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CONTAMINATION OF BEE PRODUCTS BY PESTICIDES IN COTE D'IVOIRE

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ABSTRACT

Beekeeping is an activity, that is gaining momentum in Côte d'Ivoire. However, the use of pesticides can be a source of contamination of bee products. The objective of this study was, to evaluate their presence in beekeeping products collected in cotton and cocoa production regions. Bee products (honey, pollen, wax) were sampled in beekeepers, belonging to beekeepers-farmers using Agrochemicals. The samples were analyzed by GC/MS. In cocoa production region, cypermethrin and profenofos were detected in 28-32 of the 40 samples of honey or pollen and 16-24 samples of wax. In the center, however, 80% of honey, wax and pollen samples contain metalaxyl, 58.33% honey and pollen contain chlorpyrifos and 20% of wax. Ten to 30% of the samples were contaminated with atrazine. The hazard quotient, which is the EDI/ADJ ratio, was found to be between 0.01 and 0.02 for Profenofos, metalaxyl, atrazine, and cypermethrin. It was 1.75 for chlorpyrifos. These results indicated that the occurrence of a toxic effect related to the consumption of honey from the study areas is unlikely (QD = 0.01-0.92 <1) for four of the five pesticides identified in honey. But there is a possibility of health risks due to the presence of chlorpyriphos (QD> 1).

KEYWORDS: Pollen, Pesticides, Cotton, Cocoa, Residue, Health Risk

INTRODUCTION

Agriculture is the basis of the economy of Côte D'Ivoire; it accounts for 38% of GDP and 70% of income from exports. About 66% of the population depends on agricultural activity to cover household expenses. Cocoa farming, concentrated mainly in the southern forest and in the central savanna forest transition zone, produces more than 1,400,000 tons of beans per year. Côte D'Ivoire is the world's largest producer, with about 40% of global supply. Cocoa cultivation employs about six hundred thousand planters and supports more than five million people. In addition to the income it provides to the farmers, cocoa accounts for 15% of export earnings. Cotton, for its part, is concentrated in the north of the country and represents Côte D'Ivoire's third largest export product [1]. It, thus, plays an important role in the agricultural economy of Côte D'Ivoire, by contributing to between 5 and 10% of the country's exports and generates approximately 120 billion FCFA in annual turnover [2]. [3].

However, diseases and pests in the cocoa and cotton producing regions have a negative impact on yields and could jeopardize the future of these speculations. For this reason, toxic Agrochemicals are applied to orchards to control insect pests, fungal infections and weed infestation. Cotton is the most pesticide-intensive crop, accounting for about 37% of the total value of crop protection chemicals. Cocoa farming receives few pesticides per hectare, but it consumes a large amount of pesticides due to the large area devoted to its cultivation (1.5 million ha). After these two crops, bananas, it is the third largest consumer of pesticides (12%).

Pesticides play an important role in agriculture, helping to control crop pests and diseases [4]. Although they have helped reduce crop losses and thus increased production [5], their continued and often abusive use can be a source of environmental contamination, destruction of wildlife including useful insects such as bees. Indeed, bees can be killed during the applications of these compounds on orchards. They can also transfer, during foraging, residues of pesticides from the honey-producing plants into bee products in the hives (honey, wax, pollen, etc.), which are then consumed by humans [4]. [6]. Among the useful insects, the most affected by the toxicity of pesticides are bees [7] and their exposure or the exposure of their larvae to pesticide cocktails can cause synergistic interactions that may be more toxic than each pesticide applied separately, resulting in a mortality of bees. It has led to a reduction in the population of bees, to the destruction of plant communities, and to the presence of residues in foods, leading to a significant reduction in the income of beekeepers [8]. [9]. Studies have shown that imidacloprid, an insecticide used in Côte D'Ivoire, affected communication capacity of bees, their mobility [10] and behavior during foraging [11].

Bee products have therapeutic properties and are a source of several micronutrients, and for this reason they are considered essential foods. The honey produced by bees from the nectar of honey plants, is a natural food good for the health. It is consumed by all age groups and has both nutritional and medicinal properties [12]. Indeed, it is rich in enzymatic and non-enzymatic oxidants. It is used for the treatment of throat infections, foot ulcers, eye infections, gastric ulcers and varicella [13]. These facts show the importance of the determination of pesticides in honey and honey products.

