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RESEARCH ARTICLE

ABUNDANCE AND DIVERSITY OF CULICIDAE FAUNA AT COTONOU IN SOUTHERN BENIN

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ABSTRACT

Background: To evaluate the risk of transmission of vector-borne diseases transmitted by mosquitoes, regular updates of the geographic distribution of theses vectors are required. Therefore, a study base on the abundance, richness and diversity of mosquitoes was conducted between December, 2013 and November 2014 at Cotonou, the economic capital of Benin republic.

Method: A cross seasonal entomological study on larvae and adults of mosquitoes was carried in fourteen (14) locations randomly at Cotonouin Peri-Urban (PU) and urban areas (UA). The study was based on sampling mosquito immature stages (larvae/pupae) from domestic, peri-domestic and natural water sources and reared to adults. Additional adults mosquito were collected by Human Landing Catches (HLC), Indoor Pyrethrum Spray Catches (PSC), Biogents (BG) sentinel traps and Windows traps (WT) in PU and UA in order to assess the richness of mosquito fauna in this city.

Results: Adult's mosquitoes from larvae collected in our study sites showed thirteen species of mosquitoes (including 3 *Anopheles*species, 5 *Culex*species, 4 *Aedes*species and 1 *Uranotaenia* species) belonging to 4 genera. However, when the adults came directly from HLC and the different traps cited above, 15 species of mosquitoes including 3 *Anopheles*species, 5 *Culex*species, 2 *Mansonia* species, 4 *Aedes* species and 1 *Uranotaenia* species belonging to 5 genera (*Culex*, *Aedes, Anopheles, Uranotaenia* and *Mansonia*). The abundance of the mosquito genera recorded at PU and UA varied significantly ($p<0.05$), with the *Culex* genera recording the highest abundance.

Moreover, the mosquito species recorded at PU regardless the collection methods was higher significant than those obtained at UA ($P< 0.05$).

Conclusion: The presence of *Culex, Aedes*, and *Anopheles* species highlighted by this study in the city of Cotonou is posing a serious epidemiological concern to the inhabitants of this city. Therefore, larviciding of breeding sites before the onset of the rainy season and public enlightenment on the **environmental factors** and/or human activities that encourage mosquito breeding are recommended.

INTRODUCTION

Background

Mosquitoes (*Diptera, Culicidae*) are considered one of the most relevant group of arthropods in the public health field (Schaffner *et al*., 2001 and Becker *et al*., 2010) and, like other organisms, show a direct relation to different factors such as environmental and habitat heterogeneity or host preferences (Zhong *et al*., 2003). Belongining to *Culicidae* family, mosquitoes are nuisance species as well as potential transmitters of zoonotic and human diseases such as dengue fever, yellow fever and malaria of which 1.4 million deaths are recorded yearly (ampbell-Lendrum *et al*., 2005) with 17% of all infectious diseases worldwide (Townson *et al*., 2005). According to the most recent classification of mosquitoes, the family Culicidae (Diptera) includes two subfamilies, 11 tribes, 111 genera, and 3,523 species where 150 species of the genera **Anopheles**, **Aedes** and **Culex**, are the indirect cause of morbidity and mortality among humans, more than any other group of organisms (Harbach, 2007) in the world. In context of climate change, eenvironmental changes due to human activities greatly influence the distribution and survival of many mosquito species (Dossou-Yovo *et al*., 1995; Adeleke *et al.,* 2008; Amusan *et al.,* 2005). Moreover, in many African countries as Benin, deteriorating infrastructure, poor access to health, water and sanitation services, increasing population density, and widespread poverty contribute to conditions that modify the environment in peri urban areas, which directly

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offers a suitable condition of mosquito development (Parnell and Walawege, 2011). Cotonou, the economic capital of Benin (West Africa), is located on a strip of land between the lakeNokoue and the Gulf of Guinea offers suitable condition for several species of mosquitoes development which transmitted malaria (Akogbeto, 1992; Armel Djènontin *et al*., 2010; Yadouléton *et al.,* 2010), dengue fever (Yadouleton *et al*., 2014) and other *filariasis* (Yadouléton *et al.,* 2015). Moreover, the lackofchannels of storm waterandwastewaterin many areas of this city contribute alsotothe presence of more mosquitoes breeding sites which representlarval habitats of different type of mosquitoes. Constant studies on biology and larval ecology of mosquitoes have been observed as important tools in mosquito control (Kim *et al.,* 2010) and such studies will help to determine the existing and disappearing mosquito species, relative population densities and the extent of their distribution, seasonal trends and disease infection rates. This study was designed to access the abundance and diversity of Culicidae fauna at Cotonou in southern Benin. Data from this study will help to update the mosquito fauna at Cotonou and useful for an implementation of vector control against the different species of these mosquitoes population.

