Effectiveness of three types of dual active ingredient treated nets compared to pyrethroid long lasting insecticidal nets against malaria in an area with pyrethroid-resistant mosquitoes in Tanzania: a four-arm, cluster-randomised trial

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Introduction

- Only PY-treated LLINs, but challenge with the widespread of PY resistance (Hemingway, Lancet 2016)
- **New insecticides/compounds** to manage/mitigate the impact of resistance
  - New generation LLINs (require more evidence on effectiveness)
  - Muleba, Tanzania PBO RCT net evaluation trial (*Protopopoff, N*., *Mosha J*., et al. Lancet 2018)
  - Uganda PBO study (*Staedke G et al, Lancet* 2020)
- Interim endorsement of PBO nets by the WHO
- In Tanzania piperonyl butoxide (PBO) are deployed
- Large scale evaluation of new generation LLINs
  - A cluster-randomised trial in 84 districts in North-western Tanzania

Project Tanzania

Description of product

LN brand	LN specificity: Dose AI/m^2 of netting fabric	Fibre	Manufacturer
Interceptor® (reference)	Alpha-cypermethrin 200 mg	Polyester	BASF
Interceptor® G2	Alpha-cypermethrin 100 mg **+ Chlorfenapyr 200 mg**	Polyester	BASF
Olyset™ Plus	Permethrin 800 mg **+ PBO 400 mg**	Polyethylene	Sumitomo Chemicals
Royal guard®	Alpha-cypermethrin 216 mg **+ Pyriproxyfen 225 mg**	Polyethylene	Disease Control Technologies LLC

- Pyrethroid: Neurotoxicity, Fast knock down and killing
- Chlorfenapyr: Disrupts the insect's ability to produce energy, slow killing effect.
- Pyriproxyfen: Disrupt female reproduction and fertility of eggs
- Piperonyl butoxide: Enhance the potency of the PY insecticide

Study design/ outcomes

- Four-arm, cluster-randomised trial: 21 clusters / arm

Main outcomes:

- Malaria infection prevalence by RDT in children aged 6 months to 14 years (measured at 12, 18, **24,** 30 and 36 months)
- Malaria case incidence (RDT) in children aged 6 months to 10 years (over **24 months** follow up)
- EIR and Anopheles density (over **24 months** & 36 months follow up) &

Study Site, Misungwi, Tanzania

Study Area

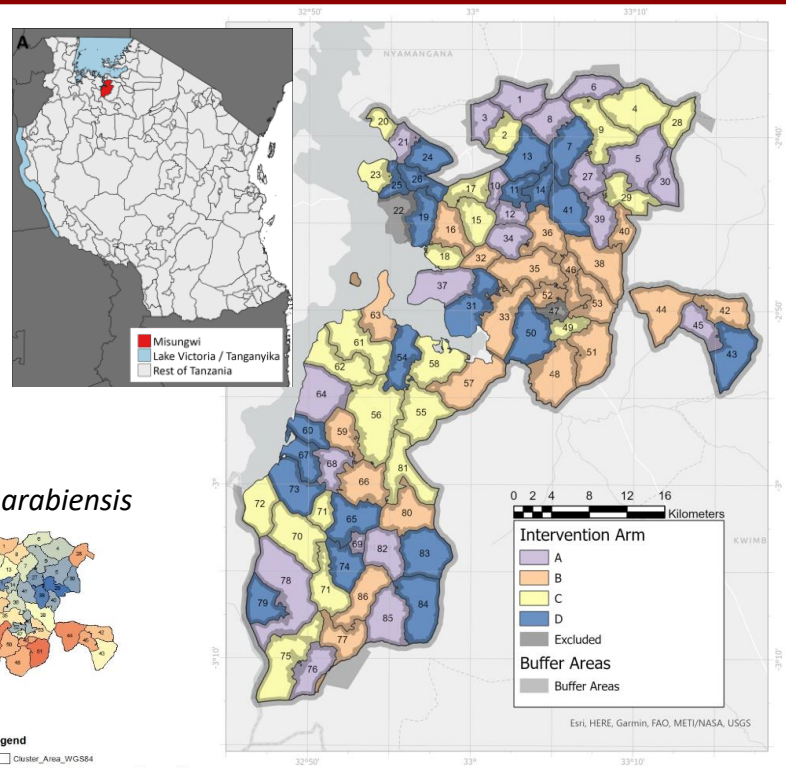
- 72 villages (84 clusters)
- 42,314 Households (study census 2018)
- 251,155 population size

Malaria:

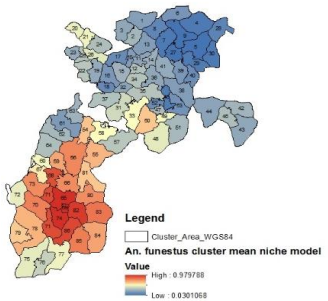
- 2017 prevalence in primary school children = 46.3% (NMCP)
- Two transmission seasons following start rainy seasons: Oct-Dec and February-May

Vector information:

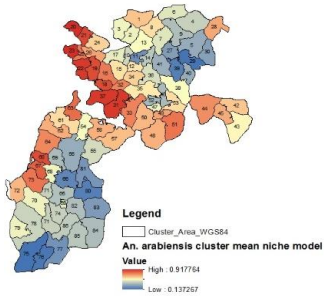
- *An.funestus* predominant in South part
- *An.gambiae* s.s. Northern part between (February-May)
- *An.arabienis* s.s. All study area
- Pyrethroid resistance Mortality < 60%



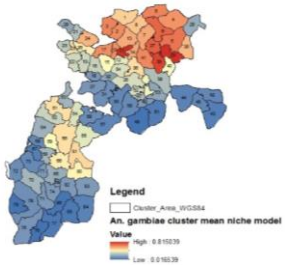
An. funestus



An. arabiensis



An. gambiae s.s.



