PRESENCE OF MYCOTOXINS (AFLATOXIN B1 AND OCHRATOXIN A) IN EDIBLE CATERPILLARS (*IMBRASIA OYEMENSIS*) CONSUMED IN WESTERN AND CENTRAL-WESTERN CÔTE D'IVOIRE

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Abstract

The "ZEGRE" caterpillars from their scientific name (*Imbrasia oyemensis*) are edible insects very rich in proteins, vitamins and mineral salts. They feed mainly on the leaves of the tree with the vernacular name "Aboudikro" (*Entandrophragma cylindricum*). It is at the foot of these trees that they are harvested and dried before being eaten or marketed. Their richness in nutrients makes them foods of choice in the diet of the forest peoples of Côte d'Ivoire. However, the unsanitary environment in which this commodity is processed and dried suggests that it may contain mycotoxins. The objective of this study was to assess the level of contamination of treated and dried caterpillars with aflatoxins and ochratoxin A (OTA). Contaminants (ochratoxin A and aflatoxin B1) were quantified in the caterpillar samples collected and dried by high-performance liquid chromatography. The results obtained reveal contamination by the mycotoxins sought at various concentrations. In the caterpillar samples from ZUENOULA, GOHITAFLA and MAN, the highest average concentrations were those of AFB1. Thus, mean loadings of aflatoxin B1 ranged from 0.280 $\mu g.kg^{-1}$ to 80.104 $\mu g.kg^{-1}$, with a mean of 24.152 $\mu g.kg^{-1}$ above the MRL of 2 $\mu g.kg^{-1}$. The OTA values ranged from 0.06 $\mu g.kg^{-1}$ to 0.740 $\mu g.kg^{-1}$ with a mean of 0.482 $\mu g.kg^{-1}$ below its MRL of 3 $\mu g.kg^{-1}$.

Introduction:-

In Côte d'Ivoire, some communities traditionally consume insects (**Foua *et al.*, 2015; Diabaté *et al.*, 2024**) including edible caterpillars. The species *Imbrasia oyemensis* (Lepidoptera Attacidae), *Macrotermes subhylinus* (Isoptera Macrotermitidae) and the species *Rhyncophorus phoenicis* or palm caterpillar are widely consumed due to their availability on the markets. Insects are already a part of the diet of people throughout Africa and the consumption of caterpillars has also been observed (**Adegbola *et al.*, 2013; Badanaro *et al.*, 2014; Malaisse and Latham, 2014; Mabossy *et al.*, 2022**). They are eaten at different stages of development as larvae or adults and with various methods of preparation (raw, fried, boiled, roasted or ground). Various published articles indicate edible caterpillars as an important resource from a food point of view (**Payne *et al.*, 2016; Mabossy *et al.*, 2017**).

Unfortunately, the hygienic conditions relating to the handling, conservation and storage of the collected caterpillars are homemade. The high heat and humidity of production areas are conducive to the proliferation of mycotoxin-secreting moulds, including aflatoxins and ochratoxin A (OTA), which can contaminate this commodity. OTA is nephrotoxic and potentially carcinogenic. It is classified in group 2B "possible carcinogen for humans" by the International Agency for Research on Cancer. Aflatoxins have mutagenic, hepatotoxic, genotoxic and immunosuppressive effects (**AFSSA, 2009**). They are classified as Group 1 and therefore proven carcinogens for humans (**IARC, 2021**). OTA and aflatoxin B1 can then be a public health problem and have become a major

concern in terms of food safety. This is particularly true in sub-Saharan Africa, where climatic conditions associated with the lack of control of hazard analysis and critical control points (HACCP) systems frequently lead to the presence of mycotoxins in foodstuffs (**Dembele *et al*., 2009; Kpan *et al*., 2019**).

In our regions, caterpillars are still a source of protein in the human diet, but little information is available on the safety and harmlessness of their consumption with regard to chemical contaminants. The objective of this study is to determine the occurrence of OTA and aflatoxin B1 in collected edible caterpillar samples.

