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

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**Research Article**

**Keywords:** PBO-pyrethroid-treated nets, durability, Bioassays, Anopheles gambiae s.l., Bertoua, Cameroon

**Posted Date:** July 22nd, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1794328/v1

## 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 (89.08% (106/119) compared to the rural area (39.73% (118/297). The majority of Olyset-Plus nets inspected 82.23% (162/197) in the rural area and 88.03% (206/234) in the urban centre were in good condition (Hole Index < 64). Only 5.58% and 6.84% of nets were badly torn in rural and urban sites respectively. Nets were washed more regularly in the urban centre. 88.10% of urban dwellers reported having washed their nets at least once compared to only 61.98% of rural dwellers. Bioefficacy tests with nets indicated a mortality rate ranging from 65.89% for unwashed nets to 86.66% 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 and soaking status (washed or unwashed, 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 on best practices concerning bed nets usage and handling during mass distribution might enhance the net durability in the field.

## Background

Long-lasting insecticidal bed nets (LLINs) and indoor residual spraying (IRS) remain the main tools for malaria prevention and control (Castellanos et al., 2021; WHO, 2021). Bed nets provides protection to individual users and to the entire community when the level of coverage of the population is high (WHO,

2011). Since 2000 the massive scale up of LLINs across sub-Saharan Africa, led to significant decrease of malaria morbidity and mortality (Bhatt et al., 2015; Griffin et al., 2016; WHO, 2020; Weiss et al., 2019). However, the situation has stalled since 2016 with many regions reporting no reduction or increase in malaria cases (WHO, 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; WHO, 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 requested (WHO, 2013).

Pyrethroid resistance is actually considered as the major cause for LLINs sub-performance (Hemingway & Ranson, 2000; WHO, 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, encountering for 39.6% of childhood mortality and up to 69% of morbidity cases in health care facilities (PNLP, 2019), pyrethroid nets are still the main preventive tool used by the population (Antonio-Nkondjio et al., 2019). It is estimated that more than 80% of household own at least a 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 vectors 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; Doumbe-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 (WHO, 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 there have been over recent years 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 not

enough information on pyrethroids + PBO nets durability. This information is highly relevant for improving malaria control strategies on 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 further deserve 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.

# Material And Methods

### 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 the 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 small rainy season from mid-March to mid-May, a small 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 degrees 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). *Anopheles gambiae* sensu lato is the main malaria vector group species. The study took place in the urban centre of Bertoua (Enia and Kano) and in a close rural location (Ndoumbi I).

### 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 and 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 (WHO, 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 greater than 25 cm). Holes with a diameter lesser than 0.5 cm were not counted. Some of the

nets Olyset Plus nets were collected for bio efficacy tests at the malaria research laboratory of OCEAC Yaoundé.

### Net sampling and mosquito collection:

Anopheline larvae were collected from aquatic suitable habitats 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. These field strains are known as pyrethroid-resistant. Larvae were fed with Tetramin® Baby fish food until pupae. Pupae were collected in plastic cups and placed in netting cages for adult emergence. Adults emerging from pupae were allowed to feed on a 10% sucrose solution until the bioassay test was conducted.

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 (WHO, 2011). Five pieces (25 cm x 25 cm) of nets were cut on different sides of each net and kept in aluminium foil at 4°C for bioassays. Five non-blood-fed adult (2 to 5 days-old) *Anopheles gambiae* s.l. females deriving from larvae collected on the field were exposed to the nets using plastic cones fixed on each piece of the netting for 3 min and transferred to observation cups. Nets bioefficacy was also tested against Kisumu (*Anopheles gambiae*) susceptible strain used as control. Test with Olyset nets were also conducted. Knockdown was recorded 60 min after exposure. Mosquitoes were after each test kept in observation and allowed to feed on a 10% sugar solution. The mortality rate was recorded 24 h after exposure.

### Data analysis

Data collected were entered into a Microsoft Excel 2013 database and cleaned from inconsistencies. 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 × no. of size-1 holes) + (23 × no. of size-2 holes) + (196 × no. of size-3 holes) + (578 × 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 (less than 100 cm² of holed area); ii) moderately damaged if 64 <HI <768; and iii) badly torn if HI >768 (more than 0.1 m² 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: ≥80% mortality or ≥95% knockdown, minimal: ≥50% mortality or ≥75% knockdown, or if the nets are not effective: <50% mortality or <75% knockdown.

# Results

### 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 1.50 (297/198) in rural area and 0.51 (119/234) in urban site. Children between 5 to 15 years old were on average 1.95 (387/198) per household in rural site and 0.67 (157/234) in urban area (Table 2). Of the 198 head of households interviewed in rural area, 63.23% had the primary school level. The majority of those interviewed in the Bertoua city centre (108/227) had the higher education level. The majority of bed nets used by the population in both urban (92.54%) and rural (99.17%) households were obtained during mass distribution campaigns organised by the Ministry of public health (Table 1).

