Bio-Efficacy of Insecticides Used for Vector Control in Busia and Tororo Districts, Eastern Uganda

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Abstract

Reductions in malaria burden worldwide coincides with the massive scale-up of malaria treatment and prevention measures, of which vector control is the major component, particularly in SSA. The key vector control interventions in Africa including Uganda, rely heavily on utilisation of long-lasting insecticidal nets (LLINs) and insecticide-based indoor residual spraying (IRS). This study assessed the bio-efficacy of insecticides used for vector control in LLINs and IRS in Busia and Tororo. Samples of Anopheles mosquito larvae were collected from various breeding grounds before rearing them in an insectary. Emerged adults were observed under a dissecting microscope and identified using standardized morphological keys after respective bioassays on used nets and sprayed walls. Independent two sample T-test was used to test for significant differences in the mean malaria vectors diversity, mean variations, mortalities by net and wall type. Results showed that mean mortalities of Anopheles mosquitoes to used brands of nets from Busia (9.86±11.35) and Tororo (9.64 ±11.12) varied insignificantly (t=0.119, p=0.906). A disaggregated analysis for each net used revealed that, the DAWA plus 2.0 registered a highest mean mortality of mosquitoes, followed by PermaNet 3.0+PBO, Olyset and PermaNet 2.0 respectively. Fludora fusion revealed highest mortalities on plastered painted wall, followed by Brick plain then mud/wattle walls at all times. The trend is similar for Actellic, but unlike Fludora fusion, Actellic exhibited mortalities lower than the 80% threshold for all the wall types and T-test (T-test P values<0.001) indicated Actellic efficacy to be significantly lower than that of Fludora fusion on all the three wall substrates. This study has shown that vector behaviour, biology and physiology need consistent monitoring and surveillance for further entomological characterisation.

Keywords: Bimodal, Bio-Efficacy, Sentinel Areas, Oscillation, Plasmodium Vectors

Background

High malaria transmission rates in Sub-Saharan Africa (SSA) are attributed to the continuous presence of effective and competent Plasmodium vectors, Anopheles gambiae complex and the Anopheles funestus group which play a key role in transmitting the most dangerous malaria parasite species Plasmodium falciparum. The core essentials that make these species highly effective Plasmodium vectors are their preference for humans as a source of blood combined with indoor resting habits and exploitation of breeding habitats created by human activities. Information of these vector innate feeding preferences and resting habits when combined with data on host accessibility, precisely forecasts the intensity of Plasmodium transmission in Uganda is perennial with two peaks in March to May and September to December consistent to the rainfall seasons, favoring mosquitoes to breed and also during which the vector biting density increases [1-8].

Reductions in malaria burden worldwide coincides with the massive scale-up of malaria treatment and prevention measures, of which vector control is the major component, particularly in SSA [9]. The core Plasmodium vector control interventions in Africa including Uganda, rely heavily on utilisation of long-lasting insecticide nets (LLINs) and indoor residual spraying (IRS) which are insecticide-based relying on four chemical classes: organochlo-
rines, pyrethroids, carbamates and organophosphates. Whereas 14 formulations belonging to these classes are approved by the World Health Organization (WHO) for use in IRS, only pyrethroids are approved for use in LLINs because of their low human toxicity, repellent properties and rapid knock down and killing effect thus the community is protected from malaria (Helinski et al., 2015). Busia district uses only LLINs while Tororo district uses both LLINs and IRS in battling against Plasmodium vectors. Although LLINs and IRS have contributed significantly to reduced clinical malaria incidences due to their efficiency in some sceneries, there is paucity of evidence regarding their effectiveness following their deployment in a given region [9-13].