There is different ways bee products can be contaminated [14] such as plants through pollen and nectar harvesting during foraging on plants that have received pesticides during application, indirect accumulation of pesticide droplets during foraging while application is undergoing. These transported pesticides can end up in the hives and could have a negative impact on the entire colony, and on bee products. Each day, about 10,000 to 25,000 working bees cover up to seven km2 to collect pollen and nectar [15].

Since 2008, the Regulation (EC) No 396/2005 of the EU has set the maximum residue levels (MRLs), for pesticides in honey at 10 to 50 ng/g. Besides that, there is a strong demand for honey and bee products free of pesticides. For this reason, the determination of pesticide residues in beekeeping products is becoming necessary in Africa. Moreover, the EU represents a potential market for small-scale producers of the continent. These compounds, by their presence in bee products, can profoundly alter the benefits derived from their consumption, if they are present in quantity unacceptable. They can have a deleterious effect on human health.

The use of pesticides in agriculture is becoming increasingly important in Côte D'Ivoire, and their non-rational use could have a negative impact on bees. Although this is not documented, some farmers, especially in cotton-producing areas, denounced the involvement of Agrochemicals in the observed colony losses. The objective of this study was to (1) determine the presence of pesticide residues in samples of bee products (honey, wax, pollen) collected in the northern and central Cote D'Ivoire, and (2) to assess the health risk arising from the consumption of contaminated products.

MATERIALS AND METHODS

Location of the Study Area

Samples of bee products were collected in the cotton and cocoa producing regions of the north and the center of Côte D'Ivoire, respectively (Figure 1). The cotton region lies between latitudes 8°30 and 10°30 North and longitude 4°and 7°East. The climate is tropical and dry, with two contrasting seasons: the rainy season extending from April to May and the dry season from November to May. Annual rainfall of 800 mm to 1200 mm is the predominant climatic factor [16]. [17], [18]. The vegetation consists of clear forests, savannah, forest galleries, and islands. The population was represented by the indigenous: Senoufo and Dioula completed by and various other socio-cultural groups from the country and from the sub-region [16]. [17].

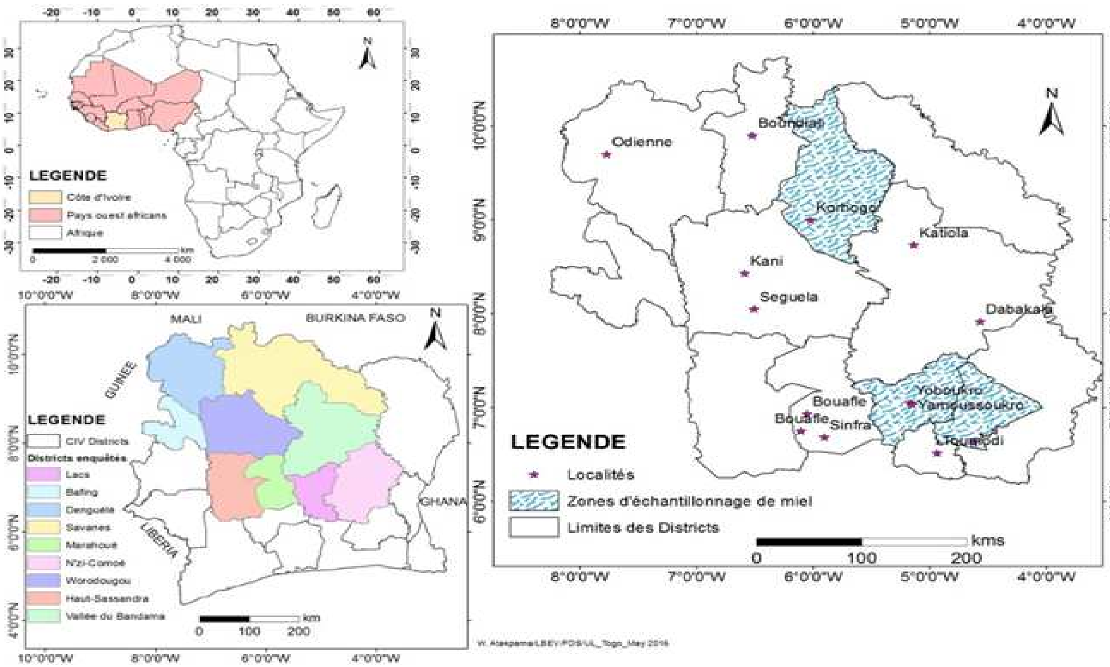

Figure 1: Carte Indiquant Les Sites De Collection Des Echantillons De Miel, Cire Et Pollen

