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Posted Date: 11 April 2024

doi: 10.20944/preprints202404.0764.v1

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Comparison of Trapping Efficacy of Locally Modified Gravid Aedes Trap and Autocidal Gravid Ovitrap for the Monitoring and Surveillance of *Aedes aegypti* Mosquitoes in Tanzania

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Simple Summary: Mosquito traps are widely used for monitoring and surveillance of mosquito vectors in many mosquito-borne diseases endemic countries.However, the costs and efficacy of traps remain a great challenge. In this study, we compared trapping efficacy of locally modified Gravid Aedes Trap (GAT) and Autocidal Gravid Ovitrap (AGO) for dengue vector (*Aedes aegypti*) in a semi field and field settings. The GAT was lined with pyrethroid-treated nets as a killing agent, while the AGO was adhered with sticky board to capture mosquitoes. We also compared the locally modified traps baited with either yeast or grass infusion with BG-Sentinel (BGS) with BG lure (a standard trap for capturing *Aedes* mosquitoes). Our findings showed that the GAT was more efficacious than the AGO in both semi-field and field settings. Additionally, there was no significant difference between yeast-baited and grass-baited GAT traps in capturing mosquitoes, although yeast was easier to use. When compared to a standard trap (BGS), GAT showed no difference in capturing *Aedes* mosquitoes in a semi-field, however, in the field setting, BGS outperformed the modified GAT.

Abstract: The study assessed the trapping efficacy of locally-modified 1) Gravid Aedes Trap(GAT) lined with insecticide-treated net (ITN) as a killing agent, 2) Autocidal Gravid Ovitrap(AGO) with sticky board in the semi-field system (SFS) and field setting. Fully-balanced Latin square experiments were conducted to compare GAT lined with ITN vs AGO, both with either yeast or grass infusion. Biogents-Sentinel (BGS) with BG-Lure and no CO_2 was used as standard trap for *Aedes* mosquitoes. In the SFS, GAT outperformed AGO in collecting both nulliparous (65% vs 49%,OR=2.22,[95%CI:1.89-2.60],p<0.001) and gravid mosquitoes (73% vs 64%,OR=1.67,[95%CI:1.41-1.97],p<0.001). Similar differences were observed in the field. Yeast and grass infusion did not significantly differ in trapping gravid mosquitoes (OR=0.91, [95%CI:0.77-1.07],p=0.250). The use of ITN improved mosquito recapture from 11% to 70% in the SFS. The same trend was observed in the field. Yeast was chosen for further evaluation in the optimized GAT due to its convenience and bifenthrin net for its resistance management properties. Mosquito density collected when using 4x GATs relative to BGS captured gravid mosquitoes 64 vs 58 (IRR=0.82,[95%CI:0.35-1.95],p=0.658) andshowed no density dependence. Deployment of multiple yeast-baited GAT lined with bifenthrin net is cost effective (single GAT<$8) compared to other traps such as BGS ($160).

Keywords: *Aedes aegypti*; Mosquito traps; Vector control; Bio-Gents Sentinel trap; Gravid *Aedes* Trap; Autocidal Gravid Ovitrap; Tanzania

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1. Introduction

Dengue fever is a rapidly growing public health concern in tropical and subtropical regions [1,2], with dramatic increase of disease incidence in the past fifty years [3,4]. This estimated increase is related to the rapid spread of highly competent dengue vectors [5] due to unplanned urbanization, climate change [1,2] and intercontinental trading [3]. There is some genetic evidence that *Aedes aegypti* mosquitoes may have been reintroduced to Africa from the Americas [6]. This reintroduction may explain the upsurge in dengue epidemics currently witnessed across the African continent [7]. Between 1990 and 2019, dengue transmission has increased by 400% in sub-Saharan Africa [8].

Currently, available options for dengue prevention primarily involve vector control and surveillance [1,2,9]. Despite the rapid spread of dengue in Africa, vector surveillance remains limited [10], underscoring the critical role of mosquito sampling tools in detecting and estimating vector species composition, biology, and ecology [11]. This information from vector surveillance is crucial for informing proactive *Aedes* control operations [9]. However, the majority of existing vector control is primarily focused on malaria vectors which may target times and places that do not overlap with *Aedes* vectors.

Various sampling tools for monitoring adult mosquitoes have been developed to provide information about the predominant vectors and the impact of the interventions [12–14]. Lethal ovitraps (gravid traps) such as Gravid *Aedes* Trap (GAT) and Autocidal Gravid Ovitrap (AGO) are among the most widely used traps for sampling *Aedes* mosquitoes and primarily designed to capture gravid mosquitoes [15]. These are passive traps that use water and organic materials to attract mosquitoes seeking a place for oviposition [16–18]. Mosquitoes are captured by either a sticky surface, oil, or insecticide lined inside the GAT [12] or an adhesive sticky board in AGO traps [19]. Both GAT and AGO are simple, lightweight and do not require electricity to function. Although their primary purpose is monitoring, they also show great promise as a control tool [20] because both traps function based on "lure and kill" strategy, effectively reducing the adult population [19,20].

Aedes mosquitoes tend to lay a single batch of eggs in multiple breeding sites through "skip oviposition" to ensure survival of at least some eggs [21,22]. The behaviour may be exploited for mosquito control through the use of lethal ovitraps. Gravid traps are advantageous because they can capture gravid *Aedes* mosquitoes which are more likely to be infected with dengue virus [23,24] due to imbibing a bloodmeal, and may therefore also be used for virus surveillance. Although gravid traps are designed for capturing gravid (eggs laying) mosquitoes, they may also capture non-gravid and non-blood fed (nulliparous) mosquitoes that are resting.

