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Resistance Profile of *Anopheles gambiae s.l.* to Insecticides in the Atlantic, Littoral and Oueme Departments of Benin in West Africa

Tatchémè Filémon Tokponnon[1,2,3*], Razaki Ossè[2,4], Justin Kossou[1,2], Rébecca Abadame[1,2], Calmette Nouwagbe[1,2], Odiline Gnacadja[1,2], Abdoul Radji[1,2], Steve Houngbe[2], Casimir Kpanou[2], Hermann Sagbohan[2], Juvenal Ahouandjinou[2], Linda Towakinou[2], Brunelle Agassounon[1,2], Zoulkifilou Sare Dabou[1,2], Lamine Baba-Moussa[5], Germain Gil Padonou[2,5], Martin Akogbeto[2]

[1]Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi, Abomey-Calavi, Bénin
[2]Centre de Recherche Entomologique de Cotonou (CREC), Cotonou, Benin
[3]Société de Biologie Médicale (SoBioMed), Cotonou, Benin
[4]Ecole de Gestion et d'Exploitation des Systèmes d'Elevage, Université Nationale d'Agriculture, Kétou, Benin
[5]Faculté des Sciences et Techniques, Université d'Abomey-Calavi, Abomey-Calavi, Bénin
Email: *filemont@yahoo.fr

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Abstract

Context: Malaria is one of the deadliest diseases in sub-Saharan Africa. Mosquitoes of the genus Anopheles are the main vectors of malaria. Several methods have been implemented to combat these mosquito vectors, including vector control. This relies on the use of long-lasting insecticide-treated mosquito nets (LLINs) and indoor residual spraying (IRS). In order to update data on the emergence of resistance in vectors, this study was conducted to assess the sensitivity of *Anopheles gambiae s.l.* to pyrethroids and chlorfenapyr in three departments in southern Benin. **Methodology:** Larval surveys were carried out in the communes of Sô-Ava, Ouidah, Cotonou, Adjarra and Adjohoun in December 2022. The larvae obtained were transported to and reared at the insectarium of the Centre de Recherche Entomologique de Cotonou (CREC). Three- to five-day-old adult females of *Anopheles gambiae s.l.* were subjected to susceptibility tests in WHO tubes with papers impregnated with deltamethrin (0.05%), permethrin (0.75%), and alpha-cypermethrin (0.05%) and also with the combination of the synergist PBO + deltamethrin and PBO + alpha-cypermethrin. Biological tests in CDC bottles were also carried out on these populations using chlorfenapyr (100 µg). PCR was then used to identify the species of the *Anopheles gambiae* complex, the frequency of the *L*1014*F and Ace*-1 *kdr* genes and finally the level of expression of detoxification enzymes (esterases, oxidases and Gluthation-S transferases) was determined for each commune. **Results:** *Anopheles gambiae s.l.* showed strong resistance to the pyrethroids used, with a mortality rate of less than 90%. On the other hand, absolute sensitivity to the synergists PBO + pyrethroids and to chlorfenapyr was recorded in all the areas studied. PCR revealed two species of the *Anopheles* complex, with *Anopheles coluzzii* predominating over *Anopheles gambiae* in the study areas. In all the sites studied, the frequency of the *kdr L*1014*F* resistance allele was very high, while the frequency of the *ace-1* resistance allele was low. Biochemical tests showed overexpression of oxidase, esterase and GST activity compared with the susceptible Kisumu strain. **Conclusion:** *Anopheles gambiae s.l* showed high resistance to the pyrethroids used and absolute sensitivity to PBO + pyrethroid synergists and chlorfenapyr. In addition, metabolic resistance and *Kdr L*1014*F* allele frequency were observed. This study therefore suggests that LLINs with dual active ingredients or with chlorfenapyr as the only active ingredient may provide better control of pyrethroid-resistant mosquitoes.

Keywords

Insecticides Resistance, *Anopheles gambiae s.l.*, Benin

1. Introduction

Malaria is an acute, febrile and potentially fatal human disease. It is caused by parasites of the genus *Plasmodium* transmitted by the bite of an infected female Anopheles mosquito. According to the WHO report, 249 million cases of malaria will be recorded in 2022, compared with 243 million in 2021 [1]. In Africa, 233 million cases of morbidity were recorded in 2022 [1], including 2,656,855 in Benin [2], compared with 1914 deaths. Due to ecological and climatic diversity, eleven parasitic species of the Plasmodium genus have currently been identified as responsible for malaria infection in humans [3]. Of these, *Plasmodium falciparum* remains the most virulent species, responsible for fatal forms of malaria. One of the methods used to prevent malaria is vector control.

