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THE PMI VECTORLINK BURKINA FASO ANNUAL ENTOMOLOGICAL MONITORING REPORT

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Contents

List of Figures

List of Tables

Acronyms

ANOVA	Analysis of Variance
CS	Capsule Suspension
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assays
HBR	Human Biting Rate
HLC	Human Landing Catch
IR	Infection Rate
IRS	Indoor Residual Spraying
IRSS	Institut de Recherche en Sciences de la Santé (Health Sciences Research Institute)
ITN	Insecticidal Treated Net
Kdr	Knockdown Resistance
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
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, the U.S. President's Malaria Initiative (PMI) VectorLink conducted spray operations from May to June (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), clothianidin and deltamethrin combination (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. In 2022, IRS was not conducted at any sites. Therefore, data presented in this report results from one-year post-IRS for Kampti and Solenzo compared to their unsprayed control districts.

To monitor changes in entomological drivers of transmission, one year post IRS and three years post ITN distribution, monthly entomological surveillance using pyrethrum spray catches (PSC) and human landing catches (HLC) were conducted in two former sprayed sites (Solenzo and Kampti), with two paired unsprayed sites (Nouna and Gaoua) and two non-sprayed sites (Karangasso-Vigué and Soumousso) where piperonyl butoxide (PBO) ITNs were distributed. Insecticide susceptibility tests were conducted in 18 sites to monitor the spread of resistance in vector populations from both PMI and National Malaria Control Program (NMCP) sentinel sites when it was safe enough for the team to collect larvae. Susceptibility tests using *An. gambiae* s.l. were conducted with pyrethroid insecticides and the PBO synergist according to World Health Organization (WHO) protocols. CDC bottle bioassays were also conducted to determine susceptibility to chlorfenapyr and clothianidin insecticides. In addition, WHO tube tests were carried out to determine susceptibility to pirimiphos-methyl.

An. gambiae was the predominant malaria vector species in the Southwest (Gaoua, Kampti, Soumousso, Karangasso-Vigué), while *An. coluzzii* was more frequent in the West-Central (Nouna and Solenzo) sites. *An. arabiensis* is increasingly being detected in the West and Southwest regions where it was formerly absent. The implication of this vector on the transmission of malaria should be investigated further in terms of biting behavior (indoors/outdoors) and its *Plasmodium* infection and insecticide resistance status.

The peak of biting activities and the high density of resting mosquitoes were observed in September in all sites. The density of *An. gambiae* s.l. collected from PSC was comparable between former IRS sites and their paired unsprayed sites, both peaking greatly in September.

Parity rates in 2022 were similar to the spray season one year after spraying but only increased in the former sprayed site of Solenzo by 16%. There was a slight reduction in the proportion of parous *An. gambiae* s.l. in former sprayed sites compared to unsprayed sites especially in Kampti where parity was reduced by almost a quarter when compared to Gaoua. These results indicate that IRS may have a slight impact on the longevity of malaria vectors and when stopped, its impact may not be sustained through the next unsprayed season. Overall, sporozoite infection rate (IR) was extremely high in Southwestern sites compared to Northern sites. Even though the IR seems to not vary significantly between former IRS sites and their unsprayed sites, the entomological inoculation rate (EIR) was increasing in the former sprayed sites except in Kampti where outdoor EIR was much lower.

The EIR was higher in the former sprayed site of Solenzo compared to Nouna both indoors and outdoors. In the two sites where PBO ITNs were distributed, EIR was not different from the previous year 2021. These results are worrying as it could change the malaria transmission patterns and jeopardize good results gathered during the three-year period of vector control monitoring. Urgent action is required in the context of high burden high impact (HBHI) by sustaining IRS campaigns (based on the rotations scheme with new insecticides) or putting in place a local district vector control plan. Nevertheless, the surveillance of entomological drivers of malaria transmissions and the vector resistance monitoring can contribute valuably to better implement effective vector control strategy based on the data gathered from project's studies of post IRS surveys.

The insecticide susceptibility tests revealed that resistance to chlorfenapyr and clothianidin is spreading quickly in many sites and could reduce IRS efficacy of these active ingredients; therefore, further investigations on the susceptibility status of the local mosquito populations are recommended. In addition, insecticides resistance data suggest the need to update the Burkina Faso National malaria control insecticides resistance management plan to inform policy.

I. INTRODUCTION

The World Health Organization (WHO) reported 247 million malaria cases and 619,000 deaths worldwide in 2021 (WHO, 2022). The number of malaria cases in Burkina Faso accounts for 3.3% of all malaria cases globally in 2021 (WHO, 2022). In Burkina Faso, malaria is endemic and remains a major public health concern. In 2021, the National Malaria Control Program (NMCP) recorded approximately 12.2 million confirmed cases of malaria and 4,355 deaths reported by health facilities (NMCP report 2021; WHO 2022). *Plasmodium falciparum* is the primary malaria parasite transmitted primarily in Burkina Faso by *Anopheles gambiae* s.l. and *Anopheles funestus* and *An. nili* in some areas (Dabiré *et al.*, 2007; Dabiré *et al.*, 2012; Hien *et al.*, 2017; Soma *et al.*, 2021; WHO, 2022). The use of ITNs remained the main tool adopted for malaria vector control in Burkina Faso. However, pyrethroid resistance within malaria vector populations has escalated across Africa and can jeopardize 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 spread quickly in Burkina Faso (Diabate *et al.*, 2004; Dabiré *et al.*, 2012; Toé *et al.*, 2015; Hien *et al.*, 2021) and broadly in West Africa; in combination with metabolic resistance 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 PMI funding, IRS has been included as a priority vector control strategy and has been implemented in targeted high burden districts annually by PMI VectorLink Burkina Faso from 2018 - 2021.

PMI VectorLink conducted spray operations in Burkina Faso in 2018 in the district of Kampti, Solenzo and Kongoussi using pirimiphos-methyl CS and clothianidin; in 2019 in Kampti, Solenzo and Kongoussi using in alternance pirimiphos-methyl, clothianidin and deltamethrin/clothianidin combination; in 2020 in Kampti and Solenzo using clothianidin and deltamethrin/clothianidin combination; and more recently in 2021 in Kampti and Solenzo using pirimiphos-methyl CS, clothianidin, and deltamethrin/clothianidin combination following a rotation scheme. Indoor residual spraying was not implemented in 2022 due to resource constraints.

Entomological monitoring was conducted annually in six sites (Kampti, Gaoua, Solenzo, Nouna, Kongoussi and Seguenega) from 2017 to 2021 and in four sites (Kampti, Gaoua, Solenzo and Nouna) in 2022. Kongoussi and Seguenega were removed in 2022 due to security reasons.

In addition to IRS, a mass distribution campaign of 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, was conducted in 2019.

1.2 KEY GOALS AND OBJECTIVES

The PMI VectorLink project, through the Institut de Recherche en Sciences de la Santé (IRSS)/ Health Sciences Research Institute, conducted entomological surveys to monitor vector bionomics and insecticide resistance monitoring during the period of high malaria transmission (June to December 2022), one-year post-IRS and two years post ITN distribution. The specific objectives of the program were to:

- Collect detailed information on mosquito densities, seasonal variations, biting rates, biting times, indoor resting densities, parity, and infection rates of malaria vectors.
- 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)) and pirimiphos-methyl 0.25 percent.
- Determine pyrethroids (permethrin, deltamethrin and alphacypermethrin) resistance intensity using the WHO tube protocol.
- Determine the susceptibility level of the main malaria vectors, *An. gambiae* s.l., to clothianidin and chlorfenapyr.
- Conduct laboratory analysis of mosquito samples to determine vector species composition, blood-meal source, and *P. falciparum* sporozoite infection rates to estimate the entomological inoculation rates (EIR) in both former IRS sites and their adjacent unsprayed control sites.
- Determine the presence of molecular markers of resistance (*kdr-w*, *kdr-e* and *Ace-1*) and their relative allele frequencies.
- Provide data, recommendations, and technical assistance for the development of the NMCP national resistance monitoring plan.

2. METHODOLOGY

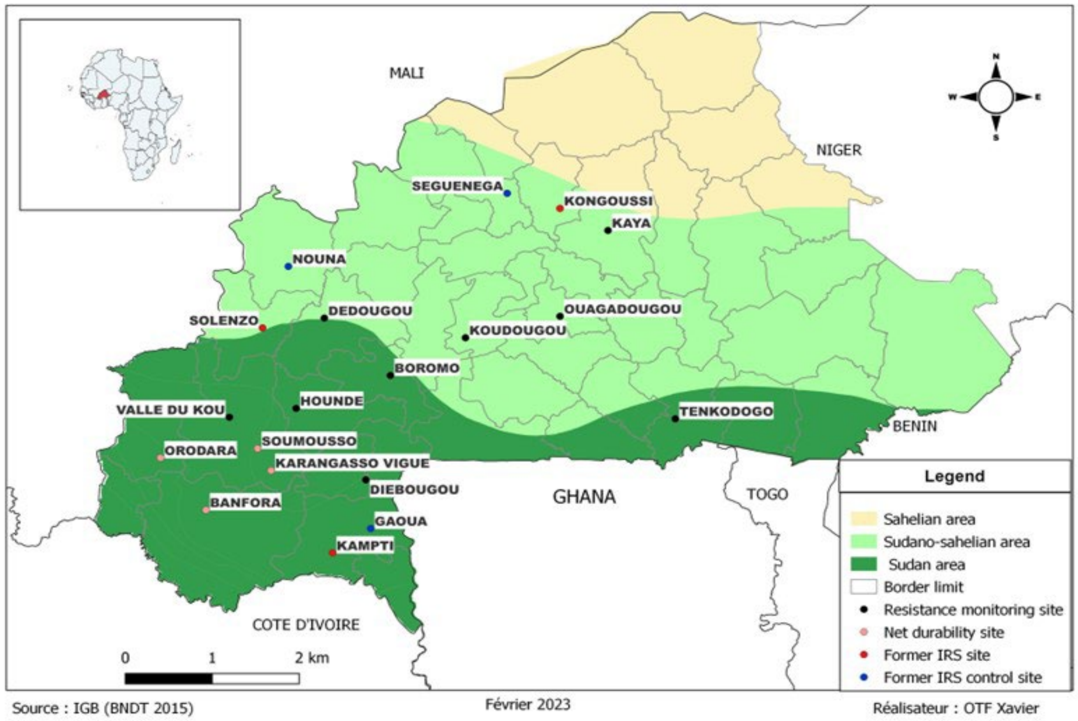
2.1 STUDY AREA

Monthly longitudinal entomological surveys were carried out during the high transmission season from June to December 2022 in six sites: two former IRS sites (Solenzo and Kampti), two paired unsprayed control sites located approximately 50km away (Nouna and Gaoua) and two unsprayed sites where PBO ITNs were distributed in 2019 (Soumousso and Karangasso-Vigué). These sites are located across the two ecological zones of Burkina Faso: Sudan (West) and Sudan-Sahelian (Centre West) ecological zones (Figure 1). The Sudan zone (Gaoua, Kampti, Soumousso and Karangasso-Vigué) is characterized by an average annual rainfalls of 1219-1273 mm in 2021 with heavy rains occurring from May to November (INSD, 2022). In the central Sudan-Sahelian zone (Solenzo, Nouna and Bena), the average annual rainfall is 826 mm in 2021, with a rainy season from June to September (INSD, 2022). Therefore, during the rainy season, conditions are suitable for *An. gambiae* s.l. proliferation. In addition, insecticide resistance monitoring sites were dispersed out in the country in order to be more representative and include all former IRS sites (IRS and their control unsprayed sites), as well as PBO ITNs sites (Figure 1).

Monthly mosquito collections were conducted to estimate entomological parameters of malaria transmission. *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 collections by HLC and PSC were conducted in two sub-locations within each site, in a more urban central site and a rural site.

