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The role of agricultural and public health insecticides in the development of the multi-resistance in *An. gambiae* in Tiassalé and Toumodi (Côte d'Ivoire)

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ABSTRACT

Recent studies have shown high level of insecticide resistance in *Anopheles gambiae* mosquitoes to almost all insecticide used in public health in Tiassalé. The selective pressures responsible for this resistance are not clearly identified. To highlight common practices which could have contributed to the emergence of resistance, a study was conducted in Tiassalé and Toumodi. A questionnaire was submitted to assess the history of insecticides used. The results of the descriptive analyses showed a high use of agricultural and public health insecticides in these cities with 97 % of users among the population. Pyrethroids appeared to be the most used insecticides in both public health and agriculture with a higher rate of usage in agriculture than in public health. The definition of measures of "good agricultural practices" ultimately to reduce the risk of cross insecticide resistance emergence should be undertaking.

Keywords: Insecticide, resistance, agriculture, public health, mosquito.

1. Introduction

Resistance of mosquito to insecticides became a public health problem when the decrease of susceptibility of *An. gambiae* to synthetic pyrethroids was observed [1]. Pyrethroids are the main insecticides currently used against mosquitoes' bites for controlling or preventing malaria. They are incorporated or coated in mosquito net or are used for Indoor Residual Spraying (IRS) [2]. Therefore, the development of resistance to pyrethroids in malaria mosquitoes appears as a major threat for malaria control. One of the major questions emerging from this situation concern the origin of the resistance. So far, two principal mechanisms were identified to be mainly involved in insecticide resistance: metabolic resistance and insecticide target site mutation including *knock down resistance* (kdr) or insensitive acetylcholinesterase (AceI).

In Burkina Faso, the frequency of *kdr* genes (*kdr*) observed in *An. gambiae* is higher in the cotton zones than the areas of garden production [3]. In Benin, the resistance of *An. gambiae* was partly due, to the pressure exerted by agricultural insecticides on larvae [1]. These data suggest that the resistance of *An. gambiae* observed in West Africa is closely linked to the insecticide treatments of fields against pests. These results suppose that, the resistance should be limited principally to the agricultural areas only, usually subjected to insecticide treatments. However, other studies in West Africa within the network "*Multilateral initiative on malaria*" showed similar frequencies of *kdr* resistance in cotton areas usually subject to insecticides as well as non-cotton areas [1].

In addition, the resistance of *An. gambiae* to Dichlorodiphenyltrichloroethane (DDT) observed in Benin was explained, in part, by IRS with the same product and dieldrin [4]. In South Africa, the IRS with DDT done previously has fostered for resistance of *An. funestus* to pyrethroids applied later [5].

In Côte d'Ivoire, mosquito resistance was observed for the first time in the region of Sassandra and involved resistance to organochlorines [6]. Prethroids and carbamates resistance were reported 30 years later in the 1990s [7-8]. Today, *An. gambiae* mosquitoes have developed resistance to almost all the four groups of insecticides family (e.g.

organochlorine, pyrethroids, carbamates and organophosphates) used in agriculture and public health in the region of Tiassalé [9]. The authors stated that, the selection pressure responsible for this multiple resistance was unclear. However, the role of agriculture was highlighted. The region of Tiassalé is an important agricultural area characterized by large farms of bananas and pineapples belonging to important agro-industrial groups and small productions like cocoa, rubber, palm oil and vegetables. The region is also characterized by irrigated rice and hydro-power production from Bandama River. Fishing and livestock production are also highly developed. Historically, Tiassalé region had a high potential for agricultural insecticides use. Furthermore, the irrigation, hydro-power and the deforestation for crop production have also changed the ecological conditions of the region, contributing to the increase of mosquitoes population throughout the year [10-11-12]. In addition, it was shown that Ivoirians mostly prefer mosquito coils (51%) and aerosol (31%) compared to the net in general (19%) to protect themselves from mosquito bites [13].

It is therefore likely that in this region, the uncontrolled use of agricultural and public health insecticides has fostered the development of the multi-resistance in *An. gambiae*. The objective of this study was to characterize agricultural practices and public hygiene which could possibly contribute to mosquito resistance to insecticides in the cities of Toumodi and Tiassalé.

