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EVALUATION OF PESTICIDE RESIDUES AND POLYCYCLIC AROMATIC HYDROCARBONS CONTAINED IN SOME INSECT SPECIES CONSUMED IN TOGO

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Abstract: This study assesses the pesticide residues and Polyclic Aromatic Hydrocarbons (PAHs) contamination risks associated with the consumption of insects in Togo. The results of this research have shown that Desethylatrazine and Diuron were two herbicides found in some species. *Acanthacris ruficornis* (Serville, 1839) (Orthoptera: Acrididae) and *Imbrasia obscura* (Butler, 1878) (Lepidoptera: Saturniidae) were found to contain Desethylatrazine at 0.320 and 0.640 $\mu g \cdot kg^{-1}$ respectively, while *Oryctes monoceros* (Olivier, 1789) (Coleoptera: Scarabaeidae) and *Macrotermes bellicosus* (Isoptera: Termitidae) contained Diuron at 0.217 and 0.532 $\mu g \cdot kg^{-1}$ respectively. Moreover, Permethrin insecticide was found only in *Rhynchophorus phoenicis* (Smeathman, 1781) (Coleoptera: Curculionidae) at 0.556 $\mu g \cdot kg^{-1}$. However, the concentration of Permethrin found in this species is low compared to the maximum residual limit recommended in animalproducts (50 $\mu g \cdot kg^{-1}$). Moreover, no species contains more than one pesticide. For PAHs, only Fluoranthene was found in processed *Cirina forda* (Westwood, 1849) (Lepidoptera: Saturniidae) (0.31 $\mu g \cdot kg^{-1}$) at a relatively low concentration compared to the threshold allowed in processed foods ($\mu g \cdot kg^{-1}$). According to our results, the insects studied had a low tendency to accumulate pesticides that could pose risks to human health and the culinary methods of insects in Togo do not lead to the formation of PAHs.

Keywords: *chemical contamination, edible insects, pesticide residues, Polycyclic Aromatic Hydrocarbons, Togo*

INTRODUCTION

For a long time, agriculture has not been interested in the insects breeding for human consumption. Apart from silkworms and bees that were domesticated centuries ago [1], it is only recently that the breeding of other insect species has attracted attention in some countries [2]. Today, it is common to find farmers in northern Thailand who locally raise and sell bamboo worms or locusts for human consumption [3]. In some countries, such as Laos, Vietnam and China, there are artisanal insect farms [1]. In Africa, the insects consumed are still harvested in the wild and are not farmed [4, 5]. However, insect farming offers many advantages over traditional animal husbandry. Insects also emit less greenhouse gases and ammonia than conventional livestock [6]. In many cases, insects can be raised on organic by-products, reducing environmental contamination while recovering waste [7]. Insect farming requires less water and land than conventional livestock [8]. In addition, insects have high reproductive rates, high resistance to disease, high adaptive capacity to ecosystems, and short life cycles [9]. They feed on a wider range of plants than conventional livestock [3]. Since insects are cold-blooded animals, they have a high conversion rate into nutrients [10]. Insects have a low risk of transmission of zoonotic infections [11]. Indeed, traditional animals in human diet are closer to humans in evolution than insects. Humans and traditional livestock have many common diseases. Recent avian and swine influenza, Ebola, Lassa fever and Covid-19 are a prime example [12]. Moreover, the outlook for the world population is that it will continue to increase over the coming decades, especially in developing countries [13]. Demand for food will increase in the world inevitably. The current environmental pressure exerted by the production of conventional animal proteins has led food markets for human and animal feed to search for alternative and innovative protein sources. Among the possible responses to food and feed adequacy problems, FAO proposes large-scale insect farming [7]. In addition to having environmental advantages, insects are also very nourishing [14, 15]. They are rich in crude proteins that constitute up to 60 % of their total body weight [15, 16]. They are also rich in minerals and vitamins [17, 18]. Studies on the nutrient composition of edible insects, which have been going on for decades, conclude that insects are a comprehensive food resource that can reduce food insecurity in tropical and subtropical regions. However, like other foods, insects can be a source of contamination if production conditions are not defined by specific standards. In fact, despite their nutritional quality, edible insects could be a source of chemical contamination (pesticide residues, antibiotics, organic pollutants, heavy metals), biological also allergens. In Togo, insects harvesting is done in various environments (farm, forest, savannah, water). Some of these environments can be polluted by household, industrial waste discharge and pesticides. In addition, some culinary methods of insects used in Africa may have the disadvantage of presenting a carcinogenic risk because of the presence of Polycyclic Aromatic Hydrocarbons (PAHs) in cooked food [19]. Yet, an inventory of scientific knowledge on the risks associated with insect consumption is missing from the international scientific literature. Research specifically on the risks of pesticide residues and PAHs in insects consumed in Togo is currently not available. As a result, there are many consumers concerns about entomophagy that are increasingly sensitive to the quality of food products. With a view to the possible development of entomophagy in mind, it is essential to conduct research on the risks associated with the consumption of insects caught in nature. The objective of this study is to assess the potential risks associated with the consumption of insects in Togo.

