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## WIDESPREAD DDT, PYRETHROIDS AND CARBAMATE RESISTANCE IN *CULEX QUINQUEFASCIATUS* POPULATIONS FROM URBAN AND PERI-URBAN AREAS IN SOUTHERN BENIN

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### ABSTRACT

*Culex quinquefasciatus*, an arboviral and filarial vector, is present in high numbers throughout southern Benin, and insecticide-resistant populations have been reported worldwide. In order to determine the insecticide resistance status of *Cx. quinquefasciatus* in southern Benin, a study was conducted from May to October 2014 where larvae collection was done during the rainy season across two districts located in urban (Agla) and peri-urban (Dandji) areas. Bioassays were performed on adults collected from the field to assess the susceptibility of *Cx. quinquefasciatus* to insecticide-impregnated papers (permethrin 0.75%, deltamethrin 0.05%, lambda-cyhalothrin 0.5%, DDT 4%, fenitrothion 1% and bendiocarb 0.1%) following WHOPEs guidelines. The presence of knock down resistance (*kdr*) and acetylcholinesterase (*ace-IR*) mutations were determined by PCR. This study showed a strong resistance to pyrethroids, DDT and carbamate in *Cx. quinquefasciatus* populations from the two study areas. However, the same populations were fully susceptible (mortality 100%) to the organophosphate fenitrothion tested. The *kdr* mutation was present in all *Cx. quinquefasciatus* populations collected in the two districts with frequency ranging from 0.65 to 0.72 respectively at Dandji and Agla. The *ace-IR* mutation was also present with frequency ranging from 0.25 to 0.3 respectively at Dandji and Agla. Conclusion: This study showed a widespread resistance to pyrethroids and carbamate with a presence of *kdr* and *ace-IR* mutations in all populations of *Cx. quinquefasciatus* from the two study areas. However, the susceptibility of these populations to organophosphate is encouraging the use of an insecticide of this family as an alternative insecticide to pyrethroids and carbamate against this mosquito in southern Benin.

**KEY WORDS:** *Culex quinquefasciatus*, Pyrethroids and Carbamate, Southern Benin.

### INTRODUCTION

The rapid urbanization in African countries has important implications on hygiene and the health of populations [1]. It represents a challenge for water and sanitation management particularly in sub-Saharan Africa. These issues have substantial public health implications, reshaping the epidemiology of both chronic and infectious diseases, with consequences worldwide [2].

Cotonou, the economic capital of Benin located in southern of Benin is in this scheme with remarkable expansion of its population with lack of storm-water drainage systems, building in hazardous areas, and unregulated housing and infrastructure development. Yadouleton et al [3] reported that it is common to find in this city wastewater collections stagnant, generated and

maintained by human practices, poorly priests gutters, open sumps or simply closed by a sheet. These collections of standing wastewater are particularly representing good breeding sites for the development of immature stages of *Culex quinquefasciatus* Say, 1823.

In West Africa, *Cx. quinquefasciatus* exploits the conditions created by tropical urbanization. This species thrives in polluted waters common in urban slum areas due to inadequate sanitation and waste disposal facilities. It's the main vector of the parasitic worm *Wuchereria bancrofti*, the agent of lymphatic filariasis (LF) [4]. They transmit many serious pathogens (filaria, West Nile virus & others) to human and animals [5-7].

Control of this vector has relied extensively on the application of insecticides [8] and hence the emergence of insecticide resistance represents a major concern for plans to control or eradicate LF [9]. However, the development of pyrethroid resistance in populations of *Cx. quinquefasciatus* has become a serious concern to the success of LF control. In the last decade, resistance to organochlorine, organophosphate and carbamate insecticides is well documented and widespread in populations of *Cx. quinquefasciatus* [10-12].

Moreover, Insecticide-treated bed nets (ITNs), which are targeted to prevent mosquitoes from feeding on people indoors, which prevents malaria transmission, are increasingly deployed in Africa as a means of malaria control and can have the added benefit of protecting people from filarial and arboviral diseases transmitted by culicine mosquitoes [13-14]. However, a study conducted by Czeher et al. [15] showed an increase level on knowck down resistance (*kdr*) in mosquitoes after a free distribution of ITNs to people against malaria. Moreover, Yadouleton et al. [3] reported in northern Benin that *Cx. quinquefasciatus* larvae are sympatric with *Anopheles* larvae. This situation certainly will strengthen the resistance status of *Cx. quinquefasciatus* against the various classes of insecticides use in public health, because *An. gambiae* has developed resistance to these insecticides [16-17].

