

RESEARCH

Open Access



Five aromatic plants against wild malaria vectors: an eco-friendly approach against *Anopheles gambiae* s.l. populations in Burkina Faso

Hamadou Konaté^{1,2,3}, Mahamoudou Balboné^{4,5}, Olivier Gnankiné^{4*} and Moussa Namountougou^{1,2,3}

Abstract

Background Vector control strategies have predominantly relied on the use of synthetic chemicals, leading to the widespread of resistance among malaria vector populations. This growing resistance has prompted increased interest in alternative control methods, particularly those based on plant-derived essential oils (EOs). The present study investigates the insecticidal efficacy of five essential oils against field populations of *Anopheles gambiae* sensu lato from Burkina Faso. The objective was also to assess the current distribution and frequency of resistance-confering mutations within these mosquito populations.

Methods Essential oils were extracted from *Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus camaldulensis*, *Lippia multiflora*, and *Ocimum americanum* plants by hydrodistillation, and their chemical composition was analyzed by gas chromatography coupled with mass spectrometry (GC–MS). The insecticidal activity of the EOs was evaluated on *Anopheles gambiae* complex populations collected from fifteen localities, following the WHO tube bioassay protocol. Species identification in *Anopheles* complex was carried out by PCR. Molecular assays targeting specific resistance genes, particularly the knockdown resistance (*kdr*) L1014F mutation, were conducted using allele-specific PCR. The KD effect and mortality rates induced by each EO were recorded.

Results GC–MS analysis revealed among the 5 EOs samples tested, monoterpenes were the dominant compounds. The KD effects of *Cymbopogon citratus*, *Cymbopogon nardus* and *Lippia multiflora* were more pronounced in all the study areas. The comparative analysis of knockdown times at 50% and 95% (KDT50 and KDT95) highlights significant differences in efficacy between the tested essential oils and permethrin. Overall, the essential oils exhibited significantly lower KDT values than permethrin, indicating a faster and potentially more effective action against *Anopheles gambiae* populations. Moreover, *L. multiflora* induced a rate of mortality of 100% in mosquitoes from fifteen localities. Statistical analysis revealed significant differences in mortality rates among the EOs tested. The potent insecticidal activity observed is likely due to the major terpenoid compounds present in these EOs. Among the mosquito populations analyzed, *Anopheles arabiensis* was predominant and exhibited high phenotypic resistance to pyrethroids in multiple locations.

Conclusion The EOs of *Cymbopogon citratus*, *Cymbopogon nardus*, and *Lippia multiflora* could be used as alternatives where pyrethroid resistance in malaria vectors has been established. These findings support the potential integration of these plant-derived oils into environmentally sustainable vector management strategies in Burkina Faso.

Keywords *Anopheles*, Resistance, Essential oils, Insecticidal activity, Mortality

*Correspondence:
Olivier Gnankiné
olivier.gnankine@ujkz.bf; olignankine@gmail.com
Full list of author information is available at the end of the article

Introduction

Vector-borne diseases such as malaria, dengue, yellow fever, and West Nile fever remain among the most significant public health challenges worldwide (Weaver, 2017). Among these, malaria stands out as the most prevalent, particularly in tropical and subtropical regions (WHO, 2024). This parasitic disease, caused by *Plasmodium* spp. and transmitted by female mosquitoes of the *Anopheles* spp, accounted for an estimated 249 million cases and approximately 608,000 deaths globally in 2022, underscoring its status as the world's leading endemic infectious disease (WHO, 2024). In Burkina Faso, malaria remains the primary cause of patient consultations and mortality in health facilities, with over 11 million cases reported annually (MS, 2023).

The primary vectors responsible for malaria transmission in sub-Saharan Africa belong to the *Anopheles gambiae* complex and the *Anopheles funestus* group, both of which contribute significantly to the region's high malaria endemicity (Costantini et al., 2009; Koffi et al., 2023; Ossè et al., 2023). Vector control remains the cornerstone of malaria prevention strategies, primarily through the use of repellents, long-lasting insecticidal nets (LLINs) impregnated with pyrethroids, and indoor residual spraying (IRS) with long-acting insecticides (WHO, 2024). However, the widespread emergence and intensification of insecticide resistance, particularly to pyrethroids, have severely undermined the effectiveness of these interventions (Ibrahim et al., 2023; Namountougou et al., 2012; Ranson et al., 2011).

One of the major drivers of insecticide resistance is the indiscriminate and large-scale application of unauthorized chemicals in both public health and agriculture (Hien et al., 2017; Nkya et al., 2014a, 2014b). Currently, all major malaria vectors across Africa exhibit resistance to at least one class of insecticides approved by the World Health Organization (WHO) (WHO, 2024). A widespread resistance of several classes of insecticides, including pyrethroids (Farenhorst et al., 2009; Somda et al., 2025), organochlorines (Gueye et al., 2020), organophosphates, and carbamates (Gueye et al., 2020; Munywoki et al., 2021) was reported. Addressing this challenge necessitates the development of new, effective and sustainable alternatives for vector control.

According to WHO guidelines, managing insecticide resistance is a critical component of effective malaria control. In this context, environmentally friendly vector control strategies have gained increasing importance. These alternatives help to reduce environmental pollution, mitigate harm to non-target organisms, and curb the progression of resistance (Nkya et al., 2014a, 2014b; Luz et al., 2020a, 2020b). Additionally, the potential

adverse effects of chemicals on human health pose a further challenge to their continued use in vector control programs. Therefore, identifying new insecticidal compounds that are both effective and less hazardous is essential for strengthening and possibly replacing current tools based on pyrethroids (Foko et al., 2018).

The plant kingdom offers promising avenues for the development of alternative control strategies (Sarma et al., 2019; Mahanta & Khanikar, 2021). Among them, plant-derived products, particularly essential oils (EOs), have emerged as potential candidates for integrated vector management (Deletre et al., 2013; Manh et al., 2020; Ejeta, Asme and Asefa, 2021; Luker et al., 2023; Soonwera et al., 2024). These natural products are known as for their low toxicity to non-target organisms (Benelli et al., 2018) and their biodegradability, making them attractive eco-friendly alternatives to chemicals. Burkina Faso's rich biodiversity, particularly in aromatic plant species, provides a valuable reservoir of essential oils with potential insecticidal activity (Ouedraogo et al., 2024).

Several EOs extracted from aromatic plants native to Burkina Faso, including *Eucalyptus camaldulensis*, *Cymbopogon nardus*, *Cymbopogon citratus*, *Lippia multiflora*, and *Ocimum americanum*, have demonstrated promising insecticidal effects against disease vectors and agricultural pests, such as those affecting cereal storage (Balboné et al., 2022a, 2022b, 2022c; Luz et al., 2020a, 2020b; Wangrawa et al., 2018). Despite this potential, relatively few studies have investigated their efficacy against wild populations of malaria vectors, even though traditional knowledge suggests they could play a significant role in mosquito control (Bossou et al., 2013; Balboné et al., 2024; Wangrawa et al., 2024). Studies carried out in the two major cities of Burkina Faso (Ouagadougou and Bobo-Dioulasso) have shown that that essential oils may offer a viable solution to pyrethroid resistance and could be integrated into current control strategies to enhance their efficacy (Gnankiné & Bassolé, 2017; Wangrawa et al., 2023). Although several studies have evaluated the insecticidal properties of essential oils from various aromatic plants acclimatized in Burkina Faso or elsewhere, none have specifically assessed their insecticidal activity on wild populations of *Anopheles gambiae* s.l. from our study sites in Burkina Faso.

Our study aims to contribute to the valorization of aromatic plant-derived strategies for the control of malaria vectors in Burkina Faso. Specifically, the study aims to (i) assess the bioefficacy of five EOs (ii) to characterize the species composition of *Anopheles* spp populations, and to (iii) determine the distribution and frequency of genetic mutations associated with insecticide resistance in these mosquitoes.

Materials and methods

Study sites

The study was conducted between June and December 2023 in eighteen sites (Fig. 1). During this period, larval collections, mosquito rearing, and susceptibility assays using essential oils (EOs) were systematically carried out. Burkina Faso is characterized by three major climatic zones: The Sahelian zone in the north receives less than 600 mm of annual rainfall, the Sudano-Sahelian zone receives between 600 and 1000 mm of rainfall over approximately four to five months, whereas the Sudanian zone located in the south part was marked by annual precipitation ranging from 1000 to 1300 mm over a period of at least five months. These distinct ecological regions influence the diversity of agro-ecological practices observed across the country, spanning from intensive crop cultivation to extensive pastoralism.

The western part of Burkina Faso represents the principal cotton-producing region, extending south part, where new cotton-growing zones have been established since 1996 (Dabiré et al., 2012) (Table 1). In addition to agricultural development, mining activities, particularly

open-pit operations, have contributed to the creation of numerous artificial water bodies, which serve as ideal breeding habitats for mosquito vectors. These varied ecological and anthropogenic factors collectively support the widespread presence of *Anopheles* species across all climatic zones. As a result, malaria transmission remains endemic throughout the country.

Plant material and extraction

All plants were harvested in July 2023 from the garden of located at the botanic garden of the Institut de Recherche en Sciences Appliquées et Technologies (IRSAT). The specimens, identified by Cyrille SINARE, were deposited in the herbarium of the 'Laboratory of Biology and Plant Ecology' of Joseph KI-ZERBO University, Ouagadougou, Burkina Faso under ID numbers: *Cymbopogon citratus* (17949), *Cymbopogon nardus* (17950), *Lippia multiflora* (17951), *Eucalyptus camaldulensis* (17971), *Ocimum americanum* (17988) in accordance with institutional, national, and international standards. The plant material consisted of the leaves of *Eucalyptus camaldulensis*, *Cymbopogon nardus*, *Cymbopogon citratus*, *Lippia multiflora*,

