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Author Details Given Below The Reference Section

Corresponding Author:
Hermann Watson Sagbohan
a) Center for Research in Entomology of Cotonou, Cotonou, Benin
b) Faculty of Sciences and Techniques, University of Abomey-Calavi, Abomey-Calavi, Benin

Efficacy of different types of impregnated mosquito nets (Conventional and new generation) on *An. gambiae* S.l. in a context of high resistance intensity in Benin, West Africa

Hermann Watson Sagbohan, Casimir D Kpanou, Clément Agbangla, Esaie Gandonou, Razaki Osse, Arthur Sovi, Aicha N Odjo, Esdras M Odjo, Serge C Akpodji, Damien Todjinou, Albert S Salako, Prudenciène Agboho, Manfred Accrombessi, Saïd Chitou, Bruno Akinro, Steve Hougbe, Linda Towakinou, Germain G Padonou and Martin Akogbeto

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Abstract

The study was executed from November 2019 to December 2020 in the different districts of the cotton zone (Kandi, Bantè, Dassa and Savè) of North Benin. All sites in this zone are characterized by heavy use of insecticides (carbamates, organophosphates and pyrethroids) against cotton pests. With the "Dipping" method, larvae of *An. gambiae* s.l. were collected. The efficacy of seven different types of LLINs incorporated with pyrethroids was evaluated on populations of susceptible Kisumu and wild mosquitoes.

After exposure of the kisumu strain to different types of conventional nets (Olyset-Net, Permanet 2.0, Yorkool, Dawa Net and Aspirational), a knock-down effect greater than 95% is induced after just fifteen (15min) minutes of observation or with the different strains in the field, a decrease in the kd and mortality rate is observed in all districts. New generation nets significantly increase the kd effect and mortality rate on field strains than conventional nets. These results show that detoxification enzymes through mixed-function oxidases are strongly implicated in the resistance of *An. gambiae* s.l. to pyrethroids in these sites.

From these results, we can say that the new generation nets (Olyset-Net plus and Permanet 3.0) are a solution for the NMCP in its fight against *An. gambiae* s.l. in these districts.

Keywords: Resistance, *knock-down*, pyrethroids, *Anopheles gambiae* and NMCP

Introduction

In recent years, several sub-Saharan African countries have based their vector control strategies on the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). The use of impregnated mosquito nets as a method of malaria prevention is adopted in most national strategies. Several studies conducted since 1988 in many countries have demonstrated the effectiveness of this tool [13, 42, 18, 7, 17, 4, 45, 32], au Kenya [39, 12, 2, 22, 33]. Thus, since 2007, Benin has integrated the use of conventional LLINs (pyrethroid-impregnated LLINs only) into its vector control program through a three-year mass distribution campaign of LLINs throughout the country. Overall, conventional LLINs impregnated only with pyrethroids are the most widely used. Indeed, this family of insecticides is the only one fully recommended by the WHO to impregnate LLINs [52, 54]. Unfortunately, resistance is a critical issue that reduces the effectiveness of LLINs.

This recurrent phenomenon of insecticide resistance in malaria vectors does not only affect Benin. Today, it is found in several countries in Central and East Africa [49, 25, 40], in West Africa [3, 14, 19, 26, 16, 38, 53], Central Africa [23, 25] and South Africa [29]. In Benin, the resistance of malaria vectors to pyrethroids first observed in Cotonou has spread to the southern and central regions of the country, but also to localities in the northern region [3, 53]. In effect, malaria vectors have developed a strong resistance to all pyrethroids used to impregnate mosquito nets including permethrin, deltamethrin and alphacypermethrin [3, 53, 20, 41, 6, 28, 9, 44, 43, 35].

However, many studies have generalized the resistance phenomenon of malaria vectors to pyrethroids in Benin [53, 46, 47]. This situation could challenge the effectiveness of conventional nets as shown in an experimental case [38], and on a small scale in the community [8]. To evaluate the potential effectiveness of this community-based control tool, the efficacy of LLINs on malaria vectors is necessary, as operational failure is likely to occur if resistance is high [51]. LLINs are critically important as they have contributed to a 68% reduction in malaria cases between 2000 and 2015 in Africa [11]. Although the community-based effectiveness of LLINs [37] is documented and not in doubt, the emergence and widespread use of vector resistance to the insecticides used could seriously undermine their effectiveness.

In addition, new generation LLINs incorporating both a pyrethroid insecticide and piperonyl butoxide (an oxidase inhibiting synergist) are one of the alternative options available. Therefore, it is important to evaluate the efficacy of these two types (conventional and PBO) of LLINs on wild populations of *An. gambiae* s.l. collected directly in the field.