Several classes of insecticides are used in Benin for this purpose. These include pyrethroids, organophosphates and carbamates. Mosquito nets are generally impregnated with pyrethroids, while non-pyrethroids (organophosphates and carbamates) are used for IRS campaigns. Because of the threat posed by insecticide resistance, there has been an urgent call for research into alternative insecticides to complement the control of resistant malaria vectors [4]. New classes of long-lasting insecticidal nets (LLINs) combining mixtures of insecticides with different modes of action acting on resistant vectors have been pre-qualified by the WHO and are already available on the market [5]. These new nets include the Interceptor G2 (IG2), which is impregnated with chlorfenapyr, a pyrole, and alpha-cypermethrin, a pyrethroid, and the other Royal Guard, which is impregnated with pyriproxyfen, a juvenile hormone, and alpha-cypermethrin [5]. Chlorfenapyr has already demonstrated its superior efficacy to standard LLINs alone, or combined with other classes of insecticides in entomological trials in West Africa [6]-[8]. This potential makes it a candidate for the control of vectors resistant to insecticides used in public health.

However, recent cases of chlorfenapyr resistance have been reported in some African countries. In Benin, Accrombessi *et al.* evaluated the efficacy of IG2 and Royal Guard nets compared with pyrethroids in Covè in 2023 through field trials. Studies are also underway and continue to be conducted on the efficacy of LLINs with dual active ingredients for continued vector control transmitted by pyrethroid-resistant vectors [9] [10].

With this in mind, for routine surveillance activities, this study was carried out to generate more data on the susceptibility of vectors to different standard and new-generation insecticides in targeted study areas in three departments of southern Benin.

2. Materials and Methods

2.1. Study Areas

The study was carried out in five communes in three different departments, namely Ouidah, Sô-Ava (Atlantic), Cotonou (Littoral) and Adjarra, Adjohoun (Ouémé). These different areas were chosen because of their insecticide resistance status, diverse ecology and the low level of urbanisation in some communes. Also in these communes, people protect themselves from mosquito bites by using impregnated mosquito nets, bombs, aerosols and coils. In addition, market garden areas have been developed, providing favourable larval habitats for the development of mosquitoes.

The commune of Ouidah covers an area of 364.00 km^2, between 6˚22'0" North, 2˚4'60" East and 6.36667 latitude and 2.08333 longitude. It is bordered to the south by the Atlantic Ocean, to the east by the commune of Abomey-Calavi, to the west by the commune of Grand-Popo and to the north by the communes of Kpomassè and Tori-Bossito. The commune of Sô-ava covers a total area of 218.00 km^2 and lies between 6˚42'43" North, 2˚29'38" East, latitude 6.46667 and longitude 2.41667. It is bordered to the north by the communes of Zè, Dangbo and Adjohoun, to the south by the commune of Cotonou, to the west by the commune of Abomey-Calavi and to the east by the lakeside commune of Aguégués.

The commune of Cotonou is the economic capital of Benin, covering an area of 79 km^2 (0.07% of the national territory) and represents the Littoral department, the smallest of Benin's twelve departments. Located at the intersection of the 6˚20 parallel North and the 2˚20' meridian East, it is bounded to the North by Lake Nokoué, to the South by the Atlantic Ocean, to the West by the commune of Abomey-Calavi and to the East by the commune of Sèmè-Kpodji. Overall, the temperature varies between 18˚C and 35˚C. Rainfall amounts to between 300 and 500 mm.

The commune of Adjarra (6˚32' north, 2˚40' east) is about 7 km from Porto-

Novo, about 38 km from Cotonou. It is bordered to the north by the commune of Avrankou, to the south by the commune of Sèmè-Podji, to the west by the commune of Porto-Novo and to the east by the Benin-Nigeria border. The commune of Adjohoun (6˚41'44" north, 2˚28'52"), in the south of the country, 32 km from Porto-Novo and 62 km from Cotonou.

These survey areas are shown on the map below (Figure 1).

