

Reviewing the evidence for and against selection of specific pyrethroids for programmatic purposes - Supplementary tables and figures

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Section 2. Molecular evidence

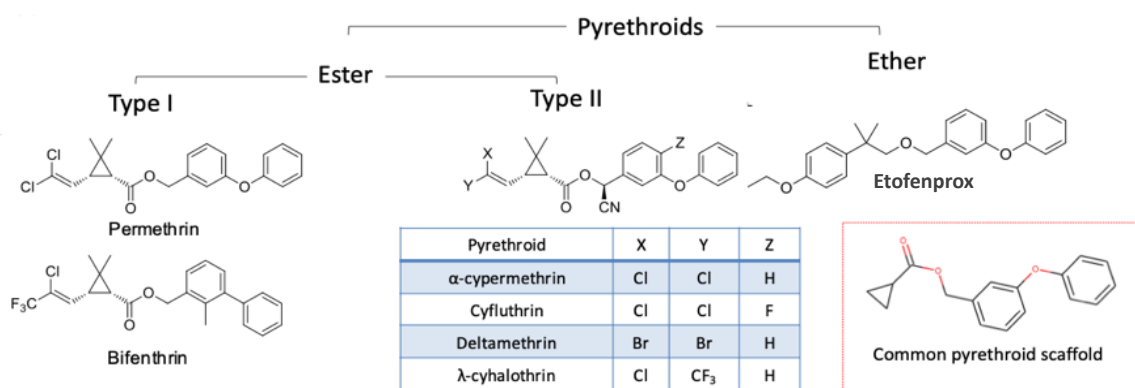


Figure S2.1. Chemical structure of pyrethroid insecticides evaluated by the WHO pesticide evaluation scheme (WHOPES) (Hougard et al., 2003). Common scaffold (JZAVZQLBHZEGQ-UHFFFAOYSA-N) of pyrethroids boxed in red identified from searching 230 million compounds available in ZINC database (<https://zinc.docking.org>).

Section 3. Lab strains

Deltamethrin dose response curves

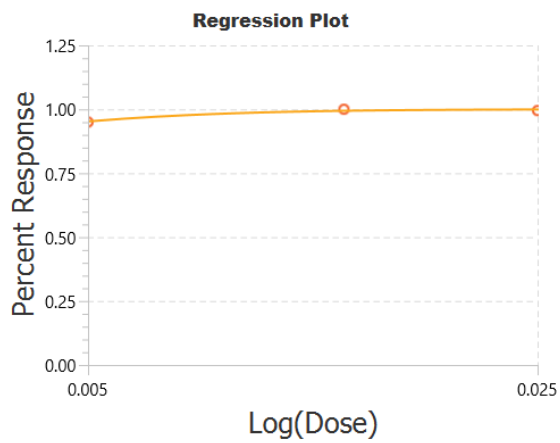
A) CDC *An. albimanus* Teco



B) Cardiff *An. gambiae* G3



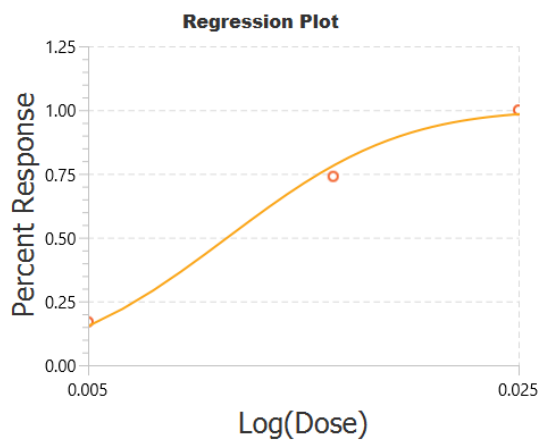
C) LIN *An. gambiae* G3



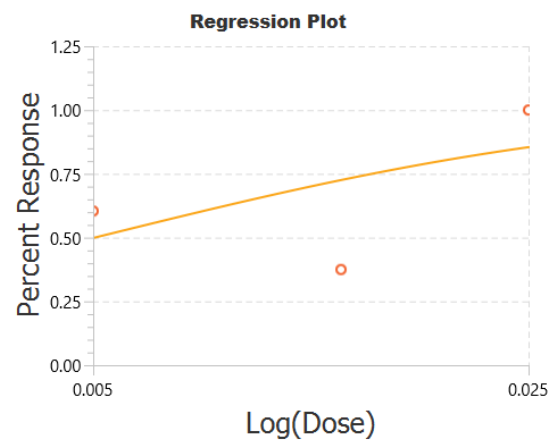
D) Mali *An. gambiae* Mopti



E) CDC *An. stephensi* Delhi



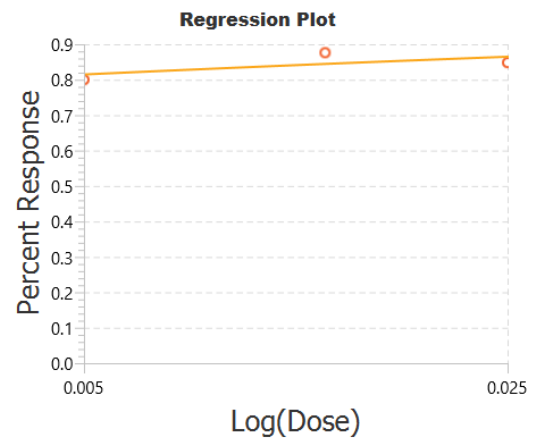
F) Cardiff *An. stephensi* St



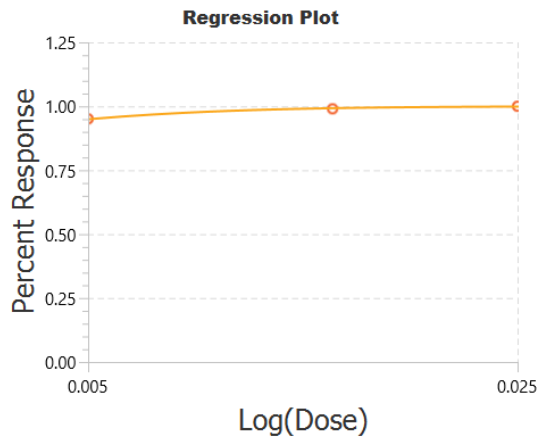
G) Cardiff *An. stephensi* Beech



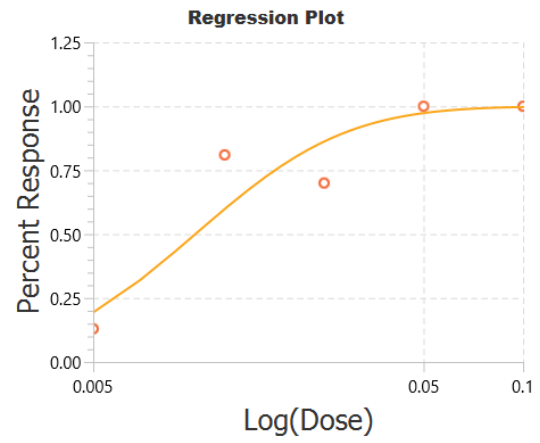
H) LSHTM *An. stephensi* Beech



I) Iran *An. stephensi* Beech



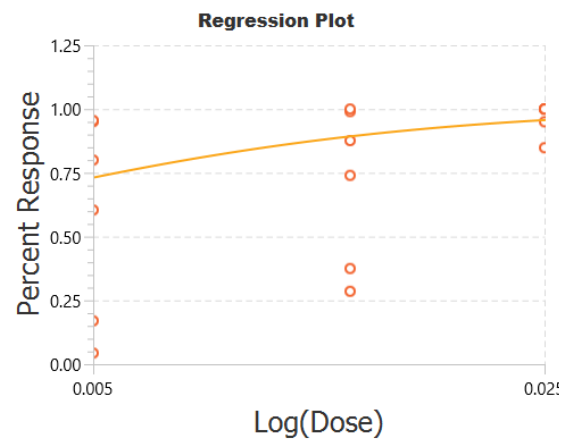
J) Pooled *An. albimanus*



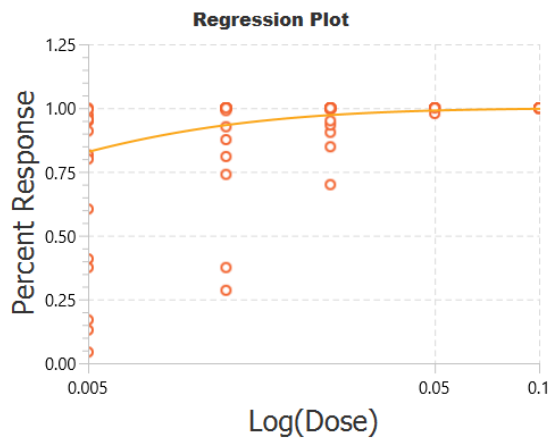
K) Pooled *An. gambiae*



L) Pooled *An. stephensi*

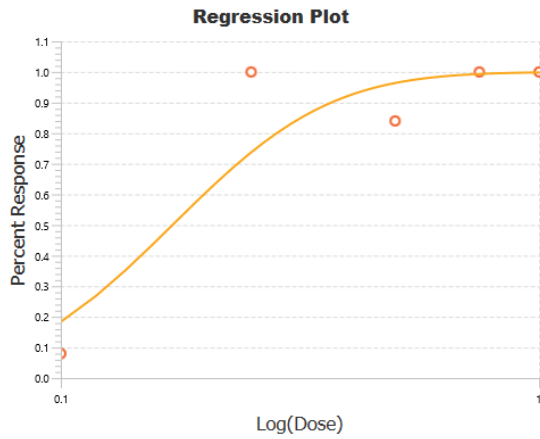


M) Deltamethrin pooled all data

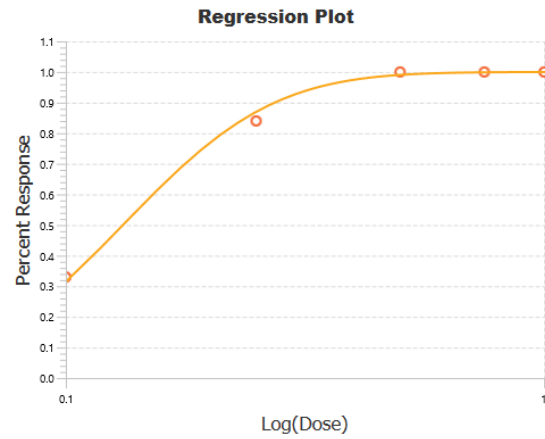


Permethrin dose response curves

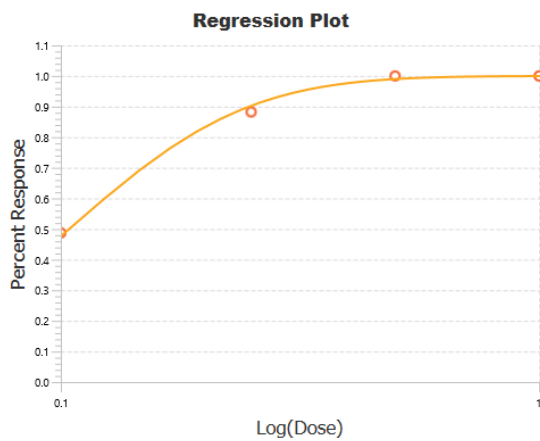
N) CDC *An. albimanus* Teco



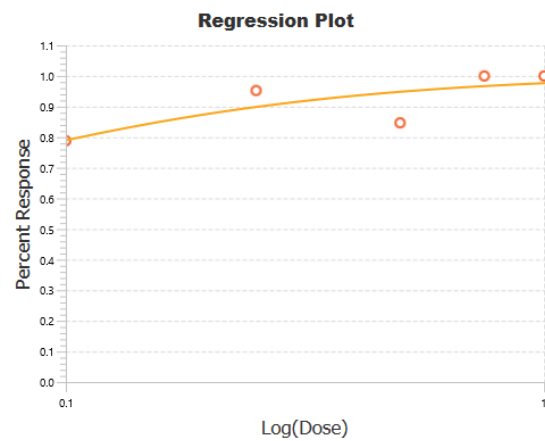
O) CDC *An. gambiae* G3



P) LIN *An. gambiae* G3



Q) LSHTM *An. gambiae* Kwa



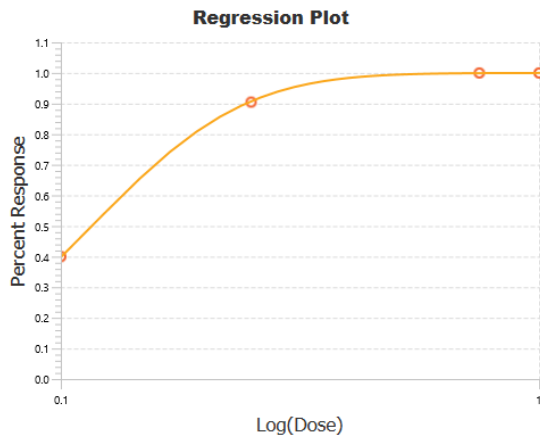
R) Mali *An. gambiae* Mopti



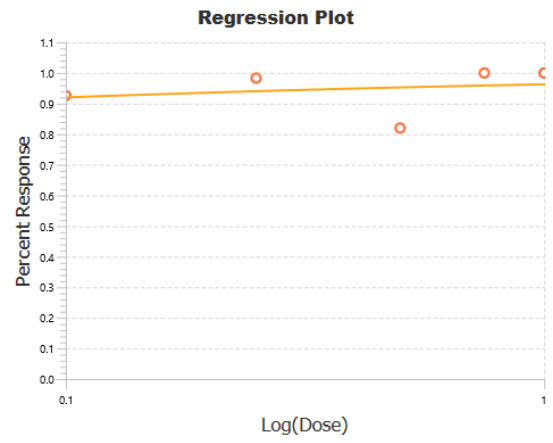
S) CDC *An. stephensi* Delhi



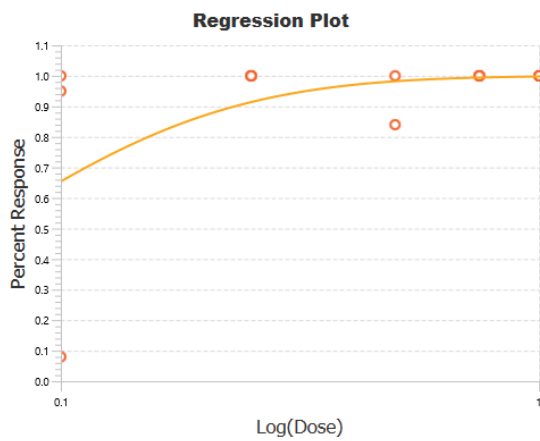
T) Cardiff *An. stephensi* Beech



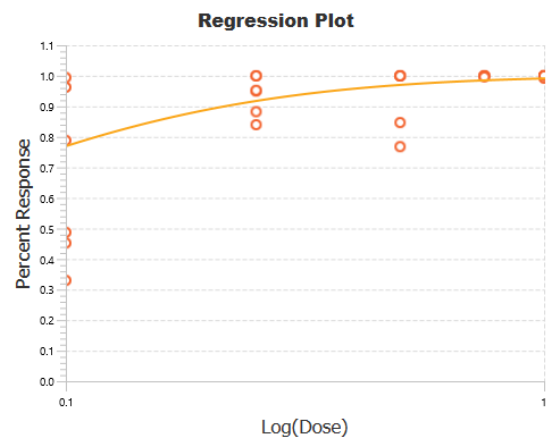
U) LSHTM *An. stephensi* Beech



V) Pooled *An. albimanus*



W) Pooled *An. gambiae*



X) Pooled *An. stephensi*



Y) Permethrin pooled all data

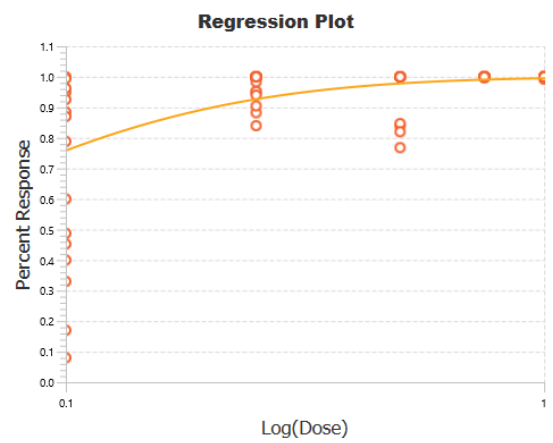


Figure S3.1. Dose response curves for deltamethrin (A – M) and permethrin (N – Y) using original raw data from the 1998 WHO multicentre study.

