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# Attracting and killing outdoor-biting malaria vectors using odour-baited mosquito landing boxes (MLB) equipped with low-cost electrocuting grids

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# Attracting and killing outdoor-biting malaria vectors using odour-baited mosquito landing boxes (MLB) equipped with low-cost electrocuting grids




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This research report has been submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of requirements for the award of the Masters of Science in Medicine (Biology and Control of African Disease Vectors) degree

**Declaration**

I, Nancy Stephen Matowo, declare that this is my own original work, and that it has not been previously submitted for any degree at any other university.

Signature:  Date: 3rd May 2015

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**DEDICATION**

To my wonderful parents for raising me to trust that everything is possible with God.

**Abstract**

**Background:** Ongoing residual malaria transmission is increasingly mediated by outdoor-biting mosquito populations, especially in communities where insecticidal interventions like indoor residual insecticides (IRS) and long-lasting insecticide treated nets (LLINs), are used. Often, the vectors are also physiologically resistant to the insecticides, making this a major against malaria elimination.

**Methods:** A recently developed odour-baited device, the mosquito landing box (MLB), was improved by fitting it with low-cost electrocuting grids to instantly kill attracted mosquitoes. An automated water-proof light sensor was also added to switch the attractant-dispensing and mosquito-killing systems on and off at dusk and dawn respectively, thus conserving energy, improving safety and removing need for frequent human-handling. MLBs fitted with one, two or three electrocuting grids, were then compared in a malaria endemic village, in south-eastern Tanzania, where vector populations are increasingly resistant to insecticides. The evaluations were done outdoors in dry and wet seasons, in 3 × 3 Latin square designs.

**Results:** Significantly more mosquitoes were killed when the MLBs had two or three grids, relative to one grid ($P<0.05$), regardless of season. During the wet season, MLBs with two or three grids killed more *Anopheles arabiensis* ($P<0.001$), but equal numbers of *An. funestu*s ($P>0.05$) compared to MLB with one grid. In the dry season, MLB with three grids killed more *An. arabiensis* ($P<0.001$), but equal numbers of *An. funestus* ($P=0.515$) compared with one grid, while MLB with two grids killed more of both *An. arabiensis* and *An. funestus* ($P<0.001$). Numbers of non-malaria mosquitoes killed, i.e. *Culex* and *Mansonia* species, also increased with higher number of grids. The MLBs were most efficient against the malaria vector, *An. arabiensis,* which were killed in higher numbers than any other single mosquito species. Of all mosquitoes, 99% were non-blood fed, suggesting host-seeking status. There was a significant influence of physical location of the devices in both seasons ($P<0.001$), the

greatest effect on malaria vectors was observed when the devices were located in the middle of the village near human dwellings, rather than at the edge of the village.

**Conclusion:** Odour-baited MLBs fitted with low-cost electrocuting grids and automated on/off switches can effectively kill outdoor-biting disease transmitting mosquitoes, including major malaria vectors, even in areas where the mosquitoes are behaviourally or physiologically resistant, and cannot be fully controlled by the current interventions like LLINS and IRS. The method is insecticides-free, hence it also has great potential for resistance busting. These devices could have potential either for surveillance or as complementary control tools, to accelerate malaria elimination efforts, particularly in communities where outdoor transmission is increasingly important.

# CHAPTER 1

## 1.0. Background

Current interventions against malaria vectors, notably long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), have been highly successful against the disease [1]. However malaria remains endemic in sub-Saharan Africa, where 81% of the 219 million new cases and 91% of the 620,000 deaths occurred yearly, according to the latest WHO World Malaria Report [1]. Despite the obvious successes and optimism expressed in current strategies, evidence suggests that in order to achieve malaria elimination as stated in the 2008 Global Malaria Action Plan [2], there are still numerous challenges that must be addressed [3].

One of these challenges is the fact that, while LLINS and IRS have effectively controlled mosquitoes that bite humans indoors and rest indoors [4], a significant proportion of malaria transmission still happens outdoors [5, 6]. This is mostly transmitted by mosquitoes that naturally bite outdoors, or those that have developed behavioural resistance (e.g. avoidance of contact with lethal insecticidal surfaces and change of their biting time), adaptive behaviour such as biting people when outdoors [7] and physiological resistance (i.e. failure to be killed after adequate contact with otherwise toxic doses of insecticides) [5]. Outdoor-biting mosquitoes today contribute significantly towards on-going residual malaria even in areas where the major malaria control tools are widely used [3, 5]. Therefore to achieve malaria elimination, complementary efforts that target outdoor-biting are essential to address this problem [3].

Various technologies have been proposed for outdoor-mosquito control, one of which is the use of devices baited with synthetic human odours to lure, trap and kill the vectors [8, 9]. While these technologies have been promoted as having great potential against malaria transmission [10], and though they have been effective against tsetse fly vectors of African trypanosomiasis [11], there is only one large scale study currently underway to demonstrate

their potential [12]. Thus, there is a need to improve and further evaluate the potential of the technology, before its benefits can be fully ascertained.

Recently an outdoor mosquito control device, named the odour-baited mosquito landing box (MLB) was developed, which can attract and kill large numbers of outdoor host-seeking mosquitoes, including the major malaria vectors, *Anopheles arabiensis* and *Anopheles funestus* [9]. During the initial tests, however, it was observed that the attracted mosquitoes spent only brief periods of time around the device [9], thus limiting the likelihood of any lethal contact even when the MLBs where covered with paint-based mixtures of highly effective organophosphates like pirimiphos-methyl emulsified concentrate. We hypothesised that the reasons for this included the avoidance responses of *An. arabiensis,* and other mosquito species on conventionally treated insecticidal surfaces that have contact irritancy, or non-contact excito-repellent effects [13]. Another possibility was that naturally, the mosquitoes gave up and left after they found no blood host. Similar behaviours have been observed in hut trials where mosquitoes enter houses to feed but escape promptly before adequate contact with sub-lethal doses of either pyrethroids or organochloride insecticides [13-15], but this behaviour was less pronounced when organophosphates were used in impregnated nets after wash [16]. In situations where vector species such as *An. arabiensis* naturally have alternative blood hosts, e.g. cattle [17, 18], and where such vectors contribute significantly to ongoing malaria transmission outdoors, it is therefore important to identify better options of instantly killing the vectors when they first make contact with the MLB.

Here, we report major improvements to the MLB technology, to address these challenges for improved efficiency, and also to conserve energy and minimize the need for human handling. These modifications included: 1) fitting low-cost electrocuting grids (EGs) onto the sides of the MLB, so as to instantly kill transient host-seeking mosquitoes and eliminate the need for insecticides especially where physiological resistance is on the rise; and 2) addition of an all-weather light-sensor which automatically switches on both the electrocuting system

and the odour-dispensing system at dusk and off at dawn, thus saving energy and reducing human handling.

### 1.1. Study Rationale

Residual malaria vectors tend to express characteristics such as outdoor-biting and physiological resistance that enable them to escape current indoor insecticidal control measures like LLINs and IRS. For example, since *An. arabiensis* has a wide host choice and can feed on either humans or cattle, indoors or outdoors depending on availability of hosts [17-19], it is a particularly difficult vector species to control using LLINs and IRS only [17, 18]. Furthermore, in some communities, such as south-eastern Tanzania, *An. arabiensis* is also behaviorally adapted to readily escape potentially lethal surfaces that are treated with insecticides [20]. My study was aimed at targeting these behaviors and contributing towards development of complementary techniques for malaria elimination in Africa.

Instead of using insecticide-based killing agents, we opted for a lure and kill technique whereby mosquitoes were attracted and instantly killed by MLBs fitted with low-cost solar powered EGs. Thus, in addition to addressing the above-mentioned challenges, we also ensured greater affordability and accessibility through use of solar energy. Using electric grids would target and kill the visiting mosquitoes instantly upon contact, even if the mosquitoes make only brief contacts. Besides, it would reduce associated labor costs and eliminate the need for insecticides, thus improving environmental safety and possible removing insecticide resistance alleles from mosquito populations. Addition of a light-based sensor to ensure that the device automatically switched on at dusk and off at dawn, also conserved energy, increased longevity of the odour lure as it is being emanated only at night, eliminated unwanted effects on daytime flying non-target insects, and also improved

the safety of the device, especially in places where children might play around, or touch the devices during the day.

### 1.2. Main Objective

The overall aim of this study was to optimize and assess efficacy of odour-baited mosquito landing boxes fitted with electrocuting grids, for luring and instantly killing outdoor malaria vectors in rural Tanzania.

#### 1.2.1. Specific objectives*:*

1. To improve and optimize the MLB by: a) automating its daily cycle of operations and b) fitting it with low-voltage, solar-powered electrocuting grids (EGs) that can instantly kill outdoor-biting malaria vectors, including members of the *An. gambiae* complex and *An. funestus* group.

2. To assess the killing efficacy of the improved MLB fitted with EGs, against free-flying wild malaria vector mosquitoes in a malaria endemic area in south-eastern Tanzania.

### 1.3. Hypotheses tested

1) The optimized MLB fitted with low-cost solar powered electric grids and automated light sensors for switching the device on and off would efficiently attract and kill transient malaria mosquitoes outdoors.

2) Increasing the number of EGs on the MLB would result in higher numbers of mosquitoes killed. Therefore, MLBs fitted with two or three grids would outperform the MLB with one grid in terms of numbers of mosquitoes collected and killed.

## CHAPTER 2

### *2.0. Literature review*

### *2.1. Current malaria situation and the success of existing interventions in Africa and Tanzania*

Malaria is a parasitic disease that is transmitted through bites of infected *Anopheles* mosquitoes. It is caused by five identifiable *Plasmodium* species (*Plasmodium falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi),* with *P. falciparum* being the major infective parasite, responsible for most malaria cases and deaths worldwide [1, 21]. The principal malaria vectors include, *An. gambiae* Giles, *An. arabiensis* Patton, and *An. funestus* Giles [21-23]. It is estimated that there are 219 million malaria cases and 627,000 deaths due to malaria annually, and that 81% of the cases and 91% of the deaths occur in sub-Saharan Africa, mostly in children under age of five years [1]. Global commitments and efforts against malaria now aim at moving from control to elimination, and eventually to eradication [24]. Recent data shows that an estimated 3.3 million lives were saved between 2000 and 2012 worldwide, equating to 42% and 49% reduction in malaria mortality globally and in Africa respectively [1]. Improved political will and financial commitments, effective diagnosis and treatment with anti-malaria drugs, as well as the scale-up of vector control interventions, mainly LLINs and IRS, are considered to be the major factors that contributed to reducing malaria burden, even in places which previously had intense transmission [1, 25, 26].