Ecological niche model

Epidemiological data collection

Cross-sectional survey: **prevalence**

45 HH selected per cluster

- Up to 2 children (6 months-14 years) are tested per HH
- Approximately 5000 children are tested at each time point

<u>Measured:</u>

- Malaria by RDT
- Anaemia
- Temperature

Cohort follow-up: **case incidence**

35 HH (year 1) and 40HH (year 2) are selected per cluster

- One child (6 months-10 years) per HH
- 2940 (cohort year 1) and 3360 (cohort year 2) children are selected and followed every 2 weeks for 1 year

<u>Measured:</u>

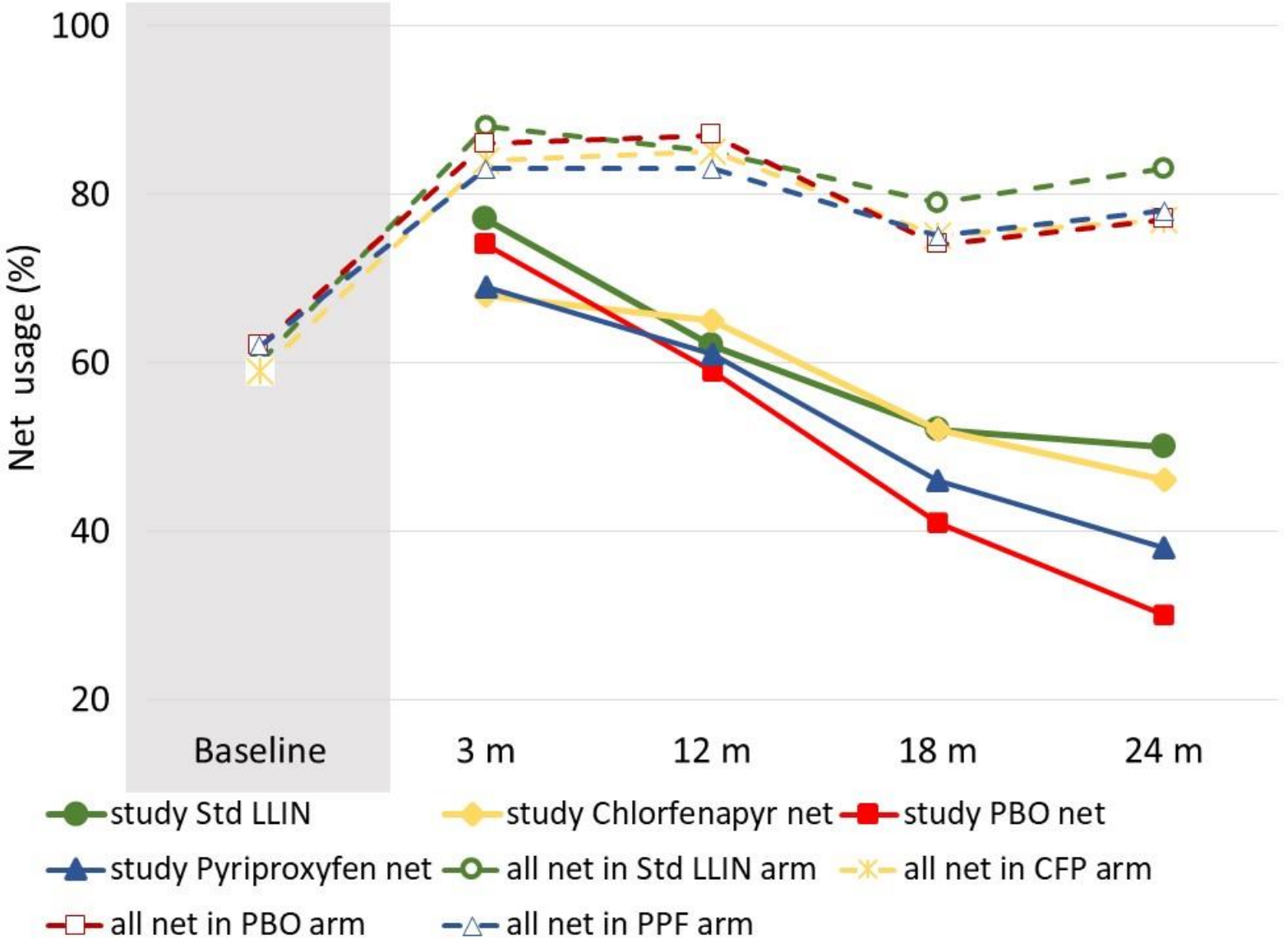
- Malaria by RDT
- Temperature

Baseline

	Std LLIN arm (Interceptor)	Chlorfenapyr arm (Interceptor G2)	PBO arm (Olyset plus)	Pyriproxyfen arm (Royal Guard)
Study cluster characteristics				
Population (all study area)	61183	60115	57631	57567
Population (core area)	43877	41748	45020	43266
Households and children characteristics baseline cross sectional survey (sept 2018)				
SES (poorest households)	28.9%	30.7%	36.6%	36.1%
LLIN use in selected children	63.6%	62.5%	63.4%	64.9%
Malaria infection prevalence	46.6%	42.7%	42.0%	46.2%
Entomological characteristics (Sept-Dec 2018)				
Mean indoor vector per house per night	5.9 (0.8-11.1)	2.8 (0-6.0)	1.9 (0.8-7.6)	4.2 (0.8-7.6)
Sporozoite rate	4.4%	2.2%	3.0%	3.3%
EIR per HH per night	0.35 (0.01-0.68)	0.04 (0-0.08)	0.07 (0.01-0.13)	0.11 (0.01-0.21)
% *An. funestus*	94.3%	95.4%	92.8%	95.0%
% *An. arabiensis* /*An.gambiae* s.l.	85%	81%	71%	84%

