

Bio-efficacy 5% Deltamethrin EaveTube insert

Materials and Methods

To test the long-lasting bio-efficacy of EaveTubes static charged netting insert, one insert was treated with 5% Deltamethrin in Wageningen (The Netherlands) and transported to Bouake (Ivory Coast). Early 2018 the insert was placed inside an EaveTube installed at eave level in a wall on the east site of a house in Bouake (Ivory Coast).

After three months the insert was removed from the wall to test for bio-efficacy against wild-type resistant malaria mosquitoes. Field collected Yaokoffikro strain *Anopheles gambiae* s.s. reared by Institute Pierre Richet (IPR) in the Bouake laboratory were used for the test. For three minutes 17 to 20 mosquitoes were released in a transparent PVC Tube with the 5% Deltamethrin insert, and in a transparent PVC Tube with a control insert (without insecticides). Mosquitoes were exposed for 3 minutes and then transferred to clean holding cups to score 1-hour knockdown and 24-hour post-exposure mortality. For both the treated and un-treated insert, two replicates were executed with 17-20 unfed females (3-6 days old) per replicate. After the tests, the insert was placed back in the same EaveTube in the wall of the house.

After twelve months the same insert was removed from the wall and WHO cone bioassays were executed at IPR. Field collected Mbe strain *Anopheles gambiae* s.l. reared by Institute Pierre Richet in the Bouake laboratory were used for the tests. The mosquitoes were exposed to the 5% Deltamethrin insert, and to a clean control insert (without insecticides) which was not used before. Mosquitoes were exposed for 3 minutes. After exposure, the mosquitoes were transferred to clean holding cups to score 1-hour knockdown and 24-hour post-exposure mortality as per WHO protocol (WHO, 2011)¹. Both the tests with treated and un-treated insert were replicated eight times with 9-13 unfed females (3-6 days old) per replicate.

After the twelve months tests in Bouake (Ivory Coast), the insert was transported to the Research Institute for Sustainable Development in France (IRD). WHO cone bioassays were executed on 13th and 14th of May 2019. Pyrethroid resistant Kdr-Kis strain *Anopheles gambiae* s.s. reared by IRD were used for the trial. The mosquitoes were exposed to the 5% Deltamethrin insert, and to a clean control insert (without insecticides) which was not used before. Mosquitoes were exposed for 1 minute or for 3 minutes, and then transferred to clean holding cups to score 1-hour knockdown and 24-hour post-exposure mortality as per WHO protocol (WHO, 2011)¹. Both the tests with treated and un-treated insert, and 1 and 3 minutes were replicated eight times with 9-11 unfed females (3-5 days old) per replicate.

Data analysis

The 3-month mortality rate of the untreated insert was between 5% and 20%. Therefore, the mortality rate was adjusted with the Abbott's formula as indicated in the WHO Guidelines (WHO, 2011).

Knockdown and mortality data are represented as the Mean rate \pm Standard Deviation (SD) for the treated insert with deltamethrin 5% and untreated insert (without insecticides). The effect of the treatment knockdown and mortality rate was done using a two-way repeated measures ANOVA, using

¹ WHO (2011) Guidelines for Monitoring the Durability of Long-Lasting Insecticidal Mosquito Nets Under Operational Conditions (World Health Organization, Geneva) Available at www.who.int/malaria/publications/atoz/9789241501705/en/.

paired t-test with a Bonferroni post-hoc analysis in R (R Development Core Team, 2020) using the “rstatix” package. For all statistical analyses, the significant P value was set at 0.05 or less.

Results

Results of the bio-efficacy tests with the EaveTubes static charged netting insert treated with 5% Deltamethrin showed significant difference for knockdown and mortality rates for both 3- and 12-month timepoints on both test locations. Furthermore, knockdown and mortality rates showed 100% efficacy for both the 1-minute and 3-minute exposure ($p < 0.0001$) (See table 1 and 2; figure 1 and 2).

Almost all mortality rates were appropriately low for the clean control samples (<5 %) and all tests were sufficiently high (>95%) for the deltamethrin-treated insert, as per WHO efficacy requirements (WHO, 2011). Mosquito mortality rate measured in the control samples three months after installation was slightly above 5% and therefore adjusted with Abbott’s formula. Difference in mortality rate between treated and untreated samples was still significant ($p=0.001$).

