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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Matowo NS, Kulkarni MA, Messenger LA, et al. Differential impact of dual-active ingredient long-lasting insecticidal nets on primary malaria vectors: a secondary analysis of a 3-year, single-blind, cluster-randomised controlled trial in rural Tanzania. *Lancet Planet Health* 2023; **7:** e370–80

Differential impact of dual-active ingredient long-lasting insecticidal nets (LLINs) on primary malaria vectors: a secondary analysis of a three-year cluster-randomized controlled trial in rural Tanzania

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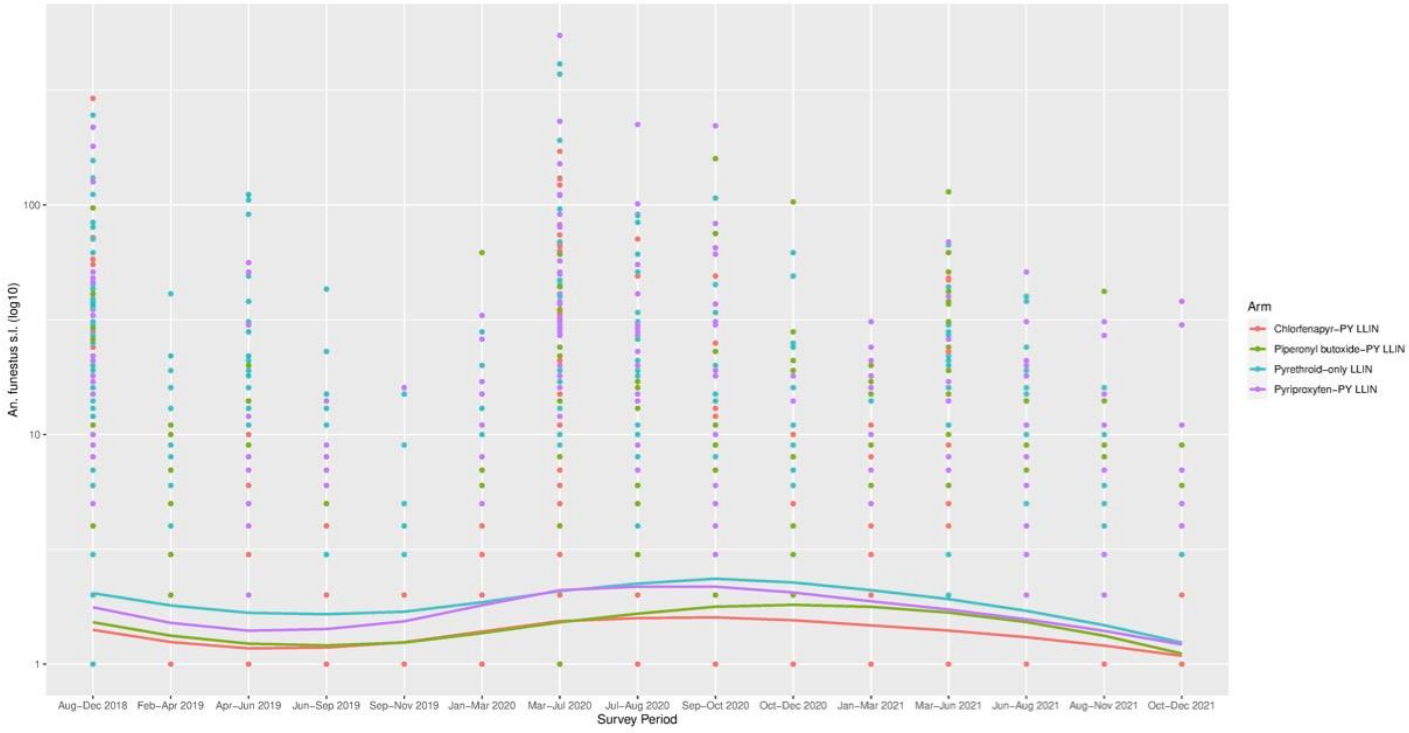
SUPPLEMENTARY APPENDIX

Contents

S1: Trends in female Anopheles species density distribution over time across the study arm



Trends of *An. funestus* population density distribution with smoothed averages over time across the treatment allocation arms defined as the orange (Chlorfenapyr-PY LLIN), green (Piperonyl butoxide-PY LLIN), blue (Pyriproxyfen-PY LLIN), and purple (Standard-Pyrethroid LLIN) with their respective 95% confidence intervals portrayed as grey shading. The plots were generated in R version 4.1.3 (R Development Core Team) using the "ggplot" packages.

