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Effect of Bendiocarb (Ficam® 80% WP) on Entomological Indices of Malaria Transmission by Indoor Residual Spraying in Burkina Faso, West Africa

Aristide Sawdetuo Hien[1], Dieudonné Diloma Soma[1], Simon Pengwende Sawadogo[1], Serge Bèwadéyir Poda[1], Moussa Namountougou[2], Georges Anicet Ouédraogo[2], Abdoulaye Diabaté[1], Roch Kounbobr Dabiré[1]*

[1]Institut de Recherche en Sciences de la Santé, Direction Regionale, Bobo-Dioulasso, Burkina Faso
[2]Université Polytechnique de Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso
Email: aristide.hien@yahoo.fr, dieusoma@yahoo.fr, sawsimp2005@yahoo.fr, sergepoda71@yahoo.fr, namountougou_d@yahoo.fr, ogeorgesanicet@yahoo.fr, npie@mail.com, *dabireroch@gmail.com

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Abstract

Context: The vector control is essential in malaria prevention strategies in several endemic countries in Africa including Burkina Faso. The high transmission of malaria occurs during the period of high vector abundance (August to October) in Burkina Faso. Therefore, a vector control strategy based on the use of indoor residual spraying targeting this period should provide effective protection against malaria. This study aimed to evaluate the effect of bendiocarb applied in indoor residual spraying on entomological parameters of malaria transmission in a pyrethroid resistance area in southwestern, Burkina Faso. **Methods:** CDC light trap and early morning collections by pyrethrum spray catches were performed monthly to determine the change in entomological parameter within malaria vector in sprayed (Diebougou) and unsprayed sites (Dano). The female's malaria vectors collected by both methods were used to determine their blood feeding pattern, biting and sporozoites rates as well as the malaria transmission risk estimated by entomological inoculation rate. **Results:** A total of 26,276 mosquitoes (13,555 anopheline and 12,721 other culicines) were collected using both CDC light trap (9158 mosquitoes) and PSC collection methods (17,118 mosquitoes) from June to December 2012. *An. gambiae* complex was the predominant species collected. *An. gambiae* was the predominant species collected (P = 0.0005), comprising 88% of the total collected and the most infected species. Malaria vectors densities were significantly lower in sprayed villages (n = 4303) compared with unsprayed villages (n = 12,569) during post-spraying period (P = 0.0012). In addition, mean human biting rate of *An. gambiae* s.l. and *An. funestus* s.l. were significantly lower in sprayed areas compared to unsprayed areas ($P < 0.05$). Overall, malaria vector transmission risk was significant four-fold lower in villages which received IRS ($P = 0.0001$) whatever the malaria vectors species (*An. gambiae* s.l. and *An. An. funestus* s.l.). **Conclusions:** The results showed that in the sprayed area (Diebougou), vector densities, human biting rates and malaria transmission risks were very lower than unsprayed areas (Dano). The findings also showed a change in vector behavior especially within *An. funestus* s.l. which became more zoophagic following IRS. The indoor residual spraying could be promoted as a control tool in areas where malaria transmission occurs during a given period of year.

Keywords

Indoor Residual Spraying, Bendiocarb, Entomological Indices, Malaria Vectors, Burkina Faso

1. Introduction

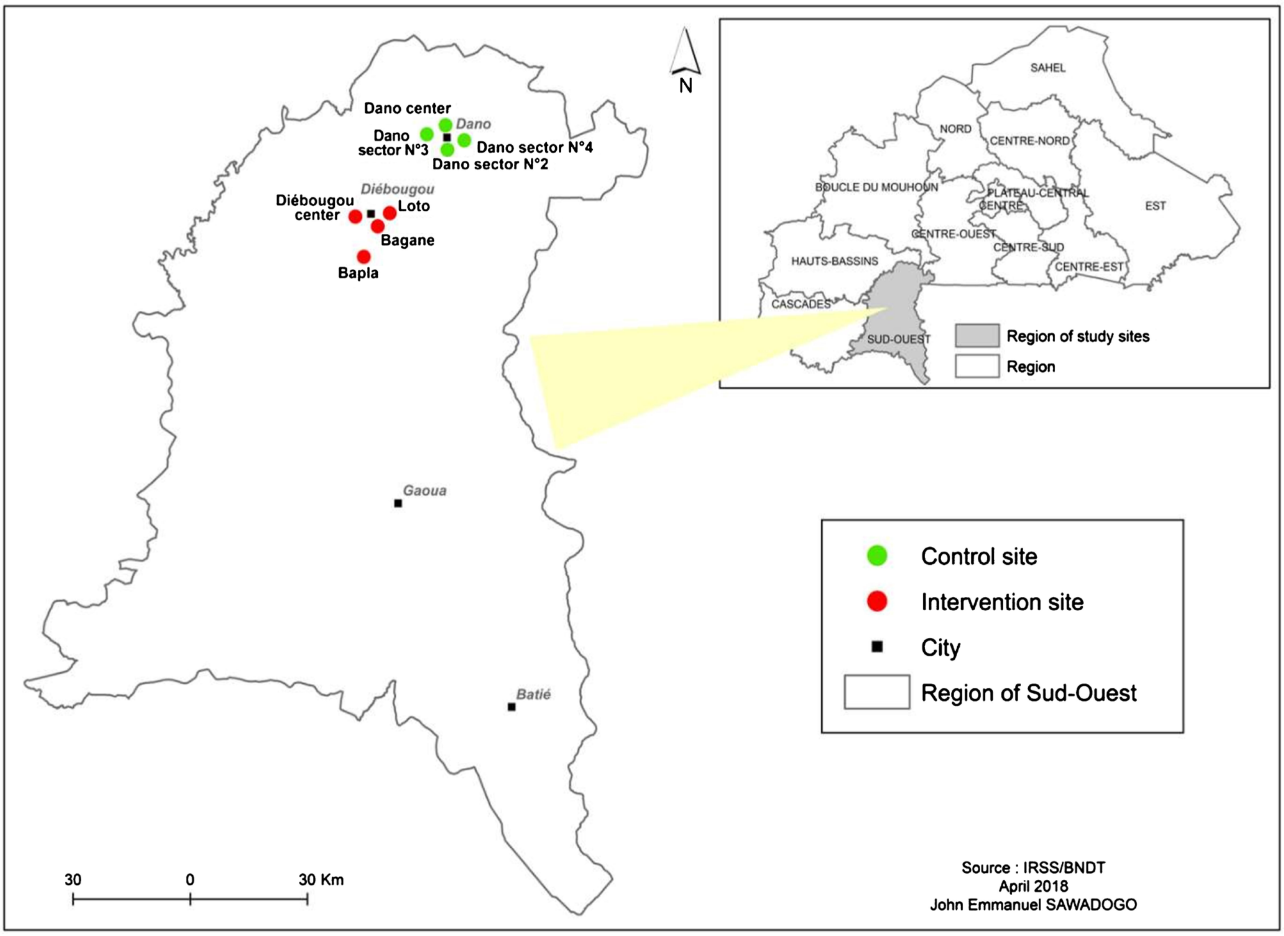
Vector control is one of the key elements of malaria control strategies [1]. In Africa, vector control relies mainly on two effective and complementary tools: long-lasting insecticidal mosquito nets (LLINs) and indoor residual spraying (IRS) [2]. Several studies have demonstrated the effectiveness of both tools in reducing the incidence of malaria-related morbidity and mortality [3] [4] in Africa [5] [6] [7] [8] [9]. However, these tools, in particular LLINs, are impregnated with insecticides from the pyrethroid family. Unfortunately, the recent evolution and the expansion of resistance to this class of insecticide in West Africa in *Anopheles gambiae* ss is a major problem for sustainability in malaria prevention in Africa [10] [11]. For this reason, the search for alternative tools using a non-pyrethroid insecticide has become a necessity [12]. Indeed, since 2006, the World Health Organization (WHO) encouraged a scale-up of IRS for vector-borne disease control, using one of several classes of insecticide that are suitable for IRS [13]. In 2008, of 108 malaria-endemic burdened countries, 44 reported the use of IRS [14]. The outcomes of indoor residual spraying towards curtailing malaria transmission are firstly to decrease the life span of vector mosquitoes and also to reduce the malaria vectors density [15]. There are several insecticide formulations currently prequalified by WHO for IRS: namely organophosphates, carbamates, pyrethroids and neonicotinoids. Moreover, the effectiveness of the IRS depends on many factors, such as the residual efficacy of the insecticide in formulations for the IRS, the feeding behavior of malaria vectors resting inside houses and susceptibility of vectors local populations to insecticide used for the IRS [16]. Residual efficacy of IRS formulations and local vector susceptibility to the insecticide used for IRS were discussed in a separate manuscript about bendiocarb efficacy on walls. The monitoring of behavioral responses of mosquitoes to insecticides is very important to the understanding of how chemicals work in the control of disease transmission [17]. Indeed, in northern Nigeria [18], IRS led to a high decrease in the total vector population and showed also a reduction in the incidence of malaria among children, the malaria parasites rate and fever, and an apparent effect on mortality of 1 - 4-year-old children. In Kenya, IRS with fenitrothion in Kisumu town [19] and the LLINs use in the south coast of Kenya [20], showed a decrease in *An. funestus* s.l and *An. gambiae* s.l. populations due to the IRS, while the high bed net coverage was followed by a much reduced human biting rate and a diminishing role of *An. gambiae* s.s. in malaria transmission.

