CONFIDENTIAL BUSINESS INFORMATION

Vector Health International Ltd
Africa Technical Research Centre
P.O. Box 15500, Arusha, Tanzania

Request title: Persistence tests of insecticide formulations on static In2Care EaveTube netting.

Purpose(s):

a. To assess the mosquitocidal efficacy of freshly treated static EaveTube netting with insecticide powder formulations against *Anopheles gambiae* (Kisumu strain)
b. To validate and compare the mosquitocidal efficacy of the insecticide-treated netting samples (that performed well at T_0) kept inside EaveTubes under field conditions for up to 18 months.

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Summary

We evaluated the efficacy of EaveTube netting treated with α-cypermethrin, β-cyfluthrin, Clothianidin, Chlorofenapyr, Delta filler 1, and Delta filler 2 against susceptible *An. gambiae* (Kisumu strain). The samples were exposed in a semi-field condition and bio-efficacy tests conducted at 3 months interval from month Zero up eighteen (18) months post installation. The efficacy in terms of knockdown lasted up to 18 months for all the AI's except for Clothianidin and Chlorofenapyr that showed very low efficacy at 18 months. Similar trends were also observed in mortality in which Clothianidin and Chlorofenapyr recorded decline in mortality from 6 months post installation all through to 18 months. Freshly treated nets exposed to mosquitoes at T_0 were also more efficacious compared to the ones exposed over time. In overall, the results from this study demonstrate that multiple of the AI's used in this technology can persist and remain efficacious overtime under semi field condition.

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1.0 Introduction

There has been significant progress in malaria control across the globe due to diverse approaches and technologies under implementation. Control of malaria transmitting mosquitoes mainly rely on two methods namely; Long-Lasting Insecticidal Nets (LLIN) and Indoor Residual Spraying (IRS). The development of new tools and insecticides that successfully target disease transmitting mosquitoes are vital to sustainable vector control.

In addition to LLINs and IRS, house improvements have shown great potential in minimizing the mosquitoes biting rates. The ventilation openings between the wall and roof the so called "eaves" have been the preferred entry point for *Anopheles* mosquitoes, and these openings have been recognized by WHO as the target sites for *Anopheles* mosquito control. The objective of this study was to evaluate the efficacy of In2Care EaveTube netting treated with the insecticides against susceptible *Anopheles* mosquitoes under semi-field environment.

In2Care EaveTubes comprise ventilation tubes with removable gauze inserts that are placed in the wall under the roof of houses where they attract malaria mosquitoes at night, block them from entering the house, and contaminate them with a lethal dose of insecticide. EaveTubes are being reviewed by the WHO as a new malaria vector control paradigm. In collaboration with In2Care, the Africa Technical Research Centre (ATRC) conducted a pilot study on EaveTube netting efficacy in Tanzanian for a period of 18 months. EaveTube netting uses In2Care's patented electrostatic coating technology to bind insecticides and transfer high doses to mosquitoes that make short contact with the netting. This high-dose transfer was shown to be very effective against highly resistant mosquito strains, enabling effective kill of pyrethroid-resistant vectors with existing pyrethroids. The static netting can also be used as a vehicle for various other active ingredients.

2.0 Material and methods

The netting materials were treated with five different insecticides namely, α-cypermethrin, β-cyfluthrin, Clothianidin, Chlorofenapyr, Deltamethrin (with filler 1, and filler 2). The details of the treatments are presented in table 1 below

Table 1: details of treated samples evaluated during the study

Sample name	Insecticide	Filler type	AI Dose	Number of samples
α-cyp	α-cypermethrin (5% w/w)	1	200 mg AI / m^2	20
β-cyfl	β-cyfluthrin (5% w/w)	1	200 mg AI / m^2	20
Chlorf	Chlorfenapyr (5% w/w)	1	200 mg AI / m^2	20
Clothianidin	Clothianidin (5% w/w)	1	200 mg AI / m^2	20
Delta_Filler 1	Deltamethrin (5% w/w)	1	200 mg AI / m^2	20
Delta_Filler 2	Deltamethrin (5% w/w)	2	200 mg AI / m^2	20

