Large-Scale Deployment of Pyriproxyfen-Treated Lehmann Funnel Entry Traps to Control Malaria Mosquito Populations

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Research

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Abstract

Background: The Lehmann Funnel Entry Trap has proven to be effective in catching and killing up to 70% of mosquitoes even in a high mosquito density setting. A medium-sized prototype was selected and deployed at large scale in Vallée du Kou 3 (VK3) in the Southwest of Burkina Faso to assess its entomological and sociological impact.

Method: Overall, 1,313 traps impregnated with Pyriproxyfen (PPF), were deployed. Of them, 12 traps were randomly selected across the intervention village compared to houses without traps in the control village, Vallée du Kou 5 (VK5). Traps were placed at the windows while doors were blocked with curtains. Mosquitoes were collected in traps and matching houses in VK3 and in houses only in VK5, for nine days per month from July to October 2015. Collected mosquitoes were morphologically identified, counted, and preserved in 80% ethanol vials for subsequent analyses, including resistance genes and female mosquito age structure. The impact of the trap on mosquito density at community level was assessed by performing a pyrethrum spray catch (PSC) with bioassays to assess the effect of PPF.

Results: Overall mosquito density was reduced by ~90% in all houses equipped with traps in VK3. At the community level, while mosquito density before intervention was 33% higher in VK3 than in VK5, it was 47% higher in VK5 after the intervention. Old female mosquito numbers increased in VK5 by 12% in October but not in VK3, indicating that the traps were cumulatively killing old females. The additional effect of PPF was to limit egg-laying, with a smaller number of eggs counted, and with a low hatching rate. Mosquitoes were highly resistant to pyrethroids with ~0.9 frequency of the kdr mutation. The trap was well accepted by the communities as 85.4% and 93.8% of interviewees in VK3 found the traps reducing mosquito bites with peaceful sleep respectively.

Conclusion: The Lehmann Funnel Entry Trap has real potential to control malaria mosquito populations and can be widely used to sustain the global effort of malaria elimination.

Background

Vector control has contributed to the reduction of malaria burden over the last few years. The number of deaths due to malaria in 2019 is estimated to be 409,000, according to a recent WHO malaria report [1] (WHO, 2020). Several vector control related factors may have contributed to this reduction, such as Indoor Residual Spraying (IRS) and the mass distribution campaigns of Insecticide Treated Nets (ITNs) [2]. However, the development of the emergence and spread of insecticide resistance in malaria vectors is threatening the future of vector control [3–5], making it clear that alternative tools are needed to sustain vector control toward malaria elimination. Thus, discovering new tools or improving the effectiveness of the existing ones should be prioritized [6].

Since the link was established between malaria and mosquitoes [7], house window screening has been used as part of malaria vector control management [8]. More recently, studies using screens at windows, ceilings, and doors have proven to be effective in reducing the number of mosquitoes entering the house.
and highlighted a consequent protection of the households against mosquito bites and malaria cases [9–12]. Whilst some studies have established a relation between malaria illness and the type and quality of dwelling construction [13, 14], other studies have demonstrated that in addition to quality of construction of the home, modifications to prevent mosquitoes from entering houses can help reducing malaria transmission by lowering human exposure to infectious bites [11, 12, 15–19]. Interestingly, the use of mosquito-repellent plants in the home has also been shown to reduce mosquito densities [20], and there is a significant reduction of anaemia in children living in homes with window screens[21].

Although these methods deny mosquitoes entry to a dwelling, mosquitoes are not killed and so they can still bite outdoors and transmit malaria parasites [7, 22–24]. To date, most malaria transmission occurs indoors, and several malaria vectors bite indoors for their blood meal in human dwellings [15, 16, 25]. However, some malaria transmission may occur outdoors [26, 27]. As such, denying mosquitoes access to indoor living spaces is a worthy cause, but killing blood-seeking (and resting-site-seeking) mosquitoes could be a more effective goal.

Other alternative tools for insecticide resistance management include the use of molecules such as pyriproxyfen 10EC (PPF), which is a juvenile hormone analog that mimics a natural insect hormone that is essential for the normal physiological growth, development, and maturation of juvenile insects. As mosquitoes are holometabolous insects, the growth regulator hormone that PPF replaces is essential for larval development and its removal leads to undeveloped miniated pupal or adult stages [27, 28]. Formerly used as a larvicide, PPF has been tested on adult mosquito species and has shown an interesting effect on female fecundity and egg fertilization [28–30]. Adding a PPF insecticide to the trap that kills immature mosquitoes may therefore increase the effects on mosquito density reduction by additionally sterilizing any mosquito encountering the net. This proposal led to the development of a new approach, which considers mosquito behaviors and typical house entry routes for mosquitoes. In this approach, Lehmann Funnel Entry traps (LFET) were tested in high and low vector density settings as a malaria vector control tool [10]. Recently three new prototypes were created and successfully tested in two ecological settings southwest and northwest of Bobo-Dioulasso, Burkina Faso [31], in view of further use against malaria vectors.