Materials and Methods

Cotton production area

The study was executed from November 2019 to December 2020 in the cotton zone, which includes the districts of Kandi, Bantè, Dassa and Savè. All sites in this zone are characterized by intensive use of insecticides (carbamates, organophosphates and pyrethroids) against cotton pests. All sites are characterized by a dry and a rainy season covering the periods from November to April and May to October, respectively. In the areas of Dassa, Savè and Bantè, the climate is sub-equatorial with an average annual rainfall of 1100 mm, while in Kandi, the climate is Sudano-Sahelian with an average annual rainfall of 1030 mm [30]. This commune is characterized by a North Sudanese type of climate with a dry season from November to April and a rainy season from May to October. The average rainfall is between 800 and 1300 mm per year. With an altitude of 200 to 300 m, Kandi is cut by steep valleys, the Sota valley to the east and the Alibori to the west. Therefore, the commune of Kandi is watered by tributaries of the Alibori and Sota rivers [1] (Figure 1).

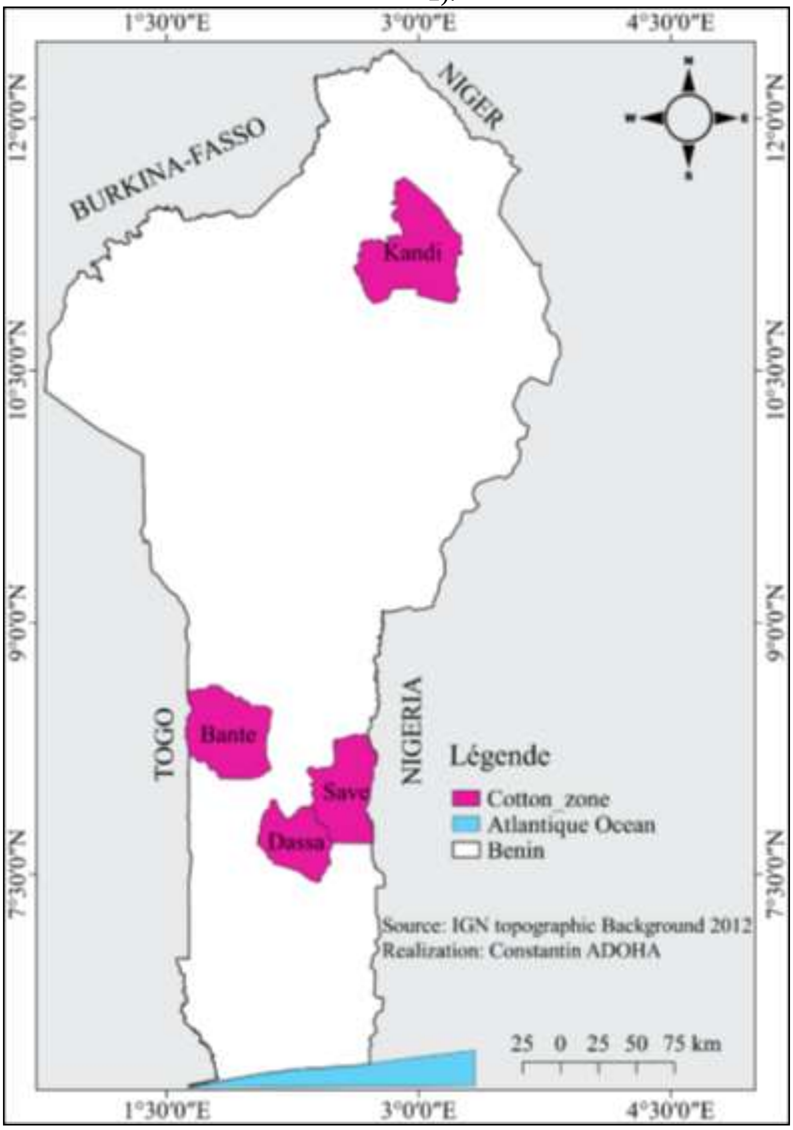

Fig 1: The sites of the cotton production zone in northern Benin

Biological Material

Larval survey

Field trips were organized to collect *An. gambiae* s.l. larvae in the four (04) sites. The "Dipping" method was used for the said collections. The collected larvae were then sent to the Entomological Research Center of Cotonou (CREC) for breeding. Pupae were subsequently sorted into a beaker and introduced into 30 cm square cages at the insectarium to ensure adult mosquito emergence. Each adult cage obtained was labeled according to the locality of larval origin and the date of adult mosquito emergence. Adults were fed honey juice (10%) for two days. Adult mosquitoes obtained from the cage were identified using a binocular magnifying glass and identification key [27]. These *An. gambiae* s.l. were then subjected to biological tests (WHO cone test) to evaluate the efficacy of the different types of LLINs.

The different types of LLINs tested

The seven different types of LLINs, which have been evaluated for their efficacy on pyrethroid-susceptible and hyper-resistant mosquito populations, are presented in the table below. These different types of LLINs are manufactured with two types of fabrics: polyester and polyethylene (Table I).