The cocoa production region, Dimbokro and Yamoussoukro are located in the forest-savannah transition zone of the V-baoulé in the center of Cote D'Ivoire. The region of N'zi-Comoé to which Dimbokro belongs It lies between 3° 50'and 5° West longitudes and 6° and 8° North latitudes. The District of Yamoussoukro is between 06°49 and 06°47 North latitude and 05°16 and 05°15 West longitude. The original vegetation consisting of fragments of mesophilic forests and wide savanna patches, is being replaced by degraded lands, due to slash-burn agriculture. The indigenous population is composed of the Akans (Agni and Baoulé), completed by other ethnic groups of Côte D'Ivoire and West African countries. As the main source of income for half the population, agriculture is the most important economic activity in the region. Cash crops consist of cocoa (17,108 tons) and coffee (3,921 tons), accounting for 50% of the total cultivated area. Rain fed rice comes second, with more than 20% of the total cultivated area, followed by: cotton (38 tons), yam (142 242 tons), cassava (26 894 tons), maize (13 009 tons), plantain (29 492 tons), groundnut Vegetable and fruit crops.

Samples Collection

On the basis of information obtained from the beekeepers' associations and field observations, four villages were identified as sampling sites: Gobgala and Samorikaha located in the north, and Yobouekro and Dimbokro in the center. For each village, five apiaries were selected on the basis of the information about beekeepers provided by the federation of beekeepers. In each apiary, eight samples of each bee product were collected, representing 40 samples per product and per site (Table 1). Samples of honey (150 g), wax (25 g) and pollen (5 g) were collected in 100 ml jars hermetically sealed and were transferred to a cooler containing dry ice. The samples were then sent to the laboratory and stored at -16 ° C before analysis.

Table 1: Number of Samples of bee Products Collected Per Site

Region	Site	Honey	Wax	Pollen
North	Gogbala	40	40	40
	Samorikaha	40	40	40
Center	Dimbokro	40	40	40
	Yobouekro	40	40	40
Total		160	160	160

Extraction

The sample processing procedure included homogenization, extraction, pre-concentration, purification and final concentration [19]. [20]. For honey, 10 g of sample were dissolved in 10 ml of water, in a falcon tube and stirred for one min in order to reduce the viscosity of the honey. Then, 100 µl of toclosmethyl (internal standard) and 25 mL of acetonitrile were added and the mixture was sonicated for 5 min. After centrifugation at 4000 rpm for 2 min, the supernatant was recovered in a vial and placed at -20 ° C overnight. The supernatant was recovered in a glass tube and passed over an SPE cartridge previously conditioned with ACN. The Eliot was subsequently concentrated by rotary evaporation to dryness, and recovered with 10 µl of ACN to be injected into GC-MS.

For wax or pollen, 2 grams previously ground in a mortar were melted in 50 ml of hexane, then put in an ultrasonic bath for 15 minutes, and centrifuged at 3500 rpm for 2 minutes. The upper phase was recovered in a 100 ml glass ampule and placed overnight in a refrigerator, at -20 °C. The unfrozen phase was rapidly recovered in a glass tube and passed twice through an SPE cartridge, previously conditioned with ACN, in order to remove the co-extracts. After evaporation to dryness, the residue was taken up in 100 µl of acetone to be injected into the GC-MS.

Determination of Pesticides

The detection and quantification of pesticides were carried out on a quadruple GC-MS (Thermo-Fisher) systematic. The injector temperature was 220 °C and the detector one was 280 °C. The sample was injected in the split less modes. A fused silica capillary TX5-MS column (30m x 0.25 mm I.D., 0.25 µm) (Resteck) was operated under the following condition. The initial temperature was 150 °C, held for 1 min, increased to 230 °C at 3 °C/min held for 5 min, then increased to 250 °C at 3 °C/min, held for 15 min.