MATARIALS AND METHODS

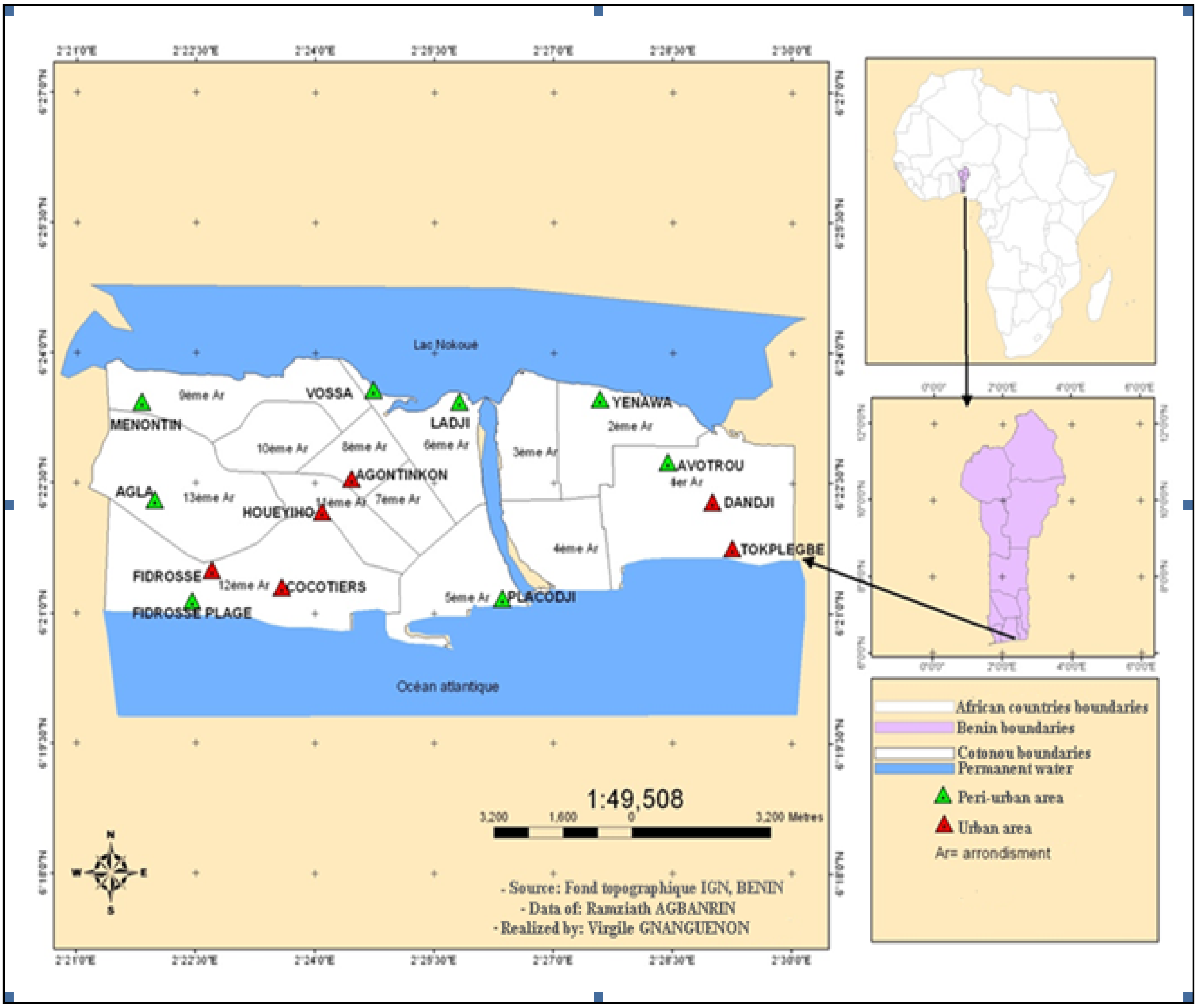
Study areas

The study was carried out in southern Benin at Cotonou, the economic capital from December 2013 to November 2014 at UA and PU (Figure 1). The choice of the study area is based on the weak level of urbanization and the presence of a lot of mosquitoes breeding sites. This city is located on a strip of land between Lake Nokoue and the Gulf of Guinea (6.2° N–6.3° N and 2.2° E–2.3° E) connected by a lagoon. This part of Benin is characterized by a tropical coastal Guinean climate with two rainy seasons (April–July and September–November). The main annual rainfall is more than 1300 mm.