In Burkina Faso, malaria was the most common cause of outpatient consultations (41.3%), hospitalizations (21.4%) and death (16.4%) in 2018 [21]. Furthermore, the resistance to at least one insecticide had been identified in 64 malaria-endemic countries according to WHO [22], including Burkina Faso [23] [24] [25] [26] [27]. Pyrethroid resistance is particularly widespread in Burkina Faso, with high frequency of voltage gated sodium channel mutations reported as long ago as 2006 [28]. However, susceptibility tests performed in 2010-2012 with insecticides belonging to carbamates class (such as 0.1% bendiocarb) have shown a high mortality rate of the local population of malaria vectors and low allelic frequencies of gene ace-1^R [28] throughout the country including the southwest (Diebougou). This insecticide molecule was chosen to be applied on walls during IRS pilot study according to results shown [28]. Indeed, the preliminary results from this study have shown a full vectors susceptibility to this insecticide. However, it was important to understand its impact on the behavior of malaria vectors in community where walls treated with this insecticide. In this context, the present study aims to evaluate the effect of a large-scale IRS using a non-pyrethroid insecticide of the carbamate family (bendiocarb) on the entomological parameters of malaria transmission in the intervention areas (Diebougou district sprayed) compared to control area used (Dano district unsprayed).

2. Methods

2.1. Study Area

Entomological surveys were performed in the health district of Diebougou (intervention or sprayed area) and covered four villages (or agglomeration) including Diebougou center (N10.96741; W003.24580), Bagane (N10.96397; W003.23422), Loto (N10.96871; W003.23477) and Bapla (N10.87638; W003.26145). Dano (control or unsprayed area) is situated 42 km from Diebougou and was utilized as an unsprayed control area. Four villages were sampled, including Dano sector 1 corresponding to the center of Dano (N11.14288; W003.05969), Dano sector 2 (N11.13802; W003.06216), Dano sector 3 (N11.16464; W003.06374) and Dano sector 4 (N11.14541; W003.05141) (**Figure 1**). Villages chosen in Diebougou and Dano districts were selected to be representative of the different settings of the



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April 2018
John Emmanuel SAWADOGO

Figure 1. Study sites.

areas (peripheral, central, sub-urban, presence of water source, etc) and had the same type of walls such as "banco" (a mixture of mud and water) and cement.

2.2. Implementation of Intervention

Indoor Residual Spraying was conducted in 2010 with funding from United States Agency for International Development/President's Malaria Initiative (USAID/PMI), and went on through 2011 in conjunction with the National Malaria Control Program (NMCP). In 2010 spray round, 33,897 structures were sprayed (with about 98.9 percent of the target area), and protected 118,691 persons including nearly 25,000 children under five and more than 2000 pregnant women. While In the spray campaign in 2011, 36,870 structures were sprayed and thus protecting 115,638 people. The spray campaign in 2012 started July 13, 2012 and lasted 21 working days. It was implemented in accordance with President's Malaria Initiative Best Management Practices [29] to ensure a high quality of spraying and the safety of the residents, spray operators and the environment. All spray operators were provided with full personal protective equipment including coveralls, gloves, boots and helmet with face visor. Spray operators used Goizper IK Vector Control compression sprayers with flat jet nozzles in order to spray onto walls and non-metal ceilings of eligible structures in IRS-targeted areas. In addition, spray operators, team leaders and spray supervisors were trained prior to spray operations. The active ingredient selected for spraying onto the walls in Diebougou was bendiocarb dosed at 80% in the wettable powder form (Ficam® 80% WP). It was applied at 400 mg of bendiocarb active ingredient/m^2 on walls of houses as recommended by the World Health Organization Pesticide Evaluation Scheme (WHOPES) [30] [31]. Prior, spray operation staff informed residents to stay out of the structure for at least two hours after IRS application.