2.1 Test wall

The treated samples (14 per treatment) were fixed on EaveTubes and affixed on a Test wall constructed by AtoZ and deployed in ATRC from 27th June 2019 to 26th December 2020. A simple test wall with roof as indicated in the picture below, (Figure 1) was used in the study. The sample arrangement is provided in table 2. The Test wall comprised 108 EaveTubes with 20 cm long 6-inch diameter PVC pipes. The test wall was placed in the ATRC compound to facilitate on-site field exposure and persistence testing. The outdoor placement exposed the test samples to relevant levels of dust under semi field conditions in Arusha, Tanzanian.

Figure 1: Test wall for exposing EaveTube nettings in a semi-field condition within ATRC compound.

Table 2: Positions of the samples on the test wall

samples	α-cyp		β-cyfl		Chlorf		Clothianidin		Delta_Filler 1		Delta_Filler 2	
T18	13	14										
T15	11	12										
T12	9	10										
T9	7	8										
T6	5	6										
T3	3	4										
T0	1	2										

*14 Samples per treatment in the test wall and two per treatment at each time point

2.2 Mosquito bioassays

Bio-efficacy tests were conducted every 3 months (T_0, T_3, T_6, T_9, T_{12}, T_{15},T_{18}), 2 netting samples (unused in previous test) per formulation were retrieved from the test wall and 2 clean netting samples from the lab (control) for WHO cone bioassays. The findings of the experiments conducted in Tanzania indicated that host-seeking anophelines contact the netting in EaveTubes in the field for only 2-3 minutes, thus this study adopted 3-minute WHO standard cone bioassays as a test method to evaluate efficacy of EaveTube samples against mosquitoes.

Three 3 - 6 day old unfed female mosquitoes *Anopheles gambiae s.s.* (Kisumu strain) from ATRC insectary were used in this study.

A total of 25 mosquitoes were exposed per sample in a cone placed on the 20 x 20 cm netting sample in the WHO-standard cone bioassay set-up during each testing period and ensuring mosquito body got contact with the netting sample within the 3 minutes of exposure.

After exposure, each group of 25 mosquitoes were placed in a clean holding cup and knockdown rates were scored at 1 hour post-exposure. The mosquitoes were then provided with access to sugar solution and mortality scored from one to seven days post-exposure exposure of the mosquitoes as per WHO cone bioassay evaluation. Mortality >80% in this study was considered to be good mosquitocidal activity of the treatments. The tested netting pieces were placed back in the wall immediately after testing.

2.3 Preparation of samples and concentration for chemical analysis

The working standards for all the active ingredients (AI's) were diluted from the stock solution as presented in the table 3 below

Table 3: Dilution and preparation of working concentration

STOCK mg/ml	mL from stock	Total Volume	Concentration mg/ml
0.5	2.4	20	0.06
0.5	1.6	20	0.04
0.5	0.8	20	0.02
0.5	0.4	20	0.01
0.5	0.2	20	0.05

Samples of 2x2 cm were cut from the treated netting, weighed, and cut into small pieces for extraction. The samples were extracted with 10 ml of organic solvent in 20 ml vials by sonication for 1 hour and filtered through 0.45 TFE membrane. A volume of 1 µl was injected into a Shimadzu GC for alpha-cypermethrin and a volume of 5 µl was injected into HPLC for the rest of the AI's.