Table S3.1. Probit analysis of deltamethrin and permethrin using original raw data from the 1998 WHO multicentre study. Analysis was conducted using PoloJR program within PoloSuite (Ver 2.1). The discriminating dose is twice the LD₉₉. Abbreviations: LD = Lethal dose, DD = Discriminating dose.

Institute	Species	Strain	Insecticide	No. concentrations test	Total Mosquitoes exposed	LD ⁹⁵	LD ⁹⁵ 95% CI	LD ⁹⁹	LD ⁹⁹ 95% CI	DD
Deltamethrin										
CDC	<i>An. albimanus</i>	Teco	Deltamethrin	5	500	0.04	0.017 - 0	0.07	0.025 - 0	0.14
Cardiff	<i>An. albimanus</i>	Panama	Deltamethrin	4	160	LD matrix not calculated	-	-	-	-
LSHTM	<i>An. albimanus</i>	Mexico	Deltamethrin	5	562	LD matrix not calculated	-	-	-	-
CDC	<i>An. gambiae</i>	G3	Deltamethrin	5	500	LD matrix not calculated	-	-	-	-
Cardiff	<i>An. gambiae</i>	G3	Deltamethrin	5	184	0.02	?	0.04	0.013 - 0.001	0.08
LIN	<i>An. gambiae</i>	G3	Deltamethrin	3	900	0	?	0.01	?	0.2
LSHTM	<i>An. gambiae</i>	Kwa	Deltamethrin	5	533	LD matrix not calculated	-	-	-	-
Mali	<i>An. gambiae</i>	Mopti	Deltamethrin	5	1525	0.02	0.013 - 0.061	0.08	0.038 - 1.042	0.16
LIN	<i>An. gambiae</i>	Kisumu	Deltamethrin	4	1400	LD matrix not calculated	-	-	-	-
CDC	<i>An. stephensi</i>	Delhi	Deltamethrin	3	300	0.02	?	0.03	?	0.06
Cardiff	<i>An. stephensi</i>	St	Deltamethrin	3	120	0.06	?	0.17	?	0.34
Cardiff	<i>An. stephensi</i>	Beech	Deltamethrin	3	128	0.03	?	0.04	?	0.08
LSHTM	<i>An. stephensi</i>	Beech	Deltamethrin	3	317	1.72	?	347.81	0.015 - 0.008	695.62

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Iran	<i>An. stephensi</i>	Beech	Deltamethrin	3	604	0.01	?	0.01	?	0.02
India	<i>An. stephensi</i>	Delhi	Deltamethrin	2	525	Excluded < 3 concentrations	-	-	-	-
<i>Species pooled</i>										
Pooled	<i>An. albimanus</i>	Pooled	Deltamethrin	-	-	0.03	0.013 - 22.116	0.06	0.022 - 10109.5039	0.12
Pooled	<i>An. gambiae</i>	Pooled	Deltamethrin	-	-	0.01	0.001 - 0.015	0.03	0.016 - 7.617	0.06
Pooled	<i>An. stephensi</i>	Pooled	Deltamethrin	-	-	0.02	0.01 - 0.001	0.06	0.018 - 0	0.12
<i>All pooled</i>										
Pooled	Pooled	Pooled	Deltamethrin	-	-	0.02	0.01 - 0.055	0.05	0.023 - 1.166	0.1
Permethrin										
CDC	<i>An. albimanus</i>	Teco	Permethrin	5	500	0.46	0.167 - 0.059	0.69	0.265 - 0.015	1.38
Cardiff	<i>An. albimanus</i>	Panama	Permethrin	4	160	LD matrix not calculated	-	-	-	-
LSHTM	<i>An. albimanus</i>	Mexico	Permethrin	5	504	LD matrix not calculated	-	-	-	-
CDC	<i>An. gambiae</i>	G3	Permethrin	5	500	0.34	0.275 - 0.457	0.5	0.38 - 0.76	1
Cardiff	<i>An. gambiae</i>	G3	Permethrin	4	162	0.25	0.242 - 0.258	0.36	0.34 - 0.372	0.72
LIN	<i>An. gambiae</i>	G3	Permethrin	4	1500	0.32	0.233 - 0.624	0.5	0.328 - 1.393	1
LSHTM	<i>An. gambiae</i>	Kwa	Permethrin	5	524	0.52	?	1.96	0.333 - 0.06	3.92
Mali	<i>An. gambiae</i>	Mopti	Permethrin	5	1425	0.52	?	0.04	?	0.08
LIN	<i>An. gambiae</i>	Kisumu	Permethrin	4	1400	LD matrix not calculated	-	-	-	-
CDC	<i>An. stephensi</i>	Delhi	Permethrin	5	500	0.26	0.252 - 0.264	0.33	0.321 - 0.34	0.66

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Cardiff	<i>An. stephensi</i>	St	Permethrin	4	160	LD matrix not calculated	-	-	-	-
Cardiff	<i>An. stephensi</i>	Beech	Permethrin	4	162	0.3	0.275 - 0.335	0.45	0.393 - 0.52	0.9
LSHTM	<i>An. stephensi</i>	Beech	Permethrin	5	522	0.42	?	26.16	?	52.32
Iran	<i>An. stephensi</i>	Beech	Permethrin	5	1063	LD matrix not calculated	-	-	-	-
India	<i>An. stephensi</i>	Delhi	Permethrin	2	500	Excluded < 3 concentrations	-	-	-	-
<i>Species pooled</i>										
Pooled	<i>An. albimanus</i>	Pooled	Permethrin	-	-	0.33	0.161 - 0	0.62	0.271 - 0	1.24
Pooled	<i>An. gambiae</i>	Pooled	Permethrin	-	-	0.36	0.218 - 1.518	0.93	0.445 - 21.446	1.86
Pooled	<i>An. stephensi</i>	Pooled	Permethrin	-	-	0.24	0.155 - 1.478	0.47	0.255 - 21.446	0.94
<i>All pooled</i>										
Pooled	Pooled	Pooled	Permethrin	-	-	0.32	0.233 - 0.544	0.73	0.452 - 2.067	1.46

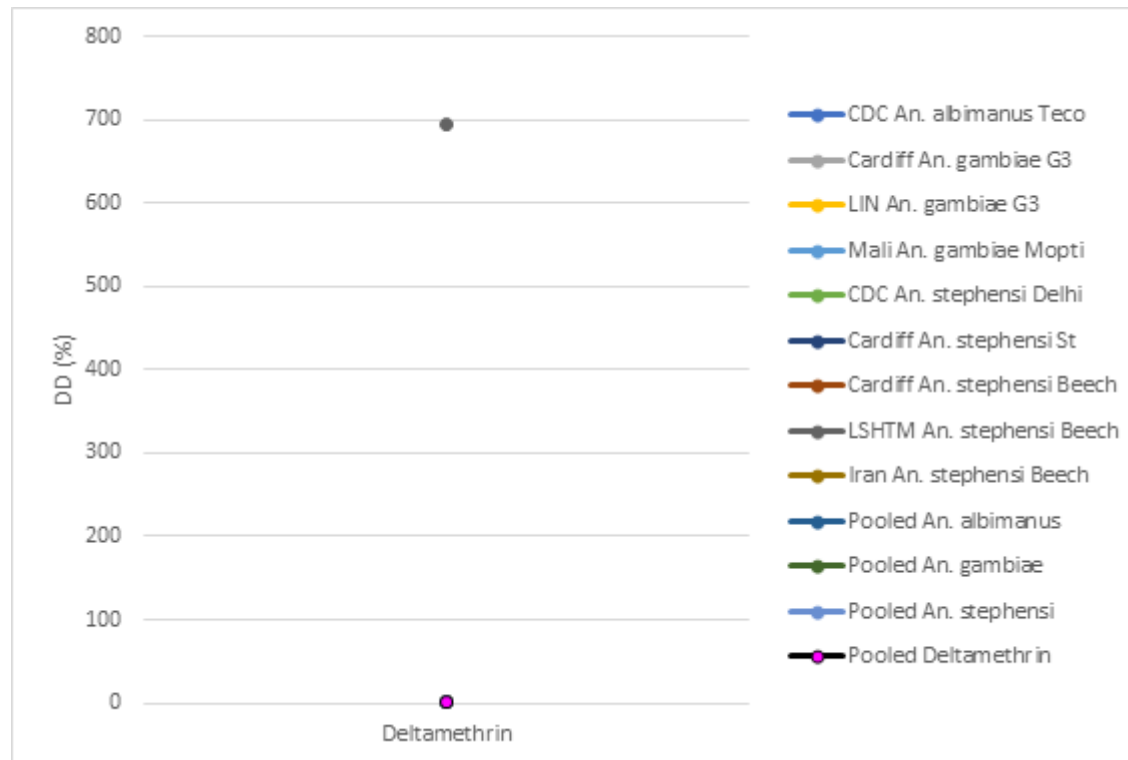


Figure S3.2. Calculated discriminating doses (%) for deltamethrin. Points show individual sites/strain combinations, and analysis pooled by species and overall. Datasets not robust enough to calculate lethal dose matrixes are excluded. Discriminating doses are set at 2 x the calculated lethal dose at which 99% (LD⁹⁹) of test mosquitoes were killed. The dashed red line represents current WHO-recommended DD for deltamethrin (0.05%).

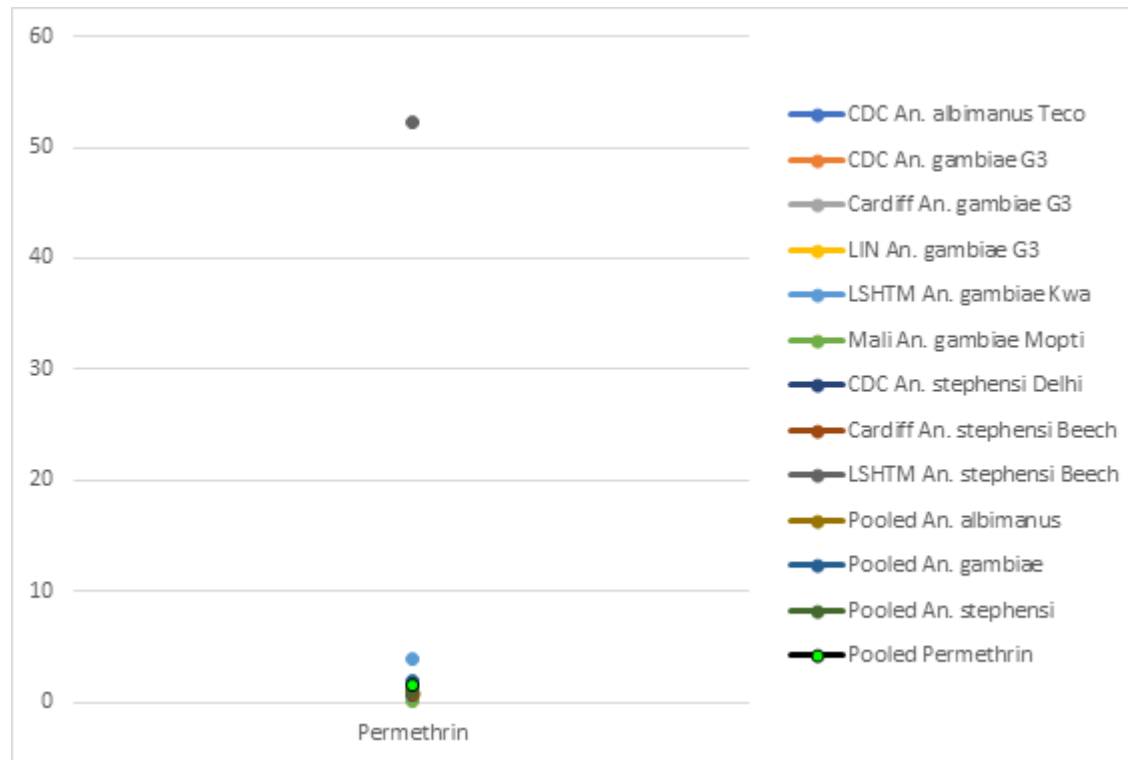


Figure S3.3. Calculated discriminating doses (%) for permethrin. Points show individual sites/strain combinations, and analysis pooled by species and overall. Datasets not robust enough to calculate lethal dose matrixes are excluded. Discriminating doses are set at 2 x the calculated lethal dose at which 99% (LD⁹⁹) of test mosquitoes were killed. The dashed red line represents current WHO-recommended DD for deltamethrin (0.75%).

Table S3.2. Summary statistics of variability in mosquito mortality following exposure to pyrethroids in standard WHO tube or CDC bottle bioassays. Mosquitoes were exposed to insecticides following the recommended methods for each test and mortality in each replicate tube or bottle was recorded 24-hours post-exposure. The strains detailed here are those maintained by the Ranson Group or LITE at LSTM, UK. Abbreviations: R = Insecticide resistant mosquito strain, S = Insecticide susceptible mosquito strain, IKR = inter-quartile range.