Due to insecurity in the Northern and Eastern parts of the country, larval collections for insecticide monitoring was not conducted in the Solenzo site as generally done the previous years.

Figure 1. Study Sites for Monthly Longitudinal Trapping by HLC and PSC (Former IRS Sites, Unsprayed Control Sites) and Sites for Resistance Monitoring



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 (VL SOP02/01). 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 Coetzee, 2020 for *Anopheles* species.

2.3 Pyrethrum Spray Catch (PSC)

A total of 20 houses were selected per sub-location (central and rural) with a total of 40 houses surveyed per site per month. The selected houses were visited in the morning between 06:00 and 09:00 am, and white sheets were laid on the floor and over furniture. Pyrethrum spray catches were conducted using 0.64 percent Pyrethrum EC aerosol insecticide (VL SOP03/01). 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 key of Coetzee, 2020. A random sample of 200 unfed *An. gambiae* s.l. per district (100 per site) per month collected from HLC were dissected to estimate the parity rate. (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 Laboratory Analyses

2.5.1 *Plasmodium falciparum* Infection Rate

All mosquitoes dissected for parity status in the field were stored in a laboratory freezer at -20°C and subsequently processed by Circumsporozoite Protein- enzyme-linked immunosorbent assay (ELISA-CSP) to determine infection rates with *P. falciparum*. The head and thorax of female *An. gambiae* s.l. specimens were used in analyses based on the protocol described in the Malaria Research and Reference Reagent Resource Centre (MR4, 2014). All samples tested for sporozoite infection rates were also identified to species level by Polymerase Chain Reaction (PCR) (Santolamazza *et al.*, 2008).

2.5.2 Origin of Blood Meal Source (Anthropophily Rate)

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

2.5.3 Molecular Identification of *An. gambiae* Complex and Characterization of Resistance Mutations (kdr L1014F/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) protocol. Species of *An. gambiae* s.l. were identified and characterized, as described by Santolamazza *et al.* (2008). Detection of knock down 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 Vgsc- 1014F West Africa *kdr*-mutation (*kdr-w*) and Vgsc-1014S East Africa *kdr*-mutation (*kdr-e*) respectively and of Weill *et al.* (2004) for the ace-1^R G119S mutation.

2.5.4 Insecticide Susceptibility Tests

An. gambiae s.l. larvae were collected from different larval habitats from 18 localities (Nouna was replaced by Dedougou for security reasons), brought to the IRSS insectary and reared to adults prior to l. The WHO tube tests (SOP06/01) were conducted to monitor insecticide susceptibility, pyrethroid resistance intensity and PBO synergists Pyrethroid resistance intensity was monitored for alpha-cypermethrin, deltamethrin and permethrin at 5x and 10x the diagnostic concentration using the WHO tube protocol. A new CDC bottle bioassay protocol was used for testing clothianidin susceptibility, and bottle bioassays were conducted with chlorfenapyr. 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 4µg/bottle
- Chlorfenapyr 100µg/bottle

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 (WHO, 2022b, 2022a).

2.6 DATA ANALYSIS

The DHIS2-based VectorLink Collect program for entomological data management has been used in Burkina Faso since 2020. The VectorLink Home Office team remotely trained and supported the IRSS entomologists and database managers on updated data workflows, including field paper collections, technical reviews, data entry, data cleaning, and analytics. This process helped to support the generation of data and assure the use of high-quality entomological data. All entomological data collected in Burkina Faso in 2022 was managed within the VectorLink Collect database. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance results. Stakeholders, including NMCP, IRSS and PMI, will have free 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 was calculated as the proportion of mosquitoes tested positive for *P. falciparum* in the head or thorax using ELISA-CSP. The 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, year). An analysis of variance (ANOVA) was performed to compare the entomological estimates (HBR, IR) between sites. Data analysis was performed using R software, version 4.2.1. For all analyses, the team used a Wilcoxon test or Chi-square test to compare the means of the independent parameters. 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 2022, a total of 15,490 *Anopheles* mosquitoes were collected in all sites: 12,263 mosquitoes by HLC and 3,227 by PSC following the same trend compared to 2021 during the spray period. *An. gambiae* s.l. (10,873; 88.7 percent) was the most abundant species collected through HLC, followed by *An. nili* with (1,063; 8.7percent), *An. funestus (*115*;* 0.9 percent), *An. pharoensis* (93; 0.8 percent), *An. rufipes* (75; 0.6 percent), *An. coustani* (42; 0.3 percent) and *An. flavicosta (*2; 0.02 percent) (Figure 2A). The *An. gambiae* s.l. (Figure 2B) also represented the most abundant species from the PSC collection with 3,196 (99.0 percent). The other species, including *An. nili, An. funestus* and *An. rufipes*, represented less than 1% (Figure 2B). The highest *Anopheles* species diversity was found in the South West region (Gaoua and Kampti) with seven species collected, whereas in the West (Soumousso and Karangasso-Vigue districts) and the Centre West (Solenzo and Nouna districts), five species were collected.

Figure 2. *Anopheles* Species Composition Based on A) HLC and B) PSC for All Sites Combined

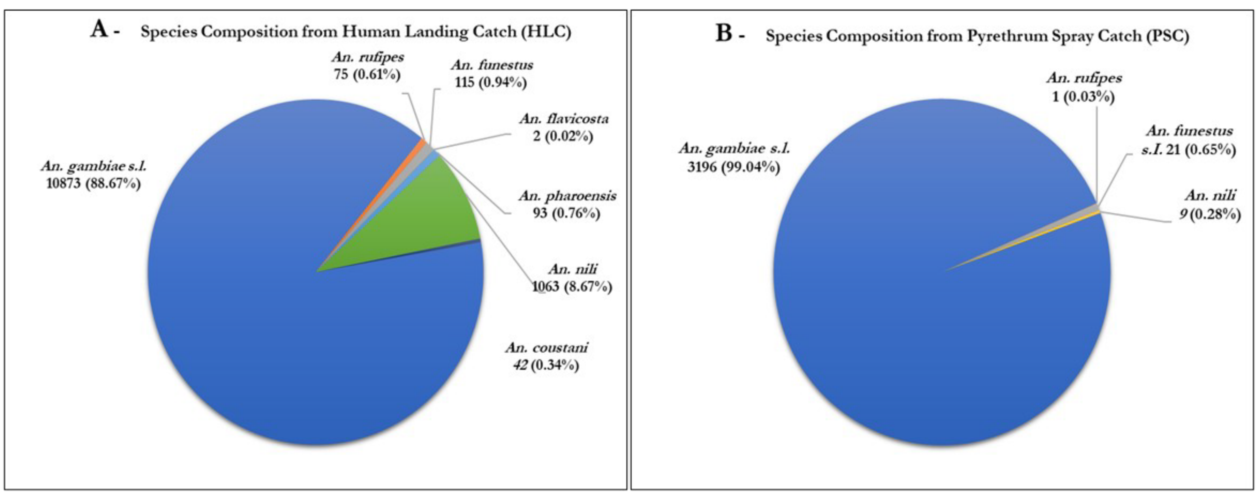
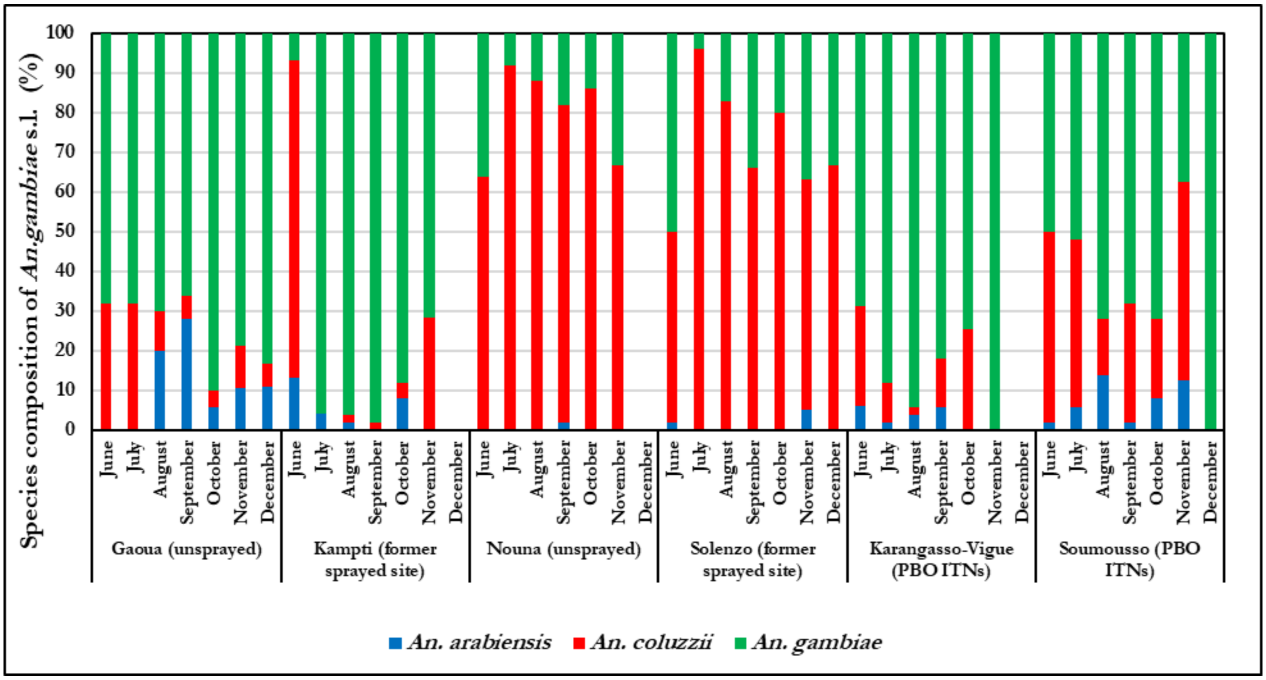


Figure 3 shows the species composition of the *An. gambiae* complex identified by PCR for all sites from June to December 2022. *An. coluzzii* was the predominant species in Nouna and Solenzo across all months except in June in Solenzo. In contrast, *An. gambiae* was predominant in Gaoua and Kampti with some intrusion of *An. coluzzii* in June and July in Gaoua and in November in Kampti. Of all species, *An. arabiensis* was most frequent in Gaoua and Kampti with proportions varying ranging between 2% and 28%. In Karangasso-Vigué (PBO ITNs sites), *An. gambiae* was the predominant species (68.7%-100%) regardless of the collection month. *An. coluzzii* was collected from June to October, reaching 25%. In Soumousso, the second PBO ITNs site, *An. gambiae* was predominating and *An. coluzzii* was well represented each month in varying proportions from 10-

50%, except in December where only *An. gambiae* was reported. In Soumousso, *An. arabiensis* was observed progressively especially in August and November with 14% and 12.5% respectively. It was formerly absent in this area few decades ago (Dabiré et al., 2007). Overall, *An. gambiae* was predominating in the West and Southwest sites in association with *An. coluzzii* in relatively high proportions in Soumousso. It is surprising but interesting to mention that *An. arabiensis* was progressively colonizing the West and Southwest regions where it was formerly absent/rare in the northern regions.