Table 1: Characteristics of the different types of LLINs used

Moustiquaires	Tissu utilisé	Produit(s) incorporé(s)
Moustiquaire non traitée	Polyester	Néant
PermaNet 2.0	Polyester	Deltaméthrine
PermaNet 3.0	Polyester	Deltaméthrine + PBO
Olyset Net	Polyéthylène	Perméthrine
Olyset Net Plus	Polyéthylène	Perméthrine + PBO
Yorkool	Polyester	Deltaméthrine
Dawa Net	Polyester	Deltaméthrine
Aspirational	Polyéthylène	Alphacyperméthrine

Bioefficacy of LLINs

The bioefficacy of the seven types of nets covered in this study, was assessed through cone tests conducted according to the WHO protocol [50]. All nets used in this study were new nets never taken out of their packaging. Thus, two standard cones were randomly attached to each side of the tested net. In each cone attached to a net, approximately 5-7 female mosquitoes (Kisumu, or wild field strains), non-gorged, and 3-5 days old are introduced for 3 minutes of exposure, making a total of 50-70 mosquitoes per net. After exposure, mosquitoes are observed for 1 hour in veiled cups with free access to sweet juice. During this observation period, the knock-down effect (number of mosquitoes dropped on the back) is recorded every 5 minutes for 60 minutes. After that, the cups with the mosquitoes are stored in racks and left for 24 hours for the mortality reading. Mosquitoes exposed to untreated nets were used as negative controls in each set of cone tests. The tests were validated only when the mortality observed in the controls was less than 5%. The different tests were performed under the following conditions: Temperature (25+/- 2°C), Hygrometry (80% +/- 10%) (Figure 2:) [50].

Fig 2: Bioeffectiveness tests of LLINs on susceptible and wild mosquitoes

Statistical analysis

The efficacy threshold for standard LLINs is set according to WHO criteria [50]. Thus, a net is said to be effective, when the mortality rate is ≥ 80% and/or the proportion of mosquitoes dropped on their backs under the impact of the insecticide (knock-down effect) ≥ 95%.

The mortality rate induced by standard LLINs and PBO LLINs is recorded and compared to each other based on the base insecticide used for LLIN impregnation.

Mortality rates of *An. gambiae* s.l. populations in PBO LLINs versus conventional LLINs were compared using the chi-square test to compare proportions. Statistical analyses were performed using R 3.3.2 software.

Results

Evaluation of the effectiveness of conventional nets on the susceptible laboratory strain Kisumu

Approximately 275 susceptible *An. gambiae* (Kisumu) are used to evaluate the efficacy of conventional nets (Olyset-Net, Permanet 2.0, Yorkool, Dawa Net and Aspirational). After 3 minutes of exposure and 60 minutes of observation of the laboratory strain Kisumu to the Olyset-Net, Permanet 2.0, Yorkool, Dawa Net and Aspirational nets, full efficacy of these nets is observed on the susceptible laboratory strain Kisumu. Note that after exposure of the kisumu strain to different types of conventional nets, a knock-down effect greater than 95% is induced by the nets after just fifteen (15min) minutes of observation. After 24 hours of observation, the mortality rates recorded are 100% for all nets tested. We can say that the Olyset-Net, Permanet 2.0, Yorkool, Dawa Net and Aspirational nets are effective on the susceptible laboratory strain Kisumu (Figure 3).

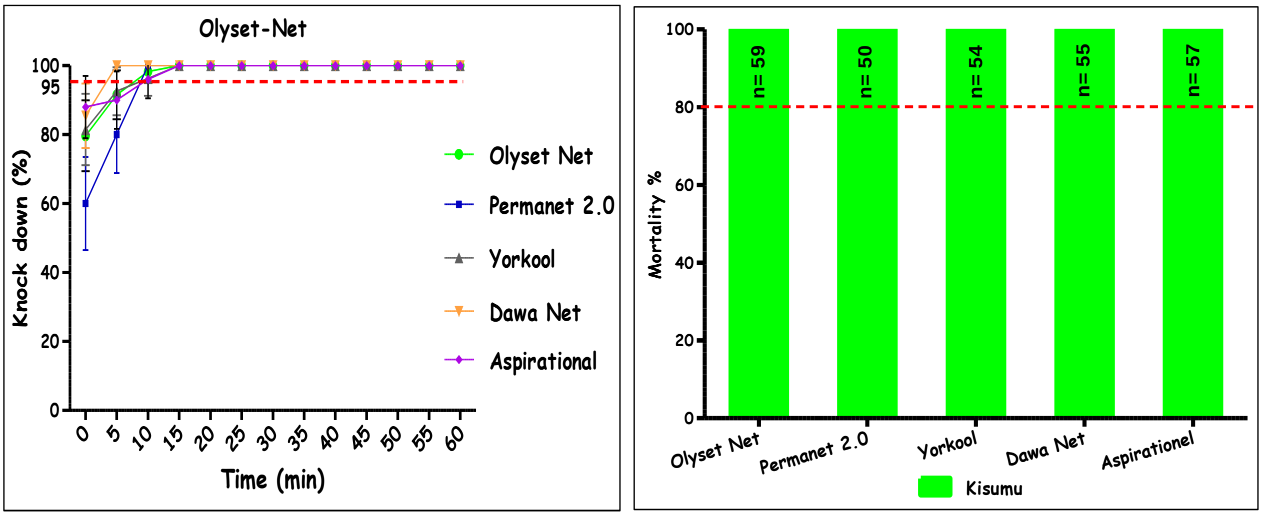

Fig 3: Efficacy of Olyset Net, Permanet 2.0, Yorkool, Dawa Net and Aspirational on the susceptible strain Kisumu

Efficacy of Olyset-Net and Olyset-Net Plus on wild *An. gambiae* s.l.

