Insecticide selection pressure affects the secondary malaria vector *Anopheles coustani* in the Vallée du Kou, Burkina Faso

Domonbabele François de Sales HIEN[1*], Arielle Bettina Sandra BADIEL[1], Koama BAYILI[1,2], Abdoulaye DIABATE[1] Etienne BILGO[1]

Abstract

Malaria remains a major public health problem in Burkina Faso and is transmitted by several *Anopheles* species. The principal vectors include *Anopheles gambiae*, *An. arabiensis*, *An. coluzzii*, and *An. funestus*, while *An. coustani*, *An. rufipes*, and *An. pharoensis* are considered secondary vectors. Vector control strategies, mainly insecticide-treated nets and indoor residual spraying, are increasingly threatened by the spread of insecticide resistance. However, most studies on resistance mechanisms have focused on primary vectors, leaving secondary vectors poorly investigated.

This study aimed to characterize the pyrethroid resistance profile of *Anopheles coustani*, a secondary malaria vector in the Vallée du Kou, Burkina Faso. Between September and October 2021, adult female mosquitoes were collected and reared under insectary conditions. Their first-generation progeny (F1) were tested using WHO bioassays with 0.75% permethrin. Molecular analyses were conducted for species identification and detection of the *kdr*-995F mutation, a key genetic marker of pyrethroid resistance.

A total of 164 *An. coustani* females were tested, showing a mortality rate of 79.79%, indicative of moderate resistance. The *kdr*-995F mutation was detected in 56.90% resistant homozygotes, 37.93% heterozygotes, and 5.17% susceptible homozygotes, corresponding to an overall allele frequency of 0.76.

These results demonstrate that *An. coustani* populations in the Vallée du Kou exhibit phenotypic resistance associated with a high prevalence of the *kdr* mutation. This first report of *kdr*-995F in *An. coustani* in Burkina Faso highlights the need to include secondary vectors in comprehensive insecticide resistance surveillance programs.

[1] Centre National de la Recherche Scientifique et Technologique (CNRST)/Institut de Recherche en Sciences de la Santé (IRSS) / Laboratoire de Recherche sur les Maladies Infectieuses et Parasitaires (LR-MIP) / Bobo-Dioulasso, BP 545, Bobo-Dioulasso, Burkina Faso.

[2] Université Nazi BONI (UNB), BP 1091, Bobo-Dioulasso, Burkina Faso.

***Corresponding author**: Domonbabele François de Sales HIEN, hiend83@yahoo.fr

DOI : https://doi.org/10.64707/revstss.v48i2.1980

Keywords: *Anopheles coustani,* pyrethroid resistance, *kdr-995F*, Vallée du Kou, Burkina Faso

Pression de sélection exercée des insecticides et impact sur le vecteur secondaire du paludisme *Anopheles coustani* à la Vallée du Kou, au Burkina Faso

Résumé

Le paludisme demeure un problème majeur de santé publique au Burkina Faso et est transmis par plusieurs espèces d'Anopheles. Les principaux vecteurs incluent *Anopheles gambiae*, *An. arabiensis*, *An. coluzzii* et *An. funestus*, tandis que *An. coustani*, *An. rufipes* et *An. pharoensis* sont considérés comme des vecteurs secondaires. Cette diversité complique la lutte antivectorielle, d'autant plus que l'efficacité des moustiquaires imprégnées et de la pulvérisation intradomiciliaire est compromise par la résistance aux insecticides. Malgré cela, les recherches se focalisent principalement sur les vecteurs primaires, laissant les vecteurs secondaires peu étudiés.

Cette étude visait à caractériser le profil de résistance aux pyréthrinoïdes de *Anopheles coustani* dans la Vallée du Kou, au Burkina Faso. Entre septembre et octobre 2021, des femelles adultes ont été collectées, élevées en insectarium, et leur descendance F1 a été soumise à des bioessais OMS avec 0,75 % de perméthrine. Des analyses moléculaires ont permis l'identification des espèces et la détection de la mutation *kdr*-995F.