S2: Per protocol analysis

Table: Post hoc per protocol analysis showing differential effects of the dual-LLINs compared to standard-PY LLIN on Anopheles species at 12, 24, 36 months, and combined after intervention

	Number of households analysed	*Total Anopheles funestus s.l.*	Density / night/ HH	DR	95%CI	p value*	*Total Anopheles gambiae s.l*	Density / night/ HH	DR	95%CI	p value*
Year 1: 2019											
Pyrethroid-only LLIN arm (ref)	511	1131	2.2	1			247	0.5	1		
Chlorfenapyr-PY LLIN arm	519	102	0.2	0.17	0.08-0.34	<0.0001	229	0.4	0.78	0.37-1.65	0.5172
Piperonyl butoxide-PY LLIN arm	510	228	0.4	0.40	0.20-0.80	0.0088	184	0.4	0.63	0.30-1.34	0.2287
Pyriproxyfen-PY LLIN arm	525	473	0.9	0.65	0.34-1.27	0.2062	216	0.4	0.73	0.35-1.55	0.4170
Year 2: 2020											
Pyrethroid-only LLIN arm (ref)	596	3309	5.6	1			1743	2.9	1		
Chlorfenapyr-PY LLIN arm	574	1519	2.6	0.28	0.16-0.51	<0.0001	2520	4.4	0.99	0.52-1.88	0.9767
Piperonyl butoxide-PY LLIN arm	458	938	2.0	0.48	0.27-0.87	0.0151	1374	3.0	1.13	0.60-2.17	0.6959
Pyriproxyfen-PY LLIN arm	520	3619	7.0	0.76	0.42-1.35	0.3450	1634	3.1	1.07	0.56-2.04	0.8325
Year 3: 2021											
Pyrethroid-only LLIN arm (ref)	430	1007	2.3	1			403	0.9	1		
Chlorfenapyr-PY LLIN arm	425	433	1.0	0.30	0.16-0.55	<0.0001	542	1.3	0.86	0.43-1.72	0.6649
Piperonyl butoxide-PY LLIN arm	235	502	2.1	0.62	0.31-1.21	0.1598	121	0.5	0.62	0.28-1.35	0.2251
Pyriproxyfen-PY LLIN arm	320	809	2.5	0.72	0.38-1.36	0.3069	418	1.3	0.85	0.41-1.77	0.6650
Overall (3 years combined)											
Pyrethroid-only LLIN arm (ref)	1537	5447	3.5	1			2393	1.6	1		
Chlorfenapyr-PY LLIN arm	1518	2054	1.4	0.25	0.16-0.39	<0.0001	3291	2.2	0.88	0.57-1.34	0.6464
Piperonyl butoxide-PY LLIN arm	1203	1668	1.4	0.50	0.32-0.77	0.0019	1679	1.4	0.82	0.53-1.27	0.3738
Pyriproxyfen-PY LLIN arm	1365	4901	3.6	0.72	0.47-1.11	0.1357	2268	1.7	0.90	0.59-1.39	0.5369

The intervention arm is compared to the standard-PY LLIN arm at each time point. LLIN=long-lasting insecticidal net. PY=Pyrethroid. DR=Density reduction ratio. DRs are adjusted for baseline cluster-level variables used in restricted randomization. *EIR are weighted to account for the proportion of mosquitoes sampled to be tested for sporozoites. We have applied a Bonferroni correction for multiplicity given the multiple comparison arms and *a p value <0·017 was considered statistically significant.

