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Effect of Piperonylbutaoxide (PBO) and S.S.S-Tributlyphosphorotrithioate (DEF) on Deltamethrin Resistant Mosquitoes of Irrigated Rice Fields in Auyo and Garunmalam Localities of Jigawa and Kano States, Nigeria

Abstract: The effect of Piperonyl-butaoxide (PBO) and S.S.S-Tributyl-phosphorotrithioate (DEF) synergist on pyrethroid deltamethrin resistant *Anopheles gambiae* sensu lato (*An. gambiae* s.l) mosquitoes in Auyo and Garunmalam Local Government Areas of Jigawa and Kano State were evaluated. *An. gambiae* s.l larvae were collected from breeding sites in rice fields of Auyo and Garunmalam LGAs and were reared. Two to three days adults were subjected to insecticides susceptibility test using CDC bioassay and biochemical assay using synergist. Batches of 20 adult mosquitoes were exposed to four Wheaton bottles coated with deltamethrin (12.5µg/ml), PBO (400µg/bottle) and DEF (125µg/bottle) insecticides and control bottles coated with acetone according to CDC protocol. *An. gambiae* s.l sub species were identified by PCR. The findings of the study establishes strong resistance to deltamethrin from both localities surveyed with a mortality rate of 45.0% and 27.0 % respectively but after synergizing with PBO and DEF, the mortality rate were found to be higher (100%) with PBO in both localities, while in combination of deltamethrin with DEF, a low gradual increase in mortality rate from 45.0 % to 62.0% in Auyo LGA and 27.0 % to 63.0% in Garunmalam LGA respectively were observed. Significant correlation in mortality rate ($p<0.001$) were also noted in both the localities. PCR showed the presence of *An. coluzzii* and *An. gambiae* giles with a predominance of *An. coluzzii* (92.3%) than *An. gambiae* giles (7.69%). Based on these findings, it may be concluded that metabolic resistance to insecticides is the biggest threat to the continued effectiveness of malaria vector control. Therefore, use of new generation ITNs impregnated with PBO is a way to mitigate the spread of malaria in these areas.

Keywords: ***An. gambiae s.l*, Deltamethrin, CDC, PBO, DEF**

INTRODUCTION

In Nigeria, vector control strategy remains one of the forefront and effective tools for controlling malaria and other insect-borne diseases. Long-lasting insecticide nets (LLINs) and indoor residual spraying (IRS) are the main methods used for malaria vector control. Pyrethroids are the only insecticides that are used for both IRS and LLINs, in the form of alphacypermethrin, bifenthrin, cyfluthrin, permethrin, deltamethrin, lambdacyhalothrin and etofenprox (Gimnig *et al.*, 2003; Yewhalaw *et al.*, 2011). Over the last several decades, it has been the chemical class of choice in agriculture and public health applications because of its relatively low toxicity to humans, rapid knock-down effect, relative longevity of 3–6 months when used as IRS, as

well as low cost. Up to date, pyrethroids remain the only insecticide family recommended by the WHO for the impregnation of bed nets (WHO, 2022).

The effect of pyrethroid resistance on malaria control has been a primary concern for over 20 years (Hemingway and Ranson, 2000). Resistance of mosquitoes to pyrethroid and other insecticides used in ITNs poses a significant problem to the long-term management and control of malaria (W.H.O, 2013, Brogdon and McAllister, 1998; Mawejje *et al.*, 2013). According to the 2021 World Health Organization Report on Malaria, resistance to pyrethroids is widespread globally and nearly three-quarters of nations reporting resistance to pyrethroids experienced high intensity resistance in 2020 (WHO, 2022).
Insecticide resistance is a genetic option that allows some individual insects the ability to survive previously lethal doses of insecticides. Over time, this results in survival of increasing numbers of resistant individual mosquitoes within the population and may render the effectiveness of insecticide applications causing failure of vector operational control. The use of agricultural pesticides may also contribute to select resistance to insecticides used for vector control. Many of the pesticides used in agriculture overlap with those relied on by public health agencies, further complicating insecticide resistance management (Diabaté *et al.*, 2002; Chouaïbou *et al.*, 2008; Yadouleton *et al.*, 2009; Nkya *et al.*, 2013; 2014).

