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Ecotypes, Diversity and Insecticide Resistance in Culex vectors of Filarial Worms Across Cameroon's Forest and Humid Savannah

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Research Article

Keywords: Culex quinquefasciatus, ecology diversity, filaria worms, Insecticides, dynamics

Posted Date: September 4th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9752423/v1

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Additional Declarations: No competing interests reported.

Abstract

Introduction:

Culex pipiens complex, vectors of filaria worms and emerging arboviruses are widely distributed in Cameroon. However, information on their infectivity, diversity and insecticide resistance profile across ecozones in the country remains limited. Understanding the pattern of resistance to pyrethroids and carbamates insecticide subclasses, as well as their eco-diversity and dynamic will support effective vector control intervention strategies.

Method:

This was a cross-sectional study conducted over 5 years (2020-2025) in the humid Savannah and forest ecological zones of Cameroon. Culex mosquitoes were collected by larva breeding (LB) and human landing catch (HLC) and identified morphologically. Sensitivity of *Cx. pipiens* complex to deltamethrin, permethrin and propoxur was assessed using the WHO insecticide susceptibility assay and its sibling species detected by multiplex diagnostic PCR. *Kdr-west*genotyping and the presence of *Wuchereria bancrofti* and *Brugia malayi* was assessed by PCR.

Results:

A total of 4749 Culex mosquitoes were collected, mainly by HLC (62.1%), with significantly ($p<0.001$) more trapped in the Forest Ecological Zone (FEZ). *Cx. quinquefasciatus* was exophagic in the FEZ and endophagic in the humid Savannah Ecological Zone (hSEZ), with peak biting period between 02:00-03:59 in both zones. *Cx. quinquefasciatus* were susceptible to 1X propoxur, exhibiting concentration-dependent mortality rates (31.1-94.0%) in the FEZ but resistant to all insecticide types and concentrations in the hSEZ. In both ecozones, there was an association ($p<0.001$) between phenotypic resistance and *Kdr-west*. *Wuchereria bancrofti* and *B. malayi* were not detected in the sample.

Conclusion:

The diversity of Culex mosquito species, insecticide resistant profiles and biting dynamics vary across ecozones. The presence of insecticide resistance necessitates continuous monitoring while implementing next generation insecticides for better vector management in Cameroon.

Introduction

Several urban environments in Africa are experiencing an increase in mosquito population density and species diversity due to unplanned construction and the lack of functional sewage and drainage systems (Hawaria et al., 2020). This has led to the emergence of stagnant water pools and puddles, which are the potential breeding habitats for species such as *Cx. quinquefasciatus* and Anopheles mosquitoes, the main vectors of *Wuchereria bancrofti* and *Brugia malayi*. In addition, some of these mosquitoes are vectors of arboviruses with potential to cause serious epidemics or pandemics. As such many countries are implementing various mosquito management interventions, with variation driven by the dynamic and

differential nature of the targeted mosquito population, including their breeding and feeding habits (Chanda et al., 2016). Though, it is conventionally accepted that Anopheles mosquitoes are mainly nocturnal feeders(Bigoga et al., 2007; Mlacha et al., 2020; Wanji et al., 2003), there are inconsistencies in the biting dynamics of Culex mosquitoes that must be considered in planning and implementing species specific interventions. While some studies suggest *Cx. quinquefasciatus* have a peak biting period from early dusk and continuously throughout the night, others claim its peak biting period is at midnight and drops immediately after (Farajollahi et al., 2011; Reni et al., 2020; Uttah et al., n.d.). Furthermore, other reports have linked the discrepancies to the opportunistic nature of these vectors, suggesting that the variation might be due to host availability (Ferraguti et al., 2023) and location(Reni et al., 2020). These discrepancies do not only complicate the control strategy but also delay the quick intervention strategies. Therefore, locally characterizing the seasonal, ecological and faunal composition, and the impact of interventions such as insecticides on arthropod disease vectors are important for strategies and interventions to control or eliminate vector borne diseases(Abiodun et al., 2016; Ayala et al., 2009; Mayi et al., 2020). Effective control of most arthropod pest populations has relied primarily on the use of pyrethroids, organophosphates, carbamates, and organochlorine insecticides. The indiscriminate use of these chemicals in various settings and agricultural practices has driven the evolution of widespread insecticide resistance (Sonhafouo-Chiana et al., 2022), threatening continental disease elimination and vector control programs. To reduce human-vector contact, especially in the fight against malaria, approaches have mainly included the community deployment of long-lasting insecticidal nets (LLINs) and indoor residual spray (IRS) (Musiime et al., 2019). However, the recent trajectory of insecticide resistance in mosquitoes (Antonio-Nkondjio et al., 2015; Corbel et al., 2007; Nchoutpouen et al., 2019a; Ochomo et al., 2017) could undermine years of efficacy from these vectors. Beyond the malaria vectors, these insecticides also impose selective pressure on other human biting mosquitoes such as those of the *Culex pipiens* complex. Unlike Anopheles mosquitoes, whose insecticide resistance profiles and bionomics have been studied extensively, the insecticide resistance profile and the relation with disease burden of *Culex* mosquitoes remain understudied in Africa. Concerningly, multiple reports suggest selection against pyrethroid insecticide classes (Adams et al., 2023), providing early evidence of increasing resistance in *Culex* spp (Nchoutpouen et al., 2019a; Poumachu et al., 2025; Vereecken et al., 2022) and Anopheles (Akuamoah-Boateng et al., 2021; Campos et al., 2022; Kwi et al., 2022). This is particularly concerning as pyrethroids type I and II are the recommended insecticides, widely used in bednets for disease control and elimination.

Mosquitoes exploit several mechanisms to render insecticides ineffective. The most widely studied mechanisms are: 1) target site resistance(Martinez-Torres et al., 1998) 2) enzymatic/metabolic resistance(Hemingway et al., 2004) 3) cuticular resistance(Jacobs et al., 2023) and 4) behavioural resistance(Zhou et al., 2020). DDT and pyrethroid insecticides target the para voltage-gated sodium channel (VGSC) in the *Cx. pipiens* complex, inducing a knockdown effect. These effects are evaded by the Culex mosquitoes following a substitution of leucine by either phenylalanine (kdr-West) or serine (kdr-East) at position 1014 in the VGSC gene, rendering the insecticide ineffective (Martinez-Torres et al., 1998). This mutation supports dual resistance to DDT and pyrethroid insecticides in areas where one or both are used to control these vectors (Clarkson et al., 2021; Danladi & Goje, 2024). Furthermore, the G119S mutation (Weill et al., 2004) in Anopheles and *Cx. pipiens* complex mosquitoes has been reported to render

organochlorine and carbamate insecticides ineffective (Binyang et al., 2022; Edi et al., 2014). This mutation counter organochlorine and carbamate insecticides from inhibiting the enzyme acetylcholinesterase (Ace-1). This has been observed in the *Cx. pipiens* Complex across West and Central African countries. Also, organophosphates (OPs) and carbamate insecticides exploit a different mechanism of action and are used as an alternative insecticide in most communities where pyrethroid resistance is confirmed (Binyang et al., 2022; Elanga-Ndille et al., 2019; Mdeni et al., 2022). Alternatively, enzyme sequestration within the metabolic pathway of mosquitoes breaks down insecticides, increasing resistance. This is enhanced by either transcriptional upregulation (Chamnanya et al., 2025; Talipouo et al., 2021) sometimes linked to gene duplication (Milesi et al., 2022; Raymond et al., 2001) leading to the over-expression of detoxification enzymes. Though several enzymes have been reported in other insects, P450 monooxygenase, glutathione-S-transferase and esterases are the main enzymes responsible for the breakdown of OPs, pyrethroids and DDT insecticides in mosquitoes (Hemingway et al., 2004).