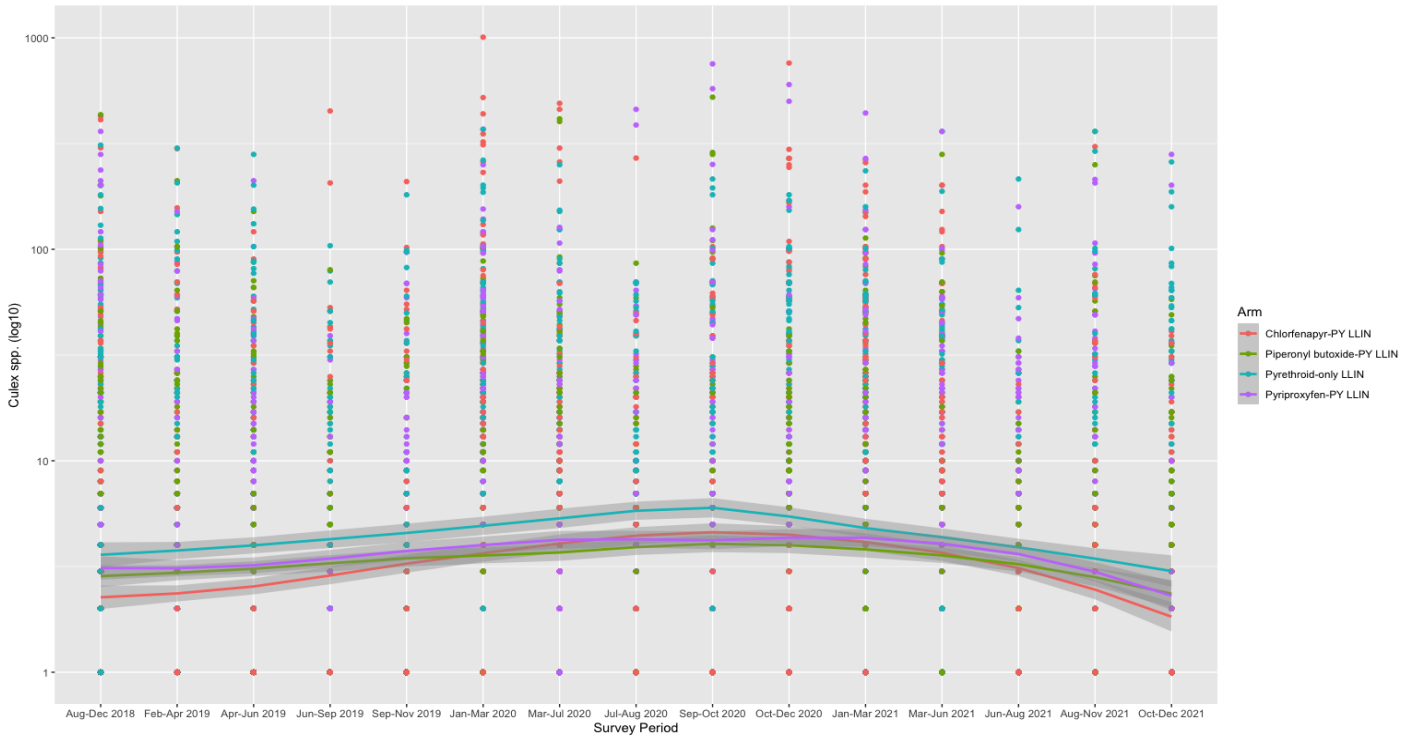
S3: Effect of dual-LLINs on non-malaria vectors

Table: Effect of dual-LLINs compared with the standard-PY LLIN on non-malaria vectors

