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Two decades of insecticide resistance in Benin: a retrospective analysis of evolution and drivers

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Abstract

Background Several studies have been conducted in Benin over the past two decades on insecticide resistance in mosquito vectors. These studies, by various authors, lead to diverse and scattered data. The present paper provides a retrospective analysis of these data to assess the current state of insecticide resistance and its evolution over two decades. Phenotypic trends were compared to mechanisms of insecticide resistance, focusing on the pyrethroids target-site mutation at codon 1014 of the voltage-gated sodium channel and the overproduction of detoxification enzymes capable of neutralizing insecticides before reaching their target.

Methods Data were collected from studies between 1996 and 1998 and from 2010 to 2024. For each selected study, the following information were extracted and organized in a Microsoft Excel spreadsheet: study year, adherence to WHO insecticide susceptibility testing protocols, mosquito species tested, study site characteristics, insecticides assessed, data source, and resistance mechanisms identified. Municipalities with data gaps exceeding five consecutive years were excluded.

Results The earliest reported cases of insecticide resistance in Benin date back to 1963, involving organochlorines. Resistance to pyrethroids was first observed in 1999, initially limited in scope. However, from 2010 to 2024, resistance to all pyrethroids spread across all regions of Benin, reaching high levels. In some municipalities, mortality rates in *Anopheles gambiae* sensu lato (s.l.) populations exposed to permethrin-treated papers fell below 10%. The frequency of the *kdr* L1014F mutation has mirrored phenotypic resistance trends, increasing from 10% homozygous resistant *(kdr/kdr)* individuals in 2011 to 90% in 2024 in the municipality of Allada. Detoxification enzymes, such as α-esterase, β-esterase, monooxygenase and glutathione *S*-transferase showed low, but steadily increasing activity between 2015 and 2024. Resistance to bendiocarb, first reported in 2012, has shown minimal progression, while resistance to pirimiphos methyl has been observed in some municipalities since 2022.

Conclusion First observed with organochlorines around the 1960s, and later with pyrethroids in 1999, insecticide resistance in mosquito vectors has continued to intensify. Over the last 20 years, it has gradually expanded, now

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affecting all ecological environments in Benin. In this context, the National Malaria Control Programme should prioritize the use of new mosquito nets for future vector control campaigns in Benin.

Keywords Resistance, Insecticide, *Anopheles gambiae*, Bénin

Background

Insecticide-Treated Nets (ITNs) and Indoor Residual Spraying (IRS) are the main tools for malaria vector control in sub-Saharan Africa [1], demonstrating significant success in reducing disease transmission [2, 3]. However, the growing resistance of *Anopheles gambiae* populations to insecticides, leads to a serious public health challenge [4, 5]. This contrast between the progress achieved in malaria control and the emergence of resistance has sparked interest among researchers, resulting in numerous studies across Africa, including Côte d'Ivoire [4, 6], Kenya [7], Benin [8, 9], Niger [10], Burkina Faso [11], Mali [12], Nigeria [13, 14], South Africa [15] and Cameroon [16]. In Benin, insecticide resistance is not a new phenomenon, with the first cases reported in 1960 involving organochlorines [dieldrin (DLD) and dichlorodiphenyltrichloroethane (DDT)] [17, 18]. Earlier, Armstrong et al. [19] documented the emergence of mosquito resistance to the same organochlorines in Nigeria. At the time, several factors were identified as drivers of insecticide resistance in West Africa. In Burkina Faso, these factors included frequent insecticide spray operations using DDT, DLD and hexachlorocyclohexane (HCH), in major cities (Ouagadougou, Bobo-Dioulasso), as well as DDT applications on cotton crops [11]. In Benin**,** similar operations in urban areas, combined with the use of DDT and DLD during the 1955–1960 malaria eradication campaign in southern regions and the application of insecticides for crop protection and domestic hygiene, were implicated [20].

The first case of pyrethroid resistance in Benin was documented in 1996 and published in 1999 [20], following earlier reports in Côte d'Ivoire [4]. Numerous other cases have been described in East Africa [7] and West Africa [8, 11, 21–23]. This study has considered factors previously described as favouring the persistence and evolution of insecticide resistance in mosquito vectors.

First, mass distribution campaigns of long-lasting insecticidal nets (LLINs) across Benin and indoor residual spraying (IRS) campaigns supported by the President's Malaria Initiative (PMI) in the departments of Ouémé, Atacora, Donga, and Alibori have drawn the attention of researchers to the emergence of resistance in Benin. Studies by Padonou et al. [24] and Ossè et al. [25] in Oueme, Aïkpon et al. [26] in Atacora, and Djègbè et al. [27], Sovi et al. [28], Agossa et al. [29],Gnanguenon et al. [30], Salako et al. [31], Kpanou et al. [32], and Sagbohan et al. [33] in various municipalities of Benin, have generated extensive data we analysed to evaluate the level and evolution of vector resistance to insecticides in Benin. Resistance intensity and its rate of spread vary across regions, influenced by the type of intervention deployed. The alternate use of LLINs and IRS could accelerate resistance to insecticides more significantly than in areas where LLINs are used alone. Notably, the cessation of IRS in Oueme (2011), Atacora (2016), and Alibori and Donga (2021) may lead to a decline in resistance levels. In the absence of selective pressure from IRS, mosquito populations may lose the resistance they have developed, offering a pathway for adaptative control strategies under the National Malaria Control Programme (NMCP). However, despite the cessation of IRS, mosquitoes may retain resistance due to other environmental factors, such as genetic background, beyond the use of impregnated materials.

In addition to insecticides used in agriculture and public health, Djouaka et al. [34] and Akogbeto et al. [5] have highlighted the role of decomposing chemical compounds in mosquito breeding sites, which exert selection pressure on larvae and contribute to the persistence and evolution of resistance in the adult stage of the mosquito, even in the absence of active insecticide deployment.

Resistance to carbamates and organophosphates were also examined. Current data on bendiocarb and pirimiphos methyl indicate suspected resistance but do not provide conclusive evidence regarding the precise susceptibility of *An. gambiae* s.l. (which will be called *An. gambiae* thereafter in the text) to these compounds. Resistance intensity, particularly the proportion of mosquitoes classified as suspected resistant, is a critical focus, as this group is most likely to gain or lose resistance under changing environmental conditions. For instance, findings on bendiocarb in Benin suggest a borderline resistance status.

Regarding pirimiphos methyl, susceptibility in *An. gambiae* populations in Atacora remains favourable [29, 31, 35]. Due to the dynamic nature of insecticide resistance, continuous monitoring is essential to detect any emerging trends of resistance to this compound. Furthermore, this study analysed the two primary mechanisms of resistance frequently observed in *An. gambiae* in Benin: target site modifications in codon 1014 of the voltage-gated sodium channel [36, 37] and metabolic resistance characterized by the overproduction of detoxifying enzymes. The allelic frequency of *kdr* mutations and the distribution of *kdr/kdr*, *kdr/kds* and *kds/kds* genotypes were assessed alongside phenotypic resistance to understand the evolutionary trajectory of resistance mechanisms.

The findings on evolution of vectors resistance to pyrethroids, carbamates and organophosphates will provide NMCP in Benin, with valuable insights to ensure targeted and effective malaria vectors control approach.

Methods

Data were collected from all 12departments in Benin and included analyses of *An. gambiae* populations conducted between 1996 and 1998, published in 1999 [20], as well as studies from 2010 to 2024 published in various sources. Thesis sources included publicly defended doctoral theses during the study period, data recorded in the database of the Centre de Recherche Entomologique de Cotonou (CREC), online bibliographic databases such as PubMed, Google, and Google Scholar, and scientific publications on vector resistance to insecticides during the study period [30, 31, 38–43].