2. Materials and methods

2.1 Survey area

A cross-sectional survey was conducted among users and distributors of agricultural and public health insecticides in the region of Tiassalé (Tiassalé and Taabo) and Toumodi (Toumodi and Djékanou), two neighboring regions with high potential for the use of agricultural and public health insecticides due to the ecological change (hydropower and irrigated lands) (Figure 1).

In all these places, we hypothesized that farmers use insecticides improperly to secure agricultural production and/or to fight against ticks (1), fishermen use insecticides to improve their production during water drops (2) and people misusing public health insecticides to protect themselves from mosquito bites, for prevention of malaria (3).

2.2 Data collection

In each zone, a survey (quantitative and qualitative information) was submitted to the population. The questionnaire was focused, on the use of insecticides in agriculture and public health, the reasons for their use, the dose employed, the timing of application, and the frequency of treatment. In addition to that, the cleaning of equipments, the place where insecticides are bought, what people know about insecticide use (information given by distributors or users), do people need to be taught about insecticide use, the security rules on the use of insecticides and management inventory before and after using insecticides.

In this study, crop producers, fishermen and herdsmen were considered as farmers. In Tiassalé the interviewees included 8 rice producers, 12 vegetable's producers, and the head of plant defense management on a banana plantation owned by an agro-industrial group. Six people in the village and one insecticide sprayer were interviewed. In the area of Taabo, 8 fishermen, 28 members of a cooperative of cocoa, one insecticide sprayer and five residents of Taabo city were interviewed.

In Toumodi, 10 herdsmen and 19 individual producers of cocoa, 10 members of a cooperative of cocoa, 3 insecticide sprayer and 4 villagers of Bringakro were interviewed. In total 124 people including 95 farmers, 8 dealers of agricultural pesticides, 1 dealer of veterinary insecticide, 5 insecticide sprayer and 15 people were interviewed throughout the study area. The particularity of the farmers interviewed in this study is the proximity of their farm to the town or village and the water points. Moreover, it is repeatedly the sale of insecticides for agricultural use is haphazardly in Côte d'Ivoire. This practice is characterized by two main factors: 1 Sellers found in stores approved that, for pesticides employees who do this job are not generally trained to and therefore are unable to advise clients. The illegal sale of insecticides (not approved by the Ministry of Agriculture) is developed in the study area. A questionnaire was subjected to 8 dealers of pesticides 5, in Tiassalé and 3 in Toumodi and one seller of veterinary one in Toumodi to visually verify this assertion.

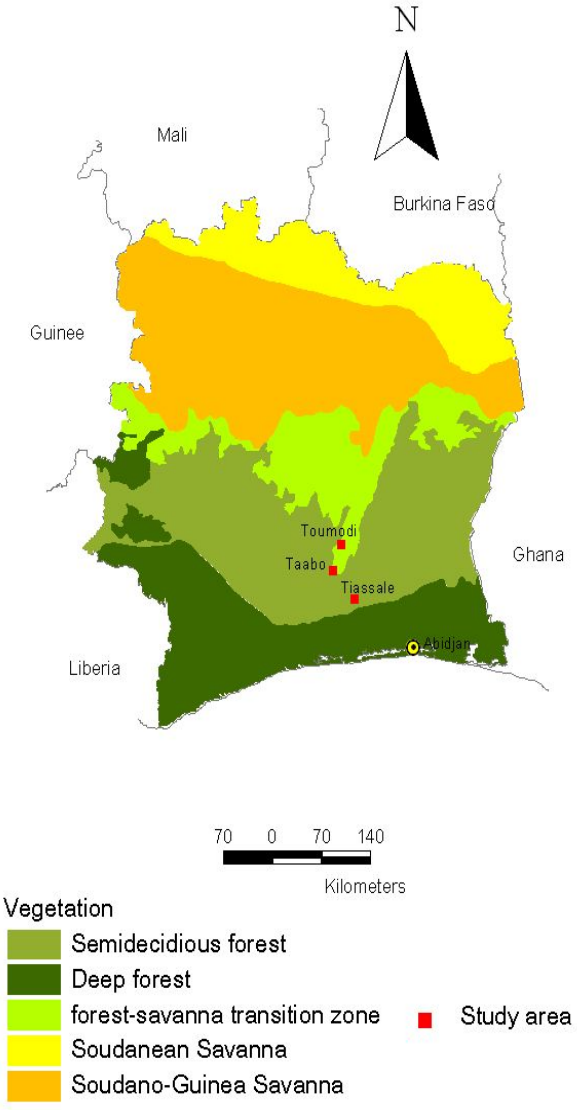

Fig 1: Map of study zone

3. Results

Investigations in Tiassalé, Taabo and Toumodi showed that the populations of these regions use mostly insecticides (Figure 2). In fact, 50% of them have used both agricultural and public health

insecticides once. Twenty seven per cent used agricultural insecticides and 20% used public health insecticides, while only 3% did not use insecticides at all. This intensive use of insecticides according to all interviewed, meets three levels of need of people (1) to protect themselves against mosquito bites and malaria, (2) guarantee agricultural production threatened by insect, pests and (3) to protect cattle against ticks.

