

Susceptibility of *Anopheles* Mosquitoes to Insecticides Used In Busia and Tororo Districts, Eastern Uganda

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Abstract

This study assessed the susceptibility of *Anopheles* mosquitoes to LLINs and IRS as vector control interventions used in Tororo and Busia district. Samples of *Anopheles* mosquito larvae were collected from various breeding grounds and reared in an insectary. Emerged adults were dissected under a stereoscopic microscope and identified using standardized morphological keys after respective bioassays on used LLINs and sprayed walls. A One-Way Analysis of Variance (ANOVA) was used to test for significant differences in the mean composition of mosquito species across the study area, mean variations, mortalities by net and wall type. Results showed that the *Anopheles gambiae sensu lato* revealed a higher mean of 78.33 ± 41.52 , compared to *Anopheles funestus* (16.42 ± 24.87) which was lonely found in Busia district. Out of four brands of nets tested for insecticides susceptibility, 99% mortality was recorded for the insecticide concentration of 80 mg/m^2 deltamethrin impregnated DAWA plus 2.0 nets. PermaNet 3.0 and Olyset recorded mortality rate of 95% and 93% at chemical concentrations of 50 mg/m^2 and 525 mg/m^2 permethrin respectively. PermaNet 2.0 recorded 67% mortality at 55 mg/m^2 permethrin. The average number of *Anopheles* mosquitoes were more susceptible to 12.5 mg/m^2 of Fludora fusion impregnated onto plastered painted walls at a rate of 100% as compared to the other types of walls after 2 weeks (6.86 ± 4.07) and 1 month of chemical spray (6.69 ± 4.01) varied insignificantly ($t = 0.175$, $P = 0.861$). Meanwhile, a disaggregated results by time of spray (T_0 and T_1) and exposure periods, showed that plastered painted wall, had higher mortalities followed by Brick Plain and Mud/Wattle wall substrates, respectively. This study has shown that vector behavior, biology and physiology need consistent monitoring and surveillance for further entomological characterization and the need for coming up with new vector control interventions.

1.0 Background

High malaria transmission rates in Sub-Saharan Africa (SSA) are attributed to the continuous presence of effective and competent *Plasmodium* vectors (Ekoko et al., 2019), *Anopheles gambiae* complex and the *Anopheles funestus* group which play a key role in transmitting the most dangerous malaria parasite species *Plasmodium falciparum* (Oguttu et al., 2017; Musiime et al., 2019). The core essentials that make these species highly effective *Plasmodium* vectors are their preference for humans as a source of blood (Sherrard-smith et al., 2019), combined with indoor resting habits (Kabbale et al., 2016), and exploitation of breeding habitats created by human activities (Gunathilaka et al., 2015; Keven, 2015). Information of these vector innate feeding preferences and resting habits when combined with data on host accessibility, precisely forecasts the intensity of *Plasmodium* transmission (Raouf et al., 2017). *Plasmodium* transmission in Uganda is perennial with two peaks in March to May and September to December (Oguttu et al., 2017), consistent to the rainfall seasons, favouring mosquitoes to breed and also during which the vector biting density increases (Raouf et al., 2017).

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 (Kleinschmidt et al., 2015). 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 (Deressa et al., 2016; Raouf et al., 2017; Antonio-nkondjio et al., 2018; Musiime et al., 2019; Rek et al., 2020), relying on four chemical classes: organochlorines, 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 (Kenea et al., 2016) 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 (Katureebe et al., 2016; Loha et al., 2019).

However, the advances made in ascertaining their efficiency are fragile due to the decreased effectiveness of the interventions (Ojuka et al., 2015; Nankabirwa et al., 2019), 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 (Kenea et al., 2016; Musiime et al., 2019). Also, these vector control approaches have been noted to be ineffective against exophagic vectors and increased resistance to pyrethroids (Yadouleton et al., 2010; Mnzava et al., 2015; Hakizimana et al., 2016; Musiime et al., 2019). This is more pertinent given the fact that elsewhere mosquitoes have become difficult to be controlled due to their change in biology, physiology and behaviour, leading to decreased efficiency of vector-control interventions (Mnzava et al., 2015; Ojuka et al., 2015; Musiime et al., 2019).

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 boarder 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 favours the proliferation of *Anopheles* mosquitoes and reproduction of the parasites within them (Oguttu et al., 2017). Additionally, limited surveillance and monitoring of mosquitoes for behavioural adaptations and changes in vector species' composition is the common challenge (Katureebe et al., 2016). 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 (Ssempiira et al., 2017).

Therefore, there was a need to assess susceptibility of *Anopheles* mosquitoes to insecticides used for vector control in the two methods, so as to ascertain their effectiveness in Busia and Tororo in order to implicate the role of variabilities of the different control frameworks in the two districts.

2.0 Materials And Methods

2.1 Description of Study Area

The study was conducted in two purposively selected districts of Busia and Tororo in Eastern Uganda depicted in Fig. 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 (Hakizimana et al., 2016; Tchouakui et al., 2021).

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 (Githinji et al., 2020). Tororo town is approximately 10 kms 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.

2.2 Research Design

This study used descriptive survey in the households whereby, semi-structured questionnaires were administered in household representative while obtaining information about the type, frequency of vector control application and nature of chemicals used in controlling *Plasmodium* vectors. The sample size obtained from the representative of each homestead was determined basing on the approach by Easterby-Smith et al., (2013) as shown below:

$$n = \left(\frac{Z^2 PQ}{\alpha^2} \right)$$

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

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

$n = 195.9216$ households which approximates to about 200 households

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

2.3 Sampling Design, Sampling Points and Sample Collection

Cluster randomized selection of sites as by Deressa et al.,(2016) was used to collect larvae in the selected sub-counties of the districts. Sampling from stagnant water around homesteads, nearby swamps and rice paddies following a method outlined by Das *et al.*,(2007). Twelve sub counties and 16 households were selected randomly among those using two or more than one interventions simultaneously. A sample size of 100 mosquito larvae was collected from each and every sub county.

2.4 Mosquito Collection

Anopheles mosquito larvae were collected using scoopers from various breeding grounds then reared in an insectary at 25 °C 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 50ml 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 (Das et al., 2007).

2.5 Species Composition Detection

Emerged adults from the reared larvae were first 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 dissected under a stereoscopic microscope and identified using standardized morphological keys as by Coetzee (2020).

2.6 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 25 *Anopheles* mosquitoes in replicates of four, were fed into used nets fitted with bioassay cones in a designated test laboratory (Staedke et al., 2019). 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 knocked down (kd) mosquitoes were scored at 3 minutes immediately after the exposure period, at which time all mosquitoes were gently transferred to holding paper cups for 1 hour, mortality was recorded and provided with 10% sugar solution soaked on cotton wool pads placed on top of the paper cups covered with net. Final mortality was recorded at 24 hours holding time based on the status of mosquitoes as no longer standing, immobile or gliding along the curvature of the paper cups (Staedke et al., 2019).