2. MATERIALS AND METHODS

2.1. Biological matrix and collection areas

The biological material that was the subject of this study is an edible caterpillar of the species I*mbrasia oyemensis* (**Figure 1A**). It is a butterfly larva of the insect class, order Lepidoptera, of the Saturnidae family. These caterpillars naturally constitute in Côte d'Ivoire the important source of animal protein for the forest peoples of Tonkpi and Marahoué, giving them a food and cultural identity (**Figure 1B**). They are the most consumed caterpillars, locally recognized under the name of "ZEGRE" and dominant in the diet of the Gouro, Guéré and Yacouba ethnic groups.

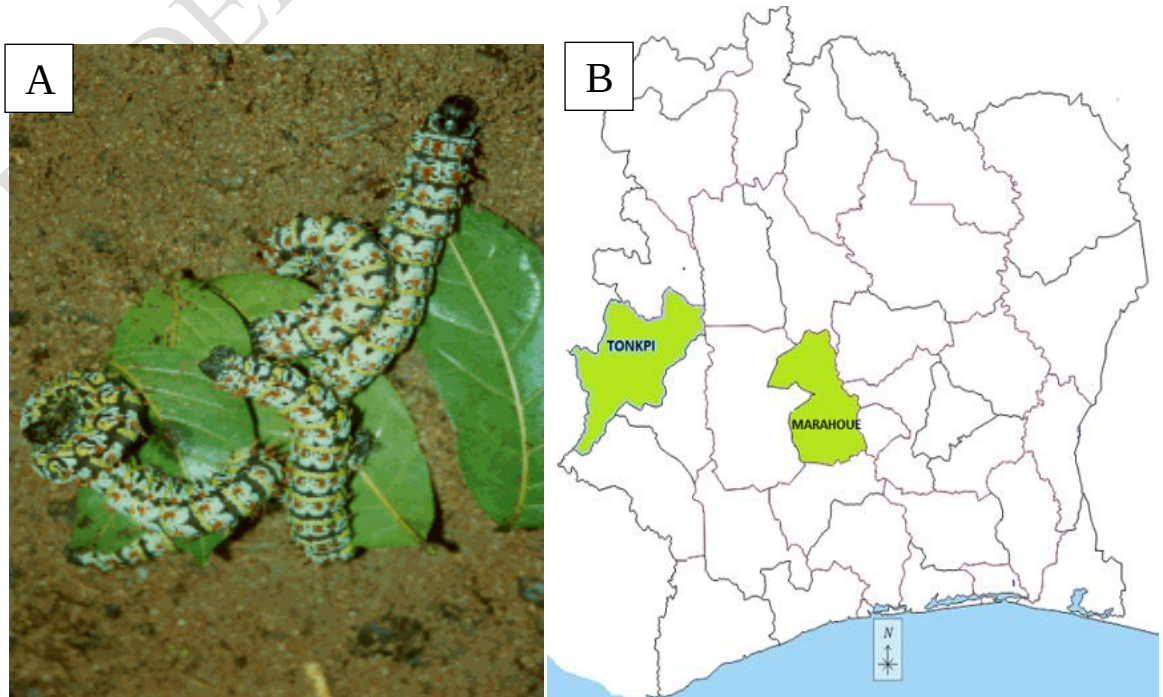

Figure 1: **A**: Caterpillars *imbrasia oyemensis*, **B**: Forest regions (caterpillar habitat)

2.2. Solvents and Reagents

OTA standards (10 µg.mL^{-1} in acetonitrile) and aflatoxins B1 (2 µg.mL^{-1} in acetonitrile) were supplied by Superlco INC (Bellefonte, USA). The ultrapure water was obtained using an ELGA (High Wycombe, England) purifier, Acetonitrile grade HPLC CHEM-LAB (Zedelgem. Belgium), Acetic acid (100 %) from Normaphur (Leuven, Belgium) and sodium hydrogen carbonate (99.7%) from Merck (Darmstadt, Germany) were used.