The keywords used to guide the literature search included "insecticide resistance", "*Anopheles*", "period" and "Benin". Excluded from the review process were articles focusing on tests on sensitive laboratory sources, bioassays conducted on *Anopheles* larvae, and modeling studies. Additionally, municipalities without data spanning five consecutive years were not included.

For each selected study, the following information were extracted and recorded in a Microsoft Excel sheet: study year, compliance with WHO standards for evaluating *Anopheles* susceptibility to insecticides, mosquito species studied, characteristics of the study site, insecticides tested, data source, and resistance mechanisms involved.

The data were analysed to assess vector resistance levels, focusing on phenotypic and molecular resistance. According to World Health Organization (WHO) criteria, a mosquito population is classified as resistant if the mortality rate is < 80% [44] or < 90% [45]. Populations are considered susceptible if the mortality rate is between 97% [44] or 98% and 100% [45]. Populations with mortality rates between 80 and 97% [44] or 90% and 97% [45] are considered suspected of resistance. Both WHO references were used to account for data spanning two periods: before and after 2013.

The *kdr* and *ace-1R* mutations were detected using the protocols described by Martinez Torrez et al. [36] and Weill et al. [46], respectively. Biochemical assays were conducted following the protocol outlined by Hemingway et al. [47].

Results

Literature search

A total of 37 scientific studies were pre-selected, among which 16 were excluded for not meeting the inclusion criteria. Ultimately, 21 documents written in French or English from 6 municipalities (Cotonou, Allada, Dassa, Parakou, Kandi, Malanville) were selected and reviewed (Table 1). The selected studies investigated the evolution of *An. gambiae* populations susceptibility rates to insecticides over the last 20 years, genotype and allele frequencies of the *kdr* L1014F and *ace-1* G119S gene mutations, and insecticide detoxification enzymes in *An. gambiae* populations.

Evolution of *Anopheles gambiae* resistance to insecticides in Benin

Data from 1999

A study by Akogbeto and Yacoubou [20] examined the susceptibility of *An. gambiae* to permethrin, deltamethrin, and lambdacyhalothrin in 15 communities along the south-north transect of Benin. The results showed that *An. gambiae* exhibited susceptibility rates below the WHO 1998 threshold (< 80%) for permethrin and lambdacyhalothrin in all communities, indicating resistance to these insecticides. For deltamethrin, mortality rates varied, reflecting resistance, suspected resistance, or susceptibility depending on the communities. Notably, in Malanville, *An. gambiae* demonstrated clear susceptibility to deltamethrin. This finding led the Centre de Recherches Entomologiques de Cotonou (CREC) to establish an experimental station in Malanville for phase 2 evaluations of insecticides and treated materials. Malanville thus became the reference site for producing a susceptible population of *An. gambiae* for laboratory studies.

Level of Anopheles gambiae resistance to pyrethroids in 2024

Figure 1 shows the results of susceptibility tests conducted in 2024 as part of resistance monitoring in 12 communities in Benin, one commune per department. The mortality rates recorded are extremely low (Fig. 1). Mortality rates registered after exposure of *An. gambiae* populations to WHO-treated papers show strong resistance to permethrin, deltamethrin and alphacypermethrin across all departments. Overall, less than 40% of mosquitoes exposed to insecticide-treated papers die within 24 h. In Cotonou, mortality rates were very low: 1.98% (2/101) for deltamethrin, 2.43% (2/82) for permethrin and 6.38% (6/94) for alphacypermethrin.

Evolution of An. gambiae resistance from 2010 to 2024

The evolution of *Anopheles gambiae* resistance was monitored in the six communities in the south (Cotonou,

Table 1 Summary of data collected in peer-reviewed papers selected for the study

References	Study year	Species	Insecticides	Locations	Topics covered
Thesis Padonou Gil Germain (2012)	2008–2010	*An. gambiae* s.l	Alpha; chlorpy Delta; bendio PM; feni	6; 7	Tool evaluation
Djègbè et al. [38]	2008–2010	*An. gambiae* *An. arabiensis*	DDT; delta Bendio PM; feni	19; 24; 5	Susceptibility & metabolic resistance
Thesis Gnanguenon Virgil [30]	2012–2015	*An. gambiae* *An. arabiensis* *An. coluzzii* *An. melas*	Delta; bendio PM; feni	1; 2; 3; 4; 5	Tool evaluation
Gnanguenon et al. [30]	2013	*An. gambiae* *An. arabiensis* *An. coluzzii*	Delta; bendio PM; feni	1; 2; 3; 4; 5	Susceptibility & metabolic resistance
Aikpon et al. [35] Agossa et al. [29]	2012 2013	*An. gambiae* s.l. *An. gambiae* s.l.	Bendio Bendio	8; 9; 10; 11 5; 8; 10	Tool evaluation Tool evaluation
Thesis Salako Albert (2020)	2016–2019	*An. gambiae* *An. coluzzii*	Perm; delta Bendio; PM	4; 12; 13; 14	Tool evaluation
Salako et al. [31]	2017	*An. gambiae* *An. coluzzii*	Perm; delta Bendio; PM	4; 12; 13; 14	Susceptibility & metabolic resistance
Thesis Kpanou Casimir D (2023)	2018–2022	*An. gambiae* *An. arabiensis* *An. coluzzii*	Perm; delta Bendio; PM	1; 2; 3; 4; 5; 19; 16; 17; 18; 3; 20; 6	Susceptibility, metabolic Resistance & kdr resistance
Assogba et al. (2020)	2013–2015	*An. gambiae* *An. arabiensis* *An. coluzzii*	Perm; bendio	19; 26; 23; 27; 28; 4; 29; 24; 30; 14; 10; 31; 32	Susceptibility & kdr resistance
Kpanou et al. (2021a)	2019	*An. gambiae* *An. arabiensis* *An. coluzzii*	Perm; delta	3; 4; 5	Susceptibility & kdr resistance
Kpanou et al. (2022)	2017	*An. gambiae* *An. arabiensis* *An. coluzzii*	Perm; delta	19; 6; 4; 5	Susceptibility & kdr resistance
Kpanou et al. (2021b)	2017–2018	*An. gambiae* *An. arabiensis* *An. coluzzii*	Perm; delta PM; bendio	1; 2; 3; 4; 5; 19; 16; 17; 18; 3; 20; 6	Susceptibility, metabolic resistance & kdr resistance
Sagbohan et al. [33]	2017–2018	*An. gambiae* *An. arabiensis* *An. coluzzii*	Perm; delta	1; 23; 25	Susceptibility, metabolic Resistance & kdr resistance
Sagbohan et al. [40, 42]	2017–2018	*An. gambiae* *An. arabiensis* *An. coluzzii*	Perm; delta	1; 2; 3; 4; 5; 19; 16; 17; 18; 3; 20; 6	Susceptibility, metabolic Resistance & kdr resistance
Thesis Sagbohan Hermann (2023)	2018–2022	*An. gambiae* *An. arabiensis* *An. coluzzii*	Perm; delta; PM	1; 2; 3; 4; 5; 19; 16; 17; 18; 3; 20; 6	Susceptibility, metabolic Resistance & kdr resistance
Zoungbédji et al. [4] Djégbe et al. [27]	2022 2013	*An. gambiae* *An. arabiensis* *An. coluzzii* *An. gambiae* *An. arabiensis* *An. coluzzii*	Perm Delta; chlor Clothi Perm Delta; bend PM	22; 20; 6; 1 12; 18; 6	Susceptibility, metabolic Resistance Susceptibility, metabolic Resistance
Thesis Zoungbédji David (2024)	2021–2023	*An. gambiae* *An. arabiensis* *An. coluzzii*	Perm Delta; chlor Clothi	22; 20; 6; 1	Susceptibility, metabolic Resistance & kdr resistance
Entomology profile report	2017; 2019; 2020	*An. gambiae* *An. arabiensis* *An. coluzzii*	Perm Delta; bend PM	1; 2; 5; 20; 16; 21; 6	Susceptibility, metabolic resistance
Database CREC	2008–2024	*An. gambiae* s.l *An. funestus* *An. melas*	DDT; mala; prop; diel; perm Delta; bend Alphacyp; feni PM; lamb; chlor Chlor; clothi	All district of Benin	Susceptibility & mechanisms resistance

Allada), centre (Dassa) and north (Parakou, Kandi, Malanville) that respect the inclusion criteria.