Au total, 164 femelles *An. coustani* ont été testées, avec un taux de mortalité de 79,79 %, indiquant une résistance modérée. La mutation *kdr*-995F était observée chez 56,90 % des homozygotes résistants, 37,93 % des hétérozygotes et 5,17 % des homozygotes sensibles, correspondant à une fréquence allélique de 0,76.

Ces résultats montrent que les populations de *An. coustani* présentent une résistance phénotypique associée à une forte prévalence de la mutation *kdr*, soulignant la nécessité d'une surveillance de la résistance incluant les vecteurs secondaires.

Mots-clés : *Anopheles coustani*, résistance aux pyréthrinoïdes, *kdr-995F*, Vallée du Kou, Burkina Faso

Introduction

Malaria is a tropical and subtropical, mosquito-borne parasitic disease that is endemic in 84 countries and causes an estimated 247 million clinical infections and 619 000 deaths annually (1). Since 2020, according to the WHO's annual report, the number of estimated malaria cases has steadily increased, and most of this increase occurred in countries in the WHO African Region with 89.7% (2). Between 2021 and 2023, deaths due to malaria were 597 000 woldwise and WHO African Region 569 000 (2). Despite this decrease, malaria remains a

public health problem worldwide especially in Africa and the number of malaria cases continuing to rise (2). In Burkina Faso, despite multiple malaria control campaigns, it remains a real human burden. The country is among the ten countries with the highest number of malaria cases and deaths worldwide, accounting for 3.1% and 2.7% respectively in 2023(3). Malaria is the leading cause of consultations, hospitalizations, and deaths in health facilities(3). The main control tools are based on vector control through long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) and malaria drugs to treat the cases. Malaria transmission dynamics within sub-Saharan African countries is highly variable due to climate and vector density. Malaria vectors are essentially divided into major and secondary vectors. In sub-Saharan Africa, the major vectors of *Plasmodium falciparum* belong to the *Anopheles gambiae* s.l. complex and the *Anopheles funestus* group (4). In Burkina Faso, *Anopheles gambiae*, *Anopheles coluzzii* and *Anopheles arabiensis* of the *Anopheles gambiae* s.l. complex, *Anopheles funestus* and *Anopheles nili* are the major malaria vectors (5). Secondary vectors in Sub-Saharan Africa are *Anopheles coustani*, *Anopheles pharoensis*, *Anopheles rufipes*, *Anopheles squamosus*, *Anopheles paludis*, *Anopheles brohieri*, *Anopheles hargreavesi*, *Anopheles hanocki* (4) (Yaw et al 2016). These species could play a relay role in malaria transmission. Their involvement in local malaria transmission has been recognized. They could contribute to increasing, prolonging and potentially maintaining malaria transmission when the main vectors are at rest or have been killed by vector control measures(6). Despite the importance role that they could play in maintaining malaria transmission, very few studies have investigated their resistance to the main insecticides used in vectors control strategies. However, vector control, one of the main strategies for combating malaria in Burkina Faso, is hampered by insecticide resistance. Monitoring must consider both major and secondary vectors. Worldwide, most insecticides are primarily used to control pests that infest cultivated plants (7); only a small minority are used against insects of medical or veterinary interest (8). The main insecticides used in malaria vector control belong to six chemical families: organochlorines, organophosphates, carbamates, pyrethroids, pyrroles and and neonicotinoids. Of these, pyrethroids remain widely used by the malaria control program in the countries due to their rapid onset of action at low doses. The aim of this study was to describe the phenotypic and genotypic resistance of *Anopheles coustani* to

pyrethroids in the Vallée du Kou. Elucidation of phenotypic and genotypic insecticide resistance in secondary vectors is critical for progress toward malaria elimination. Phenotypic resistance directly reflects the operational failure of vector control interventions after exposing mosquitoes to common insecticide. In contrast, genotypic resistance provides early warning of emerging resistance through the detection of molecular markers and resistance mechanisms before control failure becomes evident. This combined approach is essential to guide insecticide selection, manage resistance, and sustain the impact of malaria vector control strategies