After 3 minutes of exposure of the susceptible laboratory strain Kisumu to the untreated insecticide net, the knockdown rate and mortality rate after 60 minutes and 24 h of observation, respectively, was 0%. When 483 *An. gambiae* s.l. were exposed to the Olyset-Net and Olyset-Net plus, the knock-down effect induced by the Olyset-Net on *An. gambiae* s.l. varied from 70.77% (Savè) to 96.61% (Bantè). Mortality rates recorded after 24 hours of observation varied from 53.84% [(41.03 - 66.3); Savè] to 94.91% [(85.85 - 98.94); Bantè]. The knockdown effect of the Olyset-Net Plus on *An. gambiae* s.l. was higher than that of the Olyset Net in almost all communes. The knockdown and mortality rates recorded after 60 minutes and 24 hours of observation respectively were 100% in all sites. In summary, we can say that the Olyset Net was more effective on wild *An. gambiae* s.l. than the Olyset Net (Figure 4 and 5).

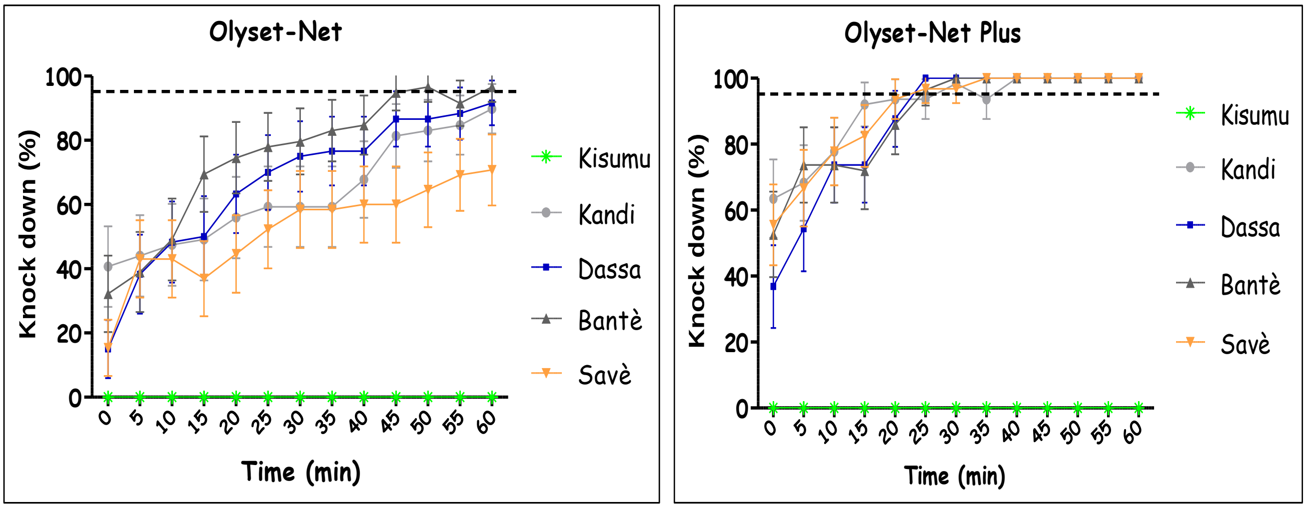

Fig 4: Knock-down effect of Olyset-Net and Olyset-Net Plus on wild *An. gambiae* s.l.

