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Resistance Status of Various Populations of *Anopheles gambiae s.l.* to Insecticides and Resistance Mechanisms Involved in Different Crop Zones of Benin (West Africa)

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Abstract

The resistance of *An. gambiae s.l.* to pyrethroids remains a major concern. The development and use of alternative insecticides seems to be the solution. At the same time, monitoring of the susceptibility of these mosquitoes to insecticides needs to be stepped up. The aim of the present study is to investigate the resistance status of various *Anopheles gambiae s.l.* populations to insecticides and the resistance mechanisms involved. Ten localities, divided into four growing zones, were studied in Benin. Larval surveys were carried out during the 2021-2023 rainy seasons. Larvae were reared at the insectarium of the Centre de Recherche Entomologique in Cotonou. Adult females aged 2 - 5 days were morphologically identified and subjected to 4 insecticides including pirimiphos-methyl 0.5%, bendiocarb 0.01%, deltamethrin 0.05% and alphacypermethrin 0.05%; and the impact of pre-exposure to PBO measured in accordance with WHO protocols. L1014F and G119S mutations in the Kdr and Ace1 genes were determined by PCR. Pyrethroid mortality rates ranged from 2.88% to 39.19% for *An. gambiae s.l.* populations in all growing areas. Pre-exposure to PBO increased pyrethroid mortality. The results also show phenotypic resistance to bendiocarb in the lagoon zone and suspected resistance in the cereal, cotton and rice-growing zones. As for pirimiphos methyl, we noted sensitivity in the cotton zone, suspected resistance in the rice zone and resistance in the cereal and lagoon zones of the different *Anopheles gambiae s.l.* populations. A significant difference was observed in the distribution of L1014F, which ranged from 76.58% to 82.33% in our crop zones, while no significant difference was observed in the distribution of G119S, which ranged

from 3.52% to 4.86%. The resistance of *An. gambiae s.l.* to pirimiphos-methyl, bendiocarb, deltamethrin and alphacypermethrin, as well as the relatively high frequency of the kdr mutation, call for the development and implementation of measures for effective insecticide resistance management.

Keywords

Anopheles gambiae s.l., Benin, Resistance, Insecticide, Growing Areas, Gene

1. Background

Malaria is one of the most widespread parasitic diseases in Africa [1] [2]. According to new estimates, there were 249 million cases and 608,000 associated deaths in 2022 [3] in sub-Saharan Africa. Benin, like many African countries, is paying a heavy price for malaria. Despite the many control measures put in place [4], the disease continues to wreak havoc, with 2,289,948 cases and 2450 deaths in 2020. The main vectors involved in transmission are *Anopheles gambiae*, *Anopheles coluzzii* and *Anopheles funestus* [5].

To combat this disease, Benin has adopted various control strategies focusing on prevention, including vector control. These include long-acting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) in some areas of Benin [6]. However, this approach is hampered by vector resistance to pyrethroid insecticides throughout Africa [7].

Two genes are involved in this resistance, in particular resistance to pyrethroids and carbamates: Kdr (or Knock down resistance, which confers resistance to pyrethroids and organochlorines by modifying the voltage-dependent sodium channel) and Ace1 (or acetylcholinesterase gene, which is the target of organophosphate and carbamate insecticides. Mutation of this gene prevents the insecticide from binding to the enzyme, which is no longer capable of degrading inter-synaptic acetylcholine [8]. Many anthropogenic actions facilitate the amplification of the phenomenon of insect resistance to insecticides. These include actions caused by agricultural practices [9] [10]. Agricultural practices are therefore at the origin of the selection of insecticide resistance in malaria vectors [11]-[13]. In Benin, the desire to increase agricultural production and productivity has led farmers to use various synthetic chemical products. Cotton crops account for almost 90% of the insecticide market and 96% of chemical fertilizers [14]. Market garden crops and maize cultivation also require the use of agricultural insecticides [15]. The chemical molecules released into the environment infiltrate the soil or run off and reach aquatic ecosystems where mosquito larvae develop [16]. According to Akogbéto *et al.* [12], it is very likely that they exert selection pressure on the larval stages of various mosquito populations. In addition, ever-increasing agricultural productivity in the face of the needs of an ever-growing human population is leading to an increase in the quantity of pesticides in agricultural environments [17]. There is every reason to believe that growers can no longer do without pesticides in a

context where the promotion of other methods of controlling crop pests has not prospered [17].