With limited data on insecticide resistance in Culex mosquitoes in Cameroon and the surge in resistance of these mosquitoes across Africa, the goal of the Global Programme to Eliminate Lymphatic Filariasis (GPELP), which somewhat relies on the use of LLINs and IRS might stall, undermining gains achieved over the last couple of decades(Davis et al., 2021). Thus, understanding the distributions, infectivity and insecticide resistance of *Culex* spp. is crucial for the monitoring their potential for supporting disease transmission. The objective of this study was to assess the diversity and insecticide resistance patterns of Culex mosquitoes and the prevalence of lymphatic filarial worms within *Cx. quinquefasciatus* across two ecological zones of Cameroon.

Materials and Methods

Study sites

This was a cross-sectional study conducted in 22 sites across 8 administrative districts within two ecological zones of Cameroon: The Forest ecological zone (FEZ); including settlements in Urban areas of Buea, Douala and Limbe as well as semi-Urban areas of Mamfe and Tiko and humid Savannah Ecological zone (hSEZ); Urban area of Kumbo and semi-Urban areas of Babessi and Mesaja (Figure 1). Besides eco-climatic differences, the selection of study sites varied along the urban-rural continuum where urban areas were predominantly characterized by residential houses and industries while the Semi-urban settings were characterized by farming activities, including rubber and cocoa plantations and fewer industries.

Adult mosquito sampling

At each collection site, four rectangular houses spaced approximately 0.5km apart, and with close proximity to mosquito breeding sites were selected. To reduce bias, clusters of houses per sites were also selected to have similar features, such as, mud bricks and metal roofing, common in the semi-urban areas in the hSEZ or cement with metal roofing in the semi-urban and urban areas in the FEZ.

A total of 80 consenting adults aged 24-40 years were recruited as mosquito collectors for human landing catches (HLC). Collections were done overnight (between 18:00 h to 06:00 h) both indoors and outdoors

simultaneously for three consecutive nights per household across 32 houses. To reduce bias due to individual differences in skills and attraction to mosquitoes, catchers were rotated between shifts of 6 hours and days of collection. Collection was done from 2020 to 2025 from September to November and March to May, which represents the rainy season, when mosquito breeding sites and density increase The mosquitoes were then sorted by genus and the culicines identified morphologically under a stereomicroscope (Olympus SZX10, Berlin, Germany) based on the WHO pictorial identification key of important disease vectors (Krambrich et al., 2024). They were then stored in labeled Eppendorf tubes containing silica gel and transported to the Molecular and Cell Biology laboratory (MCBL) at the University of Buea for downstream analysis.

Larvae collection and rearing

Culex mosquito larvae were collected from randomly selected breeding sites. The Culex larvae were identified by their long, slender siphon tubular structure projecting upward and resting on the surface of the water with their heads completely immersed in water (vertically inclined at an angle of 45 to the water surface). A 50cm^3 deep spoon was used to carefully collect different instars of larvae and pupae and placed in transparent 10L polystyrene buckets. These were transported to the insectary at the Molecular Epidemiology and Drug Resistance Unit (MEDRU) at the Faculty of Health Science in the University of Buea for further rearing. The larvae were redistributed into small transparent open mouth vessels (1000cm^3), fed with tetramine (2g/500ml) and maintained with fresh mineral water till pupation. The pupae were aspirated using a dropper and transferred into small transparent open mouth vessels containing fresh mineral water, then placed in a cage till emergence. The insectary condition was set at temperature of 24°C to 30°C and humidity 75% to 90% with a 12-hour day/night cycles (Das et al., 2007). The mosquitoes were maintained on 10% sucrose solution and libitum. Four to five days old adult mosquitoes that were morphologically verified as *Cx. pipiens* complex were then subjected to insecticide resistance testing.

WHO Insecticide Resistance bioassay Tests

Insecticide resistance bioassay tests were performed based on the WHO susceptibility test standard protocol (WHO 2022) with 4 replicates of 20 to 25 non-blood-fed female adult mosquitoes. The different insecticides (classes) and concentrations used included pyrethroids: permethrin [0.25% (1X) & 1.25% (5X)] and deltamethrin [0.025% (1X), 0.125% (5X) & 0.25% (10X)] and carbamate: Propoxur [0.1 (1X)](Table S3). For each replicate exposed to deltamethrin and propoxur insecticide-impregnated filter paper, 60 minutes interaction time was allowed while the number of immobilised (knock down) was recorded every 20 minutes for 1 hr. For permethrin, the interactive exposure period was 3 hours (180 minutes) after which, the mosquitoes were transferred into the holding tube and maintained on 10 % sucrose solution at 27 ±3^oC and 80% relative humidity. The number of dead and alive mosquitoes were recorded 24 hours post exposure and used to calculate the percentage (%) mortality [(total number of dead mosquitos after exposure/total number of mosquitoes exposed for each insecticide) X 100]. Live mosquitoes were those considered to be active 24hrs after exposure while dead mosquitoes were the nonactive ones. At the end of the experiment, the holding tubes containing live mosquitoes were placed in plaster rappers and frozen at -20^oC freezer for

4 hours to immobilize active Culex mosquitoes. The immobilized specimens were removed from holding tubes, morphologically verified and transferred into Eppendorf for storage and molecular analysis.

DNA extraction

DNA was extracted from the abdominal parts of 500 HLC (125 randomly selected per year of collection), 25 pools of 20 head-thorax segments of HLC and 308 whole LB (103 randomly selected per year) female *Culex pipiens complex* mosquitoes following the ANOSPP protocol (Korlević & Lawniczak, 2023)**.** Each individual *Culex pipiens complex* mosquito, abdominal part or pool was placed in 96-well plates and 100 µL lysis buffer (0.05% 0.05 µL Tween-20, 72.95 µL of distilled water, 200 mM 20 µL Tris pH 8, 25mM 5µL EDTA, 0.4 mg/ml 2 µL Proteinase K) added and incubated at 56°C overnight. After incubation, the lysis buffer (DNA extract) was transferred into fresh 96-well plates and stored at 4°C for further analysis. The DNA was then pipetted out and placed in a freshly prepared Eppendorf tubes for parasite detection.

