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Insecticide susceptibility status of *Culex quinquefasciatus* [Diptera: Culicidae] in Umudike, Ikwuano LGA Abia State, Nigeria

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Abstract
Larval and pupal stages of *Culex quinquefasciatus* mosquito were collected from different points within Umudike, Abia State, Nigeria, and reared to adulthood in the laboratory. The adults that emerged were tested on 4% DDT (Organochlorines); 0.1% bendiocarb (Carbamates); 0.25% Primiphos-methyl (Organophosphates); and 0.05% Deltamethrin (Pyrethroids) procured from National Arbovirus and Vector Research Institute Enugu. Twenty sugar fed female *Culex quinquefasciatus* mosquitoes aged 3-5days were used for the bioassay which was replicated four times with two control. Knockdown was recorded at five minutes, and then 10 minutes interval for 1 hour and then maintained for 24hrs post-exposure on 7% sugar solution, after which a final mortality was recorded. The Knockdown times (KDT_{50} and KDT_{90}) were determined by Probit analysis. *Culex quinquefasciatus* was resistant to all the insecticides, with 24hours post exposure percentage mortalities of 10.48, 39.20, 0.00, and 10.15 in DDT, bendiocarb, primiphos-methyl and deltamethrin respectively.

Keywords: Abia State, *Culex quinquefasciatus*, Insecticide, Nigeria, Susceptibility, Umudike

1. Introduction

Mosquitoes can be found everywhere all over the world, except at the polar regions and altitudes beyond 2000 meters. Out of about 3,500 mosquito species, 100 species are capable of transmitting diseases to humans including malaria, dengue fever, chikungunya fever, yellow fever, filariasis, Japanese encephalitis, rift valley fever, and other viral encephalitis (Kalaivani, *et al.*, 2015) [16], and of recent Zika virus. Several mosquito species belonging to the genera *Anopheles*, *Culex* and *Aedes* are vectors of these diseases (ICMR, 2003) [14].

Culex quinquefaciatus is the vector for lymphatic filariasis and some other viral encephalitis. In the *Culex* species of mosquitoes, the siphon of the larvae is often long and narrow, but it could be short and fat (Young *et al.*, 2008) [34]. There is always more than one pair of subventral tufts of hairs on the siphon, none of which is near its base (Young *et al.*, 2008) [34]. In these species the adult frequently, but not always, have the thorax, legs and wing veins covered with somber-coloured, often brown, scales. The abdomen is often covered with brown or blackish scales, although some whitish scales may occur on most segments. The adults are recognized more by their lack of ornamentation than any striking diagnostic characters. The tip of the abdomen of females is blunt. The claws of all tarsi are simple and those of the hind tarsi are very small. Microscopic examination normally shows that all tarsi have a pair of small fleshy pulvilli (Young *et al.*, 2008) [34].

World Health day Theme for 2014 was "Vector-borne diseases small creature, big threat." This implies the importance of mosquito-borne diseases and its control worldwide (WHO, 2014) [33]. In spite of innovations and revolutions in diagnostics, drug therapy, and vector control measures, the global burden of vector-borne diseases especially mosquito-borne diseases remains unacceptably high.

Vector control is a major component of the World Health Organisation (WHO) global mosquito-borne diseases intervention strategy, and it focuses primarily on indoor residual spraying and the use of Insecticide Treated Nets (ITNs). Today these control measures have setbacks, which includes insecticide resistance as well as impediments in achieving high coverage (Killeen *et al.*, 2002) [18].

Mosquitoes have developed resistance to almost all insecticides in many parts of the world, while rapid urbanization, unplanned cities and industrialization are further threatening to increase mosquito population.

There are a lot of reports on the susceptibilty status of *Culex* mosquitoes to organophosphates and carbamate insecticides in many regions of the world. The report Bisset *et al*. (1994) [7] shows that *Cx. quinquefasciatus* has proved resistant to malathion and carbamate inscecticides in the Eastern, Central and Western part of Cuba. Similarly, in Brazil, Bracco *et al*. (1997) [8] has shown that the mosquito is resistant to malathion, fenitrothion and carbamate. In the same vein Nazni *et al.* (2005) [20] examined the susceptibility of adult *Culex* mosquitoes to some insecticidal papers in Kuala Lumpur, Malaysia.