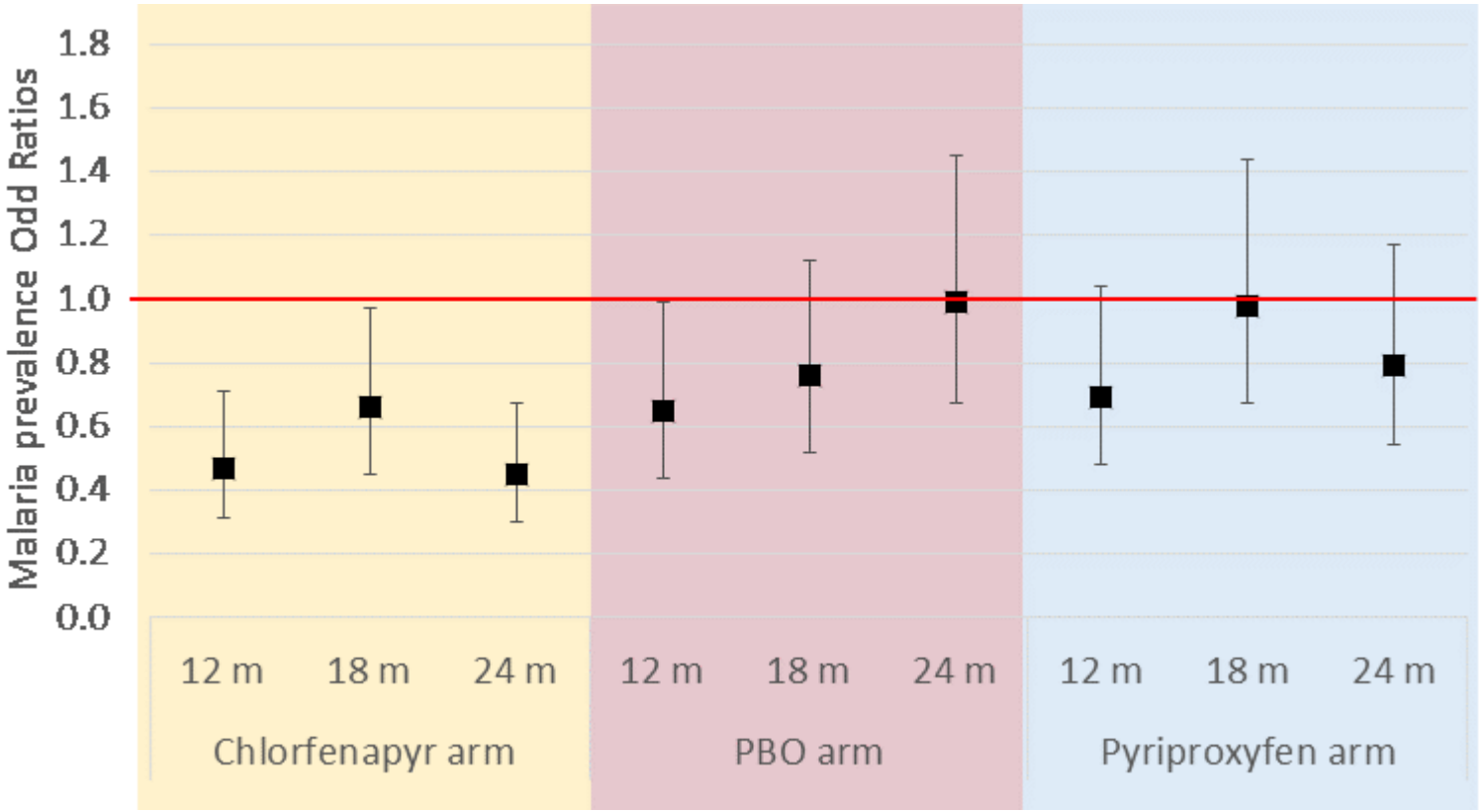
- Malaria infection prevalence, SES, LLIN use, population density and species composition similar in all 4 arms.
- Vector density and EIR higher in Std LLIN and Pyriproxyfen arm

