Journal of Parasitology and Vector Biology Vol. 3(4), pp. 59-68, November 2011
Available online at http://www.academicjournals.org/JPVB
DOI : 10.5897/JPVB11.018
ISSN 2141-2510 ©2011 Academic Journals

Full Length Research Paper

Biology *of Anopheles gambiae* and insecticide resistance: Entomological study for a large scale of indoor residual spraying in south east Benin

G. G. Padonou[1,2]*, M. Sezonlin[1], G. L. Gbedjissi[1], I. Ayi[3], R. Azondekon[2], A. Djenontin[1,2], S. Bio-Bangana[2], O. Oussou[2], A. Yadouleton[2], D. Boakye[3] and M. Akogbeto[1,2]

[1]Faculte des Sciences et Techniques de l'Universite d'Abomey Calavi, Benin.
[2]Centre de Recherche Entomologique de Cotonou (CREC), Cotonou, Benin.
[3]Parasitology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana.

Accepted 8 November, 2011

Indoor residual spraying (IRS) has been proposed by the National Malaria Control Programme of Benin in the districts of Adjohoun, Dangbo, Misserete and Seme. Up to date entomological data are unavailable in these districts. To be effective, this measure must be based on the knowledge of biology of the malaria vectors. To achieve this aim, vector collections were made in the study area. A total of 49.059 Culicidae were captured. *Anopheles gambiae s.s* accounted for 20.91% while *Anopheles funestus* accounted for only 0.007%. *An. gambiae s.s* were molecular form M, resistant to DDT and permethrin, but susceptible to deltamethrin (mortality 100%) and bendiocarb (mortality 100%). Endophilic, blood feeding and endophagic rates were respectively estimated to be 65.74%, 53.23 and 70.18%. There was no seasonal variation of longevity, but the entomological inoculation rate ranged from 0 to 0.73 infective bites/ person / night according to localities and seasons. This study confirms a high spatial heterogeneity in mosquito distribution and shows that *An. gambiae s.s* is highly endophagic and endophilic (feed and rest indoor) in south east Benin. While the vector remains susceptible to deltamethrin, high levels of *kdr* suggest that the use of pyrethrinoid for IRS may be at risk.

Key words: *Anopheles gambiae*, endophily, blood feeding, parturity, entomological inoculation rate, resistance, IRS, Benin.

INTRODUCTION

The number of deaths attributed to malaria in 2008 was estimated to 863,000 of which 89% were in the African regions (WHO, 2009). Efforts, over several years, to control malaria, have given disappointing results. A major reason for failure is the resistance of the parasite, *Plasmodium falciparum,* to drugs and of the vector, *Anopheles gambiae* to pyrethroids used on insecticide-treated (mosquito) nets (ITNs). Since Ivory Coast (Elissa et al., 1993), vector resistance to pyrethroids has been reported in Kenya, East Africa (Vulule et al., 1994), Burkina (Chandre et al., 1999), South Africa (Hargreaves et al., 2000) and Mali (Fanello et al., 2003). Others studies conducted in Benin (Akogbeto and Yacoubou, 1999; Corbel et al., 2007, Yadouleton et al., 2010) also indicated that *Anopheles gambiae* is highly resistant to pyrethroids and DDT.

The roll back malaria (RBM) partnership was established in 1998 to encourage African countries to take further measures against malaria. In response, Benin adopted a national strategy favoring large-scale, integrated control measures, including ITNs for the protection of vulnerable populations (children under 5 years and pregnant women) and indoor residual spraying

*Corresponding author. E-mail: pagergil@yahoo.fr. Tel: (229) 97289615/ (229) 64848595. Fax: (229) 21308860.

(IRS) with bendiocarb. In support of this decision, the Ministry of Health (MOH) and the president's malaria initiative (PMI) of the USA Government agreed to spread IRS on a regional basis. Initially, four districts (Adjohoun, Dangbo, Misserete and Seme) in the department of Oueme, southern Benin, were selected for IRS. This vector control strategy must be implemented on the basis of valid entomological research. No entomological data from study area was available. Recent data from the neighboring district (Cotonou) in relation with the biology of Culicidae fauna and *An. gambiae* go all the way back to 1950 (Huttel, 1950; Hamon, 1954) and 1992 (Akogbeto, 1992). Thus entomological data were collected, as reference for the monitoring and evaluation of the IRS effectiveness on the dynamic of *Anopheles* populations and their behaviour, on malaria transmission and the insecticide resistance required for a good implementation of the IRS. To achieve this aim field activities and laboratories research were carried out from January 2008 to July 2008. This article reports the results of this entomological study.

MATERIALS AND METHODS

Study area

The study area is located in Republic of Benin (West Africa) and includes four districts of the Oueme region: Adjohoun, Dangbo, Misserete and Seme (Figure 1). The four districts cover an area of 977 km2 and an estimated 64,799 households. There are 62,890 children aged <5 years in 174 villages (INSAE, 2004). From 2002 to 2006, Oueme was the region with the highest rates of malaria-associated mortality (MS, 2007). The region is characterized by a sub-equatorial type climate. There are two ecological zones: a plateau zone and a swampy zone. In the present study, the plateau zone is referred to as the ''plateau area", and the swampy zone is called "peripheral area" (Figure1). In the plateau area, mosquito breeding sites are created particularly during the rainy seasons. But the peripheral area is made up of marshy land converted to vegetable gardens. Land management in this vegetable growing area creates perfect breeding site for *An gambiae*, the main vector of malaria which is highly resistant to pyrethroids (Padonou, 2008). The practice of growing vegetable crops in all districts has resulted in consumption of fertilizers and insecticides. The insecticides used were DDT and pyrethroids until 1970 (Alphacypermethrin, Cypermethrin and Deltamethrin) after the ban of DDT. Because of a high density of mosquitoes in Oueme region, the use of individual and collective measures (aerosol cans, plates for electric diffusers and mosquito coils containing pyrethroids) to protect against mosquito bites is widespread (Akogbeto and Yacoubou, 1999). Houses in both areas are generally built in similar shape. These houses are made of either mud or cement with large eave gaps facilitating entry and exit of mosquitoes.

