

Site-specific bio-efficacy of Interceptor G2 and Royal Guard against highly pyrethroid- 1 resistant *Anopheles gambiae* s.l. in experimental hut in southwestern Burkina Faso

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Abstract

Insecticide-treated nets (ITNs) lowered malaria cases and deaths by 68% in endemic regions by 2015, but rising resistance has slowed progress. Burkina Faso faces high resistance levels challenging vector control. This study assessed the effectiveness of Interceptor G2 (IG2) and Royal Guard (RG) ITNs against highly resistant (< 10% mortality rate to alpha-cypermethrin 0.05%) *Anopheles gambiae* s.l. in Experimental Hut Trials in Tannounyan region, Burkina Faso. Among 22,234 collected mosquitoes collected over three years, 66.6% were *An. gambiae* s.l. with *Anopheles coluzzii* and *An. gambiae* s.s., dominant in Tengrela and Tiefora respectively. Unwashed IG2 ITNs induced the highest mosquito mortality rates, 57% and 34% as 72-hour mortality in Tiefora and Tengrela respectively; mortality rates were higher for *An. gambiae* than *An. coluzzii* and significantly higher in both sites compared to standard ITNs. Net washing or aging reduced the efficacy of both IG2 and RG. Only unwashed RG nets reduced blood

38 feeding success compared to standard ITNs. Follow up on the sublethal effects of the different
39 arms showed, RG ITNs drastically reduced *Anopheles gambiae* s.l. offspring number compared
40 to other nets. Results suggest, dual-ingredient nets' impact varies with local vector ecology,
41 underscoring the need for targeted strategies of deployment and monitoring.
42 **Keywords:** *Anopheles gambiae* s.l., Interceptor G2, Royal Guard, experimental hut, Burkina
43 Faso, insecticide resistance, sublethal effect.

Introduction

Malaria is a global health challenge, impacting particularly vulnerable populations. The World Health Organisation (WHO) reported 282 million malaria cases and 610 thousand deaths over all regions in 2024 (1) with more than 88% falling in sub-Saharan Africa (SSA). Vector control interventions have improved disease prevention through the availability of tools such as indoor residual spraying (IRS) (2) and insecticide-treated nets (ITNs) (3, 4). Globally, between 2004 and 2017, approximately 1.5 billion nets treated with pyrethroid insecticides – later qualified as standard nets – were sent to malaria endemic countries (5) and it is estimated that these averted 663 million clinical malaria cases by 2015 (6). Despite the efforts of national malaria control programmes, progress has largely stalled in the past decade (7) with further increases in disease incidence since 2021 (1). This change has been attributed in part to the rise of insecticide resistance in malaria vectors (8, 9). Therefore, the development of new tools and/or public health insecticides were prioritised to mitigate this growing resistance issue.

The ITN Interceptor G2 contains chlorfenapyr and a pyrethroid insecticide (alpha-cypermethrin) (10) while the Royal Guard (RG) ITN includes alpha-cypermethrin and pyriproxyfen (11). Chlorfenapyr is an N-substituted halogenated pyrrole with non-neurotoxic mode of action; combining two insecticides with different modes of action is intended to enhance the effectiveness of ITNs against pyrethroid-resistant *Anopheles gambiae* s.l. Pyriproxyfen is an insect growth regulator that mimics the natural juvenile hormone III (JH III name after Methyl (2E, 6E)-(10R)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate) by binding and impairing insect development (12). Since the first availability of dual active ingredient (dual AI) ITNs in 2018, the proportion of these ITNs deployed in Africa has increased from 2% to 70% of the total ITNs sent to SSA by the second quarter of 2025 (totalling approximately 120 million) (5). Several studies using experimental hut trials (13, 14) showed that these ITNs improved entomological outcomes. Further, cluster-randomised trials showed ITNs containing chlorfenapyr in Tanzania (15) or pyriproxyfen in Burkina Faso (16) provided better protection against clinical malaria than pyrethroid-only nets in areas with high level of pyrethroid-resistance vectors.

Vector populations in Burkina Faso have very high levels of pyrethroid resistance, caused by multiple mechanisms including metabolic and target site mutations (17-20). Experimental hut trials (EHT) with Interceptor G2 found these dual AI nets induced high levels of mortality

against pyrethroid resistant mosquitoes in Vallee du Kou (21) making it a potential resistance management tool for the country. This led Burkina Faso, through its Permanent Secretariat for Malaria Elimination (SP-Palu), to become one of the first to deploy dual AI-ITNs during a mass campaign distribution with support of the Global Fund in 2019. A total of 1,563,934 Interceptor G2 nets were distributed in the southwestern part of the country – the Tannounyan region (formerly known as Cascades region) – which was selected based on malaria prevalence and the local pyrethroid resistance profile (5). To evaluate this initial distribution, several studies were undertaken to collect epidemiological and entomological data (22), including further EHTs. Here we present the efficacy of IG2 and Royal Guard® in EHTs conducted in 2019, 2020 and 2021 against pyrethroid-resistant *An. gambiae* s.l. in the Tannounyan region of Burkina Faso.

Results

Local mosquito species composition, entry in the huts

Over the three years of EHT at both stations (Tengrela and Tiefora), a total of 22,234 mosquitoes were collected. *An. gambiae* s.l. was the most prevalent species, accounting for 66.6% (n= 14,800) of collected mosquitoes (**Supplementary Table 1**). Other taxa included *Mansoni sp* (23.9%), *Culex sp* (5.6%), *Aedes sp* (0.2%) and other *Anopheles* (3.7%).

Overall, the number of *An. gambiae* s.l. entering huts did not differ significantly between huts ($df = 5$, $\chi^2 = 5.6$, $p = 0.4$), indicating the position of the huts did not influence entry, although both treatment type and site influenced mosquito entry ($df = 7$, $\chi^2 = 62$, $p < 0.0001$). The mean number of *An. gambiae* s.l. collected per hut per night were 14.7 (95% CI: 13.4 – 16.0) in Tengrela and 5.1 (95% CI: 4.6 – 5.6) in Tiefora. Molecular analysis of 2,574 specimens at the *An. gambiae* complex member level revealed notable difference in species dominance. In Tengrela, *Anopheles coluzzii* was predominant (N = 1,580) representing 90.7% followed by *Anopheles. gambiae* s.s. with 7.9% (**Figure 1**). In contrast, in Tiefora, *An. gambiae* s.s. (67.6%) was the most prevalent species and *An. coluzzii* represented 28% (N = 994). Overall, the proportion of *Anopheles arabiensis* was very low, comprising only 1.4% in Tengrela and 4.4% in Tiefora (**Figure 1**). Temporal changes in species composition were noted between 2020 and 2021 in Tiefora with slight increases in *An. coluzzii* and *An. arabiensis* (**Figure 1**).

Further species identification of a subset of 455 *An. gambiae* s.l.– collected at larval stage and used in the bioassays for insecticide resistance profiling – confirmed *An. coluzzii* (> 75%) was

the dominant species in Tengrela over the period (2019 to 2021) of the EHTs. In Tiefora, a mixture of *An. arabiensis*, *An. coluzzii* and *An. gambiae* s.s was observed, with proportions varying by year of collection (**Figure 1**). In addition, *An. gambiae* s.s predominated in 2020; however, its proportion slightly declined in 2021 accompanied by increases in *An. coluzzii* and *An. arabiensis* (**Table 1, Figure 1**) as seen in adult population.