Most vector control interventions against malaria require active participation from the local population to ensure their complete adherence to the work [32]. Given that LFETs are indoor intervention tools, community engagement is especially important in order to build sustainable control programmes that are resilient in the face of technical, operational, and financial challenges [33]. The space in the house has multiple functions and any additional material being asked to be fitted to or within the house may require a reorganization, and so the homeowner’s full participation is required.

The objectives of this trial were to assess the impact of a large-scale deployment of PPF-treated traps in terms of mosquito density reduction, the parity status of mosquitoes in the intervention village compared to the control village, and to evaluate the community perception and acceptance of the traps as well as the physical integrity of the traps over the study period. In addition, the effects of PPF on female An.
coluzzii mosquitoes over the study period were assessed, as well as the effects of the pyrethrum catch spray to evaluate the density dynamics between both sites.

Materials And Methods

Study area

The study was carried out in Vallée du Kou (11°23' N, 4°24' W, Fig. 1), an irrigated rice field area developed in 1970. The site is characterized by wooded savannah across 1,200 ha and contains seven discrete villages. Few insecticides are used to protect the rice crops from pests, but they are extensively used in the surrounding villages in cotton fields. High mosquito density is observed in August-September, corresponding to the peak of the rainy season. An. coluzzii is predominant throughout the year and An. gambiae is observed toward the end of the rainy season (frequencies fluctuating between 5-20%). Both species are highly resistant to pyrethroids and DDT (Kdr based mechanism, (0.8-0.95) [10, 34, 35]. Two villages, Vallée du Kou 3 (VK3) and Vallée du Kou 5 (VK5), were selected as intervention (IV) and control village (CV) respectively. The study started in May 2015, with the manufacturing of traps and production of nets followed by the deployment into the field. Trap installation began in June, and mosquito collection followed in July 2015. The period May-June corresponds to the beginning of the rainy season and coincides with the increase in numbers of mosquitoes in Burkina Faso. The rainy season ends in October.

Prior to trap fabrication, a general census of the houses and windows was conducted in VK3 using a Global Positioning System (GPS) [36] to count all the inhabited houses. Traps were then produced and deployed across the entire VK3 village.

Mosquito strains

Two mosquito strains were used for the PPF bioassay tests to evaluate the effect of PPF during the trial. The first, a susceptible An. gambiae-Kisumu strain, has been maintained at the Institut de Recherche en Sciences de la Santé (IRSS) insectary under standard conditions (Temperature 27±2 °C and relative humidity 80±10%).

The other strain, resistant An. gambiae s.l. mosquitoes, were collected at larval stage from breeding sites and adult stage from traps and houses in VK3 and VK5.

Design of the traps

The medium LFET (Fig. 2) was used in this study. The trap is made of a metal frame (69 x 51 x 82.5 cm) and is fitted from the bottom to the top with a regular mosquito net to prevent any mosquitoes or other insects from escaping the trap once they have entered (see Sanou et al., 2021, [31] for more details). Every window of the inhabited houses (non-inhabited house windows were secured simply with a net, to reduce the number of refuge sites for the mosquitoes) were measured to set up bespoke trap dimensions
for manufacture. Each manufactured net covers the trap entirely, which itself fits the window to which it is secured perfectly. Each trap’s net also has a sleeve for easy access, to open and/or close each of the windows.

**Deployment of the traps in VK3**

All the traps were painted with neutral oil (Fig. 3a), labelled per dimension, and transported to VK3. Once tailors completed sewing the net (Fig. 3a), all completed trap nets were brought to the IRSS laboratory for coating according to the WHO insecticide impregnation process with Pyriproxyfen 30 mg ai /m² (PPF). For each set of dimensions according to the window, calculations were performed to ensure proper coverage and uniform standards (supplemental files 1 & 2). After net coating, all trap nets were dried overnight indoors and wrapped up into labelled sachets for easy identification.

Each household was geo-localized (Fig. 1). For trap installation, the village was divided into six line-bands from the north to the south separated by blank areas used for circulation in the village (Fig. 4d). All eaves and holes in the houses were blocked using cloth or sponge and a curtain was placed at each door by a large team made up of local and technical workers (Fig. 3b). In total, 1,313 traps were placed at the windows of houses to intercept incoming mosquitoes, and a new curtain made from regular cloth was provided to every house in VK3 to block mosquito entrance through the door. No constraints were required on the use of the doors or windows, and occupants were free to go to bed at any time.

**Bio-efficacy of PPF on fecundity and fertility of laboratory and field collected mosquitoes**

Prior to impregnating the trap net, preliminary laboratory testing with the insecticide was conducted to assess its efficacy on susceptible *An. gambiae*-Kisumu strain and resistant field *An. gambiae* s.l. mosquitoes. This was in order to find the right sterilizing dose. The test used a piece of impregnated net with 20 mg/m² or 30 mg of active ingredient (ai) /m² in WHO cylindric tubes. As a result, the impact of PPF on female fecundity and egg fertility was also assessed over the study period.

