SUSCEPTIBILITY STATUS OF MOSQUITOES (DIPTERA: CULICIDAE) TO MALATHION IN LAGOS, NIGERIA

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ABSTRACT

Mosquitoes are major vectors of infectious diseases transmitting malaria, lymphatic filariasis, yellow fever, dengue fever, zika and chikungunya virus. Resistance to DDT, pyrethriods and carbamates has been reported to different mosquito species in Nigeria. This investigation was carried out to determine the susceptibility status of mosquitoes in Lagos State, Nigeria to malathion. Mosquito larva were collected from four different Local Government Areas of Lagos, and reared to adult. Female adult mosquitoes were exposed to 5 % malathion insecticide test papers using WHO standard procedures and kits. Species identification was done using PCR assay. Suspected resistance was observed in Cx. quinquefasciatus from Kosofe and Alimosho with 24 hour mortality of 96 % and 95 % respectively. Other mosquito species and Cx. quinquefasciatus from Badagry and Ibeju-Lekki were fully susceptible 24 hours post exposure period. KDT_{50} and KDT_{95} for An. gambiae s.s ranges from 14.6 – 25.1 and 23.7 – 51.5 minutes respectively for all the location, KDT_{50} and KDT_{95} for Ae. aegypti ranges from 24.8 – 27.8 and 44.8 – 62.5 minutes respectively for all the location and KDT_{50} and KDT_{95} for Cx. quinquefasciatus ranges from 21.5 – 37.8 and 41.5 – 77.7 minutes respectively for all the location. The relatively high values of KDT_{50} and KDT_{95} in all assayed mosquito species call for urgent attention and may indicate the gradual development of malathion resistance to different mosquito species in Lagos. Regular insecticide resistance monitoring is needed and the indiscriminate use of unapproved organophosphate insecticides to be discouraged to forestall the development of malathion resistance in mosquitoes.

Keywords: Mosquitoes, Insecticide resistance, Malathion, Organophosphates, Infectious diseases

INTRODUCTION

The most important mosquito vectors of public health importance belong mainly to three genera: *Anopheles, Aedes* and *Culex.* Mosquitoes are vectors of several infectious diseases such as malaria, yellow fever, lymphatic filariasis, dengue fever and several arboviruses (Aigbodion and Uyi, 2013; Okorie *et al.*, 2014; Richards *et al.*, 2017). Chemical insecticide based vector control measures are vital in the management and control of vector-borne diseases (WHO, 2007; 2011; 2017; Prasad *et al.*, 2017). Chemical insecticides are classified as organophosphates, organochlorines, carbamates and recently, pyrethriods. The indiscriminate application of a few approved insecticides for public health usage for both agricultural pests and vector of human and livestock diseases has significantly impacted insecticide resistance making insecticides used ineffective and limiting the available option for disease control (Oduola *et al.,* 2010; Norris and Norris, 2014; Mohammed *et al.,* 2015; Riveron

et al., 2018). Insecticide resistance in mosquitoes can be as a result of one or combination of the following; behavioral changes, physiological modifications, increased activities of detoxifying enzymes and target site mutation (Bharati and Saha, 2018).

Organophosphate insecticides target and block the action of the acetylcholinesterase (AChE1), an enzyme involved in hydrolyzing the neurotransmitter acetylcholine, which prevents the cessation of the neural signal thereby leading to death of the vector by tetany (Bkhache *et al*., 2018). Malathion is one of the twelve insecticides approved by the World Health Organization for Indoor Residual Spraying (IRS) for control of malaria vector (WHO, 2006). There is currently widespread reliance on pyrethroids in mosquito control, and organophosphates remain in use for public health emergencies (Richards *et al*., 2017). The organophosphorous compounds including malathion have been widely used in vector control programme and development of resistance against these compounds has been reported in different mosquito vectors (Mekuria *et al*., 1994; Hidayati *et al*., 2011; Richards *et al*., 2017). In Nigeria, mosquitoes resistance to organochlorine, pyrethriods and carbamates insecticides have been reported (Awolola *et al*., 2007; 2009; Oduola *et al*., 2010; Oyewole *et al*., 2011). Between 1960 and 1961, The World Health Organization in conjunction with the Federal Government of Nigeria, used malathion and other organophosphate insecticides in a trial survey with the objective of evaluating on a village basis new insecticides that might be used as substitutes for DDT, y-BHC and dieldrin in malaria eradication programmes and some level of efficacy was recorded (Elliot and Barnes, 1963). Recently, World Health Organization Pesticide Evaluation Scheme (WHOPES) came out with a statement reinstating the safety of malathion in vector control provided the application and usage guideline are strictly adhere to (WHO, 2016). Few studies in Nigeria have investigated the susceptible of *Anopheles* species to malathion (Umar *et al*., 2014; Djouaka *et al*., 2016; Opara *et al*., 2017) but none was carried out in Lagos State, and also no investigation has been carried out on the susceptibility of *Cx. quinquefasciatus* and *Ae. aegypti* in Lagos State, Nigeria. Therefore the study was designed to provide information on the susceptibility status of *An. gambiae s.s*., *Cx. quinquefasciatus*, and *Aedes aegypti* in Lagos State, Nigeria.