Study net usage and all net usage in all age groups



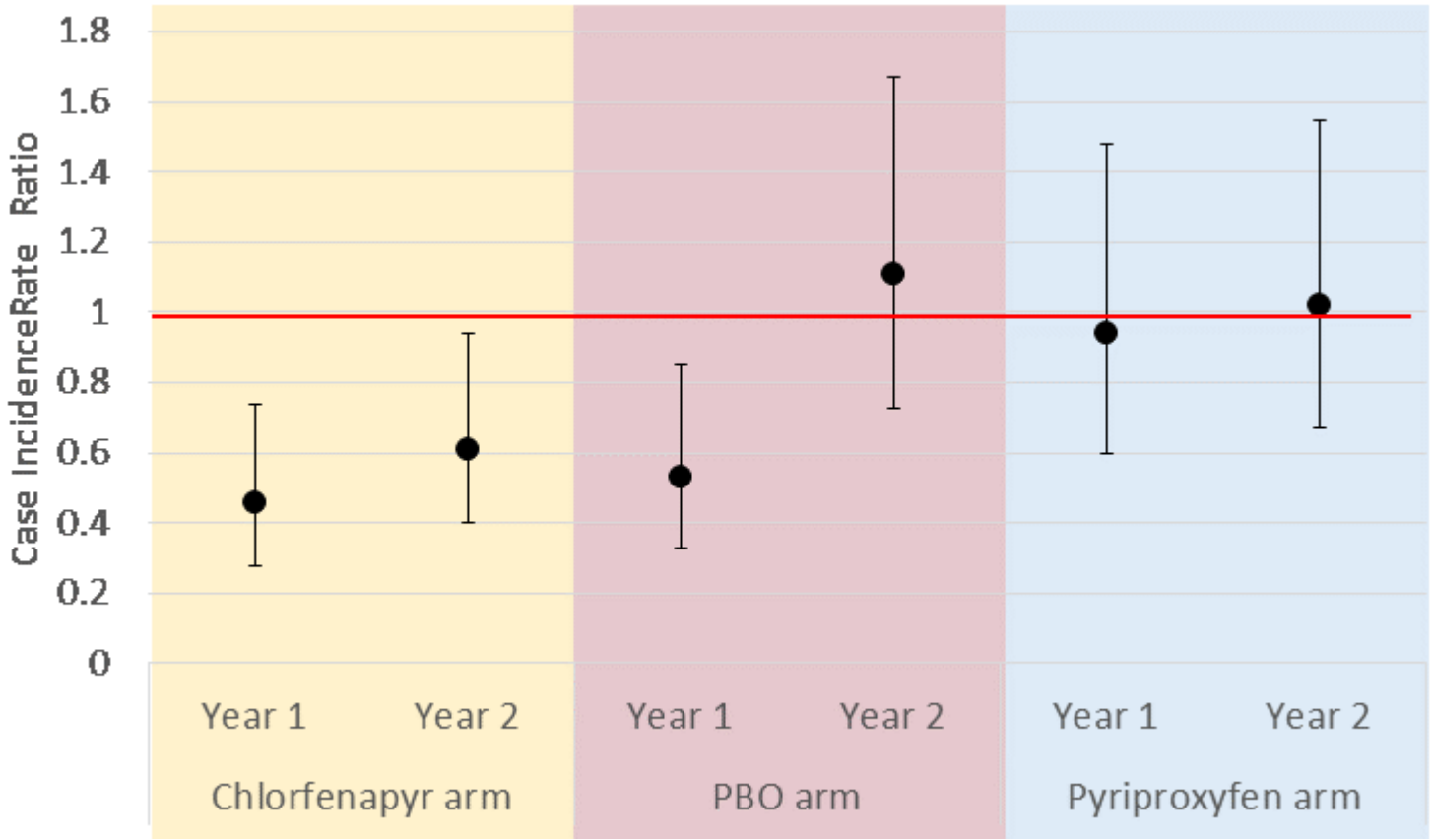
- Overall net usage increase from 60% to 80% after the net distribution and remain constant
- 3 months after distribution study net usage was between 69% and 77% but rapidly decrease to 30% to 50% at 24 months.
- PBO net usage decrease the most drastically followed by Pyriproxyfen net.

Infection prevalence (Intention to treat)



- Significant reduction in prevalence at 24 months (main end point) was only observed for Chlorfenapyr arm.
- Reduction in prevalence observed in all the intervention arms at 12 months compared to std LLIN arm but borderline for PBO and Pyriproxyfen.

Case incidence



- Overall 44% reduction in malaria incidence in children 6 months to 10 years residing in Chlorfenapyr arm compare to those in standard LLIN arm (not in table)
- 47% reduction in Malaria case incidence in year 1 in children residing in PBO arm compared to those in standard LLIN and no reduction in year 2.
- No significant reduction in malaria incidence in the Pyriproxyfen arm compared to standard LLIN.

Entomological cross sectional survey

CDC light traps

- randomly selected houses per cluster in 84 clusters (21 clusters per arm)
- Each cluster visited every quarter (32 house-CDC nights collection per cluster per year)
- Questionnaire (ODK) and direct observations
- SES, house design, LLINs coverage

Mosquito processing and molecular analysis

Morphological IDs on Anopheles species, physiological status

- Sporozoite rate: CSP ELISA on a sub-sample (10 Anopheles per species per HH) from CDC light traps
- PCR for species ID on samples confirmed positive with Plasmodium falciparum and three extra from each surveyed cluster

Resistance monitoring: Intensity bioassays

Collection

- Unfed gravid adult mosquitoes
- 1-2 clusters/intervention

Pyrethroid resistance intensity bioassays

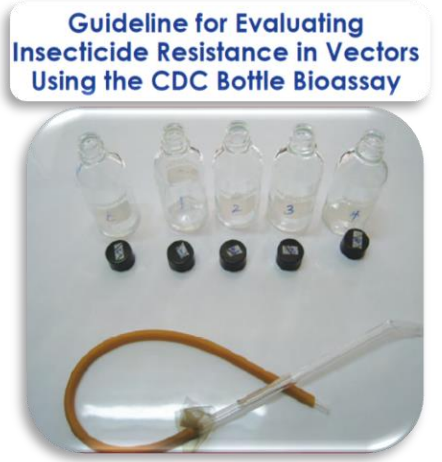
- Doses 1X, 2X, 5X and 10X for alpha-cypermethrin and permethrin
- Exposure for 30 minutes