Evolution of resistance to deltamethrin

In 2010, most populations of *An. gambiae* exposed to deltamethrin-treated papers exhibited high mortality rates, indicating susceptibility in Parakou [mortality rate (Tx) = 100%] and suspected resistance in Cotonou, Allada, Dassa and Malanville, with Tx of 97%, 92%, 90% and 95%, respectively (Fig. 2; Supplementary files, Table S1). In contrast, Kandi showed a Tx of 89% (suspicion of resistance according to WHO 1998 criteria). Between 2010 and 2017, mortality rates declined progressively, reaching low levels in all communities: 14.3% (Cotonou), 48.6% (Allada), 45.8% (Dassa), 40% (Parakou), 15.7% (Kandi), 32% (Malanville) (Fig. 2; Supplementary files, Table S1) and respectively, 11%, 23%, 27%, 26%, 14%, 11.5% in 2024, i.e. this rapid progression of resistance

Table 1 (continued)

Perm permethrin, *delta* deltamethrin, *lamb* lambda-cyhalothrin, *bend* bendiocarb, *mala* malathion, *diel* dieldrin, *chhorpi* chlorpyriphos, *prop* propoxur, *clothi* clthianidin, *chlor* chlorfenapyr, *alpha cyp* alpha cypermethrin, *feni* enitrothion, *PM* pirimiphos methyl, *chlor* chlorfenapyr, *clothi* clothianidin

Locations: 1. Allada; 2. Dassa; 3. Parakou; 4. Kandi; 5. Malanville; 6. Porto-novo; 7. Dangbo; 8. Kouande; 9.Materi; 10. Natitingou; 11. Tanguieta; 12. Gogounou; 13. Segbana; 14. Djougou; 15. Ouidah; 16. Bantè; 17. N'dali; 18. Savè; 19. Cotonou; 20. Misseretè; 21. Ouidah; 22. Ifangni

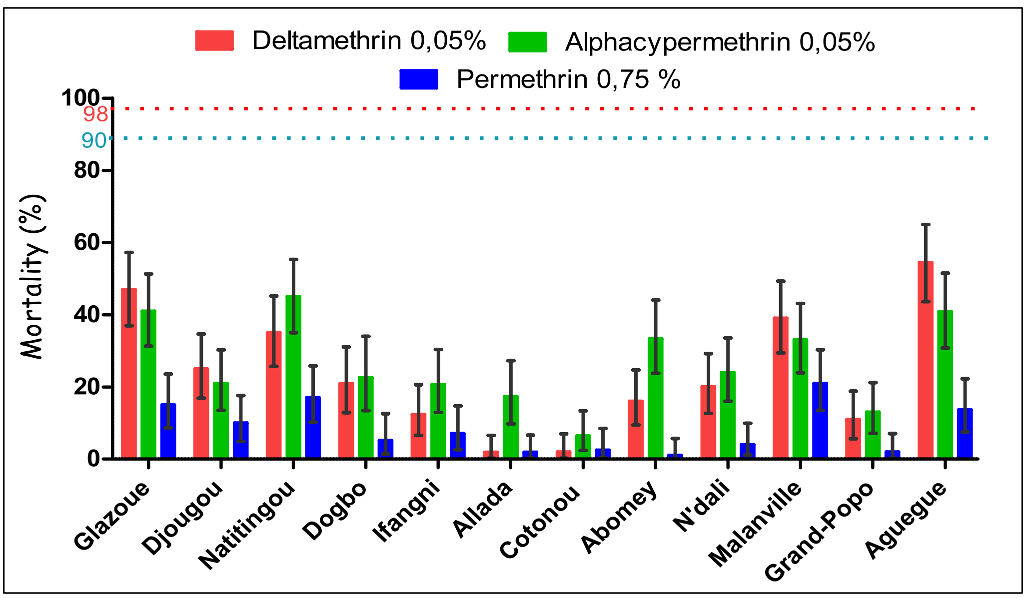

Fig. 1 Mortality rate of *Anopheles gambiae* populations after exposure to pyrethroids using the WHO tube test

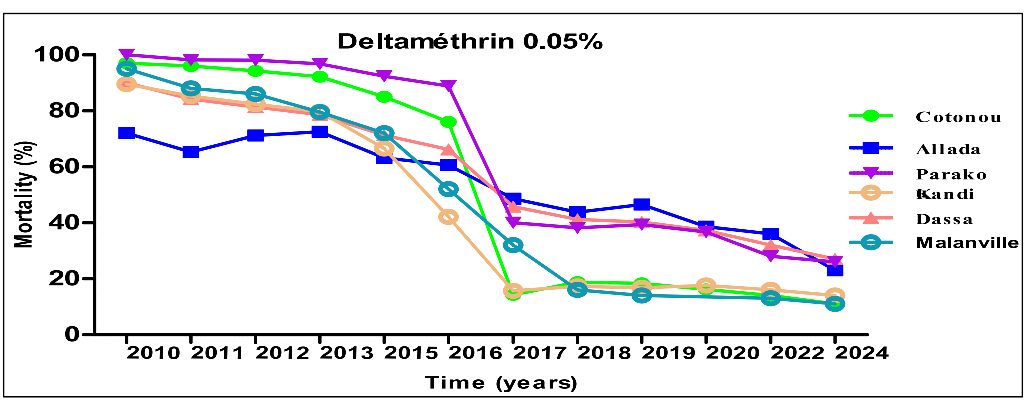

Fig. 2 Mortality rates of 6 populations *of Anopheles gambiae* after exposure to deltamethrin using the WHO tube test (2010–2024)

which reached a very high level after 14 years (from 2010 to 2024). For example, the *An. gambiae* population in Malanville, which was susceptible to deltamethrin in 1999, lost this status after 10 years (Tx = 95% in 2010 and 79.5% in 2013 (Fig. 2; Supplementary files, Table S1). Similarly, the *An. gambiae* population in Parakou, which was a susceptible population in 2010 (Tx = 100%) became resistant to deltamethrin in 2017 (Tx = 40%).

Evolution of resistance to permethrin

The resistance of *An. gambiae* to permethrin also evolved significantly between 2010 and 2024. In 2010, mortality rates ranged from 83% in Allada to 92% in Malanville, reflecting a suspicion of resistance in most study communities. Notably, in Kandi, the recorded mortality rate was 100%, indicating clear susceptibility of *An. gambiae* sto permethrin (Fig. 3; Supplementary files, Table S2). From 2013 onwards, resistance intensified progressively across the six communities. Mortality rates declined from 62.2% in 2013 to 12% in 2024 in Parakou, and from 78% in 2013 to 9% in 2024 in Malanville (Fig. 3; Supplementary files, Table S2). This steady decline in mortality rates highlights the growing resistance of *An. gambiae* to permethrin over the 14-year period.

