

# Performance of pirimiphos-methyl based Indoor Residual Spraying on entomological parameters of malaria transmission in the pyrethroid resistance region of Koulikoro, Mali.

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## Research

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# Abstract

## Background:

Vector control relies heavily on Long-lasting insecticidal nets (LLINs) and Indoor Residual Spraying (IRS) in selected districts in Mali including Koulikoro district. As part of strengthening vector control strategies in the district, IRS was initiated by the National Malaria Control Program (NMCP) with the support of the US President's Malaria Initiative (PMI) since 2008. LLINs coverage was 93.3% and 98.2% for IRS in Koulikoro, the only district of the region where IRS was supported by PMI. Following mosquito vector resistance to both pyrethroid and carbamates, organophosphate (pirimiphos-methyl) was used for the IRS campaigns of 2015 and 2016 in the district of Koulikoro. In this study, we assessed the effect of IRS on malaria transmission by comparing entomological indices in of the district of Koulikoro, where IRS was implemented and its neighbored district of Banamba, where IRS had never been implemented.

## Methods:

The study was conducted in two villages of each district (Koulikoro and Banamba). Pyrethrum spray catches and entry window trapping were used to collect mosquitoes on a monthly basis. WHO tube tests were carried out to assess mosquito susceptibility to insecticides. Mosquitoes were identified to species level by PCR and their infection to *P. falciparum* was detected by ELISA.

## Results:

Of the 527 specimens identified, *An. coluzzii* was the most frequent species (95%) followed by *An. gambiae* (4%) and *An. arabiensis* (1%). Its density was rainfall dependent in the no-IRS area, and almost independent in the IRS area. The infection rate (IR) in the no-IRS area was 0.96%, while it was null in the IRS area. In the no-IRS area, the entomological inoculation rates (EIR) was 0.21 infective bites /person month with a peak in September. High resistance to pyrethroids and carbamates and susceptibility to organophosphates was observed at all sites.

## Conclusion:

The introduction of pirimiphos-methyl based IRS in the area resulted in a significant decrease in malaria transmission. *An.gambiae s.l.*, the main malaria vector of the area, was resistant to pyrethroids and carbamates, and remained susceptible to the organophosphates.

# Background

Long-lasting insecticidal nets (LLINs) and Indoor Residual Spraying (IRS) are actively promoted as the main prevention tools for malaria control and elimination [1]. The recent wide deployment of these two control tools is considered to be responsible for the substantial reduction of the incidence and deaths related to the disease in Africa [2, 3] but they are generally not sufficient to eliminate transmission [4]

Malaria affects about 40% of the population in Mali [5]. The health services registered 2,345,481 cases of malaria including 750,973 severe cases in 2018[6]. Children under 5 years are the most affected by the disease with 33.70% of cases, followed by pregnant women (4.77%). The number of recorded deaths was 1001, with a case fatality rate of 1.33 ‰[6].

The national strategy for malaria control in Mali is based on four main interventions: i) Early diagnosis and treatment by ACTs, ii.) Seasonal Malaria Chemoprevention (SMC) in children aged 3 to 59 months (SMC), iii) Preventive Intermittent Treatment (IPT) with sulfadoxine-pyrimethamin (SP) in pregnant women and, iv) Vector control. Vector control relies heavily on LLINs at a country level and IRS in selected districts. After a mass distribution of LLINs in 2014 to achieve universal coverage of all populations at risk in 2014, demographic health surveillance study reported 93.3% coverage, 90.5% usage, and 3.6 LLINs per household in the region of Koulikoro, including Koulikoro and Banamba districts [7]. As part of the strengthening of vector control strategies in the district of Koulikoro, IRS was initiated by the National Malaria Control Program (NMCP) with the support of the US President's Malaria Initiative (PMI) since 2008. The IRS coverage was 98.2% in Koulikoro, the only district of the region where the IRS was supported by the US President malaria initiative [8]. Pyrethroid insecticides (deltamethrin and lambda-cyhalothrin) were used for the IRS during the first 4 years (from 2008 to 2011)[9]. Following resistance to these insecticides, they were replaced by a carbamate insecticide (Bendiocarb) from 2012 to 2014 campaigns. In 2015, given the short residual life of Bendiocarb on mud walls, the Mali NMCP, as well as its partners, have decided to switch to pirimiphos-methyl, an organophosphate insecticide.

With the emergence and expansion of the resistance of *Anopheles gambiae sensu lato* (*An. gambiae s.l.*), to insecticides, the fragile progress made in malaria control can be compromised [10]. The wide and long term use of pyrethroid insecticides in LLINs and the agricultural sector seems to be one of the main causes of the increasing resistance of malaria vector to these products [11, 12].

