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Intensity and different genes involved in the resistance of *An. gambiae* s.l. to pyrethroids in four districts representative of the different agricultural production zones of North Benin, West Africa

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Abstract

The different methods used for the control of *An. gambiae* s.l. have led to the deployment of several defensive weapons by these vectors. Indoor residual spraying (IRS) implemented annually in the northern part of the country, and the national distribution of long-lasting insecticide-treated nets (LLINs) carried out every 3 years are the main malaria vector control tools deployed by the NMCP. The toxicity of insecticides in these tools results from their interaction with their biological target in the insect. Thus, for the effectiveness of vector control tools in an area, the knowledge of different defense weapons of *Anopheles* present in the area is essential.

The characterization of resistance mechanisms present on samples of pyrethroid-resistant *Anopheles gambiae* s.l. was carried out in four districts of northern Benin taking into account the different agricultural production zones. The different species, resistance gene and enzyme present in these districts were investigated.

High resistance of *Anopheles gambiae* s.l. in the four communes was observed, surviving a high frequency of the *kdr* resistance gene. Mosquito mortality rates ranged from 56.80% (95% CI: 50.51 - 62.95) in Kandi, to 70.24% (95% CI: 64.61 - 75.45) in N'Dali with deltamethrin, from 57.14% (95% CI: 51.04 - 63.09) in Kandi, to 74.80% (95% CI: 68.94 - 80.06) in N'Dali with permethrin, and from 59.63% (95% CI: 51.76 - 67.17) in Kandi, to 72.90% (95% CI: 66.24 - 78.89) in Parakou with alphacypermethrin. The lowest frequency of the *kdr* gene was observed in *An. coluzzii* 64.00% (95% CI: 56.90 - 70.60) in Malanville and the highest frequency in Kandi in *An. gambiae* 88.33% (95% CI: 82.70 - 92.60). It should also be noted that *An. gambiae* s.l. from these four districts also use detoxification enzymes to eliminate pyrethroid toxicity. These are: the communes of Parakou, Kandi and N'Dali for α and β esterases ($p\alpha < 0.05$ and $p\beta < 0.05$), Kandi, Malanville and N'Dali ($p < 0.0001$) for mixed function oxidases (MFOs), and the communes of Parakou, Kandi and N'Dali for glutathione-S-transferase (GST) activities ($p < 0.05$).

Three species of *An. gambiae* s.l. are collected in the communes and in all the agricultural production areas studied with a strong involvement of several resistance mechanisms (L1014F *kdr*, MFOs, Esterases and GST).

Keywords: Resistance, gene, pyrethrinoids, *Anopheles gambiae* and *Anopheles coluzzii*

Introduction

The different methods used for the control of malaria vectors have led to the deployment of several defensive weapons by the latter. Indeed, for several years, chemical control has occupied an important place in Benin among the many strategies put in place by the National Malaria Control Program (PNLP). Indoor residual spraying (IRS) implemented annually in selected areas of the country, and national distribution of long-lasting insecticidal nets (LLINs) carried out every 3 years are the main malaria vector control tools deployed by the NMCP.

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The toxicity of insecticides results from their interaction with their biological target in the insect. Thus, the insecticide molecule comes into contact with the insect, penetrates its body, and in some cases is transformed into active metabolites that are transported to their target [8]. Unfortunately, nowadays, vector control methods based on insecticide products used in public health or agriculture are the real reasons for the selection of resistant individuals in natural vector populations [8]. Population genetics teaches us that, with each generation, a new population of resistant individuals appears, which after crossing, gives rise to other resistant individuals. It is therefore not surprising that in Benin, *An. gambiae* s.l. has developed resistance to several classes of insecticide. Overall, we distinguish four types of vector resistance to insecticides: behavioral resistance, cuticular resistance, target site modification resistance, and metabolic resistance [27]. Behavioral resistance involves a change in the behavior of the insect, which is irritated by the insecticide and flies away more or less quickly from treated surfaces [18]. Cuticular resistance refers to any chemical modification of the insect cuticle leading to a reduction in the penetration of the insecticide into the organism, which results in a better survival of the individuals [3]. These different types of resistance mechanisms remain relatively difficult to study. However, resistance by modification of the target sites leads to a change in the conformation of the target protein of the insecticide. It is explained by the substitution of one or more amino acid(s) in the protein sequence of the target protein following a non-synonymous mutation [15]. Three main targets in the nervous system have been described in the literature: mutations in acetylcholinesterase (AChE), the GABA receptor, and the voltage-dependent sodium channel. In most cases, the site conferring resistance to one or more insecticide families is conserved in a large number of unrelated species [14, 15]. On the other hand, metabolic resistance is a phenomenon that results in a decrease in the amount of insecticide reaching the target and thus an increase in the tolerance of the insect. In mosquitoes, the analysis of the molecular basis of metabolic resistance has identified three major families of detoxification enzymes [Cytochrome P450 monooxygenases (CYPs), glutathione S-transferases (GSTs) and carboxylesterases (COEs)]. In order to support the NMCP in these vector control strategies, knowledge of the different resistance mechanisms used by malaria vectors in Benin is important in order to develop strategies for the operational effectiveness of control tools.

Materials and methods

Study sites

The data used in this manuscript were collected from October 2019 to December 2020. The characterization of resistance mechanisms present on samples of pyrethroid-resistant *Anopheles gambiae* s.l. was carried out in the communes of northern Benin, taking into account the different agricultural production zones for their heavy use of insecticide.