Data on the resistance status of malaria vectors to insecticides in crop areas exist in Benin, but are outdated and need to be updated. There are several mechanisms of resistance, the most important of which are those involving a change in the target and metabolism of the insecticide. While the former is mainly characterised by the kdr and Ace1 genes, the latter is characterised by the involvement of detoxification enzymes, including cytochrome P450 (CYP) monooxygenases, glutathione S-tranferases (GST) and carboxylesterases (COE) [18]. In the present study, we considered several growing areas with different levels of pesticide use and assessed the relationship between the level of resistance developed by the vectors and the level of pesticide use. This approach will allow us to identify the main resistance mechanisms developed by vectors in different growing areas, in order to identify appropriate insecticides for better planning of vector control strategies in rural areas.

This study aims to characterize the resistance mechanisms of malaria vectors to insecticides commonly used in vector control.

2. Methods

2.1. Study Site

The study took place in Benin, a country in West Africa. It lies in the intertropical zone between the equator and the Tropic of Cancer, more precisely between parallels 6˚30' and 12˚30' north latitude and meridians 1˚ and 3˚40' East longitude [19]. It is bounded to the north by the River Niger, to the south by the Atlantic Ocean, to the west by Togo and Burkina Faso and to the east by Nigeria. It covers an area of 114,763 km^2 [19].

This study was conducted during the rainy season between 2021 and 2023 in 4 types of cropping area in Benin (**Figure 1**). These are cotton-growing areas, cereal-growing areas, rice-growing areas, lagoon areas or market garden areas.

Cereal-growing zones farming system is essentially composed of food crops such as rice, sorghum, maize, etc [20]. These are areas where insecticides and other pesticides are used sparingly. The lagoon areas are generally market-garden areas with a pseudo-development of food crops. These are areas of relative pesticide use, generally represented by fungicides and other acaricides [20]. The cotton-growing areas in northern and central Benin, are areas of high insecticide use and rice-growing areas that use little or no insecticides. We collected Anopheles larvae in N'dali and Natitingou (cotton-growing areas), Glazoué and Malanville (rice-growing areas), Boukoumbé, Dogbo and Toffo (cereal-growing areas) and Cotonou, Comè and Semè-Kpodji (lagoon and market-garden areas). These different zones constitute the agro-ecological zones of Benin and are subject to different insecticide pressures, mainly due to cultivation practices. For example, in the cotton zone, insecticide use is very high because of production, compared with other zones where cotton is not produced. It should also be noted that pyrethroid-

impregnated mosquito nets are the main vector control tools in all the different crop-growing zones.