The risk of malaria transmission due to *Plasmodium falciparum* is still overwhelmingly in Africa (Figure 1A) [27]. However, Tanzania is one of the countries that have witnessed these successes, with malaria cases reduced by more than 50% over the past decade [28]. The current under five malaria prevalence stands at 9.5% on average (Figure 1B) [29]. In the past decade, there have been particularly high child survival gains in the country [30],

resulting particularly from high coverage of LLINs [4, 26], early diagnosis [31] and prompt treatment [32]. In the new malaria strategy 2014-2020, the Tanzania National Malaria Control program has now proposed to reduce average prevalence to 5% by 2016 and to less than 1% by 2020 [33].

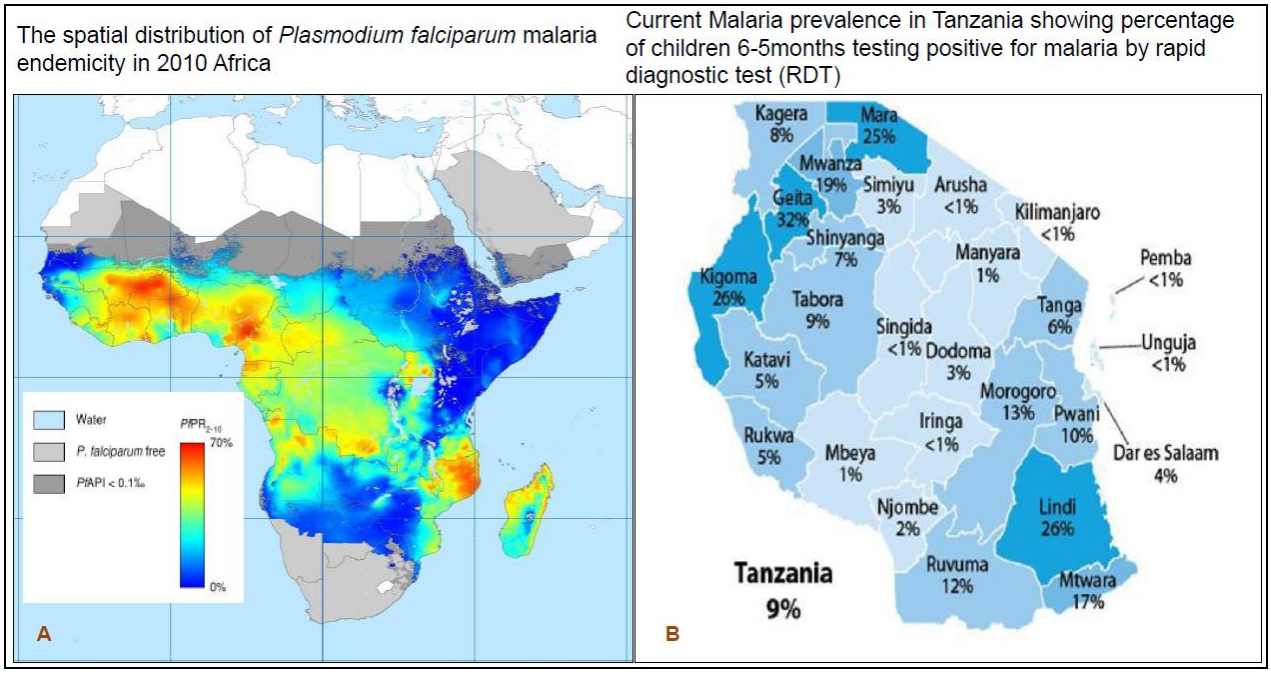



**Figure 1:** The current situation of malaria in Africa (A) and Tanzania (B). The two maps have been adapted from the Malaria Atlas Project [27], and the latest Tanzania Malaria Indicator Survey Report [34] respectively. *P. falciparum* stands for *Plasmodium falciparum*, *PfPR* stands for *Plasmodium falciparum* parasite rate and *PfAPI* stands for *Plasmodium falciparum* annual parasite incidence.

### *2.2. Challenges facing current malaria control methods*

Despite the tremendous achievements made by the current major interventions against malaria, there is still a significant level of residual transmission, even in communities with high coverage and use of LLINs alone or a combination of LLINs and IRS [1, 4, 35]. Challenges facing on-going malaria control are numerous, and include: *a*) development and spread of drug resistance in *P. falciparum* parasites [36, 37]*, b)* higher costs of the

artemisinin–combination therapy in malaria endemic countries [1], *c)* poor socio-economic status [38], and *d)* poor house structures that allow high mosquito entry points and exposure to mosquito bites indoors [39]. In addition, the prolonged applications of insecticide-based interventions are threatened by development of insecticide resistance as well as behavioural resilience of some species of mosquitoes [13, 40, 41].

Regarding vector control, one of the main reasons for incomplete control is that the current interventions (LLINs and IRS) target primarily those mosquitoes that bite humans and rest indoors. Yet, mosquito naturally spend a significant proportion of its time outdoor either seeking for nectar as a source of energy upon emergence [42, 43], oviposition sites after blood meal [44] or some mosquitoes naturally tend to bite and rest outside human dwellings, and which therefore contribute to residual malaria transmission [45] . Furthermore, mosquito host-seeking behaviour is reported to have changed due to the selection pressure caused by extensive use of these insecticide-based control methods, i.e. LLINs and IRS [6, 46].

As the mosquitoes strive to survive and reproduce using blood to develop eggs, important malaria vector species are found to be more frequently biting outdoors, starting at dusk and continuing beyond dawn, which corresponds to time periods when humans are usually also available outdoors [47, 48]. Moreover, the indoor interventions have also been shown to influence relative vector sibling species compositions [4, 49] particularly after long-term use. For example, LLINs and IRS have significantly reduced densities of *An. gambiae sensu stricto* and *An. funestus sensu stricto*, which were the major African malaria vectors and were known to bite almost exclusive indoors [4, 50].

### *2.3. Need for complementary interventions to combat residual malaria transmission and other mosquito-borne diseases*

Given the current challenges with malaria control, it is obvious that despite the successes of existing tools, we need additional new tools that could be used alongside the current ones to accelerate efforts towards elimination. Even though malaria transmission in Africa still occurs substantially indoors [51, 52], there have been gradual increases in the proportion of transmission that occurs outside, where people are neither protected by nets nor residual insecticides on indoor house surfaces [5, 53]. The Malaria Eradication Research Agenda Initiative (malERA) established that in locations with low to moderate transmission, existing tools, if used in sufficient consistency and coverage would be adequate, but that in areas where the main vectors predominantly express outdoor-feeding and outdoor-resting behaviours, new interventions are required [54].

The public health research community is already actively working on a number of these options, and a new set of criteria for evaluation of such new paradigms and tools was recently published by Vontas *et al*., [55]. To address the specific problem of outdoor-biting as a contributor to residual transmission, possible new interventions may include: *a)* use of topical and spatial repellents [56-58], *b)* developing and promoting integrated vector management (IVM) in control programmes [1, 59, 60], *c)* use of entomo-pathogens such as fungi [61], *d)* use of insect growth regulators such as pyriproxyfen, which can also be transported by mosquitoes themselves to different breeding sites [62, 63], *e)* use of larval control methods [64], *f)* use of odour baited devices that attract and either small scale trapping or killing of the vectors [9, 10, 65] or deployment of mass trapping and killing technique against both nuisance and mosquito vector populations, such as the one described previously [66-68] and *g)* environmental modification through “species sanitation”

of specific malaria breeding sites, that was successful in Malaysia and other countries [69-71].

To address the threat of insecticide resistance, the World Health Organization has recommended several approaches to improve current and future control efforts [72]. These include: *a)* combinations of two or more insecticides are applied within a single house, *b)* mixtures of two or more insecticidal compounds to form a single formulation which can be applied in a building so that the mosquito makes contact simultaneously on different chemicals with different modes of action, *c)* temporal insecticide rotations, and *d)* the mosaic approach whereby two different insecticides are sprayed in a grid pattern [72]. Moreover, in a small number of studies, additional control methods which are non-chemical, such as the use of odour-baited electric grids, have been considered as alternative means of analysing, and possibly targeting vector behaviour, while preserving efficacy of the existing insecticidal interventions [73, 74].

### *2.4. Exploiting mosquito host-seeking behaviour to address current malaria control challenges*

Mosquito blood-seeking behaviour and host preference relies on chemical cues from body emanations that consists of ammonia, lactic acid and various carboxylic acids from sweat and skin emanation, as well as carbon dioxide ($CO_2$) gas, a major constituent of breath [75]. Nevertheless, nectar/sugar still remains an essential component for the new adult female mosquitoes as a source of energy which facilitates mosquito activities such as flying as their looking for the blood hosts and oviposition sites [42, 43]. Individual human attractiveness to mosquitoes varies with amount and composition of chemical cues emanating from the body, or in sweat and breath [76, 77]. Understanding the chemical biology of the mosquitoes could therefore be of great benefit in designing methods to exploit mosquito-blood seeking

behaviour to target residual transmission [78-82]. Human body emanations, including skin odours, breath and their components such as lactic acid, ammonia and $CO_2$ gas are the principal attractant cues for the mosquitoes [79]. It is known that the highly specific odorant receptors on mosquito antennal sensillae and on the maxillary palps enable the mosquitoes to pursue their blood-hosts following concentration gradients and odour plumes [83]. Some mosquitoes species have evolved to be highly anthropophagic, and for this reason contribute substantially to disease transmission [84]. It is also well established that metabolism by bacteria residing on human skin surfaces can influence concentrations of human skin emanations, and therefore change attractiveness of individual humans to malaria vectors [85].

It is well established that by understanding the composition of different human emanations, it is possible to develop synthetic compounds or mixtures, which could be used to attract or confuse host-seeking mosquitoes, thus interrupting human-mosquito contact [82, 83]. For example, technologies based on the concept of attracting and killing of vectors have been widely evaluated and used to exploit vectors host-seeking behaviours [82]. These techniques have been useful for traps or devices designed for either sampling population densities of disease vectors through attracting and trapping, or for control purposes through attracting, trapping and killing [8, 86-91]. Odour-baited traps for controlling tsetse flies (*Glossina* species) have been highly successful [92, 93], and the technology is now also being tested against malaria mosquitoes for control and surveillance [12, 94]. Similar studies have been done against free-flying wild mosquitoes and have shown that it is possible to target larger numbers of mosquito vectors, including malaria vectors, by attracting and killing using odour-baited traps/devices [8, 9, 12].