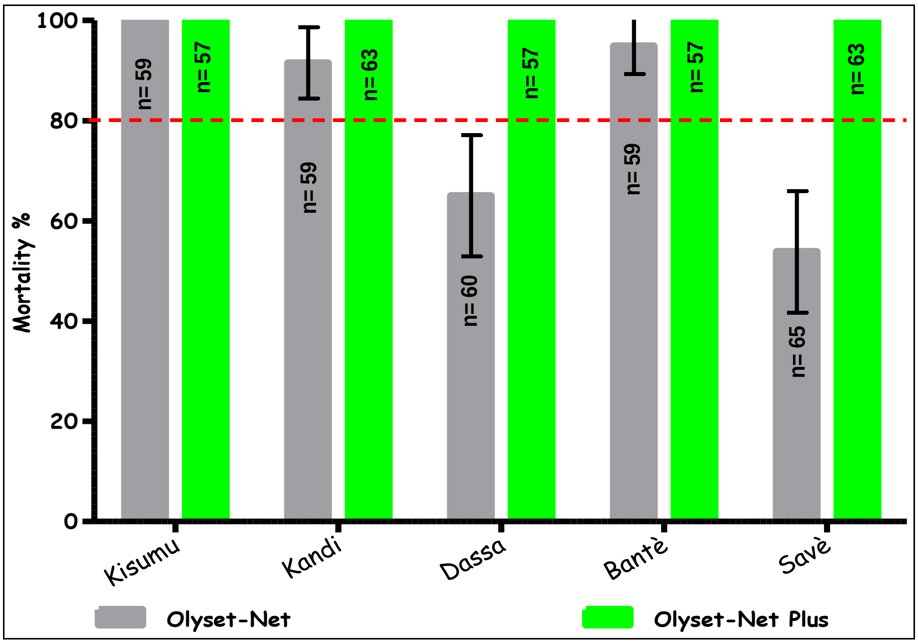

Fig 5: Mortality rate after exposure to Olyset-Net and Olyset-Net Plus on wild *An. gambiae* s.l.

Efficacy of Permanet 2.0 and Permanet 3.0 on wild *An. gambiae* s.l.

The knock-down effect induced after 3 minutes of exposure of *An. gambiae* s.l. to the Permanet 2.0 net varied from 47.76% (Kandi) to 100% (Bantè). Mortality rates recorded after 24 hours of observation ranged from 46.27% [(34 - 58.88); Kandi] to 74.19% [(61.5 - 84.47); Dassa]. As for the Permanet 3.0 net, the knock-down effect induced on submitted *An. gambiae* s.l. was slightly higher than that induced by the Permanet 2.0 net in all sites. Furthermore, after 24 hours of observation, the mortality rates of the Permanet 3.0 net on wild *An. gambiae* s.l. were statistically higher than those of the Permanet 2.0 net. In summary, we can say that the Permanet 3.0 net is more effective than the Permanet 2.0 net on *An. gambiae* s.l. in the different districts (Figure 6 and 7).

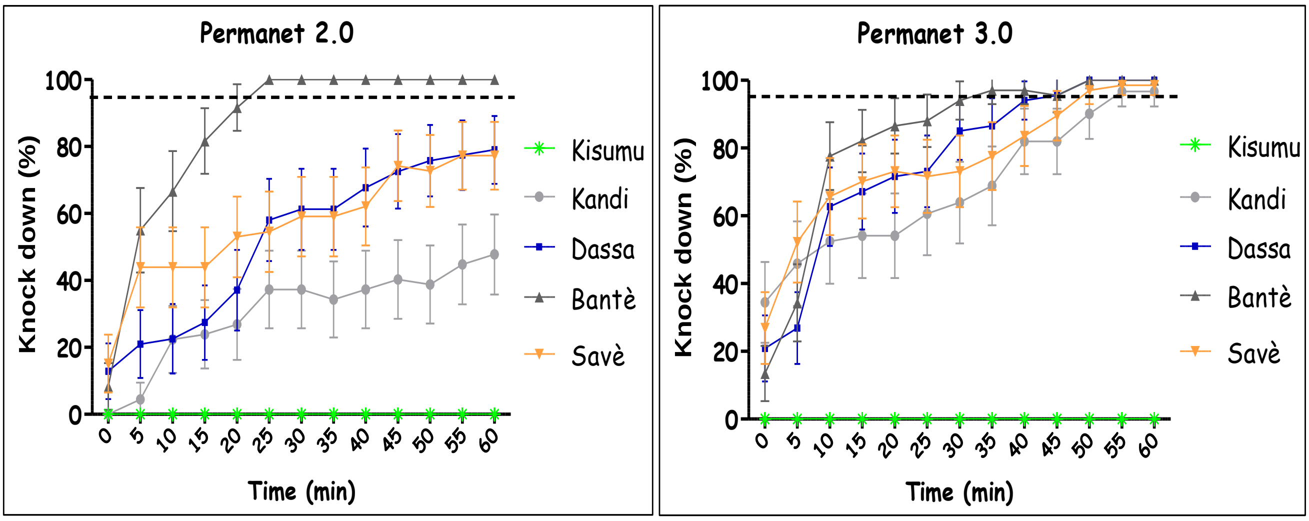

Fig 6: Knock-down effect of Permanet 2.0 and Permanet 3.0 on wild *An. gambiae* s.l.

