

Vector Control, Pest Management, Resistance, Repellents

Coverage, Usage, Physical Integrity, and Bio-efficacy of Olyset Nets in the Plateau Region, South Benin Following the 2011 Nationwide Distribution

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Abstract

The present study investigated in 8 villages of the Plateau region the coverage, usage, physical integrity, and bio-efficacy of the Olyset nets distributed nationwide by the Benin's National Malaria Control Programme in July 2011. The questionnaire administered as well as the observations made in the households allowed estimating the coverage and usage rates of the 2011 Olyset nets. While their physical integrity was assessed through standard WHO methodology, their bio-efficacy was evaluated through gas chromatography, and WHO cone testing performed with the Kisumu susceptible strain. Mosquito collections through human landing catches (HLCs) were also performed in torn nets to assess if a loss of protection of sleepers occurred as the nets fabric integrity got more damaged. Nine months postdistribution, the coverage and usage rates of the 2011 Olyset nets were 67.4% (95% CI: 65.8–68.9) and 73.3% (95% CI: 70.7–75.8) respectively. About 28% of the 2011 Olyset nets were torn. A drastic drop of the insecticide quantity on the fibers of the nets [from 7.08 µg (95% CI: 5.74–8.42) to 0.2 µg (95% CI: 0.01–0.38)] as well as mortality rates <80% were observed with most nets evaluated. Moreover, the biting rates of *An. gambiae* s.l. (Diptera: Culicidae) inside torn nets increased in line with their fabric integrity loss. These data support the conclusion that future deployment of nets in the field must be strengthened by community sensitization on their correct use in order to postpone as much as possible appearance of holes and loss of insecticidal activity and encourage repairing of torn nets.

Key words: Olyset net, coverage, usage, physical integrity, bio-efficacy

For some years, long lasting insecticidal nets (LLINs) and Indoor Residual Spraying (IRS) are the core tools used for preventing and controlling malaria transmission. In Africa, LLINs were the highest contributor (68% of cases averted) to the substantial reduction of malaria incidence between 2000 and 2015 (Bhatt et al. 2015). Similarly, reductions in malaria cases after mass distribution of LLINs conducted in Benin were documented (Damien et al. 2016, Bradley et al. 2017). Indeed, LLINs have both a physical barrier that helps reduce

the man–vector contact, and a chemical barrier (the incorporated insecticide) which repels and/or kills the mosquitoes (Curtis et al. 2003, Antonio-Nkondjio et al. 2013). A positive impact of LLINs at the community level is achieved when both high-coverage and a strong use of nets occurs (Thwing et al. 2011). For that reason, Policies at National Malaria Control Programmes (NMCPs) increasingly promote large scale distribution and universal access of the whole population to LLINs, as well as sensitization for a good use.

In July 2011, Benin's NMCP conducted with the support of the U.S. President's Malaria Initiative (PMI) a nationwide distribution campaign of a permethrin-incorporated net (Olyset net).

The present study reports the evaluation of coverage and usage rates, fabric integrity, and bio-efficacy of the Olyset nets distributed in 8 villages of the Plateau region, Southeastern Benin, at 9 months postdistribution. The study also investigated whether the efficacy of LLINs decreases as its fabric integrity is getting damaged.

Methods

Study Area

The study was conducted in 08 randomly selected villages of the Plateau region, Southeastern Benin (Fig. 1). The surveyed villages include:

- Itakpako (06° 49'44.4457" N, 02° 36'9.8110" E), Itassoumba (06° 48'23.9117" N, 02° 37'45.6711" E), and Ko-koumolou (06° 43'24.3877" N, 02° 40'21.2698" E) located in the Ifangni district,
- Djohoukollé (06° 58'33.2808" N, 02° 39'5.2337" E) and Igbola (06° 58'13.8561" N, 02° 39'4.6773" E) in the Sakété district,
- Okoofi 2 (07° 1'58.2070" N, 02° 38'48.2341" E) in the Pobè district as well as,
- Idéna 2 (07° 15'45.1192" N, 02° 32'27.5663" E) and Mowodani (07° 16'33.2686" N, 02° 33'58.7797" E) in the Kétou district.

The region has a guinean climate with two rainy seasons (March–July and September–November). The rest of the year (August and, December–February) corresponds to the dry season. The average annual rain fall was 800–1,200 mm and 1,000–1,400 mm respectively

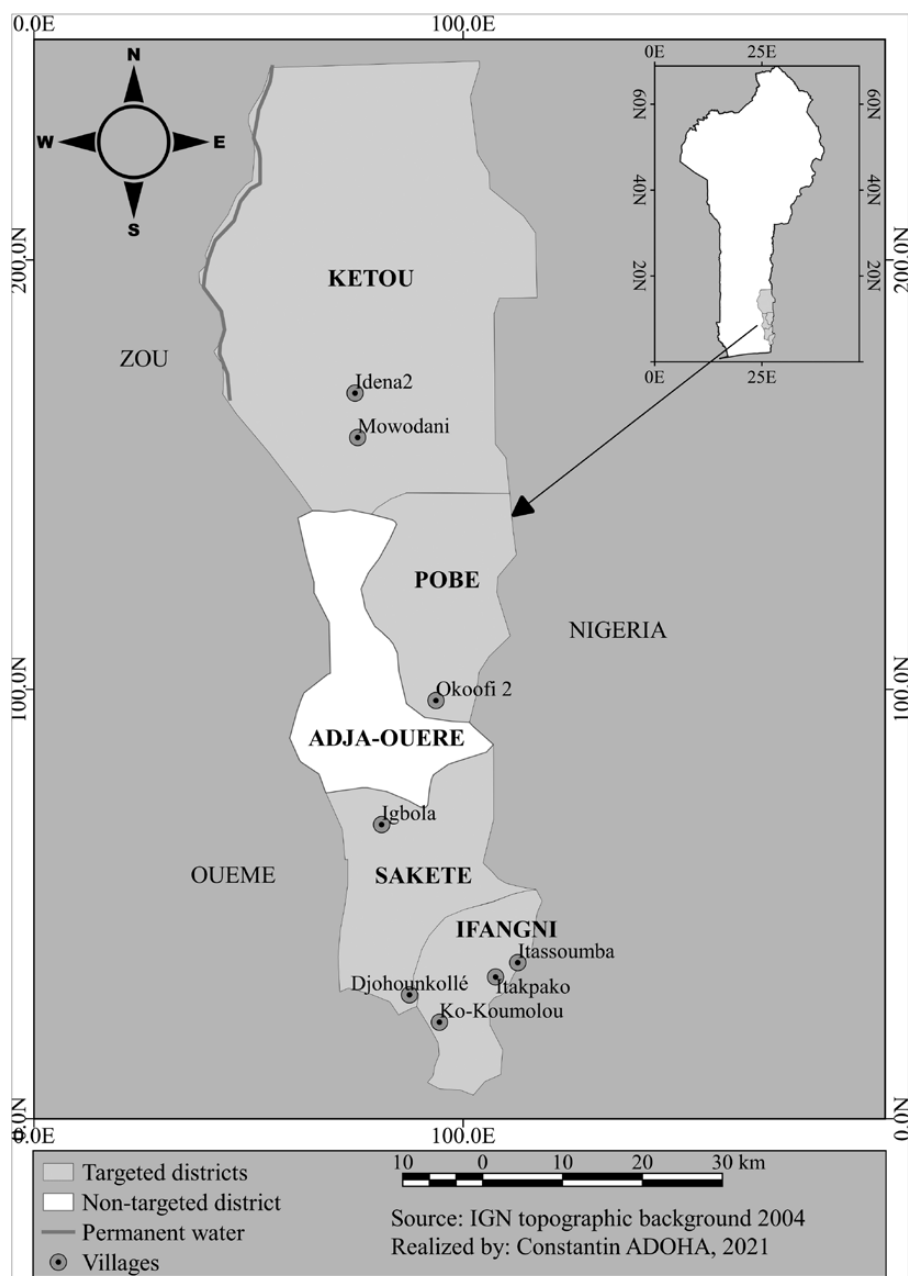


Fig. 1. Map showing the study area.

in its western and eastern part. The main activity in the Plateau region is agriculture.

Study Design

This cross-sectional study conducted at 9 months post LLINs distribution (April 2012) investigated the coverage, usage, physical integrity, and bio-efficacy of Olyset nets in 8 villages of Plateau Region, Southeastern Benin.

All these data were collected using a questionnaire digitalized on tablets (Samsung Galaxy Tab 10.1) used as data collection terminals. These tablets are directly connected to the internet via a SIM card and the data collected was then sent to a cloud server. This technique enabled ensuring good data transmission, limit errors, and avoid wasting time.

