Use of Novel Lab-Assays to Examine the Effect of Pyrethroid-Treated Bed Nets on Blood Feeding Success and Longevity of Highly Insecticide-Resistant *Anopheles Gambiae* S.l. Mosquitoes.

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Abstract

Background: There is a pressing need to improve understanding of how insecticide resistance affects the functional performance of Insecticide Treated Nets (ITNs). Standard WHO insecticide resistance monitoring assays are designed for resistance surveillance and do not necessarily provide insight into how different frequencies, mechanisms or intensities of resistance affect the ability of ITNs to reduce malaria transmission.

Methods: The current study presents some novel laboratory-based assays that attempt to better simulate realistic exposure of mosquitoes to ITNs and to quantify impact of exposure not only on instantaneous mortality, but also blood feeding and longevity, two traits that are central to transmission. The assays evaluated the performance of a standard ITN (Permanet® 2.0), a 'next generation' combination ITN that includes a resistance breaking synergist (Permanet® 3.0), and an untreated net (UTN), against field-derived *Anopheles gambiae* s.l. mosquitoes from Côte d'Ivoire exhibiting 1500-fold pyrethroid resistance.

Results: The study revealed that a standard ITN induced negligible instantaneous mortality against the resistant mosquitoes, whereas the resistance breaking net caused high mortality and a reduction in blood feeding. However, the ITNs still impacted long term survival relative to the UTN. The impact on longevity depended on feeding status, with blood-fed mosquitoes living longer than unfed mosquitoes following ITN exposure. The ITNs also reduced the blood feeding success, the time spent on the net, and blood-feeding duration, relative to the untreated net.

Conclusion: Thus, while the standard ITN did not have as substantial instantaneous impact as the resistance breaking net, it still had significant impacts on traits important for transmission. These results highlight the benefit of improved bioefficacy assays that allow for realistic exposure and consider sub- or pre-lethal effects to help assess the functional significance of insecticide resistance.

Background

In recent decades, large-scale implementation of pyrethroid-based control tools that target the adult mosquito vectors have helped reduce the burden of malaria [1]. Insecticide-treated nets (ITNs) are the most widely distributed and perhaps most important tool to date[1]. However, their extensive use has led to the evolution of insecticide resistance in many mosquito populations [2–6], and there are now mounting concerns that resistance will render ITNs ineffective and lead to a resurgence of malaria [7–9]. As yet, however, the link between emergence of different mechanisms and intensities of insecticide resistance and control failure remains unclear [10–14] which challenges the development of appropriate resistance mitigation strategies [15].

Mosquito populations are classified as resistant using standardized WHO testing procedures that measure the level of mortality within 24h of exposure to a diagnostic dose of insecticide [16]. However, this focus on instantaneous mortality ignores possible pre- or sub-lethal effects of ITNs on longevity and blood feeding success, two important parameters influencing malaria transmission potential [17].

Evidence suggests that ITNs can potentially reduce the transmission of malaria even in the absence of rapid knockdown and death, providing they reduce mosquito longevity and limit the number of mosquitoes that live long enough to enable the malaria parasite to complete its extrinsic incubation period [17–19]. This “sub-lethal” effect of insecticide might be minimal in some conditions [20] or may be enhanced by repeated exposures [17, 21]. In addition, previous studies with susceptible mosquito strains suggest that certain pyrethroid insecticides act on mosquito host-searching and blood-feeding behavior, by irritating them upon contact or repelling them prior to net contact [22, 23]. While, over short distances, the repellent effect of pyrethroid seems to impact resistant mosquito strains [24–27], it is still unclear whether resistance alters the impact of ITNs on blood feeding inhibition. In fact, some recent laboratory studies suggest that pyrethroids might even enhance host searching in resistant mosquitoes [28, 29].

This paper utilizes two novel assay methods to explore effects of ITN exposure on initial mortality, blood feeding inhibition and longevity against field-derived populations of *Anopheles gambiae* sensu lato (s.l.) from central Cote d’Ivoire that are known to exhibit intense resistance to pyrethroids [3]. The primary aim was to examine the sub/pre-lethal effects of ITNs considering different patterns of exposure. A secondary aim was to explore possible alternatives to the standard WHO assays used to determine insecticide resistance and characterize the bioefficacy of ITNs, to provide a better assessment of the functional significance of insecticide resistance.