3.0 Results

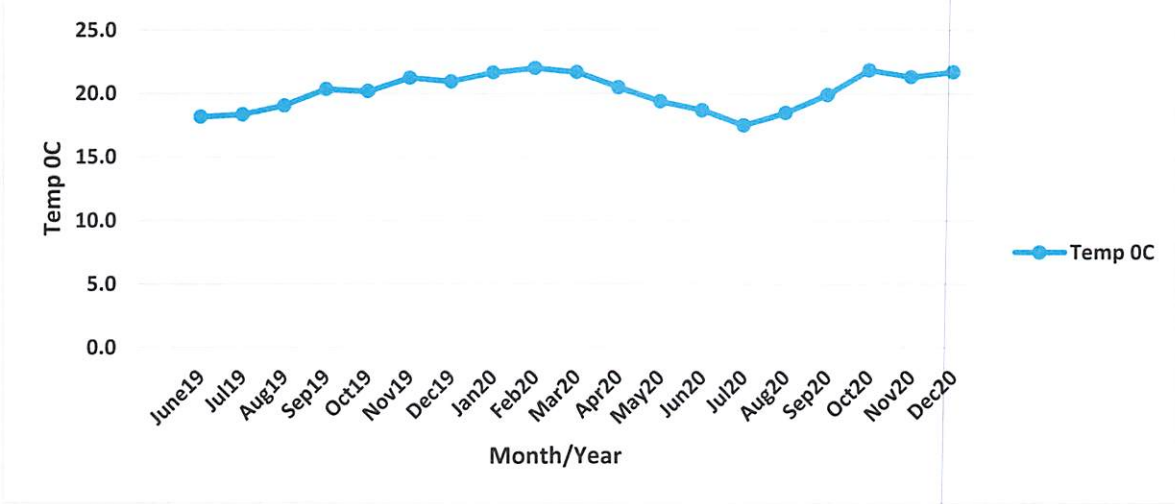
3.1 Temperature and relative humidity

Temperature and humidity were recorded during the period of the study as presented in figure 1 below.

Overall, there was minimal variation in relative humidity and temperature across the 18 months of the

study implementation. Temperature ranged between 18-23 ˚C in 2019 and 18-21˚C in 2020 while relative humidity was 59-89% in 2019 and 66-88% in 2020

a) Temperature



b) Relative humidity

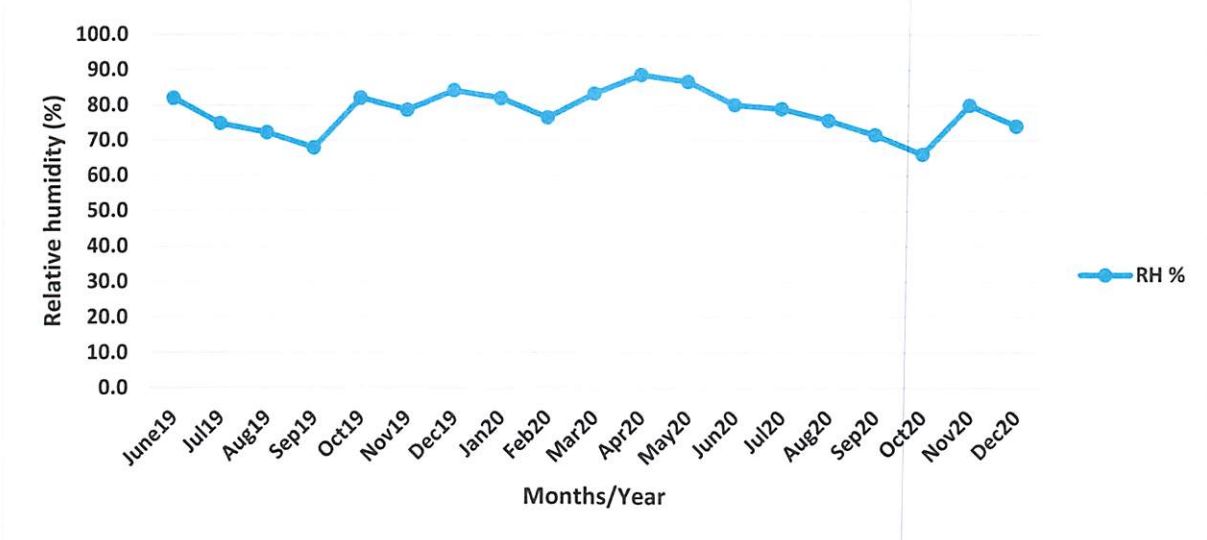


Figure 1: a) Temperature and b) Relative humidity recorded during the period of the study

