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## Durability of PBO nets (Olyset Plus®), 12 months after their distribution in Bertoua, Cameroon

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## ABSTRACT

**Background:** The rapid spread of pyrethroid resistance has led to a change in strategy, going from pyrethroid-based nets to PBO + pyrethroid-treated nets. Although these new nets may significantly improve the control of pyrethroid-resistant mosquitoes, their durability in the field remain not yet well documented. This study investigates the durability and washing resistance of Olyset-Plus nets in the city centre and rural areas of Bertoua, Cameroon. In each site, a semi-structured questionnaire was administered to at least 190 households with an Olyset-Plus net. Factors such as net use, physical integrity and bioefficacy were recorded. Bioassays were conducted on the collected nets to assess their bioefficacy and resistance to washing. They were tested against wild *Anopheles gambiae* sensus lato (s.l.). Unused nets and the Kisumu strain were used as controls. Washing and cone testing of the nets was carried out according to standard WHO protocols.

**Results:** A high rate of net use by children was recorded in the urban area (89.1% (106/119)) compared to the rural area (39.7% (118/297)). The majority of Olyset-Plus nets inspected 82.2% (162/197) in the rural area and 88% (206/234) in the urban centre were in good condition (Hole Index < 64). Only 5.6% and 6.8% of nets were badly torn in rural and urban sites respectively. Nets were washed more regularly in the urban centre. 88.1% of urban dwellers reported having washed their nets at least once compared to only 62% of rural dwellers. Bioefficacy tests with nets indicated a mortality rate ranging from 66% for unwashed nets to 86.7% for nets washed at least once. Bioefficacy varied significantly in the city of Bertoua according to net washing frequency, soaking time, soap type and drying location, whereas in the rural village, only washing(washed or

**Abbreviations:** HI, Hole Index; IRS, Indoor Residual Spraying; LLINs, Long Lasting Insecticidal Nets; OCEAC, Organisation pour la Coordination de la lutte contre les Endémies en Afrique Centrale; PBO, Piperonyl Butoxyde.

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unwashed) and soaking status (soaked or unsoaked) significantly influenced the bioefficacy of Olyset-Plus nets.

**Conclusions:** This study revealed different handling practices of bed nets in rural and urban settings which could significantly affect Olyset-Plus nets bio-efficacy and durability. Routine monitoring and sensitization of communities to best practices concerning bed nets usage and handling during mass distribution might enhance the net durability in the community.

## 1. Introduction

Long-lasting insecticidal bed nets (LLINs) and indoor residual spraying (IRS) remain the main tools for malaria prevention and control (Castellanos et al., 2021; Organization, W. H, 2021). Bed nets provides protection to individual users and to the entire community when the level of coverage of the population is high (Organization, W. H, 2011). Since 2000 the massive scale up of LLINs combined to other interventions such as IRS, Artemisinin-combination based therapy across sub-Saharan Africa led to significant decrease of malaria morbidity and mortality (Bhatt et al., 2015; Griffin et al., 2016; Organization, W. H, 2020; Weiss et al., 2019). However, malaria cases has stalled since 2016 in many regions (Organization, W. H, 2021; *World Malaria Report*, 2019). In addition to the Covid-19 pandemic affecting the delivery of malaria control interventions, rapid expansion of insecticide resistance in vector populations, low nets usage or durability could be additional factors contributing to the poor performance of bed nets-based intervention (Bamou et al., 2018; Briet et al., 2020; Graves et al., 2011; Moon et al., 2016; Organization, W. H, 2021; Russell et al., 2015). Since the combination of such factors inducing the sub-performance of vector control measures could vary from one place to another, thorough investigation of each epidemiological situation is required (Organization, W. H, 2013).

Pyrethroid resistance is actually considered as the major cause for LLINs sub-performance (Hemingway and Ranson, 2000; Organization, W. H, 2020; Ranson et al., 2011). Pyrethroids are the only insecticide family recommended for bed nets coating because of their high repellence or lethal effects on mosquitoes and low toxicity at operational doses for mammals and non-targeted organisms (Zaim et al., 2000). Both target site and metabolic base mechanisms drive mosquito resistance to pyrethroids (Hemingway et al., 2004; Ranson et al., 2000). New generation nets combining pyrethroids to compounds such as Piperonyl Butoxide (PBO) that enhances pyrethroid treated nets efficacy by inhibiting P450s detoxification enzymes, have been reported to be highly effective against pyrethroid resistant mosquitoes and are now largely recommended for mosquito control (Shono et al., 2017).

In Cameroon where malaria remains a major public health threat, counting for 39.6% of childhood mortality and up to 69% of morbidity cases in health care facilities (*PSNLP-2019-2023-CONSOLIDE-TRANSMIS.pdf*, 2024), pyrethroid nets are still the main preventive tool used by the population (Antonio-Nkondjio et al., 2019). It is estimated that >80% of household own at least one net and about 60% of the population use LLINs regularly (Antonio-Nkondjio et al., 2019). However, the level of endemicity is highly heterogeneous across the country varying from one region to the other. The situation is considered to be driven by different factors including the diversity of vector species, deforestation, urbanization, low usage of LLINs, the practice of intensive agriculture, the exploitation of lowlands for agriculture or the rapid expansion of insecticides resistance (Antonio-Nkondjio et al., 2005, 2006, 2015, 2017, 2019; Bamou et al., 2018, 2020, 2021; Doumbé-Belisse et al., 2018). The complex situation of malaria in the country calls for alternative control approaches including the scaling up of new generation nets. During the third mass distribution campaign of LLINs (over 12 million bed nets distributed), it was decided that areas experiencing high malaria burden will receive pyrethroid+PBO nets while the rest of the country will receive pyrethroid only nets. PBO nets (Olyset Plus) distributed in the East region of Cameroon, are considered to remain effective after 20 standard WHO washes under laboratory conditions and 3 years of recommended use under field conditions (Organization, W. H, 2011). Semi-fields experimental studies indicated a high bio-efficacy of the PBO-nets against pyrethroid-resistant mosquitoes compared to pyrethroid-only treated LLINs (Protopopoff et al., 2018; Shono et al., 2017; Skovmand, 2018). Although in recent years there have been many studies assessing factors affecting bed nets ownership and usage (Bamou et al., 2021; Etang et al., 2013; Fru et al., 2021; Kala Chouakeu et al., 2021; Ndo et al., 2011; Talipouo et al., 2019), however there is still insufficient information on pyrethroids + PBO nets durability. This information is highly relevant for improving malaria control strategies in the field, knowing that factors such as the frequency of washing, type of laundry soap, washing and drying practices, and daily levels of “wear and tear” can highly affect the bio-efficacy of these nets and deserve further consideration (Briet et al., 2020; Fru et al., 2021).

