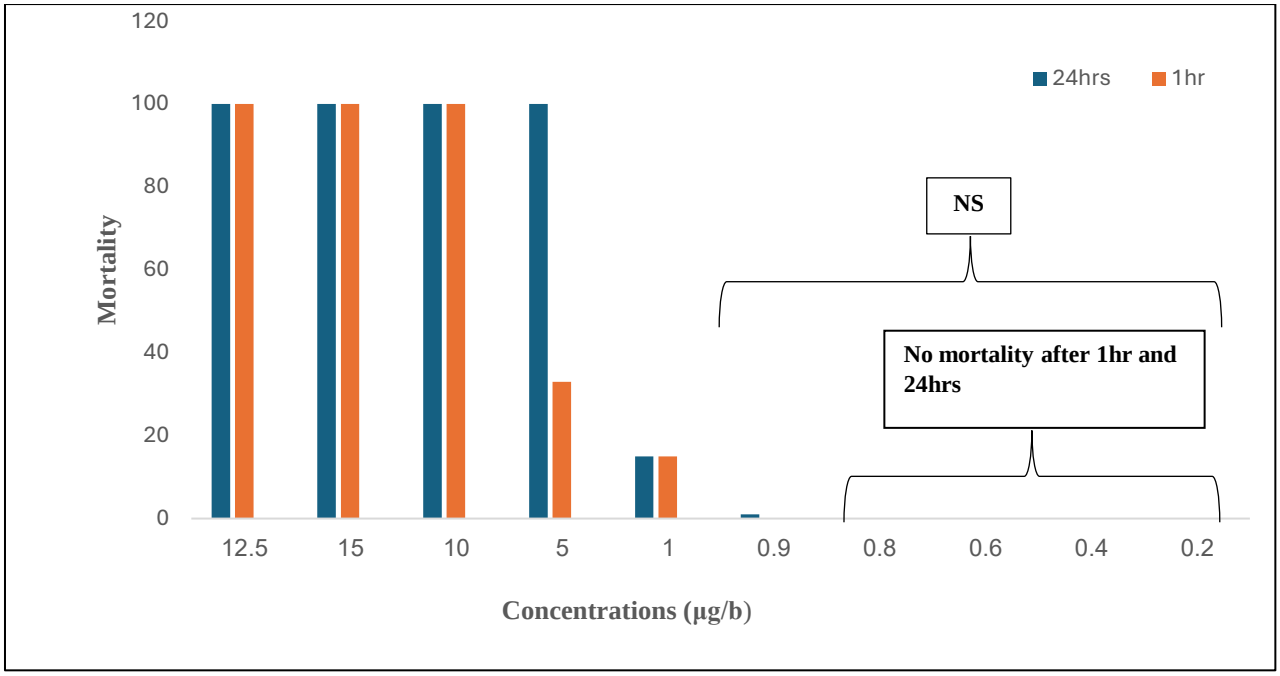

Fig. 2. Mortality Rates of Adults *Anopheles* Mosquitoes after 1hr and 24hrs Exposure to Standard, Lethal and Sub-Lethal Concentrations of Deltamethrin
(Note: NS = non-significant; P=0.005; µg/b= µg/b= Microgram per 25ml CDC bottle)

A closer look at the mortalities of the various lethal and sub-lethal concentrations after 24 hours showed that, even though not significantly different (P>0.05), no mortalities (0.00±0.00; 00.0%) were recorded at 0.8 µg, 0.6 µg, 0.4 µg and 0.2µg per bottle while (1.00%) were recorded at 0.9µg, 0 µg per bottle. These were also readily expressed in Figure 2

Table 4. Mean Activities of Detoxifying Enzymes in *Anopheles* Mosquitoes Exposed to Standard 12.5µg/b and 0.2 µg/b Deltamethrin Insecticides

Detoxifying Enzymes	0.2µg/b	12.5µg/b	Control
Monooxygenase (P450/mg protein)	61.430±0.003a	70.012±0.002b	60.201±0.001a
Esterase (µmol/min/mg protein)	50.500±1.250a	55.234±1.400b	50.200±2.300a
GST (mmol/min/mg/protein)	157.601±1.460a	199.721±1.250b	155.133±0.770a

n = 30; Subscripts with different alphabets along a row are significantly different at P<0.05