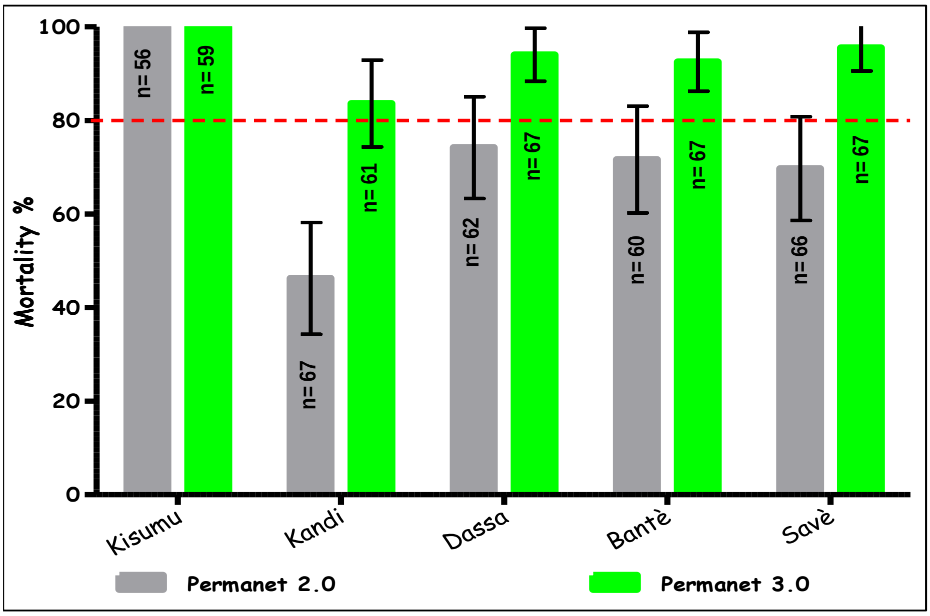

Fig 7: Mortality rate after exposure to Permanet 2.0 and Permanet 3.0 on wild *An. gambiae* s.l.

Efficacy of Yorkool, Dawa net and Aspirational on wild *An. gambiae* s.l.

A total of 774 *An. gambiae* s.l were exposed to the Yorkool and Dawa Net. After exposure to the sensitive laboratory strain Kisumu, a total efficacy of the Yorkool and Dawa nets was observed with a percentage of *kd* higher than 95% from the first twenty (20min) minutes and a mortality rate equal to 100% after 24 hours of observation. The percentage of *kd* induced by the Yorkool net on wild *An. gambiae* s.l. submitted is low except in Dassa and Bantè (100%). The mortality rates recorded after 24 hours of observation in the different districts varied from 64.81% [(50.62 - 77.32); Kandi] to 94.11% [(83.76 - 98.77); Bantè]. The Dawa Net induced on wild *An. gambiae* s.l. a more or less high kd effect in most districts. After 24 hours of observation, the mortality rates recorded with the Dawa Net ranged from 78.46% [(66.51 - 87.69); Kandi] to 98% [(89.35 - 99.95); Bantè] (Figure 8). As for the Aspirational net, the *knock-down* effect induced by the net on wild *An. gambiae* s.l. after 3 minutes of exposure and 60 minutes of observation varied from 71.43% to 98.18% respectively in Dassa and Savè districts. After 24 hours of observation, the mortality rates observed were low in the districts of Dassa and Savè. The lowest rate is observed in Savè [56.36%; (42.32 - 69.7)] and the highest rate in Kandi [74%; (59.66 - 85.37)] (Figure 8).

