COMPARATIVE STUDY ON THE SUSCEPTIBILITY STATUS OF THREE COMMON MOSQUITOES SPECIES IN MAKURDI TO EIGHT DIFFERENT INSECTICIDES USING WHO TEST TUBE BIOASSAYS

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ABSTRACT

Comparative susceptibility study of Aedes aegypti, Anopheles gambiae and Culex quinquefasciatus to eight insecticides in four classes was conducted using 2 – 5 day old laboratory reared, non-blood fed adult female mosquitoes. Standard WHO test tubes bioassay method was adopted with two thousand four hundred (2400) female mosquitoes. The study revealed that Ae. aegypti was resistant to Dichlorodiphenyltrichloroethane (DDT – an organochlorine), showed suspected resistant to Permethrin and Lambdacyhalothrin, susceptible to Deltamethrin, Alphacypermethrin, Bendiocarb, Propoxur, Pirimiphos methyl and to all the pyrethroids. An. gambiae was found to be resistant to Deltamethrin, Alphacypermethrin, Permethrin and DDT but susceptible to Lambdacyhalothrin, Bendiocarb, Propoxur and Pirimiphos-methyl. Cx. quinquefasciatus showed suspected resistant to Alphacypermethrin, Lambdacyhalothrin and Bendiocarb. Cx. quinquefasciatus was found to be highly resistant to DDT, Permethrin, Deltamethrin and Propoxur but susceptible to Pirimiphos-methyl and resistant to all pyrethroids, the Cx. quinquefasciatus had the highest level of resistance among the three species of the mosquitoes used in the study and all were resistant to DDT.

Keywords: Mosquitoes, Insecticides, Resistance, Susceptibility, Vector

INTRODUCTION

Mosquitoes are well known as annoying biting pests and vectors of disease-causing agents to humans and animals (WHO, 2012; Hill *et al*., 2013; Marshal *et al*., 2016; Shi *et al*., 2019). Mosquitoes are delicate blood sucking nematocera. Some of their unique features such as slender body, long needle-like proboscis, antennae, and long fragile legs differentiate them from other blood sucking dipteran flies (Al-Ghamdi *et al*., 2008; Alikhan *et al*., 2014; Alikhan *et al*., 2018). They have narrow wings which are sometimes covered with minute scales. Like any other biting and nuisance flies, they affect the production and performance of livestock (Mullens *et al*., 2006; Carvalho and Mello-Patiu, 2008; Taylor *et al*., 2012). They hinder grazing and caused animals to be restless. In addition to their annoyance, several species of mosquitoes are vectors for pathogens of various diseases of medical and veterinary importance (Taylor *et* *al*., 2012; Gouge *et al*., 2016). Females are particularly active biters and feed readily and consistently under most circumstances when given the opportunity (Hill *et al*., 2013). *Aedes aegypti* Linnaeus in Hasselquist, 1762 (Diptera: Culicidae) which is a native of Africa is the principal vector of Dengue virus, Yellow fever virus, Zika virus and Chikungunya virus (Holmes and Twiddy, 2003; Barret and Higgs, 2007; Kindhauser *et al*., 2016; Burt *et al*., 2017). *Anopheles gambiae* Giles, 1902 (Diptera: Culicidae) is the primary vector of malaria which account for over a million deaths and 360 millions of morbidity annually in sub-Saharan Africa (Breman *et al*., 2004; Snow *et al*., 2005; Gachelin *et al*., 2018). *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae) is a medium-sized brown mosquito that exists all over the tropics. The proboscis, thorax, wings, and tarsi appear darker than the remaining part of the body. It is responsible for the transmission of *Wuchereria bancrofti*, Rift Valley fever virus (Foster and Walker, 2002), West Nile virus (Kent *et al*., 2010) and Western equine encephalitis virus (Neira *et al*., 2014) among others.

Vector control is a very important part of the global approach for prevention of mosquitoes-associated diseases (WHO, 2017). Control measures are generally directed against only one or a few of the most important species and are usually targeted to the adults or the larvae stages (WHO, 2017; 2018). The application of insecticides is the utmost important in this effort. The use of insecticide-treated nets are the foremost and most cost effective actions against mosquitoes populations but there are set backs due to rapid development of insecticide resistance, particularly in the cities across various locations (Nwane *et al*., 2009; Antonio-Nkondjio *et al*., 2015). For effective use of insecticides against mosquitoes, it is necessary to establish their susceptibility status using World Health Organization recommended insecticides, hence the reason for this study.

MATERIALS AND METHODS

Experimental Location: The study was conducted in Makurdi Metropolis. Makurdi is the administrative head quarter of Benue State, Nigeria. Makurdi is located on latitude 7° 45″ North and longitude 8° 31″ East, which lies within the Southern Guinea Savannah region of Nigeria (Echi *et al*., 2015). The daily temperature ranges between 21.6°C in December – January and 42.6°C in February – March. The annual rainfall (April – October) ranges from 1,105 – 1,600 mm and relative humidity is highest (69 %) between August and September and lowest (39 %) between January and March (WorldWeatherOnline, 2021).

