UNDER PEER REVIEW

Original Research Article

DAILY INTAKE OF AFLATOXIN B1 AND OCHRATOXIN A FROM MAIZE GRAIN (*Zea mays* L.) DURING THE STORAGE WITH *Lippia multiflora* (VERBENACEAE) AND *Hyptis suaveolens* (LAMIACEAE) LEAVES IN COTE D'IVOIRE

ABSTRACT

The aim of this study was to monitor the sanitary quality during the storage of maize grains for 9 months in polypropylene bags containing leaves of *lippia multiflora and hyptis suaveolens* and thus to assess the risk of exposure to aflatoxin B1 and ochratoxin A. This study was carried out in the villages of Timbé and Soko respectively in the departments of Katiola and Bondoukou of Côte d'Ivoire in June 2014 to February 2015. The parameters determined were the Estimeted daily intake (EDI) of aflatoxin B1, ochratoxin A and the risk of exposure. The batches treated with *lippia multiflora* and *hyptis suaveolens* had low levels of EDI and exposure risks compared to controls independently of the study site. The leaves of *hyptis suaveolens* had a biopesticide effect much greater than *lippia multiflora* leaves and the mixture of leaves of the two plants. Indeed, the control batches varied between 0.05 ± 0.00 and 75.97 ± 1.83 ng / kg body weight / day for aflatoxin B1 and between 0.36 ± 0.00 and 57, 72 ± 1.63 ng / kg body weight / day for ochratoxin A in both localities. These values of the control batches reflect a real and high risk of exposure from 2 months of storage. For experimental batches, EDI values ranged from 0.05 ± 0.00 to 15.34 ± 0.80 ng / kg body weight / day for AFB1 and from 0.36 ± 0.00 to 7.42 ± 0.65 ng / kg body weight / day for OTA. The risks of exposure become real only after 7 months of storage. After 7 months, the leaves of *hyptis suaveolens* used individually are the most effective, followed by the leaves of *lippia multiflora* and finally the leaves eat the two plants.

These results indicate that the treatment of maize with the leaves of *lippia multiflora* and *hyptis suaveolens* inhibits insect and fungal activity and preserves the quality of the grains as well as the very low maintenance of the risk of exposure to AFB1 and OTA up to 7 months. This inexpensive and easy-to-use treatment should be popularized among farmers.

Keywords: Aflatoxin B1, ochratoxin A, Daily Intake, maize grain, storage.

UNDER PEER REVIEW

1. INTRODUCTION

Maize (Zea mays L.) is the most common staple food crop in sub-Saharan Africa. In addition, more than 300 million people in sub-Saharan Africa depend on maize as a source of food and income [1]. Maize is the second most cultivated cereal in Côte d'Ivoire after rice (Oryza spp.). Its production increases to 531,940 tons in 2007 to 680,000 tons in 2014 for a total planted area of 330,000 ha [2]. The importance of maize is due to its availability throughout the year [3]. Its nutritional benefits (rich in starch, protein, minerals) and economic (simple to produce, harvest and store crops) make it a competitive product that helps lower the prices of basic food products such as milk and meat in rural agriculture [4]. Crop problems and post-harvest maize treatments are the main problem faced by rural farmers [5-6]. Maize (Zea mays L.) is constantly exposed to the risk of developing fungi to have an ideal nutrient composition. In addition, countries with tropical and subtropical climates have favorable environmental conditions for the development of the main genotoxic fungus types, Aspergillus, Fusarium and Penicillium. Among the mycotoxins found in maize, aflatoxins, zearalenone, ochratoxin A and fuminosin B1 are detached both for the concerns expressed by the researchers because of their possible toxic effect in humans and animals, and economic reasons [7]. Aflatoxin B1 and ochratoxin A are highly toxic, teratogenic, mutagenic and carcinogenic metabolites produced by fungi of the genus Aspergillus and penicillium. Aflatoxin B1 (AFB1) and ochratoxin A (OTA) are the most common mycotoxins with the highest toxic potential of maize [8].
The Higher Council of Public Hygiene of France (Conseil Supérieur de l'Hygiène Publique de France [CSHPF], 1999) [9] and the Scientific Committee on Food (Scientific Committee on Nutrition [SCF] 1998) [10] established a tolerable daily intake (TDI) of 5 µg / kg for OTA and AFB1 in maize. To cope with the exposure of OTA and AFB1 by the consumption of maize in Cote d'Ivoire, a storage method combining the use of leaves of *lippia multiflora* and *hyptis suaveolens* and hermetic storage (triple bagging) has been developed. *Lippia multiflora* and *hyptis suaveolens* are two aromatic plants containing active insecticidal, insect repellent, fungicidal, nematicidal and rodenticidal molecules [11-14]. These natural plants limit the risk of development of resistance by pests and certain pathogenic microorganisms [15]. These plants replace pesticides which have often resulted in the presence of toxic residues on treated products and the development of resistance in pests [16]. Therefore, this study was initiated to assess the risk of exposures to AFB1 and OTA by the consumption of stored maize by the Ivorian adult.