The present study aimed to investigate the influence of washing, and handling practices of PBO nets by communities on the bioefficacy of the Olyset Plus nets 12 months after their distribution in urban and rural settings in the East region of Cameroon.

## 2. Material and methods

### 2.1. Study site

The study was conducted in Bertoua the regional capital of East Cameroon (4°34'30"north, 13°41'04"east) counting about 395,000 inhabitants. The city of Bertoua is located on the southern Cameroonian plateau, at an altitude of 400–900 m. The area is drained by river Djerem and Djadombe. The climate is subtropical with four seasons: a long dry season running from December to mid-March; a short rainy season from mid-March to mid-May, a short dry season from mid-May to mid-September and a long rainy season from mid-September to November. The area receives annually 1500 to 2000 mm of rainfall and the average temperature ranges from 23 to 30°

Celsius. The east region is one of the most affected by malaria in Cameroon with high and perennial malaria parasite transmission (Antonio-Nkondjio et al., 2019). Species belonging to the *Anopheles gambiae* complex are the main malaria vectors. The study took place in the urban centre of Bertoua (Enia and Kano) and in a close rural location (Ndoumbi I).

## 2.2. A community-based study of net utilization and net sampling

A semi-structured questionnaire was administered to some randomly selected households owning at least one Olyset Plus net. Prior to data collection, a team of 4 interviewers was trained on the purpose of the study, on how to approach respondents, obtain consent and administer the questionnaire. Interviews were conducted in French or English during face-to-face meetings with consented participants (household head, spouse, or an elder representative (of at least 18 years old) of the house). Interviews were conducted in private to reduce the influence from other people. The questionnaire was divided into three main parts. (1) The first part was to collect demographic information including locality of residence, residency area (rural or urban), level of education, and number of people in the family. (2) The second part of the questionnaire collected information on the ownership, the origin of nets, the type of nets, and frequency of usage of LLINs. (3) In the third part, information on washing practices of mosquito net, the frequency of washing, the type of soap used, the time of soaking, and the place used for drying bed nets were recorded (questionnaire in additional file). An inform consent form was signed by all participants of the study. All mosquito nets were inspected and the presence of holes on the roof or side of the net was recorded. The holes sizes were measured and classified according to WHO standard protocol (Organization, W. H., 2011) as follows: size 1 (holes of 0.5–2 cm), size 2 (holes of 2–10 cm), size 3 (holes of 10–25 cm), size 4 (holes >25 cm). Holes with a diameter lesser than 0.5 cm were not counted. Some of Olyset Plus nets were collected for bio efficacy tests at the malaria research laboratory of OCEAC (Organisation pour la lutte contre les endémies en Afrique Centrale) Yaoundé.

## 2.3. Net sampling and mosquito collection

Anopheline larvae were collected from aquatic habitats known to be suitable for *Anopheles gambiae* s.l. mosquitoes in different districts of the city of Yaoundé, pooled and reared together in the insectary of the Malaria Research Laboratory of OCEAC at a temperature of  $27 \pm 2$  °C and a relative humidity of 70–80%. These field strains are known as pyrethroid-resistant. Larvae were fed with Tetramin® Baby fish food until pupation. Pupae were collected in plastic cups and placed in netting cages for adult emergence. Emerging adults were allowed to feed on a 10% sucrose solution until the bioassay tests was conducted.

The national malaria control programme provided us with 40 new mosquito nets, which enabled us to replace what we were collecting from the population. Therefore, the bioefficacy of 40 Olyset plus nets collected in households (20 from rural area and 20 from urban area) was evaluated using WHO guidelines for cone bioassays (Organization, W. H., 2011). Five pieces (25 cm × 25 cm) of nets were cut on different sides of each net and kept in aluminium foil at 4 °C for bioassays. During the tests, 4 plastic cones were fixed to each piece of the net and 5 to 10 non-blood-fed *Anopheles gambiae* s.l. females (2 to 5 days-old) deriving from larvae collected on the field were transferred in each cones. Mosquitoes were exposed in plastic cones for 3 min and transferred to observation cups. Therefore, 100 to 200 mosquitoes were used to tests one bed net. The susceptible strain Kisumu (*Anopheles gambiae*) was used as control. Tests with Olyset nets were also conducted. Knockdown was recorded 60 min after exposure. Mosquitoes were kept in observation and allowed to feed on a 10% sugar solution after each test. The mortality rate was recorded 24 h after exposure.