The BGS is a fan operated trap with a lure to attract mosquitoes. It is a standard method that is effective for sampling host-seeking *Aedes* mosquitoes [25,26]. However, the BGS trap is costly, requires electricity and maintenance [20]. When compared to the standard trap in Brazil, GAT captured a lower number of adult mosquitoes but collected a higher number of gravid mosquitoes than BGS [27] and in Guinea, gravid traps caught a similar number of gravid, but lower number of unfed *Aedes* [28]. The optimal trap for *Aedes* sampling isn't universal across the globe [29]. This may be attributed to the differences in *Aedes* ecology [30], and most importantly the social, economic and operational constraints of different countries. Therefore, it is necessary to assess the relative trapping efficiency of the traps from an ecological, economic and operational perspective including considerations for scalability. Previous reports have evaluated the trapping efficacy of various trap types on *Ae. aegypti* mosquitoes [16,20,27,31,32]. However, there is limited data from Tanzania regarding the trapping efficacy of the *Aedes* surveillance traps for dengue vector population monitoring. Given that Tanzania is among the nations impacted by the dengue virus, where all four dengue serotypes co-circulate [33–35]. It is crucial to pinpoint a cost-effective trap for monitoring dengue vectors. This study used modified GAT and AGO traps using local materials, developed to fit the social, economic and operation modality of Tanzania. GAT were lined with insecticide treated nets (ITN) and AGO with sticky board, enhanced with yeast or grass infusion and evaluated in

 doi:10.20944/preprints202404.0764.v1

reference to BGS as a 'standard' measure of mosquito density. The evaluation was conducted in the semi-field and field settings in Bagamoyo, Tanzania.

2. Materials and Methods

Study area

Five experiments were conducted in Bagamoyo, located at 70km north of Dar es Salaam, one of the fastest growing cities in Africa [36]. Bagamoyo experiences annual rainfall ranging between 800 - 1000mm, temperature between 22-33°C and relative humidity of 73% [37]. Trap optimisation was carried out in the semi-field system (SFS) [38] of the Ifakara Health Institute (IHI) in Ifakara Ambient Chamber Test (IACT) [39] and field experiments were conducted in two commercial premises (hotels) with high densities of *Aedes* mosquitoes.

Traps and attractant development

Gravid *Aedes* Trap (GAT)

A modified GAT [13,40] (Figure 1a) is made of 1) sixteen litres bucket covered with black cloth as a base that contained 3 litres of infusion with drainage holes drilled above 3 litres capacity to prevent the trap from overfilling; 2) a translucent inverted ten litres bucket lined with net; 3) black mosquito mesh placed between the translucent bucket and the base to prevent mosquitoes from reaching the infusion; and 4) a three litres bucket with the base removed and covered with black cloth as a mosquito entrance.

Autocidal Gravid Ovitrap (AGO)

A modified AGO [20] (Figure 1b) is made of 1) a ten litre black bucket as a base that contained 3 litres of infusion with drainage holes drilled above 3 litre capacity to prevent the trap from overfilling; 2) black mosquito mesh placed between the bottom of the trap entrance and the base to prevent mosquitoes from reaching the infusion; 3) a sticky board lining (Rentokil FICS mk1, Barrettine Environmental Health) the inner walls of 3-litres black bucket; 4) three litres black bucket with the base removed which served as a trap entrance; 5) a black lid with 120-150 holes of 3 cm placed at the top of the trap entrance to prevent debris from entering the trap.

Trap infusion

To increase the attractiveness of the gravid traps (GAT and AGO), two types of infusions were made of grass or yeast. **Grass infusion**: was made by mixing 72g of dry grass and 10g of baker's yeast (*Saccharomyces cerevisiae*) into 12 litres of tap water. **Yeast infusion**: was made by mixing 22g of baker's yeast with 12 litres of tap water. Each mixture was fermented for three days and shaken once a day.

Biogents Sentinel Trap (BGS)

The BGS (BG Sentinel 2 (BioGents, Regensburg, Germany) with the BG lure cartridge without carbon dioxide was used as a standard trap and proxy of mosquito density in this study (Figure 1c). The trap is powered by 12-volt battery and comprises of a white lid with a collapsible dark blue plastic container with a flexible metal frame. The BG lure cartridge, a combination of caproic acid, lactic acid, and ammonia, which mimics human odour and lasts for 3-6 months post-opening [29]. No additional carbon dioxide was used in the traps.

Figure 1. Mosquito traps a) Gravid *Aedes* trap (GAT) b) Autocidal Gravid Ovitrap (AGO) and c) Biogents Sentinel Trap (BGS).

Mosquitoes

All SFS experiments were conducted using nulliparous (aged 3-5 days) and gravid (aged 5-8 days) female *Ae. aegypti* mosquitoes (Bagamoyo strain, established in 2015). The mosquitoes were reared according to MR4 guidelines [41] at 27±2^{0}C temperature and 75±10% humidity. Larvae were fed with cat biscuits (Whiskas, South Africa) while adults were maintained with 10% w/v sugar solution *ad libitum*. For egg-laying, female adult mosquitoes were fed with cow blood through a membrane-feeding technique. Five to eight-day-old mosquitoes were selected from the cage and fed with cow blood. The blood-fed mosquitoes reached the gravid stage after 48h. Groups of 30 gravid mosquitoes were transferred into small cages and marked with fluorescent powder for easy differentiation from the nulliparous ones. Mosquitoes (30 nulliparous and 30 gravid) were left for 1 hour to acclimatize before releasing into the experimental IACT chamber.

Experimental design and procedure

Experiment 1: comparison of trapping efficacy of Gravid Aedes Trap (GAT) against Autocidal Gravid Ovitrap (AGO) in the SFS

From June to August of 2022 a 5 x 5 balanced Latin square design in five IACT chambers over 25 nights was conducted to evaluate the trapping efficacy of i) BGS trap baited with BG lure (standard trap), ii) GAT with yeast infusion iii) GAT with dry grass infusion, iv) AGO with yeast infusion, and v) AGO with dry grass infusion were deployed into each IACT chamber (Figure 2A). Thirty nulliparous and 30 gravid *Ae. aegypti* were released into each chamber at 09:00 hours. Twenty-four hours post the release, the traps were assessed for the presence of recaptured mosquitoes according to their life stage (nulliparous or gravid). The un-trapped mosquitoes in the IACT were collected using a Prokopack aspirator [40] by first collecting dead mosquitoes on the floor then followed by the alive ones on the net walls and roof. The infusions in each GAT and AGO were changed every two weeks while the traps were rotated between chambers on nightly basis.