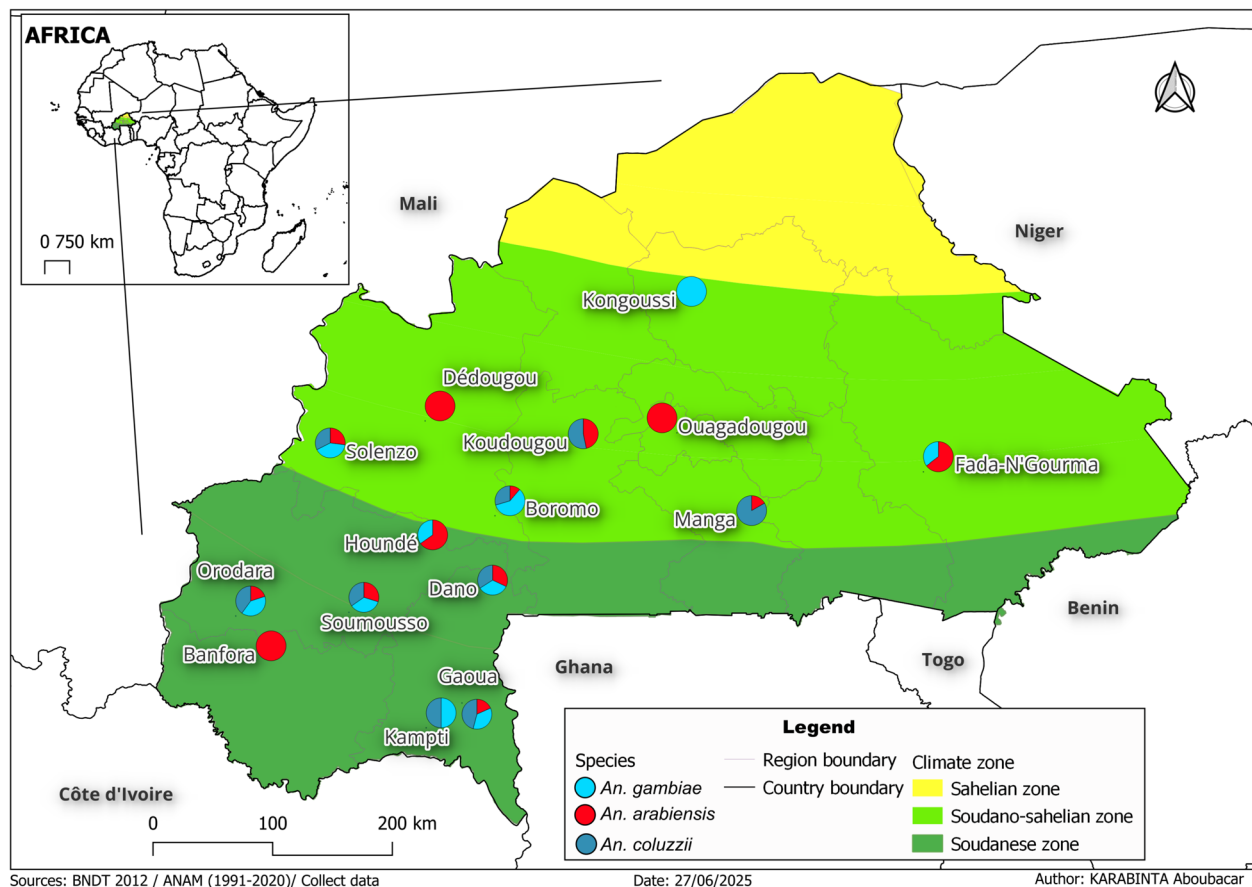


Fig. 1 Location of study sites

Table 1 Study sites

Climatic zone	Region	Study site	Geographic coordinates	Social environment	Agricultural practices
Sudanian	South-West	Kampti	10° 7' 60" N; 3° 27' 0" W	Semi-urban	Cereals, cotton
Sudanian	South-West	Gaoua	10° 40' N; 3° 15' W	Semi-urban	Cereals, cotton
Sudanian	South-West	Dano	10° 95' N; 3° 24' W	Semi-urban	Cereals, cotton
Sudanian	Hauts Bassins	Soumoussou	11° 01' N; 4° 02' W	Rural	Cotton, semi-permanent swamp
Sudanian	Hauts-Bassins	Orodara	11° 00' N; 4° 91' W	Semi-urban	Fruits, cotton
Sudanian	Cascades	Banfora	9° 54' N; 4° 21' W	Urban	Cereals, cotton
Sudanian	Boucle du Mouhoun	Solenzo	12° 11' N; 4° 05' W	Rural	Cotton, cereals
Sudanian	Boucle du Mouhoun	Dédougou	12° 26' N; 3° 28' W	Urban	Cotton, cereals
Sudano-Sahelian	Boucle du Mouhoun	Boromo	11° 75' N; 2° 92' W	Semi-urban	Cotton, cereals
Sudano-Sahelian	Kadiogo	Ouagadougou	12° 22' N; 1° 31' W	Urban	Vegetables, domestic use of insecticides
Sudanian	Hauts Bassins	Houndé	11° 30' 00" N; 3° 31' 00" W	Semi-urban	Industrial farming, irrigation system
Sudano-Sahelian	East	Fada N'Gourma	12° 05' N; 3° 55' W	Semi-urban	Cotton
Sudano-Sahelian	Central-West	Koudougou	12° 15' 09" N; 2° 21' 45" W	Urban	Industrial farming, irrigation system
Sahelian	Central-North	Kongoussi	13° 19' 33" N; 1° 32' 5" W	Semi-urban	Cereals

and *Ocimum americanum*. These five plants were chosen based on information from people interviewed in the studied regions, as well as our preliminary studies conducted in the laboratory (Balboné et al., 2022a, 2022b, 2022c).

The EOs were extracted by hydrodistillation. This method uses CLEVENGER-type apparatus (Schott DURAN, Germany) to extract the essential oils from plant materials. The plant organs used were the fresh leaves. They were cut into small pieces and weighed. Then, 300 grams of each fresh plant sample was placed in a 5-liter flask with approximately 3 liters of water. The mixture was brought to a boil during 3 hours, causing the EO to be carried away with the steam. The oil was then recovered in a device designed for this purpose. After distillation, the EO was recovered by decantation and then dried using anhydrous sodium sulfate (Na_2SO_4) to remove any residual water. The operation was repeated three times for each plant (Balboné et al., 2022a, 2022b, 2022c) studied to ensure the reliability of the results. The extracted and dried oils were stored in amber glass bottles, hermetically sealed with rubber stoppers, and refrigerated at 4°C until use for the biological tests.

Chemical composition analysis of essential oils

The phytochemical analysis was performed by gas chromatography-mass spectrometry (GC-MS) of the Eos. For this analysis, an HP 6890 GC connected to an HP5972 MSD (Hewlett Packard, Palo Alto, CA) with a Zebron ZB-5MS capillary column (30 m long, 0.25 mm internal diameter and 0.25 mm film thickness; Agilent) was used. The experimental procedures and chemical composition are detailed in Balbone et al., Balboné et al., (Balboné et al., 2022a, 2022b, 2022c).

Mosquito rearing at the insectarium

Larvae of *Anopheles gambiae* were collected from June 2023 to September 2023 from over 15 breeding sites distributed across each sampling location. The larval collection was performed using the dipping method. The collection sites included canals, brick cellars, puddles, dams, lakes, swamps, rivers, and septic tanks. The larvae were transported and reared until F0 in the insectarium of the Institut de Recherches en Sciences de la Santé/ Direction Régionale de l'Ouest (IRSS/DRO) located in Bobo-Dioulasso. In the insectarium, the larvae were fed TetraMinBaby®, fish food. After emergence, adult mosquitoes were provided with a sugar solution using a hydrophilic cotton pad soaked in 10% glucose placed in each case containing the adult mosquitoes. A random sample of 100 adult females *Anopheles* mosquitoes was euthanized, preserved on silica gel in Eppendorf tubes of 1.5 ml at −20 °C. These samples were kept at −20 °C until molecular analyses.

Susceptibility test

The susceptibility of *Anopheles gambiae* s.l populations to different EOs used was evaluated. The tests were carried out using WHO tube according to standard procedures (WHO, 2022). The impregnation of Whatman paper No. 1 was carried out according to the protocol established by N'Guessan et al. (2007) and Corbel et al. (2017). Four papers, cut into rectangles of 12 cm x 15 cm, were impregnated each with 2 ml of the solution prepared by dilution of the essential oil (EO) in acetone. The EOs were diluted to a specific concentration, and each concentration was tested in four replicates. The impregnated papers were dried in the laboratory for 10 minutes before

being wrapped in aluminum foil and stored in the refrigerator at 4 °C until the time of testing. Each treated paper was used three times. For each test, 20 to 25 females *Anopheles* aged from 2 to 5 days from F₀ were placed in an exposure tube lined. During the 1-hour exposure, the number of knocked-down mosquitoes was recorded at 30 and 60 minutes. After exposure, the mosquitoes were transferred to observation tubes and fed with 10% glucose for 24 hours. For each concentration of EO tested, 4 batches of 25 mosquitoes were exposed, added to a control batch placed in a tube containing unimpregnated paper. Additionally, a positive control batch was exposed to permethrin (0.75%). Mosquitoes were reared at a temperature of 27 ± 2 °C, a relative humidity of 70 ± 5%, 12 h of light and 12 h of darkness. At the end of the post-exposure observation period, the mortality rates were determined.

The test validation criteria for the tests were based on the mortality observed in the controls. Indeed, when the mortality in the control tubes was less than 5%, the test was considered valid. However, if this mortality was between 5 and 20%, the mortality in the treated group was corrected using the Abbott formula (1925). If the mortality in the controls exceeds 20%, the test was considered invalid and was repeated (Abbott, 1987).

Species identification and molecular analysis

The characterization of insecticide resistance mechanisms was carried out using molecular biology techniques (PCR), polymerase chain reaction. This process involves several steps, including DNA extraction, amplification, and visualization of the amplified products on a gel. The extracted DNA was used in the polymerase chain reaction for the molecular identification of species within the *An. gambiae* complex according to the protocol done by Santolamazza et al. (Santolamazza et al., 2008). Electrophoresis was performed with a 2% agarose gel stained with ethidium bromide. The expected band sizes are 479 bp for *An. coluzzii*, 249 bp for *An. gambiae*, and 223 bp for *An. arabiensis*. Moreover, PCR assays were also performed on the same genomic DNA to detect the kdr L1014 F (kdr-w) mutations according to protocols described by Martinez Torres et al. (1998). Kdr (knock-down resistance) was caused by a point mutation in the gene encoding the voltage-dependent sodium channel (CNaVdp), resulting in the substitution of one amino acid, leucine with phenylalanine (L1014F; Martinez-Torres et al., 1998) or leucine with serine (L1014S) Ranson et al., 2000 in *An. gambiae* s.l. resistant to pyrethroids and DDT (Enayati et al., 2005). This method was based on the specific amplification of sensitive and resistant alleles. Three bands of different sizes were easily resolved on a 1.5%

agarose gel, allowing for straightforward determination of the genotype of each mosquito. Results were read under ultraviolet light.

