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Optimizing a Gravid Mosquito Resting Box to Enhance Auto-dissemination of Larvicides under Semi-Field Conditions in Western Kenya

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Abstract Malaria control strategies are challenged by emergence of insecticide resistance and behavioral changes of the vector. New vector management tools are required to avert control failure. The aim of this study was to optimize a mosquito resting box that act as contamination station for auto-dissemination of novel chemicals by female *Anopheles gambiae* to their oviposition sites. In this study, cotton fabrics (red, black, blue, white), circular & rectangular boxes of different sizes were tested for resting preference. Optimal box size and shape, aligned with most attractive colour, was dusted with red fluorescent dye (larvicide proxy). Two artificial oviposition sites were set up in a screen house, one of which was treated with Cedrol, the other had tap water only. Two to three days old bloodfed mosquitoes were used for resting preference whereas gravid females were used for auto-dissemination experiments. A high resting preference was observed in red and black fabrics (28.08 ± 3.211), (28.00 ±3.922) respectively, compared to white (4.67±0.890). Choice of colour was found to influence mosquito landing ($P=0.000<0.05$). With the choice of most preferred colour, the rectangular black box (45m×30m×45m) attracted high proportion (60%) of mosquitoes. The box effectively transferred dye to the resting mosquitoes and to the oviposition site, with 67% visited oviposition site, having dye on their body. These results reveal that the black rectangular box attracted adult blood-fed and gravid mosquitoes high enough showing great potential as future malaria vector control and/or sampling tool, and is recommended for further field-based evaluation.

Keywords Mosquito resting site; *Anopheles gambiae*; Semi-field system; auto-dissemination

Background

The success of malaria mosquitoes in transmitting malaria parasite relies much on their prolific nature of life. One of the strategies of malaria control is to reduce the host-vector contact frequency which ultimately interferes with malaria transmission through infectious bites (Massebo et al., 2015). The current common frontline approaches to reduce malaria burden is the use of long lasting insecticide treatment nets (LLINs), effective treatment using ACTs, indoor residual sprays (IRS), repellent products for personal protection and larval source management (www.who.int). Aquatic larval management is effective but it is highly challenged by habitat identification, accessibility of microhabitats, hard to reach areas, labor intensity and high cost of sustainability (Mains et al., 2015).

However, the predominant tools for control of adult malaria mosquitoes (LLINs and IRS), target mainly indoor resting adult mosquitoes, and does not cover outdoor vector populations and immature stages of malaria mosquitoes (Okumu and Moore, 2011). They are highly effective when used correctly, but mosquitos are developing both behavioural and physiological insecticide resistance to this form of control (Ong and Jaal, 2015). In this regard, behavioural pattern of malaria mosquitoes towards potential host, resting site and oviposition sites has become a priority in the effort to combat the development of resistance (Edward et al., 2016). Tracking the response of mosquito to different colour tinge in an attempt to understand its behaviour, provides a powerful tool for improving vector population management (Frances and Gabriella, 2016). A mosquito resting box is a potential tool that can be used as a contamination station targeting blood-fed and gravid malaria mosquitoes. Knowledge of visually-controlled mosquito behaviour is of critical importance for designing a resting box to improve surveillance and control of disease vectors.

The resting box designed in this work can be deployed in an attract-and-kill strategy for horizontal transfer and auto-dissemination of larvicide. Use of an effective larvicide that has novel mode of action mitigates against the risk of resistance development and is useful for insecticide resistance management (Michelle et al., 2012; Russell et al., 2013). Importantly, the box can incorporate recently discovered lures for gravid mosquitoes such as Cedrol (Okal et al., 2015), leading to enhanced trapping rates of malaria mosquitoes and reduced mosquito densities. The goal of this work was to develop a cheap, passive (non-powered), easy to use and efficient tool for malaria mosquito control scalable for adoption in rural African communities. Results obtained in semi-field studies indicate a great potential for the MRB to be used as a mosquito control and surveillance tool, and future open field studies will assess the potential impact of the MRB on mosquito densities and disease transmission dynamics and also evaluate easy-to-assemble designs for use in rural African communities.