Rearing group	Assay	Insecticide	Strain	R/S	Data points	Low range	Upper range	IKR	Mean	Median	Variance	Standard Deviation
Ranson	Tube	α -cypermethrin	Kisumu <i>An. gambiae</i>	S	4	95.00	100.00	5.00	97.50	97.50	8.33	2.89
Ranson	Tube	Deltamethrin	Kisumu <i>An. gambiae</i>	S	4	100.00	100.00	0.00	100.00	100.00	0.00	0.00
Ranson	Tube	Permethrin	Kisumu <i>An. gambiae</i>	S	3	95.00	100.00	2.50	98.33	100.00	8.33	2.89
Ranson	Tube	α -cypermethrin	N'gouso <i>An. coluzzii</i>	S	8	90.00	100.00	5.00	97.50	100.00	14.29	3.78
Ranson	Tube	Deltamethrin	N'gouso <i>An. coluzzii</i>	S	4	100.00	100.00	0.00	100.00	100.00	0.00	0.00
Ranson	Tube	Permethrin	N'gouso <i>An. coluzzii</i>	S	8	84.21	100.00	0.00	98.03	100.00	31.16	5.58
Ranson	Tube	α -cypermethrin	Banfora-Sus <i>An. coluzzii</i>	S	4	92.00	100.00	5.00	95.00	94.00	14.67	3.83
Ranson	Tube	Deltamethrin	Banfora-Sus <i>An. coluzzii</i>	S	4	80.00	88.00	7.63	83.88	83.75	20.06	4.48
Ranson	Tube	Permethrin	Banfora-Sus <i>An. coluzzii</i>	S	4	68.00	92.31	13.14	79.39	78.62	114.05	10.68
Ranson	Tube	α -cypermethrin	Banfora M <i>An. coluzzii</i>	R	3	0.00	30.00	15.00	13.33	10.00	233.33	15.28
Ranson	Tube	Deltamethrin	Banfora M <i>An. coluzzii</i>	R	8	0.00	10.00	0.00	1.25	0.00	12.50	3.54
Ranson	Tube	Permethrin	Banfora M <i>An. coluzzii</i>	R	12	0.00	24.00	9.00	6.74	4.08	57.33	7.57
Ranson	Tube	α -cypermethrin	Bakaridjan	R	4	0.00	8.00	2.00	2.00	0.00	16.00	4.00

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			<i>An. gambiae</i>									
Ranson	Tube	Deltamethrin	Bakaridjan <i>An. gambiae</i>	R	8	0.00	7.69	4.81	2.40	0.00	12.42	3.52
Ranson	Tube	Permethrin	Bakaridjan <i>An. gambiae</i>	R	4	0.00	4.35	1.09	1.09	0.00	4.73	2.17
Ranson	Tube	α -cypermethrin	Gaoura <i>An. arabiensis</i>	R	4	0.00	8.00	2.00	2.00	0.00	16.00	4.00
Ranson	Tube	Deltamethrin	Gaoura <i>An. arabiensis</i>	R	8	0.00	4.00	4.00	2.00	2.00	4.57	2.14
Ranson	Tube	Permethrin	Gaoura <i>An. arabiensis</i>	R	8	0.00	12.00	2.00	2.50	0.00	22.57	4.75
Ranson	Tube	Deltamethrin	Tiefora <i>An. coluzzii</i>	R	8	0.00	7.69	4.00	2.46	2.00	8.39	2.90
Ranson	Tube	Permethrin	Tiefora <i>An. coluzzii</i>	R	8	0.00	7.69	4.38	3.09	3.92	8.00	2.83
Ranson	Tube	Deltamethrin	VK7 2014 <i>An. coluzzii</i>	R	4	4.17	13.04	3.72	8.80	9.00	13.85	3.72
Ranson	Tube	Permethrin	VK7 2014 <i>An. coluzzii</i>	R	4	0.00	8.33	5.65	5.06	5.95	16.42	4.05
Ranson	Tube	α -cypermethrin	Tiassale 13 <i>An. gambiae</i>	R	7	0.00	30.00	2.50	8.00	5.00	99.67	9.98
Ranson	Tube	Deltamethrin	Tiassale 13 <i>An. gambiae</i>	R	3	45.00	55.00	5.00	48.33	45.00	33.33	5.77
Ranson	Tube	Permethrin	Tiassale 13 <i>An. gambiae</i>	R	3	10.00	15.00	2.50	11.67	10.00	8.33	2.89
Ranson	Tube	α -cypermethrin	Fumoz <i>An. funestus</i>	R	4	0.00	4.00	1.00	3.00	4.00	4.00	2.00
Ranson	Tube	Deltamethrin	Fumoz <i>An. funestus</i>	R	24	0.00	16.67	8.08	4.68	4.00	22.06	4.70
Ranson	Tube	Permethrin	Fumoz <i>An. funestus</i>	R	6	0.00	28.00	3.07	6.62	3.85	113.40	10.65
LITE	Tube	α -cypermethrin	Fumoz <i>An. funestus</i>	R	4	0.00	10.00	3.10	5.08	5.16	16.78	4.10
LITE	Tube	Deltamethrin	Fumoz <i>An. funestus</i>	R	20	0.00	83.33	19.01	21.82	11.52	590.30	24.30
LITE	Tube	Permethrin	Fumoz <i>An. funestus</i>	R	23	0.00	86.21	42.67	25.24	9.52	847.92	29.12

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LITE	Tube	α -cypermethrin	Kisumu <i>An. gambiae</i>	S	4	100.00	100.00	0.00	100.00	100.00	0.00	0.00
LITE	Tube	Deltamethrin	Kisumu <i>An. gambiae</i>	S	12	95.24	100.00	1.04	98.89	100.00	4.02	2.01
LITE	Tube	Permethrin	Kisumu <i>An. gambiae</i>	S	12	96.14	100.00	0.00	99.68	100.00	1.24	1.11
LITE	Tube	α -cypermethrin	Moz <i>An. arabiensis</i>	S	4	88.00	100.00	6.26	95.91	97.83	32.03	5.66
LITE	Tube	Deltamethrin	Moz <i>An. arabiensis</i>	S	12	83.33	100.00	13.00	93.19	96.00	46.13	6.79
LITE	Tube	Permethrin	Moz <i>An. arabiensis</i>	S	12	92.00	100.00	4.00	97.67	100.00	10.06	3.17
LITE	Tube	α -cypermethrin	Tiassale 13 <i>An. gambiae</i>	R	8	4.00	12.50	5.13	8.13	7.42	10.61	3.26
LITE	Tube	Deltamethrin	Tiassale 13 <i>An. gambiae</i>	R	24	0.00	41.67	12.51	15.35	12.25	121.23	11.01
LITE	Tube	Permethrin	Tiassale 13 <i>An. gambiae</i>	R	25	0.00	62.50	25.00	15.97	12.00	256.38	16.01
LITE	Tube	α -cypermethrin	VK7 2014 <i>An. coluzzii</i>	R	4	0.00	8.00	2.11	3.89	3.78	10.69	3.27
LITE	Tube	Deltamethrin	VK7 2014 <i>An. coluzzii</i>	R	16	0.00	13.04	5.13	3.49	0.00	23.06	4.80
LITE	Tube	Permethrin	VK7 2014 <i>An. coluzzii</i>	R	27	0.00	8.33	0.00	1.38	0.00	7.98	2.83
LITE	Bottle	Permethrin	Fumoz <i>An. funestus</i>	R	20	0.00	100.00	50.44	46.92	42.24	1121.92	33.50
LITE	Bottle	Permethrin + PBO	Fumoz <i>An. funestus</i>	R	18	69.57	100.00	0.00	96.62	100.00	96.87	9.84
LITE	Bottle	Permethrin	Kisumu <i>An. gambiae</i>	S	20	4.00	100.00	1.67	93.58	100.00	456.84	21.37
LITE	Bottle	Permethrin + PBO	Kisumu <i>An. gambiae</i>	S	17	8.00	100.00	0.00	94.59	100.00	497.88	22.31
LITE	Bottle	Permethrin	Tiassale 13 <i>An. gambiae</i>	R	18	0.00	73.91	44.53	33.27	30.93	513.42	22.66
LITE	Bottle	Permethrin + PBO	Tiassale 13 <i>An. gambiae</i>	R	21	4.17	100.00	16.67	88.62	100.00	454.27	21.31
LITE	Bottle	Permethrin	VK7 2014	R	20	0.00	21.43	8.54	8.91	9.56	59.31	7.70

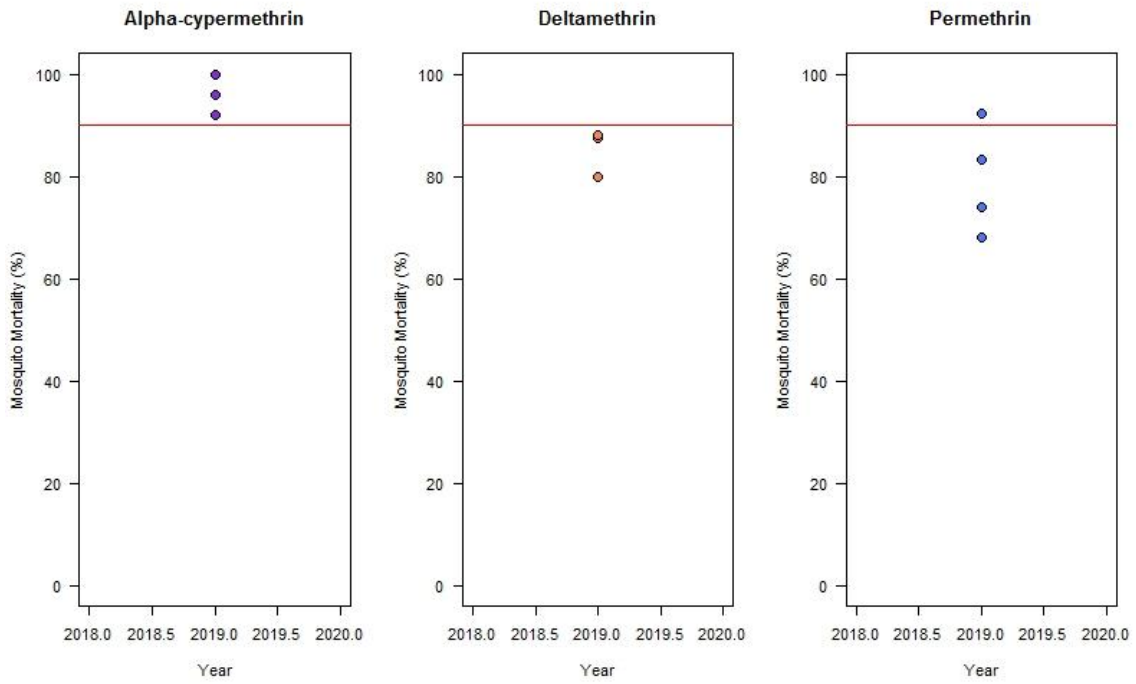
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			<i>An. coluzzii</i>									
LITE	Bottle	Permethrin + PBO	VK7 2014 <i>An. coluzzii</i>	R	20	25.00	100.00	7.73	87.06	100.00	630.80	25.12

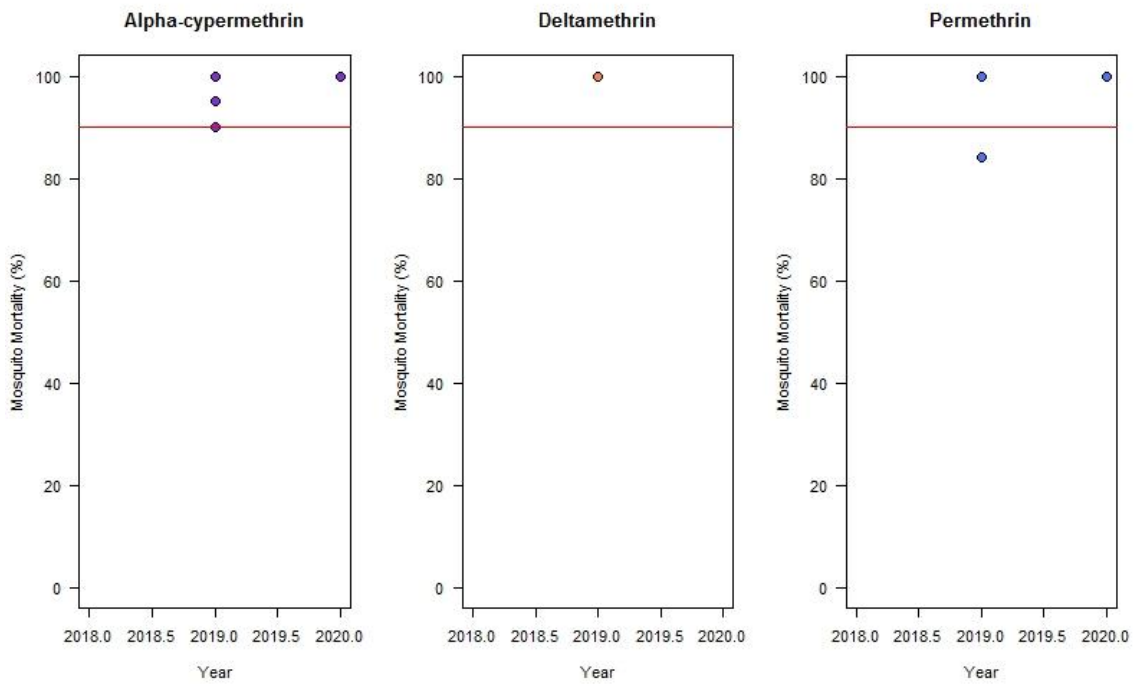
Table S3.3. P-values (Welch's t-test) comparing mean mosquito mortality following exposure to α -cypermethrin 0.05%, deltamethrin 0.05%, or permethrin 0.75% in a standard WHO tube bioassay. Values significant at the 5% level ($p < 0.05$) are highlighted in blue. Abbreviations; Delta = deltamethrin, Perm = permethrin, Alpha = α -cypermethrin.

Group	Strain	Delta v Perm	Delta v Alpha	Perm v Alpha	Delta v Perm + PBO	Perm v Perm + PBO	Alpha v Perm + PBO
Ranson	Banfora-Susceptible	0.482	0.010	0.055	-	-	-
	Kisumu	0.423	0.182	0.723	-	-	-
	N'gouso	0.351	0.104	0.829	-	-	-
	Banfora M	0.044	0.303	0.536	-	-	-
	Bakaridjan	0.446	0.870	0.706	-	-	-
	FUMOZ-R	0.680	0.254	0.451	-	-	-
	Gaoura	0.792	1.000	0.854	-	-	-
	Tiassalé 13	0.002	0.000	0.401	-	-	-
	Tiefora	0.668	-	-	-	-	-
	VK7 2014	0.223	-	-	-	-	-
LITE	Kisumu	0.251	0.082	0.339	-	-	-
	Moz	0.055	0.458	0.590	-	-	-
	FUMOZ-R	0.678	0.009	0.004	0.000	0.000	0.000
	Tiassalé 13	0.874	0.008	0.029	0.002	0.001	0.002
	VK7 2014	0.124	0.851	0.225	0.738	0.506	0.652

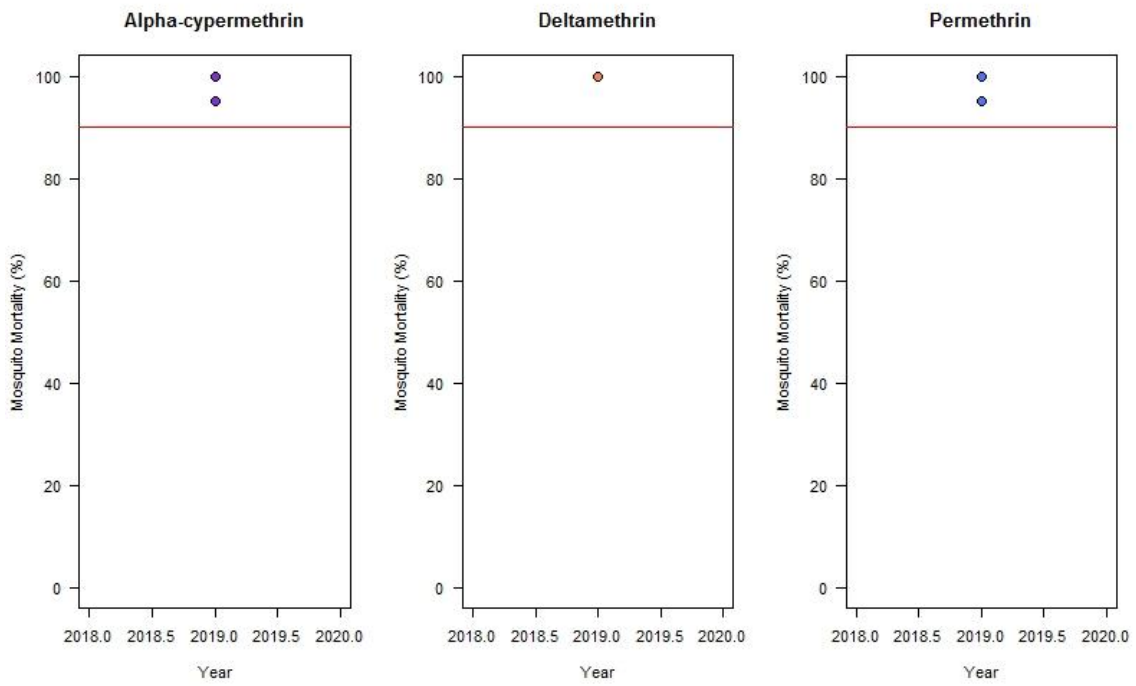
A) Banfora susceptible (Ranson group)