Molecular speciation of *Culex pipiens* complex

Molecular speciation of the *Cx. pipiens* complex subspecies was done on 808 samples (308 LB and 500 HLC) following the multiplex PCR method as previously described (Smith & Fonseca, 2004). This PCR-based assays uses polymorphisms in the second intron of the acetylcholinesterase-2 (*ace-2*) locus for the identification of sibling members: *Cx. pipiens, Cx. quinquefasciatus and Cx. pipiens pallens*). The reactions were carried out in a 25 µL reaction tube which contains 0.5 µM 2.5 µl of each primer, 12.5 µl GoTaq® Green Master Mix, 2µl DNA template and 5.5µl of nuclease free water. Thermocycler conditions were 94°C for 10 min followed by, 35 cycles of 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 1 minute, with a final elongation at 72°C for 10 minutes, and a 4°C hold. PCR amplicons were subsequently separated on a 2% agarose gel containing ethidium bromide (10 mg/ml) and visualized on a UV illuminator (TOYOBO Trans Modele TM-20) against a 100bp ladder.

Detection of the kdr west (1014F) mutation

Of the 308 Genomic DNA previously extracted and identified *as Cx. quinfuefasciatus*, a random subset of 250 samples (150 from hSEZ and 100 from FEZ) were selected and the *kdr-West* (L1014F) allele genotyped by PCR as previously described (Martinez-Torres et al., 1998). The PCR reaction conditions included initial denaturation at 94℃ for 5 mins, 35 cycles of denaturation, annealing and extension at 94℃ for 30s, 54℃ for 30s and 72℃ for 30s respectively, final extension at 72℃ for 5 mins. The reaction was set up in a total volume 15µl, consisting of 7µl GoTaq green master mix, 1µl each of Agd1 (5'-ATAGATTCCCCGACCATG-3'), Agd2 (5'-AGACAAGGATGATGAACC-3'), Agd3 (5'-AATTTGCATTACTTACGACA-3') and Agd4 (5'-CTGTAGTGATAGGAAATTTA-3) primers, 3µl nuclease-free water and 1µl of a 1 in 5 dilution of the mosquito DNA sample. The amplicons were separated on a 2% agarose gel stained with ethidium bromide and visualized using a Gel Doc (version 2.0). *Culex pipiens* complex carrying the wild type or mutant allele were ascertained based on the fragment lengths of 293bp and 195bp or 137bp respectively.

Molecular screen of *Wuchereria bancrofti and Brugia malayi*.

The 25 pools of *Cx. quinquefasciatus* DNA were used for the detection of *W. bancrofti*(Chanteau et al., 1994) and *B. malayi* (Vasuki et al., 2001) by PCR. The primer set (NV1: 5-CGTGATGGCATCAAAGTAGCG-3; NV2: 5-CCCTCACTTACCATAAGACAAC-3) for detection of *W. bancrofti* targeted a highly repeated DNA sequence (SsPI repeat), while (F (F(18-mer), 5-GCGCATAAATT-CATCAGC-3; R (23-mer), 5-GCGCAAAACTTAATTACAAAAGC-3) for *B. malayi* targeted the *HhaI* tandem DNA repeat. PCR analysis was conducted in a final reaction volume of 25 µL containing 0.5 µM 2 µL of each primer, 11.5 µL GoTaq® Green Master Mix, 4 µL DNA template and 5.5 µL of nuclease-free water. The PCR conditions were as follows: 10 min at 94°C followed by 35 cycles of denaturation during 30 s at 94°C, annealing at 54°C for 45 s, extension at 72°C for 45 s and final extension at 72°C for 10 minutes. The PCR amplicons were then separated by electrophoresis on 1.5% agarose gel and visualised under the UV illuminator (TOYOBO Trans Modele TM-20) against a 100 bp ladder. For both *W. bancrofti* and *B. malayi*, the expected band sizes were 188bp and 322bp respectively.

Data analysis

All statistical analyses and graphical visualizations were conducted using Python version 3.13. Mosquito collections were summarized by species, ecological zone, collection district, collection context (indoor/outdoor), collection time (18:00–05:59), insecticide type, and concentration tested. Species counts and proportions were compared using Pearson's Chi-square tests or Fisher's exact tests when expected cell counts were <5. Differences in species composition between ecological zones and associations between biting time and species composition were assessed using Chi-square tests. For sparse contingency tables, Fisher's exact test with Monte-Carlo simulation (10,000 replicates) was applied. Mosquito mortality rates were calculated as percentages and compared between ecological zones for the same insecticide and concentration using Chi-square or Fisher's exact tests, with odds ratios (ORs) and 95% confidence intervals reported. Dose–response effects within ecological zones were evaluated using Chi-square tests. The *Cx. pipiens* complex populations were classified as susceptible, suspected resistant or resistant if mortality rate recorded after 24 h following exposure were ≥ 98%, (>90 and <97%) or < 90% respectively (WHO 2022).Associations between ecological zone and phenotypic resistance, ecological zone and *kdr* genotype frequencies, and between *kdr* genotype and phenotypic resistance were assessed using Chi-square tests. Statistical significance was set at $p < 0.05$.

Ethical considerations

Ethical approval/clearance was obtained from the Cameroon National Ethics Committee (Ref. N^0 2020/09/1188/CE / CNERSH/ SP, 2022/12/1315/CE/CNERSH/SP and 2025/03/577/SW/CRERSH/C) while administrative authorizations were sought from the relevant regulatory authorities in all areas where sample collection by human landing catch was performed. Only individuals who tested negative after using the SD BIOLINE LF IgG4 rapid test (Wb123) and volunteered to participate by signing a written informed consent, after adequate sensitization were enrolled. Districts and village leaders were informed of the study procedure including the benefits. Verbal informed consent was obtained from village leaders where mosquito larvae were collected. All experimentations followed the Declaration of Helsinki and relevant ethical guidelines strictly.

Results

Culex species diversity

A total of 4749 Culex mosquitoes were collected in the FEZ and hSEZ between 2020 to 2025: 3518 (74.1%) from the FEZ and 1231 (25.9%) in the hSEZ of Cameroon by HLC (62.1%, 2950/4749) and LB (37.9%, 1799/4749) (Table 1). There was an association between the proportion of Culex mosquito species and the ecological zone of sampling ($p < 0.001$), with forest ecological zones showing a more diverse species composition in the FEZ compared to hSEZ (Table 2). Generally, *Cx. pipiens* complex was the most dominant species across all sites and both ecological zones (Table 2).