3.2 Bio-efficacy of EaveTube nettings against susceptible *An. gambiae* (Kisumu strain)

The results for biological evaluations done for both freshly treated EaveTube netting pieces at time T_0 and the exposed treated EaveTube nettings from time T_3 to T_{18} are presented below.

3.2.1 Mosquitocidal efficacy of freshly treated static EaveTube netting with insecticide powder formulations against *Anopheles gambiae* (Kisumu strain).

At time T_0, the freshly treated nets had high efficacy (>90%) in terms of knockdown (KD) among EaveTube nettings treated with α-cypermethrin, β-cyfluthrin, Chlorofenapyr, Delta filler 1, and Delta filler 2 against susceptible *An. gambiae* (Kisumu strain). However, for clothianidin, KD was below 20% (Figure 2a). Mortality recorded over seven days post exposure at time T_0 was 100% at 24 hours post exposure for EaveTube netting treated with α-cypermethrin, β-cyfluthrin, Chlorofenapyr, Delta filler 1, and Delta filler 2. Clothianidin attained 100% mortality at 4 days post exposure while 100% mortality in EaveTube netting treated with Chlorofenapyr was attained at 3 days post exposure (Figure 2b).

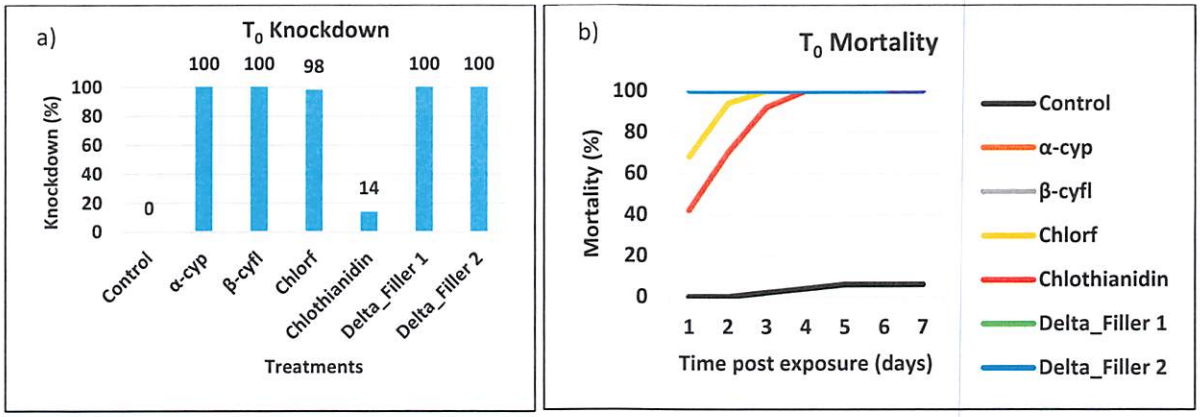


Figure 2: a) Knockdown (KD) and b) Mortality of *Anopheles gambiae* (Kisumu strain) exposed to EaveTube nettings freshly treated with different active ingredients (AI)

3.2.2 Validation and comparison of the mosquitocidal efficacy of the insecticide-treated netting samples (that performed well at T_0) kept inside EaveTubes under field conditions for up to 18 months

At 18 months under semi-field condition, KD results showed all the treatments that performed well at T_0 maintained higher efficacy except for chlorofenapyr that recorded a decline to below 20%. As expected, no KD was recorded in the treatment with clothianidin (Figure 3a). Mortalities induced by α-cypermethrin, β-cyfluthrin, Delta filler 1, and Delta filler 2 showed similar trend of efficacy. They all performed optimally and recorded mortality above 90% two days post exposure of the mosquitoes to the nettings. On the other hand, both Clothianidin and Chlorofenapyr recorded low mortalities of 76% and 72%, respectively and this was only attained at 7 days post exposure of the mosquitoes to the treatments (Figure 3b).