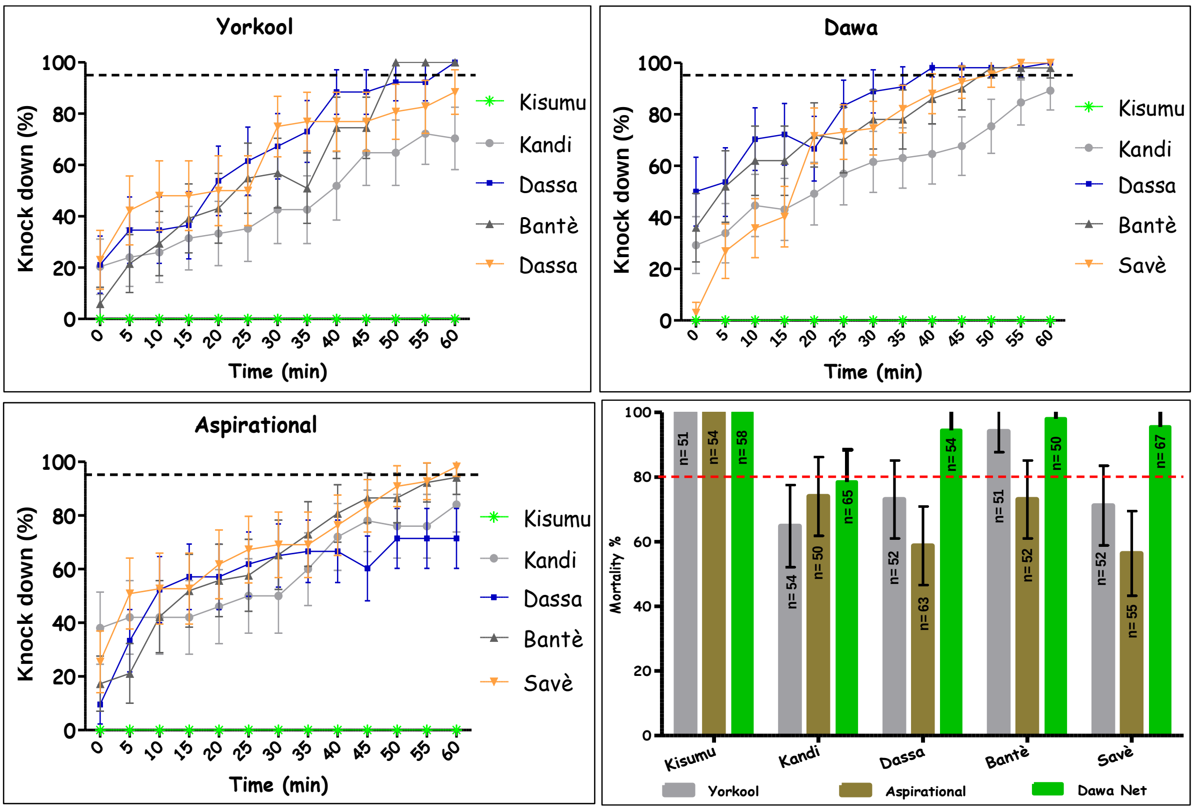

Fig 8: Knock-down effect and mortality rate after exposure to Yorkool, Dawa net and Aspirationelle on wild *An. gambiae* s.l.

Discussion

Resistance of *An. gambiae* s.l. is widespread in the different communes of Benin [3, 20, 41, 5, 28, 9, 44]. High intensity of resistance of *An. gambiae* s.l. to pyrethroids is also reported in different communes in Benin [35, 43]. Our study showed low mortality rates in some communes after bioefficacy tests with *An. gambiae* s.l. on standard nets (Permanet 2.0, Olyset net, Yorkool, Dawa net and Aspirationnelle). These results provide a small glimpse of the reality of standard nets with respect to pyrethroid-resistant *An. gambiae* s.l. The results show that pyrethroid-resistant *An. gambiae* s.l. are likely to compromise the efficacy of standard nets (nets impregnated only with pyrethroids) in the different communes of our study. These results confirm the work of N'Guessan *et al.* in 2007 who reported a decline in the efficacy of conventional nets [38]. Nevertheless, areas of the African continent with lower resistance levels to pyrethroids continue to be effective with pyrethroid-treated nets alone [15, 21, 24, 34, 36].

The low mortality rate recorded could be explained by the fact that *An. gambiae* s.l. tested under nets may have a very high level of resistance to insecticides used for LLIN impregnation (pyrethroids). These net-surviving *An. gambiae* s.l. would likely carry different genes and resistance mechanisms that could be a barrier to vector control. Recent work on malaria vector resistance has shown that the resistance of *An. gambiae* s.l. to pyrethroids has evolved over time in Benin. Today, the level of resistance of *An. gambiae* s.l. to pyrethroids is very high and widespread in Benin's different communes [43; 35]. The high resistance of *An. gambiae* s.l. in different communes of Benin may be due to the fixation of the gene coding for pyrethroid resistance in malaria vectors, since even doses ten times higher than the diagnostic dose do not abolish pyrethroid resistance in these vectors [43]. Other theories suggest that, in addition to the *kdr* gene, several different mechanisms are also involved in the resistance of *An. gambiae* s.l. to pyrethroids.

In contrast to standard nets in which low kd and mortality rates were recorded, Olyset net Plus and Permanet 3.0 nets effectively increased kd and mortality rates in the majority of the districts in our study. Exposure of *An. gambiae* s.l. to the Permanet 3.0 net showed an increase in efficacy of this net in the majority of districts and the same was true for the Olyset net plus in the different districts in our study. Note that these two nets (Olyset net plus and Permanet 3.0) are new generation nets. These nets are impregnated with a pyrethroid combined with the synergist PBO and have a slightly higher dose of insecticide than the standard net. This increase observed in the Olyset net Plus in all communes is due to the fact that all five sides of this net are impregnated with a combination of permethrin and PBO synergist, whereas in the Permanet 3.0 net, only the roof is impregnated with a combination of deltamethrin and PBO synergist. According to Stevenson *et al.* PBO inhibits P450 enzyme activity in resistant mosquito populations resulting in increased pyrethroid-induced mortality [48]. Also, the combination of permethrin with PBO in the olyset plus net fully restored the efficacy of this net in all the study sites. Detoxifying enzymes may play a crucial role in the observed resistance of *An. gambiae* s.l. of the study sites. However several studies have shown high frea quency of the *kdr* allele and the involvement of detoxifying enzymes in the resistance of *An. gambiae* sl of the study sites [43, 35, 28]. The complete restoration of the efficacy of olyset plus towards *An. gambiae* s.l. of the study sites in the presence of PBO can therefore be explained by the higher dose of permethrin in olyset plus compared to olyset net. Vincent Corbel *et al.*, 2004 demonstrated the positive dose-response effect of permethrin-treated net on resistant *An gambiae* s.l. [16]. Further study comparing the efficacy of a net impregnated with the same dose of permethrin used in olyset plus net with olyset plus net in the study sites can help shed light on this observatiThis study providesvide a glimmer of hope to the pyrethroid-based vector control strategy. The combination of the synergist PBO with pyrethroids for net impregnation significantly increased the mortality rate of *An. gambiae* s.l. in all districts. This increase can be explained by the fact that the kdr resistance gene is not the only mechanism involved in the resistance of *An. gambiae* s.l. to pyrethroids. Mixed function oxidases, GSTs or other unknown mechanisms are also involved in this pyrethroid resistance [9]. The effect induced by the PBO - pyrethroid combination clearly demonstrates that cytochrome P450 mono-oxygenases are strongly involved in resistance as they participate in the detoxification of pyrethroids in *An. gambiae* complex [31].