Material And General Methods

Mosquito populations

The study uses *An. gambiae* s.l. mosquitoes collected in natural breeding habitats around the villages of Yao Koffikro and M’be in central Côte d’Ivoire [30]. These local populations are more than 1500-fold resistant to deltamethrin relative to a standard susceptible stain (Kisumu) [3, 31]. Among other resistance mechanisms, they carry the 1014F kdr (≥ 90% fixed) and 1575Y mutations, and upregulate CYP6M2, CYP6P3 and CYP9K1 [3]. The field-collected larvae were reared at 27 ± 2°C, 60 ± 20 % RH and ambient light, in plastic boxes of 300 larvae with 1 liter of deionized water and fed daily with fish food (Tetramin™ baby) following a standardized “high food” regime described in [[32]]. Adult mosquitoes were housed in 32.5x32.5x32.5 cm mosquito cages and maintained on 10% sugar solution.

Human host preparation

The experimenter (PB) avoided tobacco, alcohol and the use of scented products for 12 hours before and during testing. Her arms were washed with unscented soap and rinsed with water the morning before a test. PB was not actively infected with any pathogen.

Bed nets

Three types of polyester netting were tested: unwashed Permanet® 2.0 (ITN), the top of Permanet® 3.0 (ITN + PBO) (Vestergaard Frandsen SA, DK), and untreated net (UTN) (Coghlan’s). The ITN is coated with 55 mg/m^2 ± 25 % deltamethrin. The ITN + PBO contains 120 mg/m^2 ± 25 % deltamethrin and 750 mg/m^2

± 25 % piperonyl butoxide (PBO) [33]. Prior to testing, fully susceptible mosquitoes (Kisumu strain) were exposed to netting samples in WHO tubes (see method below); all were killed within 24 hours when exposed to the treated nets, while the UTN killed none.

General methods

The assays used 4–5-day old adult female mosquitoes selected at random from the stock cages. Mosquitoes were assigned haphazardly to a net treatment to provide balanced sample sizes (Supplementary Information (SI); Table A). They were starved 4h prior to testing, with assays conducted in the afternoon during day light. Following exposure, feeding status was recorded; mosquitoes with a visible amount of bright red blood in their abdomen were considered as “fed”. Following exposure, mosquitoes were kept individually in plastic cups covered with untreated netting and mortality was recorded daily until all mosquitoes had died. Females had continuous access to a 10% sugar solution and to an egg laying substrate, although egg numbers were not recorded in order to minimize daily mosquito handling. All analyses and graphs were done in R© version 3.6.1. Contrasts among treatments were assessed with the multcomp package version 1.4–10 and the function glht with Tukey’s honestly significant difference test. All complete statistical analysis can be found in the SI (SI; B. Statistical analysis summaries).

Forced exposure in modified WHO tubes

This assay examined the effect of a forced exposure to an ITN (PermaNet® 2.0) on mosquito mortality and capacity to blood feed (SI. Dataset S1). The aim was partly to determine whether the highly resistant wildtype mosquitoes suffered obvious direct effects from forced contact with ITN, but also to serve as something of a range finder to determine the effects of duration exposure and relative influence of blood feeding on survival following exposure, to inform the design of the following experiment. In two experimental blocks (n_1 = 219 and n_2 = 122), mosquitoes were individually exposed to the ITN for 1-, 3- or 5-minutes using WHO tubes lined (inner wall and ends) with netting to force contact with the treated surface. During the exposure, half of the mosquitoes in each block (n_1 = 96 and n_2 = 61) had the opportunity to take a blood meal by feeding on PB’s arm through the netting; the other half could not feed, but PB held her arm 1 cm away from the tube to provide equivalent host cues. In two additional experimental blocks (n_3 = 84 and n_4 = 56), mosquitoes were exposed for 5 min only.

Variable exposure via individual feeding choice

This assay relaxed the forced contact experienced in the WHO tubes by placing mosquitoes individually in clear 180-ml plastic cups with the top covered with either ITN, ITN + PBO (top of the Permanet® 3.0), or UTN. PB’s arm was placed onto the net at the top of the cup to attract mosquitoes and enable blood feeding (SI. Dataset S2). The aim was to simulate more natural patterns and durations of contact during a 5 min exposure period. In principle, if mosquitoes were repelled by the netting or suffered irritancy following initial contact, they might have only minimal contact. On the other hand, if they were motivated to feed and were unaffected by the presence of the netting, contact could last for up to 5 min. The time mosquitoes were in contact with the net and the duration of their blood-meal were recorded, together with subsequent longevity. ITN, ITN + PBO and a UTN were compared in three experimental blocks (n_1 = 61, n_2 = 58 and n_3 = 127) while in a fourth experimental block (n_4 = 123), females were not tested against the ITN + PBO because of limited mosquito numbers.

