The effect of combined use of Mosquito Magnet Liberty Plus™ trap and insecticide treated net on human biting rates of *Anopheles gambiae* s.s. and *Culex quinquefasciatus*

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Abstract: Malaria is the most important public health problem in Sub-Saharan Africa. There is a pressing need for development and use of alternative control approaches, which will remain effective even with increasing threat of mosquito resistance to chemical control and smaller number of approved chemical insecticides. This study evaluated the effect of combined use of attractant baited Mosquito Magnet Liberty Plus™ (MM) trap and bed nets on human mosquito biting rates under semi-field conditions. Human landing catch done under holed bednets was used to assess the number of biting mosquitoes attracted to human bait. A combination of MM trap and untreated bednet reduced biting rates of *Culex quinquefasciatus* but not *Anopheles gambiae* s.s. However, combining an insecticide treated bednet (ITN) and MM trap greatly reduced biting rates of both *Cx quinquefasciatus* and *An. gambiae* s.s. Moreover, a treated bednet increased the MM trap catch of both *Cx quinquefasciatus* and *An. gambiae* s.s. The present study has shown the potential of a combination of MM trap and ITN in trapping and controlling vectors of malaria and lymphatic filariasis. Synergistic use of attractant baited traps and ITNs displays a 'push-pull' phenomenon. The findings indicate that the strategy could be incorporated in an integrated mosquito control approach to maximise the efficiency of mosquito population-reduction methods through the use of appropriately selected methods.

Key words: mosquitoes, traps, bednet, biting rate, *Anopheles gambiae, Culex quinquefasciatus*

Introduction

Malaria is the most important public health problem, causing high morbidity and mortality as well, posing a major economic burden in Sub-Saharan Africa (Hemingway *et al*., 2006). In Tanzania, malaria accounts for over 30% of the national disease burden and is responsible for more than one third of deaths among children under 5 years and up to one-fifth of the deaths among pregnant women (Mboera *et al*., 2007). In recent years mosquito vector control efforts have been biased towards the use of chemicals for residual spraying (Sharp *et al.*, 2007) and treating fabrics (Phillips-Howard *et al*., 2003, Dabiré *et al*., 2006).

Traps, which were originally meant for sampling mosquito vectors and disease surveillance (Mboera, 2005), have shown promising results in effectively attracting a large number of mosquitoes while reducing the need for live baits thus making them prospective tools for control of mosquitoes (Kline, 1999, 2006). The use of odour-baited traps followed ethical concerns over human-landing catch, a standard disease surveillance method, for exposing catchers to potentially infective mosquito bites, being exhaustive and subject to human error. Such traps include the Counterflow Geometric Traps (Kline, 1999; Mboera *et al*., 2000) and Mosquito Magnet™ traps (MM) (Kline, 2002; Ritchie *et* al., 2003). These traps utilises odour-mediated, host finding behaviours in trapping mosquitoes. To date, chemical attractants are commercially available to enhance the concentration of mosquitoes, hence to facilitate their control/removal by the traps. Different MM trap models have been evaluated successfully against a variety of mosquito species (Dennett *et al*., 2004; Siphiprasasna *et al*., 2004) for trapping and surveillance.

The threat of increasing insecticide resistance and dwindling number of approved and selected chemical insecticides; intensifies the need for development and use of alternative approaches, which will reduce reliance over chemical mosquito control. Such approaches include the removal trapping techniques in a 'push-pull' strategy. The "push-pull" strategy combines behaviour-modifying stimuli to manipulate and affect the distribution and abundance of pests or beneficial insects for pest management (Cook *et al*., 2007). In this strategy, pests are targeted and/or repelled to reduce their abundance on a protected resource (push), the pests are simultaneously attracted using attractive stimuli (pull) to areas such as traps, which will facilitate their elimination. 'Push-pull' strategy amongst other advantages may be a possible tool for resistance management.