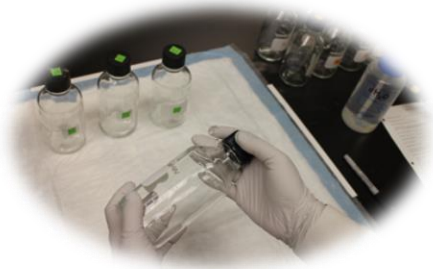
Chlorfenapyr resistance bioassays

- Dose used (100ug/bottle)
- Kisumu colony (NIMR-Mwanza) run in parallel as a positive control
- Exposure for 60 minutes
- Mortality after 72 hours

Synergist assay

- Pre-exposure for 1 hour to synergist and for another 30 minutes exposure to permethrin





Resistance monitoring: Pyriproxyfen intensity bioassays

- CDC bottle bioassays for pyriproxyfen (100ug/bottle)
- Freshly blood-fed F_0 mosquitoes
- Exposure for 1hr
- Exposed mosquitoes held for 3 days, before ovarian dissection
- Ovaries examined microscopically and egg stages (I-V) scored
- Kisumu colony (*An. gambiae* s.s from NIMR-Mwanza) run in parallel as a positive control

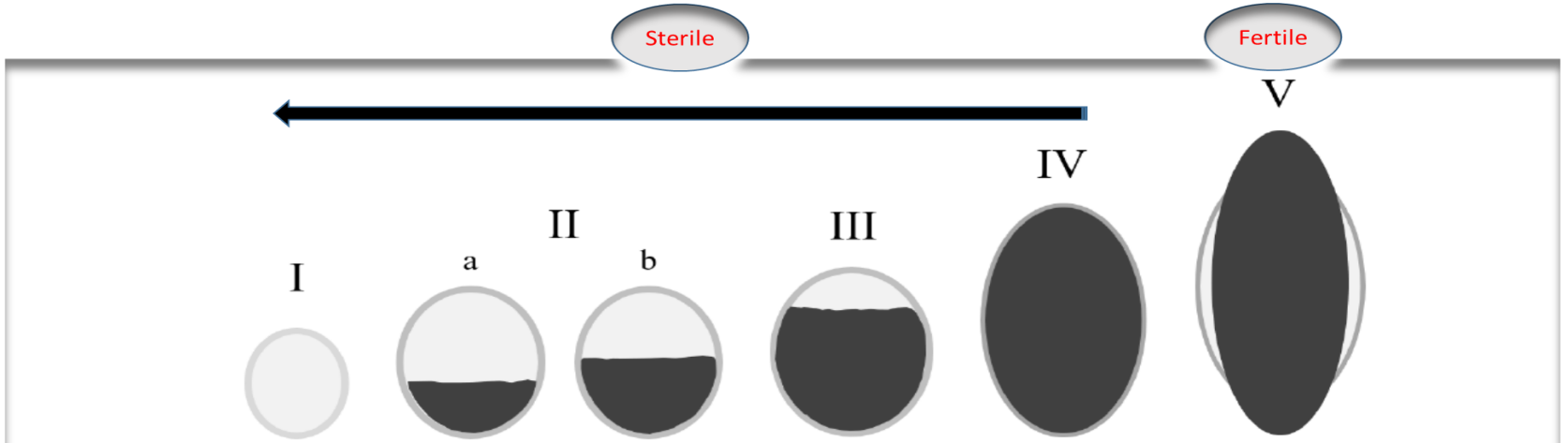


Figure 1. Christophers' Stages of Egg Development. Stage I: the primary follicle. Stage II: a) the follicle gains yolk protein, b) the follicle is approximately half comprised of yolk. Stage III: the follicle fills with yolk. Stage IV: the follicle elongates. Stage V: complete maturation of the egg, with floats.

Anopheles density & Entomological inoculation rate

	Density / night/ HH	DR*	95% CI	p value**	EIR / night/ HH	DR*	95%CI	p value**
Year 1								
Std LLIN arm (ref)	2.5	1			0.04	1		
Chlorfenapyr arm	0.7	0.33	0.19-0.58	<0.001	0.00	0.1	0.02-0.40	0.002
PBO arm	0.7	0.42	0.24-0.73	0.002	0.01	0.3	0.09-0.79	0.017
Pyriproxyfen arm	1.2	0.62	0.36-1.08	0.090	0.02	0.5	0.19-1.20	0.118
Year 2								
Std LLIN arm (ref)	7.6	1			0.09	1		
Chlorfenapyr arm	6.0	0.51	0.32-0.83	0.006	0.02	0.2	0.08-0.40	<0.001
PBO arm	4.7	0.64	0.40-1.03	0.068	0.06	0.7	0.34-1.31	0.243
Pyriproxyfen arm	9.1	0.9	0.56-1.44	0.656	0.10	0.8	0.40-1.67	0.580

