INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 4, p. 61-70, 2022

RESEARCH PAPER OPEN ACCESS

Multiple insecticide resistance in *Aedes aegypti* populations from three different setting zones in Benin

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Key words: Resistance, *Aedes aegypti*, insecticides, Dandji, Awaya, Kaoura, Benin.

http://dx.doi.org/10.12692/ijb/21.4.61-70 Article published on October 09, 2022

Abstract

Multiple insecticide resistance in *Aedes aegypti* populations from three different setting zones (Dandji, Awaya and Kaoura) in Benin was evaluated from July-October 2021 where firstly adult females aged to 2-5 were subjected to susceptible test using impregnated papers (Permethrin 0. 25%; deltamethrin 0.03%; DDT 4%, and bendiocarb 0.1%) following WHO testing protocol. Moreover, biochemical analysis was done in order to detect Mixed Function Oxydase (MFO), non-specific esterase (NSE) and glutathione-S-transferases (GST) activity in individual 5 days old adult *Ae. aegypti* that had been reared from larvae and not previously exposed to insecticides. This research showed that the wild populations of *Ae. aegypti* populations from all the study sites were fully susceptible to bendiocarb (100% of mortality). However, we noticed that all the populations of *Ae. aegypti* had developed a strong resistance to DDT with average mortalities of 16%; 20% and 26% in Dandji, Awaya and Kaoura sites respectively. Moderate resistance profiles were recorded when these mosquitoes were exposed to permethrin with average mortalities of 48% ; 54% and 62% respectively in Dandji, Awaya and Kaoura sites. For deltamethrin, only populations of *Ae. aegypti* from Kaoura were fully susceptible to this insecticide. However, 78% and 84% average mortalities were recorded respectively in Dandji and Awaya. Enzymatic activities (Glutathione-s-transferase (GST) and P450 monooxygenase) in the wild population of *Ae. aegypti* were significantly higher than the control strain SBE ($P < 0,05$). This study provides clear evidence that there is a multiple insecticide resistance in the three wild populations of *Ae. aegypti* populations from our study sites. This will jeopardise the successful of the control of *Ae. aegypti* in these districts, however, the susceptibility results of the three populations to bendiocarb shows that this insecticide appears to be a good candidate to control these wild populations in case of outbreak of dengue fever.

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Introduction

One of the main issues with public health in recent decades has been arboviruses (Weetman *et al.,* 2018; Moyes *et al.,* 2017) . Due to the size of the worldwide burden it carries and the financial losses it causes for public health systems, dengue is at the top of the list of these (Kraemer *et al.,* 2015). Previously known as "tropical flu," "red fever," or "small malaria," it is a virus that infects people and is spread by the bite of an *Aedes* mosquito, a daytime mosquito that is also home to the Flaviviridae family of dengue viruses.

Dengue fever's global distribution has been continuously growing over the past 50 years, according to information from the World Health Organization (WHO, 2016). It is presently endemic in more than 100 nations and is rife in the intertropical zone, where it poses a risk to 2/5 of the world's population. There are five distinct stereotypes of its virus (DENV), numbered from DENV-1 to DENV-4 (Fried *et al.*, 2010). A wide variety of clinical symptoms caused by DENV infection can occur, from a mild flu-like condition to the potentially lethal dengue shock syndrome (Fried *et al.*, 2010).

More than 1,500 people have died as a result of dengue outbreaks recorded in sub-Saharan Africa during the past ten years in Gabon (Abe *et al.*, 2020), Côte d'Ivoire (Diakarida *et al.*, 2019), Nigeria (Fagbami *et al.,* 2015), Senegal (Gaye *et al.,* 2021). The most recent one occurred in Burkina Faso in 2018 and was marked by approximately 1045 cases and 26 fatalities (Ouédraogo *et al.,* 2018). The ecological circumstances in certain areas of Benin, particularly those of the Awaya region in the center of the country and the Dandji district in the south (famously known for the selling of used tires), continue to be favorable for the growth of *Ae. Aegypti*, the principal dengue vector. Without a doubt, research on the bioecology of this mosquito in Benin has revealed that it is a year-round pest in the country's north and south (Anges *et al.,* 2014). In addition, the discovery of the dengue stereotype 3 virus in Japanese visitors to southern Benin (Ujiie *et al.,* 2012), and report from Anges *et al.* (2014) in Ouèsse on the detection of type G immunoglobulins, and the most recent epidemic in 2019 (with a case of dengue hemorrhagic fever followed by the subject's death) are all indications of the virus' circulation in Benin.

Despite the recent availability of an approved dengue vaccine, its usage is still restricted and protection is insufficient (WO, 2021). Given this situation, the primary method of disease management and prevention remains insecticide-based vector control. Unfortunately, it is in danger since the vector has adapted to insecticides, which has caused the rise of resistant strains all across the world (Yougang *et al.*, 2020). The two primary resistance mechanisms involved are greater insecticide metabolism, mostly as a result of increased enzyme expression, and lower sensitivity to the insecticide through diminished interactions between the insecticide and the target site via mutations.