3.1 Insecticides used in household for public health

People agree that they greatly suffer from mosquito bites. For self-protection, four (4) different methods were used (Figure 2): ITNs, aerosol sprays, mosquito coils and dual protection that combine impregnated mosquito nets and insecticide aerosols.

Throughout the study area, 20% of the population use only ITNs. In Tiassalé, users have reported to have received the nets during free distribution campaigns carried out in the framework of malaria program. Contrary to Bringakro, people paid a fixed price per unit of 3 dollars, US. The price of a net varies in general between 6 to 10 dollars. The net is used as a method of personal protection almost exclusively in small families (≤ 4 people). In this case, the family members sleep under mosquito nets. But, in the big families (≥ 5 persons), nets are placed on windows and doors of houses to protect all members of the family.

Thirteen percent (13%) of the surveyed population used aerosol insecticides spraying as a method to protect themselves against mosquitoes. These insecticides are sold under various trade names (Table 1). These aerosols have various formulations. Some have a formulation composed solely of pyrethroids (transfluthrin 0.02%, cyfluthrin + imiprothrin 0.015% 0.05% 0.1% phenothrin imiprothrin + 0.04% + 0.15% tetramethrin, allethrin 0.25% deltamethrin + 0.015%). Others have a formulation consisting of a mixture of pyrethroid (cyfluthrin 0.04%), an organophosphate (dichlorvos 1%) and a carbamate (propoxur 1%) or of the mixture of an organophosphate (dichlorvos 1%) and several pyrethroids (tetramethrin bioallethrin 0.04% + 0.2% + 0.06% permethrin) (Figure 2). For economic and financial reasons, up to 68% users underdose by spraying small quantities of insecticides. Some just select areas or room to be sprayed. Other keep the doors and windows open when spraying to avoid smell of insecticides that could cause headaches. Only 32% complied with spaying recommendations and good practices (all rooms treated, doors and windows closed for 10 to 15 min after treatment). In all cases, people treat their room between 6-8 pm to allow the removal of odor before going to the bed. The spraying frequency depends on the pressure of the mosquitoes. During periods of low infestations of mosquitoes (rainy season), the average frequency of spraying is 1-2 treatments per month and can reach 4-8 treatments per month when infestations become strong (dry season). Dealer or insecticide sprayer interviewed in Tiassalé said pyrethroids (cypermethrin 12 g/l and deltamethrin 25 g/l) are usually used in agriculture in addition to permethrin 100 g/kg for the treatment of Green spaces or garden (hotels, room) in the city when it is requested.

Five percent (5%) of the surveyed population use mosquito coils commonly called "moustico" as a method of personal or family protection against mosquito bites. The use of mosquito coils was observed only in the Taabo's fishing community. These users said that, the smoke from burning coil does not kill mosquitoes but repels them. So they prefer to keep windows and doors open at the time of treatment to repel mosquitoes out of homes. The average frequency of treatment is one spiral per night. Fishermen have also said that they use mosquito coils outdoors in the river to protect themselves from mosquito bites while fishing. Mosquito coils found in the study area have a formulation composed solely of pyrethroids (allethrin 0.30% and 0.20% of D-allethrin).

More than half of the population surveyed (52%) associated impregnated mosquito nets and insecticide aerosols to fight against mosquitoes. These users felt that the number of nets they are able to buy is not enough to protect their families generally large, then the aerosol spray comes to support the treatment only during heavy mosquitoes infestations. In these families, mostly women and children sleep under mosquito nets and men and older people spray their bedrooms with aerosol with a spraying frequency of up to 4-8 times a month.

Public health insecticides used by people to protect themselves against mosquito bites are available in the shops and supermarkets in the study area. The study revealed that these products are acquired without having information about recommendation for use. In addition, all the users generally recognize that they do not read the information on how to use the packaging prior before using them.

