Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/17855
DOI URL: http://dx.doi.org/10.21474/IJAR01/17855

RESEARCH ARTICLE

RESISTANCE STATUS IN ANOPHELES GAMBIAE AND PLASMODIUM FALCIPARUM TRANSMISSION IN TORI-BOSSITO DISTRICT, SOUTHERN BENIN, WEST-AFRICA

Armel Djènontin[1], Christophe Soares[1], Aziz Bouraima[1], Emerson Ahouandjo[1], Yao Lenucthadius Houessou[1], Owolabi Camille Tante[1], Fabrice Chandre[2], Nicolas Moiroux[2] and Cedric Pennetier[2]

1. Centre de Recherche Entomologique de Cotonou (CREC), 06 BP 2604, Cotonou (Bénin) andCentre de Recherche pour la lutte contre les Maladies Infectieuses Tropicales (CReMIT), Université d'Abomey-Calavi (UAC), BP 526, Cotonou (Bénin).
2. MIVEGEC, Université Montpellier, CNRS, IRD, Montpellier, France.

Manuscript Info

Manuscript History
Received: 15 September 2023
Final Accepted: 17 October 2023
Published: November 2023

Key words:-
Malaria, Anopheles, Plasmodium Falciparum, Transmission, Resistance, Tori-Bossito

Abstract

In prelude to a large scale trial aiming to assess the field efficacy of Fludora Fusion 562.5WP-SB, entomologic baseline data were collected in 14 villages from Tori-Bossito District in Southern Benin during the rainy season from April to May 2018. Malaria vectors aggressiveness and longevity, Plasmodium falciparum infection in malaria vectors, malaria entomologic transmission as well as insecticide resistance status in malaria vectors were determinate. Overall 2695 mosquitoes were collected during 504 persons*nights. Among mosquitoes collected, 408 were Anopheles gambiae s.l. and 43 Anopheles funestus s.l. The proportion of parous malaria vectors was 54.2% and 79.1% for An. gambiae s.l. and An. funestus s.l. respectively. A sample of 68 malaria vectors was tested for Plasmodium falciparum infection using quantitative Polymlerase Chain Reaction (qPCR). The infection rate was about 6% (4/68 vectors infected) corresponding to 1.58 infective bites per person per month. The mortality rate of An. gambiae s.l. exposed to deltamethrin 0.05%, pyrimiphos-methyl 0.25% and clothianidin at 9µg/ml was 38%, 98.86% and 100% respectively, indicating that An. gambiae s.l. was resistant to deltamethrin and susceptible to pyrimiphos-methyl and clothianidin.This study showed that An. gambiae s.l. and An. funestus s.l. are the two major vectors with a predominance of An. gambiae s.l.

Introduction:-

Each year, vector-borne diseases are responsible of more than 17% of infectious diseases provoking more than one million of death. Vector-borne diseases affect tropical and subtropical areas and especially affect the poorest populations (WHO, 2022). Among vector-borne diseases, malaria remains one of the leading causes of mortality in the world and in Sub-Saharan Africa (WHO, 2022). It is the cause of 247 million cases in the world and contributes to 619,000 deaths in 2021, most of which are recorded in Sub-Saharan Africa (WHO, 2022). In tropical regions, malaria remains the first endemic infectious disease (WHO, 2017). Malaria is due to the presence in the blood of parasites belonging to Plasmodium genus transmitted by the infecting bites of mosquitoes of Anopheles genus (WHO, 2022). In Benin, malaria is the first cause of mortality and morbidity. Malaria incidence in Benin is about

Corresponding Author:- Christophe Soares
Address:- Centre de Recherche Entomologique de Cotonou (CREC), 06 BP 2604, Cotonou (Bénin) and Centre de Recherche pour la lutte contre les Maladies Infectieuses Tropicales (CReMIT), Université d'Abomey-Calavi (UAC), BP 526, Cotonou (Bénin).

15% (Ministère de la santé, 2018).Previous studies in Benin showed the implication of Anopheles gambiae stricto sensu, Anopheles Arabiensis (Yadouleton et al., 2010) and Anopheles funestus s. l. (Djènontin et al., 2010)in malaria transmission.