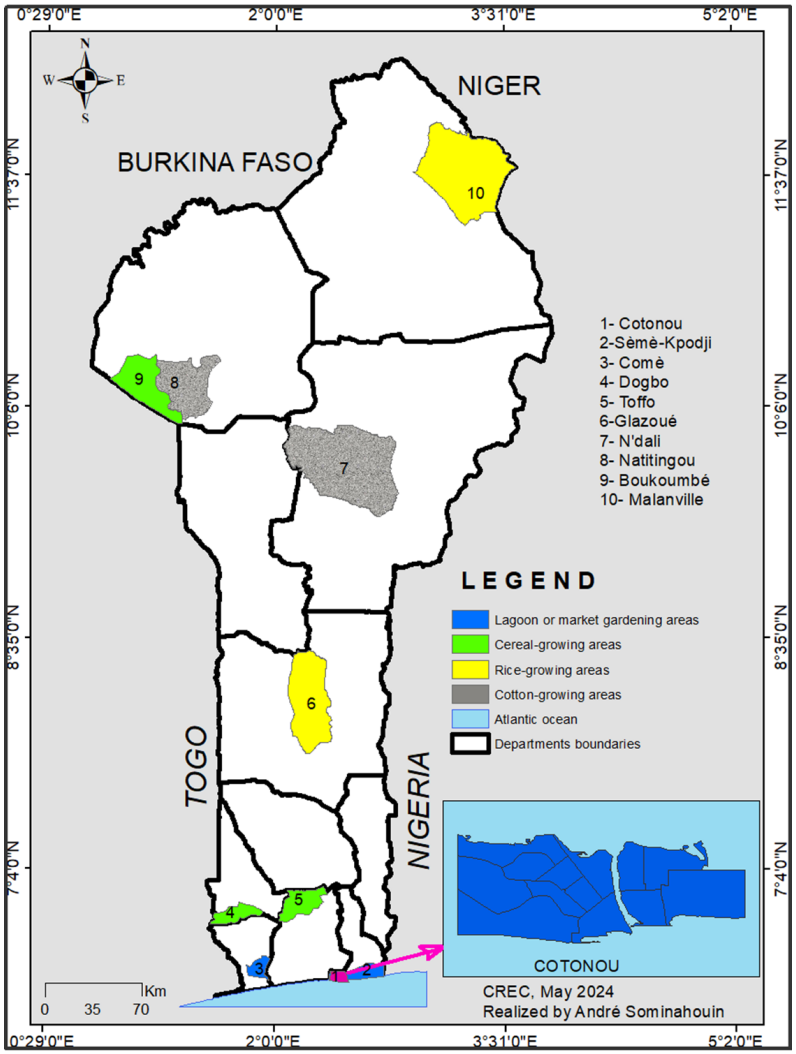

Figure 1. Geographical distribution of study sites for the period January 2021 to July 2023.

2.2. Mosquito Collection

The sampling method used to obtain imagos was the dipping method [21]. This consisted of collecting Anopheles larvae from the breeding sites using ladles. The larvae collected were transported to the insectarium of the Centre de Recherche Entomologique de Cotonou (CREC) in jars containing water from the collection sites. They were then reared until the adults emerged. The resulting imagos were fed with sugar juice. In the insectarium, the temperature was maintained at 27˚C ± 2˚C with a relative humidity of 70% ± 10%.

Morphological identification of anopheles

The female anopheles used for the tests were identified morphologically as belonging to the *Anopheles gambiae* complex using the key of Gillies and De Meillon [22] and Gillies and Coetzee [23].

Insecticides tested

Female *Anopheles gambiae s.l.* were subjected to 4 insecticides belonging to three families:

- Organophosphates: pirimiphos-methyl 0.5%;
- Carbamates: bendiocarb 0.01%; and
- Pyrethroids: deltamethrin 0.05% and alphacypermethrin 0.05%.

Insecticide sensitivity tests

Susceptibility testing was performed on fasting *An. gambiae s.l.* females aged 2 to 5 days, according to the standard WHO protocol for adults using WHO kits [24] [25]. The tests were performed at room temperature and relative humidity of 25˚C ± 2˚C and 70% ± 10% respectively. Nine tubes were used for each test. In each tube, females were introduced in batches of approximately 25 females. Each test was carried out in two stages: firstly, the batches of mosquitoes were observed for one hour in tubes fitted with unimpregnated paper (12 × 15 cm); secondly, the females were brought into contact with the insecticide in tubes fitted with impregnated paper (12 × 15 cm), while the control females were introduced into tubes fitted with unimpregnated paper. During the 60-minute exposure period, the knock-down effect was observed every 5 minutes. At the end of this period, the females were transferred to observation tubes. A cotton swab soaked in a 10% sugar solution was then placed on each observation tube to feed the mosquitoes, thus ruling out hunger as a cause of mortality. Mortality was then assessed 24 hours after exposure to the insecticide [24] [25]. The impregnated papers used came from the reference center (Vector Control Research Unit, Sains Malaysia University, Penang, Malaysia). These papers were used during their period of validity. Repetition of the tests under the same conditions and the absence of mortality in the controls enabled us to guarantee the presence of the insecticide and the quality of the impregnation of the papers used. All this confirms the results we obtained during the various tests.