Selection of Households

Not being able to know the number of mosquito nets in the households, the size of the sample to be surveyed was expressed in terms of the number of households. Based on the population data provided by the community health centers, a simple survey was performed on 100 households selected at random with a 5% margin in each study village, which makes overall 800 households surveyed for the whole study area.

Household Survey

This survey was conducted with the participation of community health workers via a questionnaire administered to the heads of households or an adult living in the surveyed households. The information collected included the types of nets found, their number, the number of people sleeping under them, and the position (place for hanging or storing) of the 2011 Olyset nets. Information on physical integrity of the Olyset nets distributed in July 2011 by the NMCP was also recorded.

Assessment of Fabric Integrity of the Olyset Nets

Following the WHO criteria (WHO 2011), the sizes of holes defined were as follows:

- S1 (0.5–2.0 cm): holes that do not allow an adult's hand thumb to pass through (S1: Size-1 hole).
- S2 (2–10 cm): holes that can allow an adult's hand thumb to pass through but not the fist (S2: Size-2 hole).
- S3 (10 cm < S3 ≤ 25 cm): holes whose size is larger than the fist of an adult's hand but smaller than a head (S3: Size-3 hole).

- S4 (>25 cm): holes that are larger than an adult's head.

The fabric integrity of Olyset nets was assessed by identifying which ones were torn and counting the number of nets having each hole size. The number of each hole size on these Olyset nets in use in the field was not counted.

Evaluation of the Olyset Nets Bio-efficacy

Gas Chromatography (GC)

In total, 20 nets randomly selected from each study village, making a total of 160 nets for the whole study area as well as 50 new and unused nets, were assessed for bio-efficacy through gas chromatography. For each Olyset net, insecticide residue samples were taken from position B (Fig. 2).

To collect the insecticide residue sample, a cap fixed to a plastic tube was covered with a 25 mm diameter lens paper manufactured by Thermo Fisher Scientific Inc., and then position B of the net was attached to an embroidery hoop having a diameter of 4 inches (Azondekon et al. 2014). The insecticide residue present on the surface of the net was then collected inside the hoop through 10 circular movements of the lens paper covering the cap (Fig. 3). The same technique was used to sample the reverse side of the net. The 2 portions of lens paper thus obtained were then cut up and stored inside a 1 ml syringe.

To extract the permethrin, 100 µl of acetone (solvent) containing 0.05 mg/ml of triphenyl phosphate (internal standard) were put on the content of the stoppered syringe. After 5 min of soaking of the sample, another 100 µl of acetone was added again. Finally, the syringe was squeezed correctly into an Eppendorf tube until the last drop (Azondekon et al. 2014).

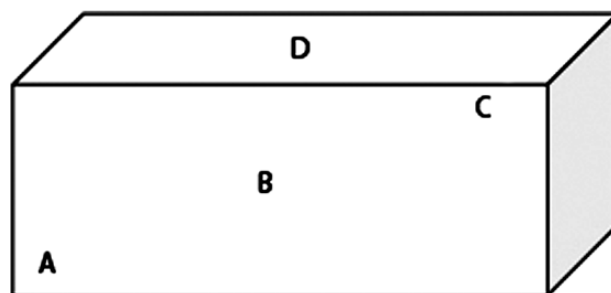


Fig. 2. Insecticide residue sampling position on the mosquito net (Source: Azondekon et al. 2014).

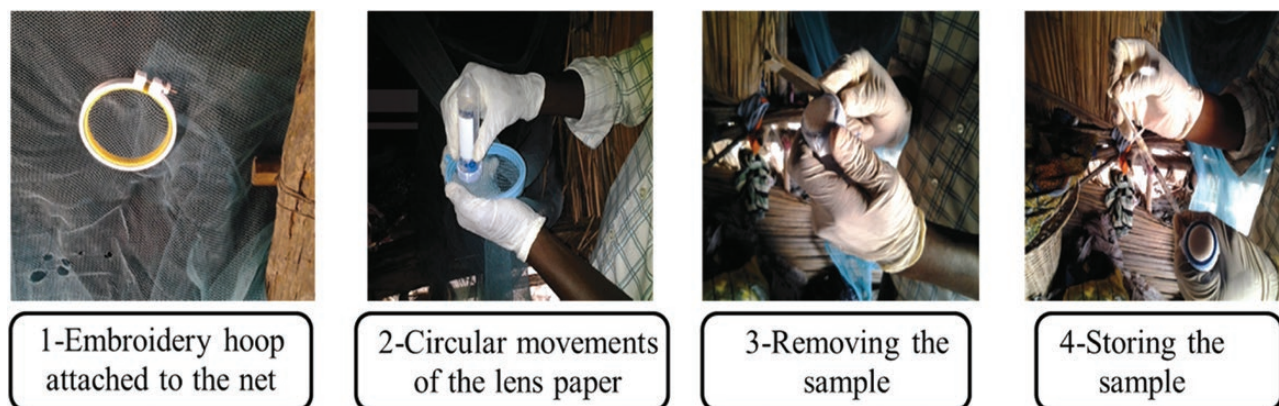


Fig. 3. Different steps for collecting the insecticide (Source: Azondekon et al. 2014).

The quantity of permethrin available on each mosquito net was then assessed using gas chromatography (GC) technology. Hydrogen was used as the carrier gas. A comparison of the obtained quantity of insecticide, was made with the efficacy threshold of 2.73 µg determined by Azondékon et al. (2014). This threshold value corresponds to the quantity of permethrin necessary to induce 80% mortality rate in the Kisumu susceptible strain. Fifty new and unused Olyset mosquito nets (control) were also tested to compare their quantity of insecticide to that of the nets in use at the different study villages.

WHO Cone Testing

To confirm gas chromatography results, fourteen other Olyset nets selected at random in seven study villages, were withdrawn and used for the WHO cone testing (WHO 2011). They were replaced in the households by fourteen other new Olyset nets.

For one mosquito net, five female specimens of the Kisumu strain (laboratory susceptible colony) aged 3–5 days were put in each of the 8 cones used for testing. These mosquitoes were exposed to the mosquito nets for 3 min and then gently removed using a mouth aspirator in the relevant cups. Kisumu strain mosquitoes were exposed to untreated (negative controls) and new Olyset (positive controls) net pieces.

All tested mosquitoes were subsequently fed with a 10% sweetened juice. At the end of the bioassays, the mosquitoes were carefully kept for observation in a room at a temperature of 25°C ± 2°C and a humidity of 80% (±4%). The mortality rates were recorded 24 h postexposure.

Ability of *An. gambiae* s.l. Carrying L1014F *kdr* Mutation to Pass Through Torn Olyset Nets

This component was conducted from May to June 2012 in the village of Itassoumba where the density of *An. gambiae* s.l. was very high, with a mean L1014F *kdr* frequency of 85%, (Sovi et al. 2013).

During this evaluation, 10 mosquito nets (4 new Olyset nets, 3 field-collected Olyset nets, and 3 new untreated nets) with or without holes, were used for mosquito collection. The approximate value of the area of holes on the mosquito nets used, was estimated using the formula of the proportionate holes index (pHI):

$$pHI = 1 \times \#S1 + 23 \times \#S2 + 196 \times \#S3 + 576 \times \#S4$$

(#S = number of a given size holes).

The torn mosquito nets that were trialed, belonged to the three condition categories defined at the time (Roll Back Malaria: Measurement of Net Durability in the Field: Current Recommended Methodology, presented in Lyon, 2012) as follows:

- pHI < 64: Good condition
- 64 ≤ pHI < 768: Still usable
- pHI > 768: To be replaced

Based on these three mosquito net condition categories, 3 Olyset nets with one per condition category were collected in the field. The first consisted of 2 size-1 and 1 size-2 holes (pHI = 25). The second consisted of 3 size-2 and 2 size-3 holes (pHI = 461) while, the third had 3 size-1, 8 size-2, and 4 size-3 (pHI = 971). Holes with size, position, and number similar to those observed on the 3 field-collected Olyset nets, were cut in the laboratory on 6 other trialed nets (3 new Olyset nets and, 3 untreated nets) so as to obtain the 3 pHIs (pHI = 25, pHI = 461, and pHI = 971) described above. The fourth new Olyset net was kept intact, with no hole. All new Olyset nets were aired before being used for the mosquito collections.