2. MATERIAL AND METHODS

2.1 Site description

The study was conducted in the villages of Timbe and Soko respectively located in the departments of Katiola (Hambol region) (8°10'N 5°40'W) and Bondoukou (Gontougou region) (8°30'N 3°20'W) in the Central North and Northeast of Cote d'Ivoire. Both localities have a humid tropical climate with 4 seasons, including 2 rainy seasons from March to July and October to November. These are interspersed with 2 dry seasons ranging from December to February and August to September. The annual rainfall ranges between 1100 and 1200 mm in Katiola and between 800 and 1400 mm in Bondoukou. The average temperatures recorded in these areas vary between 26.5°C and 33.7°C in Katiola and between 24°C and 29°C in Bondoukou, while the average humidity ranged between 60%-70% in both regions [17-18].

2.2 Plant material collection and processing

The biological material consisted of maize grains (Hybrid variety) collected in January 2014 (from the cooperatives of Timbe and Soko) and leaves of plant species *lippia multiflora* (or savannah tea) and *hyptis suaveolens* collected for their biopesticides properties. These plants are perennials and fragrant shrubs that develop spontaneously from the central to the Northern parts of the country due to the climatic conditions [13-14]. Approximately one month after harvest, maize was sun-dried and leaves of *lippia multiflora* and *hyptis suaveolens* were dried under shade and chopped.

2.3 Treatments

The implementation of the study was conducted from January to September 2014, with the participation of 2 Informal Groups (IG) of farmers. They are the IG "Sounougou" of Soko in Bondoukou and the IG "Lagnimin" of Timbe in Katiola. These farmers accustomed to preserve their maize grain in polypropylene bags in a corner of the house. Method tested in this study,

UNDER PEER REVIEW

consisted in adding of phytopesticides (5% w/w) in the polypropylene bags containing maize grains and storing on pallets in warehouses for 9 months. The steps of adding phytopesticides (*lippia multiflora* and *hyptis suaveolens*) and deposit bags on pallets constitute the principal modifications made to the method of preservation practiced by these farmers. The filling of the bags was performed by alternately as maize grains strata and phytopesticides. Thus, polypropylene bags containing 50 kg of maize grain and 5% w/w of *hyptis suaveolens* (A) or *lippia multiflora* (B) or in mixture (A+B) were stored as described below:

Treatment 1: 50 kg of maize grain + 2.5 kg of leaves of *Hyptis suaveolens* (A);

Treatment 2: 50 kg of maize grain + 2.5 kg of leaves of *Lippia multiflora* (B);

Treatment 3: 50 kg of maize grain + 1.25 kg of leaves of *Lippia multiflora* + 1.25 kg of leaves of *Hyptis suaveolens* (A+B);

Treatment 4: control (50 kg of maize grain alone).

The treatments were laid out in a randomized complete block design in each zone of study, and each treatment was replicated 3 times. Each month samples were taken for analysis.

2.4. Aflotaxins and Ochratoxin A

Chemical reagents (acetonitrile, methanol and chloroform) and standards (ochratoxin A (OTA) and aflatoxins (AFs)) were used for the study. Reagents were purchased from Carlo Erba (Spain) with analytical grade, while standards OTA and AFs were provided from Sigma (Sigma, St Louis, MO, USA).

2.4.1. Extraction and purification of OTA

100 g of the sample was crushed in a hammer mill to obtain a homogeneous fine grind. In a Nalgene jar containing 15 g of grind, 150 mL of aqueous methanol-bicarbonate 1% (m / v, 50:50) were added. The mixture was homogenized by Ultra-Turax for 3 minutes and the homogenate was centrifuged at 5000 rpm for 5 min at 4°C. The supernatant was filtered through a Whatman paper (Wathman N°4) into tubes of 25 mL. To 11 mL of filtrate were added 11 ml of saline phosphate buffered (PBS) at pH 7.3. Immunoaffinity columns brand Ochraprep and R-Biopharm were conditioned with 10 mL of PBS. Purification of 20 ml of the mixture was made on immunoaffinity columns and OTA extraction was performed with two volumes of 1.5 mL of PBS at a flow rate of 5 mL/minute. The resulting sample was packed in a chromatographic tube and the analysis of OTA was made by HPLC using the European community regulation (EC 401/2006) [19]