Pyrethroids have been used in Benin's agriculture since 1980. Therefore, it cannot be ruled out those certain insect populations, particularly *Ae. aegypti*, have not developed any evidence of resistance despite 40 years of usage. Additionally, because *Anopheles gambiae* and *Ae. aegypti* occasionally share the same breeding sites, it is likely that populations of *Ae. Aegypti* have developed resistance to the pesticides used to treat breeding sites and during indoor spraying operations. In order to be able to endure the numerous pesticide treatments brought on by the xenobiotic variables connected to their usage, it is therefore plausible that *Ae. aegypti* would have acquired a number of resistance mechanisms. To better inform future arbovirus control efforts in these 3 regions of Benin, there is a need to characterize levels of operationally-significant insecticide resistance, as well as the multiple insecticide resistance within *Ae. aegypti* populations from these three ecological areas in Benin.

Methods

Study area

The study was carried out in 3 zones in Benin. Dandji (southern Benin, 6° 24′ 18″N, 2° 22′ 31″ E) located in

urban area of Cotonou city with poor urbanisation facilities. Additionally, lots of second-hand vehicle tires from Europe and Asia, which constitute good breeding sites for *Ae. aegypti*. The locality of Awaya (central Benin, 7° 41′ 3.2″N, 2° 18′ 31″E) located in peri-urban area of Dassa city, presence of sparse forest with animals like bats, snakes and other small rodents was also for samples collection. The samples collection was extended to Kaoura (North-East of Benin, 1° 7′ 45″N, 2° 61′ 13.6″E) located in forest area of Kandi, presence of animals and birds with limited access to human activities. (Fig 1).

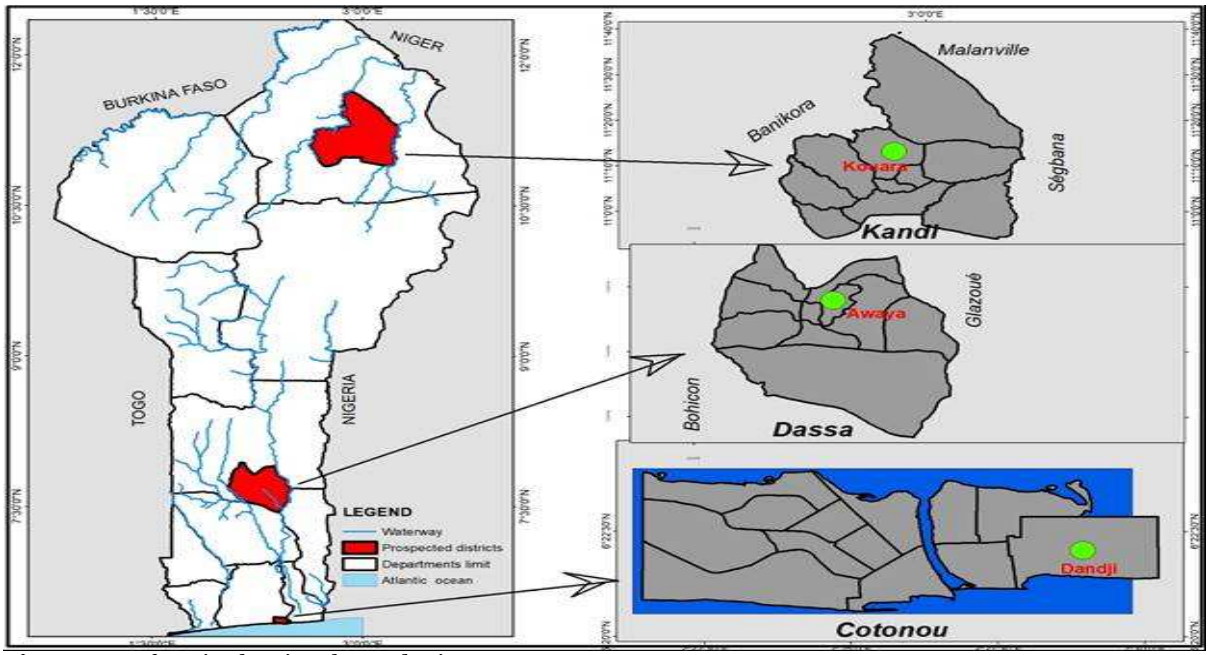

Fig. 1. Map of Benin showing the study sites.

In fact, the central and southern Benin are characterized by two rainy seasons (March- July and October-November) and two dry seasons (December-March and August-September). The mean annual rainfall is 1,500 mm in July and the temperature ranging from 23 to 32 °C. The north is characterized by a Sudanian climate with one rainy season (middle of May to October) and one dry season (November-May) with 1,300 mm as mean annual rainfall.

Mosquito collections

Larvae or pupae of *Ae. aegypti* at the 3 study sites were sampled from domestic water sources (e.g. jars, tanks), peri-domestic (e.g. tires) and natural sources (e.g. tree holes) during the rainy season and kept in plastic boxes and taken to the "Centre de Recherche Entomologique de Cotonou" (CREC) insectary for insecticide susceptibility tests.

