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REDUCED PYRETHROID MORBIDITY AND RESISTANCE OF JOINTLY EXPOSED *ANOPHELES* AND *CULEX* MOSQUITOES: IMPLICATIONS FOR MALARIA-LYMPHATIC FILARIASIS CO-INTERVENTION PLANS

| Abiodun Obembe* | Fatima Yahaya* | Adedayo Oduola |**
| Jasini Wahedi* | Samson Awolola**** |**

* Department of Zoology, Kwara State University, Malete, NIGERIA. E-mail: abiodunobembe@yahoo.com
** Department of Zoology, University of Ilorin, NIGERIA.
*** Department of Zoology, Adamawa State University, Mubi, NIGERIA.
**** Molecular Entomology and Vector Control Research Laboratory, Nigerian Institute of Medical Research, Lagos, NIGERIA.

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ABSTRACT: Emerging adult *Anopheles* and *Culex* mosquitoes migrate to human dwellings, at great risks of insecticide exposure, in search of bloodmeal. Countries like Nigeria, targeting this joint mosquito host-seeking behaviour have launched Malaria-Lymphatic Filariasis (Malaria-LF) co-intervention plans using synergistic insecticide-based control tools. However, insecticide resistance currently threatens mosquito vector control. This study investigated separate and combined pyrethroid insecticide exposure response of *Anopheles gambiae* sensu lato (s.l.) and *Culex pipiens* complex mosquitoes collected from two mixed breeding sites in Nigeria. Adult *Anopheles* and *Culex* mosquitoes reared from larval collections were exposed in separate and joint insecticide susceptibility tests to permethrin 0.75%, deltamethrin 0.05% and alphacypermethrin (0.05%) using World Health Organization (WHO) kits. The mosquitoes were identified morphologically. Time to mosquito knockdown, percentage knockdown, and mortality were monitored. The results for the different mosquito and exposure types were compared. Both mosquitoes were resistant to all pyrethroid insecticides tested. Significantly higher *Anopheles* knockdown was recorded compared to *Culex* for the joint (*Anopheles* 79-89% vs *Culex* 44-50%, $P \leq 0.011$) and separate (*Anopheles* 67-88% vs *Culex* 46-47%, $P < 0.048$) exposures respectively. Time to 95% *Anopheles* and *Culex* mosquito knock down were shorter during separate (2200.9 mins) than in joint (4513.9 mins) exposures. Faster *Anopheles* knockdown (kdt_{50} 18.7 - 68.8 mins) were recorded compared to *Culex* (kdt_{50} 22.1 - 98.8 mins). Higher but not significantly different ($P \geq 0.083$) mosquito mortalities were recorded from separate than in joint *Anopheles* and *Culex* exposures. There were no significant ($P \geq 0.072$) differences between *Anopheles* 43-77% and *Culex* 41-72% mortalities for separate and joint exposures. The speed of action and morbidity effects of the pyrethroid insecticides tested were lower during joint than in the separate mosquito exposures. Taken together, reduced pyrethroid speed of action and morbidity along with multiple mosquito resistance noted could threaten insecticide user-acceptability, performance perception and Malaria-LF co-intervention in the field.

KEY WORDS: *Anopheles*, *Culex*, joint mosquito exposure, insecticide resistance / susceptibility, vector control, malaria-lymphatic filariasis

Anopheles gambiae s.l and *Culex pipiens* mosquitoes transmit deadly parasitic and arboviral public health diseases like malaria, lymphatic filariasis (LF), Rift valley fever and West Nile Virus. These mosquito-borne diseases (MBD) threaten over 40% of global human population resulting in significant global morbidity and mortality (Jones et al., 2021). Recently, evidences of MBD re-emergence and spread have been reported in previously low-risk regions (Colon-Gonzalez et al., 2021). Indeed, the morbidity and loss of productive man hours resulting from MBD incidences take a huge toll on the economy of the rural poor in most developing countries.

Anopheles gambiae s.l mosquitoes are of major concerns especially as vectors of both malaria and LF diseases in many tropical regions (Simonsen & Mwakitalu, 2013). Preadult *Anopheles* mosquito limitation to clean rural temporary water habitats (Muirhead-Thompson, 1951) no longer holds. Like *Culex*, the principal malaria vector- *An. gambiae* s.l now adapts to organically polluted water bodies (Sattler et al., 2005; Awolola et al., 2007) in central and peri-urban areas, facilitating urban malaria. *Culex* species naturally found in these abundant urban breeding sites are the most prevalent mosquitoes in many African cities (Norris & Norris, 2011; Jones et al., 2012; Ekloh et al., 2013) and across the world (Bhattacharya & Basu, 2016). The *Culex pipiens quinquefasciatus* sibling species generates high biting nuisance against humans and animals (Antonio-Nkondjio et al., 2012) especially in unplanned urban areas (Yadouleton et al., 2015) where they breed in all types of stagnant water collections (Sattler et al., 2005). The principal malaria vector- *An. gambiae* s.l, now shares *Culex* urban breeding sites, and also serve as LF vectors in sub-Saharan Africa and parts of the western Pacific (Sasa, 1976). *Culex quinquefasciatus* drives LF transmission in urban East Africa and less so in the west Africa (Okorie et al., 2011).