Parakou (Urban market gardening zone)

The urban market gardening zone in northern Benin is represented by the commune of Parakou where pyrethroids and organophosphates are widely used on market gardening perimeters and in domestic environments for protection against infectious mosquito bites. The Parakou district is characterized by a southern Sudanese climate with a dry season (November to April) and a rainy season (May to October). The average annual rainfall is 1200 mm [21].

N'Dali (Cereal production zone)

The cereal production zone is represented by the commune of N'Dali. The crops produced here are mainly maize, yams, cassava, soybeans, groundnuts and cowpeas, and two families of insecticides are used: pyrethroids and organophosphates. The N'Dali district has a Sudano-Sahelian climate with a dry season (November to March) and a rainy season (April to October). The average annual rainfall is 1030 mm [21].

Kandi (Cotton production zone)

The cotton zone represented by the district of Kandi is characterized by the heavy use of insecticides (carbamates, organophosphates and pyrethroids) against cotton pests. This commune in northern Benin is characterized by a dry season and a rainy season covering the periods from November to April and May to October respectively. The Kandi district has a Sudano-Sahelian climate with an average annual rainfall of 1030 mm [21].

Malanville (Rice production zone)

The rice-growing zone consists solely of the town of Malanville, which has a rice-growing area of about 70 hectares. This area is characterized by a Sudano-Sahelian climate with a dry season covering the period from November to April and a rainy season from May to October. The average annual rainfall is about 750 mm per year. Malanville is an area that borders the Niger River and, is subject to flooding during periods of high water, which allows for full-time rice production with heavy use of pyrethroids and organophosphates [21].

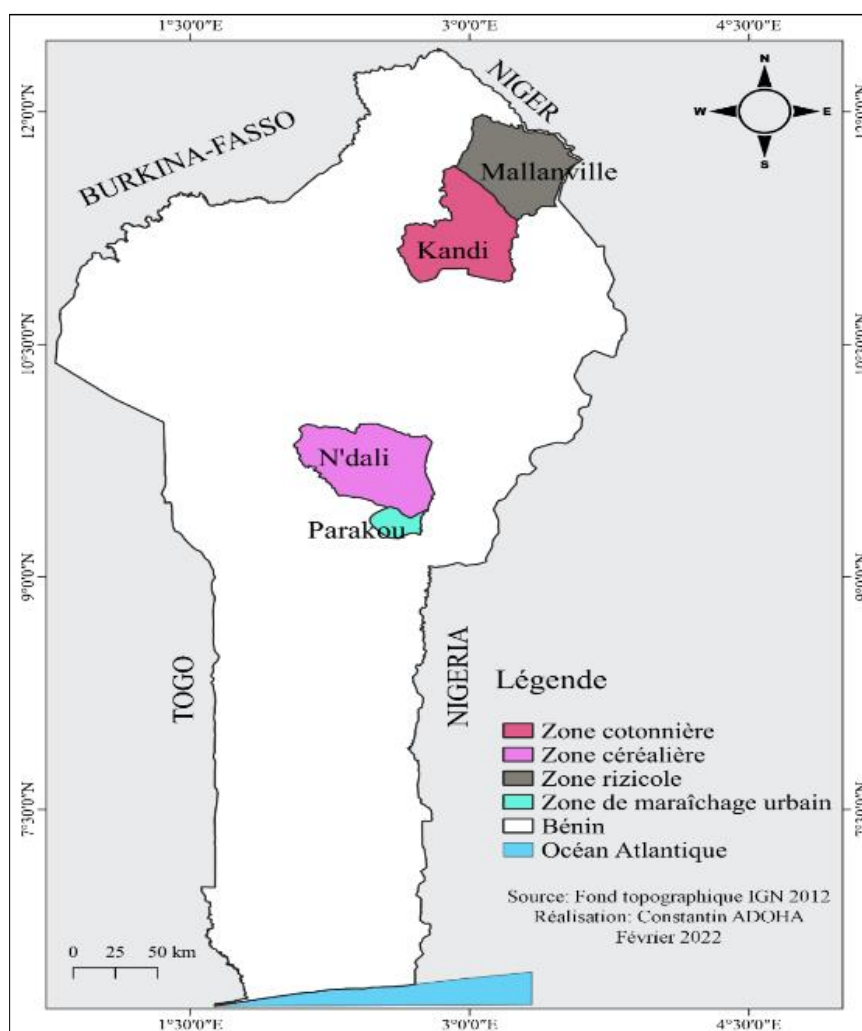


Fig 1: Map showing the different communes representative of the agricultural production zones of North Benin

Biological material

Larval survey and rearing of larvae

Larval surveys were carried out using a ladle, tubs, jars and a very fine mesh coffee filter in the four districts. Larvae from these surveys were taken to the larvarium of the Centre de Recherche Entomologique de Cotonou (CREC) where they were reared in the best conditions to optimize not only their growth but also to avoid cannibalism while feeding them cat food [24]. Each bin was covered with mosquito netting and stored in a room. Photoperiod was provided by fluorescent lamps illuminating from 6:00 PM to 6:00 AM the next day to accelerate larval development. Pupae were each time collected and placed in a 30cm square cubic cage in the insectarium to maximize the harvest of emerged adults. These caged adults were identified with a binocular magnifying glass using the identification key [16] and fed with honey juice (10%) before being used for sensitivity and biochemical tests.

