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Assessment of the Anophelinae blood seeking bionomic and pyrethroids resistance of local malaria vectors in the forest region of Southern Cameroon

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Abstract
To assess the anophelinae blood seeking bionomic and susceptibility status to pyrethroids of local malaria vectors in the forest region of southern-Cameroon, entomological surveillance, detection of *Plasmodium* infection and susceptibility to permethrin and deltamethrine was done using specific protocol. Of a total of 2,091 mosquitoes collected, 543 (25.97%) belong to anophelinae species. Malaria vectors collected included *An. gambiae*s. L., *An. moucheti, An. marshallii, An. ziemanni, An. paludis, An. coustani* and *An. nili.* Anopheline trapped using WET varied from 1.31 anophelines/trap in Nyabessan to 2.87 anophelines/trap in Olama. The densities of mosquitoes collected using CDC-LT were 3.08 (indoor) and 4.18 (outdoor) mosquitoes/trap/night in Olama whereas they were 13.44 (indoor) and 7.84 (outdoor) mosquitoes/trap/night in Nyabessan. Of the 392 anophelines screened using ELISA, 2 (0.51%) were recorded infected by *Plasmodium falciparum.* Several species including *An. gambiae*sl, *An. moucheti* and *An. nili* were found resistant or highly tolerant to permethrin and/or deltamethrin. CDC-LT and WET are not sensitive tools for monitoring mosquito populations in the forest region. The study also suggested increase tolerance of several local anopheline species to pyrethroids. These information need further consideration while designing vector control interventions in theses settings.

Keywords: Bionomics, anopheles, mosquito sampling, secondary/local vector diversity, insecticide susceptibility, Cameroon

Introduction
In Cameroon, malaria still have a devastating impact on public health and it is responsible for about one-third of outpatient consultations, 40% of morbidity cases and 18.7% of mortality cases in health care units [1]. The main control tools used by the population is LLINs [2]. However, the rapid expansion of insecticide resistance in vector populations jeopardized the effectiveness of this tool [3, 6]. In addition to insecticide resistance the complexity of the vectorial system and changes in the bionomics of vector populations are also considered to limit the effectiveness of vector control tools [7, 8].
In Cameroon, 52 anophelines species have been described and up to sixteen species take part in malaria transmission, particularly in the forest region. However, six species (*Anopheles. gambiae, An. coluzzii, An. arabiensis, An. funestus, An. moucheti* and *An. nili*) are responsible for the majority of malaria transmission accounting for about 95% of total transmission and are therefore considered as dominant or primary vectors species [4]. The remaining of the transmission (5%) is vectored by secondary malaria vectors such as *An. ovengensis, An. paludis, An. ziemanni, An. marshallii, An. rufipes*or *An. Pharoensis* [7, 11]; they have limited distribution range and contribute occasionally or seasonally to malaria transmission. In this region, LLINs is the main measure used by the population to prevent from malaria parasite transmission and over 70% of the population use treated nets regularly. It is possible that because secondary vectors display high exophilic and exophagic behaviour compare to dominant malaria vectors they could be less affected by the scaling up of indoor base interventions [12].
For most of local vector species their bionomics and susceptibility level to insecticides are still not well documented [7, 10, 13].

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In Cameroon most studies on vector bionomic conducted so far have mainly focused on species such as *An. gambiae*s. L. and *An. funestus* which are regarded as the major malaria vectors in the country whereas other species whose implications in malaria transmission (although low) should not be neglected remained largely understudied. The majority of these species are difficult to rear in the laboratory, information on their susceptibility to *Plasmodium*sp and to insecticides are not available [14]. The poor knowledge on local or secondary malaria vectors bionomic, could be detrimental for the successful elimination of malaria in Cameroon.