Experiment 2: comparison of trapping efficacy of Gravid Aedes Trap (GAT) lined with insecticide-treated net in the SFS

In November 2022 a 4 x 4 balanced Latin square design using four IACT chambers for 16 nights was conducted to evaluate the efficacy of insecticide-treated nets (ITN) as a lining for locally made GAT using the following arms i) BGS trap baited with BG lure (standard trap), ii) GAT with permethrin treated net and yeast lure iii) GAT with bifenthrin treated net and yeast lure iv) GAT with untreated net (Safi net) (Figure 2B). Fifty 5-8 days old gravid *Ae. aegypti* mosquitoes released per chamber at 10:00 hours. Twenty-four hours post the release at, all mosquitoes from each I-ACT chamber and trap were collected as described in experiment 1. Traps were rotated between the chambers on a nightly basis.

Experiment 3: evaluation of trapping efficacy of Gravid Aedes Trap (GAT) against Autocidal Gravid Ovitrap (AGO) in the field setting

Between September to December 2022 a 5x5 Latin square design as described in experiment 1 was replicated two times in each of two hotels to give 50 nights of collection per hotel. At each study site five locations were selected and marked. Each of the five traps was evaluated in each of the five locations on each site (Figure 2C), by daily rotation to account for the influence of the location on mosquito density. The traps were set at 10:00 hours and assessed for the presence of trapped mosquitoes after 24 hours. Mosquitoes collected were transported to the laboratory for morphological species identification only.

Experiment 4: evaluation of the trapping efficacy of Gravid Aedes Trap (GAT) lined with bifenthrin net in the field setting

In December, 2022 a 3 x 3 Latin square design was performed over 9 days per study site, where three traps: 1) BGS with lure (positive control), 2) GAT augmented with bifenthrin and yeast 3) GAT augmented with untreated net (negative control) and yeast were deployed in three locations at 15 m apart at each of the two study sites (Figure 2D). The traps were deployed at 10:00 hours, and left for 24 hours before collecting the trapped mosquitoes. The captured mosquitoes were transported to the laboratory for morphological species identification only. The traps were rotated between locations daily in order to account for any bias in trapping that could be influenced by location.

Experiment 5: evaluation of the efficacy of four Gravid Aedes Trap (GAT) lined with bifenthrin net baited with yeast relative to one BGS (BG-Sentinel) trap in the field setting

Between October and November, 2023, four GAT traps and one BGS trap were deployed at 10:00 hours in five different locations and then left for 24 hours. The captured mosquitoes were retrieved from the traps and categorized according to their physiological stages (non-blood fed, blood-fed and gravid) (Figure 2E). Traps were stationed for one location for three days in a testing site, 15 m apart. Then after three days, the traps were rotated simultaneously to control for locational bias (for the BGS) following a 5 x 5 Latin square design for 30 days. After deployment, captured mosquitoes were transported to the laboratory for identification of species and physiological status. Data from the four GAT were pooled.