Table 1: Temporal distribution of *Culex* Species by Human Landing Catch (2020-2025)

Year of collection	*Culex Species [n (%)]*					Total	P value
	Cx. pipiens complex	*Cx. tigripes*	*Cx. antennatus*	*Cx. duttoni*	*Cx. unidentified*		p < 0.001
2020	556 (68.4)	84 (10.3)	29 (3.6)	144 (17.7)	0 (0.0)	813	
2021	816 (78.7)	36 (3.5)	121 (11.7)	64 (6.2)	0 (0.0)	1037	
2024	469 (63.2)	168 (22.6)	96 (12.9)	0 (0.0)	9 (1.2)	742	
2025	335 (93.6)	19 (5.3)	0 (0.0)	0 (0.0)	4 (1.1)	358	

Table 2: Distribution of *Culex* species across sites and Ecological Zones

		Culex spp [n (%)]					Total	P value
Ecological zone	Collection Site	*Cx. pipiens complex*	*Cx. duttoni*	*Cx. antennatus*	*Cx. tigripes*	*Cx. Unidentified*		
FEZ	Buea	1372 (74.2)	208 (11.2)	150 (8.1)	120 (6.5)	0 (0)	1850	< 0.001)
	Douala	189 (57.5)	0 (0)	96 (29.2)	35 (10.6)	9 (2.7)	329	
	Limbe	353 (73.1)	86 (17.8)	44 (9.1)	0 (0)	0 (0)	483	
	Mamfe	784 (95.6)	0 (0)	0 (0)	36 (4.4)	0 (0)	820	
	Tiko	27 (75.0)	0 (0)	0 (0)	9 (25.0)	0 (0)	36	
Sub-Total		2725 (77.5)	294 (8.4)	290 (8.2)	200 (5.7)	9 (0.3)	3518	
hSEZ	Babessi	143 (59.8)	0 (0)	0 (0)	96 (40.2)	0 (0)	239	< 0.001)
	Kumbo	214 (82.0)	0 (0)	0 (0)	42 (16.1)	5 (1.9)	261	
	Mesaje	708 (96.9)	0 (0)	0 (0)	19 (2.6)	4 (0.6)	731	
Sub-Total		1065 (86.5)	0 (0)	0 (0)	157 (12.8)	9 (0.7)	1231	
Total		3790 (79.8)	294 (6.2)	290 (6.1)	357 (7.5)	18 (0.4)	4749	

Culicine species biting density and behavioural patterns

Mosquito density varied disproportionately across the years of collection, averaging 5.12 Culex mosquitoes/trap/night across all sites of the 2950 Culex mosquitoes trapped by HLC. The average overall biting density per year across the four-year period varied from 18.03 in 2024 to 0.73 Culex mosquitoes/trap/night in 2025 (Table 3). Furthermore, the average biting density per species was highest for *Cx. pipiens* complex and lowest for *Cx. duttoni* across the 4 years of collection (Table 3).

Table 3: Diversity of *Culex* mosquitoes collected by HLC from 2020 to 2025.

Year of collection	Culex mosquitoes/trap/night for each taxon				
	Cx. pipiens complex	*Cx. tigripes*	*Cx. duttoni*	*Cx. antennatus*	Total
2020	15.44	2.33	1.22	0.81	4.95
2021	22.67	1.00	1.78	3.36	7.20
2024	12.47	4.67	0.00	2.66	18.03
2025	0.92	0.53	0.00	0.00	0.73

In both ecozones, majority of the *Culex* mosquito species were caught outdoors (exophagic biting tendency), with *Cx. pipiens* complex dominate across all times, both indoors and outdoors in both ecozones. However, *Cx. pipiens* complex catches in the FEZ were largely outdoor-driven while the strongest pipiens peak was indoor in the hSEZ (Figure 2). In both ecozones, the dominant *Cx. pipiens* complex showed a late-night biting surge between 02:00–03:59 both outdoors and indoors (Figure 3). interestingly, the unidentified Culex species only appeared to bite at 02:00–03:59. There was a significant association between time of collection and species composition in both ecozones ($p < 0.001$ each) (Table S1 and 2).

In the hSEZ, *Cx. tigripes* was outdoor-biased with activity shown early in the evening (18:00–19:59) and near dawn (04:00–05:59). In the FEZ *Cx. antennatus* density increased steadily through the night, peaking near dawn (04:00–05:59), while *Cx. duttoni* were peaked around midnight to 03:59 and *Cx. tigripes* at dawn (04:00–05:59). For Indoor catches, the peak catch for *Cx. tigripes* was between 00:00–01:59, while *Cx. duttoni* was mainly observed between 00:00–03:59. For *Cx. antennatus,* the density was constant throughout the night, with no observable peak biting time (Figure 3).

Molecular speciation of *Culex pipiens* complex and screening of microfilaria

Of the 708 mosquitoes of the *Cx. pipiens* complex screened, only the *Cx. quinquefasciatus* sibling was detected. All samples were negative for *Wuchereria bancrofti* or *Brugia malayi.*

Insecticide resistance pattern of *Culex pipiens* complex across ecozones

Of the three insecticides tested, *Culex pipiens* complex was only susceptible to propoxur at diagnostic concentration in the FEZ while the mosquitoes were susceptible to 10X deltamethrin in the hSEZ (Figure 4). *Culex pipiens* complex were all resistant to 1X and 5X deltamethrin and permethrin in both ecozones as well as to 1X propoxur in the hSEZ and 10X deltamethrin in the FEZ(Table S3). There were significant differences in the mortality of mosquitoes exposed to 1X permethrin ($p < 0.001$), 1X deltamethrin ($p < 0.001$), 10X deltamethrin ($p = 0.04$) and 1X propoxur ($p < 0.001$) in the FEZ compared to the hSEZ (Figure 4).

KDR-west* (L1014F) mutations in *Culex quinquefasciatus

Of the 208 deltamethrin exposed *Cx. quinquefasciatus* mosquitoes successfully genotyped for the *kdr-West*, 144 and 64 were from the FEZ and hSEZ respectively. There was no statistically significant difference in the frequency of kdr genotypes between ecological zones (p=0.083) (Table 4).

Table 4: Distribution of *Kdr-West* genotypes in *Culex quinquefasciatus* across ecological zones

Ecological Zone	[n (%)]			P value
	RR	RS	SS	
FEZ	51 (35.4)	43 (29.9)	50 (34.7)	0.08
hSEZ	31 (48.4)	20 (31.3)	13 (20.3)	

FEZ = Forest Ecological zone; hSEZ = humid Savannah Ecological zone

Furthermore, in both ecological zones, there was a significant association ($p < 0.001$ each) between the kdr-west genotype and the phenotypic resistance status of the Culex mosquitoes (Table 5). In the forest (n=144), the association is statistically significant ($\chi^2(2)=24.6$, $p < 0.001$) with a moderate effect size (Cramér's V=0.397, 95% CI 0.252–0.552) while in the humid savannah (n=64), the association is likewise significant ($\chi^2(2)=16.4$, $p <0.001$) with a moderate-to-large effect (Cramér's V=0.478, 95% CI 0.281–0.681). In both zones, the patterns were concordant with a gene–dose gradient. The homozygous resistance genotype (RR) showed the highest resistant proportion followed by the heterozygous genotype (RS) and least being the homozygous susceptible genotype (SS) (Table 5). The reverse trend was observed for phenotypically susceptible culex mosquitoes, with the homozygous resistance genotype (RR) showed the least sensitive followed by the heterozygous genotype (RS) and lastly the homozygous susceptible genotype (SS) being the most susceptible.