However, the advances made in ascertaining their efficiency are fragile due to the decreased effectiveness of the interventions partially as a result of vectors’ lowered responsiveness towards the insecticides used in the control. Vector species have not only evaded exposure, but also changing of feeding from late to early biting, shifting from endophagic to exophagic, and avoiding resting on LLINs or the walls sprayed with insecticides [9, 14, 15]. Also, these vector control approaches have been noted to be ineffective against exophagic vectors and increased resistance to pyrethroids [3, 16-18]. This is more pertinent given the fact that elsewhere mosquitoes have become difficult to be controlled due to their change in biology, physiology and behavior, leading to decreased efficiency of vector-control interventions [3, 14, 17].

Despite the deployed vector control approaches, malaria status of Busia and Tororo districts is particularly high as the area is characterised by numerous and recurrent bushes, persistent stagnant water around homesteads, long rain seasons, low altitude and high temperatures. Busia and Tororo also accommodates two important border points of Busia and Malaba along the famous Trans-Africa highway, characterised by heavy traffic of people and merchandise from, through and to many other countries. All these factors favour the proliferation of Anopheles mosquitoes and reproduction of the parasites within them [2]. Additionally, limited surveillance and monitoring of mosquitoes for behavioral adaptations and changes in vector species’ composition is the common challenge [19]. Together with the fact that there is also oscillation of mosquito vectors and the human-plasmodium carriers within the area, it could explain why amidst the intensified vector control measures, the regions still experience active Plasmodium transmission, especially during the peak of malaria vector breeding season that spans the summer months [20]. Therefore, there was a need to assess the bio-efficacy of insecticides used for vector control in the two methods, so as to ascertain the effectiveness of the different control frameworks in Busia and Tororo in order to implicate the role of variabilities in the two districts.

Materials and Methods

Description of Study Area

The study was conducted in two purposively selected districts of Busia and Tororo in Eastern Uganda as depicted in Figure 1, regarded as among the sentinel regions sharing eco-epidemiological features and characterised strata of high malaria transmission with the presence of mosquito species and higher insecticide pressure [18, 21].

Figure 1: Location of Anopheles Mosquito Larvae Sampling Sites in the two Districts
Busia and Tororo districts have a stable perennial malaria transmission with malaria prevalence rates ranging from 39 to 68% (Okia et al., 2018a). Busia district is located to the southeast and lies between 0° 46′ N, 34° 0′ E of Uganda near Kenya boarder and bordering Tororo district to the north [22]. Tororo town is approximately 10 km west of the town of Malaba at the border between Uganda and Kenya, located 205 km northeast of Kampala and lies between 0° 45′ N, 34° 5′ E (Latitude: 0.692780; Longitude: 34.181655) in Eastern Uganda and lies at an average elevation of 1,278 m above sea level.

**Climate**
The rainfall patterns of Busia and Tororo is bimodal, with the first rainy season (short rains) extending from March to May and a longer rainy season extending from August to November, with annual rainfall ranging from 1520 to 1800 mm. The mean annual temperature ranges from 16.20°C to 28.70°C. Average annual precipitation is 1,494 millimeters and relative humidity ranging from 52% to 89% [23].

**Vegetation**
Riverine zones and lowlands of these districts grow rice, altitude forests, savannah mosaic, swamp, wooded savannah and grass savannah [23]. During rainy months of the year, rice gardens flood and hold water for long periods, providing potential breeding sites for Anopheles mosquitoes [2].

**Drainage**
Busia District accommodates wetlands and rivers covering a total area of 57.173sq. km, while open water, Lake Victoria, cover 36.88sq. km. The most significant permanent swamp systems are along River Lumboka to the west, forming part of the boundary with Bugiri District, and River Malaba to the north bordering Tororo District. Tororo District accommodates river Malaba, moist Combretum savanna, wetlands and swamps [23].

**Research Design**
This study took a mixed methods research approach including efficacy testing of Anopheles mosquitoes to insecticides used and retrospective data analysis for malaria cases in the study area for the past nine years (from 2012 to 2020).