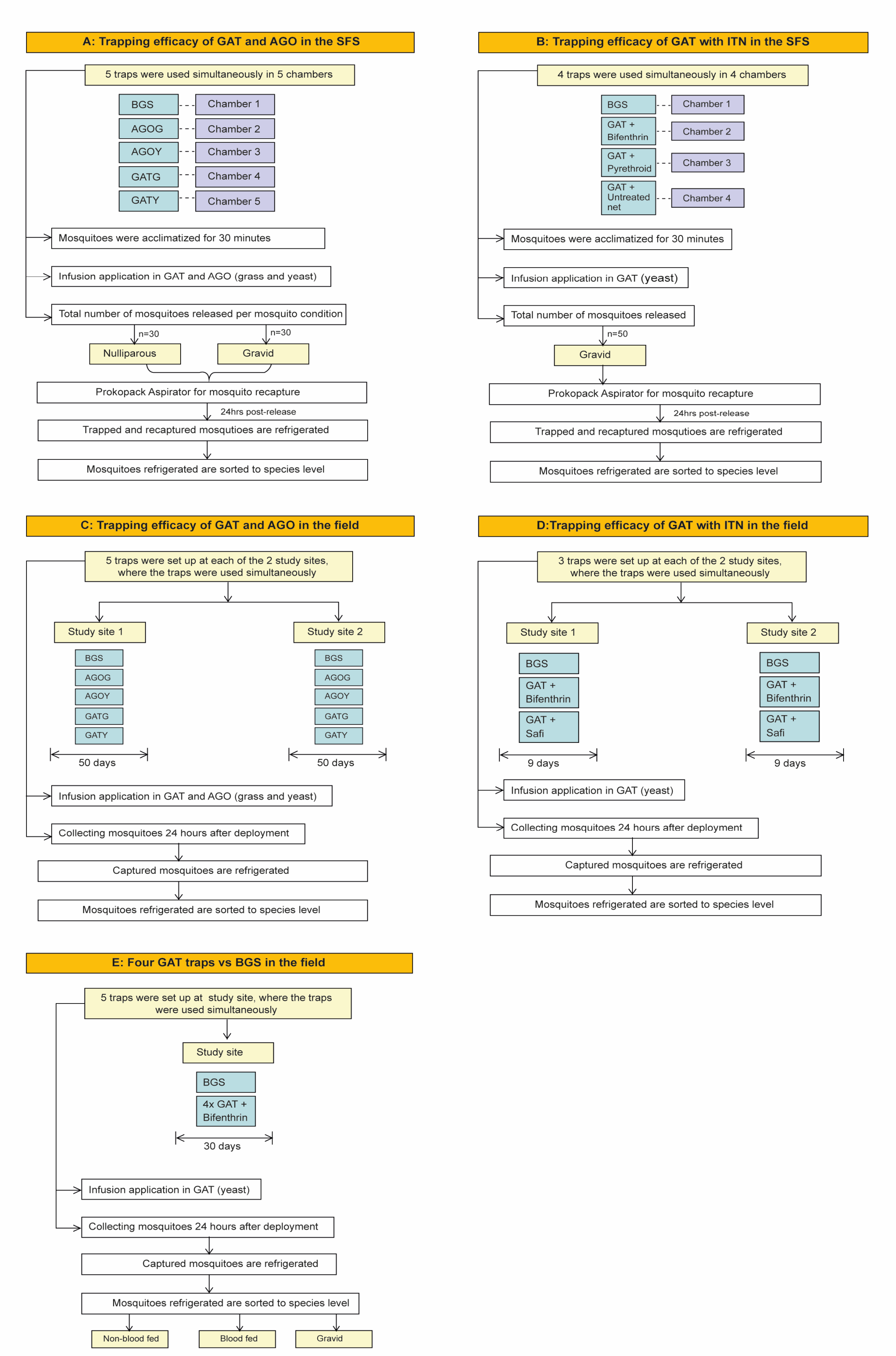

Figure 2. Study flow of experiments conducted in both SFS and field settings. In the SFS: A) 5 x 5 Latin square design in 5 chambers over 25 days. B) 4 x 4 Latin square design in 4 chambers over 16 days. In the field setting: C) 5 x 5 Latin square design was conducted in 5 locations and replicated twice over 50 days in two hotels. D) A 3 x 3 experiment was conducted in 3 locations over 9 days in each of two

hotels. E) A 5 x 5 Latin square experiment was conducted in 5 locations in the study site for over 30 days. "AGO with yeast" (AGOG), "AGO with grass infusion" (AGOY), "GAT with yeast" (GATY), "GAT with grass infusion," GATG.

Data management and statistical analysis

All data were collected into hardcopy and then double entred into Microsoft Excel to develop a dataset that were imported into STATA v.17 (Stata Corp, Texas, USA) [42] for analysis. Descriptive statistics were performed to estimate the percentage arithmetic mean with 95% confidence intervals (CI) of *Ae. aegypti* for each trap in SFS and geometric mean with 95% confidence intervals (CI) of *Ae. aegypti* for each trap in the field.

Semi-field experiments: Binomial logistic regression with mixed effects was performed to analyse the proportion of mosquitoes recaptured as the outcome. Trap types (BGS, AGO and GAT), lure (grass or yeast) and trap location (chamber) were categorical fixed effects and experimental day was a random effect as mosquito batches may vary. The same analysis was performed separately for nulliparous and gravid mosquitoes. Odds ratios (OR) with 95% CI were estimated.

Field experiments: Mixed effect negative binomial regressions were performed to compare the number of mosquitoes captured between the traps. Trap type, lure and sampling stations were categorical fixed effects and experimental day was a random effect to account for daily heterogeneity in mosquito densities. The same analysis was performed separately for non-blood fed, blood-fed and gravid mosquitoes. Incidence rate ratios (IRR) with 95% CI were estimated. Bland-altman plots were used to assess the agreement of captured female mosquitoes betweeen the BGS (standard) and GAT traps and to examine mosquito density dependence in trap performance.

3. Results

Experiment 1: comparison of trapping efficacy of Gravid Aedes Trap (GAT) and Autocidal Gravid Ovitrap (AGO) in the semi-field system

Of 7,612 mosquitoes released (both nulliparous and gravid) in the semi-field system (SFS), 66% (n=5042) were recaptured by the traps. Recapture was 70% (n=2,105) for the GAT and 56% (n=1,699) for AGO and 79% (n=2105) for BG trap (Table 1). The GAT with dry grass caught 62% (n=1077) and GAT with yeast infusion caught 57% (n=1028) of the released mosquitoes. The AGO with dry grass caught 49% (n=866) and AGO with yeast infusion caught 45% (n=833) of the released mosquitoes (Figure 3).

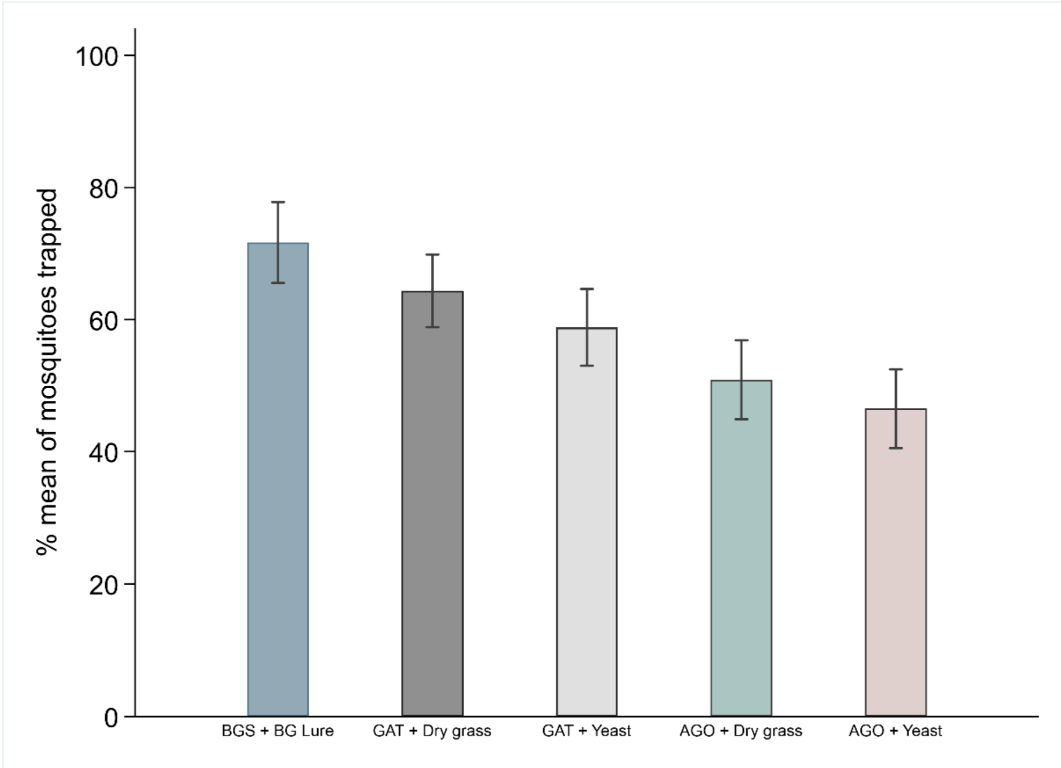

Figure 3. Percentage mean and 95% confidence interval (CI) of *Ae. aegypti* mosquitoes recaptured by three mosquito traps with their lure in the SFS.