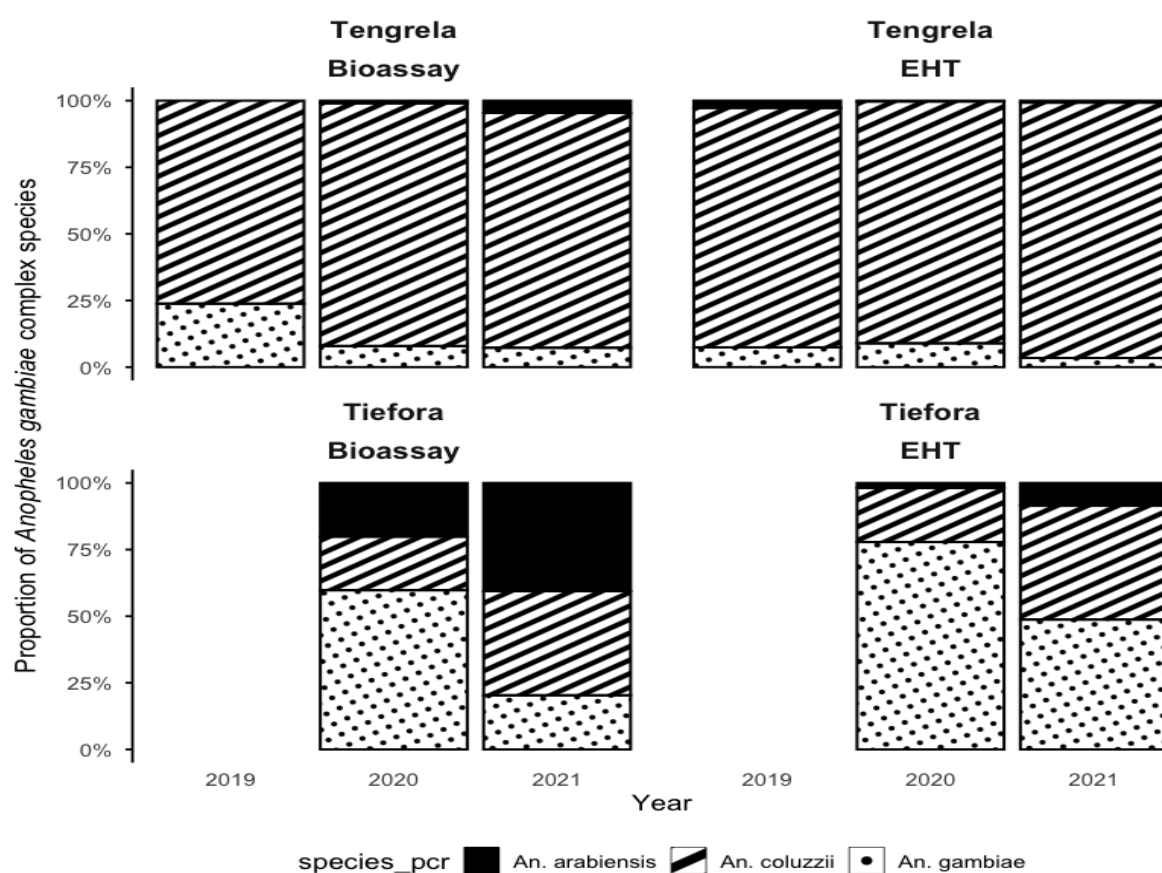


Figure 1: Histogram indicating the relative proportion of *Anopheles gambiae* s.l. sibling species from experimental huts and bioassays year and villages (Tengrela and Tiefora). Bioassay mosquitoes were collected as larvae and reared to adults in the insectary prior to insecticide testing.

Table 1: *Anopheles gambiae* complex level composition, alongside the frequency of the L995F knock-down resistance allele, as determined from bioassay data collected from the study sites (Tengrela and Tiefora) displayed per year and village.

Year	Village	Species	n_samples	N_samples	Species proportion	Freq(995F)
2019	Tengrela	An. arabiensis	0	21	0	0
		An. coluzzii	16	21	76.2 [54.9 – 89.4]	50 [33.34 – 66.66]
		An. gambiae	5	21	23.8 [10.6 – 45.1]	90 [53.28 – 98.61]
	Tiefora	An. arabiensis	nc	nc	nc	nc
		An. coluzzii	nc	nc	nc	nc
		An. gambiae	nc	nc	nc	nc
2020	Tengrela	An. arabiensis	1	101	0.99 [0.17 – 5.4]	100
		An. coluzzii	92	101	91.1 [83.9 – 95.2]	25.86 [19.9 – 32.88]
		An. gambiae	8	101	7.92 [4.07 – 14.9]	64.29 [37.63 – 84.3]
	Tiefora	An. arabiensis	32	159	20.1 [14.6 – 27]	70.59 [53.44 – 83.39]
		An. coluzzii	32	159	20.1 [14.6 – 27]	68 [53.98 – 79.38]
		An. gambiae	95	159	59.8 [52 – 67]	49.12 [40.08 – 58.23]
2021	Tengrela	An. arabiensis	5	110	4.55 [1.96 – 10.2]	20 [5.04 – 54.07]
		An. coluzzii	97	110	88.2 [80.8 – 93]	43.18 [36.06 – 50.6]
		An. gambiae	8	110	7.27 [3.73 – 13.7]	100
	Tiefora	An. arabiensis	26	64	40.6 [29.5 – 52.8]	42.5 [28.31 – 58.04]
		An. coluzzii	25	64	39.1 [28.1 – 51.3]	52 [38.35 – 65.36]
		An. gambiae	13	64	20.3 [12.3 – 31.7]	88.89 [64.78 – 97.21]

Freq(995F) = knockdown resistance 995F allele frequency in mosquitoes exposed during bioassays over the experimental hut trials periods, N_samples = number of total samples analysed by PCR, n_samples = number of sibling species found positive. nc indicates no collection for the EHT

Insecticide resistance profile in the *Anopheles gambiae* s.l. population of the study area

Bioassay results demonstrated that *An. gambiae* s.l. population from both study sites exhibit high/complete resistance to pyrethroids. The 24-hour post-exposure mortality rate to alpha-cypermethrin and deltamethrin recorded between 2019 and 2022 was overall very low (not exceeding 10% on either site to alpha-cypermethrin 0.05%) (**Figure 2**). Exposures of mosquito population from Tengrela to chlorfenapyr in both 2021 and 2022 indicate a mortality rate of >99% (**Figure 2**). However, a reduced susceptibility to chlorfenapyr was found in Tiefora population exhibiting a mortality rate of 88% (95%CI: 76.5 – 95.5) and 97.6% (95% CI: 93.8 – 99.5) respectively in 2021 and 2022 (**Figure 2**).

The frequency of the knock-down resistance 995F mutation allele (kdr-995F) was estimated from subsamples of *An. gambiae* s.l. collected as larvae for the bioassays (N = 455 samples) and those collected in the huts trial (N = 1,385 samples). Results indicated the frequency of kdr-995F varied between species and was higher in *An. gambiae* s.s in EHT compared to that from *An. arabiensis* and *An. coluzzii* (**Table 2**). Frequencies of 995F kdr from larval collections

were more variable between sites and years (**Table 1**) but overall frequency of 995F in each species was respectively 45.6% (95% CI: 34.5 – 57.0%) in *An. arabiensis*, 42.6% (95% CI: 37.9 – 47.6%) in *An. coluzzii* and 59.5% (95% CI: 51.1 – 67.4%) in *An. gambiae* s.s. No significant difference was observed between *An. arabiensis* and *An. coluzzii* regardless of the site with an Odds Ratio of 1.1 (95% CI: 0.6 – 2.1, $p = 0.9$).