Mosquitoes Sampling: The study was conducted between March – June, 2019. Samples were collected from four (North Bank, Wadata, Naka Road and Judges Quarters) locations. Various aquatic locations of immature stages of mosquitoes ranging from natural to man-made (drainage ditches, rice farms, ground pools and abandoned containers), as well as permanent and temporal sites within Makurdi metropolis were visited and examined for the presence of developmental stages (eggs, larvae and pupae) of mosquitoes. Where present, specimens were collected along with the water at the breeding sites using dippers and where necessary. They were put into clean grease-free plastic containers and transported to the Department of Veterinary Parasitology and Entomology Laboratory of Federal University of Agriculture, Makurdi for identification (adults) and rearing (larvae) using a slightly modified method of Imam *et al*. (2014).

Laboratory Rearing and Feeding: The mosquitoes were identified morphologically using standard keys (Jourdain *et al*., 2018). The larvae (L_1 to L_4) were reared separately from the pupae. They were reared in plastic cages of 30 x 50 x 20 cm^3 (length, width and depth respectively) with net covered top. Pupae were separated from the larvae using wide mouthed pipette. They were kept inside emergence cage with little water to aid adult development. The larvae consisting of L_1 to L_4 were kept in separate cages where they were fed with ground mixture of fat free biscuits (Yale Cabin) and yeast tablets at the ratio of 1:10. Each species tested was reared in separate cages to obtain pure population species. Adult were fed with 10 % sugar solution from the day of emergence until tests were carried out. Dead mosquitoes as well as those observed with any broken body parts were culled out at intervals.

Insecticide Resistance Test: A total of two thousand and four hundred (2400), eight hundred each for *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* adults unfed female mosquitoes, aged 2 – 5 days were used to determine insecticide susceptibility test using WHO test tube bioassay (WHO, 2005). One-hundred (100) adult female mosquitoes were used for each species per insecticide. The mosquitoes were exposed to four pyrethroids, two carbamates, one organophosphate and one organochlorine (Table 1). Twenty five (25) mosquitoes were introduced into each of the four WHO holding tubes using an aspirator (WHO, 2016a). Stripes of insecticide impregnated filter papers were inserted into the exposure tubes and fastened in place using appropriate clips in a vertical position. These were attached to the other side of the slide on the holding tubes. The slides were gently opened to prevent crushing of mosquitoes and were afterward closed immediately the mosquitoes have been gradually driven into the exposure tubes. The mosquitoes were left in the exposure tubes for 60 minutes and knockdown was recorded every 15 minutes beginning from 0 minute. At the end of the 60 minutes exposure time, the mosquitoes were gently returned to the holding tubes and 10 % sugar solution was provided in light cotton wools for 24 hours for each holding tube (WHO, 2016a). Percentage mortality was recorded at 24 hours as recommended (WHO, 2016a). All the bioassay tests were accompanied by negative control tests where the mosquitoes were also exposed to filter papers treated only with silicone oil for an hour and they were also supplied with a 10 % sugar meal during the recovery period (30) All tests were carried out at the standard temperature and relative humidity of 25 ± 2^{o}C and 75 ± 20 % respectively.

Data Analysis: The percentage progressive knockdown was based on the criteria that 98 – 100 % mortality indicates susceptibility of the mosquitoes; 80 – 97 % mortality indicates potential resistance that needs to be confirmed further through either biochemical or molecular assays and less than 80 % mortality implies resistance (WHO, 2016b).

RESULTS

The results of the 2400 female mosquitoes exposed to eight different insecticides in four classes revealed that *Ae. aegypti* was resistant to Dichlorodiphenyltrichloroethane (DDT – an organochlorine), showed suspected resistant to Permethrin and Lambdacyhalothrin, susceptible to Deltamethrin, Alphacypermethrin, Bendiocarb, Propoxur, Pirimiphos methyl and to all the pyrethroids (Table 1). *An. gambiae* showed resistant to Deltamethrin, Alphacypermethrin, Permethrin and DDT but susceptible to Lambdacyhalothrin, Bendiocarb, Propoxur and Pirimiphos-methyl. *Cx. quinquefasciatus* showed suspected resistant to Alphacypermethrin, Lambdacyhalothrin and Bendiocarb. *Cx. quinquefasciatus* was highly resistant to DDT, Permethrin, Deltamethrin and Propoxur, and was susceptible to Pirimiphos-methyl but resistant to all pyrethroids, The *Cx. quinquefasciatus* had the highest level of resistance among the three species of mosquitoes used and all were resistant to DDT.

DISCUSSION

The study revealed that *Cx. quinquefasciatus* had the highest resistance to most of the common insecticides in used. It was susceptible to only one (Pirimiphos-methyl) out of the eight different insecticides used. This observation was in agreement with other studies that reported of *Culex* species resistance to most of the commonly insecticides in used. Oduola *et al.* (2016), who carried out similar study on *Culex* mosquitoes in Kwara State using Deltamethrin and Lambdacyhalothrin, reported resistance. The results of the current study was at variance with Oduola *et al*. (2016) that reported susceptibility of *Culex* species to Permethrin, but in this study, resistance was observed. Ukpai and Ekedo (2018) also reported resistance of *Ae. aegypti* from Umudike, Abia State, Nigeria to Deltamethrin.