Pyrethroids resistance appears to depend mainly on the knockdown resistance target-site mutations (kdr) as well as metabolic resistance mechanisms, although other mechanisms, such as penetration resistance have also been implicated (Nkya *et al.*, 2013). Target site resistance, caused by alteration of the molecular target of the insecticide (Nkya *et al.*, 2014), and metabolic resistance, whereby mosquitoes more quickly detoxify or eliminate insecticides (Perara *et al.*, 2008), are the best-studied and most readily quantifiable mechanisms of insecticide resistance. Resistance may also develop by alterations in insecticide absorption at the point of cuticular contact, or by changes in mosquito behavior.

The emergence of insecticide resistance in *Anopheles* mosquitoes in Nigeria has enormous implications for vector control interventions in the country. Absence of available alternative insecticides for mosquito vector control has also been an issue (Coleman *et al.,* 2006). In North-Western Nigeria, data on the effect of the synergist Piperonylbutaoxide (PBO) and S.S.S-Tributylphosphorotrithioate (DEF) on pyrethroid insecticide is still insufficient.
The present study is aimed to investigate the effect of the synergist Piperonylbutaoxide (PBO) and S.S.S- Tributylphosphorotrithioate (DEF) on deltamethrin resistant anopheles mosquitoes from North-Western part of Nigeria, this will provide the National Malaria Control Programmes with a reliable data for decision- making regarding resistance management in Northern, Nigeria.

MATERIALS AND METHOD

Study Area

The study areas are located at Auyo and Garunmalam Local Government Areas of Jigawa and Kano State respectively where most farmers cultivate rice predominantly.

Auyo Local Government Area lies between latitude 12^0 21'36''N and longitude $9^0$59'8'' ^{o}E. Garun Malam lies between 11^0 41' 0''N, 8^0 22'0''E. Both localities have similar ecology with a climate mainly of Sudanese type. Petrol pumps and myriad of pesticides such as organophosphate, carbamates, pyrethroids and organochlorine (including DDT and dieldrin) are used by farmers in both Auyo and Garunmalam LGAs for crop protection (Kimmage and Adams1990).