Evaluation of the Health Risks Related to the Consumption of Honey Contaminated by Pesticides

The assessment of the toxicological significance of consumer exposure to pesticide residues found in honey was carried out by first, the hazard identification, it then by the selection of toxicological reference values (TRVs) of consumer exposure [21]. Estimates of the health risks are based on the integration of data, from residue determination and food consumption assumptions, which allows representing the actual level of residues in food consumed by local populations, considering a Body weight of 60 kg. The consumption data used are generally derived from the WHO/Global Environmental Monitoring System [22]. Given the fact that there are no data on consumption of honey in Cote D'Ivoire, we have used data from Tanzania [23] which seems to be the closest to the reality in Côte D'Ivoire.

The estimated daily intake (EDI) for pesticides related to the consumption of honey was determined by formula 1

$$EDI = CxQ\frac{F}{P} \tag{1}$$

Where EDI = Daily intake of pesticides ingested (mg/kg/d); C = Concentration of pesticide residues in honey (mg/kg); Q = Amount of honey consumed per day, (kg/day); F = Exposure frequency (F = 1); P = Body weight of the individual (kg).

The characterization of the health risk due to the consumption of contaminated honey is expressed by the hazard index (HI) determined by formula 2.

$$HI = \frac{EDI}{ADI} \tag{2}$$

Where EDI is the daily Intake ingested (mg/kg/day); and ADI, the Admitted Daily Intake (mg/kg/day), being the amount of a compound, determined on the basis of certain information, daily consumed over a lifetime, with no adverse effect on consumer's health [24]. The EDI is a realistic estimate of pesticide exposure that is calculated for each pesticide in honey in accordance with international recommendations [25]. [26].

If HI <1, the possibility of a toxic effect due to the consumption of honey is very unlikely;

If HI> 1, the possibility of a toxic effect due to the consumption of honey cannot be ruled out.

Data Analysis

The concentrations of the compounds identified by the GC/MS system were obtained using data processing software according to Equation 3.

$$C_p = \frac{S_c \times C_e V_2 \times V_f \times F}{S_e \times M_e \times V_i} \tag{3}$$

Were Cp is the concentration of active ingredient (mg kg); Sc and Se, the surface area of the peak of the compound, and of the standard respectively; Ce, the concentration of the standard (mg / l); V1 and V2, the volumes before and after purification; VF, the final volume of the extract; Me, the mass of the sample (kg); F, the dilution factor.

RESULTS

This determination focused on the molecules used in agriculture in the areas concerned by this study, through a field survey. About 20 commercial products are used to protect cotton and cocoa from pests and diseases (Table 2). Of these active ingredients, 15 were searched in the different samples. GC/MS injections of standard compounds allowed determining the retention time and the characteristic ions of each molecule. These two parameters were used in the detection and the identification of pesticides. Given the complexity of the sample matrices toclos-methyl was added as an internal standard in order to determine the extraction yield (recovery rate) which was found to be close to 75%. A calibration curve for each molecule identified in at least one sample was used to determine the limits of detection (LOD) and the limit of quantification (LOQ) (Table 3).

Table 2: Data collected on the Use of Plant Protection Products in the Cotton Production and Cacaoyère

Commercial products	Active ingredients	Utilization
Cotton		
Corporal, cafes, Polytrine, Cotovit	*Profenofos	Insecticide
Conquest, Ballistic	*Acetamiprid	insecticide
Emir	Cypermethrin/Acetamiprid	
Pyrinex quick	Deltamethrin/Chlorpyriphos	insecticide
Polytrine, Tiger	Profenofos/Cypermethrin	insecticide
Attakan	Imidacloprid/Cypermethrin	Insecticide
Procot	Carbendazim/Carbosulfan/Metalaxyl	Insecticide
Cocoa		
Grosudine, Ballistic, Banswey super	Acetamiprid/lambdacyhalothrin	Insecticide
Agrimecop, Brunex	Metalaxyl/Copper oxyde	Fungicide
Banjo star	Dimetomorph/Copper oxychloride	Fungicide
Vegetables, staple crops, and others		
Atoll ++	Isoxaflutole/Atrazine	Herbicide
Furadan	Carbofuran	insecticide
Agrimin, Calliherbe	2.4-D salt	Herbicide
Callitraz	Atrazine	Herbicide
Balt-UP, BibanaL	Glyphosate	Herbicide

*Compounds determined in the samples

Table 3: Characteristics of Determination (LOQ, LOD) of the Active Ingredients Present in Samples of Honey, Waxes and Pollen