Insecticide susceptibility test

Ae. aegypti bioassays were performed with non-blood-fed females following standards set in WHO guidelines (WHO, 2016). Tests were performed on 2-5 day-old unfed female from the first generation (F1). For each insecticide paper tested (deltamethrin (0.03%); permethrin (0.25%); DDT (4%); and bendiocarb (0.1%), four replicates of 20–25 *Ae. aegypti* were exposed for 1h. The number of knockdown (KD) mosquitoes were recorded every 10 min of exposure. For each assay, control mosquitoes were kept under similar testing conditions but with non-insecticide-impregnated papers. After 1hour of exposure, mosquitoes were transferred into holding tubes and provided with cotton wool soaked in a 10% honey solution. Mortalities were recorded after 24h and the susceptibility status of the bio-assayed mosquito population was graded according to the WHO recommended protocol (WHO, 2016). Dead and survived mosquitoes were separately kept on silica gel at -20°C for further molecular characterization. The reference laboratory strain from Benin (SBE) susceptible to all insecticides was used as

reference strain for resistance assays.

Biochemical analysis

Base on the protocol of Penilla *et al.* (1998), 80 females adults of *Ae. Agypti* 3-5 days kept at -80 degrees from each study were subjected to biochemical based on the methods to compare the levels of activity of mixed function oxidases (MFO), non-specific esterases (NSE) using α-naphtyl acetate as a substrate and glutathione S-transferases (GST) to the laboratory SBE susceptible reference strain. Individual mosquitoes were homogenized in 200 µl ml distilled water. Each of 10 ml of the homogenate was used for monooxygenase, glutathion S-transferase and protein assay. The other twenty µl ml of homogenate was used for esterases assay.

Glutathione -S-transferase (GST) assay

10 µl of each homogenate was transferred to a microplate well followed by 200 µl of the GSH/CDNB working solution which was prepared by adding 0.060g of glutathione solution(GSH) in 20 ml of Phospahte sodium buffer 0.1M and 0.013gr (in 1 ml of methanol) 1-chloro-2,4-dinitrobenzene (CDNB). The plates were read after 5 mins with the ELISA plate reader at a wave length of 340 nM.

Monooxygenase (Cytochrome p450)

Cytochrome P450 activity was determined using the heme-peroxidase assay according to the protocol described by David *et al.* (2013). Following the protocol described by Penilla *et al.* (2014) , this assay detects the elevation in the amount of heme, which is then converted into equivalent units of cytochrome P450. In addition to the protocol described by David *et al.* (2013), Eighty ml of 0.625 M potassium phosphate buffer (pH = 7.2) were added to 20 ml of mosquito homogenate together with 200 ml Tetramethyl Benzidine solution (0.011 g of 3,3',5,5' Tetramethyl Benzidine in 5 ml of 70% methanol +15 ml sodium acetate buffer 0.25 M pH = 5.0); 25 ml of 3% hydrogen peroxide were then added and the mixture was incubated for 30 min at room temperature base on the protocol described by Namountougou *et al.* (2012). The absorbance was read at 630 nm and values calculated from a standard curve of cytochrome C following the protocol described by David *et al.* (2013).

Esterase assay

20 µl of homogenated were placed in separate wells of microtitre plate. 200 µl of 0.3 mM Alpha/Beta napthyl acetate were added to each well. The plates were left at room temperature for 1 min and then added 50 µl of fast garnet. After 30 minutes, enzyme activity was determined as an *optical density* value by microplate reader at 450 nm.

Protein assay

The total protein content of individual mosquitoes was determined using the Bio –Rad Protein Assay Kit (Bio -Rad Laboratories) in order to detect the differences in size among individuals that might require correction factors for the enzyme assays

Data analysis

The populations of *Ae. aegypti* tested were classified as "resistant" if less than 90% mortality was observed, as "suspected resistant" if mortality rates were between 90% and 97% and "susceptible" for more than a 98% mortality rate (WHO, 2016).

The KD times for 50% and 95% of tested mosquitoes (KdT50 and KdT95) were estimated using a log-time probit model (Finney *et al.,* 1971) for pyrethroids and organochlorine. Biochemical assay data (enzymatic activity per mg protein, levels of MFO, NSE and GST between SBE and field populations of *Ae. aegypti* were compared using Mann-Whitney non-parametric U-test (Statistica software).

Results

Resistance to insecticides

A total of 400 females of *Ae. aegypti* collected from the 3 study sites Dandji, Awaya and Kaoura were exposed to papers impregnated with discriminating doses of permethrin (0.25%), deltamethrin (0.03%), DDT (4%) and bendiocarb (0.1%). Figures 2-4 show the insecticide resistance status of *Ae. aegypti* populations from the 3 study sites.

Table 1. Knock down time (KDT50 and KDT95) of *Ae. aegypti* populations from the 3 study sites during the 1h exposure to pyrethroids and organochlorines.