I. Materials and methods

Study site

The study took place from September to October 2021 in Bama (Vallée du Kou); (11°24'N; 4°23'W), from 30 km north of Bobo-Dioulasso. A 1,200-hectare rice-growing area has been developed in this zone since the 1970s, bringing together seven villages (9, 10). There are two seasons, a rainy season from May to October, with average annual rainfall of between 1,000 and 1,200 mm, and a dry season from November to April. Rice is grown twice a year one in the rainy season (July to November) and the other in the dry season (January to June) (11). Rice fields are permanent breeding grounds for mosquitoes due to irrigation. Depressions and rainwater puddles are additional breeding sites for *culicidae*. Malaria is transmitted throughout the year, with a peak during the rainy season. *Anopheles coluzzii* is the dominant vector. Malaria vectors are high resistance to pyrethroids and DDT with 0.8–0.9 of L1014F *kdr*-mutation as prevalent. This high level of resistance could be attributed to the use of pesticides in rice cultivation.

Mosquito collection

Blood-fed adult females of *Anopheles coustani* were collected using the double-net trap modified into a single-net trap. During the double-net trap collect method, one adult occupies one trap and according to the different experiments collected mosquitoes for six or eight hours. Participants were fully protected from mosquitoes by a small blue polyester bed net which was not treated with any insecticide and which hung over the bed to the ground. A larger untreated bed net which was also not treated with insecticide was hung over the smaller net and was raised 30 cm above the ground. Mosquitoes were caught in the ±20 cm gap between the two nets. In our context, we modify the method to a

single-net trap using cow instead of human (12). A cow of 3- -5 months old was used as a bait inside the trap from 6 pm to 5 am. The net was raised 20 cm off the ground to allow mosquitoes to enter and take their blood meal on the cow. Mosquitoes were collected from the trap between 5 and 6 am using a mouth aspirator. They were then identified morphologically according to the identification keys of Gillies and Coetzee and Gillies and de Meillon (13,14). Only mosquitoes identified as *An. coustani* were transferred to the laboratory. The live blood-fed females were group in small cages for oviposition and dead or moribund were transferred for molecular testing.

Mosquitoes breeding at the insectarium

Mosquitoes were reared at the insectarium of Centre Muraz. The females were briefly fed with 10% glucose, then placed in a group for oviposition the following day. Cups were placed inside the small cages to collecte the eggs. A small diameter opening in the net served as a channel for introducing the mosquitoes into the cages. Water was then poured over 1/3 of the cups, as mosquitoes breeding ground. Mosquitoes were removed after laying eggs for molecular testing, including those had not laid after 72 hours. The eggs were distributed on the trays with water for hatching. The larvae were fed with TetraMinBaby®. Pupae were collected each morning and transferred to 30 cm x 30 cm x 30 cm cages covered with mosquito netting. After emergence, adults (F1) were fed with 10% glucose and maintained under standard conditions of Temperature (27 ± 2ºC), Relative Humidity (75±10%) before transferred to the insecticides testing facilities for bioassays.

Bioassay test

Bioassay was carried out on 164 engorged female *Anopheles coustani* mosquitoes from the first-generation aged from the 3 to 5-day-old using permethrin 0.75% WHO impregnated papers at diagnostic dose. The test was done by following WHO protocol (14). Mosquitoes were exposed to impregnated papers for 1 hour of contact. For each test, four batches of 25±2 *Anopheles coustani* were used and KD (knock-down) assess at 5 min of intervals up to 60min. The pyrethroid-resistant laboratory strain *Anopheles coluzzii* was also tested as a control. Negative control with untreated papers were also used at the same time as treated papers. Four and two replicate tubes were respectively used for treated and untreated papers After exposure (at 27°C±2°C temperature and 75%±10% relative humidity), mosquitoes were fed

with a cotton swab soaked with 10% glucose and kept on laboratory standard conditions for 24 hours to record delayed mortality. The results were interpreted according to WHO standard operating procedure for testing the susceptibility of adult mosquitoes to insecticides in WHO tube tests (15). The results were interpreted as follows: Resistance confirmed: if mortality is <90%; Possible resistance: if mortality is ≥90% but <98% and susceptible: if mortality is ≥98%.