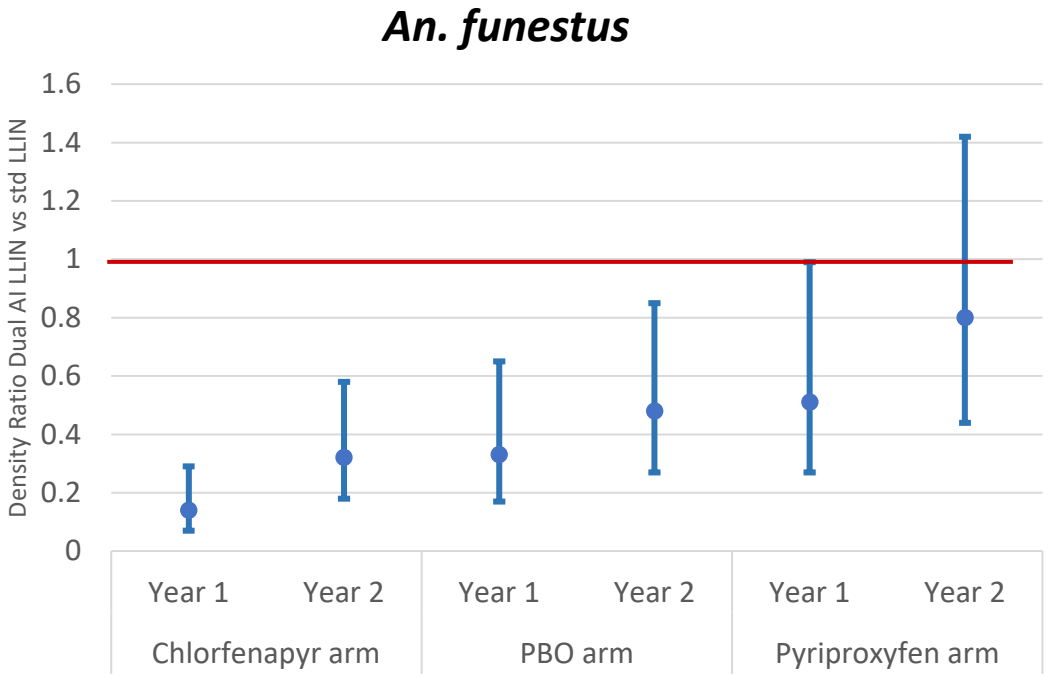
*adjusted for the baseline prevalence well as the other covariates used in the randomisation procedure
**P-value <0.017 is considered statistically significant after Bonferroni correction

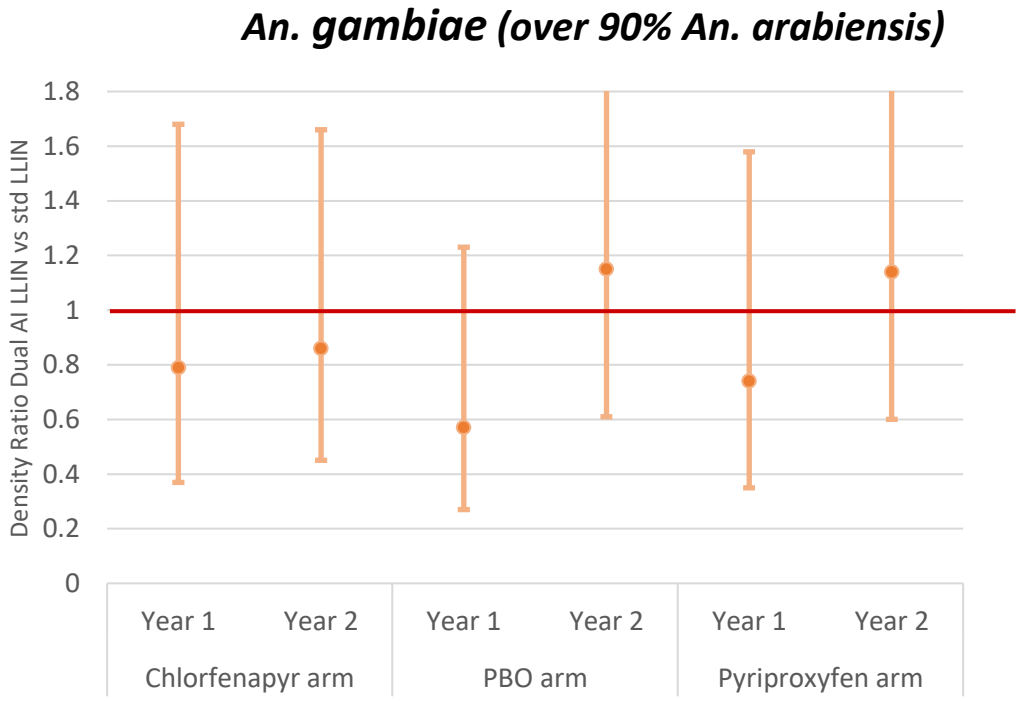
Compared to standard LLIN:

- Chlorfenapyr Net, 57% (density) and 85% (EIR) overall (year 1 and 2) reduction
- PBO net, 46% (density) and 44% (EIR) reduction
- Pyriproxyfen net, 23% (density) and 27% (EIR) reduction.