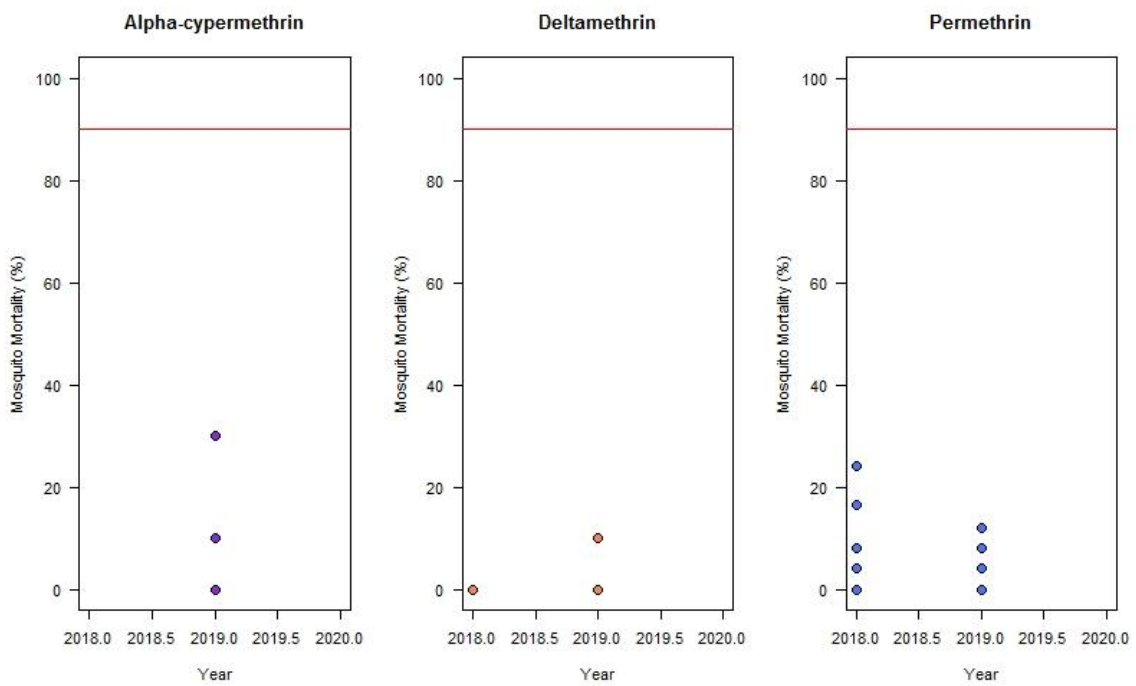
B) Kisumu (Ranson group)



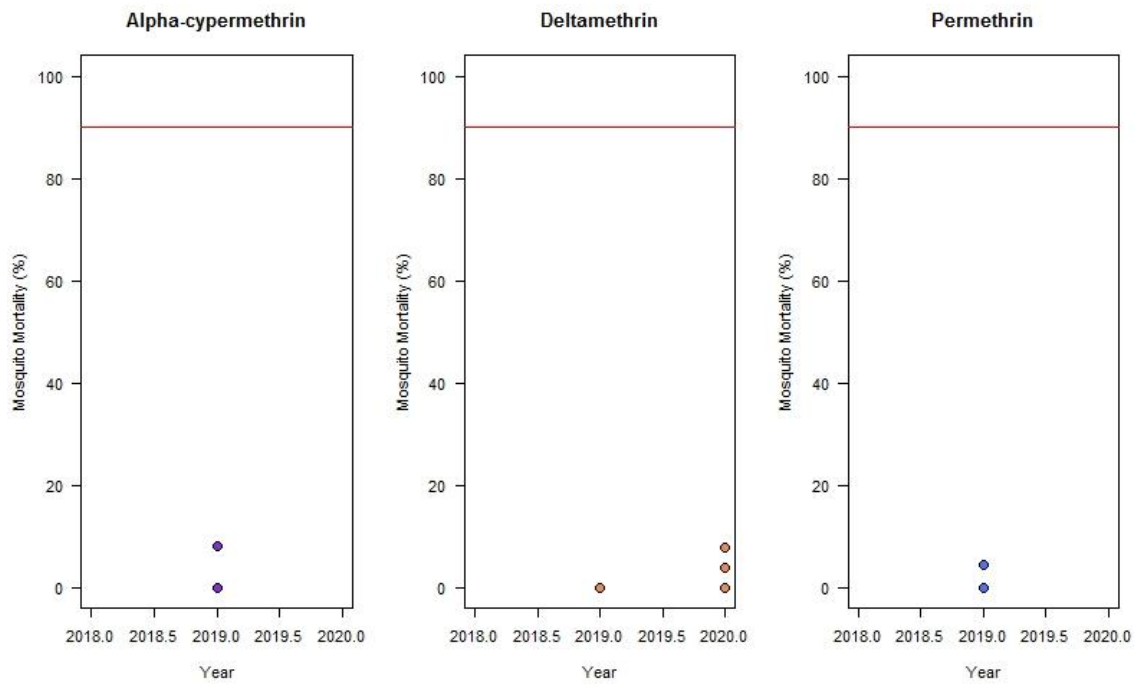
C) N'gouso (Ranson group)



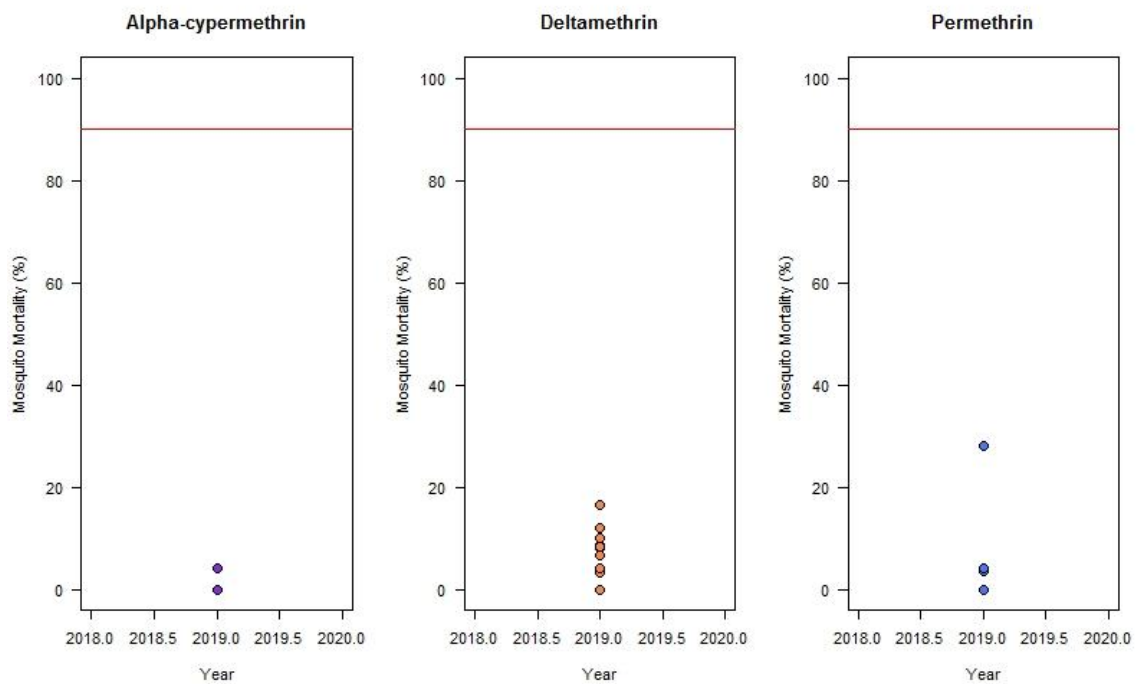
D) Banfora M (Ranson group)



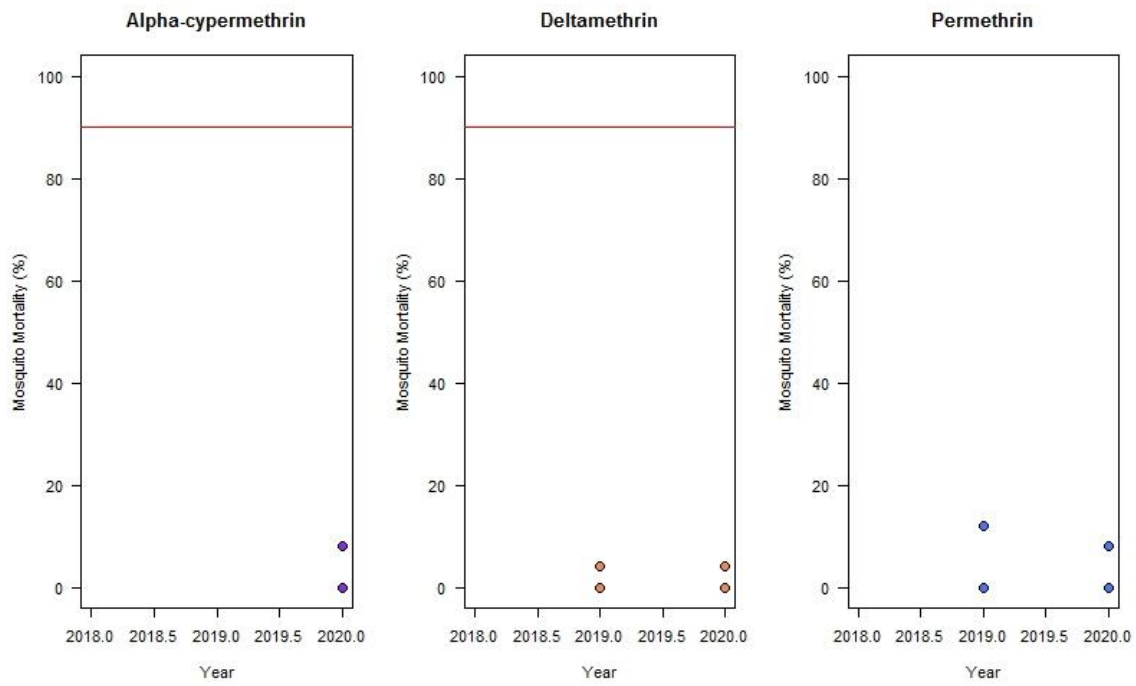
E) Bakaridjan (Ranson group)



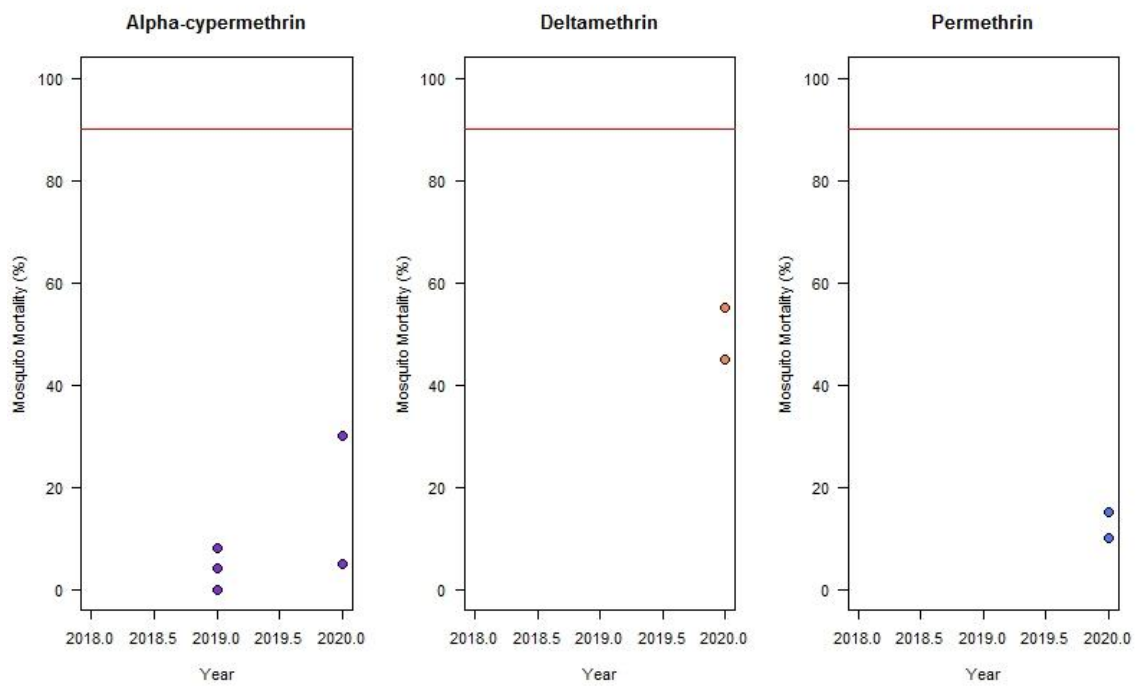
F) FUMOS-R (Ranson group)



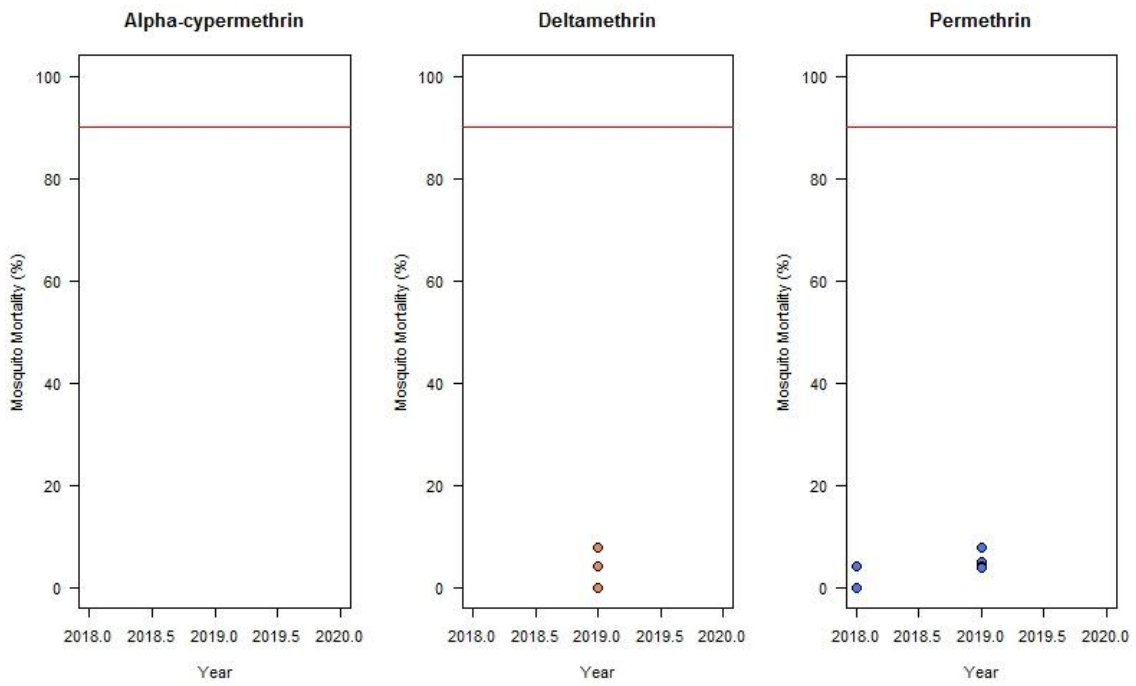
G) Gaoura (Ranson group)