Statistical analysis

a. Forced exposure in modified WHO tube assays

Using a Generalized Linear Model (GLM) with a binomial distribution, the blood feeding success of mosquitoes given access to a blood source was analysed to investigate whether the proportion that was fed depended on the duration of insecticide exposure and the experimental blocks.

The survival post exposure was analysed with a weighted Cox regression (using the R package coxphw due to the violation of the proportional hazards assumption in a Cox regression model) regarding the blood feeding categories (no access to a blood source; access to a blood source but unfed; access to a blood source and fed), the duration of insecticide exposure, the experimental blocks, and their interaction. Given the complex interaction found in this analysis and the 5-min time needed for mosquitoes to engorge blood to repletion[34], we analysed the survival post exposure for blood fed and un-fed mosquitoes separately with a weighted Cox regression including two exposure time categories (1- and 3-minutes exposure time compared to 5-minutes exposure times), the experimental blocks and their interaction. In a preliminary model for unfed mosquitoes, whether mosquitoes had access to an arm or not during exposure did not influence the longevity, thus it was not included in the final analysis.

Moreover, mosquito survival post exposure in two additional replicates was analysed together with the other experimental blocks considering mosquitoes exposed in WHO tubes for 5 min only. A weighted cox model was used to investigate the effect of the blood feeding categories, the experimental blocks, and their interaction.

b. Variable exposure via individual feeding choice

We analysed the time spent on the net with a gaussian GLM and an identity link function including the type of bed net (UTN, ITN, ITN + PBO), and the experimental blocks as nominal factors.

We analysed the time spent feeding with a gaussian GLM (for fed mosquitoes only) and the proportion of mosquitoes that fed with a binomial GLM, both including the type of bed nets, the time spent on the net, their interaction and the experimental blocks as nominal factors (the latter in interaction with the other parameters for the binomial GLM).

We analysed the survival post exposure of the mosquitoes with a weighted Cox proportional hazards model with the type of bed net, the feeding status, their interactions, and the experimental blocks as factors. Considering fed mosquitoes alone, the same analysis was done without the feeding status and adding the time spent on the net and the time spent feeding. We then repeated that analysis for unfed mosquitoes alone with the time spent on the net (summary in SI, table B.a.3).

The analysis for the time spent on the net, feeding success and survival post exposure were repeated with an additional experimental block for UTN and ITN treatments only.

Results

a. Forced exposure in modified WHO tube assays

Forced exposure to the ITN resulted in negligible mortality of highly resistant mosquitoes within 24h regardless of exposure period (note this contrasts to 100 % mortality of the susceptible Kisumu strain in pilot studies referred to in the methods) (Fig. 1). Furthermore, 84.7 (95 % Confidence interval (CI): 78.1 to 89.9) % of mosquitoes that were provided access to a blood meal were able to feed, irrespective of the duration of the exposure ($\chi^2 = 1.32$, df = 1, $p = 0.25$). Blood feeding increased mosquito longevity by approximately 4 days, with mean (± SE) survival time (post exposure at 4 days old) of blood-fed mosquitoes of 14.7 ± 0.59 days post exposure, compared with 10.5 ± 1.04 days post exposure for unfed mosquitoes having access to the arm and 10.7 ± 0.39 days post exposure for unfed mosquitoes having no access to the arm ($\chi^2 = 29.80$, df = 1, $p < 0.001$) (Fig. 1). When exposed for 1 to 3 min, fed mosquitoes lived an average of 2.8 more days post exposure than unfed mosquitoes [unfed: 11.1 ± 0.47 days; fed: 13.9 ± 0.59 days] and when exposed for 5 min, fed mosquitoes lived another 6.5 more days post exposure [unfed: 9.8 ± 0.58 days; fed: 16.3 ± 1.36 days] ($\chi^2 = 14.64$, df = 1, $p < 0.001$). While the interaction between exposure time and blood-feeding was significant on longevity, there was no significant effect of exposure duration itself ($\chi^2 = 0.56$, df = 1, $p = 0.45$). Subgroup analysis showed no influence of exposure duration on mean longevity for unfed mosquitoes ($\chi^2 = 3.20$, df = 1, $p = 0.07$). However, longer exposure to insecticide led to a longer life post-exposure for blood fed mosquitoes [13.9 ± 0.59 days for 1- and 3-min exposure and 16.3 ± 1.36 days for 5-min exposure] ($\chi^2 = 5.78$, df = 1, $p = 0.02$).