Human landing catches (HLC) remain the main sampling technique used to assess mosquito bionomics and malaria transmission patterns [9, 10, 13, 15, 17]. Although this technique commonly used across sub-Saharan Africa [18, 20] provides a good estimation of mosquito biting behaviour or of transmission patterns [15, 21, 25], the method is subjected to a certain number of limits. First, it is labour intensive; collectors have to remain alert all night long when collecting mosquitoes; a requirement which is not guaranteed neither surely fulfilled. Secondly, collectors have different attractiveness to mosquito [26, 29] and also different skill in mosquito collection [30]. All this discordances can introduce sampling bias when it comes to the evaluation of the efficacy of control interventions [31, 32]. Thirdly, HLC expose collectors to risk of infection by parasites or arboviruses, and poses ethical issues/problems [33]. Moreover it is still not known whether the use of other sampling techniques such as Centre for Disease Control light trap (CDC-LT) and window exit traps (WET) could provide further information on the bionomic of local mosquito species. Several studies across the continent have reported the high sensitivity of CDC-LT for collecting host seeking mosquitoes particularly when the traps are placed close to a person sleeping under a bed net [34, 37]. Window exit trap has also been used in different settings across the continent and is particularly appropriate for studying resting behaviours, and blood feeding preference of mosquitoes [13, 36, 38, 39].

The present study was conducted to assess whether the use of CDC LTs and WET for sampling mosquitoes could provide further information to better understand the blood seeking bionomic of mosquito population in the forest environment. The study also intended to assess the susceptibility status of local anopheline species to pyrethroid insecticides.

Materials and Methods

Ethics approval and consent to participate

The study was conducted under the ethical clearance N° 2016/01/685/CE/CNERSH/SP delivered by the Cameroon National Ethics (CNE) Committee for Research on Human Health. Authorization to carry out the study in selected houses was obtained from administration and heads of household (HH) through inform consent form.

Study sites

The study was conducted in Olama and Nyabessan (Fig 1) previously describe in Bamou *et al.* [8]. The site of Nyabessan is characterized by the presence of *An. gambiae*sl, *An. nili* and *An. moucheti* as the dominant malaria vectors. In Olama, the main vector is *An. moucheti* followed far behind by *An. gambiae*s. L. [7, 21]. Nyabessan and Olama display high and perennial malaria transmission patterns. They are located within the Congo-Guinean phytogeographic zone, characterized by a typical equatorial climate with two rainy seasons extending from March to June and from September to November. Mean annual rainfall ranges from 1,600 to 1,800 mm. Mosquito nets have been distributed in these localities by the government during mass distribution campaigns in 2011 and 2015/2016 increasing the ownership rate of LLINs to up to 90% in both sites.

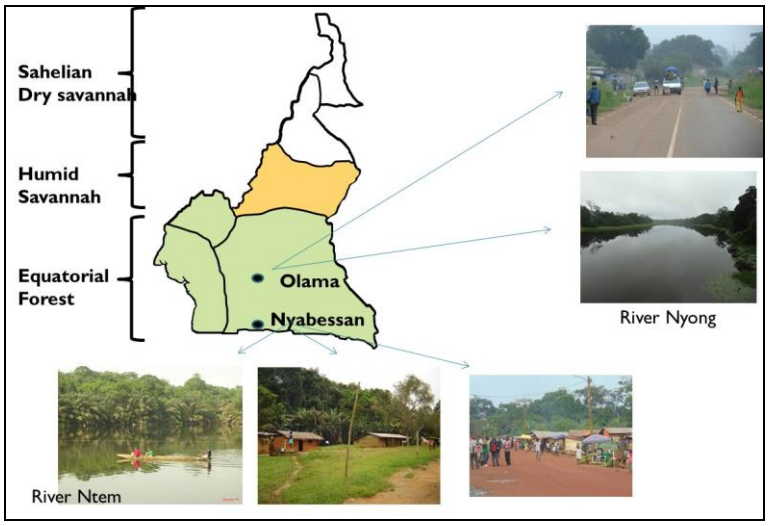

Fig 1: Study sites with their characteristics

House selection

During the study, houses were selected in both villages and permission asked to head of household. Houses were grouped according to trapping methods, ie HLC (10 houses), CDC-LTs (5 houses) and WET (5 houses). In the same group, houses were distant by 50to 100m from one another and the number of house used at each period of the study was dependent on the availability of traps.

Sampling of mosquitoes

CDC light traps and window exit traps (WET) were used to sample mosquito. CDC-LTs collections were conducted both indoor and outdoor in three selected houses per site from 1900 hrs to 0600 hrs during at least 3 consecutive nights per month. Traps were placed indoors near someone sleeping under a net at about 1.5m from the ground and outdoor in the veranda. WETs were used to collect mosquito exiting houses [23, 40, 41]

(Fig 2). They were placed on bedroom's window of five different houses (1 sleeping room was choose per house) during 2 to 3 consecutive days per site per month. 42 and 118 trap-night were used respectively for WET and CDC-LT during the study period per locality. Human landing catches were performed to collect mosquitoes used for susceptibility tests. Details on HLC are described elsewhere [8]. Briefly, mosquitoes were collected in 4 to 10 households per village for three consecutive days once in every two months using a total of 254 man-night. Collected specimen were kept in separate bags, labelled according to the site, night and hour of collection. The bags were kept in a cooler box for preservation while in the field. All volunteers consented to mosquito capture and were given free malaria prophylaxis. The study was conducted from September 2016 to November 2017 with five surveys (September & December 2016, April, August &November 2017) and all sampling techniques were deployed concurrently in separate houses.