**Effects of PPF treated traps on laboratory mosquitoes**

To assess how long a PPF treated net’s effect can last, the susceptible *An. gambiae*-Kisumu strain was used. A cage of 3-5-day old females was starved for six hours by removing the 5% glucose solution (weight/volume, w/v) prior to blood feeding at dusk. The blood feeding took place at 18:00 for 45 minutes using direct rabbit feeding in the laboratory. Only blood-fed mosquitoes were kept overnight and provided with 5% glucose solution in cotton balls for the experiments. The following morning, between 06:00 and 7:00, the mosquitoes were covered with a wet cloth (to maintain humidity) and transported by car to the field. Once in the field, one hour was given to the mosquitoes to rest prior to their release while the traps were emptied (for the monthly mosquito collection).
Around 150 susceptible An. gambiae-Kisumu blood fed females (12h after the blood meal) were then released once per month in VK3 (~50 females in each of two out of 12 monthly selected monitored traps) and in VK5 (50 females into one house). The testing was performed for 30 minutes in selected traps after which all the mosquitoes were recaptured and brought to the laboratory for oviposition (fecundity) and egg hatchability (fertility) assessment. Two releases of mosquitoes reared in the laboratory (An. gambiae Kisumu-strain in July and August) were performed over the study period.

After each release-recapture process in VK5, an indoor spray with insecticide (Kaltox Paalga, SAPHYTO, Burkina), was performed to kill the non-recaptured mosquitoes.

**Effect of PPF on field mosquitoes collected at larval stage**

To evaluate the PPF impact on field female An. gambiae s.l., mosquitoes were collected in VK3 at the larval stage and reared at the IRSS insectary until adulthood. Three-to-five-day-old females were then blood fed and exposed to the PPF-impregnated traps previously described for the susceptible An. gambiae-Kisumu mosquitoes. Blood feeding and handling were as described above. After blood feeding, fed mosquitoes (field and susceptible females) were kept under laboratory standard conditions overnight.

A total of 100 blood-fed female An. gambiae were simultaneously released in VK3 (in two selected traps in the third and fourth regular monthly collection traps) and in VK5 (50 blood-fed females into one house), once a month over the two-month trial.

**Effect of PPF on field mosquitoes collected at adult stage**

To assess the effect of PPF on field adult fecundity and fertility, field blood-fed female mosquitoes were collected from each of 12 randomly selected traps once per month over three months in VK3. Similar mosquito sampling was performed inside eight houses in VK5 and considered as control. About 25 blood-fed female mosquitoes per trap per day (VK3) and per house (VK5) were morphologically identified to species and brought back to the IRSS laboratory the same day and were allowed to lay eggs into a single cup containing Whatman filter paper and 5 ml of water for a week.

Cups were checked daily, and females that laid eggs were removed, killed, dried, and preserved into silica 1.5 ml cryotubes labelled for subsequent analysis. Eggs were counted under stereomicroscope and then hatched into rearing trays (43 × 26 × 15 cm) filled with 1 l of tap water with additional TetraMin baby fish food (TetraMin®, Germany).

Furthermore, to evaluate the impact of PPF on egg development compared to control as previously described [29, 30], about 100 field blood-fed An. gambiae s.l. were randomly collected from the monthly collection traps in VK3, and about 100 were collected from VK5 houses. The mosquitoes were kept individually in 20 ml cups with Whatman paper and 5 ml of water. Around 30 of them were dissected per day at 24h, 48h and 72h post-collection over three months.

**Entomological data collection during trap deployment**
To assess the trap performance, monthly mosquito collection was performed from 12 selected traps (trapped mosquitoes) and matching houses (indoor resting mosquitoes) in VK3, while only indoor resting mosquitoes from 8 houses were collected in VK5. Single room houses (single house with one window, one door) were randomly selected according to the geographic location (central, north, east, west and south) in both villages. Houses were far from each other, of at least 10 m to avoid human attractivity bias and were monitored over 9 days per month for both villages from July to October 2015. Mosquitoes were manually collected daily (36 collection days over the 4 months of trial.) with mouth aspirator in the traps and matching houses (for two hours) by three experienced collectors. The mosquito collection was simultaneously carried out in VK3 and VK5. Collected mosquitoes (traps versus house) were morphologically identified to genus, species, and physiological status [37], counted and a sub-sampled preserved in 80% ethanol vials for subsequent genotyping to species level and to check on the frequency of the knock down resistance \( (kdr) \) mutation.

To provide evidence of the impact of trap on mosquito density reduction at the village level, a pyrethrum spray catch (PSC) [38] was carried out simultaneously one day per month over the 4 month-trial, in 10 randomly selected houses/village (different houses from the regular study houses per village).

In addition, to assess mosquito population age structure from July to October, around 55 unfed female \( An. \ gambiae \) collected from the traps and houses were dissected and classified according to Detinova T (1962) [39].

**Socio-anthropological investigation on the use of the Lehmann Funnel Entry Traps**

*Qualitative* and quantitative surveys were conducted from March to August 2015 from the beneficiaries of LFET traps in VK3. The qualitative survey consisted of individual interviews with members of the community about their perception of the traps, and there was direct observation of trap usage. The quantitative survey covered 276 inhabitants and was based on the level of acceptance of the trap by its users, its perceived effectiveness, and the limits of the trap.

