THE PMI VECTORLINK BURKINA FASO 2021 ENTOMOLOGICAL MONITORING ANNUAL REPORT

JANUARY – DECEMBER 2021

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THE PMI VECTORLINK

BURKINA FASO

2021 ENTOMOLOGICAL

MONITORING ANNUAL REPORT

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ACRONYMS

ANOVA	Analysis of Variance
CS	Capsule Suspension
EIR	Entomological Inoculation Rate
GLMM	Generalized Linear Mixed Models
HBR	Human Biting Rate
HLC	Human Landing Catch
IR	*Plasmodium falciparum* Infection Rate
IRS	Indoor Residual Spraying
IRSS	Health Sciences Research Institute /Institut de Recherche en Sciences de la Santé
ITN	Insecticidal Treated Net
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PMI	U.S. President's Malaria Initiative
PSC	Pyrethrum Spray Catch
WG	Wettable Granules
WHO	World Health Organization
WP-SB	Wettable Powder in Water Soluble Bag

EXECUTIVE SUMMARY

The Burkina Faso 2016-2020 National Malaria Strategic Plan recommends that non-pyrethroid indoor residual spraying (IRS) should be used as a complementary vector control tool together with insecticide treated nets (ITNs) in locations where pyrethroid resistance occurs. In 2021, PMI VectorLink conducted spray operations from May 10 to June 19 (Solenzo: May 10 to June 4, 2021; Kampti: May 20 to June 19, 2021) using three insecticide formulations. The project sprayed clothianidin (SumiShield 50 WG) and a mixture of clothianidin and deltamethrin (Fludora® Fusion) in Solenzo, and Actellic 300 CS in Kampti. The project sprayed a total of 175,523 structures out of 189,425 eligible structures found by spray operators, accounting for a final spray coverage rate of 92.7 percent. Previously, a nationwide mass distribution campaign supplied 1.5 million piperonyl butoxide (PBO)-synergist PermaNet 3.0 ITNs and two million dual-active ingredient Interceptor G2 ITNs, in addition to standard pyrethroid ITNs (PermaNet^{R} 2.0, DuraNet^{R}, MagNet^{R} and Interceptor^{R}) between June and October 2019.

To monitor the impact of vector control on entomological indicators, monthly entomological surveillance using pyrethrum spray catch (PSC) and human landing catch (HLC) were conducted in two sprayed sites (Solenzo and Kampti), with two paired unsprayed sites (Nouna and Gaoua), the former IRS site of Kongoussi plus adjacent control site (Seguenega) and also two non-sprayed sites (Karangasso-Vigué and Soumousso) where PBO ITNs were distributed. Insecticide susceptibility tests were conducted in 19 sites to determine the appropriate selection of insecticides for future IRS rounds and ITN distribution campaigns. Susceptibility tests using *An. gambiae* s.l. were conducted with pyrethroid insecticides and PBO synergists according to World Health Organization (WHO) protocols. CDC bottle bioassays were also conducted to determine susceptibility to chlorfenapyr insecticide. In addition, WHO tube tests were carried out to determine susceptibility to pirimiphos-methyl and clothianidin, both of which were used for IRS in 2021. WHO cone bioassay was conducted for quality assurance of the spray campaign and monitoring of residual efficacy of sprayed insecticides.

An. gambiae was the predominant malaria vector species in the Southwest (Gaoua, Kampti, Soumousso, Karangasso-Vigué), while *An. coluzzii* was more frequent in the North-Central (Seguenega and Kongoussi) and West-Central (Nouna and Solenzo) sites.

The peak of indoor resting densities and biting rates was observed in August/September in all sites, approximately three to four months after spraying. Biting rates were far greater in the Bena site (sprayed with SumiShield WG) in Solenzo district compared to the unsprayed site of Nouna. In addition, significantly reduced biting rates were observed in Kampti (sprayed with Actellic 300 CS) compared to Gaoua (unsprayed), whereas there was no difference between Kongoussi (former IRS site) and Seguenega (unsprayed). The density of *An.*

gambiae s.l. collected from PSC was lowest in Kampti compared to Gaoua, but seemed to be higher in Solenzo (sprayed with Fludora Fusion) compared to its control site of Nouna. However, in the rural site of Bena, spayed with SumiShield, the density was slightly lower than in Nouna. *An. gambiae* s.l. biting rate and indoor resting densities trends were similar to the previous year.

There was a slight reduction in the proportion of parous *An. gambiae* s.l. in sprayed sites compared to unsprayed sites. The mean malaria sporozoite infection rate (IR) (by PCR) was extremely high in Southwestern and Northern locations, except in Nouna and Solenzo. Only Kampti (sprayed with Actellic 300CS) had the most reduced IR. The parity rate and infection rate showed similar trends as in the previous year in the sprayed sites compared to unsprayed sites. However, significant decrease in infection rate was observed in the Kampti site (sprayed with Actellic 300CS) this year compared to the previous year when it was sprayed with Fludora Fusion WP (FF).

The entomological inoculation rate (EIR) was extremely high in the Southwest (combined central+ rural sites), with Gaoua (unsprayed) having an EIR of 319 infective bites per person (i/b/p), indoors and 324 i/b/p, outdoors over the 112 collection nights (June - December 2021). The EIR was significantly reduced in the neighboring sprayed district of Kampti (Actellic 300 CS) with 140 and 124 i/b/p indoors and outdoors, respectively. The EIR was also clearly impacted by IRS in central areas with 18 i/b/p (indoors) and 48 i/b/p (outdoors) in Kampti compared to 143 i/b/p (indoors) and 161 i/b/p (outdoors) in the unsprayed site of Gaoua, over the 56 collection nights. In the two sites where PBO ITNs were distributed, the indoor EIR was 47 infectious bites per person over the 56 collection nights in the seven months in Karangasso-Vigué and Soumousso, which is higher than the other sites except the site sprayed with Actellic 300CS. The outdoor EIR for Karangasso-Vigué over the 56 collection nights reached 194 i/b/p. The PBO net results were not different from the EIR observed the previous year. Overall, IRS with Actellic 300CS appears to have better impact on EIR than the Fludora Fusion sprayed and PBO nets sites. However, the EIR still remains high especially in the endemic Southern regions in both sprayed and unsprayed sites. EIR estimate per night, month, collection nights and over the seven months, June to December, are presented in the results section and annex.

The susceptibility data showed full susceptibility of *An. gambiae* s.l. to pirimiphos-methyl in all sites, except in the sprayed site of Solenzo, and unsprayed sites of Orodara and Gaoua. There was full susceptibility of *An. gambiae* s.l. populations to clothianidin in all IRS and NMCP sites, except in the unsprayed site of Orodara that had a mortality rate of 98.8%. Therefore, all insecticide formulations containing pirimiphos-methyl and clothianidin can continue to be used in Burkina Faso for IRS on a rotational basis. The insecticide susceptibility tests revealed that *An. gambiae* s.l. were resistant to all pyrethroids tested, but pre-exposure to PBO significantly increased mosquito susceptibility to alpha-cypermethrin, deltamethrin and permethrin in all sites tested, including Karangasso-Vigue and Soumousso, where Permanet 3.0 nets were distributed in 2019. Pyrethroid resistance intensity was high in all sites for deltamethrin and alpha-cypermethrin and moderate or high for permethrin. This further highlights the wide distribution of high pyrethroid resistance intensity in Burkina Faso and the potential danger and effect on efficacy of pyrethroid-only ITNs. On a positive note, susceptibility to chlorfenapyr and clothianidin was recorded in all sites except in Vallée du Kou, a rice growing area, where *An. gambiae* s.l. were resistant to chlorfenapyr. Due to the widespread presence of pyrethroid resistance, PBO synergists and dual active ingredient-nets, such as Interceptor G2 (containing chlorfenapyr + pyrethroid), should continue to be prioritized for future ITNs distribution campaigns. Cone bioassays with a susceptible insectary strain showed that Fludora Fusion WP-SB, SumiShield WG and Actellic 300CS lasted for at least seven months in all sites (tests still ongoing). Therefore, the three insecticide formulations provided control throughout the peak malaria transmission season.

1. INTRODUCTION

The World Health Organization (WHO) reported 229 million malaria cases and 409,000 deaths worldwide in 2019 (WHO, 2020). Malaria is endemic in Burkina Faso, and the most recent National Malaria Report published in 2018 showed that malaria morbidity is still increasing, especially in children under five and pregnant women (NMCP report, 2019). In 2018, the National Malaria Control Program (NMCP) recorded approximately 11.9 million confirmed cases of malaria and 4,292 deaths reported by health facilities. This makes Burkina Faso the third most malaria affected country after the Democratic Republic of Congo and Nigeria (NMCP report, 2019). The primary malaria parasite in Burkina Faso is *Plasmodium falciparum* (Hien *et al*., 2017) which is primarily transmitted by *Anopheles gambiae* s.l. and *Anopheles funestus* (Dabiré *et al.,* 2007, 2012). The use of insecticide treated mosquito nets (ITNs) remains the main tool for malaria vector control in Burkina Faso. However, resistance to pyrethroids in malaria vectors has spread across Africa and is jeopardizing the effectiveness of this strategy (Hemingway *et al*., 2016). A common mechanism of resistance to pyrethroids, the knock-down resistance mutation (*kdr*-L1014F), emerged in Burkina Faso toward the end of the 1990's (Chandre *et al*., 1999). This *kdr*-1014F mutation (Diabaté *et al*., 2004; Dabiré *et al*., 2012; Toé *et al*., 2015) spread quickly in Burkina Faso and broadly in West Africa, and is acting in combination with metabolic resistance mechanisms that could reduce the efficacy of pyrethroid ITNs (Toé *et al*., 2015).

The national malaria strategic plan recommends that non-pyrethroid-based IRS be used as a complementary vector control tool together with ITNs in locations where pyrethroid resistance occurs. This is partly due to the availability of new non-pyrethroid IRS formulations that can provide long-lasting control of malaria pyrethroid resistant vectors. With US President's Malaria Initiative (PMI) funding, IRS was included as a priority vector control strategy and has been implemented annually since 2018. PMI VectorLink conducted spray operations from May 10 to June 19, 2021 using clothianidin (SumiShield 50 WG) and a combination of clothianidin and deltamethrin (Fludora® Fusion) in Solenzo, and Actellic 300CS in Kampti. While Kongoussi district was sprayed in 2018-2019, it did not receive IRS in 2021 due to security issues; however, entomological monitoring continued there. The project sprayed a total of 175,523 structures out of 189,425 eligible structures found by spray operators, accounting for a final spray coverage rate of 92.7 percent. In addition, a mass distribution campaign in 2019 supplied 1.5 million piperonyl butoxide (PBO)-synergist PermaNet 3.0 ITNs and two million dual-active ingredient Interceptor G2 ITNs, in addition to standard pyrethroid ITNs.

The VectorLink Burkina Faso team, in collaboration with the Institute of Health Science Research/*Institut de Recherche en Sciences de la Santé* (IRSS) conducted surveys to monitor vector bionomics and insecticide susceptibility during the period of high malaria transmission (June to December 2021). The specific objectives of the program were to:

- Collect detailed information on mosquito biting rates, biting times, indoor resting densities, seasonality, and parity rates of malaria vectors in both IRS sites and their adjacent unsprayed control sites.
- Monitor the susceptibility of *An. gambiae* s.l. to permethrin 0.75 percent, deltamethrin 0.05 percent and alphacypermethrin 0.05 percent (with and without pre-exposure to the synergist piperonyl-butoxide (PBO), bendiocarb 0.1 percent, and pirimiphos-methyl 0.25 percent.
- Determine the intensity of insecticide resistance to pyrethroids (permethrin, deltamethrin and alphacypermethrin), using the WHO tube protocol.
- Determine the susceptibility level of the main malaria vectors, *Anopheles gambiae* s.l., to two relatively new insecticides, clothianidin and chlorfenapyr.
- Conduct laboratory analysis of mosquito samples to determine vector species composition, presence of molecular markers of resistance (*kdr-w*, *kdr-e* and *Ace-1*), blood-meal source, and *P. falciparum* sporozoite infection rates.
- Provide data, recommendations, and technical assistance to the NMCP in the development of its national resistance monitoring plan.

2. METHODOLOGY

2.1 STUDY AREA

Monthly longitudinal entomological monitoring was carried out during the high transmission season from June to December 2021 in eight sites: two IRS sites (Solenzo and Kampti), two paired unsprayed control sites located approximately 50km away (Nouna and Gaoua), one former IRS site (Kongoussi) plus neighboring control site (Seguenega), and two never sprayed sites where PBO ITNs were distributed in 2019 (Soumousso and Karangasso-Vigué). Monthly mosquito collections were conducted to measure entomological parameters of malaria transmission. These sites are located across the three ecological zones of Burkina Faso: Sudanian (West), Sudano-Sahelian (West Central) and Sahelian (North) ecological zones (Figure 1). An additional site, namely Bena (sprayed with SumiShield 50WG), was added in the Solenzo district in the follow-up monitoring starting in September to provide data on its residual efficacy. The results of the mosquito densities parity rate and the residual efficacy of SumiShield 50WG in Bena are also presented in this report.

Anopheles mosquitoes were sampled using three methods: i) human landing catches (HLC), ii) pyrethrum spray catches (PSC), and iii) larval collections for insecticide resistance monitoring. Longitudinal trapping by HLC and PSC was conducted in two sub-locations within each site, in a more urban central site and a rural site. Cone bioassays were conducted on sprayed walls in the two IRS districts to measure the quality of IRS and residual efficacy.

Figure 1. Study sites for monthly longitudinal trapping by HLC and PSC (IRS sites, unsprayed control sites, former IRS site) and sites for resistance monitoring

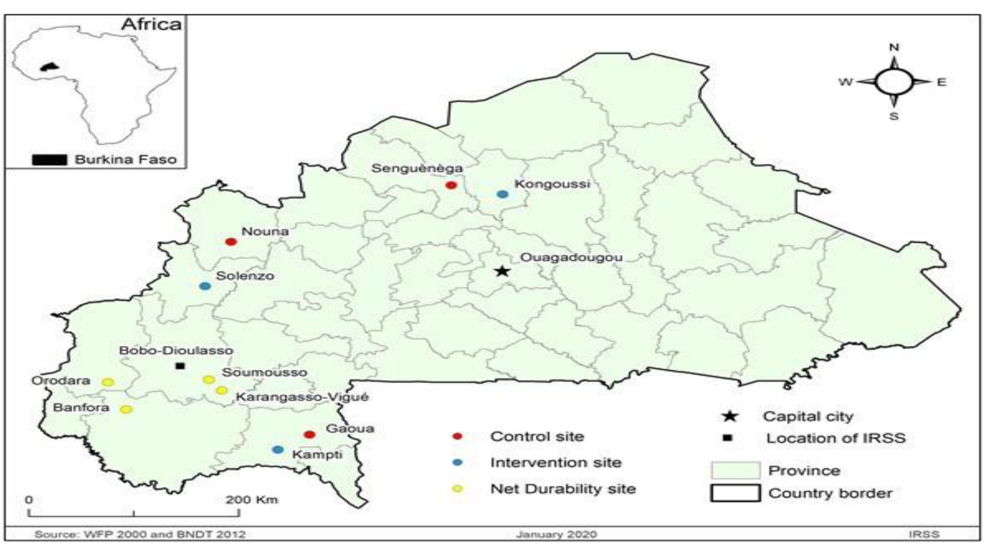
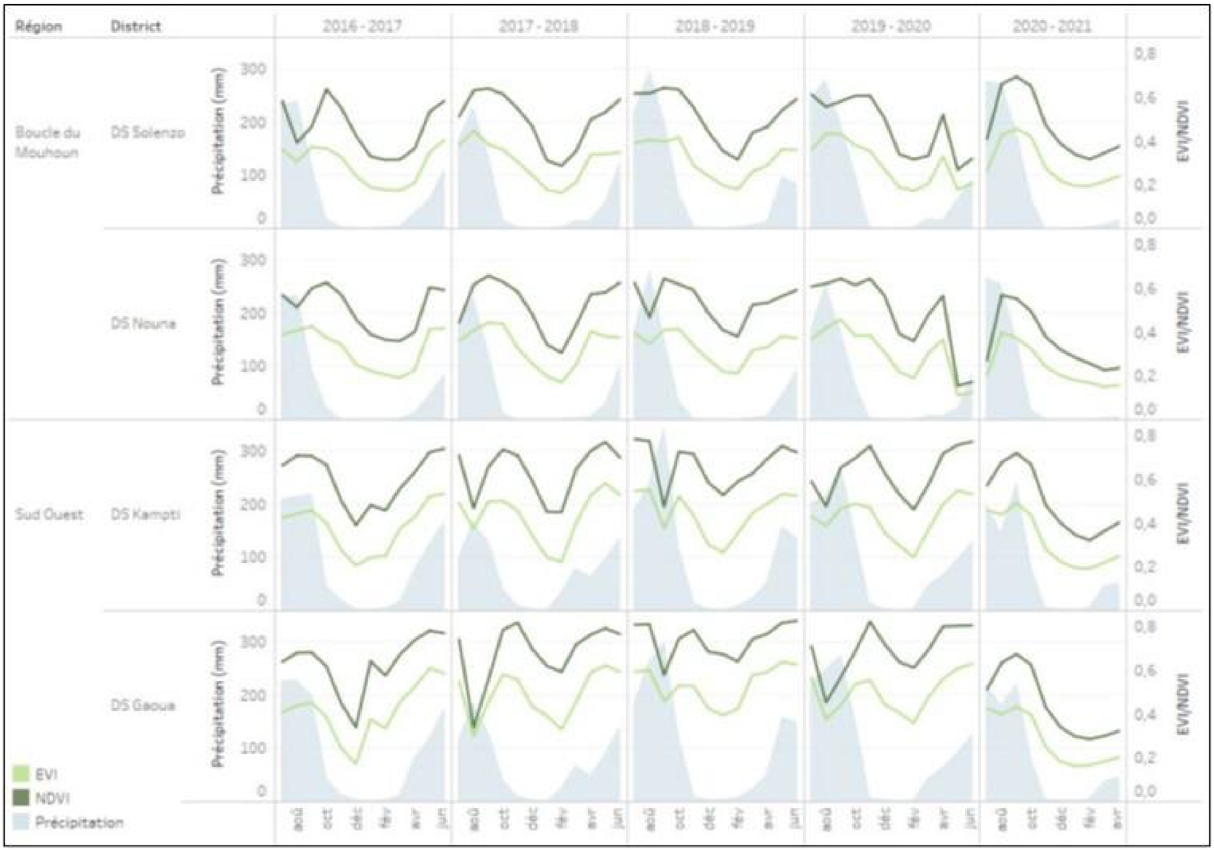


Figure 2. Average monthly precipitation (in millimeters) by district from 2016-2021



Source: Inouye et al (2020), Burkina Faso Indoor Residual Spraying 2018 Impact Evaluation.