MATERIAL AND METHODS

Choice of sampling sites of edible insect species to be analyzed

Insect collection took place from September 2016 to August 2017 at the rate of one outing per month. Three sites were chosen for the collection of each species throughout the Togolese territory depending on the localities where these species are consumed. Ten species whose nutritional quality has been determined by Badanaro *et al.* [5] were the object of sampling and chemical analysis. These species were collected from different ecological zones and ecosystems (farm, forest, savannah, water) in Togo according to their period of abundance identified by Badanaro *et al.* [5]. The insects were harvested alive and placed in an ice box for maintaining the cold chain during transport to the laboratory. Samples of the *C. forda* caterpillar boiled, roasted, sold in the market and the *M. bellicosus* termite, roasted, sold in the market were also purchased during the same periods in the same localities. Once in the laboratory, the samples were stored in a freezer.

Processing of samples in the laboratory

Frozen samples were then thawed at room temperature. Ten grams of each species from each location where sampling took place were weighed. Then, they were placed in previously numbered trays and put in an ISUZU type AS oven at 65 °C for drying. They were then ground by species in a Moulinex of General Electric Interlabs type and sifted to obtain a flour. The Moulinex was carefully washed and dried between the grinding of samples of different species. The grindings were stored in airtight containers and kept in a refrigerator for pesticide residues and PAHs analysis.

Chemical analyses

Framework

The analyses were carried out in the Biochemistry Laboratory of the Faculty of Science of the University of Lomé, Togo and the National Laboratory of Support for the Agricultural Development (LANADA), Abidjan, Côte d'Ivoire.

Standards

The individual PAH and pesticide standard solutions were obtained from Chiron AS (Germany). The PAHs standard used were: Fluoranthene, Pyrene, Benzo(k) fluoranthene, Benzo(a) pyrene, Indeno (1, 2, 3-cd) pyrilene, Benzo(a) anthracene, Benzo(b) fluoranthene. The pesticides standard used were: Desethylatrazine, Cypermethrin, Lambdacyhalothrin, Metolachlor, Ethyl chlorpyrifos, Terbutryn, Diuron, Deltamethrine, Propazine, Permehrin, Ethyl parathion, Methyl parathion.

Reagents
All the reagents used were obtained from Scharlau Chemie S.A. (Spain). Anhydrous magnesium sulfate and sodium sulfate were used. The solvents including n-hexane, dichloromethane, methanol, acetonitrile and bidistilled water were of HPLC grade (Scharlau Chemie S.A., Spain).

Extraction of pesticide residues and PAHs
Extraction was the preparatory phase of chromatographic analysis of the PAHs and the pesticide residues. During extraction, 5 grams of sample were weighed and grinded with 2 grams of magnesium sulfate ($MgSO_4$) and 2 grams of sodium sulfate (Na_2SO_4). After grinding, pesticide residues and PAHs were extracted by applying a microwave field (BP-110, Microwave Research & Applications, Illinois) in the presence of n-hexane/dichloromethane (30 mL, 50/50). This operation is repeated 3 times to optimize extraction. The organic extract was concentrated in a rotary evaporator R-114 RE (Büchi Labotfach, Switzerland) and then recuperated with 5 mL of hexane/dichloromethane. The extract is purified on a silica gel column and eluted in a vacuum with 5 mL of a mixture of n-hexane and dichloromethane (50/50). The solution obtained is filtered through a PTFE filter with a porosity of 0.45 µm and then evaporated to dryness in a rotary evaporator. The residues are recovered with 5 mL of methanol for the HPLC assay.