Evolution of resistance to bendiocarb

Resistance to bendiocarb in *An. gambiae* is a relatively recent development in Benin. Until 2012, mortality rates in four communities (Cotonou, Allada, Kandi, Malanville) were at 100%, indicating full susceptibility to bendiocarb (Fig. 4; Supplementary Files, Table S3). In two communities, mortality rates were below 100% (95% in Kandi and 90% in Parakou), suggesting a mild reduction in susceptibility to bendiocarb (suspected resistance). In 2016, the *An. gambiae* population in Dassa maintained its susceptibility to bendiocarb (Tx = 98%). However, in the previously susceptible populations, mortality rates began to decline slightly: 97% in Cotonou and Allada,

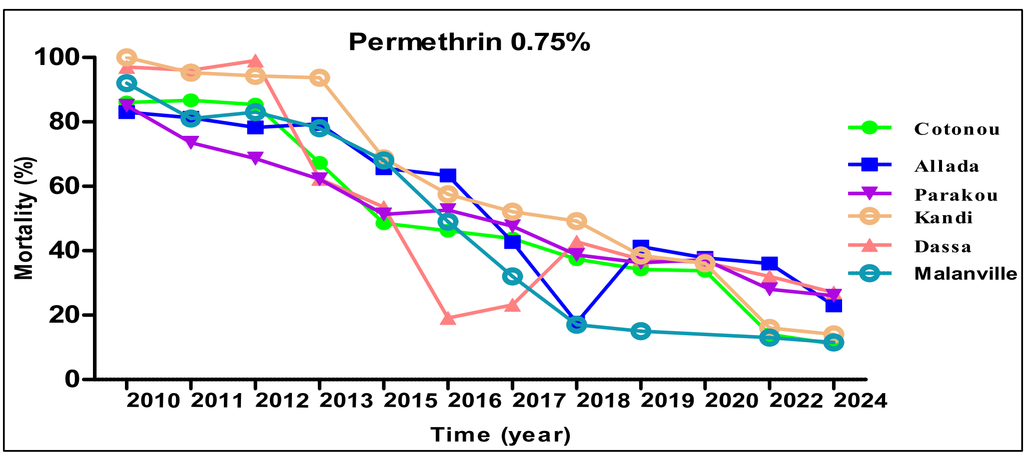

Fig. 3 Mortality rates of 6 populations *of Anopheles gambiae* after exposure to permethrin using the WHO tube test (2010–2024)

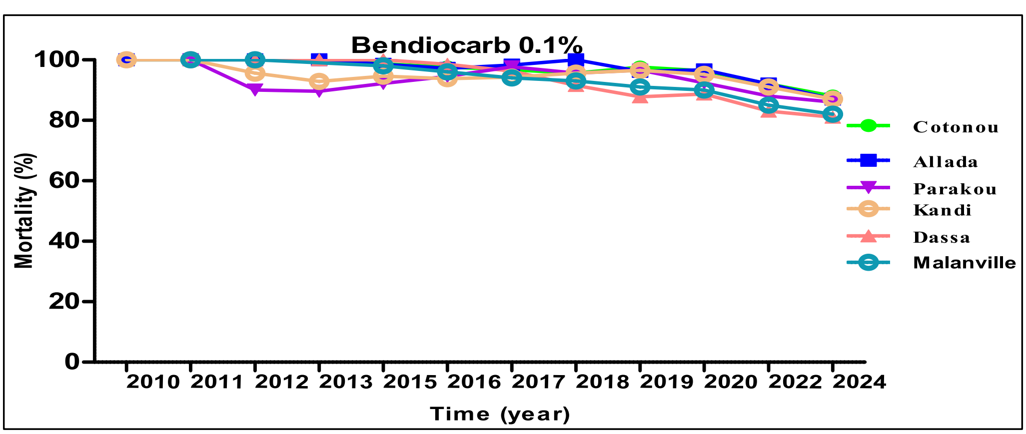

Fig. 4 Mortality rates of 6 populations *of Anopheles gambiae* after exposure to bendiocarb using the WHO tube test (2010–2024)

94% in Parakou and Malanville, and 93% in Kandi. From 2022 onwards, all six communities exhibited mortality rates below 90%, confirming resistance to bendiocarb. Mortality rates fell to 83% in Dassa, 88% in Parakou, and 85% in Malanville (Fig. 4; Supplementary Files, Table S3). This progression marks the widespread emergence of bendiocarb resistance across Benin.

Emerging resistance to pirimiphos-methyl in *Anopheles gambiae*

Pirimiphos-methyl, an organophosphate, was highly effective against *An. gambiae* populations in Benin until recently. Historically, all tested *An. gambiae* populations displayed full susceptibility to this insecticide. However, since 2022, reduced susceptibility and resistance have been observed in certain populations. Specifically, mortality rates have dropped below 90% in several locations including: Cotonou (Tx = 89%), Allada (Tx = 85%). While populations in Dassa, and Parakou still exhibit higher mortality rates (Tx = 97%), they signal a trend toward reduced efficacy of pirimiphos methyl (Fig. 5). This decline underscores the need for continuous monitoring and integrated resistance management strategies.

Mapping the distribution of resistance in *Anopheles gambiae* in 2010, 2012, 2018 and 2024 in Benin

In 2010, resistance to permethrin was observed only in the communities of Allada. By 2012, resistance expanded to deltamethrin in Allada and Parakou. By 2018, resistance to deltamethrin, permethrin, and bendiocarb became widespread across all six communities studied, though a small pocket of susceptibility to bendiocarb persisted in Allada. By 2024, resistance to all three insecticides was uniform across all study sites, underscoring the increasing challenge of vector control in Benin. This trend demonstrates the need for robust insecticide resistance management strategies to mitigate the spread of resistance (Fig. 6).

Evolution of resistance mechanisms (2010–2024)

kdr L1414F mutation frequency

The allelic frequency of the *kdr* L1014F mutation, a key marker of insecticide resistance, progressively increased across Benin from 2010 to 2024 (Figs. 7, 8, 9). Results were aggregated by department due to insufficient data at the communities level. In northern Benin (Alibori, Atacora, Borgou, Donga), the *kdr* mutation frequency rose steadily from 70–78% in 2010 to 71–95% in 2024. The department of Borgou showed the most rapid increase, with frequencies raising sharply from 33% in 2010 to 92% in 2012, reaching 95% by 2024 (Fig. 7). Similarly, in central Benin (Collines, Couffo, Mono, Zou), the *kdr* frequency exhibited a gradual upward trend. For instance, in the department of Collines, the frequency grew from 27% in 2010 to 89% in 2013, exceeding 95% by 2022. In the department of Zou, the frequency rose from below 30% in 2010 to 90% in 2024 (Fig. 8). In southern Benin (Atlantic, Littoral, Ouémé, Plateau), high *kdr* allelic frequencies were already evident by 2012, followed by a slower but steady increase over time. The *kdr* mutation frequency in these departments remained relatively stable from 2013 onward, with the department of Littoral peaking at 100% by 2020 (Fig. 9).

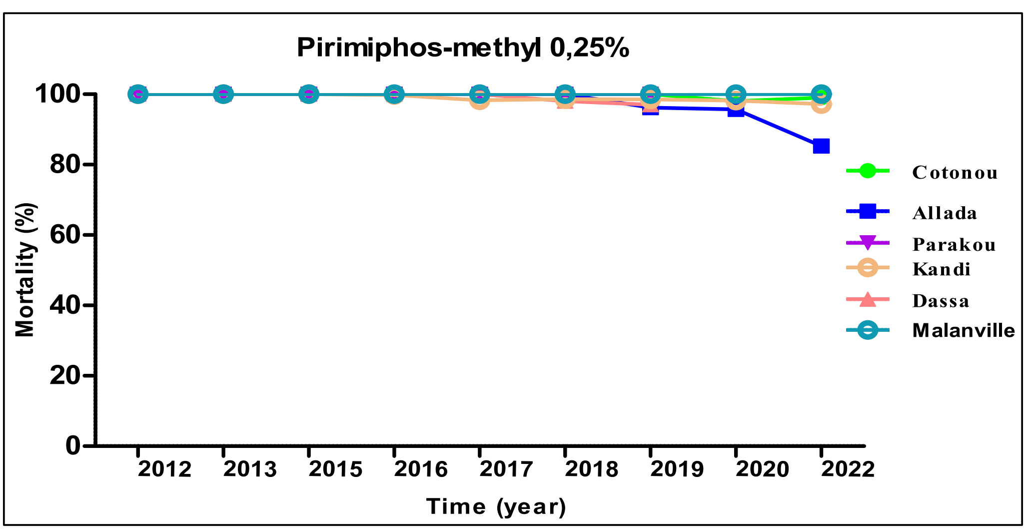

Fig. 5 Mortality rates of *Anopheles gambiae* populations after exposure to Pirimiphos-methyl using the WHO tube test (2012–2022)