The GAT with yeast recaptured an average of 10% fewer mosquitoes: 63% of nulliparous and 73% of gravid mosquitoes while the BGS recaptured an average of 73% nulliparous and 83% of gravid mosquitoes which were both statistically lower but comparable to the BGS.

The GAT had higher trapping efficacy than AGO for both nulliparous 65% vs 49% (OR=2.22, [95%CI: 1.90-2.61], p<0.001) and gravid mosquitoes 73% vs 64% (OR=1.67,[95%CI: 1.41-1.97], p<0.001).

When infusions used in the ovitraps (GAT and AGO) were compared, traps with yeast infusion recaptured a significantly lower proportion of nulliparous mosquitoes (OR=0.83 [95%CI: 0.71-0.97] p=0.018) compared to those traps with grass infusions (OR=1), and no significant difference against gravid mosquitoes (OR=0.91, [95%CI: 0.77-1.07], p=0.250) (Table 1).

The BGS had the highest trapping efficacy than any of the traps and lure combinations at trapping mosquitoes of both physiological stages in the SFS (Table 1). It recaptured more mosquitoes overall than GAT (OR=0.56, [95% CI: 0.48-0.66], p<0.001) and AGO traps (OR=0.30,[95% CI: 0.25-0.35], p<0.001), regardless of lure used in the gravid traps (Table 1).

The GAT with yeast recaptured an average of 10% fewer mosquitoes: 63% of nulliparous and 73% of gravid mosquitoes while the BGS recaptured an average of 73% nulliparous and 83% of gravid mosquitoes which were both statistically lower but comparable to the BGS at short distance (Table 1).

Table 1. Percentage means and odds ratio of nulliparous and gravid mosquitoes recaptured in semi-field system.

Trap	N	n	% Mean	OR (95%CI)	P-value	N	n	% Mean	OR (95%CI)	P-value
Overall										
BGS	1573	1238	78 (71, 85)	1						

GAT	3024	2105	69 (65, 74)	0.56 (0.48,0.66)	<0.001					
AGO	3015	1699	56 (51, 61)	0.30 (0.25,0.35)	<0.001					
	Nulliparous *Ae. aegypti*			**Gravid *Ae. aegypti***						
BGS + BG lure	761	557	73 (63,83)	1		812	681	83(73,92)	1	
GAT + Dry grass	758	519	68 (60,76)	0.79 (0.62,1.00)	0.048	751	558	74(68,81)	0.54 (0.41, 0.70)	<0.001
GAT + Yeast	761	480	63 (53,72)	0.59 (0.47, 0.75)	<0.001	754	548	73(65,80)	0.48 (0.37, 0.63)	<0.001
AGO + Dry grass	758	381	50 (41,59)	0.33 (0.26, 0.41)	<0.001	750	485	65(56,73)	0.32 (0.25, 0.41)	<0.001
AGO + Yeast	754	360	47(38,57)	0.29 (0.23, 0.36)	<0.001	753	473	63(53,72)	0.29 (0.23, 0.37)	<0.001
AGO Vs GAT										
AGO	1512	741	49 (42,55)	1		1503	958	64(57,71)	1	
GAT	1519	999	65 (59,72)	2.22 (1.90, 2.61)	<0.001	1505	1106	73(68,78)	1.67 (1.41, 1.97)	<0.001
Grass Vs Yeast										
Grass	1516	900	59(52,66)	1		1501	10	69(64,75)	1	

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Yeast	1515	840	55(48,62)	0.83 (0.71, 0.97)	0.018	1507	431021	68(61,74)	0.91 (0.77, 1.07)	0.250

N=number of mosquitoes released, n=number of mosquitoes recaptured, %mean = Percentage arithmetic mean of mosquitoes recaptured of those released, OR (95% CI) = Odds ratio with 95% Confidence interval.

Experiment 2: efficacy of Gravid Aedes Trap (GAT) lined with insecticide-treated net (ITN) against laboratory-reared Ae. aegypti in the semi-field system

A total of 3,498 mosquitoes were released and 62% (n=2,185) were recaptured. The recapture rate was substantially higher for both the GAT with a permethrin net (75%, n=700) and GAT with a bifenthrin net (68%, n=624) relative to the GAT with an untreated net (11%, n=92) . The BGS trap showed the highest recapture rate at 88% (n=769), (Table 2).

There was no significant difference in trapping efficacy between GAT with bifenthrin (OR=1) and the BGS OR=1.40, [95%CI: 0.89-2.21], p=0.144 as well as the GAT with permethrin net (69% vs 76%, OR=1.17, [95%CI: 0.85-1.61], p=0.345) in the SFS (Table 2).

Table 3. Percentage means and odds ratio of gravid female *Ae. aegypti* trapped in the semi-field system.

Trap	N	n	% Mean	OR (95% CI)	P- Value
GAT + Bifenthrin	907	624	69 (64, 74)	1	
GAT + Permethrin	927	700	76 (69, 82)	1.17 (0.85, 1.61)	0.345
GAT + Untreated	794	92	11 (9, 14)	0.02 (0.02, 0.03)	<0.001
BGS	870	769	89 (85, 92)	1.40 (0.89, 2.21)	0.144

N=number of mosquitoes released, n=Number of mosquitoes recaptured, %Mean = Percentage arithmetic mean of mosquitoes recaptured with 95% confidence interval, OR (95% CI) = Odds ratio with 95% Confidence interval.