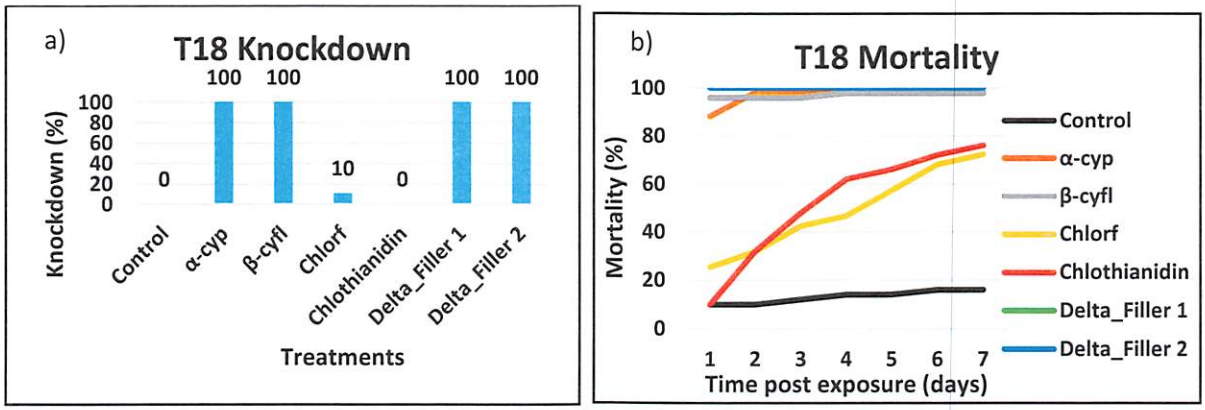
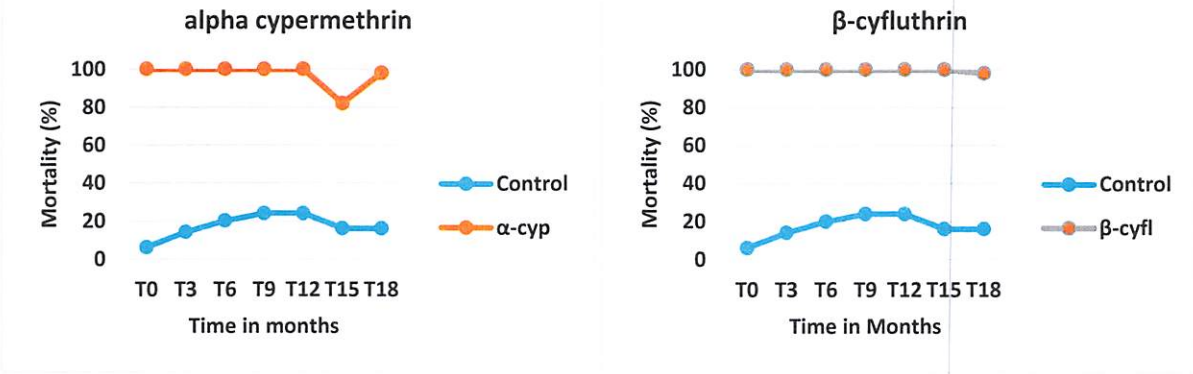


Figure 3: a) Knockdown (KD) and b) Mortality of *Anopheles gambiae* (Kisumu strain) exposed to treated EaveTube nettings at 18 moths post installation into the EaveTubes in a semi field conditions

3.2.3 Seven-day mortality trend from time T_0 to time T18

The trend recorded over time T_0 to time T_{18} for 7- day mortality showed that over the entire period, nettings treated with α-cypermethrin, β-cyfluthrin, Delta filler 1, and Delta filler 2 had higher efficacy than Clothianidin and Chlorofenapyr. The result also showed that Chlorofenapyr was the least efficacious treatment among all of them (Figure 4).