Molecular analysis

Extraction of genomic DNA from mosquitoes

Genomic DNA was extracted from whole mosquitoes. In total, genomic DNA from 576 *Anopheles coustani* females was extracted using CTAB method (16). Genomic DNA obtained was used for molecular identification of the mosquito specimens, and a part of 232 was used for ***Kdr-995F*** mutation by PCR (17).

Anopheles coustani identification by PCR

Each mosquito was identified using PCR-ITS 2rDNA. 2µl of 1:10 diluted genomic DNA was mixed in 18µl of reaction mixture. The PCR used a pair of primers labelled ITS2A and ITS2B (18).

ITS2A: 5'-TGTGAACTGCAGGACACAT-3'

ITS2B: 5'-TATGCTTAAATTCAGGGGGT-3'

PCR conditions were 5 min at 94°C for initial denaturation, followed by 35 cycles each comprising 30 seconds of denaturation at 94°C, 1 minute 30 seconds of hybridization at 55°C and 60 seconds of extension at 72°C. The last extension step is extended by holding at 72°C for 10 minutes.

Kdr-995F genotype detection by PCR

The *kdr-995F* genotype was analysed using genomic DNA from mosquito by following Martinez-Torres *et al*. (1998) protocol (17) Two pairs of primers were used for the detection of mutant genotype.

D1: 5'-ATAGATTCCCCGACCATG-3'

D2: 5'- AGACAAGGATGATGAACC-3'

D3: 5'- AATTTGCATTACTTACGACA-3'

D4: 5'- CTGTAGTGATAGGAAATTTA-3'

For each mosquito, 2µl of 1/10 diluted genomic DNA was added to 18µl of reaction mixture, bringing the final volume to 20µl. The reaction mix contained 4.1µl of ultrapure water, 4µl of 5X buffer, 0.8µl of 5 mM dNTPs, 2µl of 25 mM MgC12, 1.5µl of each of the two primers Dl (20µm) and D2 (20µm) and 2µl of each of the last two primers D3 (20µm) and D4 (20µm) and 0.5µl of Taq Polymerase 5U/µL. PCR conditions were: initial denaturation at 94°C for 10 minutes, followed by 40 cycles, each comprising 45 seconds of denaturation at 94°C, 60 seconds of hybridization at 52.4°C and 60 seconds of extension at 72°C. The expected sizes of the DNA fragments visualized under UV light were 293 bp for the common band (D1/D2), 195 bp for the resistant mutation (D1/D3), and 137 bp for the susceptible mutation (D2/D4), respectively.

Statistical analyses

All analyses were performed using R software version 4.1.2, and graph were generated using the core R package ggplot2. Allelic frequencies of the *kdr-995F* mutation were calculated using R version 4.1.2 according to the formula f(R) = (2RR + RS) / 2n.

II. Results

Identification of *Anopheles coustani*

A total of 576 mosquitoes were identified morphologically as *Anopheles coustani* females. The genomic DNA of these mosquitoes was then used for molecular analysis to confirm the morphological identification data. Thus, 524 were molecular identified as *Anopheles coustani*, i.e., 90.97% (95% CI 88.3-93.1), 47 were indeterminate, i.e., 8.16% (95% CI 6.2-10.7), and the remaining 5 were identified as belonging to species of the *Anopheles gambiae* complex, i.e., 0.87% (95% CI 0.3%-2.1%) (Table I).