Table 1: Susceptibility status of three common mosquitoes species in Makurdi to eight different insecticides

S/No	Class of Insecticide	Insecticides	Susceptibility Status of three genera of Mosquitoes species				P-value
			Total Tested (N)	*Aedes* species (% Death)	*Anopheles* species (% Death)	*Culex* species (% Death)	
A	**Pyrethroids**						0.06
1		Alphacypermethrin (0.5 %)	100	100 % (S)	66 % (R)	95 % (R)	
2		Deltamethrin (0.05 %)	100	100 % (S)	87 % (R)	72 % (R)	
3		Lambdacyhalothrin (0.05 %)	100	96 % (SR)	100 % (S)	92 % (R)	
4		Permethrin (0.75 %)	100	97 % (SR)	69 % (R)	61 % (R)	
B	**Carbamates**						0.02
5		Bendiocarb (0.1 %)	100	100 % (S)	100 % (S)	90 % (R)	
6		Propoxur (0.1 %)	100	100 % (S)	100 % (S)	52 % (R)	
C	**Organophosphate**						0.02
7		Pirimiphos-methyl (0.25 %)	100	100 % (S)	100 % (S)	100 % (S)	
D	**Organochlorine**						0.02
8		DDT (4 %)	100	79 % (R)	62 % (R)	41 % (R)	

Key: (S) = Resistant. (R) Susceptible, (SR) Suspected Resistant

High resistance of *Culex pipiens pallens* Linnaeus, 1758 (Diptera: Culicidae) to five insecticides have been reported from China (Wang *et al*., 2020).

The increasing rate of insecticides resistance by *Anopheles* species in Nigeria and Africa have spread across different regions (Djouaka *et al*., 2016 a,b; Ibrahim *et al*., 2016; Menze *et al*., 2016; Atoyebi *et al*., 2020). The results of this study was in agreement with the findings of Hammad *et al*. (2015), who reported resistance of *An. gambiae* from Sindh, Pakistan to Permethrin, Lambdacyhalothrin and Deltamethrin. Similar observation was also reported from Mali on Deltamethrin, but in variance with Lambdacyhalothrin which was effective (susceptible) against *An. gambiae* (Cisse *et al.,* 2015). Resistance of *An. gambiae* to Deltamethrin and Permethrin has also been reported from South-West Cameroon (Boussougou-Sambe *et al.,* 2018). The findings of this study were in agreement with the report of Boussougou-Sambe *et al.* (2018). Furthermore, the observation in this study disagreed with Umar *et al.* (2014), who reported resistance of *An. gambiae* of North-Western Nigeria to Lambdacyhalothrin.

The findings of this study were in partial agreement with Hakizimana *et al.* (2016), who reported resistance of *An. gambiae* from Rwanda to Permethrin and Deltamethrin. Several factors contribute to mosquito resistance to insecticides. Factors such as the type and frequency of insecticides used, anthropogenic and industrial chemical, xenobiotics as well as microbial compositions resident in mosquito breeding site and the upregulation of cuticle proteins (Nkya *et al*., 2013; Atoyebi *et al*., 2020). According to WHO (2016b), if the observed mortality rate is between 90 and 97 %, the presence of resistant genes in the vector population must be confirmed. The confirmation of resistance may be obtained by performing additional bioassay tests using the same insecticide on the same population or on the progeny (F1) of any surviving mosquitoes (reared under insectary conditions) and/ or by conducting molecular assays for known resistance mechanisms. If at least two additional tests consistently showed a mortality rate of below 98 %, then the resistance is confirmed. Therefore, additional tests are needed on *Ae. aegypti* with mortality rate of 96 % (Lambdacyhalothrin), 97 % (Permethrin) and *Cx. quinquefasciatus* 92 % (Lambdacyhalothrin), and 95 % (Alphacypermethrin).

If the mortality rate is less than 90 %, confirmation of the existence of resistant genes in the tested population with additional bioassays may not be necessary, as long as a minimum of 100 mosquitoes of each species was tested (WHO, 2016b). In the present tests, *Cx. quinquefasciatus* and *An. gambiae* showed resistance to Permethrin, as the mortality rates were 61 and 69 %, respectively. *Cx. quinquefasciatus* and *An. gambiae* also showed resistance to Deltamethrin with 72 and 87 % mortalities respectively. *An. gambiae* also showed resistance to Alphacypermethrin with mortality rate of 66 %. Therefore, no further tests may be required for confirmation of the existence of resistant genes in the tested population. However, further investigation of the mechanisms and distribution of resistance should be undertaken as recommended by WHO (2016b).

Conclusion: The study established the occurrence of most commonly used pyrethroid insecticides resistance species of mosquito in Makurdi. It is therefore, recommended that further investigation be carried out to ascertain the mechanisms and distribution of resistance.

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