Current data shows that 241 million cases and 627,000 human deaths were caused by malaria in 2020 (WHO, 2021). Global LF burden impacts 40 million chronically disabled and 80 million other people suffering from covert lymphatic changes or kidney disease (WHO, 2006). Clearly, malaria and LF exhibit distribution overlaps in Africa, south and southeast Asia, and some parts of Latin America (Manguin et al., 2010). Lymphatic filariasis elimination is currently not in view in sub-Saharan Africa probably due to control effort reliance on repeated mass administration of ivermectin or diethylcarbamazine citrate in combination with albendazole (Lupenza et al., 2021).

Vector control employed as frontline strategy against malaria has been recommended as a complementary tool to mass drug administration (MDA) in LF elimination (Lupenza et al., 2021). Combined control efforts against both diseases have been advocated due to similarities in vector species, human host, vector-control interventions, and prevalence under poor living conditions (Muturi et al., 2008; van den Berg et al., 2013). Deployments of insecticide-treated bed nets (ITNs) for malaria control have been associated with attendant reduction in LF prevalence and transmission (Lengeler, 2004).

Even so, vector control of mosquito-borne diseases using ITN and indoor residual spraying (IRS) is continuously being challenged by insecticide resistance (IR). Threats of vector control failure and disease re-emergence currently exist especially in the event of improper IR monitoring and management (Talipouo et al., 2021). *Culex* mosquito IR creates continued biting nuisance even under ITN utilization. This could trigger perceived ITN ineffectiveness and nightly user non-

compliance among human populations targeted for malaria control and elimination (Corbel et al., 2007). Implementation of a multi-disease approach involving the integration of malaria and LF vector control will require local evidence for combined data driven interventions (van den Berg et al., 2013). In Nigeria, where malaria and LF co-implementation plan has been launched (FMoH, 2014), a quarter of global malaria burden (WHO, 2021) and 70% LF infection risk (FMoH, 2013) are experienced. High pyrethroid insecticide resistance possibly limiting ITN success have also been identified in *Anopheles* (Awolola et al., 2018; Fagbohun et al., 2019; Wahedi et al., 2021) and *Culex* (Fagbohun et al., 2019; Datti et al., 2022) mosquito vectors in different regions across the country.

However, combined insecticide response of jointly exposed *Anopheles* and *Culex* mosquito vectors from mixed breeding sites have not been documented. Specifically, studies on joint insecticide susceptibility tests of *Anopheles* and *Culex* mosquitoes are not available in literature to our knowledge. This study aimed to fill this gap by conducting joint insecticide susceptibility tests of *Anopheles* and *Culex* mosquitoes collected from the same mixed breeding sites in urban Ilorin, Kwara State, Nigeria in order to determine the combined insecticide response of both mosquitoes. Aside separate exposures of *Anopheles* and *Culex* mosquito populations to recommended insecticides, joint exposures of both types of mosquitoes collected from the same mixed breeding site may give insight into their response in the event of joint insecticide encounters on the field.

METHODS

Mosquito collection and rearing

Anopheles and *Culex* mosquito third and fourth instar larvae and pupae were collected from natural mosquito breeding sites in Niger (N 08º 48.931E'004º 55.416') and Akalambi (N 08º50.645'E004º 55128') areas within Ilorin, the capital city of Kwara State, North central Nigeria. The breeding sites are located in poorly planned areas where the major river in the city (Asa River) flows through. Domestic wastes from the residents in these two areas are dumped into the flowing water body thereby forming pockets of stagnant mosquito breeding sites. The sites of mosquito collection were selected based on the presence of both *Anopheles* and *Culex* mosquitoes at ≥ 10 larvae and/or pupae density per dip. Collected mosquitoes from the two urban sites (hereafter referred to as sites 1 and 2) were transported and reared (27º-29ºC, 77-82% Relative humidity (RH)) according to standard methods (Methods in *Anopheles* research, 2015) in the insectary at the Department of Zoology, Kwara State University. Both *Anopheles* and *Culex* mosquitoes were jointly reared to adulthood using the collection water. Adult *Anopheles* and *Culex* mosquitoes emerging into the same cages were maintained on 10% sugar solution.