2.3. Apparatus

For the detection of aflatoxin B1 and OTA, a SHIMADZU HPLC line (Tokyo, Japan) equipped with a DGU-20A5 deaerator, an LC-20AT pump, a SIL-20A autosampler, a TRAY tank, a CTO-20A furnace and an RF10 AX fluorescence detector was used. A SHIMADZU electronic scale (Tokyo, Japan) with an accuracy of ± 0.01 g was used to determine the mass of the samples. A mixer (Ultra Turax, OMNI INTERNATIONAL, Kennesaw GA, USA) was used to homogenize the extracts. The extraction and purification of the extracts was possible thanks to the use of purification wells, VACUUBRAND vacuum pump (Vertheim, Germany), Aflaprep immunoaffinity columns, Ochraprep from R-BIOPHARM (Darmstadt, Germany), HETTICH centrifuge (ZAC de Montévrain. France) and filter paper, Whatman, Fisher Scientific (Paris. France).

2.4. Sampling

The composite samples were collected from caterpillar sellers in the Marahoué and Tonkpi regions respectively in the communes of ZUENOULA, GOHITAFLA and MAN in the months of September to December 2023.
A composite sample corresponds to 250 g of caterpillars collected from 5 saleswomen due to 50 g per saleswoman. In the markets of each of the municipalities, 10 composite samples were taken, packaged and sent to the laboratory. These samples were then dried in an oven at 105 °C to a constant weight before being ground into an animal powder and ready for analysis.

2.5. Determination of aflatoxin B1 and ochratoxin A content

- **Aflatoxin extraction and purification**

100 g of dried caterpillar samples were finely ground using a porcelain mortar. A quantity of 20 g of the shredded material was taken from a 200 mL flask and 100 mL of methanol/bidistilled water mixture (80/20: v/v) was added. The solution obtained was then homogenized away from light for 45 min and is then centrifuged at 5000 rpm min^{-1} for 10 min. The supernatant obtained was filtered under vacuum on Whatman N^{o}4 filter paper in an Erlenmeyer

flask.10 mL of this filtrate was collected and diluted using 40 mL of a saline phosphate buffer (PBS) solution.10 mL of the dilute solution was deposited in the Immune-Affinity (IA) column (Aflaprep) previously conditioned using 10 mL of PBS buffer at a rate of 3 mL/Min. After rinsing with 10 mL of PBS buffer, the aflatoxins were eluted with 1.5 mL of methanol and then diluted using 1.5 mL of double-distilled water to obtain the solution ready for injection into the chromatographic system.

- **OTA Extraction and Purification**

To extract the OTA, 15 g of dried caterpillar shreds were taken from a 200 mL flask. To this shredded, 150 mL of sodium methanol/hydrogen carbonate mixture (1%) in water (50/50: v/v) was added. This mixture was homogenized in a blender (Ultra Turax) for 2 min. The solution obtained was centrifuged at 4000 $rpm.min^{-1}$ for 5 min, then filtered on N° 4 Whatman paper in a 25 mL test tube. 11 mL of this filtrate was diluted with an equivalent volume of saline phosphate buffer solution (PBS). Purification was carried out on an Immuno-Affinity column (Ochraprep) conditioned using 10 mL of PBS solution at a rate of 10 $mL.min^{-1}$ using a vacuum pump (Gilson). A volume of 20 mL of the diluted extract was deposited drop by drop into the column and the solvent was discharged at a rate of 2 $mL.min^{-1}$. The column was washed with 10 mL of PBS and the OTA was unhooked by two successive washes. The first wash required 1.5 mL of a methanol/acetic acid mixture (96/4; v/v) and the second was performed using 1.5 mL of PBS at a rate of 5 $mL.min^{-1}$. The purified extract was transferred to a vial to be injected into the chromatographic system.

Mycotoxin determination was performed using the HPLC chain (SHIMADZU) whose stationary phase consisted of a reversed-phase (RP) column Shim pack VP-ODS 5 µm-C18-100 (250 L x 4.6 mm ID). The chromatographic system was pre-calibrated using separate standard solutions of aflatoxins and OTA assayed at 10 $µg.L^{-1}$. For aflatoxins. the mobile phase consisted of 40 % acetonitrile/methanol mixture (50:50; v/v) and 60 % bi-distilled water. Elution was performed in an isocratic mode at a rate of 1.5 $mL.min^{-1}$, with the oven temperature maintained at 40 °C. The injection volume was 20 µL and the detection was performed in fluorescence at the excitation wavelengths of 350 nm and emission of 450 nm. For OTA. the mobile phase was composed of 45 % acetic acid/bidistilled water mixture (2:98; v/v) and 55 % acetonitrile. Elution was performed in an isocratic mode with an elution rate of 1 $mLmin^{-1}$, with the oven temperature set at 40 °C. The excitation and emission wavelengths were 330 mn and 460 nm respectively.