A total of 725 bed nets (45.93% of the nets already used and 54.07% of those not yet used) were documented during the survey, with 314 in rural site and 411 in urban area. The average number of bednets per household was 1.59 (314/198) in rural area and 1.76 (411/234) in urban site (Table 2). Of the bed nets inspected, 108 (100%) nets in rural area were Olyset Plus nets. In the urban area, 211 (93.78%) were Olyset Plus nets and 6.22% were other nets brands including Olyset (11/225), Yoorkol (1/225), Pandanet (1/225), and permanent (1/225).

**Table 1: Educational status of households interviewed in Bertoua and origin of nets**

| | | Rural | | Urban | | Total | |
|---|---|---|---|---|---|---|---|
| **Categories** | **Characteristics** | **n/N** | **%** | **n/N** | **%** | **n/N** | **%** |
| **Head of household** | Illiterate | 9/161 | 5.59 | 12/227 | 5.29 | 21/388 | 5.41 |
| | Primary school | 100/161 | 62.11 | 19/227 | 8.37 | 119/388 | 30.67 |
| | Secondary school | 52/161 | 32.30 | 88/227 | 38.77 | 140/388 | 36.08 |
| | University level | NA | NA | 108/227 | 47.58 | 108/388 | 27.84 |
| **Origin of mosquito net** | Bought | 1/121 | 0.83 | 15/201 | 7.46 | 16/322 | 4.97 |
| | Ministry of Public Health | 120/121 | 99.17 | 186/201 | 92.54 | 306/322 | 95.03 |

N: Category number (total); n: characteristic number

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

| | | Rural | | Urban | | Total | |
|---|---|---|---|---|---|---|---|
| Categories | Characteristics | 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 | ≤5years | 297/198 | 1.5 | 119/234 | 0.51 | 416/432 | 0.96 |
| | 5-15years | 387/198 | 1.95 | 157/234 | 0.67 | 544/432 | 1.26 |
| | ≥15years | 579/198 | 2.92 | 639/234 | 2.73 | 1218/432 | 2.82 |

N: Category number (total); n: characteristic number

### Used and Handling of PBO nets Olyset Plus in Bertoua

### Bed nets usage

A total of 46.40% (586/1263) people in rural area and 90.71% (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, 89.08% (106/119) in urban area versus 39.73% (118/297) in rural area. There was also less adults, 49.22% using nets in rural area compare to urban settings (91.24%). A total of 81 participants in rural area and 23 in urban area admitted not using nets regularly. In rural area, the main reason why people were not using net was the fact that they did not own any bed net 91.36% (74/81); 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 (Table 3). The majority of Olyset Plus nets used were 6 months or one-year-old.

### 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 | 46.40 | 830/915 | 90.71 | 1416/2178 | 65.01 |
| People who slept under bed net last night according to age range | ≤5years | 118/297 | 39.73 | 106/119 | 89.08 | 224/416 | 53.85 |
| | 5-15years | 183/387 | 47.29 | 141/157 | 89.81 | 324/544 | 59.56 |
| | ≥15years | 285/579 | 49.22 | 583/639 | 91.24 | 868/1218 | 71.26 |
| | | | | | | | |
| Reason for not using nets | Heat | 0 | 0.00 | 8/23 | 34.78 | 8/104 | 7.69 |
| | Torn/Old | 7/81 | 8.64 | 10/23 | 43.48 | 17/104 | 16.35 |
| | Absence | 74/81 | 91.36 | 5/23 | 21.74 | 79/104 | 75.96 |
| | | | | | | | |
| Frequency of bed net usage last week | Not used | 26/126 | 20.63 | 4/215 | 1.86 | 30/341 | 8.80 |
| | Most nights | 8/126 | 6.35 | 10/215 | 4.65 | 18/341 | 5.28 |
| | Some nights | 4/126 | 3.17 | 5/215 | 0.93 | 9/341 | 2.64 |
| | Every night | 88/126 | 69.84 | 196/215 | 91.16 | 284/341 | 83.28 |
| | | | | | | | |
| Periods when bed nets are used | Rainy season | 2/105 | 1.90 | 14/214 | 6.54 | 16/319 | 5.02 |
| | Dry season | 0 | 0.00 | 3/214 | 1.40 | 3/319 | 0.94 |
| | All year | 103/105 | 98.10 | 197/214 | 92.06 | 300/319 | 94.04 |

Most nights: ≥5 nights, Some nights: ≤4 nights, Every night: =7 nights, N: Category number (total); n: characteristic number

### Washing practices of Olyset Plus nets

A total of 88.10% (185/210) of people interviewed in urban area versus only 38.02% (75/121) rural area reported to have washed their Olyset Plus nets at least once. Among these, 51.28% in rural area and 81.98% in urban site soaked the net before washing. The majority of bed nets were soaked for less than an hour in both sites (70.83% rural and 79.11% urban households). People in rural area mostly used the local bar soap (56.52%) to wash bed nets while in urban area, 62.37% of people used detergent powder and 1.08% used bleach for washing. Concerning the way of washing, 82.22% of people in rural area and 86.01% of urban dwellers scrubbed gently their bed nets without beating on a hard surface. After washing

the majority (52.41%) of bed nets were dried outside in the shade, while in rural area bed nets were dried equally under the shade or under the sun.