Conclusion

The level of resistance of *An. gambiae* s.l. to permethrin, deltamethrin and alphacypermethrin is very high and follows the same trend in the different districts. *An. gambiae* s.l. reduce the efficacy of standard nets on the one hand. On the other hand, the lower mortality rate of these nets could be circumvented by using new generation nets (PermaNet 3.0; Olyset Plus). The combination of the PBO synergist with pyrethroids would be the solution to circumvent at the operational level the resistance of malaria vectors to pyrethroids in the different districts of Benin. This result provides a glimmer of hope for the NMCP in its efforts to manage pyrethroid resistance in *An. gambiae* s.l. However, nets incorporating Chlorphenapire (CFP) or Piriproxyphene (PPF) have received an interim recommendation from WHO and are currently being evaluated in various randomized controlled trials (WHO., 2020) and could also be considered by the NMCP in managing resistance.

Author's Contributions

Study implementation: WHS, CDK, CA and MCA. Orientation and structuring of the various study ideas: WHS, CDK, EG, GGP, AO, RO, AT, DT, CA and MCA. La collecte des données et les tests ont été réalisés par WHS, CDK, EMO, ACS, ASS, PA and MA. Rearing of larvae to emergence was carried out by WHS, CDK, SH and LT. WHS, CA and MCA drafted the manuscript. SC and BA performed the different statistical tests. EG, CA and MCA critically corrected the content of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The basis of the data used and/or analyzed in this study are available from the Corresponding Author upon reasonable request.

Consent for publication
The findings and conclusions of the present study are from the author(s), not from the US President Malaria Initiative (PMI), the United States Agency for International Development, or the Centers for Disease Control and Prevention (CDC). Trade names used, were for identification purposes only, which does not require approval from PMI, USAID, or CDC.

Ethics approval and consent to participate: Not applicable

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Author's Details

Hermann Watson Sagbohan
a) Center for Research in Entomology of Cotonou, Cotonou, Benin
b) Faculty of Sciences and Techniques, University of Abomey-Calavi, Abomey-Calavi, Benin

Casimir D Kpanou
a) Center for Research in Entomology of Cotonou, Cotonou, Benin
b) Faculty of Sciences and Techniques, University of Abomey-Calavi, Abomey-Calavi, Benin

Clément Agbangla
a) Faculty of Sciences and Techniques, University of Abomey-Calavi, Abomey-Calavi, Benin
b) Genetics and Biotechnology Laboratory, University of Abomey-Calavi, Abomey-Calavi, Benin

Esaie Gandonou
Faculty of Agronomy, University of Abomey-Calavi, Abomey-Calavi, Benin

Razaki Osse
a) Center for Research in Entomology of Cotonou, Cotonou, Benin
b) National University of Agriculture of Porto-Novo, Porto-Novo, Benin

Arthur Sovi
a) Center for Research in Entomology of Cotonou, Cotonou, Benin
b) Faculty of Agronomy, University of Parakou, Parakou, Benin

Aicha N Odjo
Center for Research in Entomology of Cotonou, Cotonou, Benin

Esdras M Odjo
a) Center for Research in Entomology of Cotonou, Cotonou, Benin
b) Faculty of Sciences and Techniques, University of Abomey-Calavi, Abomey-Calavi, Benin

Serge C Akpodji
a) Center for Research in Entomology of Cotonou, Cotonou, Benin
b) Faculty of Sciences and Techniques, University of Abomey-Calavi, Abomey-Calavi, Benin

Damien Todjinou
Center for Research in Entomology of Cotonou, Cotonou, Benin

Albert S Salako
Center for Research in Entomology of Cotonou, Cotonou, Benin

Prudenciène Agboho
Center for Research in Entomology of Cotonou, Cotonou, Benin

Manfred Accrombessi
Center for Research in Entomology of Cotonou, Cotonou, Benin

Saïd Chitou, Bruno Akinro
Center for Research in Entomology of Cotonou, Cotonou, Benin

Steve Hougbe
Center for Research in Entomology of Cotonou, Cotonou, Benin

Linda Towakinou
Center for Research in Entomology of Cotonou, Cotonou, Benin

Germain G Padonou
a) Center for Research in Entomology of Cotonou, Cotonou, Benin
b) Faculty of Sciences and Techniques, University of Abomey-Calavi, Abomey-Calavi, Benin

Martin Akogbeto
Center for Research in Entomology of Cotonou, Cotonou, Benin