Experiment 3: field evaluation of trapping effectiveness of Gravid Aedes Trap (GAT) and Autocidal Gravid Ovitrap (AGO)

A total number of 11,397 mosquitoes were trapped. Of these, 86.2% (n=9827) were *Culex quinquefasciatus*, 13.7% (n=1,565) were *Ae. aegypti* and 0.1% (n=5) were *Anopheles gambiae s.l.*

Among the captured *Ae. aegypti*, 83% (n=1,298) were female. Most female *Ae. aegypti* were caught by BGS (71%, n=926). Of the gravid traps, GAT collected more female *Ae. aegypti* (21%, n=266) than AGO (8%, n=106), (IRR=2.58, [95%CI: 1.90-3.50], p<0.001), (Table 3). The use of yeast infusion in the GAT and AGO, captured a significantly lower number of mosquitoes (IRR=0.72, [95%CI: 0.52-0.98], p=0.037) compared to those traps with dry grass infusions (OR=1), although the difference in the absolute numbers of mosquitoes captured was marginal <0.2 per trap day (Table 3).

When comparing the ovitraps with the standard trap (Table 3), GAT caught significantly fewer mosquitoes than the BGS when baited with either dry grass (IRR=0.16, [95%CI: 0.11-0.23], p<0.001) or yeast infusions (IRR=0.13, [95%CI: 0.09-0.19], p<0.001). The same trend was observed to AGO relative to BGS with dry grass (IRR=0.07, [95%CI: 0.05-0.11], p<0.001) and yeast infusion (IRR=0.04, [95%CI: 0.03-0.07], p<0.001).

The Bland-Altman plot showed that the BGS trap consistently captured a higher number of female mosquitoes compared to GAT with yeast infusion, with a greater difference at higher mosquito density. The mean difference was 8.07 and the limit of agreement varied from -6.19 to 22.33 (Supplementary Figure S1).

Table 2. Percentage means, and incidence rate ratio (IRR) of female *Ae. aegypti* mosquitoes captured in Bagamoyo.

Trap	N	%Mean	IRR (95%CI)	P-value
AGO vs GAT				
AGO	106	1.47 (1.30, 1.68)	1	
GAT	266	1.97 (1.72, 2.25)	2.58 (1.90, 3.50)	<0.001
Grass vs Yeast				
Grass	211	1.85 (1.63, 2.11)	1	
Yeast	161	1.66 (1.42, 1.94)	0.72 (0.52, 0.98)	0.037
BGS Vs Gravid traps				
BGS + BG lure	926	6.27 (4.84,8.14)	1	
GAT + Dry grass	147	2.16 (1.80, 2.60)	0.16 (0.11, 0.23)	<0.001
GAT + Yeast	119	1.77 (1.44, 2.18)	0.13 (0.09, 0.19)	<0.001
AGO + Dry grass	64	1.49 (1.27, 1.75)	0.07 (0.05, 0.11)	<0.001
AGO + Yeast	42	1.46 (1.17, 1.84)	0.04 (0.03, 0.07)	<0.001

N=Number of mosquitoes captured, %Mean = Percentage geometric mean of mosquitoes captured daily by trap with 95% Confidence interval, IRR (95% CI) = Incidence rate ratios with 95% Confidence interval.

Experiment 4: field evaluation of Gravid Aedes Trap (GAT) lined with insecticide-treated net (ITN) against wild Ae. aegypti

A total number of 2,868 mosquitoes were trapped. Of these, 88.8% (n=2548) were *Cx. quinquefasciatus*, 11.1% (n=318) were *Ae. aegypti* and 0.1% (n=2) were *An. gambiae s.l.* Of *Ae. aegypti*, 78% (n=247) were female mosquitoes.

The use of GAT lined with a bifenthrin net resulted in a greater capture of female *Ae. aegypti* mosquitoes (2 per day per trap) compared to GAT with an untreated net (1 per day pr trap) IRR=6.19, [95%CI: 2.41-15.92], p<0.001 (Table 4).

The BGS caught mean 9 of *Ae. aegypti* mosquitoes per trap day which was greater than GAT with bifenthrin (2 per trap per day) IRR= 6.83 [95% CI: 4.12 – 11.32], p<0.001 (Table 4).

Table 4. Percentage means and Incidence rate ratio (IRR) of female *Ae. aegypti* mosquitoes captured at commercial premises in Bagamoyo.

Trap	N	% Mean	IRR (95%CI)	P-value
GAT + Untreated Vs GAT + Bifenthrin				
GAT + Untreated	5	1 (0.7,2)	1	
GAT + Bifenthrin	31	2 (1,2)	6.19 (2.41, 15.92)	<0.001
GAT + Bifenthrin Vs BGS				
GAT + Bifenthrin	31	2 (1,2)	1	
GAT + Untreated	5	1 (0.7,2)	0.16 (0.06, 0.44)	<0.001
BGS	211	9 (6,14)	6.83 (4.12,11.32)	<0.001

N=Number of mosquitoes captured, % Mean (95%CI) = Percentage geometric mean of mosquitoes captured daily by trap with 95% confidence interval, IRR (95% CI) = Incidence rate ratios with 95% Confidence interval.

Experiment 5: Evaluation of 4x Gravid Aedes Traps (GAT) traps versus BGS in the field setting

A total of 3,416 mosquitoes were collected in the field setting. Of these, 69.9% (n=2,388) were *Cx. quinquefasciatus* and 30.1% (n=1,027) were *Ae. aegypti.* Among captured *Ae. aegypti* mosquitoes 83.9% (n=862) were female of which 717 were nulliparous, 122 were gravid and 23 were blood fed.

The four GAT traps with yeast infusion lined with bifenthrin net combined (4xGAT) caught 18% (n=158) while BGS caught 82% (n=704) of *Ae. aegypti* mosquitoes (Table 5). This was significantly lower than BGS trap IRR=5.79, [95% CI: 4.08-8.21], p<0.001.

When examined by physiological state, the 4x GATs recaptured fewer non-blood fed and blood fed mosquitoes than the BGS (IRR=8.53, [95% CI: 6.11-11.91], p<0.001) and (IRR=11.4, [95% CI: 3.2-40.8], p<0.001), respectively. In contrary, the 4x GATs with yeast infusion had similar capture rate of gravid mosquitoes with the BGS (IRR=0.82, [95% CI: 0.35-1.95], p=0.658) (Table 5).

The Bland-Altman plot showed that the BGS trap captured a higher number of female mosquitoes compared to 4x GATs traps with yeast infusion lined with bifenthrin net. The mean difference was 2.52 and the limit of agreement varied from -11.74 to 16.78 (Supplementary Figure S2). Density dependence was no longer seen when 4 gravid traps were used per compound.