Glutathione-S-Transferase (GST) enzyme has the highest activities of 157.601±1.460, 199.721±1.250 and 155.133±0.770 from the mosquito populations exposed to 0.2µg/b, 12.5µg/b, and control experiments respectively compared to those recovered from monooxygenase and Esterase under the same conditions. It is quite noteworthy that the activities of enzymes increased with increase in concentration of deltamethrin they were pre-exposed to. For instance, monooxygenase enzyme has an average activity of 70.012±0.002 P450/mg protein under 12.5µg/b significantly higher (P < 0.05) than 61.430±0.003 P450/mg protein and 60.201±0.001 P450/mg protein of the same enzyme recovered from mosquito samples exposed to 0.2 µg/b and control (0 µg/b) experiments respectively (Table 4).

IV. Discussion

Lethal concentration refers to the concentration of an insecticide that causes death (mortality) in a certain proportion of the exposed population [16]. It is commonly measured as LC50 and LC90, which is the concentration that causes death in 50% and 90% of the exposed individuals respectively. However, sub-lethal concentration results in non-lethal physiological or behavioral changes, which can still impact the insect's long-term survival, reproduction, or development. These effects may include impaired movement, feeding inhibition, hormonal disruption, or reduced fertility as it was reported by [17].

Results from the present study showed that 21.5 µg/bottle, 15 µg/bottle, 5 µg/bottle, 1 µg/bottle, and 0.9 µg/bottle concentrations of deltamethrin are lethal to the mosquito population as they elicit certain percentage of mortalities in them after exposure. This is similar to the report of Adesoye *et al.* [4] that 21.5 µg/bottle, 15 µg/bottle, 5 µg/bottle, 1 µg/bottle, and 0.9 µg/bottle concentrations brings about mortality in *Anopheles gambiae* (Kisumu) exposed to permethrin insecticide.

However, their [4] report is different from the present one in that it was only 0.2 and 0.4 µg/bottle of permethrin that did not result in mortality at 24-hour mark. Meanwhile, 0.8 µg/bottle0.6 µg/bottle0.4 µg/bottle0.2 produced an insignificantly different (0%) morality with the control experiment after 24, making them all sub-lethal concentrations to the mosquito population. This disparity in result may be because wild mosquito is used for the present study as against well-established susceptible *Anopheles gambiae* (Kisumu) that was used in their study.

Standard recommended concentration of 12.5 µg/bottle of deltamethrin only brings about 27.00±0.50 (27%) mean mortality at 30 minutes mark of exposure. This means *Anopheles* mosquitoes in Suleja are resistant to the insecticide. However, the concentration elicits 100% mortality after 30 minutes. Reported spread of insecticide development across the globe [18] and most especially in Nigeria [19] has now been reported in Suleja for the first time. Increased activities of metabolic enzymes in form of cross or multiple resistance against various WHO approved insecticides against *Anopheles* mosquitoes have been previously implicated as the major threat to malaria control programmes in different parts of the world [20-23], including Nigeria [19]. The current study witnessed an increased in the activities of glutathione-S-Transferase (GST), monooxygenase and esterase metabolic enzymes in mosquitoes collected from the study area. This underlined reduction in susceptibility of the *Anopheles* mosquito population in the present test experiments. Result of the present in similar to the report [23] which reported increased metabolic activities in for of multiple-insecticide-resistance in *Anopheles* mosquito species.

Furthermore, continuous exposure of mosquitoes to various selection pressures including chemical insecticides in the principal factor that triggers the development of resistant development in *Anopheles* mosquitoes [4, 10]. In the present study, activities of enzymes increased with increase in concentration of deltamethrin (selection pressure) they were pre-exposed to. The confirms our previous finding in 2023 which suggested exposure of mosquito to chemical insecticides triggers increased activities of metabolic enzymes [4].

V. CONCLUSION

In conclusion, *Anopheles* mosquito population in has developed single, cross or multiple resistance mechanisms against deltamethrin in Suleja majorly as a result of increased metabolic enzymes in them. Continuous resistance monitoring in mosquito population is therefore recommended to promote effective vector control in Suleja metropolis.

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