Instrumental analysis
Prominence Dual HGE brand HPLC of SHIMADZU consisting of a TRAY tank, a DGU-20A5 degasser, a SIL-20A autosampler, an LC-20AT pump, a CTO-20A type oven and an UV/VIS SPD-20A detector was used for the quantification of pesticides and PAHs. The separation was achieved on Shim pack VP-ODS column (250 L x 4.6 mm) using mobile phase consist of acetonitrile: water (80 : 20, v/v) at flow rate 1 $mL \cdot min^{-1}$ and the eluent was monitored at 284 nm. Data acquisition was carried out using a computer equipped with LC solution software.

Formula for calculation of sample concentration

The concentration of the samples is given by the following formula (1):

$$C_p = \frac{S_c \times C_e \times V_f \times F}{S_e \times M_e} \quad (1)$$

where: C_p: sample concentration [$\mu g \cdot kg^{-1}$];
S_c: peak area of the sample
S_e: peak area of the standard
C_e: concentration of the standard [$\mu g \cdot mL^{-1}$]
V_f: final volume [mL]
M_e: sample volume [g]
F: dilution factor (F = 1)

Statistical analysis

All the tests were repeated three times. The data was processed using the Excel 2013 spreadsheet©.

RESULTS AND DISCUSSION

Pesticide residue content of the insects studied

Substances potentially accumulated by insects via the environment or food such as pesticides were analyzed in edible insects during this study (Table 1). The pesticide residue compositions of 10 species of edible insects analyzed showed that *B. menbranaceus*, *R. sobrina, G. impressa, G. trivittata* and *C. forda* do not contain pesticide residues. Desethylatrazine and Diuron were two herbicides found in some species. *A. ruficornis* (0.320 $\mu g \cdot kg^{-1}$) and *I. obscura* (0.640 $\mu g \cdot kg^{-1}$) contained Desethylatrazine. While Diuron has been found in *O. monoceros* (0.217 $\mu g \cdot kg^{-1}$) and *M. bellicosus* (0.532 $\mu g \cdot kg^{-1}$). In contrast, the insecticide Permethrin was only found in the species *R. phoenicis* (0.556 $\mu g \cdot kg^{-1}$). The overuse of insecticides in agricultural fields and intensive industrial activity have largely contributed to environmental contamination even in rural areas in Togo [20, 21]. These contaminants, which cannot be metabolized by living beings, accumulate in trophic chains.

Table 1. *Pesticide residues content of the main species of edible insects in Togo*

Pesticides [$\mu g \cdot kg^{-1}$]	**Average content ± Standard Deviation**									
	OM	**DS**	**RP**	**RS**	**AS**	**IO**	**GI**	**GT**	**MB**	**CF**
Desethylatrazine	ND	ND	ND	ND	0.320± 0.005	0.640± 0.005	ND	ND	ND	ND
Cypermethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lambdacyhalothrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metolachlor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl chlorpyrifos	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Terbutryn	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Diuron	0.217± 0.002	ND	ND	ND	ND	ND	ND	ND	0.532± 0.000	ND
Deltamethrine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Propazine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Permehrin	ND	ND	0.556± 0.006	ND	ND	ND	ND	ND	ND	ND
Ethyl parathion	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methyl parathion	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[1]ND means that pesticide residues were not determined. Abbreviations of the samples mean: OM: *Oryctes monoceros*; DS: *Dolyrus* sp.; RP: *Rhynchophorus phoenicis*; RS: *Rhabdotis sobrina*; AR: *Acanthacris ruficornis*; IO: *Imbrasia obscura*; GI: *Gnathocera impressa*; GT: *Gnathocera trivittata*; MB: *Macrotermes bellicosus*; CF: *Cirina forda.*