## 2.4. Data analysis

Data were entered into a Microsoft Excel 2013 database and organized. Proportions, means and frequencies were used for descriptive analysis of the data. The Chi-squared test, was used to compare frequencies between urban and rural areas. Statistical significance was set at  $P < 0.05$ . Concerning the physical integrity, the proportionate Hole Index (HI) was calculated by summing holes size for each net using the formula:  $HI = (1 \times \text{no. of size-1 holes}) + (23 \times \text{no. of size-2 holes}) + (196 \times \text{no. of size-3 holes}) + (578 \times \text{no. size-4 holes})$ . The HI was used to classify nets in different categories: good, damaged, and badly torn according to WHO criteria: i) good condition if  $HI < 64$  (<100 cm<sup>2</sup> of holed area); ii) moderately damaged if  $64 < HI < 768$ ; and iii) badly torn if  $HI > 768$  (>0.1 m<sup>2</sup> of holed area). The knockdown and mortality rate of mosquitoes were plotted according to the number of washes. These results were used to evaluate and rank the effectiveness of the nets as follow: optimal:  $\geq 80\%$  mortality or  $\geq 95\%$  knockdown, minimal:  $\geq 50\%$  mortality or  $\geq 75\%$  knockdown, or if the nets are not effective: <50% mortality or < 75% knockdown.

## 3. Results

### 3.1. Characteristics of the study population and nets distribution

Of 432 households followed in the course of the study, 234 were from Bertoua urban centre and 198 from the rural area. The average number of children under 5 years old per household was 1.5 (297/198) in the rural area and 0.5 (119/234) in the urban site. Children between 5 and 15 years old were on average 1.95 (387/198) per household in the rural site and 0.7 (157/234) in the urban area (Table 2). Of the 198 head of households interviewed in the rural area, 63.2% had been to primary school only, whilst 107 of 227 interviewed in Bertoua city centre had attended high school. The majority of bed nets used by the population in both urban (92.5%) and rural (99.2%) households were obtained during mass distribution campaigns organized by the Ministry of Public Health (Table 1).

A total of 725 bed nets were documented during the survey with 314 in the rural site and 411 in the urban area. Of the 725 bed nets,

333 (45.93%) were already used and 392 (54.07%) were not yet used. The average number of bednets per household was 1.6 (314/198) in the rural area and 1.8 (411/234) in the urban site (Table 2). A total of 333 bed nets were inspected with 108 in the rural area and 225 in the urban area. Of the bed nets inspected, 108 (100%) nets in the rural area were Olyset Plus nets. In the urban area, 211 (93.8%) were Olyset Plus nets and 6.2% were other nets brands including Olyset (11/225), Yoorkol (1/225), Pandanet (1/225), and Permanet (1/225).

### 3.2. Use and handling of Olyset Plus nets in Bertoua

#### 3.2.1. Bed nets usage

A total of 46.4% (586/1263) people in the rural area and 90.7% (830/915) in the urban centre slept under bed nets the night before the interview. The proportion of children under five sleeping under nets was, 39.7% (118/297) in the rural area versus 89.1% (106/119) in the urban area. There was also less adults, 49.2% (285/579) using nets in the rural area compare to the urban settings 91.2% (583/639). A total of 81 participants in the rural area and 23 in the urban area admitted not using nets regularly. In the rural area, 91.4% (74/81) of people did not own any bed net. In the urban area, 43.5% (10/23) of the nets were too old and torn to be used (Table 3). The majority of Olyset Plus nets used were 6 months or one-year-old.

#### 3.2.2. Washing practices of Olyset Plus nets

A total of 88.1% (185/210) of people interviewed in the urban area versus only 38% (75/121) in the rural area reported they had washed their Olyset Plus nets at least once. Among these, 51.3% in the rural area and 82% in the urban site soaked the net before washing. The majority of bed nets was soaked for less than an hour in both sites (70.8% rural and 79.1% urban households). People in the rural area mostly used the local bar soap (56.5%) to wash bed nets while in the urban area, 62.4% of people used detergent powder and 1.1% used bleach for washing. Concerning the way of washing, 82.2% of people in the rural area and 86% of urban dwellers scrubbed gently their bed nets without beating on a hard surface. In the rural area bed nets were dried equally under the shade or under the sun. While in the urban area the majority (52.4%) of bed nets were dried outside in the shade (Fig. 1).

#### 3.2.3. Physical integrity of used Olyset Plus nets

The majority of bed nets 62.4% (63/101) in the rural area were dirty, while the majority 86.8% (190/219) in the urban area were clean. The analysis of the Hole Index in the laboratory indicated that 82.2% (162/197) of bed nets in the rural area and 88% (206/234) of nets in the urban area were in good state ( $HI < 64$ ). Only 5.6% and 6.8% of nets in the rural and urban areas respectively were badly torn ( $HI > 768$ ). Participant from the urban centre indicated washing their bed nets more frequently than those in the rural area. Frequent bed nets washing was the second main cause of damage of Olyset Plus nets in the urban area (21.7%). The torn bed nets were the main type of hole recorded on bed nets in the rural area (56.8%) and the urban area (71.4%). The majority of holes was on the roof of bed nets (rural = 35.7% and urban = 33%) and the front side of the net (rural = 32.2% and urban = 28.9%) while the remaining holes were found on the other sides of nets. The majority of bed nets in the rural area (56.8%) and 49.8% in the urban area had holes between 0.5 and 2 cm. Only a few nets had holes size above 25 cm (7.7% in the rural area and 10.9% in the urban area) (Table 4).

### 3.3. Bio-efficacy of field-collected Olyset Plus nets

#### 3.3.1. Effects of washing practices

Olyset Plus nets collected on the field were classified as washed and unwashed, soaked and unsoaked. The bio-efficacy of Olyset Plus nets was ranked as optimal:  $\geq 80\%$  mortality or  $\geq 95\%$  knockdown; minimal:  $\geq 50\%$  mortality or  $\geq 75\%$  knockdown, and not effective:  $< 50\%$  mortality or  $< 75\%$  knockdown. A total of 4688 *An. gambiae* Kisumu strain and 5194 *An. gambiae* field mosquitoes were used to carry bioassays. Globally, bioassays performed with *An. gambiae* Kisumu strain using washed or unwashed Olyset Plus nets gave mortalities  $\geq 80\%$  and knockdown  $\geq 95\%$ , traducing an optimal efficacy of the nets. Whereas when field mosquitoes (wild) were exposed to the nets, a minimal efficacy of nets was recorded with mortalities  $\geq 50\%$  and knockdown  $\geq 75\%$ .