Thereafter, a man was positioned inside each of the 10 mosquito nets from 09 p.m. to 5 a.m., to collect the mosquitoes that entered it using a flashlight and sucking tubes. The mosquito nets were placed in 10 randomly selected rooms during 10 successive nights of collection. Positioning mosquito collectors in the nets was also done at random. In each room, cautions were taken so that there was only one collector under each net. Moreover, the collector did not go out of the net until the end of the collection. These measures avoided the risk of mosquitoes entering and leaving the net. In addition, they minimized the likelihood that the presence of another person in the room influences the frequency of vector bites on the collectors. The collected mosquitoes were identified the next morning using the identification key of Gillies and de Meillon (1968). The specimens of *An. gambiae* s.l. were subsequently stored at -20°C in 1.5 ml eppendorf tubes containing silica gel for further molecular analysis.

This helped evaluate the ease or the difficulty of *An. gambiae* s.l., carrying the L1014F *kdr* resistance mutation, to pass through the holes.

L1014F *kdr* Mutation Frequencies in *An. gambiae* s.l.

About 15–20 specimens of *An. gambiae* s.l. collected in each trialed mosquito net were analyzed through PCR for genotyping the L1014F *kdr* mutation according to the protocol described by Martinez-Torres et al. (1998).

Statistical Analysis

During the survey, a mosquito net was considered in use in a household, when it was found hung or tidy on the sleeping materials or, taken out of its original packaging with its user who admits to having used it the last night before the visit.

The usage rate of mosquito nets was therefore determined by dividing the number of bed nets found in use by the total number of bed nets present in the households. The coverage rate was evaluated by dividing between twice the number of Olyset nets found (for a normal coverage two people are needed for a mosquito net) by the number of people sleeping in the surveyed households. Confidence intervals for coverage and usage rates were determined using the exact binomial test.

In each village, the proportion of effective Olyset nets was obtained by dividing the number of bed nets that had a minimum quantity of insecticide of 2.73 µg (cut-off value likely to induce a mortality rate ≥80% in a WHO cone bio-assay determined by Azondékon et al. 2014) by the total number of bed nets tested. The average amount of insecticide found on the Olyset mosquito nets collected in each village is compared to that of the new and nonused ones using the t-test.

The frequencies of the L1014F *kdr* mutation of the specimens of *An. gambiae* s.l. collected in the torn mosquito nets, were calculated by the following formula: $F(kdr) = [2n.RR + n.RS] / [2(n.RR + n.RS + n.SS)]$, (n. = Number of genotype).

The human biting rates (HBR) of *An. gambiae* s.l. in the torn nets were calculated by dividing the number of specimens collected in each trialed net by the number of collector night. The incidence density ratio (IDR) was determined to first assess the evolution of the HBR according to the degree of wear (pHI) of the trialed mosquito nets and, secondly to evaluate the impact of permethrin incorporated in the Olyset nets on the HBRs. The correlation between the HBRs and the pHIs was assessed using the Pearson's linear correlation test. A linear regression was performed to assess the variability of the HBRs according to the pHIs.

Confidence intervals for L1014F *kdr* frequencies and HBRs were determined using the exact binomial test.

The comparison between mosquito nets, of the L1014F *knr* frequencies on the one hand, and the HBRs on the other hand, was carried out using a chi-square test.

All analyses were performed with the R statistical software, version 3.6.2 (R Core Team 2019).

Ethics

The protocol of the present study was reviewed and approved by the Comité National Provisoire pour la Recherche et l'Ethique en Santé (CNPERS) (Grant N° 76/MS/DC/SGM/DFRS/CNPERS/SA). All heads of surveyed households and mosquito collectors have been informed of the risks incurred over the study. They gave their written consent to participate to the study, prior to their involvement. Mosquito collectors were trained to catch mosquito before being bitten. They were vaccinated against yellow fever and received antimalarial medication whenever they suffered from malaria.

Results

Types of Mosquito Nets Encountered, Coverage, and Usage Rate in the Surveyed Households

In the study area, overall 795 households were visited and 1,599 mosquito nets were found. The Olyset nets of the 2011's campaign were the most frequent (73.7%, 95% confidence interval (CI): 71.5–75.8, $n = 1,179$). The other type of nets encountered include Permanet 2.0 (16.9%, 95% CI: 15.1–18.9, $n = 271$), untreated mosquito nets (8.8%, 95% CI: 7.5–10.3, $n = 141$), and a few other rare (0.5%, 95% CI: 0.2–0.9, $n = 8$) Olyset nets distributed through other partners. All nets combined, the mean number per household was 2 (95% CI: 1.9–2.1) for the whole study area.

Regarding the coverage rate of the 2011 Olyset nets, it ranged from 37.6% (95% CI: 33.3–42.1) at Idéna 2 to 88.3% (95% CI: 84.6–91.3) at Mowodani, with an average of 67.4% (95% CI:

65.8–68.9) for the whole study area (Fig. 4). The lowest usage rate [51.8% (95% CI: 44.0–59.5)] of this mosquito net was recorded at Ko-Koumoulou and the highest [91.2% (95% CI: 85.6–95.1)] at Itassoumba. The mean usage rate for the whole study area was 73.3% (95% CI: 70.7–75.8) (Fig. 5).

Taking into account all the mosquito nets encountered during the survey, the coverage rate varied from 66.5% (95% CI: 62.1–70.7) at Idéna 2, to 111.2% (95% CI: 100.9–122.4) at Mowodani, with an average of 91.4% (95% CI: 90.4–92.3) for the whole study area. A high availability (coverage rate > 100%) of mosquito nets was observed at Ko-Koumoulou and Mowodani (Fig. 4). The lowest usage rate [58.7% (95% CI: 52.0–65.1)] was recorded at Ko-Koumoulou and the highest [88.9% (95% CI: 83.8–92.8)] at Itassoumba. The average usage rate for all mosquito nets was estimated at 76.8% (95% CI: 74.7–78.9) (Fig. 5).

Position of the 2011's Olyset Nets in the Households

Overall, 51.8% (95% CI: 48.9–54.7) of the 2011's Olyset nets encountered in households during the survey were found hung. The nonhung nets were either tidy on the sleeping materials [17.7% (95% CI: 15.6–20.0)], or in other places [30.5% (95% CI: 27.8–33.2)] such as the dryer, suitcases, a corner of the house (Table 1).

Physical Integrity of the 2011 Olyset Nets

At nine months postdistribution, the proportion of nets found with holes ranged between 21.9% (95% CI: 15.5–29.3) at Okoofi 2 to 45.9% (95% CI: 38.0–54.0) at Itassoumba, with a mean of 28% (95% CI: 25.4–30.6) for the whole study area (Table 2).

Nets with size-1 and size-2 holes were found in majority at 50.6% (95% CI: 47.66–53.4) and 55.9% (95% CI: 53.0–58.7) respectively, while nets with size-3 holes represented 20.5% (95% CI: 18.3–23.0) of the samples surveyed (Table 2). The size-4 holes were not found during the 9 months nets assessment.