Pyrethroid resistance intensity test

To assess the intensity of insecticide resistance, WHO tube susceptibility tests were performed by exposing 2- to 5-day-old populations of *An. gambiae* s.l. to permethrin, deltamethrin, and alphacypermethrin, following the WHO protocol [30]. For the present study, we considered mosquitoes that survived the 5x dose as hyper-resistant. Thus, the 5x dose of pyrethroids (Permethrin 3.75%, Deltamethrin 0.25% and Alphacypermethrin 0.25%) was the one used for the selection

of hyper-resistant *An. gambiae* s.l. The selected hyper-resistant *An. gambiae* s.l. were grouped in cages according to the insecticide. Phenotypically resistant individuals were hyper-resistant *An. gambiae* s.l. (survived) to the 5x dose of the pooled pyrethroids are the biological material of our study [30].

Polymerase chain reaction (PCR) for the identification of molecular species within the different populations of *An. gambiae* s.l.

After the sensitivity tests, the morphological similarity of the species of the *Anopheles* complex is the origin of the confusions made during the identification of the taxa constituting it. It is therefore necessary to complete the morphological studies by molecular analyses. Thus, molecular analyses were performed on 100 mosquitoes per commune to identify the different molecular species emerging during our larval collections. Dead and live mosquitoes from the WHO tube tests previously preserved in eppendorfs containing silica-gel and cotton were sampled and the different molecular species were searched by Polymerase Chain Reaction (PCR) following the protocol of Santolamazza *et al.*, [28]. The PCR diagnostic approach used to identify the species is based on the specific and irreversible insertion of a 230 bp transposable element (SINE200) on the X chromosome of *Anopheles coluzzii* (M form) while it is absent in its twin *Anopheles gambiae* (S form). This genetically inherited feature allows

unambiguous, simple and direct recognition of the M and S molecular forms [28].

Polymerase chain reaction to identify in *An. gambiae* s.l. the *kdr* L1014F mutation involved in pyrethroid resistance

Molecular analyses were performed on 100 mosquitoes per commune to identify the different genes involved in the resistance of *An. gambiae* s.l. to insecticides. Thus, a subsample of dead and live mosquitoes obtained after exposure to the 5x dose of pyrethroids, previously preserved in eppendorf containing silica-gel and cotton was analyzed by the Polymerase Chain Reaction (PCR) technique to determine the presence of the *kdr* L1014F mutation following the protocol of Martinez-Torres *et al.*, [25].

Biochemical assays of detoxification enzymes in different populations of *An. gambiae* s.l.

In each commune surveyed, biochemical analyses were performed on at least 50 females of *An. gambiae* s.l. aged 2 to 5 days without contact with insecticide for the detection of different metabolic enzymes such as carboxylesterases (non-specific α and β esterases or COEs), cytochrome P450 monooxygenases (mixed-function oxidases or CYPs) and glutathione S-transferases (GSTs) involved in the resistance of *An. gambiae* s.l. to insecticides according to the protocol of Hemingway *et al.*, [19]. These females also come from the same population of mosquitoes used for sensitivity testing in WHO tubes in each commune. The results obtained were compared to those of the sensitive Kisumu laboratory strain of the same age (2 to 5 days).

Statistical analyses

The level of resistance of malaria vectors to the 5x dose of pyrethroids is determined according to WHO criteria [30]. When, the percentage of *An. gambiae* s.l. population mortality after 24 hours is between:

- [98-100] the intensity of resistance in the population of *An. gambiae* s.l. is low;
- [90-97] the intensity of resistance in the population of *An. gambiae* s.l. is moderate;
- [90-0] the intensity of resistance in the population of *An. gambiae* s.l. is very high.

The allelic frequencies of *kdr* L1014F were calculated as follows to assess their variability in the populations:

$$F(R) = [2n.RR + n.RS] / [2(n.RR + n.RS + n.SS)]$$

- n. is the number of mosquitoes of a given genotype;
- RR is the homozygous genotype of the resistant allele *kdr* L1014F;
- RS is the heterozygous genotype of the *kdr* L1014F,
- SS is the homozygous genotype of the sensitive allele *kdr* L1014F.

The exact binomial test was used to calculate confidence intervals for the frequencies of the *kdr* L1014F mutation.

Linear regression with analysis of variance was used to assess the variation in enzymatic activity of the different detoxification genes in each locality.

The Mann-Whitney U test was used to compare the metabolic enzyme activity between the sensitive laboratory strain (Kisumu) and those collected in the different surveyed communes.

Statistical analyses were performed using R 3.3.2 software.

Results

Intensity of resistance of *Anopheles gambiae* s.l. to pyrethroids

For this selection, *An. gambiae* s.l. adults were only exposed to 5x permethrin, alphacypermethrin and deltamethrin. Mortality in the control groups was <5%. For all surveyed communes, a total of 1117 *An. gambiae* s.l. females were exposed to Deltamethrin 5x, compared to 920 and 739 to permethrin 5x and alpha-cypermethrin 5x respectively. Overall, mosquito mortality rates varied: from 56.80% (95% CI: 50.51 - 62.95) in Kandi, to 70.24% (95% CI: 64.61 - 75.45) in N'Dali with deltamethrin, from 57.14% (95% CI: 51.04 - 63.09) in Kandi, to 74.80% (95% CI: 68.94 - 80.06) in N'Dali with permethrin, and from 59.63% (95% CI: 51.76 - 67.17) in Kandi, to 72.90% (95% CI: 66.24 - 78.89) in Parakou with alphacypermethrin. Overall, the trend observed, shows non-negligible proportions ($\geq 40\%$) of mosquitoes surviving 5x the dose of pyrethroid insecticides tested in all districts. Resistance of *An. gambiae* s.l. was very high in the different districts surveyed (Figure 2).