Two additional experimental blocks, in which the mosquitoes were all only exposed for 5 min corroborated these results. Combining all experimental blocks for the 5 min exposure showed a blood meal to extend the lifespan of mosquitoes by around 7 days post exposure [9.0 ± 0.73 days for females with no access to blood source; 9.7 ± 0.43 days for those with access to the blood source but unfed and 16.6 ± 0.87 days those with access to the blood source and fed] ($\chi^2 = 51.03$, df = 1, $p < 0.001$).

b. Variable exposure via individual feeding choice

The presence of insecticide reduced average contact time with the netting ($F = 21.97$, df = 2, $p < 0.001$; Fig. 2.a). Mosquitoes exposed to the UTN had an average contact time of 167.7 ± 13.06 sec, while those exposed to the ITN or ITN + PBO had average contact times of 121.4 ± 9.5 and 59.1 ± 9.25 sec, respectively (Tukey pairwise comparisons: $p_{ITN\text{-}UTN} = 0.005$, $p_{ITN+PBO\text{-}UTN}$ and $p_{ITN+PBO\text{-}ITN} < 0.001$).

The feeding duration showed a similar pattern between net types ($F = 45.30$, $df = 2$, $p < 0.001$; Fig. 2.b) with average times of 219.3 ± 8.82 sec, 135.4 ± 9.80 sec and 105.0 ± 15.85 sec for UTN, ITN, ITN + PBO, respectively (Tukey pairwise comparisons: $p_{ITN\text{-}UTN}$ and $p_{ITN+PBO\text{-}UTN} < 0.001$, $p_{ITN+PBO\text{-}ITN} = 0.48$). Blood feeding duration was longer for longer net contact time ($F = 72.21$, $df = 1$, $p < 0.001$), independently of the net treatment ($F = 2.36$, $df = 2$, $p = 0.10$).

The presence of insecticide reduced the percentage of mosquitoes that fed successfully. With the UTN, 60.5 (CI: 49.3 to 70.8) % of mosquitoes took a blood meal, while blood feeding rates were only 38.9 (CI: 29.1 to 49.5) % and 9.2 (CI: 3.46 to 19.0) % with the ITN and ITN + PBO treatments, respectively ($\chi^2 = 45.70$, $df = 2$, $p < 0.001$; Fig. 2.c). Blood fed females spent 3.6 more time in contact with nets than unfed ones [unfed = 59.8 ± 6.22 sec; fed = 218.7 ± 7.46 sec] ($\chi^2 = 110.22$, $df = 1$, $p < 0.001$). Contact times of the subset of mosquitoes that successfully took a blood meal showed a similar pattern between net types again with average contact times of 253.4 ± 7.49 sec, 185.4 ± 11.73 sec and 122.3 ± 13.68 sec for UTN, ITN, ITN + PBO, respectively (Tukey pairwise comparisons: $p_{ITN\text{-}UTN}$ and $p_{ITN+PBO\text{-}UTN} < 0.001$, and $p_{ITN+PBO\text{-}ITN} = 0.29$). However, unfed mosquitoes exposed to an ITN spent more time in contact with the net compared to those exposed to an UTN with average time of 36.5 ± 10.82 sec for UTN vs. 80.6 ± 10.66 sec for ITN and 52.7 ± 9.73 sec for ITN + PBO ($F = 11.97$, $df = 2$, $p = 0.002$; Tukey pairwise comparisons: $p_{ITN\text{-}UTN} = 0.04$ and $p_{ITN+PBO\text{-}UTN} = 0.88$, and $p_{ITN+PBO\text{-}ITN} = 0.24$). In one experimental block, the feeding success was slightly lower compared to the other blocks ($F = 5.63$, $df = 1$, $p = 0.02$). Thus, while there is no difference in the time spent on the net for blood fed mosquitoes, there is some variability between two experimental blocks for the contact time of unfed mosquitoes ($F = 7.92$, $df = 1$, $p = 0.005$).