**Sampling Design, Sampling Points and Sample Collection**
Cluster randomized selection of sites as by was used to collect larvae in Busitema, Sikuda, Masafu Dabani, Masaba and Buhehe for Busia, and Nagongera, Mulanda, Petta, TC Kisoko, Merikit and Molo for Tororo. Sampling from stagnant water around homesteads, nearby swamps and rice paddies following a method outlined by [10, 24]. A sample size of 500 mosquito larvae was collected from each and every sub county. In the retrospective data used, semi-structured questionnaires were administered in the form of interview to the household representative while obtaining information about the type, frequency of vector control application and nature of insecticides used. The sample size obtained from the representative of each homestead was determined basing on the approach by as shown below:

\[ n = \left( \frac{Z^2 \cdot PQ}{\alpha^2} \right) \]

Where: \( n \) = the sample number; \( Z = 1.96 \) (at 95% confidence level); \( P \) = the expected proportion of community households with information needed (15%); \( Q = 1-P \); \( \alpha \) is the margin of sampling error (5%).

Implying, \( n = \frac{1.96^2 \times 0.15(1-0.15)}{0.05^2} \)

\[ n = 195.9216 \] households which approximates to about 200 households

Thus, the estimated sample size for this study was at least 196 households [25]. Therefore, 200 households were sampled.

**Mosquito Collection**
*Anopheles* mosquito larvae were collected from seasons of February to June 2021 using scoops from various breeding grounds then reared in an insectary at 25 OC and 80 % humidity. Water in the larval container was refreshed every 2–3 days. Pupae were harvested in a plastic cup and placed within a cage (bottom 27 cm × 27 cm, top 25 cm × 25 cm, height 27 cm), in which a cotton wool soaked in 10% glucose solution was placed in a 50 ml glass flask. The cage was kept on a table in a well-ventilated insectary room. The glucose solution was changed every 2-3 days according to the mosquito rearing protocol [24].

**Malaria Vectors Diversity and Abundance Detection**
Emerged adults from the reared larvae were morphologically identified using simplified standard morphological keys adopted from Gillies & Coetzee (1987) to deduce the species present (Kabbale et al., 2016). Whilst to check and to improve on the precision of the morphological identification, emerged adult mosquitoes were observed under a dissecting microscope and identified using standardized morphological keys as by Coetzee (2020).

**Efficacy Testing on Used LLINs (PermaNet 2.0, DAWA Plus 2.0, Olyset and PermaNet 3.0)**
Using an aspirator, the reared 4-6 days non-blood fed *Anopheles gambiae* s.l mosquitoes in replicates of four, were fed into used nets fitted with bioassay cones in a designated test laboratory [26]. Four bioassay cones lined with self-adhesive tape were fixed on the net lined on a paperboard to mimic the placement of the net on bed for the assay. The mosquitoes were left in the exposure cones for 3 minutes, after which the number of knocked down (KD) mosquitoes were scored. All the exposed mosquitoes were then gently transferred to holding paper cups for 60 minutes, mortality was recorded and provided with 10% sugar solution soaked on cotton wool pads placed on top of the paper cups covered with an untreated net. Final mortality was recorded at 24 hour based on the status of mosquitoes as no longer standing, immobile or gliding along the curvature of the paper cups [26].

**Efficacy of IRS Insecticides**
In Tororo district, cone bio-assays were conducted on 3 different
sprayed wall types (plastered painted, Plain brick and mud/wattle) [27]. For each wall type, four replicates of 10 non-blood fed 4-6 day old *Anopheles* mosquitoes were exposed. Cones lined with self-adhesive tape were fixed on the sprayed walls for the assay, after which an aspirator was used to introduce the mosquitoes into the cones. The cones were placed at heights of 0.5 m, 1.0 m, and 1.5 m above the floor. The mosquitoes were left in the exposure cones for 30 minutes, after which the number of knocked down (KD) mosquitoes were scored. Final mortality was recorded at intervals of 24 hours after exposure based on the status of mosquitoes as no longer standing, immobile or gliding along the curvature of the paper cups (Hakizimana *et al*., 2016). Non-insecticides’ impregnated walls were used as the control.