Field collection

To have an idea on the richness of the mosquito fauna at Cotonou, a cross sectional entomological study was carried out from December 2013 to November 2014 in UA and PU at Cotonou, based on sampling of mosquitoes by larvae and adults collection.

Figure 1. Map of the study area showing the neighborhoods where the data was collected



Larvae of mosquito species were collected using a standard mosquito larval dipper and pipette at all sampling sites twice a month. At each sample site, mosquito larvae were sampled from wet pit latrines, blocked open drains, and polluted puddles, domestic runoffs. To maximize the number of species from larvae collection, ten artificial containers (cans, pots and discarded plastic containers) were placed and sampled twice a month at specific. Larvae and pupae collected in the different breeding sites were kept in separatelabeled bottles for each locality. Larval samples were reared up to adult emergence at the CREC (Centre de Recherche Entomologique de Cotonou, Benin) insectary for identification. Concerning the adults, collections were organized in households by using several methods:

(1) Indoor and outdoor Human Landing Catches (HLC) was performed monthly over two consecutive days (8:00 PM to 6:00 AM), in 4 randomly selected compounds;

(2) Indoor Pyrethrum Spray Catches (PSC) performed also monthly over two consecutive days (11:00 AM to 6:00 PM) in 4 other selected compounds; the same compounds in each sampling method being consistently used throughout the study.

For HLC method, collectors gave prior informed consent and received anti-malaria prophylaxis and yellow fever immunization. They were organized in teams of two for each collection point and they rotated between locations within houses every two hours. These mosquito populations were completed with those collected from Biogents (BG) sentinel trap (posed from 10:00 AM to 6:00 PM) and Windows traps (WT) pose in 4 randomly selected compounds at PU and UA from (9:00 PM to 7:00 AM). All captured mosquitoes were morphologically identified using an identification key Edwards (Edwards, 1941) and by Gillies and Meillon (Gillies and De Meillon, 1968) and stored in eppendorff tubes on silica gel. With the emergence of larvae from nests and larval surveys adults are also morphologically identified using the aforementioned key and then stored as previous mosquitoes.

Data analysis

Species diversity was assessed using index of Shannon-Weaver (H) (13), of Simpson (D1) (Simpson, 1949 and Shannon, 1948) and the diversity profile of mosquitoes was based on Renyi index (Hα) (Shannon and Weaver, 1963). This profile wasused to assessthe diversity between UA and PU areas.

Moreover, the richness (S) and relative abundance (pi = ni / N where ni = actual of species row i, N = total number) of each species were defined following Barbault index.

$$H = -\sum_{i=1}^{S} p_i \log(p_i) \quad D = \sum_{i=1}^{S} p_i^2 \quad \text{and}$$

$$D_1 = 1 - D$$

$$H_\alpha = \frac{\ln(\sum_{i=1}^{S} p_i^\alpha)}{1-\alpha} \quad (\alpha = 0; 0.25; 0.5; 1; 2; 4; 8; \infty)$$

- S is the species richness of an area (the number of species found in the area).
- $p_i = \frac{n_i}{n}$ is the relative frequency of a species, the relative abundance of the species and the total abundance.

The confidence index Shannon-Weaver and Simpson were calculated using the non-parametric bootstrap confidence interval method (Kindt *et al*., 2006). Index calculations were performed using the R vegan (DiCiccio and Efron, 1996) package.

The change in adult animals between UA and PU areas was assessed using linear regression log (Dobson, 1990).