1 Results

1.1 Visual acuity of mosquitoes to different colours

Evaluation of mud wall as resting sites (Table 1) revealed that the highest resting preference was observed in red and black fabric with a recapture proportion of (28.08 ± 3.211) and (28.00 ± 3.922) respectively, while blue had recapture proportions of 13.25 ± 2.168 and white had 4.67 ± 0.890. For fabrics anchored on metallic frames and positioned at 1.65 m and 3.7 m apart (Table 2), higher recapture rates were observed with shorter distances from the mosquito release point. At 1.65m, black colour significantly recaptured 53% of mosquitoes, compared to red (38%), blue (4%) and white (5%) (f =27.015, d.f = 3, P =0.000<0.05). When frames were positioned at 3.7 m apart from the releasing point, black fabric recaptured 43%, red 36%, blue 18% and white 3%. The difference between colours was also significant (f =14.006, d.f = 3, P=0.000<0.05).

1.2 Optimizing the size of mosquito resting box (MRB)

In the first approach, mosquito resting box (MRB) design was adopted from Mmbando et al. (2015). The design was assembled using translucent Perspex material and named Pers-Box one (PB-1). It had five louvers in both four sides and free removable top. Inside was a capsule covered with a black hydrostatic netting providing a dark hiding area for mosquitoes. During experiment, only 4% of released mosquitoes (n= 200) were found resting in it. As a result of this low resting percentage, another design was developed, adopted from Kweka et al. (2009); Pombi et al. (2014) design and named it cardboard box one (CB-1) of dimensions (30 cm×24 cm×25 cm). One of its side was fully opened (30 cm ×25 cm) as a mosquito entry point. The box caught 10% of resting mosquitoes, this was twice as much compared to Mmbando prototype design (PB-1).

Table 1 Mosquito resting preference on fabrics when displayed on the mud hut walls (MHWs) as resting site in SFS

Fabric				Attraction proportion (95% CI)		
Colour	Sum	Mean	N	(Mean ± SE)	SE	SD
Red	337	28.08	12	28.08 ± 3.211	3.211	11.123
Black	331	27.58	12	28.00 ± 3.922	3.922	13.588
Blue	159	13.25	12	13.25 ± 2.168	2.168	7.509
White	56	4.67	12	4.67 ± 0.890	0.890	3.085

Table 2 Mosquito resting preference on fabrics anchored on metallic panels positioned at 1.65m and 3.7 m apart

Metallic frames, positioned at 1.65 m apart					Metallic frames, positioned at 3.7 m apart			
Cotton Fabric	Attracted proportion (95% CI)				Attracted proportion (95% CI)			
Colour	Mean	(Mean ± SE)	SD	Recap. (%)	Mean	(Mean±SE)	SD	Recap. (%)
Black	23.88	23.88 ± 3.356	9.493	53.2	23.88	18.13 ± 2.302	6.512	43.4
Red	16.88	16.88 ± 2.263	6.402	37.6	14.88	14.88 ± 2.19	6.198	35.6
Blue	1.75	1.75 ± 0.773	2.188	3.9	7.63	7.63 ± 2.507	7.090	18.3
White	2.38	2.38 ± 0.885	2.504	5.3	1.13	1.13 ± 0.350	0.991	2.7

In the same box size pursuit, another relatively big box (45 cm L×30 cm L×45 cm H) was designed with one side fully opened (45 cm×45 cm) and named it cardboard box (CB-2). The resting increased drastically (Table 3) with a proportion of (74.25 ± 4.406), which was far much compared to PB-1 (8.13 ± 0.854), CB-1 (17.58 ± 1.699). The experiment showed that the number of mosquitoes entering the resting box is significantly dependent on the space created (Torr and Vale, 2015), for their entry into the box (F=245.644, d. f=1, p=0.000).

1.3 Optimizing box shape preferred by gravid mosquitoes.

Circular and rectangular box designs were compared, rectangular design recorded the highest number of resting mosquitoes, approximately 60% of released mosquitoes (n= 200), and circular box recorded approximately 40% (Table 4).