2.7 Efficacy Testing on IRS (Plastered painted, Brick plain and Mud/wattle walls)

The reared 4–6 days non-blood fed 10 *Anopheles* mosquitoes in replicates of four, were exposed to walls sprayed with 12.5 ug/bottle Fludora Fusion (deltamethrin + clothianidin) using bioassay cones (Pmi & Project, 2019). Bioassay cones were placed at heights of 0.5 m, 1.0 m, and 1.5 m above the floor. Cones lined with self-adhesive tape were fixed on the sprayed walls for the assay. The knocked down (kd) mosquitoes were scored at 30 minutes immediately after the exposure period, at which time all mosquitoes were gently transferred to holding paper cups for 30 minutes, mortality was recorded and provided with 10% sugar solution soaked on cotton wool pads placed on top of the paper cups covered with net. Final mortality was recorded at 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).

2.8 Statistical Analysis

To identify the *Anopheles* species composition of Busia and Tororo districts, the emerged adult mosquitoes from the reared larvae were morphologically identified using a dissecting microscope after subjecting to various susceptibility assays. The total tallies of respective *Anopheles* species composition were recorded using Microsoft Excel sheet as per the district and across larval collection zones. The data were then exported to Statistical Package for Social Sciences (SPSS) version 2.5.0. A One-Way Analysis of Variance test was carried out to test for significant differences in the mean composition of mosquito species at 95% confidence

interval. Whilst determining normality and homogeneity of variance, a Post hoc test using Turkey's was performed. To identify whether *Anopheles* species composition varied across the districts, an independent sample T-test was run in SPSS.

To determine insecticide susceptibility of *Anopheles* mosquitoes to LLINs and IRS as vector control tools used in Busia and Tororo, emerged adult *Anopheles* mosquitoes collected across larval collection zones of the districts, were exposed to four brands of used nets impregnated with different concentrations of pyrethroids used in the study area and three types of wall substrates of two weeks and one month after spray. Mortalities were recorded in Microsoft Excel based on exposure type, exposure time, holding time, and recovery time for each type of net. This was done separately for each district. The data 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 using the split by cases command for each net type in both districts and wall spray 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 split-by-cases command for different levels of exposure to nets in both districts. For wall spray in Tororo, the data were first split by 2-cases that is weeks of spray, wall type and exposure type before a One-Way ANOVA was run.

3.0 Authors Contribution Statement

F.C designed the study, collected data for research, analysed the data and manuscript writing

J.K and A.E supported in designing the study, data analysis and manuscript writing

F.K supported with the manuscript write up

All the authors reviewed the article

3.1 Ethical considerations

Clearance for this research was sought from the College of Veterinary Medicine, Animal Resources and Biosecurity (SBLS/REC/21/00018a), and the Makerere University School of Biosecurity, Biotechnological and Laboratory Sciences (SBLS 2021). During the process, I would make them aware of what the research is about and the objectives. This would help to erase doubts, know benefits that may be possible through involving them like information, dissemination of master thesis to the district authorities who may use the information while planning related activities on malaria control strategies and risks of unpleasant episodes during meeting for local people. All this informed consent made the informants to be knowledgeable about the whole research process, hence making them be at ease during interviews and the entire research process.

I also ensured ethics of anonymity and confidentiality by not exposing the identity of all those who were involved in the research process to the outside people and public and avoid the attribution of comments to identified participants and in the presentations. This was done by ensuring consciousness with every information either in words, video and photograph that was to be given by the informants. This was to ensure that nobody other than the researcher can access all the information.

4.0 Results

4.1 *Anopheles* Species Composition

According to Fig. 2, a total of 1,145 individuals of *Anopheles* mosquitoes that emerged from the reared larvae, 940 (82%) samples were *Anopheles gambiae sensu lato* collected from both districts, 197 (17%) were *Anopheles funestus* from Busia district and 1% were other *Anopheles* species unidentified; for both districts. *A. gambiae s.l* adults were more than *A. funestus* across data collection zones.

There was a significant difference in species composition on a general scale in the study area ($F = 24.79$, $p < 0.05$). *A. gambiae s.l* revealed a higher mean of 78.33 ± 41.52 , compared to *A. funestus* (16.42 ± 24.87) and other *Anopheles* species (0.67 ± 0.98) (Table 1).

Table 1
Species Composition on a General Scale in the Study Area

	N	Mean	Std. Deviation	Std. Error	F	Sig.
<i>A. gambiae s.l</i>	12	78.3333	41.52400	11.98695	24.794	0.000
<i>A. funestus</i>	12	16.4167	26.87499	7.75814		
Other <i>Anopheles</i>	12	0.6667	0.98473	0.28427		
Total	36	31.8056	43.87568	7.31261		

The Turkey's multiple comparisons test revealed significantly higher differences in the mean values of *A. gambiae s.l* than *A. funestus* and other *Anopheles* in Busia region than in Tororo ($p < 0.05$) as shown in Table 2.

Table 2
Turkey's HSD Multiple Comparisons of Species Composition in the Study Area

(I) Spp	J (Spp)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
<i>A. gambiae s.l</i>	<i>A. funestus</i>	61.91667*	11.66066	0.000	33.3038	90.5295
	Other <i>Anopheles</i>	77.66667*	11.66066	0.000	49.0538	106.2795
<i>A. funestus</i>	<i>A. gambiae s.l</i>	-61.91667*	11.66066	0.000	-90.5295	-33.3038
	Other <i>Anopheles</i>	15.75000	11.66066	0.378	-12.8629	44.3629
Other <i>Anopheles</i>	<i>A. gambiae s.l</i>	-77.66667*	11.66066	0.000	-106.2795	-49.0538
	<i>A. funestus</i>	-15.75000	11.66066	0.378	-44.3629	12.8629

*. The mean difference is significant at the 0.05 level.

According to Table 3, the mean of *Anopheles gambiae s.l* and other *Anopheles* species across the districts varied insignificantly ($t = 3.22, p = 0.159$ and $t = 0.238, p = 0.636$). The mean of *Anopheles funestus* of Busia and Tororo varied significantly ($t = 13.081, p < 0.005$).