Statistical analysis

The data obtained from the bioassay were recorded in a Microsoft Excel 2016 spreadsheet. Graphs were created using GraphPad Prism 8.0.2, while statistical analysis was conducted with R Studio, version R 4.4.1. Data analysis was performed in accordance with the standard testing protocols established by the WHO. The times required for 50% and 95% of mosquitoes to end up on their backs or sides, referred to, respectively, as knock-down times (KDT50 and KDT95), as well as their 95% confidence intervals, were estimated by probit regression using the “ecotox” package in the R software. The mortality induced by each essential oil (EO) was calculated by aggregating the total number of deceased mosquitoes across all replicates and expressing it as a percentage of the total number of individuals exposed. A control was included to validate the replicates. Mortality data analysis was carried out using a one-way analysis of variance (ANOVA), with the type of insecticide considered as the factor of interest. To determine the existence of significant differences between the various insecticides, post hoc Tukey tests were performed. To assess compliance with the assumptions of normality of residuals and homogeneity of variances, the Shapiro–Wilk test was applied to evaluate the normality of the residuals, while the Bartlett test was used to examine the homogeneity of variances. The identification and frequencies of the kdr-w mutations were calculated and analyzed and then compared between localities and species using the G test (Goudet and Raymond, 1996), with a significance threshold set at 5%.

Results

The main objective of this study was to increase knowledge of the efficacy on EOs in order to develop useful formulations against adult mosquitoes. Following preliminary screening with different concentrations on laboratory strains and various EOs, only the best concentration (1%) of various EOs was chosen for this evaluation.

Chemical composition analysis of essential oils

The percentage yields of essential oil obtained from the hydro-distillation of the leaves varied around 0.82% for *C. citratus* species and 1.7% for *O. americanum*. Data on the chemical analysis of the five EO samples tested are given in Table 2. The compounds of all these EOs were dominated by monoterpenes. The results of the gas chromatography–mass spectrometry (GC–MS) analysis

revealed among the 5 EO samples tested, monoterpenes were the major compounds. The GC–MS analysis of the *C. citratus* EO with a yield of 0.82% revealed mainly neral (44.7%) and geranial (55.2%). *Cymbopogon nardus* EO at 1.37% yield was dominated by citronellal (41.7%), geraniol (20.8%) and β -elemene (11%). Essential Oil of *E. camaldulensis*, which a yield of 1.2%, exhibits 1,8-cineole (59.55%) as the major compound. As for *L. multiflora* EO, it was predominated by β -caryophyllene (20.1%), p-cymene (14.6%) and thymol acetate (12.0%). Finally, the *O. americanum* EO contained 1,8-cineole (31.22%) and camphor (12.73%) as the major compounds.

Adulticidal activity of EOs in females of *Anopheles gambiae* s.l. Knock-down time (KDT) of essential oils tested

The results of the effect knockdown time (KDT) of EOs on mosquito populations are shown in Table 3. The data showed that the time after which the mosquitoes are knocked down varies according to both sites and EOs. Overall, the KDT (50) and KDT (90) of the EOs were more pronounced in all the study areas compared to permethrin, the positive control used in various tests. Only the EOs of *C. citratus*, *C. nardus*, and *L. multiflora* exhibit the lowest KDT on the majority of *Anopheles* mosquitoes examined (Table 3). Conversely, the EOs of *E. camaldulensis* and *Ocimum americanum* showed the lowest KDT time varying depending on the sites.

Effect of exposure to essential oils on females of *Anopheles gambiae* s.l.

The mortality rate in *Anopheles gambiae* populations was related to the EOs tested as well as the collection site (Fig. 2). All EOs exhibited mortality on the local mosquito populations collected from the study sites.

The lowest mortality of local *An. gambiae* populations was observed when the EOs of *E. camaldulensis* and *O. americanum* were tested. Overall, the highest mortality (100%) was observed with the EO of *L. multiflora* in all sites. Mortality rate was lower in Ouagadougou, Banfora, Fada, Gaoua, Houndé, Kampti, Kongoussi, Manga, Soumouso, Solenzo and Boromo with the *E. camaldulensis* EO. The same trend was observed when *O. americanum* EO was tested in Dédougou, Ouagadougou, Banfora, Fada N' Gourma, Gaoua, Houndé, Kampti, Kongoussi, Soumouso and Solenzo. Overall, there was a significant difference between the mortality rates induced by the different EOs (Kruskal–Wallis test, $p < 0.00001$).

In each locality, permethrin, the control positive exhibits the weak rate of mortality indicating the presence of resistance to these chemicals.

Distribution of species in the *An. gambiae* complex

Out of a total of 750 samples identified, 552 individuals belonged to the *Anopheles* complex and 198 were found to be negative. The 552 samples were distributed as follows: 288 individuals of *An. arabiensis*, 166 of *An. coluzzii*, and 98 of *An. gambiae*. A predominance of *An. arabiensis* was observed in nearly all study localities. The proportion of *An. coluzzii* was notably higher only in Ouagadougou, while the proportion of *An. gambiae* remained low in the majority of the investigated sites (Fig. 3).

Distribution of resistance genes in the *An. gambiae* s.l. complex Allelic

Frequency of the L995F mutation (*kdr-w* mutation)

The distribution of *kdr-w* mutations frequencies within *An. gambiae* complex populations is shown in Table 4.

Table 2 Major compounds and Yields of the essential oils of *Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus camaldulensis*, *Lippia multiflora*, and *Ocimum americanum*

Essential oils	Yields (%)	Retention index	Major compounds	Percentage (%)
<i>Cymbopogon citratus</i> (D. C.) Stapf	0.82	1242	Neral	44.7
		1268	Geranial	55.2
<i>Cymbopogon nardus</i> (Linn.) Rendle	1.37	1158	Citronellal	41.7
		1253	Geraniol	20.8
		1372	β -Elemene	11.0
<i>Eucalyptus camaldulensis</i> Linn	1.2	1034	1,8-cineole	59.55
<i>Lippia multiflora</i> Moldenke	1.3	1034	1,8-cineole	11.6
		1415	β -Caryophyllene	20.1
		1027	p-Cymene	14.6
		1355	Thymol acetate	12.0
<i>Ocimum americanum</i> Linn	1.7	1034	1,8-cineole	31.22
		1124	Camphor	12.73

Table 3 Knockdown times (KDT) at 50% and 95% of essential oils (*Cymbopogon citratus*, *Cymbopogon nardus* *Eucalyptus camaldulensis*, *Lippia multiflora* and *Ocimum americanum*) on *Anopheles gambiae* s.l populations in fifteen health districts of Burkina Faso

50 and 95% Knockdown of <i>An. gambiae</i>								
Sites	EOs	KDT50	95% CI [(LCL–UCL)]	KDT95	95% CI [(LCL–UCL)]	χ^2 (Pearson)	Slope	Intercept
Banfora	CC	5.03	[5.119–6]	5.45	[5.02–6.81]	0.66	–59.0	84.2
	CN	6.07	[5.57–6.62]	10.6	[9.07–12.4]	3.47	–9.53	12.2
	EC	16.0	[13.8–18.3]	32.0	[29.0–35.0]	3.10	0.77	3.00
	LM	6	[5–7]	21	[19–23]	1.15	0.55	2.5
	OA	17.6	[16.7–18.5]	27.5	[25.1–30.2]	308	–18.8	–
	Permethrin	74.7	[60.1–92.8]	273	[164–454]	30.5	–9.80	1.04
Boromo	CC	3.17	[2.16–4.66]	6.61	[5.35–8.16]	11.7	–4.63	9.24
	CN	4.57	[3.05– 5.42]	5.02	[4.02–6.1]	0.44	–48.2	73.0
	EC	18.2	[16.5–19.8]	35.5	[32.4–38.6]	3.67	0.82	3.73
	LM	4.57	[3.69–5.65]	5.01	[0.088–285]	0.498	–48.2	73.0
	OA	19.9	[18.7–21.1]	40.0	[34.7–46.2]	66.5	–12.6	–
	Permethrin	76.6	[58.4–100]	408	[208–800]	66.7	–7.63	0.198
Dano	CC	0	[0–Inf]	0	[0–Inf]	~0	28.8	~0
	CN	Inf	[0–Inf]	Inf	[0–Inf]	~0	28.9	~0
	EC	13.5	[11.6–15.3]	27.9	[25.0–30.7]	2.76	0.74	2.60
	LM	Inf	[0–Inf]	Inf	[0–Inf]	1.38e–10	28.8	–5.26e–9
	OA	70.9	[55.0–91.3]	316	[169–590]	18.0	–8.39	–
	Permethrin	69.5	[58.6–82.5]	200	[134–297]	43.5	–11.8	100.28
Dédougou	CC	4.96	[3.0149–5.650]	5.42	[4.98–5.96]	3.43	–53.1	76.4
	CN	5.13	[~4.86–6.36]	5.55	[5.10–6.62]	0.289	–60.9	85.8
	EC	14.1	[11.9–16.3]	28.6	[25.4–31.8]	4.05	0.78	2.80
	LM	5.13	[4.65–5.56]	9.86	[8.27–11.7]	0.16	–7.36	10.4
	OA	64.6	[58.6–80.3]	71.1	[7.59–73.6]	1.71	–129	–
	Permethrin	68.4	[55.6–84.3]	295	[168–466]	33.2	–8.84	0.89
Fada N’Gourma	CC	4.98	[0.131–190]	5.42	[0–5.56]	1.26	–55.8	80.0
	CN	5.99	[5.02–7.14]	22.0	[17.5–27.7]	42.6	–4.05	5.21
	EC	19.3	[17.2–21.4]	38.0	[35.0–41.0]	4.12	0.85	3.50
	LM	Inf	[0–Inf]	Inf	[0–Inf]	1.38e–10	29.9	–1.07e–7
	OA	358	[64.4–1990]	3007	[125–72300]	17.2	–8.14	–
	Permethrin	187	[89.1–391]	1410	[304–6500]	25.3	–7.62	1.06
Gaoua	CC	Inf	[0–Inf]	Inf	[0–Inf]	~0	28.9	0
	CN	Inf	–	Inf	–	–	–	–
	EC	21.2	[19.0–23.5]	40.6	[37.5–43.8]	4.45	0.88	3.80
	LM	4.32	[3.54–4.45]	4.79	[3.4– 5.9]	2.21	–41.5	65.3
	OA	23.9	[21.5–26.5]	89.8	[65.9–122]	11.0	–7.05	–
	Permethrin	166	[79.6–347]	606	[153–2400]	22.3	–11.6	2.25
Housd	CC	22.2	[18.9–26.1]	22.5	[121–419]	96.1	–3.94	2.93
	CN	0	–	0	–	–	28.9	8.88e–9
	EC	7.58	[5.72–10.1]	127	[67.0–240]	62.5	–2.12	2.41
	LM	0	[0–Inf]	0	[0–Inf]	1.38e–10	28.9	1.14e–8
	OA	501	[63.7–3953114]	1014	[167–726000]	7.84	–5.92	–
	Permethrin	83.2	[65.7–105]	293	[171–501]	29.3	–10.3	–
Kampti	CC	26.2	[23.6–29.1]	92.9	[68.6–126]	24.7	–7.61	5.36
	CN	47.9	[37.5–647]	61.1	[57.5–74.7]	4.03	118	– – 64,6
	EC	11.0	[9.5–12.5]	23.0	[20.5–25.4]	2.30	0.70	2.10
	LM	4.09	[3.00–5.59]	21.0	[15.7–28.3]	38.07	–2.53	4.14
	OA	153	[67.5–347]	2056	[290–14600]	7.31	–5.70	–
	Permethrin	157	[80.6–306]	607	[169–2180]	22.8	–11.0	1.96