MATERIALS AND METHODS

Study Area: The study was conducted in four Local Government Areas (LGAs) of Lagos State, Nigeria thus: Eti-Osa LGA (6º26'34"N, 3º28'29"E), situated within the southern area of Lagos State, just below the Lagos lagoon, Kosofe LGA situated at 6°45'N, 3°4'E and 35 meters' above sea level, Alimosho LGA (6°36'38"N 3°17'45"E), the largest local government in Lagos with 1,288,714 inhabitants according to NPC (2006) and Badagry LGA (6°25'N 2°53'E); a coastal town located between Metropolitan Lagos, and the border with Republic of Benin at Seme (Figure 1).

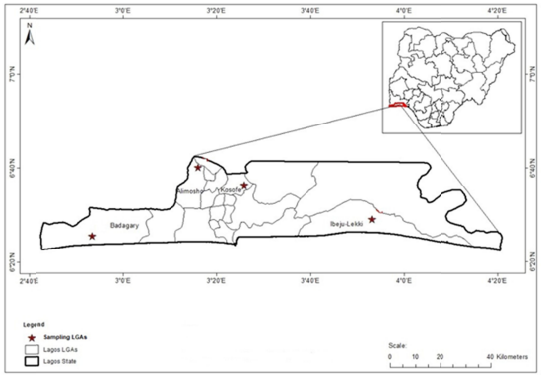

Figure 1: Map of Lagos State showing study areas

Mosquito Larva Collection and Rearing: Larvae of *Anopheles, Culex and Aedes mosquitoes* were collected between the period of July 2017 and August 2018 from various larva habitats in the four studied Local Government Areas of Lagos State, Nigeria. Mosquito samplings were performed around private houses, blocked drainages, water pots, small pools and shallow wells where the residents gave their permission. Larvae and pupae were then transferred to the insectaries and allowed to emerge to adults.

Identification and Care of Emergent Adult Mosquitoes: The immature mosquitos were poured into other plastic containers and each container labelled according to the locality and genera. Emerged adults mosquitoes were collected with the use of an aspirator and then transferred into well labelled cages. All emerged mosquitoes were fed on 10 % sugar solution imbibed in cotton wool. The differentiation of mosquito into various species was done with a dissecting microscope using the morphological identification keys, the various species of mosquitoes were identified using their wings (venation), thoracic and abdominal characteristics (Gillet, 1972; Rueda, 2004).

Insecticides Susceptibility/Resistance Assay: The tests were performed using WHO test filter paper impregnated with the insecticide for measuring insecticide susceptibility and resistance (WHO, 2016a). Non-blood fed, three days old female mosquitoes were exposed to Malathion (5 %) insecticide-treated papers in groups of 25. Each experiment consisted of four replicates. The mosquitoes were exposed for an hour with the assay cylinders in a vertical position. Knock down rates of mosquitoes were recorded at intervals for one hour and the final mortality noted after 24 hours. After the exposure, mosquitoes were transferred into tubes with untreated filter papers and allowed a 24 hours recovery period after which mortality was recorded. All the bioassays were accompanied by negative control tests where mosquitoes were exposed to filter papers treated only with silicone oil for an hour. The mosquitoes were supplied with a 10 % sugar meal during the recovery period (WHO, 2016a).

DNA Extraction: A whole mosquito was homogenized in a 1.5 ml micro-tube using 100 µl of grinding buffer with the aid of a plastic pestle. 20 µl proteinase K was added to the homogenized mosquito, the samples were incubated at 65°C in a dry bath for 30 minutes. Thereafter, the samples were placed on ice for 30 minutes and centrifuged at 14,000 xg for 15 minutes. The supernatants were then transferred to sterile micro-tube (1.5 ml); 200 µl of ice cold 100 % ethanol was added and kept at room temperature for 5 minutes. The mixture was centrifuged for 20 minutes at 14,000 xg and supernatant was carefully discarded. The pellet was washed carefully with 200 µl of 70 % ice cold ethanol, followed by 100 % ice cold ethanol. The tubes were dried using speed-vac. The DNA in the tubes were re-suspended in 100 µl of Tris-EDTA buffer (Collins *et al.*, 1987).