Field mosquito collection

The sampling was done in 4 villages (2 in the plateau area and 2 in the peripheral area) per district by window traps (WT), indoor and outdoor human landing catches (HLC), indoor pyrethrum spray catches (PSC), and by collection of larvae and pupae.

To estimate endophagy and entomological inoculating rate of *An. gambiae*, mosquitoes collection by HLC were carried out during 14 surveys from January 2008 to July 2008 (6 in the dry season and 8 in the rainy season) every month for two consecutive nights per survey (8 person-night per district per survey). Teams of collectors were rotated among the collection points on different collection nights to minimize sampling bias. Ethical clearance was granted by the Ministry of Health of Benin and by Centre de Recherche Entomologique de Cotonou (CREC). Informed consent from all volunteers was obtained before their participation in the study. Malaria prevention and curative treatments were provided to all sleepers according to World Health Organization (WHO) recommended regimen on the basis of fever and detectable *P. falciparum* parasitemia.

The exophilic behaviour of mosquito population in the 4 districts was monitored by setting a total of 32 WT with 2 traps per house. Two mosquito catching sessions in each WT were organized each month from 1800 to 0630 h. After collecting exophilic mosquitoes from the WT, pyrethrum spray catches were done to collect indoor resting mosquitoes.

Larvae and pupae were collected from breeding sites of Adjohoun, Dangbo, Misserete and Seme within the peripheral area and the plateau, using standard dipping method. Larvae and pupae were reared to adults stage at Centre de Recherche Entomologique de Cotonou (CREC) insectary and subsequently used for bioassays.

Testing insecticide susceptibility

Females *An. gambiae* aged 2 to 5 days old were exposed to WHO diagnostic dosage of various insecticides according to the standard WHO testing protocol (WHO, 1998). The following insecticides were tested: deltamethrin (0.05%), permethrin (0.75%), DDT (4%) and bendiocarb (0.1%). Mosquitoes were exposed for one hour to insecticide-treated papers and monitored at different time intervals (10, 15, 20, 30, 45 and 60 min) to record the "knock-down". Mortalities were recorded after 24 h and the susceptibility status of the population was graded according to the WHO recommended protocol (WHO, 1998). All susceptibility tests were conducted in the CREC laboratory at 26 to 29°C and 74 to 82% relative humidity.

Dead and surviving mosquitoes were separately stored in individual tubes with silicagel and preserved at -20°C in the laboratory, for further molecular characterization.

Plasmodium falciparum detection and molecular analysis of mosquito samples

Anophelines were sorted and identified to species based on morphological characters using standard identification keys (Gillies, 1968). The physiological age of adult female *An. gambiae* was determined through dissection (Detinova, 1963).The head-thoraces of females from HLCs were tested for the presence of circum-sporozoite protein (CSP) of *P. falciparum* using enzyme-linked immunosorbent assay (ELISA) (Wirtz et al., 1987). In each locality, a sub-sample of 25 to 35 *An. gambiae* of the permethrin- tested specimens were processed by PCR concomitantly for identification of species and molecular forms of *An. gambiae* (Favia et al., 2001). Detection of Leu-Phe *kdr* and *Ace-1R* mutations in the same samples were performed by PCR from genomic DNA (Martinez-Torres et al., 1998; Weill et al., 2004).

Estimation of entomological parameters

The following entomological parameters were estimated; (1) Human biting rate (HBR): the number of *An. gambiae* biting a person during