Figure 3. 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 4 shows dynamics of indoor and outdoor mean *An. gambiae* s.l. biting rate in former IRS sites and their paired unsprayed control sites one year after spraying. In general, biting rates showed similar trends both indoors and outdoors. At the beginning of the rainy season in June, the biting rates were 7.3 bites/person/night (b/p/n) and 3.6 b/p/n, respectively in Gaoua and Kampti in the Southwest region. In contrast, they were recorded at rates lower than 1 b/p/n in Nouna and Solenzo. When the rainy season progressed, the biting rates increased accordingly through the months, peaking up to > 17 b/p/n in September (indoors and outdoors) at all sites. This increase in density was consistent with the proliferation of mosquito breeding sites due to the rainfalls and could be explained by the development of human activities (development of vegetable gardening, etc.), which contributed to mosquito population abundance. One year post IRS, comparison of unsprayed sites with their paired former sprayed sites showed that average biting rates were similar in Southwest regions (Gaoua versus

Kampti; Wilcox test, $W = 5$, p= 0.45) and Northwest region (Nouna versus Solenzo; W=13, p= 0.33). This could partly be explained by the reduced residual efficacy of insecticide treated walls when spraying stopped and was particularly visible in rural areas with a significant number of malaria vectors. In the previous year (2021), the average biting rates were higher in Gaoua (unsprayed site) compared to Kampti (sprayed with Actellic 300CS) (X^2=7.01, p= 0.008), and these biting rates were higher in Solenzo (sprayed with Fludora Fusion WP-SB) compared to Nouna (unsprayed site) in all months (X^2=4.14, p= 0.04).

The comparison of the average biting rates (HBR) indoors showed a significant decrease in Solenzo in 2022 (7.2 b/p/n) compared to 2021 with 17.19 b/p/n (X^2=14.08, p= 0.0002). This could be attributed to the widespread use of personal protective equipment such as repellents and also by ITNs. However, the HBR increased in Kampti in 2022 (11.7 b/p/n) compared to 2021 (7 b/p/n) (X^2=2.79, p= 0.09). No difference was observed in Gaoua (X^2=0.35, p= 0.54) and Nouna (X^2=0.83, p= 0.36) from 2021 to 2022.

Outdoor HBR was significantly lower in Gaoua in 2022 (9.71 b/p/n) compared to 2021 (21 b/p/n) (X^2=11.95, p= 0.0005). However, there was a slight increase in Kampti in 2022 (9.9 b/p/n) compared to 2021 (7.69 b/p/n) (X^2=0.27, p= 0.59). In 2021 in Nouna, the HBR was 6.32 b/p/n compared to 6.77 b/p/n in 2022 (X^2=0.10, p= 0.74). In Solenzo, there was a decrease in 2022 (5.94 b/p/n) compared to 2021 (22.28 b/p/n); this decrease was significant (X^2=13.05, p= 0.0003). The average biting rates indoor and outdoor are presented in Annex 1. In summary, in 2022, the HBR increased in Kampti (former spray site) and decreased in Solenzo (former spray site) both indoors and outdoors, compared to 2021. This highlights the need to continuing monitoring vector densities post IRS intervention to determine changes and anticipate possible impact y in malaria transmission.

Figure 5 summarizes the *An. gambiae* s.l. biting rate collected in the two sites where nets have been distributed since 2019. For indoor collections, two peaks of biting were observed, including one in July (with 7 and 22 b/p/n in Karangasso and Soumousso respectively) and another one in September (with 26.6 and 32.5 b/p/n in Soumousso and Karangasso-Vigué, respectively) (Figure 5A). The first peak could be explained by the beginning of the rainy season in July while the second one in September could be attributed to breeding proliferation. However, for outdoor collections, both sites showed the same trends with a peak in September (Figure 5B). No difference was observed between Soumousso and Karangasso-Vigué (W= 25, p = 0.11).

Overall, low densities were reported in November and December at all sites, which corresponded to the end of the rainy season.

The average biting rates indoors and outdoors are presented in Annex 1. When comparing the HBR indoor in 2021 versus 2022, no difference was observed in Soumousso (2021: 7.82 *vs* 2022= 10.9 b/p/n; p=0.36) and Karangasso-Vigué (2021: 7.28 *vs* 2022= 6.11 b/p/n; p=0.62). However, outdoor HBR varied significantly between 2021 and 2022 both in Soumousso (X^2= 6.70, p= 0.009) and Karangasso-Vigué (X^2=6.11, p= 0.01) primarily due to the rains in 2022 and indoor ITN use.

Figure 4. 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 2022

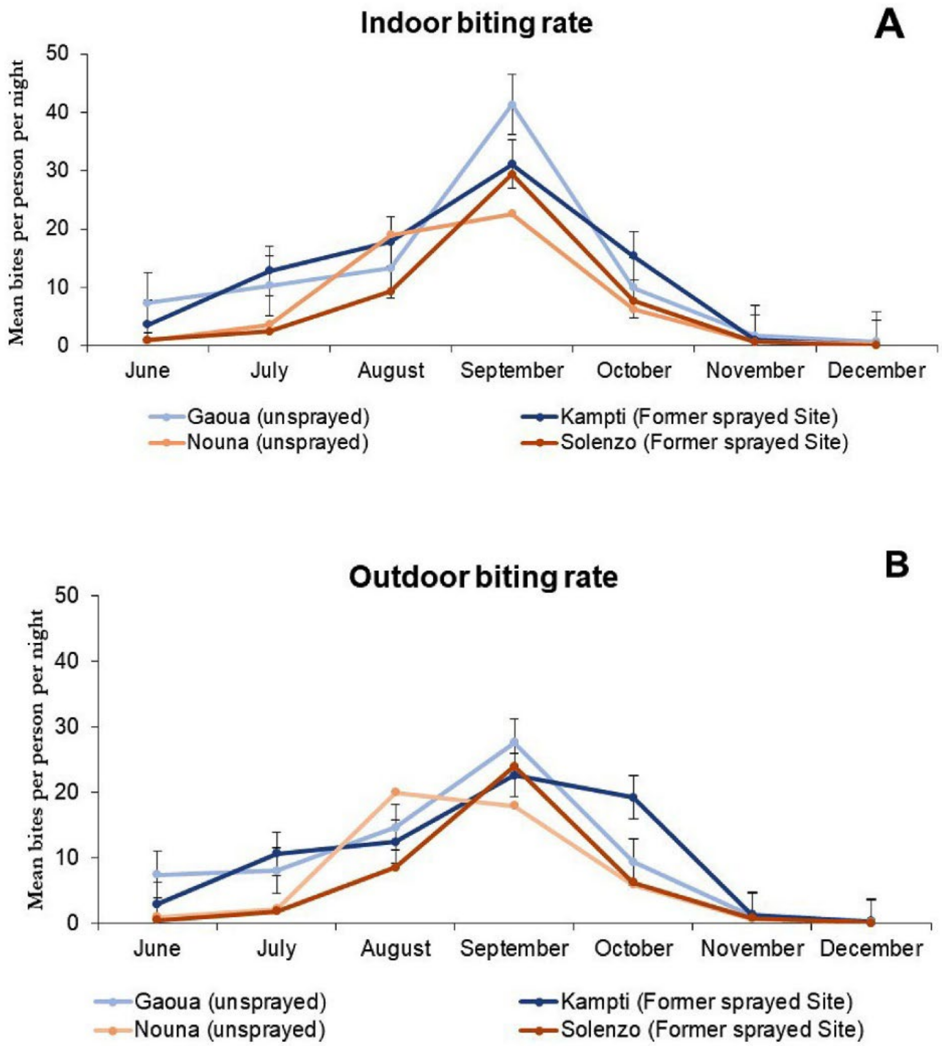
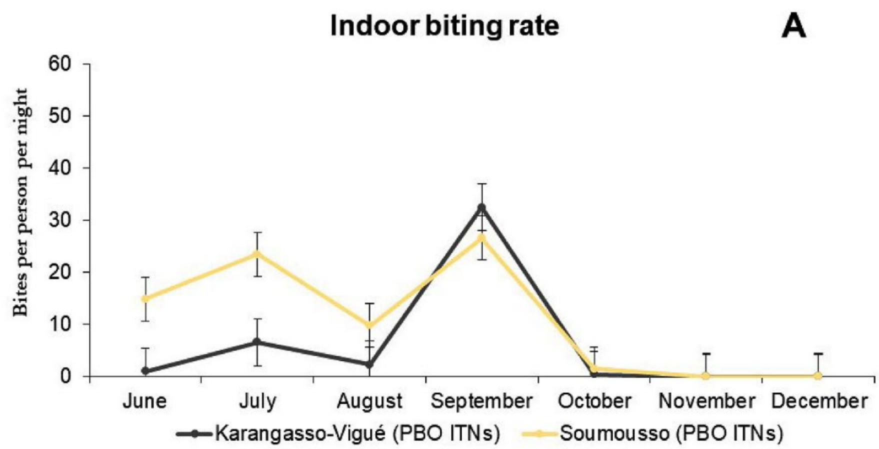
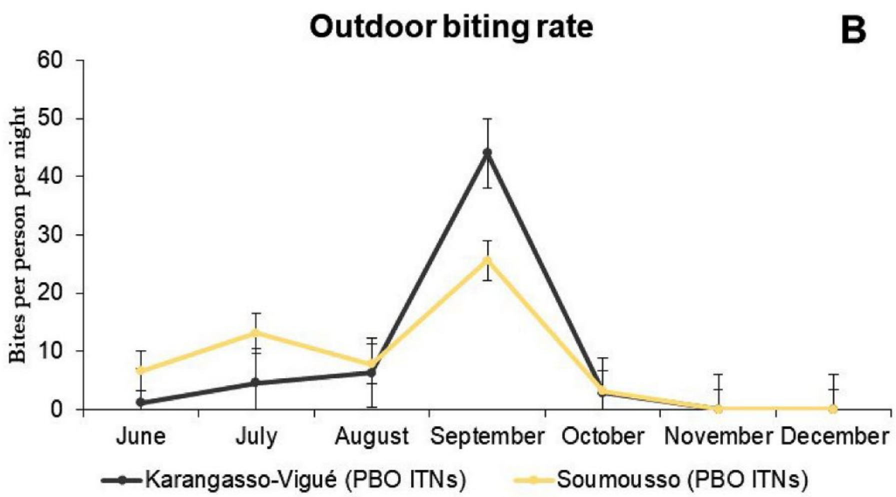


Figure 5. Mean *An. gambiae* s.l. Bites per Person per Night from Indoor (A) and Outdoor (B) HLC Collections in PBO ITN Sites from June to December 2022

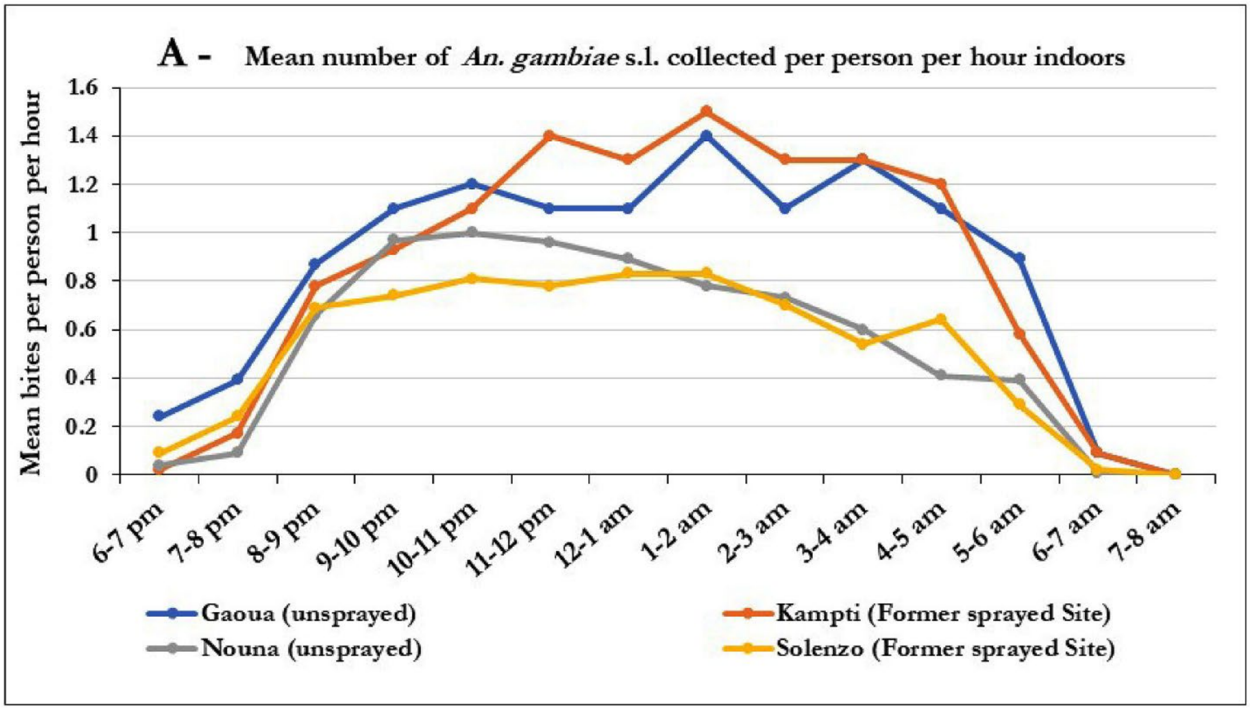




3.3 Biting Times of *An. gambiae* s.l.

Figure 6 shows the mean hourly indoor and outdoor biting times of *An. gambiae* s.l. in former IRS and unsprayed sites. The biting activities were sustained from 6pm to 7am both indoors and outdoors (Figure 6). The hourly biting cycles were marked with peaks before 3 am in all sites. In Nouna and Solenzo, biting rhythm was relatively low and decreased quickly toward 5am (Figure 6A). The outdoors biting activity followed the same trend (Figure 6B). In the two PBO ITN sites, biting started after 8pm indoors (Figure 7A) in Karangasso and Soumousso, where the team observed the same biting trends from 9pm to 6am with 1.5 b/p/n (Figure 7B).