### Physical integrity of used Olyset Plus nets

The majority of bed nets 62.40% (63/101) in rural area were dirty, while the majority 86.76% (190/219) in urban area were clean. The analysis of the Hole Index in the laboratory indicated that 82.23% (162/197) of bed nets in rural area and 88.03% (206/234) of nets in urban area were in good state (HI<64). Only 5.58% and 6.84% of nets in 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 rural area. Frequent bed nets washing was the second main cause of damage of Olyset Plus nets in urban area (21.70%). The torn bed nets were the main type of hole recorded on bed nets in rural area (56.80%) and urban area (71.40%). The majority of holes was on the roof of bed nets (rural=35.66% and urban=32.98%) and the front side of the net (rural=32.17% and urban=28.91%) while the remaining holes were found on the other sides of nets. The majority of bed nets in rural area (56.84%) and 49.79% in urban area had holes between 0.5 and 2 cm. Just few nets had holes size above 25cm (7.72% in rural area and 10.94% in urban area) (table 4).

### Table 4: Status of used Olyset Plus nets in urban and rural area

| Categories | Characteristics | Rural | | Urban | | Total | |
|---|---|---|---|---|---|---|---|
| | | n/N | % | n/N | % | n/N | % |
| Olyset nets appearance | Clean | 38/101 | 37.62 | 190/219 | 86.76 | 228/320 | 71.25 |
| | Dirty | 63/101 | 62.38 | 29/219 | 13.24 | 92/320 | 28.75 |
| | | | | | | | |
| Olyset nets state | Good | 162/198 | 81.82 | 206/234 | 88.03 | 368/432 | 85.19 |
| | Damaged | 24/198 | 12.12 | 12/234 | 5.13 | 36/432 | 8.33 |
| | Badly torn | 12/198 | 6.06 | 16/234 | 6.84 | 28/432 | 6.48 |
| | | | | | | | |
| | Fire | 3/39 | 7.69 | 3/60 | 5.00 | 6/99 | 6.06 |
| Origin of holes | Slipping | 13/39 | 33.33 | 24/60 | 40.00 | 37/99 | 37.37 |
| | Washing | 0 | 0.00 | 13/60 | 21.67 | 13/99 | 13.13 |
| | Object | 10/39 | 25.64 | 12/60 | 20.00 | 22/99 | 22.22 |
| | mix | 13/39 | 33.33 | 8/60 | 13.33 | 21/99 | 21.21 |
| | | | | | | | |
| | Burn holes | 1/37 | 2.70 | 1/49 | 2.04 | 2/86 | 2.33 |
| Type of holes | Tears | 21/37 | 56.76 | 35/49 | 71.43 | 56/86 | 65.12 |
| | Seams holes | 0 | 0.00 | 1/49 | 2.04 | 1/86 | 1.16 |
| | Holes at hanging points | 10/37 | 27.02 | 8/49 | 16.33 | 18/86 | 20.93 |
| | Mix | 5/37 | 13.51 | 4/49 | 8.16 | 9/86 | 10.47 |
| | | | | | | | |
| Position of holes | Roof | 102/285 | 35.79 | 154/466 | 33.05 | 256/751 | 34.09 |
| | width | 82/285 | 28.77 | 108/466 | 23.18 | 190/751 | 25.30 |
| | length | 92/285 | 32.28 | 135/466 | 29.97 | 227/751 | 30.23 |
| | seams | 9/285 | 3.1 | 69/466 | 14.85 | 78/751 | 10.39 |
| Size of holes | Size1 ([0,5-2cm [) | 162/285 | 56.84 | 232/466 | 49.79 | 394/751 | 52.46 |
| | Size2 ([2-10cm [) | 39/285 | 13.68 | 108/466 | 23.18 | 147/751 | 19.57 |
| | Size3 ([10-25cm [) | 62/285 | 21.75 | 75/466 | 16.09 | 137/751 | 18.24 |
| | Size4 (≥25cm) | 22/285 | 7.72 | 51/466 | 10.94 | 73/751 | 9.72 |

Mix=more than one parameter on the same bed net, cm=centimetre. N: Category number (total); n: characteristic number

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

### 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: ≥80% mortality or ≥95% knockdown; minimal: ≥50% mortality or ≥75% knockdown, and not effective: <50% mortality or <75% knockdown. Globally, bioassays performed with Kisumu using washed or unwashed Olyset Plus nets gave mortalities≥80% and knockdown≥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≥50% and knockdown≥75%.