**Bio-Efficacy Cone Assays**

To determine the bio-efficacy of the insecticides in LLINs and IRS as malaria vector control approaches used in Busia and Tororo, cone assay data (KD and mortalities) recorded in Excel sheets were exported to SPSS version 2.5.0 where an independent sample T-test was performed to test for the mean differences in mosquito mortality across the two districts. This test was repeated by net type in both districts, and wall spray type in Tororo district. One-Way Analysis of Variance was used to test for significant differences in mean variations mortalities by type of net at a 95% confidence interval. The analysis was repeated using the disaggregation-by-cases command for different levels of exposure to nets in both districts. For wall spray in Tororo, the data were first disaggregated by 2-cases that is type of insecticide and wall type before a One-Way ANOVA was run.

**Results**

**Malaria Vectors Diversity and Abundance**

Table 1 show malaria mosquito diversity and abundance results. A total of 5,180 individuals of *Anopheles* mosquitoes emerged from the reared larvae, of which 3,675 (71%) samples were *Anopheles gambiae* s.l collected from both districts and 1,505 (29%) were *Anopheles funestus*.

<table>
<thead>
<tr>
<th>Species</th>
<th>February Busia</th>
<th>February Tororo</th>
<th>March Busia</th>
<th>March Tororo</th>
<th>April Busia</th>
<th>April Tororo</th>
<th>May Busia</th>
<th>May Tororo</th>
<th>June Busia</th>
<th>June Tororo</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. gambiae S.l</em></td>
<td>199</td>
<td>254</td>
<td>313</td>
<td>447</td>
<td>428</td>
<td>222</td>
<td>519</td>
<td>308</td>
<td>566</td>
<td>419</td>
</tr>
<tr>
<td><em>A. funestus</em></td>
<td>189</td>
<td>0</td>
<td>227</td>
<td>0</td>
<td>323</td>
<td>0</td>
<td>361</td>
<td>0</td>
<td>405</td>
<td>0</td>
</tr>
</tbody>
</table>

According to Table 2, there was no significant difference between Busia and Tororo An. Gambiae abundances. The mean of An.Funestus of Busia and Tororo varied significantly (t=13.081, p<0.005).

**Table 2: Variation in Malaria Vectors Diversity and sample means between Districts**

<table>
<thead>
<tr>
<th>Malaria Vectors</th>
<th>District</th>
<th>Sample Means</th>
<th>t-test</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. gambiae s.l</em></td>
<td>Busia</td>
<td>58.33</td>
<td>2.32</td>
<td>0.159</td>
</tr>
<tr>
<td></td>
<td>Tororo</td>
<td>98.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. funestus</em></td>
<td>Busia</td>
<td>32.83</td>
<td>13.08</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Tororo</td>
<td>00.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interestingly, all the An. Funestus collected in this study came from Busia district shown in Figure 2. Of the *An. gambiae* samples collected from Busia district, 7.5% came from Masafu, 7.7% from Sikuda,……etc., and of those collected from Tororo, 8% came from Nagongera, 7.7% came from Petta. Of the *An. funestus* samples collected from Busia, Buhehe had the highest percentage (23.4%), followed by Sikuda (20%), Masaba (19%), Masafu (17.7%), Dabani (12%) and Busitema (7.8%) respectively.
percentage (23.4%), followed by Sikuda (20%), Masaba (19%), Masafu (17.7%), Dabani (12%) and Busitema (7.8%) respectively.