Insecticide susceptibility tests

Permethrin, deltamethrin and alphacypermethrin are the most common pyrethroid insecticides recommended for mosquito vector control (WHO, 2013). These pyrethroids are the insecticides specifically used for mosquito vector control in Nigeria through the deployments of permethrin, deltamethrin or alphacypermethrin insecticide treated nets (FMoH, 2013, 2014). The World Health Organization (WHO, 2013) recommends the initial testing of these

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insecticides at the WHO diagnostic concentrations (Permethrin 0.75%, Deltamethrin 0.05% and Alphacypermethrin 0.05%) as an indicator for pyrethroid resistance. Insecticide susceptibility tests were conducted with WHO test kits and test papers (Permethrin 0.75%, Deltamethrin 0.05% and Alphacypermethrin 0.05%) procured from Vector Control Research Unit, Universiti Sains Malaysia. The tests were done following standard WHO protocol (WHO, 2013). Replicates of 20 to 26, non-blood-fed, 3-5-day old, adult female *Anopheles* or *Culex* mosquitoes were separately exposed to each test paper in WHO test tubes for 1 hour (WHO, 2013). For joint exposures, the 20-26 mosquitoes exposed to each insecticide comprised 10-13, each of *Anopheles* and *Culex* mosquitoes, transferred together into each exposure tube. Available adult mosquitoes over each 3-5-day emergence period were allocated randomly to separate and joint exposure replicates. This was done to ensure that replicates of separate and joint mosquito exposures were done at the same time under the same conditions. Eight replicates of joint exposure tests were conducted for each insecticide, making a total of 24 replicates per site. Each separate exposure test had five replicates, making a total of 30 replicates per site. Numbers of knocked down mosquitoes were recorded every ten minutes during the 1-hour exposure period. Exposed mosquitoes were transferred into holding tubes supplied with 10% sugar solution for subsequent record of mortality and survival after 24 hours. Twenty to twenty-six female *Anopheles* or *Culex* or female *Anopheles* and *Culex* mosquitoes (for joint exposures) were also exposed to WHO control papers to serve as controls during each experiment. Each dead or alive mosquito was preserved in 1.5 ml Eppendorf tube containing desiccated silica gel.

Mosquito identification

Mosquitoes preserved after exposures were identified as *An. gambiae* s.l or *Culex pipiens* s.l under a dissecting microscope using standard morphological keys for *Anopheles* (Gillies & Coetzee, 1987) and *Culex* (Rueda, 2004) mosquitoes.

Data analysis

Mosquito population status was determined as resistant (<90% mortality) using percentage mortalities of exposed mosquitoes (WHO, 2013). The knock down times (KDT) for 50 and 95% of the exposed mosquitoes (KDT50 and KDT95) are the standard used to assess the speed of action of the insecticides (WHO, 2013). The KDT50 and KDT95 values were determined using log-time-probit model. Pearson's chi-squared test was used to compare the percentage knockdown of the *Anopheles* and *Culex* mosquitoes and percentage mortalities of the *Anopheles* and *Culex* mosquitoes exposed to the same insecticides. Percentage mortalities recorded from separate and joint exposures of *Anopheles* and *Culex* mosquitoes were also compared using Pearson's chi-squared test. The data were analysed using Statistical Package for Social Sciences software version 16.0 with statistical significance set at $P<0.05$.

RESULTS

Separate and joint insecticide susceptibility tests

Percentage knockdown of *Anopheles* and *Culex* mosquitoes from site 1 are presented in figure 1. Knock down of *Anopheles* were significantly higher than *Culex* for joint (*Anopheles* 79%, *Culex* 50%; $P = 0.011$) and separate (*Anopheles* 88%, *Culex* 47%; $P < 0.001$) mosquito deltamethrin exposures. Percentage

knockdown of *Anopheles* and *Culex* mosquitoes under joint (*Anopheles* 52%, *Culex* 49%; *P* = 0.765) and separate (*Anopheles* 55%, *Culex* 45%, *P* = 0.317) permethrin exposures were not significantly different. Alphacypermethrin-induced knockdown of *Anopheles* and *Culex* mosquitoes under joint (*Anopheles* 81%, *Culex* 81%) and separate (*Anopheles* 84%, *Culex* 73% *P* = 0.380) mosquito exposures were also not significantly different. Equally, for the mosquitoes collected from site 2, permethrin joint (*Anopheles* 69%, *Culex* 56%, *P* = 0.245) and separate (*Anopheles* 65%, *Culex* 51% *P* =0.262) as well as alphacypermethrin joint (*Anopheles* 69%, *Culex* 56% *P* = 0.194) and separate (*Anopheles* 65%, *Culex* 51% *P* = 0.522) exposures induced higher but not significantly different knockdown of *Anopheles* mosquitoes compared to *Culex*. However, percentage knockdown after joint (*Anopheles* 89%, *Culex* 44%, *P* < 0.001) and separate (*Anopheles* 67%, *Culex* 46%, *P* = 0.048) exposures of both mosquitoes to deltamethrin were significantly higher for *Anopheles* compared to *Culex* mosquitoes (Fig. 1).

