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The Insecticide Susceptibility Status of *Aedes aegypti* (Diptera: Culicidae) in Farm and Nonfarm Sites of Lagos State, Nigeria

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ABSTRACT. Nigeria is one of the malaria-endemic countries. In Lagos State, Nigeria, various malaria vector control programs including the use of chemical insecticides are currently being implemented. This study was designed to provide information on the susceptibility status of some nontargeted vectors such as *Aedes aegypti*. Adult *Ae. aegypti* mosquitoes from two farm sites and a nonfarm site were exposed to World Health Organization test papers impregnated with Deltamethrin (0.05%), Permethrin (0.75%), and DDT (4%) insecticides. The Knockdown time (KdT_{50} and KdT_{95}) and percentage mortality after 24 h post exposure were determined. In all the exposed mosquito populations to permethrin, mortality rate $> 98\%$ (susceptibility) was recorded, whereas mortality rates $< 95.8\%$ (resistance) and $> 98\%$ (susceptibility) to deltamethrin were observed in the nonfarm site and farm sites mosquito populations, respectively. All the mosquito populations were resistant to DDT in 2 yr. The KdT_{50} of the populations to DDT increased (60.2–69.6) in one of the farm sites and the nonfarm site (68.9–199.96), while a decrease (243–63.4) in another farm site in 2 yr. Significant difference ($P < 0.05$) in KdT_{50} was recorded between the farm and nonfarm sites *Ae. aegypti* mosquitoes in the second year after exposure to deltamethrin and DDT. An increase in KdT_{95} after exposure to deltamethrin in the first year was recorded. Higher KdT values and lower mortality rates in *Ae. aegypti* populations in the nonfarm sites are indications there are existing factors selecting for insecticide resistance outside agricultural use of insecticides.

Key Words: *Aedes aegypti*, Deltamethrin, Permethrin, DDT, insecticides Lagos

Aedes aegypti is the principal urban vector of arboviruses such as yellow fever, Dengue fever, and Chikungunya fever viruses (Gubler 2004). According to World Health Organization (WHO) (2007, 2010), one of the countries with the highest risk of yellow fever is Nigeria. Furthermore, there is a major epidemic threat in Lagos, one of the cities in Nigeria, where about one-third of the population is at risk as a result of urbanization (WHO 2010). There has been reports of isolation of arboviruses such as Chikungunya, yellow fever, West Nile from pools of *Ae. aegypti*, and mammals in Lagos (Boorman and Draper 1968; Olaleye et al. 1989). Immunizing the urban populations in this high-risk country would require vaccinating ∼100 million people (WHO 2015).

The use of insecticides in the control of the vector that transmit these arboviruses is one of the preventive measures against the disease scourge (Jirakanjanakit et al. 2007). Four classes of insecticides which include organophosphates, carbamates, organochlorines, and pyrethroids are widely employed in this vector control programs (Marcombe et al. 2009; WHO 2009). Some organophosphorous compounds such as malathion, fenitrothion, and pirimiphos methyl were used excessively in the past for the control of various vectors, but recently replaced with the pyrethroids (WHO 2001, 2009). Pyrethroids such as deltamethrin, cypermethrin, and permethrin are being used to control adult *Aedes* mosquitoes (Jirakanjanakit et al. 2007; Macoris et al. 2014). In 2006, the World Health Organization after about 30 yr of phasing out the use of DDT, again recommended its use for indoor residual spraying (IRS) in epidemic areas and in areas with constant and high malaria transmission (WHO 2008a,b).

Ae. aegypti has been reported to exhibit resistance to various insecticides (WHO 1989; Koou 2014). This situation has created some problems in vector control programs in many countries (Jamal et al. 2011).

In Africa, the use of insecticides to control agricultural insect pests is important in areas of intense agriculture (Overgaard 2006). According to WHO (1986), ∼90% of all insecticides are used for agricultural purposes. However, there are various disease vectors present in agricultural-ecological zones, which are likely to be exposed to chemicals used for controlling agricultural pests (WHO 1996). Thus, the use of these agrochemicals can contribute to the emergence of vector resistance to insecticides (Chapin and Wasserstrom 1981). Therefore, research on insecticide resistance in disease vectors should not only focus on insecticides resistance from their use in public health but also on the role agriculture plays in insecticide resistance development (Overgaard 2006). A study by Marcombe et al. (2012) reported no correlation between deltamethrin resistance and agriculture, but a correlation between agriculture and organophosphate resistance. In Africa, the role of agricultural practices in *Anopheles species* resistance to DDT and pyrethroid has been reported (Diabate 2002; Akogbeto 2005). The pyrethroids and DDT are widely used in Lagos State among subsistence farmers and rural populace [Pesticides Action Network (Pan) 2007]. Also the scaling up of IRS and Insecticide Treated Nets (ITNs) for malaria control in several African countries including Nigeria, has resulted in resistance to pyrethroids by malaria vectors (Awolola et al. 2005; Oduola et al. 2010; Ibrahim et al. 2013). In Nigeria, various studies have reported resistance of *Anopheles* species to some insecticides, including the pyrethroids and DDT (Awolola et al. 2005; Oduola et al. 2010); however, only a few studies have investigated the insecticide susceptibility status of *Ae. aegypti* to permethrin and none to DDT (Ndam et al. 2006; Kemabonta et al. 2013). In view of this, this study was designed to provide information on the insecticide susceptibility status of *Ae. aegypti* to some pyrethroids and DDT in some farm sites and a nonfarm site in Lagos State, Nigeria.