The mean climate data for the two IRS sites of Solenzo and Kampti, including the former IRS site of Kongoussi (Figure 2), showed a relative short rainy season lasting approximately four months from June to September in Kongoussi and Solenzo, and longer in Kampti, extending from April to October. The mean temperature was high throughout the year in all sites, with mean temperature above 25°C for most of the year. Therefore, during the rainy season, conditions are ideal for *An. gambiae* s.l. proliferation.

2.2 Human Landing Catch (HLC)

Human landing catches (HLCs) were carried out in each site from 06:00 pm to 08:00 am in four randomly selected houses per sub-location (eight houses total per site per month, in the same houses every month) to determine the human biting rates of malaria vector species. During each night, two HLC collectors equipped with a mouth aspirator and a flashlight, sat in each house: one indoors (living room) and the second outdoors (within two meters of the house). The following morning, mosquito identification was performed using the key of Gillies and Coetzee (1987) and Coetzee *et al.* (2020) for *Anopheles* species.

2.3 Pyrethrum Spray Catch (PSC)

Pyrethrum spray catches were conducted using 0.64 percent Pyrethrum EC aerosol insecticide. The houses were visited in the morning between 06:00 and 09:00 am and white sheets were laid on the floor and over furniture. A total of 20 houses were selected per sub-location (central and rural) with a total of 40 houses surveyed per site per month. Ten to fifteen minutes after the spraying of houses, the knocked-down mosquitoes were collected from the white sheets. Mosquitoes were put in labelled petri dishes and were later morphologically identified. All *Anopheles* females were assessed for their abdominal status (unfed, fed, half gravid and gravid) and identified to species. Female *An. gambiae* s.l. were stored in 1.5 ml labelled Eppendorf tubes for further molecular laboratory analyses.

2.4 Parity Rates of *Anopheles* Females

Anopheles mosquitoes were morphologically identified to species level using taxonomic keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987). A random sample of 50 unfed females per month collected from indoor and outdoor HLC were dissected. Ovaries were dissected to determine parity rate, by observing the coiling of ovarian tracheoles (Detinova and Gillies, 1964). All these specimens, including those dissected, were brought back to the IRSS laboratory and stored at 4°C for further laboratory analyses.

2.5 IRS quality Assurance and Residual Efficacy Monitoring (WHO Cone Tests)

The residual efficacy of insecticide on the walls was tested monthly by cone bioassays from May 2021 to December 2021 in IRS sites with WHO cone bioassays after spraying in Solenzo with SumiShield 50WG and Fludora Fusion WP-SB, and in Kampti with Actellic 300CS. Wild *An. gambiae* s.l. larvae in each IRS site were collected and reared in the insectary to the adult stage, for monthly cone bioassay in parallel with the susceptible colony *An. gambiae* "Kisumu". A total of four treated houses (two houses made of cement and two houses made of mud) were randomly selected in each district for bioassay and monitored monthly until mortality was <80 percent for two consecutive months.

Mosquitoes were exposed for 30 minutes and were then taken back to the insectary for delayed mortality assessment at 80 ± 10 percent relative humidity and 27 ± 2°C temperature. After exposure, mosquitoes were supplied with glucose solution, and mortality was recorded 24 hours post-exposure for Actellic 300CS. Mortality was recorded every 24 hours for up to seven days after exposure to walls sprayed with SumiShield 50WG and Fludora Fusion WP-SB to account for any delayed mortality.

2.6 Laboratory Analyses

2.6.1 *Plasmodium falciparum* Infection Rate

All mosquitoes that were dissected for their parity status in the field were stored in a laboratory freezer at -20°C and subsequently processed by PCR to determine infection rates with *P. falciparum*. The head and thorax of all female *An. gambiae* s.l. specimens were used for PCR analyses as described by Morassin *et al.* (2002) and adapted by Sangaré *et al.* (2013). All samples that were tested for malaria infectivity rates were also identified to species level by PCR (Santalomazza *et al.*, 2008).

2.6.2 Origin of Blood Meal Source (Anthropophily Rate)

Blood-fed females of *An. gambiae* s.l. from PSC were used to assess host preference for blood meal source. A random sub-sample of specimens were tested by PCR using sequences of human, cow, pig, donkey and sheep blood (Kent & Norris, 2005). The same DNA-extraction process was used for mosquito species identification and blood-meal source PCR.

2.6.3 Molecular identification of *An. gambiae* complex and characterization of Resistance Mutations (kdr L1014/L1014S and ace-1R)

A subsample of female *An. gambiae* s.l. were identified by PCR for species composition. Genomic DNA of mosquitoes was extracted with two percent cetyl trimethyl ammonium bromide (CTAB). Species of *An. gambiae* s.l. were identified and characterized, respectively by PCR Sine 200X 6.1 locus protocols of Santolamazza *et al.* (2008). Detection of mutations involved in insecticide resistance was also performed by PCR using the protocol of Martinez-Torres *et al.* (1998) and Ranson *et al.* (2000) for the *kdr* L1014F and L1014S mutations respectively and of Weill *et al.* (2004) for the ace-1^R G119S mutation.

2.6.4 Insecticide Susceptibility Tests

An. gambiae s.l. larvae were collected from different larval habitats from 19 localities, brought to the IRSS insectary and reared to adults prior to use in bioassays in order to assess the insecticide resistance status of adult *An. gambiae* s.l. The WHO tube tests were conducted to monitor insecticide susceptibility, pyrethroid resistance intensity and PBO synergist results using wild *An. gambiae* populations. Pyrethroid resistance intensity was monitored with alpha-cypermethrin, deltamethrin and permethrin at 5x and 10x the diagnostic concentration using the WHO tube protocol. The following insecticides were tested:

- Alpha-cypermethrin 0.05 percent, 0.25 percent, 0.50 percent
- Deltamethrin 0.05 percent, 0.25 percent, 0.50 percent
- Permethrin 0.75 percent, 3.75 percent, 7.50 percent

- Permethrin 0.75 percent + PBO 4 percent
- Deltamethrin 0.05 percent+ PBO 4 percent
- Pirimiphos-methyl 0.25 percent
- Clothianidin 2 percent
- Chlorfenapyr100µg/bottle and 200 µg/bottle (if necessary)

WHO criteria were used to classify populations as 'resistant' if less than 90 percent mortality was observed, suspected resistance if between 90-97 percent and susceptible if between 98-100 percent.

2.7 Data Analysis

The DHIS2-based VectorLink Collect program for entomological data management was used in Burkina Faso for the first time in 2020. The VectorLink Home Office team remotely trained and supported IRSS entomologists and database managers on updated data work flows, including field paper collections, technical reviews, data entry, data cleaning, and analytics, to support the generation and use of high-quality entomological data. All entomological data collected in Burkina Faso in 2021 was managed within VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results. Stakeholders including NMCP, IRSS and PMI, will have ongoing access to these results dashboards to support timely decision-making.

The human biting rate (HBR) was determined as the number of mosquitoes collected by HLC divided by the number of collector-nights (indoors and outdoors). The *Anopheles* infection rate (IR) was calculated as the proportion of mosquitoes tested positive for *P. falciparum* DNA in the head or thorax. The entomological inoculation rate (EIR) was calculated as human biting rate multiplied by IR and estimated as the number of infectious bites per human during a period (day, month, annual). An analysis of variance (ANOVA) was performed to compare the entomological estimates (HBR, IR) between sites. Data analysis was performed using R software, version 4.0.3. To analyze variables of interest (mosquito density, infectivity and entomological inoculation rates), a generalized linear mixed models (GLMM) was fitted using the glmmTMB function. In the case of the count variables, the negative binomial families such as nbinom2 was used. A difference was considered significant when the p-value was less than 0.05.

3. RESULTS

3.1 MALARIA VECTOR SPECIES COMPOSITION

From June to December 2021, a total of 27,519 *Anopheles* mosquitoes were collected in all sites: 19,481 *Anopheles* mosquitoes by HLC and 8,038 by PSC. *An. gambiae* s.l. was the most abundant species collected through HLC, with 18,368 (94.28 percent), followed by *An. nili* with 802 (4.11 percent), *An. funestus* 125 (0.64 percent), *An. coustani* 79 (0.40 percent), and *An. pharoensis* 76 (0.39 percent) (Figure 3A). The other species were found at very low percentages (> 0.1%): *An. flavicosta* (n= 19), *An. rufipes* (n=7) and *An. squamosus* (n=5). The highest *Anopheles* species diversity was found in Kampti and Gaoua districts (eight species collected) located in the South West region. However, in the Northwest region (Solenzo and Nouna districts) and Northern region (Kongoussi and Seguenega), only four species were collected in each region. From the PSC collection, *An. gambiae* s.l. was also the predominant species collected (99.82 percent, n=8024), followed by *An. rufipes* (0.14 percent, n=12) and *An. funestus* (0.01 percent, n=1) (Figure 3B).

Figure 3. *Anopheles* species composition based on A) HLC and B) PSC for all sites combined

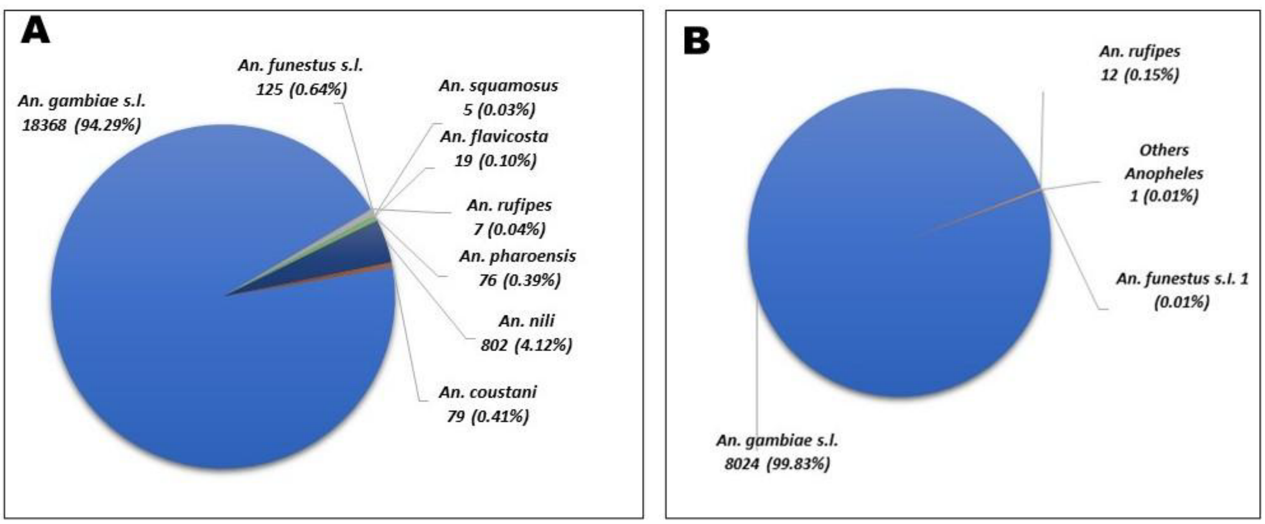
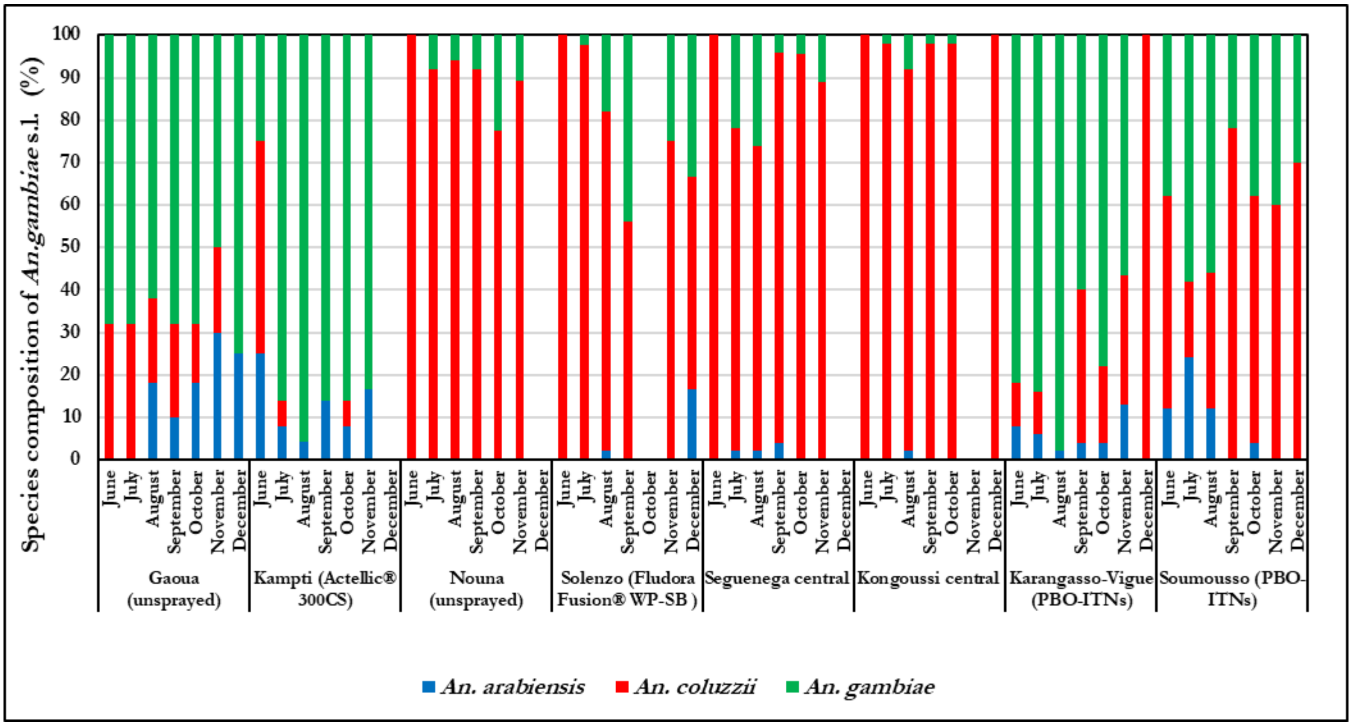


Figure 4 shows the species composition of the *An. gambiae* complex identified by PCR for all sites from June to December 2021. *An. coluzzii* was the predominant species in Nouna, Solenzo, Seguenega and Kongoussi. The majority of *Anopheles* in Kampti and Gaoua were *An. gambiae* each month, except for June in Kampti and November and December in Gaoua where more *An. coluzzii* were collected. *An. arabiensis* were found every month at low proportions in Gaoua and Kampti, but the largest proportion at 50 percent was found in Gaoua in October-November, although the proportion was low for other months. In Karangasso-Vigué (PBO net sites), *An. gambiae* was the predominant species every month, unlike in Soumousso where the proportion of *An. gambiae* and *An. coluzzii* was generally equal, with more *An. coluzzii* collected later in the year in October-December. It is interesting that the proportion of *An. arabiensis* being collected in the Southwestern regions (Kampti, Gaoua) appears to be gradually increasing compared to previous year. In summary, *An. gambiae* was the predominant malaria vector species in the Southwest (Gaoua, Kampti, Karangasso-Vigué), while *An. coluzzii* was more frequent in the Centre North (Seguenega and Kongoussi) and Centre West (Nouna and Solenzo).