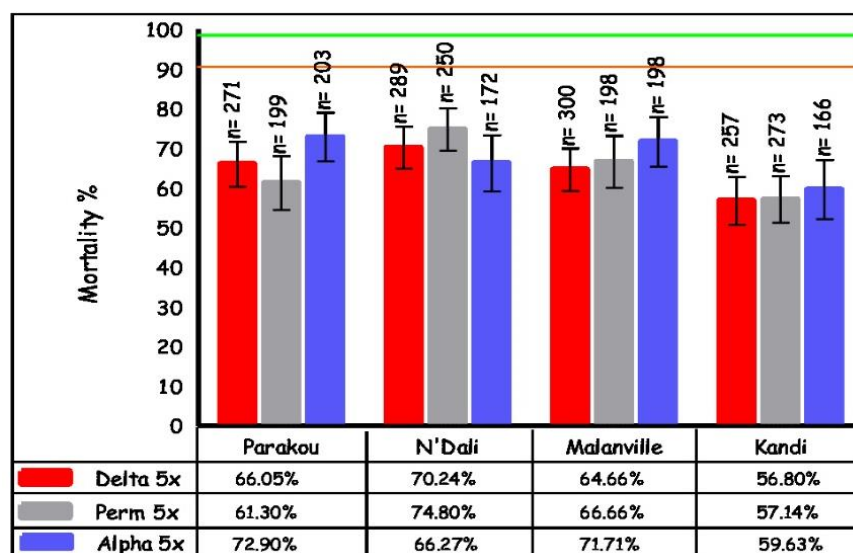


Fig 2: Mortality rate of *An. gambiae* s.l. mosquitoes after exposure to 5x dose of tested pyrethroid insecticides.

Molecular species within the different populations of *An. gambiae* s.l.

Overall, three molecular species were identified in all the communes surveyed along the South-North Benin transect: *An. gambiae*, *An. coluzzii* and *An. arabiensis*. Except for the commune of Malanville where all the Anopheles analyzed were coluzzii, an unequal distribution of *An. gambiae* s.l. was

observed with very high proportions of *An. gambiae* in the majority of communes. Thus, out of the 400 anopheles identified : *An. gambiae* is the predominant species with a percentage of 65.75% (263/400), followed by *An. coluzzii* with a percentage of 29.50% (118/400) and finally 19 *An. arabiensis* with a percentage of 04.75% (Figure 3).

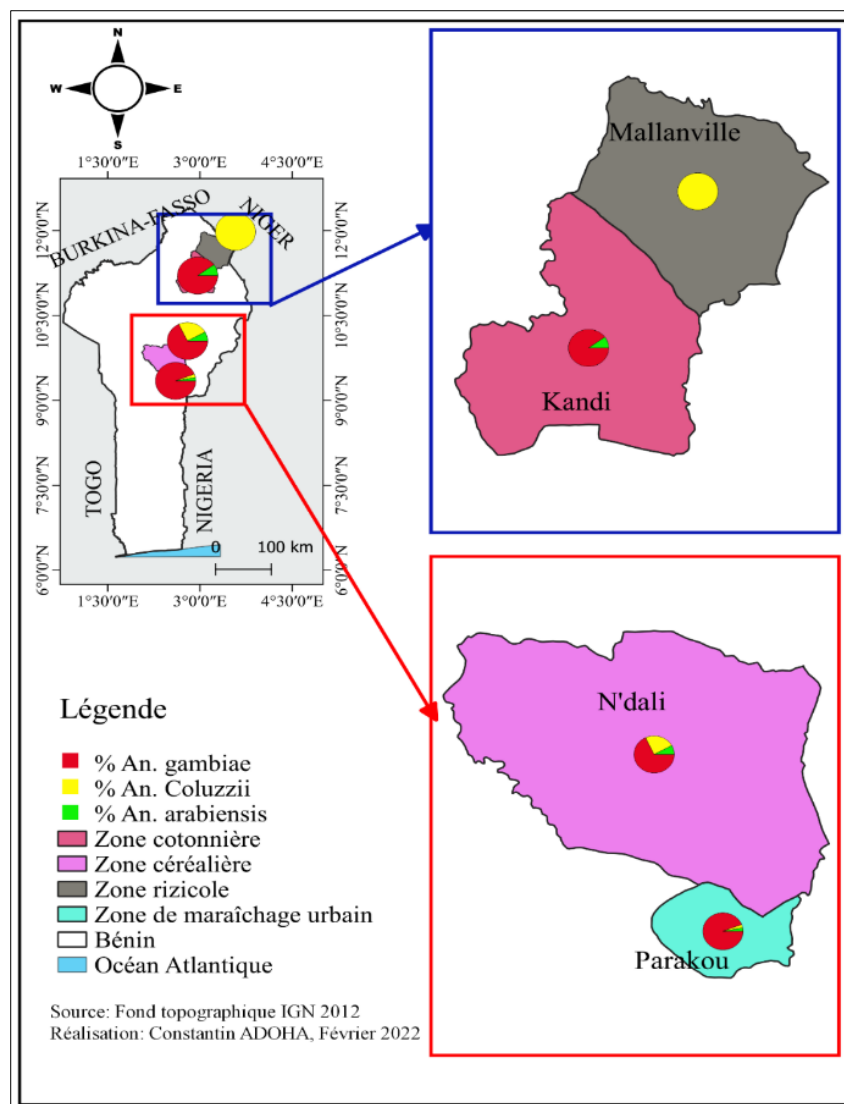


Fig 3: Distribution of the different molecular species of the *An. gambiae* s.l. complex in the districts of Benin (Parakou, N'Dali, Kandi and Malanville).