2.4.2. Extraction and purification of aflatoxins

Biological aflatoxins (B1, B2, G1 and G2) were extracted and purified from maize using the official guidelines of AOAC, 2000 [20]. To 25 g of ground maize put in an erlenmeyer flask, 100 mL of 80% methanol aqueous solution were added. The mixture was homogenized, put in darkness at room temperature for 12 h, and then filtered with a Whatman paper (Wathman N°4). Thereafter, 50 mL of the filtrate were added with 40 mL of a mixture deriving from phosphotungstic acid-zinc sulfate-water (5/15/980, w/w/v), and kept at ambient temperature for 15 min before filtration using Whatman paper. Aflatoxins were extracted from the outcoming filtrate with 3 volumes of 10 mL of chloroform. The extracts were collected into a 50 mL flask and processed with rotatory evaporator (Buchi Rotavapor R-215) at 40°C to evaporate the chloroform reagent. Finally, 0.4 mL of hydrochloric acid and 4.6 mL of bidistillated water were added to the dry extract, and the solution was filtered through filter Rezist in a chromatographic tube then passed through an immunoaffinity column (column RiDA aflatoxin, Biopharm, Germany).

2.5. Aflatoxins and OTA determination

Determination of AFs and OTA contents was achieved with high performance liquid chromatography column, using a Shimadzu liquid chromatograph (Kyoto, Japan) fitted with fluorescence detector (Table I)

UNDER PEER REVIEW

Table I: Conditions of AFB1 and OTA analysis by HPLC

	Aflatoxin B1	Ochratoxin A
Pre-column	Shim-pack GVP-ODS 10 x 4.6 mm	
Column	Shim-pack GVP-ODS, 250 mm x 4.6 mm	
Detector	Fluorescence, λ excitation : 365 nm,	Fluorescence, λ excitation : 330 nm,
	λ émission : 435 nm	λ émission : 460 nm
Phase mobile	Acetonitrile/Water/Methanol (20/20/60)	Acetonitrile/Water / Acetic acid (49/49/2)
Inject volume	20 µL	100 µl
Flow rate	1 mL/minute	
Column temperature	40°C	
Rising solvent	Methanol	Acetonitrile
Analysis duration	15 minutes	12 minutes
Limit of Detection (LD ; ng/kg)	6.18±0.55	5±0.06
Limit of quantitation (LQ ; ng/kg)	6.50±0.45	20±0.8
Recovery rate (%)	98.92±2.49	86±0.39

2.6. Estimated daily intake of aflatoxin B1 and ochratoxin A from maize grains

According to the definition of the Codex Alimentarius, the estimate of the exposure is the assessment of the quantitative exposure of the probable ingestion of chemical dangers through foods [21]. To assess ochratoxin A and aflatoxine B1 exposure, the mean level of these mycotoxins found in maize grains stored on 8 months together with the mean consumption of maize and the average body weight of individual adult were used to estimate the daily intake of OTA and AFB1 [22]. According to the National agricultural statistics of Cote d'Ivoire, the daily consumption of maize is 28.4 g per capita/day [23]. The OTA and AFB1 intake was calculated using formula 1:

$$\mathbf{EDI = (C \times Q)/ bw}$$

With:
EDI: the Estimated of OTA and AFB1 Daily Intake in pg kg^{-1} of body weight (b.w.) day^{-1};
C: the OTA and AFB1 Concentration found in maize grains stored (pg/kg);
Q, the daily consumption of maize grains (g/day);
bw, the body weight of the individual adult (70 kg).

The Estimated Daily Intakes were also expressed from the average and maximum levels of mycotoxins fixed by the European Commission (CE 2006; CE 2007 and CE 2010) [24-26] for maize "to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs" at level of 5 µg/kg for OTA and AFB1. Moreover, the estimated intakes were compared to Tolerable Daily Intake (TDI) set at 5 ng/kg bw/day for OTA and AFB1 established by WHO [27-28].
The characterization of the Risk of Exposure (RE) to aflatoxin B1 and ochratoxin A is calculated with the following formula [29]. This result allows only concluding on the potential occurrence of effects but not on their importance.

$$\mathbf{RE = (EDI) / (TDI)}$$

UNDER PEER REVIEW

If:
- RE <1 means that the exposed population is theoretically out of danger, that is, this exposed population is not likely to develop the health effects studied.
- RE> 1 means that the toxic effect can occur without it being possible to predict the probability of the occurrence of this event.