For vector control, mass distribution Long Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS) are the main tools used (WHO, 2021). Efficacy of these insecticide based tools are potentially compromised by insecticide resistance emergence in malaria vectors population, especially pyrethroid resistance (Djègbè et al., 2011; Akogbéto & Yacoubou, 1999). To face pyrethroid resistance, the combination of two active ingredients with a different modes of action is suggested, in the expectation of killing mosquitoes that are resistant to one component of the mixture by the second component. For this purpose, Bayer Environmental Science developed a new insecticide formulation for IRS named Fludora Fusion 56.25WP-SB containing 500 g/kg clothianidin and 62.5 g/kg deltamethrin. Clothianidin, is an insecticide of the neonicotinoid class which has recently been added to the WHO list of pre-qualified insecticides for IRS (WHO, 2020). To evaluate efficacy of Fludora Fusion 562.5WP-SB against mosquitoes populations in comparison with the WHO recommended insecticide actellic CS (pirimiphos-méthyl 30% CS), 14 villages were selected in Tori-Bossito District in Southern Benin. Before implementation of such evaluation, it was important to take an overview of entomological data in these villages. It was in this context that the present study was carried out, aiming to determine the aggressive density of malaria vectors, their longevity and resistance status, as well as malaria entomological transmission in selected villages.

Methods:-

Study area

The study was carried out in Tori-Bossito District. In this District, the climate is sub-equatorial with two dry seasons (August-September and December-March), and two rainy seasons (April-July and October-November). The average annual rainfall is around 1200 mm, of which 700-800 mm come in the first rainy season and 400-500 mm come in the second rainy season. The average monthly temperatures vary between 27 and 31°C. The Northern part of the health district is made of a plateau that drops into the Couffo valley and the Allada depression. The Southern is watered by several ramification arms of Toho Lake (figure 1). The study area is totally cleared of the original equatorial forest. Currently, the vegetation is characterized by bushes and isolated trees, associated with areas more or less densely populated of oil palm (Djènontin et al., 2010). Tori-Bossito District is a malaria meso-endemic area with pyrethroid-resistance vectors (Damien et al., 2010).In this District, 14 villages were selected for the trial (figure 1).

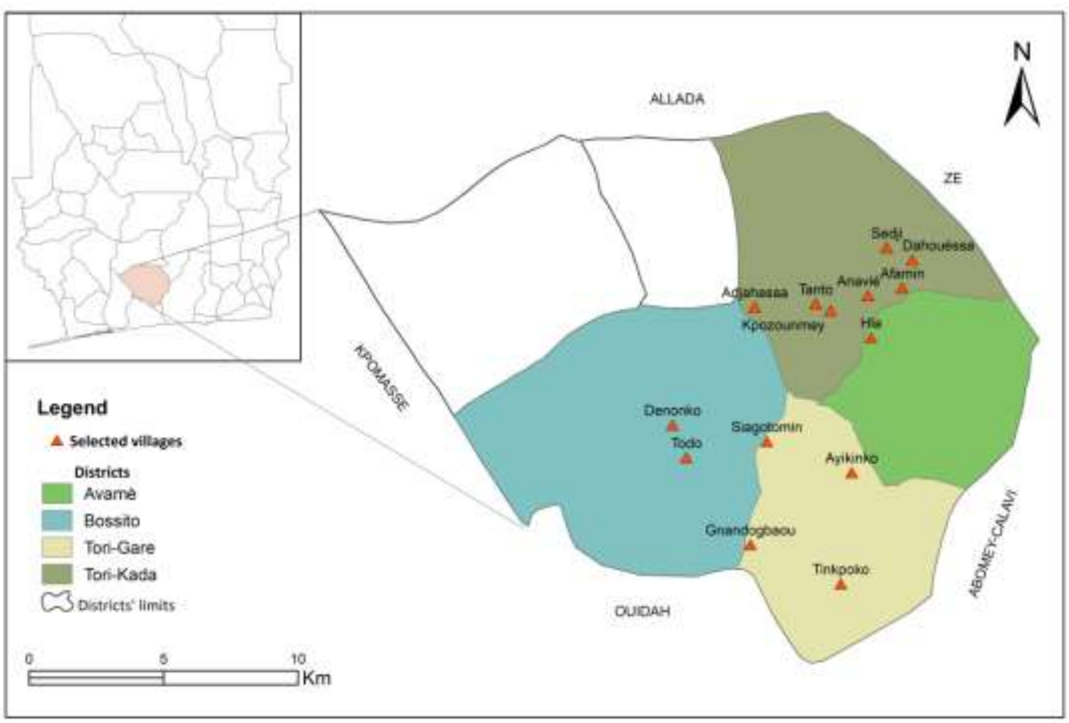

Figure 1:- Maps of area study.