Table 5: Relationship between Kdr-West genotype and phenotypic resistance across ecological zones (n=208)

Ecological zone	Kdr Genotypes	Phenotypic resistance status [n (%)]		
		Resistant	Susceptible	P value
FEZ	RR	34 (66.7)	17 (33.3)	< 0.001
	RS	25 (58.1)	18 (41.9)	
	SS	10 (20.0)	40 (80.0)	
hSEZ	RR	25 (80.6)	6 (19.4)	< 0.001
	RS	12 (60.0)	8 (40.0)	
	SS	2 (15.4)	11 (84.6)	

FEZ = Forest Ecological zone; hSEZ = humid Savannah Ecological zone

Discussion

The spatial cross sectional five-year surveillance (2020–2025) study revealed significant differentiation in Culex mosquito species composition across Cameroon's contrasting biomes. The humid savannah ecological zone supported the proliferation of two Culex mosquito species (*Cx. pipiens* complex and *Cx. tigripes*) while the forest ecological zone favored the survival of a more diverse Culex mosquito species (*Cx. pipiens* complex, *Cx. duttoni, Cx. tigripes* and *Cx. antennatus)*. The fact that, tropical forest ecological zones harbours a diverse host of culex species could be due to increase habitat heterogeneity (Mayi et al., 2020), microclimate variations (Flores Ruiz et al., 2022), high population density (Duval et al., 2023; Kwi et al., 2022) which provides breeding sites. Though the disproportionate species composition might be reflective of both genuine abundance difference and availability of breeding sites, the dominance of *Cx. pipiens* complex in both zones suggests a superior ecological plasticity and survival tendency. Additionally, the absence of *Culex duttoni* and *Cx. antennatus* in humid savannah ecological zone is indicative of a possible genetic evolutional (olfactory proteins involved in response to external stimuli) specific requirement that are incompatible with savannah's environmental conditions (host odours and wind speeds)(Nwoke et al., 1993; Ramaiah & Das, 1992).

The nocturnal behavioural patterns of different Culex species across zones and within zones is quite remarkable. Biting dynamics revealed difference amongst Culex mosquitoes host-seeking habits between ecological zones. For the savannah population, the host-seeking biting trend were preferentially endophagic as opposed to the exophagic predominant tendencies of the forest mosquito population. This was also observed across different countries in southern and western African countries (Kampango et al., 2023; Nwoke et al., 1993) and also in southern india (Ramaiah & Das, 1992). This diverse biting dynamics has profound implications for disease control, elimination strategies and transmission dynamics. Furthermore, the late-night biting surge observed for *Cx. pipiens* complex (main vector of lymphatic filariasis) was consistent in both zones. This pattern corresponded to time when most human-host were deep asleep and most vulnerable in these two ecological zones. Our results were in line with other studies conducted just before 2005 in West and East Africa showing peak biting after midnight (Pedersen & Mukoko, 2002) but in contrast to a study in Nigeria(Uttah et al., n.d.) reporting *Cx. quinquefasciatus* as an early dawn bitter. Furthermore, the significant higher average biting density recorded in 2024 compared to the other sampled years is possibly driven by favourable climatic conditions or reduced pressure exerted by insecticides and this could influence the outbreak of arboviruses and filarial worms transmitted by these vectors. In the same line, the specie-specific biting pattern suggesting temporal niche partitioning that minimizes the interspecies specific competition while maximizing continuous biting tendencies. This definitely underscores the effective surveillance progress done by vector management programs.

Cx. quinquefaciatus species have been reported across several continents (Cook et al., 2018; Corbel et al., 2007; da Moura et al., 2024; Krambrich et al., 2024) as the most prevalent sibling of the *Cx. pipiens* complex. Thus, its high prevalence provides more evidence for its high adaptability across varying environmental settings and plausible gene flow. The implications of its reduced adaptation time increase the ability of this vector to acquire any new pathogen and subsequently increasing its transmission ability. The identification of *Cx. quinquefasciatus* in these ecozones, a specie widely recognized as the primary vectors of lymphatic filariasis, West Nile Virus and Saint Louis encephalitis is crucial for the epidemiological assessment of *Cx. pipiens* complex. This is in line with previous studies carried in the forest ecological zone with bimodal

annual climate and in the outskirts of the western savannah Highlands of Cameroon in 2019 and 2025 respectively (Nchoutpouen et al., 2019b; Poumachu et al., 2025). The absence of microfilaria in all tested pools suggests very low infection prevalence in the study areas. These results were similar to previous studies in Cameroon (Bamou et al., 2018; Nchoutpouen et al., 2019) but in contrast with other studies in Africa were the presence of *Wuchereria bancrofti* was identified (Derua et al., 2015; Lupenza et al., 2021; Waje et al., 2024).

Understanding insecticide resistance patterns of Culex mosquitoes is a fundamental goal for the current challenges of vector control initiatives. *Cx. pipiens* complex population from the forest ecological zones revealed increasing mortality rates (31.1% to 94.0%) with varying concentrations of different pyrethroids insecticides while maintaining susceptibility to 1X propoxur (carbamate class). A similar trend was observed with the humid savannah Culex population with the exception of their complete susceptibility to 10X deltamethrin. Our results are in line with previous studies in Nigeria (Danladi et al., 2024), Tanzania (Edi et al., 2014) and Cameroon's savannah highland and forest ecological zone (Nchoutpouen et al., 2019; Poumachu et al., 2025; Talipouo et al., 2021). The significant concentration dependent mortality differences shown across the ecozones demonstrated that resistance is not absolute(inert) but can be overcome through intensity-based approaches thus providing a strategic option for insecticide resistance management. Additionally, the intensity bioassay results reveal that resistance is not merely a function of allele frequency, but may also involves ecological factors- possibly like temperature variations, humidity, breeding sites, nutritional status which are ecozone-specific. This might influence the physiological makeup of the vector hence modulate the expression of resistance genotype.