So far, no study was performed in Mali to assess the IRS effect on malaria transmission expressed by the entomological inoculation rate in a context of vector resistance to insecticides. Therefore, the objective of this study was to assess the effect of IRS on malaria transmission by comparing entomological indices in two localities of the district of Koulikoro, where IRS was implemented for about 9 consecutive years, to two other similar villages of its neighbored district of Banamba, where IRS had never been implemented.

## Materials And Methods

### Study areas

This study was conducted in the villages of Karadié (7.60W, 13.24N) and Koula (7.65W, 13.12N) in the district of Koulikoro and Kolondialan (7.51W, 13.49N) and N'Galamadibi (7.48W, 13.48N) in the district of Banamba (Figure 1). The climate in both districts is a typical Sudano -Sahelian savannah with two seasons: a long dry season (November to May) and a wet season (June to October) with a mean annual rainfall of 900–1200 mm. The monthly mean temperature during the rainy season varies from 29 to

33°C. *An. gambiae s.l.* is the predominant malaria vector (> 98%). Malaria transmission occurs mostly during the rainy season (June to October) with the mean monthly mosquito human biting rate reaching its peak in August and September.

LLINs and SMC for Children of less than five years old, and IPT with SP for pregnant women were ongoing control interventions in both districts. Besides this, PMI has supported nine IRS campaigns in the districts of Koulikoro (2008 to 2016), while it has never been implemented in the district of Banamba. The coverage rate for the 2016 IRS campaign was 97.14%<sup>[13]</sup>. Prior to this study, mass distribution of LLINs was performed to scale up the coverage rate to 100% in the selected study sites in both districts with the support of NMCP. Agriculture, livestock, and trade are the main economic activities in the two districts.

**Mosquito vector sampling.** In each selected village, vector populations were sampled monthly from June to November 2016 by Pyrethrum Spray Catch (PSC) at day time and Entry Windows Traps (EWT) during the night.

#### **Pyrethrum spray catches (PSC).**

In each study village, 30 sentinel houses were randomly selected from the list of households' census of each village for indoor-resting mosquitoes sampling by PSC. The sampling was done taking into account the proportion of the housing type (e.g. straw roof, mud roof, and metal roof) in each village. The PSC consisted of spraying pyrethrum in the selected houses (from 8:00 to 11:00) in the morning. Two teams of 2 entomologists (a total of 4) were operating in parallel and sampling 10-15 houses per day. The insecticide used was a mixture of Pyrethroids (Tetramethrin 0.15%) and organophosphates (Dichlorvos 1.20% and Fenitrothion 0.40%) marketed under the name "Premium®".

#### **Entry window trapping (EWT).**

Ten (10) other houses were randomly selected in each village as sentinel sites for EWT sampling. The EWT consisted of mounting on windows, traps which collected the mosquitoes entering the rooms (Figure 2). The catches were done during three successive nights per month per village. One of the local guides of the different villages was responsible for closing the traps very early in the morning (around 6:00 am) with a small curtain sewn at their opening, to prevent the trapped mosquitoes to escape. The trapped mosquitoes were removed from the traps using a mouth aspirator, held in paper cups, and the number recorded on a data sheet.

#### **Insecticide susceptibility bioassay.**

*Anopheles gambiae s.l.* larvae were collected in different types of breeding habitats in and around each study site and reared to adulthood at the Malaria Research and Training Center (MRTC) insectary at Bamako. Female adult mosquitoes of 2-5 day-olds were exposed to impregnated filter papers with deltamethrin [0.05%], bendiocarb [0.1%], and pirimiphos-methyl [0.25%]. Approximately 20-25 mosquitoes per tube with 2-6 replicates were exposed to impregnated filter papers at the diagnostic concentrations for 1 hour and then transferred to a clean holding tube supplied with a 10% sugar solution. The post-

exposure mortality rate was determined after 24 hours according to WHO standard operating procedures[14]. All the tests were carried out at  $27 \pm 1^\circ\text{C}$  and relative humidity of 70-80%.

### Sample processing.

Collected mosquitoes were identified to species level using Polymerase Chain Reaction (PCR) technique [15]. The infection rate and human blood index (HBI) were established using the Enzyme Linked-Immuno-Sorbent Assay (ELISA) techniques following the protocols of Burkot et al[16] and Beier et al [17], respectively.