Table 3
Mean Variations of Species Composition across Districts

Mosquito spp	District	N	Mean	Std. Deviation	Std. Error Mean	t-test	Sig.
<i>A. gambiae s.l</i>	Busia	6	58.33	26.01	10.62	2.322	0.159
	Tororo	6	98.33	46.44	18.96		
<i>A. funestus</i>	Busia	6	32.83	30.70	12.53	13.081	0.003
	Tororo	6	0.00	0.00	0.00		
Other <i>Anopheles</i>	Busia	6	0.50	0.84	0.34	0.238	0.636
	Tororo	6	0.83	1.17	0.48		

3.2 Mortality rates on used nets (LLINs)

According to Fig. 3, out of four brands of nets tested for insecticides susceptibility, 99% mortality was recorded for the insecticide concentration of 80 mg/m² deltamethrin impregnated DAWA plus 2.0 nets. PermaNet 3.0 and Olyset recorded mortality rate of 95% and 93% at chemical concentrations of 50 mg/m² and 525 mg/m² permethrin respectively. PermaNet 2.0 recorded 67% mortality at 55 mg/m² permethrin.

According to Table 4, disaggregated analysis for each net used revealed that, the DAWA plus 2.0 registered a highest mean mortality of mosquitos, followed by PermaNet 3.0, Olyset and PermaNet 2.0 respectively. However, these were non-significant in the mean mortalities for each of the nets under consideration ($p > 0.05$).

Table 4
Mean Variations of the most Effective Net in the two Districts

Type of net	District	N	Mean Mortality	Std. Deviation	Std. Error Mean	t-value	p-value
DAWA Plus 2.0	Busia	18	10.9	12.6	3.0	0.027	0.979
	Tororo	18	10.8	12.5	2.9		
Olyset	Busia	18	10.6	12.2	2.9	0.069	0.945
	Tororo	18	10.3	11.9	2.8		
PermaNet 2.0	Busia	18	7.3	8.5	2.0	0.100	0.921
	Tororo	18	7.0	8.2	1.9		
PermaNet 3.0	Busia	18	10.6	12.2	2.9	0.055	0.956
	Tororo	18	10.4	12.0	2.8		

On disaggregating the data by type of net and exposure time, the results revealed none of the mosquitoes died in all nets at an exposure time of 3 minutes. At holding time 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 nets 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, Olyset and PermaNet 2.0, respectively. However, the difference in mortalities caused by the nets were still not significant ($F = 0.628, P = 0.601$) as shown in Table 5.

Table 5

Mean Variations in the Type of Net that is more effective & at which Level of *Anopheles* Mosquitoes Exposure

Time Net Type		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F	p-value
Exposure time (3mins)	DAWA Plus 2.0	12	0.0	0.0	0.0	0.0	0.0		
	Olyset	12	0.0	0.0	0.0	0.0	0.0		
	PermaNet 2.0	12	0.0	0.0	0.0	0.0	0.0		
	PermaNet 3.0	12	0.0	0.0	0.0	0.0	0.0		
	Total	48	0.0	0.0	0.0	0.0	0.0		
Holding time (60mins)	DAWA Plus 2.0	12	16.3	12.0	3.5	0.0	25.0	0.752	0.537
	Olyset	12	15.6	11.5	3.3	0.0	24.0		
	PermaNet 2.0	12	10.4	7.9	2.3	0.0	18.0		
	PermaNet 3.0	12	15.7	11.6	3.3	0.0	25.0		
	Total	48	14.5	10.8	1.6	0.0	25.0		
Recovery time (24 hrs)	DAWA Plus 2.0	12	16.4	12.1	3.5	0.0	25.0	0.628	0.601
	Olyset	12	15.8	11.7	3.4	0.0	25.0		
	PermaNet 2.0	12	11.0	8.2	2.4	0.0	18.0		
	PermaNet 3.0	12	15.8	11.7	3.4	0.0	24.0		
	Total	48	14.8	10.9	1.6	0.0	25.0		

3.3 Mortality Rates on Sprayed Walls (Tororo District)

Figure 4 demonstrates that out of three wall substrates, *Anopheles* mosquitoes were more susceptible to 12.5 mg/m² of Fludora fusion impregnated onto plastered painted walls at a rate of 100% as compared to the other types of walls.

When data were disaggregated by time of spray and exposure periods, the results for both two weeks and one month duration of spray revealed higher mortalities on plastered painted wall, followed by Brick Plain and Mud/Wattle wall substrates, respectively. However, the mean differences for 30 mins exposure, 30 mins holding period and 24 hours recovery time, were not significantly different ($p > 0.05$) as depicted in Table 6.

Table 6

Mean Variations in mean When Exposed to Wall Substrates, Duration of Chemical Spray and Exposure Type

Duration	Exposure Type	Wall Type	N	Mean	Std. Deviation	Maximum	F	p-value
Two	30 mins	Brick Plain	4	6.50	4.359	9		
		Mud/Wattle	4	6.50	4.359	9	.004	.996
		Plastered Painted	4	6.75	4.573	10		
		Total	12	6.58	4.010	10		
	30 mins	Brick Plain	4	7.00	4.690	10		
		Mud/Wattle	4	6.50	4.359	9	.027	.973
		Plastered Painted	4	7.25	4.856	10		
		Total	12	6.92	4.209	10		
	24 hours	Brick Plain	4	7.25	4.856	10		
		Mud/Wattle	4	6.50	4.359	9	.048	.953
		Plastered Painted	4	7.50	5.000	10		
		Total	12	7.08	4.316	10		
One Month	30 mins	Brick Plain	4	6.75	4.573	10		
		Mud/Wattle	4	6.00	4.082	9	.038	.962
		Plastered Painted	4	6.75	4.573	10		
		Total	12	6.50	4.011	10		
	30 mins	Brick Plain	4	7.00	4.690	10		
		Mud/Wattle	4	5.75	3.862	8	.128	.881
		Plastered Painted	4	7.25	4.856	10		
		Total	12	6.67	4.119	10		
	24 hours	Brick Plain	4	7.00	4.690	10		
		Mud/Wattle	4	6.25	4.193	9	.074	.930
		Plastered Painted	4	7.50	5.000	10		
		Total	12	6.92	4.231	10		

5.0 Discussion

5.1 *Anopheles* Species Composition

The predominance of *Anopheles gambiae sensu lato* as shown in Table 1 & Fig. 2, could be attributed to the receptive lentic aquatic habitats including swamps and rice paddies, which flood and hold water for long periods during rainy months. These habitats are crucial for mosquito dynamics where many of their important life cycle processes take place (Gunathilaka et al., 2015).

The spectacular abundance of *Anopheles gambiae s.l* for Merikit as indicated in Fig. 2, could be explained by the prevailing breeding grounds of stand still small swamps that were not witnessed in other larval collection zones of Tororo district.