## CHAPTER 3

## 3.0. Methods

### 3.1. Study area

This study was carried out in Lupiro village (8.385ºS, 36.670ºE), in Ulanga district, south-eastern Tanzania (Figure 2). Lupiro village lies 300 meters above sea level, on the flood plains of the Kilombero River, approximately 26km south of Ifakara town. The area experiences two main climatic seasons, the wet season that peaks between March and June, and the dry season that peaks between August and October. The annual rainfall in Lupiro ranges between 1200mm and 1800mm, while annual mean daily temperatures range between 20ºC and 32ºC. Most of the houses in the area are constructed with clay brick walls, but incomplete with some openings on windows and doors, open eave spaces, and either iron or grass-thatched roofs. The predominant malaria vectors in Lupiro are *An. arabiensis* and *An. funestus* group. Interestingly, *Anopheles gambiae s.s.,* which historically dominated the area, is now rarely found, mainly as a result of extensive use of bed nets [4].

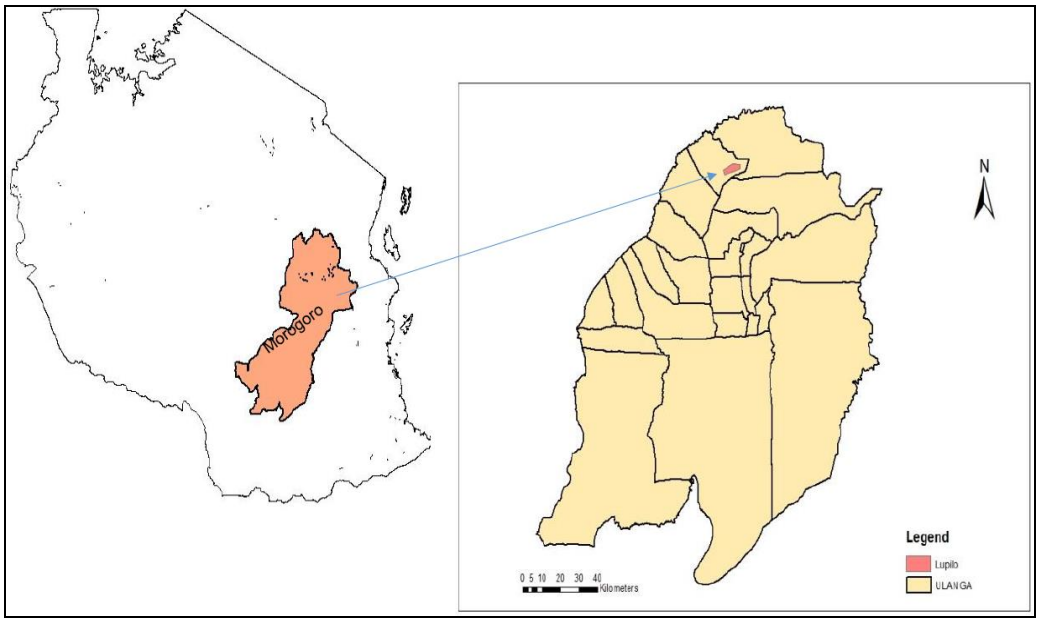



**Figure 2:** A map showing village (Lupiro) in Ulanga district, south-eastern Tanzania, where this study was conducted.

Recently, a report of standard WHO insecticide susceptibility tests showed that *An. arabiensis* was 100% susceptible to organochlorines, but had reduced susceptibility (92-98%) to pyrethroids [95], which according to the new WHO guidelines, would be classified to be ranged from susceptible to suspected resistance [96]. Over the past decade, the Kilombero valley, which was hyper-endemic in the early 2000s, has experienced more than 50% reduction in malaria prevalence and is now classified as meso-endemic [28]. Outdoor transmission is rising and is currently 20-30% [5, 53]. Interestingly, outdoor vectors here are most active at times when people are also outdoors, doing various activities (Moshi *et al.,* Unpublished and Figure 3), an observation that clearly suggests odour-baited devices that mimic outdoor humans, could provide realistic options for representatively targeting the vectors.

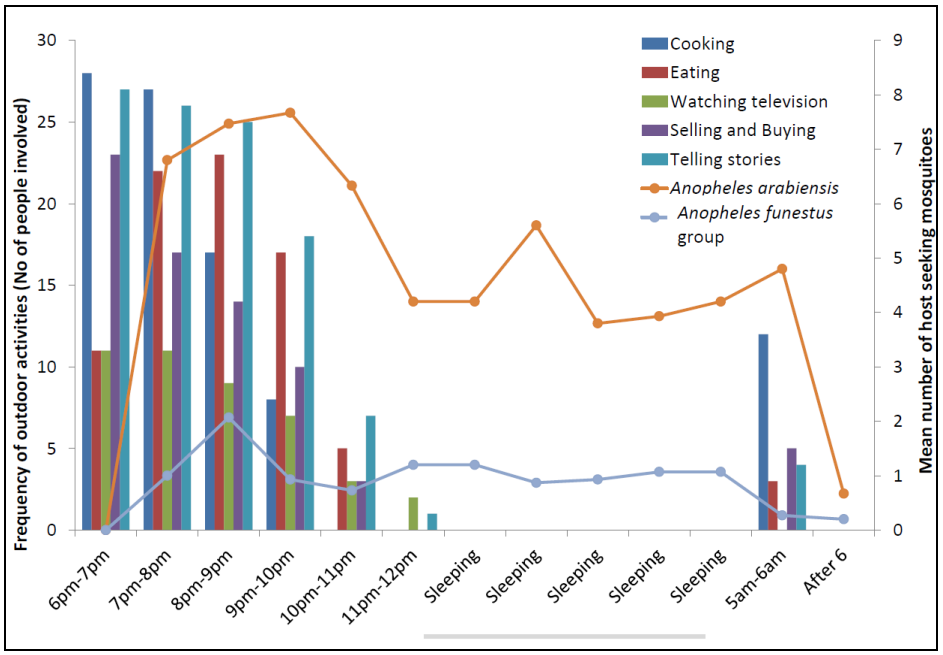



**Figure 3:** A graphical illustration of correlations between the time periods when local people are performing various outdoor tasks, and time periods when host-seeking disease-transmitting mosquitoes are also most active outdoors. Figure adapted from Matowo *et al*. [9]. The peak times of outdoor human activities were before 11:30pm, and after 5:00am, which coincided with periods when malaria vectors were also most active outdoors.

### 3.2. The Mosquito Landing Box

The MLB was designed to mimic humans sitting outside their houses (Fig. 4A and 4B), and to target disease-transmitting mosquitoes that bite outdoors so as to complement LLINs and IRS [9]. It is a wooden box measuring 0.7 × 0.7 × 0.8m, and standing on short wooden pedestals raised 10 cm above ground (Fig. 4C). All sides of the box are detachable, so that it is easy to transport and assemble onsite. Each of the four side panels of the box consist of 8 to12 louvers, which form the mosquito landing surfaces. These louvers are 1cm wide and are fixed at an angle of approximately 45° facing downwards, ensuring adjustable gaps of at least 2cm between them [9].

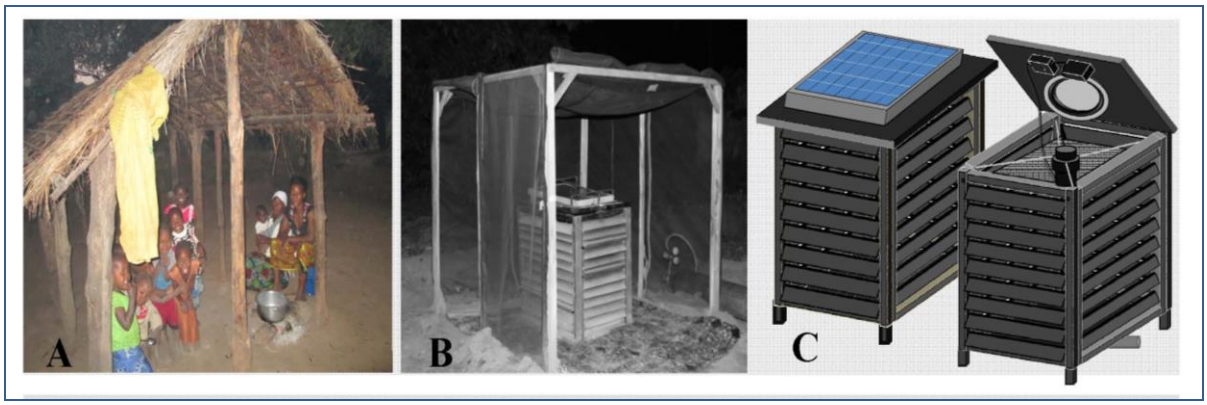



**Figure 4:** Pictures of the odour-baited mosquito-landing box (MLB). It is designed to target host-seeking mosquitoes that bite humans outside their houses, e.g. people cooking in open kitchens in rural communities **(A)**. A separate semi-open screen cage can be used to intermittently entrap and sample host-seeking mosquitoes visiting the device during experimentation **(B)**. It has a solar panel on the top **(C)**, which charges the battery for the odour-dispensing system inside it. When in use, the device can be baited with natural or synthetic mosquito attractants. Figure adapted from Matowo *et al.* [9].

More complete details of the MLB and its functionality have been provided in our previous publication [9]. The device has an attractant-dispensing unit inside it, which is made of a

short PVC pipe measuring 5.7cm diameter and 20cm length, suspended using expandable wires (Figure 5). A 12V fan is fixed at the top of this PVC pipe. This fan draws air upwards through the attractant compartment, inside which the different baits can be fitted using wire mesh, allowing airflow through the system. The upward air drawn by the fan is redirected by a deflecting dish fitted under the top cover, so that the odours come out equally from all four sides of the box (Figure 4C). Different formulations and shapes of attractants can be placed into the attractant compartment and used as a source of odour. This odour-dispensing system is powered by the energy derived from a 20W solar panel that is securely bolted on the top of the MLB [9].