RESULTS

Richness of mosquito fauna identified in urban and peri-urban areas

Thirteen species of Adult's mosquitoes coming from larvae collected in our study sites, (*Aedes aegypti, Ae. Luteocephalus, Ae. circumluteolus, Ae. vittatus, Anopheles gambiae sl, An. ziemanni, An. pharoensis, Culex decens, Cx. nebulosus Cx. quinquefasciatus, Cx. Tigripes, Cx. fatigrans and Uranotaenia bilineata)* including 3 *Anopheles* species, 5 *Culex* species, 4 *Aedes* species and 1 *Uranotaenia* belonging to 4 genera (*Culex, Aedes, Anopheles and Uranotaenia*) recorded at PU and UA (Table 1). However, when the adults came directly from the different traps described above, 15 species (*Ae. aegypti, Ae. luteocephalus, Ae. circumluteolus, Ae. vittatus, An. gambiae s.l., An. ziemanni, An. pharoensis, Cx. decens, Cx. nebulosus, Cx. quinquefasciatus, Cx. tigripes, Cx fatigrans, M. africana, M. uniformis et Ur bilineata*) of mosquitoes (including 3 *Anopheles* species, 5 *Culex* species, 2 *Mansonia* species, 4 *Aedes* species and 1*Uranotaenia* species) belonging to 5 genera (*Culex, Aedes, Anopheles, Uranotaenia* and *Mansonia)* were recorded at PU and UA.

Abundance and diversity of species culicidiennes

45,609 adult's mosquitoes have been recorded by different methods described above. Globally, mosquitoes are most abundant in PU (25,303) than in UA (20,306) (p<0.0001) In general, *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles gambiae* were the dominant species in both UA and PU areas of the city with relative abundances of 64.30%; 22.86% and 12.84% respectively (Table II). Base on the abundance of species recorded, *Cx. quinquefasciatus* is the highest species found despite the area (PU and UA) of collection. Moreover, *Cx. quinquefasciatus* is more important at PU than UA with 76.27% and 49.24% respectively as abundance (p<0.05) (Table II). The same trend was observed with the *Luteocephalus*, *Ae .vitattus, An. gambiae, An. ziemanni, An. pharoensis, Cx. tigripes, Cx.fatigrans, Cx. nebulosus*, and *M. Africana*)which were more abundant at PU than UA(p<0.05). On the other hand, *Ae. Aegypti* and *Ae. circumluteolus* were more abundant in UA than PU(p<0.05). However, *Cx. decens* and *Uranotaenia bilineata* didn't show a significant change in their relative abundance between urban and peri-urban areas (p>0.05). Moreover, Simpson index values of in UA (0.59) and PU (0.40) recorded in our study site showed the dominance of species in the PU contrary to UA where co-dominance is

Table 1. Richness of mosquito fauna recorded in Peri-urban and Urban at Cotonou

	Genus	Species	Urban area (UA)	Peri-urban area (PU)	(UA) and(PU)
Culicidae fauna identified after rearing larvae collected during larval surveys	*Aedes*	*luteocephalus*	X	X	X
		aegypti	X	X	X
		circumluteolus	X	X	X
		vittatus	0	X	X
		S_1	3	4	4
	Anopheles	*gambiae*	X	X	X
		pharoensis	X	X	X
		ziemanni	X	X	X
		S_1	3	3	3
	Culex	*tigripes*	X	X	X
		fatigans	X	X	X
		gr decens	X	X	X
		nebulosus	X	X	X
		quinquefasciatus	X	X	X
		S_1	5	5	5
	Uranotaenia	*biliniata*	X	X	X
		S_1	1	1	1
	S = 4	S_t = 13	S_t = 12	S_t = 13	S_t = 13
Culicidae fauna identified after adults sampling	*Aedes*	*luteocephalus*	X	X	X
		aegypti	X	X	X
		circumluteolus	X	X	X
		vittatus	0	X	X
		S_1	3	4	4
	Anopheles	*gambiae*	X	X	X
		pharoensis	X	X	X
		ziemanni	X	X	X
		S_1	3	3	3
	Culex	*tigripes*	X	X	X
		fatigans	X	X	X
		gr decens	X	X	X
		nebulosus	X	X	X
		quinquefasciatus	X	X	X
		S_1	5	5	5
	Mansonia	*africana*	X	X	X
		uniformis	X	X	X
		S_1	2	2	2
	Uranotaenia	*biliniata*	X	X	X
		S_1	1	1	1
	S= 5	S_t = 15	S_t = 14	S_t = 15	S_t = 15