#### 3.3.2. Effects of net soaking

Fig. 2 shows the impact of soaking on the bio-efficacy of Olyset Plus nets. Olyset Plus nets collected in the rural and urban areas

**Table 1**

Educational status of households interviewed in Bertoua and origin of nets.

Categories	Characteristics	Rural		Urban		Total	
		n/N	%	n/N	%	n/N	%
Head of household	Illiterate	9/161	5.6	12/227	5.3	21/388	5.4
	Primary school	100/161	62.1	19/227	8.4	119/388	30.8
	Secondary school	52/161	32.3	88/227	38.8	140/388	36.1
	University level	NA	NA	108/227	47.6	108/388	27.8
Origin of mosquito net	Bought	1/121	0.8	15/201	7.5	16/322	4.97
	Ministry of Public Health	120/121	99.2	186/201	92.5	306/322	95

N: total sample number, n: sample number.

**Table 2**  
Net coverage by locations and age groups in Bertoua.

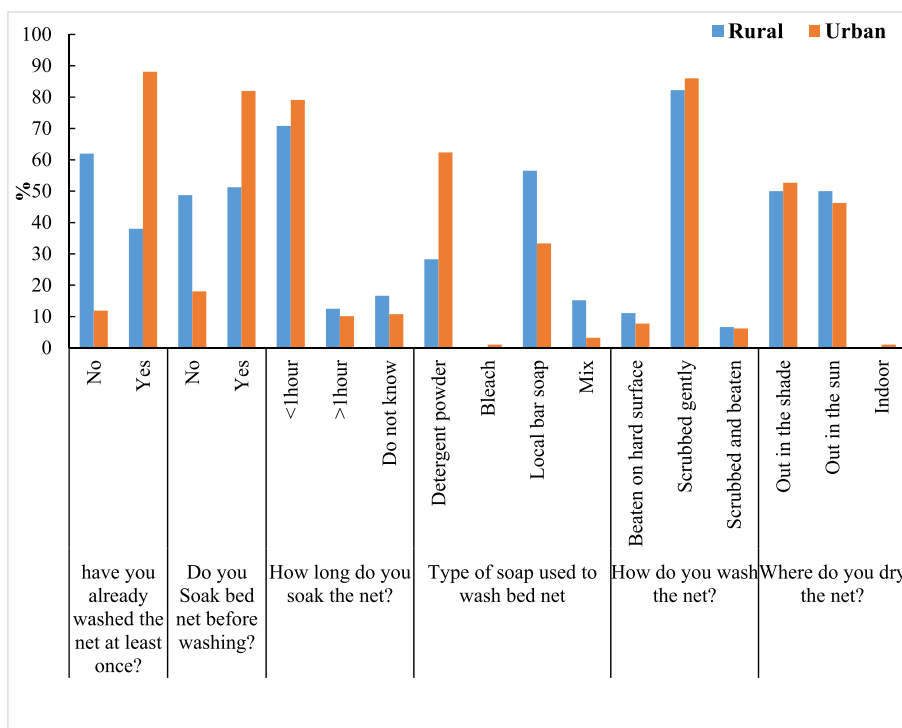
Categories	Characteristics	Rural		Urban		Total	
		n/N	Average	n/N	Average	n/N	Average
Number of head of households interviewed		198	–	234	–	432	–
Average number of Bed nets per household		314/198	1.59	411/234	1.76	725/432	1.68
Average number of people per household	<5 years	297/198	1.50	119/234	0.51	416/432	0.96
	5-15 years	387/198	1.95	157/234	0.67	544/432	1.26
	>15 years	579/198	2.92	639/234	2.73	1218/432	2.82

N: total sample number, n: sample number

**Table 3**  
Usage of bed nets in households participating in the study.

Categories	Characteristics	Rural		Urban		Total	
		n/N	%	n/N	%	n/N	%
People who slept under bed net the night before the survey		586/1263	46.4	830/915	90.7	1416/2178	65
	<5 years	118/297	39.7	106/119	89.1	224/416	53.9
People who slept under bed net last night according to age range	5-15 years	183/387	47.3	141/157	89.8	324/544	59.6
	>15 years	285/579	49.2	583/639	91.2	868/1218	71.3
	Heat	0	0.0	8/23	34.8	8/104	7.7
Reason for not using nets	Torn/Old	7/81	8.6	10/23	43.5	17/104	16.4
	Absence	74/81	91.4	5/23	21.7	79/104	76
	Not used	26/126	20.6	4/215	1.9	30/341	8.8
Frequency of bed net usage last week	Most nights	8/126	6.4	10/215	4.7	18/341	5.3
	Some nights	4/126	3.2	5/215	0.9	9/341	2.6
	Every night	88/126	69.8	196/215	91.2	284/341	83.3
Periods when bed nets are used	Rainy season	2/105	1.9	14/214	6.5	16/319	5
	Dry season	0	0.0	3/214	1.4	3/319	0.9
	All year	103/105	98.1	197/214	92.1	300/319	94

Most nights:  $\geq 5$  nights, Some nights:  $\leq 4$  nights, Every night: =7 nights, N: total sample number, n: sample number