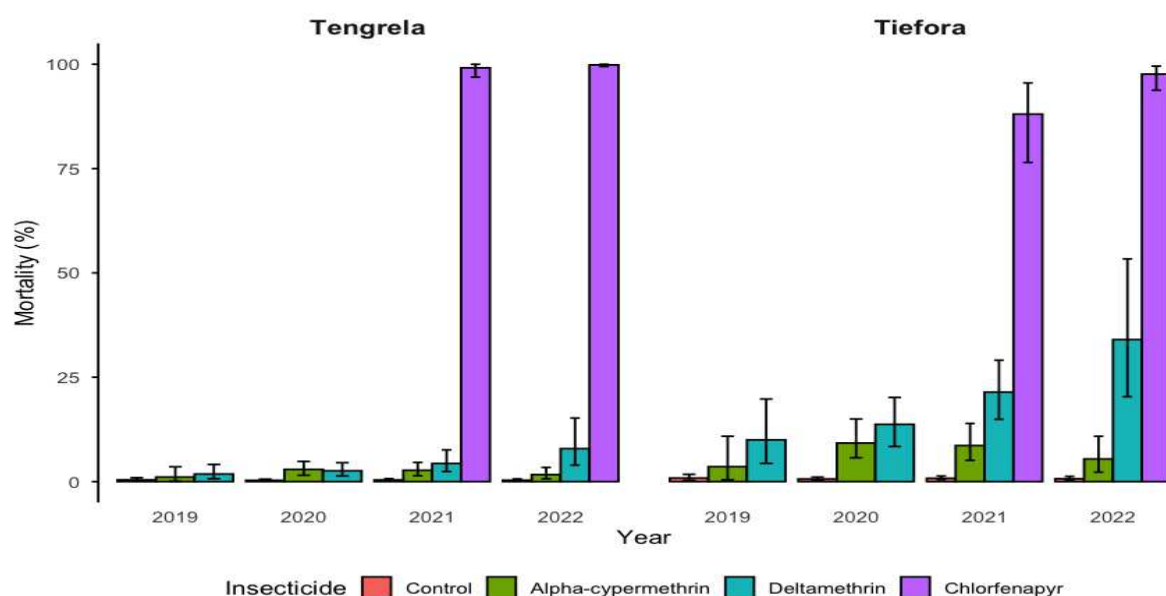


Figure 2: Histogram indicating mosquitoes mortality rates per insecticide, year and villages (Tengrela and Tiefora) where the experimental huts are located. Error bars indicate the 95% confident intervals. Mosquitoes used in the discriminating dose. Bioassays were collected as larvae from the experimental hut trial sites.

Table 2: *Anopheles gambiae* complex level composition, alongside the frequency of the L995F knock-down resistance allele, as determined in mosquitoes collected from the experimental huts on the study sites (Tengrela and Tiefora) displayed per village and species.

Species	Village	Number tested	freq(995F)
<i>An. arabiensis</i>	Tengrela	9	49.48 [27.59 – 71.57]
<i>An. coluzzii</i>	Tengrela	797	52.84 [49.85 – 55.82]
<i>An. gambiae</i> ss	Tengrela	63	78.33 [71.76 – 83.72]
<i>An. arabiensis</i>	Tiefora	31	63.10 [51.62 – 73.26]
<i>An. coluzzii</i>	Tiefora	147	57.79 [52.23 – 63.17]
<i>An. gambiae</i> ss	Tiefora	338	79.81 [76.54 – 82.73]

EHT mortality rate at 72-hour post-mosquito-collections

Mortality rate did not vary significantly with hut position ($df = 5$, $\chi^2 = 5.9$, $p = 0.61$) but was strongly influenced by treatment and village interaction ($df = 7$, $\chi^2 = 32.14$, $p < 0.0001$). Overall, mosquito mortality rate from the trials was very low, with a mean of 17.3% (95% CI: 15.7 – 19.0%) in Tengrela and 26.9% (95% CI: 24.3 – 29.8 %) in Tiefora when combining all treatment and years. At both sites, the highest mortality occurred with unwashed Interceptor G2 nets: 57.0% (95% CI: 49.6 – 64.3%) in Tiefora and 34.3% (95% CI: 29.9 – 39.0%) in Tengrela. The lowest mortality rates to treated nets were recorded for the unwashed Interceptor G1 in Tengrela (**Table 3**). However, only unwashed Interceptor G2 (both sites) and washed Interceptor G2 (Tiefora) induced significantly higher mortality than unwashed Interceptor G1 (Tengrela: OR = 3.1, 95% CI: 2.0 – 5.0, $p = 0.001$; Tiefora OR = 3.6, 95% CI: 2.0 – 6.4, $p = 0.001$). There was a large decline in mortality comparing naturally aged Interceptor G2 nets to unwashed Interceptor G2 in both sites. The odds of mortality did not differ significantly between washed and aged Interceptor G2 nets in Tengrela whereas in Tiefora with the aged Interceptor G2 nets mortality was significantly lower compared to that from the washed ones (**Table 3**).

Mortality with Royal Guard did not differ from standard Interceptor G1 nets in either site (**Table 3**). Washing reduced the mortality from Royal Guard nets at either site but not significantly (Tengrela: OR = 0.6, 95% CI: 0.3 – 1.3, $p = 0.27$; Tiefora OR = 0.7, 95% CI: 0.30 – 1.6, $p = 0.64$).

Table 3: Mortality rate at seventy-two hours post-collection from the huts and comparative Odds Ratio by contrasting treatment and sites. Estimates are derived from the generalised linear mixed effect model.

Treatment	Sites	Mortality rate [95%CI]	ITNs vs UT		ITNs vs unwashed IG1		ITNs vs unwashed ITNs		IG2_aged vs IG2_w	
			Odds.ratio [95%CI]	P-value	Odds.ratio [95%CI]	P-value	Odds.ratio [95%CI]	P-value	Odds.ratio [95%CI]	P-value
UT	Tengrela	8.08 [6.72 – 9.68]	–	–	–	–	–	–	–	–
IG1_aged	Tengrela	18.35 [14.64 – 22.76]	2.56 [1.65 – 3.97]	0.0001*	1.35 [0.80 – 2.27]	0.48	–	–	–	–
IG1_unw	Tengrela	14.24 [11.03 – 18.2]	1.89 [1.19 – 3.01]	0.0001*	–	–	–	–	–	–
IG2_aged	Tengrela	17.17 [13.75 – 21.23]	2.36 [1.53 – 3.63]	0.0001*	1.25 [0.75 – 2.06]	0.72	0.39 [0.25 – 0.61]	0.0001*	0.90 [0.54 – 1.49]	0.97
IG2_unw	Tengrela	34.31 [29.87 – 39.04]	5.95 [4.11 – 8.61]	0.0001*	3.14 [1.97 – 5.02]	0.001*	–	–	–	–
IG2_w	Tengrela	18.72 [14.98 – 23.14]	2.62 [1.69 – 4.06]	0.0001*	1.39 [0.82 – 2.35]	0.41	0.44 [0.28 – 0.68]	0.0001*	–	–
RG_unw	Tengrela	21.86 [16 – 29.13]	3.18 [1.79 – 5.65]	0.0001*	1.68 [0.89 – 3.18]	0.16	–	–	–	–
RG_w	Tengrela	13.73 [9.25 – 19.9]	1.81 [0.95 – 3.46]	0.09	0.96 [0.47 – 1.95]	1.00	0.57 [0.26 – 1.25]	0.27	–	–
UT	Tiefora	8.65 [6.75 – 11.02]	–	–	–	–	–	–	–	–
IG1_aged	Tiefora	20.08 [15.18 – 26.07]	2.65 [1.50 – 4.69]	0.0001*	0.67 [0.36 – 1.26]	0.39	–	–	–	–
IG1_unw	Tiefora	27.2 [21.23 – 34.12]	3.94 [2.26 – 6.90]	0.0001*	–	–	–	–	–	–
IG2_aged	Tiefora	29.89 [23.82 – 36.75]	4.5 [2.63 – 7.71]	0.0001*	1.14 [0.63 – 2.06]	0.96	0.32 [0.18 – 0.56]	0.0001*	0.45 [0.22 – 0.91]	0.02*
IG2_unw	Tiefora	57.01 [49.45 – 64.26]	14 [8.19 – 23.93]	0.0001*	3.55 [1.98 – 6.37]	0.001*	–	–	–	–
IG2_w	Tiefora	48.84 [38.14 – 59.66]	10.08 [5.11 – 19.87]	0.0001*	2.56 [1.24 – 5.25]	0.004*	0.72 [0.35 – 1.45]	0.67	–	–
RG_unw	Tiefora	24.67 [16.87 – 34.57]	3.46 [1.67 – 7.17]	0.0001*	0.88 [0.41 – 1.88]	0.98	–	–	–	–
RG_w	Tiefora	17.63 [12.02 – 25.11]	2.26 [1.13 – 4.51]	0.01*	0.57 [0.27 – 1.20]	0.2	0.65 [0.27 – 1.57]	0.64	–	–

ITNs = insecticide treated nets, UT = untreated net, IG1_unw = unwashed Interceptor G1 nets, IG1_w = washed Interceptor nets; IG2_unw = unwashed Interceptor G2 nets, IG2_w = washed Interceptor G2 nets; IG2_aged = aged Interceptor G2 nets; RG_unw = unwashed Royal Guard nets. RG_w = washed nets Royal Guard nets. C/= Confidence intervals with * denoting P value < 0.05.