Sites	Ecological zone	Insecticide	Number tested	KDT50 [Cl95] (min)	KDT95 [Cl95] (min)
Dandji	Urban area	DDT	100	72.2 [57.5-83.4]	152.1 [118.4-228.1]
		Permethrin	100	39.3 [32.1-58.4]	112.1 [88.2-151.9]
		Deltamethrin	100	29.5 [24.1-52.1]	112.1 [88.2-151.9]
Awaya	Peri urban area	DDT	100	79.2 [67.5-89.6]	142.1 [108.4-208.1]
		Permethrin	100	37.8 [35.1-65.6]	109.3 [88.2-151.9]
		Deltamethrin	100	27.1 [17.4-25.6]	67.1 [53.2-87.4]
Kaoura	Forest area	DDT	100	68.7 [54.5-93.4]	152.1 [118.4-228.1]
		Permethrin	100	35.0 [28.7 – 47.6]	23.5 [18.4-35.8]
		Deltamethrin	100	14.3 [15.4-18.9]	25.6 [28.2-31.5]
Control (SBE)	Susceptible reference strain	DDT	100	14.4 [16.8-20.1]	18.9 [19.8-20.1]
		Permethrin	100	14.72 [14.18–17.89]	22.16 [20.56–26.80]
		Deltamethrin	100	12.38 [12.45–13.94]	24.91 [24.11–28.71]

The *Ae. aegypti* populations from all the study sites were fully susceptible to bendiocarb (100% of mortality). However, we noticed that all the populations of *Ae. aegypti* had developed a strong resistance to DDT with average mortalities of 16%, 20% and 26% in Dandji, Awaya and Kaoura sites respectively (Fig 2). Moderate resistance profiles were recorded when these mosquitoes were exposed to permethrin with average mortalities of 48% and 54% and 62% respectively in Dandji, Awaya and Kaoura sites (Fig 3).

For deltamethrin, only populations of *Ae. aegypti* from Kaoura were fully susceptible to this insecticide. However, 78% and 84% average mortalities were recorded respectively in Dandji and Awaya (Fig 4).

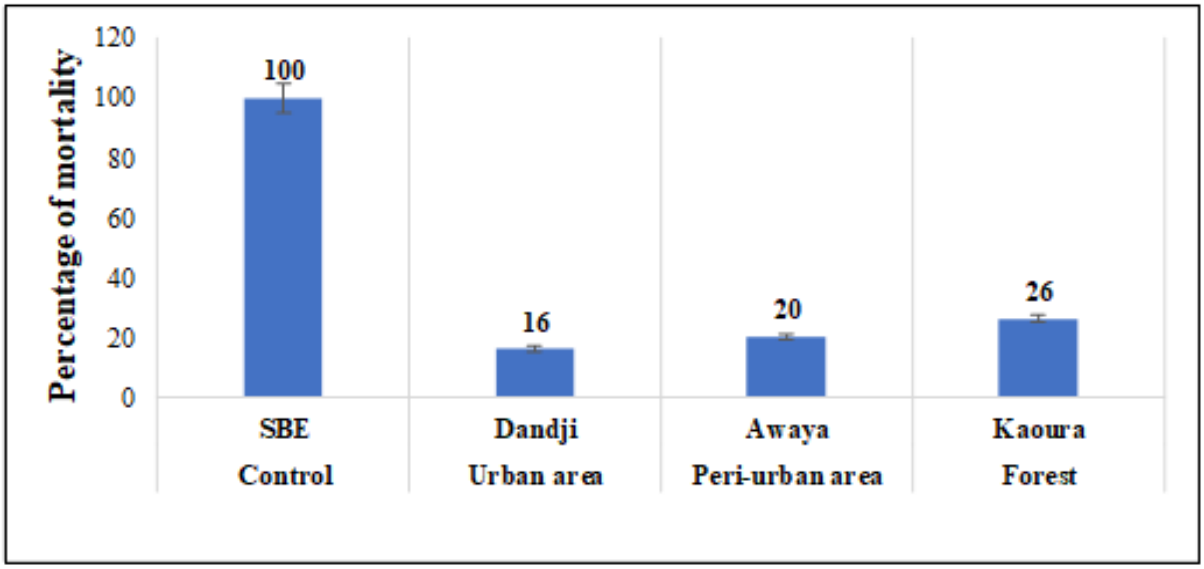

Fig 2. Results of bioassay tests of female *Aedes aegypti* from exposed to DDT.

Knock down effects

Table 1 shows the Knockdown time (KDT50 and KDT95) of *Ae. aegypti* populations from the 3 study sites during 1h exposure time to pyrethroids and organochlorines. For pyrethroids and DDT, the KdT50 and KdT95 values of the resistant populations were considerably higher 4 times compared to the control (P<0.05). However, there was no significant difference in the KDT50 and KDT95 values of the wild population of *Ae. aegypti* from Kaoura with deltamethrin compared to the control susceptible SBE strain (P>0.05).