The present study aims to assess the resistance status of *Cx. quinquefasciatus* to carbamates, pyrethroids and organochlorine and discuss the implications for vector control in southern Benin.

## MATERIALS AND METHODS

### Methods

#### Study areas

The study was carried out in southern Benin at Cotonou (6.2° N–6.3° N and 2.2° E–2.3° E) the economic capital particularly at Dandji (peripheral areas) and Agla (urban area). (Fig. 1). The choice of the two districts is based on the weak level of urbanization and the presence of a lot of mosquitoes breeding sites. These two areas of Benin are 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.

### Mosquito collections

*Cx. quinquefasciatus* larvae were collected during the rainy season. The collections were done from polluted drain. Larvae and pupae were collected using the dipping on breeding sites and then kept in separate labeled bottles for each locality. Larval samples collected were reared for adult susceptibility tests.

### Insecticide susceptibility test

125 adults females mosquitoes aged 3–5 days from each study site were exposed to diagnostic doses of various insecticides for susceptibility tests using insecticide-impregnated papers, as described by the standard WHO testing protocol [18]. The following insecticides were tested: deltamethrin (0.05%), permethrin (0.75%), DDT (4%), bendiocarb (0.1%) and fenitrothion (1%). The emphasis was also put on deltamethrin, because of a nationwide distribution of PermaNets by the National Malaria Control Program. The use of DDT is justified by the detection of cross resistance between pyrethroids and organochlorine in *Cx. quinquefasciatus* populations. The carbamate and the organophosphate are currently the alternative insecticides using for IRS in Benin [19].

For each treatment, five test tubes were used: one untreated paper as a control and four treated papers to expose mosquitoes. Control tubes contained filter papers impregnated with silicon oil (insecticide carrier) only, whereas treated papers were impregnated with diagnostic doses of insecticide plus carrier. An average of twenty-five mosquitoes was introduced into each tube. Females of *Cx. quinquefasciatus* used in this study were exposed for one hour to insecticide-treated papers. The number of knock down mosquitoes was recorded every 10 minutes. Tests with untreated papers that served as control were run in parallel. At the end of the exposure period, mosquitoes were transferred into tubes with untreated white filter papers (known as holding tubes) and allowed a 24 h recovery period. All mosquitoes were provided with 10% glucose water during the 24 h recovery period. Mortality rate was recorded after 24h.

Assayed samples were preserved individually on dessiccant (silica gel) and stored at -20°C for further analysis. All results were compared with the susceptible reference strain SLAB.

### PCR detection of the Knock-down mutation

50 survivors of mosquitoes from susceptible tests for each insecticide were used for PCR assays. In each site, each mosquito was extracted using a modified salt-extraction, with total DNA from each mosquito extraction resuspended in 50µldH<sub>2</sub>O(Kentet al. [20]). Head + thorax extractions were used to genotype samples for the Knock down resistance (*kdr*) allele, using a Polymerase chain reaction diagnostic test for detection of *kdr* "Leu-phe" mutations following the protocol described by Martinez-Torres et al. [21]. PCR conditions are as follows: each 25 µl

reaction contained 1X PCR buffer, 100 µM each dNTPs, 75 pmol CxRev primer, 75 pmol forward primer, 2.0U Taq polymerase, and 1.5 µl DNA template. A part of the DNA from the survivors mosquitoes were also screened for insensitive acetylcholinesterase (*Ace-1 R*) by the PCR-RFLP following Weill et al. [22] protocol.

**Data interpretation**

The resistant status of mosquito samples was determined according to the WHO criteria [18]. Following the WHO protocol, mortality of less than 80%, indicate resistance, while those greater than 98% indicate susceptibility. Mortality between 80%-98%, suggests the possibility of resistance that needs to be verified. The resistance allele frequency at the *kdr* and *Ace-1* locus was calculated using Genepop software (version 3.3) as described by Raymond and Rousset [23].