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With commercially available MM traps, which produce their own basic attractants and thus attracting large number of mosquitoes, and following the success achieved in tsetse control using attractant baited, insecticide-impregnated targets and traps in Africa (Torr, 1994), the use of traps in vector control (removal trapping) has received renewed attention. MM Plus™ trap is battery operated and runs on propane gas that is catalytically converted to produce carbon dioxide (CO_2), heat and water vapour. The thermoelectric generator uses excess heat from combustion to produce electricity to power the trap (Kline, 1999).

This study intended to evaluate the effect of combined use of attractant baited Mosquito Magnet Liberty Plus™ trap and bed nets on human mosquito biting rates under semi-field conditions.

Materials and Methods

Study site

Experiments were carried out in three semi-field structures located at Ubwari village (05°10' 220''S, 38°46' 733''E) in Muheza, north-eastern Tanzania between March and May 2007. The area experiences 1000mm average annual rainfall with two seasonal peaks; the main peak in March-May and a less pronounced peak in November-December. The mean temperature is 26^{0}C with cooler months between June and September and warmer months between October and May.

Experimental semi-field environment

The semi-field experimental environment ('mosquito sphere') has been constructed to simulate a local village setting (Knols *et. al*., 2002). Each sphere measures 12.2m long and 8.2m wide and 4.6m high and is covered with shade-cloth (90%) permitting entry of wind and precipitation and creating similar climatic conditions to ambient conditions. Entry into the sphere is through a double door system. A wooden door provides entrance to the sphere, after passing through a small corridor (3.0m long and 2.2m wide, 2.7m high) of similar shade, with a screened door to the outside. This prevents escape of released mosquitoes and entry of wild ones. Each sphere contains some vegetation and a traditional mud hut (2.74 x 2.74 x 1.83m) with its roof (2.56m at the apex) made of grass thatch. The house has a single door, two open-windows and a single Zanzibari-style rope bed inside that is occupied by a volunteer during experiments. Mud huts and rope beds are common in coastal village areas of Tanzania. Three mosquito spheres were used for the study.

Trapping methods

In the course of study, human landing and resting catches were used to collect mosquitoes. Mosquito Magnet Liberty Plus™ trap (referred to as "MM trap") (American Biophysics Corporation, Rhode Island, USA), was operated according to manufacturer's instructions and placed close to the entrance of spheres.

Human landing catch was done to assess the number of biting mosquitoes attracted to human bait. Volunteers were stationed inside the mud hut of each sphere and sat on bed under a bed net, equipped with a torch, an aspirator and labelled paper cups. The volunteer performed a whole night human landing catch by aspirating the biting mosquitoes into labelled paper cups. A 30-minutes resting catch was done in each sphere at daybreak of every test day to recover any mosquito left inside the sphere. The volunteers equipped with mouth aspirators and labelled paper cups collected mosquitoes resting at different places (inside the mud hut, on the shade cloth and on plant leaves) within each sphere.

Experimental procedure

MM traps were set inside spheres at 17:00h by plugging Lurex^3 attractant sachet in the plume tube according to the manufacturer's instructions and placed close to the entrance in two of the mosquito spheres. The treatments were: Sphere 1= MM trap+untreated net; Sphere 2= untreated net only; Sphere 3 = MM trap+ITN (Permanet® net, Vestergaard-Frandsen SA, Switzerland). The remaining sphere served as a control and did not contain MM trap. Traps were switched on at 18:00h and a total of 300 starved female *Anopheles gambiae* s.s. and 300 starved female *Culex quinquefasciatus* were released from the centre of each sphere by opening the release cages.

A total of three bednets, one 1.5x1.8x1.9m, long-lasting treated bednet (Permanet®) and two 1.6x1.9x2.2m, untreated bednets (Safinet®, A to Z Textile Mills, Arusha, Tanzania) were used for the study. One bed net was used in each sphere; each bed net had six, 4 x 4cm holes, two on each of the longer sides and one on each of the shorter sides. A long lasting treated net was used in spheres with MM trap only. A male volunteer (24-32 years old) was assigned to each sphere stationed inside the mud hut sat on bed under a bed net, where he collected mosquitoes attempting to bite him (human landing catch) whole night.