Table 3 (continued)**50 and 95% Knockdown of *An. gambiae***

Sites	EOs	KDT50	95% CI [(LCL–UCL)]	KDT95	95% CI [(LCL–UCL)]	χ^2 (Pearson)	Slope	Intercept
Kongoussi	CC	Inf	[0–29.1]	Inf	[0–Inf]	~0	28.9	0
	CN	Inf	–	Inf	–	–	–	–
	EC	17.5	[15.4–19.7]	34.1	[31.1–37.1]	2.98	0.79	3.20
	LM	20.0	[19.1–20.9]	29.2	[26.9–31.6]	32.8	–23.3	17.9
	OA	17.4	[16.5–18.3]	28.0	[25.6–30.8]	20.9	–17.6	–
Koudougou	Permethrin	68.3	[55.6–84.3]	75.6	[57.9–98.7]	2.10	–123	59,283
	CC	4.97	[3.09–5.24]	5.43	[4.01–7.12]	3.41	–53.4	76.6
	CN	9.53	[8.63–10.5]	21.4	[18.4–24.9]	20.2	–8.20	8.38
	EC	15.9	[14.1–17.7]	31.6	[29.0–34.1]	3.32	0.79	3.00
	LM	4.87	[3.57–11]	5.40	[4.95–6.78]	1.19	–55.5	79.8
Manga	OA	43.9	[38.3–50.3]	139	[97.6–197]	8.45	–9.68	–
	Permethrin	108	[70.2–167]	705	[257–1930]	18.0	–7.36	0.90
	CC	0	[0–Inf]	0	[0–Inf]	~0	28.9	0
	CN	5.19	[4.03–6.68]	31.5	[23.2–42.8]	4.93e+01	–2.69	3.76
	EC	14.5	[12.7–16.4]	29.0	[26.4–31.6]	3.00	0.76	2.75
Orodara	LM	Inf	[0–Inf]	Inf	[0–Inf]	1.38e-10	28.9	–7.36e-8
	OA	15.6	[14.7–16.5]	25.2	[22.7–27.9]	31.8	–16.8	–
	Permethrin	90.8	[70.3–117]	225	[129–390]	22.5	–14.7	2.28
	CC	5.13	[4.06–6.48]	27.8	[21.1–36.6]	96.4	–2.85	4.02
	CN	4.72	[3.06–6.4]	5.23	[5.06–7.4]	19.3	–44.5	6.60
Ouagadougou	EC	15.7	[14.0–17.5]	30.2	[27.8–32.9]	2.89	0.80	3.10
	LM	2.61	[1.57–4.33]	6.64	[5.63–8.27]	8.74	–3.03	7.36
	OA	23.8	[22.6–24.9]	50.3	[44.4–57.0]	233	–12.4	–
	Permethrin	7.78e7	[–3.09e30]	2.74E12	[1.64e-26–4.59e50]	20.1	–5.11	1.50
	CC	21.6	[20.1–23.2]	49.9	[41.6–59.9]	59.8	–10.8	8.10
Solenzo	CN	15.1	[14.3–16.0]	22.6	[20.6–24.8]	25.9	–19.9	16.9
	EC	12.3	[10.5–14.2]	25.8	[23.1–28.6]	3.21	0.75	2.45
	LM	18.8	[17.7–20.1]	37.3	[32.4–42.9]	102	–12.7	9.93
	OA	33.2	[29.6–37.3]	114	[82.2–157]	85.5	–40	–
	Permethrin	Inf	[0–Inf]	Inf	[0–Inf]	1.85e-10	–28.9	180,188
Soumouso	CC	0	[0–Inf]	0	[0–Inf]	~0	28.9	0
	CN	3.7	[12.4–15.1]	42.4	[34.7–51.8]	91.5	–6.82	6.00
	EC	18.7	[16.6–20.8]	36.5	[33.6–39.4]	3.88	0.83	3.40
	LM	Inf	[0–Inf]	Inf	[0–Inf]	1.38e-10	28.8	–5.26e-9
	OA	–	–	–	–	–	–	–
Soumouso	Permethrin	99.2	[65.4–96.8]	197	[127–307]	17.1	–14.2	1.90
	CC	4.32	[0–4.27e+70]	4.80	[0–5.48e+20]	1.65	–41.5	65.3
	CN	5.1	[14.3–16.0]	22.6	[20.6–24.8]	25.9	–19.9	16.9
	EC	16.8	[14.8–18.8]	33.6	[30.7–36.5]	3.52	0.80	3.10
	LM	22	[20–24]	24	[23–26]	1.82	0.67	2.8
Soumouso	OA	275	[72.4–1050]	7191	[326–158000]	8.26	–5.07	–
	Permethrin	87.9	[63.8–121]	485	[224–1050]	24.6	–7.72	0.86

CC: *Cymbopogon citratus*; CN: *Cymbopogon nardus*; EC: *Eucalyptus camaldulensis*; LM: *Lippia multiflora*; and OA: *Ocimum americanum*

The probit regressions parameters (χ^2 value of the Pearson goodness-of-fit test, slope and intercept) are indicated. No knockdown time was recorded in the control group of each mosquito strain

KDT50, KDT95 indicates Knockdown times for 50 and 95% of adult mosquitoes

Inf: infinity (means that the knockdown could not be observed within the experimental time frame, indicating a strong resistance or ineffectiveness of the insecticide)

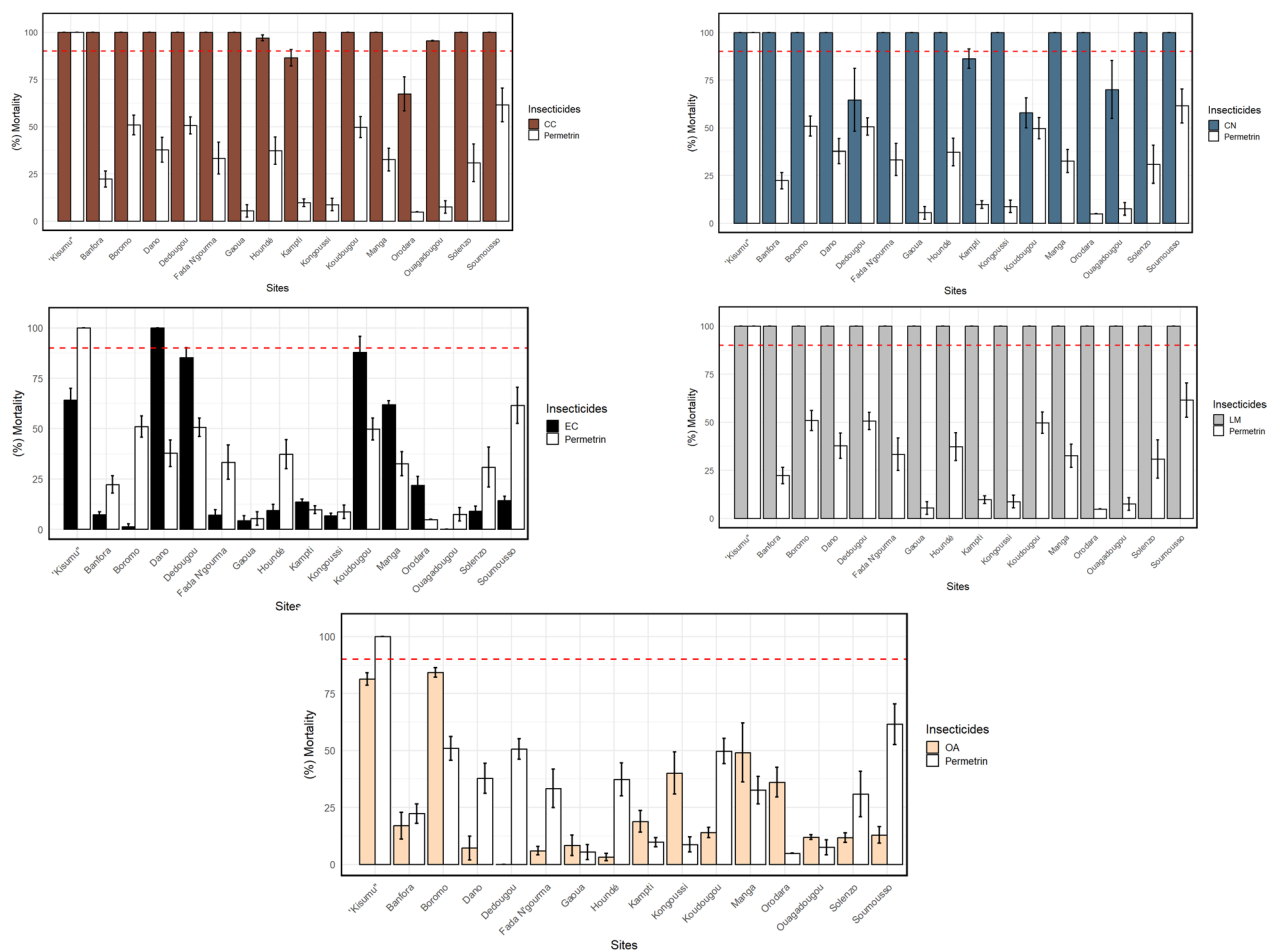


Fig. 2 Rate of mortality (%) of wild populations of *An. gambiae* s.l. in several districts of Burkina Faso exposure to essential oils. The red horizontal dotted lines indicate the resistance threshold according to the WHO. The frequency intervals of 95% are displayed. CC: *Cymbopogon citratus*; CN: *Cymbopogon nardus*; EC: *Eucalyptus camaldulensis*; LM: *Lippia multiflora*; and OA: *Ocimum americanum*

The *kdr-w* gene was observed with frequencies varying according to both species and areas. It varied from 0 to 1. Overall, the frequency of the resistant *kdr-w* allele was higher in *An. arabiensis* s.s. populations from majority of sites where it predominates.