In Nigeria, the susceptibility status of many mosquitoes in different zones to DDT and other classes of insecticides including organochlorine, organophosphate, carbamates and recently pyrethroid has been well documented (Awolola *et al*., 2005, 2007) [4, 5]. In South-west Nigeria, the first case of pyrethroid resistance in *Anopheles gambiae*, the major malaria vector, in Nigeria was documented by (Awolola *et al*., 2002) [2] and since then the phenomenon has been well established in this region (Awolola *et al*., 2003; Kristan *et al*., 2003; Awolola *et al*., 2005, 2007; Oduola *et al*., 2010, 2012) [3, 19, 4, 5, 23, 24]. In North-central Nigeria, permethrin and DDT resistance in *An. gambiae s.l* has been reported (Ndams *et al*., 2006; Olayemi *et al*., 2011) [21, 25].

The successful implementation of IRS program partly depends on availability of information on insecticides susceptibility of mosquitoes in the local environment. It is therefore imperative to periodically conduct bioassay tests to assess the susceptibility status of local mosquito species to IRS interventional insecticides.

The susceptibility of mosquitoes against insecticides has to a large extent been evaluated in the south western part of Nigeria (Olayemi *et al.*, 2011; Oduola *et al*., 2012) [25, 24]. Also resistance to the four classes of insecticides has been found previously in *An. gambiae s.l.* in southwest Nigeria (Kristan *et al.*, 2003; Awolola *et al.*, 2005, 2007; Djouaka *et al.*, 2008; Oduola *et al.*, 2010) [19, 4, 5, 9, 23]. In the Northern part of Nigeria, Ndams *et al*., 2006 [21] and Umar *et al.*, 2014 [30] have evaluated the susceptibility of various mosquito species to different insecticides, but there is dearth of information in the South-east of Nigeria. Apart from the little work in Enugu, and Ebonyi states, no documented evidence on the susceptibility status of mosquitoes to guide the procurement of IRS insecticides in the South-eastern part of Nigeria is available. Hence this study has been conducted to provide baseline data on the insecticide susceptibility status of *Culex quinquefasciatus* in Umudike, Abia State, Nigeria. It is hoped also that findings from this study will promote and improve effective vector control decision making.

2. Materials and methods

2.1 Study Area

The study was carried out in Umudike, Ikwuano L.G.A. of Abia State, South-eastern Nigeria. Ikwuano, is located in the tropical rain forest zone of Nigeria (Latitude 05°26'-5°29'N and Longitude 07°34'-7°36'E). It has a mean annual rainfall of 2238 mm, minimum and maximum temperatures of 23 and 32 °C, respectively, with a relative humidity range of 63-80% (NRCRI, 2003) [22].

Umudike is situated in Abia Central Senatorial district and is host to National Root Crops Research Institute, and Michael Okpara University of Agriculture both of which utilize agricultural pesticides.

2.2 Mosquito Larval Collection and Rearing

Immature stages of *Culex* mosquitoes (larvae and pupae) were collected from various natural breeding sites including ground pools, gutters, tyre tracks and puddles within Umudike from January to July. Water was scooped using a plastic scoop and poured into small transparent plastic bowls. A strainer was used to sieve and pool together the third and fourth instar larvae in order to have sufficient adult emergence of the same physiological age. The bowls were scrutinized for presence of unwanted organisms or predators and a pipette was used to remove any that was found. The *Culex* mosquito larvae collected were transported in well labelled plastic bottles to the insectaria in the Entomology unit of the National Arbovirus and Vector Research Institute, Enugu, where they were maintained and reared at 26±3 °C and 74±4% relative humidity to adult stage for World Health Organisation (WHO) bioassays. Larvae were fed on ground biscuits and adults were provided with 10% sugar solution. The resulting adults were identified according to the morphological keys of Gillies and Coetzee (1987) [12]. All bioassays were performed on adult females aged 3–5 days (WHO, 1998) [31].

