Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(5): 1851-1858

Received: 26-06-2020
Accepted: 05-08-2020

Armand Defo Talom
[1] University of Dshang, Vector Borne Diseases, Laboratory of the Applied Biology and Ecology Research Unit (VBID-URBEA) P.O. Box: 67, Dschang-Cameroon.
[2] International Institute of Tropical Agriculture, Yaoundé, P.O Box: 2008, Yaoundé-Cameroon

Timoleon Tchuinkam
University of Dshang, Vector Borne Diseases, Laboratory of the Applied Biology and Ecology Research Unit (VBID-URBEA) P.O. Box: 67, Dschang-Cameroon

Francis Zeukeng
[1] Subject Matter Specialist, KVK, West Kameng, Dirang, Arunachal Pradesh, India
[2] University of Buea, Faculty of Science P.O Box: 63, Buea-Cameroon

Michel Lontsi Demano
University of Dshang, Vector Borne Diseases, Laboratory of the Applied Biology and Ecology Research Unit (VBID-URBEA) P.O. Box: 67, Dschang-Cameroon

Apollin Fotso Kuate
International Institute of Tropical Agriculture, Yaoundé, P.O Box: 2008, Yaoundé-Cameroon

Gustave Leopold Lehman
University of Douala, Faculty of Science P.O Box: 24157, Douala-Cameroon

Rousseau Djouaka
International Institute of Tropical Agriculture, Cotonou 08 P.O. Box: 0932, Cotonou-Benin

Corresponding Author:
Armand Defo Talom
[1] University of Dshang, Vector Borne Diseases, Laboratory of the Applied Biology and Ecology Research Unit (VBID-URBEA) P.O. Box: 67, Dschang-Cameroon.
[2] International Institute of Tropical Agriculture, Yaoundé, P.O Box: 2008, Yaoundé-Cameroon

Susceptibility of *Anopheles gambiae s.l.* to pyrethroid insecticides in vegetable farms in the city of Yaoundé, Cameroon

Armand Defo Talom, Timoleon Tchuinkam, Francis Zeukeng, Michel Lontsi Demano, Apollin Fotso Kuate, Gustave Leopold Lehman and Rousseau Djouaka

Abstract
Agricultural practices play a major role in the selection of insecticide resistance in field populations of Anopheles mosquitoes. Here, we investigated the possible links between agricultural practice and the resistance to pyrethroid insecticides in the major malaria vector *Anopheles gambiae s.l* in the city of Yaoundé in Cameroon. Entomological surveys were conducted in the peri-urban and urban areas of Yaoundé. KAP surveys on agricultural practice and the use of pesticides in vegetable farms were conducted in all farms and the susceptibility status of female Fo *An. gambiae s.l* progeny to permethrin (type I pyrethroid) and deltamethrin (type II pyrethroid) was assessed using the WHO tubes bioassay protocol. The resistance mechanisms were assessed using synergist and kdr-genotyping assays. Our findings revealed that the application of pesticide active molecules is a common practice in vegetable farms across Yaoundé. This phenomenon has increased the development of resistance to pyrethroid insecticides in *An. gambiae sl*. The observed phenotypic resistance was higher in peri-urban sites (7% and 17% mortalities for deltamethrin and permethrin, respectively) than in urban sites (63 % and 17% mortalities for deltamethrin and permethrin, respectively). Synergist assays with PBO revealed the high implication of monooxygenase P450 enzymes in the observed phenotypic resistance pattern. The L1014F kdr-resistant allele is almost fixed in the exposed mosquito populations. These data suggest that the metabolic resistance with monooxygenase P450-enzymes might play a crucial role in the resistance of wild *An. gambiae s.l.* populations to type I and type II pyrethroid insecticides in vegetable farms across Yaoundé.

Keywords: Vegetable farming, insecticide resistance, pyrethroid molecules, *Anopheles gambiae sl.*

Introduction
Malaria falls among the deadliest infections in human history with a slight decrease in the morbidity and mortality in certain regions during the last decade. In 2018, the World Health Organization (WHO) reported 228 million cases and 405,000 deaths from malaria. Up to 90% of these cases were reported in sub-Saharan African regions [1]. Vector control strategies currently represent one of the main tools used to fight against malaria in most endemic regions across the world. The tools used include long-lasting insecticide-impregnated mosquito nets (LLINs), Indoor Residual Spraying (IRS), and larviciding which unfortunately rely on a limited number of insecticides [2]. The success of these tools has been threatened by the increase of insecticide resistance in major malaria vectors across Africa [3].
In Africa, the resistance of malaria vectors to insecticides such as DDT and pyrethroids has frequently been associated with the use of pesticides in agricultural settings [4, 5, 6, 7]. Intensive vegetable farming highly relies on the use of synthetic pesticides and agrochemicals to improve the yields of cultured crops [8, 9]. The contribution of agricultural activities such as vegetable farming in insecticide resistance selection in malaria vectors has highly been described. Malaria vectors larvae breed in water pools found around farm settings; and almost 90% of insecticides used in these farms are pyrethroids which constitute the main insecticide class used in public health interventions against malaria vectors [10, 11].
Urban and peri-urban agricultural activities have become an alternative to solve the problem of food insecurity for city dwellers during low rural cropping systems [12]. Vegetable farming has become a key component of urban and peri-urban agricultural activities in many African countries. It is one of the economic activities that meet the food and nutritional demand of the

populations while contributing to the decrease of poverty among the communities. It is also a source of employment for many young graduates and a source of income for many families [13, 14]. However, agricultural crops in vegetable farms are subjected to pest attack which damages the crops and contributes to yield loss of many tones [15, 16, 17]. Different chemical ingredients such as synthetic pesticides are being used by marked farmers to fight against pests in vegetable farms [18]. However, the misuse of these compounds has contributed to insecticide resistance in major agricultural pests such as *Helicoverpa armigera*, the cotton bollworm [19], with great impact on human health or non-target organisms and the pollution of the environment [20, 21, 22].