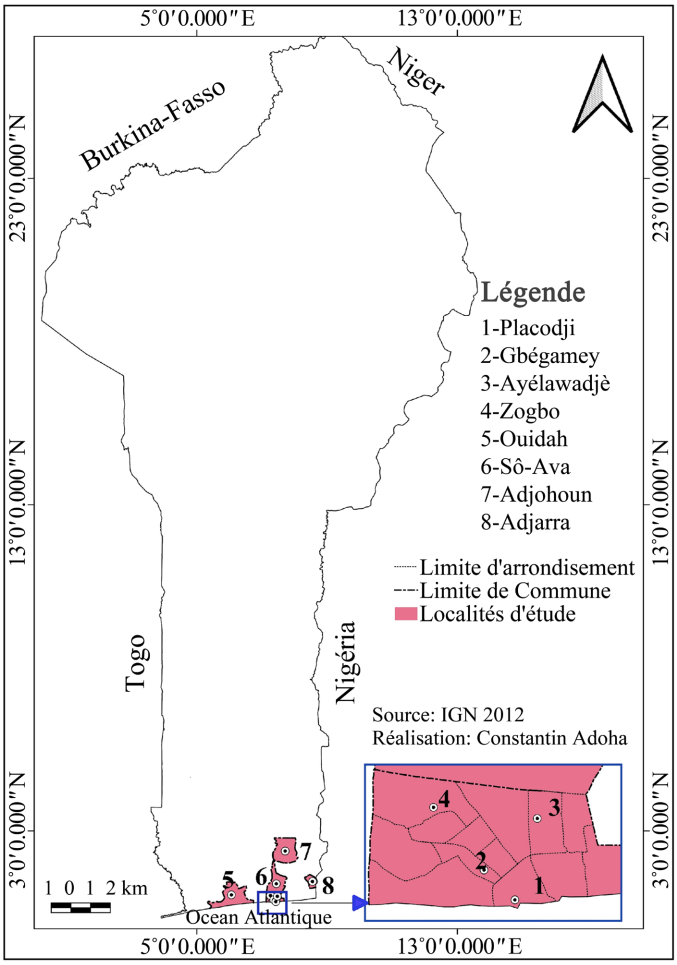

Figure 1. Map of larvae survey sites in the three departments of southern Benin.

2.2. Collection of Larvae

Larval surveys were carried out during December in the localities of Sô-ava, Ouidah, Placodji, Gbégamey, Ayélawadjè, Zogbo, Adjarra and Adjohoun in the various communes. The larvae collected were kept in labelled water tanks and sent to the insectarium of the Centre de Recherche en Entomologie de Cotonou (CREC) for rearing to the adult stage. Emerging adults were isolated from the others and fed with 10% sugar juice at a temperature of 27 ± 20˚C and a relative humidity of 75 ± 5% [11]. Morphologically identified females aged 3 to 5 days were used for sensitivity tests to various insecticides and for molecular and biochemical analyses.

2.3. Testing the Sensitivity of *Anopheles gambiae s.l.* to Insecticide

Non-gorged female mosquitoes aged between 3 and 5 days were used for the WHO tube sensitivity tests. The tests were carried out by exposing batches of 25 female mosquitoes to different doses of synergists and pyrethroids for 60 minutes. For all the tests carried out, the number of mosquitoes knocked out by the insecticide was recorded every 15 minutes. Around 25 mosquitoes exposed to unimpregnated paper served as controls. Biological tests in CDC bottles were carried out using a diagnostic dose of chlorfenapyr. After exposure, surviving mosquitoes were transferred to observation tubes where they were fed with a 10% sugar solution. Mortality rates were then determined according to the tests performed. Live and dead specimens from each locality were subjected to PCR for species identification and determination of resistance mechanisms (*kdr* and *ace-1R*) and enzymes. Table 1 shows the insecticides used and their recommended concentrations.

Table 1. Classes of insecticides tested and concentrations [12].

Classes of insecticides	Insecticides	Concentrations
Pyrethroids	Permethrin	0.75%
	Deltamethrin	0.05%
	Alpha-cypermethrin	0.05%
Pyrrole	Chlorfenapyr	100 µg
Synergists	Deltamethrin + PBO	0.05% / 4%
	Alpha-cypermethrin + PBO	0.05% / 4%

2.4. Identification of Species of the *Anopheles gambiae s.l.* Complex

A sample of 50 *Anopheles gambiae s.l.* mosquitoes per locality was selected from the populations tested and processed for molecular identification of the species *Anopheles gambiae s.l.* using the SINE PCR protocol described by Santolamazza and Wilkins [13] [14].

Molecular characterisation of *kdr (L*1014*F) and Ace*-1 resistance alleles

The *Kdr* Leu-Phe and Ace-1 G119S mutations were determined using the Martinez [15] and Weill [16] protocols respectively. After genotyping these mosquitoes, the allelic frequency of the two mutations within the mosquitoes was calculated.

2.5. Enzyme Tests

30 - 50 mosquitoes aged between 2 and 5 days and not exposed to insecticides from each target site were put to sleep and stored at −80˚C to be ground individually with 200 µl of water in a plate placed on ice to prevent degradation of the enzymes to be determined. The crushed material was then centrifuged at 12,000 rpm for two minutes. Using a multi-channel pipette, different quantities of the

supernatant were collected in two replicates in a 96-well plate for the determination of detoxification enzymes according to the protocol of Hemingway [17]. The enzymatic activities of each population were compared with those of the Kisumu strain.