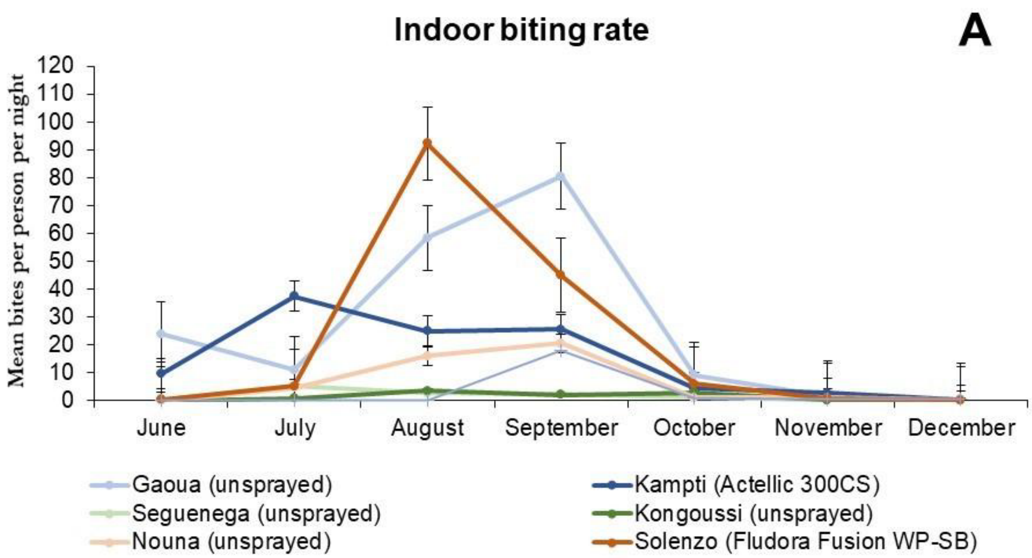
Figure 4. Species composition within the *An. gambiae* s.l. complex collected by HLC in all sites (n=50 per month/site)



3.2 *An. gambiae* s.l. Human Biting Rate (HBR)

Figure 5 presents the mean monthly indoor and outdoor biting dynamics of *An. gambiae* s.l. in all IRS sites and paired unsprayed control sites (combined data for one rural and one central sub-location). At the end of the dry season in June, when IRS application took place, the biting rates were generally low (< 1 b/p/n) in all sites except in Gaoua and Solenzo, which had mean *An. gambiae* s.l. biting rates indoors and outdoors >10 bites/person/night (b/p/n). The biting rate increased monthly after July, reaching a peak in August and September (indoors and outdoors) for all sites. Comparison of unsprayed sites with their paired sprayed sites showed that average biting rates were higher in Gaoua (unsprayed site) compared to Kampti (sprayed with Actellic 300CS) (X^2=7.01, p= 0.008). This result suggests that IRS had an impact on biting rates in this locality. In contrast, in Solenzo (sprayed with Fludora Fusion WP-SB), biting rates were higher than in Nouna (unsprayed site) in all months (X^2=4.14, p= 0.04); this could mainly be due to the presence of many breeding sites and also to human activities (mainly the development of vegetable gardening), which may not be comparable with the neighboring control site of Nouna, which may not have been detected during the baseline and selection of the control sites. In addition, no difference was observed between Nouna compared to Bena (the Solenzo rural site sprayed with SumiShield 50WG) from September to December (X^2=0.21, p= 0.64). However, the impact of IRS on biting rates appears to be marginal with consistently lower biting rates from September to December in Solenzo (sprayed with SumiShield 50WG) compared to Nouna. In Kongoussi, biting rates remained relatively low, despite the withdrawal of IRS, and were comparable to the neighboring control site of Seguenega (X^2=2.15, p = 0.14) (Annex 1). In the two PBO net sites, biting rates varied by month, reaching peak levels indoors in August with 18.87 and 26.25 bites per person per night in Soumousso and Karangasso-Vigué, respectively (Figure 6). The highest biting rates were recorded outdoors with 20.62 and 44.5 bites per person per night in Soumousso and Karangasso-Vigué respectively (Figure 6). No difference was observed between Soumousso and Karangasso-Vigué (X^2=0.59, p = 0.44).

Figure 5. Mean *An. gambiae s.l.* bites per person per night from indoor (A) and outdoor (B) HLC collections in IRS and unsprayed sites (combined central and rural) from June to December 2021



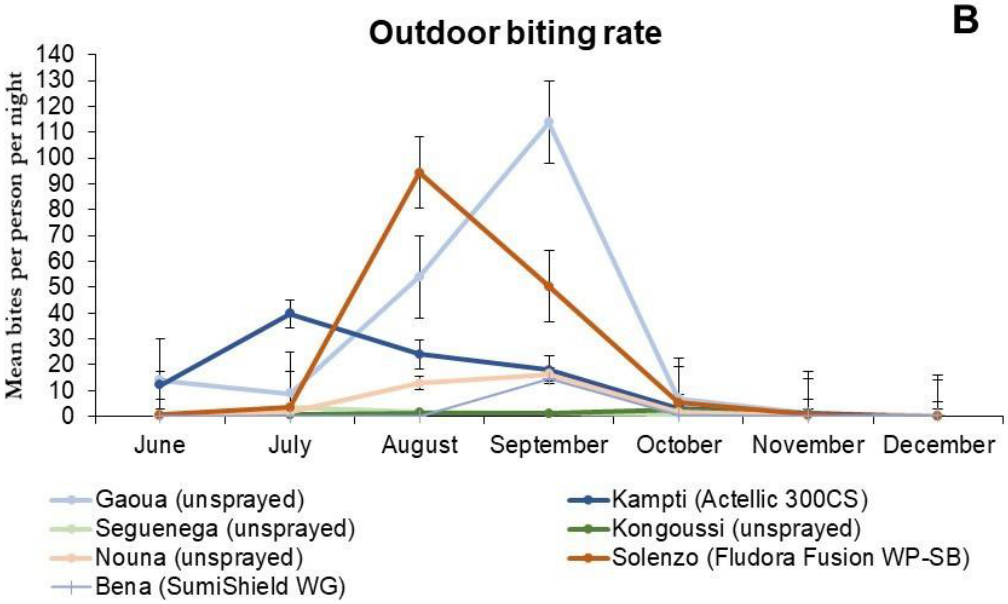
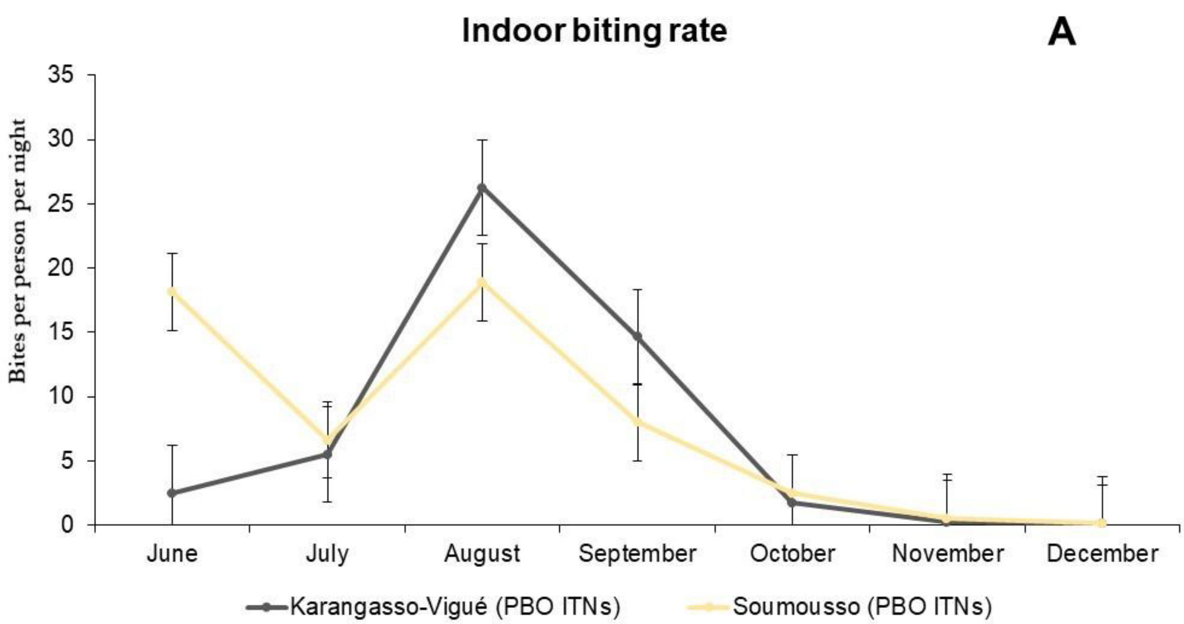
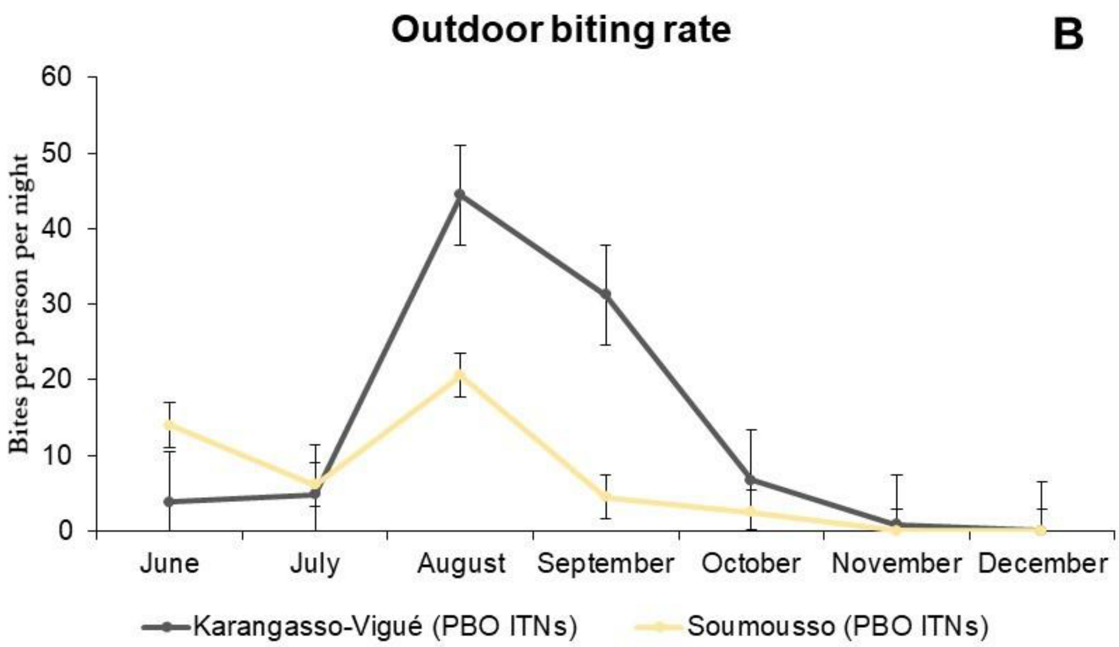


Figure 6. Mean *An. gambiae* s.l. bites per person per night from indoor (A) and outdoor (B) HLC collections in PBO ITN sites from June to December 2021





3.3 Biting Times of *An. gambiae* s.l.

Figure 7 shows the mean hourly indoor and outdoor biting times of *An. gambiae* s.l. in IRS and unsprayed sites. The trends showed a biting activity of the collected vectors from 6pm to 7am both indoors and outdoors (Figure 7). The hourly biting cycles were marked with a peak of biting activity from 11pm to 3am in Gaoua, Solenzo and Kampti. There was no clear biting pattern in the other sites as the numbers collected were low. The same trends were observed in the two PBO ITN sites with most bites starting after 9pm and peaking towards the second half of the night (Figure 8).

Figure 7. Mean number of bites of *An. gambiae* s.l. per hour from June to December 2021 (cumulated data from central and rural sites): A) indoor and B) outdoor HLC collections

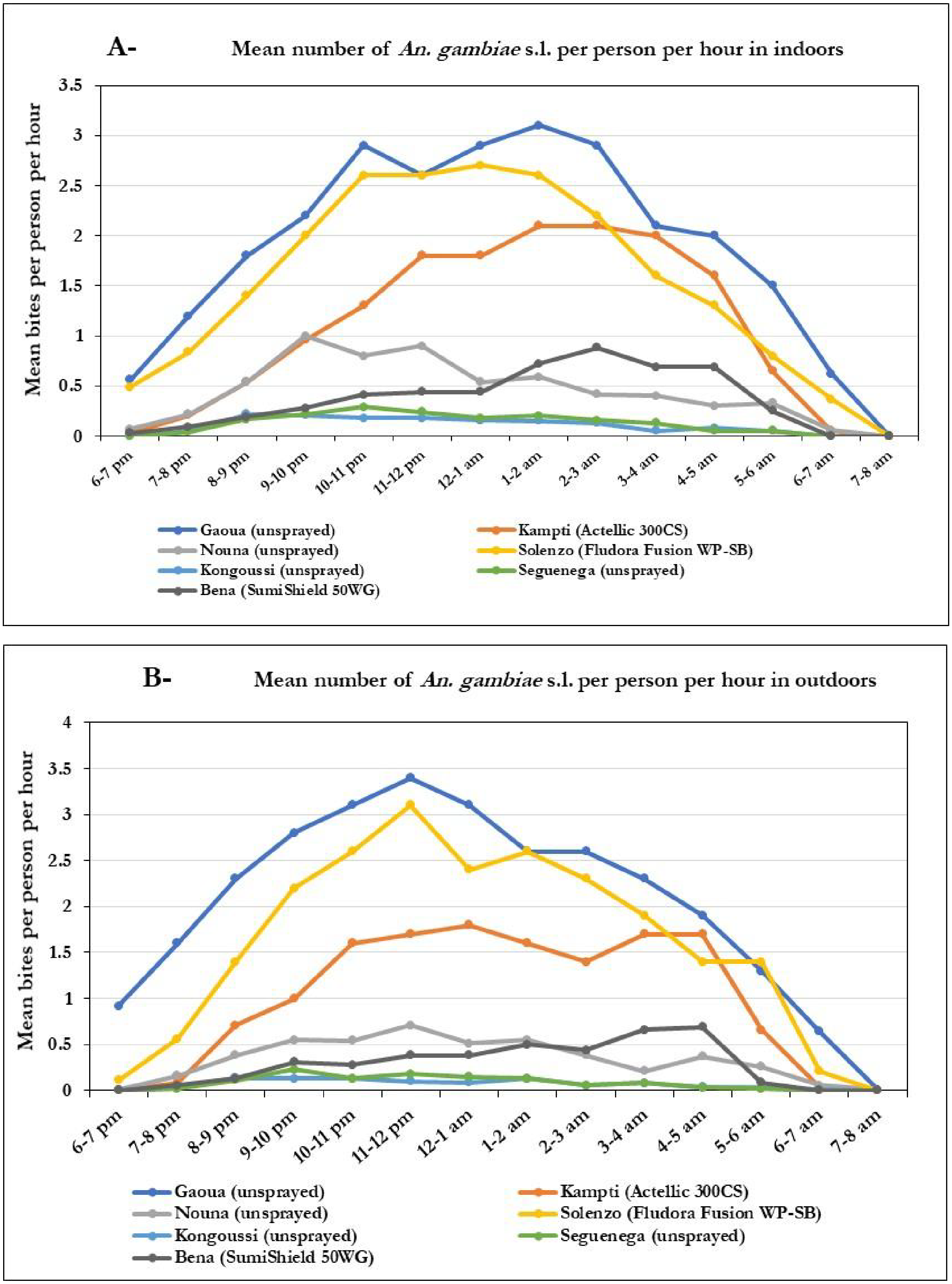
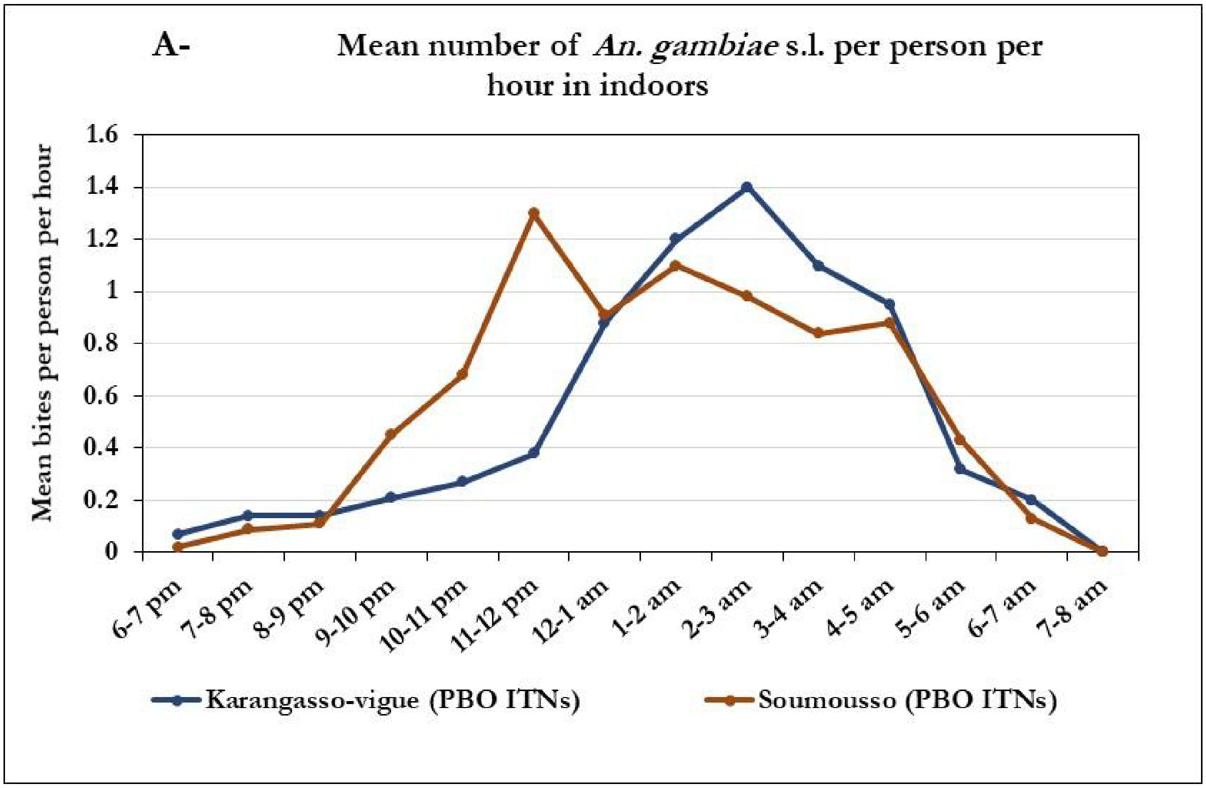
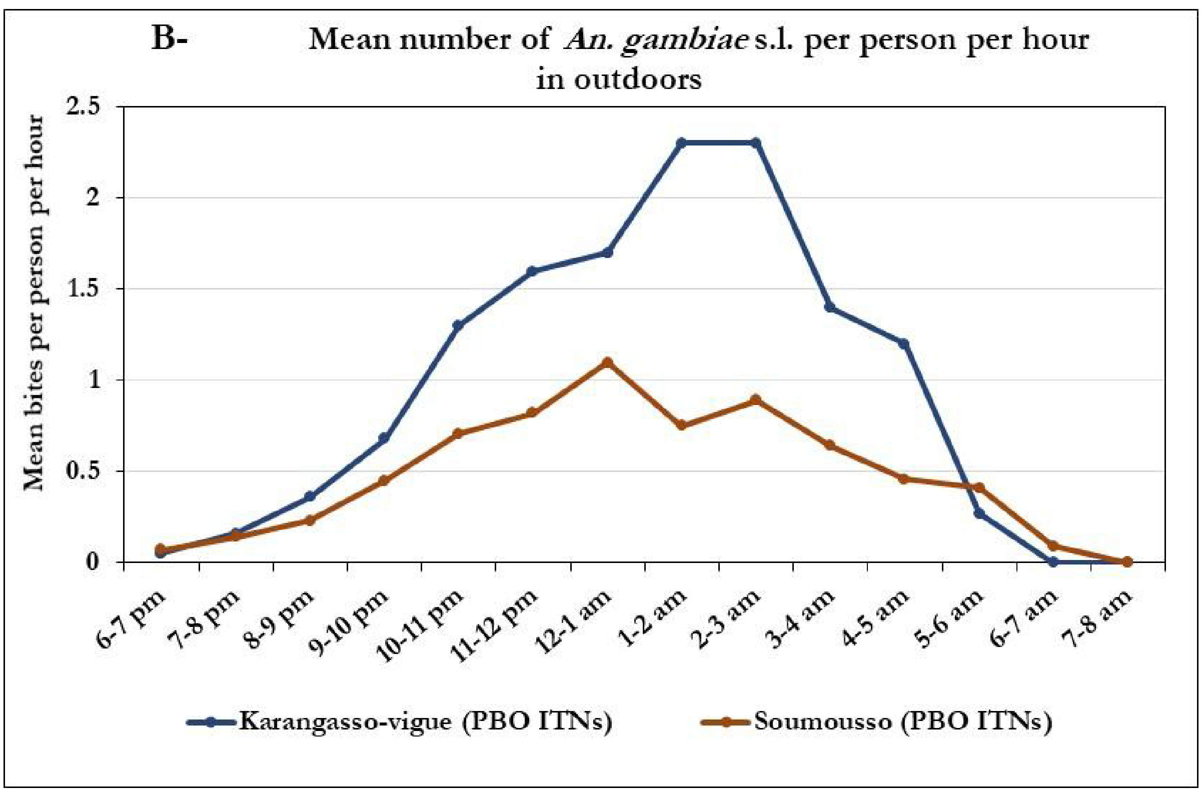