Table I: Molecular confirmation of mosquito's specimens collected in the Vallée du Kou, Burkina Faso and identified morphologically as *Anopheles coustani*

	Morphological identification of *Anopheles coustani*	Confirmation by molecular analysis		
		Confirmed samples	indeterminate	Others species
Mosquito number	576	524	47	5
Frequence (%)	-	90.97% (88.3%-93.1%)	8.16% (6.2%-10.7%)	0.87% (0.3%-2.1%)
Total	576	576		

Susceptibility status of *Anopheles coustani* to permethrin 0.75%

The rearing at the insectary produced the first generation of *Anopheles coustani* using samples identified molecularly as *Anopheles coustani*. We used a sample from this population to assess the sensitivity of the *Anopheles coustani* species to 0.75% permethrin The mortality rate of negative controls using both *Anopheles coustani* and *Anopheles* Kdr-kis was less than 5% correction of treatments mortality was not necessary. The mortality rate observed with permethrin 0.75% with *Anopheles coustani* and *Anopheles* kdr-kis was less than 90%, we got 79.79% for *Anopheles coustani*'s mortality rate and 26.28% for *Anopheles* kdr-kis' mortality rate.

Knockdown assessment

Bioassays were used to evaluate KD at regular intervals. The KD rate of mosquitoes in contact with 0.75% permethrin increased over time, reaching a maximum 60 minutes after exposure. The data in the table above was used to establish the KD curve. These curves allow the evolution of mortality rates for each strain to be assessed according to exposure time (Figure 1).

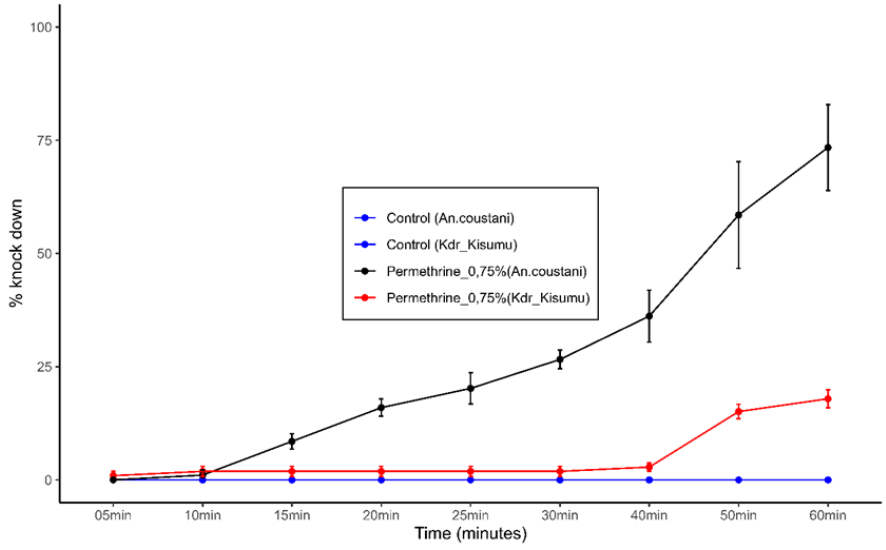

Figure 1 : Curves comparing the effect of 0.75% permethrin on Anopheles coustani and on the reference strain Anopheles kdr-kis

Kdr-995F mutation distribution

The *kdr-995F* mutation conferring cross-resistance to DDT and pyrethroids was detected in *Anopheles coustani*. Resistance genes were characterized in 232 individuals. The distribution of resistance genes in this population was as follow, 132 (56.90%) homozygous resistant RR, 88 (37.93%) heterozygous RS and 12 (5.17%) homozygous sensitive SS. The frequency of the *kdr-995F* mutation was 0.76 in *Anopheles coustani*.