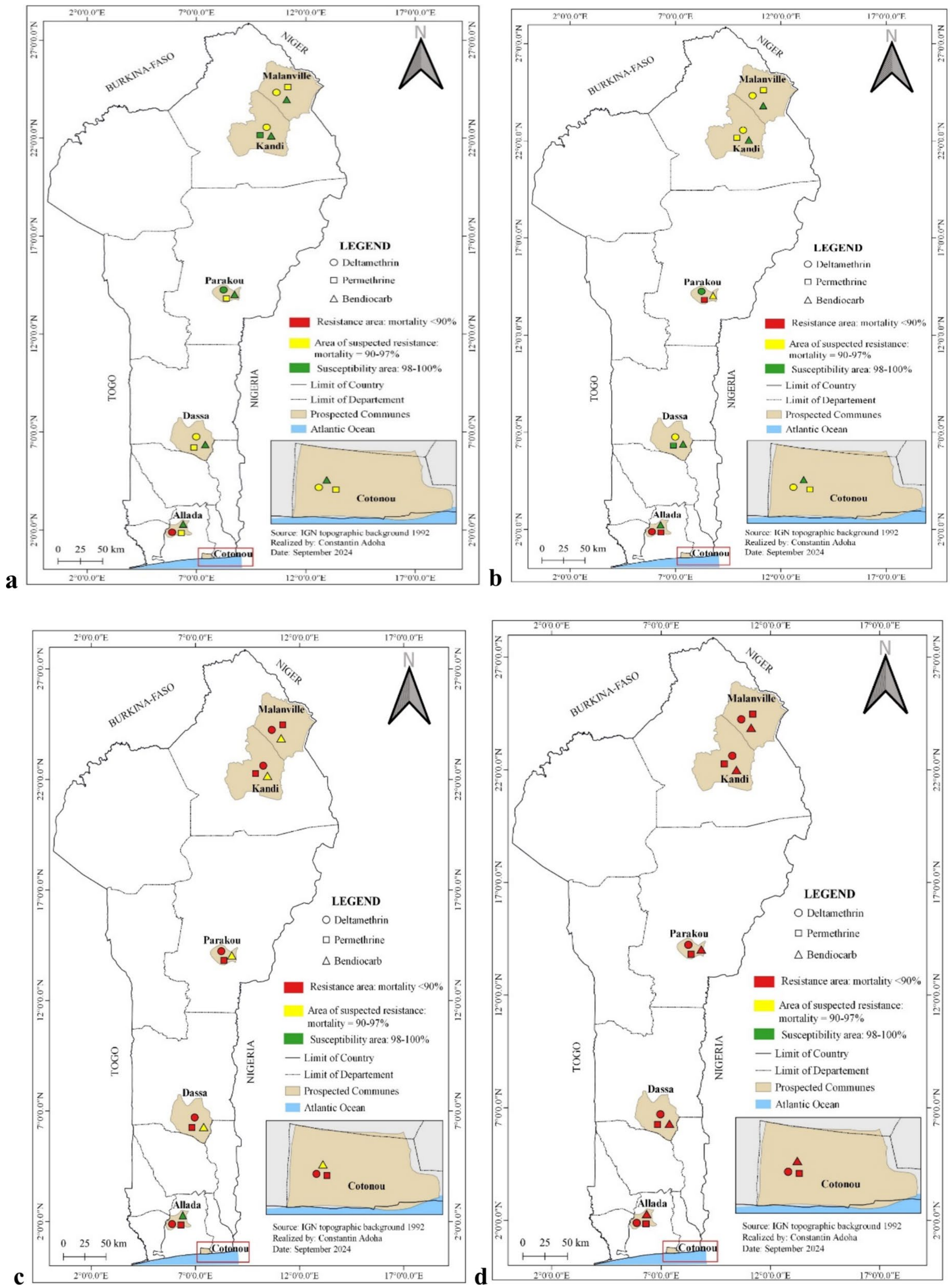

Fig. 6 Distribution of insecticide resistance in *Anopheles gambiae* in 2010, 2012, 2018 and 2024 in Benin. **a** Distribution of resistance status in 2010; **b** Distribution of resistance status in 2012; **c** Distribution of resistance status in 2018; **d** Distribution of resistance status in 2024

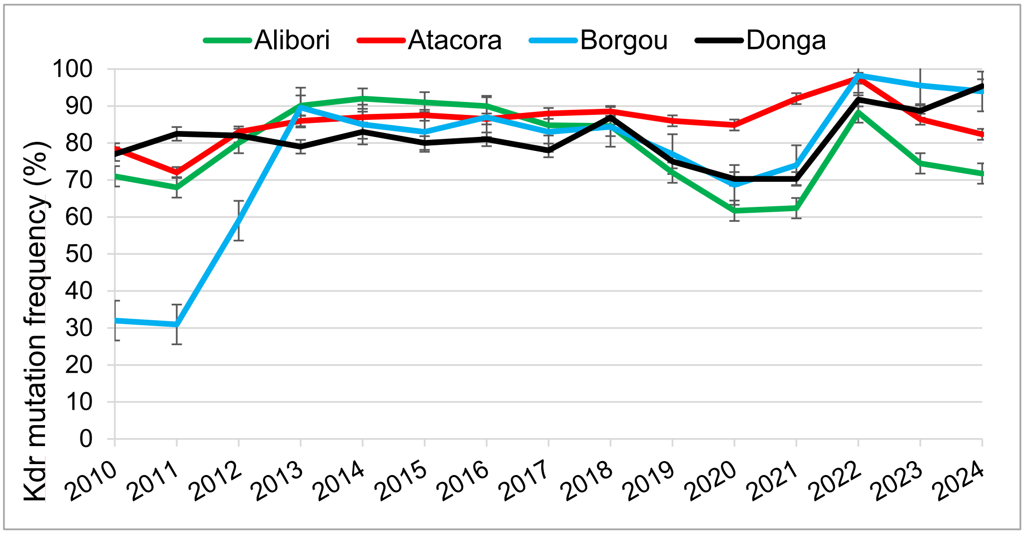

Fig. 7 Evolution of the allelic frequency of *kdr* L1014F mutation in four departments of northern Benin (Atacora, Alibori, Donga, Alibori) from 2010 à 2024