Table 5. Percentage mean and Incidence rate ratio (IRR) of female *Ae. aegypti* mosquitoes captured at the commercial premises in Bagamoyo.

Trap	N	%Mean	IRR (95%CI)	P-value
Overall				
4x GAT + Yeast	158	1.8 (1.6, 2.1)	1	
BGS	704	11.6 (7.7, 17.3)	5.79 (4.08, 8.21)	<0.001
Gravid mosquitoes				
4x GAT + Yeast	64	1.6 (1.3, 1.9)	1	
BGS	58	2.7 (1.6, 4.6)	0.82 (0.35, 1.95)	0.658
Non-blood fed mosquitoes				
4x GAT + Yeast	90	1.4 (1.2, 1.6)	1	
BGS	627	11.01 (7.5, 16.2)	8.53 (6.11, 11.91)	<0.001
Blood-fed mosquitoes				
4x GAT + Yeast	4	1 (1, 1)	1	
BGS	19	1.4 (0.9, 2.1)	11.4 (3.2, 40.8)	<0.001

N=Number of mosquitoes captured, % Mean(95%CI) = Percentage geometric mean of mosquitoes captured daily by trap with 95% confidence interval, IRR (95% CI) = Incidence rate ratios with 95% Confidence interval.

Discussion

Understanding *Aedes* vector species composition, ecology and behaviour is a crucial prerequisite for prevention of arboviral diseases. Undoubtedly, effective control cannot be achieved without having vector sampling tools which are operationally feasible, efficacious,cost effective and technologically simple to operate. This study demonstrated that a locally produced and modified GAT was a suitable tool for capturing *Ae. aegypti*, the local dengue vector. The BGS was used as a standard indicator of mosquito densities as it is an efficacious tool for sampling and monitoring *Aedes* populations in the field setting [25,26,32]. While the modified GAT did not outcompete the BGS, it gave reliable data by collecting the same species with similar numbers of mosquitoes caught in each expriment for both trap types. Data agreed with similar studies comparing BGS and gravid traps in West Africa [28]. If deployed at scale, it may prove a useful means of urban dengue vector control as

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it is a lure and kill device that has good community acceptability [43] and efficacy demonstrated in a number of settings [44].

Both GAT with yeast and drygrass infusion caught gravid and nulliparous in the SFS therefore mosquitoes may have also been attracted to the dark humid traps as a resting site or by CO_2. This may be expored in further trap optimisation. Our study found that the traps baited with drygrass outperformed traps baited with yeast. However due to conveniences yeast was selected for further evaluation. This is evident in other studies that have shown the effectiveness of yeast baited traps and ovitraps at luring different mosquito species including *Aedes* [17,18,45]. While yeast was not as attractive as fermented grass, it was far simpler to use as bakers yeast is cheap, standardised and is widely available. Yeast derived CO_2 has shown to be effective at attracting nulliparous mosquitoes [46] and yeast improves the attraction gravid mosquitoes, likely as an indicator of food availability [47].

The use of fast-acting insecticide in *Ae. aegypti* mosquito control or surveillance tools is relevant in control of arboviral diseases such as dengue fever [40,48]. The use of treated long-lasting insecticide nets as killing agent for the traps such as GAT was useful in this study and others [12]. The technique exploits the advantage that ITN are widely available, durable and wash resistant [12]. However, mosquito insecticide resistance challenges the use of pyrethroid nets [49]. This study demonstrated that bifenthrin-treated nets used in the development of GAT had nearly equal bio-efficacy to permethrin lined within the GAT traps against laboratory-reared at the SFS. Bifenthrin is a pyrethroid insecticide which is less irritant than permethrin, has temperature tolerance and effective for both susceptible and pyrethroid resistant malaria vectors [50,51] due to it structure [52]. While *Ae. aegypti* is resistant to permethrin in Dar es Salaam [49] it is less likely to develop resistance to bifenthrin and therefore bifenthrin nets were used.

In the present study, the SFS data agreed with field data although the magnitude of difference between traps was greater in the field. The results demonstrated that there is a large difference between BGS and GAT trapping efficacy for nulliparous *Ae. aegypti* mosquitoes in the field while there was a smaller observed difference in trapping efficacy for gravid *Aedes* mosquitoes as the BGS contains kairomones for host seeking mosquitoes [53]. In the SFS there was no signficant difference since the traps were tested in a confined space increasing the probability of mosquitoes interracting with the traps. The observed findings concur with Eiras et al 2021 [23] that there was no statistical difference between BGS and GAT trapping efficacy in the absence of alternative breeding sites in the simulated outdoor environment. In the SFS, where competing kairomones are not present and the radius of attraction is as important since mosquitoes are confined in close proximity to the traps, data showed slight difference in recapture between the GAT and the BGS trap. In the field setting, the same direction of effect (relative proportion of mosquitoes recaptured) as the SFS experiment was clearly observable: BGS> GAT+grass > GAT+Yeast > AGO+grass > AGO+Yeast. However, the magnitude of the difference was greater: in the field, BGS trap captured nearly 8 times more *Aedes aegypti* mosquitoes than GAT. We therefore infer that the lower performance of the GAT trap in the field may be attributed to the presence of multiple breeding sites, as it was in competition with both natural and artificial existing breeding sites that were abundant in the testing area. A similar finding was observed in a comparison of oviposition attractants for *Ae. aegypti* in the SFS and field conducted in Kenya where direction of effect was similar but magnitude of effect was different in mosquito preference for different infusions [54].