Recent studies have described the fast-increased phenomenon of insecticide resistance among malaria vectors in Cameroon [23, 24]. Factors such as intensive and misuse of pesticides in agriculture, the expansion of urban agriculture, and the pollution of the environment in urban settings have been pinpointed. Their impact on the increase of resistant mosquito populations across the country is highly considerable [25, 26].

The intervention strategies used to control agricultural pests in vegetable farming might threaten the efficacy of the tools used to fight against malaria by inducing the mechanism of cross-resistance in malaria vectors [4, 9]. A well-coordinated integrated approach between public health and agricultural activities could be sufficient to minimize the impact of crop protection activities on the development of insecticide resistance in malaria vectors. Despite the fact that pyrethroid resistance is dynamic, the influence of local selective pressure induced by agricultural practices is a piece of important information in vector management on a small scale. Here, we assessed the impact of agricultural management strategies in vegetable farming on the insecticide resistance profile of the main malaria vector *Anopheles gambiae s.l.* in the city of Yaoundé in the Centre region of Cameroon.

Materials and Methods

Study sites

The study was carried out between September 2018 and July 2019 in Yaoundé (3°51'N11°30'E), the political capital city of Cameroon. Yaoundé is located within the Congo-Guinean phytogeographic zone characterized by a typical equatorial climate with two rainy and two dry seasons. The city of Yaoundé which is mainly surrounded by hills has an annual rainfall of 1,700 mm and an altitude of 800 m. Three vegetable farms were investigated; Ezazou (3°49'36,52768"N 11°31'55,76658"E) which is located in the urban area of Yaoundé, Nkolondom (3°57'12,33741"N 11°29'39,06882"E), and Famassi (3°52'22,16880"N 11°24'38,85358"E) located in the peri-urban area of Yaoundé (Figure 1)

The vegetable farm of Nkolondom is located 7 km from the center of Yaoundé. Its hollows are crossed by a main stream, the "Ntsas" which is fed by runoffs from the various hills. Vegetable farming is the main activity here and it highly depends on input. The landscape is made up of a mosaic of plots of around 100 m^2. Small farms are mostly owned by young single people. The predominant workforce is the family.

The vegetable farm of Famassi is located in Nkolbisson, a peripheral district in the north-west of Yaoundé. This area has been relatively protected from urbanization by the presence of a chain of hills. The lowland of Nkolbisson is cultivated by professional farmers. Farmers working there are generally non native, retired or in family relationships.

The market gardening site of Ezazou is located in the fourth District of Yaoundé. It is a District undergoing urbanization although anarchic. It is a lowland that has recently been colonized by market gardening, which is the main intensive agricultural activity; one can note in this site a strong proximity of the fields with the dwellings.

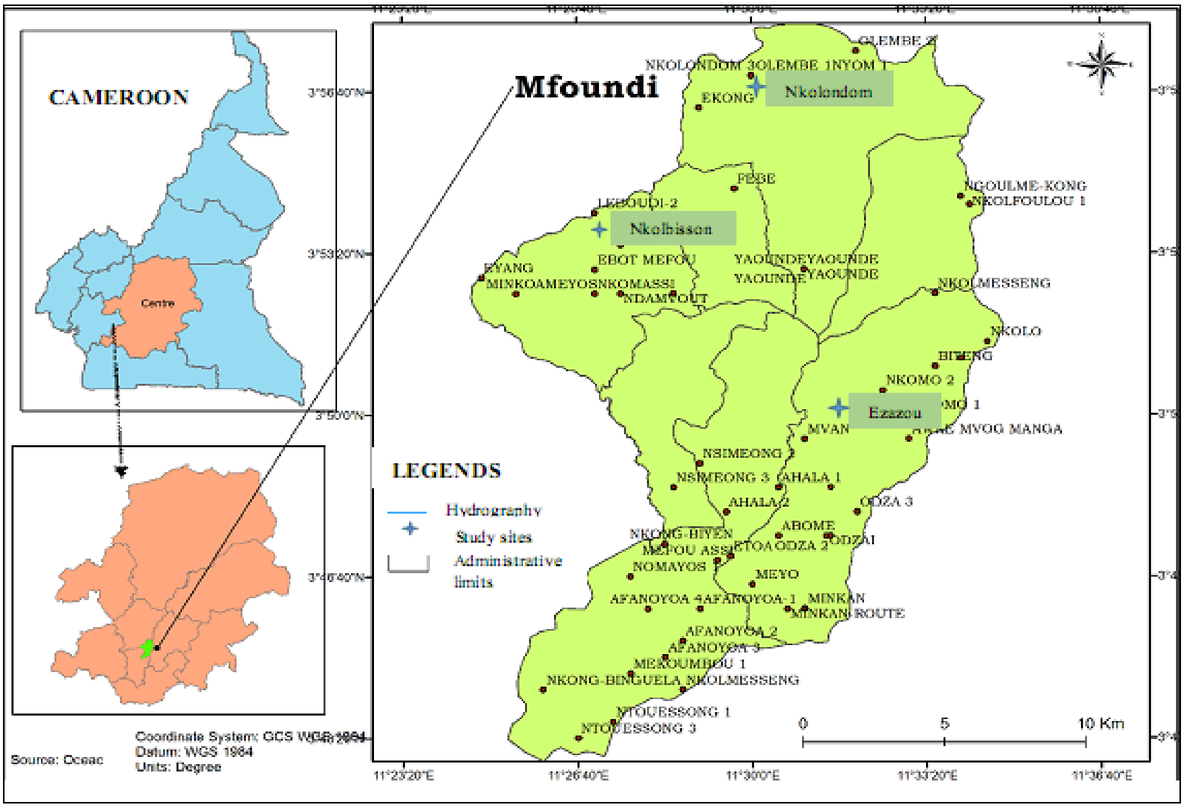

Fig 1: Study sites

Knowledge, Attitude and Practice (KAP) surveys

KAP surveys were conducted in all investigated sites. A total of 60 farmers (20 from each site) were interviewed on the agricultural system (type of speculation and mode of cultivation), the use of pesticides and agrochemicals (source, dose and frequency) in vegetable farms and the watering system. An observational datasheet was also used to collect filed observation data, and these data were matched with the information collected from the farmers.