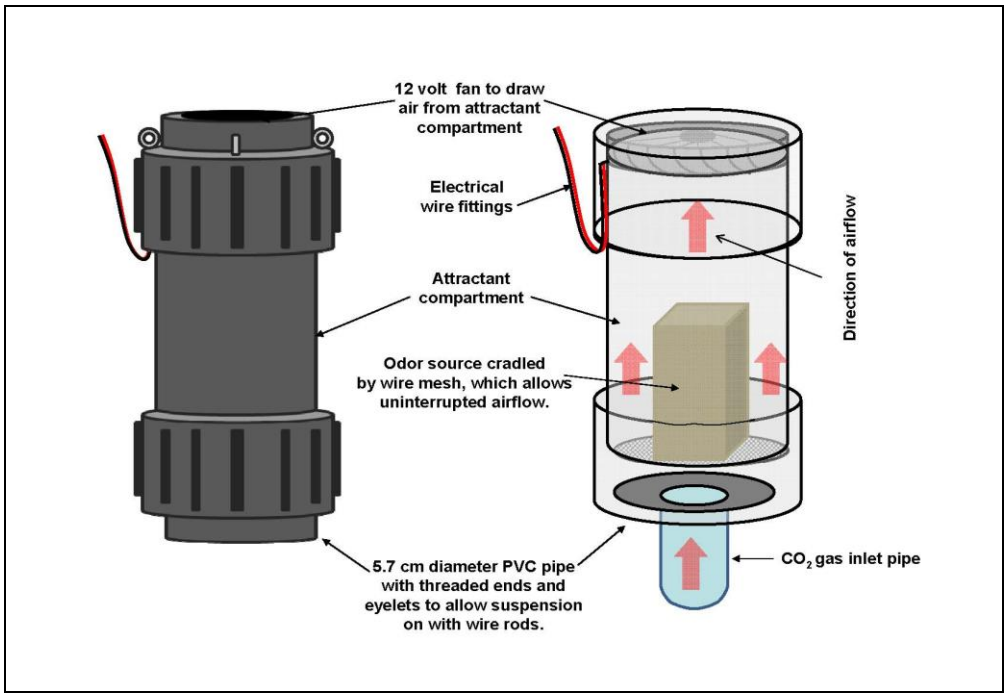



**Figure 5:** Illustration of the attractant-dispensing unit of the mosquito landing box. The unit consists of a PVC pipe (20cm long and 5.7cm diameter), inside which mosquito attractants are located and then dispersed by air currents generated from a 12-volt fan, powered by a solar-recharged battery. In this study industrial $CO_2$ gas was added into the unit through the plastic pipe, as described by Matowo *et al.,* [9], in the original description of the MLB.

### 3.3. Procedures

#### *3.3.1. Objective 1: Improvement of the Mosquito Landing Boxes by fitting low-cost electrocuting grids and light sensors that trigger automatic on/off operation of the device*

To improve efficiency and ease of use, the MLBs were fitted with solar-driven low-voltage electrocuting grids (EGs) to instantly kill attracted mosquitoes without destroying essential morphological, biochemical and molecular features for identification and pathogen monitoring. Commercially available racquet-shaped “mosquito zappers” (manufactured by *LiTian Electronic Limited Company in Yiwu City, China*), were modified and used as the EGs on the sides of the MLB (Figure 6 A, B, C, and D). The grids were supported by rectangular pieces of soft boards on each side of the box, in such a way that only a small circular portion of the soft boards, equal to the size of the grids were visibly open whenever the landing surfaces were tilted (Figure 6 B). The EGs, were inserted on the inside sections behind the louvers and powered by the same solar system, which also runs the odour-dispensing system. The louvers can be adjusted to an angle to allow attracted mosquitoes to pass through, but also provide additional protection of the grids against rainfall.

An all-weather-light sensitive sensor, which switches on the odour-dispensing unit and the EGs of the MLB at dusk and switches them off at dawn (Figure 6 E), was also fitted. This automated system ensured that: *a)* the solar battery power was saved and unused during the day, *b)* the devices were not in any way hazardous to children who may touch them during the day, and *c)* there was no daily human servicing necessary for these devices other than the role of the experimentalist collecting data.

The use of these grids was based on the hypothesis that transient behaviour of some mosquito vectors, such as *An. arabiensis* in our study area, which spend only brief periods

around such devices, can be easily targeted by the spontaneous killing mechanism using a simple and non-chemical mechanism. Similarly, addition of such a fast-acting non-insecticidal means to target malaria mosquitoes would ensure that the problem of insecticide resistance can be countered and recurrent costs minimized.




**Figure 6:** Pictorial representation of the odour-baited mosquito landing box **(A)** fitted with electrocuting grids **(B, C and D**) and an automated on/off light sensitive switch **(E)** that activates the device at dusk and stops it at dawn. The entire electric system and the odour-dispensing system are powered by a 12-volt solar rechargeable battery **(F)**, allowing for a passive but effective mosquito control and surveillance system.

### *3.3.2. Objective 2: comparative evaluation of the killing efficacy of MLBs fitted with different numbers of electrocuting grids, against wild free-flying mosquitoes*

Three MLBs (the first one fitted with one EG on only one side, the second fitted with two EGs on two sides, and the third fitted with 3 EGs on three sides) were positioned 50 - 100 meters apart, and at least 30 meters from the nearest house. A white linoleum material was placed at the bottom of each of the devices, so that any dead mosquitoes dropping down could be recovered and counted. Pedestals on water moats were used to prevent any ants from scavenging on the dead mosquitoes.

The MLBs were baited with Ifakara blend of mosquito attractants [97], a highly attractive synthetic human odour that was demonstrated in experimental hut studies to be more attractive than humans at long-range. The blend comprises hydrous solutions of ammonia (2.5%), L-lactic acid (85%), and other aliphatic carboxylic acids, namely propionic acid (C3) at 0.1%, butanoic acid (C4) at 1%, pentanoic acid (C5) at 0.01%, 3-methylbutanoic acid (3mC4) at 0.001%, heptanoic acid (C7) at 0.01%, octanoic acid (C8) at 0.01% and tetradecanoic acid (C14) at 0.01% dispensed via nylon strips, supplemented with $CO_2$ gas flowing at 500 ml/min [9, 82, 98]. The odour dispensing system was similar to that described in Figure 5.

The MLBs with one, two or three grids were compared using a 3 × 3 Latin square experiment replicated over 21 experimental nights, in three different locations across the study village (Figure 7). The experiments were done in wet season (May–June 2013) and repeated in the dry season (August-September 2014), so as to capture the vector behaviours in both seasons. The three locations were as follows: 1) position A was inside the study village approximately 150m from the edge of the village, and the device was located 30m away from the nearest household, 2) position B was in the middle of the study village, approximately 100m from the edge of the village, in an area with one human dwelling

around it, and 3) position C was at the edge of the village close to an area regularly cultivated for vegetables and rice using traditional irrigation systems.

To minimize positional bias on mosquito catches, the three MLBs were rotated nightly across the three locations. This design was meant to ensure that at the end of the 21 nights, each MLB would have been at each of the three separate locations once every three nights. The order of the rotations of the three MLBs was also randomised after every three nights, so as to counter potential effects of any cyclical variations in the natural diurnal vector densities.




**Figure 7:** Map of an aerial view of the village showing the location of the MLBs on position A, B and C. The satellite images were obtained from Google Earth.

### *3.3.3 Mosquito collections, identification and processing*

Mosquito collections were carried out in the morning, whereby electrocuted mosquitoes were individually gently removed from the EG surfaces using forceps, from the inside surfaces of the MLB and also from the raised linoleum floors. The mosquitoes were then morphologically identified and sorted to different taxa and sex. Taxonomy was done by using dichotomous keys for *Anopheles* of Africa, south of the Sahara [22]. Members of the *An. gambiae* complex and *An. funestus* group were stored separately for further individual species identification. Mosquitoes were also sorted based on whether they were blood-fed or non-blood fed, gravid, non- gravid and semi-gravid.

Female *Anopheles* mosquitoes identified as belonging to the *An. gambiae* complex and *An. funestus* group were separately pooled at a minimum of twos and maximum of tens, and stored for further examination to assess if they were infected with *P. falciparum* sporozoites, using enzyme-linked immunosorbent assay (ELISA) techniques, following procedures previously described by Beier *et al.,* [99]. To remove false positives, all the sporozoite positive ELISA lysates were boiled at 100 °C for 10minutes, so as to verify the presence of *Plasmodium* protozoan antigen, which are heat stable [100].

A subsample of the mosquitoes was also randomly selected for species identification in the molecular laboratory at Ifakara Health Institute. DNA-polymerase chain reaction (PCR) was used as a standard molecular technique for individual species identification [101]. For the *An. gambiae* complex, multiplex PCR was done following the procedures of Scott *et al.,* [102], while for the *An. funestus* group, DNA was firstly extracted [103], then PCR performed according to the procedures of Koekemoer *et al.,* [104].

### 3.4. Data analysis

Data were firstly entered into excel spreadsheets, cleaned and saved as Comma Separated Values (CSV) files. Data analysis was done using R statistical software version 3.1.1 for Windows [105]. Data were fitted using Generalized Linear Mixed Effects statistical Models (GLMMs) to describe effects of the different variables on mosquito catches. Since the data were evidently over-dispersed, we used the packages *lme4* [106], MASS and *glmmADMB* [107] to fit either the Poisson distribution models or negative binomial distribution models with log-link functions for the over-dispersed count data.

Numbers of individual mosquito species were assessed as a function of two fixed factors i.e. number of grids on the MLB and positions of the MLB, each time comparing additive versus multiplicative models, using Akaike Information Criteria (AIC) values [108]. Experimental days were treated as a random factor to account for natural nightly heterogeneity in mosquito counts. The results of the parameter estimates were log-transformed to obtain the estimated mean nightly number of mosquitoes killed by each MLB at different locations, and the associated relative rates (RR) of when compared to the reference, which was MLB with one grid, when located at the first location. The final data were summarised in tables and boxplots. Examples of the models, the R outputs, and model diagnostics are provided in appendices 8.2 and 8.3.

## CHAPTER 4

### *4.0. Results*

#### *4.1.1. Total mosquito catches and species identification*

During the experimental tests, i.e. in dry and wet seasons, a total of 9,685 mosquitoes was sampled from all the three odour-baited mosquito landing boxes fitted with electrocuting grids. Of these, there were 3533 (36%) were *Anopheles gambiae* complex, 107 (1%) were *An. funestus* group, 826 (9%) were other *Anopheles* species, 1230 (13%) were *Culex* species and 3989 (41%) were *Mansonia* species. For more specific molecular identification, DNA from sub-sample of 260 randomly selected individual mosquitoes from the *An. gambiae complex* and 57 from the *An. funestus* group were successfully amplified by PCR, to identify sibling species. Of these, 256 (98%) of the *An. gambiae s.l* were found to be *An. arabiensis* while 4 (2%) were *An. quadriannulatus*. For the *An. funestus* group, 54% (31) were found to be *An. rivulorum,* 14 (25%) were *An. funestus s.s* and 12 (21%) were *An. leesoni*. We however experienced high proportions of non-amplified mosquitoes during our molecular analysis for species identification, especially for *An. funestus* group, for which the non-amplification rate was 68%. The results presented here are therefore only for those mosquitoes for which the DNA material was successfully amplified.

Since *An. arabiensis* constituted 98% of the *An. gambiae* complex mosquitoes, the term *An. arabiensis* is used in subsequent sections of this thesis, to refer to all members of the complex.