Molecular Identification of *Anopheles gambiae s.s., Culex pipiens* and *Aedes aegypti* Mosquitoes Complex: Molecular identification of mosquito samples was carried out according the method described by Collins *et al.* (1987) for *Anopheles gambiae s.s..* Four primers including, ME (TGACCAACCCACTCCCTT GA), AR (AAGTGTCCTTCTCCATCCTA), QD (CAGA CCAAGATGGTTAGTAT), UN A (GTGTGCCCCTTC CTCGATGT), GA (CTGGTTTGGTCGGCACGTTT) were used, this was done to identify sibling species of the *An. gambiae s.s.* complex.

For *Culex,* three primers, ACEquin (5'-CCTTCTTGAATGGCTGTGGCA-3'), ACEpip (GGAA ACAACGACGTATGTACT-3' and B1246s (5'TGGA GCCTCCTCTTCACGG-3') were used to amplify at 274 bp diagnostic fragment of the entire extracted DNAs according to Smith and Fonseca (2004). This was done to identify two member of *Culex pipiens* complex which are; *Culex quinquefasciatus* and *Culex pipiens*. Gel electrophoresis was used to analyse the amplified fragments using 1.5 % agarose gel and were visualized by ethidium bromite stains under ultra violet light (UV light).

For *Aedes*; three primers AUF, AUR, AEG were amplify at 157 bp diagnostic fragment of the entire extracted DNAs. This was done to identify *Aedes aegypti*. Gel electrophoresis was used to analyse the amplified fragments using 1.5 % agarose gel and were visualized by ethidium bromite stains under UV light.

Data Analyses: Percentage progressive knockdown was computed. Insecticide susceptibility was based on the criteria that 98 – 100 % mortality indicates susceptibility; 80 – 97 % mortality implies potential resistance that needs to be confirmed via biochemical or molecular assays and < 80 % mortality implies resistance (WHO, 2016b). Regression probit was

used to compute the KDT_{50} and KDT_{95}. All data analyses were computed using Microsoft Excel version 2016 and IBM SPSS Statistics 23.

RESULTS

Susceptibility Status of *Anopheles gambiae s.s.*: *An. gambiae* s.s. collected from the four LGAs exposed to malathion were fully susceptible to malathion after 60 minutes of exposure and the 24 hours recovery period as the KDT_{50} ranged from 14.6 to 25.1 minutes and KDT_{95} ranged from 23.7 to 51.5 minutes (Table 1). Mean knockdown per time indicated that as at 30 minutes of exposure over 50 % mortality of *An. gambiae s.s.* was recorded in the locations within Ibeju-Lekki and Kosofe recording over 80 % mortality. 100 % mortality of *An. gambiae s.s.* was recorded in all locations at 60 minutes of exposure (Figure 2).

Susceptibility Status of *Aedes aegypti:* 60 minutes knockdown rates and 24 hour post-exposure mortality showed that *Ae. aegypti* populations collected from all the sampled LGAs were susceptible to malathion. The log-time probit model used to estimate the KDT_{50} and KDT_{95} values showed the highest KDT_{50} value of 27.8 minutes in Badagry LGA and lowest value of 24.8 minutes in Alimosho LGA, while the highest and lowest values of KDT_{95} were 44.8 and 62.5 minutes for Badagry and Kosofe LGAs respectively (Table 1). Percentage knockdown showed that over 50% of *Ae. aegypti* were knockdown at 30 minutes of exposure and over 95 % at 60 minutes of exposure (Figure 3).

Susceptibility Status of *Culex quinquefasciatus:* 24 hours post exposure mortality of *Cx. quinquefasciatus* indicates the possibility of resistance to malathion in Kosofe and Alimosho LGAs with mortality of 96 and 95 % respectively, while full susceptibility was recorded in Badagry and Ibeju-Lekki, KDT_{50} ranges from 21.5 to 37.8 minutes and KDT_{95} from 41.5 to 77.7 minutes (Table 1). Figure 4, shows the percentage knockdown per time of *Cx. quinquefasciatus* exposed to malathion, samples from Badagry and Ibeju-Lekki recorded over 70 % knockdown at 30 minutes of exposure while samples from Kosofe and Alimosho recorded below 30 % mortality, 100 % knockdown was recorded in samples from Badagry and Ibeju-Lekki at 60 minutes of exposure and less than 90 % was recorded in samples from Kosofe and Alimosho.