2.7. Statistical analysis

All analyses were performed in triplicate. The mean concentrations of AFB1 and OTA were calculated with their standard deviations. The corresponding proportions of daily intake of mycotoxins by maize consumption and exposure risk were calculated. The homogeneity of the mean concentrations of AFB1 and OTA was assessed by an analysis of variance by the Fischer test using SPSS version 20.0 for windows at 5% risk. The Excel 2007 software was used to build curves of evolution of the parameters over time.

3. RESULTS

3.1. Measuring the concentration of aflatoxin B1 and ochratoxin A

The evolution of the AFB1 content is different according to the treatments applied to the storage of maize grains. In the control batches, the AFB1 content increased significantly from 0.10 ± 0.00 µg/kg and 2.00 ± 0.00 µg/kg at 1 month, respectively, to 112.26 ± 2.50 µg/kg and 187.26±5.00 µg/kg at 9 months respectively for samples from Katiola and Bondoukou. For the experimental batches, this evolution is very low. Up to 7 months of storage, the AFB1 content are respectively approximately 3.30 ± 0.01 µg/kg and 8.00 ± 0.02 µg/kg for samples from Katiola and Bondoukou whatever the type of treatment applied. At 9 months of storage, we find that the AFB1 content in batch B is the lowest, followed by batch A and finally batch A + B. These AFB1 content are much lower than those of the control batches (Table 2)

The evolution of the OTA content differs according to the different lots of maize grains. In the control batches, OTA levels increased significantly from 0.80 ±0.01 µg/kg and 1.90 ± 0.01 µg/kg at 1 month to 112.25 ± 1.05 µg/kg and 142.26 ±1.31 µg/kg at 9 months respectively for samples from Katiola and Bondoukou. For the experimental batches, this evolution remains very low. Up to 7 months of storage, OTA content are approximately 11.27 ± 0.06 µg/kg and 23.79 ± 0.22 µg/kg respectively for samples from Katiola and Bondoukou. At 9 months of storage, we find that the OTA content in batch B is lowest, followed by batch A and finally batch A + B. These OTA content are much lower than those of the control batches (Table II).

Table II: Aflatoxin B1 and Ochratoxin A contents during the storage of maize grains

AFB1 (µg/kg)								
	KATIOLA				**BONDOUKOU**			
Months / Sample	1	4	7	9	1	4	7	9
C	0.10±0.00	26.25±1.25	70.26±2.56	112.26±2.50	2.00±0.00	51.24±2.05	152.25±4.55	187.26±5.00
A	0.10±0.00	2.16±0.05	3.25±0.05	15.26±	2.00±0.00	3.51±0.2	7.98±0.40	26.25±1.05
B	0.10±0.00	1.15±0.01	2.98±0.03	10.26±	2.00±0.00	2.16±0.1	5.98±0.25	16.26±0.64
A+B	0.10±0.00	2.88±0.08	4.26±0.06	21.26±	2.00±0.00	4.26±0.3	10.26±0.38	37.82±1.85
OTA (µg/kg)								
C	0.80±0.00	29.21±2.03	42.16±0.05	112.25±3.01	1.90±0.01	79.84±2.81	89.26±1.86	142.26±4.05
A	0.80±0.00	2.15±0.02	4.26±0.02	7.26±0.50	1.90±0.01	4.26±0.06	6.26±0.7	12.26±0.91
B	0.80±0.00	1.74±0.01	2.98±0.02	5.26±0.25	1.90±0.01	3.00±0.5	4.26±0.05	7.26±0.50
A+B	0.80±0.00	2.87±0.05	4.87±0.05	12.26±0.65	1.90±0.01	5.22±0.1	7.52±0.45	18.29±0.72

C: Control lots without biopesticides
A: Lot containing the dried leaves of *hyptis suaveolens*
B: Lot containing the dried leaves of *lippia multiflora*
A + B: Lot containing the mixture of dried leaves of *hyptis suaveolens* and *lippia multiflora*

UNDER PEER REVIEW

3.2. AFB1 and OTA daily intake estimated from the consumption of maize grains from Ivorian adult

The estimated daily intake (EIA) was calculated for the different concentrations of aflatoxin B1 and ochratoxin A found in the samples analyzed during the 9 months storage of maize in the two storage areas. Tables II and III present the results of the estimated intakes of aflatoxin B1 and ochratoxin A from the consumption of stored maize grains from the concentrations of AFB1 and OTA determined during the experiment.