In addition, all inconveniences reported by users of the traps were recorded by a social worker and reported for subsequent measures. A follow-up survey was conducted once the traps were installed according to the WHO indices [40], all mishandling was also reported, and actions were taken to resolve any problems raised either by social workers or users.

**Physical conditions and cleanliness of the traps**

*Immediately* after trap installation, 50 nets were sampled and were checked for their physical integrity in 50 selected households over two months. The assessment of the fabric integrity was performed by a visual examination without the removal of nets from the selected households. Observed holes were assigned to one of four size categories according to the WHO guidelines [40]: a hole size of 0.5-2.0 cm or ‘< a thumb-sized opening’; a hole size of 2.0-10.0 cm or ‘> a thumb but < a fist’; a hole size of 10-25 cm or ‘> a fist but < a head’; and, a hole size of > 2.5 cm or ‘> a head’.
The integrity parameter was quantified based on two measurements:

- The proportion of nets with any hole. The integrity of the nets was determined by counting the number of tears and holes as described: total number of coded nets with at least one hole of size (1-4) × 100 / total number of nets assessed in surveyed households.
- The proportionate holes index (pHI) for each net, calculated as the sum of the holes weighted by size for each net. For this group, the weights used to calculate the pHI were 1, 23, 196 and 576 as described below: pHI = 1 × number of size − 1 holes + 23 × number of size − 2 holes + 196 × number of size − 3 holes + 576 × number of size − 4 holes. To better correlate the hole index to an integrity status (net condition) for each sampled net, the pHI was categorized into ‘good’ (pHI ≤ 64), ‘serviceable’ (pHI ≤ 768) and ‘replace’ (pHI > 768).

The trap net dirtiness (cleanness) was also evaluated, and nets were classified and categorized into ‘clean’, ‘a bit dirty’, ‘dirty’, and ‘very dirty’. When the net was completely damaged, it was replaced by another net of the same size.

Parameters measured and statistical analysis

Descriptive data was summarized, entered, and cross-checked in Microsoft Excel 2007 (Microsoft®, New York, USA), and R-4.0.4 was used to produce tables, graphs, counts, means and standard errors.

The PPF effect on female mosquito fecundity was calculated as the mean number of egg/female that contributed to the oviposition. The fertility was measured as the mean number of larvae/female that contributed to oviposition. We used a non-parametric pairwise test, Anova-glmmTMB to compare the reduction of fecundity and fertility between the group exposed to PPF-treated net and the control group.

A Generalized Linear Mixed Models (GLMM) with a Poisson or negative binomial distribution was used to choose the suitable distribution for the collected mosquitoes in the traps and houses from VK3 and VK5 respectively. We used zero inflated Poisson mixed regression modelling to estimate the intervention (trap) effect on daily numbers of mosquitoes collected while accounting for a possible spatial variation in terms of total mosquitoes collected between VK5 and VK3. This was for a possible difference in terms of physiological status (gravid, blood fed, unfed), or for a possible temporal variation induced by monthly weather conditions (rainfall, humidity, or temperature). Therefore, two models were built including two random-intercepts, one random coefficient and zero inflation terms. The model structure is defined with trap, gonotrophic _status, status, rainfall, and humidity considered as fixed effects (fixed effects = trap + gonotrophic _status + status + rainfall + humidity) and the total number of collected female mosquitoes as response variable.

Model 1: The probability of inflation terms was constant

\[ Y \sim \text{fixed-effects} + \text{random-} (\text{month}) + \text{random (village)} + \text{zero-inflation (} \sim 1\text{)} + \text{residual error} \]

Model 2: The probability of inflation terms was dynamic depending on the trap variable
Y ~ fixed-effects + random~ (village /month) + zero-inflation (~trap) + residual error,

where Y is the total number of collected female mosquitoes.

The sub-model of model 2 was built on basic count, zero inflated and altered models to account for zero values in the data. In these models, we removed the rainfall and the humidity fixed effects to compare their Root Mean-Square Error (RMSE) and the Median Absolute Error (MAE) to choose the best model. The lower the RMSE, the better the model fits. The model with minimum AIC (Akaike information criterion) was considered as the best model to fit the data [41]. Akaike's information criterion and recent developments in information complexity were used as this considers both goodness of fit and complexity of the model, to check the performance of zero-inflated models.

To prevent our model from overfitting, the trap data was split into a training set (0.8) and a test set (0.2), and then the different models were tested on the training set and confirmed with the test set. For all analyses, the level of significance chosen was 5% (supplemental les 3 & 4).

To assess the dynamic of mosquitoes between VK3 and VK5, pyrethrum spray catch data were analyzed using a non-parametric pairwise test, Anova-glmmTMB. The allelic frequency of kdr L1014F of the collected female mosquitoes from traps was calculated following the formula: F(R)= (2n.RR+n.RS)/(2(n.RR+n.RS+n.SS)),

(\text{where } n \text{ is the number of mosquitoes of a given genotype, } RR \text{ is the homozygote resistant, } SS \text{ the homozygote susceptible, and } RS \text{ the hybrid Resistant-susceptible}), \text{ to evaluate the insecticide resistance status in VK3.}

A one-way Anova was used to compare the age distribution of mosquitoes between VK3 and VK5.