Figure 8. Mean number of *An. gambiae* s.l. bites per hour from A) indoor and B) outdoor HLC collections from June to December 2021 in Soumousso and Karangasso-Vigue

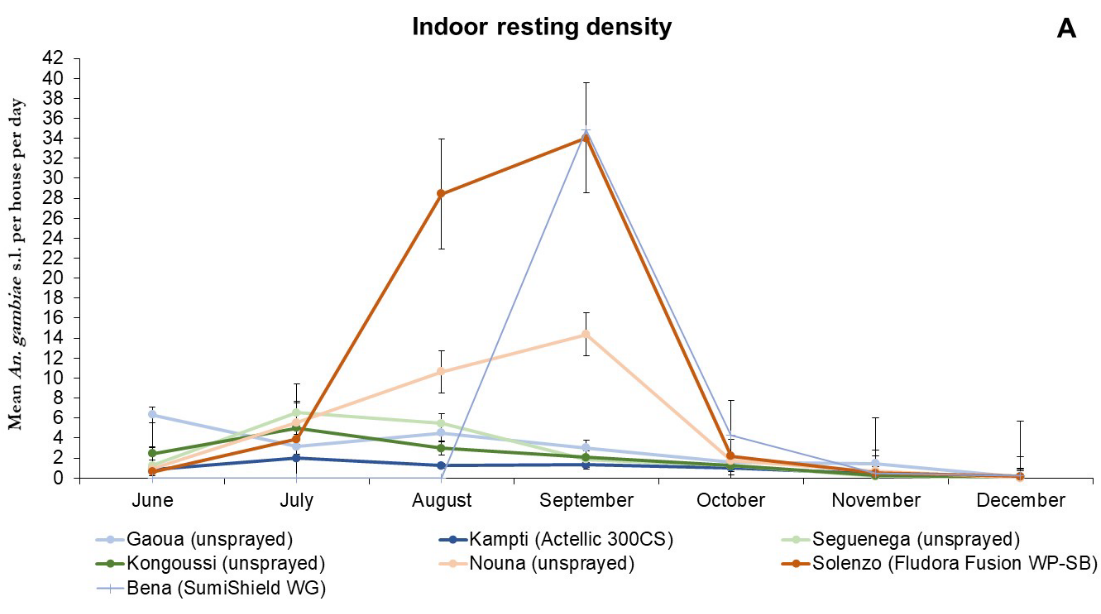


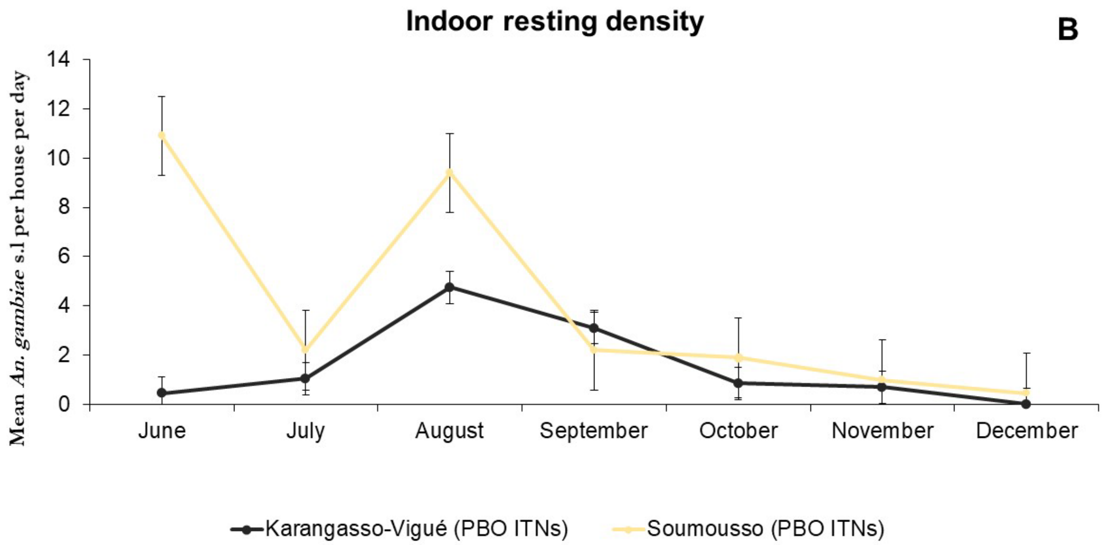


3.4 Indoor Resting Densities by PSC

The density of *An. gambiae* s.l. collected from PSC varied according to the month and the collection site (Figures 9A&B). The highest densities were observed in June in Gaoua (6.3 *An. gambiae* s.l. per house per day "/h/d") and in July in Kampti (2 *An. gambiae* s.l /h/d) (Figure 9 A). The density of *An. gambiae* s.l. was slightly lower in Kampti compared to its control site of Gaoua, where mean densities were always <1 *An. gambiae* s.l. per house per day (X^2=6.57, p = 0.01). However, except in Kampti, the comparison of unsprayed sites with their paired sprayed sites showed that mean resting density per house did not show any clear significant difference (Tukey's p > 0.12). This could be due to the effect of the Actellic 300CS in decreasing the overall densities of the *An gambiae* s.l at the Kampti site. The peak densities were observed in September in Solenzo (34 *An. gambiae* s.l /h/d), Nouna (14.3 *An. gambiae* s.l /h/d) and Bena (34.8 *An. gambiae* s.l /h/d) (Figure 9A). In fact, in the sprayed site of Solenzo (Fludora Fusion WP-SB), the densities were higher than in the paired unsprayed site of Nouna and reached a mean of nine *An. gambiae* s.l. per day compared to four in Nouna. In Kongoussi (5 *An. gambiae* s.l /h/d), where IRS was withdrawn, the resting density was similar to the neighboring control site of Seguenega (5.5 *An. gambiae* s.l /h/d) with the peak densities recorded in July (Figure 9A). The same trends were found by comparison of Soumousso to Karangasso-Vigué (X^2=3.05, p = 0.08). The highest resting densities recorded in June in Soumousso (10.9 *An. gambiae* s.l /h/d) were higher than those observed in Karangasso-Vigué in August (4.7 *An. gambiae* s.l /h/d) (Figure 9B) with similar trends to the previous year. The low densities were reported in December at all sites (Figures 9 A&B).

Figure 9. Mean *An. gambiae* s.l. collected per house per month from indoor PSC in (A) IRS and unsprayed sites (combined central and rural) and (B) PBO ITN sites





3.5 Parity Rate of *Anopheles gambiae* s.l.

Table 1 summarizes the overall parity rates from monthly dissected females between June and December 2021 by site. Overall, the parity rates did not differ significantly between sprayed and control sites in 2021 (Tukey's p > 0.25), being sometimes lower in sprayed sites, except in Bena (sprayed with SumiShield) with 74.16%. In Kampti (sprayed), the parity rate was 57.74%, which was slightly lower than that of Gaoua (control site) with 63.48%. In Kougoussi (former IRS site, last sprayed with SumiShield WG in 2018), the parity rate of 82.72% was significantly higher than its former control site of Seguenega at 72.73 %. However, in the sprayed district of Solenzo (sprayed with Fludora Fusion), it was lower (53.31%) than that of the unsprayed district of Nouna (64.04 %) (X^2 = 13.04, p = 0.003). There was no apparent difference in parity rates between the PBO-ITNs sites of Soumousso (65.33%) and Karanguasso-Vigué (66.02%) (X^2=0.05, p = 0.93).

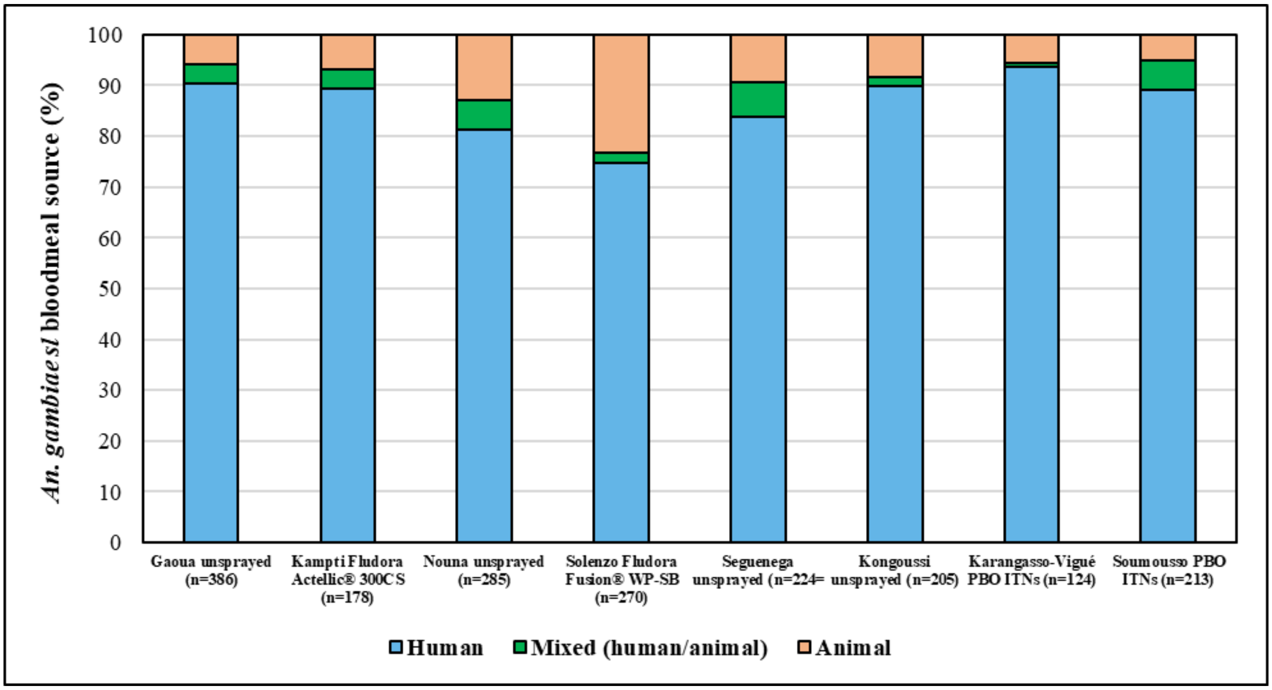
Table 1. Parity rate of *An. gambiae* s.l. females collected from sprayed sites and unsprayed sites between June and December 2021

Si	Total dissected	Parous	Non-parous	Parity rate (%)
Gaoua (unsprayed)	909	577	332	63.48
Kampti (Actellic 300CS)	795	459	336	57.74
Nouna (unsprayed)	470	301	169	64.04
Solenzo (Fludora Fusion WP-SB)	544	290	254	53.31
Bena (Sumishield WG)	89	66	23	74.16
Kongoussi (unsprayed)	180	149	31	82.78
Seguenega (unsprayed)	220	160	60	72.73
Karangasso-Vigué (PBO ITNs)	259	171	88	66.02
Soumousso (PBO ITNs)	274	179	95	65.33

3.6 *ANOPHELES GAMBIAE* S.L. BLOOD-MEAL SOURCE

An. gambiae s.l. were extremely anthropophilic in most of the study sites without any difference observed between sprayed and control sites (Figure 10). The proportion of strictly zoophagic *An. gambiae* did not reach 10%, except in Solenzo where more than 20% of *An. gambiae* population fed on animals. Mixed human and animal blood meals were very common and was no greater than 10% of samples tested.

Figure 10. Blood-meal source of *An. gambiae* s.l. collected by PSC between June and December 2021



3.7 *P. FALCIPARUM* INFECTION RATES OF *ANOPHELES GAMBIAE* S.L.

Irrespective of the vector control intervention, the *P. falciparum* infection rates (IR) of *An. gambiae* s.l. in Burkina Faso remain higher in the West and South West parts, and lowest in the Northern part as reported in previous reports. The mean infection rates (indoors and outdoors) were lower in sprayed sites (with 8.11 in Kampti and 6.39 in Solenzo sites) compared to unsprayed sites (11.03 in Gaoua and 7.46 Nouna). In Kongoussi, the mean IR was 10.05, and 9.97 in Seguenega site (Table 2). The mean infection rates did not differ significantly between sprayed and unsprayed sites (Tukey's p> 0.059). With regard to the collection point (indoor and outdoor), the mean IR did not significantly vary between sites, except in Gaoua district in relations to Kampti for indoor collection. However, the indoor mean infection rates were significantly higher in Gaoua compared to Kampti (*t-test* = 2.48, p= 0.02). The overall infection rates did not differ significantly between the two PBO TNs sites of Soumousso and Karangasso (X^2=0.21, p= 0.64). Furthermore, the infection rates were always higher in rural sites compared to central sites (Annex 2 & 3).