**RESULTS**

**Resistance status**

Figures 1 and 2 show the insecticide resistance status of *Cx. quinquefasciatus* s.l populations from the 2 districts. Following the exposure, females of *Cx. quinquefasciatus* have developed resistance to DDT in the

two districts with 17% as an average mortality. With the three pyrethroids tested, WHO diagnostic tests showed also high frequency of resistance in *Cx. quinquefasciatus* to permethrin (ranging from 25% to 35% mortality respectively at Dandji and Agla), deltamethrin (38% to 47%), lambdacyhalothrin (45% to 62%).

The same trend of resistance was observed with the carbamate and organophosphate where *Cx. quinquefasciatus* has developed resistance against these two insecticides.

**Detection of resistance genes**

A total sample of 120 mosquitoes (60 per site) was genotyped both for the *kdr* and *ace-1R* mutations. Results from this PCR showed that the *kdr* mutation was present in all *Cx. quinquefasciatus* populations collected in the two districts. The *kdr* frequency obtained in *Cx. quinquefasciatus* in the two districts is ranging from 0.65 to 0.7 respectively at Dandji and Agla (Table 1). The *Ace-1* mutation was present with frequency ranging from 0.25 to 0.3 respectively at Dandji and Agla.

There is no difference between the two frequencies of *kdr* found in *Cx. quinquefasciatus* populations from the two districts ( $P>0.05$ ). The same trend was observed statistically with *ace-1R* mutation.

**Table 1: Frequency of *Kdr* and *Ace-1R* mutations in *Culex quinquefasciatus* from Dandji and Agla in southern Benin**

Locality	<i>Kdr</i> mutation				<i>Ace-1</i> mutation			
	SS	RS	RR	F(R)	SS	RS	RR	F(R)
Dandji (60)	10	22	28	0.65	40	10	10	0.25
Agla (60)	7	22	31	0.7	38	08	14	0.3