Figure 6. Mean Number of Bites of *An. gambiae* s.l. per Hour from June to December 2022 (Cumulated Data from Central and Rural Sites): A) Indoor and B) Outdoor HLC Collections



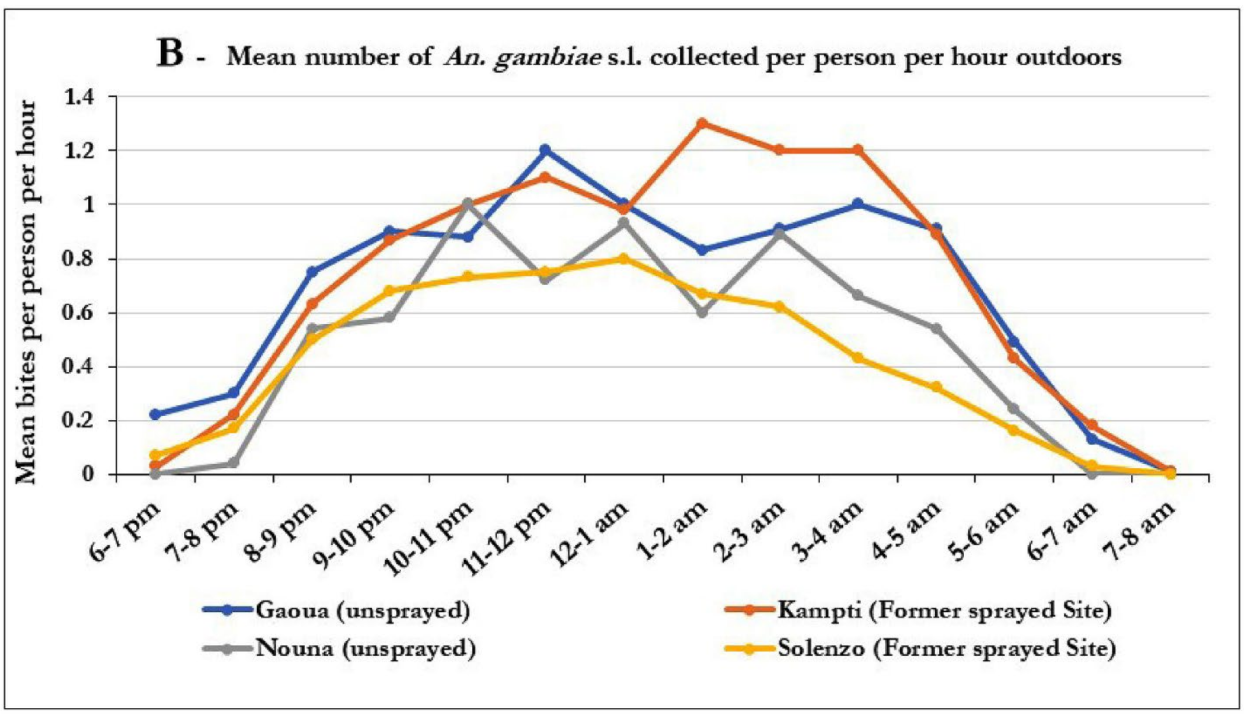
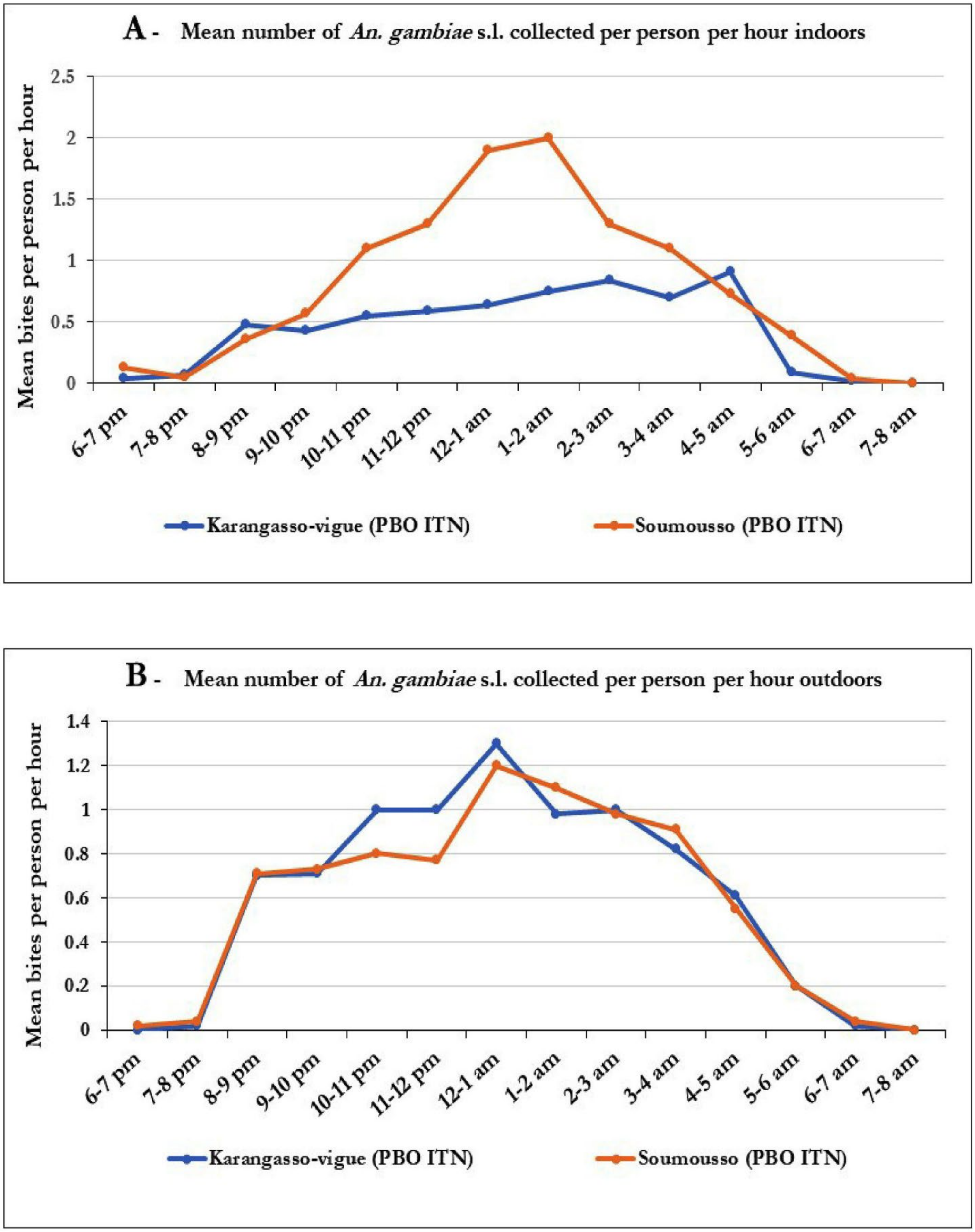


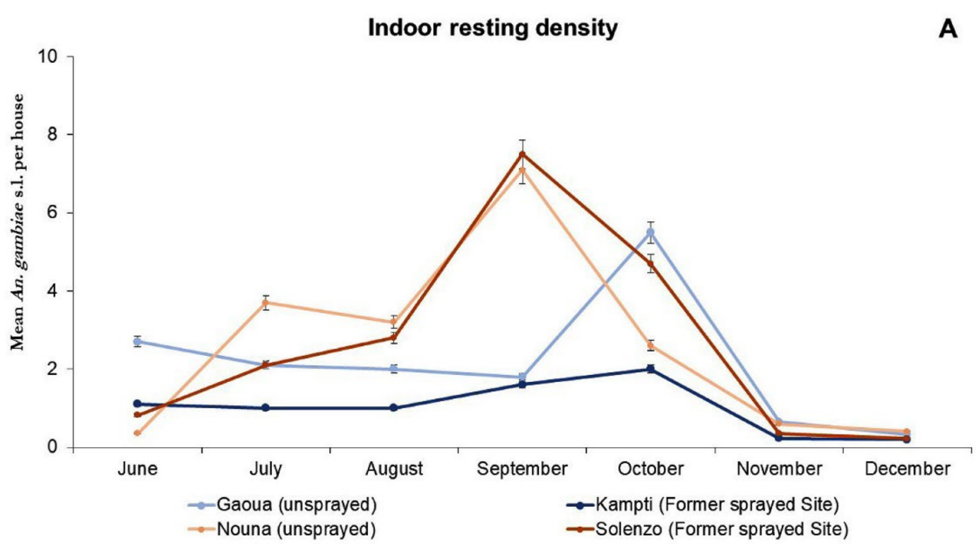
Figure 7. Mean Number of *An. gambiae* s.l. Bites per Hour from A) Indoor and B) Outdoor HLC Collections from June to December 2022 in Soumousso and Karangasso-Vigue