3.2 Insecticides used in agricultural system

3.2.1 Type of insecticide, application frequency

Most farmers (77%) used different types of insecticides to protect their crops against insect pests and / or to fight against cattle's ticks (Table 1, figure 2). Results from Tiassalé showed also that, farmers used very little insecticides against pest (insects) compared to those used against weeds grass in their farms. Some farmers said that, they only used Bastion 5G® (carbofuran 50 g/kg) to treat their vegetable nurseries against termite attack. Even though this is very rare, others have treated their rice fields with Decis 60 EC® (deltamethrin 60 g/l) just after transplanting. The major problem was grain attacks by birds and rice grains when harvested were systematically treated with Bastion 5G®. Dead birds were observed on the rice perimeter during the investigation. As for the vegetable producers, they admit not to be able to produce vegetables without using insecticides. For vegetables such as cabbage, lettuce and tomato, fields are systematically treated with pyrethroids (deltamethrin 25 g/l, Cypermethrin 50 g/l) and / or a mixture of a neonicotinoid (20 g/l) and a pyrethroid (lambda-cyhalothrin) at a frequency of 2 to 3 treatments per week. Some producers sometimes used Cypercal 200 EC® (cypermethrin 200 g/l), a cotton insecticide, pretexting that, Decis 12 EC® (deltamethrin 12.5 g/l) for vegetables becomes ineffective. Four families of insecticides are used for public health and agricultural (figure 3 and 4).

In Taabo and Toumodi, farmers interviewed are mainly cocoa, yam, cassava and plantain producers. Insecticides are not used in yam, cassava and plantain farms. Most of the cocoa farms are relatively young, but few years ago, producers recognized a drastic decrease in their cocoa yield due to damage caused by the mirids of cocoa (*Sahlbergella sp, Distantiella theobromae*) and loss of soil fertility. Their use of insecticides has become a necessity in cocoa production against bugs; stem borers and sucking pests of cocoa at a frequency of 2 treatments per year. These insecticides contained neonicotinoids (acetamiprid 20 g/l and Imidacloprid 30 and 60 g/l) or a mixture of a néonictinoïde (acetamiprid 20 g/l imidacloprid or 30 g/l) and a pyrethroid (20 g bifenthrin / l or Lambda-Cyhalothrin 15 or 30 g/l). These insecticides are sold under various trade names.

Livestock farmers in Toumodi primarily use pyrethroids (alphacypermethrin 10%) and amidine (amitraz 12.5%) to fight against cattle ticks at a frequency of 1 to 2 treatments per year

(figure 4). According to the fishermen, their production has steadily declined in recent years due to aging of fishing gear as they were hardly able to afford the high cost of fishing nets. Others think that the fish in the river have become rare because they are hiding to avoid being captured. However, no fisherman has used insecticides for fishing despite the low level of their production.

Table 1: distribution of the population according to their practical knowledge and production methods

			Farmer status	
Parameters	**Practices**	**Total respondents**	**% Cooperatives**	**% Individual farmers**
Dosage application	Recommended dose	43	74.4 (CI: 58.8-86.5)	25.6 (CI: 13.5-41.2)
	Overdose	3	66.7 (CI: 9.4-99.2)	33.3 (CI: 0.8-90.6)
	Under-dose	49	8.2 (CI: 2.3-19.6)	91.8 (CI: 80.4-97.7)
Knowledge on the insecticide	Trained	9	44.4 (CI: 13.7-78.8)	55.6 (CI: 21.2-86.3)
	untrained	74	45.9 (CI: 34.3-57.9)	54 (CI: 42.1-65.7)
	Advised	12	0 (CI: 0-26.5)	100 (CI: 73.5-100)