1.4 Mosquito contamination in the MRB and transfer of dye to artificial larval sites.

The Cedrol treated tap water (test site) recaptured 62% mosquitoes seeking an oviposition site and untreated tap water site (control) accounted for 38%. At the Cedrol treated larval site, 33% of mosquitoes visiting had partial dye, and 67% had full dye on their bodies. At the control larval site, 35% had partial dye while full dye was 65% (Table 5).

Table 3 Small (45 cm×22 cm) and large mosquito entry (45 cm×45 cm) of CB-1 and CB-2 design (30 cm×24 cm×25 cm), (45 cm×30 cm×45 cm) respectively

Box			Attraction proportion (95% CI)		
Entry space	Mean	SD	(Mean ± SE)	SE	Recap. (%)
Small entry size (45 cm×22 cm)	4.92	1.38	4.92 ± 0.398	0.398	6.0
Larger entry (45 cm×45 cm)	74.28	15.26	74.25 ± 4.406	4.406	94.0

Table 4 Mosquito resting efficiency using circular and triangular box design

Box				Attraction proportion (95% CI)			
Type	Sum	N	Mean	(Mean ± SE)	SE	SD	Recap. (%)
Rectangular	891	12	74.25	74.25 ± 4.406	4.406	15.262	60
Circular	617	12	51.42	51.42 ± 3.947	3.947	13.675	40

Table 5 Effectiveness of treated box (CB-2) in delivering dust to the resting adult female mosquito and auto-dissemination to artificial oviposition sites

Treatment site						Control site			
Artificial			Attracted proportion (95% CI)				Attracted proportion (95% CI)		
Habitat	Mean	N	(Mean ± SE)	SE	Recap. (%)	Mean	(Mean ± SE)	SE	Recap. (%)
Partial dye	19.0	12	19.0 ± 2.33	2.33	33	13.0	13.0 ± 2.02	2.02	35
Full dye	39.0	12	39.0 ± 2.33	2.33	67	23.0	23.0 ± 2.39	2.39	65
Treatment A							Control B		
	58.0	12	58.0 ± 3.14	3.41	62	36.0	36.0 ± 3.08	3.08	38

2 Discussion

Our study hypothesized that dull colour is likely to attract more mosquitoes compared to bright colour and rectangular box collect more mosquitoes than circular box with preference proportional to box size. The study provides evidence that mosquitoes perceive objects visually and visual stimuli discriminate different colour shades, a finding which is in agreement to a related study by Duffield et al. (2017).

In the colour choice experiment, four cotton fabrics of different colour value (Black, Blue, Red and White) were anchored inside the hut wall using 1 inch ordinary nails. This was intended to picture the real environment in an African hut where pieces of fabric are often used to cover the inner walls of mud-huts. The results reveal that red and black fabrics attract relatively equal mosquitoes and are most preferred. Our colour-choice experiments revealed that mosquitoes perceive colour better when it is closer and tend to lose that ability as it moves away from the dissemination station. This observation relates to the inherent mosquito behavior where they can visually recognize an object in their host seeking response (Burkot et al., 2013; Frances and Gabriella, 2016). Black fabric was therefore identified to be the most preferred colour by gravid mosquitoes and therefore fit for designing a mosquito resting box. This is in agreement with the report showing black cloth as a suitable surface that encourage mosquito landing and also effectively hold larvicide (Scholte et al., 2005).

The metallic-framed cloth experiments positioned at 1.65 m apart had the black colour as most preferred resting site (53%), followed by red at 38%, blue at 4% and white at 5%. A similar scenario was observed after when frames were 3.7 m apart although the number of mosquitoes resting was marginally reduced. It therefore implies that for more mosquitoes to get into the resting box, the MRB should be positioned closer, approximately 1 m to 3 m from the potential hiding zone or even from a potential blood host, especially when used indoors. However, this distance does not conflict the findings that approximate the maximum flight distance of mosquitoes, estimated between 50 m to 50 km because 1-3 m is within that estimate (Verdonschot and Anna., 2014), also still within the flight range reported on post-blood meal flight distance experiment estimated at 106.7 m (Greenberg et al., 2012).