As observed in the first experiment, there was negligible mortality within 24h in the UTN and ITN treatments. The ITN + PBO treatment, however, caused substantial 24h mortality of 86.1 (CI: 75.3 to 93.5) %. Beyond the instantaneous effects, insecticide exposure led to a reduction in long term survival ($\chi^2 = 146.87$, $df = 2$, $p < 0.001$) (Fig. 3). Mosquitoes exposed to an UTN had an average survival time (post exposure at 4–5 days old) of 16.7 ± 0.74 days, those exposed to the ITN 11.5 ± 0.62 days, and those exposed to the ITN + PBO just 2.3 ± 0.44 days. Blood feeding increased overall longevity ($\chi^2 = 24.53$, $df = 1$, $p < 0.001$), by approximately 6 days for the UTN and 4 days for the ITN. Nonetheless, blood fed females died more quickly after an exposure to insecticide than those exposed to a UTN [14.0 ± 1.10 days for the ITN vs. 19.0 ± 1.00 days for the UTN]. For the ITN + PBO, blood fed mosquitoes had marginally shorter lifespan than non-blood feds (average survival time post exposure of 1.0 ± 0 days and 2.5 ± 0.49 days, respectively ($\chi^2 = 10.21$, $df = 2$, $p = 0.006$). The time that blood-fed mosquitoes spent on the net and feeding duration did not influence longevity. However, fed mosquitoes exposed to an UTN had a longer life when they spend more time blood feeding which is not the case for fed mosquitoes exposed to insecticide ($\chi^2 = 6.95$, $df = 2$, $p = 0.03$).

One additional experimental block was added to an analysis comparing the effects of the ITN against the UTN only (providing 4 blocks in total for this comparison (SI, Table A)). The presence of insecticide reduced the mean time spent on the net [119.6 ± 7.73 sec for the ITN vs. 173.3 ± 10.11 sec for the UTN] (F

= 18,02, df = 1, $p < 0.001$). The ITN led to a significant reduction in blood feeding ($\chi^2 = 17.20$, df = 1, $p < 0.001$), with 38.2 (CI: 30.6 to 46.3) % blood fed mosquitoes in the ITN treatment compared with 61.9 (CI: 53.6 to 69.8) % in the UTN. Mosquitoes that spent a longer period on the net were proportionally more successful in taking a blood meal ($\chi^2 = 206.56$, df = 1, $p < 0.001$). When mosquitoes spent less than 1 min on the net, the feeding rate did not differ between ITN and UTN. However, once contact time exceeded 1 min, blood feeding increased with contact time for the UTN but did not for the ITN ($\chi^2 = 11.31$, df = 1, $p < 0.001$). There was an overall effect of the insecticide exposure on longevity ($\chi^2 = 29.34$, df = 1, $p < 0.001$). The mean survival time of unfed mosquitoes was 14.0 ± 0.83 days post exposure for the UTN and 10.0 ± 0.50 days post exposure for the ITN, while for fed mosquitoes it was 18.2 ± 0.78 days post exposure and 15.0 ± 0.88 days post exposure, respectively ($\chi^2 = 42.09$, df = 1, $p < 0.001$).

Discussion

In the forced exposure assays, a high percentage of mosquitoes were able to blood feed and contact time against the ITN for up to 5 minutes was not obviously more likely to kill mosquitoes than 1 minute. Moreover, those mosquitoes that did blood feed lived longer. Other studies have also shown that ITNs fail to cause instantaneous mortality [2, 4] or fully prevent blood-feeding [2, 23, 31] against highly resistant mosquitoes, suggesting a loss of personal protection due to resistance. However, the feeding choice exposure assays provide a slightly more nuanced picture and showed the ITN to reduce the proportion of mosquitoes that fed successfully. Unfed mosquitoes were found to spend more time in contact with the ITN than equivalent unfed mosquitoes exposed to the UTN, yet fewer mosquitoes exposed to the ITN ultimately fed. This result suggests that reduced feeding was not because mosquitoes avoided the net or were repelled by it, but more likely because contact with the insecticide reduced feeding capacity. In turn, insecticide exposure reduced the time spent on the net feeding. Spending less time on a net during blood feeding does not necessarily mean that the blood meal size is smaller and/or insufficient for malaria transmission [35]. The presence of insecticide might reduce the capacity to engorge blood [23], or on the contrary, it might motivate mosquitoes to take more blood in a shorter period in order to minimize the contact time with the treated net. A shorter time in contact with the treated net could lower the insecticide dosage received by blood feeders and this could be a behavioral adaptation of mosquitoes living in areas with high use of ITNs. Whether similar results are observable in mosquitoes infected with malaria parasites is unclear. It is known that malaria infection alters feeding rates and blood-seeking behaviours [36, 37], but more research is needed to understand whether insecticide exposure impacts vector competence [38–41] and/or the presence of malaria parasites affect expression of insecticide resistance [28, 42].