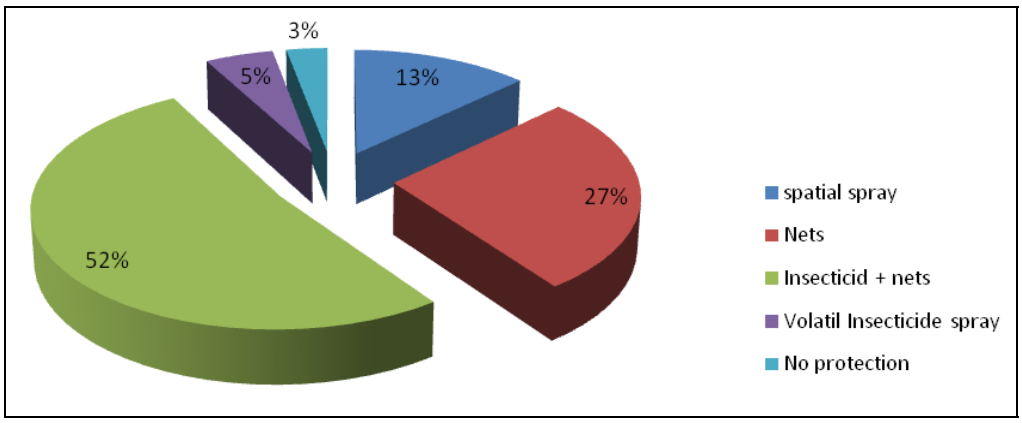

Fig 2: Proportion of household insecticide used in public health in the differents regions

3.2.2 Supply and practices by individual farmers and cooperative

Forty four percent (44%) in association and fifty four percent (54%) individual of the farmers interviewed had never received training on good practices of using insecticides. Fifty five percent (55%) of individual farmers claimed to have received training or to have been regularly advised by the agricultural extension service on insecticides. Against forty six percent (46%) of farmers train in their cooperative. However, the survey revealed that 74.4% of farmers grouped in a cooperative applied the recommended dose of insecticides against only 25.6% of individual producers who did not. The majority of individual farmers (92%) reduced the recommended dose (Table 1). Cooperative farmers reported that their cooperative provided them with phytosanitary products (fertilizers and pesticides) that they need to use in each campaign. Cooperatives buy their insecticides directly from the agro-chemical (CALLIVOIRE, PHYTO GREEN, ALL AGRO, etc.) based in Abidjan. In addition, cooperatives had each two insecticide applicators per trained agent for all the members plantations. But, individual farmers are supplied in the authorized distributors based in N'douci, Tiassalé, Taabo and Toumodi. The main complaint registered with individual farmers is the high price of pesticides and the period of the first insecticide application in cocoa plantations (July-August) coincides with the period when these producers have no money. Most individual farmers did not treat their own plantation. They needed the services of a person, usually another producer, who had a spray machine (knapsack sprayers in vegetable crops and cocoa plantation) but this one has not necessarily been trained in the application methods of insecticides. All farmers agreed that insecticides are highly toxic and dangerous to humans. They used some hygiene precautions recommended to avoid being intoxicated during the treatment. However, all applicators acknowledged that they clean their processing equipment in surface waters located in the field or near the field after treatment. No residues or unused insecticides have been observed in individual farms because they bought just what was needed for the treatment. Among cooperators product residues after treatment are transported and stored in the cooperative warehouse.

Eight out of nine insecticides vendors and veterinary assistants recognized not to have received training prior to the exercise of this business. They are employed and just committed to sell. As such, they ignored the simple instructions for their own security in the shop where the insecticides are stored. They almost never have advised farmers at the time of selling insecticides. One vendor who has received a training and formal authorization for such business acknowledgement to have informed and instructed his clients on the choice, dosage and potential symptoms. The main complaint registered with the authorized vendor was the reduced sales of prohibited and doubtful products. But, because of their low prices these insecticides became the principal choice of farmers.