III. Discussion

Malaria transmission in Africa is a dynamic, complex and constantly evolving system. Despite much work on the epidemiology and control of malaria, there are still gaps in the understanding of the ecology and biology of secondary vectors. Much of attention is being gave to the main malaria vectors, with the promotion of intensive use of long-lasting insecticide-treated nets (LLINs) and insecticide residual spray (IRS), aimed primarily to control transmission and their resistance to insecticides. However, residual malaria transmission has become a major public health problem in recent years (19). The contribution of the secondary vectors should be given greater consideration as an influence on residual malaria transmission. In their ecological study, *An. pharoensis, An. coustani* and *An. ziemanni* demonstrated that they could be anthropophilic, since they were captured in traps set by humans and carried *P. falciparum* sporozoites, as well as *P. vivax* in Ethiopia (20). Studies in sub-saharan show that it is possible for secondary vectors to become the main vectors, at a time when many African countries are moving towards malaria elimination through vector control (21). The present study objective was to describe the phenotypic and genotypic resistance of *Anopheles coustani* to pyrethroids in the Vallée du Kou. This study allows to detect for the first time the allele L1014F in *Anopheles coustani*. This specie is considered as secondary malaria vector in Burkina Faso according to its low implication in malaria transmission. The prevalence of the kdr allele L1014F mutation, which confers cross-resistance to pyrethroids and DDT on mosquitoes (22)was 0.76 in *Anopheles coustani*. Few or any, previous studies have examined the expression of this mutation in the secondary vector *Anopheles coustani*. The presence of this mutation in this specie could result from the high selective pressure induced by using pesticides in agriculture. In the Vallée du Kou, 1,200-hectare rice-growing area has been developed in this zone since the 1970s (10, 11).

The production of crops like rice, millet, and cotton requires the use of large quantities of pesticides (23) . Several studies carried out in the same rice-growing area or in neighboring rice- or cotton-growing areas have dealt with the pyrethroid resistance in Anopheles mosquitoes (24). In Côte d'Ivoire, a study carried out at five agricultural sites, the frequency of expression of the Kdr mutation varied from 0.37 in the site without agricultural insecticides to 0.95 in the site with high insecticide use in the major vectors (*Anopheles gambiae* and *Anopheles coluzzii*) (25). In addition, bioassays were performed in the present study to evaluate the susceptibility of *Anopheles coustani* to permethrin. This study revealed a mortality rate to permethrin < 90%. According to WHO standard operating procedure for testing the susceptibility of adult mosquitoes to insecticides in WHO tube tests (14), the results of the sensitivity test revealed that both populations (*Anopheles coustani* and *Anopheles* kdr-kis) are resistant to permethrin 0.75% (15). However, *Anopheles coustani* population is more sensitive to permethrin 0.75% than the reference *Anopheles* kdr-kis. The study site is a rice-growing area, which means wide used of insecticides. This result confirm also the genotype one as kdr mutation is alqo link to phenotype resistance. Most of the studies carried out in this area aimed to assess the impact of insecticide on the major malaria vectors. In Burkina Faso, insecticide susceptibility bioassays were carried out on seven natural populations of the *Anopheles gambiae* complex in western Burkina Faso during the 2016 rainy season using the WHO protocol (Namountougou et al., 2019) (26). These susceptibility tests showed that the *Anopheles gambiae* complex was multi-resistant to pyrethroids, DDT and carbamates in almost all zones(26). Mortality rates varied from 10 to 38% for pyrethroids (deltamethrin). Three (3) species of the *Anopheles gambiae* complex were identified: *Anopheles gambiae* s.s, *Anopheles coluzzii* and *Anopheles arabiensis*. Frequencies of the kdr-w mutation were widespread (0.66 to 0.98) among the three species of the complex (26).