In the optimization of the resting box size, the first design reflected Mmbando et al. (2012) prototype, but it had a capsule inside and five louvers at both four corners to let in as many mosquitoes as possible with minimal restriction. However, this design recaptured 4% of the released mosquitoes, and which was considered to be dismal. To optimize the design, small box (30 cm×24 cm×25 cm) was constructed using a cardboard box (CB), a narrow entry point was created at one of the side (The design recaptured 10% of mosquitoes and therefore not different from the first design.

When entry size was enlarged using larger box construction (45 cm×30 cm×45 cm), the box drastically increased the number of mosquito entering inside to 94%. In this experiment, the released mosquitoes were recaptured at an interval of 2 h, as from 0700 h to 1,500 h. It was observed that a high number of recaptures occurred between 0700 h to 1,100 h, and the proportion decreased towards 1,500 h.

Experiments for the preferred shape involved a rectangular and circular design based on the premise that these were representative of the major shapes in the natural environment. When both boxes were placed together in the same hut, the circular box (CB-4) recaptured approximately 40% and rectangular shape 60% of the test mosquitoes. The recapture was observed to be high in the morning and low in the afternoon. This variation in resting behavior of mosquito with respect to time, provide vital information that emphasizes on the appropriateness of setting the MRB in the morning, in order to maximize its resting preference by mosquitoes. The difference observed between the two box shapes was significant (P= 0.001) showing a preferred rectangular box over a circular shape. This finding concurs with the other studies in which most of their box designs were rectangular (Okumu et al., 2010; Pombi et al., 2014; Mmbando et al., 2015). However, the reason for this preference is not known. One striking difference of the design of the box in our study is that it was a passive box that used no power, yet previous studies had investigated boxes that were either powered by electricity, solar or a battery. Our approach was to obtain a prototype box that was easy to assemble, non-powered and sturdy for use on rural African settings.

The ability for the optimized box to act as an auto-dissemination or contamination station was demonstrated using non-toxic fluorescent dye as a proxy for larvicide, insect growth regulator or slow kill adulticide. A recent contamination station involves a sticky resting box designed by Pombi et al. (2014), that prevent resting mosquitoes from escaping out of the box to the larval site. In our approach, the intention was to demonstrate the possibility of resting mosquitoes in the MRB picking the dye, fly out of the resting box, escape from the hut and visit an artificial oviposition site installed outside the hut within a SFS (Wetoijera et al., 2014). Generally, Cedrol (oviposition attractant) treated oviposition site received highest number of oviposition site visiting mosquitoes 60% (74.25 ± 4.406) and control (tap water only) received about 40% (51.42 ± 3.947). This significant level of Cedrol attraction, concur with the result obtained by Okal et al. (2015). At the treatment site, 67% of recaptured mosquitoes were observed to have full dye and 33% had partial body dust contamination. Similarly, 65% of mosquitoes had full dye at the control site and 35% partial dye. More mosquitoes in this experiment were observed to carry full dye on their bodies, an indication of successful transfer of dye from the box to the resting insect and transfer of dust by insect to the oviposition site. Moreover, this justify that a significant quantity of dye can be transferred by mosquito to the larval habitat and it begins to answer the question of the quantity of dust a mosquito is able to transfer in an auto-dissemination system.

3 Materials and Methods

3.1 Study area

The study was conducted at the International Centre of Insect Physiology and Ecology, Thomas Odhiambo Campus, Mbita point (icipe-TOC). Icipe-TOC is in Mbita municipality on the shores of Lake Victoria (Figure 1) in Western Kenya (0° 26’ 06.19” S, 34° 12’ 5313” E; altitude 1137 m above sea Level). The campus sits on 24.5 hectares of land, 40% of which consists of experimental fields with green houses (Figure 2a; Figure 2b; Figure 2c) and landscaped buildings. The major malaria vectors in this area, which maintain holoendemic malaria, include *An. gambiae* Giles, *An. arabiensis* Patton, and *An. funestus* Giles (Kawada et al., 2011). The area has two rainy seasons; the long rains, which extend from March to June and the short rains which extend from August to December. Climatic conditions consist of temperature ranging from 17°C to 34°C. Annual rainfall ranges from 700 mm to 1,200 mm.