### Effects of net soaking

Figure 2 shows the impact of soaking on the bio-efficacy of Olyset Plus nets. Olyset Plus nets collected in rural and urban areas (unsoaked, unwashed, washed) were found to, induce a similar knockdown and mortality rate when mosquitoes of the Kisumu strain 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 (71.95±3.50%) compare to unsoaked nets (63.65±3.49) ($\chi^2$=7.228, P=0.0072). In urban area, unsoaked nets were found to induce a higher mortality rate compare to nets soaked before washing ($\chi^2$=21.6151, P<0.0001) (Figure 2b).

### 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 62.60±4.14% when washed with detergent and 64.84±3.06% 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 urban area induced a higher mortality rate when bed nets were washed with local bar soap (70.42±2.94%) compare to when they were washed with other detergent (54.86±3.19%) or when they were unwashed (46.98±4.32%, $\chi^2$= 41.920, P<0.0001) (Figure 3 b).

### Effects of drying practices

Olyset Plus nets collected in rural area which were dried outdoor in the shade induce a mortality rate of 64.21±4.44% to field mosquitoes similar to nets dried under the sun (63.50±4.09%) ($\chi^2$= 3.190, P = 0.0741). However, in urban area, Olyset Plus nets dried under the sun induced a lower mortality rate to field mosquitoes (62.72±2.14%), compare to those dried out in the shade (78.43±7.98%), ($\chi^2$=8.009, P = 0.0047) (Figure 4 a, b).

### Effects of number of washing time

Olyset Plus nets were washed a maximum of 20 times in rural sites and 5 times in urban sites. Bed nets that have never been washed induced a lower mortality rate (65.89±3.42%) compare to those washed up to 10 times (86.66±6.50%) ($\chi^2$=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) (figure5b).

# Discussion

The spread of pyrethroid resistance in mosquito populations threatens to undermine malaria control efforts. The management of pyrethroid resistance could be significantly enhance 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 now 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 PBO nets efficacy have been assessed in different field trials (Birhanu et al., 2019; Protopopoff et al., 2018), yet little is known of the durability of PBO nets 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 net treated with permethrin (20 g/kg + 25%) and PBO (10 g/kg + 25%) across the whole net (WHO, 2003, 2012). A high proportion of PBO nets in both the urban centre and rural area were in good state supporting good handling of nets by the population. However low usage rate of bed nets in rural area compare to urban settings was recorded and could result from the low nuisance of mosquitoes during this particular season (dry season). 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 compare 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 rural setting. It also came out from the study that the bio-efficacy of bed nets was also 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 use 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 affect directly 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 to frequent and different washing practices, the use of some aggressive detergent or harsh washing practices could affect the integrity and 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 outdoor 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 underestimates the real amount of insecticide washed from LLINs compared to traditional washing practices in the community (Atieli et al., 2010a, b). 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 & Rowland, 2018; Skovmand, 2018). The low bioefficacy of Olyset plus nets recorded above the 3rd wash is in contradiction with studies conducted so far and deserve further investigations.

# Conclusions

The study highlighted the high insecticide efficacy of Olyset Plus nets collected on the field. However, inappropriate washing and handling practices in rural and urban settings were found to significantly affect bed nets bio-efficacy and durability. 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.

# 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

# Declarations

**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

**Availability of data and material:** Data generated on behalf of this study are included in this published article.

**Competing interests:** The authors declare that they have no competing interests.

**Funding:** This work received financial support from National Institute for Health Research (NIHR) [56566 / 6565], as part of Royal Society of Tropical Medicine & Hygiene's (RSTMH) 2020 small grants programme in Public Health and Tropical Medicine.

*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

**Authors' contribution: DLN**: Conceptualization, Project Supervision, Methodology, Investigation, Visualization, Data Curating, Formal Analysis, Writing – Original Draft Preparation, Funding Acquisition; **EK**: Conceptualization, Investigation, Methodology Visualization, Writing – Original Draft Preparation; **INNS**: Investigation, Review & Editing; **PNM:** Investigation, Review & Editing; **NAKC:** Review & Editing; **PAA**: Writing – Review & Editing; **CAN**: Conceptualization, Project Administration, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing. All authors read and approved the final manuscript. **DLN** and **CAN** are guarantors of the paper.