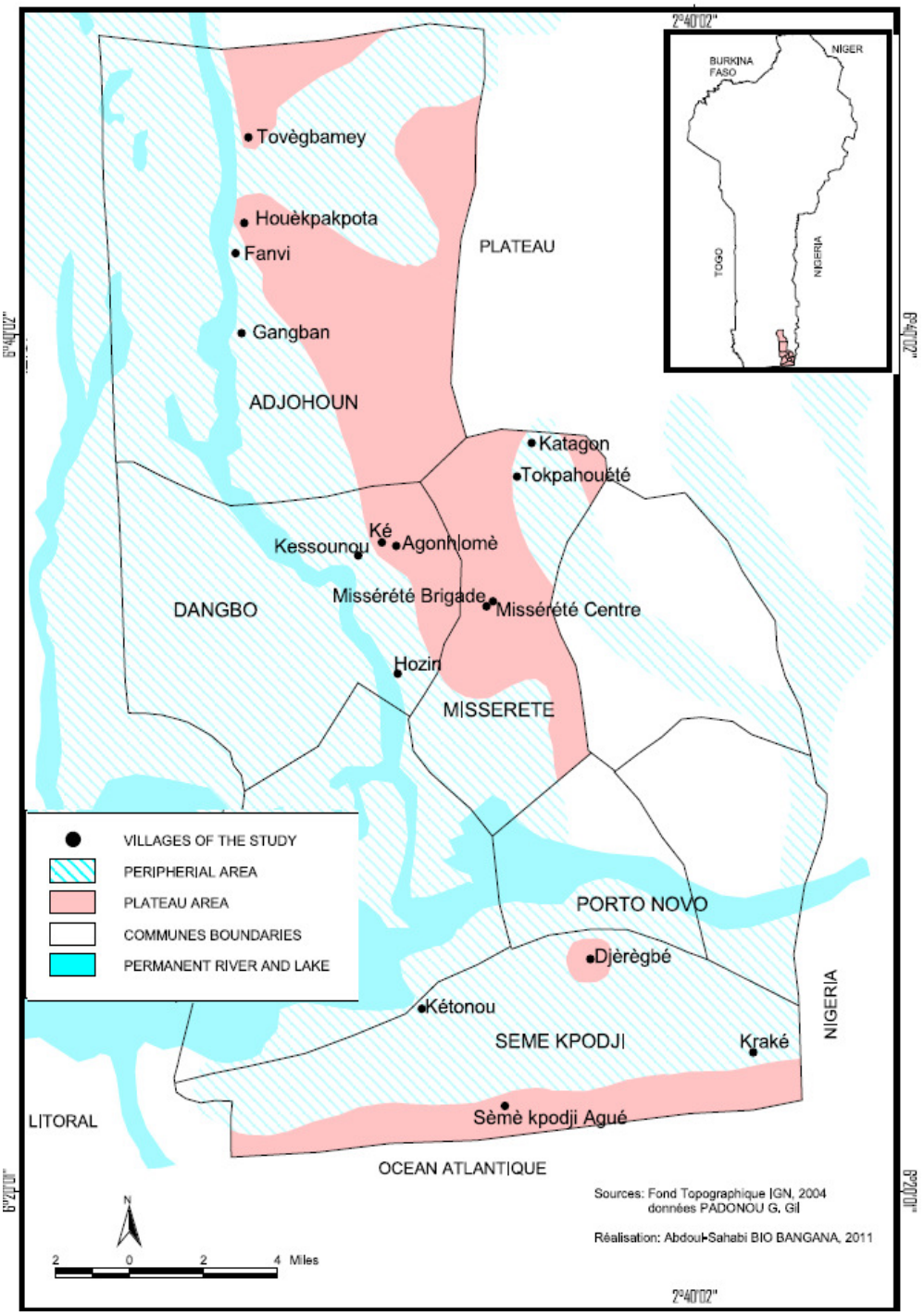

Figure 1. Map of study area.

a given time period (bites/p/t) (time being night, month or year). (2) Endophily rate: the number of *An.gambiae* at rest divided by the total number collected by PSC and the window trap converted to a percentage. (3) Blood-feeding rate: the percentage of blood fed mosquitoes collected divided by the total number collected by indoor PSC and the window trap. (4) Parous rate: the percentage of the number of *An.gambiae* having laid eggs at least once, divided by the total of *An.gambiae* females dissected. (5) The sensitivity of *An. gambiae* insecticide was defined according to WHO protocol (WHO, 1998). (6) Sporozoite rate: a ratio of the total number of mosquitoes infected divided by the total number of *An.gambiae* dissected. (7) Entomological inoculation rate (EIR): the number of infective bites per person per night is calculated, by multiplying the number of *An.gambiae* bites per person per night by

Table 1. Diversity of mosquitoes species collected at Adjohoun, Dangbo, Misserete and Seme, during the dry (January to March 2008) and rainy (April to July 2008) seasons (2 catch session per month).

Species	Adjohoun		Dangbo		Misserete		Seme		Total
	Nb Ds	Nb Rs	Nb Ds	Nb Rs	Nb Ds	Nb Rs	Nb Ds	Nb Rs	
Anopheles gambiae	124	1219	432	1966	198	1414	388	4518	10259
Anopheles funestus	0	1	0	1	0	1	0	0	3
Anopheles pharoensis	73	124	337	119	6	7	14	39	719
Anopheles ziemanni	0	0	9	4	0	0	0	5	18
Anopheles coustani	0	1	3	0	0	0	5	0	9
Culex quinquefasciatus	238	370	781	836	14	396	342	205	3182
Culex decens	30	150	187	1804	13	219	874	1640	4917
Culex nebulosus	197	706	100	1417	0	1306	1178	537	5441
Culex thalassius	0	88	41	13	0	0	0	0	142
Culex fatigans	0	0	0	0	0	7	0	0	7
Aedes aegypti	18	15	20	44	25	36	4	7	169
Aedes palpalis	1	15	0	16	3	108	0	0	143
Mansonia africana	7353	2404	7603	2815	1065	452	1793	542	24027
Mansonia uniformris	5	2	3	2	1	2	3	5	23
Total	8039	5095	9516	9037	1325	3948	4601	7498	49059

Nb Ds : Number in dry season ; Nb Rs: Number in rainy season.

the sporozoïte rate.

Statistical analysis

Normality was tested by a Shapiro-Wilk test and we used a rank sum test, Kruskal-Wallis to compare the variables of density, endophagy and aggressivity between localities, between ecological zones and between seasons. ANOVA was used for the analysis of the means of endophily, gravidity and parturity. Comparison of the *kdr* frequency among localities was performed using Fisher's exact test with Genepop software. The significance level was set at 5%.

RESULTS

Species diversity

Four mosquito genera, comprising 14 species, were collected and identified taxonomically: 4 *Anopheles*, 5 *Culex*, 3 *Aedes* and 2 *Mansonia* (Table 1). The Culicidae fauna was present during the dry and rainy seasons and at all localities. However, its composition and size vary. Total culicidae captured was 49,059 of which 38,051 (77.56%) are *Mansonia africana, Culex nebulosus, C. quinquefasciatus* and *C. decens*. The Anophelines included *An. gambiae s.l,* representing 20.91% of all Culicidae, *An. funestus,* representing only 0.007%, and *An. pharoensis*, *An. coustani* and *An. ziemani,* at very low percentages.