Frequency of the *kdr* L1014F mutation in *An. gambiae* s.l. in the different communes

The frequency distribution of the L1014F allele of the *kdr* resistance gene is high in all the communes surveyed during our study. This frequency of the L1014F mutation of the *kdr*

gene does not differ significantly from one species to another. The lowest frequency of the *kdr* resistance gene was observed in Malanville in *An. coluzzii* (64%; 95% CI: 56.9-70.6) and the highest in Kandi in *An. gambiae* s.s. (88.33%; 95% CI: 82.7 - 92.6) (Table I and Figure 4).

Table 1: Allelic frequencies of the *kdr* L1014F mutation in molecular species of the *An. gambiae* s.l. complex collected in different districts of Benin.

Districts	Species	N tested (%)	<i>kdr</i> L1014F genotypes			Fréq (%)	95%CI
			1014F 1014F	1014L 1014F	1014L 1014L		
Parakou	<i>An. arabiensis</i>	03	02	01	00	83.33	[35.9 - 99.6]
	<i>An. coluzzii</i>	03	02	00	01	66.66	[22.3 - 95.7]
	<i>An. gambiae</i> s.s.	94	60	27	07	78.19	[71.6 - 83.9]
	Total	100	64	28	08	78.00	[71.6 - 83.5]
Kandi	<i>An. arabiensis</i>	10	06	03	01	75.00	[50.9 - 91.3]

	<i>An. gambiae s.s.</i>	90	77	05	08	88.33	[82.7 - 92.6]
	Total	100	83	08	09	87.00	[81.5 - 91.3]
Malanville	<i>An. coluzzii</i>	100	45	38	17	64.00	[56.9 - 70.6]
	Total	100	45	38	17	64.00	[56.9 - 70.6]
N'Dali	<i>An. arabiensis</i>	06	04	01	01	75.00	[42.8 - 94.5]
	<i>An. coluzzii</i>	15	08	05	02	70.00	[50.6 - 85.3]
	<i>An. gambiae s.s.</i>	79	43	26	10	70.88	[63.1 - 77.8]
	Total	100	55	32	13	71.00	[64.1 - 77.2]
Study area	<i>An. arabiensis</i>	19	12	05	02	76.31	[59.7 - 88.5]
	<i>An. coluzzii</i>	118	55	43	20	64.83	[58.3 - 70.9]
	<i>An. gambiae s.s.</i>	263	180	58	25	79.46	[75.7 - 82.8]
	Total	400	247	106	47	75.00	[71.8 - 77.9]

N: number, *kdr*: knock down resistance, Freq: frequencies of the L1014F *kdr* mutation, CI: confidence intervals.