This study provided scientific proof of insecticides effects on secondary vectors, in this case *Anopheles coustani*. Secondary vectors are therefore able to resist to pyrethroids in the same way as major vectors. This study has limitations that should be considered when interpreting the results. First, the investigation was conducted at a single site (Vallée du Kou), which limits the extrapolation of the findings to other ecological settings in Burkina Faso where *Anopheles coustani* may experience different insecticide selection pressures. Second, although

phenotypic resistance to permethrin was demonstrated, resistance intensity assays and synergist bioassays (e.g., PBO tests) were not performed, preventing discrimination between metabolic and target-site resistance mechanisms. Third, the genotypic analysis focused on the *kdr-995F* mutation only, while other resistance markers (e.g., *kdr-995F*, N1575Y, or metabolic resistance genes) were not investigated and may contribute to the observed resistance phenotype. Fourth, the study did not assess seasonal variation or temporal trends in resistance, which could influence both allele frequency and phenotypic susceptibility. Finally, although *Anopheles coustani* is considered a secondary vector, the study did not evaluate its vector competence or contribution to malaria transmission, limiting conclusions regarding the epidemiological impact of insecticide resistance in this species.

Conclusion

The present study revealed that *Anopheles coustani* develops phenotypic resistance associated with a high frequency of the kdr mutation, the main mechanism of resistance to pyrethroids. The selection pressure for pyrethroid resistance in the Vallée du Kou is therefore not limited to major vectors. However, vector control in its current form only targets major vectors; it would therefore be important to involve secondary vectors, in particular *Anopheles coustani*, in this control.

Acknowledgements

We thank all volunteers for participating in this study as well as the local authorities for their support. We are very grateful to all the students and technicians at the IRSS who provided valuable assistance for the experiments of this study.

Author contributions

A.B.S.B, K.B and D.F.S.H conceived and designed the present study and conducted the experiments, with E.B. providing supervision and overseeing the study; and D.F.S.H carried out the data analysis; A.B.S. B drafted the manuscript; E.B., K.B and A.D provided critical revisions to the manuscript. All authors reviewed and approved the final version of the manuscript

Consent for publication

All authors concur with the submission presented by the corresponding authors.

Competing interests

The authors declare no competing interests

References

1. Poespoprodjo JR, Douglas NM, Ansong D, Kho S, Anstey NM. Malaria. In *The Lancet* 2023 Vol. 402, Issue 10419, pp. 2328–2345).
2. World Health Organization. World Malaria Report 2023a. World Health Organization: Geneva, Switzerland, 2023a
3. World Health Organization. World Malaria Report 2023b. World Health Organization: Geneva, Switzerland, 2023b
4. Lord CC, Woolhouse MEJ, Heesterbeek JAP, Mellor PS. Vector-borne diseases and the basic reproduction number: a case study of African horse sickness. *Medical and Veterinary Entomology* 1996, *10*(1), 19–28.
5. Plowe CV. The evolution of drug-resistant malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2009, *103*(1), S11–S14.
6. Wanji S, Tanke T, Atanga SN, Ajonina C, Nicholas T, Fontenille D. *Anopheles* species of the mount Cameroon region: biting habits, feeding behaviour and entomological inoculation rates. *Tropical Medicine & International Health* 2003, *8*(7), 643–649.
7. Araújo MF, Castanheira EMS, Sousa SF. The Buzz on Insecticides: A Review of Uses, Molecular Structures, Targets, Adverse Effects, and Alternatives. In *Molecules* 2003, (Vol. 28, Issue 8).
8. Quêlennec G. *Pratique de la lutte contre les arthropodes d'intérêt médical* 1974.
9. Diabate A, Baldet T, Chandre F, Akoobeto M, Guiguemde TR, Guillet P, Hemingway J, Small GJ, Hougard JM. The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae* sl in Burkina Faso. *The American Journal of Tropical Medicine and Hygiene* 2002 *67*(6), 617–622.
10. Robert V, Ouari B, Ouedraogo V, Carnevale P. An ecologic study of adult and larval *Culicidae* in a rice field of Kou Valley, Burkino Faso. *Acta Tropica*1988, *45*(4), 351–359.