EHT blood-feeding inhibition

Overall, blood-feeding success differed significantly across treatment and sites ($df = 7$, $\chi^2 = 67.4$, $p < 0.0001$; **Table 4**). Only washed Royal Guard nets had lower blood-feeding rates than standard Interceptor G1. The estimated blood-feeding success rate in the presence of unwashed Interceptor G2 was lower in Tengrela (26.9%, 95% CI: 21.6 – 33.0%) than Tiefora (53.2%, 95% CI: 43.1 – 62.9%). Pairwise comparisons indicated the odds of blood-feeding success was similar between unwashed Interceptor G2 nets and either washed or aged Interceptor G2 nets in Tiefora. However, in Tengrela, this success was significantly lower with unwashed Interceptor G2 nets compared with both washed and aged Interceptor G2 nets. No significant differences were detected between aged and the washed Interceptor G2 nets at either site.

Blood-feeding success was significantly lower with washed compared to unwashed Royal Guard nets in Tiefora (OR = 0.10, 95% CI: 0.03 – 0.4, $p = 0.0001$) whereas no difference was detected in Tengrela.

Table 4: Blood feeding rates from mosquito collected in the huts and comparative Odds Ratio by contrasting treatment and sites. Estimates are derived from the generalised linear mixed effect model.

Treatment	Sites	Blood feeding rate [95%CI]	ITNs vs UT		ITNs vs unwashed IG1		ITNs vs unwashed ITNs		IG2_aged vs IG2_w	
			Odds.ratio [95%CI]	P-value	Odds.ratio [95%CI]	P-value	Odds.ratio [95%CI]	P-value	Odds.ratio [95%CI]	P-value
UT	Tengrela	50.65 [45.11 – 56.16]	–	–	–	–	–	–	–	–
IG1_aged	Tengrela	36.07 [28.5 – 44.4]	0.55 [0.32 – 0.94]	0.02*	1.14 [0.59 – 2.18]	0.9713	–	–	–	–
IG1_unw	Tengrela	33.16 [25.68 – 41.61]	0.48 [0.28 – 0.84]	0.003*	–	–	–	–	–	–
IG2_aged	Tengrela	41.31 [33.07 – 50.06]	0.69 [0.4 – 1.18]	0.30	1.42 [0.75 – 2.69]	0.54	1.91 [1.05 – 3.46]	0.03*	1.05 [0.53 – 2.08]	0.99
IG2_unw	Tengrela	26.95 [21.62 – 33.04]	0.36 [0.22 – 0.58]	0.001*	0.74 [0.41 – 1.36]	0.63	–	–	–	–
IG2_w	Tengrela	40.2 [31.9 – 49.1]	0.66 [0.38 – 1.14]	0.21	1.35 [0.69 – 2.67]	0.71	1.82 [1.01 – 3.29]	0.04*	–	–
RG_unw	Tengrela	31.57 [21.66 – 43.51]	0.45 [0.22 – 0.94]	0.02*	0.93 [0.41 – 2.12]	1.00	–	–	–	–
RG_w	Tengrela	50.79 [37.7 – 63.78]	1.01 [0.47 – 2.16]	1.00	2.08 [0.88 – 4.9]	0.13	2.24 [0.84 – 6.03]	0.17	–	–
UT	Tiefora	54.73 [47.72 – 61.56]	–	–	–	–	–	–	–	–
IG1_aged	Tiefora	57.08 [46.93 – 66.68]	1.1 [0.58 – 2.1]	0.99	2.11 [1.08 – 4.97]	0.02*	–	–	–	–
IG1_unw	Tiefora	36.48 [27.53 – 46.48]	0.48 [0.25 – 0.91]	0.02*	–	–	–	–	–	–
IG2_aged	Tiefora	55.59 [45.54 – 65.2]	1.04 [0.55 – 1.96]	1.00	2.18 [1.03 – 4.6]	0.04*	1.10 [0.53 – 2.31]	0.99	0.64 [0.24 – 1.70]	0.69
IG2_unw	Tiefora	53.15 [43.14 – 62.9]	0.94 [0.5 – 1.78]	1.00	1.97 [0.94 – 4.17]	0.09	–	–	–	–
IG2_w	Tiefora	66.21 [51.45 – 78.37]	1.62 [0.66 – 3.95]	0.55	3.41 [1.29 – 9.02]	0.005*	1.73 [0.66 – 4.53]	0.5	–	–
RG_unw	Tiefora	41.33 [27.44 – 56.75]	0.58 [0.24 – 1.44]	0.45	1.23 [0.46 – 3.26]	0.97	–	–	–	–
RG_w	Tiefora	6.49 [3.33 – 12.25]	0.06 [0.02 – 0.16]	0.001*	0.12 [0.04 – 0.36]	0.001*	0.10 [0.03 – 0.34]	0.001*	–	–

ITNs = insecticide treated nets, UT= untreated net, IG1_unw = unwashed Interceptor G1 nets, IG1_w = washed Interceptor G1 nets, IG2_unw = unwashed Interceptor G2 nets, IG2_w = washed Interceptor G2 nets, IG2_aged= aged Interceptor G2 nets, RG_unw = unwashed Royal Guard nets. RG_w = washed Royal Guard nets. CI = Confidence intervals with * denoting $p < 0.05$.

***Anopheles coluzzii* mortality relative to *An. gambiae* s.s in the presence of different nets**

Analysis of mortality 72 hours post-collection of *An. gambiae* s.l. from both sites indicates a significant association between net treatment types and the mosquito species mortality ($df = 7$, $\chi^2 = 23.9$, $p = 0.001$). Across both site, *An. gambiae* s.s. exhibited the highest mortality in the presence of unwashed Interceptor IG2 nets (53.2%, 95% CI: 44.9 – 61.3%) and *An. coluzzii* showed higher rate with washed Interceptor G2 nets (42.1%, 95% CI: 34.8 – 49.8%) (**Figure 3**). Pairwise comparisons showed no evidence of difference in mortality between *An. coluzzii* and *An. gambiae* s.s, when collected in huts with both Interceptor G1 and Royal Guard nets

regardless of their washing status (**Figure 3**). However, *An. coluzzii* showed significantly lower mortality than *An. gambiae* s.s in the presence of aged Interceptor G2 (OR = 0.4, 95% CI: 0.2 – 0.9, $p = 0.02$) and unwashed Interceptor G2 nets (0.5, 95% CI: 0.3 – 0.7, $p = 0.001$; **Figure 3**).