Synergistic bioassays

In order to assess the involvement of detoxification enzymes in resistance to deltamethrin and alphacypermethrin (insecticides used in the impregnation of mosquito nets in Benin), tests with synergists were carried out with pre-exposure to 4% PBO (piperonyl butoxide), an inhibitor mainly of mono-oxygenases. This test was carried out in three stages using three batches of 20 to 25 non-gorged *An. gambiae s.l.* females aged between 2 and 5 days. The three batches were divided as follows: 1 batch to be tested with insecticide (deltamethrin 0.05% or alphacypermethrin 0.05%) alone, 1 batch to be tested with synergist followed by insecticide and 1 batch serving as a control exposed to unimpregnated paper. Initially, the batches of females were observed for one hour in tubes fitted with unimpregnated paper (12 × 15 cm). Secondly, 2 batches of 20 to 25 females were each introduced into a tube lined with paper impregnated with synergist for 1 hour. Finally, the females were transferred to the exposure tubes. The transfer was carried out as follows: the batch to be tested with synergist and then with insecticide was transferred to the tube containing insecticide, the batch to be tested with insecticide was transferred to a tube containing paper impregnated with insecticide and the

control batch was transferred to a tube containing unimpregnated paper. After 1 hour exposure, the three batches were transferred back into their respective observation tubes supplied with a 10% sugar water solution and maintained for 24 hours before recording mortality. It should be noted that these tests were carried out at a temperature of 25˚C and a humidity of 70%.

Sensitivity test validity criteria

The susceptibility of *Anopheles gambae s.l.* populations was interpreted according to WHO criteria [25]. The knock-down effect of the insecticide was assessed during the exposure period, which lasted 1 hour, and its lethal effect was assessed 24 hours after the exposure period. The interpretation criteria used were as follows:

- Mortality rate between 98% - 100%: population sensitive to the insecticide tested

- Mortality rate between 90% - 97%: decrease in sensitivity within the population; suspected resistance

- Mortality rate below 90%: population resistant to the insecticide tested

For tests with synergists, the validity criteria were applied in accordance with previous recommendations [25]-[27]. For the validation of the results, the mortality of the controls must be less than 5%. When it is between 5% and 20%, Abbot's formula is used to correct the mortality of exposed mosquitoes. However, if the mortality is greater than 20%, the test is cancelled.

2.3. Determination of the Kdr and Ace1 Mechanisms Involved

2.3.1. Determination of the L1014F Mutation in the Kdr Gene

The L1014F resistant allele of the kdr gene was determined using the protocol of Martinez-Torres *et al.* [28]. This diagnostic PCR test uses four oliogonucleotides or primers (Agd1, Agd2, Agd3, Agd4) and a Taq polymerase to amplify resistant or susceptible alleles on a DNA fragment coding for the voltage-gated sodium channel in each mosquito tested. The Agd1/Agd2 primer pair identifies the kdr gene by amplifying a 293-bp product as a control. The Agd3/Agd1 primer pair associates only with the resistance part of the kdr gene to amplify a 195 bp fragment. The Agd4/Agd2 pair associates only with the sensitive part of the gene, amplifying a 137 bp fragment. The nucleotide sequences of these primers are as follows: Agd1: 5'-ATAGATTCCGACCATG-3'; Agd2: 5'-ACAAGGATGATGAACC3'; Agd3: 5'-AATTTGCATTACTTACGACA-3'; Agd4: 5'-CTGTAGTGATAGGAAATTTA-3'

2.3.2. Determination of the G119S Mutation of the Ace1 Gene

For the Ace1 marker, PCR diagnosis of the G119S mutation was carried out according to the protocol of Weill *et al.* [29] using two primers with the following nucleotide sequences: Ex3AGdir: 5'-GATCGTGGACACCGTGTTCG-3' Ex3AGrev: 5'-AGGAT GGCCCGCTGGAACAG-3'

2.4. Data Analysis

In this study, data were entered into Excel 2010 and analysed using R software

(version 3.3.3) [30]. The Chi2 test was used to compare mortality rates. A probability value of p less than or equal to 0.05 was considered significant. The frequencies of the L1014F and G119S allele of the Kdr and Ace1 gene were determined for each growing zone. Frequency confidence intervals were calculated using the binomial formula. The proportion test [30] in R software, version 3.3.3 was used to compare the distribution of the different species and the frequency of L1014F alleles within these species in the different agro-ecosystems.