Endophilic and blood feeding rate

Endophily ranged from 60.9% at peripheral of Adjohoun to 73.3% at Misserete. The overall comparison of different rates showed no significant difference (Table 2). Endophily was comparable from one locality to another as well as between ecological zones. More than half (53.2%) of the *An. gambiae* caught were blood fed and 38.8% were gravid. Significant variation (Table 2) in blood feeding rate was observed between localities and according to ecological zones (Table 2). In the plateau area blood feeding rate was significantly lower at Misserete (50.7%), Adjohoun (52.4%), Dangbo and Seme (54.3%) than all other localities (Table 2). However, it was higher at peripheral of Dangbo where 70.2% of mosquitoes blood fed. Blood fed females were more common than gravid females throughout the study area (Table 2).

Endophagy

A total of 896 HLC sessions yielded 9,068 *An. gambiae*. 6,364 *An. gambiae* were captured by indoor human landing catch versus 2,704 *An. gambiae* collected outdoors. Endophagy rate was estimated at 70.18% (Table 3), that is approximately 3/4 of the *An. gambiae* sampled preferred to take their meals inside the houses. This relatively high degree of endophagy ranged between 59.78% (Misserete peripheral) and 79.57% (Dangbo plateau) (Table 3) and characterized all localities. There was no significant difference between localities and between ecological zones (Table 3). This requires a prevention strategy against aggressive mosquitoes inside

Table 2. Endophily, blood feeding and gravid behaviour of *Anopheles gambiae* in Adjohoun, Dangbo, Misserete and Seme districts (May to July 2008).

Locality	Total	Npsc	N gravid	N blood fed	Parameter					
					Endophily rate (%)		Gravid rate (%)		Blood feeding rate (%)	
					Mean	[95 %CI]	Mean	[95 %CI]	Mean	[95 %CI]
Adjohoun pl	84	58	26	44	69.45^{a}	[59.07-79.82]	30.75^{a}	[24.09-37.40]	52.38^{ab}	[50.37-53.62]
Dangbo pl	84	53	31	46	63.86^{a}	[51.98-75.73]	36.59^{ab}	[33.91-39.27]	54.33^{ab}	[50.36-58.29]
Misserete pl	84	52	29	43	62.12^{a}	[53.33-70.91]	34.19^{ab}	[30.43-37.94]	50.66^{a}	[44.85-56.47]
Seme pl	388	250	142	151	64.38^{a}	[57.59-71.16]	36.60^{ab}	[29.66-43.54]	54.33^{a}	[43.74-64.92]
Adjohoun per	91	55	41	61	60.94^{a}	[52.48-69.40]	44.72^{cd}	[41.44-47.99]	66.66^{cd}	[61.89-71.43]
Dangbo per	82	50	38	57	61.87^{a}	[55.25-68.50]	46.93^{d}	[41.43-52.43]	70.16^{d}	[62.05-78.27]
Misserete per	288	210	120	180	73.30^{a}	[66.95-79.64]	41.83^{bcd}	[39.00-44.66]	62.16^{bcd}	[58.10-66.22]
Seme per	90	55	35	52	61.38^{a}	[52.19-70.57]	39.02^{cd}	[32.81-45.22]	58.33^{abc}	[49.19-67.47]
Total	1191	783	462	634	65.74	[62.12-67.20]	38.79	[36.86-40.79]	53.23	[55.99-61.17]

pl: plateau; per : peripheral; C I: 95% Confidence Interval; psc: pyrethrum spray catch; for a same parameter of the table values in column with different superscript were significantly different ($p<0.05$).

Table 3. Human biting rate (HBR) and endophagy of *An. gambiae* by locality and ecological zone.

Locality	Total number	Number of man night	Total number inside	Endophagy (%)
Adjohoun pl	272	112	200	73.52^{a}
Dangbo pl	328	112	261	79.57^{a}
Misserete pl	136	112	88	64.70^{a}
Seme pl	1692	112	1080	63.82^{a}
Adjohoun per	896	112	592	66.07^{a}
Dangbo per	1904	112	1375	72.21^{a}
Misserete per	1104	112	660	59.78^{a}
Seme per	2736	112	2108	77.04^{a}
Total	9068	896	6364	70.18
Ecological zone				
pl area	2428	448	1629	67.09^{a}
per area	6640	448	4735	71.31^{a}

pl: plateau; per : peripheral; for a same parameter of the table values in column with different superscript were significantly different ($p<0.05$).

houses.

Lifespan and infectivity

The parous rate for *An. gambiae* ranged from 68.18 to 80.06% (Table 4a, b). The average parous rate was 77.10% with variations from one locality to another. At Seme peripheral, the parous rate appeared higher (80.06%) but comparable with other localities (Table 4b). At Adjohoun peripheral the rate was lower ($p < 0.05$). There was no seasonal variation in longevity. The entomological inoculation rate (EIR) ranged from 0 to 0.73 infective bites per person per night (Tables 4a, b). Projecting this rate throughout the study period, it appears that a man received theoretically between 14.7 and 21 infective bites in 7 months (January to July). However, EIR was higher in Seme (94.5 to 153.3 infective bites in 7 months) and Adjohoun peripheral (100.8 infective bites in 7 months). These infections were obtained between April and July during the main rainy season. Between January and March (main dry season) EIR was zero except Seme plateau and Dangbo plateau (Table 4a) where one person received respectively 3.6 and 1.8 infective bites in three months.