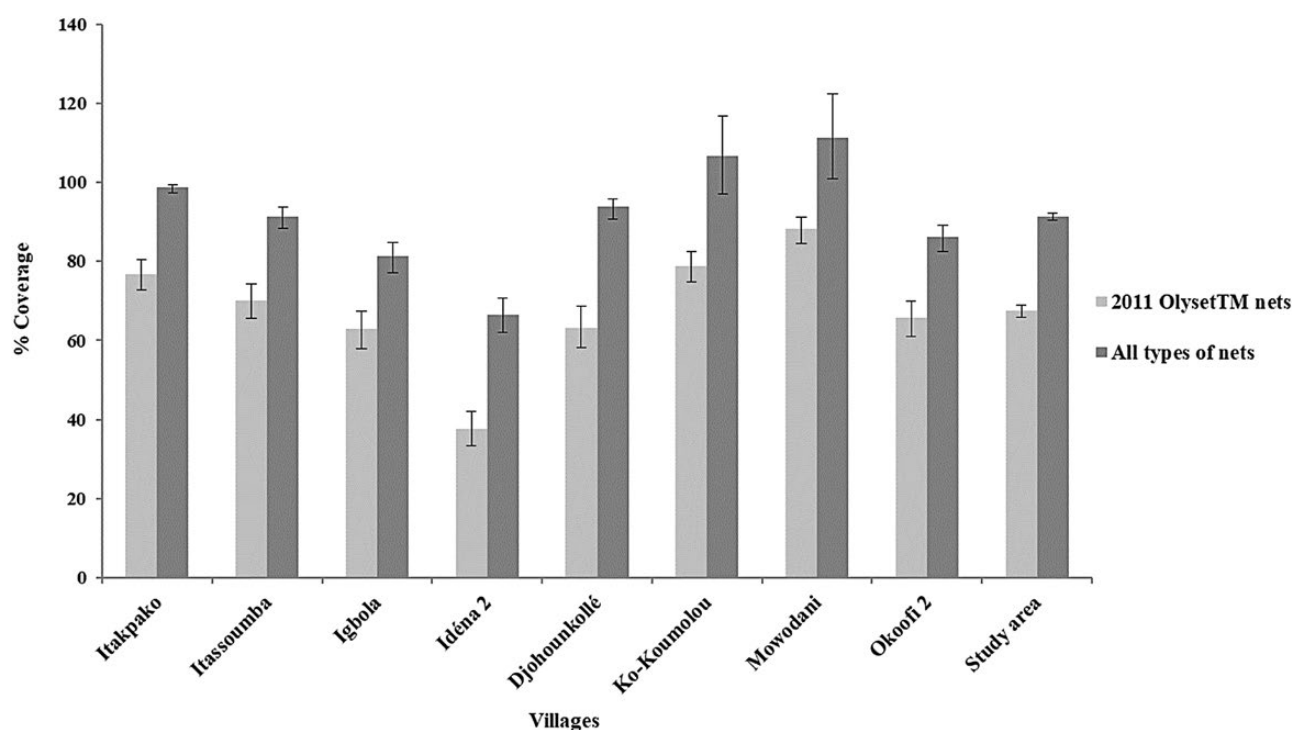


Fig. 4. Coverage rate of the 2011 Olyset nets and all types of nets encountered in the study area.

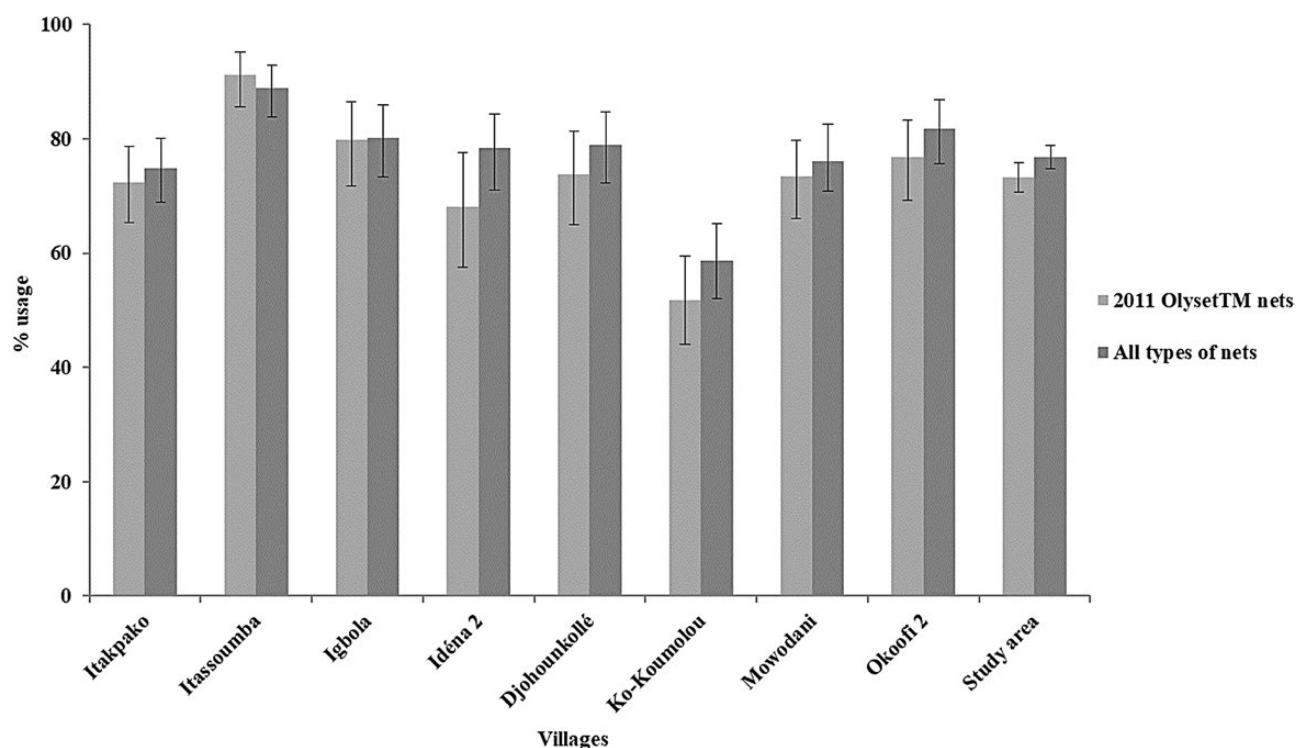


Fig. 5. Usage rate of the 2011 Olyset nets and all types of nets encountered in the study area.

Table 1. Position of the 2011 Olyset nets in the households

Indicators	Itakpako	Itassoumba	Igbola	Idéna 2	Djohounkollé	Ko-Koumoulou	Mowodani	Okoofi 2	Total
Trialed nets									
N	188	159	129	91	122	170	169	151	1,179
Hanged on the sleeping materials									
N	102	122	68	42	50	63	77	87	611
% (95% CI)	54.3 (46.8–61.5)	76.7 (69.4–83.1)	52.7 (43.7–61.6)	46.2 (35.6–56.9)	41 (32.2–50.3)	37.1 (29.8–44.8)	45.6 (37.9–53.4)	57.6 (49.3–65.6)	51.8 (48.9–54.7)
Tidy									
On the sleeping materials									
N	28	21	33	20	33	28	26	20	209
% (95% CI)	14.9 (10.1–20.8)	13.2 (8.4–19.5)	25.6 (18.3–34.0)	22.0 (14.0–31.9)	27.0 (19.4–35.8)	16.5 (11.2–22.9)	15.4 (10.3–21.7)	13.2 (8.3–19.7)	17.7 (15.6–20.0)
On other places									
N	58	16	28	29	39	79	66	44	359
% (95% CI)	30.9 (24.3–38.0)	10.1 (5.8–15.8)	21.7 (14.9–29.8)	31.8 (22.5–42.5)	31.9 (23.8–41.0)	46.5 (38.8–54.3)	39.1 (31.7–46.8)	29.1 (22.0–37.1)	30.5 (27.8–33.2)

N: number, CI: confidence interval.

Bio-efficacy of Olyset Nets, 2011

Mean Quantity of Insecticide Available on the Surface of Olyset Nets Using GC

For the insecticide quantification through GS, of the 210 tested Olyset nets, 50 were new, and unused (selected immediately after distribution), while 20 nets under use were withdrawn from each of the 8 study villages at 9 months postdistribution.

Results obtained show a clear decline of the mean quantity of insecticide in all villages, after only 9 months of community use compared to the new and unused nets (control) (Fig. 6).

Overall, the mean quantity of permethrin for nets sampled from all 8 villages combined was 0.2 µg (95% CI: 0.01–0.38), against 7.08 µg (95% CI: 5.74–8.42) for the new and unused ones.