Traps were switched off and retrieved at 06:00h in the morning. The collections from traps and human landing catches were placed in the freezer to kill mosquitoes which were later identified and counted. A 30-minute resting catch was done in each sphere at daybreak of every test day to recover any mosquitoes left inside the sphere. Individuals equipped with mouth aspirators collected mosquitoes resting at different places (inside the mud hut, on the shade cloth and on plant leaves) within each sphere. The number of mosquitoes caught in each sphere with each method (MM trap, human landing catch, resting catch) was recorded. In these experiments the procedure was repeated for nine test nights. To limit effects of differences between test-nights, volunteers and sphere location, volunteers and treatments were rotated so that each treatment was tested in each sphere three times following a 3x3 Latin Square design.

Data analysis

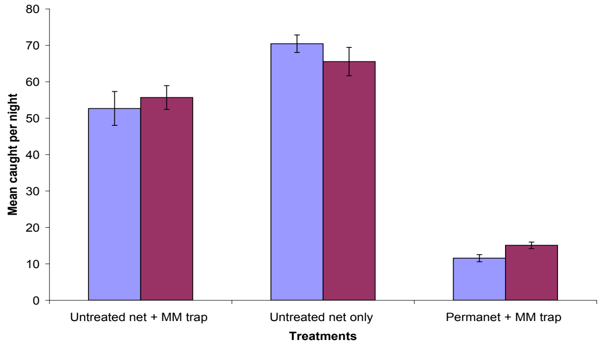
Data was analysed using Analyse-it™ programme for Microsoft Excel statistical software. Counts from each treatment were checked for normality using the Shapiro-Wilk (W) test. Datasets were then compared for homogeneity of variance and either subjected to Analysis of Variance or an independent t-test (using Welch's approximation for unequal variances) to investigate the difference between the mean numbers of each species caught with each treatment. The Mann-Whitney (U) test was used for non-parametric data to determine any significant differences between treatments.

Ethical consideration

Full verbal explanation of the study was given to volunteers who participated in night study collections. Verbal consent was then obtained from volunteers before commencement of the study.

Results

Of 8100 *Cx quinquefasciatus* and *An. gambiae* s.s. released, 77.9% and 74.1% were recovered respectively. Compared to the control (untreated net only), combination of MM trap and untreated bednet significantly reduced biting rates of *Cx quinquefasciatus* (t-test, P=0.0037) but not *An. gambiae* s.s. (t-test, P=0.0695). However, combining an ITN and MM trap markedly reduced the biting rates of both *Cx quinquefasciatus* and *An. gambiae* s.s. (t-test, P<0.0001) compared to the control (untreated net only) (Figure 1).

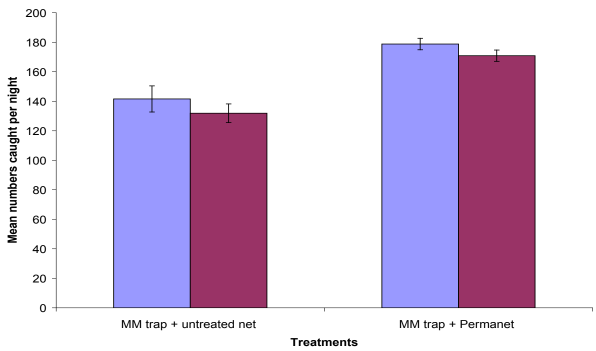


Error bars represent the standard error.

An. gambiae s.s. Cx. quinquefasciatus

Figure 1: Human biting rates of *Culex quinquefasciatus* and *Anopheles gambiae s.s.*

The use of ITN significantly increased the MM trap catch of both *Cx quinquefasciatus* (t-test, P=0.0014) and *An. gambiae* s.s. (t-test, P<0.0001) than when untreated net was used (Figure 2).



Error bars represent the standard error

An. gambiae s.s. Cx. quinquefasciatus

Figure 2: MM trap catch of *Culex quinquefasciatus* and *Anopheles gambiae s.s.*

Although not significant (t-test, P>0.05), more *Cx quinquefasciatus* than *An. gambiae* s.s. were caught in the MM traps. Significantly more mosquitoes were caught resting in the control treatment (untreated net only), than from the rest of the treatments (Table 1).