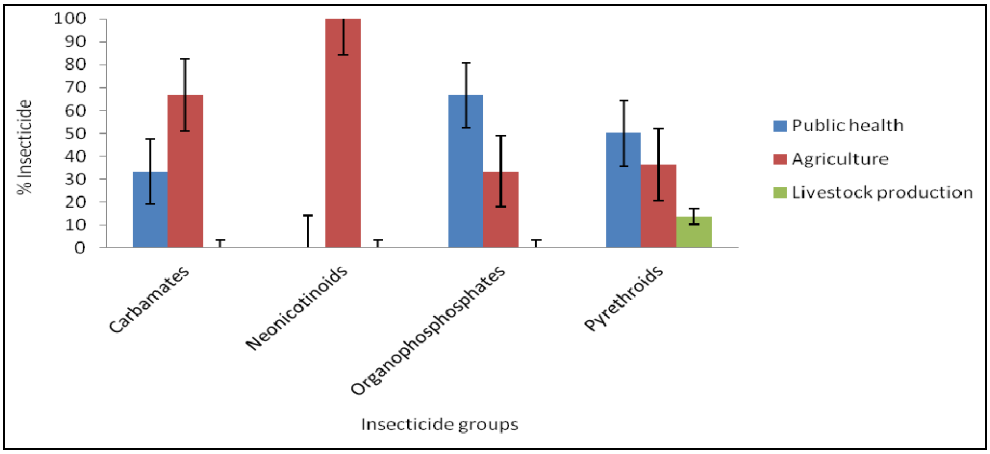

Fig 3: Proportion of families of insecticides used in different sectors

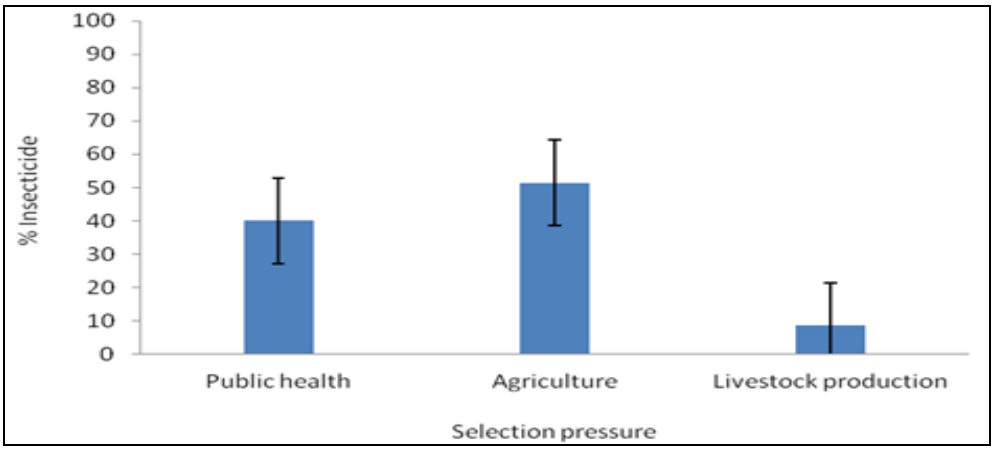

Fig 4: Proportion of insecticides used in the different sectors of activities

4. Discussion

4.1 Joint area of agricultural and public health insecticides

The current study confirmed the intensive use of agricultural and public insecticides Tiassalé and Toumodi regions with 97%. Pyrethroids are the main insecticides used in public health. Permethrin is used for impregnating mosquito nets [2], while allethrin and d-Allethrin are the main active ingredients released by the burning of mosquito coil. A wide range of pyrethroids are used to reduce vector density in houses by spraying aerosols insecticides. Insecticides used on vegetable crops and in cocoa farms were in general pyrethroids even if they are sometimes mixed with neonicotinoids. Pyrethroids are also used in livestock against ticks infestation. These results showed that pyrethroids are the insecticides used in public health and agriculture to fight against pests. However, the significant difference is that pyrethroids concentration used in agriculture is stronger than those used in public health [14].

4.2 Role of agricultural and public health practices in the selection of resistant mosquitoes to insecticides

The deforestation and irrigated space for rice production increased the eggs laying space for mosquito and their spread to the fields. Small irrigated rice fields, small vegetable gardens and small rivers that cross the cocoa fields serve as the main breeding sites for mosquito larvae [1-13]. These mosquito larvae are directly exposed to insecticide application [15]. These treatments put a pressure on mosquito larvae. But the concentrations of pyrethroids used in agriculture are higher than those used in public health. So even if the use of agricultural insecticides is controlled. The application of pyrethroids in higher concentration indirectly on mosquito's larvae can result in the development of mosquito population which eventually tolerates lower concentrations of pyrethroids used in public health. The survey revealed many poor agricultural practices, particularly in individual farms. Some individual farmers, by the lack supervision, apply non-recommended doses with a high frequency of treatment, particularly in vegetable crops (2-3 treatments per week). Others, for economic reasons, dilute the recommended dose in order to treat their entire field. In addition, applicators clean the processing equipment (knapsack sprayers and atomizers) directly into the small water in the field or nearly to their fields, releasing particles of active materials that come into direct contact with the mosquito's larvae that develop. Such practices exert pressure on mosquito larvae and eventually lead to the development of mosquito populations that can tolerate small concentration of insecticides which they were previously sensitive to [15]. It is clear that even if poor agricultural practices contribute to the development of resistance, extending the range of mosquito's fields would also be an important factor which can contribute to the selection of resistance.