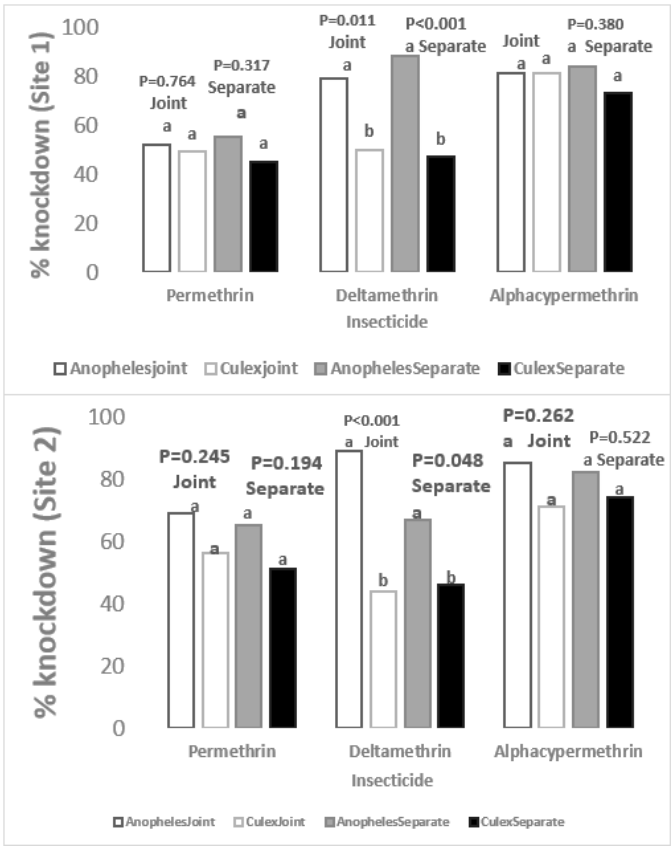


Figure 1. Percentage knockdown of separate and jointly exposed *An. gambiae s.l* and *Cx. quinquefsciatus* mosquitoes from Sites 1 and 2 in urban Ilorin. Knockdown values with different letters under the same insecticide are significantly different (P < 0.05).

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Percentage mortality and the time to 50 and 95% knockdown (kdt_{50} and kdt_{95}) during separate and joint exposures of *Anopheles* and *Culex* mosquitoes from the two sites are presented in table 1. Lower kdt_{50} and kdt_{95} values indicate faster knockdown. Largely, knock down times (kdt) of *Anopheles* (kdt_{50} 18.7 - 51.1; kdt_{95} 65.7 – 295.7) and *Culex* (kdt_{50} 22.1 - 98.8; kdt_{95} 78.2 – 249.7) mosquitoes were lower indicating faster knockdown during separate compared to the joint *Anopheles* (kdt_{50} 20.4 - 68.8, kdt_{95} 118.2 – 810.6) and *Culex* (kdt_{50} 22.8 - 77.3, kdt_{95} 151.9 – 656.4) mosquito exposures. In fact, the total time to 95% knockdown of both mosquitoes during the joint exposures (kdt_{95} 4513.9 mins) was more than twice the time required in the separate exposures (2200.9 mins). Generally, lower kdt values indicating faster *Anopheles* mosquito knockdown (kdt_{50} 18.7 - 68.8) were recorded in both separate and joint pyrethroid insecticide exposures compared to *Culex* (kdt_{50} 22.1 - 98.8). Accordingly, percentage mortalities of *Anopheles* (43-77%) and *Culex* (43-72%) mosquitoes were mostly higher for the separate than for the joint exposures (*Anopheles* 44-68%; *Culex* 41-69%) respectively. However, there were no significant differences between *Anopheles* and *Culex* mosquito mortalities for both separate ($P \geq 0.154$) and joint ($P \geq 0.072$) mosquito exposures. Differences between separate and joint exposure mosquito mortalities were not significant for both *Anopheles* ($P \geq 0.083$) and *Culex* ($P \geq 0.252$) mosquitoes. In all, the percentage mortalities of *Anopheles* and *Culex* mosquitoes from the two sites were all lower than the 90% confirmed resistance benchmark (Table 1).

Table 1. Knockdown times and percentage mortality of pyrethroid exposed *Anopheles* and *Culex* mosquitoes in Urban Ilorin, Kwara State.

Site	Insecticide	Exposure type	Mosquito	N	KDT_{50} (min)	KDT_{95} (min)	Mortality (%)	*P* value	Status
	Deltamethrin	Joint	*Anopheles*	100	32.5	118.2	44	0.166	Resistant
			Culex	100	61.4	372.5	58		Resistant
		Separate	*Anopheles*	111	18.7	65.7	55	0.154	Resistant
			Culex	116	22.1	78.2	71		Resistant
		Anopheles	Joint	100	32.5	118.2	44	0.269	
			Separate	111	18.7	65.7	55		
		Culex	Joint	100	61.4	372.5	58	0.252	
			Separate	116	22.1	78.2	71		
Site 1	Permethrin	Joint	*Anopheles*	106	64.5	698.9	63	0.726	Resistant
			Culex	100	75.2	656.4	67		Resistant
		Separate	*Anopheles*	100	51.1	295.7	45	0.200	Resistant
			Culex	112	35.3	249.7	58		Resistant
		Anopheles	Joint	106	64.5	698.9	63	0.083	
			Separate	100	51.1	295.7	45		
		Culex	Joint	100	75.2	656.4	67	0.421	
			Separate	112	35.3	249.7	58		
	Alphacypermethrin	Joint	*Anopheles*	110	20.4	225.2	63	0.602	Resistant
			Culex	109	22.8	151.9	69		Resistant
		Separate	*Anopheles*	100	27.9	164.3	77	0.564	Resistant
			Culex	102	32.6	169.8	70		Resistant
		Anopheles	Joint	110	20.4	225.2	63	0.237	
			Separate	100	27.9	164.3	77		
		Culex	Joint	109	22.8	151.9	69	0.932	