3. Results

3.1. Sensitivity of *Anopheles gambiae s.l.* to Pyrethrinoids (Deltamethrin and Alphacypermethrin) in Crop-Growing Zones

The resistance of vectors to insecticides was monitored in the different growing zones (cereal-growing zones, rice-growing zones, lagoon zones and cotton-growing zones) using the WHO tube test (Table 1). Mortality rates were below 40% for both insecticides in all four crop zones, indicating high resistance to both pyrethroids. There was no significant difference in the susceptibility level of *Anopheles gambiae s.l.* populations in three of the four growing zones (cotton, cereals and rice). However, in the lagoon zone, the level of susceptibility of *Anopheles gambiae s.l.* populations to deltamethrin was significantly higher than to alphacypermethrin (Figure 2).

Table 1. Results of sensitivity tests on *Anopheles gambiae s.l.* to pyrethroids.

Growing zones	Deltamethrin			Alphacypermethrin		
	Number	Mortality (%)	IC 95%	Number	Mortality (%)	IC 95%
Cereal-growing areas	158	24.05	17.61 - 31.48	136	20.59	14.14 - 28.36
Cotton-zones	148	39.19	31.28 - 47.54	141	32.62	24.97 - 41.02
Rice-growing areas	129	32.56	24.57 - 41.36	125	26.4	18.92 - 35.03
Lagoon areas	228	23.68	18.32 - 29.74	139	2.88	0.78 - 7.2

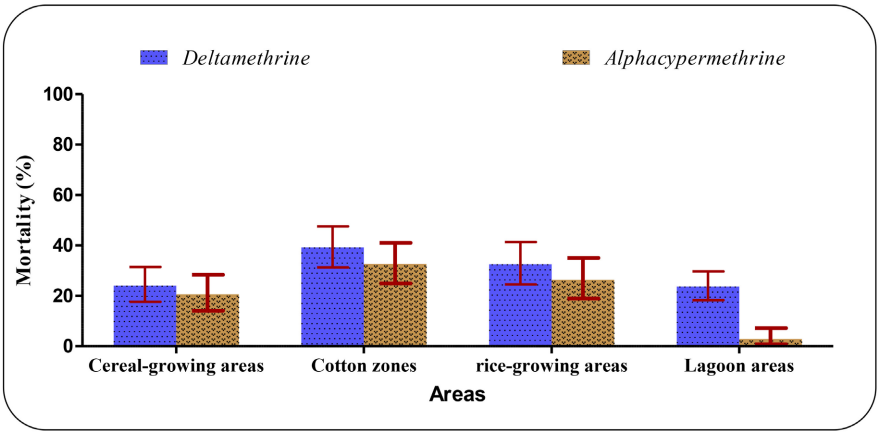

Figure 2. Mortality rate observed in *Anopheles gambiae s.l.* after exposure to pyrethroids (Deltamethrin and alphacypermethrin) in different growing zones.

3.2. Synergistic Dosages

Pre-exposure of *An. gambiae s.l.* to PBO induced a significant increase in mortality in the different populations tested with alphacypermethrin and deltamethrin alone, indicating resistance management due to the action of the PBO synergist. This means that MFOs are partially involved in the phenotypic resistance of *An.gambiae s.l.* to pyrethroids. No significant differences were observed between the mortality rates of *An. gambiae s.l.* populations in the different growing zones (Table 2 and Figure 3).

Table 2. Results of sensitivity tests on *Anopheles gambiae s.l.* to pyrethroids + PBO.

Growing zones	Deltamethrin + PBO			Alphacypermethrin + PBO		
	Number	Mortality (%)	IC 95%	Number	Mortality (%)	IC 95%
Cereal-growing areas	47	44.70	30.17 - 59.88	61	50.82	37.69 - 63.86
Cotton-zones	147	93.88	89.55 - 97.62	137	91.24	95.19 - 95.39
Rice-growing areas	68	85.29	74.61 - 92.71	64	79.68	67.77 - 88.71
Lagoon areas	198	83.33	77.39 - 88.24	100	88	79.97 – 93.64