2.3 Insecticide Susceptibility Test

Insecticide susceptibility tests were carried out using the WHO standard procedures and test kits for adult mosquitoes (WHO, 1998) [31]. Four types of WHO bioassay test papers impregnated with recommended diagnostic concentrations of 4% DDT (Organochlorines); 0.05% Deltamethrin (Pyrethroids); 0.1% bendiocarb (Carbamates); and 0.25% Primiphos-methyl (Organophosphates) procured from National Arbovirus and Vector Research Institute, Enugu, were used for the bioassay. Tests were carried out using 3-5 day old, sugar-fed female *Culex quinquefaciatus* mosquitoes. A maximum of 100 female mosquitoes in four replicates were tested for each insecticide. Accordingly, 4% DDT (Organochlorines); 0.05% Deltamethrin (Pyrethroids); 0.1% bendiocarb (Carbamates); and 0.25% Primiphos-methyl (Organophosphates) impregnated paper strips were each introduced into 4 exposure tubes and rolled to line with the wall of the tube and fastened into position by a wire clip for each of the insecticides, while one control was lined with plain sheet of paper. A pre-test was performed by carefully introducing 20 female *C.quinquefaciatus* mosquitoes into the four holding tubes with an aspirator and allowed to stand for 1hour. Thereafter, the mosquitoes were transferred into the exposure tubes through a hole on the lid that separates the holding tube and the exposure tube. The exposure tubes were then set upright with the screen-end up and allowed to stand for 1 hour. Records of mortalities were taken at intervals of 0, 15, 20, 30, 40, 50 and 60 minutes. The mosquitoes were then carefully transferred back to the holding (recovery) tubes and kept for 24 hours during which they were fed with 7% sucrose solution. Records of final mortality were taken after 24 hours and the susceptibility status of the population was graded according to WHO recommended protocol (WHO, 2013) [32]. Dead and survived mosquitoes from this bioassay were separately kept in clearly labelled 1.5 ml Eppendorf tubes containing silica gel, for preservation. All susceptibility tests were carried out at 26±3°C temperature and 74±4% relative humidity.

2.4 Data Interpretation and Analysis

The 24 hours percentage mortality of each insecticide was calculated as the proportion of mosquitoes that died after 24 hours and the total number of mosquitoes exposed using 95% confidence intervals. Mortality rate in the control tubes were not above 5%, and hence were not corrected using Abbott formula (Abott, 1987) [1]. The resistance status of the *Culex quiquefasciatus* mosquito samples were determined according to WHO criteria (WHO, 2013) [32]. Mortality rates of less than 80% indicated full resistance while those greater than 98% indicated full susceptibility. Mortality rates between 80-98% suggested the possibility of resistance that needs to be clarified. The Knock down data was subjected to probit analysis using statistical software (Statsdirect, 2013) [28] to compute the KDT_{50} and KDT_{90} (Time taken to knock down 50% and 90% of the exposed mosquitoes) and their 95% confidence intervals. Analysis of Variance (ANOVA) was also used to compare the mortalities across the insecticides and Least Significant Difference (LSD) was used to separate the means.

3. Results and Discussions

The KDT_{50} and KDT_{90} results of female *Culex quinquefasciatus* mosquitoes exposed to DDT (organochlorine), bendiocarb (carbamate), primiphosmethyl (organophosphate), and deltamethrin (pyrethroid), as well as the percentage mortality recorded after 24 hours exposure period are presented in Table 1. The highest KDT_{50} was recorded in bendiocarb (50 mins.), followed by deltamethrin (40 mins.), and then primiphosmethyl and DDT recorded 30 minutes each. There was no mortality 24 hours after exposure of the *Culex* mosquitoes to primiphosmethyl, while there was the greatest mortality of 39.20% for bendiocarb, followed by DDT (10.48%) and then deltamethrin (10.15%). Comparatively the percentage mortalities of *Culex quinquefasciatus* across DDT, primiphosmethyl and deltamethrin did not differ significantly (P>0.05), but differed significantly (P<0.05) between them and bendiocarb.