2.6. Data Analysis

The criteria used to determine the resistance of *Anopheles* are those of the WHO presented in **Table 2** below.

Table 2. Determination of the prevalence of the resistance phenotype.

Mortality rate	Interpretation of results
Mortality ≥ 98	Sensitive
Mortality between 90 - 97	Possible resistance
Mortality < 90	Resistance confirmed

For the frequency of the L1014F *kdr* allele, we assessed the variability in the population using the following formula:

$F(R) = [(2nRR + nRS)/(2(nRR + nRS + nSS))] \times 100$ with R the frequency of resistance, n: the number of the corresponding genotype, RR: the homozygous resistance genotype, RS: the heterozygous resistance genotype and SS: the homozygous susceptibility genotype [18]. The Man-Whitney U test will be used to compare metabolic enzyme activity between the laboratory-susceptible strain (Kisumu) and those collected in the different health zones of Cotonou. The analyses were carried out using GraphPad Prism 5.

3. Results

3.1. WHO Tube Sensitivity Test

The results of the sensitivity tests on WHO tubes and CDC bottles are shown in the tables below:

Using the interpretation criteria for WHO tube sensitivity tests, *Anopheles gambiae s.l.* populations in the eight localities showed a mortality rate of less than 90% 24 hours after exposure to the diagnostic dose of permethrin (0.75%). This indicates a high level of resistance in these localities. Mortality rates ranged from 4% to 12% in Ayélawadjè, Gbégamey and Placodji, and from 15% to 35% in Adjarra, Adjohoun, Ouidah and Sô-Ava. Populations of *Anopheles gambie s.l.* in Adjarra, Adjohoun, Ouidah and Sô-Ava exposed to the synergist PBO + Permethrin showed a mortality rate of between 36.66% and 44.23%, while in Ayélawadjè, Gbégamey and Placodji, the rates varied between 17.6% and 44.23%. This could indicate the partial involvement of monooxygenases in the expression of the resistant phenotype observed and probably other resistance mechanisms at work in the populations tested (**Figure 2**).

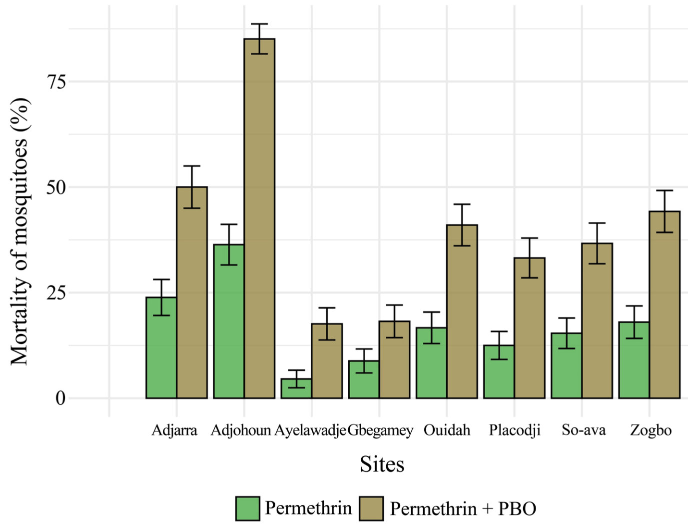

Figure 2. Graph of sensitivity tests in WHO tubes of *Anopheles gambiae* in the study areas after exposure to permethrin and permethrin + PBO.

Using the interpretation criteria for sensitivity tests in WHO tubes, *Anopheles gambiae s.l.* populations in the various localities showed a mortality rate of less than 90% 24 hours after exposure to the diagnostic dose of deltamethrin (0.05%). This indicates strong resistance in these localities. Mortality rates ranged from 18% to 25% in Adjohoun, Gbégamey and Placodji, while mortality rates in Adjarra, Ayélawadjè, Ouidah and Sô-Ava ranged from 27% to 63%. In addition, populations of *Anopheles gambiae s.l.* in Ayélawadjè, Gbégamey and Placodji exposed to the synergist PBO + Deltamethrin showed a mortality rate of between 94.11% and 100%, while for the communes of Adjarra, Adjohoun, Ouidah and Sô-Ava these rates varied between 79.2% and 100%. This indicates the inhibitory effect of PBO synergists on the target enzymes for detoxification of insecticides such as pyrethroids in certain localities (**Figure 3**).