Mosquito collection and morphological identification

Aquatic stages (larvae and pupae) of anopheles' mosquitoes were collected in semi-permanent and permanent larval habitats in and around the vegetable farms using the WHO dipping method [27]. Collected mosquitoes were reared in laboratory conditions (25±2°C, 70-80% RH) and emerged adult mosquitoes were subjected to WHO test tube assays. Another portion of adult anopheles' mosquitoes were subjected to molecular speciation.

Molecular speciation of *An. gambiae* S.L Complex

One hundred mosquito samples were randomly selected from each study site and subjected to DNA extraction using the Livak protocol [28]. The DNA extracts were then amplified for the molecular speciation of *An. gambiae* complex using the SINE200 PCR technique described by Santolamazza *et al.* [29].

Insecticide susceptibility assays

Susceptibility tests to pyrethroid class insecticides (permethrin 0.75% and deltamethrin 0.05%) were conducted using the WHO test tube assays [30]. Prior to the bioassays, *An. gambiae*-Kisumu, a reference laboratory susceptible strain, was used to test the efficacy of insecticide-impregnated papers. Non-blood fed adult, 2-5 days old female Fo progeny of *An. gambiae s.l* field populations were exposed to the diagnosis dose (1X) of each insecticide according to WHO protocol. A total of 120-150 mosquito samples were used to assess the susceptibility of these insects to each insecticide. The knock-down effect was read 60 minutes post-exposure to the insecticide impregnated papers and the morality was recorded after 24 hours. Dead and alive mosquitoes were kept separately in dry Eppendorf tubes containing silica gel for further molecular analysis.

PBO synergist assays

To investigate the possible implication of cytochrome P450 enzymes in the observed resistance to pyrethroids, 2-5 days old females Fo *An. gambiae s.l.* progeny were pre-exposed to 4% piperonylbutoxide (PBO) for 1 h and then to each insecticide (permethrin 0.75% and deltamethrin 0.05%) according to WHO standard protocol [30]. Exposed mosquito populations were treated as described above for each insecticide tested.

KDR genotyping

For each insecticide, equal numbers of live and dead mosquitoes were subjected to DNA extraction according to the bioassay. The genotype at the kdr locus (for both kdr-west and kdr-east mutations) was determined using TaqMan real time PCR technique according to the protocol described by Bass *et al.* [49].

Statistical analysis

Data from this study were computed in SPSS 20.0 software. The resistance status of exposed mosquito populations was classified according to WHO criterion [30] as: mortality rate ≥ 98% for susceptible populations; 98% <mortality> 90% for suspected resistant populations and mortality < 90% for resistant populations. Chi-square contingency tests were used to test the association of kdr with bioassay survivorship for each insecticide; and to establish the difference between KAP categorical data. A two-sided p value < 0.05 was considered statistically significant.

Ethics statement

This study received an ethical authorization from the IITA internal review board (IRB). Farmer participation into the study was strictly voluntary and each participant provided a verbal consent prior to field activities and administration of questionnaires through interviews.

Results

Characteristics of vegetable farms in Yaounde

Vegetable farmers in Yaoundé had different educational levels including primary school (17/60), secondary school (39/60) and university training (4/60). In regard to agricultural practices in investigated farms, lettuce was the common vegetable found. Parsleys, leeks and celeries were common in Nkolondom and Ezazou vegetable farms, whereas cabbage and carrot were specific to Famassi vegetable farm. The use of synthetic pesticides was very common in investigated sites and most farmers from Nkolondom and Famassi (peri-urban area) acquired these products from the local markets; while those from Ezazou (urban area) got their inputs from suitable local stores. Up to 70% and 75% of farmers from Ezazou respectively use the recommended dose of pesticide (5 mL/10 L) and the required frequency (every 14 days) for the application of these products in the farm. Only 55% of farmers used the recommended dose of pesticides for crops protection in Nkolondom, and 35% respect the frequency of application of the chemicals in the farm. The respect of these pesticide utilization norms significantly decreased in Famassi (table1).

Table 1: Agricultural practice and characteristics of vegetable farms investigated in the city of Yaoundé.

Parameters	Nkolondom	Famassi	Ezazou
Nature of the farm	Peri-urban	Peri-urban	urban
Agricultural system	Vegetable farm	Vegetable farm	Vegetable farm
Main vegetable speculations	Parsley, leek, celery	cabbages, carrots, lettuce	Parsley, leek, celery, lettuce
Irrigation system	Swamps, wells	Swamps, Borehole	Swamps, Borehole
Source of pesticides used	Local market	Local market	Plant protection product store
Pesticides used	Pesticide, herbicides, fungicides	Pesticide, herbicides fungicides	Pesticide, herbicides fungicides
Agrochemicals used			
Active ingredients used	Pyrethroids, glyphosates, Copper sulfate	Pyrethroids, glyphosates, Copper sulfate	Pyrethroids, glyphosates, Copper sulfate
Recommended frequency of Pyrethroids' application (Every 14 days)	7/20 (35%)	6/20 (30%)	17/20 (85%)
Recommended dose of Pyrethroids' application (5 mL/10 L)	11/20 (55%)	7/20 (35%)	16/20 (75%)

Molecular identification of *An. gambiae s.l* complex.