In public health, the use of agricultural insecticides such as Cypercal 50 EC (cypermethrin 50 g/l) and Decis 25 EC (deltamethrin 25 g/l) in the disinsectisations of green spaces could

allow selection of resistance of *A. gambiae*. The efficacy of the spraying product depends on both spatial accessibility to the vector (mosquito) then the period in which operations are conducted. Applications of aerosols are only effective if they are applied when mosquitoes are most active (flying) [2]. Anopheles mosquitoes are most active during the night between 2:00 and 3:00 am [13]. But insecticides spray takes place in the evening between 6:00 and 8:00 pm, inside homes that are not usually rows. Only few flying mosquitoes are affected. In addition, applications of aerosols have no residual effect [2]. For volatile insecticides (burning coil insecticides), the efficacy of treatment is the optimal dosage defined as the minimum amount necessary to produce the desired effect. The actual dose of a diffuser is the concentration of insecticide in the treated air volume while the dosage is the number of mosquito coils to use for the size of the room [2]. The treatment applied the mosquito coils users are not efficient because they recognize that the product does not kill mosquitoes. The loss of efficacy of these treatments (mosquito coils and aerosols) is much faster when the doors and windows are open at the time of treatment, thus favoring mosquitoes escapes out of treatment areas Such practices when repeated, can lead to resistance phenomenon.

4.3 Practical answers needed in insect control

In general, farmers don't know the concentration of pesticides to spray due to the lack of supervision. Some individual farmers make the effort to take information from agriculture extension agents, but pesticides vendors are unable to provide advice during sales. Although good agricultural practices are applied by cocoa farmers within cooperatives, most producers did not know and even not expressing the need for this information. But certified cooperative must have at least two regularly trained applicators on good agricultural practices. Although this method can now allow better treatment of cocoa plantations, but the limited number of applicators by cooperative does not make the requirement of sustainable agricultural practices [16]. To mobilize the main actors in agricultural sectors, on the threat posed by the development of resistance to insecticides in agriculture as in public health, it is necessary to improve knowledge of the users of insecticides and phytosanitary practices. Training sessions and information of users, extension services and distributors of insecticides, and producers should be considered. The main complaint registered with the authorized distributor of phytosanitary products is unfair competition from unlicensed products in Côte d'Ivoire. It is possible that widespread use of these products with low quality for a while has contributed to the emergence of resistance. It is therefore imperative to fight against the illegal sale of these products by applying the regulations on the use of phytosanitary products in Côte d'Ivoire [16].

As far as public health in Côte d'Ivoire concerned, mosquito coils and aerosol sprays are widely used as the ways of 82% users to fight against mosquitos [13]. However, no optimal dosage, frequency and periods of application are clearly indicated on the packaging as observed with agricultural insecticides. One of the consequences of lacking such information is the misuse of the products. The best period and effective aerosol spray in space would have been between 2:00 and 3:00 am when the mosquitoes are more active. As defined, the optimal dosage of insecticides volatile diffuser (number of mosquito coils to use for the size of the room to produce the desired effect) is almost unknown for a user of mosquito coils. Application for household use does not seem to be the most appropriate thing to effectively fight against mosquitoes. The development of control methods more suitable tracking information campaigns and training will make treatment more effective and thus reduce the risk of emergence of resistance in mosquitoes [17]. After several years of pyrethroids use in agriculture and public health, insects, including mosquitoes, develop resistance to these products. While the synthesis of insecticides based on the mixture of different families of molecules is to obtain a synergistic effect that prevents the emergence of resistance [18], it is time to think of a new molecule in public health in order to replace those already existing.

5. Conclusion

Irrigated lands have become the preferred place of mosquitoes [19]. With the development of peri-urban agriculture and irrigation systems, the risk of emergence of resistance of mosquitoes will increase due to the continuous use of insecticides throughout the year to secure agricultural production [20]. Prevention and integrated and sustainable management of resistance involve good collaboration between researchers (agronomists, public health and sociology) and agro-chemical and / or retailers responsible for the distribution of insecticides and producers on a combined program agriculture – health using the one health framework. These programs should lead to the establishment of a network of regional monitoring insect populations (disease vectors and pests of culture), assessing the risk and sensitivity to key insecticides popularized. Such programs will define measures of good agricultural practices (cultivation techniques, varietal resistance, insecticide treatment threshold, natural control, etc.).

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