2.3. Mosquito Sampling and Identification

Following IRS, the researche institute (Institut de Recherche en Sciences de la Santé, IRSS) monitored the efficacy of IRS on entomological indicators of malaria transmission. Monthly collections were conducted to determine mosquito species composition, biting rates and indoor resting densities. The malaria vector populations dynamic was monitored in each of the eight selected villages (four sprayed and four unsprayed controls) using indoor and outdoor CDC light trap collection (CDC LT) and pyrethrum spray catch (PSC). Two months of baseline collections were conducted from June 2012 to July 2012 (just a week before IRS application launched July 13, 2012) in both Dano and Diebougou districts. The relatively short baseline period of data collection was due to the long dry season in the study area from November until May, during which *Anopheles* densities and malaria transmission is low. Subsequent monthly entomology surveys were conducted from the end of July after IRS until December 2012.

Indoor CDC LT were installed about 1.5 m above the ground next to an occupied (bait) untreated mosquito net. Unbaited light traps were also hung outdoors at the same height, approximately 10 - 20 m away from the houses. CDC light trap was the preferred trapping method due to concerns about potential disease transmission risks during human landing catch. Moreover, several studies [32] [33] [34] showed a comparability of CDC light trap catch size compared with HLC for different *Anopheles* species. For that, we used the CDC light trap data collection to estimate the Human biting rate in all manuscript.

In each village, CDC light trap collections were conducted in indoors and outdoors between 20:00 pm and 06:00 am for a total of four nights per month. Four randomly selected houses were sampled each month, resulting in 16 trap-nights indoors and 16 trap-nights outdoors per month, per site. In addition, mosquitoes resting indoor were collected by pyrethrum spray catches (PSC) in four randomly selected houses in each village once per month. PSC was performed by laying white sheets on the floor and furniture before spraying a commercial aerosol consisting of 0.64% Pyrethrum EC and 0.75% chlorpyrifos ethyl. PSC collections were performed from 06:00 to 09:00 am. The mosquitoes sampling was carried out in the same bedrooms and at the same frequency during the intervention period.

2.4. Laboratory Processing

All anophelines were separate and assigned to species based on morphological characters using standard identification keys [35]. Legs of each *An. gambiae* s.l. female collected using CDC light trap and PSC methods were tested by PCR for molecular identification of *An. gambiae* complex species [36]. Aliquots of DNA extracted from PCR positive specimens of *An. gambiae* s.s. were subjected to PCR assays for identification of the molecular "M" and "S" forms [37] that are currently *An. gambiae* and *An. coluzzii.* The heads and thoraces of host-seeking females were tested by enzyme-linked immunosorbent assay-circumsporozoite protein (ELISA-CSP) for *Plasmodium falciparum* detection using the protocol of Wirtz *et al.,* [38]. The blood meals source from freshly fed females collected using early morning collections (PSC method) were used to assess *An. gambiae* s.l. host preference. Therefore, a random selection of 30 specimens per month and per district were tested by a direct ELISA bloodmeal source detection [39] using anti-host immunoglobulin G (IgG) conjugated against human, bovine, pig, donkey and sheep blood. Unfortunately, parity rates could not be assessed because females died while in traps and were too dry for dissection. All the mosquito samples collected were stored individually in numbered vials with desiccant.

2.5. Data Analysis

The mean human biting rate (HBR) was calculated for each specie collected by CDC LT by dividing the total number of captured specimens by the total person-nights for the collection period. The mean indoor resting density (IRD) was defined as the total number of mosquitoes (per species) collected by PSC divided by the total number of rooms sampled. The circumsporozoite (CSP) rate was calculated as the proportion of mosquitoes infected with *P. falciparum* sporozoites. The malaria vectors anthropophilic rate was calculated as the proportion of female mosquitoes with human blood out of the total tested for blood-meal source. The entomological inoculation rate (EIR) was calculated by multiplying the HBR indoor/outdoor and the CSP rate.

All the measured parameters were computed and analysed using the free software GraphPad 5.0 version. Data were compared with the Pearson chi^2 or Fisher exact tests and odds ratio were calculated to determine the impact of IRS in study sites. All calculations were expressed with the statistically significant threshold set at $P \leq 0.05$.

3. Results

3.1. Density and Species Composition of Malaria Vectors

From June to December 2012, a total of 26,276 mosquitoes (13,555 anopheline and 12,721 other culicines) were collected using both CDC light trap (9158 mosquitoes) and PSC collection methods (17.118 mosquitoes). In addition, 9404 mosquitoes were collected in Diebougou (sprayed area) between June and December (**Table 1**) whose 3040 mosquitoes collected in baseline (June-July 2012) and 6364 mosquitoes during post-spraying period (Augut-December) compared to unsprayed area with 16,872 collected mosquitoes whose 4303 mosquitoes at baseline and 12,569 mosquitoes in post-spraying period (P = 0.0012). According to species composition, *An. gambiae* s.l. (6934%) and *An. funestus* s.l. (2416%) were the most predominant *Anopheline* species collected in sprayed area (Diebougou) compared to 45% *An. gambiae* s.l., 19% *An. funestus* s.l., and 36% others *Culicine* (*Culex* sp, *Aedes* sp., …) in unsprayed area (Dano). Their proportion were significantly reduced between sprayed and unsprayed areas (P = 0.039). In addition, there was a greater number of culicids collected in sprayed areas compared to unsprayed areas certainly due to impact of IRS. Overall, the total number of collected mosquitoes in sprayed areas (6364 mosquitoes) compared to unsprayed area (12,569 mosquitoes) was significant (P = 0.001) after spraying period.

During the post IRS study period, indoor resting densities of malaria vectors were significantly lower in sprayed villages (n = 1798) compared with unsprayed villages (n = 8607) with P = 0.0051 (**Table 1**). a significant difference was observed for total A*nopheline* catch by indoor CDC LT, with 1527 in the unsprayed area compared with 623 in the sprayed area (P = 0.0069). When broken down to species, *An. funestus* s.l. indoor resting (PSC) and host-seeking (CDC light trap) densities (CDC light trap: sprayed = n = 166 vs unsprayed n = 521 with P = 0.004; PSC: sprayed n = 99 vs unsprayed n = 2136; P = 0.0079) and *An. gambiae* s.l. indoor resting densities (PSC: sprayed n = 1076 vs unsprayed n = 494,411; P = 0.0005 were significantly lower in sprayed sites compared with control villages (**Table 1**).