Figure 2: Malaria Vectors Diversity and Abundance across Data Collection Sites in Busia and Tororo Districts

**Mosquito Mortalities in LLINs Cone Assays**

According to Table 3 and Fig.3, out of four brands of LLINs tested for insecticides efficacy, 99% mortality was recorded for the deltamethrin 80 mg/m² impregnated DAWA plus 2.0 LLINs in Busia and 98% in Tororo. PermaNet 3.0 +PBO (84 mg/m²) and Olyset (525 mg/m²) permethrin respectively recorded mortalities of 94% and 88% in Busia, and mortalities of 92% and 84% in Tororo. PermaNet 2.0 recorded 68% mortality at 55 mg/m² permethrin in Busia, and mortalities of 62% in Tororo. According to Table 3, DAWA plus 2.0 (t=0.027; p >0.05), PermaNet 3.0 +PBO (t=0.100), Olyset (t=0.069) and PermaNet 2.0 (t=0.055).

<table>
<thead>
<tr>
<th>Type of net</th>
<th>District</th>
<th>Chemical type</th>
<th>Chemical conc. (mg/m²)</th>
<th>Mean mortality (%)</th>
<th>Std. Deviation</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAWA Plus 2.0</td>
<td>Busia</td>
<td>Deltamethrin</td>
<td>80</td>
<td>99</td>
<td>0.6</td>
<td>0.027</td>
<td>0.979</td>
</tr>
<tr>
<td></td>
<td>Tororo</td>
<td>Deltamethrin</td>
<td>80</td>
<td>98</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olyset</td>
<td>Busia</td>
<td>Permethrin</td>
<td>525</td>
<td>88</td>
<td>2</td>
<td>0.069</td>
<td>0.945</td>
</tr>
<tr>
<td></td>
<td>Tororo</td>
<td>Permethrin</td>
<td>525</td>
<td>84</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PermaNet 2.0</td>
<td>Busia</td>
<td>Deltamethrin</td>
<td>55</td>
<td>94</td>
<td>1.7</td>
<td>0.100</td>
<td>0.921</td>
</tr>
<tr>
<td></td>
<td>Tororo</td>
<td>Deltamethrin</td>
<td>55</td>
<td>93</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PermaNet 3.0+PBO</td>
<td>Busia</td>
<td>Permethrin</td>
<td>84</td>
<td>68</td>
<td>3</td>
<td>0.055</td>
<td>0.956</td>
</tr>
<tr>
<td></td>
<td>Tororo</td>
<td>Permethrin</td>
<td>84</td>
<td>62</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As can be observed in Figure 3, the DAWA plus 2.0 registered a highest mean mortality of mosquitoes in both districts, followed by PermaNet 3.0 +PBO, Olyset and PermaNet 2.0 in that order; and there was no significant difference in the efficacy of the LLINs between study districts.
On disaggregating the data by type of net and time, the results revealed none of the mosquitoes died in all LLINs at the exposure time of 3 minutes. At holding time (knockdown) of 60 minutes, higher mortalities were registered by DAWA plus 2.0, followed by PermaNet 3.0, Olyset and PermaNet 2.0 respectively. However, the difference in mortalities caused by the LLINs were not significant (F=0.752, p=0.537). Under recovery time (24 hours), higher mosquito mortalities were further registered by DAWA plus 2.0, followed by PermaNet 3.0+PBO, Olyset and PermaNet 2.0, respectively. However, the difference in mortalities caused by the LLINs were still not significant (F=0.628, P=0.601) as shown in Table 4.