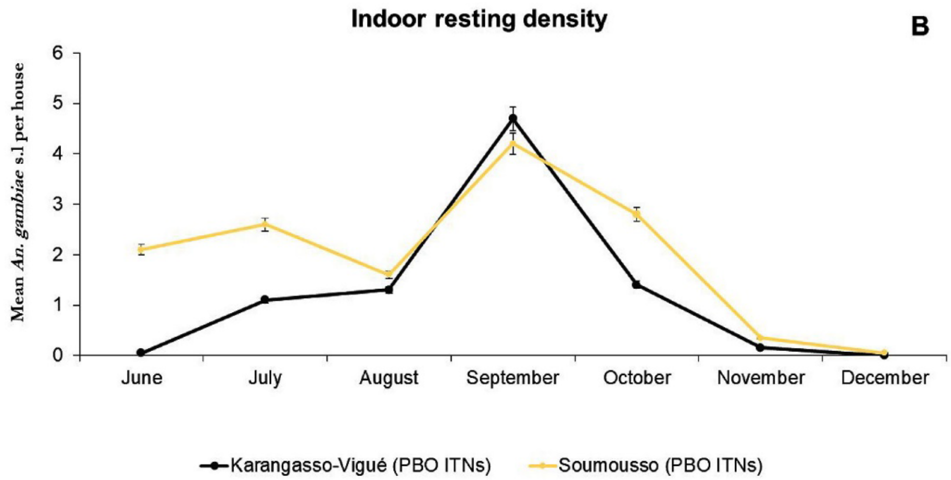


3.4 Indoor Resting Densities by PSC

The density of *An. gambiae* s.l. collected from PSC varied according to the month and the collection site (Figures 8A&B). The highest densities were observed in October in Gaoua with 5.5 *An. gambiae* s.l. per house per day (/h/d) and 2 *An. gambiae* s.l./h/d in Kampti (Figure 8A). In the previous year, the highest densities were observed during the early rainy season in June. The mean densities of *An. gambiae* s.l. remained slightly lower in the previously sprayed site of Kampti (1.01 *An. gambiae* s.l./h/d) compared to its control site in Gaoua (2.1 *An. gambiae* s.l./h/d) (W= 28, p= 0.007). In the Centre West region, highest densities were observed in September in Nouna (7.1 *An. gambiae* s.l /h/d) and Solenzo (7.5 *An. gambiae* s.l /h/d); however, the mean resting densities in Solenzo (2.5 *An. gambiae* s.l /h/d) were comparable to its control site of Nouna (2.6 *An. gambiae* s.l./h/d) (*W*=3, p = 0.43) (Figure 8A). Comparing the data from last year, the densities in Solenzo (sprayed site) were higher (34 *An. gambiae*/h/d) than in the paired unsprayed site of Nouna (14.3 *An. gambiae* s.l /h/d). Also, in the previous year, except in Kampti district (sprayed with Actellic 300CS), the comparison of unsprayed sites with their paired sprayed sites had showed that mean resting density per house did not differ significantly (Tukey's p > 0.12). Similarly, the resting densities recorded in September in Soumousso (4.2 *An. gambiae* s.l /h/d) did not differ significantly (*W*=20, p=0.05) from those observed in Karangasso-Vigué (4.7 *An. gambiae* s.l /h/d) (Figure 8B). The lowest densities were reported in December at all sites (Figures 8 A&B).

Figure 8. 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 2022 per site. Overall, more than 50% of females dissected were parous regardless of the site. The parity rates increased in Solenzo in 2022 compared to 2021 by 16% one year post-IRS but did not differ significantly in Solenzo (67.65%) and Nouna (67.96%) ($X^2 = 0.10$, $p = 0.91$). In contrast, in Kampti (sprayed site), the parity rate was still significantly lower (56.92%) compared to the control site of Gaoua (71.49%) ($X^2 = 8.10$, $p = 0.005$) during the 2021 spray year. No clear differences were reported for the PBO ITN-distributed sites of Soumousso (64.74%) and Karangasso-Vigué (53.82%) (X^2=0.06, $p = 0.92$).

Table 1. Parity Rate of *An. gambiae* s.l. Females Collected from Sprayed Sites and Unsprayed Sites between June and December 2022

Site	Total Dissected	Parous	Non-Parous	ParityRate (%)
Gaoua (unsprayed	677	484	193	71.49
Kampti (former sprayed site)	852	485	367	56.92*
Nouna (unsprayed)	437	297	140	67.96
Solenzo (former sprayed site)	606	410	196	67.65
Karangasso-Vigué (PBO ITNs)	301	162	139	53.82
Soumousso (PBO ITNs)	156	101	55	64.74

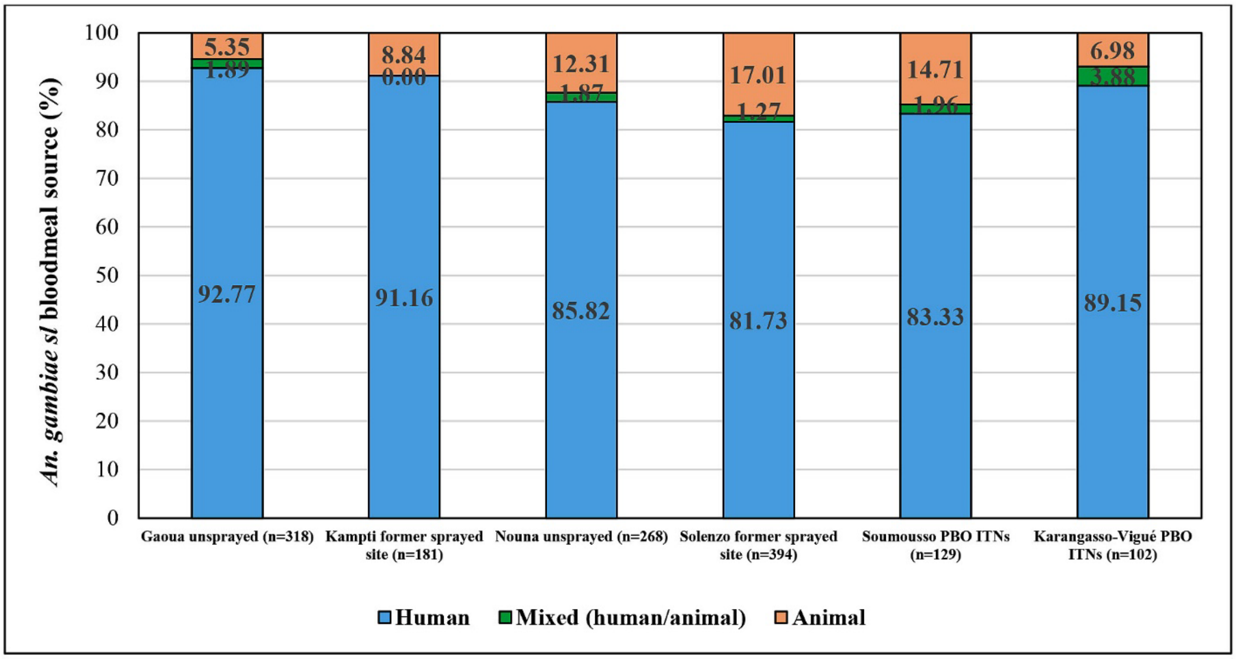
*Statistically different from the comparison site

3.6 *Anopheles gambiae* s.l. Blood-Meal Source

An. gambiae s.l. were extremely anthropophilic in most of the study sites without any differences observed between sprayed and control sites (Figure 9). The proportion of exclusive zoophagic *An. gambiae* was less than

10%, regardless of the site. However, the trends to the zoophily (animal blood meals, > 12%) were more marked in Solenzo, Nouna and Soumousso compared to other sites (Gaoua, Kampti and Karangasso-Vigue < 9%), probably due to the abundance of livestock in these sites. Mixed human and animal blood meals were found in all sites, except in Kampti site but less than 5% overall.

Figure 9. Blood-Meal Source of *An. gambiae* s.l. Collected by PSC Between June and December 2022



3.7 *P. falciparum* Infection Rates in *Anopheles gambiae* s.l.

Irrespective of the vector control intervention, the *P. falciparum* infection rates (IR) of *An. gambiae* s.l. in Burkina Faso remained higher in the Western and Southwestern parts, as reported in previous reports. In Gaoua, 10.03% of *An. gambaie* sl tested were infected with *P. falciparum* with no significant difference compared to Kampti (13.27%) (X^2=1,01, p= 0.30). This was also the case between Solenzo (former IRS site) and Nouna (unsprayedwith mean IR of 5.39% and 2.86% respectively) (X^2=0.78, p= 0.37). Overall, the mean infection rates did not differ significantly between sprayed and unsprayed sites (Tukey's p> 0.05) one-year post-IRS. These findings could be explained by cumulative data (central+rural) from rural sites where the IR and HBR had been reported to be higher than that of the central sites. The previous annual report showed that when rural and central data are disaggregated, the high impact of IRS was mostly observed in central sites (Mean IR: 18.02% in Gaoua versus 14.6% in Kampti; 12.75% in Nouna versus 7.97% in Solenzo). Annex 2 shows an increase in infection rates in rural sites compared to the central sites. Furthermore, the overall infection rates did not differ significantly between the two PBO ITNs sites of Soumousso and Karangasso (X^2=0.73, p= 0.39).

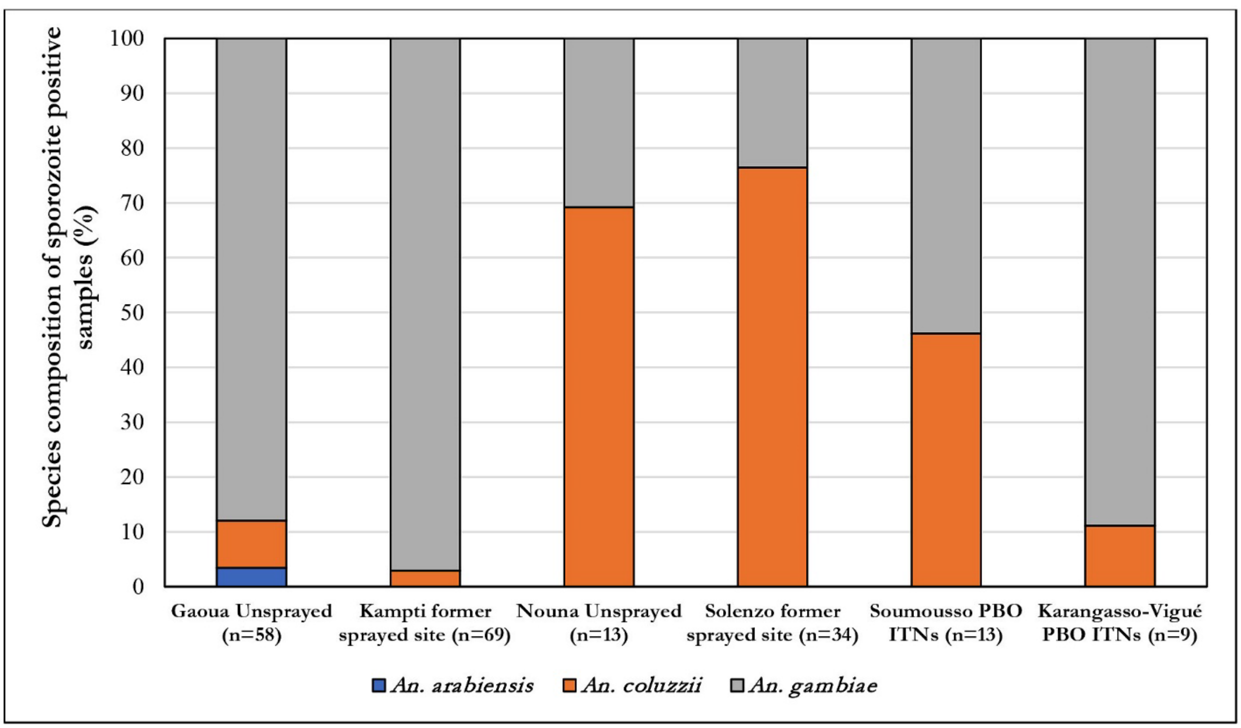
Table 2. *Plasmodium falciparum* Infection Rate Recorded from Indoor and Outdoor HLC-Collected *An. gambiae* s.l. per Site from June to December 2022

Site	Infection Rate Indoors	Infection Rate Outdoors	Total Positive
Gaoua (unsprayed)	**8.85** (30/339)	**11.76** (28/238)	**10.03** (58/578)
Kampti (former sprayed site)	**11.49** (33/287)	**15.45** (36/233)	**13.27** (69/520)
Nouna (unsprayed)	**2.44** (7/287)	**3.57** (6/168)	**2.86** (13/455)
Solenzo (former sprayed site)	**7.03** (31/441)	**1.59** (3/189)	**5.39** (34/630)
Soumousso (PBO ITNs)	**5.59** (9/161)	**4.04** (4/99)	**5** (13/260)
Karangasso-Vigué (PBO ITNs)	**5.15** (5/97)	**3.57** (4/112)	**4.31** (9/209)
Overall Mean	**7.13** (115/1612)	**7.79** (81/1039)	**7.39** (196/2651)