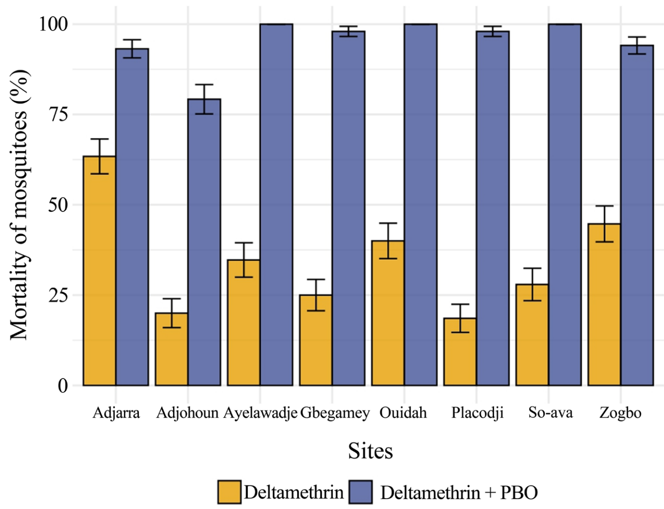

Figure 3. Graph of sensitivity tests in WHO tubes of *Anopheles gambiae* in the study areas after exposure to deltamethrin and deltamethrin + PBO.

According to the interpretation criteria for WHO tube sensitivity tests, *Anopheles gambiae s.l.* populations in the eight localities showed a mortality rate of less than 90% 24 hours after exposure to the diagnostic dose of alpha-cypermethrin (0.05%). This indicates a high level of resistance in these localities. Mortality rates ranged from 1% to 4% in Ayélawadjè, Gbégamey, Placodji and Zogbo, while mortality rates in Adjarra, Adjohoun, Ouidah and Sô-Ava ranged from 15% to 48%. In addition, populations of *Anopheles gambiae s.l.* in Ayélawadjè, Gbégamey, Placodji and Zogbo exposed to the synergist PBO + Alpha-cypermethrin showed a mortality rate of between 79.68% and 100%, while for the communes of Adjarra, Adjohoun, Ouidah and Sô-Ava these rates varied between 80.88% and 100%. This indicates the inhibitory effect of PBO synergists on the target enzymes for detoxification of insecticides such as pyrethroids in most localities (**Figure 4**).

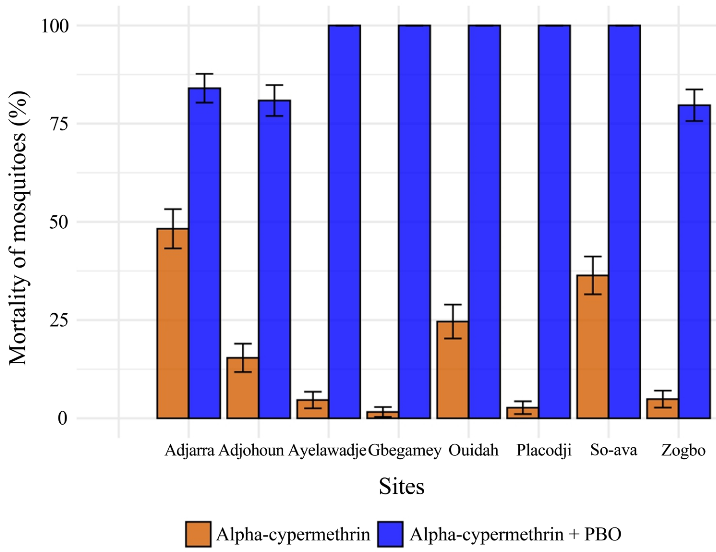

Figure 4. Graph of sensitivity tests in WHO tubes of *Anopheles gambiae* in the study areas after exposure to alpha-cypermethrin and alpha-cypermethrin + PBO.

In CDC bottle tests, *Anopheles gambiae s.l.* showed a mortality rate of 100% within 48 hours of exposure to the diagnostic dose of 100 µg/ml chlorfenapyr in all the localities studied with the exception of Adjohoun (98%). This proves the absolute sensitivity of these mosquitoes to chlorfenapyr.

3.2. Species Identification and Allele Frequency *Kdr-West* and *Ace-1R* Genes

The results of the species identification revealed two species of *Anopheles gambiae s.l* with a predominance of *Anopheles coluzzii* in Adjohoun (93.33%), Sô-ava (96.71%), Ouidah (96.55%) and Zogbo (90%). In Adjarra, Placodji, Gbégamey and Ayélawadjè, *Anopheles coluzzii* was the only species (100%) of the *Anopheles gambiae* complex recorded.