**Statistical analysis.** The data were entered in Microsoft Excel and analyzed in SPSS version 22, STATA version 10, and GraphPad Prism 7. The following entomological parameters were calculated: vector density per room, human biting rate (HBR), infection rate (IR), entomological inoculation rate (EIR), human blood index (HBI), and parity rate (PR). The density of malaria vector was calculated as the average number of indoor resting mosquitoes per room; the HBR as the average number of mosquito bites received by a sleeper per time unit (blood-fed and half gravid mosquito/number of room sleepers); the IR corresponds to the proportion of *An. gambiae s.l.* carrying sporozoites; the HBI as the proportion of females mosquito having human blood in their guts; the PR was calculated as the percentage of parous females relative to the total number of mosquitoes dissected (parous + nulliparous), and the EIR was calculated as the total number of infectious bites per human per time unit. The Chi-square test was used to compare the HBR, HBI, and PR.

Mosquito mortality rates 24 hours after exposure to the insecticide-impregnated filter papers were calculated by dividing the number of dead mosquito by the number exposed to the insecticide.

- Mortality  $\geq 98$  indicate mosquitoes were susceptible,
- Mortality between 90 and 97% indicate mosquitoes were suspected to be resistant,
- Mortality  $< 90\%$  indicate mosquitoes were resistant.

## Results

### Molecular species composition of *An. gambiae s.l.*

A total of 2270 female specimens of *An. gambiae s.l.* were collected over the study period, 2000 by PSC, and 270 mosquitoes by EWT. *An. gambiae s.l.* was the only malaria vector collected in both areas. Molecular identification of *An. gambiae s.l.* by PCR showed that *An. gambiae s.l.* was composed of three species in all the study sites except for N'Galamadibi where we did not observe *An. arabiensis* (Table 1). In the IRS area, *An. gambiae s.l.* consisted of *An. coluzzii* 95.88% (93/97), *An. gambiae* 2.06% (2/97) and *An. arabiensis* 2.06% (2/97) and in the no-IRS area, it consisted of *An. coluzzii* 94.12% (409/430), *An. gambiae* 4.42% (19/430) and *An. arabiensis* 0.47% (2/430) (Table 1).

### The density of *Anopheles gambiae s.l.* per room

The mean density of *An. gambiae s.l.* per house over the study period was 7.18 times higher (4.88/0.68) in the no-IRS area (4.88, 1756/360) than in the IRS area (0.68, 244/360). However, there were monthly variations in *An. gambiae s.l.* density (Table 2). It increased with rainfall in the no-IRS area, while it remained low regardless of the variations in rainfall in the IRS area (**Figure 3**). The highest density was observed in August (16.27, 968/60) in the no-IRS area. In the IRS area, the peak was observed in September (1.27, 95/60) two months after the IRS was done.

### **Parity rate of *Anopheles gambiae s.l.***

The parity rate was estimated from samples collected by EWTs (Table 3). The mean parity rate in the IRS area (96.42%, 54/56) was lower compared to the no-IRS area (98.36%, 120/122) with a statistically borderline significant difference (P = 0.4190).

### **Human biting rate of *Anopheles gambiae s.l.***

Using PSC, the overall mean monthly human biting rate (MHBR) of *An. gambiae s.l.* was significantly higher in the no-IRS area (20.41, 95%CI = 20.20–20.62) than in the IRS area (3.03, 95%CI = 2.95–3.12). There were monthly variations in both areas (Figure 4). In the IRS area, the peak of the MHBR (7.47, 95%CI = 7.15–7.80) was observed in September (> 2 months after IRS), while it was observed in August in the no-IRS area (66.00, 95%CI = 65.09–66.92). In both areas, the lowest MHBR was observed in June (Figure 4).

### **Infection rate (IR) and Entomological inoculation rates (EIR) in *An. gambiae s.l.***

Specimens of *An. gambiae s.l.* collected from PSC and EWT were screened to look for the *P. falciparum* CSP antigen. The mean infection rate of *An. gambiae s.l.* in the no-IRS area was 0.96% (16/1670). In the IRS area, the null IR observed may be likely due to the low number of *An. gambiae s.l.* specimens tested (n = 253). In the no-IRS area, mean EIR was 0.21 infective bites/person/month with a peak in September at 0.75 infective bites per person per month (Figure 4 and Table 4).

### **Human blood index**

The HBI (PSC) shows that the mosquitoes were highly anthropophilic in both areas. The average HBI in the IRS area was 74.27% (127/171) was significantly low ( $\chi^2 = 19.09$ ; P < 0.001) compared to the no-IRS area 86.90% (1042/1199). However, monthly variations in these rates were observed in both areas (Table 5). It is only in October that significant-high HBI was recorded in the no-IRS area (97.62%). In August HBI in the no-IRS area and the IRS area were almost similar (88.51 vs 88.89%).