**Fig. 1.** Washing practices in Bertoua urban and rural area (Mix = more than one type of soap).

**Table 4**  
Status of used Olyset Plus nets in the urban and rural area.

Categories	Characteristics	Rural		Urban		Total	
		n/N	%	n/N	%	n/N	%
Olyset nets appearance	Clean	38/101	37.6	190/219	86.8	228/320	71.3
	Dirty	63/101	62.4	29/219	13.2	92/320	28.8
Olyset nets state	Good	162/198	81.8	206/234	88	368/432	85.2
	Damaged	24/198	12.1	12/234	5.1	36/432	8.3
	Badly torn	12/198	6.1	16/234	6.8	28/432	6.5
	Fire	3/39	7.7	3/60	5.0	6/99	6.1
Origin of holes	Slipping	13/39	33.3	24/60	40.0	37/99	37.4
	Washing	0	0.0	13/60	21.7	13/99	13.1
	Object	10/39	25.6	12/60	20.0	22/99	22.2
	mix	13/39	33.3	8/60	13.3	21/99	21.2
Type of holes	Burn holes	1/37	2.7	1/49	2	2/86	2.3
	Tears	21/37	56.8	35/49	71.4	56/86	65.1
	Seams holes	0	0.0	1/49	2	1/86	1.2
	Holes at hanging points	10/37	27	8/49	16.3	18/86	20.9
Position of holes	Mix	5/37	13.5	4/49	8.2	9/86	10.5
	Roof	102/285	35.8	154/466	33.1	256/751	34.1
	width	82/285	28.8	108/466	23.2	190/751	25.3
	length	92/285	32.3	135/466	30	227/751	30.2
Size of holes	seams	9/285	3.1	69/466	14.9	78/751	10.4
	Size1 ([0,5-2 cm ])	162/285	56.8	232/466	49.8	394/751	52.5
	Size2 ([2-10 cm ])	39/285	13.7	108/466	23.2	147/751	19.6
	Size3 ([10-25 cm ])	62/285	21.8	75/466	16.1	137/751	18.2
	Size4 ( $\geq 25$ cm)	22/285	7.7	51/466	10.9	73/751	9.7

Mix = more than one parameter on the same bed net, cm = centimetre. N: total sample number, n: sample number

(unsoaked, unwashed, and washed) were found to, induce a similar knockdown and mortality rate when mosquitoes of the Kisumu strain ( $n = 3181$ ) were exposed to these nets (chi-squared ( $P > 0.05$ )). Olyset Plus nets from rural area soaked before washing were found to kill more mosquitoes ( $72 \pm 3.50\%$ ) compared to unsoaked nets ( $63.7 \pm 3.49\%$ ) ( $\chi^2 = 7.228$ ,  $P = 0.0072$ ). In the urban area, unsoaked nets were found to induce a higher mortality rate compared to nets soaked before washing ( $\chi^2 = 21.6151$ ,  $P < 0.0001$ ) (Fig. 2b).

### 3.3.3. Potential effects of type of soap used

The average mortality rate of field mosquitoes when exposed to Olyset Plus nets collected from the rural area was  $62.6 \pm 4.1\%$  ( $n = 524$ ) when washed with detergent and  $64.8 \pm 3.1\%$  ( $n = 933$ ) when washed with local soap bar. These mortality rates were not significantly different from those induced by unwashed nets ( $\chi^2 = 0.241$ ,  $P = 0.6238$ ). Olyset Plus nets collected in the urban area induced a higher mortality rate when bed nets were washed with local bar soap ( $70.4 \pm 2.9\%$ ) compared to when they were washed with other detergent ( $54.9 \pm 3.2\%$ ) or when they were unwashed ( $47 \pm 4.3\%$ ,  $\chi^2 = 41.920$ ,  $P < 0.0001$ ) (Fig. 3b).

### 3.3.4. Effects of drying practices

Olyset Plus nets collected in the rural area which were dried outdoor in the shade induce a mortality rate of  $64.2 \pm 4.4\%$  to field mosquitoes similar to nets dried under the sun ( $63.5 \pm 4.1\%$ ) ( $\chi^2 = 3.190$ ,  $P = 0.0741$ ). However, in the urban area, Olyset Plus nets dried under the sun induced a lower mortality rate to field mosquitoes ( $62.7 \pm 2.1\%$ ), compared to those dried out in the shade ( $78.4 \pm 8\%$ ), ( $\chi^2 = 8.009$ ,  $P = 0.0047$ ) (Fig. 4 a, b).

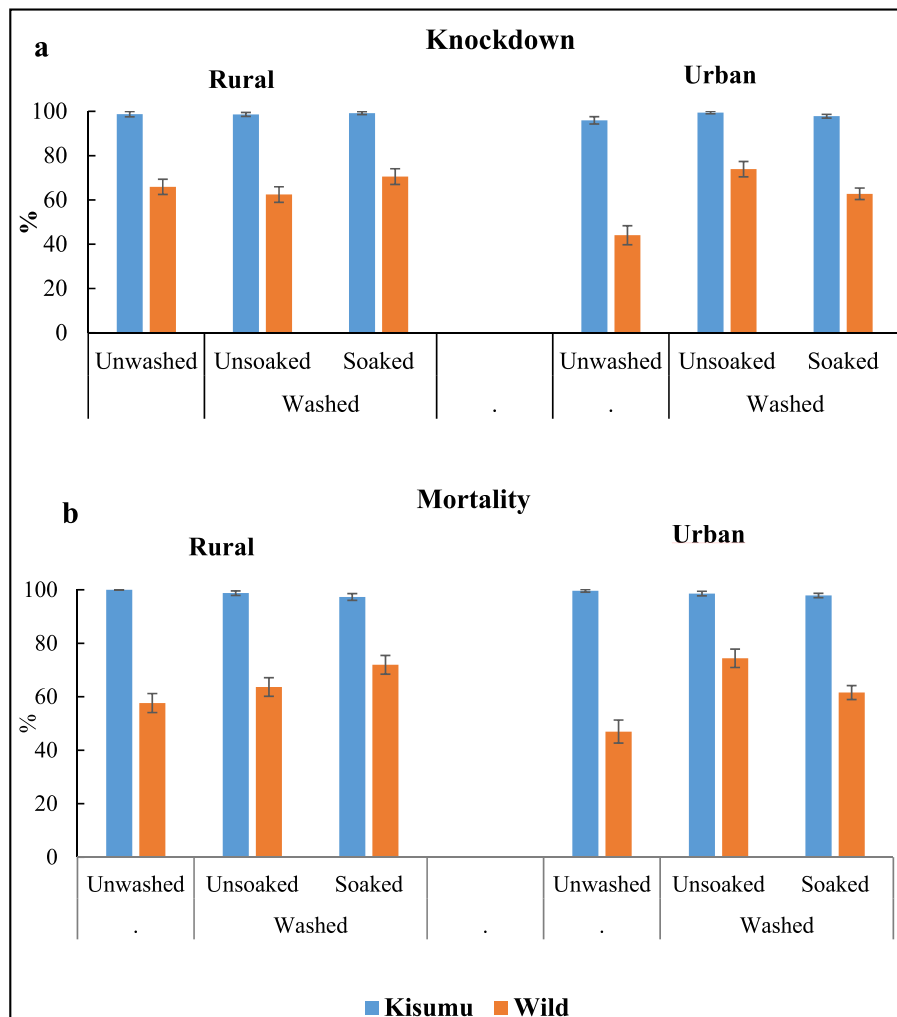
### 3.3.5. Effects of number of washing time