This study also reports that GAT is more efficacious than AGO at capturing laboratory-reared and wild *Ae. aegypti* mosquitoes that is in alignment with another study in Florida [31]. Although GAT and AGO are lethal ovitraps that are both used for mosquito surveillance and control of *Aedes* mosquito species, in this study, we hypothesized that differences in the design and the size of the traps may have resulted in higher trapping in the GAT as has been observed in another study [55] although the amount of infusion was the same in all traps. The GAT is larger and had a more obvious entrance [56], and the addition of the ITN killed mosquitoes to enhance their retention and reduce predation by ants which is useful when using the traps for surveillance.

 doi:10.20944/preprints202404.0764.v1

The BGS demonstrated greater trapping efficacy of wild *Aedes* species than GAT and AGO in the current study as has also been observed in other studies [16,28]. The observed difference can be attributed to the fact that BGS targets host-seeking at distances up to 10 metres [57] through visual and olfactory cues, while both GAT and AGO attract gravid mosquitoes that need to lay eggs [23] also through olfactory and visual cues [58], but in an area where there were competing oviposition sites. Competing resources will influence both blood feeding and oviposition behaviour [59]. The BGS operates based on a counter flow principle that helps disperse attractant molecules to enhance the radius of mosquito detection and attraction. It sucks mosquitoes that are in proximity to the trap's lid with downstream airflow generated by a fan [26]. The GAT and AGO are passive traps which trap only mosquitoes that enter voluntarily into the trap [20,40].

The longer the GAT trap stayed outdoor at one location in the field setting, the more mosquitoes were captured. When GAT traps were stationed in one location in the testing area for three days, GAT traps collected similar number of gravid mosquitoes with BGS trap. Furthermore, GAT trapping performance increased when multiple traps were deployed and stationed at one location for three days in a field setting, as opposed to utilizing a single GAT trap that remained for only one day at one location. Both the BGS and the GAT caught similar mosquito densities in repeated tests and estimates of mosquito density were precise with both traps. Importantly, when four GAT were deployed, they showed no density dependence, therefore it appears that they may be used interchangeably with the BGS. Further large-scale longitudinal assessment is ongoing to verify these data under operational settings.

Despite the relatively greater catch of the BGS trap compared to GAT in the field, its cost and operational requirements (such as the need for electricity) hinder its adaptation for use in mass trapping programs for surveillance or control. This limitation is particularly evident in low and middle-income countries with limited health budgets and in areas without regular access to electricity. The GAT is substantially less expensive (less than $8(single GAT) while BGS is $150) [16] and doesn’t need power or electricity to operate, captures the same species as BGS [60] therefore it can be considered as an alternative for use in mosquito mass trapping programs particularly in countries will low resources like Tanzania. Additionally, GAT is selective in attracting gravid mosquitos [32], which is advantageous for dengue virus surveillance, as gravid mosquitoes will have taken a blood meal and are therefore more likely to be dengue virus positive than nulliparous mosquitoes. Ovitraps have been successfully used for dengue monitoring in Malaysia [61] and Singapore [62] and is predictive of dengue cases in Indonesia [63]. There are several studies from Africa also showing that ovitraps may be useful for dengue vector monitoring from Reunion [64], Cameroon [65], Ghana [66] and Tanzania [67].

The GAT trap demonstrated good trapping efficacy in both the SFS, and at the field setting, especially when multiple traps were deployed. The SFS proved useful for trap optimisation as results in both SFS studies were reflective of the same studies repeated in field settings. The SFS is useful for these kinds of experiments as mosquito density, species and physiological status can be selected so data is more cost effective to collect and larger sample sizes improve precision of estimates. Nevertheless, field studies of optimised traps are still warranted due to the interplay of mosquitoes and traps over space and competition with other kairomones that may affect results.

This study examined the efficacy of mosquito traps for *Ae. aegypti* monitoring and surveillance in outdoor commercial settings at one location, more research is needed to investigate if the modified traps work as well in different locations in urban setting. To get a better understanding of how well do the trap works, further studies such as longitudinal surveys are recommended.

Conclusion

This study addresses the gap in the need to improve dengue vector surveillance for epidemiologic investigations using locally modified traps that are less costly yet efficacious. GAT with yeast infusion lined with bifenthrin net is a potential trap for *Ae. aegypti* surveillance for dengue control based on convenience in making it. Althogh, it had lower performance than BGS in the field but when four traps were deployed the trapping efficacy increased and there was no density dependence in mosquito catches between the two methods. Further larger longitudinal studies are recommended to assess the GAT trap performance for operational use.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, **Additional File 1: Figure S1.** Bland-Altman plot showing the mean difference (y-axis) plotted against the difference and the (x-axis) average value between the BGS and GAT + Yeast infusion to capture adult female *Ae. aegypti* mosquitoes in the field setting. **Additional File 2: Figure S2.** Bland-Altman plot showing the mean difference (y-axis) plotted against the difference and the (x-axis) average value between the BGS and four GAT + Yeast infusion to capture adult female *Ae. aegypti* mosquitoes in the field setting.

Author Contributions: JJM, FCT, JM and SJM conceptualized and designed the experiments. JJM, MSM, JBM and JM collected data. JBM created the study flow and conducted image capturing. JJM, FCT, SJM and OGO analysed data. JJM, MSM and FCT wrote the paper. JJM, MSM, JM, JBM OGO, SJM and FCT reviewed and contributed to the final draft. All authors read and approved the final manuscript.

Funding: This study is supported by VCPTU, and IHI Training and Capacity Building Department.

Data Availability and Materials: Data will be made available on request from the authors.

Acknowledgement: We would like to thank the Vector Control Product Test Unit (VCPTU) and Training department of Ifakara Health Institute for funding this research. VKA Polymers for the provision of bifenthrin treated net. Also, we would like to extend our gratitude to the hotel owners who granted us permission to conduct this study on their premises. Furthermore, we are grateful to Dr. Seth Irish for his professional advice and support regarding mosquito sampling and the VCPTU team for their support during the time of research implementation.

Conflict of Interests: JJS, MSM, JM, JBM, OGO, SJM, and FCT conduct evaluations of vector control products for a number of vector control product manufacturers. The other authors declare that they have no competing interests.

Ethical Approval and Consent to Participate: Written informed consent forms were provided to the Hotel owners for their voluntary permission to work on their premises. The National Institute of Medical Research (NIMR) (NIMR/HQ/R.8a/Vol.IX/4179) and the Institute Review Board of IHI (IHI/IRB/No:19-2022) approved the study.

Consent for publication: Permission to publish has been granted by Tanzania National Institute of Medical Research (NIMR) NIMR/HQ/P.12/VOL.XXXV/158.

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