Mosquitoes collection

Mosquitoes collection was carried out by human volunteers in 6 sentinel habitations in each selected villages during the rainy season from April to May 2018. Mosquitoes were captured indoors and outdoors in each sentinel habitation by two different volunteers. Volunteers were trained by the research team and have signed an informed consent form. Mosquitoes were collected between 10 p.m. and 6 a.m. on 3 consecutive nights. A total of 504 persons*nights of mosquitoes collection were carried out. All mosquitoes collected were identified using morphological identification key (Gillies & De Meillon 1968). Malaria vectors were conditioned for further tests in laboratory. Immature stages of malaria vectors were also collected in selected villages and reared in laboratory. Adults obtained were used for bioassays.

Mosquitoes dissection

The ovaries of anophelines were dissected under binocular loupe to assess whether the tracheoles are coiled (unparous female) or uncoiled (parous female). The tracheoles was observed by microscopy. The proportion of parous females is a proxy of the probability of daily survival of mosquitoes in the population.

Plasmodium falciparum detection in malaria vectors

The main malaria parasite in the study area is Plasmodium falciparum. Quantitative polymerase chain reaction (qPCR) was used to detect parasite DNA in the heads and thoraxes of a sub-sample of malaria vector collected from all villages.

Bioassays

Bioassays were performed on female adults from reared Anopheles gambiae s.l. immature stages. Tube tests was carried out according to standard WHO protocol in order to determine the resistance status of malaria vectors to deltamethrin 0.05% and pyrimiphos-methyl 0.25%. The CDC Atlanta bottle test was used to determine vector susceptibility to clothianidin at 9µg/ml (WHO, 2017).

Data analysis

Proportion of parous malaria vectors was determined by dividing the number of parous mosquitoes by the total of mosquitoes dissected. The infection index in malaria vectors was calculated by dividing the number of infected mosquitoes by the total tested by qPCR. The EIR was calculated according to the formula: EIR = HBR x (s), with HBR (Human biting rate) the number of bites per person per month. It is expressed as the number of infecting bites per man per month.

Mosquito susceptibility and resistance to insecticides have been defined according to WHO (WHO, 2017) criteria:

1. When mortality is ≥ 98%, vectors are susceptible,
2. When mortality is between 90-97% there is a suspicion of resistance in the vector population,
3. When mortality is < 90%, resistance is confirmed.

Results:-

Mosquitoes density and malaria vectors longevity

A total of 2695 mosquitoes were collected. The most abundant mosquitoes were Mansonia africana (35%), Culex gr decens (30%). Malaria vectors collected were Anopheles gambiae s.l. (14%) et Anopheles funestus s.l. (1%). Others species as Anopheles pharoensis, Culex quinquefasciatus, Culex annulioris, Aedes aegypti, Aedes vittatus and Aedes palpalis were collected but represent less than 2% of the mosquitoes collected (table I). The proportions of parous malaria vectors, indicating vectors longevity were on average 54% and 79% respectively for Anopheles gambiae s.l. and Anopheles funestus s.l. (table I).

Table I:- Mosquitoes density and malaria vectors longevity.

Villages	*An. gambiae*	*Parous An. gambiae*	% Parous *An. gambiae*	*An. funestus*	*Parous An. funestus*	% Parous *An. funestus*
Adjahassa	9	5	56	0	0	NA
Afamin	34	5	15	1	0	0
Anavié	24	6	25	6	3	50
Ayikinko	4	3	75	0	0	NA
Dahouessa	33	2	3	0	0	NA
Denonko	24	17	71	0	0	NA
Gnandogbahou	14	13	93	0	0	NA
Hla	10	4	40	12	11	92
Kpozounmey	63	42	67	6	5	83
Sèdji	11	0	0	1	0	0
Siagotomin	4	4	100	0	0	NA
Tanto	91	50	55	13	11	85
Tinkpoko	19	12	63	2	2	100
Todo	68	58	85	2	2	100
Total	**408**	**221**	**54**	**43**	**34**	**79**

Plasmodium falciparum infection in malaria vectors and entomological inoculation rate (EIR)

From the sample of 68 vectors tested by qPCR for Plasmodium falciparum detection, 4 individuals were infected, corresponding to 6%. The entomological inoculation rate (EIR) was 1.58 infecting bites per person per month.