Olyset Plus nets were washed a maximum of 20 times in the rural sites and 5 times in the urban sites. Bed nets that have never been washed induced a lower mortality rate ( $65.9 \pm 3.4\%$ ) compared to those washed up to 10 times ( $86.7 \pm 6.5\%$ ) ( $\chi^2 = 31.315$ ,  $P < 0.0001$ ). Similar observation was made with bed nets collected in both rural and urban areas ( $\chi^2 = 49.690$ ,  $P < 0.0001$ ) (Fig. 5b).

## 4. Discussion

The spread of pyrethroid resistance in mosquito populations threatens to undermine malaria control efforts. The management of pyrethroid resistance could be significantly enhanced through the use of bed nets impregnated with both pyrethroid and synergists such as Piperonyl butoxide (PBO) which act by enhancing the potency of insecticides. PBO nets represent a new tool with the capacity to affect pyrethroid resistant mosquito populations (Boussougou-Sambe et al., 2017; Menze et al., 2020; Pennetier et al., 2013; Shono et al., 2017). Although the efficacy of PBO nets have been assessed in different field trials (Birhanu et al., 2019; Protopopoff et al., 2018; Lukole et al., 2022) yet there is limited informations on their life span and their washing resistance in communities. The present study was conducted to assess the durability and the effect of repeated washing on Olyset plus nets bioefficacy one year after their distribution to communities in Cameroon. Olyset Plus nets manufactured by Sumitomo Chemical are a polyethylene nets treated with

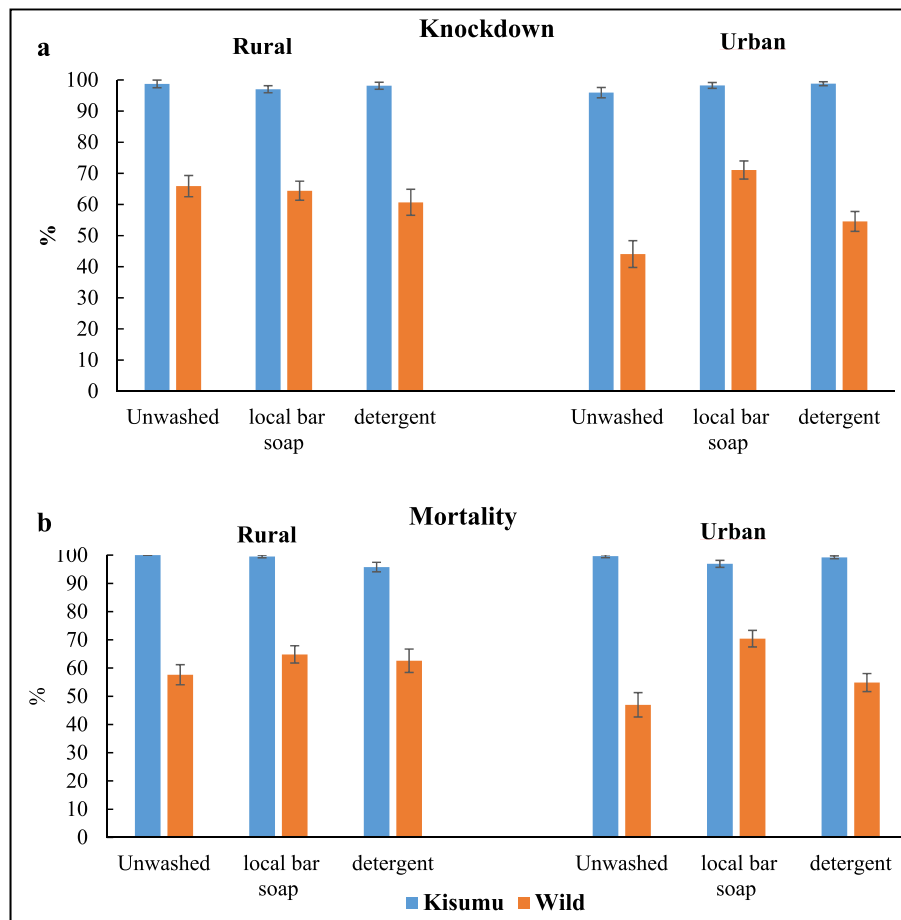




**Fig. 2.** Impact of the soaking (a, b) on the bioefficacy of Olyset Plus nets against field *An. gambiae* and Kisumu strain (a = Knockdown, b = Mortality).

permethrin (20 g/kg + 25%) and PBO (10 g/kg + 25%) across the whole net (Organization, W. H, 2003; Organization, W. H, 2012). A high proportion of PBO nets in both the urban centre and the rural areas were in good state supporting good handling of nets by the population. However low usage rate of bed nets in the rural areas compare to urban settings was recorded. In rural area, the main reason why people were not using net was the fact that they did not own any bed net, while in urban area, the main reason pushing people not to use nets was the fact that their nets were old and torn or the sensation of excessive heat when sleeping under a bed net. In Cameroon bed net usage rate is estimated to be close to 60% (Antonio-Nkondjio et al., 2019) and stress the need for regular sensitization campaigns towards communities.