### **The resistance of *An. gambiae s.l.* to insecticides**

Results showed high resistance to deltamethrin in the four study sites (Figure 5) with mortality rates of 15%, 20%, 24.5%, and 41% respectively in Karadié (N= 100), N'Galamadibi (N= 150), Kolondialan (N= 150) and Koula (N= 200). *An. gambiae s.l.* was resistant to bendiocarb (Figure 5) in all study sites with

mortality rates of 56%, 73%, 79%, 69% respectively in Koula (N=150), Karadié (N=100), N'Galamadibi (N=200) and Kolondialan (N=200). Susceptibility to pirimiphos-methyl was observed in all study sites.

## Discussion

In this study, we compared entomological indices of malaria transmission of the district of Koulikoro, where IRS was implemented for about 9 consecutive years, to the neighboring district of Banamba where the IRS was never been implemented. *An. gambiae s.l.* was the only malaria vector encountered in both study areas and consisted of three species: *An. coluzzii*, *An. arabiensis* and *An. gambiae*. The finding is consistent with *An. gambiae s.l.* species identified in Mali[18]. *An. coluzzii* was the most abundant species in both areas as generally reported [9, 19, 20] and can be found everywhere in the country and during all seasons in Mali [21].

*An. gambiae s.l.* mean densities per room over the study period were lower in the IRS area compared to the no-IRS area with monthly variations. Similar in both areas before the application of the IRS, *An. gambiae s.l.* densities significantly increased trough the rainiest period (July to September) in no-IRS compared to the IRS area, then became comparable at the end of the rainy season. Many studies have demonstrated the association between *An. gambiae s.l.* abundance and rainfall [22-24] in arid water-limited areas where its breeding is almost exclusively rain-dependent. The observed difference in *An. gambiae s.l.* densities between the two areas could be explained by the residual effect of the IRS campaign [25, 26].

The parity rate in the IRS-area was lower compared to a no-IRS area with a borderline statistically significant. From one month after the IRS, parity rates were decreasing in the IRS-area while increasing in the no-IRS area. This is in line with the ultimate goal of IRS which is to reduce the lifespan of mosquitoes, hence the parity rate. A similar observation was made in neighbor Senegal during a pirimiphos-methyl based IRS campaign [26].

Control tools (LLIN and IRS) deployed in both areas are known to limit mosquitoes' access to human blood sources. However, *An. gambiae s.l.* was found very anthropophilic in both areas even though the overall HBI was significantly high in the no-IRS area. Several studies conducted across Africa have shown that the long term use of LLIN or IRS alone or combined lead to changes in the biting behavior of *An. gambiae s.l.* from inside to outside biting [25, 27-29]. In addition, based on our experience in the field, the human population spends much more time outside than inside because of the heat as the study area is located in a Sahelian eco-climatic zone of Mali. Thus, in both areas, mosquitoes may have feed outside before a portion goes to rest inside. In the IRS area, resting places are limited explaining the lower densities and HBR in this area compared to the no-IRS area. The peak of HBI occurred earlier in the IRS area probably because of the effect of the decline observed in mosquitoes 'life expectancy one month after the application of the IRS.

In the IRS area, we could not detect transmission as measured by EIR because none of the tested specimens of *An. gambiae s.l.* by ELISA was infected, while in the no-IRS area, the transmission was

typically seasonal with the peak observed at the end of the rainy season. The non-detection of transmission in the IRS area can be attributed to the low number of mosquitoes we collected probably because of the effects of the IRS. Another explanation could be because IRS may have shortened the life expectancy of the mosquitoes population to accomplish the complete parasite development in mosquito to ensure a transmission as shown by the parity rate. A limitation of this study has been that we could not use human landing collection (HLC) to estimate indoor and outdoor transmission. Nevertheless, the PSC and EWT were consistently used across all sites.

Susceptibility testing performed show high and moderate phenotypic resistance respectively to deltamethrin and bendiocarb in all four study sites. The similarity in the resistance status of populations of *An. gambiae s.l.* in the two areas could be due to their closeness (separated by a 7 Km distance). Indeed, mosquitoes can actively migrate up to 2-7 km [30, 31]. Moreover, malaria vectors can migrate at long-distance in the Sahel (up to 300 km) by the wind as reported by a recent study in the same region of Koulikoro where this study occurred[32]. Also, previous studies have shown the same trend of resistance in *An. gambiae s.l.* population, low resistance to bendiocarb in some from sentinel sites of the NMCP far away from the IRS areas [9, 20]. The emergence and spread of resistance of *An. gambiae s.l.* to different classes of insecticides used in vector control could be due to their massive use of pesticides and herbicides in agriculture for the protection of crops (cotton, cereals, oilseeds, and market gardening) and the use of bendiocarb in IRS campaigns between 2012 and 2014. [33-38]. We observed a difference in resistance level between the two families (carbamates and organophosphates) of insecticide. Resistance to bendiocarb does not imply systematic resistance to pirimiphos-methyl even though they share the same resistance gene. These results confirm those observed in several African countries which had shown that the presence of G119S does not systematically confer resistance to organophosphates[39, 40].