Figure 2 presents monthly molecular species data for *An. gambiae* s.l. collected

Table 1. Seasonal variation of major vectors densities in sprayed (Diebougou) and unsprayed (Dano) sites.

Samples	CDC indoor collections							CDC outdoor collections							Pyrethrum indoor collections							Total	%
Sprayed area	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**		
An. gambiae s.l.	33	76	213	106	76	40	22	8	35	28	2	3	2	18	234	317	338	335	141	140	122	2289	24.34
An. funestus s.l.	0	0	27	47	33	28	31	5	15	62	9	31	36	41	10	11	8	11	20	35	25	485	5.16
Others *Culicine*	256	220	678	246	309	206	92	15	131	433	37	203	183	135	809	865	667	149	588	191	217	6630	70.50
Total	289	296	918	399	418	274	145	28	181	523	48	237	221	194	1053	1193	1013	495	749	366	364	9404	100.00
Unsprayed area	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Total**	**%**
An. gambiae s.l.	74	218	640	244	72	36	14	17	31	21	14	2	2	4	469	777	1926	1160	1337	323	198	7579	44.92
An. funestus s.l.	15	98	94	109	158	110	50	7	25	2	3	3	4	26	118	244	256	547	557	468	308	3202	18.98
Others *Culicine*	150	263	413	198	172	159	170	128	77	25	20	28	17	74	1145	447	839	844	275	323	324	6091	36.10
Total	239	579	1147	551	402	305	234	152	133	48	37	33	23	104	1732	1468	3021	2551	2169	1114	830	16872	100.00

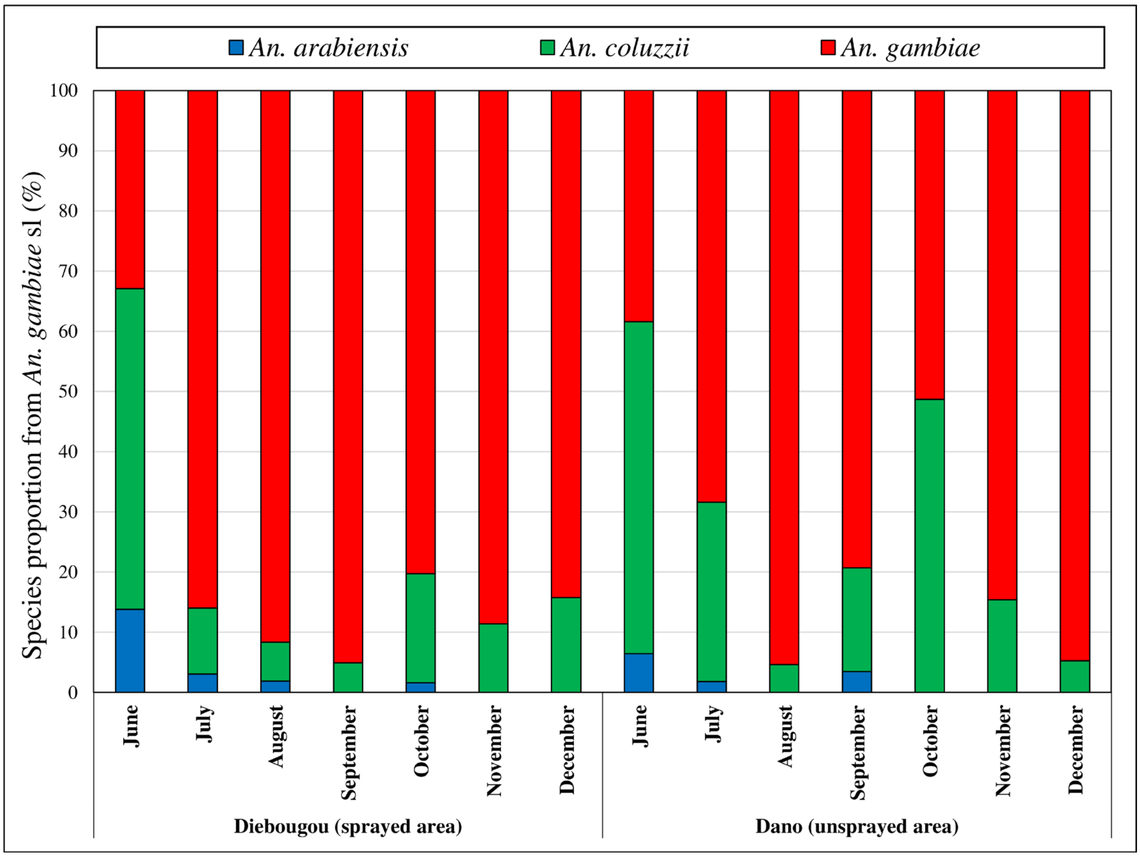

Figure 2. Species composition within the *An. gambiae* complex in sprayed (Diebougou) and unsprayed areas (Dano).

using CDC light trap in sprayed (Diebougou) and unsprayed areas (Dano). More than 80 percent of *An. gambiae* s.l. collected in Diebougou were *An. gambiae* while Dano had relatively similar frequencies of both species (*An. gambiae* and *An. coluzzii*) in June, July and October with the other months dominated by *An. gambiae*. *An. arabiensis* proportion was relatively higher in Diebougou from June to August and a low proportion in October whilst in Dano this specie was found at the beginning of season (June-July) but also in September. The frequency of *An. arabiensis* was higher in June in both sites (Figure 2). Across the study areas, *An. gambiae* s.s. was the predominant species from the complex (P = 0.0005), comprising 88% (1145/1301) out of total collected and 70% of those from CDC LT (582/831), compared with 23% (194/831) *An. coluzzii*. *Anopheles arabiensis* was the least frequent species (55/831). There were no apparent changes in species composition in the IRS site following spraying when compared to the unsprayed control.