Sensitivity, kdr genotype, species and molecular forms of *Anopheles gambiae*

Bioassays using non-insecticidal papers showed the control mortality rate in wild populations from each locality to be less than 5%. Overall *An. gambiae* showed

Table 4a. Parous rate and entomological inoculation rate of *An. gambiae* s.l. collected by HLC in the plateau area of Adjohoun, Dangbo, Misserete and Seme (south of Benin).

Locality		Dry season		Rainy season		January to July 2008	
		January to March 2008		April to July 2008			
Adjohoun plateau	N Total	65		207		272	
	Parturity %	81.67[a]		77.25[a]		79.41[b]	
	HBR	1.35	[74.08-89.26]	3.23	[71.68-82.82]	2.43	[75.74-83.07]
	N (ELISA)	58		70		128	
	S%	0		5.71		3.12	
	EIR (b/m/n)	0		0.18		0.07	
Dangbo plateau	N Total	48		280		328	
	Parturity %	84.67[a]		75.75[a]		79.87[b]	
	HBR	1	[70.99-98.35]	4.375	[60.25-91.25]	2.93	[71.39 -88.34]
	N (ELISA)	48		89		137	
	S%	2.08		4.49		3.64	
	EIR (b/m/n)	0.02		0.19		0.1	
Misserete plateau	N Total	38		98		136	
	Parturity %	76[a]		66[a]		70.58[ab]	
	HBR	0.79	[73.52-78.48]	1.53	[54.31-77.69]	1.21	[63.56 -77.59]
	N (ELISA)	38		78		116	
	S%	0		0		0	
	EIR (b/m/n)	0		0		0	
Seme plateau	N Total	100		1592		1692	
	Parturity %	84.67[a]		76.25[a]		80[b]	
	HBR	2.08	[78.42-90.92]	24.88	[68.63-83.87]	15.11	[74.49 -85.50]
	N (ELISA)	52		114		166	
	S%	1.92		3.5		3.01	
	EIR (b/m/n)	0.04		0.87		0.45	

N Total: Total number; HBR: Human bite rate; N(ELISA): Number tested by ELISA CSP; S%: Sporozoite index; EIR (b/m/n): Entomological inoculating rate (infected bite/man/night); for a same parameter of the table, values in the dry season and in the rainy season which carry different letters in exposant were significantly different ($p<0.05$).In the column of January to July 08 values which carry different letters in exposant were significantly different ($p<0.05$) from one locality to other locality.

susceptibility to bendiocarb and deltamethrin was observed everywhere: 100% of *An. gambiae* tested were dead (Table 5). PCR has revealed 100% of mosquitoes tested were *Anopheles gambiae s.s.* M form. The frequency of the *kdr* alleles was 79.35% and frequency of *Ace-1* was 0% (Table 6). Furthermore, the survival rate obtained among the *An. gambiae s.l.* populations tested with permethrin was correlated with the frequency of the *kdr* allele (correlation coefficient $R^2 = 0.96$) as shown in Figure 2.

DISCUSSION

The present study provides entomological baseline data in the 4 districts before IRS in Benin. In this study, 14 species of Culicidae: 4 *Anopheles*, 5 *Culex*, 3 *Aedes* and 2 *Mansonia* were collected. In south east Benin (Cotonou and Porto Novo) Huttel (1950) and Hamon (1954) had identified the same species in varying proportions, but without reporting the presence of *Culex fatigans, Cx. quinquefasciatus* and *Aedes palpalis*. The factor that could explain the presence of these vectors is urbanization (Hamon et al., 1967). Another author, Akogbeto (1992), reported 8 species in Southern Benin with *Mansonia sp.*, *An. gambiae s.l.*, *Culex thalassius* and *Culex. gr. decens.* Moreover, Djenontin et al. (2010) revealed 28 different species of Culicidae with *M. africana*, *C. gr. decens*, *C. quinquefasciatus*, *C. nebulosus*, *Anopheles nili*, *An. funestus s.s.* and *Anopheles leesoni* in southwest Benin. The variability in ecological diversity noted by different authors is probably

Table 4b. Parous rate and entomological inoculation rate of *An. gambiae* s.l. collected by HLC in the peripheral area of Adjohoun, Dangbo, Misserete and Seme (south of Benin).

Locality		Dry season January to March 08		Rainy season April to July 08		January to July 08	
Adjohoun peripheral	N Total	59		837		896	
	Parturity %	69.67[a]	[65.87-73.46]	67[a]	[61.34-72.66]	68.18[a]	[65.37 - 70.98]
	HBR	1.23		13.08		8	
	N (ELISA)	59		170		229	
	S%	0		1.17		0.87	
	EIR (b/m/n)	0		0.15		0.07	
Dangbo peripheral	N Total	384		1520		1904	
	Parturity %	76.33[a]	[64.08-88.59]	71.25[a]	[61.49-81.01]	73.77[ab]	[68.31 - 79.22]
	HBR	8		23.75		17	
	N (ELISA)	109		170		279	
	S%	0		4.7		2.86	
	EIR (b/m/n)	0		1.11		0.48	
Misserete peripheral	N Total	160		944		1104	
	Parturity %	75.67[a]	[70.50-80.84]	74.75[a]	[58.30-91.20]	75.2[ab]	[68.33 -82.06]
	HBR	3.33		14.75		9.86	
	N (ELISA)	46		93		139	
	S%	0		1.07		0.71	
	EIR (b/m/n)	0		0.15		0.07	
Seme peripheral	N Total	288		2448		2736	
	Parturity %	83[a]	[74.04-91.96]	77.75[a]	[69.49-86.01]	80.06[b]	[75.29 - 84.82]
	HBR	6		38.25		24.43	
	N (ELISA)	76		124		200	
	S%	0		4.83		3	
	EIR (b/m/n)	0		1.84		0.73	

N Total: Total number; HBR: Human bite rate; N(ELISA): Number tested by ELISA CSP; S%: Sporozoite index; EIR (b/m/n): Entomological Inoculating Rate (infected bite/man/night); for a same parameter of the table, values in the dry season and in the rainy season which carry different letters in exposant were significantly different ($p<0.05$).In the column of Jan.-Jul. 08 values which carry different letters in exposant were significantly different ($p<0.05$) from one locality to other locality.