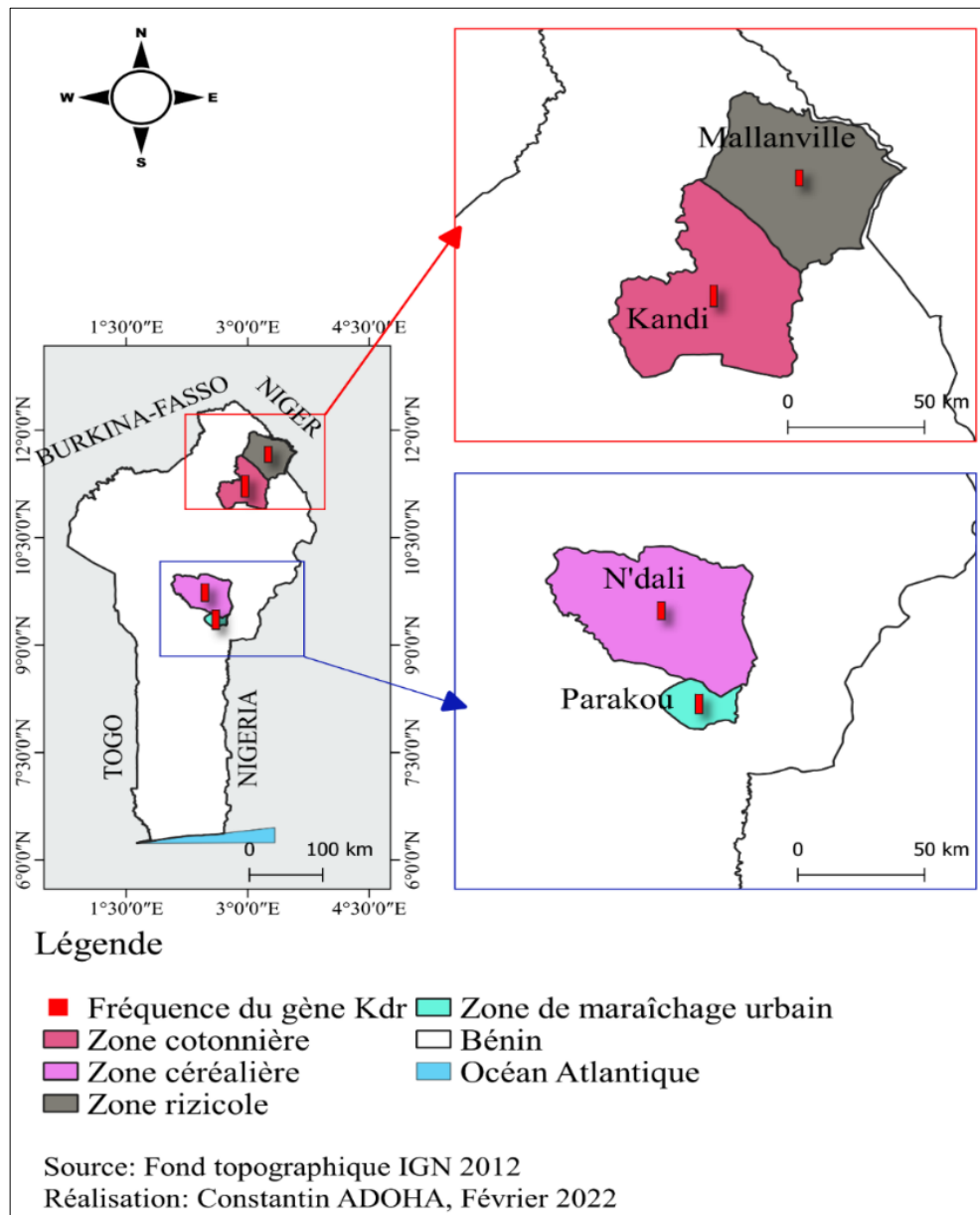


Fig 4: Map showing the proportion of the frequencies of the L1014F allele of the *kdr* gene in the different districts.

Allelic frequencies of the *kdr* L1014F mutation in dead and live *An. gambiae* s.l. to pyrethroids in different district

Regardless of the commune area, similar frequencies of the L1014F mutation of the *kdr* resistance gene were observed in dead and live specimens of *An. gambiae* s.l. obtained after exposure to deltamethrin 5x except in the communes of

N'Dali and Kandi where there was a significant difference between the frequencies of dead and live. Note that, the total frequency of the L1014F mutation of the *kdr* resistance gene is high in live *An. gambiae* s.l. to deltamethrin 5x than in dead *An. gambiae* s.l. to deltamethrin 5x (Table II).

Table 2: Allelic frequencies of the L1014F mutation of the *kdr* gene in dead and living specimens of *An. gambiae* s.l. in the different districts

Districts	Status	N tested	KDR L1014F Genotypes			Fréq (%)	95%IC
			1014F	1014L	1014L		
			1014F	1014F	1014L		
Parakou	Mort	50	28	14	08	70.00	[60,02 - 78,76]
	Vivant	50	36	14	00	86.00	[77,63 - 92,13]
N'Dali	Mort	50	18	21	11	57.00	[46,71 - 66,86]
	Vivant	50	37	11	02	85.00	[76,47 - 91,35]
Malanville	Mort	50	21	18	11	60.00	[49,72 - 69,67]
	Vivant	50	24	20	06	68.00	[57,92 - 76,98]
Kandi	Mort	50	36	07	07	79.00	[69,71 - 86,51]
	Vivant	50	47	01	02	95.00	[88,72 - 98,36]
Totale	Mort	200	103	60	37	66.50	[61,64 - 71,11]
	Vivant	200	144	46	10	83.50	[79,49 - 87,00]

N: number, *Kdr*: knock down resistance, Fréq: frequencies of the L1014F *kdr* mutation, CI: confidence intervals.

Enzyme activities of *An. gambiae* s.l. in the different populations

Compared to the susceptible strain from the Kisumu laboratory, nonspecific α and β esterases were overexpressed

in the *An. gambiae* s.l. populations from the communes of Parakou, Kandi, and N'Dali ($p\alpha < 0.05$ and $p\beta < 0.05$) (Figure 5).

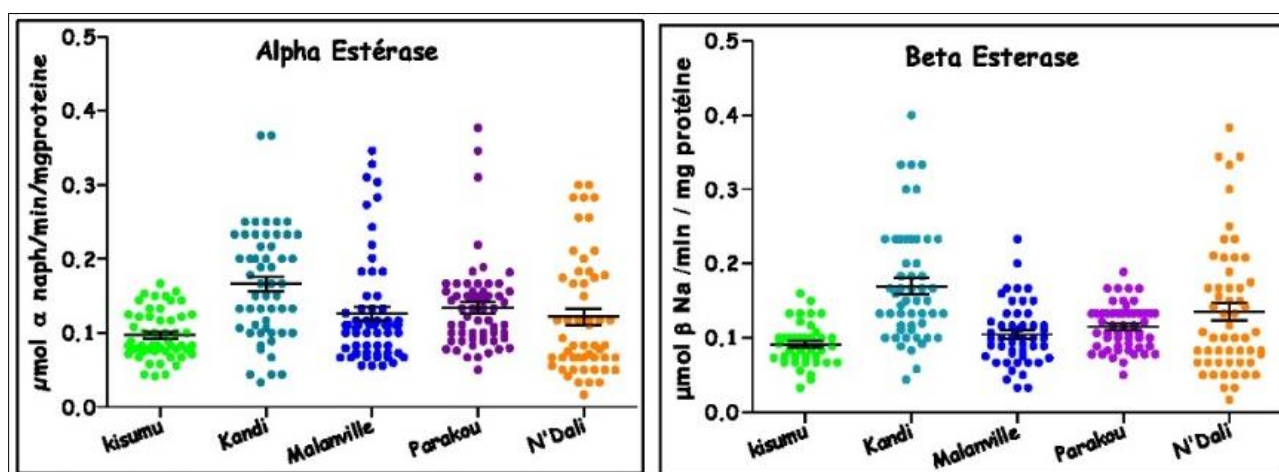


Fig 5: Activities of nonspecific esterases (α and β) in *An. gambiae* s.l. collared in different districts.