Table 1: Knockdown assessment and percentage mortality 24 hours after exposure of *Culex quinquefasciatus* mosquitoes exposed to four insecticides

Insecticides	Mortality after 24 hours (%)	KDT_{50} (Mins)	KDT_{90} (Mins)
DDT (Organochlorine)	10.48^{b}	30^{b}	60^{a}
Bendiocarb (Carbamate)	39.20^{a}	50^{a}	60^{a}
Primiphosmethyl (Organophosphate)	0.00^{b}	30^{b}	60^{a}
Deltamethrin (Pyrethroid)	10.15^{b}	40^{c}	60^{a}

Figures with same letters in columns are not significantly different (P>0.05)

Table 2.below shows the resistance status of the mosquito to the different insecticides, based on the WHO (2013) [32] grading of resistance/susceptibility to inseciticides.

Table 2: Susceptibility status* of *Culex quinquefasciatus* exposed to four different insecticides

Insecticides	Class	*Culex quinquefasciatus*
DDT	Organochlorine	Resistant
Bendiocarb	Carbamate	Resistant
Primiphosmethyl	Organophosphate	Resistant
Deltamethrin	Pyrethroid	Resistant

*WHO scoring for resistance (WHO, 2013) [32]

The present study presents for the first time, baseline data on the susceptibility status of *Culex quinquefaciatus* to World Health Organization Pesticide Evaluation Scheme (WHOPES) approved indoor residual spray (IRS) insecticides in Umudike, Abia State, Southeastern Nigeria to guide procurement of IRS in the State.

During the knockdown assessment for *C. quinquefasciatus,* the highest KDT_{50} at 50 minutes was recorded in bendiocarb, although it caused the greatest mortality 24hours after exposure (Table 1). This was followed by deltamethrin (40 mins), with 30 mins recorded for DDT and primiphosmethyl each (Table 1). There was no significant difference (P>0.05) between the KDT_{50} value of DDT and primiphosmethyl. But there was significant difference between them and bendiocarb, as well as between them and deltamethrin. There was also significant difference (P<0.05) between the KDT_{50} value of bendiocarb and that of deltamethirin. For the KDT_{90} values, they were all the same for the four insecticides (60 mins), hence they were not significantly different (P>0.05).

The results of the knockdown assessment showed that the tested insecticidal papers induced knockdown of the adult *C. quinqufasciatus* mosquito, suggesting that knockdown mechanisms could be operating in the local mosquito populations of Umudike. This confirms earlier studies which indicates the knockdown effects of impregnated papers against mosquitoes in Nigeria (Awolola *et al*., 2005; 2007: Oduola *et al*., 2010; Olayemi *et al*., 2011; Ibrahim *et al*., 2014; Umar *et al*., 2014) [4, 5, 23, 25, 13, 30]. The knockdown of the mosquitoes exposed to insecticidal papers indicates the presence of knock down resistance (KDR) mechanism (Kristan *et al.*, 2003; Awolola *et al*., 2007; Ibrahim *et al.*,2014; Umar *et al.*, 2014) [19, 5, 13, 30] operating in populations of *C. quinquefasciatus* mosquitoes at Umudike. This could have been responsible for the level of resistance displayed by these mosquitoes to the various insecticides evaluated.

Using the WHO (2013) [32] criteria for insecticides susceptibility or resistance assessment of mosquitoes, the 24hour post exposure results indicate that *C. quinquefasciatus* was resistant to DDT (10.48%), bendiocarb (39.20%), primiphosmethyl (0.00%) and deltamethrin (10.15%) (Table 1 and 2). This is in agreement with other researchers who have lamented on the growing resistance of many mosquito species to DDT (Sunaiyana *et al.*, 2006) [29]. This is also in agreement with other documented evidence of *Culex* mosquitoes resistance to DDT (Somboon *et al*., 2003; Nazni *et al.*, 2005; Sunaiyana *et al.*, 2006) [27, 20, 29]; bendiocarb (Bracco *et al*., 1997[8]; Nazni *et al*., 2005[20]) [8, 20]; primiphosmethyl (Bisset *et al*., 1994[7]; Bracco *et al*., 1997[8]; Nazni *et al*., 2005[20]) [7, 8, 20]; and deltamethrin (Nazni *et al.*, 2005) [20]. Nazni *et al.* (2005)