Proportion of Effective Olyset Nets Using GC

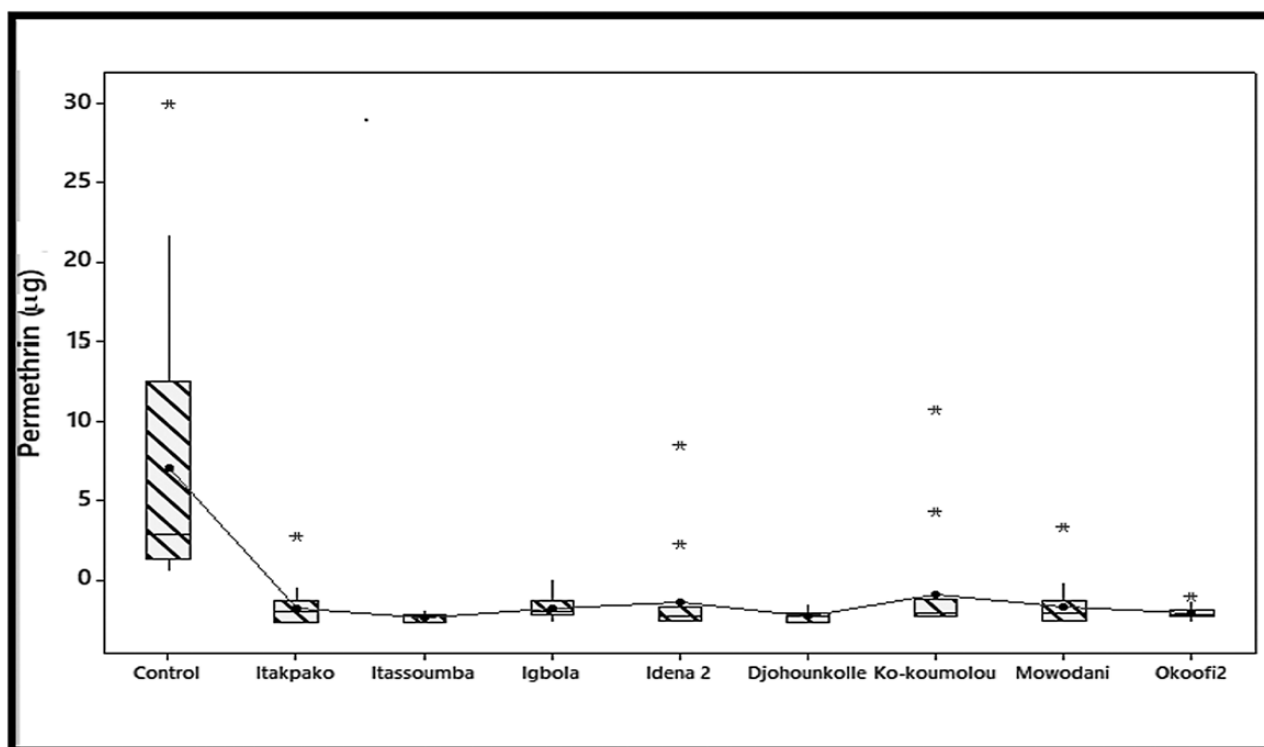
Figure 7 shows the number of new-unused (control) and field-collected Olyset nets that were effective or not.

Of the 50 new and unused Olyset nets tested immediately after distribution, 49 met the efficacy criteria of ≥ 2.73 µg permethrin on surface, corresponding to an efficacy rate of 98% (95% CI:

Table 2. Proportion of Olyset nets with any holes

Indicators	Itakpako	Itassoumba	Igbola	Idéna 2	Djohoukollé	Ko-Koumolou	Mowodani	Okoofi 2	Grand total
Trialed LLINs, N	188	159	129	91	122	170	169	151	1,179
Holed LLINs, N	45	73	40	23	34	32	50	33	330
Holed LLINs, % (CI)	23.9 (18.0–30.7)	45.9 (38.0–54.0)	31 (23.2–39.7)	25.3 (16.7–35.5)	27.9 (20.1–36.7)	18.8 (13.2–25.5)	29.6 (22.8–37.1)	21.9 (15.5–29.3)	28 (25.4–30.6)
LLINs with size 1 holes, N	30	148	51	76	94	41	119	37	596
LLINs with size 1 holes, % (CI)	16.0 (11.0–22.0)	93.1 (88.0–96.5)	39.5 (31.0–48.5)	83.5 (74.3–90.5)	77.0 (68.6–84.2)	24.1 (17.9–31.3)	70.4 (62.9–77.2)	24.5 (17.9–32.2)	50.6 (47.66–53.4)
LLINs with size 2 holes, N	97	124	70	41	81	63	98	85	659
LLINs with size 2 holes, % (CI)	51.6 (44.2–59.0)	78.0 (70.7–84.2)	54.3 (45.3–63.1)	45.1 (34.6–55.8)	66.4 (57.3–74.7)	37.1 (29.8–44.8)	58.0 (50.2–65.5)	56.3 (48.0–64.3)	55.9 (53.0–58.7)
LLINs with size 3 holes, N	54	60	20	15	18	17	18	40	242
LLINs with size 3 holes, % (CI)	28.7 (22.4–35.8)	37.7 (30.2–45.8)	15.5 (9.7–22.9)	16.5 (9.5–25.7)	14.8 (9.0–22.3)	10.0 (5.9–15.5)	10.7 (6.4–16.3)	26.5 (19.6–34.3)	20.5 (18.3–23.0)

N: number, CI: confidence interval.

**Fig. 6.** Mean quantity of permethrin available in new and field collected Olyset nets (the control box plot shows the mean quantity of permethrin on new Olyset nets. The other box plots indicate the mean quantity of permethrin on Olyset nets withdrawn from each study village after 9 months of use).

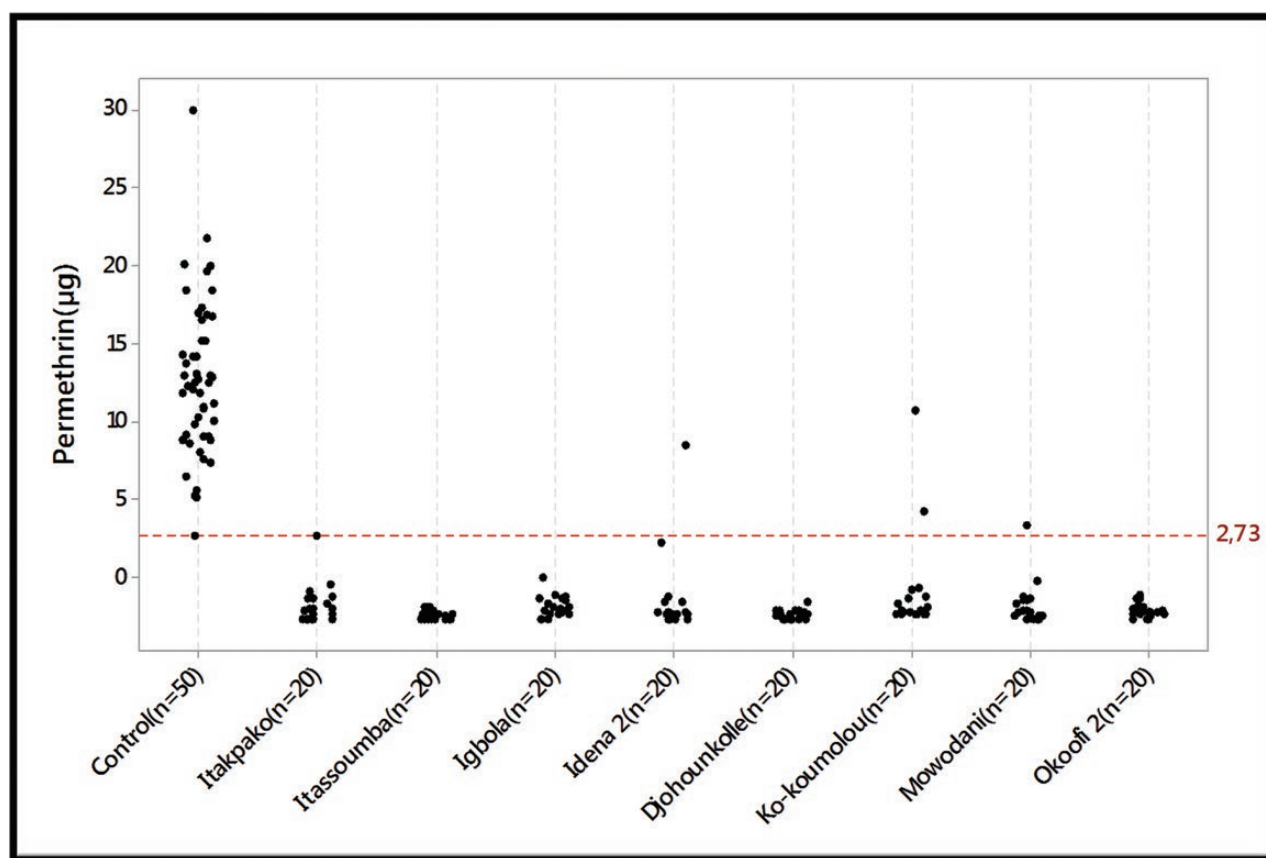


Fig. 7. Scatter plot showing permethrin quantity on surface of 210 Olyset nets (The horizontal dotted line shows the efficacy threshold of 2.73 µg. The control indicates the new Olyset nets).

89.4–99.9). Regarding the nets withdrawn after 9 months of usage in the field, only 4 out of a total of the 160 tested were effective, representing 2.5% (95% CI: 0.7–6.3) of the field-tested nets. Among the 4 mosquito nets still effective, 2 were found at Ko-Koumolou, 1 at Idéna 2, and 1 at Mowodani (Fig. 7).