Reduction in EIR mainly driven by density except for Chlorfenapyr net (Sporozoite rate 0.8% vs 1.8% (std LLIN) OR: 0.48 (95%CI: 0.24–0.95), p value: 0.035)

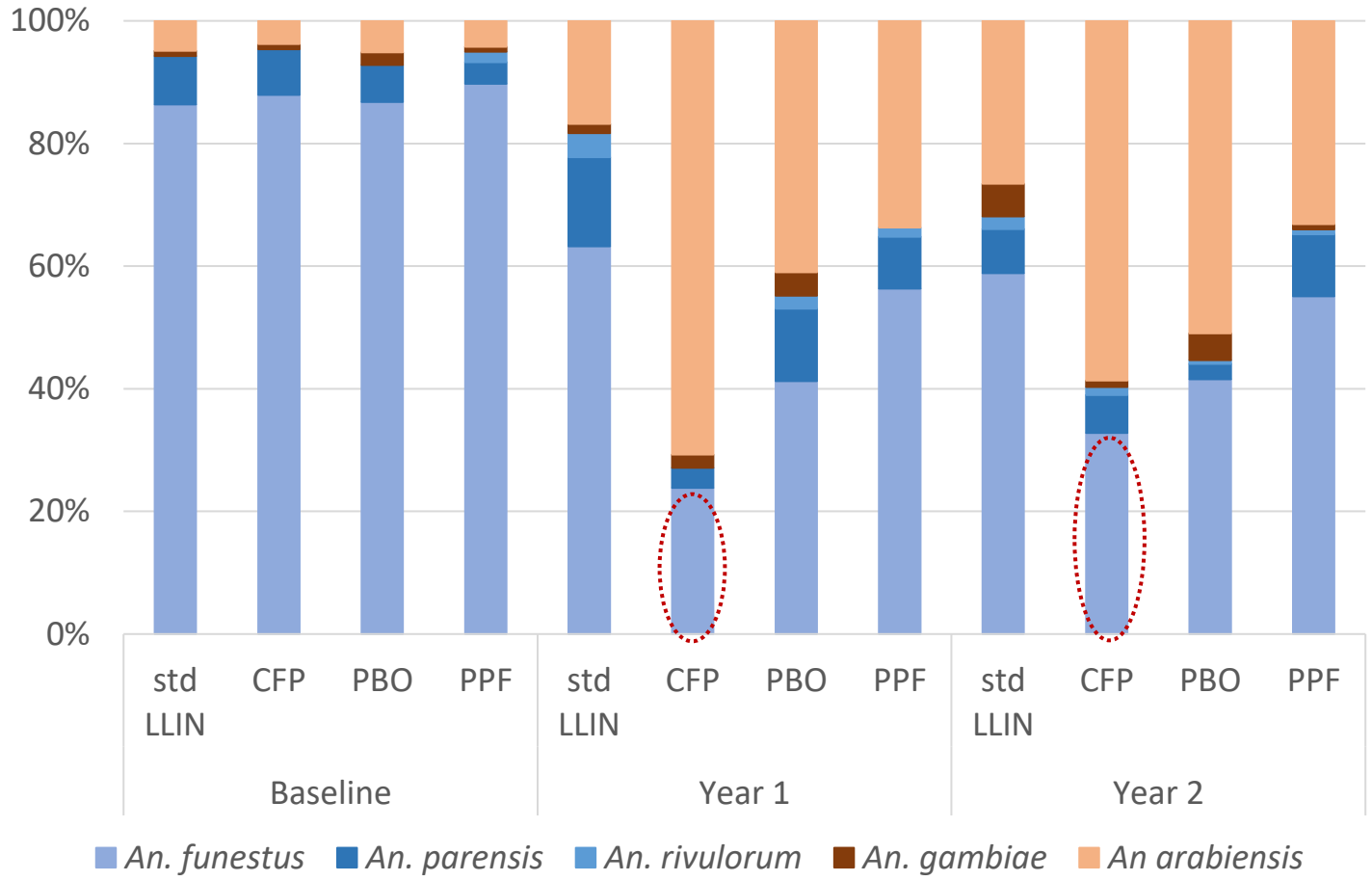
An. funestus and *An. gambiae* density reduction





- *An. funestus* well controlled by Chlorfenapyr and PBO net over 2 years and Pyriproxyfen net in year 1.
- The impact of the dual ai nets was not significant on *An. gambiae* s.l (mainly *An. arabiensis*)

Species composition



- *An. funestus* is the main vector in the study area
- Relative proportion of *An. funestus* vs *An. gambiae* is lower in year 1 and 2 than baseline
- In year 1 and 2 proportion of *An. funestus* the lowest in the Chlorfenapyr arm