Active ingredient	Honey		Wax		Pollen	
	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Atrazine	6	20	-	-	9	15
Chlorpyrifos	4	15	9	20	10	34,5
Cypermethrin	3,8	93,3	10	30	3,8	93,3
Metalaxyl	10	26	8	25	4	14
Profenofos	9	20	6	20	8	20

Level of Pesticides in Sampled Bee Products

Five molecules of pesticide were identified in the samples: atrazine, cypermethrin, chlorpyriphos, metalaxyl and profenofos (Fig 2). According to the survey of farmers, these pesticides are the most used by them. Cypermethrin is a synthetic pyrethroids insecticide widely used in cotton growing area for the control of phytophagous and sucking insects such as Helicopervera Armigera. Depending on the period, it is used alone or in combination with other insecticides (cypermethrin-profenofos 36-300 g/ha). Profenofos is used as an alternative to pyrethroids in the control system in order to avoid the appearance of resistance to insecticides [27]. Metalaxyl is a fungicide used in combination with copper oxide for the control of brown rot of cocoa pods [28]. As for chlorpyrifos, it is used for the control of termites that attack young and adult cocoa trees. Atrazine, is an herbicide of the S-triazines family; it is highly appreciated by farmers, because of its efficacy in the control of weeds. It is mainly used in food crops and seems to induce a mulch effect that ameliorate soil moisture and help return nutrients to the soil.

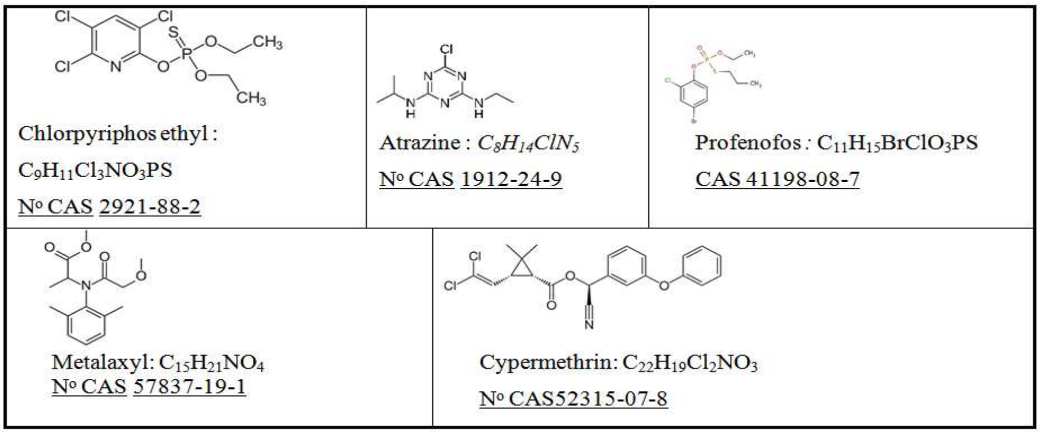

Figure 2: Structure, Formula and Registration Number (CAS) of the Pesticides Determined in the Different

The results showed that in the north, in cotton cropping system, two molecules were determined in the samples, namely profenofos and cypermethrin, which are insecticides. Of the 80 samples taken, four samples of honey and 16 samples of wax did not contain any of these two compounds. On the other hand, all the 80 pollen samples were contaminated with pesticides. Concerning the samples collected in the cocoa producing area, three molecules were found: metalaxyl, chlorpyriphos, and atrazine. Among the 80 samples, eight honey samples and seven wax samples were found to be free from contamination; pollen samples were found to contain residues of these molecules.

Generally, regardless of the cropping zone, the number of positive samples was the same for honey and pollen and relatively less for wax samples. Cypermethrin and profenofos were determined in 28-32 of the 40 honey or pollen samples, that is 70-80% of the samples and 16-24 samples of wax, or 40-60% of the samples in the cotton zone. In the two localities in the center of the country, ie Yobouekro and Dimbokro, 80% (32/40) samples of honey, wax and pollen were contaminated with metalaxyl. Chlorpyriphos contamination was higher in honey and pollen (28/48) than in wax (8/40) in both localities. Atrazine is less present, approximately 4 to 12 out of 40 samples or 10 to 30% of the samples.