Regardless of the exposure pattern/duration there was negligible mosquito mortality within 24h of contact with an ITN. However, this standard 24h assessment [16] misses potential long-term effects of exposure. Data from both assays show reduced long term survival following exposure to a standard ITN, consistent with the delayed mortality for highly insecticide resistant mosquitoes reported elsewhere [17]. The experiments also highlight the fact that the standard WHO test procedures for evaluating resistance and measuring the bio-efficacy of ITNs [16, 43] are weak indicators of how ITNs ultimately determine malaria transmission risk, and hence for understanding the functional significance of insecticide resistance. The WHO resistance assay uses tubes (as used in the initial assays here) to force mosquitoes into contact with filter paper treated with diagnostic doses of insecticide for 1h [44–46].The WHO ITN bioefficacy assay uses cones to force mosquitoes into contact with ITNs for 3 minutes [47–49]. Neither assay simulates how mosquitoes contact ITNs during host searching and blood feeding in nature [26]. According to data acquired by Diop et al. [50], mosquitoes exposed to insecticide tend to bounce on the ITN until they decide to probe and take a blood meal. How long they choose to stay on the net depends on the level of toxicity of the net as well as on the presence of a host [51]. The cup assay used in the current study provides a potential method to allow for more realistic patterns of contact with an ITN. The data reveal that the repellent and irritant effects of deltamethrin [52, 53], and especially in combination with the synergist PBO [54], reduces the time spent on the treated net but does not completely prevent mosquitoes from biting through it. Interestingly, limited repellency may help maximize the sublethal toxic effects of insecticide against them, which would increase ITN efficacy [17, 19].

The data also highlight the importance of blood feeding in the evaluation of insecticide resistance. In general, those mosquitoes that blood fed survived insecticide exposure better than those that did not (the exception being in the ITN + PBO exposure). Whether this is because those mosquitoes able to feed during contact with an insecticide are the most resistant and robust individuals, or whether blood feeding itself enhances expression of insecticide resistance is unclear. The fact that the longevity of mosquitoes having no access to a blood source was similar to the longevity of mosquitoes failing to blood feed despite the access to a blood source suggests the effect is more to do with blood feeding than individual variation (i.e. it would be expected that the overall survival of mosquitoes with no access to blood should be greater as these mosquitoes represent a mixed population that includes the potentially more robust individuals). In addition, blood meal ingestion induces oxidative stress leading to increased metabolic activity [55, 56], which could in turn result in higher expression of detoxification enzymes [57].

Conclusion

Overall, the combination of delayed mortality and antifeedant effects suggests that ITNs retain at least some functionality above and beyond a simple physical barrier, even against mosquitoes with 1500-fold resistance. These effects do not mean that ITNs are as effective against resistant mosquitoes as they are against susceptible ones. Moreover, the Permanet® 3.0 (ITN + PBO) induced much greater mortality and feeding inhibition than the standard ITN, suggesting improved control potential of resistance breaking nets in areas of high insecticide resistance. Nonetheless, the results provide further evidence to support why ITNs can continue to contribute to reduced malaria transmission in the face of insecticide resistance. This is likely to be especially so in areas with high effective coverage (i.e. high ownership and use) of ITNs as even small effect sizes at individual level can lead to large overall effect sizes when multiplied up to community level [8, 31].

Abbreviations

CI
Confidence Interval
ITN
Insecticide-treated net (Permanet® 2.0)
ITN + PBO
Insecticide-treated net with PBO (Permanet® 3.0 roof)
GLM
Generalized Linear Model
PB
Priscille Barreaux
PBO
piperonyl butoxide
UTN
untreated net
WHO
World Health Organization

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All datasets generated, used and analyzed in this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

P.B. and M.B.T. conceived the study, P.B. developed the methodology, performed all the behavioural assays, monitored the mosquitoes for longevity, performed the statistical analysis, prepared the figures and wrote the draft; R.N'G. gave access to the laboratory space and his team collected the larvae in the field; all authors reviewed and edited the draft and M.B.T. provided financial support.