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

Figure 10 summarizes the *Anopheles* species proportion that were infected with *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 predominant malaria vector species in Solenzo (76.5%) and Nouna (69%). Low proportions of infected *An. arabiensis* were detected in Gaoua

Figure 10. Species Composition of *P. falciparum* Infected Female *An. gambiae* s.l. Collected from June to December 2022 per Site



3.8 Entomological Inoculation Rate

The entomological inoculation rate was calculated for the period of June through December 2022 (Table 3). The EIR was higher in the Southwest and Western regions. It was significantly higher in Kampti (former IRS site) with 161 infectious bites per person (ib/p) over the 120 collection nights indoors (central and rural sites combined), compared to Gaoua (unsprayed) which had 126 infected bites per person over the same nights (X^2 =4.27 p=0.03). However, the opposite was observed outdoors, where EIR was significantly reduced in Kampti to 268 ib/p over the same collection period, while it was recorded at 445 ib/p for the control site of Gaoua. Nevertheless, the Kampti outdoor EIRs were higher than the outdoor ones. The EIR was still higher in Solenzo (former sprayed site) (Table 3) compared to Nouna (the unsprayed control area) with 86 versus 20 ib/p for Nouna (X^2=41.09, $p < 0.0001$) indoors following the same trend outdoors with 238 versus 30 i/b/p (X^2=161.43, $p < 0.000001$) (Table 3). In the two PBO ITN sites, the EIR increased even more in Soumousso but did not differ from that of Karangasso indoors (X^2=1.88, p=0.16) in the previous year. In contrast, the EIR was significantly higher in Soumousso with 58 i/b/p compared to Karangasso with 36 ib/p (X^2=5.15, p=0.02) over the 56-night collections (Table 3). In summary, the EIR was significantly higher in Kampti indoors compared to Gaoua, contrary to the 2021 EIR which was significantly lower outdoors. In Solenzo, there was up to four-fold infective bites indoors (86 ib/p versus 20 ib/p) and more than eight-fold infective bites outdoors (248 ib/p versus 30 ib/p) compared to Nouna. Regardless of the site (former sprayed or control), the outdoor transmissions were exceptionally high in Gaoua and surprisingly high in Solenzo. This could be explained by the use of the mass ITNs distributed between 2019 and 2020 at least in Gaoua by reducing exposure and contact with people sleeping under ITNs deployed indoors. In Solenzo, the rotation or the alternance of new insecticides for IRS reduced the indoor biting whereas the exceptional rainfall grew up mosquitoes' density that increasing outdoor transmission.

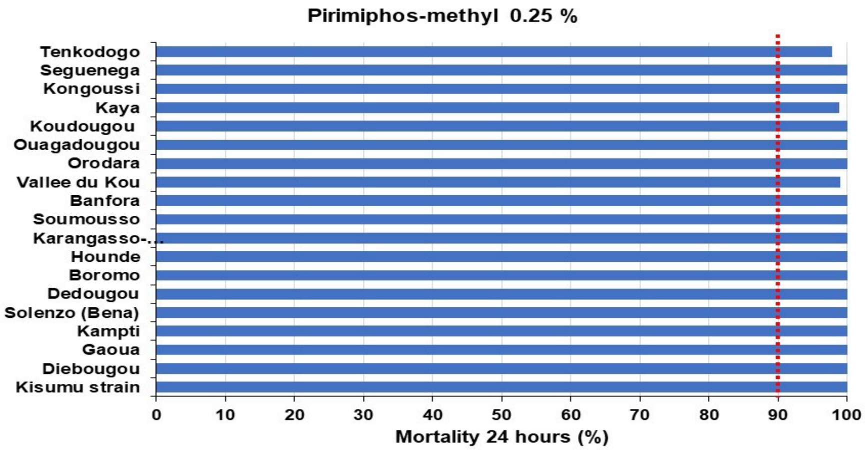
Table 3. Entomological Inoculation Rate (June to December 2022) from Combined Central and Rural Data

	Gaoua (unsprayed)	Kampti (former sprayed site)	Nouna (unsprayed)	Solenzo (former sprayed site)	Karangasso-Vigué (PBO ITNs)	Soumousso (PBO ITNs)
Total *An. gambiae* s.l. collected in indoor (HLC)	1,433	1,415	849	1,207	342	472
HLC trap-nights	120	120	112	168	56	56
HBR per night	11.94	11.79	7.58	7.18	6.11	8.43
Total *An. gambiae* s.l. tested by PCR (HLC)	339	287	287	441	97	161
Number *An. gambiae* sl infected	30	33	7	31	5	9
Sporozoite rate (%)	8.8	11.4	2.44	7.03	5.15	5.59
EIR per night	1.05	1.34	0.18	0.51	0.31	0.47
EIR indoor June to December 2022 (trap- nights) (Calculated using mean sporozoite rate)	**126**	**161**	**20**	**86**	**17**	**26**
Total *An. gambiae* s.l. collected in outdoor (HLC)	1,152	1,191	757	998	469	550
HLC trap-nights	120	120	112	168	56	56
HBR per night	9.6	9.92	6.76	5.94	8.37	9.82
Total *An. gambiae* s.l. tested by PCR (HLC)	238	233	455	630	209	260
Number *An. gambiae* sl infected	28	36	13	13	9	13
Sporozoite rate (%)	10.03	13.27	2.86	5.39	4.31	5
EIR per night	3.71	2.23	0.27	1.42	0.64	1.03
EIR outdoor June to December 2022 (trap-nights) (Calculated using mean sporozoite rate)	**445**	**268**	**30**	**238**	**36**	**58**

3.9 Insecticide Susceptibility Data

The susceptibility tests results with 0.25 percent pirimiphos-methyl in WHO tubes against wild *An. gambiae* s.l. are presented in Figure 11. In all-monitoring sites, *An. gambiae* s.l. populations were susceptible to pirimiphos-methyl with mortalities of 98.9-100 percent, except in Tenkodogo site, where *An. gambiae* s.l. populations exhibited a possible resistance to pirimiphos-methyl with a mortality of 97.8% that warrant further confirmation.

Figure 11. Results of Susceptibility Tests with 0.25 Percent Pirimiphos-Methyl in WHO Tube Assays Against Wild *An. gambiae* s.l. Collected as Larvae from 18 Sites.



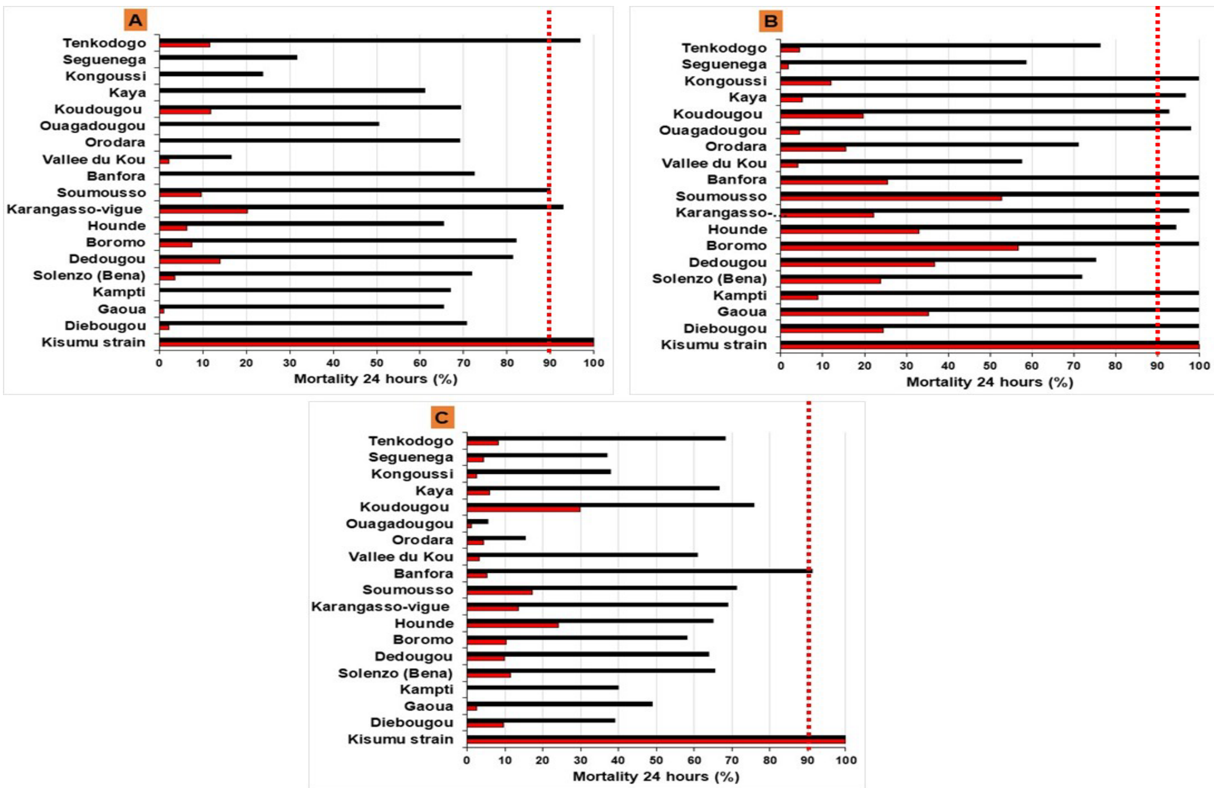
Note: Red dashed line represents 90% mortality threshold.

The susceptibility tests results 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 18 sites (Figure 12).

Pre-exposure to PBO followed by exposure to pyrethroid insecticides resulted in increased mortality rates compared to pyrethroids alone. The mortality rates were lower than 90 % in almost all sites (15/18 sites) after pre-exposure to PBO 4% + alpha-cypermethrin 0.05% (Figure 12A). However, mortality between 90 – 97% was reported in three sites, including two PBO ITNs sites (Soumousso and Karangasso-vigue) (Figure 12A). In the other monitoring sites, the mortality rate varied between 50 and 82% (Figure 12A).
In contrast, the effect of PBO was greater with deltamethrin 0.05% exposure. The results showed mortality rates > 50% in six sites: Vallée du Kou, Seguenega, Orodara, Dedougou, Solenzo and Tenkodogo (Figure 12B). In Koudougou, Hounde, Kaya and Karangasso-vigue, mortality rates ranged between 92 - 97.7%, reaching 98 - 100% in the other sites with (Figure 12B). When considering PBO 4% + permethrin 0.75%, the mortality was <50% in Ouagadougou, Orodara, Seguenega, Kongoussi, Diebougou, Kampti and Garoua (Figure 12C). The highest mortality rates were observed in Banfora (91.3%), Koudougou (75.9%), Soumousso (71.3%) and Karangasso-vigue (70%) (Figure 12C).

Overall, pre-exposure to PBO followed by alpha-cypermethrin and/or permethrin resulted only in increased mortality rates > 60% in most sites. However, the effect of the PBO was more significant with deltamethrin resulting in increased mortality of up to 90% in most sites. These findings indicate that metabolic resistance (presence of oxidases) was involved in the resistance phenotypic observed. In addition, it was noted that the mortality rate (< 90%) pre-exposure to PBO did not restore susceptibility in many sites to alpha-cypermethrin and permethrin, except in Tenkodogo and Karangasso-Vigue (figures 12A and 12C). Therefore, it could be assumed that ITNs using deltamethrin + PBO could be more suitable to control pyrethroid-resistant vector populations compared to pyrethroids ITNs alone. The results shows that deltamethrin +PBO ITN are likely to be more suitable in Burkina Faso compared to ITN with alphacypermethrin + PBO or Permethrin +PBO. These results suggest that metabolic and knock down resistance mechanisms are sustaining the resistance patterns in Burkina Faso; hence indicating that more research activities in genomic are crucial to better address this situation.