The absence of *Anopheles funestus* in Tororo district depicted in Fig. 2 could be explained by the complementary effectiveness of the vector control interventions of LLINs & IRS under use in this region. The synergistic control strategy of LLINs plus IRS could have led to a shift in mosquito vector dynamics, whereby the most susceptible to a specific vector-control measure becomes less common (Musiiime et al., 2019). Similar studies by Abong'o et al. (2020) conducted in Migori Kenya reflects the same whereby, LLINs and IRS reduced indoor *Anopheles* densities, shifts in vector species composition, changes in the time and location of mosquito biting, and changes in host selection, and increases in early exophily. However, this could not rule out the fact that there could be other prevailing ecological or human factors in Busia district responsible for their proliferation such as increased permanent breeding sites. The *A. funestus* that dominated in Busia district is known to breed all year round and prefer permanent, stagnant water bodies, while *Anopheles gambiae sensu lato* breed in temporary human created water bodies including rice paddies, pools, puddles, construction sites and hoof prints which were more prevalent in Tororo than Busia district (Kabbale et al., 2013; 2016; Oguttu et al., 2017).

In addition, the LLINs and IRS have also been associated with changes in sympatric *Anopheles* species composition. In Uganda, Kenya and elsewhere, sustained vector control has not only resulted in reductions in transmission intensity, but also changes in *Anopheles* species composition, their behaviour, biology and density (Mawejje et al., 2021)

5.2 Efficacy Testing on Used Nets (LLINs)

The efficiency of DAWA plus 2.0 net compared to other brands of nets used in the two districts in deterring *Anopheles* mosquitoes depicted in Fig. 3, could be credited to the insecticides' selected active ingredients durability and bio-efficacy in killing the mosquitoes over time (Okia et al., 2013; Opiyo & Paaijmans, 2020; Tangena et al., 2020). 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 Okia et al. (2013). Similar studies by Staedke et al. (2020) 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.

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 (Kenea et al., 2016), the insecticides selected as per active ingredient, bio-efficacy over time and durability (Opiyo & Paaijmans, 2020; Tangena et al., 2020). 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 inform selection of appropriate interventions, this is pertinent to the consistent optimal bioefficacy of DAWA plus 2.0 indicating that this net represents a viable option for areas with pyrethroid-resistant *Anopheles* species (Okia et al., 2013; Raouf et al., 2017).

5.3 Efficacy Testing on IRS

The bio-efficacy of plastered painted walls performing best when compared to brick plain and mud/wattle walls (Pmi & Project, 2019), could be credited 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 (Opiyo & Paaijmans, 2020).

The effectiveness of IRS relies on: the biology and behaviour of mosquito vectors based on their biting and resting behaviour as well as their susceptibility status to insecticides (Tangena et al., 2020), the insecticides selected based on residual bio-efficacy over time (Opiyo & Paaijmans, 2020). There is substantial variation in the duration of action: induced mortality, inhibited blood-feeding between same study conducted in Ethiopia by Deressa et al. (2016) with the same product. Some of these differences are attributed to procedural differences such as wall-type (Russell et al., 2013), though others will reflect true differences in the behaviours and susceptibility of local mosquito populations (Sherrard-smith et al., 2018).

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-pyrethroid IRS products are available with different modes of action (Abong'o et al., 2020a), this is pertinent to their long lasting residual activity that achieve broadly equivalent reductions in malaria burden across Africa (Abong'o et al., 2020b; Kleinschmidt et al., 2018). Similar studies in Zanzibar by Haji et al., (2015) 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 longer residual life efficiency of up to one year when used in mosaics.

6.0 Conclusions

The predominantly abundant species recorded in this study were *Anopheles gambiae sensu lato*. This species has developed resistance to insecticides as well as change in its behaviour and biology which poses special attribute promoting *Plasmodium* transmission. The presence of *Anopheles funestus* in Busia and not in Tororo district is attributed to the synergistic *Plasmodium* vector control strategies played by LLINs & IRS which led to dynamic species shift. For optimal results larval source management using chemical or microbial larvicides,

combined with environmental management, could be used to improve control, especially in areas of high transmission.

Indoor residual chemical sprays on walls and DAWA plus 2.0 LLIN net proved to be more efficient in deterring *Anopheles* mosquitoes. These findings suggest that the impact of LLINs and IRS on the primary malaria vectors (*Anopheles gambiae s.l* and *Anopheles funestus*) may be affected by the behaviour of these mosquito populations. Current vector control interventions are effective against *Plasmodium* vectors, but will not lead to elimination of the disease unless additional tools are included as supplementary interventions.

7.0 Recommendations

The absence of *Anopheles funestus* in Tororo district is probably due to effectiveness of the vector control interventions attributed to IRS under use in the region, while *Anopheles gambiae sensu lato* being the abundant strain in the study area is due to their change biology and behaviour enabling their proliferation in presence of insecticides. However, further studies are needed in order to monitor the impact of long-lasting insecticidal nets and indoor residual sprays on vector density, behaviour and composition to help supplement epidemiological data.

Supplementing LLINs with IRS were effective in deterring mosquitoes. Although, in order to understand the combined benefits of LLINs and IRS, *Plasmodium* transmission dynamics models predicting their effectiveness should be availed as these tools in combination give seemingly contradictory results. This is pertinent given the fact that the area still experience high malaria cases.

Molecular study should be carried out in order to distinguish the specific species in the *Anopheles gambiae* complex responsible for the *Plasmodium* mediation in the study area. This baseline characterisation 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.

Declarations

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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.

Questionnaire for household interviews about vector control interventions (LLINs and IRS)

Dear respondent,

My name is Faith Chemutai. I am a Master's Degree student from Makerere University carrying out an academic study on **'Susceptibility of *Anopheles* Mosquitoes to Insecticides Used in Tororo and Busia Districts, Eastern Uganda'**

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.

Signature..... (If willing to participate)

If they are not willing to participate in the interview, state their reason.

.....

ID no.:

Sub County:Parish:Village:

References

1. Abong'o, B., Gimnig, J. E., Torr, S. J., Longman, B., Omoke, D., Muchoki, M., ter Kuile, F., Ochomo, E., Munga, S., Samuels, A. M., Njagi, K., Maas, J., Perry, R. T., Fornadel, C., Donnelly, M. J., & Oxborough, R. M. (2020a). Impact of indoor residual spraying with pirimiphos-methyl (Actellic 300CS) on entomological indicators of transmission and malaria case burden in Migori County, western Kenya. *Scientific Reports*, *10*(1), 1–14. <https://doi.org/10.1038/s41598-020-61350-2>