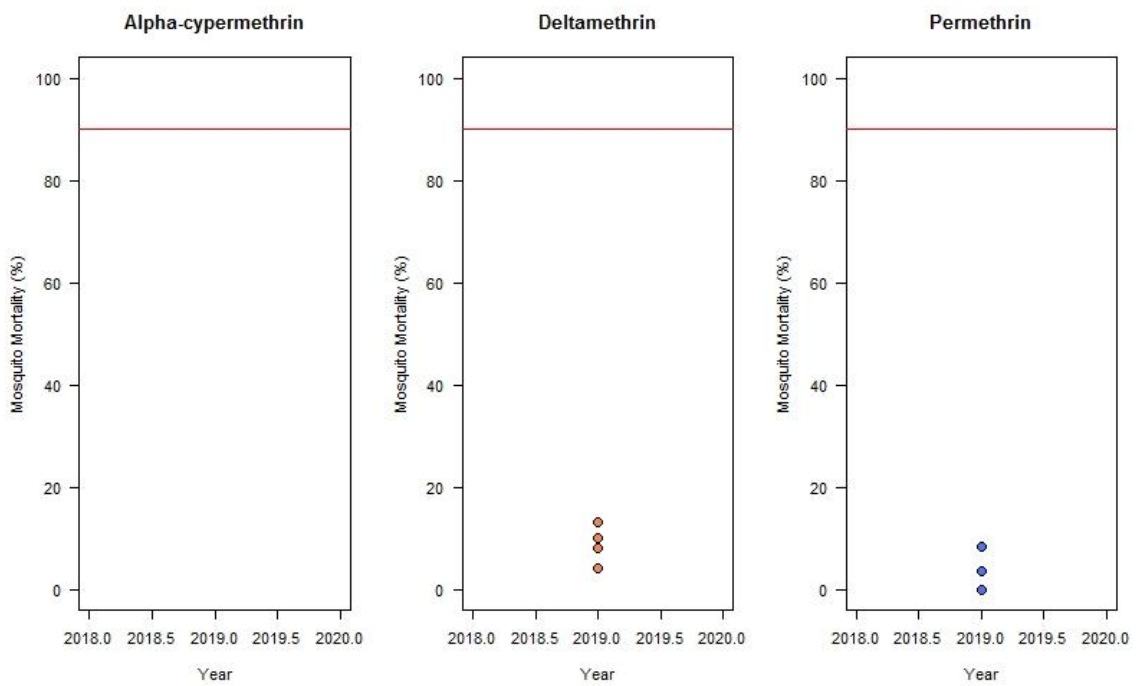
H) Tiassalé 14 (Ranson group)



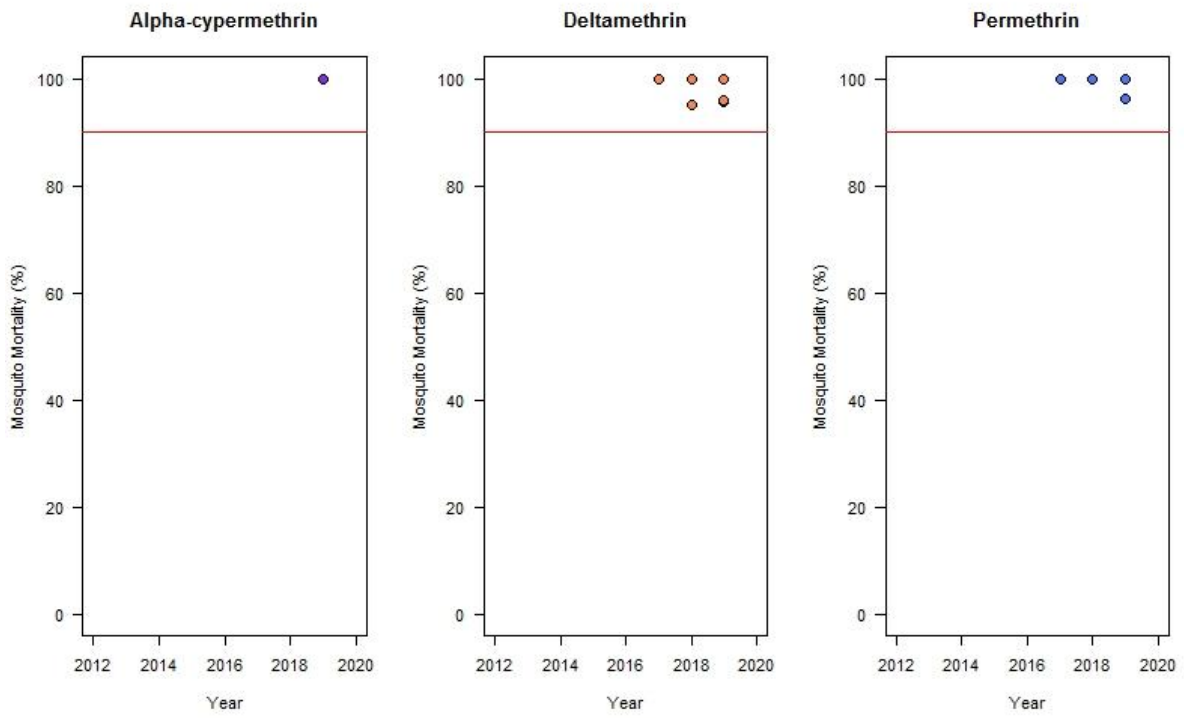
I) Tiefert (Ranson group)



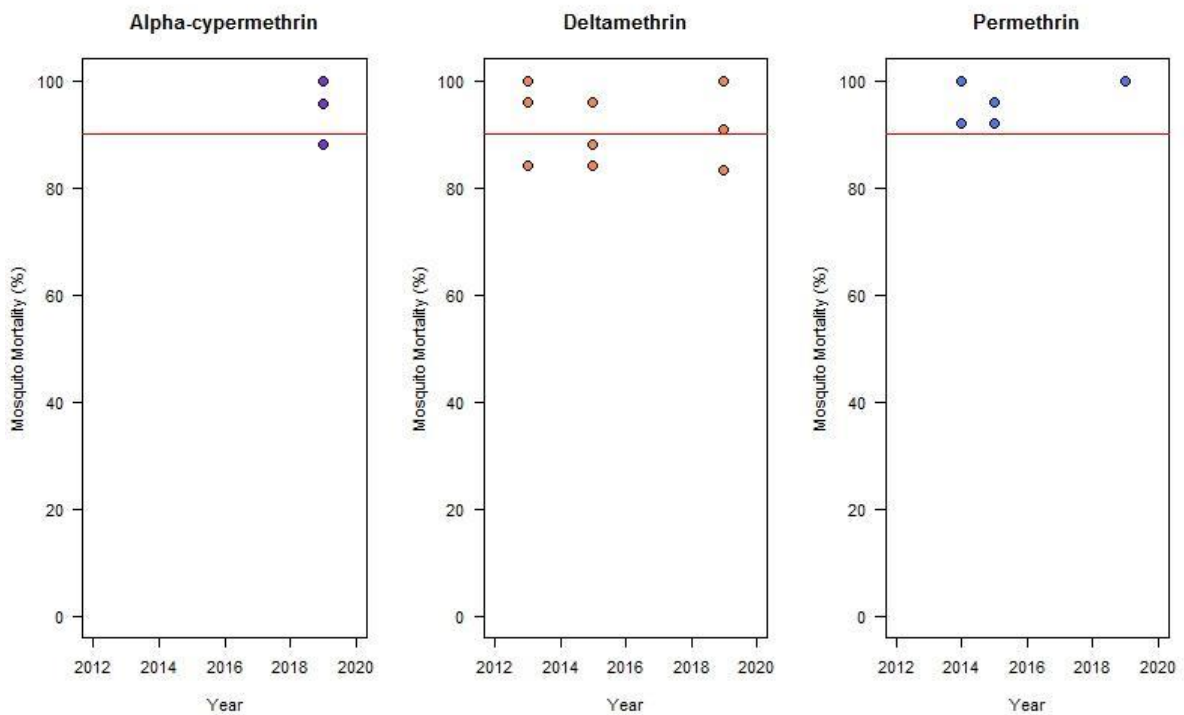
J) VK7 2014 (Ranson group)



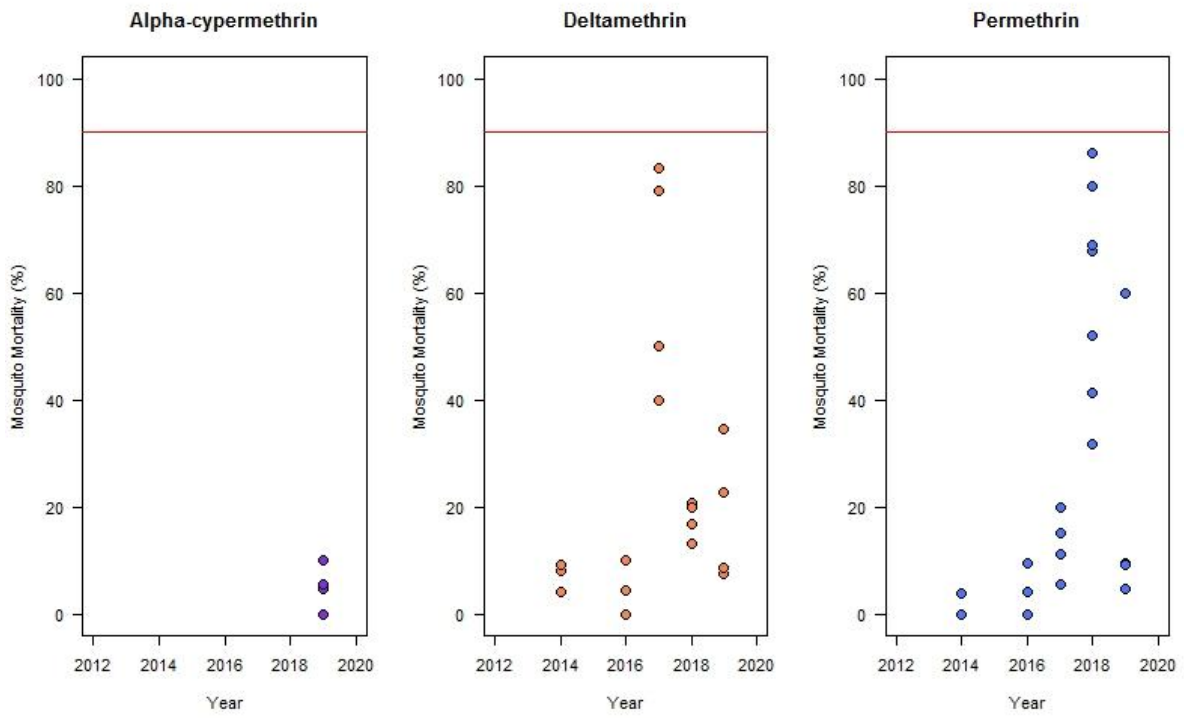
K) Kisumu (LITE)



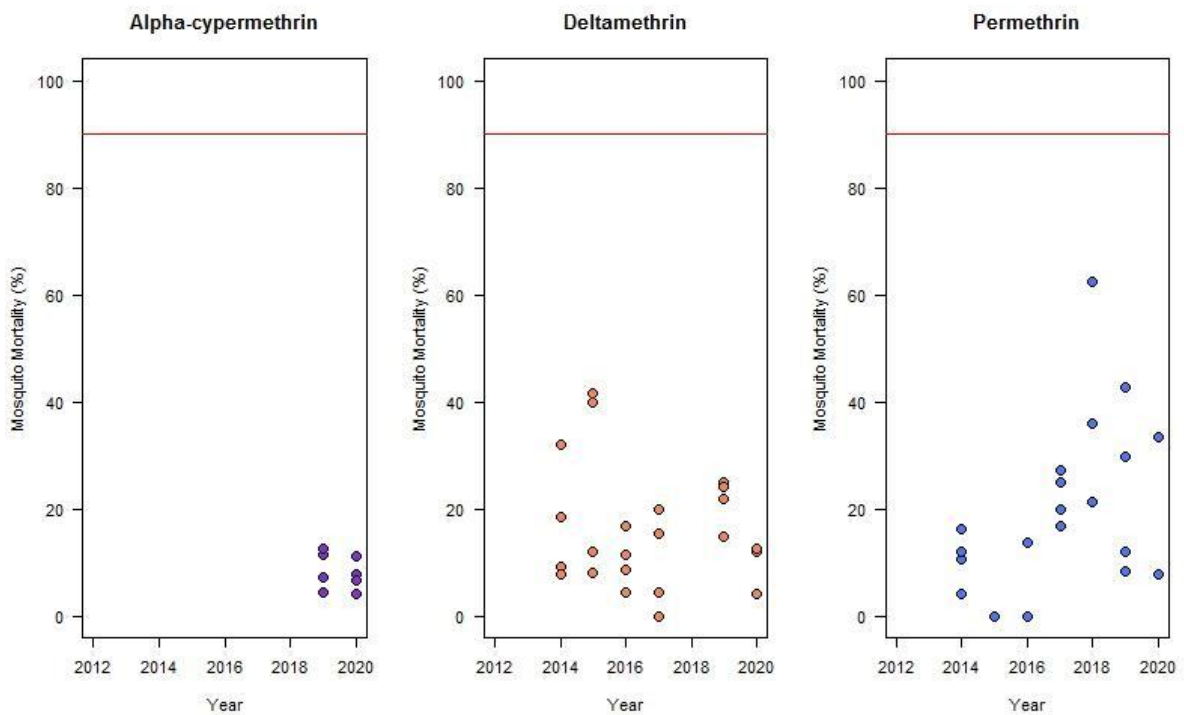
L) Moz (LITE)



M) FUMOS-R (LITE)



N) Tiassalé 13 (LITE)



O) VK7 2014 (LITE)

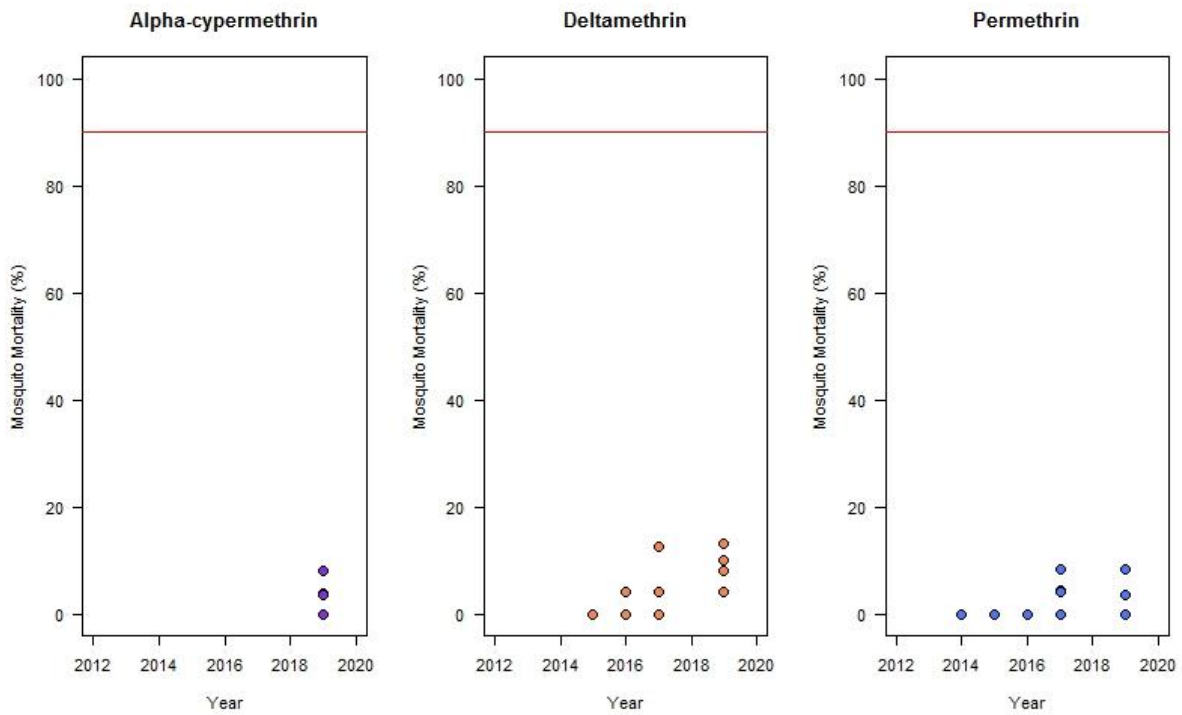


Figure S3.4. Mosquito mortality over time following exposure to pyrethroids in a standard WHO tube bioassay. Ranson group (A- J) and LITE (K – O) mosquito strains were exposed to deltamethrin 0.05%, permethrin 0.75% and α -cypermethrin 0.05% in a standard 1-hour WHO tube bioassay, and their 24-hour mortality was recorded. Coloured circles indicate each individual replicate tube.