The *Kdr-west* (L1014F) mutation experimental analysis revealed a unique insight in to the molecular background of *Culex pipiens* complex pyrethroid type I and II resistant and its ecological distribution. Though this isn't the first time resistance in both pyrethroids type are reported in Cameroon (Nchoutpouen et al., 2019; Poumachu et al., 2025),this is actually the first time an ecological component has been exploited in insecticide resistance pattern for culex mosquitoes. While there seems to be no statistically significant differences between the *kdr-west* genotype frequencies registered in the two ecological zones, the observed trends provide important biological patterns that warrant further investigation. The lower frequencies of homozygous susceptible genotypes (SS) (20.3% vs 34.7%) in the humid savannah coupled with the higher frequencies of resistant genotypes (RR) (48.4% vs 35.4%) is suggestive of an ongoing selection pressure for resistance alleles in these environments. This observed patterns is similar to previous reports on the insecticide pattern of *Anopheles gambiae* complex across East (Mwagira-Maina et al., 2021), central (Antonio-Nkondjio et al., 2015; Boussougou-Sambe et al., 2018; Nkemngo et al., 2020) and west (da Cruz et al., 2021; Perugini et al., 2024) Africa. This pattern is consistent with higher pesticide selection pressure from continuous use in agriculture in most sampling sites within the humid savannah ecological zones, coupled with increased use of LLINs widely exploited for malaria vector control. Also, though metabolic resistance wasn't assessed in our studies, the species might be exploiting metabolic detoxification P450 enzymes and GST (Saha et al., 2025; Samra et al., 2012) as possible insecticide evasion mechanisms.

Furthermore, the strongest associationbetween the *Kdr-west* genotype and the phenotypic resistance within both ecological zones confirms the functional significance of the L1014F mutation in Cameroon's *Cx. quinquefasciatus* populations. Additionally, the gene-dose effect, which shows an increase in the Culex mosquito phenotypic resistance profile as the resistance genotype increases from SS to RS to RR, demonstrates classic additive inheritance patterns typical of Kdr mutations. This is similar to several studies observed in Cameroon and across Africa and Asia (Abbasi & Daliri, 2024).However, the incomplete penetrance (RR individuals showing only 66.7% -80.6% resistance) indicates that additional genetic and environmental modulators are possibly affecting resistance gene expression. The multi-factorial nature of insecticide resistance has important implications for resistance management strategies, as targeting a single mutation as the primary mechanism exploited by *Cx. quinquefasciatus* may prove insufficient for a long-term control strategy. Though other insecticide resistance mechanisms might be contributing to the resistance patterns of these Culex mosquitoes, based on our study, the association between kdr genotype and phenotypic resistance reveals that *Kdr-west* is a major contributor of insecticide resistance in Culex mosquitoes across the ecozones.

In general, the molecular speciation of *Cx. pipiens* complex and the bioassay of *Cx. quinquefasciatus* is necessary to assess resistance to pyrethroid and carbamate insecticides: vital for disease control and elimination. The increased resistance patterns of *Cx. quinquefasciatus* to varying concentration of deltamethrin and permethrin across the different ecological zones of Cameroon requires constant surveillance.

Conclusion

The survey of Culex species diversity, infectivity, biting dynamic and insecticide resistance spanning across years and ecological zones in Cameroon provides critical evidence for vector-borne disease control and resistance management. The absent of filarial worms in the dominant *Cx. quinquefasciatus* across the different ecozones should be treated with causation while their significant insecticide resistant patterns to pyrethroid insecticides presents a substantial challenge for current vector control strategies. The strong association between the *kdr-west* genotype and phenotypic resistance coupled with ecological zone-specific specie dynamic patterns necessitates a tailored approached to vector control and elimination. Thus, implications of these findings extend beyond immediate vector control and provides a base line for fundamental questions regarding adaptations, specie evolution and human-vector interaction. As urbanization and climate change continue to alter Africa's landscape, understanding vector responses to these changes becomes increasingly important for human health and achieving goals related to Vector-borne disease elimination.

Abbreviations

PCR

Polymerase chain reaction

Cx

Culex

LLINs

Long Lasting Insecticidal Nets

ITNs

Insecticide Treated Nets

Kdr

Knockdown Resistance

FEZ

Forest Ecological

hSEZ

humid Savannah Ecological Zone

Declarations

Acknowledgements

We thank the participants from the various communities who made this study possible, Chiefs, Quarter Heads, administrative and health personnel who assisted with this work.

Clinical trial

Not applicable.

Author’s contributions

PNK: Surveyed the collection areas, coordinated the study, performed field and laboratory experiments and drafted the manuscript. SBE: performed field survey and laboratory experiment. ANE: performed field survey and laboratory experiment. ALW: performed data analysis and reviewed the manuscript: NKW: performed field and laboratory experiments: BAE: performed field and laboratory experiments. BKR: performed field survey and collection. JED: performed field survey. TK: reviewed the manuscript. BSA: reviewed the manuscript.JDB contributed reagents and materials. TOA: designed and coordinated the study, restructured the manuscript. AA: Coordinated the study, provided reagents, funding and substantial improvement information in manuscript. All authors read and approved the final manuscript.

Funding

Kwi Pilate Nkineh was supported by a DELTAS II Africa grant (DEL-22-014: Awandare). This research was funded in whole by Science for Africa Foundation to the Developing Excellence in Leadership, Training and Science in Africa (DELTAS Africa) programme [DEL-22-014] with support from Wellcome and the UK Foreign, Commonwealth & Development Office and is part of the EDCPT2 programme supported by the European Union. The work was also supported by the Pan African Malaria Genetic Epidemiology Network (PAMGEN)—Human Heredity and Health in Africa (H3Africa), grant number H3AFull/17/008. The funders have no role in designing the study, collecting and analysing the data, deciding to publish, and preparing the manuscript.

Data availability

All Data is provided within the manuscript.

Ethical approval and consent to participate

Consent was obtained from all participants. Ethical clearance and approval was obtained from the Comite National D'ethique de la Research pour la Sante Humaine (CNERSH) (Ref. N^0 2020/09/1188/CE/CNERSH/SP, 2022/12/1315/CE/CNERSH/SP and 2025/03/577/SW/CRERSH/C) while administrative authorization was obtained from the Northwest and Southwest Regional delegation of Public Health, District Medical officers and Chief Medical officers/Chiefs/Quarter Heads in charge of the district and health facilities or community respectively. Only individuals who volunteered to participant by signing a written informed consent after adequate sensitization about the study objectives, risks and possible benefits were enrolled. All experimentations followed the Declaration of Helsinki and relevant ethical guidelines strictly.

Competing interests

The authors declare no competing interests.