Concerning mixed function oxidases (MFO), an overexpression was observed in the populations of Kandi, Malanville and N'Dali ($p < 0.0001$) compared to the susceptible strain of Kisumu. Conversely, the expression of this enzyme in the commune of Parakou ($p > 0.05$) was similar to that of

the susceptible Kisumu strain (Figure 6). The mean glutathione-S-transferase (GST) activities observed in the Parakou, Kandi and N'Dali populations were higher than those observed in the susceptible Kisumu strain ($p < 0.05$) (Figure 6).

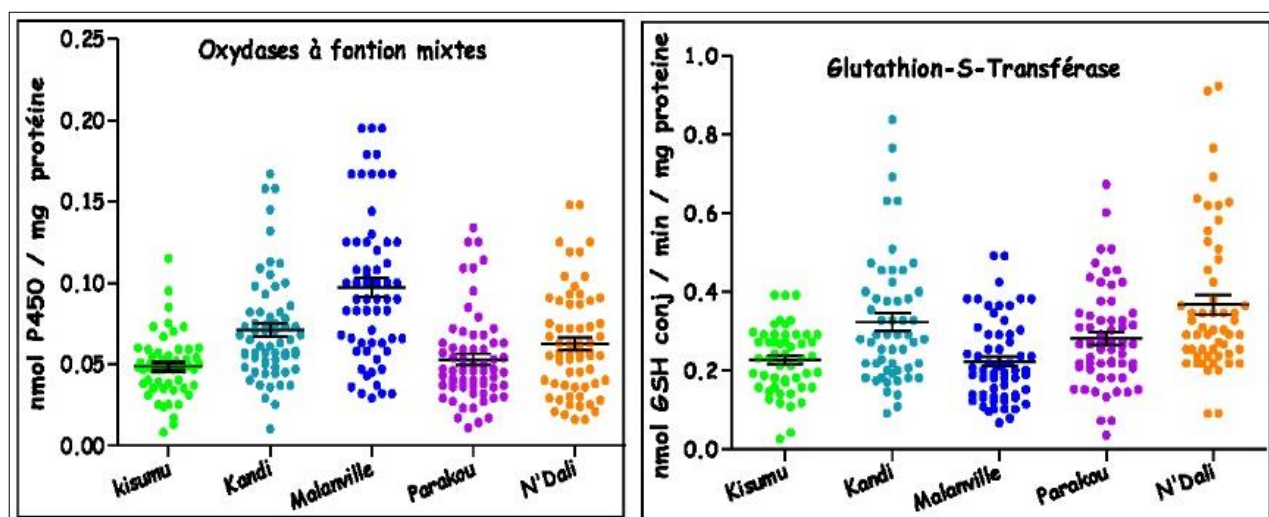


Fig 6: Activities of mixed-function oxidases, and glutathione-S-transferase in *An. gambiae* s.l. collared in different districts

Discussion

The data from the present study show the presence of three species of the gambiae complex in the different communes of Northern Benin. The distribution of these species in these communes is uneven with a predominance of *An. gambiae* s.s. in most communes except Malanville where all the species collected are *An. Coluzzii*. Overall, the cumulation of the different species in all the communes gave 65.75% of *An. gambiae*, 29.50% of *An. coluzzii* and finally 04.75% of *An. arabiensis*. These results corroborate the research work of Djogbenou *et al.*,^[12] conducted in 30 different localities in Benin and confirm the wide distribution of this Anopheles species (*An. gambiae* s.s.) in West Africa^[10, 12]. In 2014 in Benin, Aikpon *et al.*, in the department of Atacora (North - West Benin) and Gnanguenon *et al.*,^[17] along the South - North transect, more precisely in the localities of Allada, Dassa, Parakou, Kandi and Malanville collected mainly the species *Anopheles coluzzii* and *Anopheles gambiae* in variable proportions. Similar results were also obtained by Djègbè *et al.*, in 2011 in the communes of Malanville, Cotonou, Bohicon and Tori-Bossito^[11]. The high presence of *An. coluzzii* in the commune of Malanville and *An. gambiae* s.s. in the communes of Kandi, Parakou and N'Dali can be explained by the period and especially by the types of sites where these larvae were collected. According to Mbida *et al.*, *An. coluzzii* prefers permanent roosts while *An. gambiae* s.s. is infatuated with temporary ones^[26]. In the north, *An. gambiae* s.s. is the majority species collected during our study. The dominance of *Anopheles gambiae* s.s. in this area of the country is related to some extent to the terrain, which represents the most mountainous part of the national geography. A small rainfall in this area would allow the installation of temporary breeding sites because of the rocky structure of the soil and subsoil that would not favor the infiltration of rainwater. Previous work in this environment has well revealed that the predominance of *An. gambiae* and *An. coluzzii* species is variable and depends on the time of sample collection^[2]. These two sister species were found in almost all ecological facies even though seasonal variation was found due to the bio-ecology of each species. We can assume that the adaptation of *An. gambiae* and *An. coluzzii* to their different environments is the result of a very advanced speciation, confirming their status as a taxon in its own right.