#### *4.1.2. Tests conducted in wet season*

A total of 4,986 mosquitoes was collected from all three odour-baited MLBs equipped with EGs during the first round of the tests, i.e. 21 nights in wet season. Of these, there were 1,541 *An. arabiensis (*31%*)*, 38 *An. funestus* (1%), 356 mosquitoes belonging to any other *Anopheles* species such as *An. coustani* (7%), 554 *Culex* species (11%), and 2,497

*Mansonia* species (50%). *Anopheles arabiensis* was therefore the most predominant malaria vector collected in this study, but also the most prominent individual species collected. Of all the mosquitoes of any species caught, 99% were non-blood fed, suggesting that they were host-seeking mosquitoes.

The estimated mean numbers of mosquitoes killed by each of the MLBs are shown in (Table 1 and 2). The number of mosquitoes collected increased concurrently with increase in number of grids on the MLBs (Table 1 and 2). The MLB fitted with three grids killed significantly more *An. arabiensis* (RR = 1.59 [1.37-1.84], P<0.001), but significantly fewer of the other *Anopheles* species (RR = 0.41 [0.30-058], P <0.001), and fewer *Mansonia* mosquitoes (0.73 [0.65-0.82], P <0.001), than the MLB with just one grid. The MLB with 3 grids also killed more *An. funestus* (RR = 1.56 [0.64-3.81], P = 0.335), and more *Culex* mosquitoes 1.90 [0.98-2.59], P =0.329), than the MLB with one grid, though not by a statistically significant margin for either taxa. Similarly, the MLB fitted with two grids killed significantly more *An. arabiensis* (RR = 1.59 [1.37-1.84], P <0.001), and more *Culex* mosquitoes (RR = 1.89 [1.03-2.64], P < 0.005) than the MLB fitted with one grid. However, the number of *An. funestus* killed by this MLB with two grids were similar to the MLB with one grid (RR= 0.95 [0.36-2.52], P = 0.913).

We also observed statistically significant effects of location on number of mosquitoes of different species collected. When any of the MLBs, regardless of number of grids, was at position C (i.e. at the edge of the village close to an area regularly cultivated for vegetables and rice using traditional irrigation systems), we collected at least 4 times more mosquitoes of all species combined than at position A (i.e. inside the study village approximately that was 150m from the edge of the village). Specifically, at position C, we collected 5.4 times more *An. funestus* (RR = 5.37 [2.04-14.15], P <0.001), 5.3 times more of the other *Anopheles* mosquitoes (RR = 5.26 [3.83-7.23], P < 0.001), 4.2 times more *Culex* mosquitoes (RR = 4.23 [3.28-5.46], P < 0.001) and 5.4 times more *Mansonia* mosquitoes (RR = 5.43

[4.79 -6.14], P < 0.001) than at position A. However there were significantly fewer *An. arabiensis* mosquitoes sampled when the MLB was at position C relative to position A (RR = 0.70 [0.62-0.80], P<0.001). Similarly, we collected more of the non-malaria mosquitoes (*Culex* (RR = 1.68 [1.26 -2.24], P <0.001), other *Anopheles* species (RR =1.18 [0.81-1.73], P =0.39) and *Mansonia* (RR = 1.63 [1.41 -1.88), P <0.001)) at position B in the middle of the village (approximately 75 metres from the edge of the village, in an area with one human dwelling near it), but fewer *An. arabiensis* (RR = 0.83 [0.72-0.94, P = 0.01) and same number of *An. funestus* (RR=0.99 [0.29-3.46], P =0.99) compared to position A.

**Table 1:** Estimated mean number of *Anopheles* mosquitoes collected per night from the odour-baited mosquito landing boxes (MLBs) fitted with one, two or three electrocuting grids during the wet season or dry season tests.

| | **No. Grids on MLB** | ***Anopheles arabiensis*** | | | ***Anopheles funestus*** | | | **Other *Anopheles*** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean [95% CI] | RR [95% CI] | P value | Mean [95% CI] | RR [95% CI] | P value | Mean [95% CI] | RR [95% CI] | P value |
| Round 1 (Wet season) | One Grid | 13.82 [9.90-19.28] | REF | REF | 0.15 [0.05-0.45] | REF | REF | 1.51 [0.73-3.13] | REF | REF |
| | Two Grids | 21.97 [18.95-25.47] | 1.59 [1.37-1.84] | <0.001 | 0.14 [0.05-0.36] | 0.95 [0.36-2.52] | 0.913 | 1.14 [0.12-10.71] | 0.75 [0.08-7.09] | 0.049 |
| | Three Grids | 36.04 [9.16-141.76] | 2.61 [0.66-10.26] | <0.001 | 0.23 [0.09-0.55] | 1.56 [0.64-3.81] | 0.335 | 0.63 [0.45-0.88] | 0.41 [0.30 -058] | <0.001 |
| | | | | | | | | | | |
| | **No. Grids on MLB** | ***Anopheles arabiensis*** | | | ***Anopheles funestus*** | | | **Other *Anopheles*** | | |
| | | Mean [95% CI] | RR [95% CI] | P value | Mean [95% CI] | RR [95% CI] | P value | Mean [95% CI] | RR [95% CI] | P value |
| Round 2 (Dry season) | One Grid | 17.67 [15.64 -19.96] | REF | REF | 0.03 [0.01-0.14] | REF | REF | 0.21 [0.09-0.47] | REF | REF |
| | Two Grids | 32.65 [29.07-36.67] | 1.85 [1.65-2.08] | <0.001 | 0.13 [0.06-0.31] | 4.79 [2.08-11.02] | <0.001 | 0.49 [0.32-0.73] | 2.35 [1.56-3.53] | <0.001 |
| | Three Grids | 28.79 [25.54-32.45] | 1.63 [1.45-1.84] | <0.001 | 0.02 [0.01-0.05] | 0.73 [0.29-1.85] | 0.515 | 0.40 [0.26-0.63] | 1.95 [1.25-3.04] | 0.003 |

The RR stands for relative rate while REF stands for a reference category. All the estimations were generated using Generalized Linear Mixed Effects Models in R [105].

**Table 2:** Estimated mean number of non-malaria mosquitoes and overall number of mosquitoes collected per night from the odour-baited mosquito landing boxes (MLBs) fitted with one, two or three electrocuting grids during the wet season or dry season tests.

| | **No. Grids on MLB** | ***Culex* species** | | | ***Mansonia* species** | | | **All mosquito species combined** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean [95% CI] | RR [95% CI] | P value | Mean [95% CI] | RR [95% CI] | P value | Mean [95% CI] | RR [95% CI] | P value |
| Round 1 (Wet season) | One Grid | 2.61 [1.79-3.81] | REF | REF | 14.66 [10.87-19.75] | REF | REF | 20.24 [14.72-27.84] | REF | REF |
| | Two Grids | 4.39 [2.68-4.88] | 1.89 [1.03-2.64] | 0.029 | 12.08 [10.85-13.45] | 0.82 [0.74-0.92 | <0.001 | 99.36 [82.35-119.89] | 4.91 [4.07-5.92] | <0.001 |
| | Three Grids | 3.39 [2.56-4.15] | 1.90 [0.98-2.59] | 0.329 | 10.68 [9.50-12.01] | 0.73 [0.65-0.82] | <0.001 | 41.53 [34.37-50.19] | 2.05 [1.69-2.48] | <0.001 |
| | | | | | | | | | | |
| | **No. Grids on MLB** | ***Culex* species** | | | ***Mansonia* species** | | | **All mosquito species combined** | | |
| | | Mean [95% CI] | RR [95% CI] | P value | Mean [95% CI] | RR [95% CI] | P value | Mean [95% CI] | RR [95% CI] | P value |
| Round 2 (Dry season) | One Grid | 1.93 [1.24-2.99] | REF | REF | 1.59 [1.01-2.51] | REF | REF | 19.07 [13.77-26.41 | REF | REF |
| | Two Grids | 2.78 [2.24-3.44] | 1.44 [1.16-1.79] | <0.001 | 2.05 [1.65-2.54 | 1.29 [1.04-1.59] | 0.02 | 30.02 [27.77-32.45] | 1.57 [1.46-1.70 | <0.001 |
| | Three Grids | 3.70 [2.97-4.62] | 1.92 [1.54-2.39] | <0.001 | 4.16 [3.32-5.21] | 2.61 [2.08-3.27] | <0.001 | 33.05 [30.49-35.82] | 1.73 [1.59-1.88] | <0.001 |

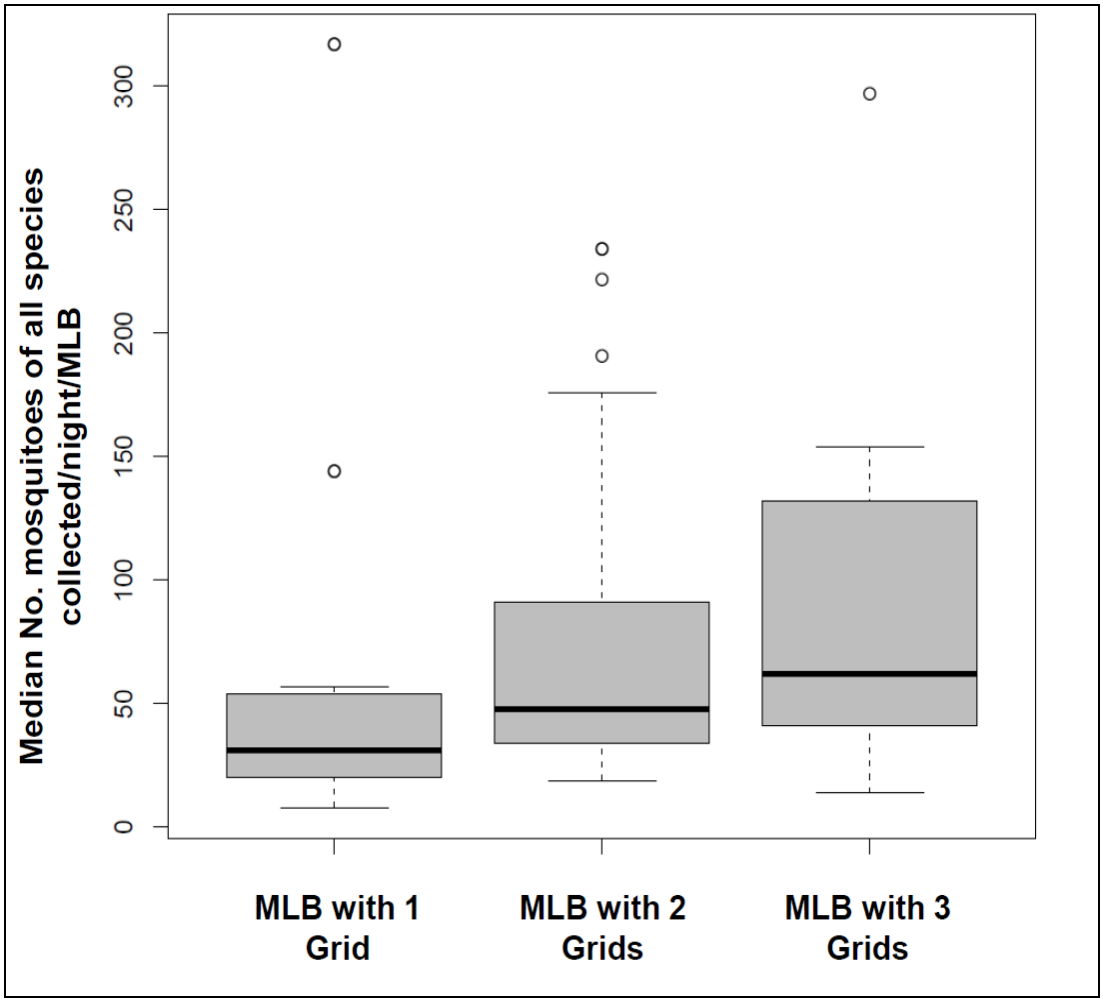
The RR stands for relative rate while REF stands for a reference category. All the estimations were generated using Generalized Linear Mixed Effects Models in R [105].