3.2. Malaria Vectors Monthly Biting and Resting Behaviour Following IRS

3.2.1. Baseline Data

In Dano (unsprayed), in June 2012, indoor human biting rate of *An. gambiae* s.l. was estimated at 4.6 bites per person per night by CDC light trap collection and

13.6 bites/person/night in July 2012 (**Figure 3(a)**). However, in Diebougou *An. gambiae* s.l. human biting rates of by indoor CDC light trap were found to be lower at 2 and 5 b/p/n indoors in June and July respectively. The *An. funestus* s.l. indoor human biting rate was less than 1 b/p/n (**Figure 4(a)**). A similar trend was recorded for indoor resting densities, with Dano having approximately double the catch size of Diebougou (**Figure 5(a)** & **Figure 5(b)**). The highest resting densities by indoor PSC collection with a mean value in July reaching 49 *An. gambiae* s.l. per house per night in Dano (**Figure 5(a)**). The catch size was generally low in outdoor CDC light trap collections in both sites (**Figure 3(b)** & **Figure 4(b)**).

3.2.2. Post-Spraying Data

A summary of mean biting rates is presented in **Figure 3** for *An. gambiae* s.l. and **Figure 4** for *An. funestus* s.l. In addition, the number of mosquitoes collected by month and by site is summarized in **Table 2** & **Table 3**.

In the period post-IRS (August to December) the mean indoor biting rate per person per night (b/p/n) was significantly highest in the unsprayed sites (mean =

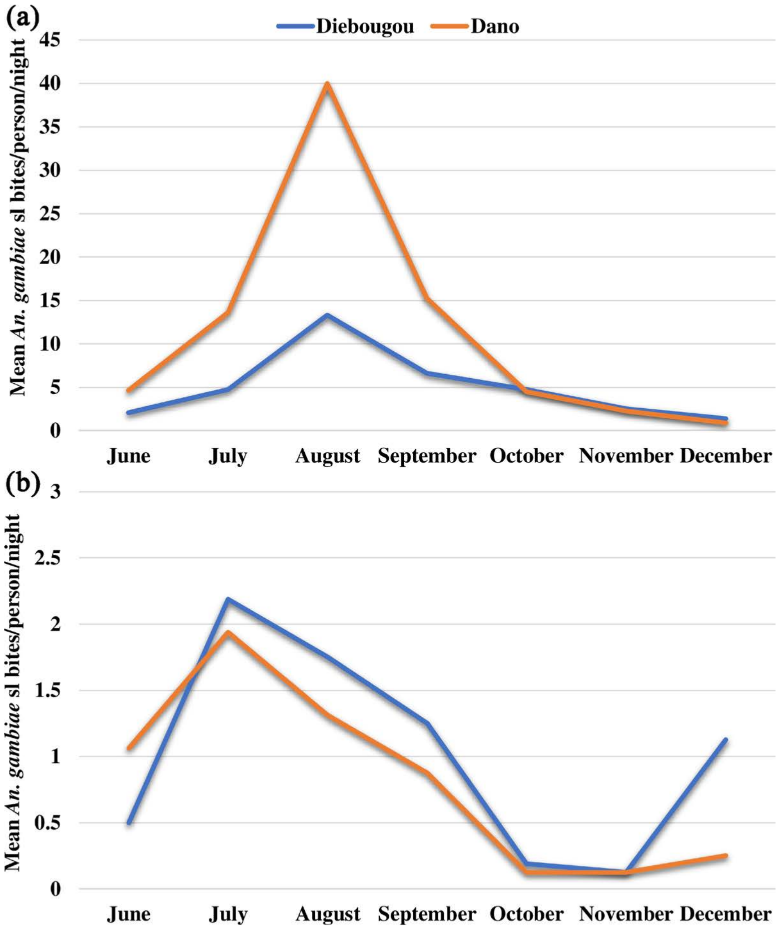

Figure 3. Mean A*n. gambiae* s.l. bites per person per night collected by CDC LT in sprayed (Diebougou) and unsprayed areas (Dano) in (a) Indoor collection and (b) Outdoor collections before and after spraying.

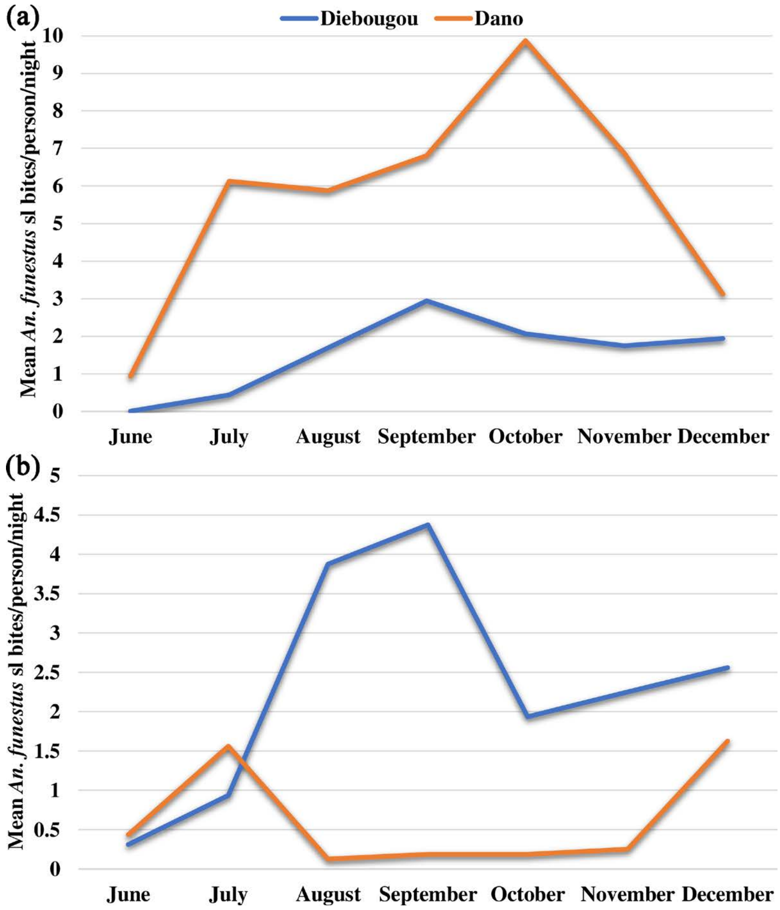

Figure 4. Mean *An. funestus* s.l. bites per person per night collected by CDC LT in sprayed (Diebougou) and unsprayed areas (Dano) in (a) Indoor collection and (b) Outdoor collections before and after spraying.