[20] examined the susceptibility of adult *Culex* mosquitoes to some insecticidal papers in Kuala Lumpur, Malaysia. In the field strains from Ampang Hill and Pantai Dalam, cyfluthrin was the most effective among all the insecticides. Field strain of *C. quinquefasciatus* showed 0% mortality against WHO 4% malathion and 5% DDT discriminating dosage. This indicates that this species is highly resistant to malathion and DDT. The resistance ratio of the insecticides in descending order was DDT > malathion >fenitrothion > propoxur >permethrin > lambdacyhalothrin >cyfluthrin (Nazni *et al.,* 2005) [20]. Sunaiyana *et al.,* (2006) [29] in their own work in Baan Suan community, Nonthaburi province, Thailand, discovered that adults of *C. quinquefasciatus* from Baan Suan community were highly resistant to DDT, deltamethrin, fenitrothion and permethrin with the percentage mortality of 0%, 11.0%, 21.2%and 10.1%, respectively.

Culex mosquitoes are mostly outdoor feeders and breeders (Ndams *et al.,* 2006) [21]. They also breed in very dirty aquatic environments (gutters, very dirty water pools) where they stand the risk of being exposed to agricultural insecticidal runoffs. All these would have exerted enough pressure for the development of resistance in *Culex*. There was no significant difference (P>0.05) in the effects of DDT, primiphosmethyl and deltamethrin on the *Culex* mosquitoes, while there was significant difference between their effects and that of bendiocarb (P<0.05) (Table 1).

The multiple resistances of *C. quinquefasciatus* mosquitoes in Umudike to the tested pyrethroid, organophosphate and organochlorine insecticides may have grave implications for the mosquito control programmes. It may compromise the efficacy of interventions and potentially lead to the failure of IRS and ITNs based vector control (Awolola *et al*., 2008; Umar *et al*., 2014) [6, 30]. Some of these insecticides would have been used in IRS hence leading to resistance. It is established that prior exposure of mosquitoes to insecticides may induce selection pressure (Kerah-Hinzoumbe *et al*., 2008) [17]. Pyrethroids-based aerosols and coils are used for control of mosquitoes and domestic pests and it might contribute to the development of resistance as reported elsewhere (Kristan *et al*., 2003) [19]. The farmers in the community also use deltamethrin for agricultural crop protection. Previous researchers have reported that exposure of mosquitoes to crop protection insecticides could result to development of insecticide resistance (Etang *et al*., 2003; Awolola *et al*., 2007; Philbert *et al*., 2014) [11, 5, 26]. LLINs have been in use for protection against mosquitoes for a long time in Umudike, and this may induce selections to pyrethroid insecticides. Previous studies revealed that the use of LLINs could result in development of insecticides resistance in *Anopheles* mosquitoes (Kabula *et al.,* 2011; Umar *et al*., 2014) [15, 30].

4. Conclusion

The resistance to bendiocarb recorded in *Culex* in this study raises a special concern for National mosquito Control programs which, because of high resistance to pyrethroids and DDT, are currently introducing bendiocarb based IRS for mosquito control in West African countries (Djouaka *et al*., 2011) [10]. Therefore in the search of National mosquito control programs for a suitable replacement to pyrethroid for IRS as part of an insecticide management strategy, all of these four insecticides may not be the right insecticide for *Culex* control in Umudike, South east Nigeria. It is hence necessary that the mechanism behind this resistance displayed by *Culex. quinquefasciatus* mosquitoes in Umudike be investigated. Routine surveillance of insecticide susceptibility/resistance in wild mosquito population is also advocated in line with integrated vector control strategy in Umudike.

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