2. Abong'o, B., Gimnig, J. E., Torr, S. J., Longman, B., Omoke, D., Muchoki, M., ter Kuile, F., Ochomo, E., Munga, S., Samuels, A. M., Njagi, K., Maas, J., Perry, R. T., Fornadel, C., Donnelly, M. J., & Oxborough, R. M. (2020b). Impact of indoor residual spraying with pirimiphos-methyl (Actellic 300CS) on entomological indicators of transmission and malaria case burden in Migori County, western Kenya. *Scientific Reports*, *10*(1). <https://doi.org/10.1038/s41598-020-61350-2>
3. Antonio-nkondjio, C., Sandjo, N. N., Awono-ambene, P., & Wondji, C. S. (2018). *Implementing a larviciding efficacy or effectiveness control intervention against malaria vectors: key parameters for success*. 1–12. <https://doi.org/10.1186/s13071-018-2627-9>
4. Back, R. (2010). *1. The burden of malaria Malaria kills children in*. 17–23.
5. Coetzee, M. (2020). Key to the females of Afrotropical Anopheles mosquitoes (Diptera: Culicidae). *Malaria Journal*, 1–20. <https://doi.org/10.1186/s12936-020-3144-9>
6. Collins, E., Vaselli, N. M., Sylla, M., Beavogui, A. H., Orsborne, J., Lawrence, G., Wiegand, R. E., Irish, S. R., Walker, T., & Messenger, L. A. (2019). The relationship between insecticide resistance, mosquito age and malaria prevalence in *Anopheles gambiae* s. l. from Guinea. *Scientific Reports, November 2018*, 1–12. <https://doi.org/10.1038/s41598-019-45261-5>
7. Control, M., Swiss, E., & Winter, T. P. H. (2016). *Vector Control Strategies and Successes ... and challenges*.
8. Das, S., Garver, L., & Dimopoulos, G. (2007). *Protocol for Mosquito Rearing (A. gambiae)*. <https://doi.org/10.3791/221>
9. Deressa, W., Loha, E., Balkew, M., Hailu, A., Gari, T., Kenea, O., Overgaard, H. J., Gebremichael, T., Robberstad, B., & Lindtjørn, B. (2016). Combining long-lasting insecticidal nets and indoor residual spraying for malaria prevention in Ethiopia: study protocol for a cluster randomized controlled trial. *Trials*, 1–16. <https://doi.org/10.1186/s13063-016-1154-2>
10. Easterby-Smith, M., Thrope, R., Jackson, P., Thorpe, R., & Jackson, P. (2013). Management research. In *Sage*. <https://doi.org/10.1097/01.brs.0000207258.80129.03>
11. Ekoko, W. E., Ambene, P. A., Bigoga, J., Mandeng, S., Piameu, M., Nvondo, N., Toto, J. C., Nwane, P., Patchoke, S., Mbakop, L. R., Binyang, J. A., Donnelly, M., Kleinschmidt, I., Knox, T., & Mbida, A. M. (2019). Patterns of anopheline feeding / resting behaviour and Plasmodium infections in North Cameroon, 2011–2014 : implications for malaria control. *Parasites & Vectors*, 1–12. <https://doi.org/10.1186/s13071-019-3552-2>
12. Fullman, N., Burstein, R., Lim, S. S., Medlin, C., & Gakidou, E. (2013). *Nets, spray or both ? The effectiveness of insecticide-treated nets and indoor residual spraying in reducing malaria morbidity and child mortality in sub-Saharan Africa*. 1–10.
13. Githinji, E. K., Irungu, L. W., Ndegwa, P. N., Machani, M. G., Amito, R. O., Kemei, B. J., Murima, P. N., Ombui, G. M., Wanjoya, A. K., Mbogo, C. M., & Mathenge, E. M. (2020). *Species Composition, Phenotypic and Genotypic Resistance Levels in Major Malaria Vectors in Teso North and Teso South Subcounties in Busia County, Western Kenya. 2020*.
14. Godfray, H. C. J. (2013). Mosquito ecology and control of malaria. *Journal of Animal Ecology*, *82*(1), 15–25. <https://doi.org/10.1111/1365-2656.12003>
15. Gunathilaka, N., Abeyewickreme, W., Hapugoda, M., & Wickremasinghe, R. (2015). Species Composition and Diversity of Malaria Vector Breeding Habitats in Trincomalee District of Sri Lanka. *BioMed Research*

International, 2015. <https://doi.org/10.1155/2015/823810>

16. Haji, K. A., Thawer, N. G., Khatib, B. O., Mcha, J. H., Rashid, A., Ali, A. S., Jones, C., Bagi, J., Magesa, S. M., Ramsan, M. M., Garimo, I., Greer, G., Reithinger, R., & Ngondi, J. M. (2015). Efficacy, persistence and vector susceptibility to pirimiphos-methyl (Actellic® 300CS) insecticide for indoor residual spraying in Zanzibar. *Parasites & Vectors*, 1–7. <https://doi.org/10.1186/s13071-015-1239-x>
17. Hakizimana, E., Karema, C., Munyakanage, D., Iranzi, G., Githure, J., Tongren, J. E., Takken, W., Binagwaho, A., & Koenraadt, C. J. M. (2016). Susceptibility of *Anopheles gambiae* to insecticides used for malaria vector control in Rwanda. *Malaria Journal*, 1–11. <https://doi.org/10.1186/s12936-016-1618-6>
18. Helinski, M. E. H., Nuwa, A., Protopopoff, N., Feldman, M., Ojuka, P., Oguttu, D. W., Abeku, T. A., & Meek, S. (2015). Entomological surveillance following a long-lasting insecticidal net universal coverage campaign in Midwestern Uganda. *Parasites & Vectors*, 1–11. <https://doi.org/10.1186/s13071-015-1060-6>
19. Indicator, M. (2009). *Uganda Bureau of Statistics and ICF International Macro, 2010. Uganda Malaria Indicator Survey 2009. Calverton, Maryland, USA: UBOS and ICF International Macro.*
20. Kabbale, G. F., Anne, M. A., John, B. K., Enock, M., Anne, K., Anges, Y., & Ambrose, W. O. (2016). Molecular identification of *Anopheles gambiae sensu stricto* Giles (formerly *Anopheles gambiae* Savannah Form) in Kamuli District, Uganda. *African Journal of Biotechnology*, 15(39), 2124–2131. <https://doi.org/10.5897/ajb2016.15444>
21. Kabbale, F. G., Akol, A. M., Kaddu, J. B., & Onapa, A. W. (2013). Biting patterns and seasonality of *Anopheles gambiae sensu lato* and *Anopheles funestus* mosquitoes in Kamuli District, Uganda. *Parasites & Vectors*, 6(340), 1–9.
22. Katureebe, A., Zinszer, K., Arinaitwe, E., Rek, J., Kakande, E., Charland, K., Kigozi, R., Kilama, M., Nankabirwa, J., Yeka, A., Mawejje, H., Mpimbaza, A., Katamba, H., Donnelly, M. J., Rosenthal, J., Drakeley, C., Lindsay, S. W., Staedke, S. G., Smith, L., ... Dorsey, G. (2016). *Measures of Malaria Burden after Long-Lasting Insecticidal Net Distribution and Indoor Residual Spraying at Three Sites in Uganda: A Prospective Observational Study. December 2014*, 1–22. <https://doi.org/10.1371/journal.pmed.1002167>
23. Kenea, O., Balkew, M., Tekie, H., Gebremichael, T., Deressa, W., Loha, E., Lindtjørn, B., & Overgaard, H. J. (2016). Human-biting activities of *Anopheles* species in south-central Ethiopia. *Parasites & Vectors*, 1–12. <https://doi.org/10.1186/s13071-016-1813-x>
24. Keven, J. B. (2015). *The host-feeding ecology of mosquito vectors in the Anopheles punctulatus (Diptera: Culicidae) species complex in a malaria endemic province of Papua New Guinea. May.* <https://doi.org/10.13140/RG.2.1.1693.3285>
25. Kigozi, S. P., Kigozi, R. N., Sebuguzi, C. M., Cano, J., Rutazaana, D., Opigo, J., Bousema, T., Yeka, A., Gasasira, A., Sartorius, B., & Pullan, R. L. (2020). *Spatial-temporal patterns of malaria incidence in Uganda using HMIS data from 2015 to 2019.* 1–14.
26. Kleinschmidt, I., Bradley, J., Knox, T. B., Mnzava, A. P., Kafy, H. T., Mbogo, C., Ismail, B. A., Lines, J., & Donnelly, M. J. (2018). *Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study.* 18(June). [https://doi.org/10.1016/S1473-3099\(18\)30172-5](https://doi.org/10.1016/S1473-3099(18)30172-5)
27. Kleinschmidt, I., Mnzava, A. P., Kafy, H. T., Mbogo, C., Bashir, A. I., Bigoga, J., Adechoubou, A., Raghavendra, K., Knox, T. B., Malik, E. M., Nkuni, Z. J., Bayoh, N., Ochomo, E., Fondjo, E., Kouambeng, C., Awono-ambene,