	Number of households analysed	Female *Culex species* collected	Density / night/ HH	DR	95%CI	p value*	Total all mosquito collected	Density / night/ HH	DR	95%CI	p value*
Year 1: 2019											
Pyrethroid-only LLIN arm (ref)	670	6630	9.9	1			9100	13.6	1		
Chlorfenapyr-PY LLIN arm	671	3741	6.2	0.36	0.20-0.66	0.0009	4937	7.4	0.36	0.22-0.61	0.0001
Piperonyl butoxide-PY LLIN arm	672	3820	5.7	0.66	0.36-1.19	0.1634	4738	7.1	0.56	0.34-0.92	0.0237
Pyriproxyfen-PY LLIN arm	672	3741	5.6	0.53	0.29-0.96	0.0359	5378	8.0	0.55	0.33-0.91	0.0206
Year 2: 2020											
Pyrethroid-only LLIN arm (ref)	838	13909	16.6	1			21640	25.8	1		
Chlorfenapyr-PY LLIN arm	840	15541	18.5	0.58	0.33-1.01	0.0535	22162	26.4	0.51	0.32-0.82	0.0053
Piperonyl butoxide-PY LLIN arm	840	8780	10.5	0.50	0.29-0.85	0.0115	13421	16.0	0.52	0.32-0.83	0.0058
Pyriproxyfen-PY LLIN arm	840	12639	15.0	0.50	0.29-0.86	0.0128	21553	25.7	0.59	0.37-0.95	0.0283
Year 3: 2021											
Pyrethroid-only arm LLIN (ref)	840	10027	11.9	1			13431	16.0	1		
Chlorfenapyr-PY LLIN arm	840	7941	9.5	0.60	0.34-1.05	0.0748	10653	12.7	0.49	0.30-0.79	0.0036
Piperonyl butoxide-PY LLIN arm	840	5773	6.9	0.72	0.42-1.26	0.2516	8226	9.8	0.60	0.38-0.96	0.0341
Pyriproxyfen-PY LLIN arm	840	8312	9.9	0.94	0.54-1.64	0.8395	11697	13.9	0.74	0.46-1.19	0.2172
Overall (all three years combined)											
Pyrethroid-only LLIN arm (ref)	2348	30566	13.0	1			44171	18.8	1		
Chlorfenapyr-PY LLIN arm	2351	27627	11.8	0.52	0.33-0.81	0.0037	37752	16.1	0.46	0.31-0.67	<0.0001
Piperonyl butoxide-PY LLIN arm	2352	18373	7.8	0.61	0.39-0.95	0.0273	26385	11.2	0.56	0.38-0.81	0.0020
Pyriproxyfen-PYLLIN arm	2352	24692	10.5	0.63	0.41-0.99	0.0431	38628	16.4	0.63	0.43-0.92	0.0150

The intervention arm is compared to the standard-PY LLIN arm at each time point. LLIN=long-lasting insecticidal net. PY=Pyrethroid. DR=Density reduction ratio. DRs are adjusted for baseline cluster-level variables used in restricted randomization. *EIR are weighted to account for the proportion of mosquitoes sampled to be tested for sporozoites. We have applied a Bonferroni correction for multiplicity given the multiple comparison arms and *a p value <0·017 was considered statistically significant.

S4: Trends in female Culex species density distribution over time across the study arm



Plots showing smoothed averages female *Culex* population density over time across the treatment allocation arms defined as the orange (Chlorfenapyr-PY LLIN), green (Piperonyl butoxide-PY LLIN), blue (Pyriproxyfen-PY LLIN), and purple (Standard-Pyrethroid LLIN) with their respective 95% confidence intervals portrayed as grey shading. The plots were generated in R version 4.1.3 (R Development Core Team) using the "ggplot" packages