Discussion

The growing resistance to synthetic compounds presents a significant obstacle to malaria vector control efforts worldwide, particularly across the African continent. Overall, in fifteen (15) sites investigated, when permethrin tested, a pyrethroid molecule, *Anopheles* populations exhibited a rate of mortality below 90% showing the presence of resistant populations to this chemical commonly used in vector control strategies.

Also, the current study highlighted the implication of molecular resistance in mosquitoes' populations collected in investigated sites. Here, molecular data revealed widespread presence of the *kdr* L995F mutation at

frequencies higher than previously reported, particularly in *An. arabiensis*, across Dano, Soumouso, Hounde, Solenzo, Orodara, and Ouagadougou. The resistance to pyrethroids, often linked to the *kdr* mutation, is likely driven by the widespread deployment of LLINs and the use of pyrethroids in agricultural practices (Dabiré et al., 2009; Hien et al., 2017; Namountougou et al., 2012). This contrasts with earlier findings that noted higher *kdr* frequencies in *An. coluzzii* and *An. gambiae* s.s. and lower frequencies in *An. arabiensis* (Dabiré et al., 2012; Hien et al., 2017). High *kdr* frequencies in *An. gambiae* were recorded in Dano, Gaoua, Kampti and Soumouso, suggesting an intensification of resistance selection. In Dano, *An. coluzzii* and *An. gambiae* reached frequencies up to 0.77, slightly lower than the 0.86 reported by Soma and colleagues (Soma et al., 2021). In Solenzo, near the Malian border, all three-sibling species exhibited elevated *kdr* frequencies, consistent with findings found by Keita et al. (2020). Conversely, Gaoua showed a decline

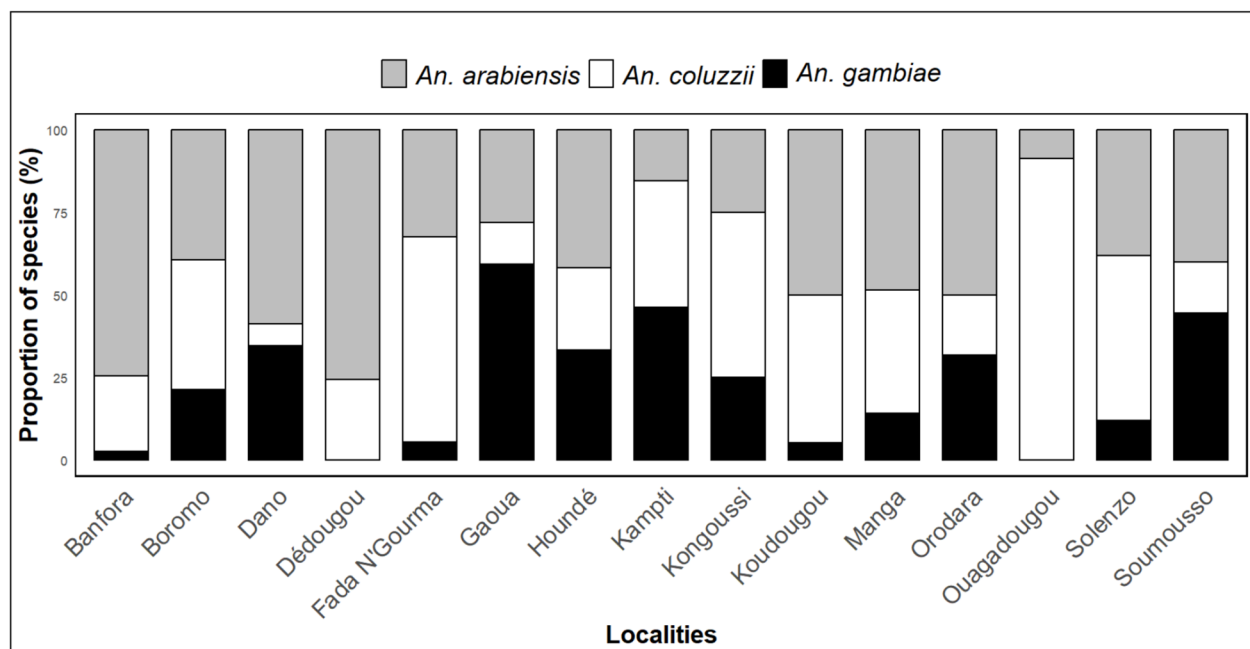


Fig. 3 *Anopheles gambiae* complex species composition, in Burkina Faso

in *kdr* frequency relative to earlier studies (Namountougou et al., 2019). This heterogeneity underscores the need for continuous surveillance. The improper use of LLINs repurposed for fishing or crop protection, as reported in previous studies (Keïta et al., 2020; Nkya et al., 2014a, 2014b; Oumbouke et al., 2020), may further exacerbate resistance, highlighting the urgency of reinforcing proper LLIN utilization and exploring EO-based alternatives for effective vector control.

Pyrethroid resistance calls into the seek of alternatives of chemical resistance as the use of molecules from Plants as Essential oils (EOs). The present study assessed the insecticidal efficacy of five essential oils (EOs), *Cymbopogon citratus* (DC.) Stapf. and *Cymbopogon nardus* (L.) Rendle (Poaceae), *Eucalyptus camaldulensis* Dehnh. (Myrtaceae), *Lippia multiflora* Moldenke (Verbenaceae), and *Ocimum americanum* L. (Lamiaceae), against wild-type adult populations of *Anopheles gambiae* s.l. Previous investigations by Balboné et al., (2022a, 2022b) demonstrated promising outcomes with these EOs on laboratory strains (Kisumu and VK7), suggesting their potential utility as alternatives in the control of natural vector populations. The chemical composition of essential oils reveals a predominance of oxygenated monoterpenes, notably neral (44.7%) and geranial (55.2%) in *Cymbopogon citratus*. For *Cymbopogon nardus*, the main constituents are citronellal (41.7%) and geraniol (20.8%), while β -elemene constitutes low proportion (11.0%). In the essential oil of *Eucalyptus camaldulensis*, 1,8-cineole predominates with

about 60%, a typical characteristic of this species. *Lippia multiflora* has a more varied composition, including 1,8-cineole (11.6%), β -caryophyllene (20.1%), p-cymene (14.6%), and thymol acetate (12.0%), suggesting multiple potential insecticidal properties. As for *Ocimum americanum*, it exhibits a high concentration of 1,8-cineole (31.22%) and camphor (12.73%). These results are in accordance with those reported by Wangrawa in Burkina Faso, who also observed a strong presence of monoterpenes in these essential oils (Wangrawa et al., 2024). They also corroborate data obtained in Benin, where essential oils from the *Cymbopogon* and *Eucalyptus* genera have been shown to have a high monoterpene content (Nonviho et al., 2010; Bossou et al., 2013). Our results revealed that all five EOs have induced both knockdown (KD) and adulticidal effects, although the intensity of these effects varied by compound and geographical location.

In general, EO-induced knockdown was more pronounced than that caused by permethrin, regardless the site. The strong KD effects obtained with *C. nardus* can be attributed to high concentrations of active constituents such as geranial, neral, and monoterpenes, as previously reported by Bossou et al. (2013), who observed 100% KD at 0.77% EO concentration (Bossou et al., 2013). The KD effect of *L. multiflora* EO remained robust across all sites, consistently reaching 100%, likely due to its high monoterpenes content (Bossou et al., 2013; Wangrawa et al., 2015). This is in line with previous reports that monoterpenes induce strong KD effects in

Table 4 Allelic frequencies at the *kdr* locus in wild *An. gambiae* s.l. populations from sites in Burkina Faso

Genotypes								
Sites	Species	RR	RS	SS	N	f(1014F)	[95%CI]	p(HW)
Banfora	<i>An. arabiensis</i>	1	13	2	16	0.46	[0.28; 0.65]	0.81
	<i>An. gambiae</i>	0	0	0	0	–	–	–
	<i>An. coluzzii</i>	0	0	0	0	–	–	–
Boromo	<i>An. arabiensis</i>	1	2	4	7	0.29	[0.11; 0.56]	0.41
	<i>An. gambiae</i>	3	0	1	4	0.75	[0.32; 0.96]	0.49
	<i>An. coluzzii</i>	3	3	2	8	0.56	[0.33; 0.77]	0.88
Dano	<i>An. arabiensis</i>	9	3	3	15	0.70	[0.49; 0.85]	0.51
	<i>An. gambiae</i>	7	3	1	11	0.77	[0.53; 0.91]	0.80
	<i>An. coluzzii</i>	7	3	1	11	0.77	[0.53; 0.91]	0.80
Dédougou	<i>An. arabiensis</i>	6	6	14	26	0.46	[0.33; 0.60]	0.88
	<i>An. gambiae</i>	0	0	0	0	–	–	–
	<i>An. coluzzii</i>	0	0	0	0	–	–	–
Fada N’Gourma	<i>An. arabiensis</i>	2	2	0	4	0.75	[0.26; 0.97]	0.31
	<i>An. gambiae</i>	0	0	1	1	0	–	–
	<i>An. coluzzii</i>	3	6	2	11	0.68	[0.43; 0.86]	0.85
Gaoua	<i>An. arabiensis</i>	2	1	2	5	0.40	[0.15; 0.72]	0.72
	<i>An. gambiae</i>	7	2	0	9	0.89	[0.65; 0.98]	0.35
	<i>An. coluzzii</i>	1	0	0	1	1.00	–	–
Houndé	<i>An. arabiensis</i>	6	1	1	8	0.81	[0.49; 0.95]	0.55
	<i>An. gambiae</i>	0	0	3	3	0	–	–
	<i>An. coluzzii</i>	2	0	3	5	0.40	[0.15; 0.72]	0.53
Kampti	<i>An. arabiensis</i>	0	0	0	0	–	–	–
	<i>An. gambiae</i>	2	0	0	2	1.00	–	–
	<i>An. coluzzii</i>	3	0	0	3	1.00	–	–
Kongoussi	<i>An. arabiensis</i>	0	0	0	0	–	–	–
	<i>An. gambiae</i>	1	0	0	1	1.00	–	–
	<i>An. coluzzii</i>	0	0	0	0	–	–	–
Koudougou	<i>An. arabiensis</i>	9	0	3	12	0.88	[0.62; 0.97]	0.38
	<i>An. gambiae</i>	0	0	0	0	–	–	–
	<i>An. coluzzii</i>	6	0	1	7	0.93	[0.66; 0.99]	0.84
Manga	<i>An. arabiensis</i>	1	2	2	5	0.50	[0.21; 0.79]	0.74
	<i>An. gambiae</i>	0	0	0	0	–	–	–
	<i>An. coluzzii</i>	1	0	0	1	1.00	–	–
Orodara	<i>An. arabiensis</i>	1	3	0	4	0.88	[0.37; 0.99]	0.40
	<i>An. gambiae</i>	1	1	0	2	0.75	–	0.75
	<i>An. coluzzii</i>	1	1	0	2	0.75	–	0.75
Ouagadougou	<i>An. arabiensis</i>	3	2	6	11	0.50	[0.27; 0.73]	0.45
	<i>An. gambiae</i>	0	0	0	0	–	–	–
	<i>An. coluzzii</i>	0	0	0	0	–	–	–
Solenzo	<i>An. arabiensis</i>	5	4	3	12	0.71	[0.47; 0.88]	0.65
	<i>An. gambiae</i>	5	2	1	8	0.81	[0.53; 0.96]	0.94
	<i>An. coluzzii</i>	8	5	3	16	0.78	[0.59; 0.91]	0.99
Soumousso	<i>An. arabiensis</i>	10	7	2	19	0.70	[0.51; 0.85]	0.79
	<i>An. gambiae</i>	5	2	1	8	0.81	[0.53; 0.96]	0.94
	<i>An. coluzzii</i>	11	5	2	18	0.86	[0.67; 0.96]	0.78