3.2 Mosquito preparation

Laboratory reared female *Anopheles gambiae* mosquito, Mbita strain (hematophagous) with ability to transmit malaria parasite during their feeding on the suitable susceptible host were used (Olasemi et al., 2011). The larval stages were fed on Tetramin fish baby food. Temperature and relative humidity in the insectary (colony room) varied between 26°C and 30°C and 67%~73%. Mosquitoes (2~3 days old) were held in a 30 × 30 × 30 cm netting cage, maintained on 6% glucose solution given as energy source *ad libitum* using absorbent paper wicks soaked in 25-ml vials. A piece of cotton (50 × 25 cm) saturated with distilled water, positioned on top of the cage twice a day, ensured that mosquitoes remained hydrated throughout. Mosquitoes were fed on blood for 15 min from a human arm by volunteer staff at 0600 h after starving them for 6 h before the blood-meal. Mosquitoes were left undisturbed for 10 minutes after the blood meal.

3.3 Mosquito identification and collection

Gorged female mosquitoes were selected through visual inspection for experiment using mouth aspirator and transferred to a paper cup. Females were presumed gorged when they had distended abdomen, an indication of successful blood feeding.

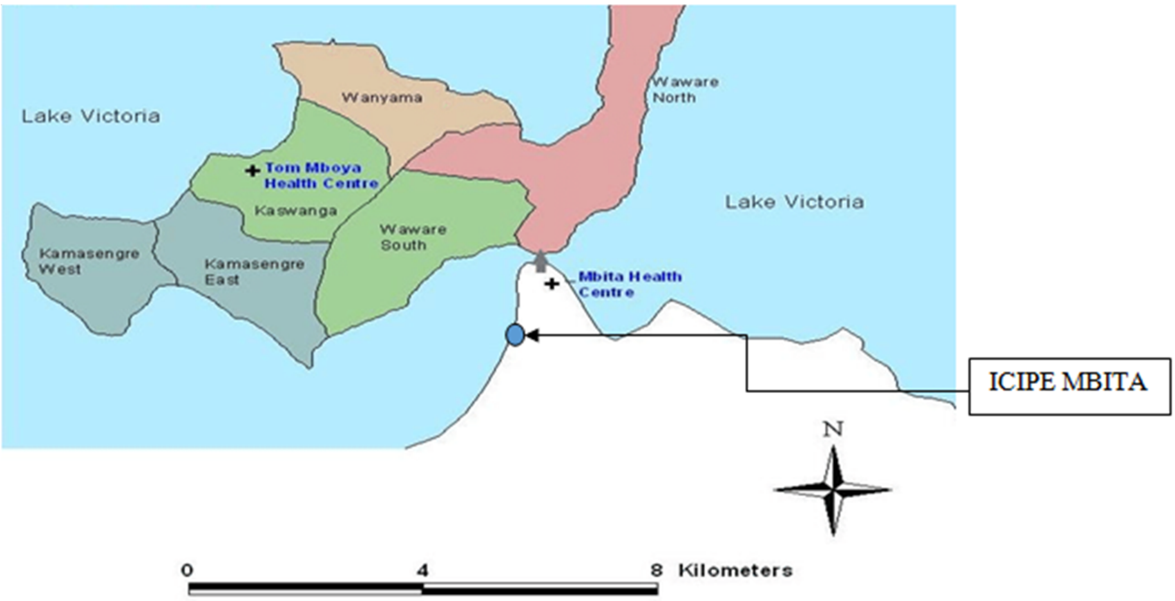

Figure 1 Location where research was conducted under semi-field conditions, Western Kenya

a) Semi field system(SFS) with hut inside b) Mud walled hut inside SFS c) Open SFS (No hut inside)