Unwashed Olyset Plus nets were found to display a lower bioefficacy against wild mosquitoes (low mortality rate) compared to nets washed at least once. Such a low efficacy could result from the presence of dirt at the nets surface reducing the diffusion of insecticide. The influence of dirt and fume on the efficacy of LLINs have been reported in previous studies (Etang et al., 2013). People residing in the city of Bertoua were found to wash their bed nets more regularly than those from the rural setting. This could result from the fact that in urban areas water is piped into people's houses whereas in rural areas it is available at manually operated pumps or from wells, making it a much more precious commodity. It also came out from the study that the bio-efficacy of bed nets was affected by the frequency of washing, the duration of soaking, and the type of soap used for washing nets. Soaking bed nets before washing allows removing much dirt from the bed nets. Different soap or detergent used for washing nets were also documented and could affect bed nets efficacy. It is possible that the combination of soaps could enhance insecticide release, but this was also reported to damage bed nets or to directly affect insecticide compounds released (Vatandoost et al., 2009). The inspection of nets permitted the detection of many holes in some of the nets. Some of the holes resulted from harsh washing practices. Indeed, although LLINs could resist frequent and different washing practices, the use of some aggressive detergent or harsh washing practices could affect the integrity and



**Fig. 3.** Bioefficacy of Olyset Plus net washed with different type of soap in urban and rural area (a = Knockdown, b = Mortality).

durability of nets. Other practices such as the drying of nets under sun light, or playing under the nets by children were found to effect bed nets durability. Drying bed nets outdoors under the shade conserve their efficacy better than when they are dried under the sun. UV light from sunlight is known to degrade pyrethroids (Kayedi et al., 2008; Ouattara et al., 2013; Snow et al., 1988). It is rather possible that standard WHOPEs washing protocol could underestimate the real amount of insecticide washed from LLINs compared to traditional washing practices in the community (Atieli et al., 2010a, 2010b). Different poor usage practices not all documented in this study, could be influencing bed nets durability and physical integrity and are consistent with previous studies conducted on the same subject (Fru et al., 2021; Graves et al., 2011; Mboma et al., 2021; Moon et al., 2016).

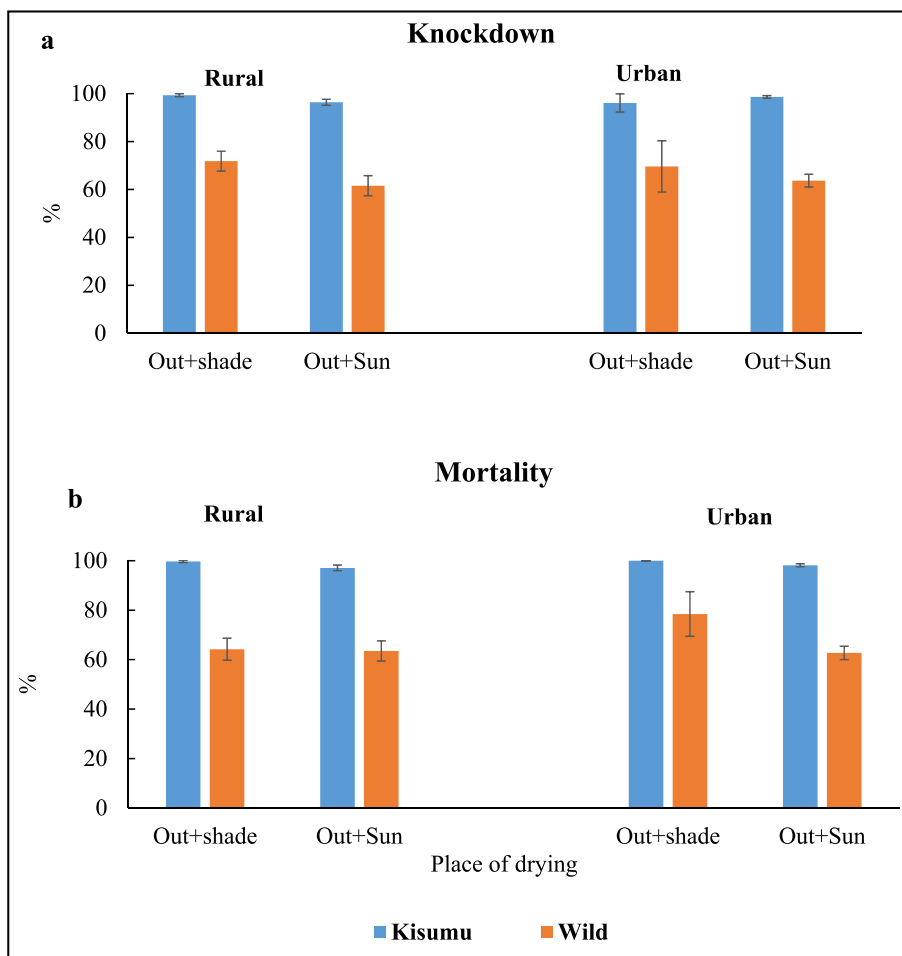
Laboratory experiments assessing the bioefficacy of Olyset Plus nets compare to Olyset nets (bed net without PBO) indicated a high efficacy of Olyset Plus nets compare to Olyset nets during the first wash. Olyset plus nets are considered to have a great availability of permethrin on the surface of nets fibers compare to Olyset (Etang et al., 2013; Protopopoff and Rowland, 2018; Skovmand, 2018). The low bioefficacy of Olyset plus nets recorded after the third wash is in contradiction with studies conducted to date; this may be due to differences in handling and washing practices between communities, which merit further study.