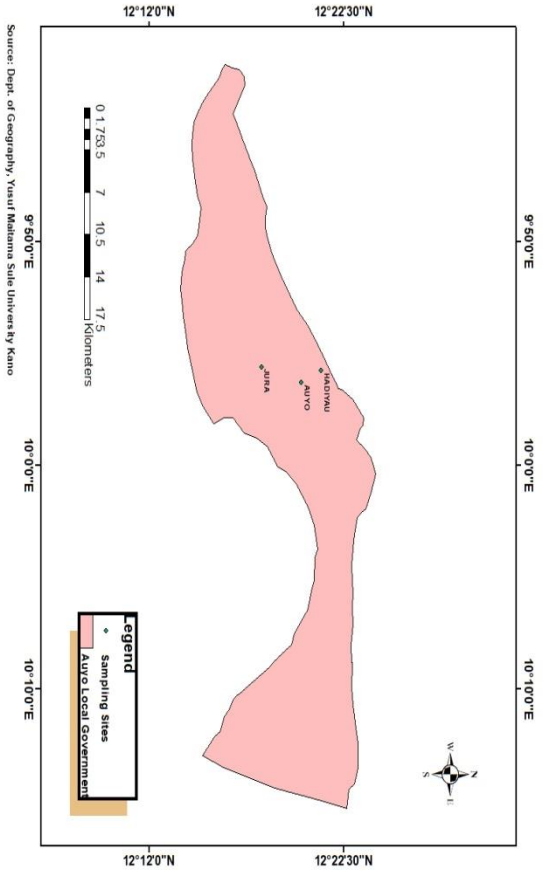

Fig 3.1: Map of Auyo Local Government Area Showing the Sampling Sites

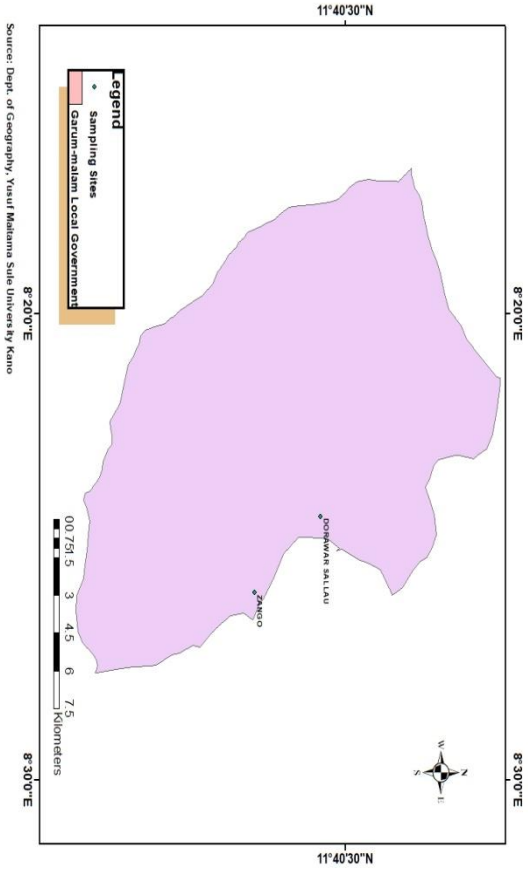

Fig 1: Map of Garunmalam Local Government Area Showing the Sampling Sites.

CDC Stock solution Preparation and Coating

The stock solutions were prepared based on the CDC protocols, 2010. An appropriate amount of technical grade deltamethrin (12.5mg), Piperonyl butoxide (400mg) as well as S.S.S tributyl-phosphorotrithioate (125mg) synergist was diluted in acetone to make1 litre (1000ml) each of total solution. Therefore, each ml of the stock solution contains a desired amount of the insecticide in µg/ml (Brogdon and Chan, 2010). The stock solutions were kept in amber coloured bottles and labelled appropriately with the name, concentration, and date of preparation of the insecticide. They were then kept in the refrigerator at 4^{o}C. Prior to the bioassay they were taken out of the refrigerator for 1 hour. The solutions were swirled gently to mix it before use. Four labelled 250ml Wheaton bottles were coated with 1 ml of the stock solutions, and a labelled control bottle coated with 1 ml of acetone following CDC protocol (Brogdon and Allister, 1998).

Mosquito Collection and Rearing

Anopheles Mosquito larvae were collected during the peak rainy season in August from three rice fields within the localities of Auyo (Hadiyau, Jura and Sabuwar Auyo) and Garunmalam (Dorawar Sallau, Zango and Garunmalam) using classical dipping method as describe by Robert *et al.* (2002). Larvae of *An. gambiae s.l* complex were transported to an insectary at Aminu Kano Teaching Hospital, Kano. The larvae were maintained under standard insectary condition (25-28^{0}c) temperature and 70-80% Relative humidity (Das and Dimopoulos, 2007). The larvae were fed with Tetramin baby fish food once every two days. The adults that emerged were fed with

10% sucrose solutions which were mixed at subsequent experiment.

Bioassay for Insecticide Susceptibility

Unfed twenty 2-3 days female *An. gambiae* s.l mosquitoes were aspirated into four 250ml Wheaton bottles coated with deltamethrin insecticide stock solution (12.5µg/bottle) and a control bottle coated with acetone only. Mosquitoes were exposed to insecticide- treated bottles for two hours and the number of dead or alive mosquitoes were monitored at different intervals of time (15, 30, 35, 40, 45, 60 75, 90, 105, 120 minutes) based on the CDC protocols, 2010.

Biochemical Assay using Synergist

Unfed twenty 2-3 days female *An. gambiae s.l* mosquitoes were exposed to four replicates and one control bottles of PBO (400µg/bottle), and DEF(125µg/bottle) synergist respectively for one hour and then tested for insecticide resistance with deltamethrin (12.5µg/bottle) using the CDC bottle bioassay. The numbers of dead or alive mosquitoes were monitored at intervals of different time (0, 10, 20, 30, 40, 50, 60 min) (Brogdon and Allister, 1998; CDC, 2010).

Morphological Identification

All dead and alive female *An. gambiae* s.l mosquitoes were morphologically identified to specie level at Centre for Infectious Disease Research laboratory, Bayero University, Kano at Aminu Kano Teaching Hospital. This was done using morphological characters of Gillies and Coetzee (1987) under x 20 Zeiss light microscope.