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Figures

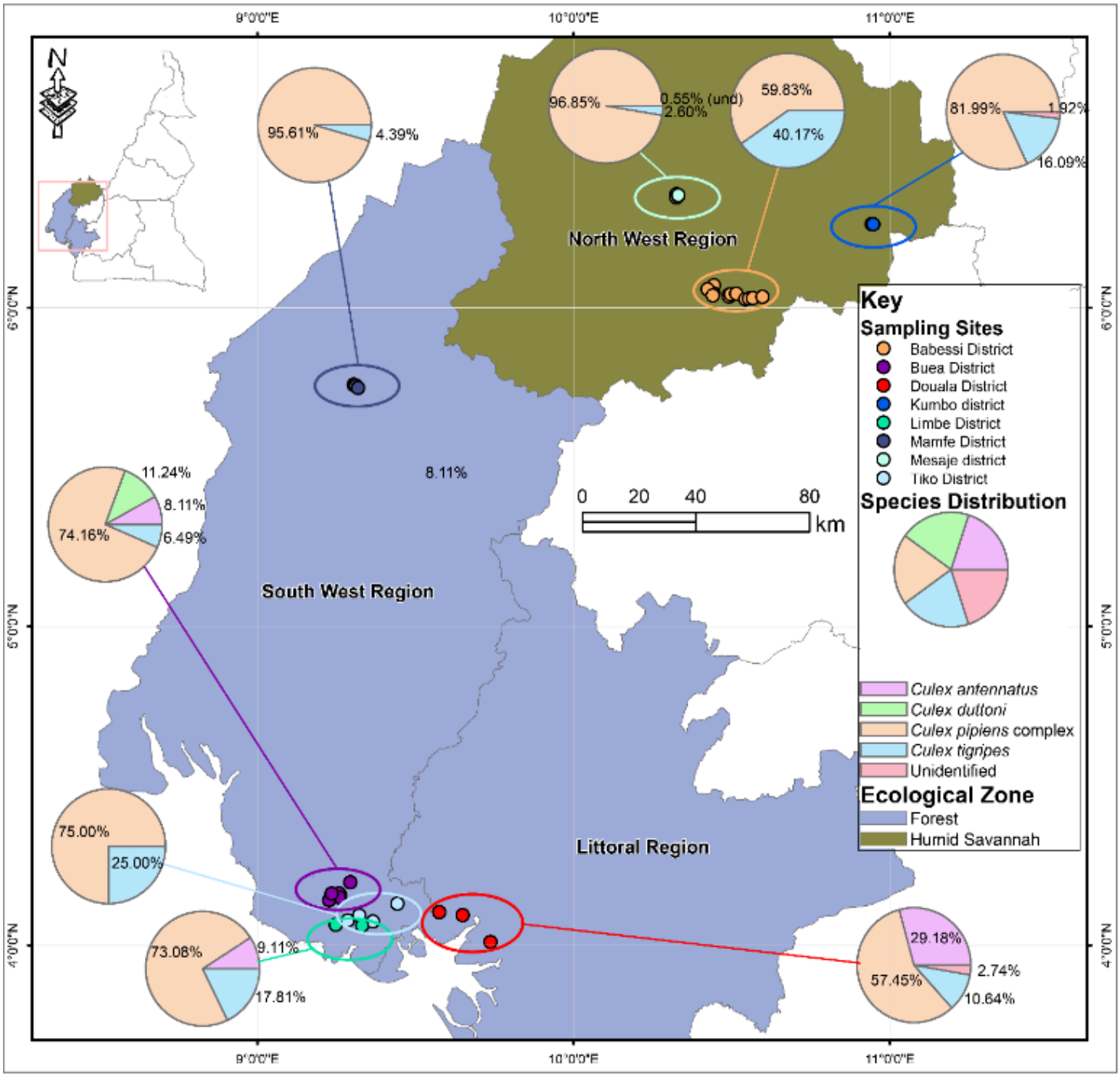


Figure 1

Map showing mosquito sampling sites in Cameroon differentiated ecologically. Collection sites are shown as different coloured points, and proportion of *Culex*spp. per study site indicated as pie charts defined by colours as shown in the legend.

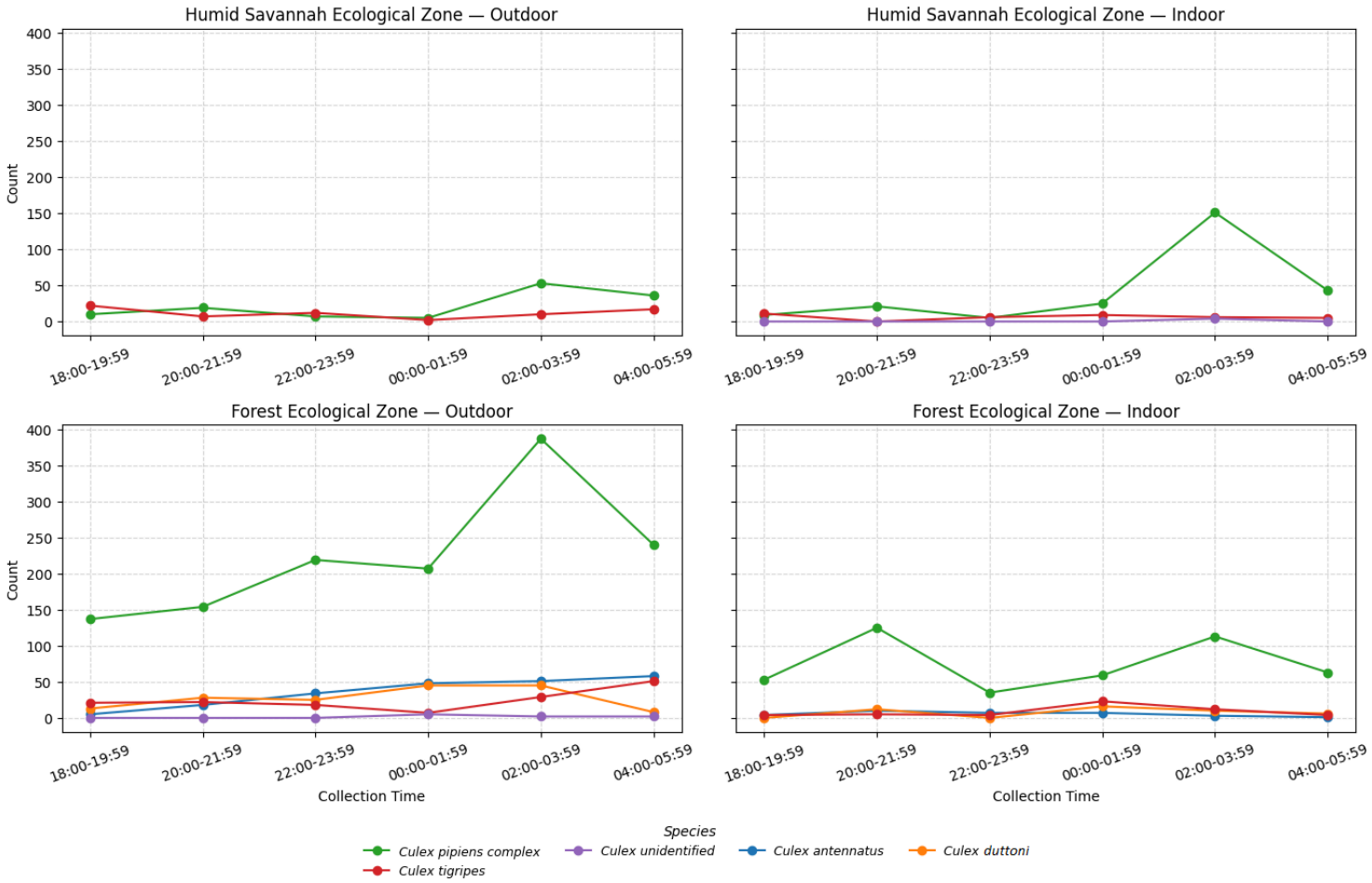


Figure 2

Culex mosquito counts indoor and outdoor at different times in hSEZ and FEZ.