Resistance status in malaria vectors

The knock-down (KD) rates of female adults Anopheles gambiae s.l. exposed to deltamethrin 0.05%, pyrimiphos-methyl 0.25% and clothianidin 9µg/ml were 48.67%, 88.64% and 96.02% respectively. Mortality rates were 38%, 98.86% and 100% respectively (table II), indicating that An. gambiae s.l. was resistant to deltamethrin and susceptible to pyrimiphos-methyl and clothianidin in the study area.

Table II:- Knock-down (KD) and mortality rates of Anopheles gambiae s.l. exposed to different insecticides.

Insecticides tested	N tested	N KD	% KD	N dead 24 h	% 24 h mortality
Control	300	0	0	4	1.33
Deltamethrin	300	146	48.67	114	38
Pyrimiphos-methyl	264	234	88.64	261	98.86
Clothianidin	502	482	96.02	502	100

Discussion:-

Results showed that Anopheles gambiae s.l. and Anopheles funestus s.l. were the main vectors in study area. A study conducted in the same study area about a decade earlier had reported the same vector species (Djènontin et al., 2010). This similarity in results is due to the environmental conditions that continue to favour the presence of malaria vectors. Climatic factors in the area favour the emergence of breeding sites after the first rains, providing ideal sites for the oviposition of females and the development of Anopheles larvae. The density of Anopheles gambiae s.l. has increased considerably over the years taking account the results found by Djènontin et al. (2010) in the same study area. This increase can be explained by the demographic growth of the human population, which, through their anthropic action facilitate the multiplication of Anopheles positive breeding sites. Moreover, Anopheles gambiae s.l. seems the main vector of malaria in the study area. A study carried out in Northern Benin revealed the great implication of this vector species in malaria transmission (Yadouleton et al., 2018). Consequently, Anopheles gambiae s.l. is the most abundant malaria vector in Benin and effectively ensures the transmission of malaria (Djègbè et al., 2019; Yadouleton et al., 2018). Furthermore, the rarity of Anopheles funestus s.l. may be due to the period during which collections were made (beginning of the rainy season). The beginning of the rainy season is a good period for fieldwork in the study area. This preparation would have destructed the larval breeding sites of Anopheles funestus s.l. which is an ombrophilous species requiring plant shelter (Pages et al., 2007).On the other hand, the low numbers of Anopheles funestus s.l. can be explained by the destruction of positive breeding sites for this species, attributed to the urbanization of the study area. This could also be the case for Anopheles nili, which was present in the study area ten years previously (Djènontin et al., 2010).

Concerning vectors longevity, a considerable variability was observed between villages for two vectors species. However, Anopheles gambiae s.l. had a lower vector longevity than Anopheles funestus s.l. in the study area. In many cases, Anopheles funestus s.l. has been implicated in malaria epidemics (Cohuet et al., 2004). The high longevity of Anopheles funestus s.l. suggest that this vector would have a more dangerous epidemiology than Anopheles gambiae in the study area if it is abundant. The contribution of Anopheles funestus s.l. to malaria transmission in the study area has been demonstrated (Djènontin et al., 2017; Djènontin et al., 2010) and also in Cameroon (Cohuet et al., 2004).

The entomological inoculation rate was 1.58 infecting bites per person per month (i.e. 18.96 bites/pers/year). This result confirms previous studies carried out in the same health zone (Djènontin et al., 2010; Damien et al., 2010).However, our results do not corroborate those obtained in a study carried out in another district in the Northern of the country (18.96 pi/pers/year vs 182 pi/pers/year) (Yadouleton et al., 2018). Consequently, the level of malaria endemicity varies from the Southern to the Northern of Benin.