The results of the polymerase chain reaction (PCR) also showed a high frequency of the *Kdr-west* mutation in the eight localities; the allelic frequency for

Anopheles gambiae in Adjohoun, Sô-Ava, Ouidah and Zogbo was 100%, 75%, 100% and 83.33% respectively. The same result was observed for *Anopheles coluzzii* with Adjarra 85%, Adjohoun 73.21%, Sô-ava 85.41%, Ouidah 75%, Placodji 93.33%, Gbégamey 83.33%, Ayelawadje 85% and Zogbo 83.33%. On the other hand, the allele frequency of *Ace*-1 was very low for all species (*Anopheles coluzzii* and *Anopheles gambiae*). For example, the allele frequency of *Ace*-1 in *Anopheles coluzzii* in Adjarra, Sô-Ava and Gbégamey was 4%, 0% and 6.66% respectively. The frequency for *Anopheles gambiae* in Adjohoun was 50% (**Table 3** and **Table 4**).

Table 3. Statistics for the *Kdr* mutation.

Mutation	Localities	Species	Tested	Homozygote mutation (RR)	Heterozygote mutation (RS)	Homozygote wild type (SS)	Allele frequency R	Allele frequency S	pval_
L1014F	Adjarra	*An. coluzzii*	30	22	7	1	0.85	0.15	0.50
	Adjohoun	*An. coluzzii*	28	17	7	4	0.73	0.27	0.06
		An. gambiae	2	2	0	0	1	0	1
	So-ava	*An. coluzzii*	24	18	5	1	0.85	0.15	0.40
		An. gambiae	4	2	2	0	0.75	0.25	1
	Ouidah	*An. coluzzii*	28	17	8	2	0.75	0.25	0.57
		An. gambiae	1	1	0	0	1	0	1
	Placodji	*An. coluzzii*	30	27	2	1	0.93	0.07	0.10
	Gbegamey	*An. coluzzii*	30	21	8	1	0.83	0.16	1
	Ayelawadje	*An. coluzzii*	30	22	7	1	0.85	0.15	0.50
	Zogbo	*An. coluzzii*	27	19	7	1	0.83	0.17	0.55
		An. gambiae	3	2	1	0	0.83	0.16	1

Table 4. Statistics for the *ace*-1 mutation.

Mutation	Localities	Species	Tested	Homozygote mutation (RR)	Heterozygote mutation (RS)	Homozygote wild type (SS)	Allele frequency R	Allele frequency S	pval_
G119S	Adjarra	*An. coluzzii*	25	0	2	23	0.04	0.96	1
	Adjohoun	*An. coluzzii*	26	0	2	24	0.04	0.96	1
		An. gambiae	1	0	1	0	0.5	0.5	1
	So-ava	*An. coluzzii*	24	0	0	24	0	1	1
		An. gambiae	4	0	0	4	0	1	1
	Ouidah	*An. coluzzii*	28	0	3	25	0.05	0.95	1
		An. gambiae	1	0	0	1	0	1	1
	Placodji	*An. coluzzii*	28	0	0	28	0	1	1
	Gbegamey	*An. coluzzii*	30	0	4	26	0.07	0.93	1
	Ayelawadje	*An. coluzzii*	30	0	2	28	0.03	0.97	1
	Zogbo	*An. coluzzii*	27	0	1	26	0.02	0.98	1
		An. gambiae	3	0	0	3	0	1	1

3.3. Enzyme Tests

Biochemical tests showed that the activity of non-specific esterases (*α* and *β*) was significantly high in Sô-Ava, Ouidah, Adjarra, Adjohoun, Placodji, Zogbo, Ayélawadjè and Gbégamey compared with the Kisumu reference strain (P < 0.05). GST activity was high in Adjarra, Sô-Ava, Placodji (P < 0.0001) and Zogbo (P < 0.0005) compared with the Kisumu reference strain. In Ayélawadjè (P < 0.223), Adjohoun (0.8310), Ouidah (0.0384) and Gbégamey (P < 0.0721), this activity was similar to that of Kisumu. With regard to oxidases, the level of expression in the localities was significantly higher than the Kisumu reference strains (P < 0.0001) in Sô-Ava, Placodji, Ayélawadjè and Zogbo, Gbégamey and Adjohoun (0.0002). On the other hand, Ouidah (0.0062) and Adjarra (0.0506) were similar to Kisumu (**Figure 5**).