X = presence, S1 = Number of species in the genus, S = Number of genus, St = Total species

Table 2. Abundance Culicidae in urban and peri-urban areas of Cotonou

Genus	Species	Peri-urban area		Area urban		Total		P
		AB	AB(%)	AB	AB(%)	AB	AB(%)	
Aedes	*luteocephalus*	79	0,33	22	0,11	101	0,23	0.0004
	aegypti	**2187**	9	**7781**	40,3	9968	22,86	<0.0001
	circumluteolus	41	0,17	61	0,32	102	0,23	0.002
	vittatus	10	0,04	0	0	10	0,02	0.0108
Anophele	*gambiae*	**2839**	14,56	**2285**	11,93	5124	12,84	<0.0001
	pharoensis	163	0,67	98	0,51	261	0,6	0.03312
	ziemanni	96	0,4	29	0,15	125	0,29	<0.0001
Culex	*tigripes*	319	1,31	146	0,76	465	1,07	<0.0001
	fatigans	77	0,32	23	0,12	100	0,23	<0.0001
	gr.decens	33	0,14	25	0,13	58	0,13	0.9627
	nebulosus	264	1,09	167	0,87	431	0,99	0.02312
	quinquefasciatus	**18535**	76,27	**9507**	49,24	28042	64,3	<0.0001
Mansonia	*africana*	544	2,24	214	1,11	758	1,74	<0.0001
	uniformis	3	0,01	0	0	3	0,01	0.2573
Uranotenia	*biliniata*	114	0,47	89	0,46	203	0,47	0.9778
	Abundance	**25303**	100	**20306**	100	45609	100	<0.0001
	Simpson_1-D	0,40[0,39-0,44]		0,59[0,59-0,6]		0,53[0,52-0,53]		
	Shannon_H	0,95[0,93-0,97]		1,11[1,1-1,13]		1,1[1,08-1,11]		

AB = Abundance ; AB (%) = Relative Abundance

observed between species (Table II). Similarly, the Shannon index values (1.11) in UA and PU (0.95) showed that diversity is more often in UA compared to the PU (Table II).

Profile of diversity between urban and peri-urban area

The diversity profile of mosquitoes in PU areas is dominant, when theα values are $\leq$ 0.5 (Figure2). Moreover, base on the profile values both in PU and UA, results from our study sites showed that the profile value of mosquitoes (H0 = 2.56) in PU area is higher significant in species than what we got (H0 = 2.68) in UA.

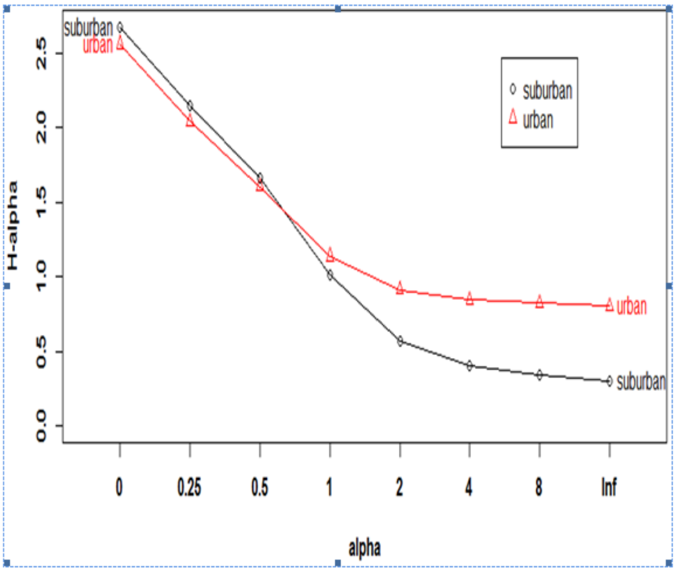


Figure 2. Diversity Profile of mosquitoes based on the series H-alpha Renyi between suburban (peri-urban) areas and urban areas at Cotonou.