Figure 2 Semi field systems (SFS) where experiments were conducted

3.4 Experimental design and procedure

3.4.1 Visual acuity of mosquitoes to different colours

a) Fabrics anchored on mud hut walls as mosquito resting sites

Experimental design

Four (4) coloured cotton fabrics were randomly selected (red, blue, black and white) for use in the mosquito visual acuity experiment. We assessed mosquito resting behaviour in two experimental settings; (i) anchored fabrics on the hut in a semi field system (Figure 2a), the hut walls made of mud (Figure 2b) and (ii) anchored fabrics on the metallic frames set inside an open SFS (Figure 2c). The 3 m x 3 m mud hut had 4-equal sides at equidistance and the four randomly collected cotton fabrics of different colors (red, blue, black and white) were pinned on each side of the wall (4-walls) inside the hut using 1 inch ordinary nails (Figure 3a; Figure 3b; Figure 3c). Across experimental replicates, fabrics were anchored on rotation and in consistent height to eliminate positional bias.

Procedure

Insectary reared (2-3 days old) freshly blood fed *A. gambiae* s.s malaria mosquitoes (n= 200) were collected from the insectary cage into a paper cup at 1,830 h and released at the Center of the hut at 1,900 h then left undisturbed for 2 h. Recovery of released mosquitoes was done in 2hr intervals post release, precisely at 0900 h, 1,100 h, 1,300 h and 1,500 h. Mosquitoes resting on the coloured sheets were recaptured using a mouth aspirator and transferred into respective color-coded cups.

b) Fabrics anchored on metallic frames (placed at 1.65 meters and 3.7 meters apart) as mosquito resting sites in open SFS

Experimental design

To eliminate potential bias due to hut-SFS experiment, we used an open screen house (12 m x 7 m, no hut inside, Figure 2c). Four (4) metallic frames of dimension 1.83 m×1.22 m were constructed and coloured cotton fabrics were anchored on frames, fastened at every corner to create a flat surface (Figure 4a; Figure 4b; Figure 4c). The frames were positioned inside an open screen house in two arrangements, first at 1.65 m and then 3.7 m apart (Figure 4d).

a) 1st Section of hut walls with anchored coloured fabrics inside to create resting sites

b) 2nd Section of hut walls with anchored coloured fabrics inside to create resting sites

c) 3rd Section of hut walls with anchored coloured fabrics inside to create resting sites; Hobo data loga at the middle corner

Figure 3 Fabrics of different colours pinned on the inside walls of the hut, within the SFS

a) Red fabric anchored on a frame

b) Blue fabric anchored on a frame

c) Black fabric anchored on a frame

d) frames arranged inside SFS, insect releasing point at the center

Figure 4 A setup of panels that allows for equal distribution of light and equidistant rotations of the panels

Procedure

Insectary reared (2-3 days old) freshly blood fed *A. gambiae s.s* malaria mosquitoes (n=200) were released at the central point inside the open screen house (2.2 m from each frame) at 0700 h then left undisturbed for a period of 2 h. In 2 h intervals, mosquitoes that were resting on the sheets were recaptured using mouth aspirator and transferred into colour coded collection cups. Rotation of frames was done in each replicated experiments to eliminate bias to an experimental side of the screen house (SFS).

3.4.2 Optimizing the size of mosquito resting box (MRB) and the preferred entry point

Different box designs were constructed and evaluated to establish the preferred resting box size for blood fed mosquitoes. To assess entry point preference, the cardboard material was used because it is easy to manipulate, cheap and readily available, but final design would use Perspex or any other material deemed appropriate.

Experimental design

A prototype box adapted from Mmbando et al. (2015) was assembled using translucent Perspex material and an inner capsule fitted with black electrostatic netting and tested in open SFS. For entry point preference, a small box (30 cm L×24 cm W×25 cm H) named cardboard box one (CB-1) was constructed; having one of its side fully opened for mosquito entry (30 cm×12 cm). The inner walls lined with black cotton fabric. The box was suspended at 0.85 m from the ground (to avoid predation by ants) inside the hut.

Procedure

In optimizing the box size, blood fed laboratory reared (2-3 days old) mosquitoes (n=200) were released 2 meters from the MRB at 0700 h in 12 consecutive experimental nights. The release positions were varied on the three sides of the screen house and recaptured mosquitoes recorded.