			Separate	102	32.6	169.8	70		
	Deltamethrin	Joint	*Anopheles*	114	28.9	139.1	64	0.859	Resistant
			Culex	108	77.3	455.6	62		Resistant
		Separate	*Anopheles*	114	28.5	128.9	70	0.289	Resistant
			Culex	117	60.9	237.8	58		Resistant
		Anopheles	Joint	114	28.9	139.1	64	0.604	
			Separate	114	28.5	128.9	70		
		Culex	Joint	108	77.3	455.6	62	0.715	
			Separate	117	60.9	237.8	58		
Site 2	Permethrin	Joint	*Anopheles*	112	30.9	159.9	59	0.072	Resistant
			Culex	107	49.9	242.1	41		Resistant
		Separate	*Anopheles*	110	40.7	239.9	43	-	Resistant
			Culex	116	98.8	236.8	43		Resistant
		Anopheles	Joint	112	30.9	159.9	59	0.113	
			Separate	110	40.7	239.9	43		
		Culex	Joint	107	49.9	242.1	41	0.827	
			Separate	116	98.8	236.8	43		
	Alphacypermethrin	Joint	*Anopheles*	111	68.8	810.6	68	0.863	Resistant
			Culex	109	50.1	483.5	66		Resistant
		Separate	*Anopheles*	100	27.9	164.3	72	-	Resistant
			Culex	100	32.6	169.8	72		Resistant
		Anopheles	Joint	111	68.8	810.6	68	0.735	
			Separate	100	27.9	164.3	72		
		Culex	Joint	109	50.1	483.5	66	0.610	
			Separate	100	32.6	169.8	72		

N- number of mosquito samples exposed, KDT- knock down time, Min- minutes

DISCUSSION

This study determined combined insecticide response of *Anopheles* and *Culex* mosquitoes jointly exposed in insecticide susceptibility tests. Lower knock down time (kdt_{50} and kdt_{95}) values indicate faster knockdown. Lower knock down times and higher percentage knockdown of *Anopheles* than *Culex* mosquitoes in both separate and joint pyrethroid insecticide exposures indicates higher insecticide vulnerability of *Anopheles* compared to the *Culex* mosquitoes. *Culex quinquefasciatus* is notoriously difficult to control with pyrethroid treated nets (Corbel et al., 2007) which offers little or no protection against pyrethroid-resistant *Cx. quinquefasciatus* once insecticide-treated nets (ITNs) become holed (Irish et al., 2008).

Lower percentage knockdown of *Culex* mosquitoes observed during the separate exposures in this study have also been reported in other studies in Nigeria (Fagbohun et al., 2019). This result indicates that the mosquitoes from the study area may elicit reduced morbidity/knockdown when exposed to these pyrethroid insecticides in the field. Users of insecticide-treated nets are discouraged when *Culex* mosquitoes that have developed resistance to the insecticide on the nets are able to enter holed nets to bite the sleepers (Corbel et al., 2007). This is because sustained motivation for ITN utilization is driven more by mosquito bite prevention than malaria prevention (Pulford et al., 2011). Therefore, absence of rapid *Culex* mosquito knockdown response to pyrethroid insecticide exposures on the field may negatively impact user acceptability,

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perceived efficacy and sustained uptake of vector control tools with these insecticides. While studies reporting *Anopheles* (Awolola et al., 2018; Fagbohun et al., 2019; Wahedi et al., 2021) separately from *Culex* (Oduola et al., 2016; Fagbohun et al., 2019; Datti et al., 2022) mosquito resistance abound in Nigeria and Africa (Norris & Norris, 2011; Jones et al., 2012; Yadouleton et al., 2015; Talipouo et al., 2021), those containing separately exposed *Anopheles* and *Culex* mosquito insecticide susceptibility/resistance (Corbel et al., 2007; Fagbohun et al., 2019) together in the same report are scanty. Like that of Fagbohun et al. (2019), our study found phenotypic resistance in both *Anopheles* and *Culex* mosquitoes exposed separately to pyrethroid insecticides. Pyrethroid resistance demonstrated by both mosquito species may be due to selection pressure from widespread utilization of pyrethroid ITNs facilitated by massive periodic ITN distribution campaigns in the city over the years (Obembe et al., 2014a,b). Indeed, widespread long-lasting insecticidal net (LLIN) utilization have been linked with increasing knockdown resistance in *An. gambiae* from Niger (Czeher et al., 2008) and *Culex quinquefasciatus* from Zambia (Norris & Norris, 2011). More attention should be paid to assessing the level of resistance of different species of mosquitoes especially *Culex* species known for biting nuisance (Omotayo et al., 2022). Higher KDT_{50} values in *An. gambiae* field mosquito population could give an early indication of the involvement of *kdr* mechanism of resistance (Chandre et al., 1999).