### *4.1.3. Tests conducted in dry season*

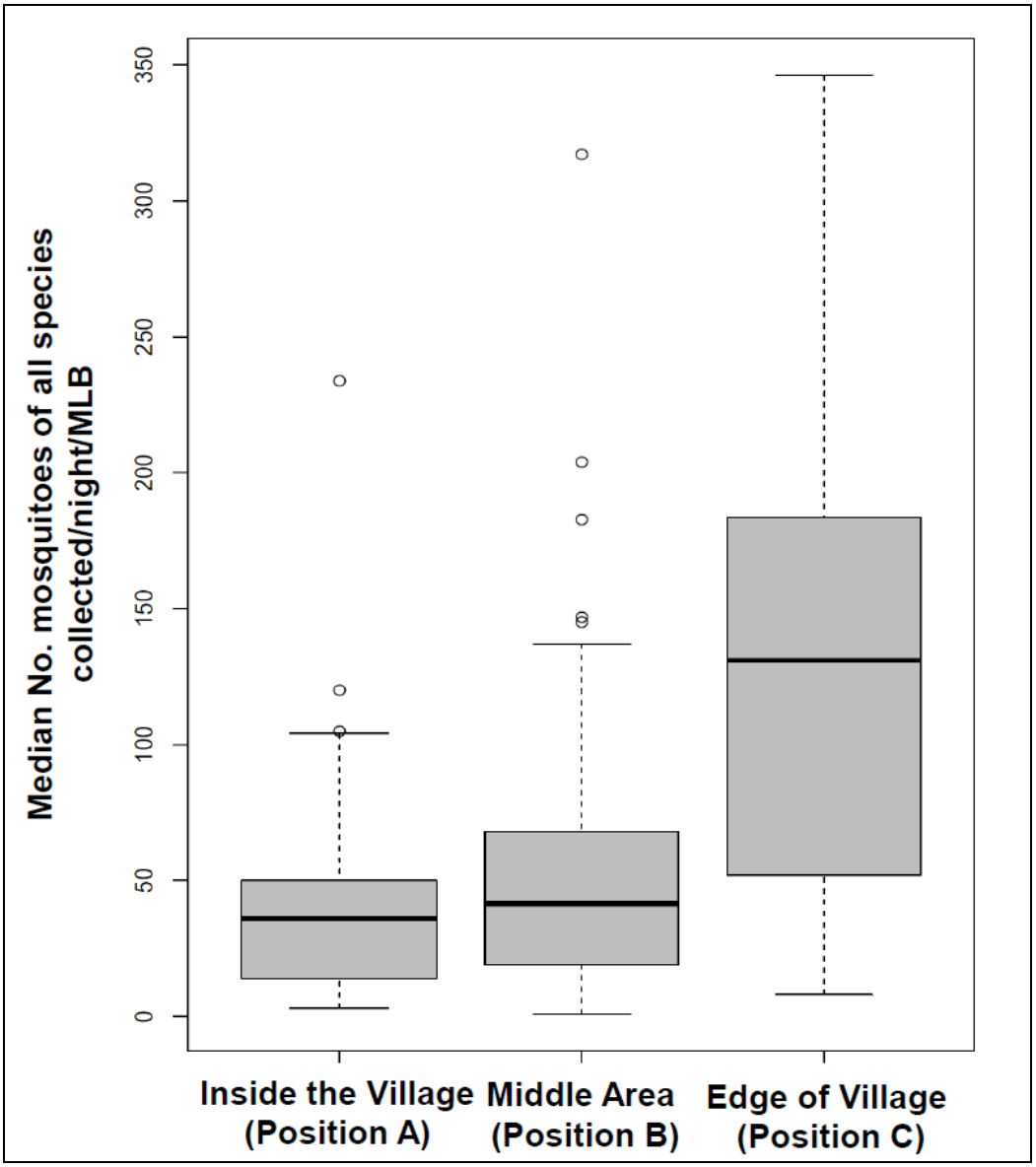
Results were generally similar to those of the wet season and are given in (Table 1 and 2). Overall, 4,699 mosquitoes were collected from all the three odour-baited MLBs fitted with electric grids during the dry season tests. Of these 1992 (42%) were *An. arabiensis*, 69 (2%) were *An. funestus*, 470 (10%) were other *Anopheles* species, 676 (14%) were *Culex* species and 1492 (32%) were *Mansonia* species. Relative to the MLB with one grid, significantly more mosquitoes (all species combined) were collected at the MLB with three grids (RR =1.73 [1.59-1.88], P <0.001) or the one with two grids (RR =1.57 [1.46-1.70], P <0.001). Regarding effect of position, we also observed that relative to position A (centre of the village), 3.8 times more mosquitoes of all species were collected when MLBs were at position C (RR = 3.84 [3.53-4.17], P <0.001), and 1.8 times more when MLBs were at position B (RR = 1.83 [1.68-2.00], P < 0.001).

Upon morphological identification, we determined that the MLB with three grids killed significantly more *An. arabiensis*, (RR = 1.63 [1.45-1.84], P <0.001), more *Culex* mosquitoes (RR =1.92 [1.54-2.39], P <0.001) and more *Mansonia* mosquitoes (RR =4.16 [3.32 -5.21], P < 0.001) than MLB with one grid. There was however no significant difference in numbers of *An. funestus* caught from the MLB with three grids compared to the MLB with one grid. The MLB with two grids also killed more *An. arabiensis* (RR=1.85 [1.65-2.08], P <0.001), more *An. funestus* (RR =4.79 [2.08-11.02], P<0.001) more *Culex* (RR = 1.44 [1.16 -1.79], P <0.001) and more *Mansonia* mosquitoes (RR =1.29 [1.04 -1.59], P < 0.02), compared to the MLB with one grid.

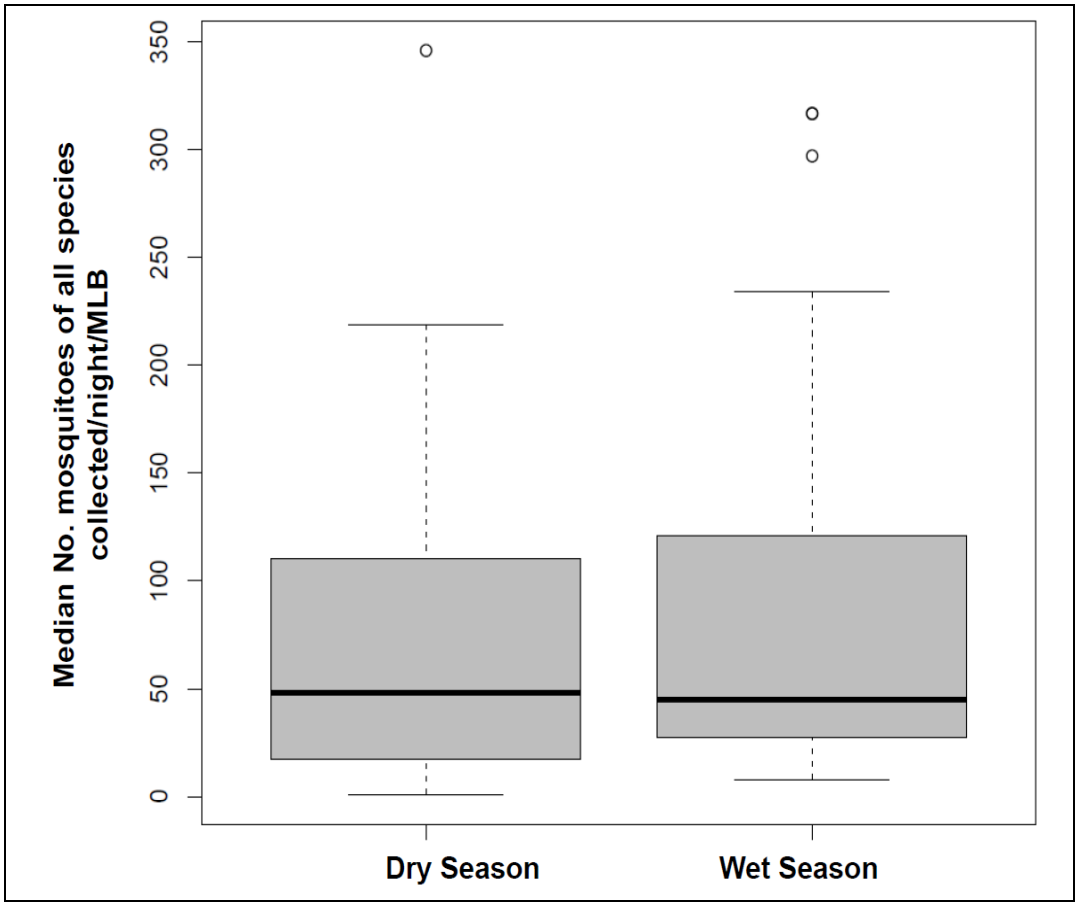
Interestingly, there was no difference in number of mosquitoes collected between wet season (first round) and dry season (second round) except for *Mansonia* species, for which there were higher numbers in the wet season relative to the dry season (RR=1.80 [1.17-2.79], P<0.007). Figure 8-10 show the variations in mosquito collections by number of grids, position and seasons.




**Figures 8:** Comparison of numbers of mosquitoes (all species combined) killed by odour-baited mosquito landing boxes fitted with one, two, or three electrocuting grids on the sides.




**Figures 9:** Comparison of numbers of mosquitoes (all species combined) that were collected when the odour-baited mosquito landing boxes were located at the three different positions.




**Figures 10:** Comparison of number of mosquitoes (all species combined) that were collected during dry and wet seasons of the experimental trials.