All anopheles mosquito specimens collected from the study sites belonged to *An. gambiae s.l.* complex. *An. gambiae s.s.* constituted the main species found in Nkolondom (100%) and Famassi (96%), while *An. Coluzzii* (95%) was the main species found in Ezazou vegetable farm (Table2).

Table 2: *An. gambiae s.l.* species composition from vegetable farms in the city of Yaounde.

Study sites	Total number analyzed (n)	*An. coluzzii* (%)	*An. gambiae s.s.* (%)
Ezazou	100	95	5
Famasi	100	4	96
Nkolondom	100	0	100

Susceptibility of *An. gambiae s.l.* to deltamethrin and permethrin

A total of 1,375 *An. gambiae s.l* field populations were exposed to pyrethroid insecticides. These mosquito populations exhibited different resistant profiles from one vegetable farm to another after exposure to permethrin (X^2 =11.292; D.F. = 2; P=0.0035) and deltamethrin (X^2 =59.408; D.F. =2; P<0.0001). The mortalities recorded were 7%, 13% and 17.85% respectively for Nkolondom, Famassi and Ezazou after exposure to permethrin, a type I pyrethroid insecticide (figure 2), suggesting a resistance to permethrin in all vegetable farms investigated in Yaoundé. (X^2=11.292; D.F. = 2; P=0.0035). An equal resistance profile was recorded for deltamethrin, a type II pyrethroid, with mortality rates of 17.8%, 21.4% and 64 %, respectively for Nkolondom, Famassi and Ezazou (figure 3). The assays revealed different resistant profiles between urban and peri-uban vegetable farms for deltamethrin ($\chi2$= 59.408; P-value < 0.0001) and not permethrin ($\chi2$ = 1.436; P-value = 0.224); Suggesting different factors implicated in resistance selection to deltamethrin according to the type of vegetable farm.

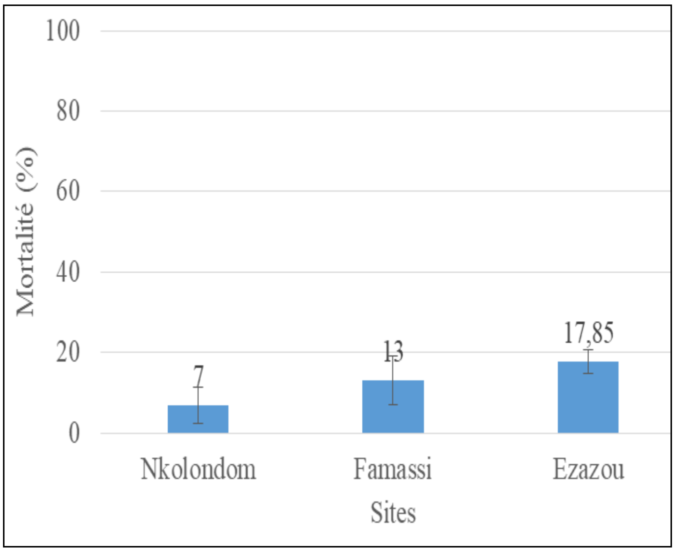

Fig 2: Susceptibility of field populations of *An. gambiae s.l.* to permethrin according to vegetable farms in the city of Yaoundé.

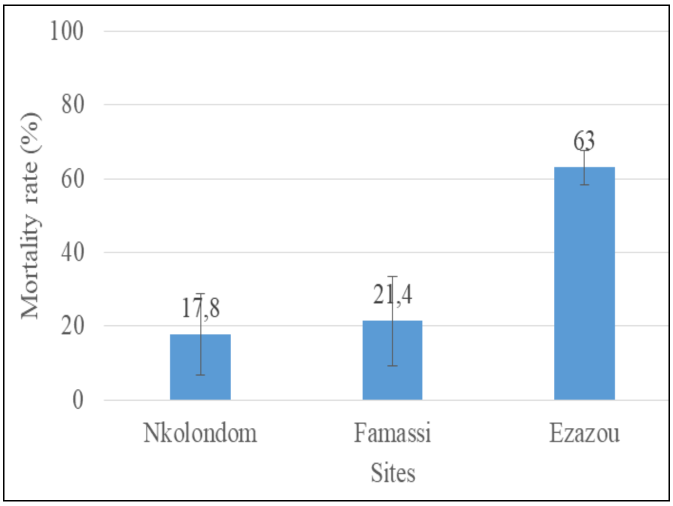

Fig 3: Susceptibility of field populations of *An. gambiae s.l.* to deltamethrin according to vegetable farms in the city of Yaoundé.

Susceptibility of *An. gambiae s.l* to PBO

After exposure to PBO, a partial recovery of the susceptibility to permethrin was recorded in all vegetable farms with mortality rates of 76.39% in Nkolondom, 51.58% in Famassi and 69.83% in Ezazou (figure 4). In addition, a partial recovery of the susceptibility to deltamethrin was recorded in all vegetables with mortality rates of 71.67% in Nkolondom, 60.05% in Famassi and 85.14% in Ezazou (figure 5). These data suggest that monooxygenase P450 enzymes is not the only resistance mechanism involved in the resistance to pyrethroid insecticides in investigated vegetable farms; and that additional mechanisms such as target site mutations (kdr mutations) might be involved.