Preservation of Adult Mosquito

Dead and Live mosquitoes were preserved individually in 1.5 ml eppendorf tubes with silica gel to avoid Dna-cross contamination for further molecular identification (WHO, 1998).The tubes were labelled based on the tested insecticide and its concentration and whether the individual mosquito was dead or alive after post-exposure (WHO, 1998).

Mosquito Species Identification

Species and Molecular forms identification of members of *An. gambiae s.l* complex that are dead and alive after exposure to deltamethrin insecticide were confirmed using Polymerase Chain Reaction. Livak method was used to extract genomic DNA from mosquitoes (Livak, 1984). The species identity and the molecular forms were obtained according to the SINE 200 PCR method (Santomalazza *et al.*, 2004).

Interpretation of Bioassay Result

The status of the resistance mosquito samples from set 2 was determined based on CDC (Brogdon and Allister, 1998; Brogdon and Chan, 2010).

i. The susceptibility thresholds at 30 minutes diagnostic time for pyrethroids and carbamates are: 100% mortality indicates full susceptibility of the population.
ii. Less than 100% mortality indicates that the population is considered resistant.

Statistical Analysis

Binomial exact test of proportion was used to compare the percentage Mortality rate at 30 minutes. Pearson's correlation (r) was used to investigate the relationship between mortality rate of *An. gambiae* s.l mosquitoes of Auyo and Garunmalam Local Governments Areas before and after synergising with PBO. These were estimated using the SPSS statistical software (Version 20).

RESULT AND DISCUSSION

Table 1 shows mortality rate of *An. gambiae* s.l mosquitoes to the pyrethroid deltamethrin insecticides using CDC bioassay, and the result shows that in both Auyo and Garunmalam LGAs, *Anopheles gambiae* s.l. mosquitoes were both found to be resistant to deltamethrin insecticides with a mortality rate of 45.0% and 27.0% respectively.

Table 1: Mortality Rate of *An. gambiae* s.l Mosquitoes to Deltamethrin Insecticides using CDC Method

Localities	Insecticides	Number Tested	%Mortality at 30 min	RS	Resistance Status
Auyo	Deltamethrin	100	45.0	R	Resistance
Garunmalam	Deltamethrin	100	27.0	R	Resistance

RS- Resistance Status; S- Susceptible; R- Resistance

Table 2 shows result of mortality rate of *An. gambiae* s.l mosquitoes before and after synergizing deltamethrin insecticide with PBO. The result shows that after synergizing PBO with deltamethrin, the mortality rate were higher than the one recorded with deltamethrin alone with mortality rate increasing from 45.0% to 100% and 27.0% to 100% respectively.

Table 2: Mortality Rate of *An. gambiae s.l* Mosquitoes before and after synergizing Deltamethrin insecticide with PBO

Localities	Mortality at 30 min (Delta)	RS	%Mortality at 30 min (Delta+PBO)	RS	Resistance Status
Auyo	45.0	R	100	S	Resistance
Garunmalam	27.0	R	100	S	Resistance

RS- Resistance Status; S- Susceptible; R- Resistance

Table 3 shows result of mortality rate of *An. gambiae* s.l mosquitoes before and after synergizing deltamethrin insecticide with DEF. The result shows that the combination of DEF synergist with deltamethrin insecticide reveals a low gradual increase in mortality rate from 45.0 % to 62.0% in Auyo LGA and 27.0 % to 63.0% in Garunmalam LGA respectively.

Table 3: Mortality Rate of *An. gambiae* s.l Mosquitoes before and after synergizing Deltamethrin insecticide with DEF

Localities	%Mortality at 30 min (Delta)	RS	%Mortality at 30 min (Delta+DEF)	RS	Resistance Status
Auyo	45.0	R	62.0	R	Resistance
Garunmalam	27.0	R	63.0	R	Resistance

RS- Resistance Status; S- Susceptible; R- Resistance

Table 4.4: Shows that there was significant correlation ($P < 0.001$) between mortality rate of *An. gambiae* s.l mosquitoes collected from Auyo and Garunmalam LGAs after exposure to deltamethrin insecticides. Significant correlation ($P < 0.001$) was also noted between the two localities using PBO and DEF synergy.

Table 4: Relationship between Mortality Rate of *An. gambiae* s.l Mosquitoes of Auyo and Garunmalam LGAs using Deltamethrin Insecticides based on CDC and Synergist Assays.