Apart from reporting separate exposures of both mosquito species, our study represents a first report on joint exposures of *Anopheles* and *Culex* mosquitoes to our knowledge. None of the pyrethroid insecticides induced up to 90% knockdown of the mosquitoes exposed during both separate and joint exposures. Nevertheless, the significantly higher percentage *Anopheles* knockdown than *Culex* recorded only for deltamethrin suggests higher morbidity performance effects of the insecticide against *Anopheles* compared to *Culex* mosquitoes. Despite the slightly higher mosquito mortalities during separate than in joint exposures, the absence of any significant difference ($P > 0.05$) suggests that the exposure types (separate or joint) had no substantial impact on mortalities of the mosquitoes. However, time required for mosquito knock down were shorter by half during separate than in joint exposures. Perhaps, longer time periods were required to overcome the differential responses from the two mosquito types compared to single mosquito response type in the separate exposures. The implication of the reduced speed of action for Malaria-LF co-implementation plan is that the current pyrethroid insecticides deployed for vector control may not elicit rapid actions against the vectors of these diseases in the event of joint exposures encounters as compared to separate encounters in real field situations. This represents an important finding considering that the speed of action is a critical insecticide user-acceptability and performance index. If ITNs impregnated with these pyrethroid insecticides no longer effect rapid knockdown of mosquitoes due to the development of resistance, users of such ITNs may discard the nets and even reject free future net distribution. In addition, percentage *Anopheles* mosquito mortalities were not significantly different from those of *Culex* indicating confirmed resistance (< 90%) either in the separate or joint mosquito exposures. The widespread mosquito resistance results obtained here and in several other studies in Nigeria (Awolola et al., 2018; Fagbohun et al., 2019; Wahedi et al., 2021; Datti et al., 2022) suggests the potential ineffectiveness

of the use of pyrethroid insecticide-treated nets as part of the co-intervention plan against *Anopheles* and *Culex* mosquitoes transmitting malaria and LF. The broad implication of this findings is that regions with similar mosquito species and multiple pyrethroid insecticide resistance patterns should avoid the continuous use of ITNs with insecticides to which the mosquitoes have developed resistance. Integrated vector management targeting larval and adult peculiarities of both mosquitoes is recommended rather than the currently practiced reliance on ITN alone for the implementation of the Malaria-LF vector control co-intervention plan.

CONCLUSION

This study demonstrates decreased speed of insecticide action and mosquito morbidity effects during joint *An. gambiae* s.l and *Cx. quinquefasciatus* pyrethroid insecticide exposures compared to separate mosquito exposures. Indicatively, the pyrethroid vector control insecticides tested may not elicit rapid actions required against the vectors in the event of joint exposures encounters as compared to separate encounters in the field. This represents a significant finding for further exploration considering that user-acceptability and performance perception is based on speedy insecticidal morbidity actions and not eventual mortality. Multiple pyrethroid resistance in both mosquitoes represents a threat to the Nigerian Malaria-LF co-intervention plan involving pyrethroid ITN deployment for control of both mosquitoes in the study area. Alternative ITNs such as the dual active ingredients or synergist-incorporated insecticide-treated nets could be deployed as replacements for the current pyrethroid ITNs used for control of mosquito vector and Malaria-LF transmission in the study area.

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Conflict of interest
There is no conflict of interest between the authors.

Author's contribution
AO conceived the project, obtained funding, and wrote the manuscript. FNY and AO coordinated larval collections and insecticide exposure experiments. AOO, JW and STA assisted with the interpretation of results and production of the final draft of the manuscript. All authors approved the final draft of the manuscript.

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LITERATURE CITED

Antonio-Nkondjio, C., Defo-Talom, B., Tagne-Fotso, R., Tene-Fossog, B., Ndo, C., Lehman, L. G. *et al.* 2012. High mosquito burden and malaria transmission in a district of the city of Douala, Cameroon. BMC Infect Dis., 12 (1): 1–8.

Awolola, T. S., Adeogun, A., Olakiigbe, A. K., Oyeniyi, T., Olukosi, Y. A., Okoh, H. *et al.* 2018. Pyrethroids resistance intensity and resistance mechanisms in *Anopheles gambiae* from malaria vector surveillance sites in Nigeria. PLoS ONE, 13 (12): e0205230.

Awolola, T. S., Oduola, A. O., Obansa, J. B., Chukwurar, N. J. & Unyimadu, J. P. 2007. *Anopheles gambiae* s.s. breeding in polluted water bodies in urban Lagos, southwestern Nigeria. J. Vector Borne Dis., 44 (4): 241–244.

Bhattacharya, S. & Basu, P. 2016. The southern house mosquito, *Culex quinquefasciatus*: profile of a smart vector. J. Entomol. Zool. Stud., 4 (2): 73–81.

Chandre, F., Darriet, F., Manguin, S., Brengues, C., Carnevale, P. & Guillet, P. 1999. Pyrethroid cross-resistance spectrum among populations of *Anopheles gambiae s.s.* from Cote D'Ivoire. J. Am. Mosq. Cont. Assoc., 15: 53-59.