DISCUSSION

Effective mosquito control entails the regular monitoring of insecticide resistance status of native mosquito species. The objective of this study was to assess the susceptibility status of the three main vectors of mosquito-borne human infectious diseases in Lagos State, Nigeria to malathion. Apart from *Cx. quinquefasciatus* from Alimosho and Kosofe, the rest mosquito species showed full susceptibility to malathion. Previous studies in West Africa have reported the susceptibility of various malaria vectors to organophosphate insecticides; in Northern Nigeria, *An. gambiae*, *An. funestus* and *An. nilli* (Umar *et al*., 2014), *An. gambiae s.l*. in Southsouth Nigeria (Opara *et al*., 2017) and *An. funestus* in Southwestern Nigeria (Djouaka *et al*., 2016). In Benin republic susceptibility of *An. gambiae* to malathion has been reported by (Corbel *et al*., 2007). Susceptibility of *An. gambiae* to malathion in Cameroon has also been reported (Antonio-Nkondjio *et al*., 2016; Boussougou-Sambe *et al*., 2018). The 24 hours percentage mortality recorded for *An. gambiae s.s.* in this study is similar to that previous studies in west Africa (Corbel *et al*., 2007; Umar *et al*., 2014; Boussougou-Sambe *et al*., 2018). Most KDT_{50} and KDT_{95} values recorded in this current study is similar to pervious reports in Nigeria by (Umar *et al*., 2014; Opara *et al*., 2017) though some few higher values were recorded in this study indicating that there might by a gradual tendency toward malathion resistance in *An. gambiae s.s* in Lagos State, malathion resistance to *An. maculipennis* has been reported in Turkey (Akiner, 2014)

Aedes aegypti collected from the studied LGAs in Lagos State for this study showed susceptibility to malathion. There has been a paucity of information to insecticides resistance status of *Aedes* in Nigeria and West Africa.

Table 1: Log-time probit model used to estimate the KDT_{50} and KDT_{95} values and percentage mortality of mosquito exposed to malathion in Lagos State, Nigeria

Location	Mosquito species	Number exposed	KDT_{50} (95% cl)	KDT_{95} (95% cl)	Mortality (%)	Status
Kosofe	*An. gambiae s.s.*	100	18.4 (16.1-21)	26.7 (22.8-39.4)	100	S
	Ae. aegypti	100	25.3 (23.7-26.9)	62.5 (56.1-71.3)	100	S
	Cx. quinquefasciatus	100	36 (29.9-42.8)	67.8 (54-111.8)	96	SR
Alimosho	*An. gambiae s.s.*	100	25.1 (23.8-26.5)	51.5 (47.2-57.2)	100	S
	Ae. aegypti	100	24.8 (23.4-26.4)	56.8(51.5- 64.1)	100	S
	Cx. quinquefasciatus	100	37.8 (33.2-43.4)	77.7 (62.9-113)	95	SR
Ibeju-Lekki	*An. gambiae s.s.*	100	14.6 (12.5-16.7)	23.7 (20.1-34.1)	100	S
	Ae. Aegypti	100	26 (24.6-27.3)	50 (46.1-55)	100	S
	Cx. quinquefasciatus	100	21.5 (20.2-22.7)	45.2 (41.3-50.4)	100	S
Badagry	*An. gambiae s.s.*	100	23.5 (21.2-25.8)	48.7 (42.3-59.4)	100	S
	Ae. Aegypti	100	27.8 (25.4 30.3)	44.8 (40-53)	100	S
	Cx. quinquefasciatus	100	23.3 (22.1- 24.5)	41.5 (38.5-45.4)	100	S

Note: Mortality of >98 % indicates susceptibility, 97 – 90% indicates suspected resistance and <90% resistant (WHO, 2016a); S: susceptible, SR: suspected resistance, R: resistant

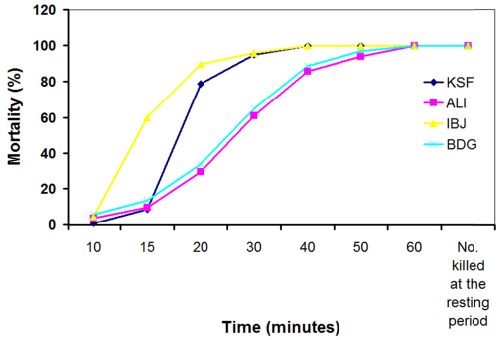

Figure 2: Percentage knockdown per time of *Anopheles gambiae* s.s. exposed to malathion (5%) in Lagos State, Nigeria. Note KSF: Kosofe LGA, ALI: Alimosho LGA, IBJ: Ibeju-Lekki, BDG: Badagry