In the entry point preference experiments, blood fed *An. gambiae s.s* (n= 200) were released 1 m from the box at 0700 h. Recapture was done at an interval of 2 hours up to 1700 h. CB-1 was modified to create a bigger box (45cmL×30cmW×45cmH) and named it cardboard box two (CB-2). One of its sides was fully exposed (45 cm×45 cm) for mosquito entry and inside lined with black cotton fabric. All other experimental conditions remained similar to the former experiment.

3.4.3 Compare rectangular and circular shapes of the mosquito resting box

Experimental design

A prototype box shape adapted from Sikulu et al. (2009) and Kweka et al. (2009) was used as a baseline to evaluate the optimal shape of a mosquito resting box (MRB) for freshly blood fed and gravid *An. gambiae s.s.* Boxes (rectangular and circular) were assembled using cardboard material and inner surface fitted with black cotton fabric (Figure 5a; Figure 5b). The MRBs were positioned individually in the SFS at a corner and rotated in the four corners daily to eliminate the aspect of positional bias.

Procedure

For every experimental day, freshly bloodfed insectary reared (2-3 days old) mosquitoes (n= 200) were released at the center of the SFS at 0700 h. The observations were done in an interval of 2 hours from 0900 h up to 1700 h.

a) Rectangular box, one side opened b) Circular box, one side opened c) OviART Trap set up in SFS , Dugassa et al., 2013 d) Sucking fan, a section of OviART, Dugassa et al., 2013

Figure 5 Optimised mosquito resting boxes and a setup of artificial oviposition sites in the SFS

3.4.4 Evaluating mosquito contamination in MRB and auto-dissemination of tracking to artificial oviposition sites

Experimental design

The optimized rectangular mosquito resting box (Figure 5 a) was dusted with 5 g red fluorescent dye to create a contamination station. The fluorescent dye in this experiment was used as a proxy for a larvicide. Artificial mosquito oviposition sites were placed at the back corners of the screen house by digging in two (n= 2) black troughs of 15 liters and filled them with 10 liters of tap water (Figure 5c; Figure 5d). Cedrol (5 ppm) was added in artificial oviposition site A (Treatment) as an oviposition attractant while site B had tap water only (Control). Sodium chloride (150 g/L) was dissolved in both sites just 10 to 20 minutes before the beginning of experiment to facilitate the release of odour from artificial oviposition sites.

Procedure

Laboratory reared adult gravid *A. gambiae* mosquitoes (n= 200) were released near the MRB at 1,700 h, mosquitoes were given time to enter the box, the box was then closed with a mosquito netting cover and left for 2 hours. All other mosquitoes in the hut were vacuumed out except those in the MRB. The closed hut windows were opened and the netting box cover removed to allow mosquitoes resting in the box to freely fly out to visit artificial oviposition and resting sites within the screen house. An OviART gravid mosquito trap developed by Dugassa et al. (2013), was used to collect gravid mosquitoes that quested on the artificial oviposition sites outside the hut (Figure 5c; Figure 5d). The experiment was left to run till the following day at 0800 h. Mosquitoes found in the collection chamber of the OviART trap were put in the freezer for 20 minutes to die. Mosquitoes contaminated with dye were categorized as partial (when they had dye on wings and legs) and as full (when they had dye on legs, wings and body).

3.5 Statistical analysis

Data entry and validation was done using MS-excel 2010 version. The multiple comparisons of four colours and four hut wall was done by Post Hoc analysis using Turkey's test and one-way ANOVA by using SPSS version 20.0. Preferred resting box shape (rectangular or circular) was determined using independent samples t-test. All values were expressed as mean ± standard error (SE). The dye contamination conditions of mosquitoes that visited oviposition sites were presented in percentages for each oviposition site.

Authors' contributions

MBO designed the study, carried out the experiments, collected the data, performed the statistical analysis and interpreted the data, wrote and edited the manuscript. PO participated in mosquito culture and data collection. FMW participated in supervision and guidance during the study. DRS participated in the study design and supervised the study. JMM participated in the study design, supervision, manuscript writing, and coordination of the study. All authors read and approved the final manuscript.

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