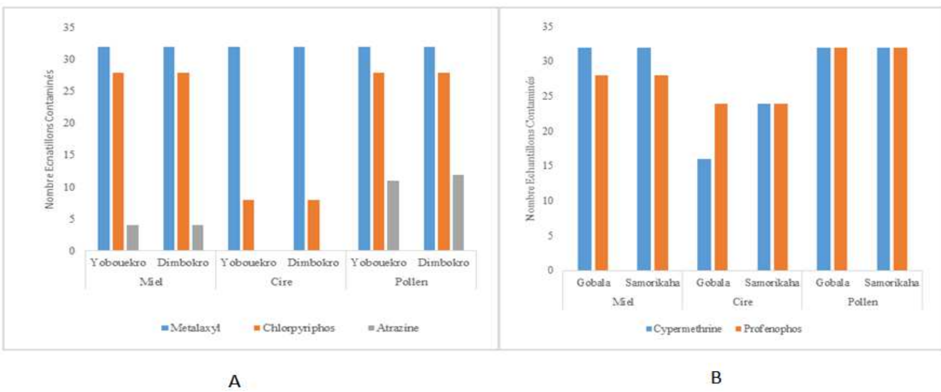

Figure 3

Figure 3: Number of bee products contaminated by pesticides found in the samples in the cocoa (A), and cotton (B) prodoction regions

Health Risks Related to the Consumption of Honey

The concentration of the five chemicals identified in honey samples were consigned in table 5 along with the LMRs of these chemicals. Among the five chemicals, only cypermethrin has a very high concentration, 6 times the LMR. Atrazine and Chlorpyriphos concentration found in honey are below the limit set, for the LMR with chlorpyriphos presenting a level 90%, below the LMR.

Table 4: Estimated Daily Intake (EDI) and ADJs of Pesticides in Honey Collected in the North and Center of the Country

Active ingredient	LMR (mg/kg)	ADI* (mg/kg bw./j)	C (mg/kg)	Q (kg/day)	P (kg)	EDI (mg/kg bw/day)	Health risk (DJI/DJA)
Profenofos	0,05	0,004	0,053	0,90	60	0,000795	0,20
Metalaxyl	0,05	0,080	0,06	0,90	60	0,0009	0,01
Atrazine	0,05	0,005	0,03	0,90	60	0,00045	0,09
Chlorpyriphos	10,00	0,001	0,11	0,90	60	0,00165	1,65
Cypermethrin	0,05	0,050	0,305	0,90	60	0,004575	0,92

Established by the Codex Alimentarius Committee on Pesticide Residues, the Joint FAO / WHO Meeting on Pesticide Residues (JMPR), The US Environmental Protection Agency (USEPA) and The European Food Safety Authority (EFSA)

For the five pesticides identified in honey samples, one, cypermethrin has a concentration higher than the limit set for the maximum level of residue; the other pesticides presented level around their LMR or very far below the LMR (chlorpyriphos). These results indicated that, because of cypermethrin, the consumption of honey sampled from these regions mays pose health risks. However, because of the low level of honey consumed by the populations, the risk of adverse effect seems to be derisory. When considering le health risk hazard, the results indicated that there was a risk for chlorpyrifos poisoning (HI = 1.65> 1), and chronic exposure to chlorpyrifos may lead to a cumulative decrease in cholinesterase activity at a critical level. Chlorpyrifos is a very lipophilic compound that bounds to the acetylcholinesterase of the central nervous system of muscles and red blood cells. This molecule is one of the seven bee-killing pesticides on the list of substances to be banned in priority established by Greenpeace [29]. A similar study, carried out in Egypt found a HI values of 0.0091 for chlorpyriphos, and 0.050 for profenofos [30]; which were well below those found in this study.

Although the results of this study showed that the total intake of pesticides through the consumption of honey taken from the four localities rarely exceeds the MRLs, it is important to bear in mind that the disturbances to which the body is confronted, as a result of food contamination can be of various kinds (neurological, endocrine, immune system, cancers, reproductive disorders, etc.). That is why; the contributions to the health risks due to the other ingredients in the diet of populations should be assessed. Indeed, some of our food, due to the increasing use of pesticides, can be contaminated. For example, a study carried out in the Niayes area in Senegal, showed the presence of pesticides in vegetables (tomato, eggplant, chilli, cabbage, onion and lettuce) at levels of 0.011 to 0.035 mg/kg; often above the MRL [31]. [32], also determined pyrethroids in vegetable in Nieky, in southeastern Côte d'Ivoire. Similarly, studies in Benin have shown the presence of pesticide residues in cassava tubers at levels below MRLs; Glyphosate (0.134 - 0.128 µg/kg), profenofos (0.115 - 0.128 µg/kg) and cypermethrin (0.133 and 0.153 µg/kg). Considering that, it is desirable that a regular monitoring program be established in order to minimize the contamination of foodstuffs by pesticides. For this reason, the

WHO had advocated that each country should be able to carry out studies to determine the dietary composition of populations; which should make it possible to estimate possible levels of pesticides used in agriculture in food in our country.