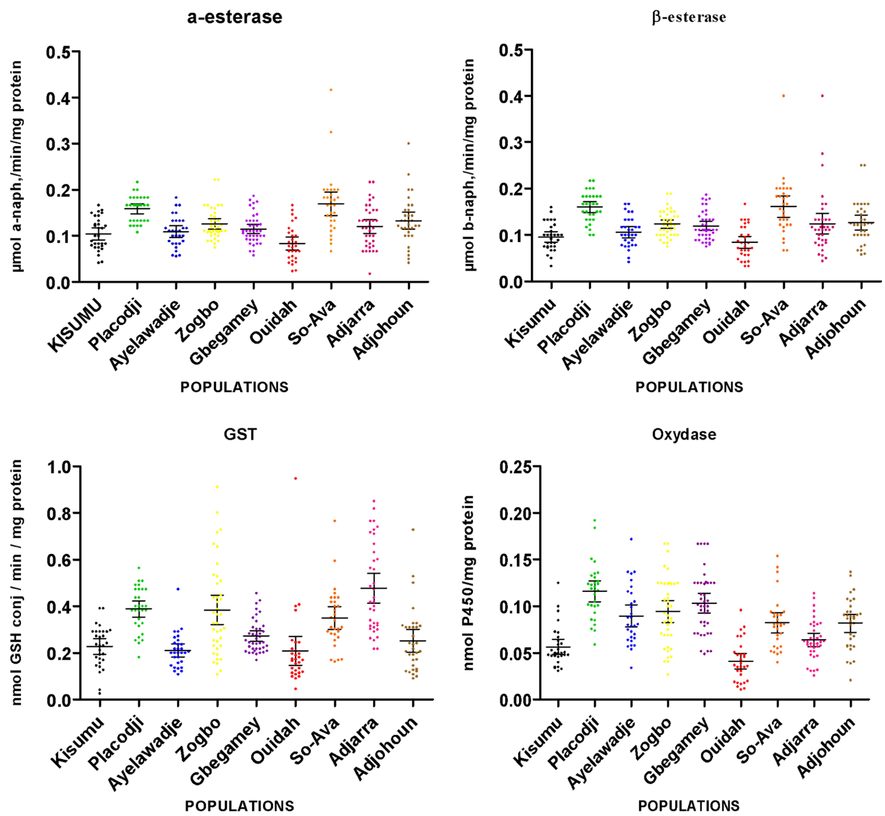

Figure 5. Graph showing the level of expression of non-specific esterases (alpha and beta), GSTs and oxidases in *Anopheles gambiae s.l.* in the study zones.

4. Discussion

The strong resistance of mosquitoes to insecticides has led the WHO to recommend entomological surveillance as an integral part of vector control management. This will enable updates to be made and informed decisions to be taken on the choice of insecticides. The study showed that *Anopheles gambiae s.l.* is highly resistant to pyrethroids, with a mortality rate of less than 90%. On the other hand, absolute sensitivity to PBO-pyrethroid synergists and chlorfenapyr was recorded in all the areas studied. Molecular analysis revealed two species of the *Anopheles* complex, with *Anopheles coluzzii* predominating over *Anopheles gambiae* in the study areas. In addition, in all the sites studied the frequencies of the *kdr L1014F* resistance allele were very high, while those of the *ace-1* resistance allele were low. Biochemical tests showed overexpression of oxidase, esterase and GST enzymatic activity compared with the sensitive Kisumu strain. High levels of resistance to permethrin, deltamethrin and alpha-cypermethrin were observed in *Anopheles gambiae s.l* in the study localities. These results could be attributed to the excessive use of insecticides and pesticides in vegetable-growing areas to control crop pests. This agricultural practice is a factor in the selection of resistant insects, not only among pests, but also among mosquitoes that transmit malaria. Previous studies by authors in Benin [18]-[20] confirm the presence of this resistance. In Côte d'Ivoire in 2020, Kouassi *et al.* [21] reported this selection of resistance within *Anopheles* populations. Similarly, Apetogbo *et al.* [22], in Togo in 2022.

However, populations of *Anopheles gambiae s.l* in contact with pyrethroids (deltamethrin and alpha-cypermethrin) combined with the synergist PBO showed total sensitivity with 100% mortality in most of the study areas. This synergist is therefore capable of neutralising insecticide detoxification enzymes in vectors using metabolic resistance. In addition, the appearance of a resistant phenotype detected in certain localities could be due to other resistance mechanisms in the population tested. These results confirm the recommendations made to the WHO on new insecticides to be incorporated into mosquito nets to reduce human-vector contact by the work of Oumbouke *et al.* [23] in 2019 and Sagbohan *et al.* in 2021 [18].