- H. P., Etang, J., Akogbeto, M., Bhatt, R., ... Donnelly, M. J. (2015). Design of a study to determine the impact of insecticide resistance on malaria vector control: a multi – country investigation. *Malaria Journal*, July. <https://doi.org/10.1186/s12936-015-0782-4>
28. Loha, E., Deressa, W., Gari, T., Balkew, M., Kenea, O., Solomon, T., Hailu, A., Robberstad, B., Assegid, M., Overgaard, H. J., & Lindtjørn, B. (2019). Long – lasting insecticidal nets and indoor residual spraying may not be sufficient to eliminate malaria in a low malaria incidence area: results from a cluster randomized controlled trial in Ethiopia. *Malaria Journal*, 1–15. <https://doi.org/10.1186/s12936-019-2775-1>
29. Mawejje, H. D., Kilama, M., Kigozi, S. P., Musiime, A. K., Kamya, M., Lines, J., Lindsay, S. W., Smith, D., Dorsey, G., Donnelly, M. J., & Staedke, S. G. (2021). Impact of seasonality and malaria control interventions on Anopheles density and species composition from three areas of Uganda with differing malaria endemicity. *Malaria Journal*, 20(1). <https://doi.org/10.1186/s12936-021-03675-5>
30. Mnzava, A. P., Knox, T. B., Temu, E. A., Trett, A., Fornadel, C., Hemingway, J., & Renshaw, M. (2015). Implementation of the global plan for insecticide resistance management in malaria vectors: progress, challenges and the way forward. ???, 1–9. <https://doi.org/10.1186/s12936-015-0693-4>
31. Musiime, A. K., Smith, D. L., Kilama, M., Rek, J., Arinaitwe, E., Nankabirwa, J. I., Kamya, M. R., Conrad, M. D., Dorsey, G., Akol, A. M., Staedke, S. G., Lindsay, S. W., & Egonyu, J. P. (2019). Impact of vector control interventions on malaria transmission intensity, outdoor vector biting rates and Anopheles mosquito species composition in Tororo, Uganda. *Malaria Journal*, 1–9. <https://doi.org/10.1186/s12936-019-3076-4>
32. Nankabirwa, J. I., Briggs, J., Rek, J., Arinaitwe, E., Nayebare, P., Katrak, S., Staedke, S. G., Rosenthal, P. J., Rodriguez-barraquer, I., Kamya, M. R., Dorsey, G., & Greenhouse, B. (2019). *Persistent Parasitemia Despite Dramatic Reduction in Malaria Incidence After 3 Rounds of Indoor Residual Spraying in Tororo, Uganda*. 219. <https://doi.org/10.1093/infdis/jiy628>
33. Ochomo, E., Bayoh, N. M., Kamau, L., Atieli, F., Vulule, J., Ouma, C., Ombok, M., Njagi, K., Soti, D., Mathenge, E., Muthami, L., Kinyari, T., Subramaniam, K., Kleinschmidt, I., Donnelly, M. J., & Mbogo, C. (2014). Pyrethroid susceptibility of malaria vectors in four Districts of western Kenya. *Parasites and Vectors*, 7(1), 1–9. <https://doi.org/10.1186/1756-3305-7-310>
34. Oguttu, D. W., Matovu, J. K. B., Okumu, D. C., Ario, A. R., Okullo, A. E., Opigo, J., & Nankabirwa, V. (2017). Rapid reduction of malaria following introduction of vector control interventions in Tororo District, Uganda : a descriptive study. *Malaria Journal*, 1–8. <https://doi.org/10.1186/s12936-017-1871-3>
35. Ojuka, P., Boum, Y., Denoed-Ndam, L., Nabasumba, C., Muller, Y., Okia, M., Mwanga-Amumpaire, J., De Beaudrap, P., Protopopoff, N., & Etard, J. F. (2015). Early biting and insecticide resistance in the malaria vector Anopheles might compromise the effectiveness of vector control intervention in Southwestern Uganda. *Malaria Journal*, 14(1), 1–8. <https://doi.org/10.1186/s12936-015-0653-z>
36. Okia, M., Hoel, D. F., Kirunda, J., Rwakimari, J. B., Mpeka, B., Ambayo, D., Price, A., Oguttu, D. W., Okui, A. P., & Govere, J. (2018a). Insecticide resistance status of the malaria mosquitoes: Anopheles gambiae and Anopheles funestus in eastern and northern Uganda. *Malaria Journal*, 1–12. <https://doi.org/10.1186/s12936-018-2293-6>
37. Okia, M., Hoel, D. F., Kirunda, J., Rwakimari, J. B., Mpeka, B., Ambayo, D., Price, A., Oguttu, D. W., Okui, A. P., & Govere, J. (2018b). Insecticide resistance status of the malaria mosquitoes: Anopheles gambiae and