Collins, F. H., Mendez, M. A., Rasmussen, M. O., Mehaffey, P. C., Besansky, N. J. & Finnerty, V. A. Ribosomal RNA gene probe differentiates member species of the *Anopheles gambiae* complex. Am. J. Trop. Med. Hyg., 37 (1): 37–41.

Colon-Gonzalez, F. J., Sewe, M. O., Tompkins, A. M., Sjodin, H., Casallas, A., Rocklov, J. *et al.* 2021. Projecting the risk of mosquito-borne diseases in a warmer and more populated world: a multi-model, multi-scenario intercomparison modelling study. The Lancet Planetary Hlth., 5 (7): e404–414.

Corbel, V., N'Guessan, R., Brengues, C., Chandre, F., Djogbenou, L., Martin, T. *et al.* 2007. Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. Acta Trop., 101: 207–216.

Czeher, C., Labbo, R., Arzika, I. & Duchemin, J. B. 2008. Evidence of increasing Leu-Phe knockdown resistance mutation in *Anopheles gambiae* from Niger following a nationwide long-lasting insecticide-treated nets implementation. Malar. J., 7: 189.

Datti, J. A., Mukhtar, M. M., Usman, M. T., Abba, B. I., Kurfi, B. G. & Ibrahim, S. S. 2022. Evaluation of efficacy of insecticides and long-lasting insecticidal nets for control of *Culex quinquefasciatus* Say populations from Northern Nigeria. Advances in Entomol., 10: 186-203.

Ekloh, W., Oppong, G., Adinortey, M. B., Stiles-Ocran, J. B. & Hayford, D. 2013. Susceptibility of *Culex quinquefasciatus* populations to deltamethrin in the Sefwi area of the western region of Ghana. Eur. J. Exp. Biol., 3: 72–79.

Fagbohun, I. K., Oyeniyi, T. A., Idowu, T. E., Otubanjo, O. A. & Awolola, T. S. 2019. Cytochrome P450 mono-oxygenase and resistance phenotype in DDT and deltamethrin-resistant *Anopheles gambiae* (Diptera: Culicidae) and *Culex quinquefasciatus* in Kosofe, Lagos, Nigeria. J. Med. Entomol., 56: 817-821.

Favia, G., Della Torre, A., Bagayoko, M., Lanfrancotti, A., Sagnon, N. F., Touré, Y. T. *et al.* 1997. Molecular identification of sympatric chromosomal forms of *Anopheles gambiae* and further evidence of their reproductive isolation. Insect Mol. Biol., 6 (4): 377-383.

FMoH, Guideline for Malaria-Lymphatic Filariasis Co-implementation in Nigeria. [Online]. 2013. Available: https://www.health.gov.ng/doc/guidelines-malaria-lf-co-implementation-nigeria-.pdf [2 October 2022].

FMoH, National Malaria Strategic Plan 2014-2020. [Online]. 2014. Available: https://www.health.gov.ng/doc/NMEP-Strategic-Plan.pdf [2 October 2022].

Gillies, M. T. & Coetzee, M. 1987. A supplement to the Anophelinae of Africa South of the Sahara. Pub. South Afri. Inst. Med. Res., 55: 1-143.

Irish, S. R., N'Guessan, R., Boko, P. M., Metonnou, C., Odjo, A., Akogbeto, M. *et al.* 2008. Loss of protection with insecticide-treated nets against pyrethroid-resistant *Culex quinquefasciatus* mosquitoes once nets become holed: an experimental hut study. Parasit Vectors, 1: 17.

Jones, R. T., Ant, T. H., Cameron, M. M. & Logan, J. G. 2021. Novel control strategies for mosquito-borne diseases. Phil. Trans. Royal Soc. B, 376 (1818): 20190802.

Jones, C. M., Machin, C., Mohammed, K., Majambere, S., Ali, A. S., Khatib, B. O. *et al.* 2012. Insecticide resistance in *Culex quinquefasciatus* from Zanzibar: implications for vector control programmes. Parasit. Vectors, 5 (1): 78.

Lengeler, C. 2004. Insecticide-treated bednets and curtains for preventing malaria. Cochrane Database Syst. Rev., 2: CD000363.

Lupenza, E., Gasarasi, D. B. & Minzi, O. M. 2021. Lymphatic filariasis, infection status in *Culex quinquefasciatus* and *Anopheles* species after six rounds of mass drug administration in Masasi District, Tanzania. Infect. Dis. Poverty, 10 (1): 1–1.

Manguin, S., Bangs, M. J., Pothikasikorn, J. & Chareonviriyaphap, T. 2010. Review on global co-transmission of human *Plasmodium* species and *Wuchereria bancrofti* by *Anopheles* mosquitoes. Infect Genet. Evol., 10: 159–177.

Martinez-Torres, D., Chandre, F., Williamson, M., Darriet, F., Bergé, J., Devonshire, A. L. *et al.* 1998. Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae* s.s. Insect Mol. Biol., 7: 179-184.