**Aknowledgements:** We are grateful to the population of Bertoua for their participation to the study.

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# Supplementary

Additional File is not available with this version

# Figures

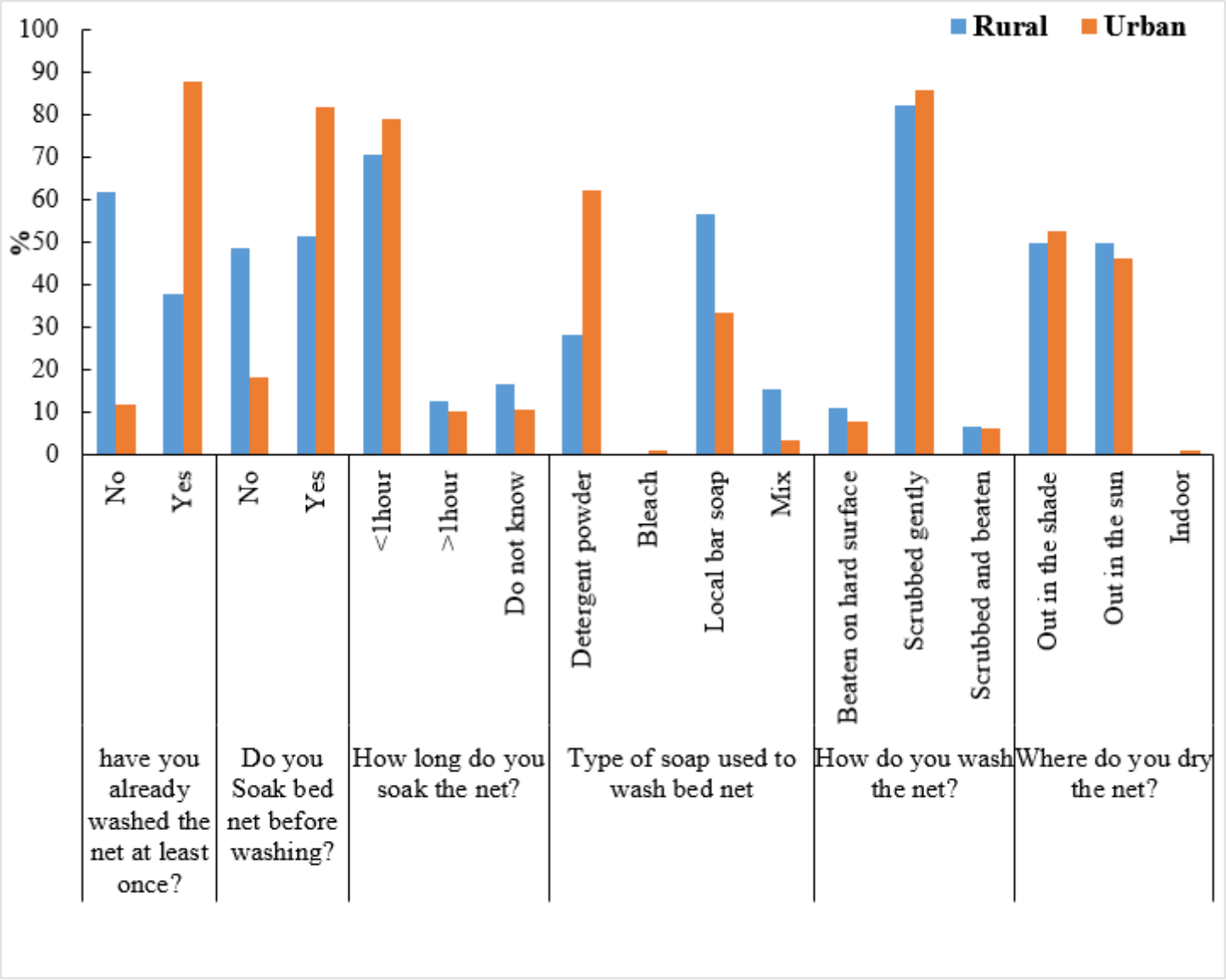



**Figure 1**

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

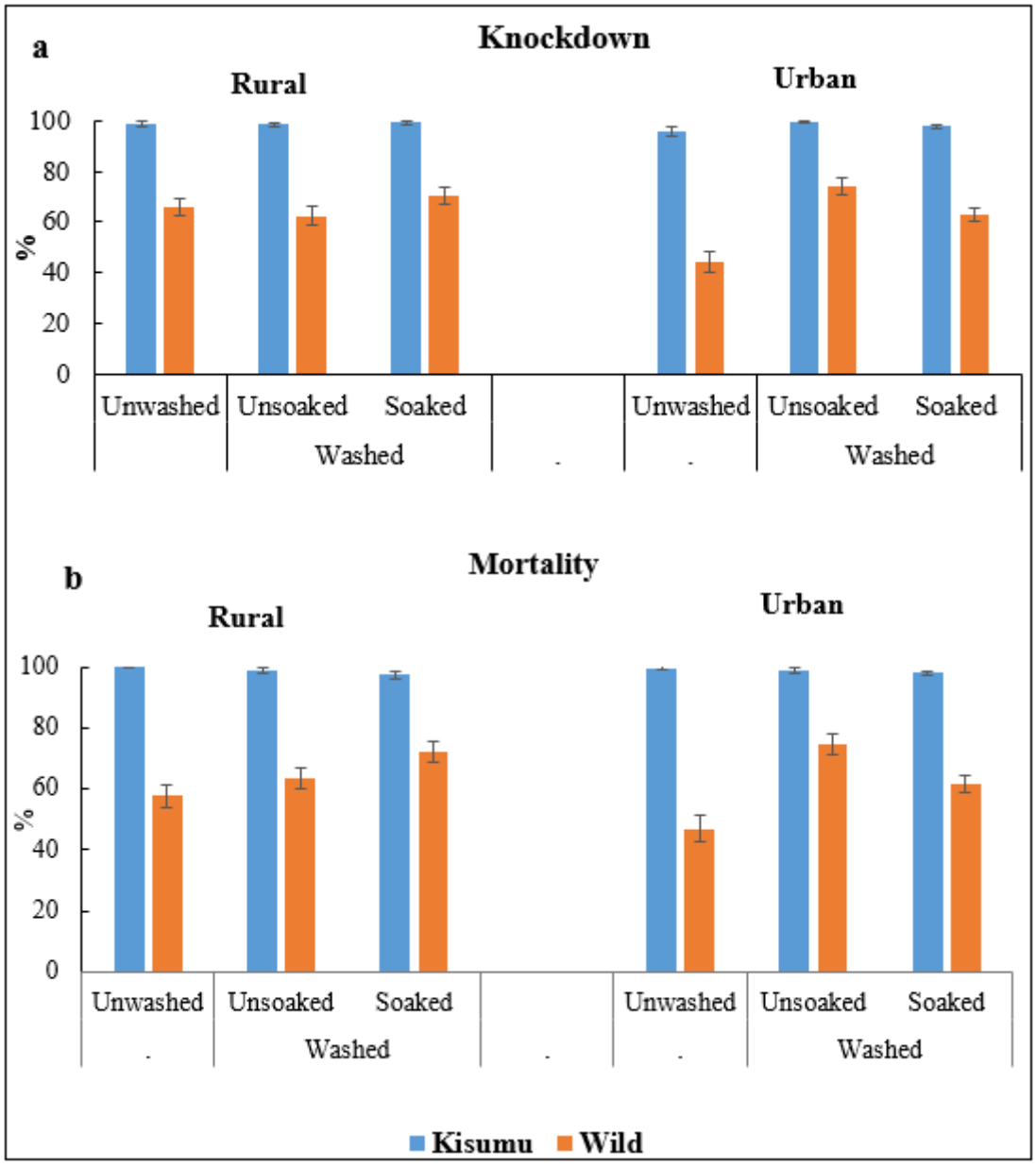
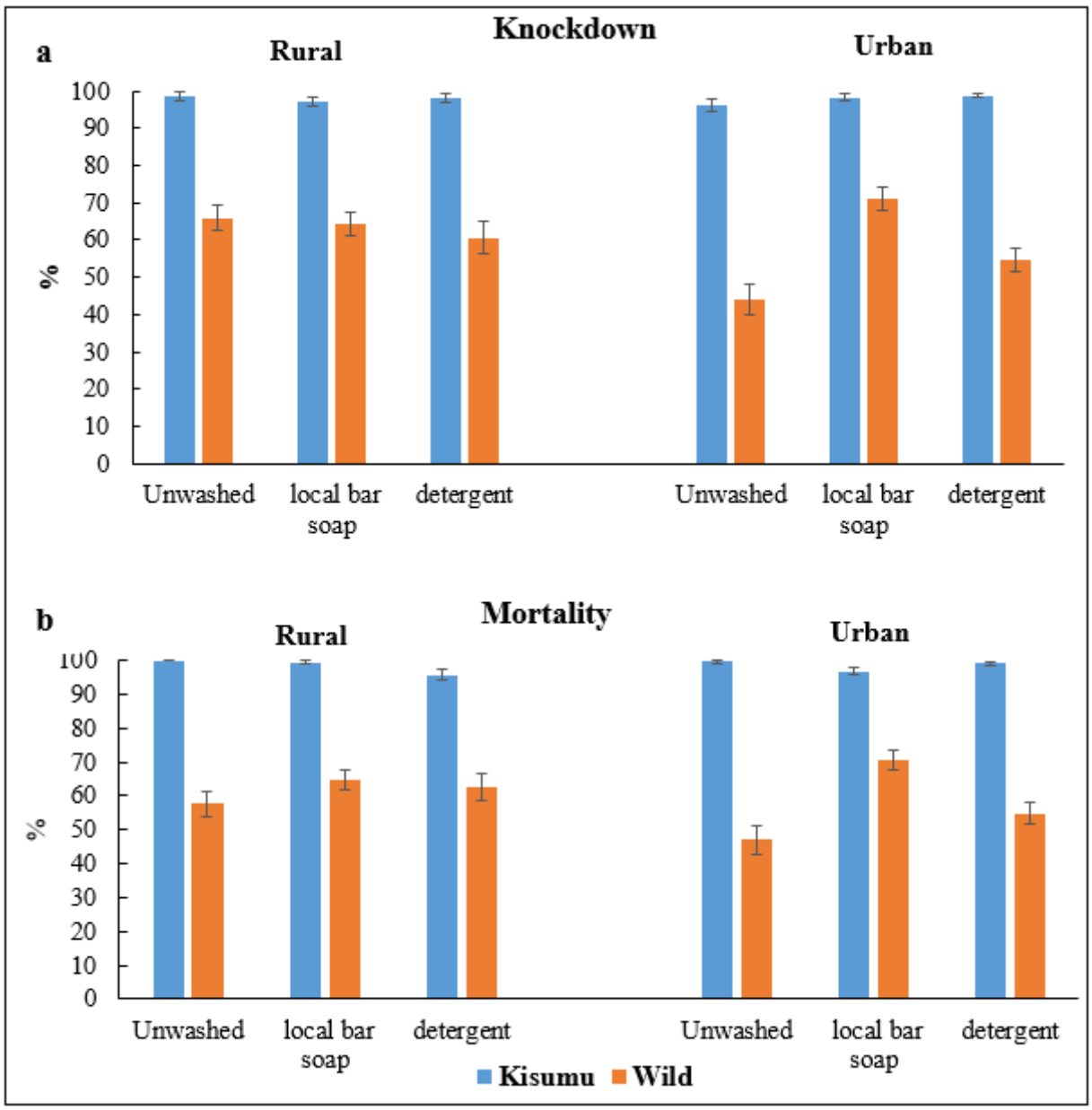