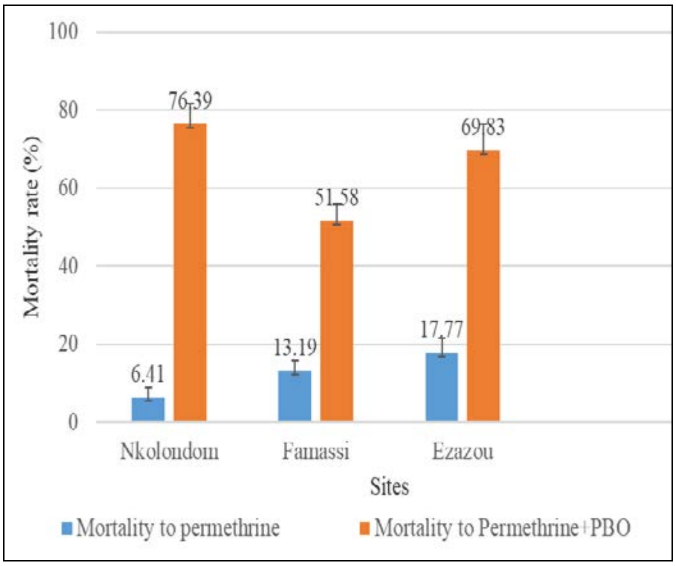

Fig 4: Susceptibility of field populations of *An. gambiae s.l.* to permethrin and PBO+ permethrin in investigated vegetable farms in the city of Yaoundé.

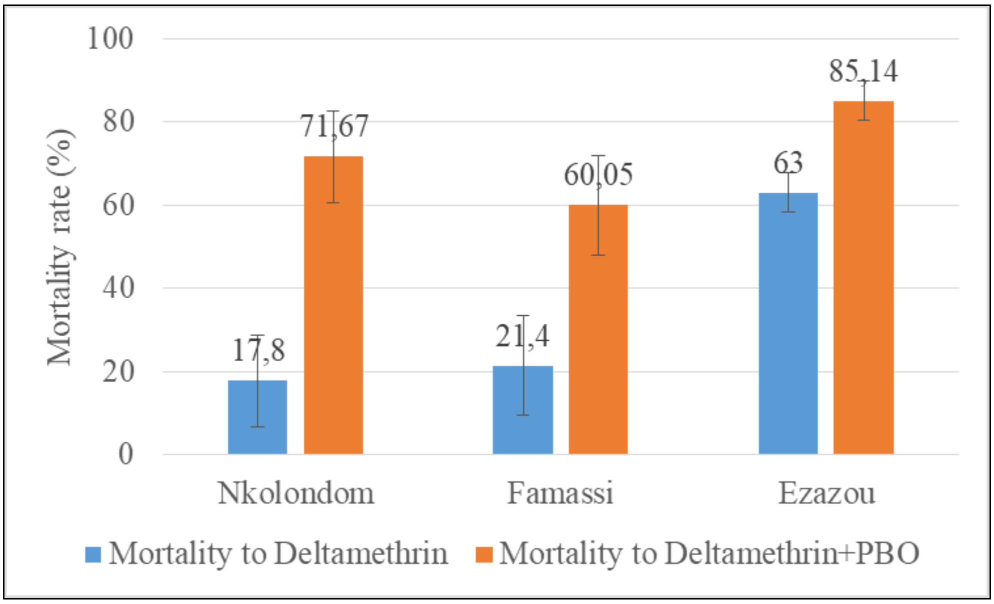

Fig 5: Susceptibility of field populations of *An. gambiae s.l.* to deltamethrin and PBO+ deltamethrin in investigated vegetable farms in the city of Yaoundé.

Kdr genotyping in *An. gambiae s.l.*

The analysis of dead and live mosquito specimens revealed that the resistant kdr 1014F allele is close to fixation in all sites in mosquitos tested to permethrin (0.90, 1 and 1 respectively in Ezazou Famassi and Nkomondom) and deltamethin (0.94, 0.96 and 1, respectively in Ezazou Famassi and Nkomondom) A strong association was observed between the presence of the kdr 1014F-resistant allele and the survivorship of the mosquito populations either to permethrin and deltamethrin (OR=7.66; P<0.0001 and OR= 10.4; P<0.0001 respectively).

Table 4: Frequencies of kdr-west L1014F alleles in field An. gambiae s.l populations exposed to pyrethroid insecticides (permethrin and deltamethrin).

investigated farm	Insecticide	Phenotype	L1014F Alleles			N	2N	frequency Genotypic			Allelic frequency		OR	P-Value
			RR	RS	SS			f(RR)	f(RS)	f(SS)	f(R)	f(S)		
Ezazou	Permethrin	Alive	21	4	0	25	50	0.8	0,2	0	0,9	0,1		
		Dead	2	23	0	25	50	0.08	0,92	0	0,54	0,56	7,66	<0.0001
	Delthamethrin	Alive	22	3	0	25	50	0.088	0.12	0	0.94	0.06		
		Dead	2	23	0	25	50	0.79	0.21	0	0.6	0.4	10.44	<0.0001
Famassi	Permethrin	Alive	25	0	0	25	50	1	0	0	1	0		
		Dead	1	24	0	25	50	0.07	0,93	0	0,53	0,47	NA	NA
	Delthamethrin	Alive	24	1	0	25	50	0.92	0.08	0	0.96	0.04		
		Dead	0	25	0	25	50	0	1	0	0.5	0.5	9.20	<0.0001
Nkolondom	Permethrin	Alive	25	0	0	25	50	1	0	0	1	0		
		Dead	0	25	0	25	50	0	1	0	0,5	0,5	/	/
	Delthamethrin	Alive	25	0	0	25	50	1	0	0	1	0		
		Dead	1	24	0	25	50	0.04	0.69	0	0.52	0.48	/	/

N: number of mosquito analyzed; OR: odd ration; f: allelic frequency, RR: resistant mosquito; RS: suspected resistant mosquito; SS: susceptible mosquito**.**

Discussion

The frequency of vegetable farms in most urban and peri-urban areas in Africa is increasing due to unemployment and rural exodus [31]. Urban and peri-urban agriculture stands as an alternative to solve the problem of unemployment and food insecurity in many capital cities in Africa [12]. The application of chemical compounds in vegetable farms is often very random, contributing to several agricultural and public health challenges such as pest resistance and the development of resistance in non-targeted organisms, such as mosquito species of public health interest. Although the pesticides used in vegetable farms are usually obtained from illegal markets with unknown origins, their misuse could be attributed to the low educational level of the farmers who mostly have a primary educational level, as well as the lack of technicity in the application of agricultural activities, mainly in peri-urban areas. Quantitative data from this study revealed that most of the farmers do not know the activity of the products they apply on the crops. Moreover, these products are relatively used according to previous application by relatives or other farmers. The main farmer's interest is to intensify the productivity of the crops and raise money for routine subsidence. It is therefore recommended to intensify the capacity building of the vegetable farmers mainly on the use of pesticides (frequency and dose) and personal protection during application of these products in the farms.