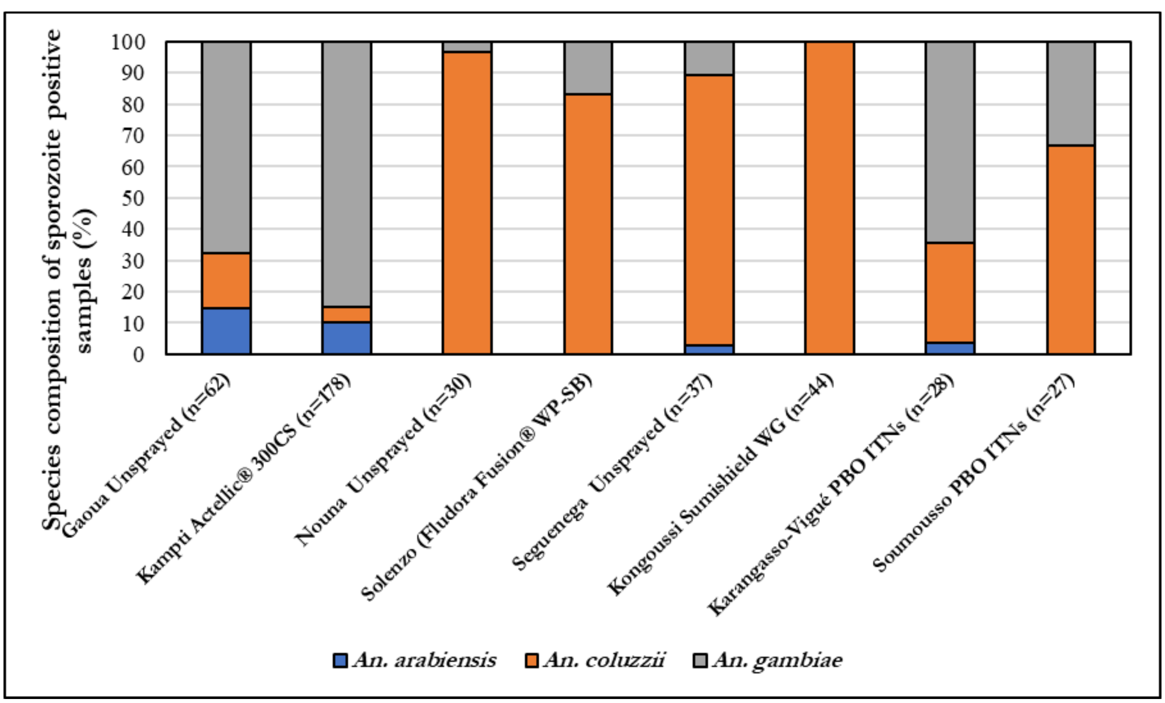
Table 2. ***Plasmodium falciparum*** **infection rate recorded from indoor and outdoor HLC-collected** ***An. gambiae*** **s.l. per site from June to December 2021**

Site	Infection rate indoors	Infection rate outdoors	Total positive
Gaoua (unsprayed)	10.78 (33/306)	11.33 (29/256)	11.03 (62/562)
Kampti (Actellic 300CS)	8.30 (22/265)	9.73 (11/113)	8.11 (40/493)
Nouna (unsprayed)	6.57 (19/289)	3.31 (5/151)	7.46 (30/402)
Solenzo (Fludora Fusion WP-SB)	7.72 (23/298)	4.38 (7/160)	6.39 (30/458)
Seguenega (unsprayed)	9.42 (26/276)	11.58 (11/95)	9.97 (37/371)
Kongoussi (unsprayed)	10.98 (38/346)	6.52 (6/92)	10.05 (44/438)
Karangasso-Vigué (PBO ITNs)	11.57 (14/121)	9.15 (14/153)	10.22 (28/274)
Soumousso (PBO ITNs)	10.80 (15/161)	7.34 (8/109)	9.47 (27/285)
Overall Mean	9.34 (194/2077)	8.62 (104/1206)	9.08 (298/3283)

Note: IR value in red in the table; IR = positive/number tested)

Figure 11 summarized the *Anopheles* species proportion that were infected by P. *falciparum* sporozoites. *An. gambiae* was the most common malaria vector transmitting *Plasmodium* in the Southwest sites (Gaoua, Kampti, Karangasso-Vigué and Soumousso) with proportions ranging from 30-85% of the infected mosquitoes. *An. coluzzii* was the main vector species transmitting malaria in Solenzo, Nouna, Kongoussi and Seguenega. Low proportions of infected *An. arabiensis* were detected in Gaoua, Kampti and Soumousso located in the Southwestern area and Seguenega in the Northern area.

Figure 11. Species composition of ***P. falciparum*** **infected female** ***An. gambiae*** **s.l. from June to December 2021**



3.8 ENTOMOLOGICAL INOCULATION RATE

The entomological inoculation rate was calculated for the period of June through December 2021 (Table 3). The EIR was higher in the Southwest and Western regions. It was particularly higher in Gaoua (unsprayed) with 319 infectious bites per person (ib/p) over the 112 collection nights indoors (central and rural sites combined), compared to Kampti (sprayed with Actellic 300 CS) which had 140 infected bites per person over the same nights (X^2 =6.39, p=0.01). The same trend was observed outdoors, where EIR was significantly reduced in Kampti with 124 ib/p over the same collection period while it is 324 ib/p for the control site of Gaoua. When extracting only for the central sites, the impact of IRS was more visible with reduced values of EIR indoors with 18 infected bites per person in Kampti compared to 143 infected bites per person in Gaoua (Table 4). However, the EIR was surprisingly higher in Solenzo (sprayed with Fludora Fusion), with 186 infectious bites per person over 112 nights compared to Nouna (the unsprayed control area) with 46 infectious bites per person (X^2=37.92, $p < 0.0001$) (Table 3). In Kongoussi (where IRS was withdrawn), the EIR estimated indoors was the lowest with 18 infectious bites per person and did not differ from that of Seguenega with 19 infectious bites (X^2=0.26, p = 0.60). In the two PBO nets sites, the EIR was similar indoors (X^2=0.21, p=0.64) but the outdoor EIR was higher in Karagansso-Vigué with 194 infected bites per person compared to Soumousso with 28 infected bites (X^2=128, $p< 0.0001$) over the 56 nights collection (Table 4). The entomological inoculation rate of *An. gambiae* s.l. per night, per month per the collection nights and over the seven months is presented in Annex 4 for the IRS and controls sites and in Annex 5 for the PBO nets sites.

Table 3. Entomological inoculation rate (June to December 2021) from combined central and rural data

	Gaoua (unsprayed)	Kampti (Actellic 300CS)	Nouna (unsprayed)	Solenzo (Fludora Fusion WP-SB)	Seguenega (unsprayed)	Kongoussi (unsprayed)
Total *An. gambiae* s.l. collected indoors (HLC)	2965	1684	698	2405	194	164
HLC collection nights	112	112	112	112	112	112
HBR per night	26.47	15.04	6.23	21.47	1.73	1.46
Total *An. gambiae* s.l. tested by PCR (HLC)	306	265	289	298	276	346
Total number positive to *P. falciparum*	33	22	19	23	26	38
Sporozoite rate (%)	10.78	8.3	6.57	7.72	9.42	10.98
EIR per night	2.85	1.25	0.41	1.66	0.17	0.16
EIR indoor - June to December 2021 (112 nights) (Calculated using mean sporozoite rate)	**31**	**14**	**4**	**18**	**1**	**1**
Estimated indoor EIR from June to December 2021 (214 days)	**610**	**268**	**88**	**355**	**36**	**34**

	Gaoua (unsprayed)	Kampti (Actellic 300CS)	Nouna (unsprayed)	Solenzo (Fludora Fusion WP-SB)	Seguenega (unsprayed)	Kongoussi (unsprayed)
Total *An. gambiae* s.l. collected outdoors (HLC)	3193	1570	527	2480	127	108
HLC collection nights	112	112	112	112	112	112
HBR per night	28.51	14.02	4.71	22.14	1.13	0.96
Total *An. gambiae* s.l. tested by PCR (HLC)	256	228	113	160	95	92
Total number positive to *P. falciparum*	26	18	11	7	11	6
Sporozoite rate (%)	10.16	7.89	9.73	4.37	11.58	6.52
EIR per night	2.89	1.11	0.46	0.97	0.13	0.062
EIR outdoor - June to December 2021 (112 nights) (Calculated using mean sporozoite rate)	**324**	**124**	**52**	**109**	**15**	**7**
Estimated outdoor EIR from June to December 2021 (210 days)	**618**	**23**	**9**	**208**	**28**	**13**

Table 4. Entomological inoculation rate (June to December 2021) from central sites (sprayed and unsprayed sites)

	Gaoua (Unsprayed)	Kampti (Actellic 300CS)	Nouna (Unsprayed)	Solenzo (Fludora Fusion WP-SB)	Seguenega (Unsprayed)	Kongoussi (Unsprayed)	Karangasso-Vigué (PBO ITNs)	Soumousso (PBO ITNs)
Total *An. gambiae* s.l. collected indoor (HLC)	1055	392	477	963	149	88	408	438
HLC trap-nights	56	56	56	56	56	56	56	56
HBR per night	18.84	7	8.52	17.19	2.66	1.57	7.28	7.82
Total *An. gambiae* s.l. tested by PCR (HLC)	170	106	158	135	146	173	121	176
Total number positive to *P. falciparum*	23	5	5	5	15	18	14	19
Sporozoite rate (%)	13.53	4.72	3.16	3.70	10.27	10.40	11.57	10.79
EIR per night	2.55	0.33	0.27	0.63	0.27	0.16	0.84	0.84
EIR indoor June to December 2021 (56 nights) (Calculated using mean sporozoite rate)	**143**	**18**	**15**	**35**	**15**	**9**	**47**	**47**
Estimated indoor EIR from June to December 2021 (214 days)	**546**	**71**	**58**	**135**	**58**	**34**	**180**	**180**
Total *An. gambiae* s.l. collected in outdoor (HLC)	1176	431	354	1248	101	53	737	382
HLC trap-nights	56	56	56	56	56	56	56	56
HBR per night	21	7.69	6.32	22.28	1.80	0.95	13.16	6.82
Total *An. gambiae* s.l. tested by PCR (HLC)	124	90	60	76	78	46	153	109
Total number positive to *P. falciparum*	17	10	5	4	10	1	14	8
Sporozoite rate (%)	13.71	11.11	8.33	5.26	12.82	2.17	26.41	7.34

	Gaoua (Unsprayed)	Kampti (Actellic 300CS)	Nouna (Unsprayed)	Solenzo (Fludora Fusion WP-SB)	Seguenega (Unsprayed)	Kongoussi (Unsprayed)	Karangasso-Vigué (PBO ITNs)	Soumousso (PBO ITNs)
EIR per night	2.88	0.85	0.53	1.17	0.23	0.02	3.47	0.5
EIR outdoor June to December 2021 (56 nights) (Calculated using mean sporozoite rate)	**161**	**48**	**30**	**65**	**13**	**1**	**194**	**28**
Estimated indoor EIR from June to December 2021 (214 days)	**616**	**182**	**113**	**250**	**49**	**4**	**742**	**107**

3.9 Residual Efficacy of SumiShield 50WG, Fludora Fusion and Actellic 300CS against Susceptible *An. gambiae* "Kisumu" and Wild *An. gambiae* s.l.

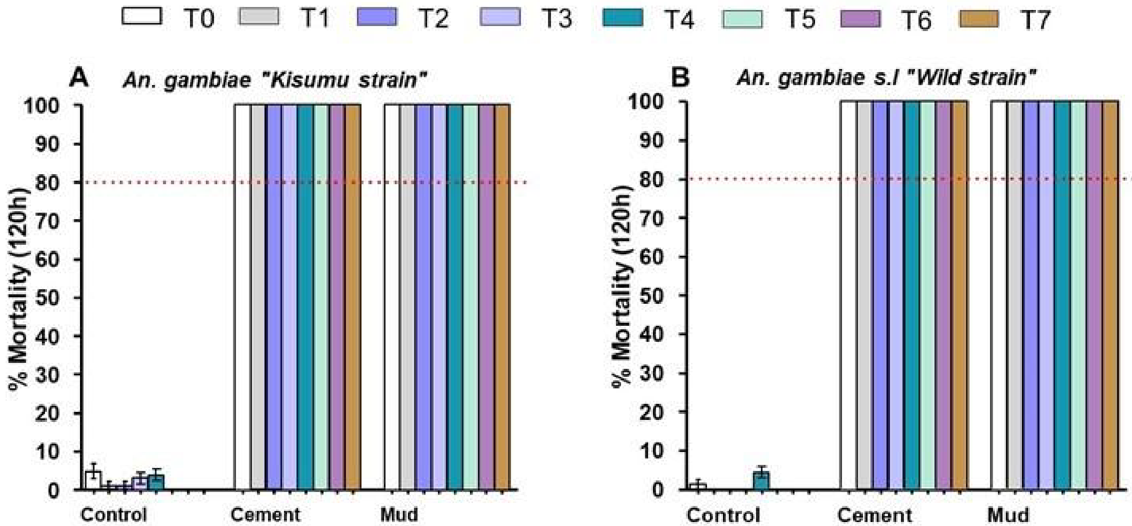
The mortality rates of control bioassays on unsprayed walls were less than five percent for all tests.

In Solenzo district, mortality rates (120h) with Fludora Fusion® WP-SB was 100 percent over a seven-month period with walls made of mud and cement for both Kisumu and wild *An. gambiae* s.l. (Figure 12). These results were similar in the areas sprayed with SumiShield 50WG on painted cement and mud walls, with mortalities of 100 percent from four months to seven months post-IRS (Figure 13).

In Kampti district, the mortality rates (24h) with Actellic 300CS was 100 percent over a seven-month period with walls made of mud and cement for Kisumu strain (Figure 14).

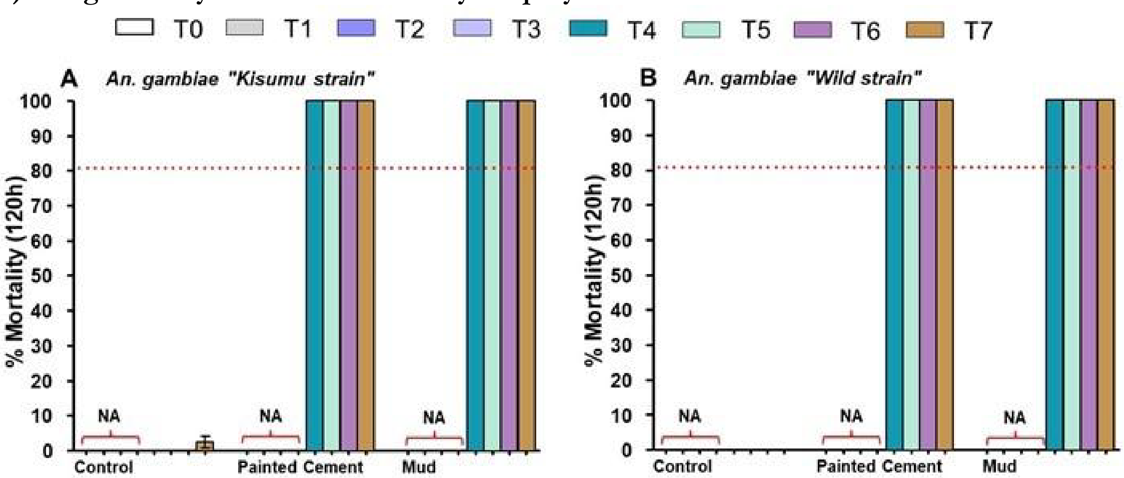
Results of cone bioassay show that all insecticide formulations (SumiShield 50WG, Fludora Fusion WP-SB and Actellic 300CS) sprayed were effective during the seven months of monitoring (May to December 2021). Residual efficacy will continue to be monitored until mortality is <80 percent for two consecutive months.

Figure 12. Mean mortality rates (96h) of *Anopheles gambiae* "Kisumu" (A) and wild *Anopheles gambiae* s.l (B) using monthly WHO cone bioassay on sprayed walls with Fludora Fusion in Solenzo district



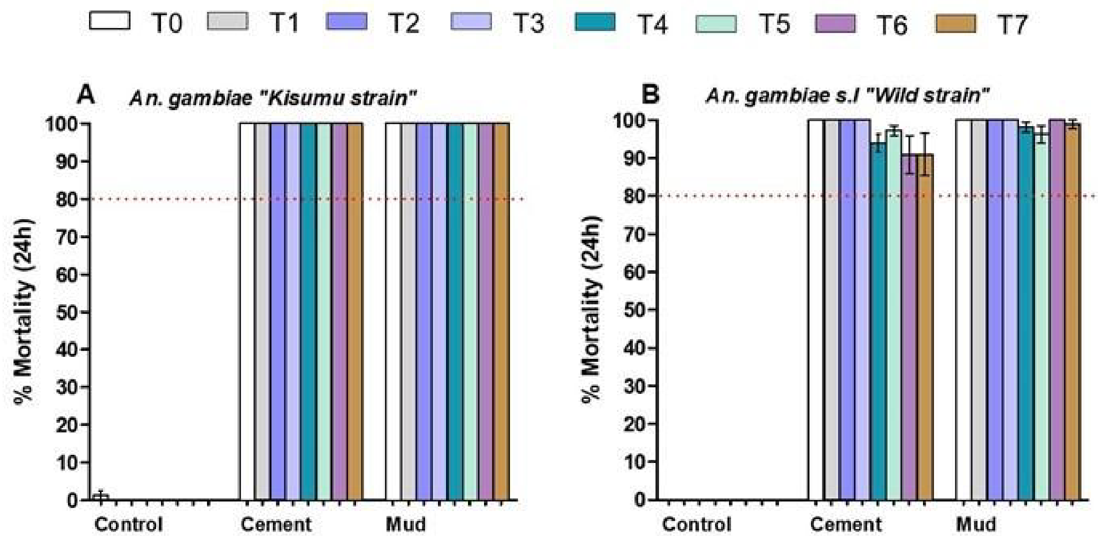
Note: Error bars represent the 95% confidence interval. Red dashed line represents the WHO threshold of 80% mortality. T0 to T7: = 0 to 7 months after spraying.