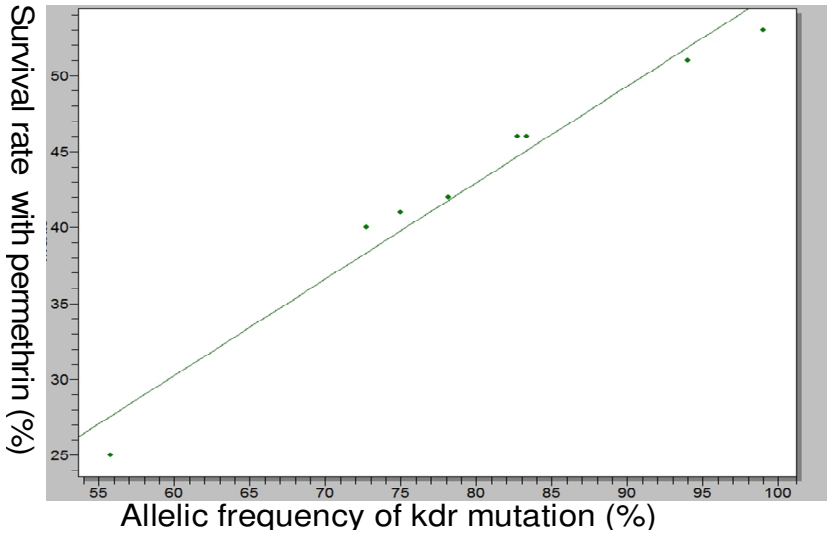

Figure 2. Correlation between *kdr* frequency and survival rates with permethrin.

Table 5. Percentage of dead *Anopheles gambiae* observed after 1 hour exposure to 0.75% permethrin, 0.01% bendiocarb, 4% DDT and 0.05% deltamethrin in the localities of Adjohoun, Dangbo, Misserete and Seme (south of Benin) from January to July 2008.

Locality	Permethrin				DDT				Deltamethrin			Bendiocarb		
	N	% Mort		St	N	% Mort		St	N	Mort (%)	St	N	Mort (%)	St
		Mean	[95% CI]			Mean	[95% CI]							
Adjohoun pl	100	60.00^{b}	[54.80- 65.20]	R	100	32.00^{b}	[26.80-37.20]	R	100	100	S	100	100	S
Dangbo pl	100	54.00^{ab}	[45.78- 62.22]	R	100	20.00^{a}	[14.80- 25.20]	R	100	100	S	100	100	S
Misserete pl	100	49.00^{a}	[42.91- 55.09]	R	100	22.00^{ab}	[13.78- 30.22]	R	100	100	S	100	100	S
Seme pl	100	47.00^{a}	[40.91- 53.09]	R	100	19.00^{a}	[12.91- 25.09]	R	100	100	S	100	100	S
Adjohoun per	100	75.00^{c}	[68.91- 81.09]	R	100	30.00^{b}	[21.78- 38.22]	R	100	100	S	100	100	S
Dangbo per	100	59.00^{b}	[52.91- 65.09]	R	100	23.00^{ab}	[13.45- 32.55]	R	100	100	S	100	100	S
Misserete per	100	58.00^{b}	[49.78- 66.22]	R	100	26.00^{ab}	[17.78- 34.22]	R	100	100	S	100	100	S
Seme per	100	54.00^{ab}	[45.78- 62.22]	R	100	25.00^{ab}	[18.91- 31.09]	R	100	100	S	100	100	S
Total	800	57	[54.12- 59.38]	R	800	24.62	[22.49- 26.76]	R	800	100	S	800	100	S

pl: plateau; per : peripheral; N: Number tested; C I : 95% confidence interval ; St: status; R: resistance; S: susceptible; mort : mortality ; For each insecticide, for a same parameter, values which carry different letters in exposant were significantly different *(p<0.05)*.

Table 6. Species identification, molecular forms (Mf) and frequency of the *kdr* and *Ace-1* genotypes (RR,RS,SS) in *Anopheles gambiae s.l.* females collected in the localities of Adjohoun, Dangbo, Misserete and Seme (south of Benin) from January to July 2008.

Locality	Species	Mf	Kdr mutation				Ace-1 mutation			
	An. gambiae s.s	M Form	RR	RS	SS	*F (Kdr)*	RR	RS	SS	*F (Ace-1)*
Adjohoun pl	24	24	15	5	4	72.91^{a}	0	0	24	0
Dangbo pl	28	28	20	5	3	80.35^{a}	0	0	28	0
Misserete pl	28	28	24	4	0	92.85^{b}	0	0	28	0
Seme pl	25	25	24	1	0	98^{bc}	0	0	25	0
Adjohoun per	26	26	07	15	4	55.78^{a}	0	0	26	0
Dangbo per	30	30	17	12	1	76.66^{a}	0	0	30	0
Misserete per	28	28	16	12	0	78.57^{a}	0	0	28	0
Seme per	29	29	25	0	4	86.20^{ab}	0	0	29	0
Total	218	218	148	54	16	80.16	0	0	218	0

pl: plateau; per : peripheral; for a same parameter of the table values which carry different letters in exposant were significantly different *(p<0.05)*.