For the control batches, estimated AFB1 intake increased significantly during the 9 month storage period from 0.05 ± 0.00 and 0.81 ± 0.03 ng / kg body weight / day for the Katiola and Bondoukou area at one month storage to 45.54 ± 1.50 and 75.97 ± 1.83 ng / kg body weight / day at 9 months storage for the Katiola and Bondoukou area respectively. The estimated values for AFB1 in the Katiola area range from 0.05 ± 0.00 ng / kg body weight / day at the start of storage to 6.19 ± 0.03; 4.16 ± 0.02 and 8.62 ± 0.03 at 9 months storage respectively for experimental batches A, B and A +B. The estimated values for AFB1 in the Bondoukou area range from 0.81 ± 0.03 ng / kg body weight / day at the start of storage to 10.65 ± 0.64; 6,60 ± 0,02 and 15,34 ± 0,80 at 9 months storage respectively for experimental lots A, B and A+B. The estimated intakes of AFB1 in the Katiola area are lower than those of Bondoukou aera.

For the control batches, estimated OTA intakes increased significantly during the 9 month storage period from 0.36 ± 0.00 and 0.80 ± 0.02 ng / kg body weight / day for the Katiola and Bondoukou area at one month storage to 45.54 ± 1.20 and 57.72 ± 1.63 ng / kg body weight / day at 9 months storage for the Katiola and Bondoukou area respectively. Estimated values for OTA in the Katiola area range from 0.36 ± 0.00 ng / kg body weight / day at the start of storage to 2.94 ± 0.01; 2.13 ± 0.01 and 4.97 ± 0.02 at 9 months storage respectively for experimental batches A, B and A+B. Estimated values for OTA in the Bondoukou area range from 0.80 ± 0.02 ng / kg body weight / day at the start of storage to 4.97 ± 0.14; 2.94 ± 0.01 and 7.42 ± 0.65 at 9 months storage respectively for experimental lots A, B and A+B. The estimated intakes of OTA in the Katiola area are lower than those of Bondoukou (**Table III**)

Table III: Estimated intakes of Aflatoxin B1 in Ivorian adults for consumption of stored maize

Lots	Month	EDI (ng/kg bw/day)		TDI recommended by SCF (ng/kg bw/day)
		AFB1 (Katiola)	AFB1 (Bondoukou)	
Control	1	0.05±0.00	0.81±0.03	5
	4	10.65±0.64	20.79±0.84	
	7	28.50±0.65	61.77±1.02	
	9	45.54±1.50	75.97±1.83	
A	1	0.05±0.00	0.81±0.03	
	4	0.88±0.02	1.42±0.07	
	7	1.32±0.06	3.24±0.12	
	9	6.19±0.20	10.65±0.76	
B	1	0.05±0.00	0.81±0.03	
	4	0.47±0.01	0.88±0.03	
	7	1.21±0.03	2.42±0.06	
	9	4.16±0.02	6.60±0.41	
A+B	1	0.05±0.00	0.81±0.03	
	4	1.17±0.01	1.73±0.01	
	7	1.73±0.01	4.16±0.02	
	9	8.62±0.03	15.34±0.50	

C: Control lots without biopesticides
A: Lot containing the dried leaves of hyptis suaveolens
B: Lot containing the dried leaves of lippia multiflora
A + B: Lot containing the mixture of dried leaves of hyptis suaveolens and lippia multiflora
EDI: Estimated Daily Intake
TDI: Tolerable daily Intake
SCF: Scientific Committee on Food

UNDER PEER REVIEW

Tableau IV: Estimated intakes of Ochratoxin A in Ivorian adults for consumption of stored maize

Lots	Month	EDI (ng/kg bw/day)		TDI recommended by SCF (ng/kg bw/day)
		OTA (Katiola)	OTA (Bondoukou)	
Control	1	0.36±0.00	0.80±0.02	5
	4	11.85±0.40	32.39±0.96	
	7	17.10±0.48	36.21±1.05	
	9	45.54±1.20	57.72±1.63	
A	1	0.36±0.00	0.80±0.02	
	4	0.87±0.00	1.73±0.01	
	7	1.73±0.04	2.54±0.03	
	9	2.94±0.05	4.97±0.14	
B	1	0.36±0.00	0.80±0.02	
	4	0.71±0.01	1.22±0.01	
	7	1.21±0.02	1.73±0.01	
	9	2.13±0.01	2.94±0.01	
A+B	1	0.36±0.00	0,80±0.02	
	4	1.17±0.01	2.12±0.01	
	7	1.98±0.01	3.05±0.03	
	9	4.97±0.02	7.42±0.65	