Figure 13. Mortality rate (120h) of *Anopheles gambiae* "Kisumu" (A) and wild *Anopheles gambiae* s.l (B) using monthly WHO cone bioassay of sprayed walls with SumiShield® 50WG in Solenzo district



Note: Error bars represent the 95% confidence interval. Red dashed line represents the WHO threshold of 80% mortality. T0 to T7: = 0 to 7 months after spraying. NA: no test; this site was not included in the workplan, but was added to cover all insecticides sprayed, which explains the tests carried out from T4.

Figure 14. Mean mortality rates (24h) of *Anopheles gambiae* "Kisumu" (A) and wild *Anopheles gambiae* s.l (B) using monthly WHO cone bioassay on sprayed walls with Allelic® 300CS in Kampti district.

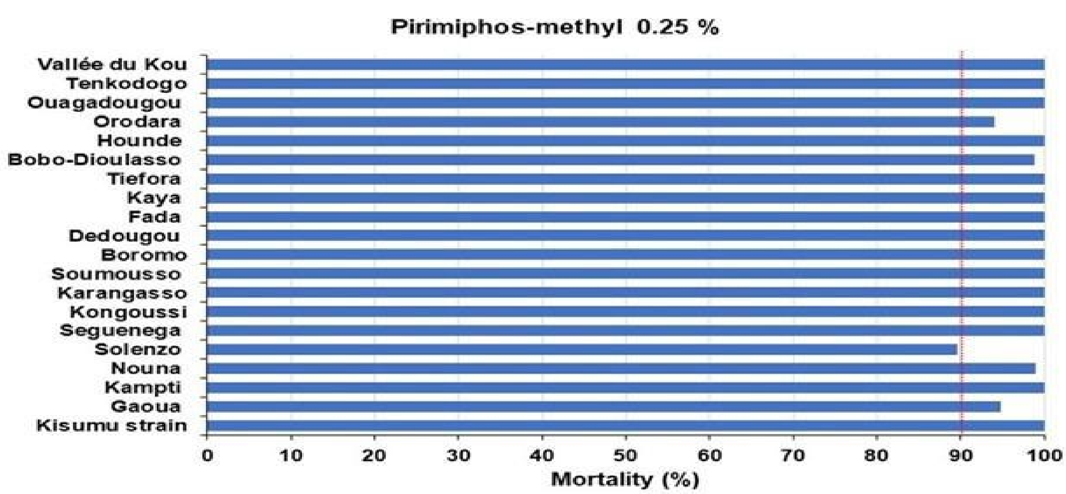


Note: Error bars represent the 95% confidence interval. Red dashed line represents the WHO threshold of 80% mortality. T0 to T7: = 0 to 7 months after spraying.

3.10 Insecticide Susceptibility Data

The results of susceptibility tests with 0.25 percent pirimiphos-methyl in WHO tubes against wild *An. gambiae* s.l are presented in Figure 15. In Solenzo (IRS site), *An. gambiae* s.l. was resistant to pirimiphos-methyl with a mortality of 89.58% (Figure 15). There was possible resistance in Gaoua and Orodara (unsprayed sites) with a mortality of 94%. In the other monitoring sites, *An. gambiae* s.l. populations were susceptible to pirimiphos-methyl with mortalities of 98-100 percent.

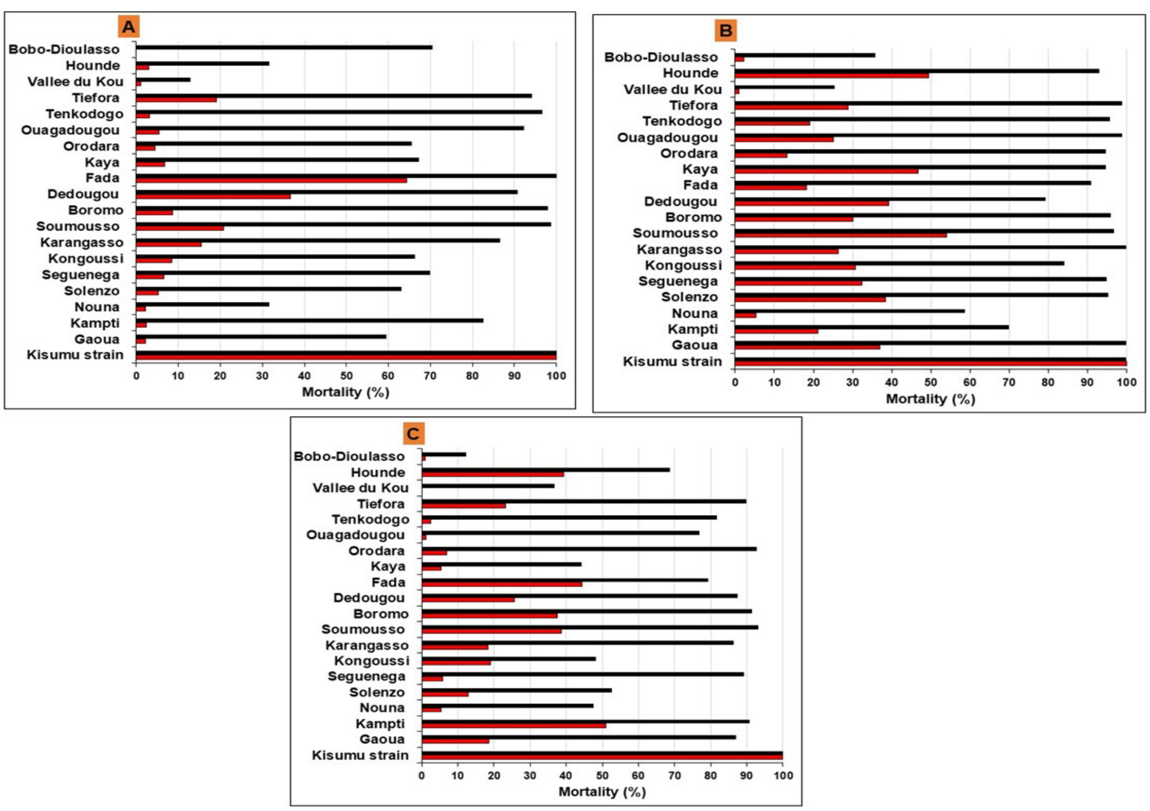
Figure 15. Results of susceptibility tests with 0.25 percent pirimiphos-methyl in WHO tubes against wild *An. gambiae* s.l. collected as larvae from 19 sites.



*Red dashed line represents 90% mortality threshold.

The results of susceptibility tests performed with pyrethroid insecticides (alpha-cypermethrin 0.05%, deltamethrin 0.05% and permethrin 0.75%) showed that *An. gambiae* s.l. were resistant in all 19 sites (Figure 16). Pre-exposure to PBO followed by exposure to pyrethroid insecticides resulted in much greater mortality rates compared to pyrethroid alone. Mortalities were < 50 % with pre-exposure to PBO 4% + alpha-cypermethrin 0.05% in three sites: Nouna, Houndé and Vallée du Kou (Figure 16 A). However, mortality between 90 - 97% were recorded in four sites (Ouagadougou, Tenkodogo, Dédougou and Tiéfora). Mortality was over 98% in three sites: Soumousso, Boromo and Fada. With PBO 4% + deltamethrin 0.05 percent, mortality was < 50% in the Vallée du Kou and Bobo-Dioulassso. However, in Gaoua, Karangasso, Tiéfora and Ouagadougou, there was higher mortality rates (>98%; Figure 16 B). With PBO 4% + permethrin 0.75%, mortalities was < 50% in Kongoussi, Kaya, Vallée du Kou and Bobo-Dioulasso (Figure 16 C). The highest mortalities were observed in Soumousso (93.2%), Orodara (92.8%), Boromo (91.3%) and Kampti (90.8%). Overall, pre-exposure to PBO followed by alpha-cypermethrin, deltamethrin or permethrin resulted in a partial or full restoration of susceptibility with mortalities > 80% in most sites. These results suggest that ITNs with PBO, such as PermaNet 3.0, could be more effective on pyrethroid-resistant vector populations than pyrethroids only ITNs. In addition, these findings indicate that metabolic resistance mechanisms (presence of oxidases) were involved in the phenotypic resistance observed.

Figure 16. Results of synergist tests with (A) alpha-cypermethrin 0.05% with or without PBO 4%, (B) deltamethrin 0.05% with or without PBO 4% and (C) permethrin 0.75% with or without PBO 4%



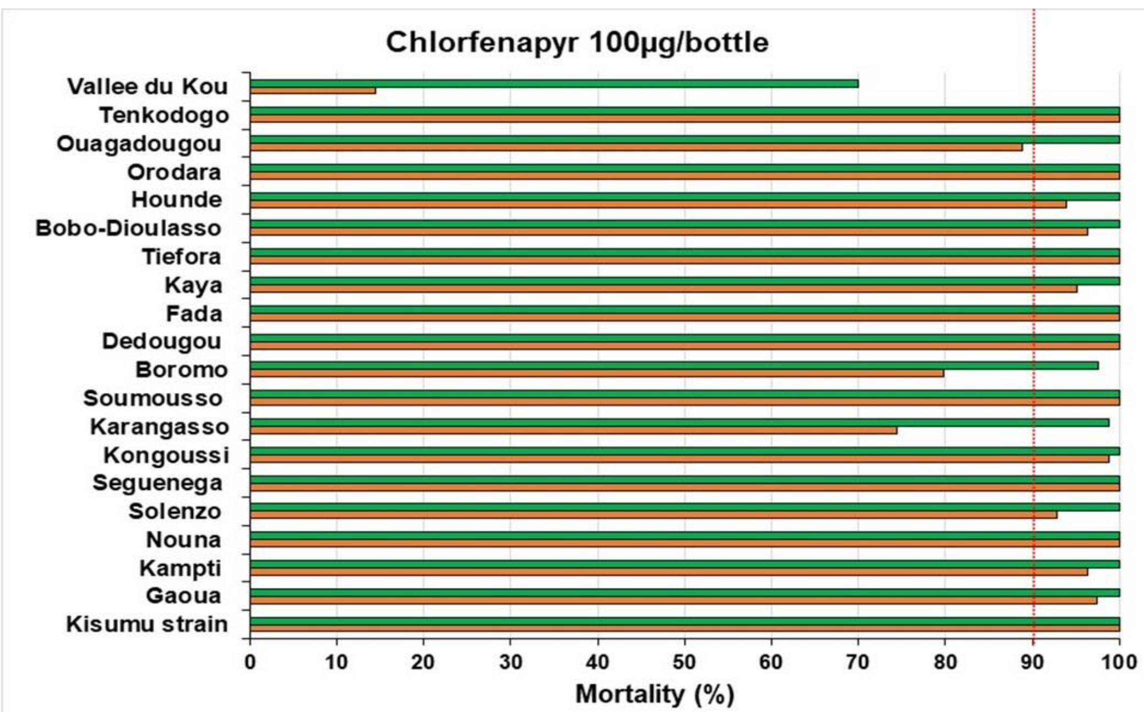
Note: Insecticide only (red bars); PBO 4% + insecticide (black bar)

Figure 17 show the results of susceptibility tests performed on *An. gambiae* s.l. against chlorfenapyr using the diagnostic dose of 100µg/bottle, while figure 19 shows results for the 200 µg/bottle dose for sites where mortality was <100% with the 100µg/bottle dose (Figure 18).

Mortalities of *An. gambiae* s.l. exposed to chlorfenapyr at the diagnostic dose of 100 µg/bottle after 24 hours were > 90% in most sites: except in Karangasso (74.4%), Boromo (79.8%), Ouagadougou (89.9%) and Vallée du Kou (14.4%). At the diagnostic time, after 72h post exposure, the *An. gambiae* s.l. population of Vallée du Kou showed a resistance (70% mortality) to chlorfenapyr, and a possible resistance was observed in Boromo with a mortality of 97.6%. However, in the other sites, a sensitivity to chlorfenapyr of 98.8-100% was observed (Figure 17). There was 100% mortality at the 200 µg/bottle in three sites: Boromo, Vallée du Kou and Karangasso-Vigue (Figure 18). These results suggest that ITNs containing chlorfenapyr could be used in most sites in Burkina Faso. However, monitoring and research of resistance mechanisms in sites where chlorfenapyr is used as a control tool, should be encouraged.

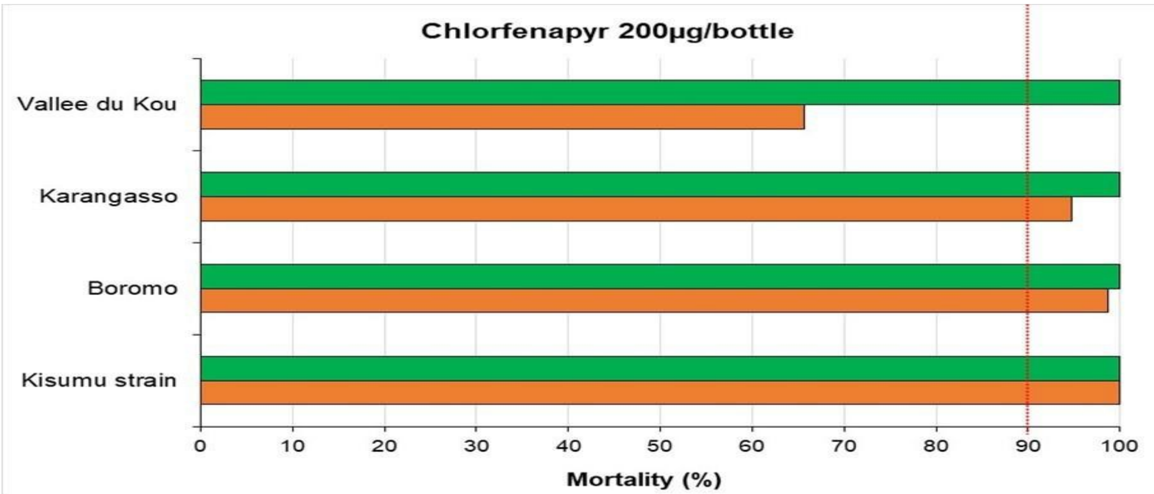
Mortality rates were > 90% in all sites after exposure with 2% clothianidin (Figure 19). After five days (120h), *Anopheles* populations showed susceptibility to clothianidin with 100% mortality in all sites (including IRS sites with clothianidin) except in Orodara where mortality was 98.8% (Figure 19). This suggests that SumiShield WG and Fludora Fusion WP-SB could be used in rotation during IRS campaigns.

Figure 17. Results of susceptibility tests with chlorfenapyr 100µg/bottle in CDC bottle bioassays against *An. gambiae* s.l. in 19 sites.



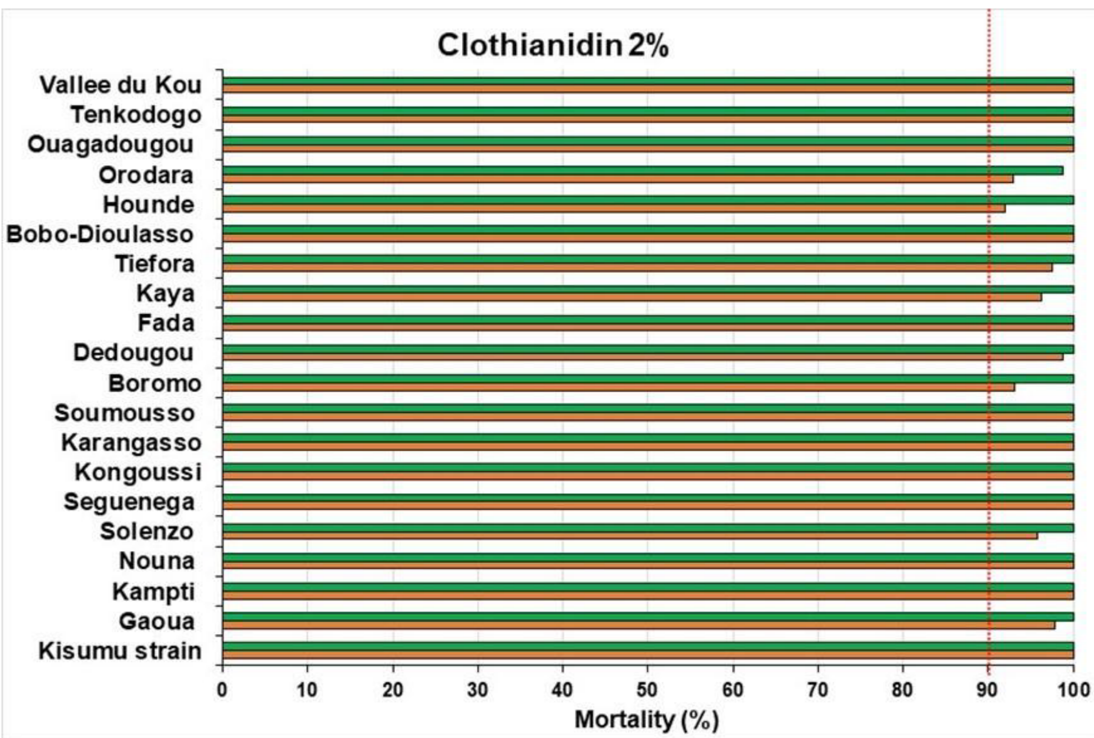
Note: Mortality at 24h (orange bars) and mortality at 72h (green bar)

Figure 18. Results of susceptibility tests with chlorfenapyr 200µg/bottle in CDC bottle bioassays against *An. gambiae* s.l. in three sites.