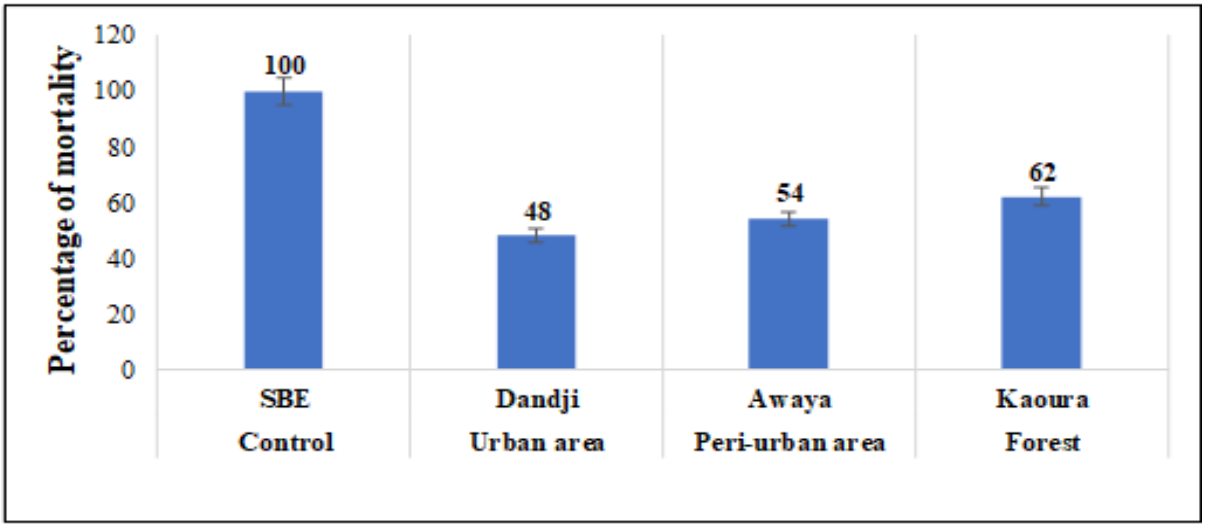

Fig 3. Results of bioassay tests of female *Aedes aegypti* from exposed to Permethrin.

Enzymatic resistance

Results from this study showed a significant high level of monooxygenase P450 (Figure 5) and Glutathione-S-Transferase (Fig 6) activities from the wild populations of *Ae. aegypti* from the three study sites compared to the susceptible SBE (P<0.05).

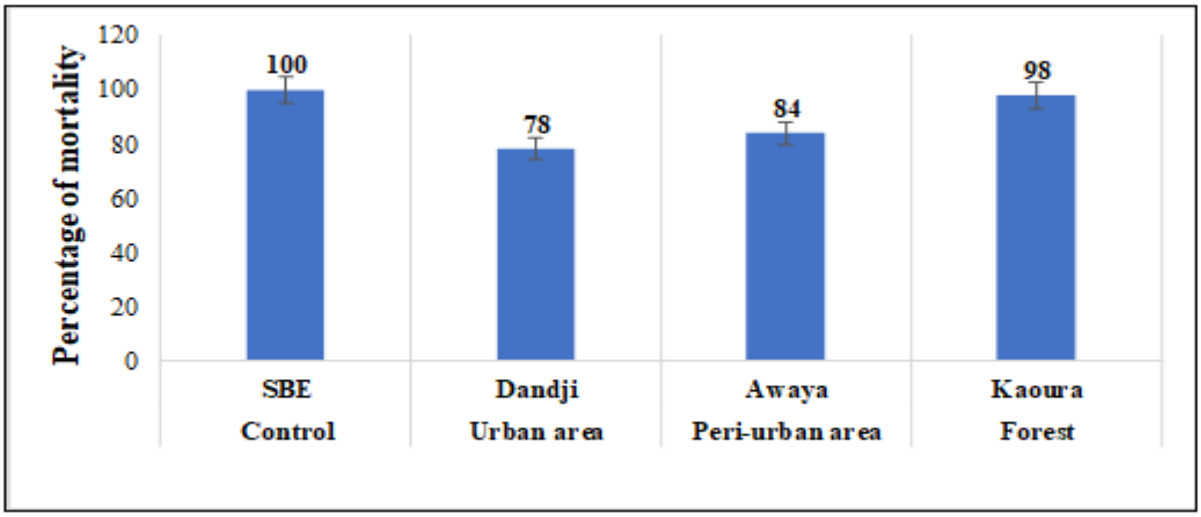

Fig. 4. Results of bioassay tests of female *Aedes aegypti* from exposed to deltamethrin.

However, there is no significant difference in the level of Esterase activity (α and β-Naphthyl) (Fig 7) from the wild populations of *Ae. Aegypti* from the 3 collection points of the study site compared to the susceptible SBE (P>0.05).

Discussion

The findings of our study indicate that *Ae. aegypti* is resistant to organochlorines and pyrethroids in the three study sites. *Ae. aegypti's* resistance to these insecticides is undoubtedly explained by the usage of

bombs and coils in Awaya, Dandji and Kaoura households to reduce culicidial and other insect pests, which is similar to the findings of Akogbéto *et al.* (2005). These authors showed that in Benin, the use of coils and bombs day and night is common to fight insects, especially mosquitoes. These coils and bombs are often composed of several mixtures of synthetic products belonging to the pyrethroid family.