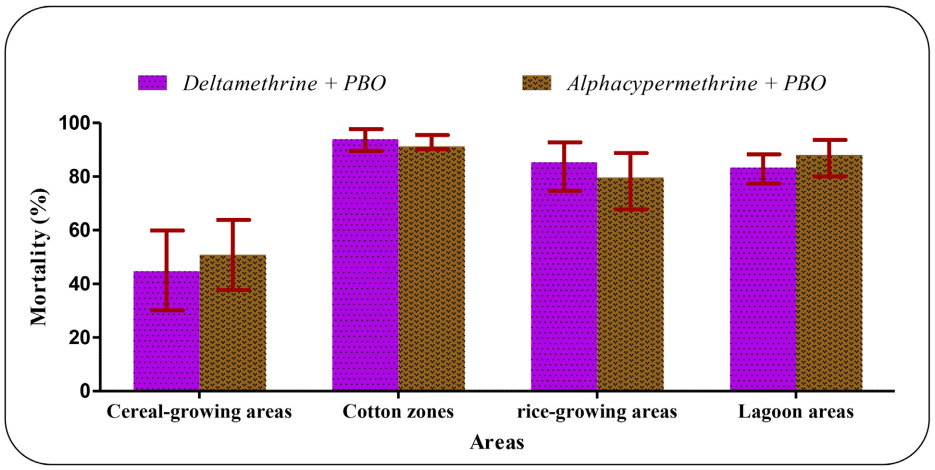

Figure 3. Mortality rate observed in *Anopheles gambiae s.l.* after exposure to pyrethroids + PBO (Deltamethrin + PBO and alphacypermethrin + PBO) in different growing zones.

3.3. Sensitivity of *Anopheles gambiae s.l.* to Organophosphates and Carbamates in Different Crop Zones

Table 3 shows the mortality rates observed in *Anopheles gambiae s.l.* in the different growing zones. The results show phenotypic resistance to bendiocarb in the lagoon zone and suspected resistance to this insecticide in the cereal, cotton and rice-growing zones. As for pirimiphos methyl, there was sensitivity in the cotton-growing zone, suspected resistance in the rice-growing zone and resistance in the cereal-growing and lagoon-growing areas of the different populations of *Anopheles gambiae s.l.* (Figure 4).

Table 3. Results of sensitivity tests on *Anopheles gambiae s.l.* to carbamates and Organophosphates.

Growing zones	Bendiocarb			Pirimiphos méthyl		
	Number	Mortality (%)	IC 95%	Number	Mortality (%)	IC 95%
Cereal-growing areas	20	95	75.12 - 99.87	50	86	73.26 - 94.18
Cotton-zones	49	97.95	89.14 - 99.94	48	100	92.60 - 100
Rice-growing areas	62	96.77	88.82 - 99.60	43	97.35	87.71 - 99.94
Lagoon areas	166	87.35	81.31 - 91.99	221	85.97	80.68 - 90.26

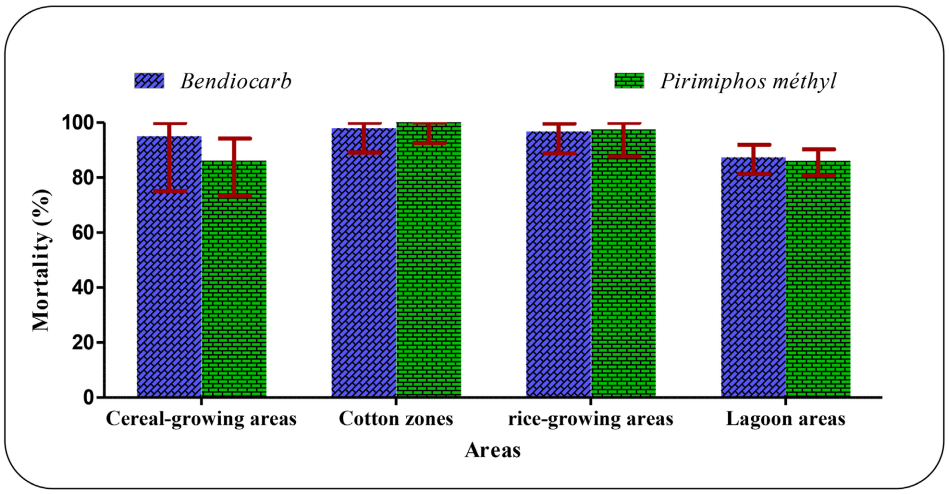

Figure 4. Mortality rate observed in *Anopheles gambiae s.l.* after exposure to carbamates (bendiocarb) and organophosphates (pirimiphos methyl) in different growing zones.