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Figures

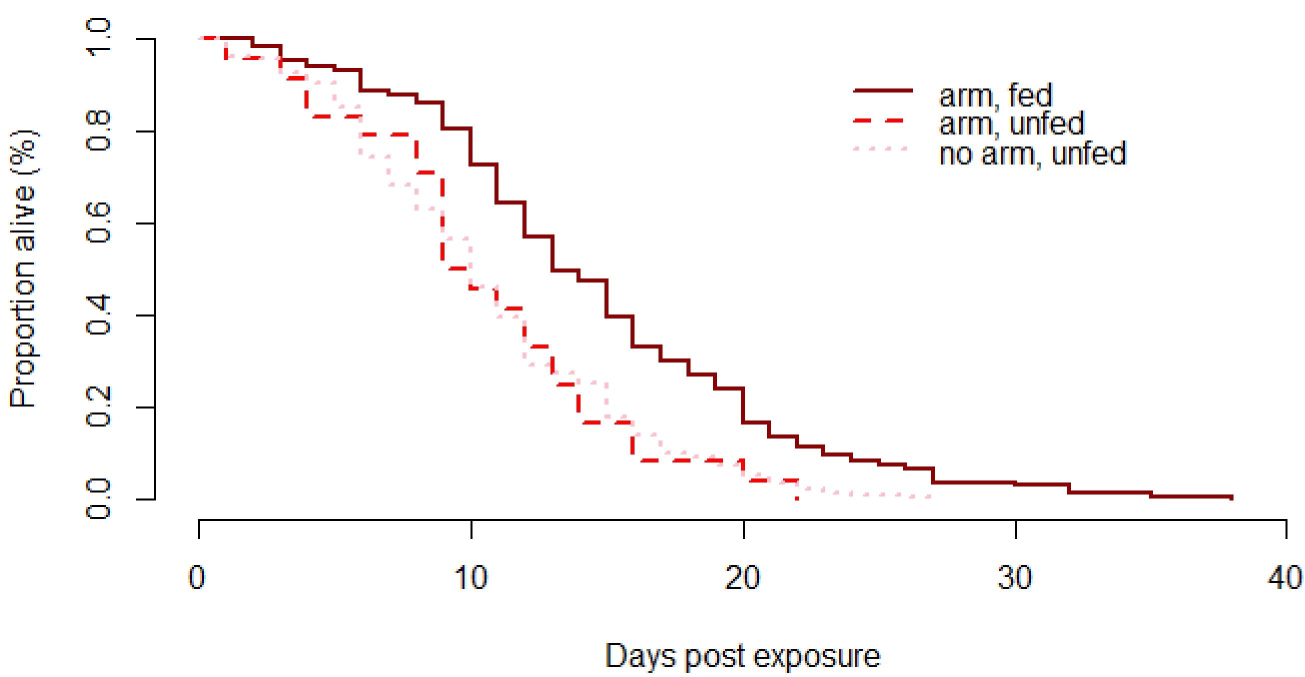

Figure 1

Survival curves for mosquitoes exposed in World Health Organization bioassays tubes against a Permanet® 2.0 for 1, 3 or 5 minutes and with or without access to a human host (arm). The 3 lines represent the survival curves of mosquitoes that took a blood meal when they had access to a human arm (solid dark red line), mosquitoes that did not take a blood meal while having access to a human arm (dotted red line) or mosquitoes with no access to a blood source (smaller dotted pink line). The experimental blocks 3 and 4 are not represented in this figure as there is no data for exposure times 1min and 3min.