Despite the lack of baseline data and the use of human landing collection, coupled with the low number of study sites and mosquitoes collected and tested in the IRS area, our results show the effects of pirimiphos-methyl based IRS on entomological parameters of malaria transmission in the context of pyrethroids resistance.

## Conclusions

IRS using pirimiphos-methyl has been successful in reducing substantially entomological parameters of malaria transmission in the area of pyrethroids resistance of Koulikoro, Mali. However, additional studies with baseline data extended to more sites are needed to better assess the impact of pirimiphos-methyl based IRS on malaria transmission. As the IRS intervention was ended after the 2016 campaign, further studies are needed to determine the rebound effect and monitor the potential emergence of organophosphate resistance for better management of insecticide resistance.

## List Of Abbreviations

Indoor residual spraying (IRS)

Long-lasting insecticidal nets (LLINs)

Intermittent Preventive Treatment (IPT)

Seasonal Malaria Chemoprevention (SMC)

The Ministry of Health (MOH)

The National Malaria Control Program (NMCP)

Preventive Intermittent Therapy (IPT)

Case report forms (CRFs)

Rapid diagnostic test (RDT)

University of Sciences, Techniques and Technologies of Bamako (USTTB)

Human biting rate (HBR)

Infection rate (IR)

Entomological inoculation rate (EIR),

Human blood index HBI)

Parity rate (PR)

## Declarations

### Ethical considerations

The protocol of this project has been approved by the Ethics Committee of FMPOS/USTTB under the letter **N°2014/51/CE/FMPOS**. The research activities related to this protocol were carried out in accordance with good clinical research practice in humans and good laboratory practice as set out in the international conventions (Helsinki Declaration; International Conference on the Harmonisation of Good Practice in Biomedical Research). All our researchers were trained in good clinical and laboratory practice during the research. In the field, the community (administrative, customary authorities) was informed of all aspects of the study.

**Consent for publication:** Not applicable

**Availability of data and materials:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests:** The authors declare that they have no competing interests

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### **Authors' contributions**

**MK:** has worked on the research hypothesis, data collection and analysis and write the manuscript.

**NS:** has worked on has worked on the research hypothesis and manuscript writing.

**FK:** has worked on data collection and sample processing.

**BT:** has worked on data collection and sample processing.

**BC:** has worked on data collection.

**SFT:** Correct and approved the latest version before submission.

**NS:** Correct and approved the latest version before submission.

**SD:** worked on the research hypothesis, correct and approved the latest version before submission

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## Tables

Table 1: Species composition of three members of the *Anopheles gambiae sensu lato* in four localities in Mali in 2016.

Areas	Localities	<i>An. arabiensis</i>		<i>An. coluzzii</i>		<i>An. gambiae</i>		Total
		N	%	N	%	N	%	
IRS area	Koula	1	1.72	55	94.83	2	3.45	58
	Karadié	1	2.56	38	97.44	0	0.00	39
	Total	2	2.06	93	95.88	2	2.06	97
no-IRS area	Kolondialan	2	0.68	278	95.21	12	4.11	292
	N'Galamadibi	0	0.00	131	94.93	7	5.07	138
	Total	2	0.47	409	95.12	19	4.42	430

Table 2 : Variation of mean monthly density of *An. gambiae* s.l. per room in IRS and no-IRS areas from June to November 2016

Months	IRS area			no-IRS area		
	# collection rooms	Mean density	95% CI	# collection rooms	Mean density	95% CI
June 16	60	0.38	0.20—0.57	60	0.15	0.05—0.25
July 16	60	1.10	0.60—1.60	60	7.02	4.67—9.36
August 16	60	0.62	0.10—1.14	60	16.27	12.60—19.93
September 16	60	1.27	0.79—1.75	60	4.43	3.07—5.79
October 16	60	0.52	0.25—0.78	60	1.07	0.58—1.56
November 16	60	0.18	0.07—0.29	60	0.33	0.08—0.58
TOTAL	360	0.68	0.52—0.84	360	4.88	3.92—5.84

Table 3 : Variation of the parity rate in IRS area and no-IRS area from June to November 2016.

Month	IRS area			No-IRS area		
	Dissected	Parous	% PR*	Dissested	Parous	% PR
June 16	0	0	0.00	0	0	0.00
July 16	16	16	100	25	25	100
August 16	8	7	87.50	87	85	97.70
September 16	20	19	95.00	7	7	100
October 16	7	7	100	0	0	0.00
November 16	5	5	100	3	3	100
Mean rate	56	54	96.42	122	120	98.36

\*PR: Parity rate

Table 4: Variation of the infection rate in IRS area and no-IRS area from June to November 2016.