The CDC bottle tests carried out in this study showed absolute sensitivity (100%) of *Anopheles gambiae s.l* populations in the two areas studied to chlorfenapyr (100 µg), a pyrrole-based insecticide recently recommended by the WHO. These results confirm that there is no cross-resistance with other insecticides used in public health. It is one of the new recommendations for insecticides used to eliminate resistant malaria vectors. It was demonstrated by Oxborough *et al.* [24] in 2021 that the discriminating concentration for controlling resistant vectors was 100 or 200 µg/ml. However, resistance to chlorfenapyr (100 µg) was detected by Tchouakui *et al.* [25] in 2023 in *Anopheles gambiae s.l* in the Democratic Republic of Congo (Kinshasa), Ghana (Obuasi) and Cameroon (Mangoum and Nkolondom).

This study shows the predominance of *Anopheles coluzzii* over *Anopheles gambiae* in the study areas. This predominance is explained by the permanent nature of the breeding sites, which are always irrigated, which favours the proliferation of *Anopheles* [26]. This has been confirmed by previous studies carried out by the authors Ossè *et al.* 2023 [3], Tokponnon *et al.,* 2023 [27], Djègbè *et al.* 2011 [26], and Gnanguenon *et al.* 2015 [28] in southern Benin.

In addition to the high prevalence of insecticide resistance in *Anopheles gambiae s.l,* the *kdr-west* allele was found at high frequencies ranging from 73.21% to 100%. These results are very similar to those of Amoudji *et al.* 2019 [29], Djègbè *et al.* 2019 [30] and Kpanou *et al.* 2022 [11]. A study by Tokponnon *et al.* 2023 [27] revealed an allelic frequency of *kdr* mutations of 84% in the Ouémé region, 90% in the Atlantic region and 98% in the coastal region. This high frequency of *Anopheles gambiae s.l* in these departments could be explained by the selective pressure of insecticides as well as by protective measures taken against adult mosquito bites, agricultural insecticide run-off and the combined action of the two [31].

Pyrethroid resistance is also associated with a low and variable frequency of the *Ace-1* gene in the different localities, *i.e.* from 3% to 6% in the Atlantic and Littoral regions and from 3% to 50% in the Ouémé region. The high rate observed in Ouémé could be explained by the small sample size obtained during the study, which makes it impossible to confirm this increase with certainty. These results are in line with those reported by Salako *et al.* in 2018 [32] and Kpanou *et al.* in 2021 [33] showing that the frequency of the *Ace-1* gene is increasing in Benin. It is therefore imperative to carry out continuous monitoring of insecticides by identifying the insecticide associated with this resistance in order to monitor the evolution of the gene.

The biochemical data from this study revealed esterase overproduction in *Anopheles gambiae s.l.* in the localities of Sô-Ava, Ouidah, Adjarra, Adjohoun, Placodji, Zogbo, Ayélawadjè and Gbégamey. Overexpression of GST was also noted in Adjarra, Sô-Ava, Placodji and Zogbo. This could explain the insecticide sequestration mechanisms in which these enzymes are involved and hence the resistance conferred to pyrethroids in these mosquitoes. These data are consistent with previous work by [11] [18] [34]. Furthermore, our results also showed strong expression of oxidases in Sô-Ava, Placodji, Ayélawadjè, Zogbo, Gbégamey and in Adjohoun. These data demonstrate their involvement in the detoxification of pyrethroids [32] [33] to which mosquitoes are resistant. The level of expression of these detoxification enzymes should be a warning sign for all those involved in vector control. New, more effective strategies must therefore be adopted for the rational use of pesticides, both in agriculture and in public health.

5. Conclusion

This study confirms the existence of resistance in *Anopheles gambiae s.l.* mosquitoes to pyrethroids (deltamethrin, permethrin and alpha-cypermethrin) and absolute sensitivity to chlorfenapyr and pyriproxifen. It also corroborates previous studies on the fact that *Anopheles coluzzii* is the predominant species in all these localities, but cohabits with *Anopheles gambiae.* PCR showed that the allelic frequency of the *kdr* L1014F mutation is increasing, with overexpression of esterases, GSTs and oxidase, implying metabolic resistance.

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Authors' Contributions

TFT, RO, JK, RA, CN, OG, AR and MA designed the research; TFT, SH, JK, RA, CN, OG, AR and CK conducted data collection; all authors conducted data analysis. JA, LT, BA, LB, GGP, BA and JK coded the data; TFT, RO, JK, RA and ZSD led the drafting with substantive input from MA, JK BA and RO in the results section; all authors revised the manuscript.

All authors read and approved the final manuscript.

Ethics Approval

The study was conducted in accordance with the Declaration of Helsinki.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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