Section 4. Field populations

b. Analysis of insecticide divergence and data noise in the field

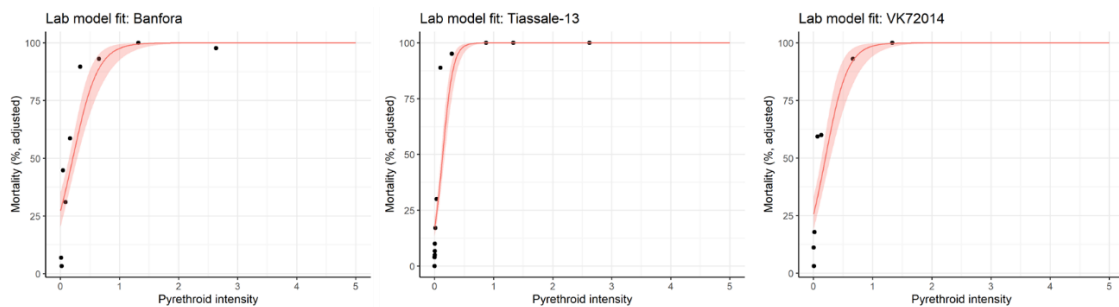


Figure S4.1. Illustration of three resistance intensity bioassays conducted in controlled laboratory conditions at LITE. Black points denote individual mortality estimates whilst red solid line shows the best fit dose response curve. Shaded red region indicates the 95% uncertainty around this best fit line.

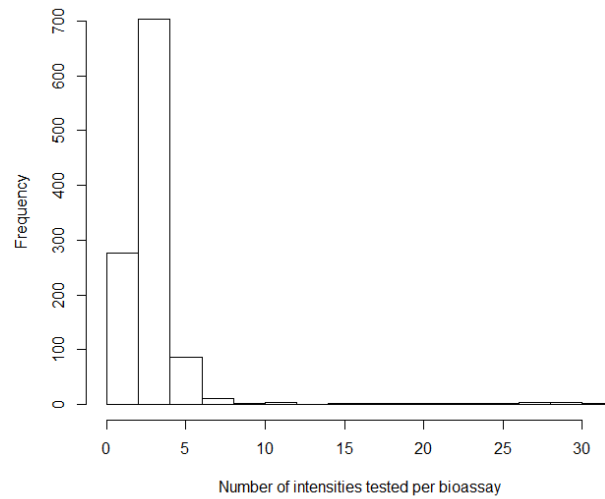


Figure S4.2. Histogram showing the number of different insecticide doses conducted in each resistance intensity bioassay. On average 2.8 doses were done in each assay with a high number of different doses conducted in a small number of locations.

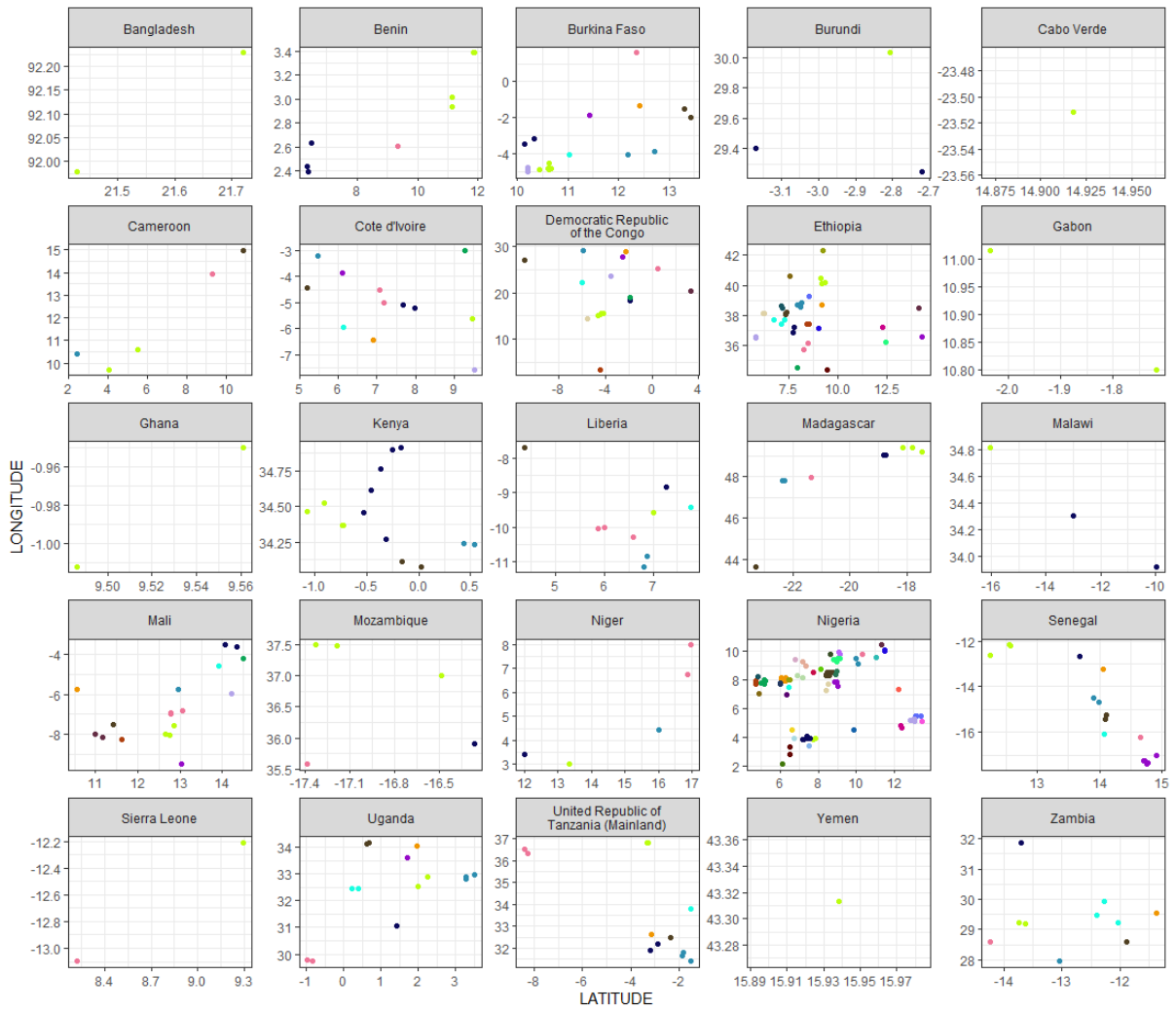
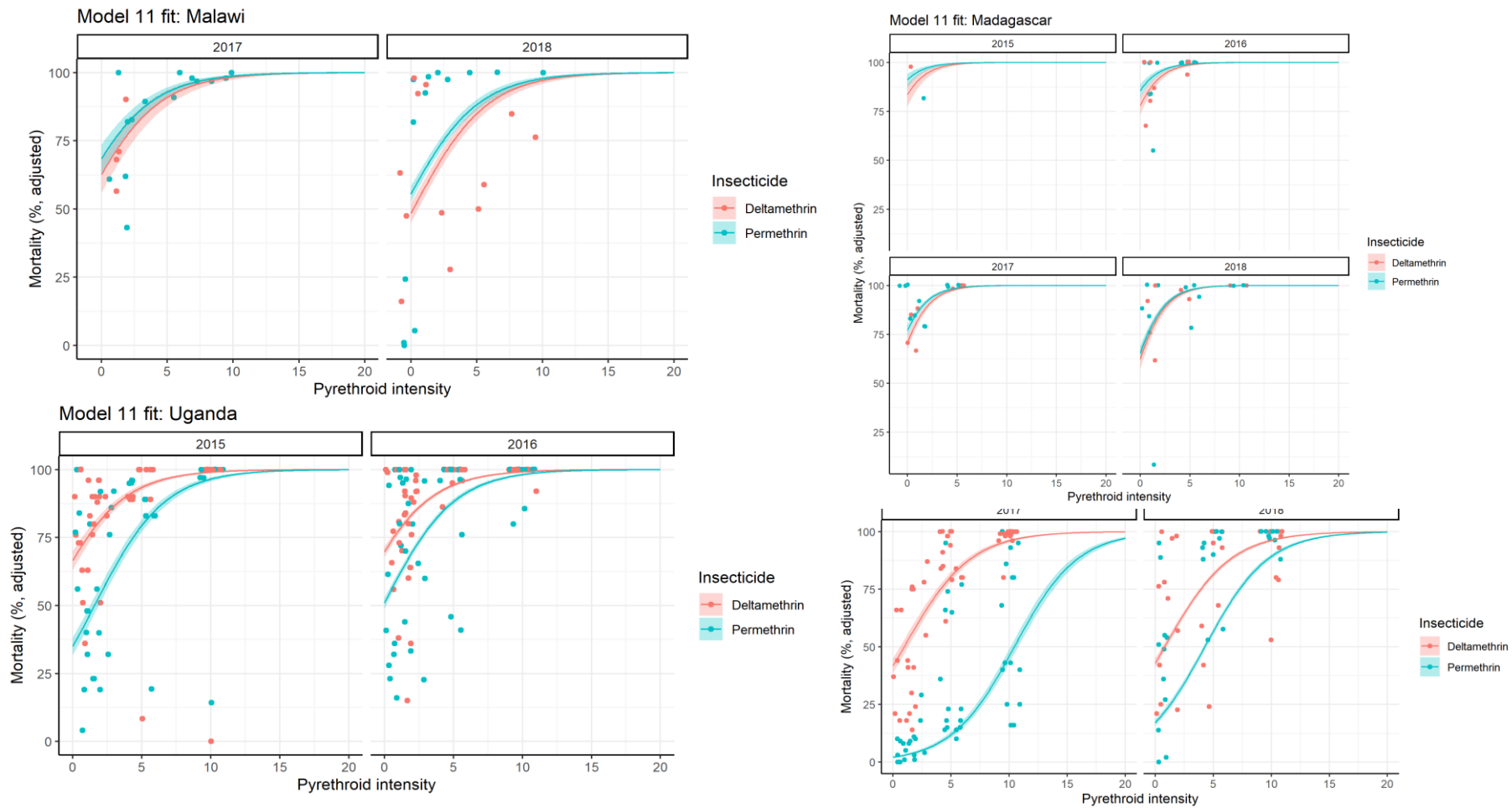


Figure S4.3. Geographical location of the different resistance intensity bioassays used in the analyses. Each panel shows a different country with each point identifying a sampling point for the resistance intensity assay (note some sites are relatedly sampled over multiple years). Point colour indicates sample locations which are grouped into a single cluster. Each cluster has a maximum distance of 50km between all sampling locations. Scale varies for each country.

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Figure S4.4. Dose response curves for different countries with data over 4 years from 2015-2018. Red points indicate mosquito mortality estimates observed in different clusters induced by either permethrin (green) or deltamethrin (red) for different insecticide concentrations (1 being the discriminating dose concentration). Coloured lines indicate the best fit dose response curve which was allowed to vary between insecticides and over time. Individual assays are grouped together in clusters (Table 5.1), pooling data from the same calendar year which were conducted within a 50km radius (Figure S5.3). Additional data are provided in Figure 5.4 and 5.5 of the main text.



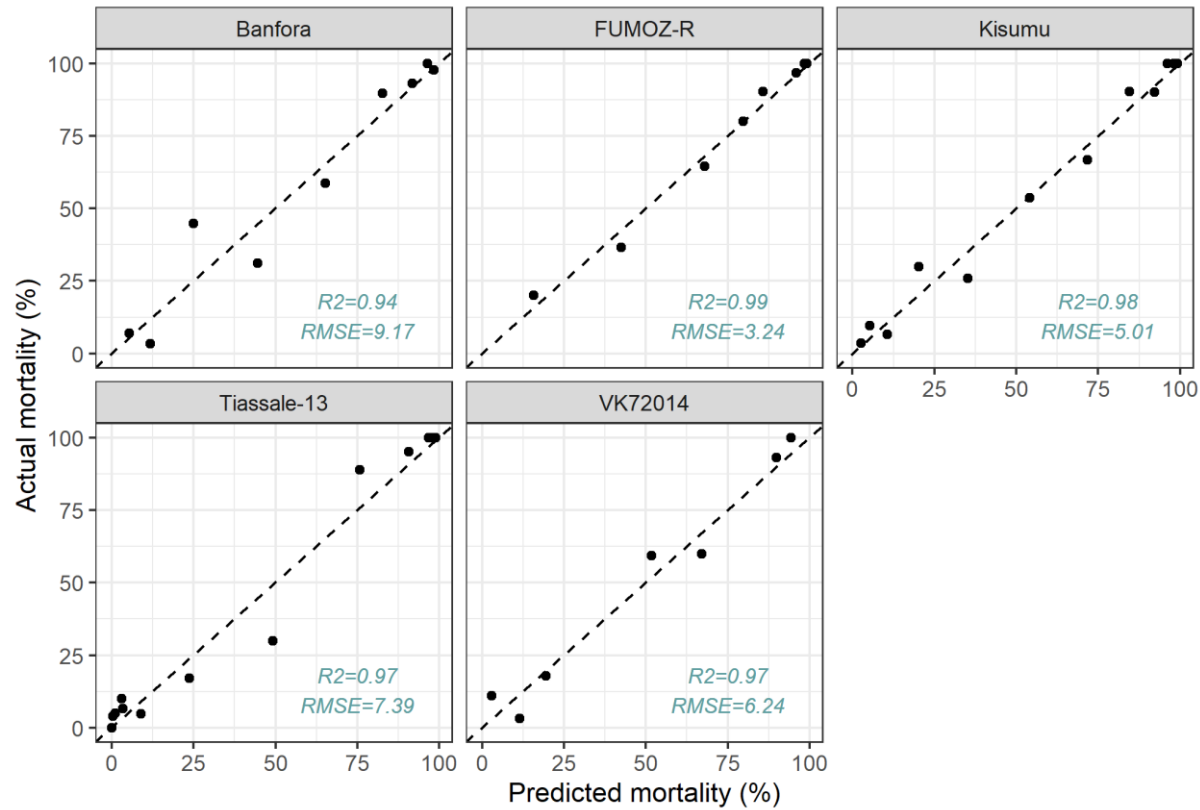


Figure S4.5. Measurement error of individual resistance intensity bioassays for different laboratory strains from LITE. This graph summarizes how accurately the dose-response curve fits the observed data (illustrated in Figure S1). RMSE denotes the percentage root mean squared error. Each panel indicates a different mosquito strain.

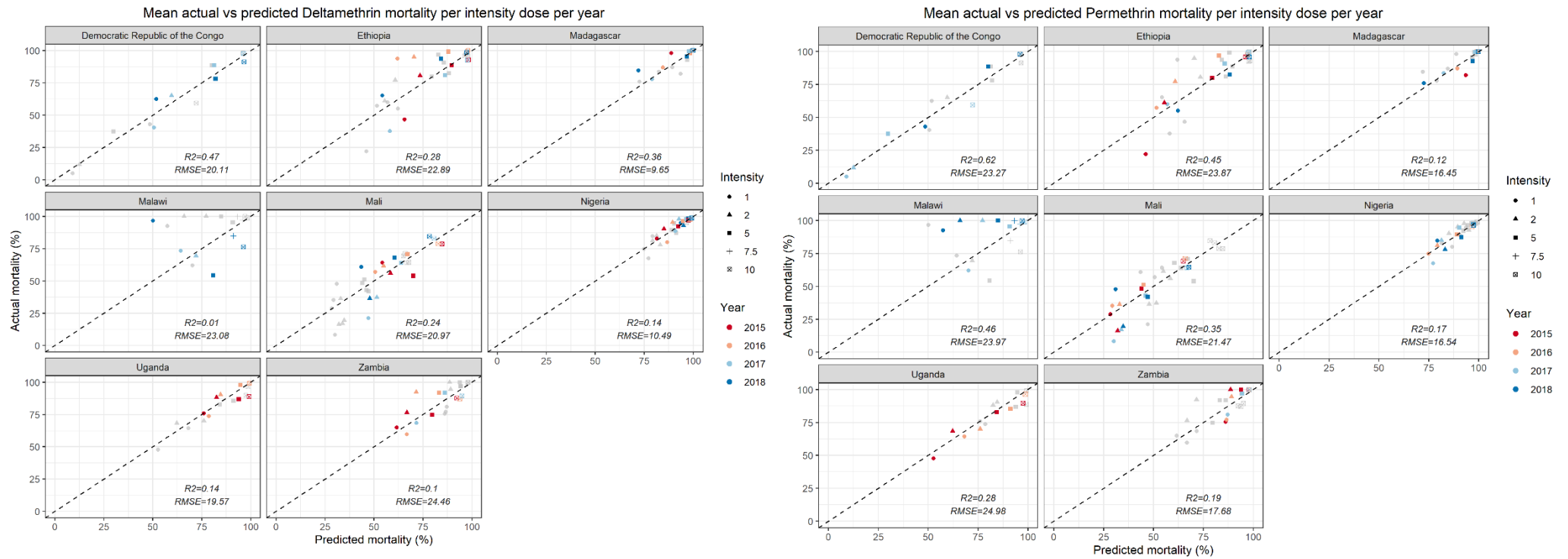


Figure S4.6. Measurement error of cluster resistance intensity bioassays for wild caught mosquitoes. This graph summaries how accurately the dose-response curve fits the average mortality of the different clusters. Points denote different concentrations (shape) and years (colours). RMSE denotes the percentage route mean squared error. Each panel indicates a different mosquito strain. Left panel shows deltamethrin whilst right panel shows permethrin assays. Each plot shows different countries.