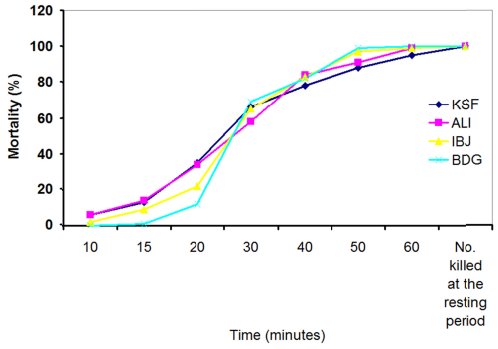

Figure 3: Percentage knockdown per time of *Aedes aegypti* exposed to malathion (5%) in Lagos State, Nigeria. Note KSF: Kosofe LGA, ALI: Alimosho LGA, IBJ: Ibeju-Lekki, BDG: Badagry

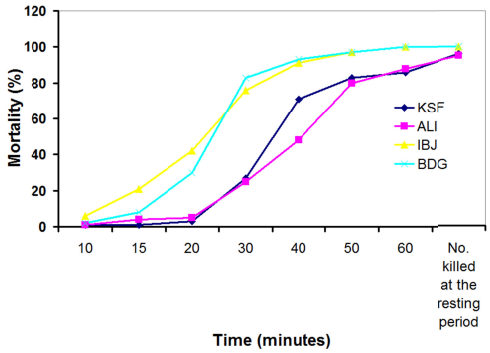

Figure 4: Percentage knockdown per time of *Culex quinquefasciatus* exposed to malathion (5%) in Lagos State, Nigeria. Note KSF: Kosofe LGA, ALI: Alimosho LGA, IBJ: Ibeju-Lekki, BDG: Badagry

The susceptibility status of *Ae. aegypti* to DDT, permethrin and deltamethrin was assessed by (Ayorinde *et al.*, 2015) in Lagos, Nigeria. Similar to result from this study, malathion susceptibility has been reported in *Ae. aegypti* in Yaounde, Cameroon (Kamgang *et al.*, 2017). Conversely, malathion resistance has been reported to Ae aegypti in Kuala Lumpur, Malaysia (Ishak *et al.*, 2015), West Bengal, India (Bharati and Saha, 2018), *Ae. albopictus* in different states in USA (Richards *et al.*, 2017) and different *Aedes* species in South Carolina, USA (Mekuria *et al.*, 1994).

After 24 hours post exposure period full susceptibility was recorded from *Cx. quinquefasciatus* from Badagry and Ibeju-Lekki

LGAs of Lagos State, but possible resistance to malathion was recorded in Kosofe and Alimosho LGAs with mortality of 96 and 95 % respectively. This result is similar to the report from five different site in Benin Republic (Corbel *et al.,* 2007). *Cx. quinquefasciatus* susceptibility to malathion has been reported in Macha, Zambia after 24 hours exposure period(Norris and Norris, 2014). High level of malathion resistance has reported in *Cx. pipiens* in Morocco (Bkhache *et al.*, 2018), *Cx. quinquefasciatus* larva in West Indies (Delannay *et al.*, 2018), *Cx. pipiens* larva in Western Iran (Ghorbani *et al.*, 2018). Relative high KDT_{50} and KDT_{95} ranging from 21.5 – 37.8 minutes and 41.5 – 77.7 minutes was recorded in the surveyed locations. The indiscriminate domestic use of dichlorvos or 2,2-dichlorovinyl dimethyl phosphate (DDVP) an organophosphate insecticide for the control of mosquito and other household pests (Ogwuche and Aginde, 2014; Eze *et al.*, 2018), could has could have contributed to the possible resistance found in *Cx. quinquefasciatus* in Kosofe an Alimosho LGAs of Lagos, this could also have accounted for the high KDT_{50} and KDT_{95} recorded in all the mosquito species in this current study.

Conclusion: This may be the first study assessing the susceptibility status of *Ae. aegypti* and *Cx. quinquefasciatus* in Lagos State to malathion. The suspected resistance in *Cx. quinquefasciatus* from the two urban LGAs and the relatively high values of KDT_{50} and KDT_{95} in all assayed mosquito species calls for urgent attention. The widespread use of DDVP for domestic purpose should be discouraged as it may not only cause deleterious effect on human health but lead to the insecticide resistance in other organophosphate insecticides approved by WHO for use in public health. Further research into malathion resistance development and resistance mechanism should be carried out in the study area.

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