Methods in *Anopheles* research. 2015. MR4 2015 Edition CDC Atlanta GA, USA.

Muirhead-Thompson, R. C. 1951. Mosquito behavior in relation to malaria transmission and control in the tropics. London: Edward Arnolds & Co.

Muturi, E. J., Jacob, B. G., Kim, C. H., Mbogo, C. M. & Novak, R. J. 2008. Are coinfections of malaria and filariasis of any epidemiological significance? Parasitol. Res., 102: 175–181.

Norris, L. C. & Norris, D. E. 2011. Insecticide resistance in *Culex quinquefasciatus* mosquitoes after the introduction of insecticide-treated bed nets in Macha, Zambia. J. Vector Ecol., 36 (2): 411–420.

Obembe, A., Anyaele, O. O. & Oduola, A. O. 2014a. Lessons from the implementation of LLIN distribution campaign in Ilorin Kwara State, Nigeria. BMC Public Hlth., 14: 514.

Obembe, A., Oduola, A., Popoola, K. & Ajao, A. 2014b. Community knowledge and practices as regards malaria in Ilorin City: implications for the elimination plan of the National Malaria Elimination Program. Asian Pacific J. Trop. Dis., 4 (1): 670-676.

Oduola, A. O., Obembe, A., Adelaja, O. J. & Ande, A. T. 2016. Surveillance and insecticide susceptibility status of culicine mosquitoes in selected communities utilizing long-lasting insecticidal nets in Kwara State, Nigeria. Animal Res. Intl., 13 (3): 2483–2491.

Okorie, P. N., McKenzie, F. E., Ademowo, O. G., Bockarie, M. & Kelly-Hope, L. 2011. Nigeria *Anopheles* vector database: an overview of 100 years' research. PLoS One, 6: e28347.

Omotayo, A. I., Dogara, M. M., Sufi, D., Shuaibu, T., Balogun, J., Dawaki, S. *et al.* 2022. High pyrethroid-resistance intensity in *Culex quinquefasciatus* (Say) (Diptera: Culicidae) populations from Jigawa, North-West, Nigeria. PLoS Negl. Trop. Dis., 16 (6): e0010525.

Pulford, J., Hetzel, M. W., Bryant, M., Siba, P. M. & Mueller, I. 2011. Reported reasons for not using a mosquito net when one is available: a review of the published literature. Malar. J., 10: 83.

Rueda, L. M. 2004. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. Zootaxa, 589: 1–60.

Sasa, M. 1976. Human filariasis: a global survey of epidemiology and control. Tokyo: University of Tokyo.

Sattler, M. A., Mtasiwa, D., Kiama, M., Prenji, Z., Tanner, M., Killeen, F. *et al.* 2005. Habitat characterization and spatial distribution of *Anopheles* sp. Mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. Malar. J., 4: 4.

Scott, J. A., Brogdon, W. G. & Collins, F. H. 1993. Identification of single specimens of the Anopheles gambiae complex by the polymerase chain reaction. Am. J. Trop. Med. Hyg., 49 (4): 520-529.

Simonsen, P. E. & Mwakitalu, M. E. 2013. Urban lymphatic filariasis. Parasitol. Res., 112: 35–44.

Smith, J. L. & Fonseca, D. M. 2004. Rapid assays for identification of members of the *Culex* (*Culex*) *pipiens* complex, their hybrids, and other sibling species (Diptera: culicidae). Am. J. Trop. Med. Hyg., 70 (4): 339–345.

Talipouo, A., Mavridis, K., Nchoutpouen, E., Djiappi-Tchamen, B., Fotakis, E. A., Kopya, E. *et al.* 2021. High insecticide resistance mediated by different mechanisms in *Culex quinquefasciatus* populations from the city of Yaounde´, Cameroon. Sci. Reports, 11 (1): 1–1.

van den Berg, H., Kelly-Hope, L. A. & Lindsay, S. W. 2013. Malaria and lymphatic filariasis: the case for integrated vector management. Lancet Infect. Dis., 13: 89–94.

Wahedi, J. A., Ande, A. T., Oduola, A. O. & Obembe, A. 2021. Bendiocarb resistance and, *kdr* associated deltamethrin and DDT resistance in *Anopheles gambiae s.l.* populations from North Eastern Adamawa State, Nigeria. Ceylon Journal of Science, 50 (1): 63-73.

WHO. 2006. Informal consultation on preventing disability from lymphatic filariasis. WHO, Geneva, Wkly Epidemiol. Rec., 81: 373–383.

WHO, Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. [Online]. 2013. Available: http://www.africairs.net/wp-content/uploads/2012/08/Test-procedures-for-insecticide-resistance-monitoring-WHO.pdf [2 October 2022].

WHO, World Malaria Report. [Online]. 2021. Available: http://apps.who.int/iris/rest/bitstreams/1398397/retrieve [2 October 2022].

Yadouleton, A., Badirou, K., Agbanrin, R., Jost, H., Attolou, R., Srinivasan, R. *et al.* 2015. Insecticide resistance status in *Culex quinquefasciatus* in Benin. Parasit Vectors, 8 (1): 17.