**Table 4: Variation in mosquito mortalities post exposure to LLINs**

<table>
<thead>
<tr>
<th>Time</th>
<th>Net Type</th>
<th>Mean Mortalities</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Minimum</th>
<th>Maximum</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure time</td>
<td>DAWA Plus 2.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Olyset</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PermaNet 2.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PermaNet 3.0+PBO</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>0.0</strong></td>
<td><strong>0.0</strong></td>
<td><strong>0.0</strong></td>
<td><strong>0.0</strong></td>
<td><strong>0.0</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holding time</td>
<td>DAWA Plus 2.0</td>
<td>16.3</td>
<td>12.0</td>
<td>3.5</td>
<td>0.0</td>
<td>25.0</td>
<td>0.752</td>
<td>0.537</td>
</tr>
<tr>
<td>jpeg</td>
<td>Olyset</td>
<td>15.6</td>
<td>11.5</td>
<td>3.3</td>
<td>0.0</td>
<td>24.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>jpeg</td>
<td>PermaNet 2.0</td>
<td>10.4</td>
<td>7.9</td>
<td>2.3</td>
<td>0.0</td>
<td>18.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>jpeg</td>
<td>PermaNet 3.0+PBO</td>
<td>15.7</td>
<td>11.6</td>
<td>3.3</td>
<td>0.0</td>
<td>25.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>14.5</strong></td>
<td><strong>10.8</strong></td>
<td><strong>1.6</strong></td>
<td><strong>0.0</strong></td>
<td><strong>25.0</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery time</td>
<td>DAWA Plus 2.0</td>
<td>16.4</td>
<td>12.1</td>
<td>3.5</td>
<td>0.0</td>
<td>25.0</td>
<td>0.628</td>
<td>0.601</td>
</tr>
<tr>
<td>jpeg</td>
<td>Olyset</td>
<td>15.8</td>
<td>11.7</td>
<td>3.4</td>
<td>0.0</td>
<td>25.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>jpeg</td>
<td>PermaNet 2.0</td>
<td>11.0</td>
<td>8.2</td>
<td>2.4</td>
<td>0.0</td>
<td>18.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mosquito Mortalities Post Exposure to Treated Walls in Tororo District

Figure 4 and 5 show mortalities for Actellic® 300 CS and Fludora fusion on three wall types respectively. Fludora fusion revealed highest mortalities on plastered painted wall, followed by Brick plain then mud/wattle walls at all times.

The trend is similar for Actellic, but unlike Fludora fusion, Actellic exhibited mortalities lower than the 80% threshold for all the wall types and T-test (T-test P values<0.001; Table 5) indicated its efficacy to be significantly lower than that of Fludora fusion on all the three wall substrates.
The concentration of deltamethrin in DAWA plus 2.0 (80 mg/m²) is consistent to DAWA plus 2.0 versus Olyset. The differential 2.0 compared to PermaNet 3.0 respectively. Similar explanation than permethrin explains the improved bioefficacy of DAWA plus relative superior performance of deltamethrin active ingredient and bio-efficacy in killing the mosquitoes over time [30-32]. The be credited to the insecticides‘ active ingredients residual effect used in the two districts in deterring Anopheles mosquitoes could served for DAWA plus 2.0 net compared to other brands of nets plus having the highest mortalities. The higher mortalities ob served for DAWA plus 2.0 net compared to other brands of nets used in the two districts in deterring Anopheles mosquitoes could be credited to the insecticides‘ active ingredients residual effect and bio-efficacy in killing the mosquitoes over time [30-32]. The relative superior performance of deltamethrin active ingredient than permethrin explains the improved bioefficacy of DAWA plus 2.0 compared to PermaNet 3.0 respectively. Similar explanation is consistent to DAWA plus 2.0 versus Olyset. The differential concentration of deltamethrin in DAWA plus 2.0 (80 mg/m²) is responsible for its relatively better performance compared to PermaNet 2.0 (55 mg/m²) by [30]. Similar studies by on comparisons between synergised LLIN and non-synergised LLIN reflects the same phenomena to this study whereby PermaNet 3.0 performed significantly better than PermaNet 2.0 [12].

The efficiency of LLINs relies on: the biology and behaviour of mosquito vectors based on their biting and resting behaviour as well as their susceptibility status to insecticides the insecticides selected as per active ingredient, bio-efficacy over time and durability [13, 31, 32]. Though the greater the overlap in activity time between mosquitoes biting and resting indoors and people being indoors, but not under bed nets could suggest why high malaria status in the region is still recorded (Ekoko et al., 2019).