Table S4.1. Summary of resistance intensity data used in analyses.

Country	Number of individual assays (with multiple intensities per assay)			
	In dataset		Assay pairs for analysis	
	Deltamethrin	Permethrin	Deltamethrin	Permethrin
Benin	43	42	10	10
Burkina Faso	488	0	0	0
Burundi	0	8	0	0
Cabo Verde	3	0	0	0
Cameroon	16	11	4	3
Cote d'Ivoire	35	34	9	9
Democratic Republic of the Congo	84	83	27	28
Ethiopia	149	154	44	45
Gabon	6	4	2	2
Ghana	10	0	0	0
Kenya	6	53	3	17
Liberia	44	3	14	1
Madagascar	24	35	10	15
Malawi	16	27	5	6
Mali	280	288	87	88
Mozambique	0	14	0	0
Niger	17	12	5	3
Nigeria	474	513	147	141
Senegal	44	36	15	11
Sierra Leone	5	0	0	0
Uganda	94	87	27	25
United Republic of Tanzania - mainland	18	51	6	16
Zambia	88	70	22	21
OVERALL	1944	1525	437	441

Table S4.2. Summary of the experimental hut trial studies used in the systematic review. Studies with an * did not provide blood-feeding data but were included in mortality and deterrence comparison.

Study	No. of EHTs	Reference	Study site
1	2	Agossa <i>et al.</i> , (2014)	Akron, Benin Malanville, Benin
2	1	Allossogbe <i>et al.</i> , (2017)	Cové, Benin
3	1	Ketoh <i>et al.</i> , (2018)	Kolokopé, Togo
4	1	Malima <i>et al.</i> , (2008)	Muheza, Tanzania
5	1	Mosha <i>et al.</i> , (2008)	Lower Moshi, Tanzania
6	1	N'Guessan <i>et al.</i> , (2010)	Akron, Benin
7*	2	Okumu <i>et al.</i> , (2013)	Lupiro, Tanzania
8	2	Toe <i>et al.</i> , (2018)	Vallée du Kou, Burkina Faso Tengrela, Burkina Faso
9	1	Guillet <i>et al.</i> , (2001)	Yaokoffikro, Côte d'Ivoire
10	1	Miller, Lindsey and Armstrong, (1991)	Wali Kunda, The Gambia
11	1	Darriet <i>et al.</i> , (1991)	Yaokoffikro, Côte d'Ivoire

c. Investigating whether differences between pyrethroids identified by molecular studies (Section 2) can be detected in wild mosquito populations

Relative divergence in resistance to pyrethroids within wild populations of malaria vectors – additional validation

The data subsets used for the repeat correlation analyses were not independent of each other and, in the most extreme example, the α -cypermethrin vs etofenprox data were identical in each repeat because the full dataset for this pair totalled 42 data points only. We, therefore, conducted a second set of correlation analyses for those pyrethroid pairs with the highest data volumes (all combinations of α -cypermethrin,

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deltamethrin, λ -cyhalothrin and permethrin as well as deltamethrin vs etofenprox) using independent subsets of the randomised data where no individual data point was included in more than one subset.

Two sets of correlation coefficient were generated, the first incorporating 13 pyrethroid pairs using randomly selected data subsets (main text), and the second incorporating the 7 most commonly tested pairs using randomly selected unique data subsets. Each set of values was ranked from most divergent to most closely correlated (Tables S4.3 and S4.4). The rankings were identical for those pairs included in both sets of analyses with one exception; (the rankings of deltamethrin vs etofenprox and λ -cyhalothrin vs α -cypermethrin were reversed, however, the values for these pairs were very similar to each other in both sets of analyses) so the second analysis provides additional validation of the results presented in the main text.

Table S4.3. Ranking based on correlation coefficients using randomly selected subsets of test result pairs.

Rank	Pyrethroid pair	N=42 drawn from a pool of:	Times correlation analysis repeated	Mean r
1	D vs L	597	20	0.783
2	P vs C	62	20	0.761
3	P vs L	484	20	0.729
4	D vs P	1278	20	0.710
5	D vs A	243	20	0.783
6	D vs C	65	20	0.681
7	P vs A	197	20	0.672
8	P vs E	68	20	0.586
9	D vs E	81	20	0.557
10	L vs A	154	20	0.555
11	A vs E	42	20	0.507
12	L vs C	62	20	0.495
13	L vs E	54	20	0.421

Table S4.42 Ranking based on correlation coefficients using randomly selected subsets of unique test result pairs.

Rank	Pyrethroid pair	N=40 drawn from a pool of:	Times correlation analysis replicated	Mean r
1	D vs L	597	14	0.818
2	P vs L	484	12	0.716
3	D vs P	1278	20	0.698
4	D vs A	243	6	0.696
				0.634
5	P vs A	197	4	0.634
6	L vs A	81	2	0.585
7	D vs E	154	3	0.539

The most closely correlated pair is ranked first and the most divergent last. A is α -cypermethrin, C is cyfluthrin, D is deltamethrin, E is etofenprox, L is λ -cyhalothrin, P is permethrin, and r is Pearson's correlation coefficient. Pyrethroid pairs used in both analyses have been coloured to aid comparison of the two rankings.

The results from the 2018 geostatistical study further validated these rankings for the three most tested pyrethroids (Tables S4.5 and S4.6).

Table S4.5. Ranking based on correlation coefficients that used predicted values for west Africa. **Table S4.6** Ranking based on correlation coefficients that used predicted values for east Africa.

Rank	Pyrethroid pair	N=42 drawn from a pool of:	Times correlation analysis repeated	Rm	Rank	Pyrethroid pair	N=42 drawn from a pool of:	Times correlation analysis repeated	rm
1	D vs L	643	20	0.814	1	D vs L	643	16	0.799
2	D vs P	510	20	0.693	2	P vs L	510	12	0.734
3	P vs L	246	20	0.691	3	D vs P	1391	20	0.706

The most divergent pair are ranked first and the most closely correlated last. D is deltamethrin, L is λ -cyhalothrin, P is permethrin, and rm is the posterior mode of the Pearson's correlation coefficient. Pyrethroid pairs have been coloured to aid comparison with the other rankings.

Table S4.7. Correlations in resistance to different pyrethroids in *An. funestus* subgroup samples

	N	r
Deltamethrin vs λ -cyhalothrin	46	0.818*
Permethrin vs λ -cyhalothrin	26	0.786*
Deltamethrin vs permethrin	113	0.608*

Significant results (at the 0.05 level with a Bonferroni correction) are denoted by *.

Table S4.8. Correlations in resistance to different pyrethroids four African malaria vector species

Deltamethrin vs λ-cyhalothrin	N	r
<i>Anopheles arabiensis</i>	28	0.946*
<i>Anopheles coluzzii</i>	18	0.863*
<i>Anopheles coluzzii/gambiae</i>	19	0.603
<i>Anopheles gambiae</i>	19	0.418
<i>Anopheles funestus</i>	24	0.865*
Permethrin vs λ-cyhalothrin		
<i>Anopheles arabiensis</i>	31	0.859*
<i>Anopheles coluzzii</i>	14	0.740*
<i>Anopheles coluzzii/gambiae</i>	17	0.790*
<i>Anopheles gambiae</i>	4	Not tested
<i>Anopheles funestus</i>	16	0.845*
Deltamethrin vs permethrin		
<i>Anopheles arabiensis</i>	116	0.840*
<i>Anopheles coluzzii</i>	48	0.793*
<i>Anopheles coluzzii/gambiae</i>	63	0.714*
<i>Anopheles gambiae</i>	75	0.782*
<i>Anopheles funestus</i>	69	0.726*

Significant results (at the 0.05 level with a Bonferroni correction) are denoted by *.

Table S4.9. Correlations in resistance to deltamethrin and non-pyrethroid insecticides for four African malaria vector species

Deltamethrin vs DDT	N	Correlation
<i>Anopheles arabiensis</i>	107	0.808*
<i>Anopheles coluzzii</i>	69	0.506*
<i>Anopheles coluzzii/gambiae</i>	62	0.545*
<i>Anopheles gambiae</i>	43	0.370
<i>Anopheles funestus</i>	48	-0.071
Deltamethrin vs bendiocarb		
<i>Anopheles arabiensis</i>	97	-0.224
<i>Anopheles coluzzii</i>	22	0.133
<i>Anopheles coluzzii/gambiae</i>	28	0.218
<i>Anopheles gambiae</i>	22	0.073
<i>Anopheles funestus</i>	35	0.439
Deltamethrin vs fenitrothion		
<i>Anopheles arabiensis</i>	55	0.170
Deltamethrin vs malathion		
<i>Anopheles arabiensis</i>	37	0.084
<i>Anopheles funestus</i>	29	0.061
Deltamethrin vs pirimiphos-methyl		
<i>Anopheles arabiensis</i>	34	0.246
Deltamethrin vs propoxur		
<i>Anopheles arabiensis</i>	34	0.249

Significant results (at the 0.05 level with a Bonferroni correction) are denoted by *.

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Figure S4.7. Prevalence of the pyrethroid resistance phenotype in sympatric populations of malaria vectors.

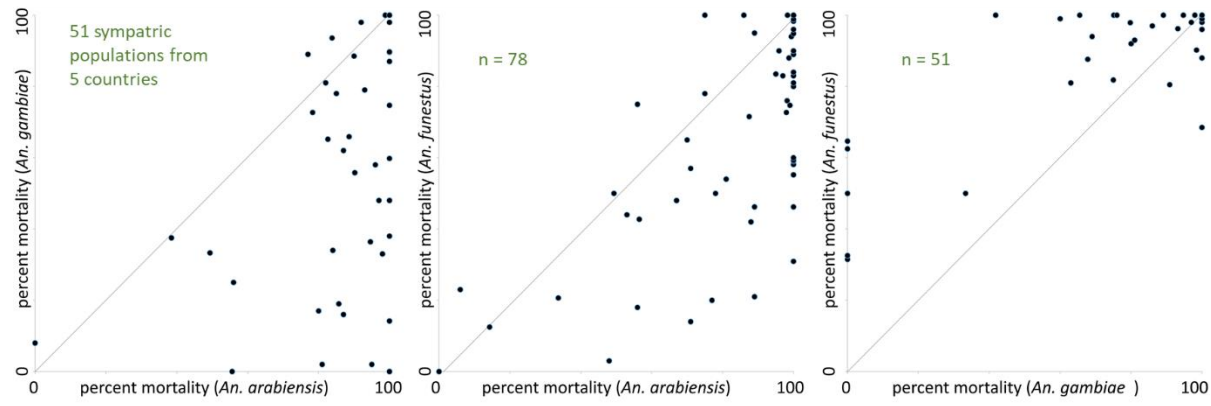


Table S4.10. Knockdown concentration (KC₅₀) and resistance ratio (RRKC₅₀) of five pyrethroids against *Aedes aegypti* females from Veracruz, Mexico

Insecticide	<i>Aedes aegypti</i>	KC ₅₀ (s)	KC ₅₀ (R)	KC ₅₀ - 95% CI	RRKC ₅₀	χ ²
Permethrin	New Orleans	0.45	-	0.37 - 0.51	1	13.51
	Panuco	-	2.3	1.71 - 2.96	10.4	12.3
	Tantoyuca	-	1.5	1.27 - 1.70	6.7	15.2
	Poza Rica	-	8.3	7.15 - 9.57	37.7	8.3
	M. de la Torre	-	5.6	4.61 - 6.58	25.4	12.7
	Veracruz	-	2.7	2.23 - 3.14	12.1	4.2
	Coatzacoalcos	-	2.9	2.48 - 3.45	13.4	11.2
	Cosoleacaque	-	0.7	0.57 - 0.8	3.1	5.8
Deltamethrin	New Orleans	0.021	-	0.017 - 0.024	1.0	10.1
	Panuco	-	0.1	0.046 - 0.089	7.0	0.7
	Tantoyuca	-	0.0	0.015 - 0.020	1.9	7.1
	Poza Rica	-	0.1	0.130 - 0.160	16.2	0.0
	M. de la Torre	-	0.0	0.014 - 0.026	2.1	19.0
	Veracruz	-	0.2	0.132 - 0.177	17.1	5.4
	Coatzacoalcos	-	0.2	0.149 - 0.189	18.7	7.5
	Cosoleacaque	-	0.0	0.023 - 0.038	3.3	15.6
α-Cypermethrin	New Orleans	0.0016	-	0.0013 - 0.0018	1.0	15.2
	Panuco	-	0.1	0.113 - 0.177	128.2	25.4
	Tantoyuca	-	0.0	0.032 - 0.070	42.7	18.0
	Poza Rica	-	0.5	0.385 - 0.560	420.0	1.9
	M. de la Torre	-	0.4	0.307 - 0.428	328.2	16.3
	Veracruz	-	0.2	0.204 - 0.297	222.7	9.8
	Coatzacoalcos	-	0.4	0.344 - 0.562	395.5	10.8
	Cosoleacaque	-	0.1	0.089 - 0.167	110.9	8.0
λ-Cyhalothrin	New Orleans	0.005	-	0.0002 - 0.001	1.0	15.0
	Panuco	-	0.0	0.010 - 0.019	20.0	7.2
	Tantoyuca	-	0.0	0.009 - 0.029	22.9	5.7
	Poza Rica	-	0.1	0.070 - 0.120	128.6	11.6
	M. de la Torre	-	0.0	0.014 - 0.019	22.9	6.3
	Veracruz	-	0.1	0.060 - 0.120	122.9	7.4
	Coatzacoalcos	-	0.1	0.080 - 0.180	171.4	6.4

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	Cosoleacaque	-	0.0	0.030 - 0.060	64.3	21.6
Bifenthrin	New Orleans	0.21	-	0.170 - 0.210	1.0	45.2
	Panuco	-	0.1	0.060 - 0.140	1.7	8.8
	Tantoyuca	-	0.2	0.140 - 0.230	3.3	8.4
	Poza Rica	-	1.0	0.900 - 1.200	19.3	9.2
	M. de la Torre	-	0.2	0.127 - 0.187	2.8	15.8
	Veracruz	-	4.4	3.870 - 4.970	81.5	20.1
	Coatzacoalcos	-	0.4	0.310 - 0.600	8.0	16.0
	Cosoleacaque	-	1.3	1.060 - 1.600	24.4	19.5

KC₅₀, 50% knockdown concentration in micrograms per bottle, 95% CI in parentheses. RRKC₅₀, resistance ratio: KC₅₀ resistant strain/KC₅₀ New Orleans susceptible strain.