Insecticide resistance

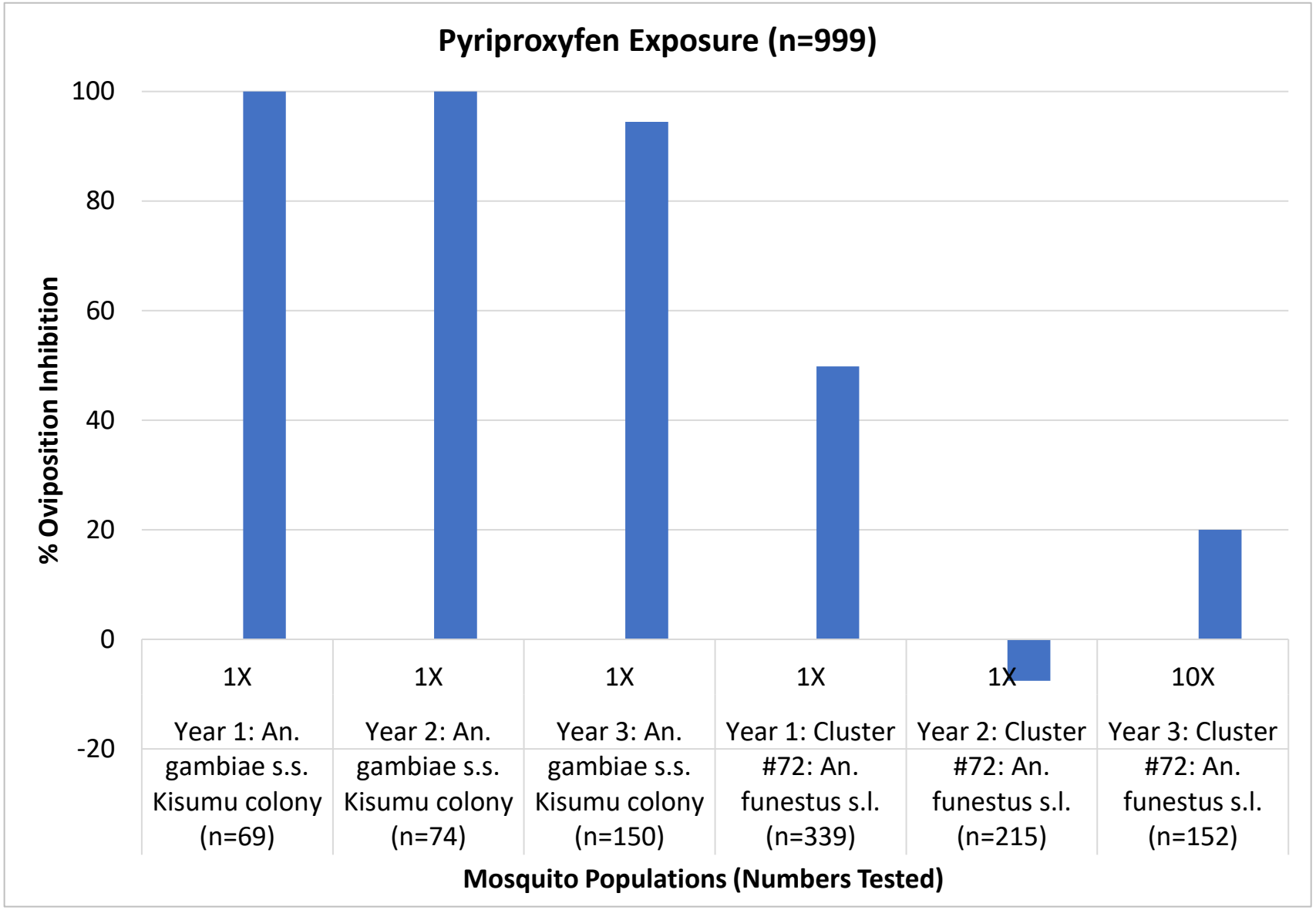
Baseline

- Alpha-cypermenthrin resistance: 43.7% *(An. funestus* s.l) to 59.4% (*An. gambiae* s.l)
- Permethrin resistance: 40% (*An. funestus* s.l) to 56.5% (*An. gambiae* s.l)
- *Kdr*-East fixed in *An. gambiae* s.s and CYP6M2, CYP6P3, CYP6P4 and CYP9K1 were modestly upregulated

Post-intervention

- Increase in PY resistance in *An. funestus* s.l. in all arms except in IG2:
  - Average mortality 71.7% and 84.8% after exposure to 10 x diagnostic concentration of alpha-cypermethrin and permethrin
- Decrease in PBO synergism in all intervention arms except IG2
- High level of chlorfenapyr susceptibility to the tentative diagnostic doses (99.8% *An. funestus* s.l.)

Pyriproxyfen resistance



- Oviposition inhibition to PPF was moderate to low in year 1 and absent by year 2 and 3
- Investigation ongoing to assess pyrethroid and PPF cross resistance mechanisms
  - CYP6M2, CYP6P2, CYP6P3, CYP6P5, CYP6Z2 and CYP9JS previously shown to metabolise PPF in vitro
  - CYP6P3, CYP6P4 and CYP9K1 (previously identified in Muleba), overexpressed in *An. gambiae* s.s. in Misungwi during baseline

Conclusions

The **chlorfenapyr**-pyrethroid treated net (Interceptor G2), is more effective than standard pyrethroid-only LLINs over 2 years of use.

Effect on incidence and entomological outcome lower in year 2

- Decrease net usage (textile durability)
- 80% reduction of chlorfenapyr content

The trial confirmed the superior effectiveness of **PBO**-pyrethroid nets (Olyset plus) compared with standard pyrethroid LLINs, but over a limited period of 12 months.

- Rapid drop in Olyset plus usage could partly explain the lack of effectiveness in year 2.
- Poor textile durability of the nets is likely to be the main factor of usage reduction.

Pyrethroid treated nets with **pyriproxyfen** (Royal Guard), did not provide significant additional protection against malaria compared with standard pyrethroid-only LLIN.

- Low bio-efficacy of PPF could explain the results as well as decrease net usage over time and/or PPF resistance in the main malaria vector *An. funestus*.

Acknowledgements

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- Jacklin Martin (Textile durability and bio-efficacy)
- Manisha Kulkarni (Ecological niche model)
- Charles Thickstun (Map and cluster delineation)

Collaborators

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- PORALG
- RMO Mwanza, DMO Misungwi, Malaria Focal Person, DC Misungwi, DED and CHMT representatives
- Community in Misungwi
- Field technicians

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