Table 1. Exposure of *Aedes aegypti* mosquitoes to Deltamethrin (0.05%) from 2011 to 2012

Site	% Mortality (sample size) 2011	% Mortality (sample size) 2012	Susceptible status	KdT_{50} 2011	KdT_{50} 2012	KdT_{95} 2011	KdT_{95} 2012
Badagry (farm site)	100 (80)	100 (100)	Susceptible	10.8 (9.5–11.8)	12.5 (11.1–13.7)	18.3 (16.6-21.6)	20.5 (18.9–22.8)
Ikorodu (farm site)	98.7 (80)	100 (100)	Susceptible	8.8 (5.5–11.1)	13.4 (10.7–15.8)	20.4 (17.9-24.1)	28.8 (24.7–34.6)
Ebute Metta (nonfarm site)	95.8 (80)	91.7 (100)	Tolerance	13.2 (10.2–15.8)	15.6 (14.0–17.2)	38.8 (33.8-46.6)	34.4 (31.8–38.5)

Materials and Method

Study Area. The study was carried out in two farm sites and one non-farm site from three local government areas in Lagos State. Lagos State is situated in South-western Nigeria. It is Nigeria's largest city, principal economic, industrial, and cultural centre; it is also Nigeria's chief seaport. There are six farm settlements in three of the 20 Local government areas in Lagos. The two farm sites studied are Odogunyan farm settlement in Ikorodu local government area (03° 53′ E, 06° 37′ N) and Ajara farm settlement in Badagry local government area (3° 13′ E, 6° 27′ N). Larvicidal application and IRS which are part of Lagos State roll-back malaria program that started in 2010 took place in Badagry and Ikorodu Local government areas. The nonfarm site is Ebute Metta situated in Lagos Mainland local government area (3° 38′ E, 6° 48′ N). Ebute Metta is one of the towns where distribution of long lasting insecticide nets started in 2010 (Lagos State Ministry of Health 2012).

Mosquito Larval Collection and Rearing. The larvae and pupae of *Ae. aegypti* were collected from tyres and other containers in the farm and nonfarm settlements from July to September, 2011 and July to September 2012 using standard methods (O'Malley 1995). The larvae and pupae were collected from the containers using a dipper and transferred to a plastic bottle using a wide mouthed pipette. They were transported to the insectary of the Molecular Entomology and Vector control laboratory of the Nigerian Institute of Medical Research, Yaba, Lagos State and reared to adult stage at $28 \pm 2°C$ and $72 \pm 5\%$ relative humidity (RH).

Mosquito Identification. The mosquitoes exposed in the tests were examined under the dissecting microscope (Olympus SZ 40). They were further identified to species level using morphological (pictorial) keys for identification by Rueda (2004).

Insecticide Susceptibility Tests. Insecticide susceptibility tests were performed using the WHO standard procedures and test kits for adult mosquitoes (WHO 1998). Tests were carried out using 2–5-d-old, nonblood fed adult female *Ae. aegypti* mosquitoes. For each test, 3–4 replicates of 20–25 females were exposed to DDT (4%), deltamethrin (0.05%), and permethrin (0.75%) insecticide-impregnated test papers in the test tubes for 1 h. The number of mosquitoes knockdown after 5, 10, 15, 20, 25, 30, 40, 50, and 60 min was recorded. Mosquitoes were then transferred into holding tubes and supplied with a 10% sugar solution and kept at 27–28°C and 70–80% RH. Mortality was recorded 24 h after exposure. Controls were also set up by exposing a group of mosquitoes to untreated papers. The tests results were discarded if mortality in the control group was over 20% but corrected using Abbort's formula (Abbott 1987) if mortality was between 5 and 20%. Percentage mortality after 24 h post exposure of the mosquito populations was also determined for each insecticide. Exposure to DDT and Deltamethrin was carried out in 2011 and 2012 while exposure to permethrin was carried out in 2012.