3. RESULTS AND DISCUSSION

3.1. Contamination status of caterpillars with ochratoxin A (OTA) and aflatoxin B1 (AFB1)

The results obtained give the level of impregnation of the OTA and AFB1 tracks. They show that most of the samples analyzed were contaminated and that their contamination levels varied depending on the contaminant and the collection area. **Figure 3** shows the percentage of samples in which the various mycotoxins were detected with concentrations above the limit of quantification (LOQ). It indicates that more than half of the samples collected and analyzed contain OTA and AFB1. Aflatoxin B1 was detected in 83.3 % of all samples. Its presence is more pronounced in GOHITAFLA (100 %) and MAN (90 %). ZUENOULA accounts for 60 % of the contaminated samples.

OTA contamination appears to be uniform with a slight increase in MAN samples. Indeed, it was detected in 70 % of the samples in the commune of MAN and 60 % in each of the communes of ZUENOULA and GOHITAFLA.

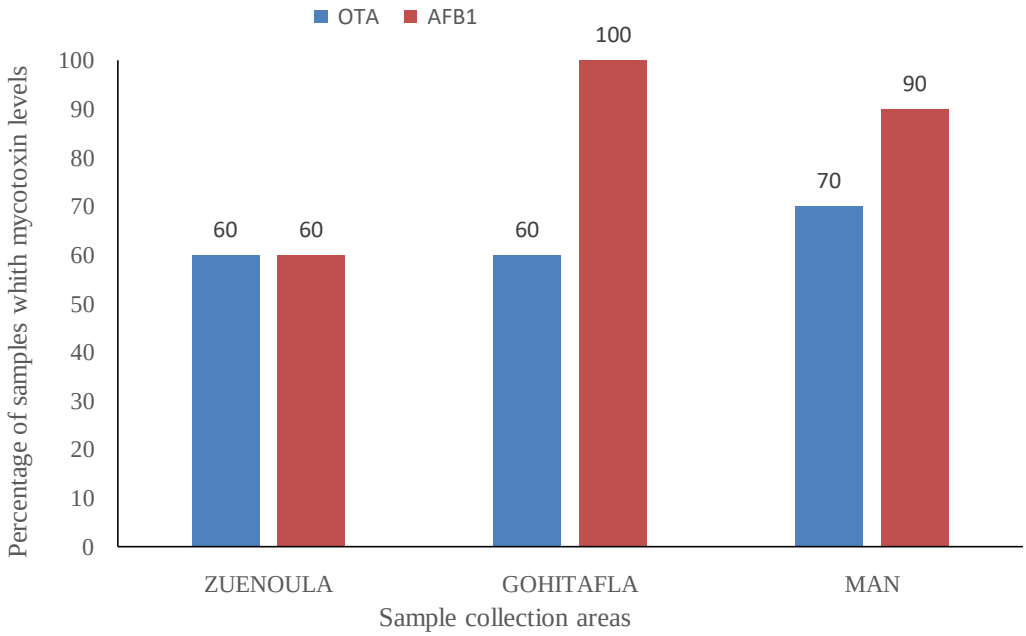

Figure 3: Percentage of samples with mycotoxin levels (OTA and AFB1) above the limit of quantitation (LOQ)

The results presented in **Tables 1** and **2** show the average content, maximum, minimum and median concentrations of OTA and AFB1 measured in dried caterpillar samples.