Table 1: ***Culex quinquefasciatus*** **and** ***Anopheles gambiae s.s*** **recaptured in the morning resting catch**

Treatments	Mean number caught ± SE	
	Cx quinquefasciatus	*An. gambiae* s.s.
MM trap + untreated net	39.7 ± 3.86[a]	43.8 ± 2.96 [a]
Untreated net only	174.3 ± 6.00[b]	162.7 ± 9.46 [b]
Trap + ITN	32.1 ± 2.94[c]	23.9 ± 1.98 [c]

Values in the column with different superscript letters are statistically different ($P<0.0001$)

Discussion

From the results, a combination of Mosquito Magnet Liberty Plus™ (MM) trap and an untreated bednet successfully reduced biting rates of *Cx quinquefasciatus*. Unlike the control (untreated net only), MM trap baited with a commercial attractant, produced a set of odour (CO_2, lactic acid and ammonia and additional heat and moisture) to which mosquitoes were attracted and trapped. In this setup on the other hand, moderate human mosquito biting rates were recorded as a result of mosquito attraction to human bait and entry through holed bednet. Mosquito attraction by ammonia (Smallegange, *et al*., 2005), carbon dioxide (Mboera *et al*., 1997; Costantini *et al*., 1998; Dekker *et*. *al*., 2005), heat and moisture (Khan & Maibash, 1966) and lactic acid (Murphy *et al*., 2001; Dekker *et al*., 2002; Bernier *et al*., 2003) is well documented. According to Bernier *et al*. (2003) lactic acid in synergy with other human odours, has a crucial role in mosquito attraction.

In this study, *An. gambiae* s.s. was not affected by the use of untreated bednet and MM trap possibly suggesting their higher affinity for humans. This and the fact that relatively fewer *An. gambiae* s.s. were caught in baited traps, indicate that Lurex[3] is likely to be more suitable as an attractant for *Cx quinquefasciatus* than for *An. gambiae* s.s. or possibly *An. gambiae* s.s. had difficulties following the odour plume released from the traps. A different lure combination may therefore, be required to increase the trapping efficiency of *An. gambiae* s.s. In the absence of a trap (control) all mosquitoes were attracted to human bait contributing to higher biting rates recorded. In this case mosquitoes which could not locate holes in the untreated bed nets and therefore not accessing human bait were recaptured in the morning resting catches, which explains higher number of resting mosquitoes collected in the control treatment.

Highest reduction in human mosquito biting rates was effected through a combination of MM trap and insecticide treated bednet. Insecticide treated bednet repelled mosquitoes, which could otherwise be attracted into the net for a blood meal. Repelling effect of treated bednets has been shown by Dabirè *et al*. (2006). In addition to the repelling effect, insecticide treated bednets have also been shown to produce high mortalities (Msangi *et*. *al*., 2008). In this view therefore, reduction in mosquito bites in the present study is also likely to be a result of mortality of mosquitoes which landed on the treated net. Using an insecticide treated bednet provided a repelling effect to mosquitoes, which were then attracted and trapped by a baited MM trap (a push-pull effect).

Despite under utilization of push pull strategy in the control of insects of medical importance (Cook *et al*., 2007), the strategy has been shown to be effective against crop pests (Cowles & Miller, 1992; Nalyanya *et al*., 2000). Our findings indicate that the strategy could be incorporated in an integrated mosquito control approach to maximize the efficiency of mosquito population–reduction methods through the use of appropriately selected methods.

The present study has highlighted the potential of trapping systems in mosquito control. From the results, it is possible therefore to divert mosquitoes to other available alternative hosts or a removal trapping system by the use of insecticide treated bed nets through universal repelling effect. This could be of paramount importance especially now that insecticide treated bednets are highly advocated for their proven efficacy on reducing morbidity and mortality due to malaria. In conclusion the use of attractant baited traps and insecticide treated bed nets demonstrates a 'push-pull' system. Even when not treated, a bed net when used with a MM trap successfully reduced mosquito biting rates. Moreover, higher reductions in biting rates were achieved through combining insecticide treated bed net and MM trap.

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