Control: Lot containing no leaves with biopesticide effect;
A: Lot containing the dried leaves of *hyptis suaveolens*;
B: Lot containing the dried leaves of *lippia multiflora*;
A + B: Lot containing the mixture of dried leaves of *hyptis suaveolens* and *lippia multiflora*
EDI: Estimated Daily Intake;
TDI: Tolerable daily Intake;
SCF: Scientific Committee on Food

3.3. Aflatoxin B1 and ochratoxin A Exposure Risk during storage of maize

The ratio of Estimated Daily Intake (EDI) to Tolerable Daily Intake (TDI) was determined to assess the risk of exposure to consumers. Compared to the TDI, for the control batches in the two storage zones, from 2 months of storage, the risk of exposure in AFB1 becomes real because EDI > TDI. For experimental batches A, B and A + B the risk of exposure to AFB1 is very low up to 7 months of storage in the two experimental zones because the EDI is lower than the TDI. Beyond the 7 months storage, all the experimental batches present a real risk of exposure in AFB1 except lot B of the zone of Katiola. (Fig. 1 and 2).
Regarding the risk of exposure to ochratoxin A for the Ivorian adult, in the two storage areas, the control batches show high risks of exposure after 2 months of storage because EDI becomes higher than the TDI. For experimental batches A, B and A + B the risk of exposure to OTA is low over the 9 months of storage in the two experimental zones with the exception of the lot A+B in the Bondoukou area. Indeed, the risk of OTA exposure in the lot A+B of the Bondoukou zone at 9 months of storage is 1.48 ng / kg of body weight / day greater than 1 ng / kg of body weight / day (Fig. 3 and 4).
The proportion of samples with EDI > TDI, 16.66% and 18.75% were exposed to high risk of exposure to AFB1 in the Katiola and Bondoukou areas respectively and 0% and 8.33% which pose high risks of OTA exposure in the Katiola and Bondoukou areas, respectively.