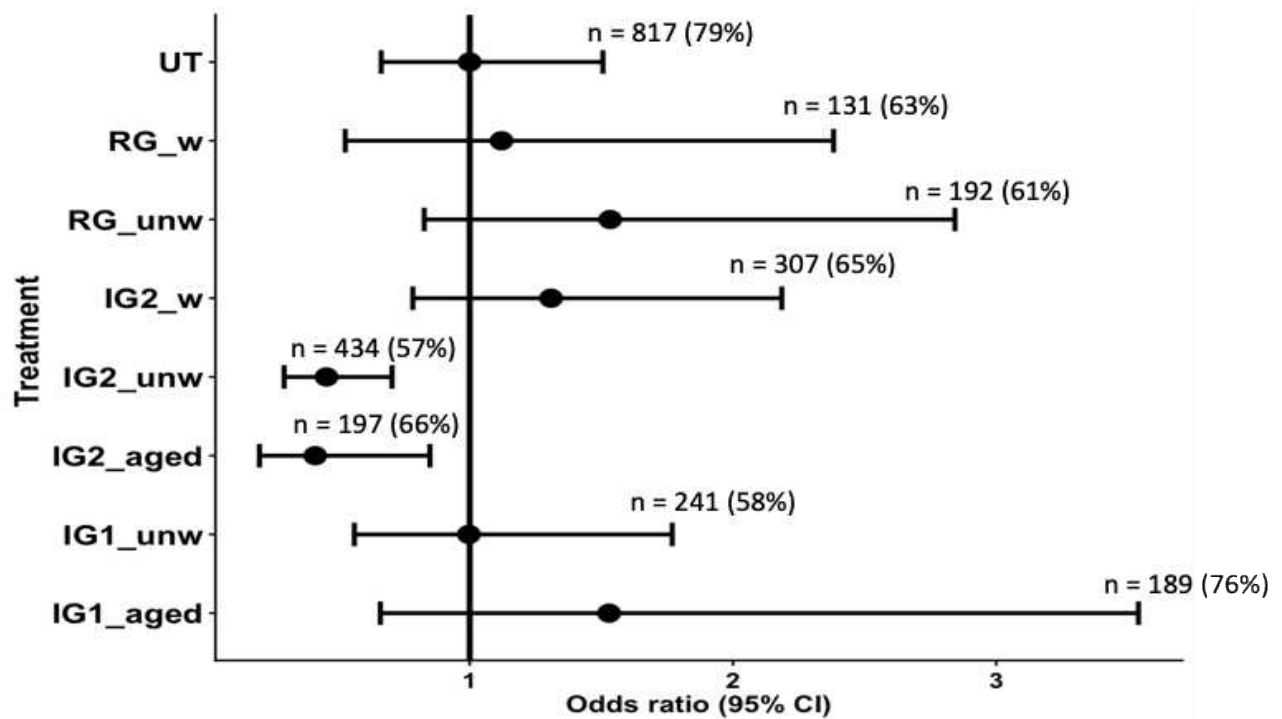


Figure 3: Comparative Odds ratio related (*An. coluzzii*/*An. gambiae* s.s.) to the seventy-two-hour mortality rate of *Anopheles gambiae* complex members according to the treatments over sites. Error bars indicate the 95% confidence interval. **n** indicates the number of mosquitoes tested (subsample of dead and alive at 72-hours post-collection) and figures inside brackets indicate the proportion of *An. coluzzii*. **UT, IG1, IG2** and **RG** respectively indicate the untreated, interceptor G1, interceptor G2

Sublethal effects on *An. gambiae* s.l. oviposition and fertility

A total of 866 fully blood-fed female *An. gambiae* s.l were selected from collection in the huts and sent to the insectary to allow ovipositing. Oviposition rate varied between villages ($df = 1$, $\chi^2 = 9.6$, $p = 0.002$) and between treatments ($df = 7$, $\chi^2 = 24.7$, $p < 0.001$). Overall, female mosquitoes from Tiefora were more likely to lay eggs compared to those from Tengrela (OR = 0.5 [95% CI = [0.36 – 0.80], $p = 0.002$). The highest oviposition rate occurred with the untreated net (32.9% 95% CI: 25.6 – 41.1%) while the lowest rate was recorded with unwashed Royal Guard (7.2% 95% CI: 2.9 – 16.7%; **Table 5**). There was no significant difference in the

oviposition rate between washed and unwashed Royal Guard nets. Further, no significant difference was found between unwashed Interceptor G1 and other treatment arms (**Table 5**). Fecundity did not vary between villages ($df = 1$, $\chi^2 = 0.6$, $p = 0.46$) but differed between treatments ($df = 7$, $\chi^2 = 66.1$, $p < 0.0001$). On average, 96 eggs (95% CI: 86 – 105) were laid per individual female mosquito over villages and treatments. The highest number were found in huts with untreated nets (148 eggs, 95% CI: 136 – 160). As expected, female *An. gambiae* s.l. laid fewer eggs when collection was conducted in huts with either unwashed or washed Royal Guard nets (**Table 5**). There was no significant difference in the number of eggs laid by *An. gambiae* s.l. collected from huts with unwashed or washed Royal Guard nets (OR = 0.7, 95% CI: 0.3 – 2.0). Significantly more eggs were laid in huts with unwashed Interceptor G2 nets than either washed (OR = 0.9, 95% CI: 0.6 – 1.3) or aged (OR = 0.9, 95% CI: 0.7 – 1.3) Interceptor G2 nets. No difference was found (OR = 1.1, 95% CI: 0.8 – 1.6) between washed and aged IG2 nets.

Fertility also differed significantly between treatments ($df = 7$, $\chi^2 = 126.9$, $p < 0.0001$), but not between villages ($df = 1$, $\chi^2 = 3.0$, $p = 0.08$). In huts with untreated nets, only about 40% of laid 148 eggs developed into adults (59 adults, 95% CI: 49 – 71). The mean number of adults emerged from individual female was lowest with – both unwashed and washed – Royal Guard nets (**Table 5**). As for the fecundity, no significant differences were detected when comparing the unwashed Interceptor G2 nets to either washed (OR = 0.9, 95% CI: 0.4 – 1.9) or aged (OR = 0.9, 95% CI: 0.4 – 2.0) ones nor between washed and aged Interceptor G2 nets (OR = 1.4, 95% CI: 0.7 – 2.9). However, when comparing unwashed Interceptor G1 (taken as reference) to unwashed Royal Guard nets, a significant reduction in offspring number (OR = 0.1, 95% CI: 0.03 – 0.5; **Table 5**) was observed in Royal Guard nets. There was no significant difference (OR = 0.9, 95% CI: 0.2 – 4.9) in the mean number of progenies from female collected in huts provided with unwashed versus washed Royal Guard nets.

Table 5: Oviposition, fecundity and fertility rates from mosquito collected in the huts displayed by treatment. Estimates are derived from the generalised linear mixed effect model.

Treatment	Total for oviposition	Nb Ovipo	Oviposition rate [95%CI]	Number of eggs per treatment	Mean number of eggs per female [95%CI]	Number of emerged female per treatment	Mean number of emerged female per FO [95%CI]
UT	209	76	32.89 [25.6 – 41.11]	11284	148.08 [136.27 – 159.89]	2240	58.98 [48.83 – 71.24]
IG1_aged	113	23	17.87 [11.24 – 27.2]	2510	108.64 [87.22 – 130.06]	223	20.61 [14.52 – 29.25]
IG1_unw	99	29	24.06 [15.66 – 35.1]	3486	120.42 [101.37 – 139.47]	268	19.94 [14.6 – 27.25]
IG2_aged	181	33	17.06 [11.33 – 24.87]	3539	108.29 [90.23 – 126.35]	429	29.26 [21.83 – 39.2]
IG2_unw	92	19	16.55 [9.85 – 26.46]	2183	114.57 [91.04 – 138.11]	197	22.67 [15.45 – 33.27]
IG2_w	59	17	23.06 [13.09 – 37.37]	1677	97.95 [73.01 – 122.88]	161	20.31 [13.51 – 30.53]
RG_unw	61	6	7.18 [2.9 – 16.72]	224	36.52 [5.39 – 78.44]	9	2.43 [1.05 – 5.6]
RG_w	52	8	16.71 [7.77 – 32.33]	218	30.16 [6.9 – 67.21]	9	2.37 [1.11 – 5.05]

UT= untreated net, IG1_unw = unwashed Interceptor G1 nets, IG1_w = washed Interceptor G1 nets; IG2_unw = unwashed Interceptor G2 nets, IG2_w = washed Interceptor G2 nets; IG2_aged= aged Interceptor G2 nets; RG_unw = unwashed Royal Guard nets. RG_w = washed nets Royal Guard nets. CI = Confidence intervals, Nb Ovipo = Number of females that laid eggs.

Deterrence and exophily rates

Comparison within treatment (unwashed versus washed or aged within same net type) indicated no significant difference in deterrence and exophily, with the exception of Tengrela, where the washed Royal Guard reduced mosquito entry by about 45% compared to their unwashed counterparts (OR = 0.6, 95% CI: 0.3 – 0.9, $p = 0.01$) (**Supplementary Table 2**). Moreover, mosquitoes exiting from the huts (exophily) was unaffected by hut position ($df = 5$, $\chi^2 = 0.4$, $p = 0.99$) and did not differ significantly between sites ($df = 1$, $\chi^2 = 1.0$, $p = 0.31$). However, a treatment-by-site interaction was significant ($df = 7$, $\chi^2 = 16.3$, $p = 0.02$). In evaluating exophily within-treatments (unwashed versus aged or washed IG2 nets and washed and unwashed Royal Guard nets), no significant differences were observed between net conditions tested at either site (**Supplementary Table 3**).