- Anopheles funestus in eastern and northern Uganda. *Malaria Journal*, 17(1), 157.
<https://doi.org/10.1186/s12936-018-2293-6>
38. Okia, M., Ndyomugenyi, R., Kirunda, J., Byaruhanga, A., Adibaku, S., Lwamafa, D. K., & Kironde, F. (2013). Bioefficacy of long-lasting insecticidal nets against pyrethroid-resistant populations of *Anopheles gambiae* s.s. from different malaria transmission zones in Uganda. *Parasites and Vectors*, 6(1), 1–10.
<https://doi.org/10.1186/1756-3305-6-130>
39. Opiyo, M. A., & Paaajmans, K. P. (2020). “We spray and walk away”: Wall modifications decrease the impact of indoor residual spray campaigns through reductions in post-spray coverage. *Malaria Journal*, 19(1).
<https://doi.org/10.1186/s12936-020-3102-6>
40. Perugini, E., Guelbeogo, W. M., Calzetta, M., Manzi, S., Virgillito, C., Caputo, B., Pichler, V., Ranson, H., Sagnon, N. F., Torre, A., & Pombi, M. (2020). Behavioural plasticity of *Anopheles coluzzii* and *Anopheles arabiensis* undermines LLIN community protective effect in a Sudanese – savannah village in Burkina Faso. *Parasites & Vectors*, 1–10. <https://doi.org/10.1186/s13071-020-04142-x>
41. Pmi, T. H. E., & Project, V. (2019). *THE PMI VECTORLINK PROJECT ANNUAL ENTOMOLOGY REPORT*.
42. Profi, V. (2016). *Tororo District*.
43. Raouf, S., Mpimbaza, A., Kigozi, R., Sserwanga, A., Rubahika, D., Katamba, H., Lindsay, S. W., Kapella, B. K., Belay, K. A., Kanya, M. R., Staedke, S. G., & Dorsey, G. (2017). *Resurgence of Malaria Following Discontinuation of Indoor Residual Spraying of Insecticide in an Area of Uganda With Previously High-Transmission Intensity*. 65, 453–460. <https://doi.org/10.1093/cid/cix251>
44. Rek, J., Musiime, A., Zedi, M., Otto, G., Kyagamba, P., Rwatooro, J. A., Arinaitwe, E., Nankabirwa, J., Staedke, G., Drakeley, C., Rosenthal, P. J., Kanya, M., Dorsey, G., & Id, J. K. (2020). *Non-adherence to long-lasting insecticide treated bednet use following successful malaria control in Tororo, Uganda*. 1–14.
<https://doi.org/10.1371/journal.pone.0243303>
45. Russell, T. L., Beebe, N. W., Cooper, R. D., Lobo, N. F., & Burkot, T. R. (2013). *Successful malaria elimination strategies require interventions that target changing vector behaviours*. 1–5.
46. Sherrard-smith, E., Grif, J. T., Winskill, P., Corbel, V., Pennetier, C., Djénontin, A., Moore, S., Richardson, J. H., Müller, P., Edi, C., Protopopoff, N., Oxborough, R., Agossa, F., Guessan, R. N., Rowland, M., & Churcher, T. S. (2018). *Systematic review of indoor residual spray efficacy and effectiveness against Plasmodium falciparum in Africa*. <https://doi.org/10.1038/s41467-018-07357-w>
47. Sherrard-smith, E., Skarp, J. E., Beale, A. D., Fornadel, C., Norris, L. C., & Moore, S. J. (2019). *Mosquito feeding behavior and how it influences residual malaria transmission across Africa*. 116(30).
<https://doi.org/10.1073/pnas.1820646116>
48. Ssempiira, J., Nambuusi, B., Kissa, J., Agaba, B., Makumbi, F., Kasasa, S., & Vounatsou, P. (2017). The contribution of malaria control interventions on spatio-temporal changes of parasitaemia risk in Uganda during 2009–2014. *Parasites and Vectors*, 10(1), 450. <https://doi.org/10.1186/s13071-017-2393-0>
49. Staedke, S. G., Kanya, M. R., Dorsey, G., Maiteki-sebuguzi, C., Gonahasa, S., Yeka, A., Lynd, A., Opigo, J., Hemingway, J., & Donnelly, M. J. (2019). *LLIN Evaluation in Uganda Project (LLINEUP) – Impact of long-lasting insecticidal nets with, and without, piperonyl butoxide on malaria indicators in Uganda : study protocol for a cluster-randomised trial*. 1–13.

50. Tangena, J. A. A., Hendriks, C. M. J., Devine, M., Tamaro, M., Trett, A. E., Williams, I., Depina, A. J., Sisay, A., Herizo, R., Kafy, H. T., Chizema, E., Were, A., Rozier, J., Coleman, M., & Moyes, C. L. (2020). Indoor residual spraying for malaria control in sub-Saharan Africa 1997 to 2017: An adjusted retrospective analysis. In *Malaria Journal* (Vol. 19, Issue 1). BioMed Central Ltd. <https://doi.org/10.1186/s12936-020-03216-6>
51. Tchouakui, M., Mugenzi, L. M. J., Menze, B. D., Khaukha, J. N. T., Tchapgga, W., Tchoupo, M., Wondji, M. J., & Wondji, C. S. (2021). *Pyrethroid Resistance Aggravation in Ugandan Malaria Vectors Is Reducing Bednet Efficacy*. 2–13.
52. Tukei, B. B., Beke, A., & Figueroa, H. L. (2017). Assessing the effect of indoor residual spraying (IRS) on malaria morbidity in Northern Uganda: a before and after study. *Malaria Journal*, 1–9. <https://doi.org/10.1186/s12936-016-1652-4>
53. Were, V., Buff, A. M., Desai, M., Kariuki, S., Samuels, A., Ter Kuile, F. O., Phillips-Howard, P. A., Patrick Kachur, S., & Niessen, L. (2018). Socioeconomic health inequality in malaria indicators in rural western Kenya: Evidence from a household malaria survey on burden and care-seeking behaviour. *Malaria Journal*, 17(1). <https://doi.org/10.1186/s12936-018-2319-0>
54. West, P. A., Protopopoff, N., Wright, A., Kivaju, Z., Tigererwa, R., Mosha, F. W., Kisinza, W., Rowland, M., & Kleinschmidt, I. (2014). Indoor Residual Spraying in Combination with Insecticide-Treated Nets Compared to Insecticide-Treated Nets Alone for Protection against Malaria: A Cluster Randomised Trial in Tanzania. *PLoS Medicine*, 11(4). <https://doi.org/10.1371/journal.pmed.1001630>
55. Yadouleton, A. W., Padonou, G., Asidi, A., Moiroux, N., Bio-Banganna, S., Corbel, V., N'guessan, R., Gbenou, D., Yacoubou, I., Gazard, K., & Akogbeto, M. C. (2010). *Insecticide resistance status in Anopheles gambiae in southern Benin*. <http://www.malariajournal.com/content/9/1/83>