The columns provide information on the genotypes (RR, RS, SS), the total number of samples (N), the frequency of the 1014F genotype (f(1014F)), the 95% confidence interval ([95% CI]), and the *p*-value for the Hardy–Weinberg test (p(HW))

SS: susceptible homozygote; **RS:** heterozygote; **RR:** resistant homozygote;

various mosquito species, including *Aedes aegypti*, *Culex quinquefasciatus*, and several *Anopheles* species (Bossou et al., 2013; Wangrawa et al., 2024). Conversely, *O. americanum* EO was generally less effective than the others, as also observed in prior studies (Badolo et al., 2019; Wangrawa et al., 2024). Indeed, Balboné et al. (2022b) further reported a lower KD effect of *Ocimum* EO compared to *Cymbopogon* species on resistant mosquito strains (Balbone, et al., 2022b), which may due to differences in concentrations and exposure duration. For example, Wangrawa et al. (2018) used higher concentrations (1.82% for *Hyptis suaveolens*, 1.39% for *O. canum*) and a longer exposure time (90 min) compared to our study (1% EO, 60 min) (Wangrawa et al., 2018).

The comparative analysis of knockdown times (KDT50 and KDT95) reveals significant differences in efficacy between the tested essential oils and the reference insecticide, Permethrin. In the majority of sites, the essential oils CC, CN, LM, EC, and OA exhibit lower KDT50 and KDT95 values than those of Permethrin, indicating a faster action against *Anopheles gambiae* populations. For example, in Banfora, the KDT50 for CC (5.03 min) is about 15 times lower than that of Permethrin (74.7 min), with an even greater difference for KDT95 (5.45 min versus 273 min). However, some exceptions are observed. In sites such as Dano, Fada N'Gourma, Ouagadougou, and Soumouso, the essential oil OA shows knockdown times that are sometimes higher or comparable to those of Permethrin, suggesting reduced efficacy or the existence of specific resistance to this extract in these populations. Finally, the presence of infinite knockdown values at certain sites indicates a marked resistance to some essential oils, contrasting with more sensitive responses observed elsewhere, highlighting the need for a localized approach in managing resistance.

The adulticidal effects of the tested EOs varied according to the study sites. *Cymbopogon nardus* and *C. citratus* EOs significantly outperforming permethrin, which showed reduced efficacy across most sites. Lower EO-induced mortality observed in sites such as Kampti, Orodara, Koudougou, and Ouagadougou may reflect elevated detoxification enzymes activity in local mosquito populations in addition to molecular mechanism as previously shown by Namountougou et al. (2019).

Mortality rates from *C. nardus* EO were generally higher than those induced by permethrin, corroborating its recognized potential as an effective insecticide (Ríos, Stashenko and Duque, 2017). Despite such variability, *L. multiflora* EO remained effective at all sites.

Overall, statistical analyses confirmed that *L. multiflora* EO was the most effective with potential implications for future vector control strategies. Additionally, the high efficacy of *E. camaldulensis* EO in areas such as

Dano and Manga supports its potential use in localized interventions. These results align with previous findings demonstrating that *E. camaldulensis* formulations can outperform permethrin (Baeshen & Baz, 2023; Dua et al., 2010). Similarly, the efficacy of *O. americanum* compared to permethrin at several sites may depend on formulation specifics or distinct modes of action. Nonetheless, site-specific variability suggests the influence of environmental and ecological factors on EO effectiveness. Sites such as Dano and Koudougou, for example, showed a rate of mortality reaching to 100% with *E. camaldulensis* EO, consistent with results reported by Balboné et al. (2022b). Interestingly, Bossou et al. (2013) also reported susceptibility in resistant *An. gambiae* strains in Benin when tested with *E. citriodora* and *E. tereticornis* at higher concentrations (2.80% and 3.37%, respectively).

Our data further underscore that increasing EO concentration could enhance efficacy for both *E. camaldulensis* and *O. americanum* EOs, which showed promising results even at the tested 1% level. Additionally, the sustained efficacy of *C. nardus*, *C. citratus*, and *L. multiflora* suggests these botanicals hold strong potential for incorporation into integrated vector management programs.

Moreover, PCR-based analyses enabled species-level identification within the *An. gambiae* complex, updating the distribution of *An. gambiae*, *An. coluzzii*, and *An. arabiensis* across Burkina Faso's climatic zones. In several localities, Banfora, Dano, Dédougou, Houndé, Koudougou, Manga, and Orodara, *An. arabiensis* emerged as the predominant species, contrasting earlier reports by Namountougou et al. (2012), which found *An. gambiae* s.s. to be dominant (Namountougou et al., 2012). This shift may reflect the ecological adaptability of *An. arabiensis*, particularly in arid environments with limited water sources, where its zoophilic tendencies provide a selective advantage (Chabi et al., 2024; Diallo et al., 2021).

Conversely, *An. coluzzii* was predominant in Fada N'Gourma, and *An. gambiae* in Gaoua and Soumouso. The presence of *An. coluzzii* in irrigated and agricultural areas such as Boromo, Kongoussi, and Ouagadougou aligns with findings from Côte d'Ivoire (Chabi et al., 2024), where rice cultivation provides ideal breeding conditions (Hien et al., 2017; Yao et al., 2022).

Conclusion

The study elucidates the insecticidal effects of essential oils derived from *Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus camaldulensis*, *Lippia multiflora*, and *Ocimum americanum* against malaria vectors. Additionally, molecular analyses reveal a wide distribution of resistance alleles, notably the *kdr* L1014F mutation. In light of these results, the essential oils of *Cymbopogon nardus*, *Cymbopogon citratus* and *Lippia multiflora*

could be potential adulticides, promising alternatives to pyrethroid resistance in malaria vector control strategies. Future research should prioritize phase 3 evaluations, focusing on optimizing essential oil formulations and assessing their long-term residual effects.

Abbreviations

CC	<i>Cymbopogon citratus</i>
CN	<i>Cymbopogon nardus</i>
EC	<i>Eucalyptus camaldulensis</i>
LM	<i>Lippia multiflora</i>
OA	<i>Ocimum americanum</i>
EOs	Essential oil

Acknowledgements

We are indebted to "Institut de recherche en Sciences Appliquées et technologiques" (IRSAT) for providing us with essential oils. Hamadou Konaté is the recipient of a PhD studentship of University Joseph KI-ZERBO.

Author contributions

H.K., M.N., and O.G. designed the study. M. B. critically supervised the study. H.K. and M.B. carried out the laboratory experiments. H.K., M.B., O.G., and M.N. analyzed and interpreted the data and drafted the manuscript. O.G. and M.N. revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

Funding for this study was provided partly by the TWAS 18–163 RG/BIO/AF/AC_G–FR3240303649 and the "Centre d'excellence Africain (CEA) en Innovations biotechnologiques pour l'élimination des maladies à transmission vectorielle."

Data availability

No datasets were generated or analyzed during the current study.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Human or animal rights

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Centre MURAZ/Institut National de Santé Publique, Bobo-Dioulasso, Burkina Faso. ²Unité de Formation Et de Recherche en Sciences de La Vie Et de La Terre, Université Nazi BONI, Bobo-Dioulasso, Burkina Faso. ³Institut de Recherche en Sciences de La Santé (IRSS), Bobo-Dioulasso, Burkina Faso. ⁴Unité de Formation en Sciences de la Vie et de la Terre, Université Joseph KI-ZERBO, Ouagadougou, Burkina Faso. ⁵Centre Universitaire de Manga, Université Norbert ZONGO, Koudougou, Burkina Faso.

Received: 1 July 2025 Accepted: 4 October 2025

Published online: 29 October 2025

References

Abbott, W.S. (1987) 'A method of computing the effectiveness of an insecticide. 1925', *Journal of the American Mosquito Control Association*, 3(2), pp. 302–303.