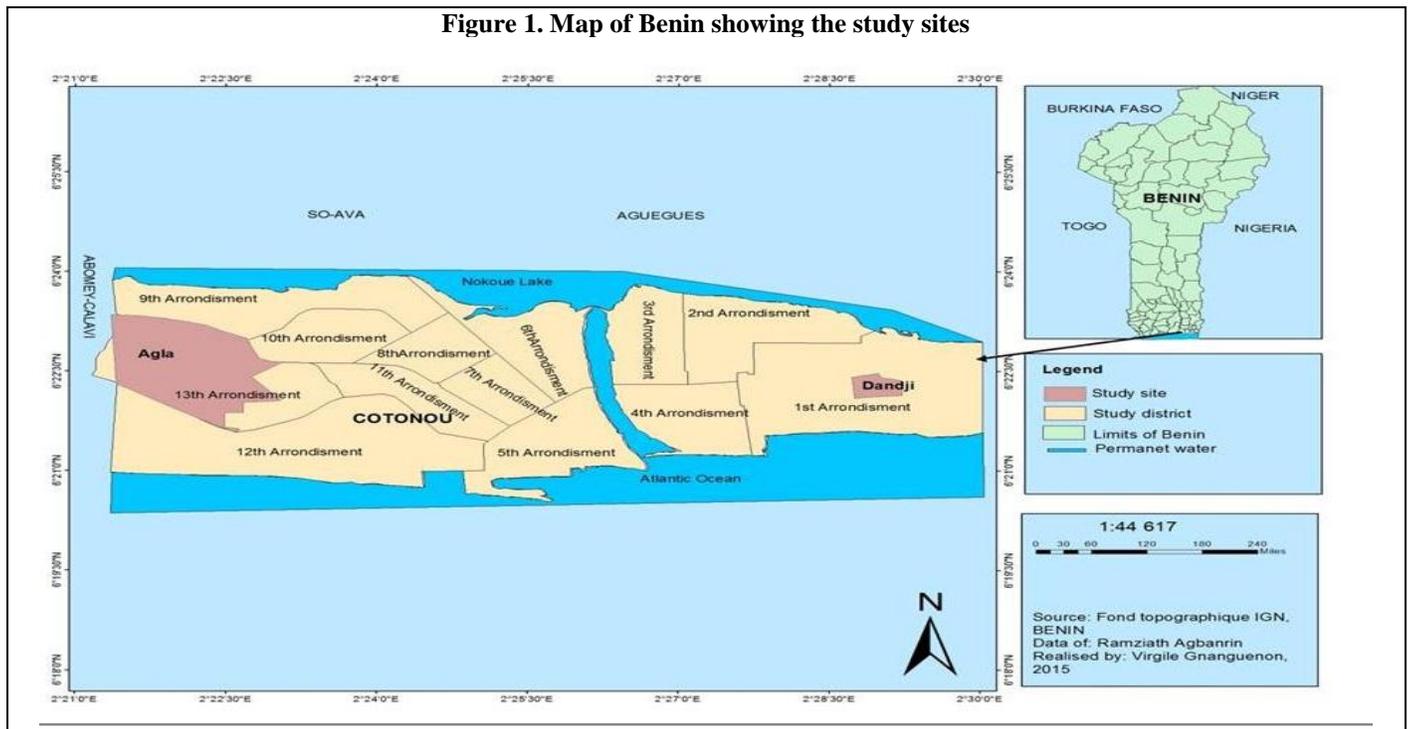
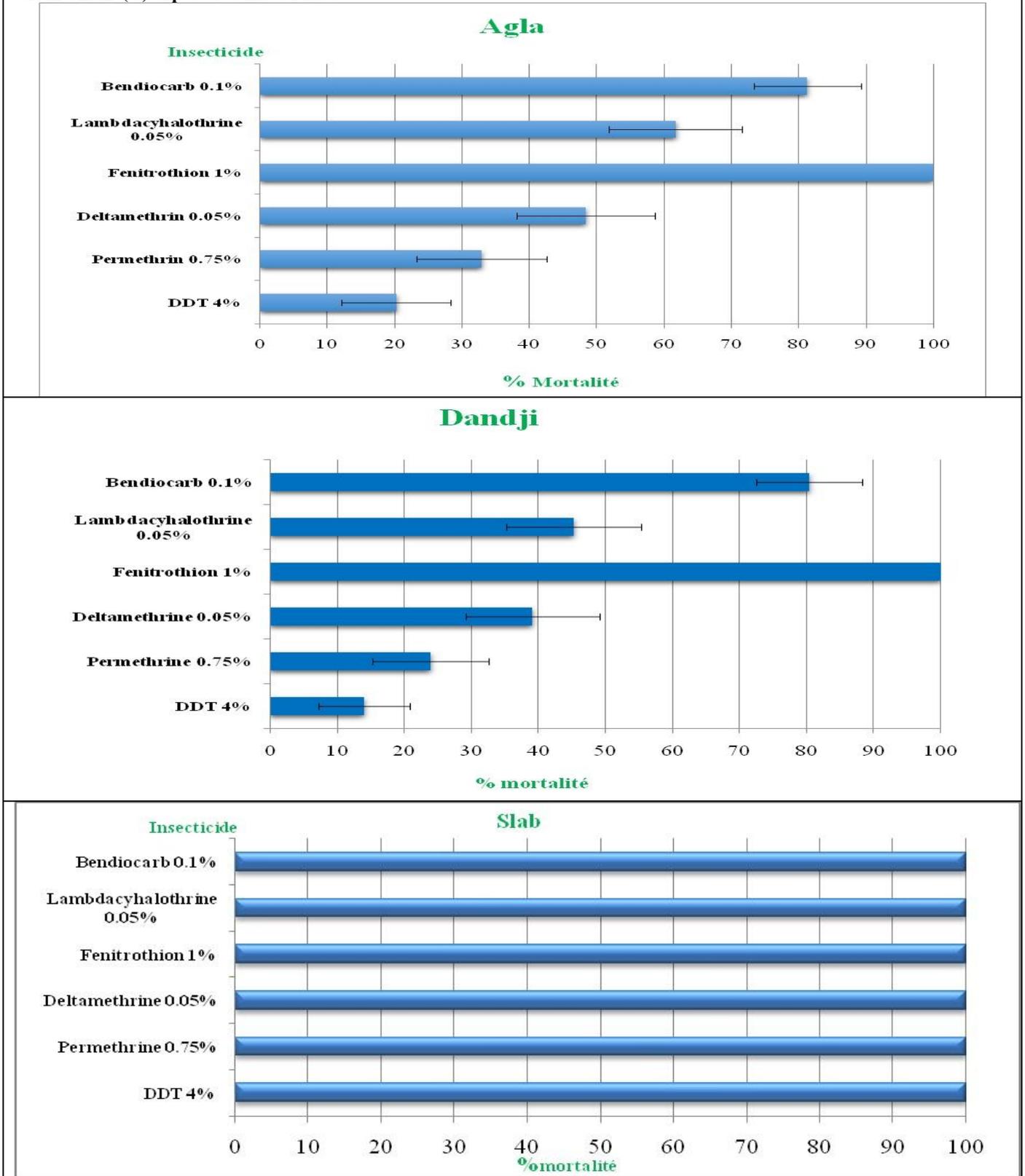