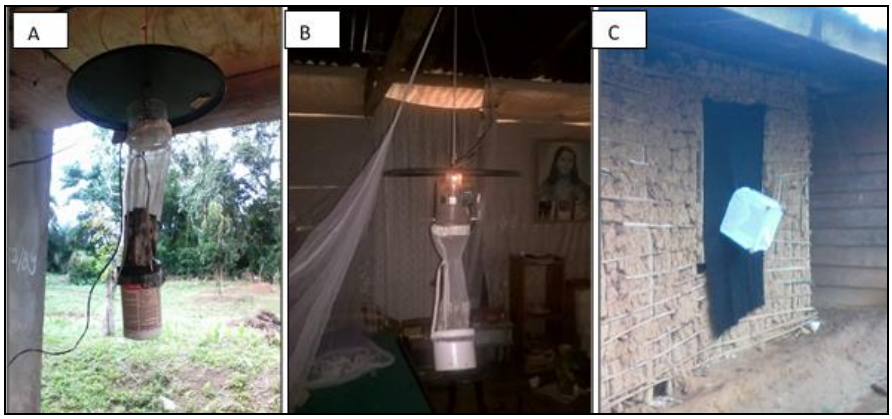

Fig 2: Image showing traps in place, CDC-LT set outdoors in the veranda of the house (A) and indoors near the bed net in sleeping room and WET fixed on window of sleeping room (C).

Mosquito processing

Mosquitoes collected were sorted by genus and identified up to species level based on morphological identification keys [42, 43]. Members of the *An. gambiae* complex collected using CDC-LT, WET and HLC were identified using the molecular diagnostic tools previously described [44]. DNA was extracted from a mosquito leg and/or wing and used for analysis. The blood feeding status of mosquitoes collected using WET and CDC-LT was checked, and mosquitoes were classified as blood fed, unfed or gravid [45]. All anophelines were put in Eppendorf tube containing silicate and stored at -20°C for further analysis. The head and thoraces of anophelines collected using CDC-LTs and WET were screened for the presence of *Plasmodium* Circumsporozoite antigen using Enzyme Linked Immunosorbent Assays (ELISA) technique [46, 49]. The blood meal origin of blood fed mosquitoes collected using window exit trap and CDC-LT were also checked using ELISA technique according to Beier *et al.* [50]. Human, bovine, goat, dog, pig and chicken blood antigens were tested as these are the potential hosts present in the area.

Insecticide susceptibility tests

Because it is difficult in the forest to collect larvae of local anopheline species to carry susceptibility tests and that CDC-LT and WET collect few or death mosquitoes, HLC were practiced to collect large sample of adult anopheline of different species for susceptibility tests. Once collected, females anophelines were identified morphologically to the species level and non-blood fed females of each species were selected and kept in cages. Female anopheline, were grouped according to species and kept in cages with access to 10% glucose solution at room temperature for more than 4h. The females were later exposed to 0.05% deltamethrin and 0.75% permethrin impregnated papers according to WHO protocol [51]. Mosquitoes exposed to untreated papers were used as control. The insecticide-impregnated papers used were supplied by the University of Sains in Malaysia. The quality of the papers was tested by exposing the reference susceptible strain (Kisumu colony) to the papers. Mosquitoes were exposed for 60 minutes with knock-down estimated every 10 minutes. Mortality was recorded 24 hours after exposure. Mortality rate was calculated by expressing the percentage of total number of dead mosquitoes from all replicates for each type of insecticide.