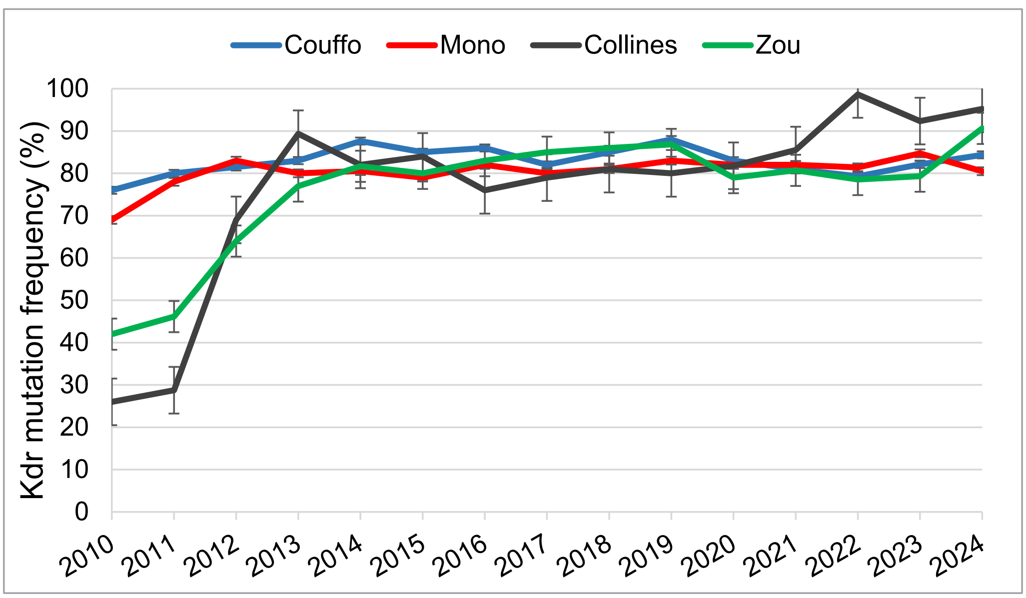

Fig. 8 Evolution of the allelic frequency of *kdr* L1014F mutation in four departments of central Benin (Collines, Couffo, Mono, Zou) from 2010 to 2024

ace-1 G119S mutation frequency

The analyses reveal that the *ace-1* G119S mutation has contributed minimally to resistance mechanisms, but its frequency has shown a gradual increase over time. In Atacora, Alibori, Borgou, and Donga, mutation frequencies of approximately 1–2% in 2010 rose to 5–7% by 2024 (Fig. 10). Similarly, in the departments of Collines, Couffo, Mono, and Zou, *ace-1* frequencies increased from 0% in 2016 to 3–8% in 2024 (Fig. 11). A comparable trend was observed in southern Benin, including the Atlantic, Littoral, Ouémé, and Plateau departments, where the frequency rose from 0% in 2016 to 6% in 2024, particularly in Littoral (Fig. 12). Collectively, Figs. 10, 11, and 12 illustrate a steady progression of the *ace-1* G119S mutation in all 12 departments from 2017 onwards.

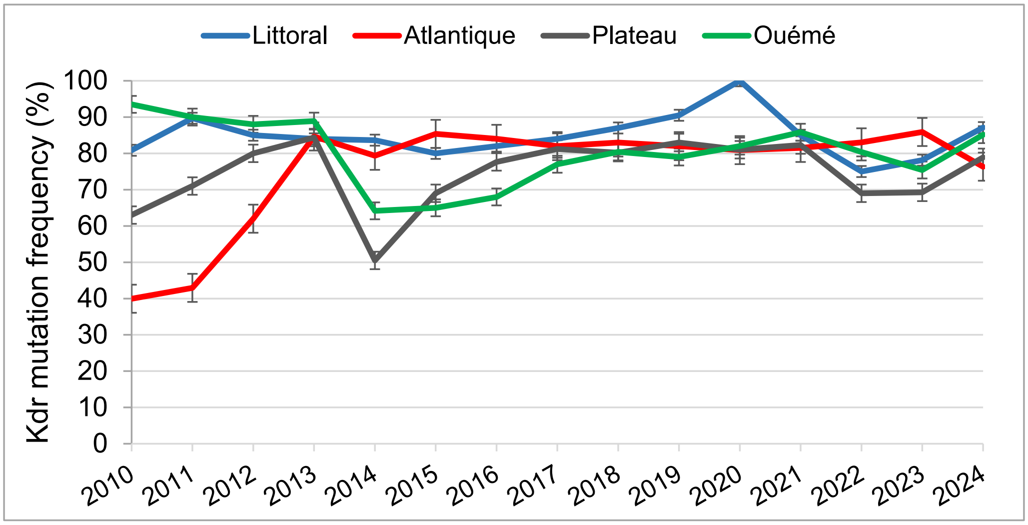

Fig. 9 Evolution of the allelic frequency of *kdr* L1014F mutation in four departments of southern Benin (Atlantic, Littoral, Ouémé, Plateau) from 2010 to 2024

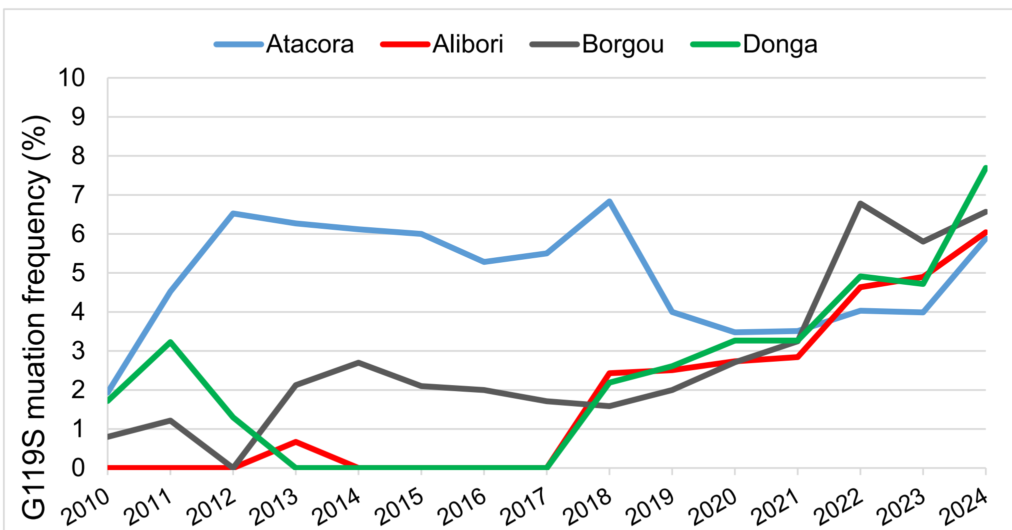

Fig. 10 Evolution of the allelic frequency of *ace-1* G119S mutation in four departments of northern Benin (Atacora, Alibori, Donga, Alibori) from 2010 to 2024

Frequencies of RR, RS and SS genotypes of the *kdr* gene from 2010 to 2024

The frequencies of *An. gambiae* carrying the homozygous resistant (RR) genotype of the *kdr* gene has significantly increased over time. Between 2011 and 2012, RR frequencies were below 80% but rose sharply in subsequent years, surpassing 80% and reaching over 90% in several communities. Notably, RR frequency reached 100% in Cotonou by 2022 and 90% in Allada by 2024. Conversely, the frequency of homozygous susceptible (SS) individuals has drastically declined, with SS genotypes now almost entirely absent across all departments in Benin (Fig. 13).

Evolution of detoxification enzymes in different populations of *An. gambiae*

Figure 14 illustrates the progressive increase in insecticide detoxification enzymes from 2015 and 2024. In 2015, detoxification enzymes such as α-esterase, β-esterase, monooxygenase, and Glutathione *S*-transferase were present in most of the study communities, albeit at varying levels. For example, the average proportion of α-esterase

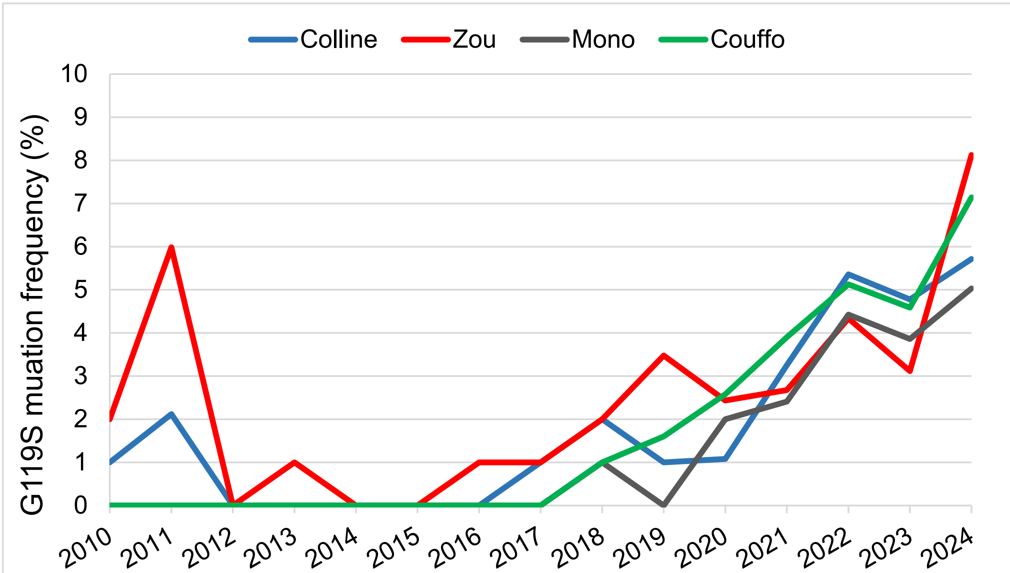

Fig. 11 Evolution of the allelic frequency of *ace-1* G119S mutation in four departments of central Benin (Collines, Zou, Mono, Couffo) from 2010 to 2024