Discussion

Our study, conducted in West African-style experimental huts following WHOPES guidelines, evaluated the efficacy of Interceptor G2 (chlorfenapyr + alpha-cypermethrin) and Royal Guard

(pyriproxyfen + alpha-cypermethrin) against pyrethroid-resistant *An. gambiae* s.l. Two sets of huts were located in two villages (Tengrela and Tiefora), in southwestern Burkina Faso, 40 km apart with similar agricultural practices. In addition to immediate mortality and blood-feeding rates in *An. gambiae* s.l., oviposition, fecundity, and fertility were measured as outcomes to assess entomological efficacy.

The vector populations at the study sites have distinct species composition of *An. gambiae* complex members and exhibit high levels of pyrethroid resistance. The presence of *An. arabiensis*, *An. coluzzii* and *An. gambiae* s.s were noted in different proportions with Tengrela having predominantly *An. coluzzii* across the period, whereas Tiefora saw a decline in *An. gambiae* s.s proportions with an increase in *An. coluzzii* in the last year (2021) of the study. Bioassays conducted during the hut trials showed that *An. gambiae* s.l. from both sites exhibited very low mortality when exposed to diagnostic dose of alpha-cypermethrin, in line with previous findings of high pyrethroid resistance in this area (18, 23). Studies across the country have similarly documented level of resistance, likely resulting from a combination of different mechanisms including target site mutations and metabolic resistance (17, 19, 20). Here, we reported a high frequency of the kdr-995F alleles in both sites and across all the three species with *An. gambiae* s.s. carrying higher frequency aligning with results from other west African country (24). High levels of mortality were observed after exposure of mosquitoes from both experimental hut sites to chlorfenapyr as described elsewhere (18). although full susceptibility was only observed in Tengrela and reduced susceptibility in Tiefora

Results from the current study indicates lower mortality and efficacy of the Interceptor G2 nets compared to results from a study undertaken in 2014 in Valley-du-Kou (21). In the current study, the mortality was less than 60% in the presence of Interceptor G2 nets regardless of whether the ITNs were unwashed, washed or operationally aged. From the study in Valley-du-Kou, using the same style of experimental huts, the mortality rates registered in the presence of both unwashed and washed Interceptor G2 nets were approximately 80% (21). Valley-du-Kou has ecology and agricultural activities are similar to Tengrela – one of the sites used in the current study – with *An. coluzzii* being also the most dominant species (25). Results from the discriminating dose bioassay suggested somewhat lower chlorfenapyr mortality in Tiefora than Tengrela, but it is unclear whether this is sufficient to explain the substantially lower mortality observed in the experimental hut trials. A recent meta-analysis indicated substantial

heterogeneity in the EHT mortality caused by pyrethroid-chlorfenapyr-based nets, with some association with the mortality induced by pyrethroid-only net, suggesting the potential for some cross resistance (26), though measurement error in the complex entomological assay or other factors (outlined below) could have caused the result.

At both locations, Tengrela and Tiefora, unwashed Interceptor G2 nets achieved the highest mortality rate, indicating enhanced efficacy of this intervention under optimal (unwashed) conditions. This outcome is consistent with expectation for dual-active ingredient nets in areas where vectors exhibit pyrethroid resistance (14, 27) and the higher performance of unwashed Interceptor G2 compared to unwashed Interceptor G1 across all sites underscores the added value of using chlorfenapyr in the Interceptor G2 nets for increasing mortality in the pyrethroid-resistant *An. gambiae* s.l. population (28). Chlorfenapyr acts as a pro-insecticide which requires bioactivation into its active form of tralopyril through metabolism in *An. gambiae* s.l. via cytochrome P450 enzymes actions (29). The notable difference in the mortality between the two sites indicates that the performance of Interceptor G2 may be affected by local vector population characteristics – resistance mechanisms or behavioural differences. Since the net washings were standardised, this observation also applies to the variation in odds of mortality between unwashed and washed Interceptor G2 nets across sites. Variation in efficacy seen between aged and washed nets may be attributable to difference in their handling. The aged nets were obtained from residents (in the field) after two years of use, without regard to the specifics of their aging or washing conditions, whereas the washed nets were processed under standardised laboratory conditions.

Overall, the vector blood-feeding success showed efficacy was dependent on both treatments and sites. The estimated blood-feeding success rate was higher in Tiefora than that estimated from Tengrela indicating possible associations with *An. gambiae* complex member species composition, blood-feeding behaviour and livestock characteristics (30). None of the dual Active Ingredient nets reduced blood feeding beyond what was observed with unwashed Interceptor G1, indicating that the alpha-cypermethrin content was responsible for this personal protection, not the additional active ingredient. This may be due to a baseline level of repellency by pyrethroids – avoidance in mosquitoes – associated with inhibiting short-term blood-feeding.

Pairwise comparisons indicated the odds of blood-feeding success did not differ between either washed or aged Interceptor G2 nets compared to the untreated ones in Tiefora. This is in line with several previous studies on the performance of Interceptor G2, demonstrating that despite multiples washes and ageing Interceptor G2 maintains its efficacy as personal protection tool (14, 21, 31). The 20 washes are used as a proxy for long-term household use although ageing may still differ due to handling and insecticide depletion over time. Similarity, observation at both locations between the washed and aged Interceptor G2 nets, with respect to blood feeding success, supports the WHO proxy for aging nets. It should be noted that only two aged Interceptor G2 nets – from different households – were used at each site in this study, and the treatment of these nets by the households is not known.

Together, both mortality rate and blood-feeding success of *An. gambiae* s.l. in the presence of unwashed Interceptor G2 in Tiefora compared to those from Tengrela indicate heterogeneity in the effects of this net, implying site-dependent effect in Interceptor G2 nets efficacy. Several factors including local mosquito species composition, their feeding behaviour and insecticides resistance profile may collectively contribute to such observed discrepancies in performance of this ITN. Our analysis indicated that the mortality to Interceptor G2 is species-specific with *An. gambiae* s.s. being more likely to die in the presence of both unwashed and aged Interceptor G2 nets compared to *An. coluzzii*. This observation may be associated with insecticide resistance mechanisms as showed to vary between species. Previous study has shown that *An. gambiae* s.s. homozygous for kdr-995F allele experienced higher mortality after exposure to chlorfenapyr (32). Thus, the current higher mortality in the Tiefora population may be possibly due to the high frequency of the kdr-995F mutation seen in *An. gambiae* s.s. However, several mechanisms are involved in the local vector resistance to insecticide including other target site mutations such as I1527T, V402L (both within the Voltage-gated sodium channels) (33) that are known to be present in *An. coluzzii* populations in this region, and differences in metabolic resistance mechanisms in both population (20, 33). Therefore, the current data does not enable any predictions on the role of kdr-L995F mutation or other resistance mechanisms in the response to dual active ingredient nets.

Secondly, the feeding success was higher in Tiefora compared to that from Tengrela. *An. gambiae* s.s. predominant in Tiefora is more anthropophilic compared to *An. coluzzii* (30),

though it is unclear whether this blood-feeding behaviour is altered by the host availability (34).

Additionally, the difference in the feeding success between Tengrela and Tiefora may be associated with potential higher-flying activities and more frequent net contact, as mortality increases with increased net contact. Previous studies have shown that the activity during host seeking may depend on type of net-treatments in place and also the resistance profile in of *An. gambiae* s.l. (35). Further, increased activity has been linked with enhanced efficacy of chlorfenapyr, as its activation into tralopyril (its toxic compound) by the cytochrome P450 enzymes (29) is potentially associated with increased movement (36).