Figure 2: Mortality rates and resistance status of *Culex quinquefasciatus* from Agla, Dandji and the susceptible reference strain SLAB (C) exposed to insecticides



## DISCUSSION

This study showed that *Cx. quinquefasciatus* populations from Dandji and Agla respectively at urban and peri-urban areas in southern Benin have developed resistance to organochlorine (DDT), pyrethroids (permethrin, deltamethrin, lambda-cyhalothrin) and carbamate (bendio-carb), but susceptible to organophosphate (fenitrothion).

Results from this study showed that the pattern of resistance of *Cx. quinquefasciatus* to insecticides within a class of insecticide was similar across the two collection areas. However, the mortality rate was higher significant with the organochlorine than to other classes of insecticides ( $P < 0.05$ ).

Among the three pyrethroids tested (permethrin, deltamethrin and lambda-cyhalothrin), mortality rate with permethrin and deltamethrin was more important than the rate obtained with lambda-cyhalothrin. This can be explained by the massive free campaign of bed nets (Olyset and Permanet) distributed since 2006 until now which were impregnated with permethrin or deltamethrin insecticide. This was supported by Czeher et al. [15] who reported a high level of mortality of *An. gambiae* with the increasing of *ldr* resistance mutation in from Niger following a nationwide long lasting insecticide-treated nets implementation. This widespread resistance to DDT and pyrethroids observed in our two study sites can be also explained by a longstanding massive use of DDT house-spraying in several districts of the country during the WHO malaria eradication program in the 1950s [24].

Additionally, our larvae collections were done in agriculture areas particularly in vegetable farming (Agla) and in cereal agriculture (Dandji) where pyrethroids have been extensively used for crop protection [25]. In West Africa, the increased rate of pyrethroid resistance and *ldr* mutation frequency were often attributed to the massive agricultural use of DDT and pyrethroids [26- 27]. Similar finding has been reported by Wilding et al. [28] among *Cx. quinquefasciatus* mosquitoes from Accra. Akogbeto et al. [29] in Benin reported that during the treatments, insecticides in cereal/ vegetable fields are washed into mosquito breeding sites which exerted a huge selection pressure on mosquito larval population and contributed to the emergence of insecticide resistance in *Cx. quinquefasciatus*. This confirms once again, the role of agricultural in the selection of insecticide in mosquitoes. In other hand, massive use of domestic insecticides such as

mosquito coils or aerosol sprays in many households in developing countries particularly in Benin can be an additional selective pressure favouring pyrethroid resistance in urban and peri-urban areas. This has been reported by several authors [27, 30].

The resistance against carbamate and the presence of *ace-1R* mutation observed in *Cx. quinquefasciatus* populations in the two study areas can be explained by the fact that some of the pesticides belong to carbamate family were not recommended in vegetable farms and in cereal agriculture and were used in an improper manner to control agriculture pests [31].

Similar results of resistance to carbamate have also been observed in *Culex* mosquitoes in northern Benin and from Ghana's neighbouring countries [3, 32].

Our results showed also that *Cx. quinquefasciatus* populations from the two collection areas are fully susceptible to fenitrothion (organophosphate). This can be explained by the fact that in these areas, organophosphorous insecticides were not used. This is particularly relevant to strengthen vector control campaigns using Indoor Residual Spraying based on organophosphate as alternatives to pyrethroids/carbamate, which is currently used by the National Malaria Control Program in several areas of Benin.