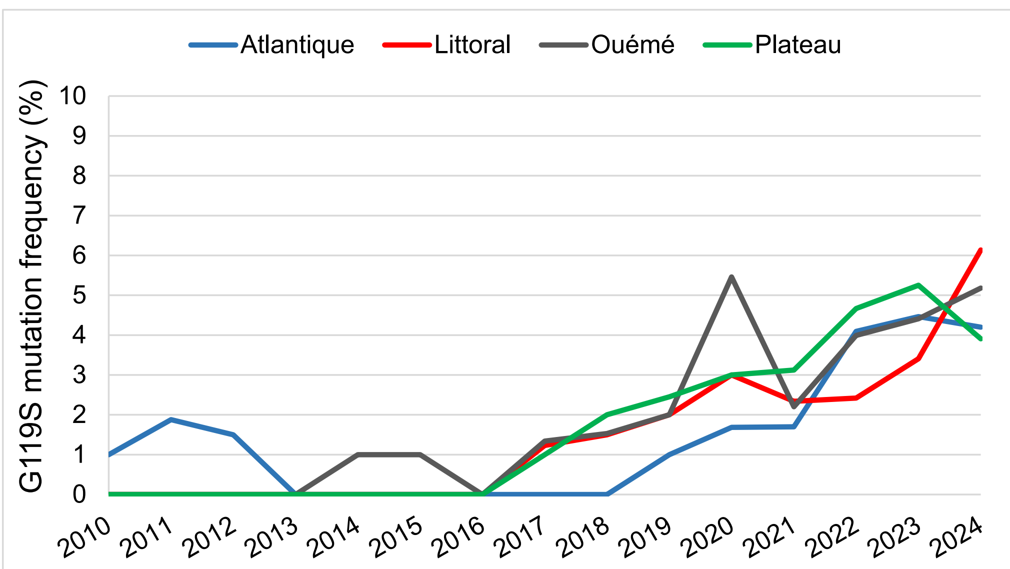

Fig. 12 Evolution of the allelic frequency of *ace-1* G119S mutation in four departments of southern Benin (Atlantic, Littoral, Ouémé, Plateau) from 2010 to 2024

in Malanville increased from 0.100 min/mg/protein in 2015 to 0.141 min/mg/protein in 2020 and 0.174 min/mg/protein in 2024. Similarly, in Cotonou, the average proportion of monooxygenase (MFO) rose from 0.230 min/mg/protein in 2015 to 0.388 min/mg/protein in 2020 and 0.580 min/mg/protein in 2024. In Allada, the average proportion of Glutathione S-transferase (GST) increased from 0.167 min/mg/protein in 2015 to 0.181 min/mg/protein in 2020, reaching 0.325 min/mg/protein in 2024.

Discussion

Vector resistance to pyrethroids, first reported in Benin in 1999, has spread rapidly across the country, reaching a very high level of intensity over the past two decades. In some communes, such as Cotonou, Allada, Dogbo,

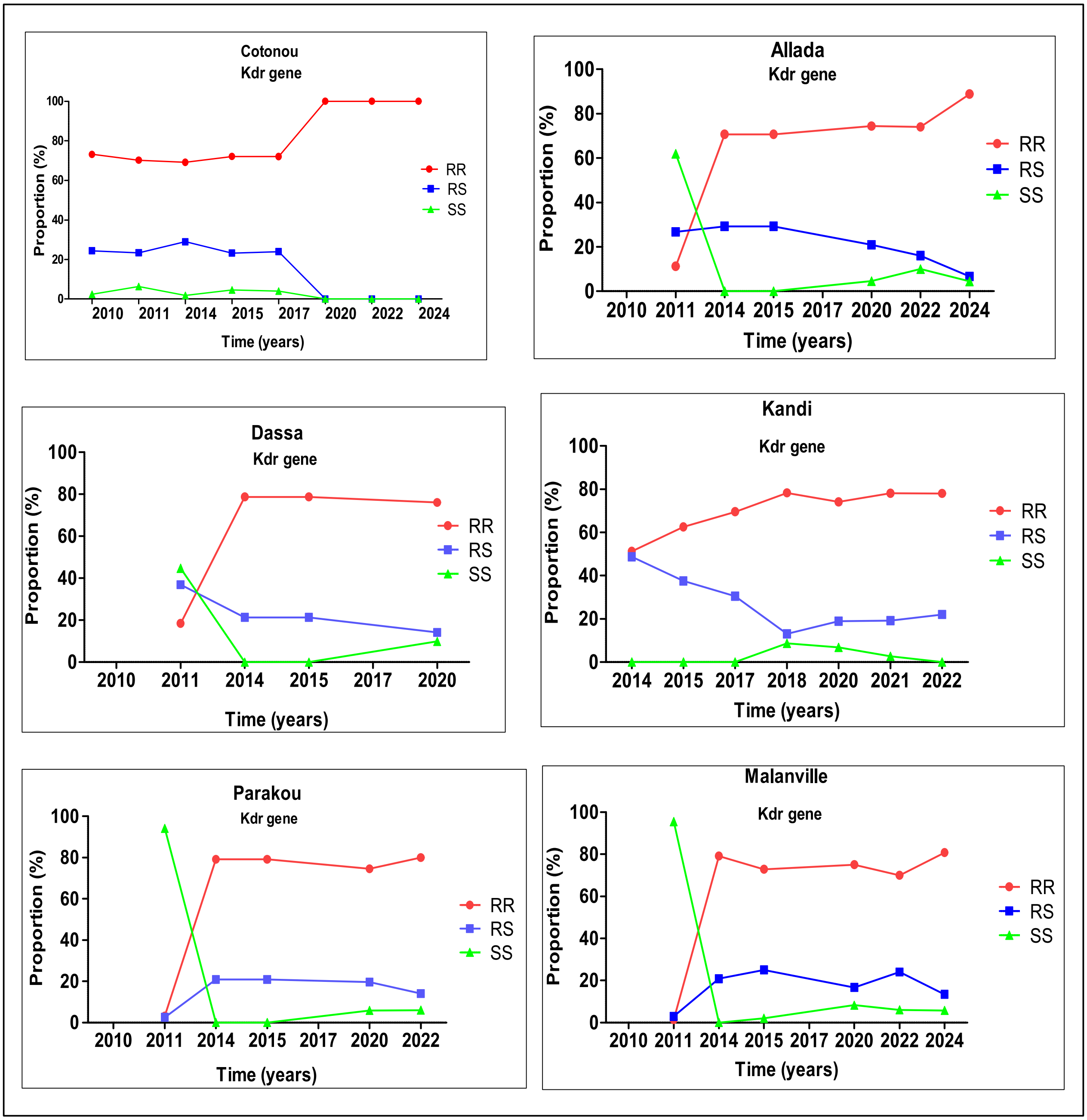

Fig. 13 Evolution of the allelic frequency of *kdr* L1014F genotypes (RR, RS, SS) in six municipalities of Benin from 2010 to 2024

N'dali and Grand-Popo, the mortality rate of *Anopheles gambiae* populations exposed to permethrin-treated papers dropped to less than 10%. The origins of this resistance, initially attributed to insecticide spray operations in major cities during the colonial era and the use of insecticides in agriculture and public health, cannot fully explain the observed trends, as these operations were often limited in scope and time. Insecticide resistance that emerged earlier has been sustained and exacerbated by continued insecticide pressure from factors such as the introduction of insecticidal mosquito nets in Benin approximately 20 years ago and indoor residual spraying campaigns conducted in Ouémé (2008–2010) [24], Atacora (2011–2016) [26], Alibori and Donga (2017–2021) [31].