The vectors collected in this study area are susceptible to pyrimiphos-methyl and clothianidin area according to WHO criteria (WHO, 2017) which is not the case in another Southern district where they are resistant to pyrimiphos-methyl (40% mortality) (Djènontin et al., 2017). Moreover, in the study area, vectors are resistant to deltamethrin (pyrethroids). The resistance observed is in line with the results of various studies conducted throughout Benin (Bouraima et al., 2023 ; Djègbè et al., 2019; Gnanguenon et al., 2015; Sovi et al., 2014;Djègbè et al., 2011; Djènontin et al., 2010) and in Africa (Kodindo et al., 2018 ; Antonio-Nkondjio et al., 2017 ; Konan et al ., 2011). This requires others formulations to control vector resistance to pyrethroids.Indeed,pyrethroids are the main insecticides used to impregnate nets distributed in mass in all malaria transmission contexts (WHO, 2021). The universal use of LLINs contributes to the development of vector resistance to this class of insecticides (Tokponnon et al., 2014). In addition, the use of insecticides of the same class for agricultural purposes in the study area would also be a source of resistance selection.

Conclusion:-

This study showed that malaria transmission occurs in Tori-Bossito District with Anopheles gambiae and Anopheles funestus as two major vectors. Vectors are resistant to deltamethrin. This acquisition of field entomological data is essential to better estimate malaria risk stratification in order to the field evaluation implementation.

Acknowledgements:-

We thank Bayer Environmental Science for funding this study. We also thank people of study area for their collaboration andlocal authorities at various levels.

Authors contributions

AD, CP, NM and FC designed this study. CS, AB, and EA conducted the fieldwork and laboratory tests. AD and SC drafted the manuscript. All authors read and corrected the manuscript.

Ethical clearance

The study was submitted to the National ethical committee of Benin and clearance (N°110 du 5/04/2018) was obtained in April 2018.

References:-

1. Akogbéto, M. and Yacoubou, S (1999): Resistance of malaria vectors to pyrethroids used for impregnated bednets, Benin, West Africa Bull Soc Pathol Exot., 92: 123–130.
2. Antonio-Nkondjio, C., Sonhafouo-Chiana, N., Ngadjeu, CS., Doumbe-Belisse, P., Talipouo, A., Djamouko-Djonkam, L., Kopya, E., Bamou, R., Awono-Ambene P., Wondji,CS (2017): Review of the evolution of insecticide resistance in main malaria vectors in Cameroon from 1990 to 2017. Parasit Vectors., 10 : 471-473.
3. Bouraima, A., Djènontin, A., Dossou, Y., Houessou, L., Soares, C., Anato, M., Zinsou, B., Dechavanne, C., Clain, J., Massougbodji, A. and Cottrel, G (2023): Measuring entomological parameters before implementing a study on asymptomatic carriers of Plasmodium falciparum in the Zè District in southern Benin. Malar J., 22-24.