Data analysis

The density of mosquitoes collected was estimated by dividing the total number of mosquitoes collected by the total number of trap-day used. The human blood index was estimated by dividing the number of blood meal taken on human over the total number blood tested. Infection rates were calculated as total number of females *Anopheles* found infected by *Plasmodium falciparum* Circumsporozoite Protein (CSP) antigens over the total number of mosquitoes screened. Bioassay data was scored according to WHO guidelines, with mortality >98% regarded as susceptible, 90 - 98% mortality considered as suspected resistant pending further tests while populations with <90% mortality were considered as resistant. Statistical comparisons and 95% confidence interval estimation were performed using MedCalc V14.8.1 software.

Results

Species composition and abundance

A total of 2,091 mosquitoes belonging to five genera were collected using both CDC LT and WET. The collection consisted of 1548 culicines (74.03%) and 543 Anophelines (25.97%). Anopheline species collected included *An. gambiae*s.l.(n = 107), *An. moucheti (n* = 311),*An. marshalli* (n = 11), *An. nili* (n = 42), *An. paludis* (n = 54) and *An. ziemanni* (n = 12). Culicines species recorded were respectively *Culex*sp (n = 1223), *Mansonia*sp (n = 321), *Aedes*sp (n = 1) and *Coquilletidia*sp (n = 3) (Table 1).

Table 1: Mosquito fauna composition and relative abundance in Olama and Nyabessan

Species	Olama	Nyabessan	Total	%
An. Gambiae sl	4	103	107	5,12
An. Moucheti	199	112	311	14,87
An Marshallii	11	0	11	0,53
An. paludis	6	48	54	2,58
An. ziemanni	12	0	12	0,57
An nili	0	48	48	2,30
Total anophelines	232	311	543	25,97
Culex sp	265	958	1223	58,49
Mansonia sp	151	76	227	10,86
Aedessp	0	1	1	0,05
Coquilletidia sp	1	2	3	0,14
Total culicines	417	1131	1548	74,03
Over all	649	1442	2091	100,00

Of the six anopheline species collected, *An. moucheti* was the most abundant representing more than half of the total anophelines sampled (311/543). The composition and abundance of catches varied with the study site. In Olama, five species including *An. moucheti*, *An. gambiae* sl, *An. marshallii*, *An. paludis* and *An. ziemanni* were collected while in Nyabessan, only four species including *An. moucheti*, *An. gambiae*sl, *An. paludis* and *An. nili* were found. *An. gambiae*sl and *An. moucheti* were the most abundant species in Nyabessan whereas, *An. moucheti*was the predominant species in Olama.

Within culicines, *Mansonia* sp was abundant in Olama while *Culex*sp was predominant in Nyabessan. A subsample of culicines was identified using morphological taxonomic key of Jupp (Jupp, 1996). Out of a total of 191 *Culex*sp processed, five *Culex* species were identified: *Culex perfuscus* (68.72%; n=145), *Cx. quinquefasciatus* (5.68%; n=12), *Cx. antennatus* (1.89%; n=4), *Cx. poicilipes* (8.53%; n=18), *Cx. duttoni* (8.53%; n=18), and *Cx. pipiens* (6.68%; n=14). *Mansoniauniformis* (63.43%; n=144) and *Ma. africana* (36.56%; n=83) were found in both study sites. One *Aedesal bopictus* was recorded in Nyabessan.

Molecular identification of sibling species

A total of 428 specimens (420 in Nyabessan and 8 in Olama) of the An. gambiae complex were analysed to detect the presence of members of the complex using SINE PCR. Both *An. gambiae*and *An. coluzzii*were found. *An. gambiae* was predominant in the two sites and represented 75% of the sample in Olama and 55% in Nyabessan (Fig 3).