Our findings showed that the mean number of both eggs and adults emerging per female were lowest with Royal Guard nets regardless of whether the nets were washed or not, implying a sublethal effect on mosquito reproductive capacity. These observations align with previous findings from experimental huts trials using Royal Guard nets (13, 27). Additionally, research involving field-collected *An. gambiae* s.l. from the same area, sampled before and after the deployment of Olyset Duo nets – another pyrethroid-pyriproxyfen combination net, demonstrated significant reduction in offspring (37), likely attributable to pyriproxyfen. However, exposure of *An. gambiae* s.l. to this Olyset Duo nets – in cone bioassays – showed that effects of Pyriproxyfen on fertility in the durability study dissipated after one month. As this result pertains to a distinct type of net, the persistence of the fertility-reducing effect may vary with Royal Guard nets (37). Though, the similarity between unwashed and washed Royal Guard nets suggests that this sublethal reproductive effect remain even after washing. Despite having little effect on 72 hour mortality – as expected and potentially due to resistance to the alpha-cypermethrin – Royal Guard nets could be an effective tool in the study areas for reducing mosquito density but also longevity (37), and subsequently malaria transmission.

There are several limitations in the current study. Despite aged nets being in good physical condition when collected, their handling history – including the washing frequency over the two-years of use in the field which may potentially or partially explain some of the discrepancies in the results between sites - was not collected. Further, the species identification and the kdr-995F mutation genotyping provided only partial insight into the underlying resistance mechanisms. The assessment of the metabolic profile of the *An. gambiae* s.l. and other target site mutations that might have clarified cross-resistance between pyrethroid and chlorfenapyr was beyond the scope of this study. The full

characterisation of these population is being processed and will be published elsewhere. Additionally, two rounds of EHTs were conducted each year; however, mosquitoes were not collected under the same treatment arms across year and round. Consequently, evaluating changes in the parameters over time (year) is challenging.

Conclusion

The current findings demonstrated that the performance of Interceptor G2 and Royal Guard nets exceeded standard ITNs for at least one entomological outcome, although performance was reduced by artificial and operational aging in high-pyrethroid resistance settings. The efficacy of these nets is attributed to the addition of chlorfenapyr or pyriproxyfen, compounds that are not affected by conventional resistance mechanisms. This feature positions these nets as promising tools for managing pyrethroid-resistant vector populations. However, the current findings demonstrate that nets performance is influenced not only by product characteristics but also by site-specific ecological factors. Thus, these findings support tailored evidence-based strategic deployment of nets and evaluation of vector control tools across different transmission settings to ensure accurate operational effectiveness. More investigation is needed to elucidate the potential site-specific performance of the pyrethroid-pyrrole nets observed in the current study.

Methods

Study site

Two experimental stations were used for the trials: Tengrela (longitude: 10.2078; latitude: - 4.9788) and Tiefora (longitude: 10.6241; latitude: - 4.5533 (**Figure 4**)). Both are situated in the Tannounyan regions in the Southwestern part of the country, approximatively 450 km from the Capital, Ouagadougou. The two sites are located about 40 km apart, with Tiefora positioned around 25 km to the main city of the region, Banfora, and Tengrela approximately 15 km from Banfora. The two sites share similar geographical features and experience comparable climatic conditions. Both have access to permanent water bodies such as swamps. However, Tiefora is characterised by numerous temporary ponds, whereas the water sources in Tengrela feature more agricultural irrigation. Agricultural activities include significant cultivation of vegetables and rice on both sites. Cotton is significantly produced in Tiefora while sugar cane field dominates an edge of Tengrela. According to the Agence Nationale de la Météorologie of Burkina Faso, annual rainfall at these sites ranged from 1,049 to 1,157 mm.

The average temperature was 27. °C with observed values ranging between 23 °C and 33°C. During the study periods, the average humidity was 78%, with a range from 43 to 96%. *An. coluzzii* is the most abundant species in Tengrela throughout the year. In contrast, Tiefora hosts a mix of *An. arabiensis*, *An. gambiae* s.s and *An. coluzzii* with their proportion varying depending on the sampling period (23). Both sites display high level of insecticide resistance; with mortality rates measured 24 hours after exposure to 15-times the diagnostic dose of deltamethrin not exceeding 87% (23). This resistance is attributed to target site mutations (as L995F, I1527T) and the presence of cytochrome P450 enzymes (38).

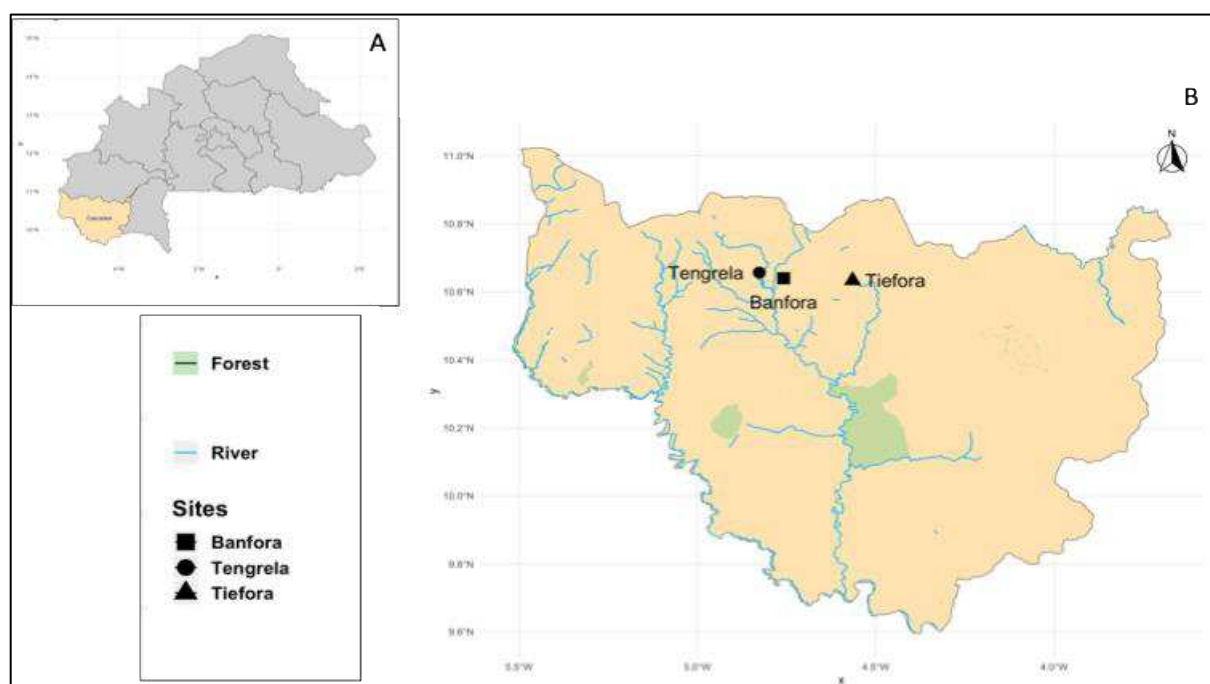


Figure 4: Map of the study sites, A) the map of the country indicating the Cascades region in yellow and, B) is the Cascades region indicating the villages (Tengrela and Tiefora) where the experimental huts are located and the main city of the region.

Insecticide resistance profiling in *Anopheles gambiae* s.l. from study area

During the experimental hut trials, larvae were collected from various breeding sites and pooled according to collection sites. These pools were then reared into adulthood. During the larval stage, baby fish food (TetraMin®, Tetra GmbH, Germany) was provided as nourishment, while adult mosquitoes were maintained on a 10% sugar solution. All rearing took place in an insectary set to 27 ± 2 °C temperature, $85 \pm 10\%$ relative humidity, and a 12:12 hour dark-light cycle. Unfed F₀ female *An. gambiae* s.l. aged 2 - 5 days were selected for bioassays. These adults *An. gambiae* s.l. were exposed for one hour to filter paper coated with 0.05% of each alphacypermethrin and deltamethrin using WHO cylindrical tubes (39), following established

WHO susceptibility testing guidelines: mosquitoes from the same batch were exposed to untreated papers, serving as controls. Additionally, a group of these mosquitoes were exposed to 100 µg of chlorfenapyr per bottle using WHO bottle assay guidelines (39). After exposure all mosquitoes were transferred into untreated paper cups for observations. Mortalities were recorded under standard laboratory conditions at 24 hours post-exposure to alphacypermethrin and deltamethrin, and 72 hours for exposed mosquitoes to the chlorfenapyr.