WHO Cone Testing

Figure 8 shows results from WHO cone testing performed with exposition of mosquitoes of the Kisumu susceptible strain to 14 (2 per village except Mowodani) other randomly selected Olyset nets. Overall, only 1 net collected from Okoofi 2 succeeded in inducing a mortality rate of 80% (28/35), which equals to an efficacy rate of 7.1% (95% CI: 0.2–33.8, 1/14).

No mortality occurred in the negative controls, while it was 98.7% (95% CI: 93.2–99.9) in the positive controls.

Variation in the HBRs of *An. gambiae* s.l. in Mosquito Nets With Increasing pHIs

Figure 9 shows the variation in the HBR of *An. gambiae* s.l. in different types of mosquito nets with increasing pHIs. Inside the new and nontorn Olyset net, no mosquito specimen was collected.

With the untreated nets, the HBR was 2.6 (95% CI: 1.7–3.9, $P < 0.0001$) and 3.6 (95% CI: 2.1–4.7, $P < 0.0001$) times higher with those of condition categories 2 (pHI = 461) and 3 (pHI = 971) respectively, compared to that of condition category 1 (pHI = 25) (Fig. 9).

A similar trend was observed for the new Olyset nets. The HBR was significantly higher in the condition categories 2 (Incidence

Density Ratio (IDR) = 3.5, 95% CI: 1.9–6.5, $P < 0.0001$) and 3 (IDR = 6.3, 95% CI: 3.5–11.3; $P < 0.0001$) compared to the condition category 1 (Fig. 9).

With the field collected Olyset nets, similar HBRs were obtained between those of condition categories 1 and 2 (IDR = 0.87, 95% CI: 0.6–1.3, $P = 0.6$). At the opposite, a higher HBR was observed in the condition category 3 (IDR = 2.1, 95% CI: 1.5–3.0, $P < 0.0001$) than in the condition category 1 (Fig. 9).

Overall, a decisive and positive correlation of 0.87 [0.50–0.97] was observed between the HBRs of *An. gambiae* s.l. collected inside the nets and the pHIs ($P = 0.002$). The linear regression analysis performed shows that 72.9% of the variability in the HBRs was due to the pHIs of the trialed nets (Fig. 10).

Efficacy of the Insecticidal Barrier in the New Olyset Nets Belonging to the 3 Condition Categories

The collections of *An. gambiae* s.l. inside torn-untreated nets and torn-new Olyset nets allowed us to assess the efficacy of the insecticidal barrier in each of the 3 condition categories of mosquito nets.

With the condition category 1 nets, a significantly lower HBR was observed in the new Olyset net compared to the untreated mosquito net (IDR = 0.41, 95% CI: 0.21–0.77, $P = 0.007$). A similar trend was observed with the condition category 2 mosquito net (IDR = 0.55, 95% CI: 0.38–0.79, $P = 0.001$) (Table 3).

At the opposite, with the condition category 3 nets, the HBRs were similar between the new Olyset net and the untreated mosquito net (IDR = 0.80, 95% CI: 0.60–1.08, $P = 0.16$) (Table 3).

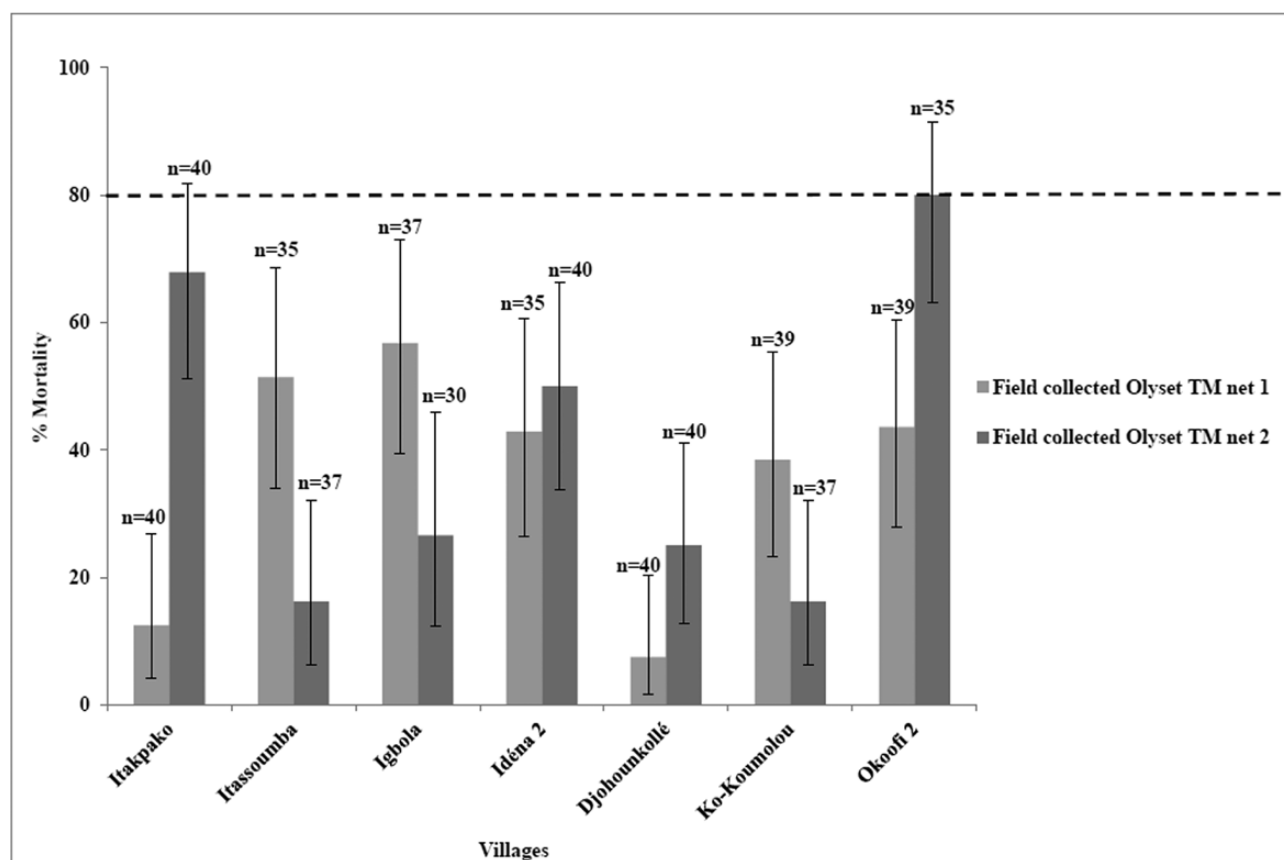


Fig. 8. WHO cone testing results (the black dotted line shows the efficacy threshold of 80% mortality rate, n = number of tested specimens of the Kisumu strain).