Locality	r (Correlation coefficient)	r (Correlation coefficient)
Auyo and GarunMallam Deltamethrin	0.964	0.964
Deltametrin + PBO Auyo and GarunMallam	0.987**	0.987**
Deltametrin + DEF Auyo and GarunMallam	0.996**	0.996**

*, **- Significant *P < 0.05, ** P < 0.001

Table 5 shows result of species and molecular forms identification. Approximately 92.3% of the mosquitoes in this study were found to be *An. coluzzii* and 7.69% were found to be *An. gambaie* giles respectively. *An. coluzzii* were found to be more in abundance in Auyo LGA (53.84%) than in Garunmalam LGA (38.46%). On the other hand, *An. gambaie* giles were identified in Auyo LGA and none was detected in Garunmalam LGA.

Table 5: Species and Molecular Forms Identification of *An. gambiae s.l* mosquitoes

Localities	Species	No. Identified %	No. Identified (%)
Auyo	*An. coluzzii*	53.84	53.84
	An. gambiae giles	7.69	7.69
Garunmalam	*An. coluzzii*	38.46	38.46
	An. gambiae giles	0.00	0.00
	TOTAL=99.99		**TOTAL=99.99**

DISCUSSION OF RESULTS

Deltamethrin resistance observed among *An. gambiae* s.l in this study (Table 1) confirmed previously reported pyrethroid resistance from Central and South-Western Nigeria (Adeogun *et al.*, 2018; Awolola *et al.*, 2007). The result also corroborate with report from Lome and kovie areas of Southern Togo (Koffi *et al.*, 1997). Pyrethroid resistance may be due to frequent and indiscriminate use of pyrethroids for domestic, agricultural and for malaria vector control. Similarly exposure to Xenobiotic pollutants encountered by aquatic stages of natural populations of malarial vector species in agricultural and domestic environment often selected for resistance to various insecticides (Yayo *et al.*, 2020). The increased susceptibility of the mosquitoes to the pyrethroid suggested the possible implication of metabolic enzymes in resistance development.

PBO has been established as inhibitor of cytochrome P450s, an enzyme known to involve in pyrethroid metabolism. This result (Table 2) is in accordance with the report of Corbel *et al.* (2007) who reported the increased susceptibility of mosquitoes to pyrethroid following PBO exposure in Southern Benin. Other synergist S.S.S-Tributlyphosphorotrithioate is an inhibitor of another insecticide metabolising enzyme esterases. Exposures of Anopheles populations from Auyo and Garunmalam to this chemical were found to reduce resistance to deltamethrin insecticide (Table 3). This could be as a result of inhibitory effect of DEF on pyrethroids metabolising enzymes esterases. This is in accordance with the

work of Aizoun *et al*. (2013). However, metabolic based resistance is not always automatic as other studies reported insignificance association of pyrethroids resistance with cytochrome P450s and esterases. Some studies revealed presence of multiple resistance mechanism in some mosquito's population from southern Benin (Corbel *et al*., 2007).

Results of species and molecular identification revealed that *An. gambiae* s.l were identified in the study area as the major malaria vector. The predominance of *An. coluzzii* from collections in the field surveyed (Table 5) is in correspondence with the observation prior made by Ibrahim *et al.* (2014) in Auyo and Bunkure LGAs, who reported that *An. coluzzii* (Mopti chromosomal form) is more in abundance in an ecological area with a more permanent breeding site from the irrigation system. The result of this study is in contrast to what was found by Awolola *et al.* (2005) who find out that *Anopheles coluzzii* are found across the mangrove forest and transitional ecotypes with a pure collection of *An. gambiae* giles in the guinea and Sudan savannah part of Nigeria.

CONCLUSION

Based on these findings, it may be concluded that *An. gambaie colluzzii is* the dominant vector specie in the study area and are highly resistant to pyrethroids. Synergy assay revealed the probable roles of metabolic based resistance in the population.

REFERENCES

Aïzoun N, Aïkpon R, Padonou GG, Oussou O, Oké-Agbo F, Gnanguenon V, Ossè R, Akogbéto M (2013a). Mixed-function oxidases and esterases associated with permethrin, deltamethrin and bendiocarb resistance in *An. gambiae* s.l in the south-north transect Benin, West Africa. *Parasite Vectors*, ***6***:223.