## CONCLUSION

This study confirmed the widespread of pyrethroid and carbamate resistance in *Cx. quinquefasciatus* populations in southern Benin. However, the susceptibility of these populations to organophosphate is encouraging the use of an insecticide of this family as an alternative insecticide to pyrethroid and carbamate against mosquito in southern Benin.

## AUTHORS' CONTRIBUTIONS

AR contributed to design of the study and conceived the protocol, proceed data analysis and interpretation. AM contributed to the study design, provided funding and coordination. PGG, YA, AIR, OR, AA, AF, BK, AR, AB contributed in the study design and in the implementation of this research. PGG, YA, AM contributed to manuscript drafting. All authors read and approved the final manuscript.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## REFERENCES

1. Phillips DR. Urbanization and human health. *Parasitology*, 106, 1993, 93–107.
2. Vlahov D, Galea S. Urban health, a new discipline. *Lancet*, 362, 2003, 1091-92.
3. A. Yadouléon, K. Badirou, R. Agbanrin, H. Jöst, R. Attolou, R. Srinivasan, G. Padonou M. Akogbéto. Insecticide resistance status in *Culex quinquefasciatus* in Benin. *Parasites and Vectors*, 8, 2015, 17-29.
4. Jayasekera N, Kalpage KS & De Silva CS. The significance of low density microfilariemia in the transmission of *Wuchereria bancrofti* by *Culex (Culex) quinquefasciatus* Say in Sri Lanka. *Transaction of the Royal Society of Tropical Medicine and Hygiene*, 85, 1991, 250–254.