The use of agricultural pesticides may have a deep impact on the development of resistance in wield populations of anopheles mosquito. This study revealed that *An. gambiae s.l* mosquito from larval habitats found in vegetables farms in urban and peri-urban areas in Yaoundé are totally resistant to type I and type II pyrethroid insecticides. This data is consistent with previous observations described in the city of Yaoundé by Antonio-Nkondjio *et al.* [32, 33] and in other African capitals with *An. Arabiensis* [34, 35, 36]. The reduced susceptibility of malaria vectors to pyrethroids is a major concern for the National Malaria Control Program and a major threat for the effectiveness of the vector control tools such as LLINs and IRS. In fact, pyrethroid insecticides such as lambda-cyhalothrin which is mainly used in vegetable farms constitute the same class of molecules with deltamethrin and permethrin which are used in public health intervention tools (LLINs and IRS). This common utilization of pyrethroid insecticides in both agricultural and public health interventions has generated the phenomenon of cross-resistance in *An. gambiae s.l* as found in this study. In addition, the use of different types of xenobiotics (herbicides, fungicides, fertilizer or other insecticides) could also impact the metabolic system of mosquito larvae, leading to a broad spectrum of tolerance to several insecticides; and the selection of resistance during future generations of insect populations [37, 38, 39]. The present study demonstrated a cause-and-effect relationship between the agricultural system and the development of resistance in malaria vectors in a small scale, and may favor the evolution of resistance [4, 25, 32, 41].

The kdr-west L1014F-resistant mutation which confers resistance to pyrethroids and organochloride insecticide classes (DDT) was detected in all vegetable farms investigated in this study. Although the resistant 1014F allele of this mutation is already fixed in two vegetable farms, it is almost fixed in the third site. Suggesting that mosquito species carrying this mutation could not by-stand the effect of pyrethroid insecticides and that the presence of this mutation could not always be associated with the survivorship of the insects. Additional resistant mechanisms are therefore involved in the survivorship of *An. gambiae s.l* populations collected from vegetables farms in Yaoundé. Among these mechanisms, we have the metabolic mechanisms of monooxygenase P450 enzymes as revealed by PBO synergist assays in this study. This finding is in concordance with other studies in western Africa [42, 43]. In contrast, previous studies have shown weak or absence of a significant association between the kdr mutation and survivorship phenotype in *An. arabiensis* in Sudan [35, 44, 45]. The kdr-east 1014S mutation was not investigated in this study because of its very low proportion in resistant mosquito populations from Central African regions [46]. The strong resistance to pyrethroids observed and the high frequency of resistance genes are major threats on the efficacy of mosquito nets. The identification of individual mosquitoes harboring two types of resistance genes (e.g., kdr + metabolic resistance) appears to be an additional hinder to current vector control tools, and if this feature is widely spread, the effectiveness of malarial intervention tools will be intensively decreased and new tools should be envisaged.

The effective resistance management strategies which depend on the population of insects should also consider the principal features of insecticide resistance selection pressures among the insect populations. Thus, the spread of insecticide resistance could be a result of the weakness of current vector control tools and the misuse of pesticides in agriculture [9, 47]. Other factors such as xenobiotics or heavy metals have also been incriminated [10]. Vegetable farming represents the main source of food and income in most sub-Saharan African countries [48]. Farmers commonly apply chemicals which serve as pest control strategies to increase their yield [48]. Fighting against immature stages of mosquitoes is very crucial in vector control strategies to interrupt the development of adults. However, the implementation of larviciding is somehow very complex and it requires important resources to be effective. An integrated approach could be required to target the agricultural pests and mosquito insects found in the same farm, and to stop the spread of resistance in major insects of agricultural and public health interests.

Conclusion

An. gambiae s.l. mosquito populations have developed high resistance to type I and type II pyrethroid insecticides in vegetable farms across Yaounde. The kdr L1014F mutation is almost fixed in these mosquito populations and P450 enzymes play a crucial role in the observed resistance pattern. These findings are consistent with the use of agricultural insecticide in investigated vegetable farms and which contribute to insecticide resistance selection in malaria vectors. A coordination between the integrated vector control program and the Global Plan for Insecticide Resistance Management (GPIRM) should be achieved for a successful implementation of rational resistance management strategies against malaria vectors at small scale.