Awolola, T.S., I.O. Oyewole, C.N.. Amajoh, E.T. Idowu, M.B. Ajayi, A. Oduola, O.U. Manafa, K. Ibrahim, L.L. Koekemor and M. Coetzee, (2005). Distribution of the molecular forms of *An. gambiae* s.l and pyrethroid knock down resistance gene in Nigeria. *Acta Tropica*, **95:** 204-209.

Awolola, T. S., Adeogun, A., Olakiigbe, A. K., Oyeniyi, T., Olukosi, Y. A., Okoh, H., ... and Amajoh, C. N. (2018). Pyrethroids resistance intensity and resistance mechanisms in *Anopheles gambiae* from malaria vector surveillance sites in Nigeria. *Plos one*, **13**(12), e0205230.

Brogdon W, Chan A (2010). *Guidelines for Evaluating Insecticides Resistance in Vectors using the CDC Bottle Bioassay/Methods in Anopheles research.* Second edition CDC Atlanta USA: CDC technical report. 343.

Brogdon WG, Mc Allister JC (1998): Simplification of adult mosquito bioassay through the use of time-mortality determination in glass bottles. *Journal of American Mosquito Control Association*, **14 (2)**: 159-164.

Brogdon WG, Mc Allister. JC. (1998) Insecticide resistance and vector control. *Emerging Infections Diseases*, **4**: 606-613.

Brogdon WG, MCAllister JC, Vulule J (1998). Heme peroxidise activity measured in single mosquitoes identifies individual expressing the elevated oxidase mechanism for insecticide resistance. *Journal of American Mosquito Control Association*, **13**: 233-237.

Coleman, M., Sharp, B., Seocharan, I., and Hemingway, J. (2006). Developing an evidence-based decision support system for rational insecticide choice in the control of African malaria vectors. *Journal of Medical Entomology*, **43**(4), 663-668.

Chouaïbou MS, Etang J, Brévault T, Nwane P, Kérah-Hinzoumbé C, Mimpfoundi R, (2008) The dynamics of insecticide resistance in the malaria vector *An. gambiae* s.l from an area of extensive cotton cultivation in Northern Cameroon, *Tropical Medicine and International Health*, **13**:1–11.

CDC (2010): Methods in *Anopheles* Research. Second Edition /CDC Technical Report, 343.

Corbel, V., R. N'Guesssan, C. Brengues, F. Chandre, L. Djogbenou, T. Martin, M. Akogbeto, J.M. Hougard and M. Rowland, (2007). Multiple insecticide resistance mechanisms in *An. gambiae* s.l and *Culex quinquefasciatus* from Benin, West Africa. *Acta Tropica*, **101**:207-216.

Diabaté A, Baldet T, Chandre F, Akogbéto M, Guiguemde TR, Darriet F.(2002). The role of agricultural use of insecticides in resistance to pyrethroids in *An. gambiae* s.l in Burkina Faso. *American Journal of Tropical Medicine and Hygiene*, **67**:617–622.

Das S, Garver L, Dimopoulos G (2007): Protocol for mosquito rearing (*An.gambiae*). *Journal of Visualized Experiments*, (issue 5) 221. doi: 103791/221.

Gillies, M. T. and Coetzee, M. (1987). A supplement to the *Anophelinae* of Africa south of the Sahara. *Pub. South African Institute of Medical Research*; **55**: 1-143.

Gimnig, J.E., Kolczak, M.S., Hightower, A.W., Vulule, J.M., Schoute, E., Kamau, L.,Phillips-Howard, P.A., ter Kuile, F.O., Nahlen, B.L. and Hawley, W.A. (2003). Effect of permethrin-treated bed nets on the spatial distribution of malaria vectors in western Kenya**.** *American Journal of Tropical Medicine and Hygiene*, **68:** 115-1.

Hemingway J, Ranson H. (2000) Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology*; **45**: 371–91.

Kimmage K, Adams WM (1990): Small-scale farmer-managed irrigation in Northern Nigeria. *Geoforum* , **21**(4):435-443.

Koffi, A. A., Darriet, F., N'Guessan, R., Doannio, J. M. C., and Carnevale, P. (1999). Évaluation au laboratoire de l'efficacité insecticide de l'alpha-cyperméthrine sur les populations d'*Anopheles gambiae* de Côte d'Ivoire résistantes à la perméthrine et à la deltaméthrine. *Bull Soc Pathol Exot*, **92**, 62-66.