5. Turell M.J, O'Guinn M.L, Dohm D.J, Jones J.W. Vector competence of North American mosquitoes (Diptera, Culicidar) for West Nile Virus. *J. Med. Entomol*, 38, 2001, 130–134.
6. Cui F, Lin L.F, Qiao C.L, Xu U, Marquine M, Weill M, Raymond M. Insecticide resistance in Chinese populations of the *Cx. pipiens* complex through esterase overproduction. *Entomol. Exp. Appl*, 120, 2006, 211–220.
7. Kasai S, Komagata O, Tomita T, Sawabe K, Tsuda Y, Kurahashi H, Ishikawa T, Motoki M, Takahashi T, Tanikawa T, Yoshida M, Shinjo G, Hashimoto T, Higa Y, Kobayashi M. PCR-based identification of *Cx. pipiens* complex collected in Japan. *Jpn. J. Infect. Dis*, 61, 2008, 184–191
8. McCarroll L & Hemingway J. Can insecticide resistance status affects parasite transmission in mosquitoes? *Insect Biochemistry and Molecular Biology*. 2002, 32, 1345–1351.
9. Ottesen EA, Duke BO, KaramM& Behbehani K. Strategies and tools for the control / elimination of lymphatic filariasis. *Bulletin of the World Health Organization*, 75, 1997, 491–503.
10. Daaboub J, Ben Cheikh R, Lamari A, Ben J.I, Feriani M, Boubaker C, Ben Cheikh H. Resistance to pyrethroid insecticides in *Cx. pipiens pipiens* (Diptera, Culicidae) from Tunisia. *Acta Trop*, 107, 2008, 30–36.
11. Curtis CF, Pasteur N, Organophosphate resistance in vector populations of the complex of *Culex pipiens* L. (Diptera, Culicidae). *Bull Entomol Res*, 71, 1981, 153-156.
12. Corbel V, N'Guessan R, Brengues C et al. Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. *Acta Tropica*, 101, 2007, 207–216.
13. Kilian A, Byamukama W, Pigeon O, Atielli F, Duchon F, Phan C. Long-term field performance of a polyester-based long-lasting insecticidal mosquito net in rural Uganda. *Malaria J*, 7, 2008, 49.
14. D'Alessandro U, Olaleye BO, McGuire W, et al. Mortality and morbidity from malaria in Gambian children after the introduction of an impregnated bednet program. *Lancet*, 345, 1995, 479-83.
15. Czeher C, Labbo R, Arzika I, Duchemin JB. Evidence of increasing Leu-Phe knockdown resistance mutation in *Anopheles gambiae* from Niger following a nationwide long-lasting insecticide-treated nets implementation. *Malar J*, 7, 2008, 189.
16. Aikpon R, Agossa F, Ossè R, Oussou O, Aizoun N, Oké-Agbo F, et al. Bendiocarb resistance in *Anopheles gambiae* s.l. populations from Atacora department in Benin, West Africa, a threat for malaria vector control. *Parasit Vectors*, 6, 2013, 192.
17. Padonou GG, Sezonlin M, Ossé R, Aizoun N, Oké-Agbo F, Oussou O, et al. Impact of three years of large scale Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs) interventions on insecticide resistance in *Anopheles gambiae* s.l. in Benin. *Parasit Vectors*, 5, 2012, 72.
18. WHO. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. *Geneva, World Health Organization*, 2013.
19. Aikpon R, Sèzonlin M, Tokponon F, Okè M, Oussou O, Oké-Agbo F, et al. Good performances but short lasting efficacy of Actellic 50 EC Indoor Residual Spraying (IRS) on malaria transmission in Benin, West Africa. *Parasit and Vectors*, 7, 2014, 256.
20. Kent RJ, Thuma PE, Mharakurwa S, Norris DE. Seasonality, blood feeding behavior, and transmission of *Plasmodium falciparum* by *Anopheles arabiensis* after an extended drought in southern Zambia. *Am J Trop Med Hyg*, 76, 2007, 267–74.
21. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, et al. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* s.s. *Insect Mol Biol*, 7, 1998, 179–84.
22. Weill M, Malcolm C, Chandre F, Mogensen K, Berthomieu A, Marquine M, et al. The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. *Insect Mol Biol*, 13, 2004, 1–7.
23. Raymond M, Rousset F. Genepop (version 12), a population genetics software exact tests and ecumenicism. *J Heredity*. 86, 1995, 248–9.
24. Joncour G, Lutte anti-palustre au Dahomey. *Rapport no 13*, Ministère de la Santé Publique, 1959
25. Yadouléon AW, Martin T, Padonou G, Chandre F, Alex A, Djogbenou L, Dabiré R, Aikpon R, Glitoh I, Akogbeto MC, Cotton pest management strategies on the selection of pyrethroid resistance in *Anopheles gambiae* populations in northern Benin. *Parasites and Vectors*, 4, 2011, 60.
26. Yadouléon AW, Padonou G, Asidi A, Moiroux N, Sahabi B, Corbel V, N'guessan R, Gbenou D, Yacoubou I, Kinde G, Akogbeto MC, Insecticide resistance status in *Anopheles gambiae* in southern Benin. *Malar J*, 9, 2010, 83.
27. Diabate , A, Baldet, T, Chandre, F, Akogbéto, M, Guiguemde, R.T, Darriet, F, Brengues, C, Guillet, P, Hemingway, J, Graham, J. S, Hougard, J.M The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae* s.l. in Burkina Faso. *Am J Trop Med Hyg*, 67, 2002, 617–622
28. Wilding CS, Smith I, Lynd A, Yawson AE, Weetman D, Paine MJ, et al. A cis-regulatory sequence driving metabolic insecticide resistance in mosquitoes, functional characterisation and signatures of selection. *Insect Biochem Mol Biol*, 42, 2012, 699–707
29. Akogbeto, M.C, Djouaka, R.F, Kinde-Gazard, D.A. Screening of pesticide residues in soil and water samples from agricultural settings. *Malar.J*, 2006, 5, 22.

30. Elissa, N, Mouchet, J, Riviere, F, Meunier, J.Y Yao, K. Resistance of *Anopheles gambiae* s.s. to pyrethroids in Cote d'Ivoire. *Ann. Soc. Belge. Med.Trop*, 73, 1993, 291–294.
31. Yadouleton, A.W, Asidi, A, Djouaka, R.F, Braima, J, Agossou, C.D, Akogbeto,M.C. Development of vegetable farming, a cause of the emergence of insecticide resistance in populations of *Anopheles gambiae* in urban areas of Benin. *Malar. J*, 8, 2009, 103.
32. Chandre F, Darriet F, Darder M, Cuany, A, Doannio, J.M.C,Pasteur, N, Guillet, P. Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Medical and Veterinary Entomology*, 12, 1997, 359–366.