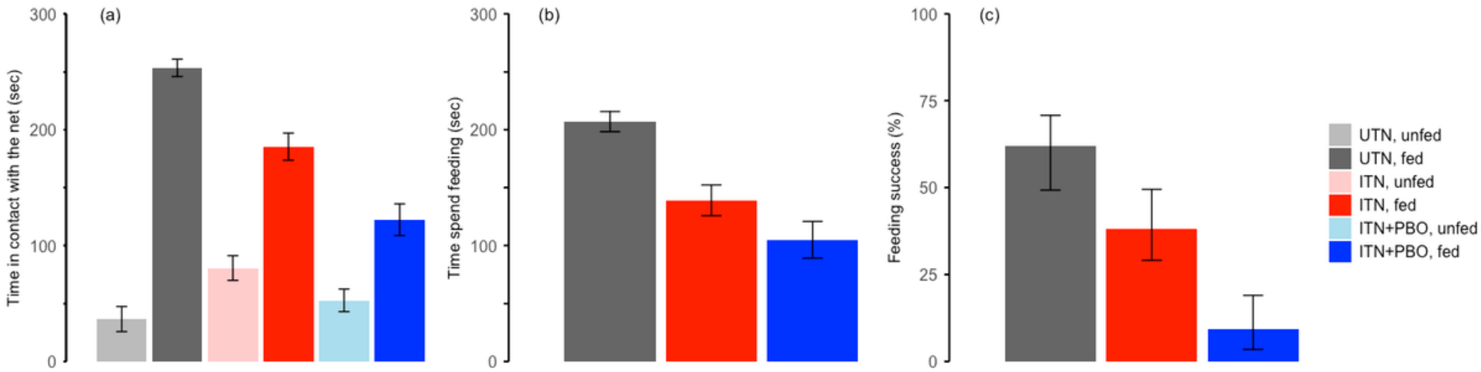

Figure 2

Panel representing the (a) mean time (in seconds) spent on the net, (b) mean time (in sec) spent taking a blood meal and (c) percentage of feeding success (in %) for mosquitoes exposed in a plastic cup against a Permanet® 2.0 (ITN), a Permanet® 3.0 (ITN+PBO), or an untreated net (UTN) for 5 min and with access to a human host. The black and grey bars represent respectively mosquitoes exposed to an UTN that did or did not take a blood meal. The red and pink colors represent respectively mosquitoes exposed to an ITN that did or did not take a blood meal. The blue and light blue and blue colors represent respectively mosquitoes exposed to an ITN+PBO that did or did not take a blood meal. The replicate 4 is not represented in this figure as there is no data for ITN+PBO. ± standard error bars are shown in the figures 2.a and 2.b and 95 % confidence intervals are shown in the figure 2.c.

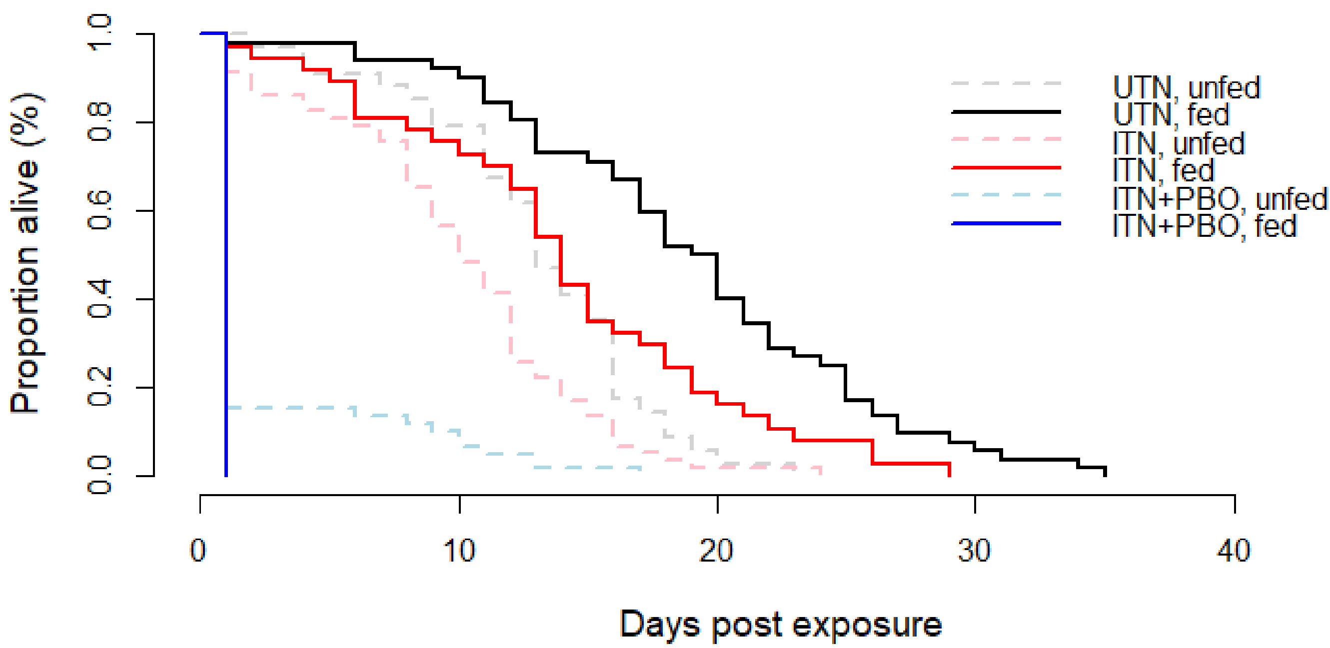

Figure 3

Survival curves for mosquitoes exposed in a plastic cup against a Permanet® 2.0 (ITN), the roof of a Permanet® 3.0 (ITN+PBO), or an untreated net (UTN) for 5 min and with access to a human host. The dotted lines and light colors represent the survival of mosquitoes that did not take any blood meal even though they had access to a human arm, the solid lines and darker colors represent mosquitoes that successfully fed. The blue lines represent mosquitoes exposed to an ITN+PBO, the red lines mosquitoes exposed to an ITN, the black lines mosquitoes exposed to an UTN. The experimental block 4 is not represented in this figure as there is no data for ITN+PBO.

Supplementary Files

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- 3.Additionalfile2.B.Statisticalanalysissummaries.docx
- 4.Additionalfile3.C.Statisticalanalysis.docx
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- 6.Additionalfile5.DatasetS2.csv
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