Furthermore, physical integrity data were recorded, and descriptive statistics (mean, median, interquartile range) were used to compare pH1 values. Based on the pH1 score, nets were assigned to one of three condition categories and comparisons between hole indices of different categories were performed using a wilcoxon test.

Weather related temporal measures such as the mean temperature, the mean relative humidity and rainfall, were collected from Burkina Faso's meteorological station (http://www.meteoburkina.bf).

**Results**

**Bio-efficacy of PPF on fecundity and fertility**

Through the preliminary laboratory testing, the PPF dose 30 mg/m² ai showed a reduction of fecundity and egg fertility of female mosquitoes and was then selected to impregnate all of the trap nets.
Blood-fed female *An. gambiae*-Kisumu strain released into the traps for 30 minutes showed a significant higher reduction of fecundity (mean number of eggs per female) in VK3 as compared to VK5 (egg Control / (PPF_30mg/m^2) rate: 1.34, se: 0.091, df: 174, t-ratio: 4.318, p < 0.0001) over the course of the study. Similarly, a greater female fertility reduction (mean number of larvae per female) was observed in VK3 as compared to VK5 (larvae Control / (PPF_30mg/m^2) rate: 0.681, se: 0.078, df: 174, t-ratio: -3.358, p < 0.0001).

With *An. gambiae* mosquitoes collected at the larval stage and then blood-fed in a laboratory, there was a greater fecundity and fertility reduction in VK3 than in VK5, (egg Control / (PPF_30mg/m^2) rate: 1.45, se: 0.041, df: 214, t-ratio: 13.005, p < 0.0001 and larvae Control / (PPF_30mg/m^2) rate: 3.27, se: 0.026, df: 214, t-ratio: 14.750, p < 0.0001).

With blood-fed female *An. gambiae* collected from the traps, the fecundity reduction in VK3 and VK5 was similar (egg Control / (PPF_30mg/m^2) rate: 1.01, se: 0.009, df: 1147, t-ratio: 1.292, p = 0.1967), while a greater reduction in fertility was observed between VK3 and VK5, (larvae Control / (PPF_30mg/m^2) rate: 1.29, se: 0.019, df: 1147, t-ratio: 17.034, p < 0.0001) (Table 1). Furthermore, of 100 field blood-fed *An. gambiae* mosquitoes (collected monthly from the traps in VK3 and dissected after 24h, 48h, and 72h), 80 females had non-developed ovaries compared to normal ovaries of females from VK5 (Fig. 5).

Table 1: PPF effects on *Anopheles gambiae* Kisumu and field mosquitoes

**Effect of traps on mosquito population reduction (Trap performance)**

A total of 24,100 and 15,650 *An. gambiae* mosquitoes were collected over the study period in VK3 (from selected traps and matched houses) and in houses in VK5, respectively. Out of this, 90% (21,653/24,100) of female mosquitoes were collected from the traps as compared to 10% (2,447/24100) from the matching houses respectively in VK3.

Overall, 24,100 *An. gambiae* mosquitoes (including unfed, blood-fed and gravid females) were removed over nine days per month of collection in the selected traps in the village of VK3.

The removal on average through the selected traps ranged from 0.95 ± 0.19 to 31.76 ± 4.40 (means ± se) dead mosquitoes, from 8.96 ± 0.92 to 54.99 ± 5.34 live mosquitoes caught in trap, and 2.77 ± 0.43 to 10.18 ± 2.12 live mosquitoes collected indoor in VK3, compared to between 1.65 ± 0.18 and 21.44 ± 2.02 live mosquitoes collected indoors in VK5 (Table 2). No dead mosquitoes were collected from the houses in both sites. The daily mosquito removal averaged 201 per day per trap in VK3, which was 263,913 mosquitoes removed per day.

There were significantly fewer mosquitoes collected from the houses in VK3 as compared to VK5, regardless of the gonotrophic status (unfed, df = 0.4964, χ² = 2175, p < 0.0001, blood fed, df = 0.3525, χ² = 1193.2, p < 0.0001, and gravid, df = 0.1439, χ² = 665.27, p < 0.0001) (Table 3).
Figure 6 shows the gonotrophic status and distribution of *An. gambiae* females over the study period in VK3 and VK5 according to a daily mosquito catch. The number of unfed female mosquitoes caught was higher from day four to day eight, and there was a slightly lower number of blood-fed females from day four to day six compared to the gravid mosquitoes collected over the study time course (Fig. 6a). Figure 6b shows that a higher number of unfed females was collected in August in VK3, and September in VK5. The number of blood-fed mosquitoes in VK3 was higher than that seen in VK5, while the number of gravid mosquitoes in both villages was quite similar over the study period (Fig. 6b).