### *4.2. Plasmodium infection rates in the sampled mosquitoes*

A total of 1,230 individual mosquitoes from the *An. gambiae* complex and *An. funestus* group were tested for *P. falciparum* circumsporozoite protein using an advanced ELISA technique that involved boiling the ELISA lysate for 10 minutes at 100°C, to exclude any false positives [109]. Of this 0.84% (i.e. 8 out of 958) from *An. gambiae s.l,* all of which were *An. arabiensis* were found to be sporozoite positive before boiling. However after boiling the sporozoite rate dropped to 0.2% (i.e. 2 out of 958). None of the *An. funestus,* in which half were *An. rivulorum,* were found to be positive with malaria parasites.

# CHAPTER 5

## 5.0. Discussion

In Africa, malaria transmissions still occurs substantially indoors [51, 52], but the proportion that occurs outdoors is increasing, particularly in communities where the indoor interventions are extensively used [6, 46]. Odour-baited technologies for attracting and killing disease vectors, e.g. by using lethal insecticide targets, have been considered as one of the options against outdoor-biting malaria vectors [8]. Such technologies must however be designed in such a way as to avoid known vector control challenges such as the development of insecticide resistance after prolonged use, as well behavioural resilience or avoidance by some vector species or populations [13, 110].

Here, a recently developed odour-baited MLB [9] was optimized by fitting it with EGs so that it could offer a quick-acting and non-chemical control mechanism against outdoor-biting malaria vectors, even if these vectors spend only brief periods on the device. The work was motivated by observations that effective odour-baited mosquito control device needs to be low cost, environmentally friendly, non-hazardous to the people, easy to maintain, robust and efficient against target vectors. Therefore the improved MLB that is described here was aimed at meeting these criteria.

Electrocuting grids (EGs) have been widely used in studying vector behaviours such as flight, oviposition and host seeking responses of both tsetse fly and mosquito vectors [74, 111-115]. The technique has also been deployed at household level and in commercial areas such as restaurants and bars, for trapping and killing houseflies and other nuisance flying insects that are visually attracted to light [116, 117]. However, such commercially available EGs are not regularly used outdoors in local rural settings [73], due to high costs and the need for electrical power supply. Another limitation of these EG devices is the fact

that most of the existing versions are not robust under natural settings and not simple to construct locally especially in rural communities [73].

The present study involved adaptation of an existing mosquito control device, the MLB [9], by fitting it with low-cost, commercially available EGs, to effectively target and kill host-seeking mosquito populations, that where these mosquitoes are deemed less responsive to insecticide treated surfaces due to behavioural avoidance characteristics [7, 13, 19] or physiological resistance [5, 6]. The simple type of EGs that we used here, is also widely used domestically for killing flying insects including mosquitoes, just by swatting the flies mid-air, and was readily adapted here as a component of the MLB for passive control and surveillance. The grids, as fitted in the improved MLB, are powered by the same solar panel that also powers the odour-dispensing unit. Both the electrical system and the odour-dispensing units are automated by a light sensor, to improve its feasibility for use at the local settings, and to maximize the community acceptability (Moshi *et al*., unpublished).

The number of mosquitoes collected increased concurrently with the increase number of grids in the MLB. Significantly more mosquitoes of all species were killed by the three-grid MLB relative to one-grid MLB, and slightly more by the two-grid MLB than the one-grid MLB, a trend which might be attributed to the increase in mosquito contact surface areas when the number of grids is increased (Figure 8). *Anopheles arabiensis* was the only member of the *An. gambiae* complex identified and although this represents 98% of the total sample size, it provides an indication of the species present in the study area. This could be attributed to the fact that these collections were all outdoors, we also have extensive evidence from previous indoor and outdoor studies that *An. gambiae s.s*, which was formerly the dominant sibling species in this complex, is now nearly extinct [4].

The majority of the other *Anopheles* mosquitoes caught during the experiments were morphologically identified as *An. coustani* group. Since vector species such as *An. arabiensis* seek blood hosts both outdoors and indoors, depending on availability of the

hosts [19, 118], the efficiency of the MLB against this species suggests that the device can be effective as a complementary and non-chemical option of targeting them. Interestingly some of *An. funestus s.s*., that are known to feed and rest mainly indoors, were caught outdoors. However, it is unknown what proportion of the population the outdoor collections represent as indoor collections were not conducted as part of this study. Since *An. funestus* is also known to be a major contributor to the on-going residual malaria transmission in this part of Tanzania, this observation further justifies the need of supplementary interventions to target these species that are increasingly biting outdoors. These results are also in line with a previous study reported by Russell and other researchers in Tanzania [5], as well as a recent study in west Africa by Moiroux *et al* [46, 119].

The ELISA assays determined that the sprozoite rate was 0.84% for *An. arabiensis* before boiling, but only 0.2% was confirmed positive after boiling. False positive results are known to occur with samples that feeds on animals, such as *An. parensis* and *An. marshallii* group [109], and in this study, boiling reduced false positivity to (0.2 %). The change in sporozoite rate might also be due to technical errors during boiling or a different *Plasmodium* species that could not be detected. None of the *An. funestus,* in which half were *An. rivulorum,* were found positive with malaria parasite. However it is advisable that more collections and analyses should be done since this species has been previously found infectious with malaria sporozoites [120-122], particularly in areas with widespread indoor interventions [122]. No ELISA was done for other *Anopheles* species, the majority of which were *An. coustani*. However, the MLB killed significant numbers of this vector, which is also one of the known secondary vectors that contribute malaria transmission especially in areas where the major malaria vectors are interrupted by the existing control interventions. Reports have showed that this *Anopheles* species could indeed contribute significantly in outdoor transmission of malaria [123] as well as a vector of Rift Valley fever virus during the disease epidemics [124]. This suggests that the MLB could potentially be used as an outdoor

intervention to target these mosquito species and complement the existing indoor control tools.

Though the main target of this study was malaria mosquitoes (for which the estimated mean catches are shown in (Table 1), it was also important to assess overall impact of the devices on all mosquito species combined, including the culicines. This is particularly important as the biting densities of non-malaria mosquitoes could influence people's perception on whether a malaria control intervention is effective or not [125].

The differences due to location are important when determining optimal positions necessary to achieve maximum impact on vector densities. For example, at least four times more mosquitoes of all species were collected when the MLB (regardless of number of grids) was placed at position C, which was at the edge of the village, nearest to vector breeding sites, relative to when the device was positioned at A, which was 150 meters away from the nearest mosquito breeding site and inside the village (Figure 9). The present results match the findings of many previous reports on strong associations between high number of adult mosquito densities and distance from nearest aquatic breeding sites [126, 127]. Similar observations have also been made of relationships between increase in malaria cases in houses which are near mosquito breeding sites, suggesting that for the general mosquito control interventions should be either more focused on the households close to the breeding sites, or located in such a way that it is possible to intercept mosquitoes flying between these aquatic sites and human dwellings [128, 129].

Unexpectedly, the was no difference in number of mosquito catches at the end of the wet season relative to the dry season except for *Mansonia* species that increased significantly at the end of the wet season when the study was performed. The lack of difference between seasons could be due to the fact that the study area is a rice growing area, with consistently high vector densities throughout the year [130, 131]. Moreover, some species, such as *An. arabiensis,* tend to maintain their population size throughout most of the year even when the

breeding sites are dry, since their eggs can remain dormant to resist desiccation [132]. Also, the persistent number of *An. funestus* during the dry season may be due to their preference for breeding in permanent water bodies and the fact that they can breed in hidden, small, man-made habitats [133].

Even though we did not conduct test for insecticide resistance in this study, the experiments described in this thesis were conducted in an area where previous WHO susceptibility tests have shown that the local malaria vector populations no longer have complete susceptibility to pyrethroids that are commonly used insecticides [134].

The mosquito lure used here has previously been demonstrated to attract similar proportions of mosquito species as humans [82]. Even though during these experiments the synthetic lure used [82], was manually prepared by hand and dispensed using locally available nylon strips [98], we envisage that eventually the blend could be improved and packed into simple long-lasting pellets that can be widely used, hence reducing cost and time for labour. Similar procedures are already being implemented by commercial manufacturers such as Biogents (BG) Ltd (Germany), for large-scale use against the dengue vector, *Aedes aegypti* [135]. No other insects were found stuck to the grids or on the linoleum, suggesting that the MLB baited with the synthetic lures that we used here, would selectively work predominantly against disease-transmitting mosquitoes, thus improving environmental friendliness. Moreover, in this study, nearly all female mosquitoes collected at any of the positions, were not blood fed, suggesting that they were in a host-seeking state or looking for resting sites after emerging. Additional studies should be done to determine if non-odour- baited MLB also acts as attractive outdoor resting boxes. As an effective control tool against such vector populations, the MLB would be effective against outdoor-biting vectors, which otherwise perpetuate residual malaria even where indoor interventions like LLINs are already common.

We have demonstrated the potential of MLB with EGs against malaria vectors and non-malaria vectors outdoors. However we envisage that in the future the device can also be

deployed as a surveillance tool to measure vector population densities and disease transmission outdoors. Given that the MLB was baited with synthetic human lures, the technology may also eventually be used as an alternative to the current practice of sampling potentially infectious mosquitoes by using human volunteers, a technique commonly referred to as human landing catches (HLC), but which many consider to be unethical, as it increases the risk of the volunteers being bitten by infectious mosquitoes [136].

In addition to attracting and killing potentially infectious and nuisance mosquitoes, the MLB has the potential of supplying lighting to households in rural communities. This concept has already been demonstrated by scientists at Ifakara Health institute (Okumu *et al*., unpublished), in rural Tanzania communities where mains electricity coverage is still below 5%. In those demonstrations, nine specially-designed experimental huts and two village houses (Figure 11) were supplied with solar-powered lighting systems, using the same solar panel that powers the odour-dispensing unit and electrocuting grids in the devices. If used this way the devices can improve livelihoods by controlling disease vectors and providing basic lighting, thus reducing risks associated with common rural light sources like kerosene lamps as depicted on the bottom right panel of figure 11. We expect that the fact that these devices can provide energy for basic lighting, pupils' home-study and mobile phone charging, improves opportunities for acceptability and sustainability of this technology in rural and remote communities.