UNDER PEER REVIEW

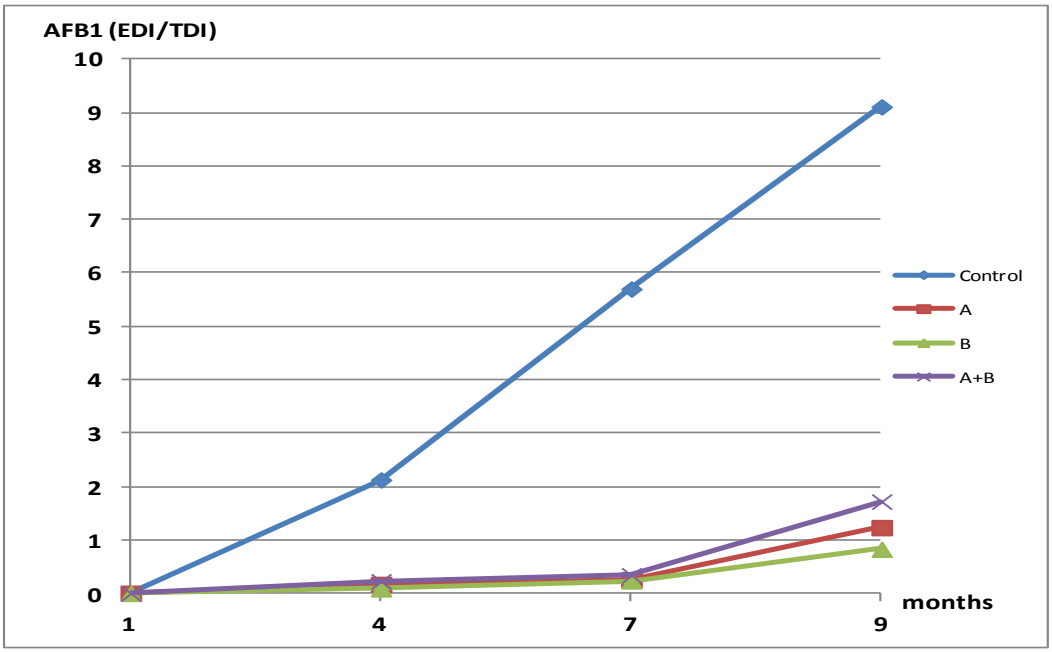

Fig.1: Aflatoxin B1 Exposure Risk during storage of maize grains at Katiola

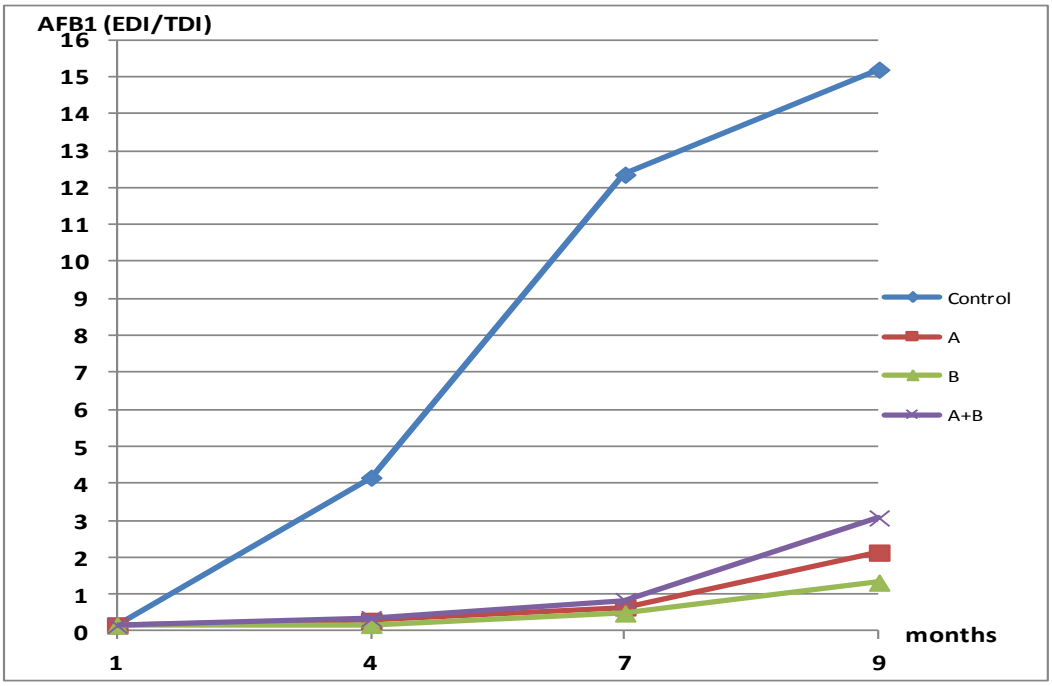

Fig.2: Aflatoxin B1 Exposure Risk during storage of maize grains at Bondoukou

Control: Lot containing no leaves with biopesticide effect;
A: Lot containing the dried leaves of *hyptis suaveolens*;
B: Lot containing the dried leaves of *lippia multiflora*;
A + B: Lot containing the mixture of dried leaves of *hyptis suaveolens* and *lippia multiflora*
EDI: Estimated Daily Intake;
TDI: Tolerable daily Intake;