During the study, other mosquito species including *An. coustani, An. pharoensis, Culex sp, Mansonia sp* and *Aedes* (1169 in traps vs 89 in house in VK3, and 707 collected from the house in VK5) (Table 4) were also collected.

Table 2: Descriptive table of gonotrophic status of *An. gambiae* female mosquitoes in VK3 and VK5 over the study period

Table 3: Comparison and proportions of *Anopheles gambiae* female mosquitoes collected per house in VK3 and VK5 over the study period

Table 4: Numbers of other mosquito species caught in trap versus house in VK3 and VK5 over the study period

**Species and insecticide resistance identification**

*Anopheles coluzzii* (formerly *An. gambiae* form M) was identified as the only *Anopheles* species in VK3 and VK5. Out of 512 sub-samples analyzed, PCR revealed that most of the mosquitoes were highly resistant to pyrethroids and DDT 0.9 (*kdr* based mechanism), as shown in Table 5.

Table 5: Repartition of *Anopheles coluzzii* in traps and houses and allelic frequencies of the *kdr* mutation from Vallée du Kou, in overall mosquitoes collected over the study period.

**Pyrethrum spray catch with Kaltox Paalga**

The PSC with Kaltox Paalga showed that mosquito density in VK5 was significantly higher than that of VK3 (rate=0.662 se= 0.0113, df=176, t-ratio= -24.131, p < 0.0001) (Table 6).

Table 6: Pyrethrum Spray Catch data from VK3 and VK5

**Dissection of female mosquitoes on site and in the laboratory**

The dissection of the ovaries indicated that mosquito population kept a stable age structure as compared to the beginning of the intervention with about ~2% (1/55) of the population being parous females in VK3
and VK5 ($\chi^2 = 0.00016532$, df = 1, p-value = 0.9897). So, toward the end of the intervention, 12% (7/58) of the females were parous in VK3 as compared to ~3% (3/60) in VK5 ($\chi^2 = 1.7509$, df = 1, p-value = 0.1858) (Table 7).

Table 7: Number of unfed females parous and nulliparous from traps, dissected in both sites over the study period

### Social perceptions on the Lehmann Funnel Entry Trap in VK3

#### Acceptance and benefits attached to the Lehmann Trap

Qualitative and quantitative data showed a good acceptance level of the Lehmann Funnel Entry Trap by the interviewees. About 85.4% (223/276) of the beneficiaries declared that the trap had reduced the number of mosquitoes in the house. Trap users gave positive feedback during the interviews. For instance, one of the interviewees stated: "When you check the trap, there are a lot of mosquitoes like that. If all these mosquitoes should enter the home! Good gracious!" Table 10 shows the benefits gained from the deployment of the trap in VK3. Importantly, 98.55% of the respondents (i.e. 272 of 276) claim to have had peaceful sleep since setting up the traps in their homes, while 98.91% of the respondents believe that the trap will help to reduce malaria cases.

Table 8. Different benefits of the Lehmann Funnel Entry Trap in VK3

#### Perceptions of the effectiveness of the trap

Over 90% (248/276) of the users of the Lehmann Funnel Entry Trap claimed that it effectively protected against mosquitoes. The qualitative data backed-up the widespread appreciation of the mosquito trap, as shown in the following words of one beneficiary: “At night I sit in my house, and I'm not bothered by mosquitoes. When I feel sleepy, I go under the net. There are days I often forget to use the mosquito net, etc.”

#### Constraints related to the use of the trap

Even though the medium trap we manufactured and used was half the size of the original trap [10], some interviewees found the new trap to be too large and taking up too much space in the house. In addition, fixing the traps and the curtains sometimes resulted in damage, with some cracks in the walls of homes following installation. Furthermore, the movements of inhabitants through the entrance door led to curtain damage and subsequently repairs or replacements were sought through the course of the study.

#### The desire to keep the trap after the period of the entomological study

Most of the beneficiaries (97.1% or 268/276) stated that they appreciate and would like to keep their trap after the entomological study. Only one inhabitant demonstrated a lack of interest, however in this case
the inhabitant did not reject the tool but simply failed to reply to the questionnaire. Most respondents to the questionnaire found the prospect of paying 30 USD per trap to be costly.

**Physical conditions and cleanness of the of the traps during trial**

The proportion of nets with at least one hole was ~76% over the two months of study. Moreover, the number of holes was 141 and higher in the second month of survey than 91 holes in the first month. The mean of Hole index was significantly higher in the second month of follow up, 2.86 (95% CI =2.079 - 3.641) as compared to the first month 1.82 (95% CI =1.244 - 2.396; p = 0.0298). The number of holes based on the location of the net (entry window nets, 69 holes) was similar to that of rear window nets (74 holes) (p > 0.05). The data showed a greater number of holes in category hole-1 than that observed in the two other groups (categories hole-2 and hole-3) (121:20:1 respectively) (p < 0.0001). The location of holes in the net was explored during the second month of follow-up. Of the 141 counted holes, about 53 were found on the front of the nets, 38 on the seams, 30 on the length, and 20 on the width. There were more holes in the nets placed at window entry of the houses than in nets placed on rear windows, but this difference was similar for both kinds of nets. Twenty five percent of the holes were found along the length and width of the net followed by 10% and less than 1% respectively on the front side and the seams of the net. The median proportionate hole index (pHI) was 3.5 with IQR (2-27.5). The pHl for each net showed a good net utilization as 66% (33/50) of nets were found in a good condition (pHI ≤ 64) and only 1% were found as still serviceable (pHI ≤ 768 – serviceable). About 75% of the nets were clean. The dirty nets were the ones installed on the windows of houses built with bricks made of mud. Moreover, the dirtiest nets were located at the front of the house. At the end of each survey, nets with several holes were replaced by new ones of the same dimensions.