3.4. Resistance Mechanisms Involved

Table 4. Frequency of the L1014F allele of the *Kdr* gene in *An. gambiae s.l.* per zone.

Growing Zones	Number tested	1014F/1014F) N (F %)	1014F/1014L N (F %)	1014L/1014L N (F %)	L1014F (F %)
Cotton-zone	284	183 (64.44%)[a]	69 (24.30%)[a]	32 (11.27%)[a]	(76.58)[a]
Rice-growing areas	453	319 (70.42)[ab]	93 (20.53)[abc]	41 (9.05)[ac]	(80.68)[ac]
Cereal-growing areas	686	527 (76.82)[b]	110 (16.03)[b]	49 (7.14)[ac]	(84.84)[b]
Lagoon areas	645	456 (70.70%)[ab]	150 (23.26%)[ac]	39 (6.05%)[c]	(82.33)[bc]

Frequencies with the same letter in the columns do not differ significantly from one locality to another. N: Number of genotypes; (F %): frequency; homozygous resistant genotype 1014F/1014F; heterozygous genotype 1014F/1014L; susceptible homozygous genotype 1014L/1014L.

Table 5. Frequency of the G119S allele of the Ace1 gene in *An. gambiae s.l.* per zone.

Growing Zones	Number tested	119S/119S (F %)	119G/119S (F %)	119G/119G (F %)	G119S (F %)
Cotton-growing zone	284	4 (1.41%)[a]	12 (4.23%)[a]	268 (94.37%)[a]	(3.52)[a]
Rice-growing zone	453	0 (0%)[b]	44 (9.71%)[b]	409 (90.29%)[a]	(4.86)[a]
Cereal-growing zone	686	0 (0%)[b]	60 (8.75%)[ab]	626 (91.25%)[a]	(4.37)[a]
Lagoon zone	645	0 (0%)[b]	50 (7.75%)[ab]	595 (92.25%)[a]	(3.88)[a]

Frequencies with the same letter in the columns do not differ significantly from one locality to another. (F %): frequency; homozygous resistant genotype 119S/119S; heterozygous genotype 119S/119G; susceptible homozygous genotype 119G/119G.

Table 4 and Table 5 show the allelic frequencies of the Kdr L1014F gene and the Ace1 G119S gene in *Anopheles gambiae s.l.* in different cropping zones in Benin. Kdr L1014F gene frequency values are high (above 76%) in all cropping zones: the highest value is observed in the cereal-growing areas (84.84%) and the lowest value in the cotton-zone (76.58%). A comparison of these different frequencies reveals a significant difference between the allelic frequencies observed (Table 4). As for the frequency of the Ace 1 gene, the values observed in the different cropping zones were generally low (below 5%). The highest value was observed in the rice-growing zone with a frequency of 4.86% and the lowest value was observed in the cotton-zone with a frequency of 3.52%. No significant difference was observed between the allele frequencies of the Ace1 gene in the different cropping zones (Table 5).

4. Discussion

This study clearly established pyrethroid resistance in *Anopheles gambiae s.l.* for all the different crop zones. This resistance had already been demonstrated by Akogbéto *et al.* [31] and Sagbohan *et al.* [32]. According to the latter author, the resistance of Anopheles populations to pyrethroids probably contributes to the decline in efficacy of pyrethroid-impregnated mosquito nets (deltamethrin, permethrin, alphacypermethrin, etc.…) in Benin and to the stagnation in the reduction in the number of cases of malaria in the world in general and in Benin in particular. Several factors may contribute to the increase in resistance in certain areas, such as cotton-growing areas and food-growing areas, where pesticides are used extensively to increase agricultural production. The pesticides used contribute to environmental pollution and at the same time encourage the selection of resistant individuals in vector populations, as the insecticides used in agriculture contain practically the same active ingredients as those used in public health for indoor spraying or impregnating mosquito nets [33] [34]. Although the overuse