Month	IRS area			No-IRS area		
	N* tested	N positive	% IR*	N tested	N positive	% IR
June 16	21	0	0.00	8	0	0.00
July 16	64	0	0.00	374	2	0.53
August 16	35	0	0.00	945	3	0.31
September 16	92	0	0.00	256	9	3.52
October 16	31	0	0.00	65	2	3.08
November 16	10	0	0.00	22	0	0.00
Mean rate	171	0	0.00	1670	16	0.96

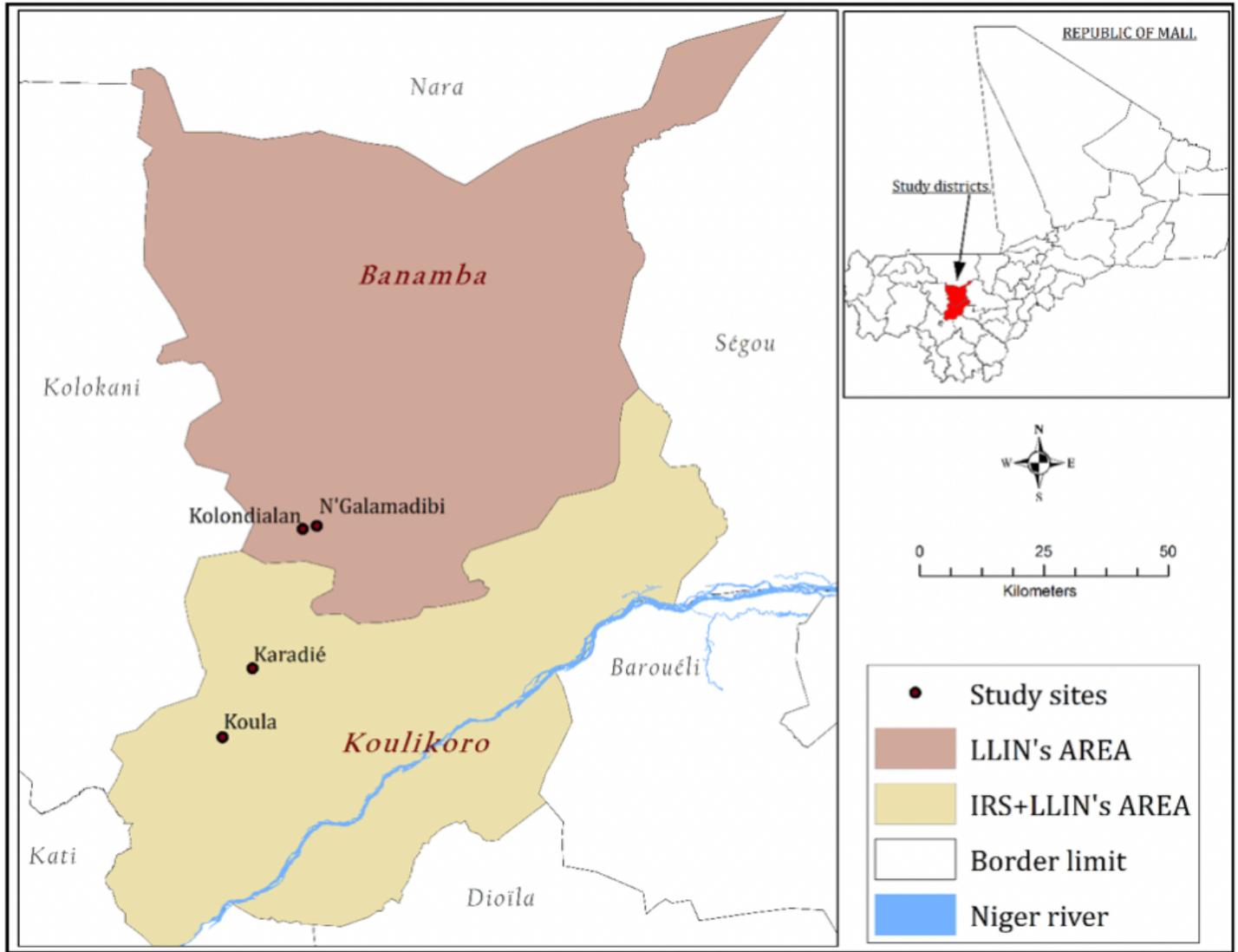
\*N: number, \*IR: infection rate

Table 5: Variation of the human blood index in IRS area and no-IRS area from June to November 2016.