based on sampling techniques used, periods of study, study area and population dynamics of mosquitoes under external pressure. The presence of *An. gambiae s.l* in all localities during the rainy season and dry season could be explained by the permanent presence and the abundance of small

collections of natural waters. In contrast, the low presence of *An. funestus* in Adjohoun, Dangbo and Misserete could be justified by the richness of the soil in organic matter and human activities that pollute larval breeding sites and makes them less favorable to its multiplication; while this absence in Seme was likely due to soil salinity because of the proximity of the sea. Unlike to studies conducted in Benin (Akogbeto, 1992) and Cameroun (Atangana et al., 2009), in dry season (January, February, March) more Culicidae were captured (Adjohoun: 61.20%; Dangbo: 51.29%) than in rainy season (April, May, June and July) (Adjohoun: 38.8%; Dangbo: 48.71%). This could be explained by the overflow of the Oueme River enlarged by rains from the northern region of Benin. When the waters recede, small pockets of water form, creating favorable breeding sites of *M. africana*, *Culex sp* and the *An. gambiae* in December, January and February. But, the strong tendency for the endophily of *An. gambiae* (Mouchet, 1957) noticed in all localities could be justified by factors including the scarcity of outdoor shelters and the proximity of many breeding sites in the residential area. The endophagy could be justified by the juxtaposition of habitat because of communal lifestyle and the average number of 2.9 persons per bedroom facilitating the taking of human blood meal.

During the study period, the EIR estimated by extrapolation to 164.5, 175.2 and 266.45 infective bites per man per year, respectively for Seme plateau, Dangbo peripheral and Seme peripheral is higher compared to those mentioned by Akogbeto et al. (1992) in southern Benin. In this study, the determination of the sporozoite was not done by ELISA for the presence of the CS protein, but was based on the detection in salivary glands by microscopy which might account for the lowest levels of sporozoite. However these EIRs are similar to those described by Adja et al. (2006) in the savannah zone of Côte d'Ivoire (EIR = 410 infective bites per year) where *An. gambiae*, *An. funestus* and *An. nili* were involved in malaria transmission and in western of Cameroon, where EIRs were estimated at 100.8 infective bites / man / year (Atangana et al., 2009). The number of infective bites per man per year, estimated at 25.55 (Adjohoun plateau, Adjohoun peripheral and peripheral Misserete), 36.5 (Dangbo plateau) and 0 (Misserete plateau) are significantly lower than the values for similar areas in south Benin in 1990 (Akogbeto et al., 1992). These low levels of the EIR are may reflect the widespread use of mosquito coils and promotion of ITNs. Despite these low EIR, the IRS is needed. Because an EIR less than 20 infective bites per man per year is sufficient to maintain a transmission of malaria (Akogbeto, personal communication). The variability of EIR observed in the 4 localities according to the seasons, could be justify by the rarity of *An. gambiae* in dry season.

From the perspective of molecular tools, data analysis confirmed that *An. gambiae* M form is the principal vector of malaria in the study area. The absence of the S molecular form showed that it must have been the ecological characteristics of the department of Oueme that did not support its selection. The high frequency of *kdr* observed in *An. gambiae* population has confirmed previous studies in south of Benin (Akogbeto and Yacoubou, 1999; Corbel et al., 2007; Yadouleton et al., 2010). The correlation that we observed between the frequency of the *kdr* allele and the survival rate following exposure to permethrin suggests that the substitution type Leu1014 Phe in *An gambiae* may be a contributing mechanism underlying this resistance (Ranson et al., 2000). This mutation is justified by the fact that the study area is dominated by farming activities and subjected to selection pressure exerted by the pesticides used by farmers to protect their crops (Akogbeto et al., 2005). The promoting of the use of ITNs encouraged by WHO, might have also contributed to this selection pressure of pesticides. The difference between mortality rate to DDT (22.62%) and permethrin (57%) of the study area could be explained by the involvement of other mechanisms resistance such as metabolic resistance (Corbel et al., 2007). The absence of resistance to bendiocarb has confirmed the previous studies carried out by Akogbeto et al. (2010) and Yadouleton et al. (2010).

Conclusion

The present study provides useful information on the mosquito species composition in south east of Benin and supports the feasibility of IRS as an anti-vector malaria control intervention in Adjohoun, Dangbo, Misserete and Seme. *An. gambiae* is highly endophagic, endophilic (feed and rest indoor) and resistant to permethrin and DDT. The choice of pyrethrinoid treatment of houses is not desired. A possible alternative is the use of bendiocarb, to which we have observed a good sensitivity in the case of *An. gambiae*. In case this product is held there is hope that malaria transmission will be reduced drastically.

ACKNOWLEDGEMENTS

We are grateful to the PMI (President Malaria Initiative) which supported financially this study through USAID and RTI. The authors would like to thank Dr N'Guessan Raphaèl of London School of Hygiene and Tropical Medicine in Cotonou for his helpful suggestions and correction made to the manuscript. We also thank populations of Adjohoun, Dangbo, Seme, and Misserete for their collaboration.

REFERENCES

Adja AM, N'Goran KE, Kengue P, Koudou GB, Touré M, Koffi AA, Tia E, Fontenille D, Chandre F(2006). Transmission vectorielle du paludisme en savane arborée à Gansé en Côte d'Ivoire. Med. Trop., 66: 449-455.