Note: Mortality at 24h (orange bars) and mortality at 72h (green bar)

Figure 19. Results of susceptibility tests with 2% clothianidin in WHO tube tests against *An. gambiae* s.l. in 19 sites.



Note: Mortality at 24h (orange bars) and mortality at 5 days (green bar)

Resistance Intensity (WHO Tube Bioassay)

The results of resistance intensity tests for *An. gambiae* s.l. exposed in WHO test tubes to alpha-cypermethrin at 5x and 10x the diagnostic dose showed high resistance intensity in all sites, except in Ouagadougou where moderate resistance status was observed (Table 5). The trends were similar to those of deltamethrin with high resistance intensity in all sites except in Fada N'Gourma where moderate resistance status was observed (Table 6). The results were more variable for permethrin, with high resistance intensity in 11 sites and moderate intensity in eight sites (Table 7).

Table 5. Mortality of *An. gambiae* s.l. after 24h post exposure to 5x and 10x concentrations of alphacypermethrin in WHO bioassays and status of resistance intensity

Sites	Alphacypermethrin diagnostic concentration (%)				Status
	5x (0.25%		10x (0.5%)		
	n	% mortality	n	% mortality	
Kisumu strain	100	100.00	NA	NA	Susceptible
Gaoua	81	13.58	98	65.31	High
Kampti	97	24.74	88	48.86	High
Nouna	93	5.38	103	25.24	High
Solenzo	101	11.88	93	64.52	High
Seguenega	83	28.92	91	60.44	High
Kongoussi	86	9.30	96	41.67	High
Soumousso	86	43.02	108	67.59	High
Karangasso	81	37.04	96	82.29	High
Boromo	83	34.94	76	47.37	High
Kaya	93	63.44	87	88.51	High
Orodara	102	25.49	99	41.41	High
Ouagadougou	88	80.68	82	100.00	Moderate
Tiéfora	84	38.10	102	50.98	High
Vallée du Kou	82	71.95	84	72.62	High
Bobo-Dioulasso	74	14.86	89	31.46	High
Houndé	104	53.85	99	57.58	High
Dédougou	94	60.64	103	80.58	High
Fada N'Gourma	98	73.47	95	78.95	High
Tenkodogo	81	61.73	94	78.72	High

NA (Not applicable): 98–100 percent mortality at 5x dose indicates a low resistance intensity. Not necessary to assay at 10x dose: not tested.

Table 6. Mortality of *An. gambiae* s.l. after 24h post exposure to 5x and 10x concentrations of deltamethrin in WHO bioassays and status of resistance intensity

Sites	Deltamethrin diagnostic concentration (%)				Status
	5x (0.25%)		10x (0.5%)		
	n	% mortality	n	% mortality	
Kisumu strain	100	100	NA	NA	Susceptible
Gaoua	96	53.13	90	70.00	High
Kampti	65	84.62	85	97.65	High
Nouna	91	40.66	92	44.57	High
Solenzo	107	49.53	103	78.64	High
Seguenega	84	77.38	91	81.32	High
Kongoussi	88	54.55	96	77.08	High
Soumousso	99	64.65	108	81.48	High
Karangasso-V	94	71.28	100	72.00	High
Boromo	95	72.63	92	85.87	High

Sites	Deltamethrin diagnostic concentration (%)				Status
	5x (0.25%)		10x (0.5%)		
	n	% mortality	n	% mortality	
Kaya	90	60.00	97	81.44	High
Orodara	92	66.30	88	80.68	High
Ouagadougou	83	54.22	97	94.85	High
Tiéfora	95	81.05	100	86.00	High
Vallée du Kou	93	36.56	89	14.61	High
Bobo-Dioulasso	90	28.89	101	37.62	High
Dédougou	84	85.71	90	90.00	High
Houndé	90	73.33	89	74.16	High
Fada N'Gourma	89	79.78	83	100.00	Moderate
Tenkodogo	99	76.77	105	86.67	High

NA (Not applicable): 98–100 percent mortality at 5x dose indicates a low resistance intensity. Not necessary to assay at 10x dose: not tested

Table 7. Mortality of *An. gambiae* s.l. after 24h post exposure to 5x and 10x concentrations of permethrin in WHO bioassays

Sites	Permethrin diagnostic concentration (%)				Status
	5x (3.75%)		10x (7.5%)		
	n	% mortality	n	% mortality	
Kisumu strain	100	100.00	NA	NA	Susceptible
Gaoua	86	54.65	95	88.42	High
Kampti	92	51.09	88	98.86	Moderate
Nouna	88	64.77	97	85.57	High
Solenzo	97	52.58	95	87.37	High
Seguenega	82	56.10	83	92.77	High
Kongoussi	86	70.93	97	91.75	High
Soumousso	99	66.67	94	98.94	Moderate
Karangasso-V	83	87.95	83	100.00	Moderate
Boromo	98	84.69	85	100.00	Moderate
Kaya	102	68.63	101	91.09	High
Orodara	83	86.75	89	97.75	High
Ouagadougou	92	91.30	98	98.98	Moderate
Tiéfora	88	85.23	102	98.04	Moderate
Vallée du Kou	81	29.63	77	68.83	High
Bobo-Dioulasso	91	37.36	82	65.85	High
Dédougou	92	78.26	96	91.67	High
Houndé	98	95.92	92	98.91	Moderate
Fada N'Gourma	87	91.95	86	100.00	Moderate
Tenkodogo	104	73.08	101	95.05	High

NA (Not applicable): 98–100 percent mortality at 5x dose indicates a low resistance intensity. Not necessary to assay at 10x dose: not teste

3.11 Distribution of Allele Frequencies of KDR (L1014F and L1014S) and *Ace-1R* Mutations

The allele frequency of the West African kdr-L1014F mutation showed high variations (Annex 7) in *An. gambiae* populations, but was particularly high in Northwest (Boromo, Solenzo, Nouna), Centre (Ouagadougou), Southwestern (Gaoua, Kampti and Orodara) and Northern (Seguenega) sites, reaching frequencies between 0.55 and 1. The kdr-L1014S mutation was only found in *An. gambiae* and *An. coluzzii* in low frequency (< 0.30) in Solenzo, Ouagadougou, Kampti, Gaoua, Kongoussi and Seguenega (Annex 6). The occurrence of these two mutations simultaneously in the same populations of *An. gambiae* s.l. indicates the existence of multiple resistance mechanisms (L1014F, L1014S and metabolic resistance). The *ace*-1R G119S mutation was reported both in *An. gambiae* and *An. coluzzii* populations at lowest frequencies in Northern sites (Kongoussi) and Southwestern sites (Kampti, Gaoua, Soumousso) (Annex 7).

4. CONCLUSIONS

Fludora Fusion WP-SB and SumiShield WG sprayed in Solenzo and Actellic 300 CS in Kampti lasted for at least seven months (cone bioassay ongoing until April 2022). There was a clear difference in biting rates for Kampti (Actellic 300CS) and Gaoua (unsprayed); however, this was not the case in Solenzo vs. Nouna, where biting rates were surprisingly higher in sprayed area of Solenzo in August and September.

When broken down to sub-locations, there appears to be a more consistent impact of IRS in sprayed central sub-locations compared to unsprayed areas, but not in rural sites where biting rates were higher. This is probably due to the intensive rainy period from August to October, increasing the occurrence of suitable breeding sites in those settings.

The density of *An. gambiae* s.l. collected from PSC was clearly more reduced in Kampti (Actellic 300CS) compared to its control site of Gaoua. However, this was not the case in Solenzo, particularly in August-September, where the resting densities were higher in Solenzo compared to the unsprayed control site of Nouna.

There was no apparent reduction in parity rates of *An. gambiae* s.l., except in Kampti with reduced parity rate compared to Gaoua. Conversely in Nouna, the parity rate was lower than that of Solenzo (sprayed). The result was comparable to 2020.

The sporozoite rates were lower in Kampti (sprayed) compared to Gaoua (unsprayed) where it was still extremely high. The sporozoite rates were highest in the Southwest and were considerably lower in the Northern sites, such as Kongoussi and Seguenega (despite both being unsprayed).

IRS appears to have had an important and increasing impact on the entomological inoculation rate in 2021 in Kampti compared to its unsprayed control site with reduced infective bites both indoors and outdoors. However, in Solenzo (sprayed with Fludora Fusion), the EIR was higher in the sprayed site compared to its control site of Nouna. EIR in Kongoussi and Seguenega remained the lowest at both central and rural sites.

In the two sites where PBO ITNs were distributed, the EIR was similar indoors with 180 i/b/p in Soumousso and 47 i/b/p in Karangasso-Vigue. The team has no pre-distribution data to determine the impact of PBO nets, but the EIR values recorded in this current report were similar to the EIR reported in 2020.

Insecticide susceptibility tests revealed that *An. gambiae* s.l. were resistant to all pyrethroids tested, but pre-exposure to PBO increased mosquito susceptibility to alpha-cypermethrin, deltamethrin and permethrin in all sites tested, including Karangasso-Vigue and Soumousso where Permanet 3.0 nets were distributed in 2019. Susceptibility to chlorfenapyr was recorded in all sites except in Vallée du Kou where *An. gambiae* s.l. populations were resistant to chlorfenapyr. These results support the distribution of PBO-combined nets and/or dual insecticide impregnated nets such as permethrin + piriproxifen or chlorfenapyr with alphacypermethrin IG2 nets in Burkina Faso instead of pyrethroid-only ITNs.

Susceptibility data of insecticides used for IRS showed that there was full susceptibility of *An. gambiae* s.l. to clothianidin observed in all sites tested, including both IRS locations. Therefore, insecticide formulations containing clothianidin can continue to be used in Burkina Faso for IRS on a rotational basis.

Susceptibility to pirimiphos-methyl was also recorded in most sites nationwide. However, reduced mortalities or resistance were observed for the first time in three sites: Orodara, Solenzo and Gaoua. Therefore, continuous monitoring is crucial to avoid the spread of pirimiphos-methyl large spectrum resistance.

Overall, IRS has an impact on malaria vector biting and resting densities particularly in Kampti. However, it had minor impact in Solenzo in 2021, which was not the case in 2020 where EIR was substantially reduced in this IRS site.

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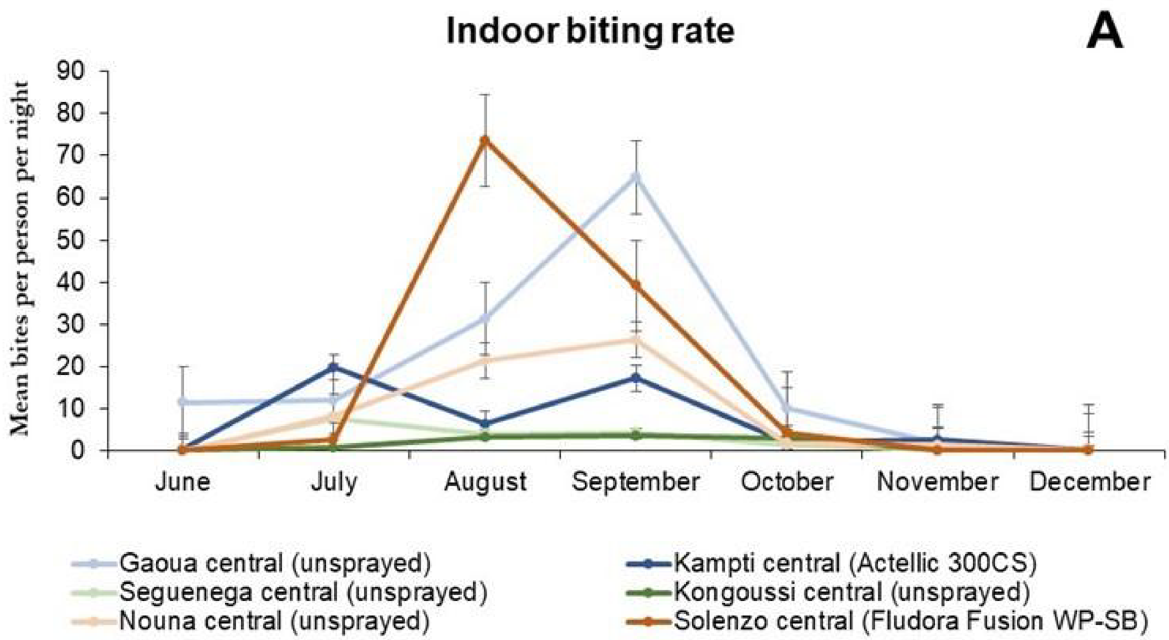
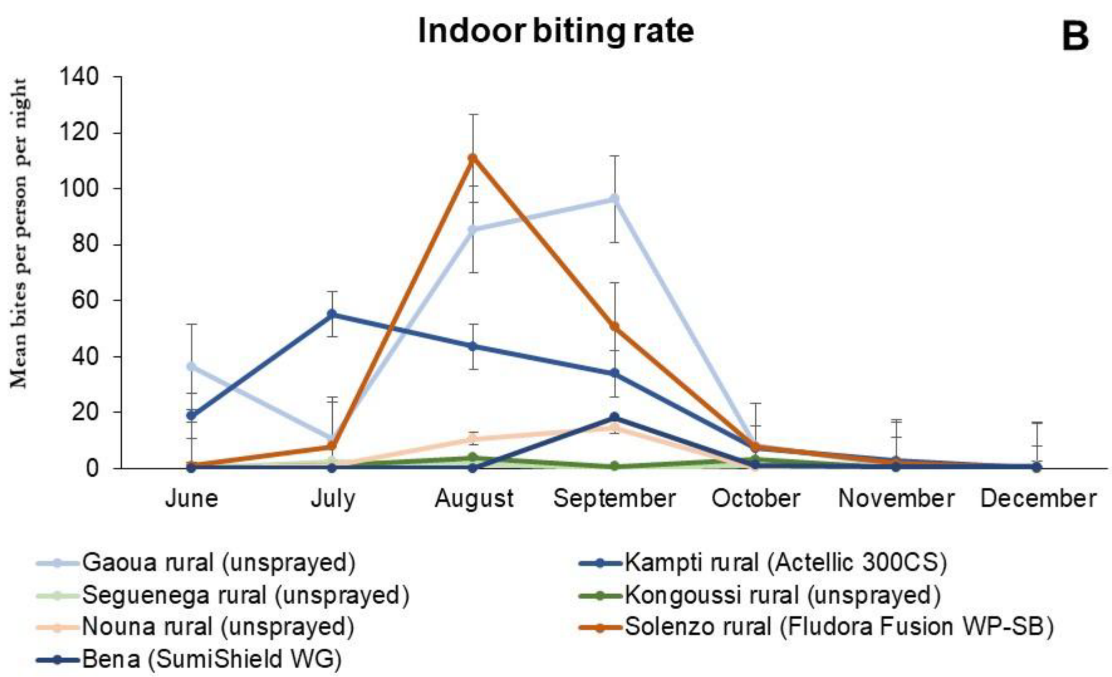
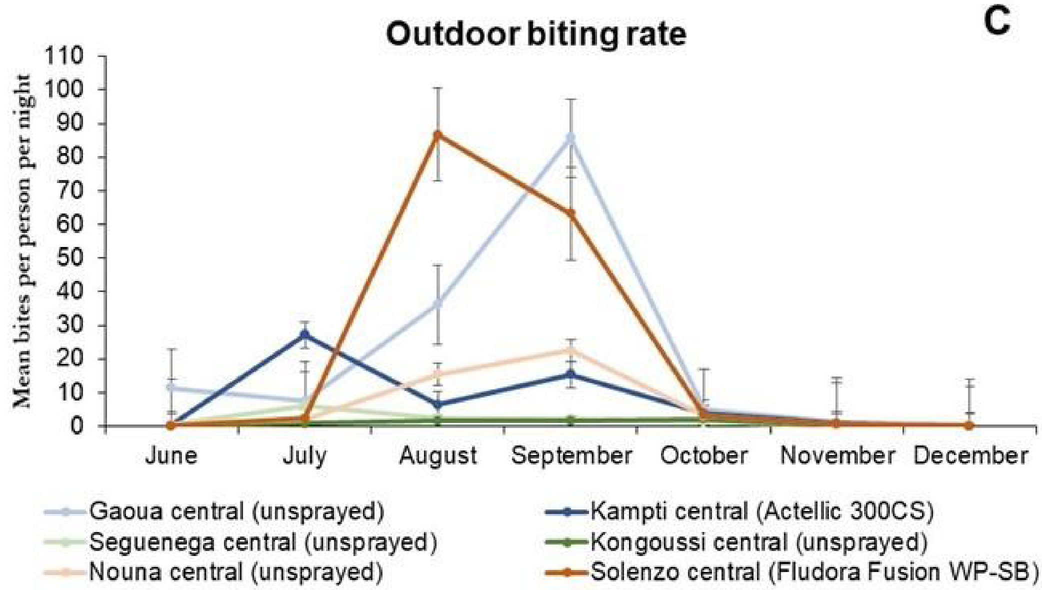
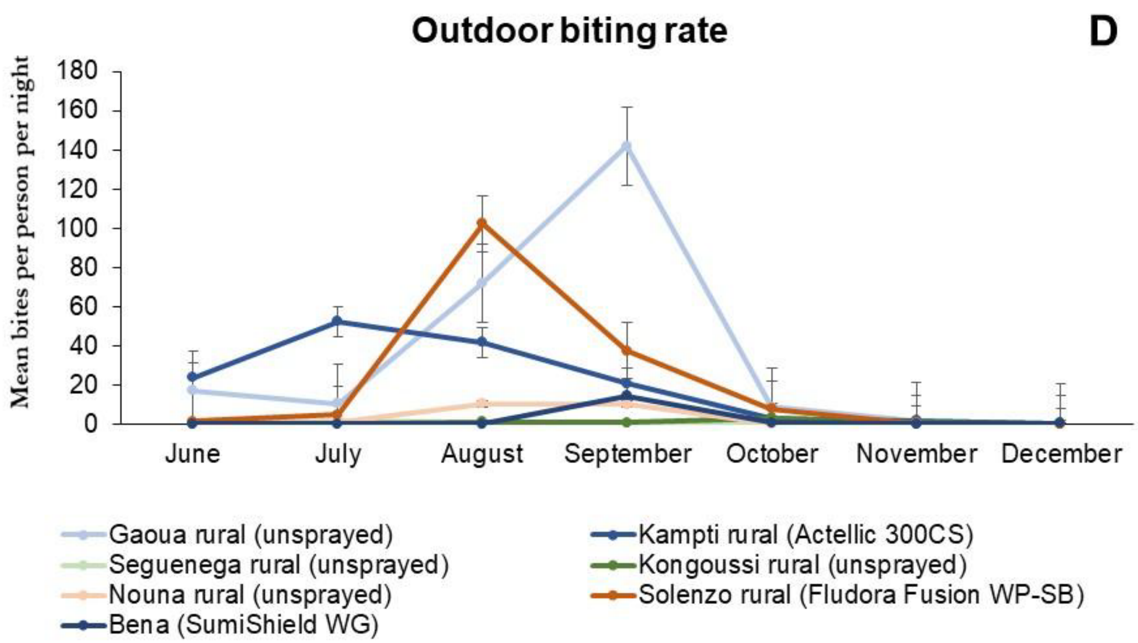
ANNEX