Interpretation of Data and Statistical Analysis. Percentage knockdown and percentage mortality to the three insecticides for the mosquitoes from each of the study locality were determined. The resistance/susceptibility status of the mosquitoes were evaluated using WHO (1998). Mortality rates <80% at 24 h post exposure indicated resistance, >97% indicated susceptibility and mortality rates between 80 and 97% indicated that resistance is suspected. For the probit analysis, knockdown times for 50% and 95% of the test population were estimated by the log time probit model using the Microsoft Excel Software

Table 2. Exposure of *Aedes aegypti* mosquitoes to Permethrin (0.75%) in 2012

Site	% Mortality (sample size)	Susceptibility status	KdT_{50}	KdT_{95}
Badagry	100 (80)	Susceptible	15.05 (12.9–17.1)	33.0 (29.4–37.9)
Ikorodu	100 (80)	Susceptible	13.2 (11.2–14.8)	23.4 (21.3–26.3)
Ebute Metta	100 (80)	Susceptible	15.6 (9.4–21.9)	43.2 (30.8–71.2)

2007. Unpaired sample *t*-test was also used to determine the significant difference in mortality rates between each of the insecticides used by using the SPSS software (version 15.0).

Result

Exposure to Deltamethrin. Populations of *Ae. aegypti* from Badagry and Ikorodu farm sites were susceptible to deltamethrin with mortality rate between 98 and 100% after 24 h recovery period in 2011 and 2012. Tolerance to deltamethrin was observed in *Ae. aegypti* population from the nonfarm site with mortality rate between 80 and 97% after 24 h recovery period in 2011 and 2012. The log-time probit model used to estimate the KDT_{50} & KDT_{95} values (percentage knockdown with time) for deltamethrin at the farm and nonfarm sites indicated values were within the mandatory 60 min recommended by the World Health Organization. Mortality rate of the populations from the farm site compared with the population from the nonfarm site to deltamethrin was not significant ($P > 0.05$) in 2011 and 2012. The highest KdT_{50}, 3.2 (10.2–15.8) and KdT_{95}, 38.8 (33.8–46.6) values were recorded at the nonfarm site in 2011. In 2012, the same trend was observed. The highest KdT_{50}, 15.6 (14.0–17.2) and KdT_{95}, 34.4 (31.8–38.5) were also observed at the nonfarm site (Table 1).

Exposure to Permethrin (0.75%). A 24-h post exposure mortality rate of 100% indicating susceptibility to permethrin was observed in all the farm and nonfarm sites populations (Table 2). The log-time probit model used to estimate the KDT_{50} & KDT_{95} values (percentage knockdown with time) for permethrin at the farm and nonfarm sites populations indicated values were within the mandatory 60 min recommended by the World Health Organization. The highest time to knockdown 50% (15.6 min) and 95% (43.2 min) of the populations was observed at the nonfarm site. A significant difference ($P < 0.05$) in KdT_{95} values between the farm and nonfarm sites populations was recorded.

Exposure to DDT (4%). In the first year, populations of *Ae* aegypti from the farm sites were tolerant to DDT with mortality rate between 80 and 97% after 24 h recovery period. The populations from the nonfarm site were resistant, mortality <80%. In the second year of exposure, populations of *Ae. aegypti* from Ikorodu farm site were still tolerant to DDT with mortality between 80 and 97%. However, decrease in mortality was recorded in populations from Badagry farm site (status changed to resistance) and the nonfarm site (status remained as resistance), with mortality <80% indicating resistance.

When mortality of the farm site population (Badagry) was compared with the nonfarm site population, a significant difference ($P < 0.05$) was recorded in 2012.

Table 3. Exposure of *Aedes aegypti* mosquitoes to DDT (4%) in 2011 and 2012

Sites	% Mortality (sample size) 2011	% Mortality (sample size) 2012	Status	KdT_{50} 2011	KdT_{50} 2012	KdT_{95} 2011	KdT_{95} 2012
Badagry	81.44 (80)	54 (100)	T/R	60.2 (49.7–77.4)	69.6 (62.9–81.2)	306.5 (168.0–1888.0)	185.4 (140.3–290.9)
Ikorodu	83.75 (80)	85.1% (100)	T/T	243.0	63.4 (59.7–68.9)	1091.0	111.1 (95.3–142.7)
Ebute Metta	65.5 (80)	23.5 (100)	R/R	68.9 (60.9–105.1)	199.9	167.0 (108.0–1089.0)	796.4