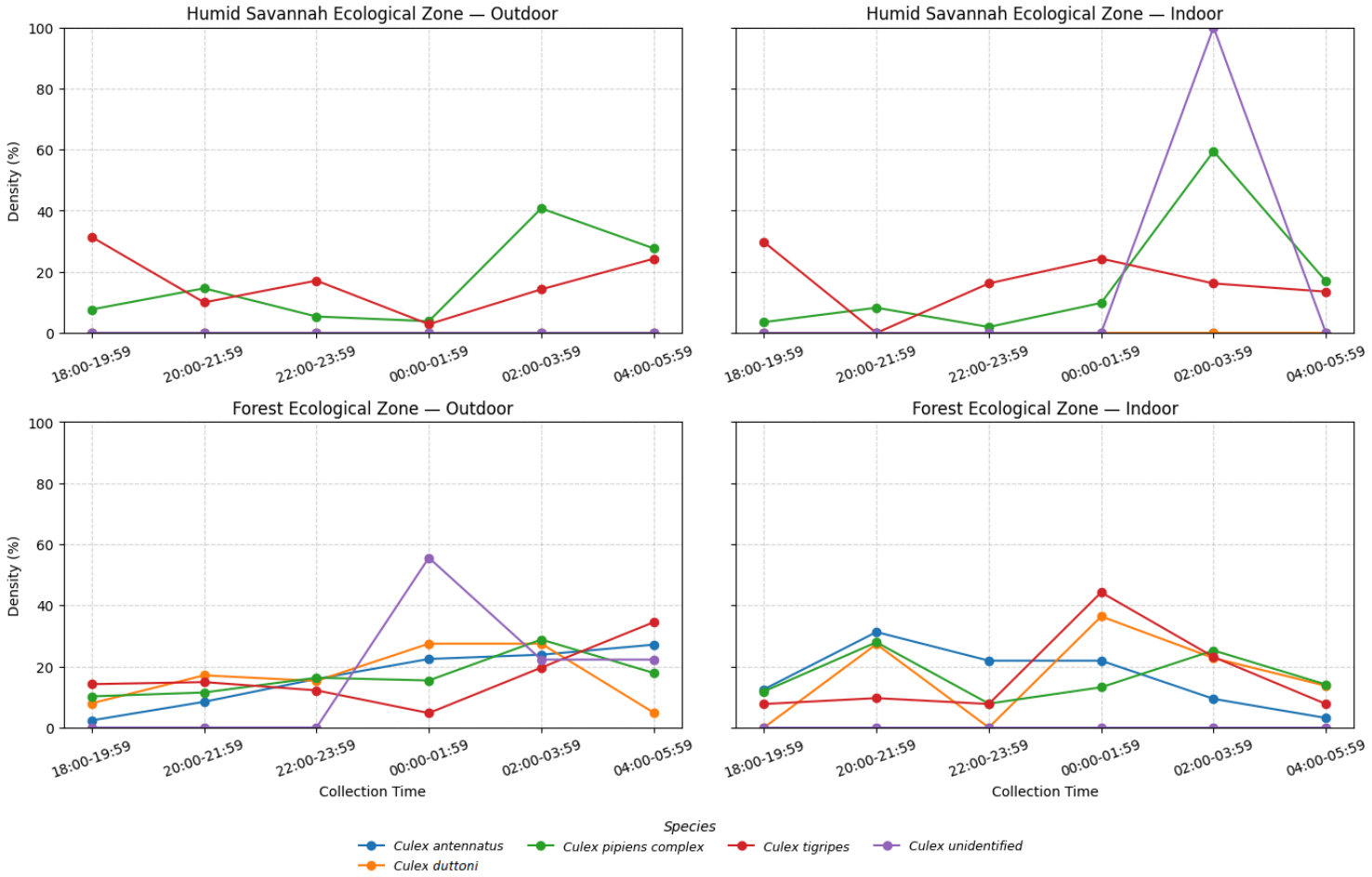


Figure 3

Proportion of *Culex*mosquitoes trapped indoor and outdoor at different times in hSEZ and FEZ.

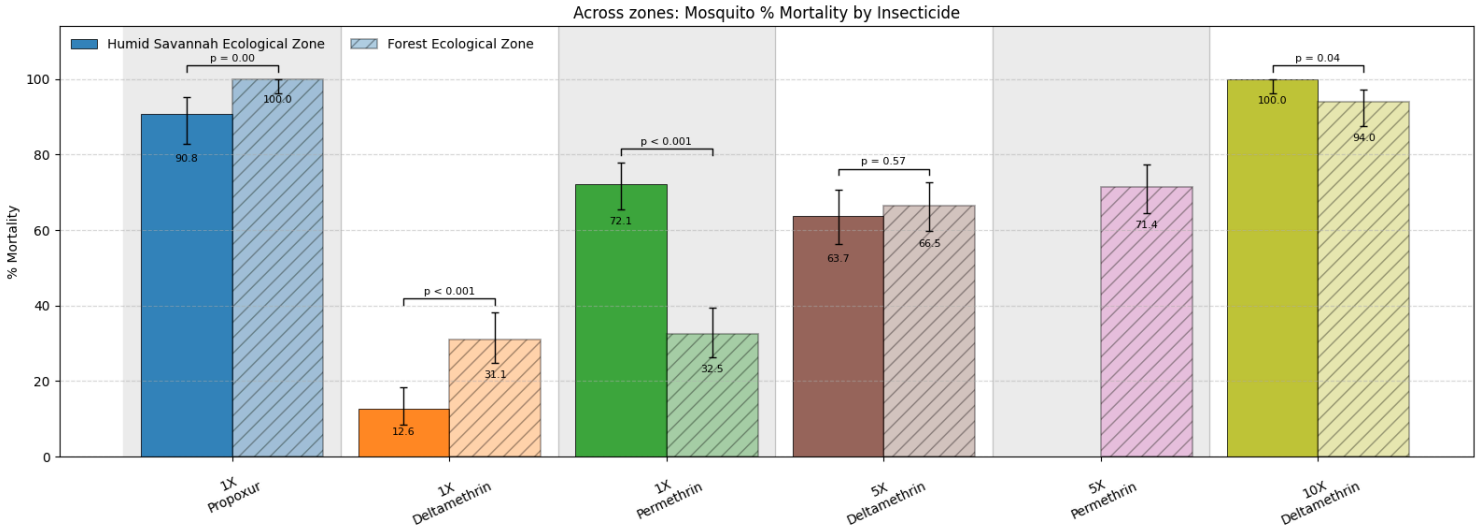


Figure 4

Mortality of *Cx. pipiens* complex populations in the humid Savannah and Forest ecological zones following exposure deltamethrin, permethrin and propoxur.

Supplementary Files

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