Annex 1. Nightly biting rate of *An. gambiae* s.l. indoor collections in central (A) and rural (B) sites and outdoor collections in central (C) and rural (D) sites from June to December 2021 from HLC in the IRS and control sites






Outdoor biting rate
C
Mean bites per person per night
110
100
90
80
70
60
50
40
30
20
10
0
June
July
August
September
October
November
December
Gaoua central (unsprayed)
Kampti central (Actellic 300CS)
Seguenega central (unsprayed)
Kongoussi central (unsprayed)
Nouna central (unsprayed)
Solenzo central (Fludora Fusion WP-SB)


Outdoor biting rate
D
Mean bites per person per night
180
160
140
120
100
80
60
40
20
0
June
July
August
September
October
November
December
Gaoua rural (unsprayed)
Kampti rural (Actellic 300CS)
Seguenega rural (unsprayed)
Kongoussi rural (unsprayed)
Nouna rural (unsprayed)
Solenzo rural (Fludora Fusion WP-SB)
Bena (SumiShield WG)

Annex 2. Mean infection rate of *An. gambiae* s.l. collected by HLC (indoor + outdoor) collections from June to December 2021 in the IRS and control central and rural sites.

Infection rate of *An. gambiae* s.l. from central sites						
Months	**Gaoua central (Unsprayed)** (N=283)	**Kampti Central (Actellic 300CS)** (N=178)	**Nouna Central (Unsprayed)** (N=196)	**Solenzo Central (Fludora® Fusion WP-SB)** (N=163)	**Seguenega Central (unsprayed)** (N=201)	**Kongoussi Central (unsprayed)** (N=191)
June	6 (3/50)	0 (0/3)	0 (0/7)	0 (0/3)	0 (0/26)	3.3 (1/33)
July	33.33 (13/39)	6 (3/50	4 (2/50)	0 (0/7)	4 (2/50)	0 (0/50)
August	20 (10/50)	10 (3/30)	6 (3/50)	0 (0/50)	2 (1/50)	4 (2/50)
September	2 (1/50)	10.2 (5/49)	2 (1/50)	4 (2/50)	8 (4/50)	2 (1/50)
October	34 (17/50)	33.33 (15/45)	50 (18/36)	22.44 (11/49)	13.33 (2/15)	0 (0/6)
November	16.16 (1/6)	0 (0/1)	33.33 (1/3)	0 (0)	25 (1/4)	0 (0)
December	15.78 (6/38)	0 (0)	0 (0)	0 (0/4)	0 (0/6)	0 (0/2)
Mean infection rate % (P/N)	**18.02 (51/283)**	**14.6 (26/178)**	**12.75 (25/196)**	**7.97 (13/163)**	**4.97 (10/201)**	**2.09 (4/191)**

Infection rate of *An. gambiae* s.l. from rural sites						
Month	**Gaoua rural (unsprayed)** (N=265)	**Kampti Rural (Actellic 300CS)** (N=241)	**Nouna rural (unsprayed)** (N=194)	**Solenzo Rural (Fludora® Fusion WP-SB)** (N=228)	**Seguenega Rural (unsprayed)** (N=174)	**Kongoussi Rural (unsprayed)** (N=164)
June	18 (9/50)	10,52 (4/38)	0 (0)	0 (0/19)	0 (0/20)	0 (0/11)
July	36 (18/50)	26 (13/50)	2 (1/50)	2 (1/50)	5 (5/50)	4,34 (2/46)
August	52 (26/50)	26 (13/50)	2 (1/50)	4 (2/50)	2 (2/50)	4,08 (2/49)
September	16 (8/50)	6 (3/50)	0 (0/50)	8 (4/50)	5 (5/50)	6 (3/50)
October	46 (23/50)	48 (24/50)	33,33 (11/33)	6 (3/50)	1 (1/4)	0 (0/5)
November	50 (2/4)	33,33 (1/3)	27,27 (3/11)	25 (1/4)	0 (0)	50 (1/2)
December	18.18 (2/11)	0 (0)	0 (0)	20 (1/5)	0 (0)	100 (/1)
Mean infection rate % (P/N)	**33.20 (88/265)**	**24.06 (58/241)**	**8.24 (16/194)**	**5.26 (12/228)**	**7.47 (13/174)**	**5.48 (9/164)**

Annex 3. Infection rates of *An. gambiae* s.l. females to *P. falciparum* from indoor and outdoor collections in PBO-ITNs sites in 2021.

Sporozoite rate of *An. gambiae* s.l. from indoor and outdoor HLC collections		
Months	**Karangasso-Vigué**	**Soumousso**
June	0 (0/50)	0 (0/50)
July	28 (14/50)	18 (9/50)
August	2 (1/50)	6 (3/50)
September	6 (3/50)	6 (3/50)
October	14 (7/50)	14 (7/27)
November	12.5 (2/16)	26.31 (5/19)
December	20 (1/5)	25 (1/4)
Total	**10.33% (28/271)**	**10.25% (28/273)**

Annex 4. Nightly and monthly entomological inoculation rate of *An. gambiae s.l.* collected through HLC collection from June to December 2021 in the IRS and control central and rural sites.

Overall EIR per night and month of *An. gambiae* s.l. from central sites												
Months	**Gaoua Central (unsprayed)**		**Kampti Central (Actellic 300CS)**		**Nouna Central (unsprayed)**		**Solenzo Central (Fludora® Fusion WP-SB)**		**Seguenega Central (unsprayed)**		**Kongoussi Central (unsprayed)**	
	Mean EIR per night	Mean EIR per month	Mean EIR per night	Mean EIR per month	Mean EIR per night	Mean EIR per month	Mean EIR per night	Mean EIR per month	Mean EIR per night	Mean EIR per month	Mean EIR per night	Mean EIR per month
June	0.22	6.6	0	0	0	0	0.01	0.3	0.02	0.6	0	0
July	2.87	88.97	3.41	105.71	0.12	3.72	0	0	0.3	9.3	0.1	3.1
August	3.33	103.23	0.29	8.99	0.85	26.35	0	0	0.77	23.87	0.19	5.89
September	1.2	36	0.34	10.2	1.8	54	2.84	85.2	0.47	14.1	0.11	3.3
October	0.77	23.87	0.34	10.54	0.03	0.93	0.22	6.82	0.04	1.24	0.04	1.24
November	0.26	7.8	0.33	9.9	0.03	0.9	0	0	0	0	0	0
December	0.02	0.62	0	0	0	0	0.02	0.62	0	0	0	0
Mean Monthly EIR		38.16		20.76		12.27		13.28		7.02		1,93
Estimated EIR, June to Dec. 2021 in central sites (214 days) ib/p/7months		**267.09**		**145.34**		**85.90**		**92.94**		**49.11**		**13.53**

Overall EIR per night and month of *An. gambiae* s.l. from rural sites												
Months	**Gaoua rural (unsprayed)**		**Kampti Rural (Actellic 300CS)**		**Nouna Rural (unsprayed)**		**Solenzo Rural (Fludora® Fusion WP-SB)**		**Seguenega Rural (unsprayed)**		**Kongoussi Rural (unsprayed)**	
	Mean EIR per night	Mean EIR per month	Mean EIR per night	Mean EIR per month	Mean EIR per night	Mean EIR per month	Mean EIR per night	Mean EIR per month	Mean EIR per night	Mean EIR per month	Mean EIR per night	Mean EIR per month
June	0	0	0.32	9.6	0	0	0.07	2.1	0	0	0.003	0.09
July	2.26	70.06	7.6	235.6	0.04	1.24	0.65	20.15	0	0	0.067	2.077
August	0	0	1.49	46.19	0.64	19.84	0	0	0.2	6.2	0.32	9.92
September	6.66	199.8	3.69	110.7	3.37	101.1	7.18	215.4	0.01	0.3	0.15	4.5
October	1	31	0.74	22.94	0	0	0.45	13.95	0.09	2.79	0.19	5.89
November	0	0	0.09	2.7	0	0	0.12	3.6	0	0	0	0
December	0.21	6.51	0	0	0	0	0.07	2.17	0	0	0.09	2.79
Mean Monthly EIR		43.91		61.10		17.45		36.77		1.33		3.61
Estimated EIR, June to Dec. 2021 in rural sites (214 days) ib/p/7months		**307.37**		**427.73**		**122.18**		**257.37**		**9.29**		**25.27**

Annex 5. Monthly entomological inoculation rate of *An. gambiae* s.l. collected through indoor and outdoor HLC collections from June to December 2021 in PBO-ITNs sites

EIR per month of *An. gambiae* s.l.		
Months	**Karangasso-Vigué**	**Soumousso**
June	14.38	47.89
July	3.17	16.08
August	0	0
September	74.09	18.22
October	18.17	5.77
November	2.81	1.5
December	0	0.42
Mean Monthly EIR	16.09	12.84
Overall EIR (indoor + outdoor) for 7 months	112	90

Annex 6. Distribution and frequency of the L1014F and L1014S knockdown resistance (kdr) alleles of *An. gambiae* s.l. from PMI and NMCP sites in Burkina Faso in 2021.

Species	Sites	N	Genotypes 1014L 1014L	1014L 1014F	1014F 1014F	f(L1014F)	p(HW)	1014L 1014L	Genotypes 1014L 1014S	1014S 1014S	f(1014S)	(HW)
An. arabiensis	Kampti	9	1	8	0	**0.444**	0.0549	9	0	0	**0.000**	-
	Gaoua	15	3	12	0	**0.400**	0.0270	15	0	0	**0.000**	-
	Solenzo	14	5	2	7	**0.571**	0.0098	14	0	0	**0.000**	-
	Nouna	10	4	5	1	**0.350**	1.0000	10	0	0	**0.000**	-
	Kongoussi	13	6	5	2	**0.346**	0.5772	13	0	0	**0.000**	-
	Seguenega	12	2	6	4	**0.583**	1.0000	12	0	0	**0.000**	-
	Orodara	1	1	0	0	**0.000**	-	1	0	0	**0.000**	-
	Soumousso	27	12	15	0	**0.278**	0.1405	27	0	0	**0.000**	-
	Ouagadougou	32	16	10	6	**0.344**	0.1119	32	0	0	**0.000**	-
	Boromo	47	19	10	18	**0.489**	0.0000	47	0	0	**0.000**	-
	Kaya	12	7	4	1	**0.250**	1.0000	12	0	0	**0.000**	-
An. Coluzzii	Kampti	1	0	1	0	**0.500**	-	1	0	0	**0.000**	-
	Gaoua	21	1	5	0	**0.417**	0.3954	6	0	0	**0.000**	-
	Solenzo	21	4	6	11	**0.667**	0.1401	20	1	0	**0.024**	-
	Nouna	33	11	12	10	**0.485**	0.1599	32	1	0	**0.015**	-
	Kongoussi	25	14	7	4	**0.300**	0.1429	25	0	0	**0.000**	-
	Seguenega	11	5	5	1	**0.318**	1.0000	11	0	0	**0.000**	-
	Orodara	3	1	1	1	**0.500**	1.0000	3	0	0	**0.000**	-
	Soumousso	6	3	2	1	**0.333**	1.0000	6	0	0	**0.000**	-
	Ouagadougou	2	1	0	1	**0.500**	0.3338	2	0	0	**0.000**	-
	Boromo	2	0	1	1	**0.750**	-	1	1	0	**0.250**	-
	Kaya	13	5	8	0	**0.308**	0.2430	13	0	0	**0.000**	-
An. gambiae	Kampti	40	0	33	7	**0.588**	0.0000	39	1	0	**0.013**	-
	Gaoua	29	1	24	4	**0.552**	0.0006	27	2	0	**0.034**	1.0000
	Solenzo	15	5	2	8	**0.600**	0.0066	14	1	0	**0.033**	-
	Nouna	7	2	2	3	**0.571**	0.4398	7	0	0	**0.000**	-
	Kongoussi	12	4	6	2	**0.417**	1.0000	11	1	0	**0.042**	-
	Seguenega	27	8	11	8	**0.500**	0.4393	24	3	0	**0.056**	1.0000
	Orodara	46	0	40	6	**0.565**	0.0000	46	0	0	**0.000**	-
	Soumousso	17	2	13	2	**0.500**	0.0622	17	0	0	**0.000**	-
	Ouagadougou	16	1	0	15	**0.938**	0.0337	12	4	0	**0.125**	1.0000
	Boromo	1	0	0	1	**1.000**	-	1	0	0	**0.000**	-
	Kaya	25	3	22	0	**0.440**	0.0001	25	0	0	**0.000**	-

N: number of mosquitoes; f(1014F): frequency of the 1014F resistant *kdr* allele; f(1014S): frequency of the 1014S resistant *kdr* allele; p(HW): probability of the exact test for goodness of fit to Hardy-Weinberg equilibrium; '-': not determined

Annex 7. Allelic and genotypic frequencies at the ace-1 locus in *An. gambiae* s.l. populations from PMI and NMCP sites in Burkina Faso in 2021.

			Genotypes				
			119G	119G	119S		
Species	Sites	N	119G	119S	119S	f(119S)	p(HW)
An. arabiensis	**Kampti**	19	19	0	0	**0.000**	-
	Gaoua	8	8	0	0	**0.000**	-
	Solenzo	2	2	0	0	**0.000**	-
	Nouna	3	3	0	0	**0.000**	-
	Kongoussi	0	-	-	-	**-**	-
	Seguenega	0	-	-	-	**-**	-
	Orodara	1	1	0	0	**0.000**	-
	Soumousso	21	21	0	0	**0.000**	-
	Ouagadougou	16	16	0	0	**0.000**	-
	Boromo	16	16	0	0	**0.000**	-
	Kaya	12	12	0	0	**0.000**	-
An. coluzzii	**Kampti**	-	-	-	-	**-**	-
	Gaoua	2	2	0	0	**0.000**	-
	Solenzo	39	39	0	0	**0.000**	-
	Nouna	37	37	0	0	**0.000**	-
	Kongoussi	22	21	1	0	**0.023**	-
	Seguenega	9	9	0	0	**0.000**	-
	Orodara	1	1	0	0	**0.000**	-
	Soumousso	4	4	0	0	**0.000**	-
	Ouagadougou	1	1	0	0	**0.000**	-
	Boromo	3	3	0	0	**0.000**	-
	Kaya	13	13	0	0	**0.000**	-
An. gambiae s.s	**Kampti**	31	26	5	0	**0.081**	1.0000
	Gaoua	40	37	3	0	**0.037**	1.0000
	Solenzo	9	9	0	0	**0.000**	-
	Nouna	10	10	0	0	**0.000**	-
	Kongoussi	28	24	4	0	**0.071**	1.0000
	Seguenega	41	41	0	0	**0.000**	-
	Orodara	48	48	0	0	**0.000**	-
	Soumousso	25	23	2	0	**0.040**	1.0000
	Ouagadougou	33	33	0	0	**0.000**	-
	Boromo	31	31	0	0	**0.000**	-
	Kaya	25	25	0	0	**0.000**	-

N: number of mosquitoes; f (119S): frequency of the 119S resistant *ace1* allele; p(HW): probability of the exact test for goodness of fit to Hardy-Weinberg equilibrium; '-': not determined