The log-time probit model used to estimate the KdT_{50} (percentage knockdown with time) for DDT at one of the farm site (Badagry) and the nonfarm site populations indicated values were within the mandatory 60 min recommended by the World Health Organization in the first year. However, the log-time probit model used to estimate the KdT_{50} & KdT_{95} values (percentage knockdown with time) for DDT in another farm site (Ikorodu) and & KdT_{95} values for DDT in all the populations were unrealistic being greater than the mandatory 60 min recommended by the World Health Organization. The time (minutes) taken to knockdown 50% of the population increased at one of the farm sites, Badagry (60.2–69.6) and the nonfarm site, Ebute Metta (68.9–199.96) from 2011 to 2012. There was a decrease in KdT_{50} at Ikorodu, a farm site, (243–63.4) from 2011 to 2012. The same trend was observed with the KdT_{95} to DDT at the various sites (Table 3). A Significant difference ($P < 0.01$) in KdT_{50} was recorded between mortality to DDT at the farm sites and nonfarm site in the second year (2012).

Discussion and Conclusion

This study demonstrates the occurrence of susceptibility of permethrin insecticides in populations of *Ae.* Aegypti in farm sites and nonfarm sites, Lagos, Nigeria. Previous studies have also reported susceptibility of *Ae. aegypti* to permethrin in urban areas of Nigeria and Senegal (Ndam et al. 2006; Dia et al. 2012; Kemabonta et al. 2013). Other studies have reported resistance of populations of *Ae. aegypti* in other parts of the world (Prapanthadara et al. 2002; Srisawat et al. 2012). The time to knockdown 50 and 95% of populations was higher in the nonfarm site compared with the farm sites. This finding suggest the use of permethrin in the control of *Ae. aegypti* in both farm and nonfarm sites in Lagos State, Nigeria. However, constant surveillance to monitor resistance levels of *Ae. aegypti* especially in the nonfarm sites in Lagos, Nigeria.

Ae. aegypti populations in the farm sites were susceptible to deltamethrin but tolerance recorded at the nonfarm site. This was also similarly reported by Ahmad et al. (2007) in Indonesia. The occurrence of deltamethrin resistance in a nonfarm site (urban area) supports the hypothesis of possible existence of other factors selecting for insecticide resistance outside the usage of agricultural insecticides (Oduola et al. 2012). The insecticides recommended for IRS, ITNs in Nigeria include the pyrethroids (WHO 2012). The use of pyrethroid in impregnated bed-net and for domiciliary spraying for malaria control have been suggested to be responsible for resistant of some *Ae. aegypti* populations to pyrethroid in Thailand (Jirakanjanakit et al. 2007). The use of pyrethroid for bed net and domiciliary spraying for malaria in Lagos State could have resulted in the resistance recorded to deltamethrin by the vector in the nonfarm site. Studies by Ayorinde et al. (2014) observed the use of pyrethroid-based mosquito repellent in urban areas of Lagos State. The constant use of this pyrethroid-based repellent could also have contributed to resistance to pyrethroid suspected in the nonfarm site of Lagos State.

Resistance to DDT was observed in both farm and nonfarm sites in this study. Similar reports in Cameroon and some countries in Latin America (Harris et al. 2010; Kamgang et al. 2011). The resistance to DDT may not be unconnected with the historical use of this insecticide in vector control activities in Nigeria. The extensive use and abuse of DDT for agricultural purposes could have contributed immensely to the development of resistance in insect pests (Oduola et al. 2010; Ibrahim et al. 2013). IRS of DDT for malaria control has been reported to favor the selection of DDT resistance in *Anopheles* as well as in *Aedes*. The Contamination of larval breeding places by insecticides used in agriculture has also been shown to select for DDT resistance in malaria vectors (Kamgang et al. 2011). This Insecticide has been used in the past for agricultural purpose and IRS programs carried out in these areas which could have resulted in selection of DDT resistance in *Anopheles* as well as *Aedes* mosquitoes (Awolola et al. 2005, 2007; Oduola et al. 2010, 2012). Co-occurrence of both DDT and deltamethrin resistance among populations of *Ae. aegypti* population in the nonfarm site suggest a similar mechanism modulating for both pyrethroid and DDT resistance since both insecticides act on the same target site. However, the disparity in mortalities observed in deltamethrin exposure compared with DDT in the nonfarm sites populations suggest that the *knockdown resistance gene* alone may not be responsible for the resistance observed in these populations.

Conclusion

Resistance of this vector group to DDT (at all sites) and deltamethrin in the urban sector reveals that theses insecticides cannot be used alone but with other insecticides with different target sites under an integrated vector control management.

Acknowledgments

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