CONCLUSIONS

This study showed that, on high agricultural production areas, such as cotton growing and cocoa growing areas, the use of pesticides is a common practice. It confirms that honey, wax and pollen were contaminated by pesticides, on the basis of samples collected from two agricultural production areas. Four insecticides and one herbicide were determined in 40-70% of the samples. This demonstrates that, bees are faced with numerous threats, particularly due to our agricultural model, with the increasing use of pesticides: loss of natural habitats and biodiversity, increase in diseases and parasites. It also showed, the possible exposure to multiple pesticides and residues in honey, pollen and nectar. The strategies for protecting bees and other insects, on which our agriculture and horticulture depend, and which are a vital component of the ecosystems, must therefore, be taken into account a variety of factors.

The obtained results represent only a snapshot of the residues present in bee products, in two agricultural production areas of Cote d'Ivoire, because of the limited sample size and time period covered by the study, it does not show the importance of the contamination of bee products, by pesticides. However it has the merit of showing the possible contamination of bee products on two different geographical zones, with different agricultural activities. In general, EDI for pesticides, detected in honey are below EDI values, indicating that, the consumption of these honey products presents a very low contribution to the toxicological risks. We suggest, however, a regular monitoring for the determination of pesticide residues in honey, at the national level in order to protect the health of consumers and ensure irreproachable quality of honey, for export. Moreover, to avoid contamination, pesticides should be used outside the flowering periods or at least, not during the foraging periods of the bees. Contamination could also be avoided, by installing apiaries more than 3 km from agricultural plots, treated with plant protection products.

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Table 5: Level of Pesticides Detected in Honey, Wax and Pollen Samples Based on Sampling Locations (n = 40)

Bee product	Village	Metalaxyl		Chlorpyriphos		Atrazine		Profenofos		Cypermethrin	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Honey	Yobouekro	0,05-0,07	0,06±0,03	0,01-0,6	0,11±0,201	0,03-0,03	0,03±0,0	ND	ND	ND	ND
	Dimbokro	0,05-0,06	0,059±0,003	0,01-0,6	0,104±0,208	0,03-0,03	0,03±0,0	ND	ND	ND	ND
	Gobala	ND	ND	ND	ND	ND	ND	0,01-0,06	0,053±0,018	0,01-0,6	0,305±0,185
	Samorikaha	ND	ND	ND	ND	ND	ND	0,01-0,06	0,053±0,018	0,01-0,6	0,305±0,186
Wax	Yobouekro	0,06-0,04	0,07±0,09	0,11-0,02	0,06±0,109	ND	0,000	ND	ND	ND	ND
	Dimbokro	0,04-0,07	0,059±0,008	0,02-0,05	0,035±0,016	ND	0,000	ND	ND	ND	ND
	Gobala	ND	ND	ND	ND	ND	ND	0,03-0,06	0,053±0,011	0,02-0,05	0,035±0,013
	Samorikaha	ND	ND	ND	ND	ND	ND	0,03-0,63	0,33±0,220	0,02-0,06	0,042±0,014
Pollen	Yobouekro	0,04-0,07	0,06±0,011	0,04-0,7	0,23±0,294	0,01-0,04	0,02±0,013	ND	ND	ND	ND
	Dimbokro	0,04-0,07	0,063±0,011	0,04-0,7	0,37±0,353	0,01-0,04	0,023±0,013	ND	ND	ND	ND
	Gobala	ND	ND	ND	ND	ND	ND	0,01-0,07	0,058±0,022	0,04-0,7	0,37±0,285
	Samorikaha	ND	ND	ND	ND	ND	ND	0,01-0,11	0,067±0,028	0,04-0,7	0,37±0,279

ND, not found at the level of detection (LD) (Table 3)