Comparative effectiveness evaluations using local vector populations such as presented in this study provide valuable data to in form selection of appropriate interventions, this is pertinent to the consistent optimal bio-efficacy of DAWA plus 2.0 indicating that this net represents a viable option for areas with pyrethroid-resis tant Anopheles species [7, 30].

### Bio-efficacy of Actellic® 300CS and Fludora Fusion IRS

The bio-efficacy of plastered painted walls performing best regardless of insecticide type when compared to brick plain and mud/wattle walls has been observed in other studies and could be attributed to the oil emulsions combined with paint in the plastered wall enabling it to retain the chemical for a longer period of time hence higher residual activity [27, 31]. The effectiveness of IRS relies on: the biology and behavior of mosquito vectors based on their biting and resting behavior as well as their susceptibility status to insecticides the insecticides selected based on residual bio-efficacy over time [27, 32]. There is substantial variation in the duration of action: induced mortality, inhibited blood-feeding between same studies conducted in Ethiopia by with the same product. Some of these differences are attributed to procedural differences such as wall-type though others will reflect true differences in the behaviors and susceptibility of local mosquito populations [10, 33, 34].

The recent consistent change of insecticide type from bendiocarb to Actellic® 300CS and to Fludora fusion in Tororo district, means that for the first-time multiple non-parathyroid IRS products are available with different modes of action this is pertinent to their long lasting residual activity that achieve broadly equivalent reductions in malaria burden across Africa [28, 35, 36]. Similar studies in Zanzibar by demonstrated bendiocarb to have a shorter residual life span on sprayed surfaces of two to three months when compared to Actellic® 300CS and Fludora fusion, which have a
Conclusions
Anopheles gambiae sensu lato is still predominant in both districts despite of employment of two different control strategies, and in addition Anopheles funestus was caught in different areas of Bussia district. This suggests that the control strategies in place are not sufficient. More strategies are required to combat the problem. Both LLINs and IRS proved to have effect on Anopheles mosquitoes, but catching high numbers of endophilic mosquitoes suggest that the impact of LLINs and IRS on the primary malaria vectors (Anopheles gambiae s.l and Anopheles funestus) may be affected by change of behaviour of these mosquito populations. This leads to the conclusion that the current vector control interventions are effective against Plasmodium vectors, but will not lead to elimination of the disease unless additional tools are employed.

Recommendations
Molecular study should be carried out in order to distinguish the specific species in the Anopheles gambiae complex and Anopheles funestus group responsible for the Plasmodium mediation in the study area. This baseline characterization will provide a background of insecticide resistance mechanisms in mosquito populations in the different clusters, to enable effective management of insecticide resistance and at the same time facilitate continued vector control efforts.

Further studies are needed to quantify the individual contribution of each method when LLINs and IRS are deployed in combination. Additionally, to characterize the impact of long-lasting insecticidal nets and indoor residual sprays on vector density, behavior and species composition for accurate epidemiological modelling.

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Authors Contribution Statement
F.C conceived the study, collected data for research, analysed the data and manuscript writing
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Ethical considerations
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Competing Interests
The Authors declares that they have no competing interests in terms of financial or non-financial status.

Data Availability
The Mosquito Larvae Collection
The larvae were collected from the study area and reared in the laboratory. Respective bioassays were undertaken on used sleeping nets and indoor residual sprays. After bio-efficiency tests they were stereoscopically dissected and morphologically identified under dissecting microscope.

I would like to ask you some questions regarding plasmodium vector control in your area. I am specifically asking about the type of control intervention you practice. The information collected from this interview will assist the decision makers with proper planning. Participation in this interview is voluntary. You may answer questions that you only desire, stop at any time or decline the interview. If you go on with the interview, the results captured in this interview will be treated confidentially. We will not disclose your name or details at any one moment and expect the interview to last 20-35 minutes. Thank you for sparing your valuable time to answer this questionnaire.

References
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