Figure 12. 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%

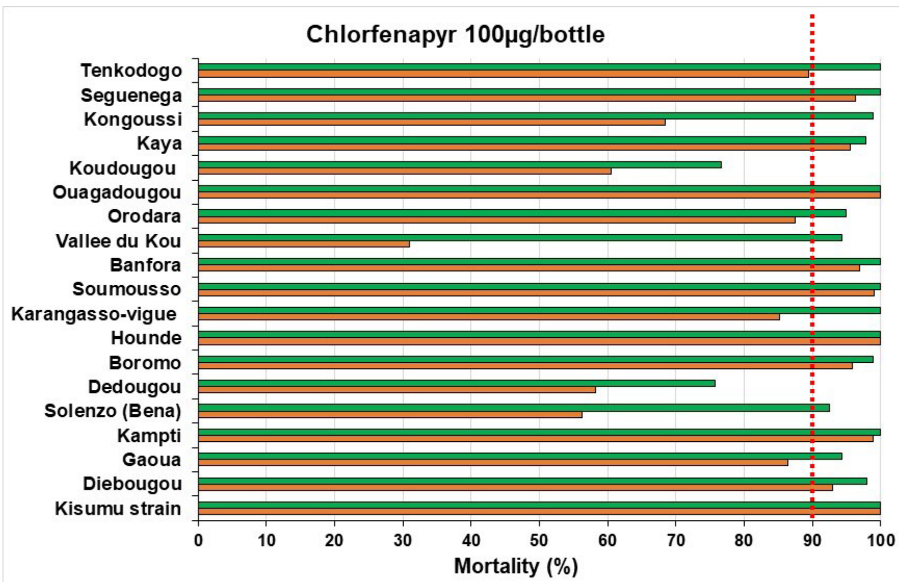


Note: Insecticide only (red bars); PBO 4% + insecticide (black bar); Red dashed line represents 90% mortality threshold.

Figure 13 shows the results of susceptibility assays performed on *An. gambiae* s.l. from 18 sites against chlorfenapyr, using the diagnostic dose of 100 µg/bottle. The mortality rates of *An. gambiae* s.l. exposed to its diagnostic dose after 24 hours reached 98% in four out of the 18 sites tested: Kampti, Soumousso, Houndé and Ouagadougou. After 72h post exposure, the *An. gambiae* s.l. populations from Dedougou (75.8% mortality) and Koudougou (76.7% mortality) showed resistance to chlorfenapyr. Further tests should be conducted to confirm these results. Suspected resistance was recorded in Solenzo, Vallée du Kou, Gaoua, Orodara and Kaya with mortality ranging from 92.5% to 97.8 %. However, in the other sites (13 out of 18), susceptibility to chlorfenapyr with 98-100% mortality was clearly reported (Figure 13). With regards to these results, ITNs containing chlorfenapyr + pyrethroids could be valuable in most sites in Burkina Faso. However, further tests should be conducted to ascertain the mechanisms of resistance against chlorfenapyr.

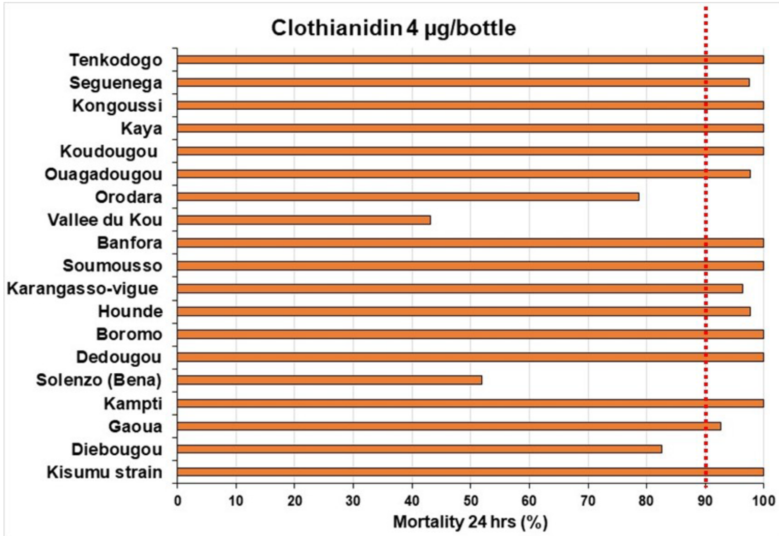
Bioassays showed resistance to clothianidin 4µg/bottle in four sites: Solenzo, Vallée du Kou, Orodara and Diébougou with a mortality rate < 90 % 24h post exposure (Figure 14). Possible resistance was reported in the localities of Gaoua, Karangasso vigue, Seguenega, Hounde and Ouagadougou with mortality ranging between 92%-97.7% (Figure 14). *Anopheles* populations showed susceptibility to clothianidin with 100% mortality in other sites (Figure 14). These results highlight the necessity for early monitoring of clothianidin resistance in order to alert policy makers to better scale up the resistance management plan.

Figure 13. Results of Susceptibility Tests with Chlorfenapyr 100µg/Bottle in CDC Bottle Bioassays Against *An. gambiae* s.l. in 18 Sites.



Note: Mortality at 24h (orange bars) and mortality at 72h (green bar); Red dashed line represents 90% mortality threshold.

Figure 14. Results of Susceptibility Tests with Clothianidin 4 ug/Bottle in CDC Bottle Bioassays Against *An. gambiae* s.l. in 18 Sites.



Note: Red dashed line represents 90% mortality threshold.

3.10 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 diagnostic doses showed high resistance intensity in all sites (Table 4). The WHO intensity tests with deltamethrin revealed high resistance intensity in all sites, except in Diebougou, Gaoua and Banfora where moderate resistance status was observed (Table 5). Results were more variable for permethrin, with high resistance intensity in 11 sites and moderate in seven sites (Table 6).

Table 4. 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
	% Mortality 5x (0.25%)	% Mortality 10x (0.5%)	
Kisumu strain	100	NA	Susceptible
Diebougou	35.2	65.2	High
Gaoua	35.3	58.1	High
Kampti	27.9	52.4	High
Solenzo (Bena)	25.9	51.3	High
Dedougou	42	53.1	High
Boromo	65	84.4	High
Hounde	32.3	63.6	High
Karangasso-vigue	51.5	70.8	High
Soumousso	64.8	82.5	High
Banfora	67.4	88.4	High
Vallee du Kou	7.1	13	High
Orodara	9.5	41.6	High
Ouagadougou	44.9	64.4	High
Koudougou	53.2	89.7	High
Kaya	5.6	27.5	High
Kongoussi	10.1	20.8	High
Seguenega	38.3	55.8	High
Tenkodogo	75.3	93.1	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 5. 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
	% Mortality 5x (0.25%)	% Mortality 10x (0.5%)	
Kisumu strain	100	NA	Susceptible
Diebougou	89.1	100	Moderate
Gaoua	96.9	98.9	Moderate
Kampti	81.1	96	High
Solenzo (Bena)	37	80	High
Dedougou	43	57.5	High
Boromo	80	86.7	High
Hounde	44.6	52.9	High
Karangasso-vigue	43.8	68.9	High
Soumousso	71	86.3	High
Banfora	93.9	100	Moderate
Vallee du Kou	33.7	75	High
Orodara	36.8	79.5	High
Ouagadougou	83.1	96.5	High

Sites	Deltamethrin Diagnostic Concentration (%)		Status
	% Mortality 5x (0.25%)	% Mortality 10x (0.5%)	
Koudougou	60.9	83.3	High
Kaya	97.9	96.9	High
Kongoussi	34.4	67	High
Seguenega	47.1	62.5	High
Tenkodogo	71.9	89.7	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 Permethrin in WHO Bioassays

Sites	Permethrin Diagnostic Concentration (%)		Status
	% Mortality 5x (3.75%)	% Mortality 10x (7.5%)	
Kisumu strain	100	NA	Susceptible
Diebougou	85.1	97.6	High
Gaoua	84	100	Moderate
Kampti	73.5	100	Moderate
Solenzo (Bena)	61.7	87.7	High
Dedougou	54.3	70.7	High
Boromo	48.3	87.5	High
Hounde	53.7	88.9	High
Karangasso-vigue	80.8	96.5	High
Soumousso	82.8	100	Moderate
Banfora	97.7	100	Moderate
Vallee du Kou	86.7	98.9	Moderate
Orodara	48.8	97.8	High
Ouagadougou	36	91.8	High
Koudougou	54.3	69.9	High
Kaya	75.8	100	Moderate
Kongoussi	83.2	95.7	High
Seguenega	89	98.9	Moderate
Tenkodogo	64.9	94.7	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

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 variations (Annex 4) in *An. gambiae* populations. These frequencies were moderate in the Western (Soumousso, Karangasso vigue, Banfora and Orodara), Southwestern (Gaoua and Kampti), Northwestern (Dedougou, Solenzo and Boromo), Centre (Ouagadougou) and Northern (Kaya, Seguenega and Kongoussi) sites, with frequencies between 0.25 and 1. In the Central East region in Tenkodogo, the allele frequency of kdr-L1014F mutation was > 0.35 (Annex 3). The kdr-L1014S mutation was found in the three species of *An. gambiae s.l* populations in low frequency (< 0.20) (Annex 4). 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 not reported in *An. gambiae* s.l. collections (Annex 5). However, this mutation was reported both in *An. gambiae* and *An. coluzzii* populations at lowest frequencies in Northern (Kongoussi) and South Western (Kampti, Gaoua), and Western sites (Soumousso) in the previous years. On the other hand, the kdr-L1014S mutation has not been reported in *An. arabiensis*.

4. CONCLUSION

This report presented data collected one-year post-IRS in former sprayed sites (Kampti and Solenzo) and their paired unsprayed sites (Gaoua and Nouna). The entomological surveys were also extended to PBO-ITN sites located in the western part of Burkina Faso near Bobo-Dioulasso namely Kanrangasso-vigue and Soumousso. The data showed that the mean biting rates did not differ significantly in Kampti compared to Gaoua even though they were slightly higher in some months in Gaoua. The same trend was observed between Nouna and Solenzo. When broken down to sub-locations, biting rates from rural sites seem higher compared to central sites as shown in the previous report. 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, indicating that the IRS treated walls were still efficacious although those in Solenzo did not differ from Nouna (unsprayed control site).

There was no apparent reduction in parity rates of *An. gambiae* s.l., except in Kampti when compared to Gaoua. The parity rate in Nouna was similar to Solenzo (sprayed). These results were comparable to the 2021 results. Overall, the sporozoite rates were higher in the Southwest (Gaoua, Kampti, and Solenzo) compared to those in Soumousso, Karangasso-Vigué and Nouna.

When comparing Kampti, a former IRS site to its control site of Gaoua, it appeared that no difference was observed between the two, suggesting that transmission increased when the effect of IRS reduced; whereas overall, it was clearly reduced during the spray month. Surprisingly in Solenzo, a former IRS site, the transmission was higher than its control site of Nouna.

The EIR value was high in the Southwest sites (Kampti and Gaoua) and moderate in Nouna and Solenzo. However, when comparing each post-IRS site to its control site, the cumulated EIR (indoor and outdoor) was lower in Kampti (former IRS) compared to Gaoua (control). The situation was reversed in Solenzo where the EIR was always high either indoors or outdoors compared to its control site of Nouna.

In the two PBO ITN sites, the EIR was always higher indoors in Soumousso compared to Karangasso-Vigue, reaching respectively 26 ib/p versus 17 ib/p indoors and 58 ib/p versus 36 ib/p outdoors.

Insecticide susceptibility tests revealed that *An. gambiae* s.l. were resistant to the three pyrethroids insecticides tested, but pre-exposure to PBO significantly increased mortality rate to alphacypermethrin, deltamethrin and permethrin in all sites tested. Full susceptibly was not restored with permethrin and alpha cypermethrin (except with deltamethrin in eight sites.