Further studies, including those by Akogbeto et al. [5] and Djouaka et al. [34], highlight the role of environment pressures, such as chemicals in mosquito breeding

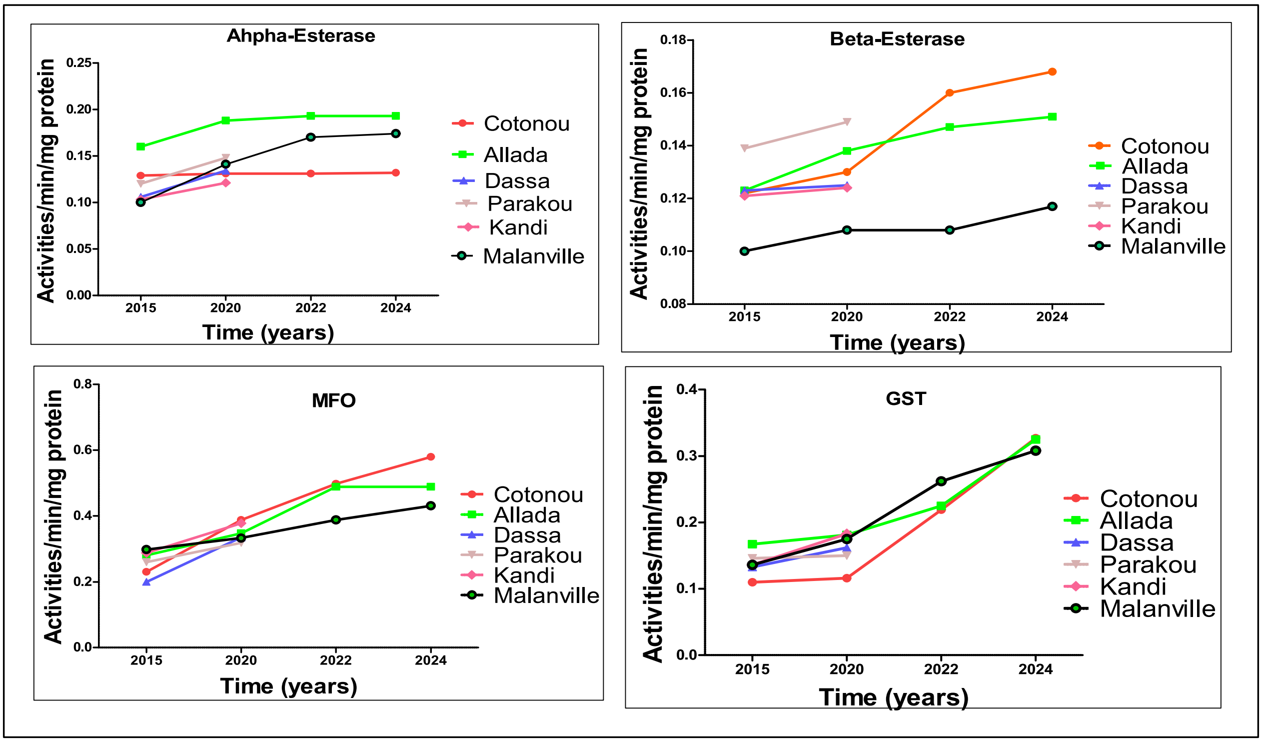

Fig. 14 Evolution of detoxification enzymes in *Anopheles gambiae* in six municipalities of Benin in 2015 to 2024

sites, which exert constant pressure on mosquito larvae, have potentially triggered resistance or intensify existing resistance. The factors contributing to insecticide resistance in mosquito vectors are diverse, encompassing environmental, behavioural, and genetic components. For example, Coetzee et al. [48] reported that 40 years of indoor insecticide spraying in Cookwe, South Africa, did not induce insecticide resistance in *Anopheles arabiensis*, in contrast to the resistance observed in *Anopheles funestus* in Kwazulu/Natal. Similarly, Nigatu et al. [49] found that populations of *An. arabiensis* characterized by the chromosomal inversion 2Rb were more resistant to DDT than their 2Rb+ counterparts. These examples demonstrate the complexity of vector resistance and underscore the difficulty of reversing it once initiated. This diversity of contributing factors likely explains the persistent increase in vector resistance to insecticides since its emergence in Benin.

The progression and intensification of insecticide resistance in malaria vectors in Benin are closely linked to the development of specific resistance mechanisms, two of which have been well studied. The first mechanism involves target-site modification, specifically at codon 1014 of the voltage-gated sodium channel [36, 37]. The second mechanism is metabolic resistance, characterized by the overproduction of detoxification enzymes that degrade insecticides before they reach their target. From 2010 to 2024, the allelic frequency of the *kdr* L1014F resistance gene mutation has steadily increased in *An. gambiae* mirroring phenotypic resistance trends. Although metabolic resistance plays a secondary role compared to target-site modifications, detoxification enzymes such as α-esterase, β-esterase, monooxygenase and glutathione S-transferase are significantly present in some municipalities and have steadily increased in activity between 2015 and 2024. The cross-resistance and multiple observed in the different populations of *An. gambiae* warns of a future operational failure of current vector control strategies in Benin if a strategic resistance management plan based on WHO guidelines is not put in place [50].

Compared to pyrethroids, the development of *An. gambiae* resistance to bendiocarb in Benin has been slower. Initially selected in 2007 for indoor spraying in Ouémé [24, 51], bendiocard was highly effective against pyrethroid-resistant mosquitoes. However, by 2010, cases of suspected resistance emerged [35], prompting the Benin National Malaria Control Program to abandon bendiocarb and replace it with pirimiphos-methyl for IRS campaigns in Atacora. After this change, data from 2010 to 2024 indicate limited progression in resistance to bendiocarb. Pirimiphos-methyl, regarded as a cornerstone product for IRS campaigns, has been alternated with other commercial products such as Fludora Fusion^{R}

(Clothiandin 500 g/kg and deltamethrin 62.5 g/kg), then Sumishield[R] 50 WG (clothianidin) [52, 53]. However, resistance to pirimiphos-methyl has been observed in some communes in Benin over the past 2 years, raising concerns about its continued efficacy.

Conclusion

At the current stage of insecticide resistance in Benin, complementary vector control strategies are critical. Physical vector control strategies, including environmental sanitation and the elimination of mosquito breeding sites, should be integrated into existing interventions. This approach requires the active involvement of local authorities and grassroots communities to enhance the overall effectiveness of vector control efforts.

Abbreviations

An	*Anopheles*
IRS	Indoor residual spraying
LLINs	Long-lasting insecticidal nets
NMCP	National Malaria Control Programme
WHO	World Health Organization

Supplementary Information

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Additional file 1.

Additional file 2.

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Author contributions

The study was designed and its protocol written by HZS, RAO, LB and MA. Data collection was performed by HZS, SH, CK, KDK, ZA, CA, BY, AS, and EO. MJA and LT were performed Molecular analyses. HZS, CK, AS, FT and RAO wrote the manuscript. HZS and BA performed the statistical analysis of the data. RAO, RA, GGP and MA have revised the manuscript.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

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