Livak KJ (1984): Organization and mapping of a sequence on the Drosophila melanogaster X and Y chromosomes that is transcribed during spermatogenesis. *Genetics*, **107**(4):611-634.

Mawejje, H. D., Wilding, C. S., Rippon, E. J., Hughes, A., Weetman, D., and Donnelly, M. J. (2013). Insecticide resistance monitoring of field-collected An. gambiae s.l populations from Jinja, eastern Uganda, identifies high levels of pyrethroid resistance. *Medical and Veterinary Entomology*, **27**(3), 276-283.

Nkya TE, Akhouayri I, Kisinza W, David JP.(2013) Impact of environment on mosquito response to pyrethroid insecticides: facts, evidences and prospects. *Insect Biochemistry and Molecular Biology*;**43**:407–16.

Nkya, T. E., Akhouayri, I., Poupardin, R., Batengana, B., Mosha, F., Magesa, S., ... and David, J. P. (2014). Insecticide resistance mechanisms associated with different environments in the malaria vector *Anopheles gambiae*: a case study in Tanzania. *Malaria Journal*, **13,** 1-15.

Nkya T, Poupardin R, Laporte F, Akhouayri I, Mosha F, Magesa S.(2014). Impact of agriculture on the selection of insecticide resistance in the malaria vector *An. gambiae s.l*: a multigenerational study in controlled conditions. *Parasite Vectors*, **7**:480.

Perera MDB, Hemingway J, Karunaratne SP, (2008). Multiple insecticide resistance mechanisms involving metabolic changes and insensitive target sites selected in

anopheline vectors of malaria in Sri Lanka. *Malaria Journal*, **7**:168.

Robert V, Le Goff G, ArieyF, Duchemin JB (2002): A possible alternative method for collecting mosquito larvae in rice fields. *Malaria Journal*, .**1**:4.

Santolamazza F, della Torre A, Caccone. (2004). A Short report: A new polymerase chain reaction-restriction fragment length polymorphism method to identify *Anopheles arabiensis* from *An. gambiae* and its two molecular forms from degraded DNA templates or museum samples. *American Journal of Tropical Medicine and Hygiene*, **70**:604-606.

Sulaiman SI, Yayo AM, Tukur Z, Helen I, Charles SW (2014): High frequency of kdr L1014F is associated with pyrethroid resistance in *Anopheles coluzzii* in Sudan savannah of Northern Nigeria. *BMC infectious Diseases*, **14**:441.

WHO (1998): Report of the WHO Informal Consultation: Tests procedures for insecticide resistance monitoring in malaria vectors, bio-efficiency and persistence of insecticides on treated surfaces. Geneva: World Health Organization: *Parasitic Diseases and Vector Control, Prevention and Eradication, (CPE);* **43**. WHO/CDS/CPC?MAL/98.12.

World Health Organization (WHO). (2013). *World Malaria report.* In WHO Global Malaria Programme. Geneva, Switzerland.

World Health Organization. (2022). *World malaria report* 2022. World Health Organization.

Yadouléton A, Martin T, Padonou G, Chandre F, Asidi A, Djogbénou L.(2011). Cotton pest management practices and the selection of pyrethroid resistance in *An. gambiae s.l* population in Northern Benin. *Parasite Vectors*, **4**:60.

Yadouleton AW, Asidi A, Rousseau FD, Braïma J, Agossou CD, Akogbeto MC. (2009) Development of vegetable farming: a cause of the emergence of insecticide resistance in populations of *An. gambiae* s.l in urban areas of Benin. *Malar J* ;**8**:103.

Yayo A. M., Ado A , Safiyanu M, Hemingway J. (2020). Selection for and biochemical characterization of DDT resistance in laboratory strains of *Anopheles arabiensis*. *African Journal of Biotechnology* 1-10.

Yewhalaw, D., Wassie, F., Steurbaut, W., Spanoghe, P., Van Bortel, W., Denis, L., ... and Speybroeck, N. (2011). Multiple insecticide resistance: an impediment to insecticide-based malaria vector control program. *PloS one*, **6**(1), e16066.