Figures



Figure 1

Location of *Anopheles* Mosquito Larvae Sampling Sites in the two districts

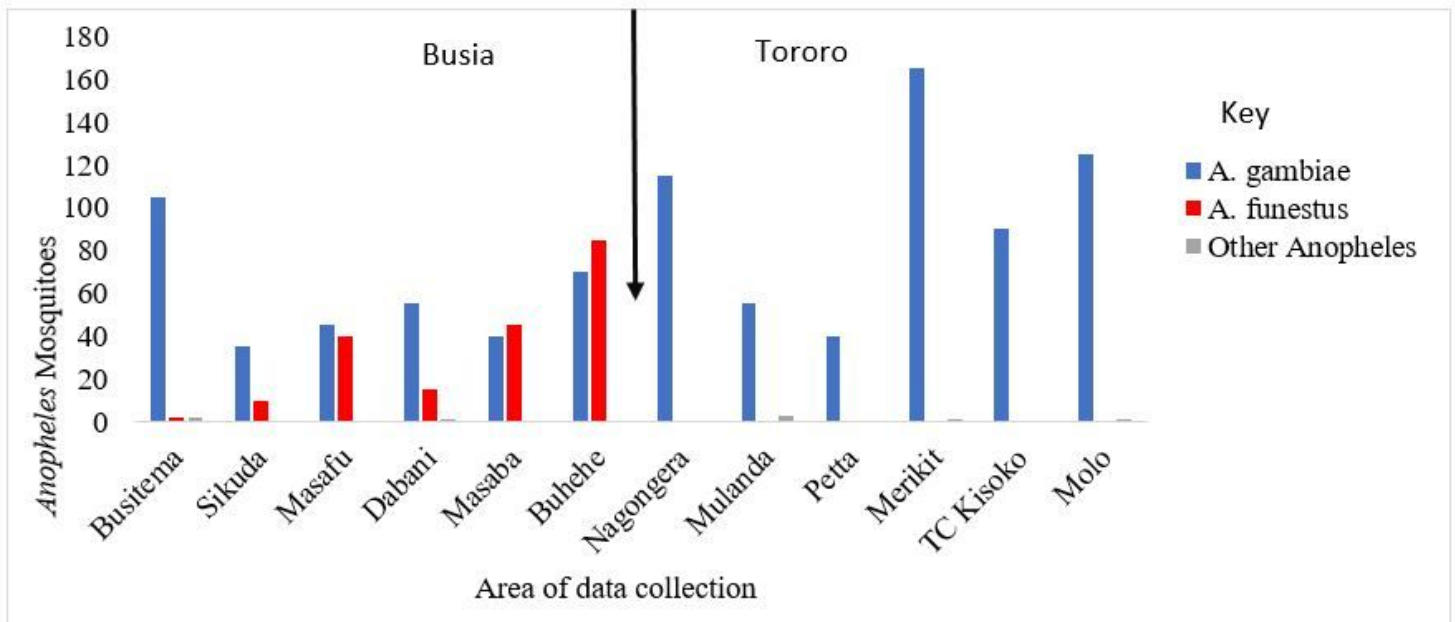


Figure 2

General Species Composition across Data Collection Zones in Busia and Tororo Districts



Figure 3

Percentage Mortality Rates on Used Nets (LLINs) of Busia and Tororo District

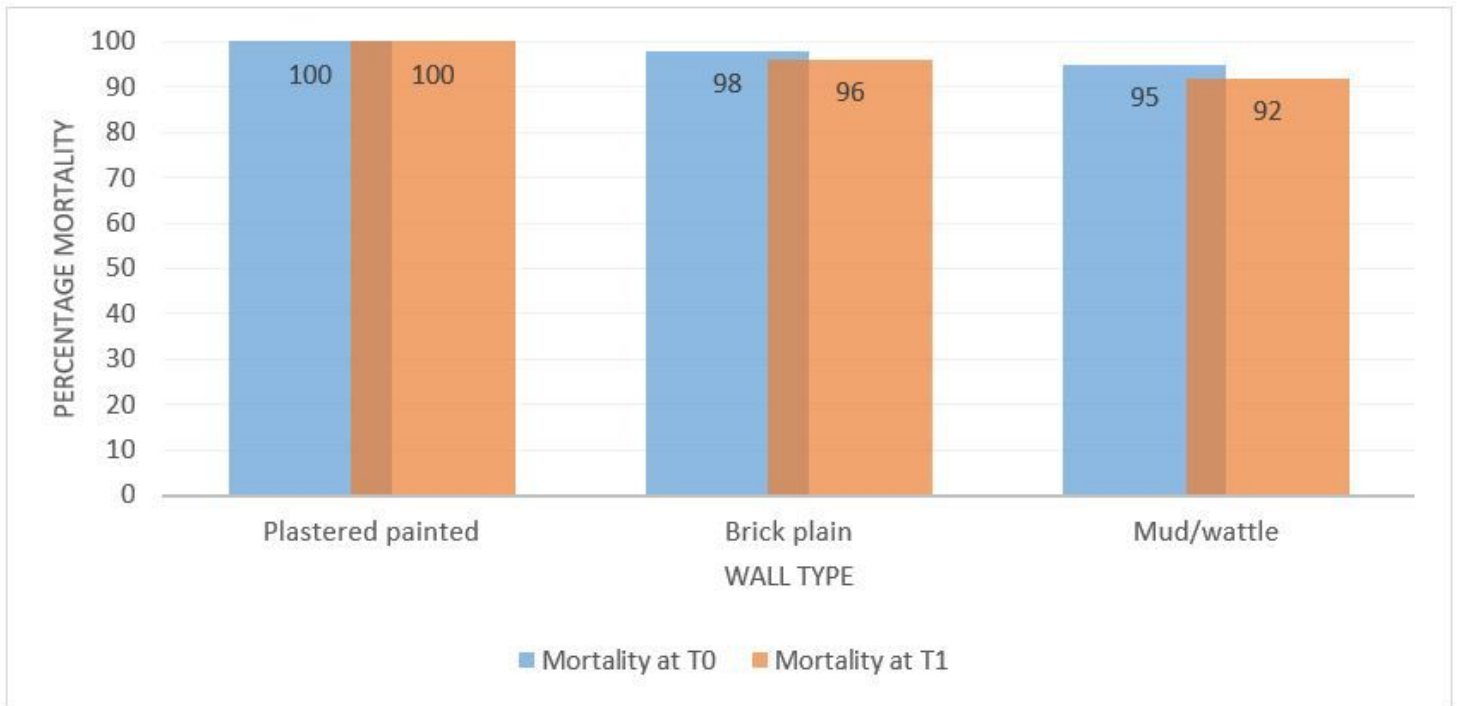


Figure 4

Susceptibility of *Anopheles* mosquitoes on Walls after two Weeks & one Month of Chemical Spray