4. Cohuet, A., Simard, F., Wondji, C., Antonio-Nkondjio, C., Awono-Ambene, H. and Fontenille, D (2004): High malaria transmission intensity due to Anopheles funestus (Diptera: Culicidae) in a village of Savannah-Forest transmission area in Cameroon", J Med Ent., 41: 901–905.
5. Damien, GB., Djènontin, A., Rogier, C., Corbel, V., Bangana, SB., Chandre, F., Akogbéto, M., Kindé-Gazard, D., Massougbodji, A. and Henry, MC (2010) "Malaria infection and disease in an area with pyrethroid-resistant vectors in southern Benin», Malar J., 9: 380.
6. Demba, KK., Kana-Mbang, A., Moundai, T., Nakebang, FA., Yangalbe-Kalnone, E., Mahamat, OA., Mallaye, P. et Kerah-Hinzoumbe, C (2018): Sensibilité de Anopheles gambiae s.l. et Culexquinquefasciatus à divers insecticides en milieu urbainà N'Djamena, Tchad. Med Santé Trop., 28: 154-157.
7. Djègbè, I., Boussari, O., Sidick, A., Martin, T., Ranson, H., Chandre, F., Akogbéto, M. and Corbel, V (2011): Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of L1014S kdr mutation in Anopheles gambiae from West Africa", Malar J., 10: 261.
8. Djègbè, I., Tokponnon, F., Gbankoto, A., Tchigossou, G., Djossou-Hessou, D., Dossou, C., Yessoufou, A. Akogbéto, M., Djogbénou, L. et Djouaka, R (2019): Typologie des gîtes larvaires et résistance des vecteurs du paludisme à la deltaméthrine dans les milieux urbains et ruraux du département de l'Atlantique au Sud du Bénin: données préliminaires. Eur Scien J., 15: 171.
9. Djènontin, A., Bio-Bangana, S., Moiroux, N., Henry, M., Boussari, O., Chabi, J., Ossè, R., Koudénoukpo, S., Corbel, V., Akogbéto, M. and 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.
10. Djènontin, A., Zogo, B., Ahlonsou, J., Bouraima, A., Ibikounle M., Courtin, D. and Pennetier, C (2017): Mosquitoes fauna diversity, Plasmodium falciparum infection and insecticide resistance status in malaria vectors in a lagoon area in Southern Benin, West Africa Int J Multi Cur Res., 5: 23.
11. Gillies, MT. and De Meillon, B (1968): The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region). Johannesburg: South Afri Inst Med Res.
12. Gnanguenon, V., Agossa, FR., Badirou, K., Govoetchan, R., Anagonou, R., Oke-Agbo, F., Azondekon, R., AgbanrinYoussouf, R., Attolou, R., Tokponnon, FT., Aïkpon, R., Ossè, R. and Akogbéto, MC (2015): Malaria vectors resistance to insecticides in Benin: current trends and mechanisms involved", Parasit Vectors., 8: 223.
13. Konan., KG., Koné, AB., Konan, YL., Fofana, D., Konan, KL., Diallo, A., Ziogba, JC., Touré, M., Kouassi, KP. And. Doannio, JMC (2011): Résistance de Anopheles gambiae s.l. aux pyréthrinoïdes et au DDT à Tiassalékro, village de riziculture irriguée en zone sud forestière de Côte-d'Ivoire. Soc Path Exo., 104: 303-306.
14. Ministère de la Santé du Bénin (2018). Plan national de développement sanitaire 2018-2022., 82.
15. Pages, F., Orlandi-Pradines, E. et Corbel, V (2007): Vecteurs du paludisme: biologie, diversité, contrôle et protection individuelle Vectors of malaria: biology, diversity, prevention, and individual protection", Med Malar Inf., 37: 153–161.
16. Sovi, A. Djègbè, I., Soumanou, L., Tokponnon, F., Gnanguenon, V., Azondékon, R., Oké-Agbo, F., Okè, M., Adéchoubou, A., Massougbodji, A., Corbel, V. and Akogbéto, M (2014): Microdistribution of the resistance of malaria vectors to deltamethrin in the region of Plateau (southeastern Benin) in preparation for an assessment of the impact of resistance on the effectiveness of Long Lasting Insecticidal Nets (LLINs): BMC Inf Dis., 14: 103.
17. Tokponnon FT., Ogouyémi, AH., Sissinto, Y., Sovi, A., Gnanguenon, V., Cornélie, S., Adéothy, AA., Ossè, R., Wakpo, A., Gbénou, D., Oke, M., Kinde-Gazard, D., Kleinschmidt, I., Akogbéto, MC. and Massougbodji, A (2014): "Impact of long-lasting, insecticidal nets on anaemia and prevalence of Plasmodium falciparum among children under five years in areas with highly resistant malaria vectors", M.J., 13: 76.
18. WHO. World malaria report. Geneva (2022): World Health Organization.
19. WHO.Guidelines for malaria vector control. Geneva (2021): World Health Organization.
20. WHO. List of pre-qualified products. Geneva (2020): World Health Organization.
21. WHO. World malaria report. Geneva (2017): World Health Organization.
22. WHO. Procedures for testing insecticide resistance in malaria vector mosquitoes - second edition. Geneva (2017): World Health Organization.
23. Yadouleton, A., N'Guessan, R., Allagbé, H., Asidi, A., Boko, M., Osse, R., Padonou, G., Kindé, G. and Akogbéto, M (2010): The impact of the expansion of urban vegetable farming on malaria transmission in major cities of Benin. Parasit Vectors., 3: 118.
24. Yadouleton, A., Aïkpon, R., Houndeton, G., Aboubacar, S., Ursins, F., Tchibozo, C., Agolinou, A. et Akogbéto, M (2018): Données entomologiques préliminaires pour la mise en place d'une pulvérisation intra-domiciliaire à grande échelle dans la commune de Corpargo au Nord-Est du Bénin. Int J Biol Abd Chem Siences, 12: 1993-2003.