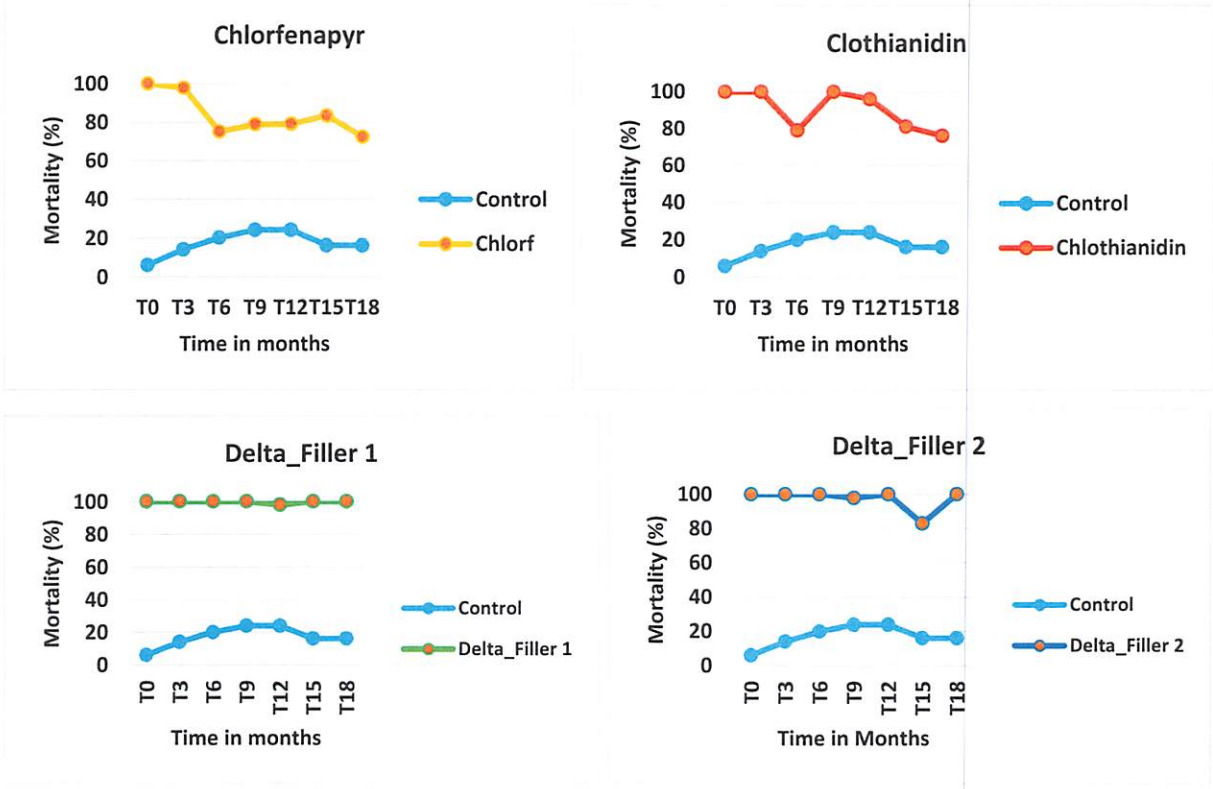


Figure 4: Seven-day post exposure mortality of *Anopheles gambiae* (Kisumu strain) exposed EaveTube nettings treated with different AI's from time T_0 to T18 moths post installation of the nettings in the EaveTubes in a semi field condition