Study arms and mosquito collection procedure

Experimental hut design

The trials were conducted using two sets of six (06) experimental huts, each designed in the West African style as described in the WHO Pesticide Evaluation Scheme (WHOPES) guidelines for laboratory and field-testing of long-lasting nets [9]. Prior to study initiation, the huts were refurbished to repair gaps that could potentially allow mosquitoes to escape.

Experimental hut trials (EHT) were conducted over three consecutive years (2019 – 2021) with two rounds (round 1 run from late August to September/October and round 2 from late September/October to earlier November) per year coinciding with the rainy season. The ITNs used were Interceptor G2, Royal Guard (RG), Interceptor G1 (IG1) and untreated nets (**Supplementary Table 4 and 5**). Four Interceptor G2 and two Interceptor G1 nets previously used by residents for two years (here referred to as aged nets), were also collected (from different households) and tested as part of the study in 2021.

Nets preparation for experimental hut testing

Nets were washed following the WHOPES established protocols (40). Interceptor G1 and Interceptor G2 nets were prepared using a one-day wash interval (41), while the Royal Guard nets had three-days wash interval (11). Washing was performed in 2g/l of soap (Savon de Marseille) prepared with dechlorinated water. Nets were manually rotated at 20 rotation per minute for three (03) minutes followed by a four-minute resting period and then rinsed twice in clean dechlorinated water.

Before trials, each net was intentionally holed with two holes (4 cm X 4 cm) on each of the longer sides and one on each of the small sides to simulate worn nets following guidelines (40).

Experimental hut trial procedure

The trials were conducted as single-blinded studies, each spanning 36 collection nights. Prior to each trial, a two-week baseline collection was conducted after hut refurbishment using untreated nets only. This ensured the huts were free from contamination, provided an estimate of baseline vector density. prior to the trials. Each study arm occupied a hut six days per week. The allocation of the arms to huts was randomised on the first day and rotated weekly following a 6 x 6 Latin Square Design (LSD) to control for hut position bias. At each site, six volunteers over 18 years old from local community served as sleepers. To minimise variation in huts and individual attractiveness, sleepers rotated nightly following an LSD scheme. On the seventh day each week nets were removed, and the huts were thoroughly cleaned, ventilated, and dried.

Sleepers entered the huts at 20:00 after which supervisors opened the windows. At 05:00 the following morning, supervisors closed the windows and sleepers collected mosquitoes from inside. Collections were carried out sequentially from inside the nets, then the room area and finally in the veranda with each compartment processed and kept separately. Mosquitoes from each hut and compartment were stored separately as alive or dead. Live mosquitoes were provided with 10% sugar solution, transported to the on-site laboratory and morphologically identified to species following establish keys (42, 43).

All live *An. gambiae* s.l. were systematically monitored with continued access to sugar solution. Fully blood-fed female split into two cohorts. The first cohort was observed for three days (72 hours) – as other remaining live collected mosquitoes – in the laboratory whereas the second cohort, consisting of blood-fed and gravid mosquitoes, was maintained individually in paper cups lined with a water-moistened cotton pad and watered-filter-paper for oviposition. Oviposition was checked daily, and eggs were monitored for larval emergence. Eggs and larvae were pictured using a smartphone and later counted using a software “Eggy” for digital image counting. Larvae were then maintained at room temperature, fed with baby fish food (TetraMin®, Tetra GmbH, Germany) and monitored until reaching adulthood.

Laboratory processing

At the end of the trial, a subset (including both live and dead) of the *An. gambiae* s.l. specimens from the experimental hut underwent molecular analysis for the species identification at complex members level and knockdown resistance (kdr), specifically the kdr-L995F mutations detection. Genomic DNA was extracted from individual sample using the DNAzol method (44).

For species identification, one microliter of the extracted DNA was used in a species-specific polymerase chain reaction (PCR) (45) with the resulting bands visualised on a 2% Tris–Acetate–EDTA agarose gel. Taqman real time-PCRs were undertaken to assess the presence of 995F mutations (kdr-L995F), following the methods in (46).

Statistical analysis

All statistical analysis were conducted using R software (47) . The following outcomes were measured from the EHT as primary outcomes: (i) blood feeding rate (the proportion of blood-fed mosquitoes collected in each arm), (ii) mortality rate at 72-h post-exposure/collection (the proportion of mosquitoes dead after 72 h considering each arm), (iii) fecundity (the average number of eggs laid per blood-fed mosquito).

The secondary outcomes comprised: (iv) species-specific mortality after collection in hut in presence of treatment, (v) deterrence (proportion of reduction entry in treated arms relative to the control arm), (vi) induced exophily (the proportional of reduction in mosquitoes found in exit traps relative to the untreated nets), (vii) oviposition rate (the proportion of alive blood-fed mosquitoes that laid eggs), and (viii) fertility (the mean number of adults that emerged per surviving blood-fed mosquito).

These data were used to compare: (i) untreated nets versus ITNs, (ii) standard unwashed IG1 versus other unwashed ITNs (iii) and (iv) unwashed ITNs versus both washed and aged ITNs of the same brand in addition to washed IG2 versus aged IG2. Assessment of the sublethal effects – focusing on oviposition, fecundity and fertility – were done using second cohort mosquito (blood-fed and gravid collected alive from the huts). This comparison used data aggregated over all sites to minimise potential biases that could arise from small sample sizes when analysing by individual site and treatment.

Data from discriminating dose bioassays were used for estimating the 24-hour mosquito mortality rates to the different dose of both alphacypermethrin and deltamethrin. Mortality rates were also estimated at 72-hours post-exposure to chlorfenapyr. Then the mosquito population was classified as pyrethroid-resistant when the mortality rate after 24-hours or 72-hours post-exposure to pyrethroid is $\leq 90\%$ but susceptible if this rate is $\geq 98\%$ (39, 48).

Unless otherwise stated statistical analysis was undertaken using generalised linear mixed-effect models. Proportional outcomes including the mortality, blood-feeding, exophily and oviposition, were analysed using a logit link function through binomial distribution.

Deterrence, as count data, was estimated using a negative binomial distribution. Though, fertility and fecundity were fitted using a Conway-Maxwell Poisson (COM–Poisson) generalised linear mixed-effect model to account for dispersion. The treatment effects were estimated as odd ratio or rate ratio (for fertility and fecundity) “emmeans package” in R, with 95%. Fixed effects in the full model included each study arm, hut, sleepers and the experimental station site, while random effects accounted for variation in observation and collection dates. The significance of the fixed effect terms in final models was determined using the MuMIn package (49) based on the Akaike information criterion (AIC). The Model fit/uniformity and dispersion were estimated with the DHARMA R package (50). Generalized linear mixed models (GLMM) were fitted using the ‘lme4’ package (51) (version 1.1-34) for R. Further, samples of morphological identified *An. gambiae* s.l. from the EHT (collected dead and live) were used for *An. gambiae* complex members’ level species-specific mortality rate. In addition, molecular-analysed samples from EHT were also used to determine the *kdr*-995F alleles frequency (46).

Ethical clearance

Ethical approval for this study was obtained from the Institutional Bioethics Committee (N°2019/000007_MS_SG_CNRFP/CIB of 18th July 2019) and LSTM Research Ethics Committee (19-038). Prior written informed consents were acquired from each volunteer sleeping in the hut before their involvement in the research.

Abbreviations

IG1: Interceptor G1

IG2: Interceptor G2

ITNs: Insecticide treated nets

OR: Odds ratios

RG: Royal Guard net

SSA: Sub Sahara Africa

UT: untreated nets

WHO: World Health Organization

Data accessibility

All data are provided as Figures and Supplementary materials

Author's contributions

AS, MWG, SNF, PMc, TC, HR and **GF** designed the study. **AS, MT, CF, BS** conducted the field data collection, **MGB, LDZ and SZ** did the molecular analysis in the labs. **AS** performed the data analysis, interpreted the results and drafted the manuscript. **WMG, NFS, TC, PMc, HR** and **GF** provided comments upon the manuscript. **TC, IJS, DD, JJ** and **AM** contributed to the data analysis. All authors reviewed and approved the final version of the manuscript.

Competing interests

All authors declare no competing interests

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