A high frequency of the *kdr* L1014F mutation was observed in all communes and agricultural production zones of Benin. This frequency observed within *An. gambiae* s.l. varies from one commune to another and from one agricultural production zone to another. Ten years after the study by Djègbè *et al.*,^[11] an increase in *kdr* L1014F frequency was observed in Malanville from 40% to 64%. Despite this increase, the commune of Malanville still retains its status as a commune with a low frequency of the *kdr* L1014F mutation. The *kdr* Leu-Phe mutation, the main mechanism of resistance to pyrethroids in West Africa, was initially detected in cotton areas in populations of the S molecular form at relatively high frequencies, but is now almost fixed in this form and has started to expand in the M molecular form by introgression^[31]. Pyrethroid/organochlorine residues found in soils could favor the selection of the L1014F mutation of the *kdr* gene and it is tempting to conclude that agricultural intensification using these pesticides could be at the origin of the increase in this frequency^[31]. Local practices, both in agriculture and in vector control, can therefore rapidly impact the dynamics of

pyrethroid resistance alleles. This high heterogeneity in agricultural practices would probably also explain the variations in *kdr* gene L1014F allele frequencies observed between vector populations^[6]. Numerous studies have shown the involvement of pesticides used in agricultural and market gardening perimeters in the selection of resistance^[1, 9, 31]. This resistance is observed not only in urban areas and in rural areas characterized by cotton and market gardening, but also in rural areas with traditional cereal farming that does not require the use of agricultural insecticides or fertilizers^[31]. Recently, a study in the city of Bobo-Dioulasso in Burkina-Faso reported a high frequency of *kdr* in *An. arabiensis* collected from household waste polluted breeding sites^[23]. In Cameroon in the city of Yaoundé, mosquito populations in this city have been reported to express high resistance to DDT, pyrethroids and carbamates, with resistance mechanisms including the *kdr* allele (West and East *kdr*)^[4, 5, 7]. The relatively high frequencies indicate that the *kdr* L1014F resistance gene allele is still under selection even though vectors are developing other resistance mechanisms (metabolic resistance) that make them more resistant to pyrethroids^[13, 32].

Biochemical analyses carried out on the same populations of *An. gambiae* s.l. compared to the susceptible strain Kisumu show that detoxification enzymes (non-specific esterases, mixed function oxidases and glutathione-S-transferase) are also involved as a defense weapon of *An. gambiae* s.l. against pyrethroids. The overexpression of mixed-function oxidases observed in most of the *An. gambiae* s.l. populations in our study clearly shows that cytochrome P450 mono-oxygenases are strongly involved in pyrethroid resistance in some communes (Kandi, Malanville and N'Dali) because they are involved in pyrethroid detoxification in *An. gambiae* s.l.^[22]. The high activity of Glutathione-S-Transferases in wild populations of *An. gambiae* s.l. in the localities of Parakou, Kandi and N'Dali may play a major role in pyrethroid resistance due to detoxification of lipid peroxidation by-products induced by pyrethroids^[20]. Thus, these enzymes (MFOs and GSTs) could increase the phenotypic resistance to pyrethroids due to the *kdr* L1014F mutation by broadening the spectrum of resistance to independent compounds^[29]. Furthermore, these results reinforce the idea that there may be other mechanisms in *An. gambiae* s.l. besides *kdr*, oxidases and GSTs that are involved in pyrethroid resistance of this vector.

Conclusion

Overall, three species of *An. gambiae* s.l. are collected in the communes and in all agricultural production areas studied with a strong involvement of several resistance mechanisms (L1014F *kdr*, MFOs, Esterases and GST). The species encountered are *An. gambiae*, *An. coluzzii* and *An. arabiensis*. The L1014F mutation of the *kdr* gene is very high in *An. gambiae* s.l. from different communes of Benin associated with a strong overexpression of pyrethroid detoxification enzymes. These results show the very high level of resistance of malaria vectors and thus give a strong signal to vector control programs that detoxification enzymes are strongly involved in the resistance of *An. gambiae* s.l. to pyrethrinoids.

Author's Contributions

WHS, CDK, GGP, AC et MCA conceived the study. WHS, CDK, GGP, EG, AT, RO, AO, CA et MCA has participated

in the design of the study. Entomologic data was collected by WHS, CDK, AO, BY, AC, ASS, ID, AF et AS. Bioassays and laboratory analysis was carried out by WHS, CDK et ABS. WHS, CA et MCA drafted the manuscript. Statistical data analysis by SC. EG, CA et MCA critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests

Availability of data and materials

The data used and/or analyzed in this study are available from the corresponding author on reasonable request.

Consent for publication

The findings and conclusions in this manuscript are those of the author(s) and do not necessarily represent the official views of the United States Centers for Disease Control and Prevention (CDC), the United States Agency for International Development (USAID), or the United States President's Malaria Initiative (PMI). Use of trade names is for identification only and does not imply endorsement by the CDC, USAID, PMI, or the US Department of Health and Human Services.

Ethics approval and consent to participate

Not applicable

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