Table 1 : Average, maximum, minimum and median OTA concentrations in the zone-dried caterpillars

	OTA content (µg.kg^{-1}) in the caterpillar *imbrasia oyemensis*		
	LD = 0.009 (µg.kg^{-1}); LQ = 0.027 (µg.kg^{-1})		
	ZUENOULA	GOHITAFLA	MAN
Average content	0.305	0.462	0.299

Maximum	0.515	0.740	0.355
Minimum	0.060	0.155	0.255
Median	0.300	0.488	0.295
Overall average content ($\mu g.kg^{-1}$)	0.482		

LOD: Limit of detection; LOQ: limit of quantification

Table 2: Average, maximum, minimum and median concentrations of AFB1 in the zone-dried caterpillars

	Aflatoxin B1 content ($\mu g.kg^{-1}$) of the caterpillar *imbrasia oyemensis*		
	LD = 0.001 ($\mu g.kg^{-1}$); LQ = 0.005 ($\mu g.kg^{-1}$)		
	ZUENOULA	GOHITAFLA	MAN
Average content	0.653	32.325	39.478
Maximum	1.155	52.000	80.000
Minimum	0.290	21.350	18.900
Median	0.573	27.600	25.050
Overall average content ($\mu g.kg^{-1}$)	24.150		

LOD: Limit of detection; LOQ: limit of quantification

The OTA levels found in the samples analyzed from the three zones ranged from 0.060 to 0.740 $\mu g.kg^{-1}$ with an overall average of 0.482 $\mu g.kg^{-1}$. Those of aflatoxin B1 (AFB1) ranged from 0.290 to 80.000 $\mu g.kg^{-1}$ with an overall mean of 24.150 $\mu g.kg^{-1}$.

The distribution of OTA grades in the prospected areas is shown in **Figure 4**. It was found that the average concentrations of AFB1 were 2.14, 70 and 132 times higher than those of OTA in the samples of ZUENOULA, GOHITAFLA and MAN respectively. The GOHITAFLA zone leads for OTA with 0.462 $\mu g.kg^{-1}$, followed by ZUENOULA with 0.305 $\mu g.kg^{-1}$ and MAN with 0.299 $\mu g.kg^{-1}$.

As far as AFB1 is concerned the MAN zone is in the lead with 39.478 $\mu g.kg^{-1}$. followed by the GOHITAFLA zone with 32.325 $\mu g.kg^{-1}$ and the ZUENOULA zone with 0.653 $\mu g.kg^{-1}$.