S5: Spatial differences of effectiveness of dual-AI LLINs

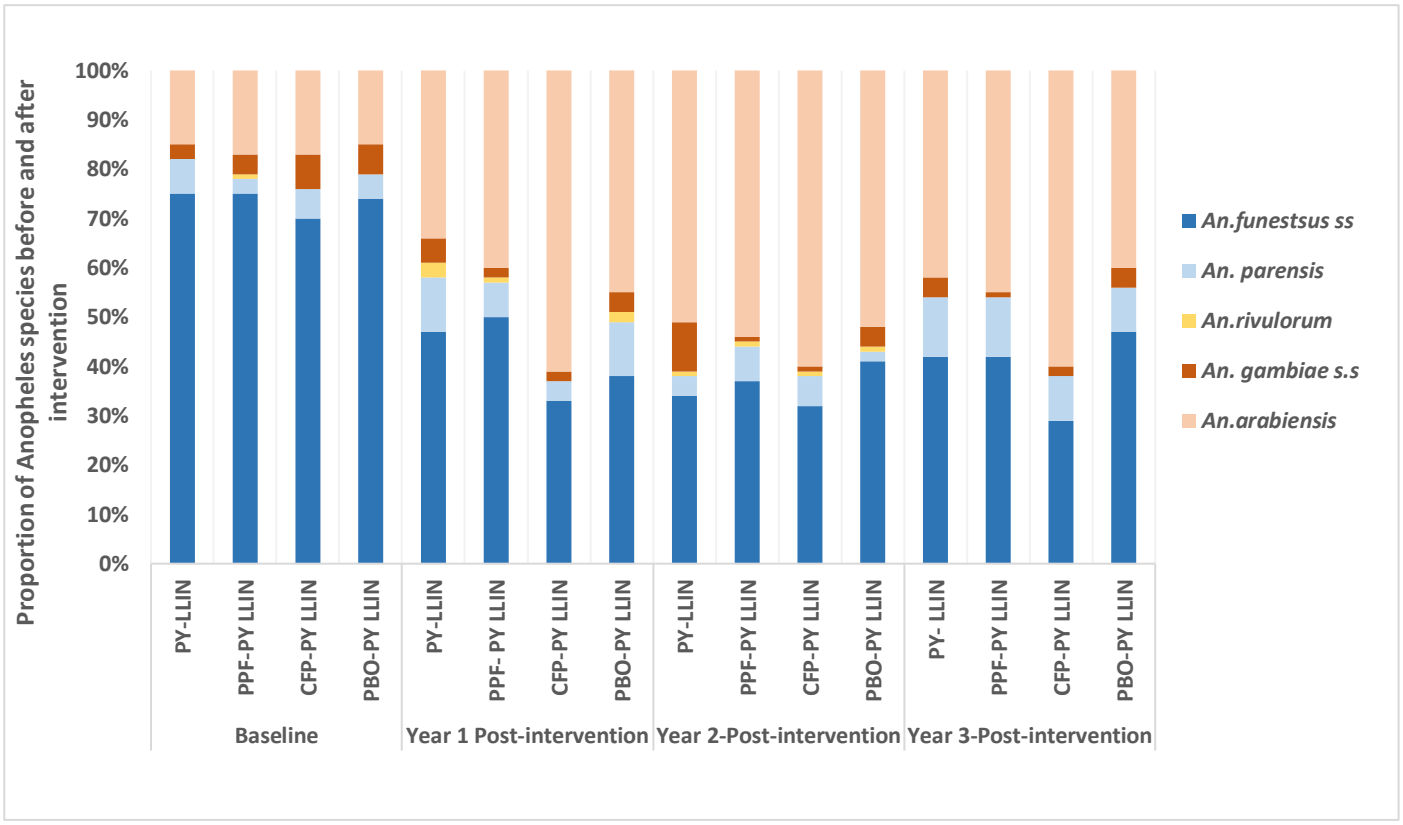
Table: Spatial heterogeneity of effectiveness of dual-AI LLINs on malaria vectors species