Month	IRS area			No-IRS area			P-Value
	N* tested	N positive	% HBI* (95%CI)	N tested	N positive	% HBI* (95%CI)	
June 16	15	8	53.33	2	0	0.00	<i>0.169</i>
July 16	46	29	63.04	266	212	79.70	<i>0.015</i>
August 16	18	16	88.89	670	593	88.51	<i>0.960</i>
September 16	66	58	87.88	205	186	90.73	<i>0.502</i>
October 16	17	12	70.59	42	41	97.62	<i>0.002</i>
November 16	9	4	44.44	14	10	71.43	<i>0.206</i>
Mean rate	171	127	74.27	1199	1042	86.91	<i>0.0001</i>

N\* = Number; HBI\* = human blood index

## Figures



**Figure 1**

Map of Mali showing the different study sites



Figure 2

Field technician and local guide mounting an entry window trap

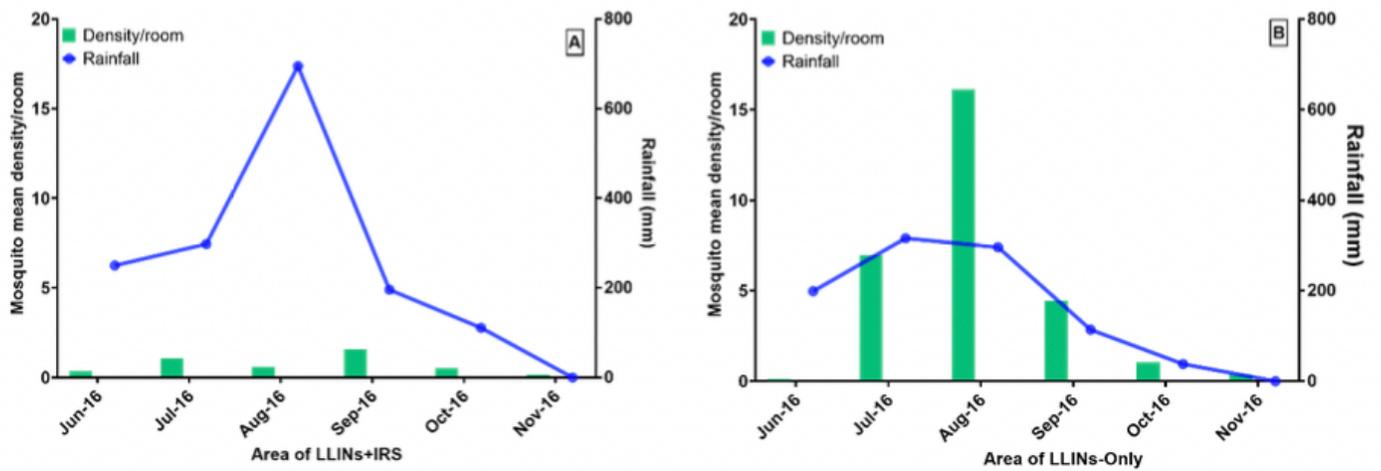


Figure 3

Variation in *An. gambiae* s.l. density (green bars) and rainfall (blue line) in areas of IRS and no-IRS from June to November 2016.

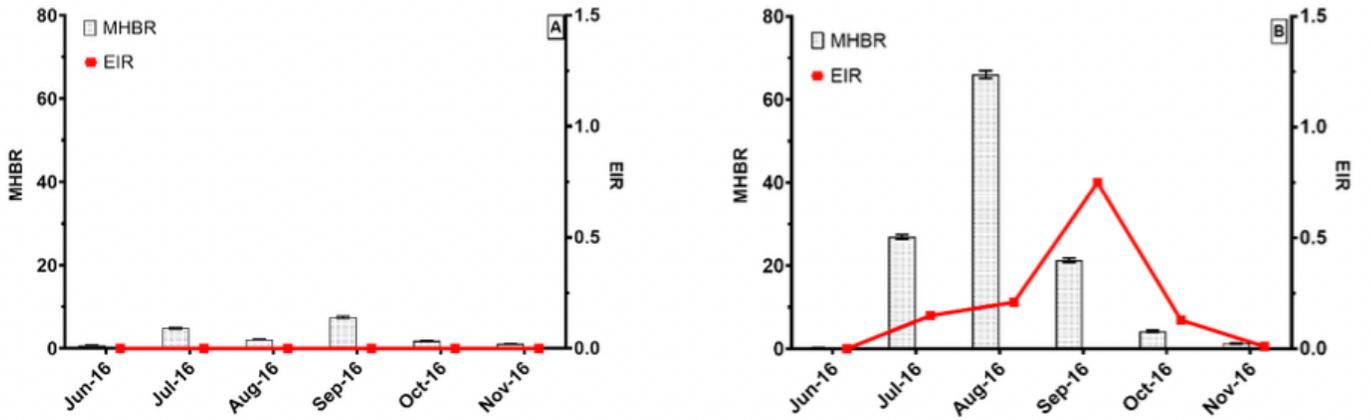


Figure 4

Monthly variation in *An. gambiae* s.l. man biting rates (gray bars) and entomological inoculation rates (Red line) in areas of IRS (A) and no-IRS (B) from June to November 2016.