Susceptibility to chlorfenapyr was recorded in almost all sites except in Dedougou and Koudougou where *An. gambiae* s.l. populations were resistant to chlorfenapyr. Resistance was suspected in five sites including: Gaoua, Solenzo, Vallée du Kou, Orodara and Kaya (< 97% mortality). The rapid development of resistance to chlorfenapyr is of concern and warrant further investigation.

Resistance to clothianidin was detected in four sites (Diebougou, Solenzo, Vallée du Kou, Orodara), while suspected resistance (90-97%) was detected in five sites (Gaoua, Karangasso, Houndé, Ouagadougou and Seguenega). This is the first case of clothianidin resistance; last year, mosquitoes from all tested sites demonstrated full susceptibility.

Susceptibility to pirimiphos-methyl (PM) was recorded in most sites. though there was reduced mortality in Tenkodogo, Kaya and Vallée du Kou.

Given these results, it is crucial to implement resistance monitoring and management strategies such as introduction of rotation scheme with new and efficient molecules or combining tools (ITNs and IRS) aimed at enhancing the impact of vector control tools.

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Annex

Annex 1: Average Biting Rates

Sites	Average Biting Rates Indoors 2021	Average Biting Rates Indoors 2022	Comparison 2021 vs 2022
Gaoua	18.83	12.07	X=0.35, p=0.54
Kampti	7	11.7	X=2.79, p=0.09
Nouna	8.51	7.58	X=0.83, p=0.36
Solenzo	17.19	7.2	X=14.08, p=0.0002
Karangasso-Vigué	7.28	6.11	X=0.81, p=0.62
Soumousso	7.82	10.9	X=0.82, p=0.36

Sites	Average Biting Rates Outdoors 2021	Average Biting Rates Outdoors 2022	Comparison 2021 vs 2022
Gaoua	21	9.71	X=11.95, p=0.0005
Kampti	7.69	9.9	X=0.27, p=0.59
Nouna	6.32	6.77	X=0.10, p=0.74
Solenzo	22.28	5.94	X=13.05, p=0.0003
Karangasso-Vigué	13.16	6.11	X=6.11, p=0.01
Soumousso	6.82	10.9	X=6.70, p=0.009

Annex 2: Mean Infection Rate of *An. gambiae* s.l. Collected by HLC (Indoor + Outdoor) Collections from June to December 2022 in the Former 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 (Former Sprayed sites)** (N=233)	**Nouna Central (Unsprayed)** (N=259)	**Solenzo Central (Former Sprayed Site)** (N=166)
June	11.54 (3/26)	7.14 (1/14)	0 (0/25)	0 (0/3)
Jul	16 (8/50)	8 (4/50	4 (2/50)	0 (0/26)
August	10 (5/50)	8 (4/50)	0 (0/50)	0 (0/35)
September	2 (1/50)	8 (4/50)	2 (1/50)	0 (0/50)
October	4 (2/50)	4 (2/50)	8 (4/50)	0 (0/30)
November	0 (0/28)	7.14 (1/14)	0 (0/15)	0 (0/19)
December	0 (0/18	20 (1/5)	5.26 (1/19)	0 (0/3)
Mean infection rate % (P/N)	**6.71 (19/283)**	**7.29 (17/233)**	**3.09 (8/259)**	**0 (0/166)**

Infection Rate of *An. gambiae* s.l. from Rural Sites				
Months	**Gaoua Rural (Unsprayed)** (N=305)	**Kampti Rural (Former Sprayed Sites)** (N=287)	**Nouna Rural (Unsprayed)** (N=194)	**Solenzo Rural (Former Sprayed Site)** (N=228)
June	16 (8/50)	14 (7/50)	0 (0/12)	61.54 (24/39)
July	22 (11/50)	36 (18/50)	8 (2/25)	0 (0/100)
August	28 (14/50)	38 (19/50)	0 (0/50)	3.53 (3/85)
September	2 (1/50)	10 (5/50	0 (0/50)	4 (4/100)
October	8 (4/50)	2 (1/50)	6 (3/50)	2 (2/100)
November	2.63 (1/38)	8.33 (2/24)	0 (0/6)	3.22 (1/31)
December	0 (0/17)	0 (0/13)	0 (0/3)	0 (0/8)
Mean infection rate % (P/N)	**12.79 (39/305)**	**18.12 (52/287)**	**2.55 (5/196)**	**7.33 (34/464)**

Annex 3. Infection Rates of *An. gambiae* s.l. Females to *P. falciparum* from Indoor and Outdoor Collections in PBO-ITN Sites in 2022.

Sporozoite Rate of *An. gambiae* s.l. from Indoor and Outdoor HLC Collections		
Months	**Karangasso-Vigué (PBO ITNs)**	**Soumousso (PBO ITNs)**
June	6.25 (1/16)	10 (5/50)
July	2 (1/50)	8 (4/50)
August	4 (2/50)	2 (1/50)
September	2 (1/50)	0 (0/50)
October	7.69 (3/39)	4 (2/50)
November	25 (1/4)	12.5 (1/8)
December	0 (0/0)	0 (0/2)
Mean infection rate % (P/N)	**4.31 (9/209)**	**5 (13/260)**

Annex 4. 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 2022

			Genotypes					Genotypes				
Species	**Sites**	**N**	**1014L 1014L**	**1014L 1014F**	**1014F 1014F**	**f(L1014F)**	**p(HW)**	**1014L 1014L**	**1014L 1014S**	**1014S 1014S**	**f(1014S)**	**p(HW)**
An. arabiensis	**Kampti**	1	0	1	0	**0.5**	-	1	0	0	**0**	-
	Gaoua	38	7	27	4	**0.461**	0.0205	37	1	0	**0.013**	-
	Solenzo	1	1	0	0	**1**	-	1	0	0	**0**	-
	Dedougou	35	17	16	2	**0.286**	0.6878	32	3	0	**0.043**	1
	Kongoussi	-	-	-	-	**-**	-	-	-	-	**-**	-
	Seguenega	11	4	2	5	**0.545**	0.06	11	0	0	**0**	-
	Banfora	35	12	17	6	**0.414**	1	35	0	0	**0**	-
	Orodara	2	0	2	0	**0.5**	1	2	0	0	**0**	-
	Soumousso	17	6	11	0	**0.324**	0.1063	14	3	0	**0.088**	1
	Karangasso Vigue	21	7	14	0	**0.333**	0.047	20	1	0	**0.024**	-
	Ouagadougou	2	1	1	0	**0.25**	-	2	0	0	**0**	-
	Boromo	29	11	6	12	**0.517**	0.002	29	0	0	**0**	-
	Tenkodogo	9	4	3	2	**0.389**	0.494	9	0	0	**0**	-
	Kaya	4	1	3	0	**0.375**	1	4	0	0	**0**	-
An. coluzzii	**Kampti**	-	-	-	-	**-**	-	-	-	-	**-**	-
	Gaoua	-	-	-	-	**-**	-	-	-	-	**-**	-
	Solenzo	49	18	15	16	**0.48**	0.0107	45	4	0	**0.041**	1
	Dedougou	6	3	3	0	**0.25**	1	6	0	0	**0**	-
	Kongoussi	49	0	43	6	**0.561**	0	47	2	0	**0.02**	1
	Seguenega	12	2	9	1	**0.458**	0.2372	11	1	0	**0.042**	-
	Banfora	6	3	2	1	**0.333**	1	6	0	0	**0**	-
	Orodara	1	0	1	0	**0.5**	-	1	0	0	**0**	-
	Soumousso	5	1	3	1	**0.5**	1	5	0	0	**0**	-
	Karangasso Vigue	-	-	-	-	**-**	-	-	-	-	**-**	-
	Ouagadougou	3	1	1	1	**0.5**	1	3	0	0	**0**	-
	Boromo	3	1	2	0	**0.333**	1	3	0	0	**0**	-
	Tenkodogo	9	3	5	1	**0.389**	1	9	0	0	**0**	-
	Kaya	9	1	8	0	**0.444**	0.0564	9	0	0	**0**	-
An. gambiae	**Kampti**	47	3	38	6	**0.532**	0	45	2	0	**0.02**	1
	Gaoua	12	1	9	2	**0.542**	0.2387	11	1	0	**0.042**	-
	Solenzo	-	-	-	-	**-**	-	-	-	-	**-**	-
	Dedougou	9	3	6	0	**0.333**	0.4558	7	2	0	**0.111**	1
	Kongoussi	1	0	1	0	**0.5**	-	1	0	0	**0**	-
	Seguenega	27	4	16	7	**0.556**	0.4441	24	3	0	**0.056**	1
	Banfora	9	2	6	1	**0.444**	0.5392	6	3	0	**0.167**	1
	Orodara	47	4	40	3	**0.489**	0	41	6	0	**0.064**	1
	Soumousso	28	0	27	1	**0.518**	0	25	3	0	**0.054**	1
	Karangasso Vigue	29	1	19	9	**0.638**	0.0458	29	0	0	**0**	-
	Ouagadougou	45	17	8	20	**0.533**	0.0001	45	0	0	**0**	-
	Boromo	18	6	5	7	**0.528**	0.0706	18	0	0	**0**	-
	Tenkodogo	31	13	14	4	**0.355**	1	29	2	0	**0.032**	1
	Kaya	35	3	29	3	**0.5**	0.0002	33	2	0	**0.027**	1

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 5. Allelic and Genotypic Frequencies at the Ace-1 Locus in *An. gambiae* s.l. Populations from PMI and NMCP Sites in Burkina Faso in 2022

Species	Sites	N	Genotypes 119G 119G	119G 119S	119S 119S	f(119S)	p(HW)
An. arabiensis	**Kampti**	11	11	0	0	**0**	-
	Gaoua	8	8	0	0	**0**	-
	Solenzo	-	-	-	-	-	-
	Dedougou	40	40	0	0	**0**	-
	Kongoussi	-	-	-	-	-	-
	Seguenega	1	1	0	0	**0**	-
	Banfora	33	33	0	0	**0**	-
	Orodara	2	2	0	0	**0**	-
	Soumousso	18	18	0	0	**0**	-
	Karangasso Vigue	7	7	0	0	**0**	-
	Ouagadougou	32	32	0	0	**0**	-
	Boromo	16	16	0	0	**0**	-
	Tenkodogo	16	16	0	0	**0**	-
	Kaya	13	13	0	0	**0**	-
An. coluzzii	**Kampti**	7	7	0	0	**0**	-
	Gaoua	6	6	0	0	**0**	-
	Solenzo	46	46	0	0	**0**	-
	Dedougou	8	8	0	0	**0**	-
	Kongoussi	23	23	0	0	**0**	-
	Seguenega	40	40	0	0	**0**	-
	Banfora	4	4	0	0	**0**	-
	Orodara	2	2	0	0	**0**	-
	Soumousso	11	11	0	0	**0**	-
	Karangasso Vigue	16	16	0	0	**0**	-
	Ouagadougou	2	2	0	0	**0**	-
	Boromo	2	2	0	0	**0**	-
	Tenkodogo	10	10	0	0	**0**	-
	Kaya	16	16	0	0	**0**	-
An. gambiae	**Kampti**	32	32	0	0	**0**	-
	Gaoua	36	36	0	0	**0**	-
	Solenzo	4	4	0	0	**0**	-
	Dedougou	2	2	0	0	**0**	-
	Kongoussi	27	27	0	0	**0**	-
	Seguenega	9	9	0	0	**0**	-
	Banfora	13	13	0	0	**0**	-
	Orodara	46	46	0	0	**0**	-
	Soumousso	21	21	0	0	**0**	-
	Karangasso Vigue	27	27	0	0	**0**	-
	Ouagadougou	16	16	0	0	**0**	-
	Boromo	32	32	0	0	**0**	-
	Tenkodogo	24	24	0	0	**0**	-
	Kaya	21	21	0	0	**0**	-

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