	Number of households analysed	An. vector species	Density / night/ HH	DR	95%CI	p value**	*An. funestus s.l*	Density / night/ HH	RR	95%CI	p value**	*An. gambiae s.l*	Density / night/ HH	DR	95%CI	p value**
Southern clusters																
Pyrethroid-only LLIN arm (ref)	1118	7643	6.8	1			5801	5.2	1			1842	1.7	1		
Chlorfenapyr-PY LLIN arm	1008	3353	4.3	0.30	0.19-0.50	<0.0001	1685	2.2	0.21	0.12-0.38	<0.0001	1668	2.1	0.63	0.33-1.20	0.4301
Piperonyl butoxide-PY LLIN arm	784	2953	3.8	0.46	0.27-0.80	0.0057	1992	2.5	0.45	0.24-0.86	0.0152	961	1.2	0.72	0.35-1.49	0.3825
Pyriproxyfen-PY LLIN arm	783	7433	7.4	0.47	0.25-0.90	0.0229	5483	5.4	0.45	0.21-0.97	0.0420	1950	1.9	0.71	0.30-1.67	0.1565
Northern clusters																
Pyrethroid-only LLIN arm (ref)	1230	2650	2.2	1			1503	1.2	1			1147	0.9	1		
Chlorfenapyr-PY LLIN arm	1568	3828	2.4	0.60	0.39-0.93	0.0213	1213	0.8	0.37	0.22-0.63	<0.0001	2615	1.7	0.93	0.53-1.66	0.8158
Piperonyl butoxide-PY LLIN arm	1568	3225	2.1	0.67	0.44-1.02	0.0594	1329	0.9	0.54	0.33-0.88	0.0141	1896	1.2	0.88	0.50-1.53	0.6421
Pyriproxyfen-PY LLIN arm	1344	3520	2.6	1.22	0.78-1.89	0.3873	1657	1.2	1.15	0.68-1.94	0.6117	1863	1.4	1.33	0.74-2.40	0.3378

The intervention arm is compared to the standard-PY LLIN arm at each time point. LLIN=long-lasting insecticidal net. PY=Pyrethroid. DR=Density reduction ratio. DRs are adjusted for baseline cluster-level variables used in restricted randomization. *EIR are weighted to account for the proportion of mosquitoes sampled to be tested for sporozoites. We have applied a Bonferroni correction for multiplicity given the multiple comparison arms and *a p value <0·017 was considered statistically significant.

S6: Anopheles species composition

The figure shows Anopheles sibling species composition at baseline and each time point post-intervention



S7: Maxent model

Table: Model validation statistics for Maxent species distribution models of *An. funestus* and *An. arabiensis* comparing pilot predicted species distribution to post-intervention species occurrence by study arm and year.

Species	Validation test dataset	Arm	AUC	Standard error of AUC	Fractional predicted area	Test omission rate
An. funestus	Baseline	n/a	0.788	0.017	0.59	0.078
	Year 1	Pyrethroid-only LLIN arm (ref)	0.809	0.014	0.59	0.075
	Year 1	Chlorfenapyr-PY LLIN arm	0.792	0.019	0.59	0.038
	Year 1	Pyriproxyfen-PY LLIN arm	0.852	0.013	0.59	0.045
	Year 1	Piperonyl butoxide-PY LLIN arm	0.874	0.011	0.59	0.018
	Year 2	Pyrethroid-only LLIN arm (ref)	0.781	0.011	0.59	0.086
	Year 2	Chlorfenapyr-PY LLIN arm	0.759	0.012	0.59	0.061
	Year 2	Pyriproxyfen-PY LLIN arm	0.835	0.01	0.59	0.044
	Year 2	Piperonyl butoxide-PY LLIN arm	0.875	0.007	0.59	0.011
An. arabiensis	Baseline	n/a	0.8	0.023	0.684	0.025
	Year 1	Pyrethroid-only LLIN arm (ref)	0.812	0.023	0.684	0.058
	Year 1	Chlorfenapyr-PYLLIN arm	0.734	0.026	0.684	0.029
	Year 1	Pyriproxyfen-PY LLIN arm	0.729	0.023	0.684	0.014
	Year 1	Piperonyl butoxide-PY LLIN arm	0.747	0.024	0.684	0
	Year 2	Pyrethroid-only LLIN arm (ref)	0.73	0.019	0.684	0.051
	Year 2	Chlorfenapyr-PY LLIN arm	0.731	0.02	0.684	0.079
	Year 2	Pyriproxyfen-PY LLIN arm	0.756	0.017	0.684	0.021
	Year 2	Piperonyl butoxide-PY LLIN arm	0.708	0.02	0.684	0.081

The fractional predicted area and test omission rate are based on the minimum training presence threshold (0.102 for *An. funestus* and 0.155 for *An. arabiensis*). A p-value<0.05 indicates that test points are better predicted by the model than by random prediction with the same fractional predicted area.