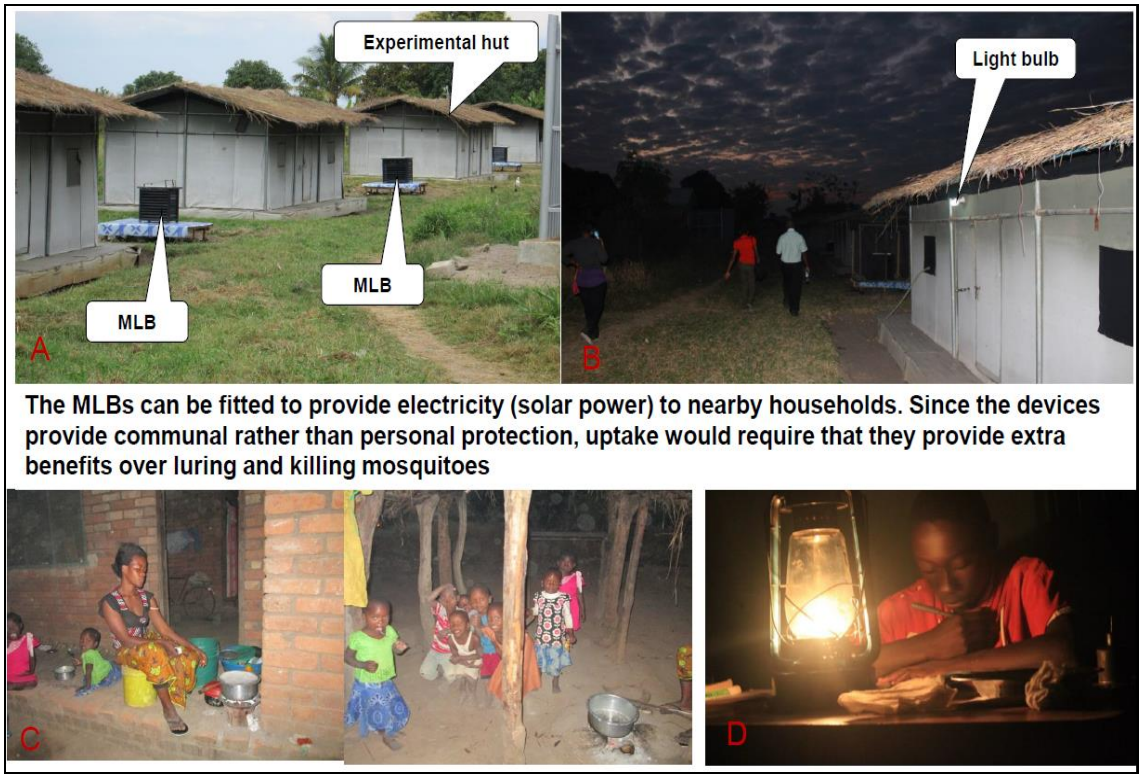



**Figure 11:** Pictorial representation of how mosquito landing boxes (MLBs) fitted with grids and automated light sensor **(A)** can be used to provide basic lighting in rural households. Here, this concept was demonstrated by fitting MLBs onto nine experimental huts **(A and B)** and two local houses **(C)** located in a malaria endemic village in rural Tanzania. This would reduce risks associated with common rural light sources, such as kerosene lamps **(D)**.

One of the main limitations of this device is the need for $CO_2$ gas, which was a major component of the mosquito lures that we used. In this study we used industrial carbon dioxide gas, delivered in pressurized cylinders, and dispensed through calibrated flow metres (Glass Precision Engineering Ltd., United Kingdom) through gas inlet pipe as described in Figure 5. The $CO_2$ gas is an important component of mosquito lures and is necessary for activating mosquitoes and synergizing other lure components [137, 138]. While industrial $CO_2$ can be effective for experimental and demonstration purposes, it is

logistically difficult to use, expensive, and usually not feasible on a large-scale in rural areas in sub-Saharan Africa. However, there are now a number of alternative options including $CO_2$ gas generated from yeast-sugar fermentation [139] or yeast-molasses fermentation [140]. For field applications, either of the above alternatives, or the use of host odours suctioned directly from human dwellings [9, 88, 141] could be considered.

Another limitation was that we experienced high proportions of non-amplified mosquitoes during our molecular analysis for species identification, especially for *An. funestus* group, for which non-amplification rate was 68%. Though we were unable to immediately determine causes of these poor amplification rates, we hypothesise that the most likely explanation is that the specimens were morphologically mis-identified, since the collection method may have morphologically damaged the specimens, making identification difficult. However, there may be other possibilities that cannot also be excluded, such as problems arising from suboptimal specimen handling, preservation and storage conditions, and problems arising during DNA extraction processes. Nevertheless, plans are underway to repeat the PCR assays in separate molecular laboratory, to determine what the main cause of the low amplification rates could be.

### 5.1. Conclusion and recommendations

This study has shown the newly improved odour-baited mosquito landing box (MLB), which is fitted with affordable electrocuting grids (EGs) and automated sensors, could be used to target outdoor-biting mosquitoes, including major malaria vectors. This non-chemical technology instantly kills host-seeking mosquitoes that naturally bite outdoors or those that have changed behaviours from indoor-biting, and now increasingly bite outdoors due to selection pressure from the indoor insecticide-based interventions like LLINS and IRS. In addition, this method could be effective against vector populations that are physiologically

resistant to insecticide-based interventions. The use of electric grids will eliminate the need for applying insecticides to the device, and ensure high killing efficacy even if the mosquitoes are behaviourally or physiologically resistant. Thus the tools may allow us to preserve effectiveness of the existing interventions such as LLINS and IRS, by removing resistance alleles from the population using an entirely new insecticide-free means of killing mosquitoes. Moreover, the MLBs with EGs can offer an integrated vector control approach and a feasible method for both controlling and effectively monitoring densities and infectiousness of disease-transmitting mosquitoes. Other than the malaria vectors, the device can also simultaneously target non-malaria mosquitoes such as *Culex* and *Mansonia* species that are potential vectors of lymphatic filariasis and arboviruses, and are also major sources of biting nuisance to humans. Reducing nuisance biting will increase consumer satisfaction with this method.

Further studies are recommended to assess and compare the monitoring efficacy of the MLB equipped with grids with other existing tools, as well as the potential effects of the tool on malaria transmission and incidence rates in communities. We also suggest comparative evaluation studies using MLBs with grids to determine species variations on outdoor collections relative to indoor mosquito populations.

## 6.0. Ethical consideration

Ethical review and approval was granted by the institutional review board of Ifakara Health Institute (Ref: IHI/IRB/NO.030) and The Medical Research Coordinating Committee at the National Institute of Medical Research in Tanzania (Ref: NIMR/HQ/R.8a/Vol.IX/1222). An ethics waiver was provided from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg (Appendix 8.1).

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## 8.0. Appendices

### Appendix 8.1. A copy of Ethical Waiver

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)

Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

Ref: W-CJ-100510-1

10/05/2010

***TO WHOM IT MAY CONCERN:***

**Waiver:** This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

**Investigators:** **Professor Maureen Coetzee**

**Project title:** **Research on mosquitos.**

**Reason:** This waiver covers all research using mosquitos and mosquito parasites by Professor Coetzee, her staff and students as long as no humans or human tissues are involved. The waiver lasts 5 years and may be renewed.

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2010 -05- 10
JOHANNESBURG

Professor Peter Cleaton-Jones
Chair: Human Research Ethics Committee (Medical)

copy: Anisa Keshav, Research Office, Senate House, Wits

**Appendix 8.2. Statistical models and model outputs**

**Poisson Distribution Models and Negative Binomials Distribution Models**

The following are examples of how R analysis was performed, showing a summary of the models used during analysis and their outputs. Here we report only models which were used for malaria vectors during round one of the experimental tests. Also we included a summary figures showing residual distribution to assess model fit.

***Model for Anopheles arabiensis (data from round 1)***

Model n6<-glmer

(*Arabiensis*~treatment*position+(1|day),family=poisson(link=log),data=obj2r1)

**Model R output summary:**

```
Fixed effects:

                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      2.71865    0.17714  15.348  < 2e-16 ***
treatmentGrid 2                  0.37061    0.14508   2.555  0.01063 *
treatmentGrid 3                  0.77002    0.13980   5.508 3.63e-08 ***
positionB                       -0.43105    0.15862  -2.718  0.00658 **
positionC                       -0.44066    0.17957  -2.454  0.01413 *
treatmentGrid 2:positionB        0.33453    0.23558   1.420  0.15559
treatmentGrid 3:positionB        0.33180    0.23471   1.414  0.15745
treatmentGrid 2:positionC       -0.01688    0.23704  -0.071  0.94323
treatmentGrid 3:positionC        0.26568    0.25581   1.039  0.29901
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

***Model for Anopheles funestus (data from round 1)***

Model n7<-glmer

(*Funestus*~treatment*position+(1|day),family=poisson(link=log),data=obj2r1)

**Model R output summary:**

Fixed effects:

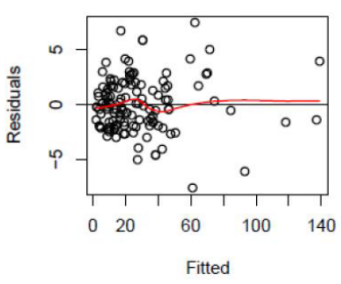
| | Estimate | Std. Error | z value | Pr(>\|z\|) | |
|---|---|---|---|---|---|
| (Intercept) | -2.856 | 1.089 | -2.623 | 0.00873 | ** |
| treatmentGrid 2 | 1.995 | 1.326 | 1.504 | 0.13259 | |
| treatmentGrid 3 | 1.235 | 1.300 | 0.950 | 0.34194 | |
| positionB | 1.437 | 1.297 | 1.107 | 0.26815 | |
| positionC | 2.687 | 1.195 | 2.248 | 0.02459 | * |
| treatmentGrid 2:positionB | -3.307 | 1.916 | -1.726 | 0.08428 | . |
| treatmentGrid 3:positionB | -1.356 | 1.735 | -0.782 | 0.43452 | |
| treatmentGrid 2:positionC | -2.287 | 1.597 | -1.432 | 0.15211 | |
| treatmentGrid 3:positionC | -1.018 | 1.609 | -0.633 | 0.52693 | |

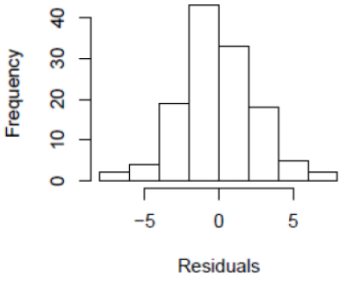
---

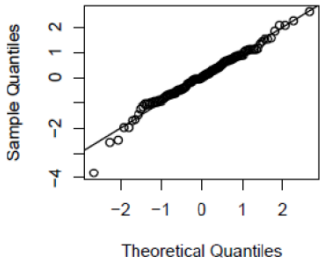
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

## Appendix 8.3. Model diagnostics (included only for malaria vectors for round 1)

Model n6<-glmer(*Arabiensis*~treatment*position+(1|day),family=poisson(link=log),data=obj2r1)




Model n7<-glmer(*Funestus*~treatment*position+(1|day),family=poisson(link=log),data=obj2r1)