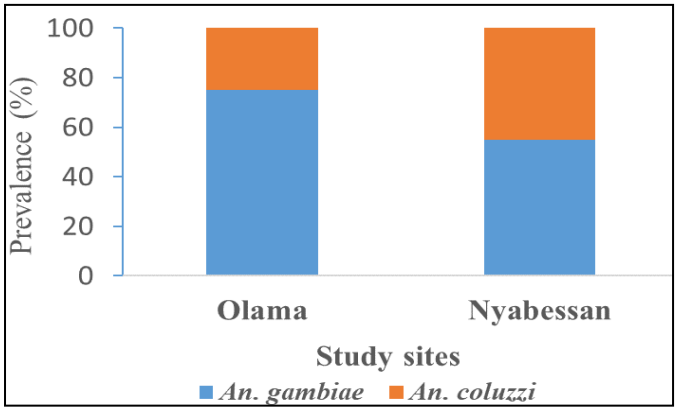

Fig 3: composition of the *An. gambiae* complex in Olama and Nyabessan

CDC light traps collections

A total of 1,784 mosquitoes corresponding to 15.12 mosquitoes/night were collected using 118 CDC-LTs trap-nights in each locality (59 indoor and 59 outdoor). The average density of anophelines collected with this tool vary from 1.15 mosquito per trap-night (*mosq*/trap) indoor to 0.83 mosq/trap-night outdoor in Olama whereas in Nyabessan the density was 3.39 mosq/trap-night indoor and 0.95 mosq/trap-night outdoor. *Culex* sp was the most abundant species collected using CDC-LT in both villages followed by *An. moucheti* and *Mansonia* sp.

A total of 373 anophelines including six species: *An. gambiaesl*, *An. moucheti*, *An. marshallii*, *An. paludis*, *An. ziemanni*and *An. nili* were collected with CDC-LT. Of the 373 anophelines collected, 19 were blood fed. *Anopheles* densities were significantly higher indoors (n=268; 71.85%) compared to outdoors (n=105; 28.15%) in both sites ($P<0.05$). However, species such as *An. paludis* and *An. ziemanni* were mainly collected outdoors in both sites (Table 2).

Table 2: Distribution of mosquitoes collected using CDC light traps in Olama and Nyabessan

		Indoor		Outdoor	
Sites	Species	N	n/trap	N	n/trap
Olama	*An. gambiae*s.l.	0	0.00	1	0.02
	An. moucheti	63	1.06	24	0.41
	An. marshallii	5	0.08	6	0.10
	An. paludis	0	0.00	6	0.10
	An. ziemanni	0	0.00	12	0.20
	Culex sp	80	1.36	155	2.63
	Mansoniasp	32	0.54	43	0.73
	*Coquelletidia*sp	1	0.02	0	0
	Total	181	3.07	247	4.19
Nyabessan	*An. gambiae*s.l.	67	1.14	10	0.17
	An. moucheti	80	1.36	7	0.10
	An. nili	36	0.62	9	0.15
	An. paludis	17	0.28	30	0.51
	Culex sp	585	9.92	352	5.97
	*Mansonia*sp	11	0.19	55	0.93
	*Coqueletidia*sp	2	0.03	0	00
	Total	793	13.44	463	7.85
Overall		979	8.30	710	6.02

N: number of collected mosquito; n/trap: number of mosquito per trap

Window exit traps collections (WET)

To assess the resting behaviour of indoor feeding mosquitoes, 42 WET traps-night were used to collect mosquitoes in each locality. A total of 307 mosquitoes were collected using this method; 170 anophelines and 137 culicines. Anophelines collected included *An. gambiae*sl, *An. moucheti, An. paludis* and *An. nili. An. moucheti* (n=112; 97.4%) was the most abundant species collected using WET in Olama whereas in Nyabessan, the most abundant in catches were both *An. gambiae*sl (45.45%; n =. 25) and *An. moucheti* (47.27%; n =. 26). Within culicines, *Mansonia*sp (71.70%; n=. 76) was the most common in Olama, whereas*Culex*sp (87.09%; n= 27) was the most abundant in Nyabessan. The majority of mosquitoes collected using WET were unfed (n=156; 91.17%) (Table 3).

Table 3: Composition and blood feeding status of mosquitoes collected using Window Exit Traps in Olama and Nyabessan

Sites	Species	N Collected	N/trap	Unfed	Blood fed
Olama	*An. gambiae*sl	3	0.07	3	0
	An. moucheti	112	2.67	105	7
	Total Anopheles	115	2.87	108	7
	Culex sp	30	0.71	26	4
	*Mansonia*sp	76	1.81	74	2
	Total Culicines	106	2.52	100	6
Nyabessan	*An. gambiae*s. L.	26	0.62	25	1
	An. moucheti	25	0.59	20	5
	An. paludis	1	0.02	1	0
	An. nili	3	0.07	2	1
	Total Anopheles	55	1.31	48	7
	Culex sp	27	0.64	23	4
	*Mansonia*sp	4	0.09	4	0
	Total Culicines	31	0.74	27	4
	Overall	307	3.65	283	24

N: number; n/trap: number per trap

Identification of blood meal source

A total of 33 *Anopheles* mosquito fully blood fed from both CDC-LT and WET were analysed by Elisa technique to determine blood meal source. These included *An. paludis* (n=2), *An. nili* (n=3), *An.gambiae*sl (n=8) and *An. moucheti* (n=20). All mosquitoes tested were found to have fed on humans.