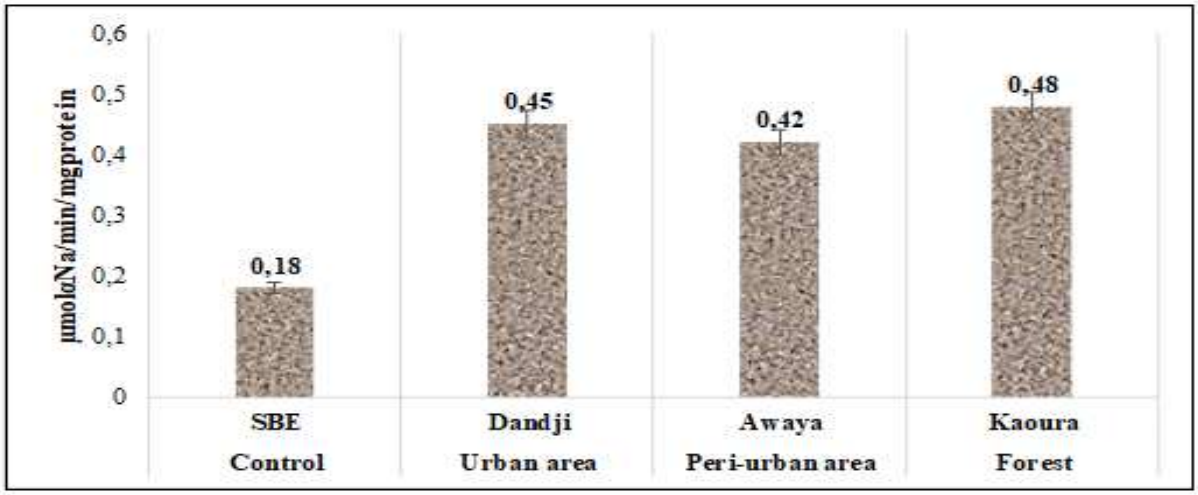

Fig 5. Mixed Function Oxidases activity of *Ae.aegypti* populations from the study sites compared to the control.

Because the impregnated materials that are currently the main tool available to national mosquito control programs in African countries have lost their chemical barrier properties, the study of resistance in dengue vectors, particularly the *Ae. aegypti* populations, is once again on the agenda in Africa. Admitting that pyrethroids, which are utilized in public health for impregnating LLINs, are also being used in agriculture against agricultural pests in an unregulated manner is the most coherent approach to explain this scenario.

Aedes spp. mosquitoes in particular have developed pesticide resistance as a result of this predicament. Since the 1990s, several reports have documented numerous instances of *Ae. aegypti* resistance.

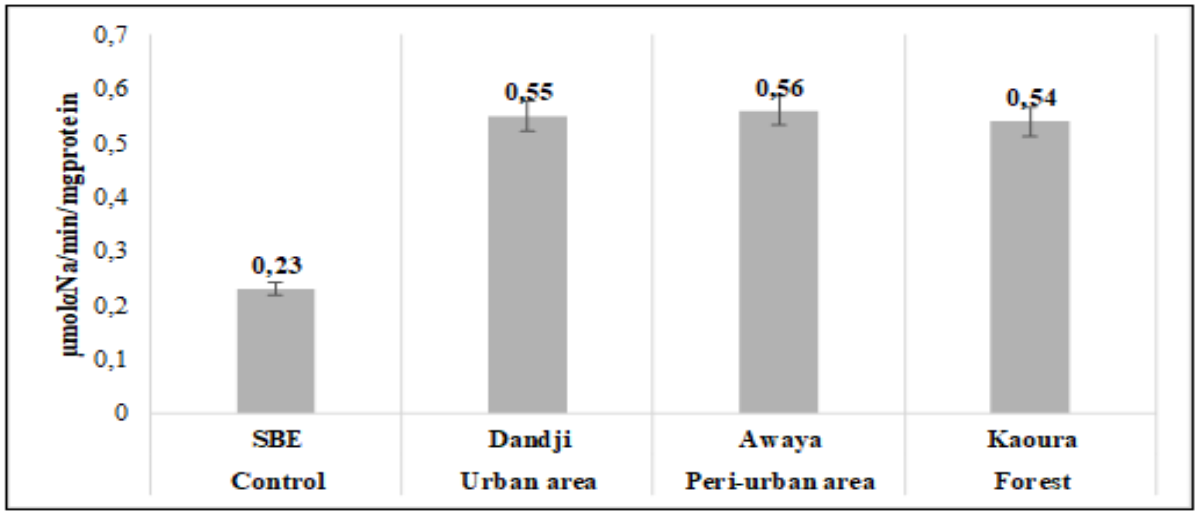

Fig. 6. Gluthation activity of *Ae.aegypti* populations from the study areas compared to the control.

The first cases were noted in Thailand with the appearance of resistance of *Ae. aegypti* to DDT and a year later to dieldrin (Ponlawat *et al.,* 2005). Over the last decade, the emergence of *Ae. aegypti* resistance to the various insecticides used in public health, in particular organochlorines and pyrethroids, has been

reported in several African countries such as in Gabon (Abe *et al.* 2020), Côte d'Ivoire (Diakarida *et al.,* 2019), Nigeria (Fagbami *et al.,* 2015), Senegal (Gaye *et al.,* 2021). This selection of *Ae. aegypti* for resistance to pyrethroids and organochlorines could be explained by agriculture practices where many insecticides are used for crop protection and where Ae. Aegypti has it breeding site. such activities in urban, peri urban areas and forest directly led to an improper use of insecticides to control vegetable pests, or other insects such as mosquitoes thus exerting a huge selection pressure on mosquito larval population. The susceptibility of *Ae. aegypti* to bendiocarb from the three study sites is a hopeful sign for the National Mosquito Control Programme, which had chosen this insecticide as an alternative to pyrethroids for indoor spraying in many districts of the country.