Table S4.11 Toxicity (LC₅₀) and resistance ratio (RRLC₅₀) of five pyrethroids against *Aedes aegypti* females from Veracruz, Mexico

Insecticide	<i>Aedes aegypti</i>	LC ₅₀ (s)	LC ₅₀ (R)	LC ₅₀ - 95% CI	RRLC ₅₀	χ ²
Permethrin	New Orleans	0.22	-	0.15 - 0.27	1.0	7.6
	Panuco	-	0.62	0.43 - 0.83	2.8	4.6
	Tantoyuca	-	1.24	1.05 - 1.44	5.6	28.8
	Poza Rica	-	7.31	4.78 - 10.71	33.2	47.3
	M. de la Torre	-	4.84	3.94 - 5.74	22.0	10.6
	Veracruz	-	2.41	2.03 - 2.83	11.0	3.0
	Coatzacoalcos	-	1.96	1.64 - 2.30	8.9	26.5
	Cosoleacaque	-	0.43	0.35 - 0.51	2.0	7.1
Deltamethrin	New Orleans	0.009	-	0.003 - 0.015	1.0	9.9
	Panuco	-	0.023	0.016 - 0.031	2.6	7.5
	Tantoyuca	-	0.017	0.014 - 0.018	1.9	7.1
	Poza Rica	-	0.15	0.13 - 0.17	16.7	9.6
	M. de la Torre	-	0.001	0.0004 - 0.002	0.1	23.1
	Veracruz	-	0.159	0.126 - 0.197	17.7	16.1
	Coatzacoalcos	-	0.175	0.156 - 0.195	19.4	4.1
	Cosoleacaque	-	0.025	0.019 - 0.033	2.8	20.7
α-Cypermethrin	New Orleans	0.0011	-	0.0009 - 0.0013	1.0	43.1

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	Panuco	-	0.171	0.135 - 0.222	155.5	11.9
	Tantoyuca	-	0.051	0.032 - 0.0825	46.4	12.6
	Poza Rica	-	0.743	0.595 - 0.972	675.5	9.6
	M. de la Torre	-	0.467	0.373 - 0.624	424.5	10.8
	Veracruz	-	0.32	0.259 - 0.408	290.9	7.6
	Coatzacoalcos	-	0.579	0.448 - 0.776	526.4	10.8
	Cosoleacaque	-	0.005	0.008 - 0.014	4.5	20.1
λ -Cyhalothrin	New Orleans	0.0007	-	0.0002 - 0.001	1.0	31.0
	Panuco	-	0.006	0.003 - 0.114	8.6	26.4
	Tantoyuca	-	0.001	0.0004 - 0.002	1.4	3.6
	Poza Rica	-	0.109	0.081 - 0.143	155.7	15.3
	M. de la Torre	-	0.006	0.0042 - 0.0074	8.6	18.8
	Veracruz	-	0.047	0.029 - 0.073	67.1	28.5
	Coatzacoalcos	-	0.138	0.084 - 0.243	197.1	9.4
	Cosoleacaque	-	0.019	0.010 - 0.033	27.1	44.4
Bifenthrin	New Orleans	0.054	-	0.04 - 0.071	1.0	21.8
	Panuco	-	0.033	0.019 - 0.056	0.6	5.7
	Tantoyuca	-	0.147	0.113 - 0.188	2.7	7.0
	Poza Rica	-	0.835	0.737 - 0.947	15.5	37.9
	M. de la Torre	-	0.131	0.110 - 0.158	2.4	18.0
	Veracruz	-	1.908	1.508 - 2.027	35.3	37.1
	Coatzacoalcos	-	0.413	0.281 - 0.608	7.6	20.9
	Cosoleacaque	-	1.358	1.082 - 1.722	25.1	22.7

LC₅₀, 50% lethal concentration in micrograms per bottle, 95% CI in parentheses. RRLC₅₀, resistance ratio: LC₅₀ resistant strain/LC₅₀ New Orleans susceptible strain.

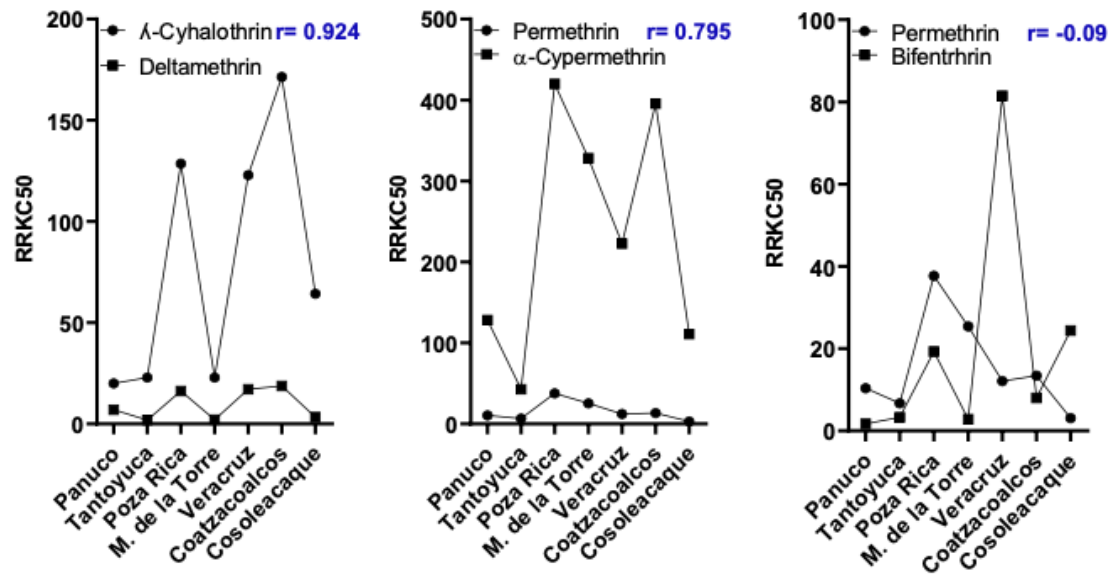


Figure S4.8. Examples of the relationships between RRK₅₀ for three pairs of pyrethroids against *Aedes aegypti* from seven locations in Mexico.

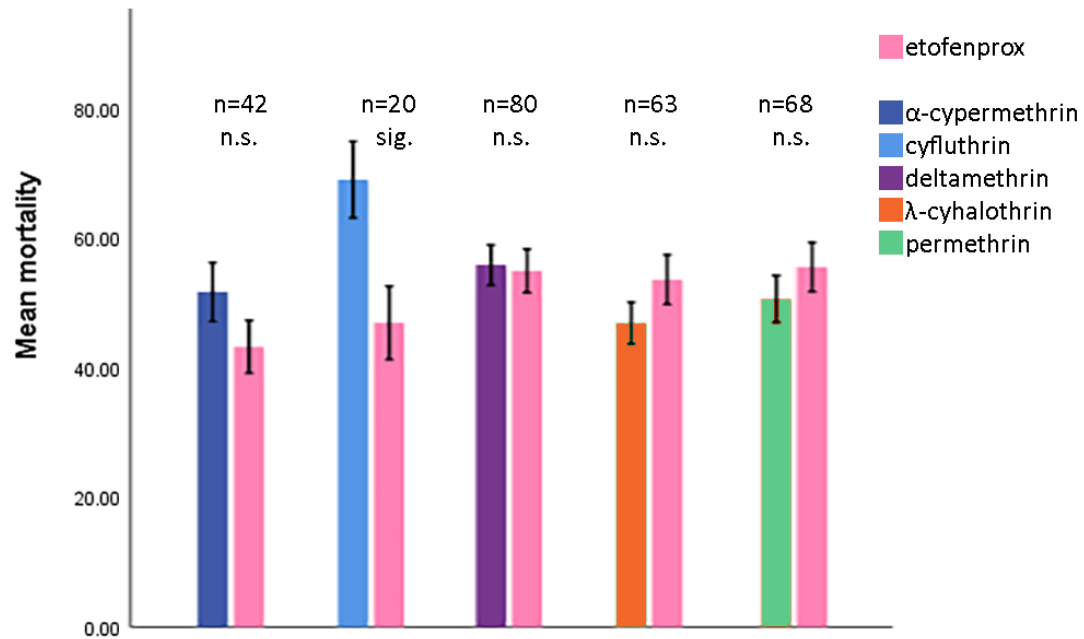
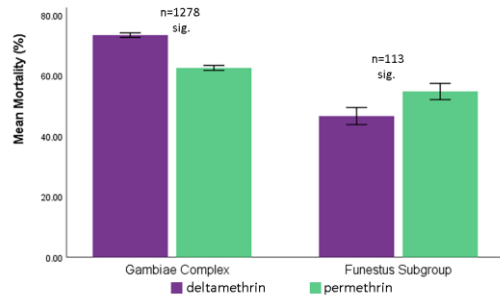
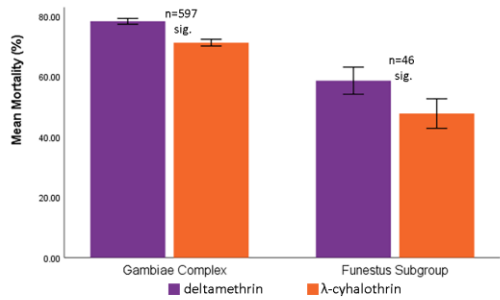


Figure S4.9. Pairwise comparison of resistance to etofenprox with resistance to five other pyrethroids, measured using standard WHO susceptibility tests. Significant (at the 0.05 level with a Bonferroni correction) results from a paired-sample t-test are denoted 'sig.', non-significant results are denoted 'n.s.', and 'n' is the number of pairs in each set.

Deltamethrin vs Permethrin Resistance in Malaria Vector Complexes



Deltamethrin vs λ -Cyhalothrin Resistance in Malaria Vector Complexes



Permethrin vs λ -Cyhalothrin Resistance in Malaria Vector Complexes

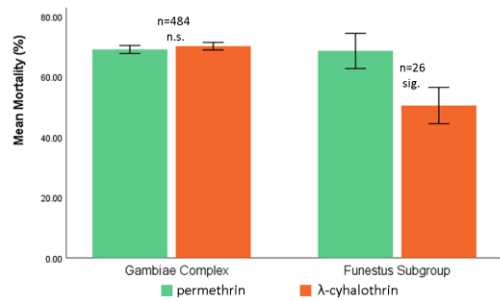


Figure S4.10. Comparison of resistance to three pyrethroids in the *An. gambiae* complex and *An. funestus* subgroup. Significant results (at the 0.05 level with a Bonferroni correction) from a paired-sample t-test are denoted 'sig.', non-significant results are denoted 'n.s.', and 'n' is the number of pairs in each set.

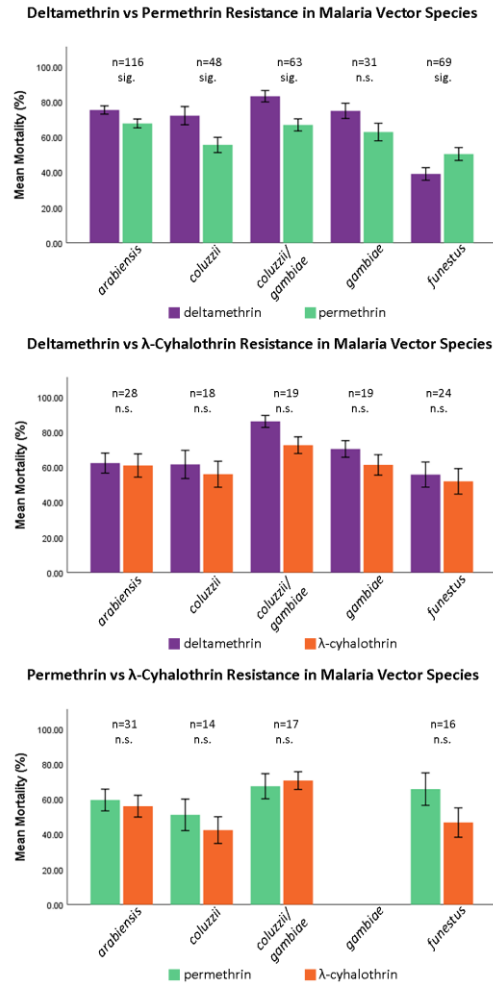


Figure S4.11 Comparison of resistance to three pyrethroids in four malaria vector species. Two species, *An. coluzzii* and *An. gambiae*, were previously classified as one and results for this former species are classified ‘coluzzii/gambiae’. Significant results (at the 0.05 level with a Bonferroni correction) from a paired-sample t-test are denoted ‘sig.’, non-significant results are denoted ‘n.s.’, and ‘n’ is the number of pairs in each set.

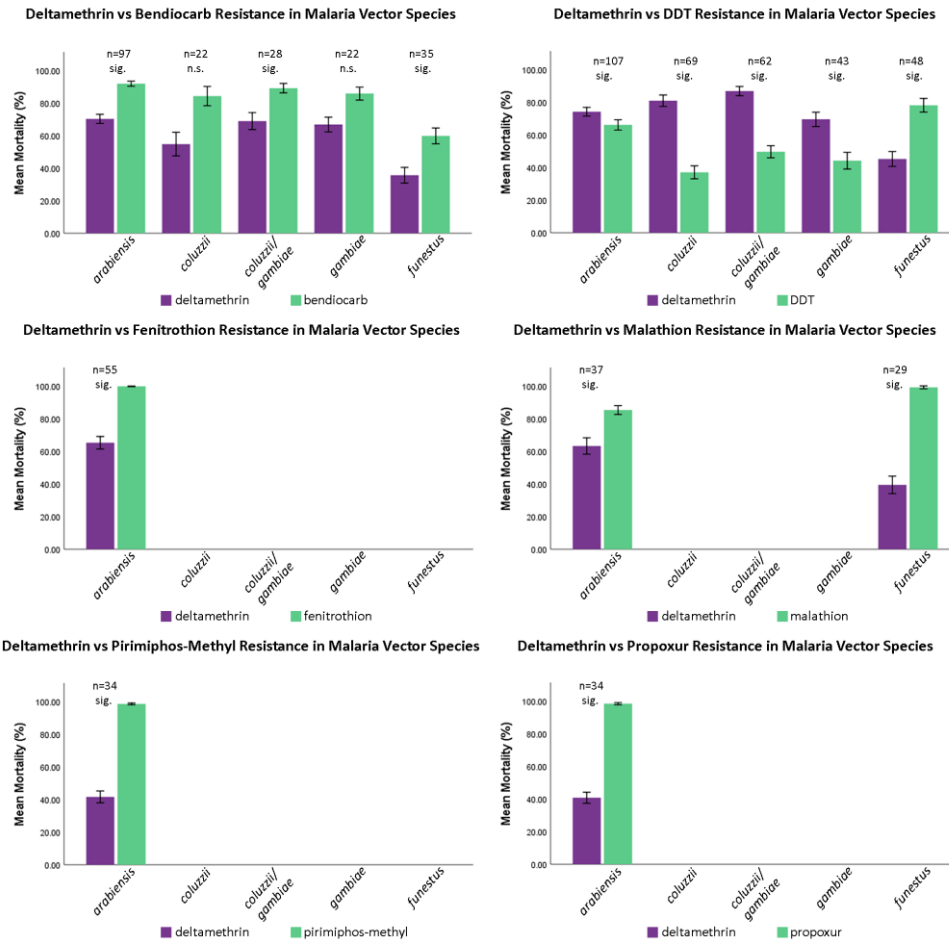


Figure S4.12. Comparison of resistance to deltamethrin and six insecticides from other classes. Two species, *An. coluzzii* and *An. gambiae*, were previously classified as one and results for this former species are classified ‘coluzzii/gambiae’. Significant results (at the 0.05 level with a Bonferroni correction) from a paired-sample t-test are denoted ‘sig.’, non-significant results are denoted ‘n.s.’, and ‘n’ is the number of pairs in each set.