Akogbéto M (1992). Etude des aspects épidémiologiques du paludisme côtier lagunaire au Bénin. Thèse de doctorat, Université de Paris XI.
Akogbéto M, Chippaux JP, Coluzzi M (1992). Le paludisme urbain côtier à Cotonou (République du Bénin). Rev. Epidemiol. Sante. Publique., 40: 233-239.
Akogbéto M, Djouaka R, Noukpo H (2005). Use of agricultural insecticides in Benin. Bull. Soc. Pathol. Exot., 98: 400-405.
Akogbéto M, Yakoubou S (1999). Résistance des vecteurs du paludisme vis-à-vis des pyréthrinoïdes utilisés pour l'imprégnation des moustiquaires au Bénin, Afrique de l'Ouest. Bull. Soc. Pathol. Exot., 92: 123-130.
Akogbéto MC, Padonou GG, Gbénou D, Irish S, Yadouleton A (2010). Bendiocarb, a potential alternative against pyrethroid resistant *Anopheles gambiae* in Benin, West Africa. Malar. J., 9: 204.
Atangana J, Fondjo E, Fomena A, Tamesse LJ, Patchoké S, Ndjemai MN, Hamadou NMN, Prosper ABN (2009). Seasonal variation of malaria transmission in Western Cameroon highlands: Entomological and clinical investigations. J. Cell. Anim. Biol., 3: 033-038.
Chandre F, Darriet F, Manga L, Akogbeto M, Faye O, Mouchet J, Guillet P (1999). Status of pyrethroid resistance in *Anopheles gambiae* sensu lato. Bull. WHO., 77: 230-234.
Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbenou L, Martin T, Akogbeto M, Hougard JM, Rowland M (2007). Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. Acta. Trop., 101: 207-216.
Détinova TS (1963). Méthode à appliquer pour classer par groupe d'âge les diptères présentant une importance médicale. Org. Mond. Santé. Sér. Mono., 47.
Djènontin A, Bio-Bangana S, Moiroux N, Henry MC, Bousari O, Chabi J, Ossè R, Koudénoukpo S, Corbel V, Akogbéto M, Chandre F (2010). Culicidae diversity, malaria transmission and insecticide resistance alleles in malaria vectors in Ouidah-Kpomasse-Tori district from Benin (West Africa): A pre-intervention study. Parasit Vectors, 3: 83.
Elissa N, Mouchet J, Rivière F, Meunier JY, Yao K (1993). Resistance of *Anopheles gambiae* s.s. to pyrethroids in Côte d'Ivoire. Ann. Soc. Belg. Med. Trop., 73: 291-294.
Fanello C, Petrarca V, della Torre A, Santolamazza F, Dolo G, Coulibaly M, Alloueche A, Curtis CF, Touré YT, Coluzzi M (2003). The pyrethroid knock-down resistance gene in the *Anopheles gambiae* complex in Mali and further indication of incipient speciation within *An. gambiae s.s* . Insect. Mol. Biol., 12: 241-245.
Favia G, Lanfrancotti A, Spanos L, Siden Kiamos I, Louis C (2001). Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae s.s.* Insect. Mol. Biol., 10: 19-23.
Gillies MT, De Meillon B (1968). The Anophelinae of Africa south of the Sahara. South. Afr. Inst. Med. Res. Johannesburg, 54: 1-343.
Hamon J, Burnett G, Adam J P, Rickenbach A, Grjebine A (1967). *Culex pipiens* fatigans Wiedemann, Wuchereria bancrofti Cobbold, et le développement économique de Afrique tropicale. Bull. Org. mond. Santé, 37: 217-237.
Hargreaves K, Koekemoer LL, Brooke BD, Hunt RH, Mthembu J, Coetzee M (2000). *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. Med. Vet. Entomol., 14: 181-189.
Huttel J (1950). Note sur la répartition des moustiques dans le Bas-Dahomey. Bull. Soc. Pathol. Exot., 43: 563-566.
INSAE(2004). Fichier village Ouémé. Porto-Novo. 29.
Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, Guillet P, Pasteur N, Pauron D (1998). Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae s.s.* Insect. Mol. Biol., 7: 179-184.
Ministère de la Santé (2007). Annuaire des statistiques sanitaires 2006.
Mouchet J, Gariou J (1957). Exophilie et exophagie d'*Anopheles gambiae* Giles 1902, dans le sud Cameroun. Bull. Soc. Pathol. Exot., 3*:* 446-461.
Padonou GG (2008). Evaluation en cases expérimentales de l'efficacité de quelques insecticides pyréthrinoïdes, organophosphorés et carbamate en pulvérisation intradomiciliaire. Mémoire de Master d'entomologie appliquée, Université d'Abomey Calavi.
Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH (2000). Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. Insect Mol Biol., 9: 491-497.
Vulule JM, Beach RF, Atieli FK, Roberts JM, Mount DL, Mwangi RW (1994). Reduced susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin-impregnated bednets and curtains in Kenya. Med. Vet. Entomol., 8: 71-75.
Weill M, Malcolm C, Chandre F, Mogensen K, Berthomieu A, Marquine M, Raymond M (2004). The unique mutation in *Ace-1* giving high insecticide resistance is easily detectable in mosquito vectors. Insect. Mol. Biol., 13: 1-7.
World Health Organization (WHO) (1998). Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Document WHO/CDS/CPC/MAL/98.12. World Health Organization, Geneva.
World Health Organization (WHO) (2009). World Malaria Report. World Health Organization, Geneva.
Wirtz RA, Zavala F, Charoenvit Y, Campbell GH, Burkot T, Schneider I, Esser KM, Beaudoin RL, Andre RG (1987). Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. Bull. WHO., 65: 39-45.
Yadouleton AW, Padonou G, Asidi A, Moiroux N, Banganna S, Corbel V, N'guessan R, Gbenou D, Yacoubou I, Gazard K, Akogbeto MC (2010). Insecticide resistance status in *Anopheles gambiae* in southern Benin. Malar. J., 9: 83.