Plasmodium Infection in *Anopheles* mosquitoes

A total of 392 anophelines collected using both CDC LT (n=232) and WET (n=160) were screened for the presence of *Plasmodium* infections. One *An. gambiae*s. L. and *one An. moucheti* were found infected in Nyabessan (Table 4) representing 0.51% of the total mosquito screened. From CDC-LT collection 4.34% (1/24) of *An. gambiae*s while 1.72% (1/57) of *An. moucheti* were infected in Nyabessan. No mosquito collected using WET was recorded infected.

Table 4: Circumsporozoite infection rate of anopheline species collected using Window Exit Traps and CDC-LT in Olama and Nyabessan

	CDC LT			WET		
	Tested	Infected	% (95% CI)	Tested	Infected	% (95% CI)
Olama						
*An. gambiae*s.l.	1	0	0 (0-369)	2	0	0 (0-184.4)
An. moucheti	66	0	0 (0-5.6)	98	0	0 (0-3.8)
An. paludis	3	0	0 (0-122.9)	-	-	-
An. ziemannii	15	0	0 (0-24.5)	-	-	-
Total	85	0	0 (0-4.35)	100	0	0 (0-3.9)
Nyabessan						
*An. gambiae*s.l.	23	1	4.34 (0.11-24.2)	27	0	0 (0-13.7)
An. moucheti	57	1	1.72 (0.04-9.8)	26	0	0 (0-14.2)
An. paludis	25	0	0 (0-14.8)	2	0	0 (0-184.4)
An. nili	42	0	0 (0-8.8)	5	0	0 (0-73.8)
Total	147	2	1.36 (0-2.5)	60	0	0 (0-6.15)
Overall	232	2	0.86 (0.1-3.1)	160	0	0 (0-2.3)

Insecticide susceptibility

Adult mosquitoes collected after human landing catches were tested to assess their susceptibility to both permethrin and deltamethrin. A total of 3561 mosquitoes belonging to seven anopheline species were exposed. This include *An. gambiae*s.l., *An. nili, An. moucheti, An. paludis, An. ziemanni, An. coustani*and *An. marshallii.* In Olama, all species tested were recorded susceptible to permethrin 0.75%. *An. moucheti* and *An.marshallii* showed increase tolerance to deltamethrin 0.05%. In Nyabessan, almost all species were recorded displaying reduced susceptibility (mortality rate varying 80-94%) to deltamethrin 0.05% (Table 5).

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Table 5: Mortality rate of Anopheles species exposed to deltamethrin (0.05%) and permethrin (0.75%) in Olama and Nyabessan

		Permethrin 0.75%				Deltamethrin 0.05%			
Villages	**Species**	**N**	**n**	**%**	**Status**	**N**	**n**	**%**	**Status**
Olama	*An. moucheti*	458	449	98%	S	422	405	96%	SR
	An. paludis	73	73	100%	S	39	39	100%	S
	An. marshallii	126	124	98%	S	77	68	88%	R
	An. ziemanni	53	53	100%	S	-	-	-	-
	An. coustani	41	41	100%	S	-	-	-	-
Nyabessan	*An. moucheti*	380	304	80%	R	246	206	84%	R
	An. paludis	225	212	94%	SR	37	36	97%	SR
	An. nili	59	51	86%	R	28	28	100%	S
	*An. gambiae*s. L.	438	54	12%	R	828	351	42%	R

N: number of mosquitos used for bioassay; n: number of death mosquito after 24h post exposure; %: mortality rate; R: resistance; SR: suspected or possible resistance; S: susceptible; Status: resistance status according to WHO