Results show that the 1-year-old 5% Deltamethrin insert still killed 98,8% (SD \pm 3.5) of the highly resistant Mbe mosquitoes and 100% of the highly resistant Kdr-Kis mosquitoes ($p < 0.0001$).

Table 1. Mean knockdown rates (\pm standard deviation) of *Anopheles gambiae* mosquitoes after 1- and 3-minute exposure on 3-month and 12-month field-exposed static charged netting insert sample treated with 5% deltamethrin and control inserts (without insecticides). Trials executed at IPR and IRD, as shown in the table. N is number of mosquitoes.

Time tested after installation (months)	Research location	Exposure time (minutes)	<i>Anopheles gambiae</i> s.s. strain	N		Knockdown rate (%) \pm SD		P-value
				5% Deltamethrin	Untreated	5% Deltamethrin	Untreated	
3	IPR	3	Yaokoffikro	39	35	79.6 \pm 6.5	0	0.0370
12	IPR	3	Mbe	86	86	68.7 \pm 9.8	0	< 0.0001
12	IRD	3	Kdr-Kis	85	83	100 \pm 0.0	0	< 0.0001
12	IRD	1	Kdr-Kis	82	83	100 \pm 0.0	0	< 0.0001

Table 2. Mean mortality rates (\pm standard deviation) of *Anopheles gambiae* mosquitoes after 1- and 3-minute exposure on 3-month and 12-month field-exposed static charged netting insert sample treated with 5% deltamethrin and control inserts (without insecticides). Trials executed at IPR and IRD, as shown in the table. N is number of mosquitoes.

Time tested after installation (months)	Research location	Exposure time (minutes)	<i>Anopheles gambiae</i> s.s. strain	N		Mortality rate (%) \pm SD		P-value
				5% Deltamethrin	Untreated	5% Deltamethrin	Untreated	
3	IPR	3	Yaokoffikro	39	35	100 \pm 0.0 ⁱ	5.7 \pm 0.2	0.0010
12	IPR	3	Mbe	86	86	98.8 \pm 3.5	2.2 \pm 4.1	< 0.0001
12	IRD	3	Kdr-Kis	85	83	100 \pm 0.0	2.4 \pm 4.4	< 0.0001
12	IRD	1	Kdr-Kis	82	83	100 \pm 0.0	2.4 \pm 4.4	< 0.0001

i. Adjusted with Abbott’s formula.

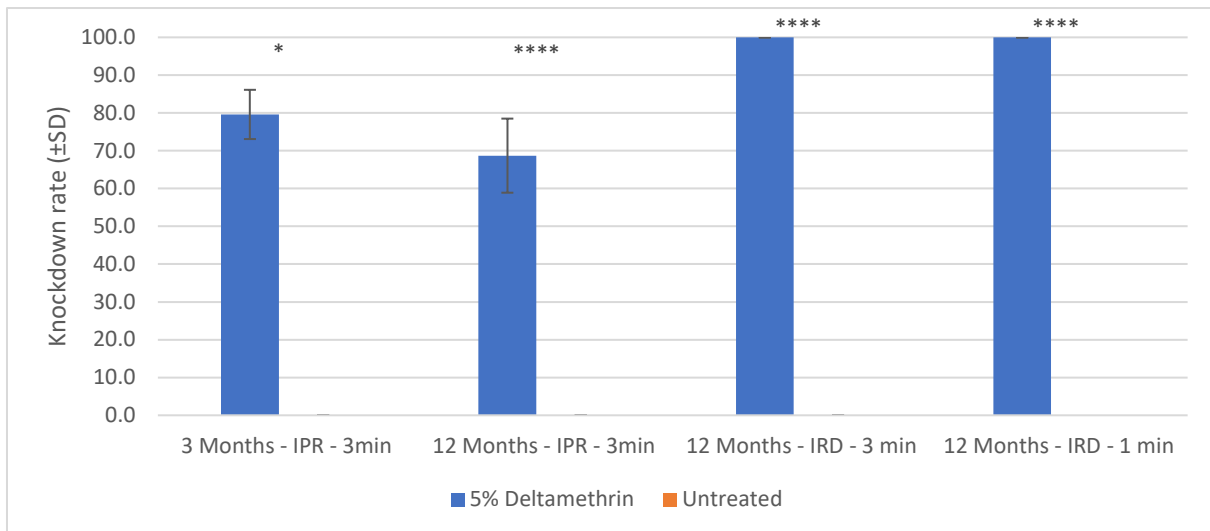


Figure 1. Mean knockdown rates (\pm SD) of *Anopheles gambiae* mosquitoes after 1- and 3-minute exposure on 3-month and 12-month field-exposed static charged netting insert sample treated with 5% deltamethrin and control inserts (without insecticides). Trials executed at IPR and IRD, as shown in the figure. The significance level per test moment is indicated above the graph as * when $P < 0.05$, and as **** for $p < 0.0001$.