11. Robert V, Petrarca V, Coluzzi M, Boudin C, Carnevale P. Etude des taux de parturité et d'infection du complexe *Anopheles gambiae* dans la rizière de la vallée du Kou, Burkina Faso. *Le Paludisme En Afrique de l'Ouest* 1991, 17.

12. Tangena J-AA, Thammavong P, Hiscox A, Lindsay SW, Brey PT. The human-baited double net trap: an alternative to human landing catches for collecting outdoor biting mosquitoes in Lao PDR. *PLoS One*, 2015. vol. 10, no 9, p. e0138735.

13. Gillies MT, Coetzee M. A supplement to the *Anophelinae* of Africa South of the Sahara. *Publ S Afr Inst Med Res*1987, *55*, 1–143.

14. Gillies MT, De Meillon B. *The* Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region) 1968

15. World Health Organization. Standard operating procedure for testing insecticide susceptibility of adult mosquitoes in WHO bottle bioassays. 2022.

16. Murray MG, Thompson W. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 1980, *8*(19), 4321–4326

17. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, Guillet P, Pasteur N, Pauron D. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* ss. *Insect Molecular Biology* 1998, *7*(2), 179–184.

18. Collins, F. H. et Paskewitz, S. M. A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic Anopheles species. *Insect molecular biology*, 1996. vol. 5, no 1, p. 1-9.

19. Carnevale, Pierre et Manguin, Sylvie. Review of issues on residual malaria transmission. *The Journal of Infectious Diseases*, 2021, vol. 223, no Supplement_2, p. S61-S80.

20. Tsegaye A, Demissew A, Hawaria D, Getachew H, Habtamu K, Asale A, Yan G, Delenasaw Y. Susceptibility of primary, secondary and suspected vectors to Plasmodium vivax and Plasmodium falciparum infection in Ethiopia. *Parasites & Vectors*, 2022, vol. 15, no 1, p. 384.

21. Afrane YA, Bonizzoni M, Yan G. *Secondary malaria vectors of sub-Saharan Africa: threat to malaria elimination on the continent?*. IntechOpen, 2016.

22. Dabiré KR, Diabaté A, Namountougou M., Toe KH, Ouari A, Kengne P, Bass C, Baldet T. Distribution of pyrethroid and DDT resistance and the L1014F kdr mutation in *Anopheles gambiae* sl from Burkina Faso (West Africa). *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2009. *103*(11), 1113–1120.

23. Mayi MPA, Antonio-Nkondjio C, Bamou R, Damiani C, Cappelli A, Djiappi-Tchamen B, Djamouko-Djonkam L, Nejad IKM, Pichler V, Ricci I, Favia G. First detection of kdr-995F allele in *Anopheles ziemanni* and *Anopheles pharoensis* in Cameroon and distribution of the allele in members of the *Anopheles gambiae* complex. *Parasites and Vectors* 2024. *17*(1).

24. Wielgosz B, Mangheni M, Tsegai D, Ringler C. Malaria and Agriculture A Global Review of the Literature With a Focus on the Application of Integrated Pest and Vector Management in East Africa and Uganda Environment and Production Technology Division 2012.

25. Tia E, Chouaibou M, Gbalégba CNG, Boby AMO, Koné M, Kadjo AK. Distribution des espèces et de la fréquence du gène Kdr chez les populations d'*Anopheles gambiae* ss et d'*Anopheles coluzzii* dans cinq sites agricoles de la Côte d'Ivoire. *Bulletin de La Société de Pathologie Exotique* 2017. *110*(2), 130–134.

26. Namountougou M, Soma DD, Kientega M, Balboné M, Kaboré DPA, Drabo SF, Coulibaly AY, Fournet F, Baldet T, Diabaté A. Insecticide resistance mechanisms in *Anopheles gambiae* complex populations from Burkina Faso, West Africa. *Acta Tropica 2019*. *197*, 105054.