6.55 bites per person per night from August to December) compared to sprayed sites (mean = 3.18 bites per person per night) in *An. gambiae* s.l. ($P = 0.015$). furthermore, the peak from indoor biting density of *An. gambiae* s.l. occurred in August with about 40 bites per person per night in Dano (unsprayed) and decreased progressively to December, when it was less than 5 b/p/n towards the end of the rainy season (Figure 3(a)). The similar pattern was observed in the intervention area but with less than 15 b/p/n of *An. gambiae* s.l. The human biting rate and mean number of *An. gambiae* s.l. per house from indoor collections (CDC LT and PSC) in sprayed sites was half a time lower compared to unsprayed sites (Odds ratio$_{\text{(CDC LT)}}$ = 0.51 with 95% CI: [0.34 - 0.67] and $P = 0.001$ and Odds ratio$_{\text{(PSC)}}$ = 0.30 with 95% CI: [0.21 - 0.43] and $P = 0.0025$. Outdoor biting rates were particularly low in both sites, with a mean of <3 bites per person per night (Figure 3(b)). But, the exposure to mosquito bites outdoors was slightly, but more increased in Diebougou (sprayed area) after treatment compared to Dano, the unsprayed area but the difference was not significant ($P > 0.05$).

Similar results were observed in *An. gambiae* s.l. and *An. funestus* s.l. biting

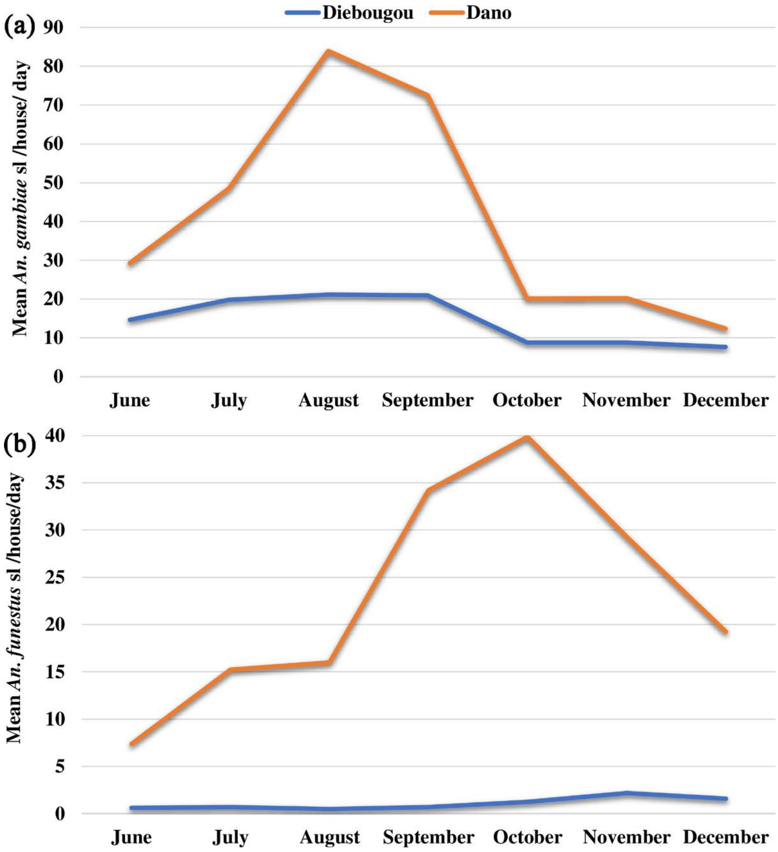

Figure 5. Mean number of mosquitoes /houses from indoor PSC collection in sprayed (Diebougou) and unsprayed areas (Dano) in (a) *An. gambiae* s.l. and (b) *An. funestus* s.l. before and after spraying

rates (Figure 4) in PSC collection (Figure 5(b)) with a mean biting rate of 2 b/p/n in Diebougou compared with 4 b/p/n in Dano during the post-spraying period August-December (Odds ratio$_{\text{(indoors CDC LT)}}$ = 0.28 with 95% CI: [0.11 - 0.35] and P = 0.035 (Table 3).

3.3. *Plasmodium falciparum* Circumsporozoite and Entomological Inoculation Rates (EIR) from *An. gambiae* s.l. and *An. funestus* s.l.

The results of CSP-ELISA assays and entomological inoculation rate of *An. gambiae s.l.* and *An. funestus* s.l. are presented in Table 2 and Table 3 respectively. Overall, 2051 *An. gambiae* s.l. and 1072 *An. funestus* s.l. specimens were screened for the circumsporozoite protein from June to December 2012 in the two areas. The sporozoites and entomological inoculation rate (EIR) were calculated by grouping the indoor and outdoor collections of *An. gambiae* s.l. and *An. funestus* s.l. due to low number sampled sporozoites rates detection. So, during the post-IRS period (August-December), the mean sporozoites rate differed between unsprayed and sprayed areas for CDC LT method. The average sporozoites rates were significantly different (more than 2-fold) between the unsprayed areas (average sporozoite rate = 15.82%; 95% CI: [8.94 - 23.49]) and the

Table 2. Monthly *An. gambiae* s.l. sporozoite rate and entomological inoculation rate from Dano unsprayed area and Diebougou (sprayed area) from June to December, 2012.

	June	July	August	September	October	November	December	2012 Total
Dano (unsprayed area)								
Total *An. gambiae* s.l. (CDC-LT) collected	91	249	661	258	74	38	18	1389
CDC trap-nights (indoors + outdoors)	32	32	32	32	32	32	32	224
HBR per night	2.84	7.78	20.66	8.06	2.31	1.19	0.56	6.2
Total *An. gambiae* s.l. tested by CSP	78	45	44	14	21	27	13	242
Sporozoites rate	0	6.7	13.6	14.2	9.5	11	30.8	7
EIR p/night	0	0.521	2.809	1.145	0.219	0.131	0.173	0.714 (mean)
EIR p/month*	0	15.64	84.28	34.35	6.591	3.92	5.19	21.42 (mean)
Dano 5-month EIR post-IRS August-December 2012 = 134 infectious bites per person								
Diebougou (sprayed area)								
Total *An. gambiae* s.l. (CDC-LT) collected	41	111	241	108	79	42	40	662
CDC trap-nights (indoors + outdoors)	32	32	32	32	32	32	32	224
HBR per night	1.28	3.47	7.53	3.38	2.47	1.31	1.25	2.95
Total *An. gambiae* s.l. tested by CSP	42	49	56	25	114	62	30	378
Sporozoites rate	0	6.1	5	8	14	3.2	0	5.2
EIR p/night	0	0.212	0.377	0.27	0.346	0.042	0	0.1778 (mean)
EIR p/month*	0	6.35	11.29	8.1	10.36	1.26	0	5.34 (mean)
Diebougou 5-month EIR post-IRS August-December 2012 = 31 infectious bites per person								

Table 3. Monthly *An. funestus* s.l. sporozoite rate and entomological inoculation rate from Dano (unsprayed area) and Diebougou (sprayed area) from June to December, 2012.