- Badolo, A., Sombié, A., Pignatelli, P. M., Sanon, A., Yaméogo, F., Wangrawa, D. W., Sanon, A., Kanuka, H., McCall, P. J., & Weetman, D. (2019). Insecticide resistance levels and mechanisms in *Aedes aegypti* populations in and around ouagadougou, Burkina Faso. *PLoS Neglected Tropical Diseases*, 13(5), 1–17. <https://doi.org/10.1371/journal.pntd.0007439>
- Baeshen, R. S., & Baz, M. M. (2023). Heliyon safsafs on the mortality and development of two vector-borne mosquito species, *Culex pipiens* and *Aedes aegypti*, in the laboratory and field. *Heliyon*, 9(5), 16378. <https://doi.org/10.1016/j.heliyon.2023.e16378>
- Balboné, M., D. D. Soma, M. Namountougou, S. F. Drabo, H. Konate, O. Toe, K. Bayili, G. B. Meda, R. K. Dabiré, and O. Gnankine (2022) 'Essential Oils From Five Local Plants : An Alternative Larvicide for *Anopheles gambiae* s. l. (Diptera : Culicidae) and *Aedes aegypti* (Diptera : Culicidae) Control in Western Burkina Faso', *Front. Trop. Dis.* 3:853405. <https://doi.org/10.3389/td.2022.853405> 3(July), pp. 1–7.
- Balboné, M., Gnankine, O., Namountougou, M., Soma, D. D., Drabo, S. F., Romba, R., Bassolé, I. H. N., & Dabire, R. K. (2024). 'The excito-repellent activity of five essential oils extracted from local plants against dengue and malaria vectors in Burkina Faso', *Biologia*, 79(8), 2495–2503. <https://doi.org/10.1007/s11756-024-01738-x>
- Balboné, M., Sawadogo, I., Soma, D. D., Drabo, S. F., Namountougou, M., Bayili, K., Romba, R., Meda, G. B., Nebié, H. C. R., Dabire, R. K., Bassolé, I. H. N., & Gnankine, O. (2022c). Essential oils of plants and their combinations as an alternative adulticides against *Anopheles gambiae* (Diptera: Culicidae) populations. *Scientific Reports*, 12(1), 1–10. <https://doi.org/10.1038/s41598-022-23554-6>
- Balboné, M., Soma, D. D., Drabo, S. F., Namountougou, M., Konaté, H., Meda, G. B., Sawadogo, I., Romba, R., Bilgo, E., Nebié, R. C. H., Bassolé, I. H. N., Dabire, R. K., & Gnankine, O. (2022a). Alternatives to Pyrethroid Resistance: Combinations of *Cymbopogon nardus* and *Ocimum americanum* essential oils improve the bioefficiency control against the adults' Populations of *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, 59(6), 2102–2109. <https://doi.org/10.1093/jme/tjac148>
- Benelli, G., Jeffries, C. L., & Walker, T. (2018). Acute and sub-lethal toxicity of eight essential oils of commercial interest against the filariasis mosquito *Culex quinquefasciatus* and the housefly *Musca domestica*. *Industrial Crops and Products*, 112, 668–680. <https://doi.org/10.1016/j.indcrop.2017.12.062>
- Bossou, A. D., Mangelinckx, S., Yedomonhan, H., Boko, P. M., Akogbeto, M. C., De Kimpe, N., Avlessi, F., & Sohounhloue, D. C. K. (2013). 'Chemical composition and insecticidal activity of plant essential oils from Benin against *Anopheles gambiae* (Giles)', *Parasites & Vectors*. <https://doi.org/10.1186/1756-3305-6-337>
- Chabi, J., Edi, C. V. A., Kouassi, B. L., Gbalegba, C. N. G., Kouassi, A. E., Kouame, J. K. I., Kadio, Y. K. A., Yokoly, F. N., Golou, L. B., Gouaméné, J., Assamoi, J.-B., Tia, E., Yapo, R. M., Konan, L. Y., N'Tamon, R. N., Koffi, A. A., Tanoh, A. M., Ging-Cissé, N., Zinzindohoué, P., ... Kirby, M. (2024). Level of involvement of four selected cytochrome P450s (CYPs) in pyrethroid-resistant *Anopheles gambiae* (s.s.) and *Anopheles coluzzii* across Côte d'Ivoire. *Current Research in Parasitology and Vector-Borne Diseases*. <https://doi.org/10.1016/j.crpvbd.2024.100223>
- Corbel, V., Fonseca, D. M., Weetman, D., Pinto, J., Achee, N. L., Chandre, F., Coulibaly, M. B., Dusfour, I., Grieco, J., Juntarajumnong, W., Lenhart, A., Martins, A. J., Moyes, C., Ng, L. C., Raghavendra, K., Vatandoost, H., Vontas, J., Muller, P., Kasai, S., ... David, J. P. (2017). International workshop on insecticide resistance in vectors of arboviruses, December 2016, Rio de Janeiro. *Brazil. Parasites & Vectors*, 10(1), 278.
- Costantini, C., Badolo, A., & Ilboudo-Sanogo, E. (2009). Living at the edge : biogeographic patterns of habitat segregation conform to speciation by niche expansion in *Anopheles gambiae*. *BioMed Central*. <https://doi.org/10.1186/1472-6785-9-16>
- Dabiré KR, Diabaté A, Namountougou M, Toé KH, Ouari A, Kengne P, et al. Distribution of pyrethroid and DDT resistance and the L1014F kdr mutation in *Anopheles gambiae* s.l. from Burkina Faso (West Africa). *Trans R Soc Trop Med Hyg.* 2009;103:1113–20.6/s13071-017-2224-3.
- Dabiré, R. K., Namountougou, M., Sawadogo, S. P., Yaro, L. B., Toe, H. K., Ouari, A., Gouagna, L.-C., Simard, F., Chandre, F., Baldet, T., Bass, C. and Diabate, A. (2012) 'Population dynamics of *Anopheles gambiae* s.l. in Bobo-Dioulasso city: Bionomics, infection rate and susceptibility to insecticides', *Parasites and Vectors*, 5(1), pp. 1–9. Available at: <https://doi.org/10.1186/1756-3305-5-127>.

- Dabiré, K. R., Diabaté, A., Namountougou, M., Toé, K. H., Ouari, A., & Kengne. (2009). Distribution of pyrethroid and DDT resistance and the L1014F kdr mutation in *Anopheles gambiae* s.l. from Burkina Faso (West Africa). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 103(11), 1113–1120.
- Deletre, E., Martin, T., Campagne, P., Bourguet, D., Cadin, A., Menut, C., Bonafos, R., & Chandre, F. (2013). Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* mosquito. *PLoS ONE*, 8(12), 1–10. <https://doi.org/10.1371/journal.pone.0082103>
- Diallo, M., Hamid-Adiamoh, M., Sy, O., Sarr, P. C., Manneh, J., Ndiath, M. O., Gaye, O., Faye, O., Konaté, L., Sesay, A. K., Assogba, B. S., & Niang, E. A. (2021). Evolution of the pyrethroids target-site resistance mechanisms in senegal: Early stage of the vgsc-1014f and vgsc-1014s allelic frequencies shift. *Genes*, 12(12), 1–13. <https://doi.org/10.3390/genes12121948>
- Dua, V. K., Pandey, A. C., & Dash, A. P. (2010). Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indian Journal of Medical Research*, 131(3), 434–439.
- Ejeta, D., Asme, A., & Asefa, A. (2021). Insecticidal effect of ethnobotanical plant extracts against *Anopheles arabiensis* under laboratory conditions. *Malaria Journal*. <https://doi.org/10.1186/s12936-021-04004-6>
- Enayati, A. A., Ranson, H., & Hemingway, J. (2005). Insect glutathione transferases and insecticide resistance. *Insect Molecular Biology*, 14, 3–8.
- Farenhorst, M., Mouatcho, J. C., Kikankie, C. K., Brooke, B. D., Hunt, R. H., Thomas, M. B., Koekemoer, L. L., Knols, B. G. J. et Coetzee, M. (2009). Fungal infection counters insecticide resistance in African malaria mosquitoes, 106(41), pp. 17443–17447.
- Foko D.G.A., Bobo N.M.P., Nyegue M.A., et Tamesse J.L. (2018) 'Chemical composition and biocidal properties of Clausena anisata (Rutaceae) essential oil against developmental stages of the malaria vector *Anopheles*', *essencejournal.com* FDG Aurelie, BNM Pierre, NM Ascension, TJ Lebel American Journal of Essential Oils and Natural Products, 2018-essencejournal.com, (ISSN: 2321–9114), p. 9. Available at: <https://www.essencejournal.com/pdf/2018/vol6issue1/PartA/5-4-7-762.pdf>.
- Gnankiné, O., & Bassolé, I. L. H. N. (2017). 'Essential oils as an alternative to pyrethroids' resistance against anopheles species complex giles (Diptera: Culicidae): *Molecules*. <https://doi.org/10.3390/molecules22101321>
- Gueye OK, Tchouakui M., Dia AK, Faye MB, Ahmed AA, Wondji MJ, Nguiou DN, Mugenzi LMJ, Tripet F., Konaté L., Diabate A., Dia I., Gaye O., Faye O., Niang EA, and Wondji CS (2020) 'Insecticide Resistance Profiling of *Anopheles coluzzii* and *Anopheles gambiae* Populations in the Southern Senegal : Role of Target Sites and Metabolic', pp. 1–17.
- Hien, A. S., Soma, D. D., Hema, O., Bayili, B., Namountougou, M., Gnankine, O., Baldet, T., Diabaté, A., & Dabire, K. R. (2017). Evidence that agricultural use of pesticides selects pyrethroid resistance within *Anopheles gambiae* s. l. populations from cotton growing areas in Burkina Faso, West Africa. *PLoS ONE*, 12(3), 1–15.
- Ibrahim, S. S., Muhammad, A., Hearn, J., Weedall, G. D., Nagi, S. C., Mukhtar, M. M., Fadel, A. N., Mugenzi, L. J., Patterson, E. I., Irving, H., & Wondji, C. S. (2023). Molecular drivers of insecticide resistance in the Sahelo-Sudanian populations of a major malaria vector *Anopheles coluzzii*. *BMC Biology*, 21(1), 1–23. <https://doi.org/10.1186/s12915-023-01610-5>
- Keita, M., Kané, F., Thiero, O., Traoré, B., Zeukeng, F., Sodio, A. B., Traoré, S. F., Djouaka, R., Doumbia, S., & Sogoba, N. (2020). Acetylcholinesterase (ace-1R) target site mutation G119S and resistance to carbamates in *Anopheles gambiae* (sensu lato) populations from Mali. *Parasites and Vectors*, 13(1), 1–9. <https://doi.org/10.1186/s13071-020-04150-x>
- Koffi, A. A., Camara, S. A. A., Ludovic, P. O., Welbeck, A. W., Rosine, Z. T., Sternberg, E. I. Z., Yapo, F. D. H. A., Koffi, F. M. A. S. B., Cook, J. T., & Guesson, M. B. N. (2023). *Anopheles* vector distribution and malaria transmission dynamics in Gbèkè region, central Côte d'Ivoire. *Malaria Journal*, 22(1), 1–12. <https://doi.org/10.1186/s12936-023-04623-1>
- Luker, H. A., Salas, K. R., Esmaeili, D., Holguin, F. O., Bendzus-Mendoza, H., & Hansen, I. A. (2023). Repellent efficacy of 20 essential oils on *Aedes aegypti* mosquitoes and *Ixodes scapularis* ticks in contact - repellency assays. *Scientific Reports*. <https://doi.org/10.1038/s41598-023-28820-9>
- Luz TR SA, Mesquita LSS, Amaral FMM, and Coutinho D. F. (2020) 'Essential oils and their chemical constituents against *Aedes aegypti* L. (Diptera: Culicidae) larvae', *Acta Tropica*, 212(September), p. 105705. Available at: <https://doi.org/10.1016/j.actatropica.2020.105705>.
- Luz, T. R. S. A., Mesquita, L. S. S., Amaral, F. M. M., & Coutinho, D. F. (2020b). Exploring essential oils of Slovak medicinal plants for insecticidal activity: The case of *Thymus alternans* and *Teucrium montanum* subsp. *jailae*. *Food and Chemical Toxicology*. <https://doi.org/10.1016/j.fct.2020.111203>
- Mahanta, S. and Khanikar, B. (2021) 'Heliyon Mosquitocidal activity of twenty-eight plant essential oils and their binary mixtures against *Culex quinquefasciatus*, (Diptera : Culicidae)', *Heliyon*, 7 (November 2020), p. e06128. Available at: <https://doi.org/10.1016/j.heliyon.2021.e06128>.
- Manh HD, and Tuyet OT (2020) 'The mosquito larvicidal activity of essential oils from *Cymbopogon* and *Eucalyptus* species in vietnam', *Insects*, 11(2), pp. 1–7. Available at: <https://doi.org/10.3390/insects11020128>.
- Martinez-Torres, D., Chandre, F., Williamson, M. S., Darriet, F., Bergé, J. B., Devonshire, A. L., Guillet, P., Pasteur, N., & Pauron, D. (1998). Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* s.s. *Insect Molecular Biology*, 7(2), 179–184. <https://doi.org/10.1046/j.1365-2583.1998.72062>
- Ministère de la Santé (2023) *annuaire statistique 2022*. Ouagadougou.
- Munyoki DN, Kokwaro ED, Mwangangi JM, Muturi EJ et Mbogo CM (2021) 'Insecticide resistance status in *Anopheles gambiae* (s.l.) in coastal Kenya', *Parasites and Vectors*, 14(1), pp. 1–10. Available at: <https://doi.org/10.1186/s13071-021-04706-5>.
- N'Guessan, R., Corbel, V., Akogbéto, M., & Rowland, M. (2007). Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerging* <https://doi.org/10.3201/eid1302.060631>.
- Namountougou, M., Simard, F., Baldet, T., Diabate, A., Ouedraogo, J. B., Martin, T., et al. (2012). Multiple insecticide resistance in *Anopheles gambiae* s.l. populations from Burkina Faso, West Africa. *PLoS ONE*, 7(11), Article e48412.
- Namountougou, M., Soma, D. D., Kientega, M., Balboné, M., Kaboré, D. P. A., Drabo, S. F., Coulibaly, A. Y., Fournet, F., Baldet, T., Diabaté, A., Dabiré, R. K., & Gnankine, O. (2019). Insecticide resistance mechanisms in *Anopheles gambiae* complex populations from Burkina Faso, West Africa. *Acta Tropica*, 197, Article 105054. <https://doi.org/10.1016/j.actatropica.2019.105054>
- Nkya TE, Poupardin R, Laporte F, Akhouayri I, Mosha F, Magesa S, Kisinza W., and David J.-P. (2014) 'Impact of agriculture on the selection of insecticide resistance in the malaria vector *Anopheles gambiae* : a multigenerational study in controlled conditions', 7(1), pp. 1–12. Available at: <https://doi.org/10.1186/s13071-014-0480-z>.
- Nkya, T. E., Akhouayri, I., Poupardin, R., Batengana, B., Mosha, F., Magesa, S., Kisinza, W., & David, J. P. (2014b). Insecticide resistance mechanisms associated with different environments in the malaria vector *Anopheles gambiae*: A case study in Tanzania. *Malaria Journal*, 13(1), 1–15.
- Nonviho, G., Wotto, V. D., Noudogbessi, J., Avlessi, F., Akogbeto, M. et Sohounhoulou, D. C. K. (2010) 'Original research paper insecticidal activities of essential oils extracted from three species of Poaceae on *Anopheles gambiae* spp, major vector of malaria', 11(4), pp. 411–420.
- Ossé, R. A., Tokponnon, F., Padonou, G. G., Glitho, M. E., Sidick, A., Fassinou, A., Koukpo, C. Z., Akinro, B., Sovi, A., & Akogbéto, M. (2023). Evidence of transmission of *Plasmodium vivax* 210 and *Plasmodium vivax* 247 by *Anopheles gambiae* and *An. coluzzii*, major malaria vectors in Benin/West Africa. *Insects*. <https://doi.org/10.3390/insects14030231>
- Ouedraogo WJ, Yerbanga RS, Meda R., Ouedraogo JB, and Ouedraogo GA (2024) 'Revue des Plantes Médicinales à Potentiel Aromatique du Burkina Faso Medicinal Plants of Aromatic Potential from Burkina Faso : a Review', 2(August), pp. 1–14.
- Oumboulo, W. A., Pignatelli, P., Barreaux, A. M. G., Tia, I. Z., Koffi, A. A., Ahoua Alou, L. P., Sternberg, E. D., Thomas, M. B., Weetman, D., & N'Guessan, R. (2020). Fine scale spatial investigation of multiple insecticide resistance and underlying target-site and metabolic mechanisms in *Anopheles gambiae* in central Côte d'Ivoire. *Scientific Reports*, 10(1), 1–13. <https://doi.org/10.1038/s41598-020-71933-8>
- Ranson, H., Jensen, B., Vulule, J. M., Wang, X., Hemingway, J., & Collins, F. H. (2000). Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance