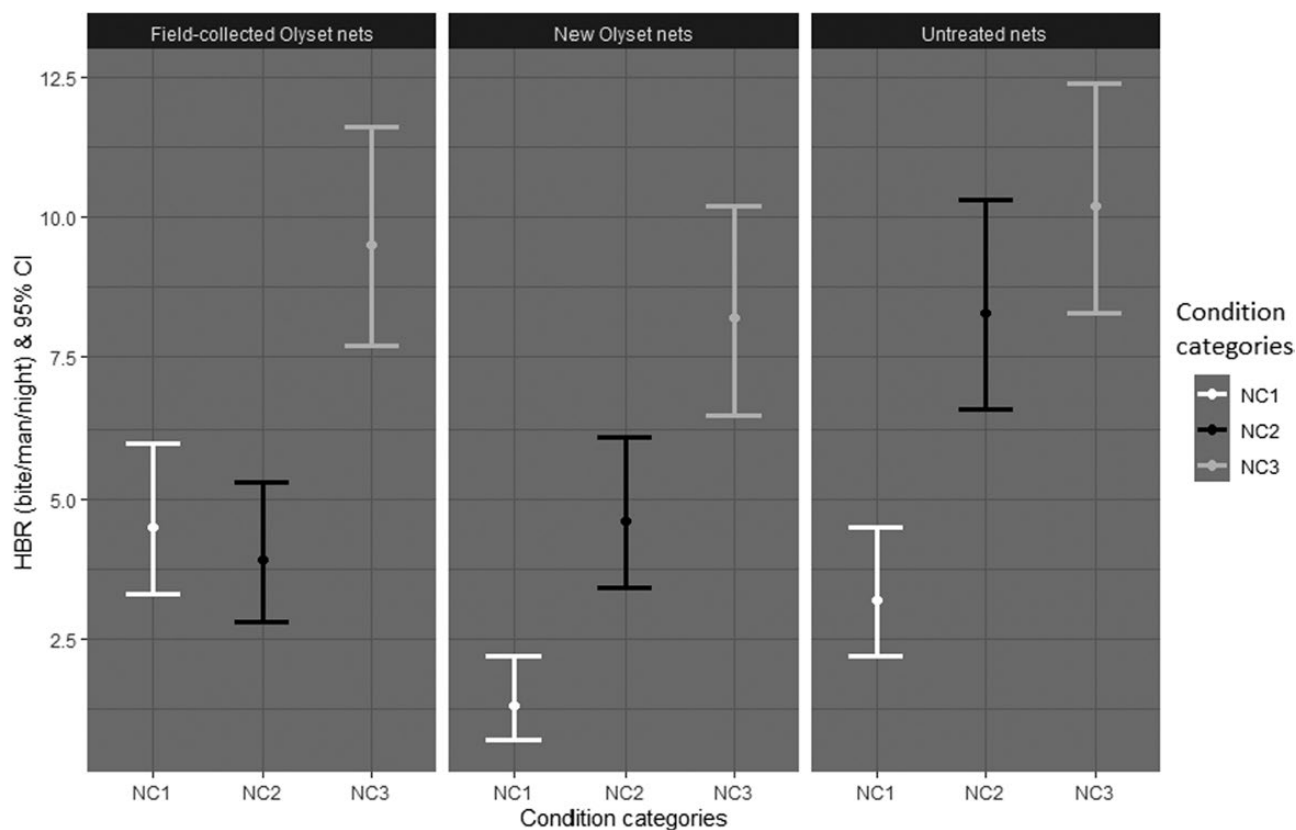


Fig. 9. Variation in the HBRs of *An. gambiae* s.l. in different mosquito nets with increasing pHIs (NC1: net of condition category 1 with pH = 25, NC2: net of condition category 2 with pH = 461, NC3: net of condition category 3 with pH = 971).

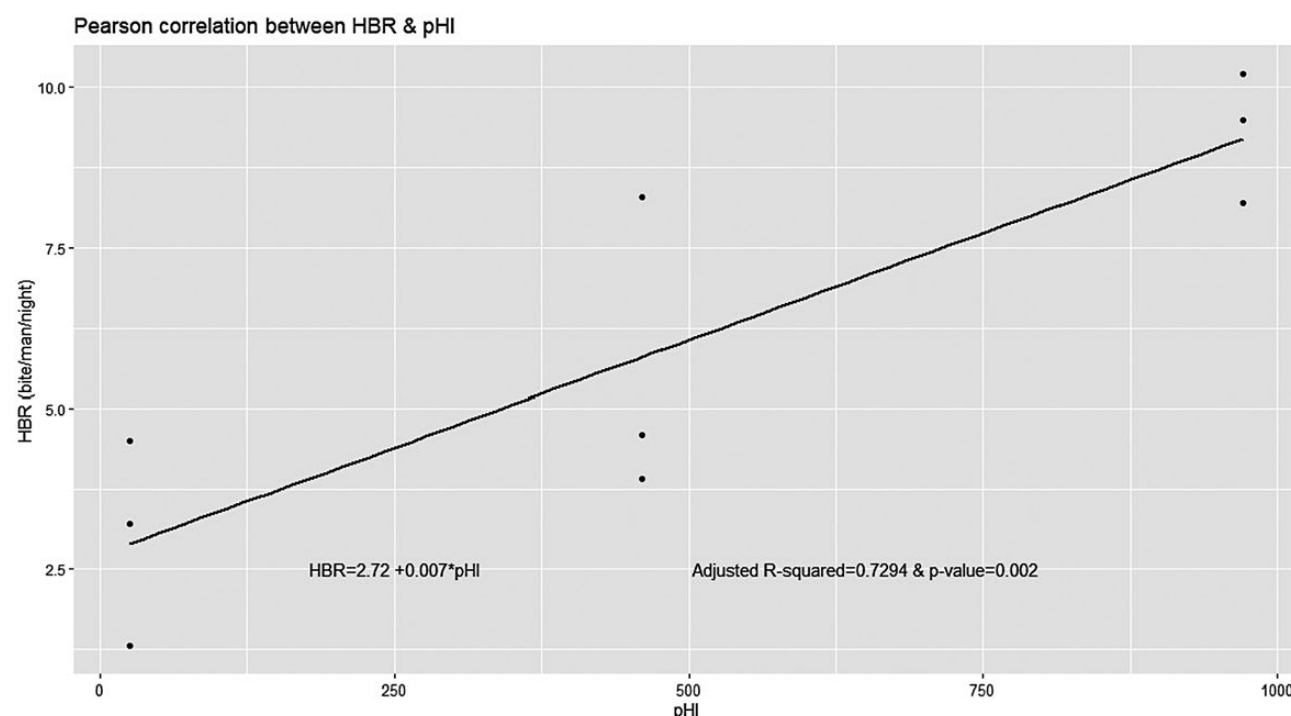


Fig. 10. Pearson correlation between the HBRs of *An. gambiae* s.l. and the pHIs of trialed mosquito nets.

Table 3. Efficacy of the insecticidal barrier in new Olyset nets with different pHIs

Net of condition catégories	Type of nets	N of <i>An. gambiae</i> s.l.	Man night	HBR (b/m/n)	IDR	CI-95% (IDR)	P
NC1 (pHI = 25)	Untreated nets	32	10	3.2	1	—	—
	New Olyset nets	13	10	1.3	0.41	0.21–0.77	0.007
NC2 (pHI = 461)	Untreated nets	83	10	8.3	1	—	—
	New Olyset nets	46	10	4.6	0.55	0.38–0.79	0.001
NC3 (pHI = 971)	Untreated nets	102	10	10.2	1	—	—
	New Olyset nets	82	10	8.2	0.80	0.60–1.08	0.16

NC: nets of condition category, N: number of, HBR: human biting rate, b/m/n: bite/man/night, IDR: incidence density ratio, CI: confidence interval.

Frequency of the L1014F *kdr* Mutation in *An. gambiae* s.l. Collected in Torn Nets

In total, 114 specimens of *An. gambiae* s.l. collected inside both torn-new Olyset nets, and torn-untreated nets were genotyped through PCR. No susceptible homozygous (SS) mosquito was identified (Table 4).

Overall, a higher frequency of the L1014F *kdr* mutation (92.7%, 95% CI: 86.2–96.8) was obtained with mosquito collected in the new Olyset nets, compared to that (82.2%, 95% CI: 74.1–88.6) of mosquito collected in the nontreated mosquito nets (Table 4).

In addition, the frequency of homozygous resistant (RR) mosquitoes entering new Olyset nets (85.4%, 95% CI: 73.3–93.5) was higher compared to that obtained with mosquito harvested in the nontreated mosquito nets (64.4, 95% CI: 50.8–76.4) (Table 4).

Discussion

After the nationwide distribution of Olyset nets in July 2011, the present study carried out nine months later in the Plateau region (South Benin), shows that the coverage rate was below expectation (Coverage rate \geq 80%). In addition, a drastic drop of the bio-efficacy of these mosquito nets out of which, a nonnegligible proportion was torn after only nine months of community use was observed.

In fact, the coverage rate of the freshly distributed Olyset nets in the study area was 67.4% (95% CI: 65.8–68.9) at 9 months postcampaign, which might suggest that the objective of at least 80% coverage targeted by the NMCP was not reached at the time of the investigation. Besides, the net coverage was much better and estimated at 91.4% (95% CI: 90.4–92.3) when considering all types of mosquito nets found in surveyed households. From our observations in the households, some people used the mosquito nets they had beforehand, so as to keep for later the new ones freely provided to them during the July 2011 campaign.