This is the case of the herbicides (Desethylatrazine and Diuron) found in certain insects consumed in Togo. Herbicides are phytosanitary substances with the property of killing plants. The presence of Desethylatrazine and Diuron in the insects studied would be due to their diet. Researchers have shown that edible insects absorb pesticides from plants growing in areas treated with herbicides [22, 23]. In addition to these herbicides, the insecticide Permehrin has been found in *R. phoenicis*. However, its concentration in this species is below the maximum residual limit (50 $\mu g \cdot kg^{-1}$) for food of animal origin [24]. Unfortunately, the accumulation of pesticides by humans at the end of the trophic chain can have adverse consequences on their health. Indeed, several pathologies result from pesticide intoxication. Cancer is the most well-known health risk associated with the use of pesticides [25]. Neurotoxic and reprotoxic effects resulting from pesticide intoxication are also known [26, 27]. Residues of more than one pesticide (multiple residues) have not been detected in the species studied as is often the case in foodstuffs. Generally, the same pesticide-contaminated foodstuff contains more than one pesticide [28]. Consequently, chronic intoxication with pesticides is likely to be sustained because of the probable additionality of pesticides [29]. The insect species studied therefore have a low tendency to accumulate pesticides.

Polycyclic aromatic hydrocarbon (PAHs) content of the studied insects

Polycyclic aromatic hydrocarbons were also analyzed in this study (Table 2).

Table 2. *PAHs content of the processed and sold edible insect species in Togo*

Polycyclic Aromatic Hydrocarbons [$\mu g \cdot kg^{-1}$]	Average content ± Standard Deviation			
	Raw *M. bellicosus*	**Processed *M. bellicosus***	**Raw *C. forda***	**Processed *C. forda***
Fluoranthene	0	0	0	0.31±0.000
Pyrene	0	0	0	0
Benzo(k) fluoranthene	0	0	0	0
Benzo(a) pyrene	0	0	0	0
Indeno (1, 2, 3-cd) pyrilene	0	0	0	0
Benzo (g, h, i) pyrilene	0	0	0	0
Benzo(a) anthracene	0	0	0	0
Benzo(b) fluoranthene	0	0	0	0

The results showed that whether raw or processed *M. bellicosus* did not contain PAHs. Raw *C. forda* did not contain PAHs also. But, processed *C. forda* contained only Fluoranthene. However, the concentration of Fluoranthene found in processed *C. forda* was very low (0.31 $\mu g \cdot kg^{-1}$) compared to the threshold allowed in food (1 $\mu g \cdot kg^{-1}$) [30]. The consumption of insects cooked and sold in Togo does not present therefore a risk of contamination with PAHs. Indeed, according to researchers [30, 31], some insect cooking methods used in Africa may have the disadvantage of presenting a carcinogenic risk because of the presence of toxic substances such as Heterocyclic Aromatic Amines (HAMs), Polycyclic Aromatic Hydrocarbons (PAHs), acrylamide, chloropropanols and furans that may be formed by chemical reactions between constituents of the insects themselves, or between constituents of the insects and other ingredients.

CONCLUSIONS

This study made it possible to assess the sources of chemical contamination linked to the consumption of insects in Togo. In this work, the roasting techniques used in Togo for cooking insects did not lead to the formation of PAHs. The insects studied had a low tendency to accumulate pesticides because the species do not contain pesticides or contain them at low concentrations. In addition, there were no species containing multiple residues. However, the collection of insects in nature for human consumption is not recommended due the lack of sanitary control. For the development of entomophagy and its sustainability it is necessary to establish rules that govern the practice, throughout the production chain. It should be noted that the consumers expect to be protected against risks of all kinds that can be observed throughout the chain, from the primary producer to the consumer. Protection will only be ensured if all links in the chain function in an integrated manner and if food control systems take into consideration each link. The use of animal husbandry, by creating a chain with its own requirements and expectations, seems to be the most suitable solution for mass production. An integrated approach of this type will enable consumer protection, effectively stimulate the production and food processing industries and the marketing of insects.

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