**Discussion**

The main objective of this study was to show the potential of the Lehmann Funnel Entry Trap to control malaria vectors in insecticide-resistant mosquito population settings. In addition, one challenge of this study was to produce as many LFETs as was necessary to cover the entire intervention village of VK3. A total of 1,313 traps were deployed in VK3, which contributed to a reduction of indoor mosquito density of 90% throughout the four months of the trial. Traps have proven to be effective in suppressing *An. gambiae* species and have furthermore successfully caught a variety of mosquito species including species that might have bitten the villagers if there were no traps deployed. Traps protected the population of the village from indoor mosquito biting, in addition to the impact of other malaria control methods already in use such as bed nets, IRS, repellent plants, etc. This could have consequently reduced malaria transmission in the village. This study is consistent with a recent study in Gambia showing that improved ventilation using window screening with netting that creates cooler houses at night can reduce malaria mosquito house entry [42]. Additionally, traps removed and killed a proportion of older outdoor mosquitoes from the village, which may have impacted on outdoor malaria transmission, as was recently shown in other studies [24, 43–45]. Successful implementation of LFET traps as a malaria tool may
focus on the direct monitoring of the adult mosquito population, as this provides a realistic picture of the effects of intervention [46, 47]. As part of this direct monitoring, the PSC results indicated that traps have significantly reduced mosquito population density at the community level in the intervention village VK3, when compared to the control village, VK5.

Interestingly, the dissection of the ovaries of mosquitoes collected indicated that the mosquito population had the same age structure at the beginning of the intervention (~2% female parous) in both villages. However, toward the end of the intervention, 12% of the females were scored parous in the control village against ~3% in the intervention village, indicating that the trap was cumulatively extracting and killing old females capable of transmitting malaria in the intervention village. Furthermore, adult dissection results showed that 80% of the females dissected failed to develop their ovaries as previously reported [29, 30, 47]. That the LFETs were impregnated with PPF increased the performance of the trap by sterilizing any female mosquito that came into contact with the trap net, preventing them from having offspring and consequently decreasing the malaria vectors’ population in the village. For this trial, mosquitoes (susceptible and field larvae reared into laboratory) exposed to PPF-treated nets over the study time course showed a fewer number of eggs per female and a lower egg hatch rate in the intervention village as compared to the mosquitoes from the control village. However, the fecundity of the mosquitoes collected from the traps in the intervention village was similar to the control mosquitoes. This may be due to the short contact time with the net experienced by some mosquitoes, as the entry rate and time spent on the trap’s netting are not evenly spread and similar for all mosquitoes. The impact of PPF on other trapped mosquito species was not assessed in this study. Nevertheless, recent studies have demonstrated a PPF sterilizing effect on Aedes spp, and Culex spp etc. when transported and spread by mosquito tarsi to breeding sites [47]. Consequently, this route could have impacted other mosquitoes within and around the LFET trial site.

In this study, the mosquito population was exclusively composed of An. coluzzii (the former M molecular form) in Vallée du Kou, which is in alignment with the previous studies [29, 48]. The kdr L1014F mutation conferring resistance to pyrethroids and to DDT was found in the samples analyzed to be very high (~0.9) when the study was carried out. This result is consistent with previous studies reporting a high level of resistance in mosquito populations due to the extensive use of pesticides in rice and cotton fields in the village [34, 49, 50]. In this study, environmental factors such as rainfall, humidity, and temperature had a great impact on mosquito densities. VK3 is one of the seven discrete villages of the whole Vallée du Kou (VK1 to VK7). The village is close to its sister villages, VK2 and VK4, on its western and eastern sides respectively, all surrounded by rice fields. It is clear that the poor isolation of VK3 has contributed to its sustained high mosquito density. It has been shown that mosquitoes can disperse up to two kilometers (2 km) looking for blood meal [51] and so this could have impacted on mosquito density in VK3.