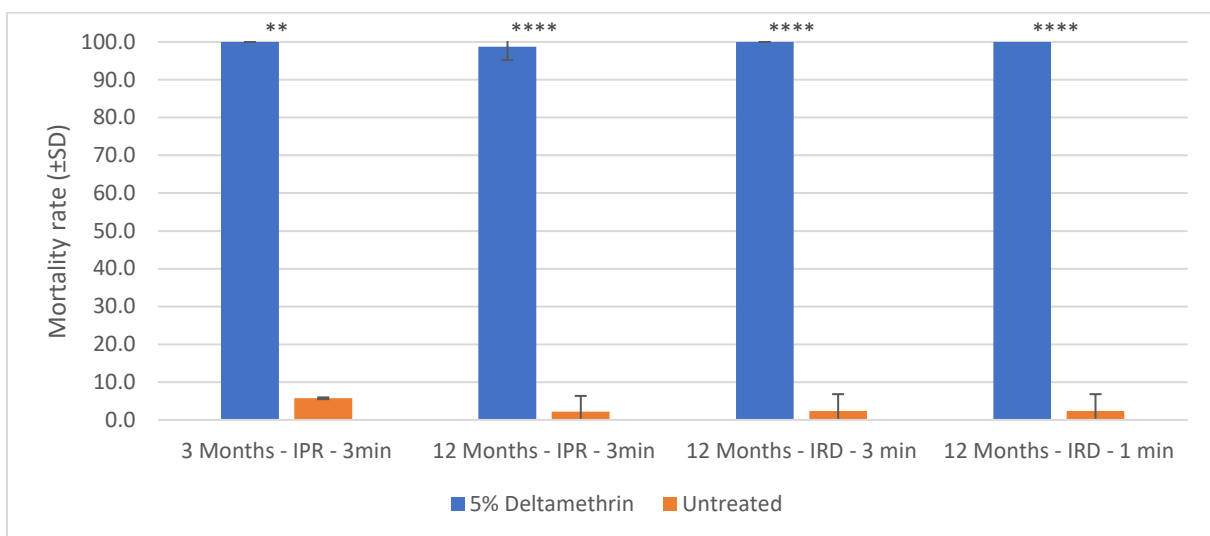


Figure 2. Mean mortality rates (\pm SD) of *Anopheles gambiae* mosquitoes 24 hours after 3-minute exposure on 3-month and 12-month field-exposed static charged netting insert sample treated with 5% deltamethrin and control inserts (without insecticides). Trials executed at IPR and IRD, as shown in the figure. The significance level per test moment is indicated above the graph as ** for $P < 0.001$, and as **** for $p < 0.0001$.

Discussion

Although only one field exposed insert treated with 5% Deltamethrin was used for the tests, high mosquito knockdown and mortality after 12 months was confirmed by two independent laboratories. Both 1-minute and 3-minute exposure showed high mortality of pyrethroid resistant Mbe and Kdr-Kis strain *Anopheles gambiae* s.s. mosquitoes. These results show that the transfer of deltamethrin powder by the static charged netting (that is used in EaveTubes), retains its effectiveness even after 12 months of field exposure. The long-lasting 5% Deltamethrin insecticide formulation will enable EaveTube insert retreatment once per year, compared to the 4-monthly retreatment of beta-cyfluthrin inserts used in a trial in Cote d'Ivoire (Sternberg et al. 2021)². Lower frequency of insert retreatment will significantly benefit the cost-effectiveness of future interventions with EaveTubes.

² Sternberg ED, Cook J, Alou LPA, Assi SB, Koffi AA, Doudou DT, et al. Impact and cost-effectiveness of a lethal house lure against malaria transmission in central Côte d'Ivoire: a two-arm, cluster-randomised controlled trial. *Lancet*. 2021;397: 805–815. doi:10.1016/S0140-6736(21)00250-6