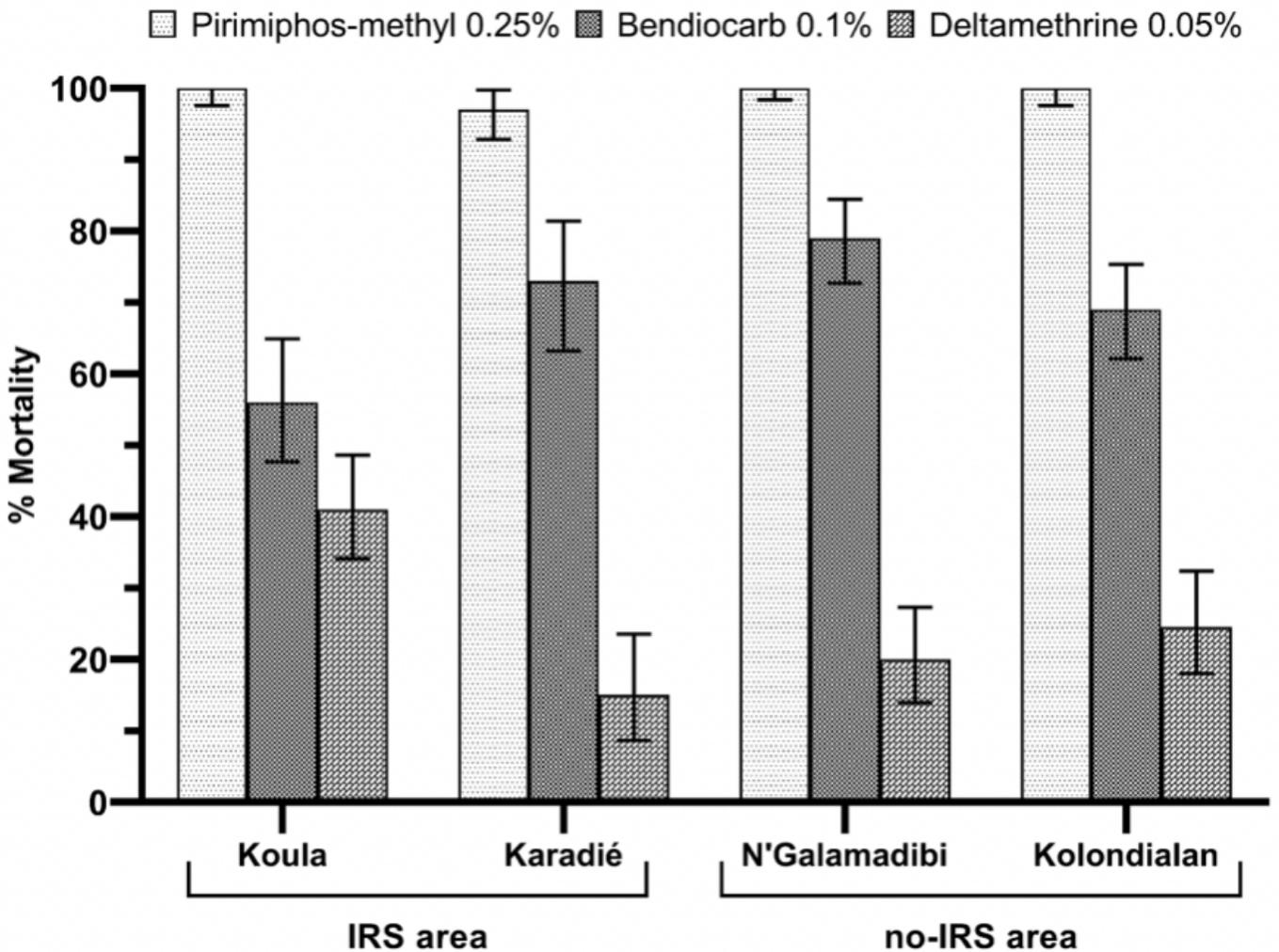


Figure 5

Observed 24 hours mortality (%) of *An. gambiae* s.l. following 60 mn exposition to Pyrethroids (Deltamethrin), Carbamates (Bendiocarb) and Organophosphates (Primiphos-methyl) in the selected study sites using WHO standard bioassay test.