of insecticides in agriculture and public health is the main reason reported [13], our results clearly show that even in areas where fewer insecticides are used, the level of resistance appears to be higher than that observed in areas where insecticides are used extensively. This shows that the insecticidal pressure exerted by the use of insecticides on populations of *Anopheles gambiae s.l.* is not the only cause in the evolution of resistance in vectors to pyrethrinoids. Furthermore, several researchers have demonstrated the ineffectiveness of pyrethroid-based products against Helicoverpa armigera, the main cotton pest [35]. This situation is leading cotton growers to prefer neonicotinoid-based insecticides, which are more effective but of dubious origin [36]. This could lead to a reduction in the insecticidal pressure exerted on resistant populations of *Anopheles gambiae s.l.* and the emergence of susceptible individuals.

Tests carried out with pyrethroids + a synergist induced significantly higher mortality than that observed for pyrethroid alone, confirming the partial involvement of mixed-function oxidases (MFOs). These results are similar to those observed by Salako *et al.* [37] and Sagbohan *et al.* [32]. Although the PBO synergist bioassays did not induce full susceptibility of *An. gambiae s.l.* populations, the significant increase in susceptibility suggests that PBO LLINs could be an alternative for the control of pyrethroid-resistant mosquitoes.

Tests on the susceptibility of *Anopheles gambiae s.l.* to carbamates (bendiocarb) and organophosphates (pirimiphos-methyl) showed that *Anopheles gambiae s.l.* was resistant to bendiocarb irrespective of the cropping zone, whereas for pirimiphos-methyl, only the cotton zone showed optimum susceptibility to *Anopheles gambiae s.l.* populations. In the cereal and lagoon zones, we observed resistance of *Anopheles gambiae s.l.* populations to pirimiphos-methyl. The latter is the insecticide that Benin has been using for indoor residual spraying for several years and which is jealously guarded. This resistance could compromise its effectiveness and future use in Benin.

On the other hand, *An. gambiae s.l.* shows high frequencies of the kdr L1014F mutation in all cultivated areas. This confirms the results of Gnanguenon *et al.* [38] obtained in several sites located on the North-South transect of Benin. Our results show that L1014F frequencies are significantly higher in the cereal and lagoon zones compared with the cotton zone. This result seems paradoxical given that cotton production, for which demand has never ceased to increase, uses greater quantities of pesticides. In reality, the explanation lies in the type of insecticide used in cultivation practices in recent years. Several studies based on surveys and observations [39] have shown that cotton growers have long since abandoned the use of pyrethroids, which they consider ineffective, in favour of neonicotinoids, while the use of pyrethroids in cereal production is increasing. This has certainly contributed to increasing selection pressure on *An. gambiae s.l.* larvae in cereal-growing areas. Finally, with regard to the frequency of the Ace1 mutation, it is low in all growing zones and does not seem to differ significantly from one growing zone to another.

5. Conclusion

This study showed that *Anopheles gambiae s.l.* populations are resistant to pyrethroids (deltamethrin and alphacypermethrin) and carbamates (bendiocarb) This resistance appears to be significantly higher in the cereal-growing and lagoon areas (market-garden areas) than in the cotton-growing areas. This is also the case for the kdr L1014F frequency, one of the mutations responsible for the phenotypic resistance observed. This situation is due to the use of neonicotinoids in cotton production, whereas pyrethroids are used more intensively in vegetable and cereal production. In addition, in cereal and lagoon (market-garden) areas, populations of *Anopheles gambiae s.l.* are resistant to pirimiphos-methyl. Given that this insecticide is the best organophosphate used in Benin for indoor residual spraying, we can't help wondering what the future holds for this product in IRS in the coming years.

Recommendations

Author Contributions

CA, GGP and MCA designed the study. SA, AF, ZCK, AS, EO, OO Performed the experiment. SA, ZCK, AF and MCA analysed the data. SA wrote the manuscript. ZCK, AF and MCA reviewed the manuscript critically. All authors read and approved the final manuscript.

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Conflicts of Interest

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

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