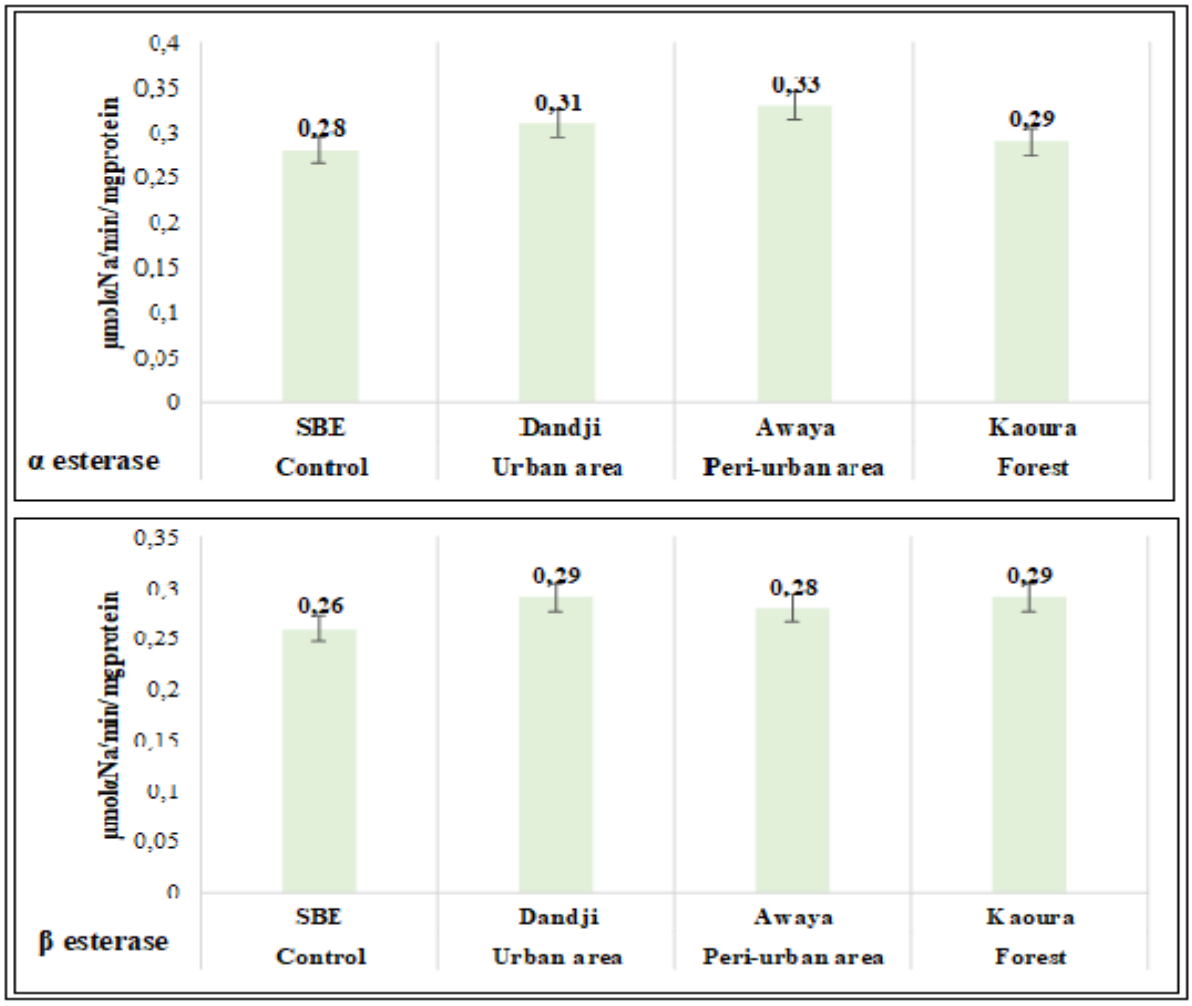

Fig. 7. Esterase activity of *Ae.aegypti* populations from the study site compared to the control.

Additionally, the high glutathione-S-transferase activity seen in *Ae. aegypti* wild populations from the three study sites supports the significant resistance of this mosquito to DDT shown in our research and is similar to the findings of Toé *et al.* (2022). The results of our phenotypic tests are further supported by the significant alpha and beta esterase activity seen in all *Ae. aegypti* populations at the three study sites. The widespread usage of pyrethroids in the three study sites is the sole explanation for the elevated esterase activity found in all *Ae. aegypti* populations there. Due to the high frequency of these metabolic genes in *Ae aegypti* populations, it would be crucial to measure them over time using the qPcr method. Our study has demonstrated for the first time in Benin the existence of significant esterase and glutathione-S-transferase (GST) activity in *Ae. aegypti* populations that are resistant to organochlorines and pyrethroids.

To further characterize *Ae. aegypti's* resistance to these two types of insecticides, it would be required to utilize the qPCR method to search for the different mechanisms which can explain the *Ae. Aegypti's* resistance to pyrethroids and DDT in these three study sites. It will be very useful for better controlling this vector in case of outbreak.

Acknowledgements

We are very grateful to the householders around all the different collection sites for their participation. We are also grateful to our lab technicians DANSI SOCLO Flocas, SAGBOHAN Zita and ZOLA Jacques for their help.

Conflict of interest

No conflict of interest to declare.