- to DDT and pyrethroids. *Insect Molecular Biology*, 9(5), 491–497. <https://doi.org/10.1046/j.1365-2583.2000.00209.x>
- Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z., & Corbel, V. (2011). Pyrethroid resistance in African anopheline mosquitoes: What are the implications for malaria control? *Trends in Parasitology*, 27(2), 8.
- Ríos, N., Stashenko, E.E. and Duque, J.E. (2017) 'Evaluation of the insecticidal activity of essential oils and their mixtures against *Aedes aegypti* (Diptera: Culicidae)', *Revista Brasileira de Entomologia*, (RBE-168), p. 5.
- Santolamazza, F., Mancini, E., Simard, F., Qi, Y., Tu, Z., & Della Torre, A. (2008). Distribution of knock-down resistance mutations in *Anopheles gambiae* molecular forms in west and west-central Africa. *Malaria Journal*, 8, 8.
- Sarma R, Adhikari K, Mahanta S, Khanikar B. (2019) 'Insecticidal activities of Citrus aurantifolia essential oil against *Aedes aegypti* (Diptera: Culicidae)', *Toxicology Reports*, 6, pp. 1091–1096. Available at: <https://doi.org/10.1016/j.toxrep.2019.10.009>.
- Soma DD, Zogo BM, Some A, Tchikoi BN, Hien DF de S., Pooda HS, Coulibaly S., Gnambani JE, Ouari A., Mouline K., Dahounto A., Ouédraogo GA, Fournet F., Koffi AA, Pennetier C., Moiroux N., and Dabiré RK (2020) 'Anopheles bionomics, insecticide resistance and malaria transmission in southwest Burkina Faso : A pre-intervention study', pp. 1–21. Available at: <https://doi.org/10.1371/journal.pone.0236920>.
- Soma, D. D., Zogo, B., Hien, D. F., De, S., Hien, A. S., & Kabor' EDA, Kientega M, Ouedraogo AG, Pennetier C, Koffi AA, Moiroux N, Dabir' ERK,. (2021). Malaria vectors diversity, insecticide resistance and transmission during the rainy season in peri-urban villages of south-western Burkina Faso. *Malaria Journal*, 20(1), 1–11. <https://doi.org/10.1186/s12936-020-03554-5>
- Somda, Z., Zandrè, N., Wangrawa, D. W., Toé, H. K., Sombié, A., Saiki, E., Fukumoto, S., Sakurai, T., Sanon, A., McCall, P. J., Kanuka, H., Weetman, D., & Badolo, (2025). 'High pyrethroid resistance is associated with high frequencies of 1014F and 1014S kdr mutations in *Anopheles arabiensis* (Diptera: Culicidae) from Ouagadougou. *Burkina Faso*', *Journal of Medical Entomology*, 62(2), 381–388. <https://doi.org/10.1093/jme/tjae135>
- Soonwera, M., Mounthipmalai, T., Puwanard, C., Sittichok, S., Sinthusiri, J., & Passara, H. (2024). Adulticidal synergy of two plant essential oils and their major constituents against the housefly *Musca domestica* and bioassay on non-target species. *Heliyon*, 10(5), Article e26910. <https://doi.org/10.1016/j.heliyon.2024.e26910>
- Wang Rawa, D. W., Badolo, A., Guelbéogo, W. M., Ki Endrébeogo, M., Nèbié, R. C. H., Sagnon, N., & Et Sanon, A. (2015). Biological activities of four essential oils against *Anopheles gambiae* in Burkina Faso and their *in vitro* inhibition of acetylcholinesterase. *International Journal of Biological and Chemical Sciences*, 9(2), 793. <https://doi.org/10.4314/ijbcs.v9i2.19>
- Wangrawa DW, Badolo A, Ilboudo Z et al (2018) 'Insecticidal Activity of Local Plants Essential Oils Against Laboratory and Field Strains of *Anopheles gambiae* s.l. (Diptera : Culicidae) From Burkina Faso', pp. 1–10.
- Wangrawa D. W., Odero J. O., Baldini F., Okumu F., and Badolo A. (2024) 'The Essential Oils Compounds of *Lippia multiflora* Moldenke and *Cymbopogon schoenanthus* (L.) Spreng Repel and Affect the Survival of the Maize Pest *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Larvae', *Psyche: Journal of Entomology*, 2024. Available at: <https://doi.org/10.1155/2024/1202962>.
- Wangrawa, D. W., Waongo, A., Traoré, F., Ilboudo, Z., Upshur, F., Borovsky, D., Lahondère, C., Badolo, A., & Sanon, A. (2023). Insecticidal and Anti-Feeding Activities of *Cymbopogon schoenanthus*, *Lippia multiflora*, and *Ocimum americanum* Essential Oils Against Larvae and Pupae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Acta Scientific Agriculture*. <https://doi.org/10.31080/asag.2023.07.1315>
- Weaver, V. M. (2017). 'Cell and tissue mechanics: The new cell biology frontier'. *Molecular Biology of the Cell*, 28(14), 1815–1818.
- WHO (2022) Standard operating procedure for determining the ability of PBO to restore susceptibility of adult mosquitoes to pyrethroid insecticides in WHO tube tests, World Health Organization. Available at: <https://www.who.int/publications/i/item/9789240043855>.
- WHO (2024) World malaria World malaria report report. Genève. Available at: <https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2024>
- Yao, F. A., Millogo, A.-A., Epopa, P. S., North, A., Noulain, F., Dao, K., Drabo, M., Guissou, C., Kekele, S., Namountougou, M., Ouedraogo, R. K., Pare, L., Barry, N., Sanou, R., Wandaogo, H., Dabire, R. K., McKemey, A., Tripet, F., & Diabaté, A. (2022). Mark-release-recapture experiment in Burkina Faso demonstrates reduced fitness and dispersal of genetically-modified sterile malaria mosquitoes. *Nature Communications*, 13(1), 1–11. <https://doi.org/10.1038/s41467-022-28419-0>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.