References

1. WHO. World Malaria Report, 2018.
2. Raghavendra K, Barik T, Reddy B, Sharma P, Dash P. Malaria vector control: from past to future. Parasitol Res., 2011; 108:757-79.
3. WHO. World Malaria Report 2017. Geneva: World Health Organization, 2018.
4. Diabaté A, Baldet T, Chandre F, Akogbeto M, Guiguemde R, Darriet F *et al.* Role of agricultural use of insecticides in resistance to pyre- throids in Anopheles gambiae s.l. in Burkina Faso. Am J Trop Med Hyg. 2002; 67:617-22.
5. Yadouleton AW, Asidi A, Rousseau FD, Braïma J, Agossou CD, Akogbeto MC. Development of vegetable farming: a cause of the emergence of insecticide resistance in populations of Anopheles gambiae in urban areas of Benin. Malar J. 2009; 8:10
6. Matowo J, Kulkarni MA, Mosha FW, Oxborough RM, Kitau JA *et al.* Biochemical basis of permethrin resistance in Anopheles arabiensis from Lower Moshi, north-eastern Tanzania. Malar J. 2010; 9:1475-2875.
7. Ranson H, Abdallah H, Badolo A, Guelbeogo WM, Kerah-Hinzoumbé C *et al.* Insecticide resistance in Anopheles gambiae: data from the first year of a multi-country study highlight the extent of the problem. Malar J 2009; 8:299.
8. Khambay BPS, Jewess PJ. Pyrethroids. In: Gilbert, L.I., Gill, S.S. (Eds.), Insect Control: Biological and Synthetic Agents. Elsevier Academic Press, 2010, 1e29.
9. Nkya TE, Akhouayri I, Poupardin R, Batengana B, Mosha F, Kisinza W et al. Insecticide resistance mechanisms associated with different environ- ments in the malaria vector Anopheles gambiae: a case study in Tanzania. Malar J. 2014; 13:28.
10. Defo-talom A, Essoung MA, Gbankoto A, Tchigossou G, Akoton R, Bio-Bangana *A et al.* A preliminary analysis on the effect of copper on Anopheles coluzzii insecticide

resistance in vegetable farms in Benin. Scientific Report 2020; 10:6392.

11. Chouaïbou MS, Fodjo BK, Fokou G, Allasane OF, Koudou BG, David JP *et al*. First report of the East African mutation in an kdr Anopheles mosquito in Côte d'Ivoire gambiae Wellcome Open Research 2017; 2:8.
12. Assogba-Komlan F, Anihouvi P, Achigan E, Sikirou R, Boko A, Adje C *et al.* Pratiques Culturales et Teneur en Eléments Antinutritionnels (Nitrates et pesticides) du Solanum macrocarpon au Sud du Bénin. Africain Journal of Food, Agriculture, Nutrition and Development. 2007; 7:1-21.
13. James B, Atcha-Ahowé C, Godonou I, Baimey H, Goergen G, Sikirou R *et al*. Gestion intégrée des nuisibles en production maraîchère : Guide pour les agents de vulgarisation en Afrique de l'Ouest. Institut international d'agriculture tropicale (IITA), Ibadan, Nigeria. 2010, 120.
14. Yolou I, Yabi I, Kombieni F, Tovihoudji PG, Yabi JA, Paraïso AA *et al*. Maraîchage en milieu urbain à Parakou au Nord-Bénin et sa rentabilité économique. Int. J. Innov. Sci. Res. 2015; 19(2):290-302.
15. Tchuinte GMS. Etude socio-économique des systèmes de production maraîchère en zones urbaines et périurbaine de la ville de Djougou (Département de la Donga). Mém. Ing. Agron., FSA, Univ. Abomey-Calavi, Cotonou, 2005, 141
16. Mondédji AD, Nyamador WS, Amévoin K, Abbey GA, Ketoh GK. Analyse des caractéristiques sociodémographiques et identification des perceptions des distributeurs de pesticides et des consommateurs sur l'utilisation d'extraits botaniques dans la gestion des insectes ravageurs des cultures maraîchères au sud du Togo. Bulletin de l'IFAN Cheikh Anta Diop, Série A, 2014b; 53(2):135-150.
17. Kanda M, Akpavi S, Wala K, Djaneye-Boundjou G, Akpagana K. Diversité des espèces cultivées et contraintes à la production en agriculture maraichère au Togo. Int. J. Biol. Chem. Sci.,2014; 8(1):115- 127.
18. Traoré A, Ahoussi KE, Aka N, Traoré A, Soro N. Niveau de contamination par les pesticides des eaux des Lagunes aghien et potou (sud-est de la côte d'ivoire) Int. J. Pure App. Biosci. 2015; 3(4):312-322 ISSN: 2320 – 7051.
19. Tossou E, Tepa-Yotto G, Kpindou OKD, Sandeu R, Datinon B, Zeukeng F *et al*. Susceptibility Profiles of Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) to Deltamethrin Reveal a Contrast between the Northern and the Southern Benin. Int. J. Environ. Res. Public Health 2019; 16:1882.
20. Xiang H, Nuckols JR, Stallones LA. Geographic information assessment of birth weight and crop production patterns around mother's residence. Environ Res., 2000; 82(2):160-167.
21. Levario-Carrillo M, Amato D, Ostrosky-Wegman P, Gonzalez-Horta C, Corona Y, Sanin LH. Relation between pesticide exposure and intrauterine growth retardation. Chemosphere, 2004; 55(10):1421-1427.
22. De Siqueira MT, Braga C, Cabral-Filho JE, Augusto LGDS, Figueiroa JN, Souza AI. Correlation between pesticide use in agriculture and adverse birth outcomes in Brazil: an ecological study. Bull Environ. Contam. Toxicol., 2010; 84 6):647-651.
23. Antonio-Nkondjio C, Sonhafouo-Chiana N, Ngadjeu CS, Doumbe-Belisse P, Talipouo A, Djamouko-Djonkam L, *et al.* Review of the evolution of insecticide resistance in main malaria vectors in Cameroon from 1990 to 2017. Parasit Vectors. 2017; 10:472.
24. Menze BD, Wondji MJ, Tchapga W, Tchoupo M, Riveron JM, Wondji CS. Bionomics and insecticides resistance profiling of malaria vectors at a selected site for experimental hut trials in central Cameroon. Malar J., 2018; 17(1):317.
25. Chouaïbou MS, Etang J, Brévault T, Nwane P, Kérah-Hinzoumbé C, Mimpfoundi R *et al.* The dynamics of insecticide resistance in the malaria vector Anopheles gambiae s.l. from an area of extensive cotton cultivation in Northern Cameroon. Trop Med Int Health. 2008; 13:1-11.
26. Gillies MT, De Meillon B. The anophelinae of Africa south of the Sahara. Pub. lSouth African Institute Med. Res. 1968; 54:343.
27. WHO. The World Health report, 2003.
28. Livak KJ. Organization and mapping ofa sequence on the Drosophila melanogaster X and Y chromosomes that is transcribed during spermatogenesis.Genetics. 1984; 107:611-634.
29. Santolamazza F, Mancini E, Simard F, *et al*.: Insertion polymorphisms of SINE200 retrotransposons within speciation islands of Anopheles gambiae molecular forms. Malar J. 2008; 7:163.
30. WHO. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. World Health Organisation Technical Report Series. 2nd edn. Geneva: World Health Organization, 2013.
31. Akogbeto M, Djouaka R, Kinde-Gazard D. Screening of pesticide residues in soil and water samples from agricultural settings. Malar. J. 2006; 5:22
32. Antonio-Nkondjio C, Fossog BT, Ndo C, Menze-Djantio B, Togouet SZ, Awono-Ambene P *et al*. Anopheles gambiae distribution and insecticide resistance in the cities of Douala and Yaoundé (Cameroon): influence of urban agriculture and pollution. Malar J. 2011; 10:154.
33. Antonio-Nkondjio C, Simard F. Highlights on Anopheles nili and Anopheles moucheti, Malaria Vectors in Africa. In Anopheles Mosquitoes: New Insights into Malaria Vectors; Manguin, S., Ed. In Tech :Rijeka (HR), Croatia, 2013.
34. Seidahmed OM, Abdelmajed MA, Mustafa MS, Mnzava AP. Insecticide susceptibility status of the malaria vector Anopheles arabiensis in Khartoum city, Sudan: differences between urban and periurban areas. East Mediterr Health J 2012; 18:769-776. PubMed: 22891527.
35. Himeidan YE, Muzamil HM, Jones CM, Ranson H. Extensive permethrin and DDT resistance in Anopheles arabiensis from eastern and central Sudan. Parasit Vectors, 2011; 4:154.
36. Yewhalaw D, Wassie F, Steurbaut W, Spanoghe P, Van Bortel W *et al*. Multiple insecticide resistance: an impediment to insecticide- based malaria vector control program. PLOS ONE, 2011; 6:e16066.
37. Nkya TE, Akhouayri I, Kisinza W, David JP. Impact of environment on mos- quito response to pyrethroid insecticides: facts, evidences and prospects. Insect Biochem Mol Biol, 2013.
38. Poupardin R, Riaz MA, Vontas J, David JP, Reynaud S. Transcription profiling of eleven cytochrome P450s potentially involved in xenobiotic metabolism in the mosquito Aedes aegypti. Insect Biochem Mol Biol. 2010;