References

Weetman D, Kamgang B, Badolo A, Moyes CL, Shearer FM, Coulibaly M. 2018. *Aedes* mosquitoes and *Aedes*-borne arboviruses in Africa: current and future threats. Int J Environ Res Public Health **15,** 220.

Moyes CL, Vontas J, Martins AJ, Ng LC, Koou SY, Dusfour I, Raghavendra K, Pinto J, Corbel V, David JP. 2017. Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. PLOS Neglected Tropical Diseases **11,** e0005625.

Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W. 2015. The global distribution of the arbovirus vectors Aedes aegypti *and* Ae. albopictus. eLife **4,** e08347.

WHO. 2016. Monitoring and managing insecticide resistance in Aedes mosquito populations. Interim guidance for entomologists: World Health Organization.

Fried JR, Gibbons RV, Kalayanarooj S, Thomas SJ, Srikiatkhachorn A, Yoon IK, Jarman RG, Green S, Rothman AL, Cummings DA. 2010. Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. PLOS Neglected Tropical Diseases **4,** e617.

Abe H, Ushijima Y, Loembe MM, Bikangui R, Nguema-Ondo G, Mpingabo PI. 2020. Re-emergence of dengue virus serotype 3 infections in Gabon in 2016–2017, and evidence for the risk of repeated dengue virus infections. International Journal of Infectious Diseases **91(3),** 129–36.

Diakarida Fofana, Jean Michel Vianney Beugré, Genevieve Lydie Yao-Acapovi, and Sevidzem Silas Lendzele. 2019. Risk of Dengue Transmission in Cocody Abidjan, Ivory Coast). Journal of Parasitology Research **2,** 49-54.

Fagbami AH, Onoja AB. 2018. Dengue haemorrhagic fever: An emerging disease in Nigeria, West Africa. Journal of Infection and Public Health **11(6),** 757–762.

Gaye A, Ndiaye T, Sy M. 2021**.** Genomic investigation of a dengue virus outbreak in Thiès, Senegal, in 2018. Scientific Reports **11,** 10321.

Ouédraogo S, Benmarhnia T, Bonnet E, Somé P-A, Barro AS, Kafando Y. 2018. Evaluation of effectiveness of a community-based intervention for control of dengue virus vector, Ouagadougou, Burkina Faso. Emerging Infectious Diseases **24,** 1859–1867.

Anges Yadouleton, Ramziyath Agbanrin C. Vodounon G, padonou Badirou K. 2014. Seasonal distribution of *Aedes aegypti* in southern Benin: a risk of dengue virus transmission to urban populations. International Journal of Innovation and Applied Studies **9(2),** 648–654.

Ujiie Moi ML, Kobayashi T, Takeshita N, Kato Y, Takasaki T, Kanagawa S. 2012. Dengue virus type-3 infection in a traveler returning from Benin to Japan. Journal of Travel Medicine's **19,** 255-7.

World Health Organization. 2021. Global strategy for dengue prevention and control 2012–2020. 2021.

Yougang AP, Kamgang B, Bahun, TAW, Tedjou AN, Nguiffo-Nguete D, Njiokou F. 2020. First detection of F1534C knockdown resistance mutation in Aedes aegypti (Diptera: Culicidae) from Cameroon. Infectious Diseases of Poverty **9,** 152.

World Health Organization. 2016. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2nd ed. 48p. http://www.who.int/iris/handle/10665/250677.

Ridde V, Carabali M, Ly A, Druetz T, Kouanda S, Bonnet E. 2014. The need for more research and public health interventions on dengue Fever in Burkina Faso. PLOS Neglected Tropical Diseases **8,** e2859. http://dx.doi.org/10.1371/journal.pntd.0002859

Penilla RP, Rodriguez AD, Hemingway J, Torres JL, Arredondo-Jimenez JI, Rodriguez MH. 1998. Resistance management strategies in malaria vector mosquito control. Baseline data for a large-scale field trial against *Anopheles albimanus* in Mexico. Med Vet Entomol **12(1),** 217–233.

David JP, Ismail HM, Chandor Proust A, Paine MJ. 2013. Role of cytochrome P450s in insecticide resistance: Impact on the control of mosquito-borne diseases and use of insecticides on Earth. Philosophical Transactions of the Royal Society B: Biological Sciences **3681612),** 201-204.

Namountougou M, Simard F, Baldet T, Diabaté A, Ouédraogo JB, Martin T. 2012. Multiple Insecticide Resistance in *Anopheles gambiae* s.l. Populations from Burkina Faso, West Africa. PLoS ONE **7(11),** e48412.

Finney DJ. 1971. Probit analysis. Cambridge University Press Cambridge.

Akogbéto M, Djouaka R, Noukpo H. 2005. Use of agricultural insecticides in Benin. Bulletin de la Société de Pathologie Exotique **98,** 400–405.

Ponlawat J, Scott G, Harrington LC. 2005. Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. Journal of Medical Entomology **42,** 821-825.

Toé HK, Zongo S, Guelbeogo MW, Kamgang B, Viana M, Tapsoba M. 2022. Multiple insecticide resistance and first evidence of V410L kdr mutation in Aedes (Stegomyia) aegypti (Linnaeus) from Burkina Faso. Medical and Veterinary Entomology **36(3),** 309–319.