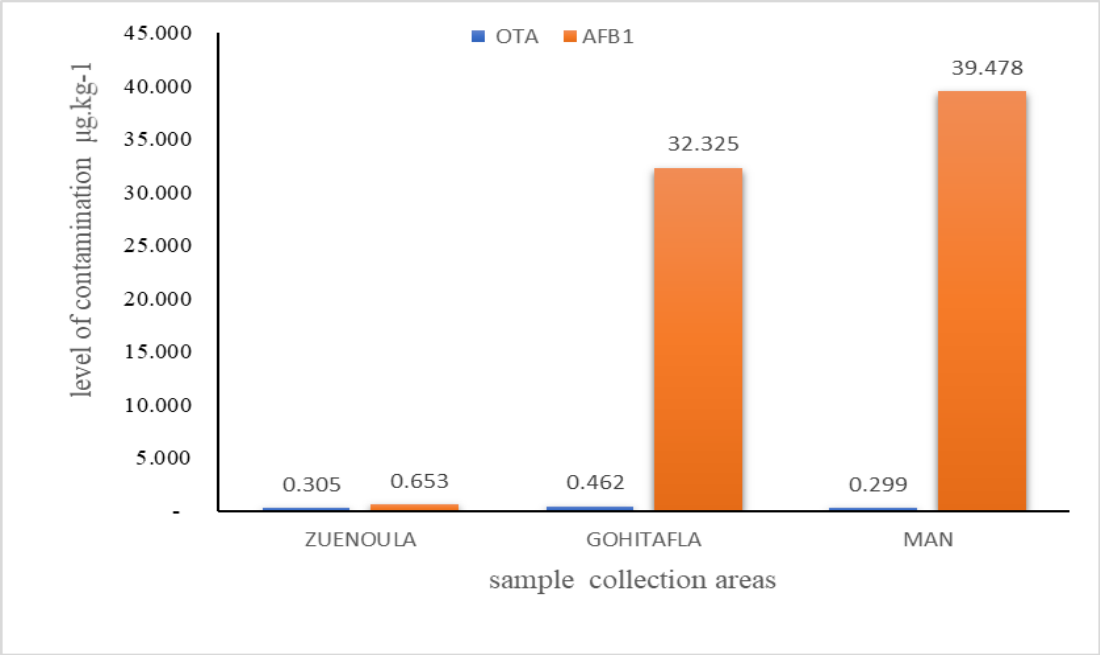

Figure 4: AFB1 and OTA content in the caterpillar *imbrasia oyemensis*

3.2. Comparative study between the level of mycotoxins presents in caterpillars with MRL

Figures 5 and **6** show the comparison of the average concentrations of OTA and AFB1 measured in the different collection areas with European standards. They are respectively 3 µg/kg for OTA and 2 µg/kg for AFB1 (**Regulation (EC) No 1881 / 2006**).