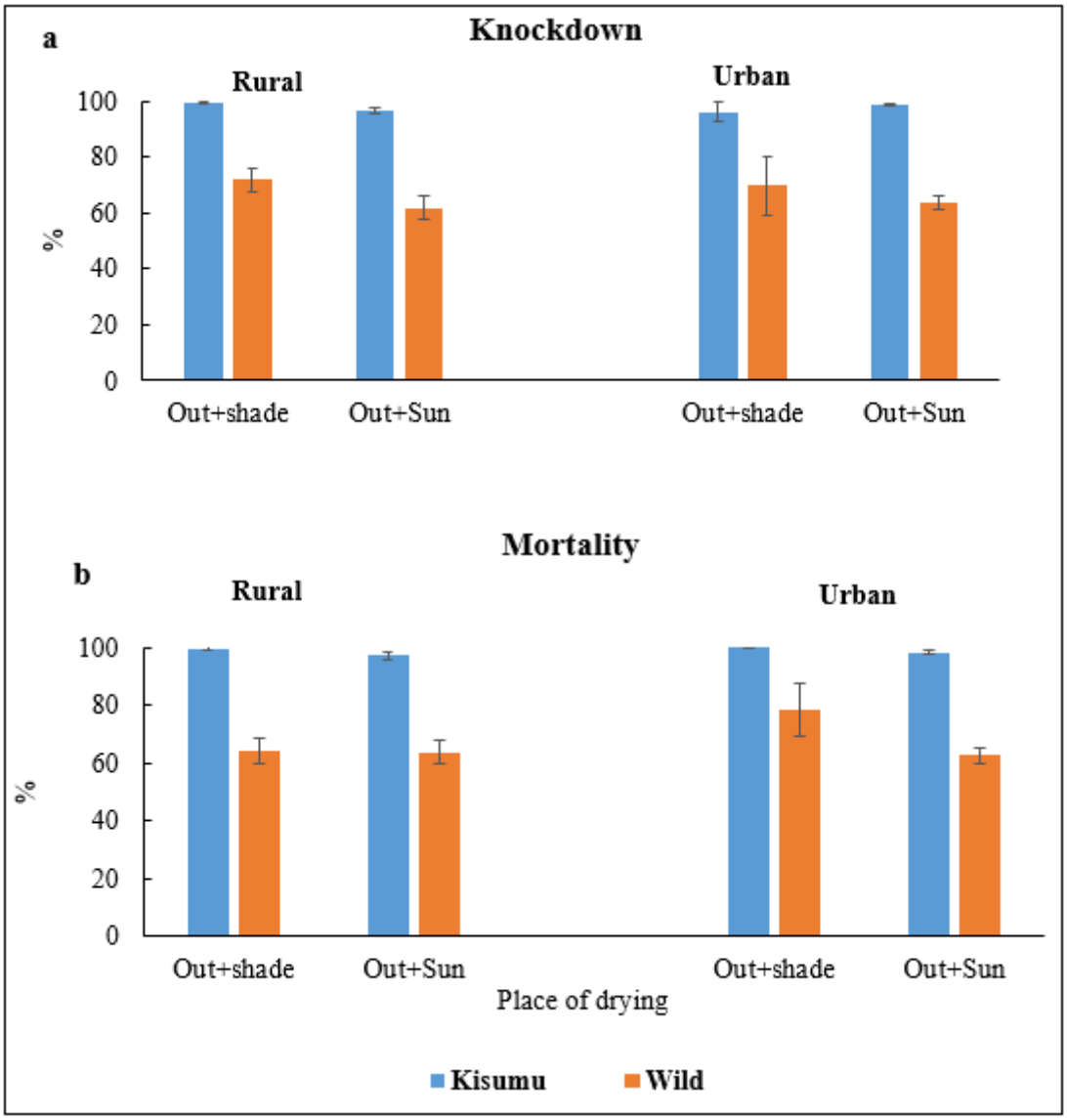

Figure 2

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




## Figure 3

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