As previously stated, most vector control interventions against malaria require active participation by the local population [32]. As such, a social follow-up was undertaken during the study. Most of the interviewees acknowledged the utility and the important role of the trap in capturing mosquitoes, as the
trapping effect was visible. The LFET has proven efficacy in controlling highly resistant malaria vectors. A recent study completed in the same ecological setting in Burkina Faso has shown that newer branded nets struggled to kill mosquitoes in this area, which makes it clear that additional intervention tools are needed to control resistant mosquito populations [52]. The fact that the trap can further help to control the mosquito population makes it very attractive. Once installed, the trap requires minimal changes in human behavior, and unlike personal protection tools such as bednets, it protects anyone that is sleeping within the dwelling. Furthermore, it has been shown that space inside the house has multiple functions [52]. When one brings a new item in the house that may impair the use of the space as it has been established, it becomes difficult to integrate the new item in the functions and space of the house. As such, community acceptance of the trap is crucial. Acceptance was high as the beneficiaries could see mosquitoes trapped every day in the morning and as a result, they felt that the trap could help reduce the disease burden. Importantly, most of the interviewees acknowledged that the trap reduced mosquito biting and allowed them to sleep peacefully, which is one of the most important criteria that impacts on the acceptability of conventional intervention tools such as bed nets [52]. The high acceptance of the LFET may explain the overall physical good condition of the traps seen at one to two months post-deployment.

Although the retail price of a single trap was estimated to be too high by the villagers, it is worth noting that they all wanted to keep the trap after the evaluation, meaning that the trap is very useful to them. It is common that most households receive malaria preventive methods, including bed nets, for free in Burkina Faso [53]. A cost-effectiveness study has not yet been done but it is important to note that a single trap installed in a house protects anyone sleeping within that house.

**Conclusion**

The Lehmann Funnel Entry Trap has proven efficacy in controlling highly resistant malaria vectors. The additional effect of PPF also enhanced its efficacy. The acceptance of the trap was high, as the beneficiaries could see the number of mosquitoes trapped in it every day, and it was acknowledged that the trap reduced mosquito biting and allowed peaceful sleep. Although the deployment of the LFET across an entire village showed a positive impact, a trial in an isolated site with a high mosquito population could better show the suppressing effect of the trap. No survey data on parasitemia and malaria cases were collected during this trial and so a trial with malaria epidemiological end points could link the performance of the trap against malaria. Nevertheless, there is growing evidence that trap installation in VK3 may reduce malaria transmission [54].

Assuming that the size of the trap can be further reduced and the price to the user can be lowered, LFETs may represent a viable business opportunity to entrepreneurs. The next step is to build a business plan and work alongside local window manufacturers to help them to start manufacturing the traps at a large scale. Efforts will be devoted toward the national malaria control program in Burkina Faso to integrate the Lehmann Funnel Entry Trap into the malaria control toolbox.
Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated and analyzed during this study are available on Github: https://github.com/RogerSANOU/Lehmann-trap-dataset.git.

Authors’ contributions

RS and HM carried out the field work, participated in the analysis of the data, and wrote the original manuscript. AO and AAM carried out the social field and geographic information system work respectively and revised the manuscript. ASH carried out the physical integrity work and revised the manuscript. SPS, KB, SAM, AMGB, LPT and RKD revised the manuscript. BDS carried out the statistical analyses. AD designed the trap and the study and supervised the entire work. All authors have read and approved the final manuscript.

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Tables

Due to technical limitations, tables 1 to 8 are only available as a download in the Supplemental Files section.

Figures

Figure 1

Map of Vallée du Kou and its different sectors or villages
Figure 2

1- Dimensions of the trap, front and side view. (a) Inserts front view: 69 cm wide × 165 cm high; 13.3 cm long × 11.2 cm wide (small opening of the funnel). (b) Inserts side view: 51 cm depth of the trap, 70 cm long × 54 cm diagonal (large opening of the funnel); 10 cm distance of the small opening of the funnel from the backside of the trap (Diabaté et al. 2013); (c) original prototype 1 outside view inside a house, 2-(a) Dimensions of the trap, front and side view. Inserts front view: 69 cm wide × 82.5 cm high; 13.3 cm long × 11.2 cm wide (small opening of the funnel). Inserts side view: 51 cm depth of the trap, 70 cm long × 54 cm diagonal (large opening of the funnel); 10 cm distance of the small opening of the funnel from the backside of the trap.; (b) Prototype 2 outside view inside a house, 3-Lehmann Trap within a house (a) and out view through window (b), (c), a curtain at the door

Figure 3

Local window manufacturers (a) and tailors producing the traps (a) and field entomology working local young people recruited to install traps (b)

Figure 4

Maps of VK3 village. (a) map of the houses distribution, (b) number of windows, (c) human density and (d) distribution of the households and colored bands the scheme of traps’ installation
Figure 5

(a): normal ovary from the control VK5, 10X, 24 h post contact, (b): normal egg in development from the control VK5, 24h post contact at 20X, (c): impact of PPF on egg development from VK3 24h post exposure at 20 X

Figure 6

*Anopheles gambiae* females gonotrophic status per day of mosquito catch (a) in both VK3 and VK5 villages and (b) summary of catches over the study period
Figure 7

Model validation graph on test set (actual and predicted values) (a) for Zero Inflated Poisson (ZIP) and (b) for zero Inflated Poisson Mixed Models, (ZIPMM)

Figure 8

Ten deciles test set (actual and predicted values) for ZINB.png prediction of mosquito catches in VK3

Supplementary Files
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- LFET2Supplementalfile.pptx