19:185-93.
39. Riaz MA, Poupardin R, Reynaud S, Strode C, Ranson H, David JP. Impact of glyphosate and benzo[a]pyrene on the tolerance of mosquito larvae to chemical insecticides. Role of detoxification genes in response to xenobi- otics. Aquat Toxicol. 2009; 93:61-9.
40. Chouaïbou MS, Fodjo BK, Fokou G, Ouattara AF, Koudou BG, David JP *et al*. Inluence of the agrochemicals used for rice and vegetable cultivation on insecticide resistance in malaria vectors in southern Côte d'Ivoire. Malar J, 2016; 15:426.
41. Yadouleton A, Thibaud M, Padonou G, Chandre F, Asidi A, Djogbenou L, *et al*. Cotton pest management practices and the selection of pyrethroid resistance in Anopheles gambiae population in Northern Benin. Parasit Vectors. 2011; 4:60.
42. Diabaté A, Baldet T, Chandre F, Dabire KR, Simard F *et al*. First report of a kdr mutation in Anopheles arabiensis from Burkina Faso, West Africa. J Am Mosq Control Assoc 2004; 20:195-196.
43. Donnelly MJ, Corbel V, Weetman D, Wilding CS, Williamson MS *et al*. Does kdr genotype predict insecticide-resistance phenotype in mosquitoes? Trends Parasitol, 2009; 25:213-219.
44. Abdalla H, Matambo TS, Koekemoer LL, Mnzava AP, Hunt RH *et al*. Insecticide susceptibility and vector status of natural populations of Anopheles arabiensis from Sudan. Trans R Soc Trop Med Hyg. 2008; 102:263-271.
45. Matambo TS, Abdalla H, Brooke BD, Koekemoer LL, Mnzava A *et al*. Insecticide resistance in the malarial mosquito Anopheles arabiensis and association with the kdr mutation. Med. Vet. Entomol, 2007; 21:97-102.
46. Amen N, Fadel AN, Ibrahim SS, Tchouakui M, Ebai T, Wondji M *et al*. A combination of metabolic resistance and high frequency of the 1014F kdr mutation is driving pyrethroid resistance in Anopheles coluzzii population from Guinea savanna of Cameroon. Parasites Vectors. 2019; 12:263.
47. Protopopof N. *et al*. Significant increase in kdr in Anopheles gambiae is associated with an intensive vector control intervention in Burundi highlands. Trop. Med.Int.Health, 2008; 13:1479-1487.
48. NEPAD. African agriculture, transformation and outlook. NEPAD, 2013.
49. Bass C, Nikou D, Blagborough AM, Vontas J, Sinden RE, Williamson MS, *et al*. PCR-based detection of Plasmodium in Anopheles mosquitoes: a comparison of a new high-throughput assay with existing methods. Malar J. 2008; 7:177.