3.3 Residual content of active ingredients in the nettings at 18 months (T_{18})

For alpha-cypermethrin, clothianidin and deltamethrin filler 1, about half of the starting amount of AI was still remaining after 18 months. The amount of betacyfluthrin, chlorfenapyr and deltamethrin filler 2 remaining was 24.5 mg/m^2, 1.6 mg/m^2 and 5.4 mg/ m^2 corresponding to 18 %, 1.1% and 15.7 % of the original amount. The results are summarized in figure 5 and table 3

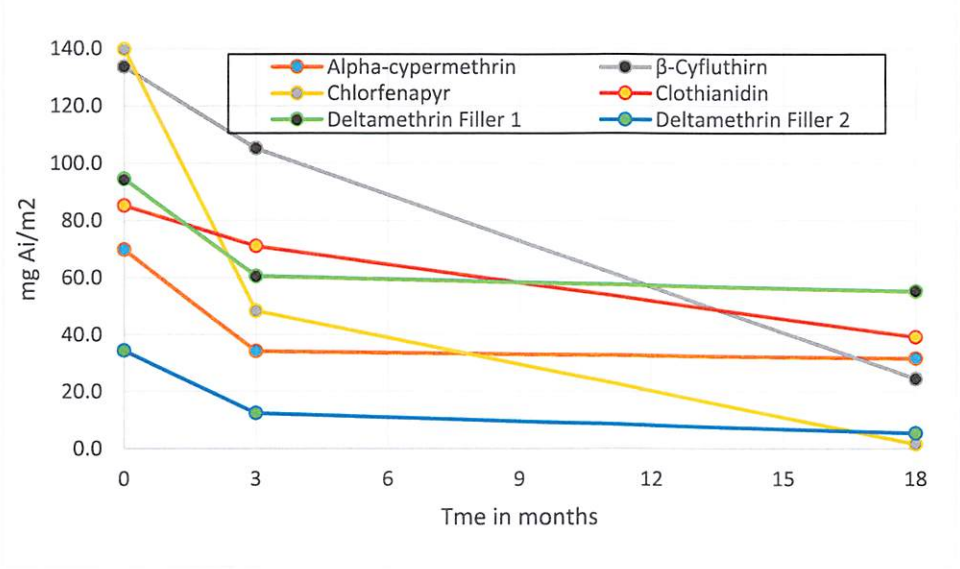


Figure 5. Residual AI content in EaveTube netting samples after 18 months trial at ATRC

Table 3: Amount of AI in mg/m2 and percentage remaining on EaveTube after 18 months exposure

Sample Name	Content mg/m^2			Percentage AI remaining
	0 Months	3 Months	18 Months	
Alpha-cypermethrin	69.6	34.1	31.7	45.5
β-Cyfluthirn	133.6	105.1	24.5	18.3
Chlorfenapyr	139.6	48.4	1.6	1.1
Clothianidin	85.0	70.9	39.2	46.1
Deltamethrin Filler 1	94.2	60.4	55.2	58.6
Deltamethrin Filler 2	34.3	12.3	5.4	15.7

4.0 Discussion and Conclusion

The efficacy of EaveTube nettings treated with α-cypermethrin, β-cyfluthrin, Delta filler 1, and Delta filler 2 persisted under semi-field conditions for up to 18 months post installation of the EaveTubes. This was demonstrated by their high efficacy (>90% kill) against susceptible *An. gambiae* (Kisumu strain) at T18 when the study was terminated. However, efficacy of Clothianidin and Chlorofenapyr declined at three months post installation indicating potential loss of the AI on the surface of the nets. Although the percent mortality kept on fluctuating over time, perhaps due to variation in mosquito behaviour during the test, or levels of dust on net surface, it was notable that the efficacy among the active ingredients that performed high across the months remained so at most testing points. From the chemical analysis results, it was notable that chlorfenapyr was a poor candidate for treatment of EaveTube netting as it does not persist over time.

In conclusion, the persistence of multiple of the AI's on the netting materials for over 18 months demonstrate that this technology may play a key role in controlling mosquito bites by minimize the entry of mosquitoes into houses and subsequently contribute to the control of malaria.

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