DISCUSSION

The Abundance and diversity of Culicidae fauna study conducted at Cotonou, Benin showed 13 species of adult's mosquitoes coming from larval sampling methods and 15 species of adults mosquitoes coming from the different traps and HLC. These results were found in previous study conducted by Padonou et al. (Padonou *et al*., 2012). Hower, Hüttel et *al*. (Huttel, 1950) and Hamon (Hamon, 1954) found the same results but with absence of *Cx. fatiguing, Cx quinquefasciatus and Cx. decens*. The absence of some species can be explained by the climate change as it was highlighted by Akram et al. (Matthys *et al*., 2010). In fact, 20 years ago, a study conducted by Akogbeto et al. (Akogbeto, 1992) in southern Benin reported only eight species (*Anopheles gambiae slAn.coustani, An.pharoensis, Ae.aegypti Culex Thalassius, Cx gr.decens, Mansonia uniformis and Ma.africana*) compared to 13 or 15 species found in our current study.In the same line, in 2010 Lingenfelser et al. (Lingenfelser *et al*., 2010) identified 24 species of Culicidae fauna in southern Benin while Djenontin et al. (Armel Djènontin *et al*., 2010) identified 28 species. A part from the effect of the climate change on the dynamic of mosquitoes which can explain the number of species got from several studies, sampling methods and the period of mosquitoes collection can also affect the number of species. Furthermore, the presence of three mosquitoes genera at all sampled habitats in this study showed the abilities of mosquitoes to breed at all habitat types throughout the year in the southern of Benin. This result indicated a high potential for malaria, yellow fever and bancroftian filariasis in the city throughout the year. The persistence of the three mosquito genera (Table I) in this part of the country could be explained by the increased and varied human activities, urbanization and other related anthropogenic factors that create different natural and artificial habitats as sources of water for oviposition and breeding sites for several mosquitoes species. Also, the increase of mosquitoes found in PU compared to UA can be explained by several factors such as the poor economic condition, low literacy levels, poor sanitation levels, indiscriminate disposal of wastes (including discarded household materials, cans, plastic and metal containers), abundant numbers of abandoned construction sites, uncontrolled domestic run-offs and poor maintenance of gutters and drainages by people leaving at PU compared to UA. These findings were reported by many authors (Dossou-Yovo *et al*.,1995; Aigbodion and Odiachi, 2003; Adeleke *et al*., 2008; Impoinvil *et al*., 2008). During our collection, *Cx. quinquefasciatus* was more abundant at PU than at UA. This can be explained by the fact that in many sub-Saharan towns, unplanned urbanization and lack of waste management lead to widespread water collection, thus favoring the proliferation of *Cx. quinquefasciatus*. (Amarasinghe *et al.,* 2011). However, the concentration of administrative and economic infrastructure at UA (cotonou) offers a good condition of development for Aedes spp as we found in our study site. In fact, the presence of *Aedes* particularly *Ae. Aegypti* collected can be explained by the existence ofsecond hand tires from Europe and Asia which are stored and sold in southern Benin (Yadouleton *et al*., 2014). These tires were the most productive *Ae. aegypti* larvae breeding sites and thus, independently of season. This result confirmed what was found in the Central African Republic (Kamgang *et al*., 2013) and in southern Cameroon (Kamgang *et al*., 2010). In fact, the advantages of this second hand tire trade of are considerable. They contribute to the improvement of living conditions by supplying income and employment to urban populations. However, the positive economic and social impact of this trade is limited by the fact that these tires are good breeding sites for *Ae. aegypti,* and therefore contribute to the development of large mosquito populations in these areas. Despite their massive presence in the tires, some larvae were found also in abandoned peri-domestic containers and discarded tanks. This finding is in agreement with observations made in Cameroon (Kamgang *et al.,* 2013), where peri-domestic containers represented the bulk of the containers infested by *Ae. aegypti* or *Ae. albopictus*.

Conclusion

The presence of *Culex, Aedes*, and *Anopheles* species highlighted by this study in the city of Cotonou is posing a serious epidemiological concern to the inhabitants of this city. Therefore, larviciding of breeding sites before the onset of the rainy season and public enlightenment on the**environmental factors** and/or human activities that encourage mosquito breeding are recommended.

Competing interests

The authors declare that they have no conflict of interest.

Authors' contributions

AR carried out field experiments, collected, analysed, interpreted data and wrote the first draft of the manuscript.

GGP, **AY, BK, AE, SV, GV, RA** helped with the field activities. **AR, GGP**, **AY** and **MA** conceived and designed the study, supervised fields collection, data analysis and interpretation, revised the manuscript and gave final approval for the version to be published. All authors read and approved the final manuscript.

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