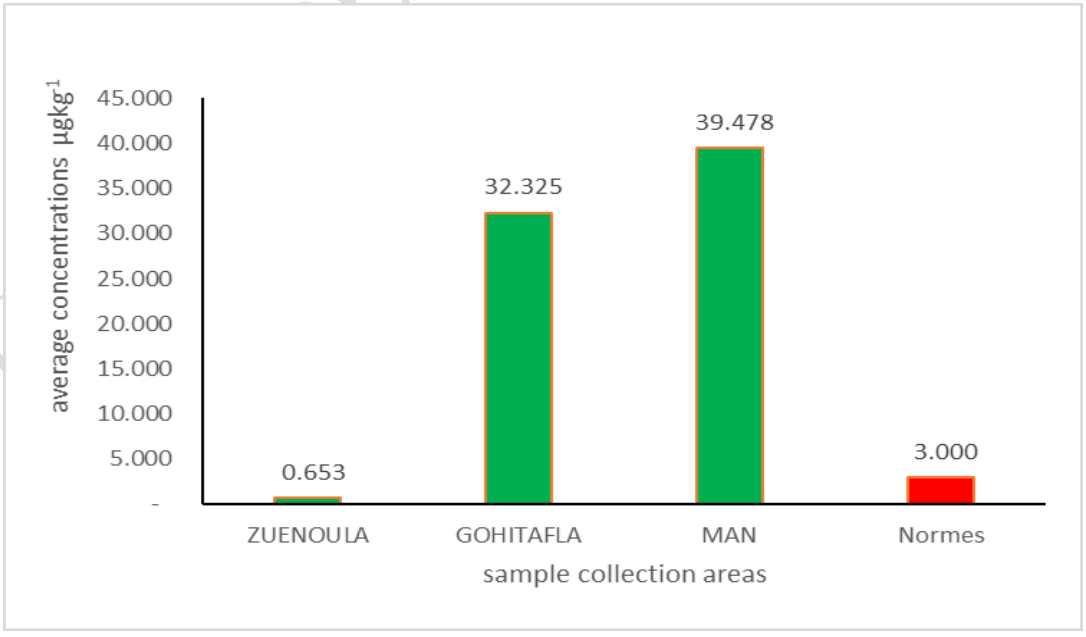

Figure 5: Comparison of AFB1 contents with the standard

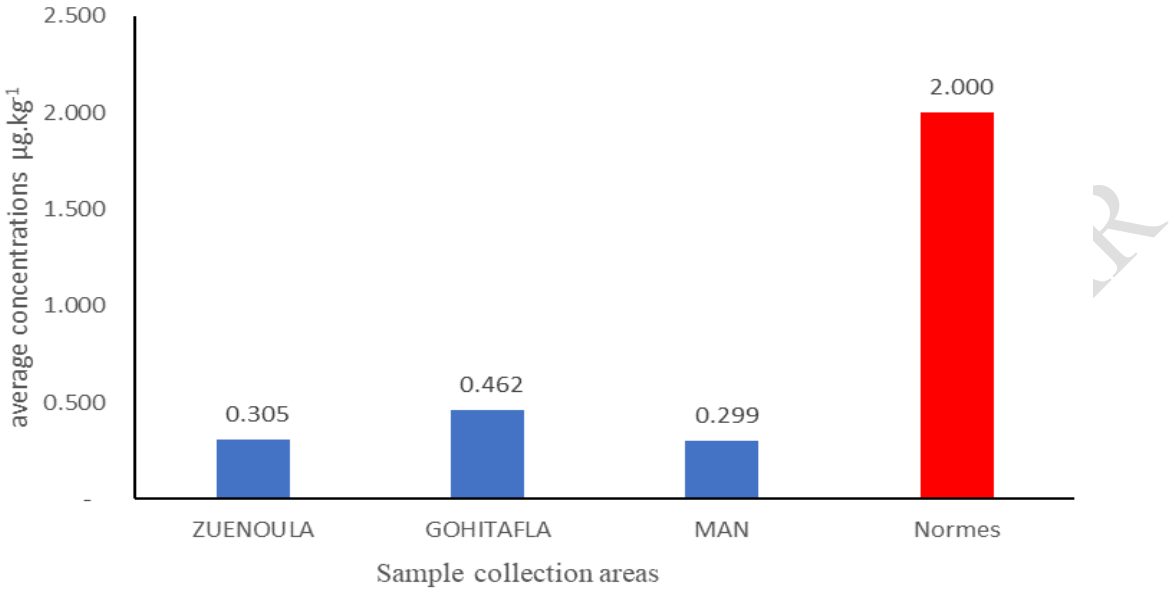

Figure 6: Comparison of OTA levels with the standard

It can be seen that the levels obtained for OTA are below the current standard. On the other hand, with the exception of the municipality of ZUENOULA. the aflatoxin B1 levels in the other municipalities are more than 10 times higher than the standard.

This contamination of the caterpillars by mycotoxins, in particular OTA and aflatoxins, could come from an initial contamination by the leaves and the soil on the one hand, and from recontamination linked to poor drying and storage conditions on the other. Caterpillars can also be infected by pathogenic moulds such as *Aspergillus Penicillium* via their diet (**Devkota & Schmidt, 2000; Durst *et al*., 2010; Belluco *et al*., 2013**). **Simpanya *et al*. (2000)** isolated fungal flora including mycotoxin-producing species from caterpillar samples and the most frequent isolates were the mycotoxin-producing *Aspergillus Penicillium* and *Fusarium*.

It is clear that drying insects in the sun or over a wood fire, even if it limits the growth of most micro-organisms, seems to be ineffective in humid areas such as Côte d'Ivoire. Dried insects are sensitive to moisture, which can promote mould growth and mycotoxin production (**Amadi *et al*., 2005**). Edible insects are also contaminated elsewhere such as at home. Imbrasia belina larvae sold in the markets of Gaborone (capital of Botswana) were studied by **Mpuchane *et al*. (1996)**. The results showed the presence of aflatoxins in some samples and in several of them, with a level of up to 50 µg/kg. In a Belgian study, mycotoxins (aflatoxins, OTA, etc.) were also detected in mealworms (Tenebrio molitor) and migratory locusts (Locusta migratoria), both fresh and freeze-dried or frozen (**Stoops *et al*., 2014**). Researchers **Paepe *et al*. (2019)** and **Charlton *et al*. (2015)** have detected mycotoxins such as OTA and aflatoxins in edible insects. But not at levels that are of public health concern. However, being thermostable, these mycotoxins are not eliminated during the cooking of contaminated food and can be a problem when these caterpillars are consumed regularly.

CONCLUSION

The objective of this study was to verify the contamination of *Imbrasia oyemensis* "ZEGRE" caterpillars by mycotoxins (OTA and AFB1) and to evaluate their content. The analysis of the collected samples made it possible to determine their mycotoxin loads. The concentrations found remain relatively low (0.00-1.30 µg/kg) and below its MRL for OTA. unlike those for AFB1. Caterpillars remain marginal sources of OTA intake in the human diet compared to other food products such as cereal products (maize. rice) and spices. However. the average concentrations of AFB1 were higher than the corresponding standard. with the exception of the ZUENOULA region.

We note that beyond the nutritional benefits of eating caterpillars, mycotoxins are a very current problem in terms of the quality and safety of this food. Although it is exceptional to be exposed to toxic doses in a single ingestion of contaminated caterpillars. the chronic effects (repeated exposure to low or even very low doses) are the most feared because of dietary habits and the persistence of these toxins.

A study of the dietary exposure of caterpillar consumers to mycotoxins complementing this study will make it possible to characterize the major health risks.

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