## Figure 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).

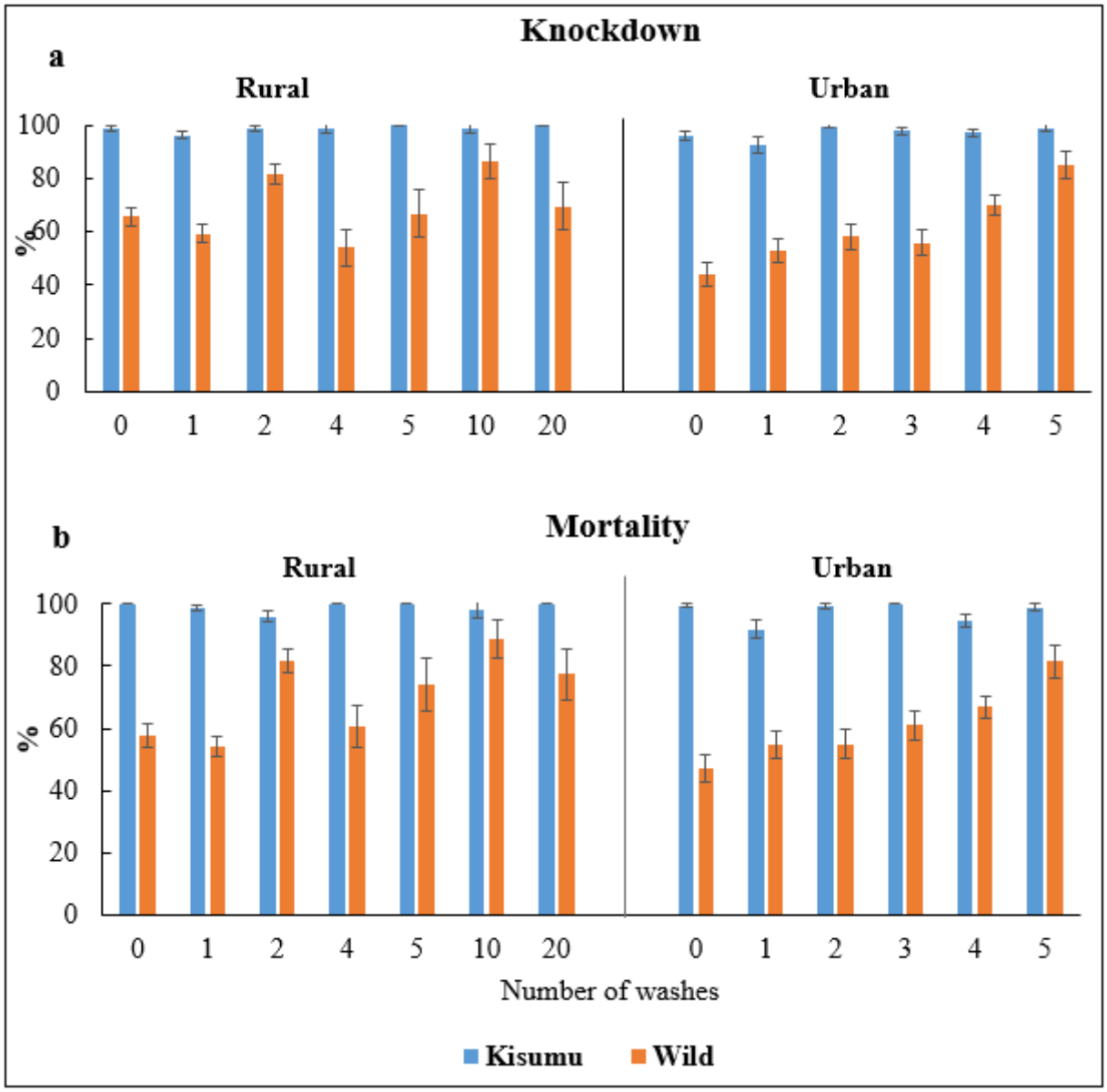



Figure 5

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