Among the surveyed villages, the highest usage and loss of physical integrity rates in the 2011 Olyset nets were recorded in Itassoumba, a village characterized by the presence of a water-course and a large market-gardening perimeter with several fish farming ponds. In fact, this major environmental modification operated at Itassoumba for agricultural production purposes created numerous breeding sites conducive to an increased proliferation of *An. gambiae* s.l. mosquito (Sovi et al. 2013). This high density of mosquito would probably have forced the populations to a strong use of mosquito nets to protect themselves against mosquito bites. Also, it is generally accepted that, the more the mosquito nets are used, the more likely they are to tear. In addition, the closeness of

Table 4. Frequency of the L1014F *kdR* mutation in *An. gambiae* s.l. collected in torn-untreated nets and torn-new Olyset nets

Mosquito nets	N tested	RR	RS	SS	F(L1014F <i>kdR</i>), 95% CI	X ²	df	p	F(RR), 95% CI	X ²	df	P
Untreated nets	59	38	21	0	82.2, 74.1–88.6	4.7	1	0.03	64.4, 50.8–76.4	5.6	1	0.02
New Olyset nets	55	47	8	0	92.7, 86.2–96.8				85.4, 73.3–93.5			

N: number of specimens of *An. gambiae* s.l., F(L1014F *kdR*): frequency of the L1014F *kdR* mutation expressed in percentage, F(RR): frequency of the RR genotype expressed in percentage, CI: confidence interval.

a watercourse to Itassoumba would have likely favored much more frequent washing of the 2011 Olyset nets, which accelerated the loss of their physical integrity. This phenomenon had been previously documented in Késsounou and Malanville, two sites bordered by a river (Azondékon et al. 2014). This observation further shows that the longevity of mosquito nets strongly depends on the place where they are used. Additionally, other factors such as: the lifestyle of the users of the mosquito nets (awkward use of sharp objects near the mosquito nets, proximity to meal cooking fire), the number of people sleeping under these mosquito nets could also explain the proportion of 28% (95% CI: 25.4–30.6) of torn Olyset nets, obtained after only 9 months of use. This worrying loss of physical integrity rate could be effectively avoided in the future through information, education and communication sessions on a better use of mosquito nets arranged for the community during distribution campaigns, as previously mentioned by Ndjinga et al. (2010) in the Democratic Republic of Congo. The majority of the nets had size 1 and 2 holes. These holes are set to widen within time of use, thereby facilitating massive entry of mosquitoes inside the nets. As the mosquito nets tear, the resulting decrease in their efficacy is confirmed by data gathered after mosquito collections conducted inside the torn mosquito nets. The repairing of holes should then be encouraged as it would postpone as much as possible the loss of efficacy of the nets.

Furthermore, limitations for the present study include the fact of not having counted the number of each hole size on the Olyset nets in use in the field so as to estimate their pHIs and the condition category they belong to, as well as the frequency of their washing that was not recorded.

The main objective of the National Malaria Control Program through the 2011 nationwide distribution of Olyset nets was to reduce the incidence, prevalence, and transmission of malaria in a context of generalized resistance of malaria vectors to insecticides. According to a trial conducted in Benin between 2007 and 2008 in the health area of Ouidah–Kpomassè–Tori Bossito where malaria vectors are pyrethroid-resistant (Djènontin et al. 2010), the use of long lasting insecticidal nets (LLINs) at a threshold greater than 60% had a significant impact on the malaria incidence (Moiroux et al. 2012). Considering that malaria vectors were also pyrethroid-resistant in the Plateau region (Sovi et al. 2014), one could theoretically estimate that the usage rate of 73.3% (95% CI: 70.7–75.8) of the 2011 Olyset nets in the present study, would induce a positive impact on the incidence, prevalence, and transmission of malaria in the study area. However, epidemiological data are required, to support this hypothesis.

Regarding the position of the 2011 Olyset nets in the households, there is overall a significant difference between the proportion of nets found hung [51.8% (95% CI: 48.9–54.7)] and the usage rate [73.3% (95% CI: 70.7–75.8)]. This difference is explained by the fact that some mosquito nets in use in the households were tidied up by the populations, early in the morning when they woke up to have more space in the houses during the day. The proportion of mosquito nets in use but not hung is estimated at 21.5% (95% CI: 19.1–24.0).

Apart from the physical integrity, the bio-efficacy of the 2011 Olyset nets was also assessed using the gas chromatography. The position B from which the insecticide residue was taken is the best one, as it is located in the middle of the mosquito net. In fact, many owners often put various objects on the roof of the net while the bottom is often folded under the sleeping materials (mat or mattress). This usually causes a loss of insecticide molecules in these 2 positions (Azondékon et al. 2014, Nanguenon et al. 2014). The use of the gas chromatography technology has indeed revealed a drastic drop in the quantity of insecticide (below the efficacy threshold of 2.73 µg) on the surface of most field-collected Olyset nets compared to the new ones, after only nine months of use. Little permethrin quantity was therefore available on the fibers of the field collected Olyset nets. This trend obtained with gas chromatography was confirmed by data from WHO cone testing (mortality rates <80% with the majority of the nets). Apart from the frequency of nets washing which could be incriminated (Maxwell et al. 2006, Atieli et al. 2010) in this loss at short-term of the bio-efficacy of the field-collected nets, other factors to investigate through observation of population behaviour over time, could likely be involved.

A review of experimental hut trials data by Irish et al. (2014) shows that the more the area of holes on the nets is large, the more the blood feeding rate of mosquitoes is high, which suggests a strong association between the level of loss of the physical integrity and the mosquito biting rate. The results of the present study confirm this hypothesis insofar as the biting rate increased as the mosquito nets were more torn. Consequently, the significant correlation obtained between the biting rates of *An. gambiae* s.l. and the pHIs, shows that the more holes in a mosquito net, the less it protects. This observation raises again the need that households repair their bed nets.

The crucial role played by the insecticide barrier in reducing the man–vector contact was demonstrated by Nanguenon et al. (2013). The same trend is observed in the present study with the torn nets (insecticide-treated vs untreated) of the condition category 1 on the one hand and, the condition category 2 on the other hand. However, with torn nets of the condition category 3, no reduction in the frequency of biting of *An. gambiae* s.l. was observed with the Olyset net compared to the untreated one. This observation suggests that the insecticidal barrier does no longer plays its role effectively when the level of loss of physical integrity of the insecticide-treated net reaches a very high threshold.

The frequencies of the L1014F *kdR* mutation and homozygous resistant (RR) mosquitoes were higher in the new Olyset nets than in the untreated ones. This result suggests that the carriage the R resistant allele provides the mosquito an advantage when passing through the insecticidal barrier. It further suggests that the more mosquitoes carry the R resistant allele, the less the excito-repellency effect of LLINs occurs.

Next generation LLINs that the WHO is urging manufacturers to develop have aroused high expectations in controlling insecticide-resistant malaria vectors. Next generation LLINs that are currently undergoing trials combine a pyrethroid insecticide with either piperonyl butoxide (Olyset plus, Permanet 3.0), or pyriproxyfen

(Olyset duo, Royal guard), or chlorfenapyr (Interceptor G2). However, only pyrethroid-piperonyl butoxide nets received an interim recommendation from the WHO (WHO 2017). This is subsequent to a cluster randomized controlled trial conducted in Tanzania (Protopopoff et al. 2018) reporting Pyrethroid-piperonyl butoxide nets performing better than pyrethroid only nets. For that, the WHO recommended the use of pyrethroid-piperonyl butoxide nets in areas where malaria vectors display high-moderate resistance intensity due (at least partly) to mixed-function oxidases (WHO 2017). Given the next generation LLINs did not receive WHO full recommendation so far—the lack of high quality RCT evidence for some of them—their higher per unit cost, pyrethroid only nets such as the Olyset nets which have been evaluated in the present study are still widely procured and distributed in sub-Saharan Africa. Indeed, 57% (50,760,921 out of 89,528,562) of all LLINs shipped to sub-Saharan Africa in the first half of 2021 were pyrethroid only LLINs (Alliance for Malaria Prevention 2021). Thus, despite an increased push for next generation LLINs, data on coverage, usage, physical integrity, and bio-efficacy of pyrethroid-only LLINs such as Olyset nets remain relevant for informing procurement and deployment decisions for NMCPs. In addition to this, there are growing concerns regarding the physical and insecticidal durability of next generation LLINs. Historical data on physical integrity and bio-efficacy of pyrethroid-only LLINs are therefore increasingly useful, to serve as a reference point for community evaluations of next generation LLINs.

Conclusion

Factors that might limit the efficacy of the 2011 Olyset nets include the holes found at the surface of the nets as well as the low quantity of insecticide present on their fibers, only 9 months after their use in the community. These results suggest that public awareness sessions should be included in future distribution campaigns. Such activities should educate populations to prioritize the use of newly distributed mosquito nets to the detriment of old ones and, correct use of these mosquito nets. Over time, particular emphasis must be placed on the repair of torn mosquito nets by the households. Such repairs would contribute somewhat to maintaining bed net efficacy already compromised by the resistance of vectors to insecticides.

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Author Contributions

Conception and design of the work: AS, VG, RA, and MCA. Acquisition of data: AS, VG, RA, FOA, SH, and ASS. Analysis and interpretation of data: AS, VG, RA, and BA. Drafting and substantial revision of the manuscript: AS, ASS, RG, RO, FT, GGP, and MCA. All authors read and approved the final version of manuscript.

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