Discussion

The present study's objective was to assess whether the use of CDC-LT and WET could provide additional information on the bionomics of blood seeking local malaria vectors in the south Cameroon equatorial forest region and also assess the susceptibility level to pyrethroids of different anopheline species. A high species diversity was found and was in line with previous works [8]. *Anopheles moucheti* was the most abundant species in Olama while *An. gambiae*s. l. was commonly found in Nyabessan. Absence of stagnant water bodies considered as *An. gambiae* sl in addition of no deforestation could explain the low density of *An. gambiae* sl while the presence of natural breeding site of An. moucheti, the river Nyong, explained the presence in density of this species. Breeding sites although up to six different anopheline species were recorded, their densities were far lower compared to those recorded using HLC in the same settings previously [8]. The following support the low efficiency of CDC-LT and WET methods for sampling mosquitoes in the forest environment. CDC-LTs attract mosquitoes only through visual stimuli and this could explain the low efficiency of this sampling technique compared to human landing collections where mosquitoes are attracted by both visual and chemical stimuli [52, 54]. High anopheline catches were recorded indoor in both sites with CDC-LTs. The following findings which suggest high endophilic behaviour of anophelines, are in contrast with previous works [8] and could be explained by the fact that, CDC light traps perform less outdoors because of the influence of winds or because they also attract a high number of insects which by flying around the trap could divert mosquitoes from being trapped. Low mosquito densities were recorded using WET and could come from the fact that houses in these villages are poorly constructed with many entry/exit points for mosquitoes such as eaves or holes in walls reducing the efficacy of WET. Yet WET provided interesting findings indicating that many mosquitoes getting in rooms do not usually feed and get outdoor to have a blood meal. This information likely suggests high protection provided by bed net. In Nyabessan and Olama, over 90% of the population in the two villages were reported owning at least a net and >70% indicated using nets regularly [8]. The fact that many mosquitoes enter houses stresses the need to incorporate in the control arsenal additional tools such as IRS in order to reduce the density of indoor seeking mosquitoes as previously recommended [55]. Few mosquito exiting houses were blood fed. All blood fed females collected with WET were all found with human blood and was consistent with the high anthropophilic behaviour of forest mosquitoes [11, 21]. Samplings conducted in dry savanna settings of northern Cameroon with WET recorded a high number of anophelines which fed on different hosts [13]. Although WET efficacy could be affected by variation in house design, and behaviour of both mosquitoes and humans [56], this technique simple to implement could be used as a routine monitoring tool for malaria vector surveillance in poor resource communities yet the design still need to be improved to increase its efficiency.

Two infected mosquitoes were recorded using CDC LT and none with WET. Previous studies in the same settings using HLC recorded >5 time more infected mosquitoes by *Plasmodium* with up to six species detected infected [8]. The following highlights limits of alternative sampling techniques sensitive in measuring human exposure to malaria transmission in the forest region. In Ifakara (Tanzania), malaria transmission was found to be undetectable using both CDC LTs and Suna Traps compared to HLC [57]. The sensitivity of alternative tools in malaria epidemiological studies might vary according to species, sites and seasons [35, 57], the following deserve further investigations.

Several anopheline species including *An. gambiae*sl, *An. moucheti, An. nili, An. marshallii* were recorded fully resistant or more tolerant to permethrin and/or deltamethrin. The following findings were in line with insecticide resistance expansion across Cameroon [58, 60]. This is the first time that species such as *An. moucheti*, *An. marshallii* and *An. nili* are reported resistant to pyrethroids. Although adult mosquitoes deriving from HLC were screened this seems not to have biased greatly our evaluation. It is possible that the resistance level in local malaria vectors could be greater than reported since mosquitoes of different ages were screened. It is still not known which mechanisms are responsible for increase tolerance in these mosquito species. The emergence of insecticide resistance in local malaria vectors could come from the intensification of LLINs use across the country. In addition to treated nets the population used spray or burn natural repellent to protect themselves from mosquito bites [59]. Due to the spreading out of insecticide resistance to other anopheline species thorough surveillance need to be implemented to monitor anopheline species bionomics and performance of control interventions. It is rather not known the influence of the rapid emergence of insecticide resistance on local malaria vectors fitness and competence to transmit malaria parasite; the following still deserve further investigations.

Conclusion

The study provided updated information on the bionomics of local malaria vectors in the forest region of Cameroon and supports the fact that additional sampling techniques need to be evaluated for vector surveillance activities. Moreover, the

study also suggested that in addition to *An. gambiaes.* L., more species are now becoming resistant to pyrethroids. This information has to be taken into consideration when developing plan for insecticide resistance management and malaria elimination in Cameroon.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Conceived and designed the study protocol: CAN, participated in field and laboratory activities: BR, KE, DDL, AAP, TT, CAN; critically revised the manuscript: KE, AAP, TT, NF; Interpreted, analysed data and wrote the paper: BR, CAN with contribution of other authors. All the authors read and approved the final version.

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