The present study has these as limits: it did not quantify the amount of residual PBO on the nets this would have permitted to assess the effect of different washing or handling practices. Also since the study was a field assessment, it is possible that some factors not measured may have introduced some bias. A controlled study in the laboratory may have been more indicated to assess the effect of different washing practices.

## 5. Conclusions

The study highlighted the high insecticide efficacy of Olyset Plus nets collected in the field. However, inappropriate washing and handling practices such as soaking bed nets, frequent harsh washing, sun drying, were found to significantly affect bed nets bio-efficacy and durability in rural and urban settings. Therefore, the association of LLIN mass deployment programs with a routine monitoring and sensitization of communities on best practices concerning bed nets usage and handling could help improve vector control programs and insecticides resistance management. Since nets are not surviving the 3 years recommended by the WHO this may be of significant





**Fig. 4.** Drying practices and bioefficacy of the Olyset Plus net in urban and rural area (Out-shade = Outdoor in the shade, Out-sun = Outdoor in the sun, a = Knockdown, b = Mortality).

concern in the future given the state of the World at the moment (climate change and the different wars now taking place). It is likely that the provision of nets will no longer be assured. The roll out of malaria vaccines also means that there could be a change in emphasis in the future despite the reality that vector control is key to malaria control or elimination and all this needs to be considered.

#### Ethics approval and consent to participate

The study was conducted under the ethical clearance N°2020/04/1209/CE/CNERSH/SP delivered by the Cameroon National Ethics (CNE) Committee for Research on Human Health.

#### Consent for publication

Not applicable.

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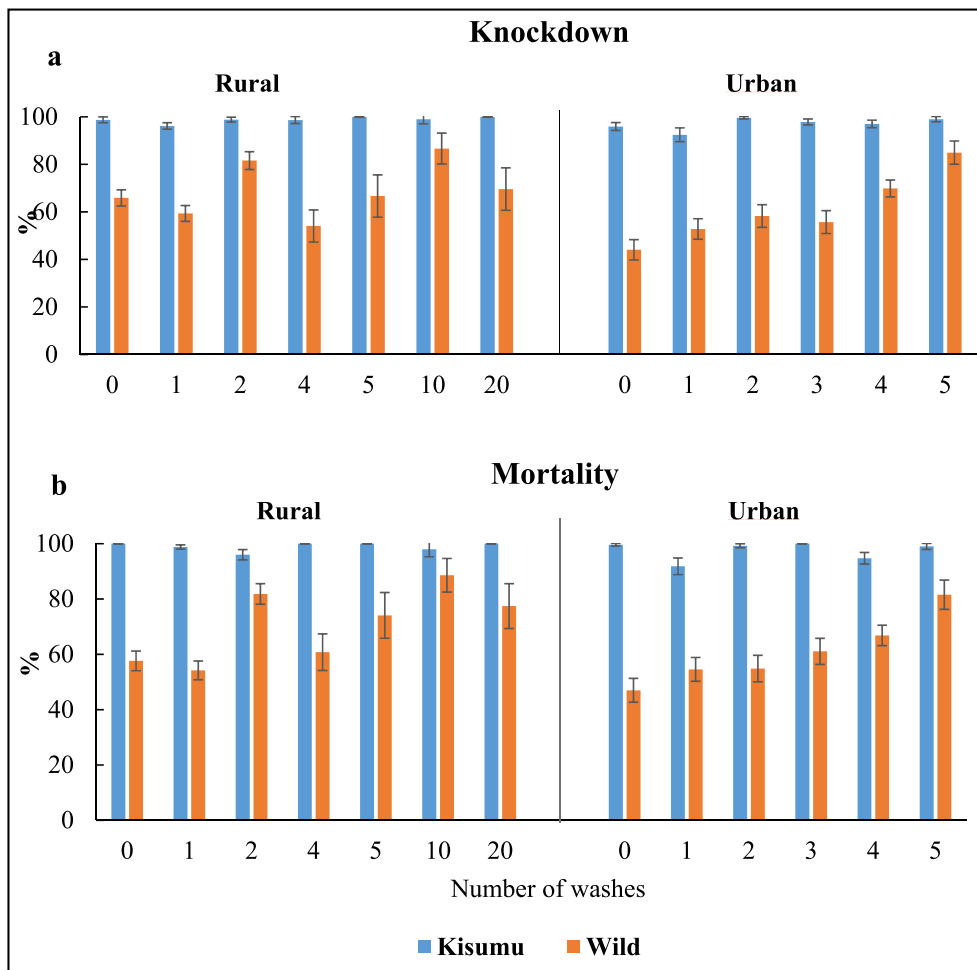


Fig. 5. Bioefficacy of field collected bed nets according to the number of washings (a = Knockdown, b = Mortality).

participation to the study.

#### CRediT authorship contribution statement

**Diane Leslie Nkahe:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Writing – original draft. **Edmond Kopya:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – review & editing. **Nasser Idriss Ngangue Siewe:** Writing – review & editing, Investigation. **Paulette Ndjeunia Mbiakop:** Investigation, Writing – review & editing. **Nelly Armanda Kala Chouakeu:** Investigation, Writing – review & editing. **Rémy Mimpfoundi:** Writing – review & editing. **Sévilor Kekeunou:** Writing – review & editing. **Parfait Awono-Ambene:** Writing – review & editing. **Christophe Antonio-Nkondjio:** Conceptualization, Formal analysis, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

#### Declaration of competing interest

None.

#### Data availability

Data generated on behalf of this study are included in this published article.

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