	June	July	August	September	October	November	December	2012 Total
Dano (unsprayed area)								
Total *An. funestus* s.l. (HLC) collected	22	123	96	112	161	114	76	704
CDC trap-nights (indoors + outdoors)	32	32	32	32	32	32	32	224
HBR per night	0.69	3.84	3	3.5	5.03	3.56	2.38	3.14
Total *An. funestus* s.l. tested by CSP	18	78	77	24	58	23	26	304
Sporozoites rate (%)	0	1.3	1.3	4.2	5.2	0	0	1.97
EIR p/night	0	0.05	0.039	0.147	0.262	0	0	0.061 (mean)
EIR p/month*	0	1.5	1.17	4.41	7.85	0	0	1.83 (mean)
Dano 5-month EIR post-IRS August-December 2012 = 13 infectious bites per person								
Diebougou (sprayed area)								
Total *An. funestus* s.l. (HLC) collected	5	15	92	56	64	64	72	368
CDC trap-nights (indoors + outdoors)	32	32	32	32	32	32	32	224
HBR per night	0.16	0.47	2.88	1.75	2	2	2.25	1.64
Total *An. funestus* s.l. tested by CSP	8	11	50	44	43	37	16	209
Sporozoites rate (%)	0	9.1	0	0	2.3	2.7	0	1.43
EIR p/night	0	0.043	0	0	0.046	0.054	0	0.023 (mean)
EIR p/month*	0	1.2796875	0	0	1.38	1.62	0	0.69 (mean)
Diebougou 5-month EIR post-IRS August-December 2012 = 3 infectious bites per person								

sprayed areas (average sporozoites rate = 6.05%; 95% CI: [3.509 - 12.59]) (t = 2.475; df = 9 with P = 0.022) (Table 2). The highest sporozoites rates were observed in Dano in August (average sporozoite rate = 13.6%; 95% CI: [9.68 - 17.33]) and September reaching an average of 14.2%. The similar trends were also observed in *An. funestus* s.l. sporozoites rate (Table 3) but in lowest proportions (average sporozoite rate = 1.97%; with 95% CI: [0.13 - 2.16] in unsprayed areas and average sporozoites rate = 1.47% with 95% CI: [0.37 - 2.01] in sprayed area) but the differencewas not significant (P = 0.051).

The major contributor to the EIR, both in the control and intervention areas, was *An. gambiae* s.l. (70%). The indoor EIR reached 134 infective bites/person during the five-month post-IRS in the unsprayed area Dano. IRS appears to have reduced the EIR four-fold in the sprayed area (31 infective bites /person) after spraying with P = 0.0001. *An. funestus* s.l. contributed also to the transmission in the two areas, with the similar results (EIR reduced 4-fold in sprayed area) compared to *An. gambiae* s.l. after spraying (mean EIR in Dano = 13 bi/p/n vs mean EIR in Diebougou = 3 bi/p/n with P = 0.003).

3.4. *An. gambiae* s.l. and *An. funestus* s.l. Blood Meal Sources

The results presented in Figure 6 included data from indoor PSC collections, for *An. gambiae* s.l. and *An. funestus* s.l. from the two areas. Irrespective of the sampling month, the proportion of *An. gambiae* s.l. blood-fed on human was highest, reaching more than 80% of the total of 335 females analysed, both in the sprayed and unsprayed areas. No female was recorded blood-fed only on animals. For *An. funestus* s.l. the feeding patterns were quite different, especially in

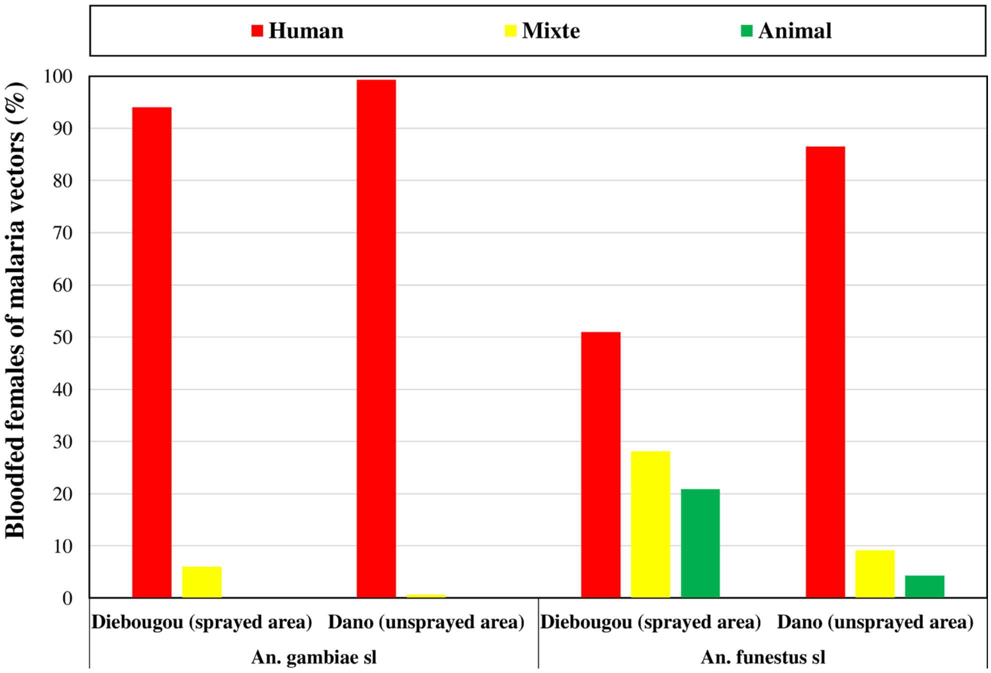

Figure 6. Proportion of *An. gambiae* s.l. and *An. funestus* s.l. blood-fed on humans, animals or mixed from sprayed area (Diebougou) and unsprayed area (Dano).

the sprayed area (Diebougou) where females showed a large range of hosts. Out of 132 *An. funestus* s.l. females analysed for their blood-fed origin, about 20% - 40% had taken a mixed bloodmeal (human, bovine and goat). A potential effect of the IRS on *An. funestus* s.l. was that the proportion of human blood meals decreased being replaced by animal and mixed blood meals.

4. Discussion

The IRS carried out in the Diébougou district of had a positive impact because it was able to protect 115,639 inhabitants including 23,118 children under 5 years and 2188 pregnant women who are the most vulnerable in thr country. The study showed that two species *An. gambiae* s.l. and *An. funestus* s.l. were predominant vectors of malaria transmission in study areas [28] collected using CDC LT and pyrethrum spray catches. Our findings confirmed again the *An gambiae* specie predominance among *An. gambiae* complex members in this part of Burkina Faso [29]. In addition, the results also indicated that the *An. gambiae* s.l. entomological inoculation rate was 4-fold lower in sprayed area compared to the unsprayed area, after the implementation of the IRS primarily due to a lower indoor biting rate and a significant decrease of malaria vectors sporozoite rates. However, the indoor resting density of vectors declined in the sprayed houses following IRS, but increased slightly in October, probably due to the relatively short residual duration of bendiocarb indicated in a separate manuscript. This drastic drop could be also due to the lethal effect of bendiocarb on the anophelines resistant to pyrethroids [40]. The biting rates observed outdoors were slightly higher in intervention areas compared to control areas and may be an early sign of biting behavior change. In conclusion IRS did not reduce the endophily behaviour from that of the baseline but had significantly reduced the density of mosquitoes resting indoors in sprayed area compared to the unsprayed area. In addition, EIR might have been impacted by the observed outdoor biting behaviour, and the reduced residual efficacy of the insecticide after September. The overall indoor biting rate of *An. funestus* s.l. was twofold greater in the unsprayed area. Indeed, the susceptibility status, taxonomy, and the role of *An. funestus* s.l. in malaria transmission was well documented in similar areas at west (Lena) and southwestern Burkina Faso (Soumousso) [41] [42].

The use of vector control tools and behaviors of the host would be the main factors that modify the behavior of human blood feeding observed on *An. gambiae* s.l. Indeed, recent studies showed that the long-term indoor application of residual insecticides contributes towards an increased tendency for outdoor feeding among malaria vector populations [43] [44]. The treatment had a great positive impact within *An. funestus* s.l. decreasing the human host-seeking in interventions sites compared to control sites. It is probably due to behavior change that this species is opting to go outside to seek a bloodmeal. It is important to highlight the exophagic host seeking activities and the exophilic behaviour that resulted in the search for blood in animals and mixed meals analyzed which more developed by *An. funestus* s.l. in the intervention area than in the control area where this vector remains mainly endophagic and endophilic. Indeed, after treatment there is less of *An. funestus* s.l. collected in intervention area compared to control area. Moreover, the exophagic host seeking recorded in intervention area was more pronounced than that obtained with *An. gambiae* s.l. Gillies and De Meillion [35] stated "…*Funestus* shows a closer adaptation to human dwellings than any other African anopheline. In many areas it spends the greater part of its adult life in houses, which has made it one of the most vulnerable of species to attack with residual insecticides". This statement is clearly valid in this case. Such a clear response to indoor insecticides makes the emergence of insecticide resistance in this species all the more likely [45]. Moreover, the results of impact of IRS on malaria transmission by *An. gambiae* s.l. indicated that the transmission was lower compared to the control area, after the implementation of the IRS, where the biting rate of *Anopheles* dropped drastically. This drastic drop is due to the lethal effect of bendiocarb on the anophelines resistant to pyrethroids [40] even though such efficacy did not last more than three months (discussed in a separate manuscript). The biting rates observed in outdoors were higher in intervention areas compared to control areas assuming a less pronounced behavior change of vectors.

In conclusion IRS did not reduce the endophily behavior from that of the baseline though it had significantly reduced the density of mosquitoes resting indoors compared to the control area. In addition, the findings have also shown *An. gambiae* s.l. were particularly anthropophilic in the two areas with few cases of mixed blood meals and no pure animal blood meals identified. This feeding pattern was the inverse for *An. funestus* s.l. in the intervention area where more females showed a large plasticity of the host range (zoo-anthropophilic). The results have shown that *An. gambiae* specie (former S-form) was the major malaria vector species biting in the southwestern region in Burkina Faso. Indeed, this corroborates previous reports [27] [28] of the *Anopheline* distribution in Burkina Faso, which explained the abundance of *An. gambiae* species by the ecological characteristics in this area.

5. Conclusions

The pilot study of IRS with bendiocarb appeared to have a significant impact on malaria transmission in the sprayed areas, as measured by EIR. Indeed, the results illustrated that IRS was strong enough to reduce mosquito abundance, sporozoite rate and EIR in pyrethroids resistance areas. However, the baseline period indicated intrinsic differences in biting rates between Dano and Diebougou before IRS.

The findings also showed a change in vector behavior, with *An. funestus* s.l. becoming more zoophagic after IRS. Furthermore, the residual efficacy of IRS did not last more than three months. In areas of high transmission, other insecticides with a longer life span covering the malaria transmission season need to be explored, in combination with LLINs. These results indicate not only the additional benefit of IRS strategy as a complementary intervention to LLINs but also the need to find new classes of insecticides (non-pyrethroids) to be used within the framework of the resistance management to insecticides published by WHO in 2012. These management strategies include insecticides rotations applied in IRS, mosaics spraying and also combinations of insecticides for better management of resistance to insecticides and in particular to pyrethroids, the only insecticide recommended for LLINs impregnation.

Ethics Approval and Consent to Participate

Ethical approval for this study was granted by the Ethical Committee of the Ministry of Health in Burkina Faso. The mosquito collectors gave prior informed consent and they were vaccinated against yellow fever. They were also subjected to regular medical check-ups with preventive treatments of malaria.

Authors' Contributions

KRD designed the study and drafted the manuscript. ASH, DDS and SPS performed field and laboratory activities: ASH, DDS, SPS, MN and KRD analysed the data; all authors drafted, revised the final version of the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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List of Abbreviations

IRS: Indoor Residual Spraying
LLIN: Long-lasting insecticidal nets
IRSS: Institut de Recherche en Sciences de la Santé
WP: Wet powder
NMCP: National Malaria Control Program
CDC: Center for Disease Control and Prevention
PSC: Pyrethrum spray catch
HBR: Human biting rate
b/p/n: Number bites per person per night
IRD: Indoor resting density
EIR: Entomological inoculation rate
WHOPES: World Health Organization Pesticide Evaluation Scheme
CSP: Circumsporozoite protein
ELISA-CSP: Enzyme-linked immunosorbent assay-circumsporozoite protein
HBR: Human biting rate
EIR: Entomological inoculation rate