UNDER PEER REVIEW

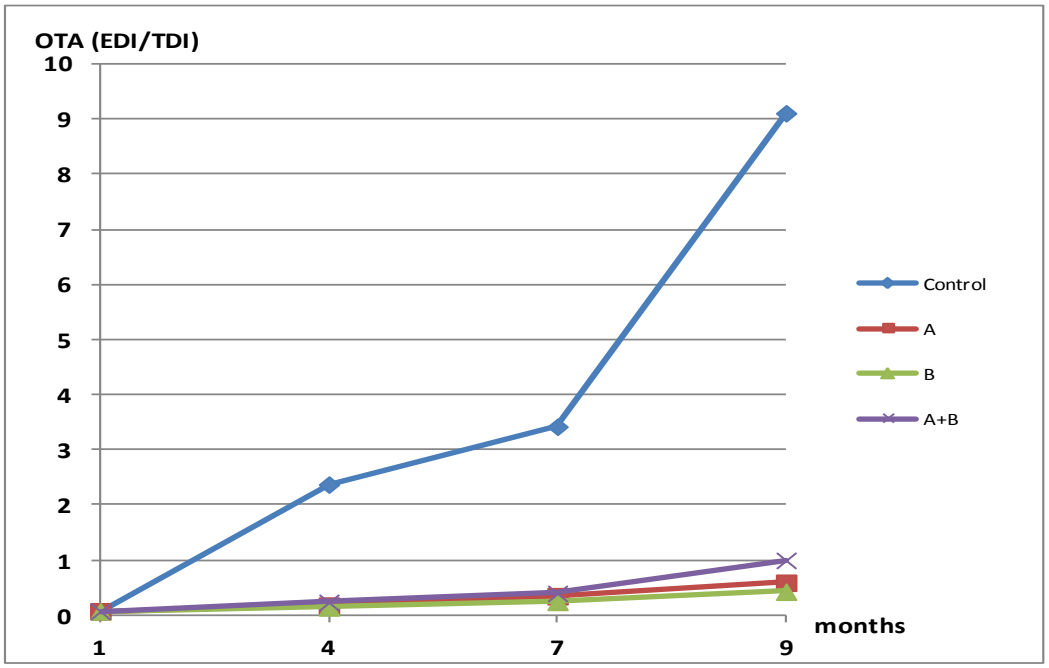

Fig.3: Ochratoxin A Exposure Risk during storage of maize grains at Katiola

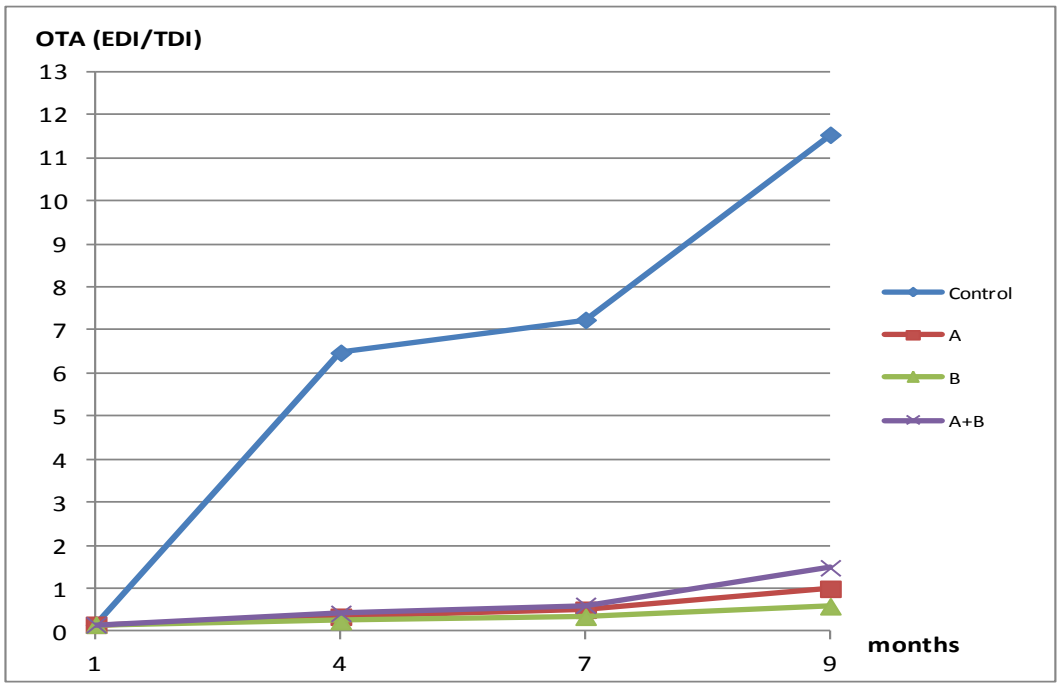

Fig.4: Ochratoxin A Exposure Risk during storage of maize grains at Bondoukou

Control: Lot containing no leaves with biopesticide effect;
A: Lot containing the dried leaves of *hyptis suaveolens*;
B: Lot containing the dried leaves of *lippia multiflora*;
A + B: Lot containing the mixture of dried leaves of *hyptis suaveolens* and *lippia multiflora*
EDI: Estimated Daily Intake;
TDI: Tolerable daily Intake;

UNDER PEER REVIEW

4. DISCUSSION

The maize storage system, which combines triple bagging and dried leaves of *lippia multiflora* and *hyptis suaveolens*, has helped to maintain sanitary quality and thus reduce the risk of exposure to mycotoxins (AFB1 and OTA) during storage.

In the control batches, the estimated intakes of Aflatoxin B1 in adult Ivorian are high and well above the Tolerable Daily Intake (TDI) recommended by the European Commission's Scientific Committee on Food (SCF) after two months of storage. This constitutes a real risk of exposure to AFB1. The same is true of the estimated intakes of ochratoxin A (OTA). This means that if the maize conservation in these two areas is done without treatment, the consumer populations are exposed to the harmful effects of these two mycotoxins (AFB1 and OTA). AFB1 and OTA are the basis of various problems such as nutritional deficiencies, immunosuppression, liver cancer, mutagenic and teratogenic effects [30-33].

For the different experimental batches, the estimated intakes of AFB1 and OTA remain below the Tolerable Daily Dose (TDI) over a 7-month storage period. In these experimental batches, the dried leaves of *lippia multiflora* and *hyptis suaveolens* are used as biopesticides. The leaves of *lippia multiflora* and *hyptis suaveolens* used in this study have positively influenced the sanitary quality of the stored maize grains and thus reduced the risk of exposure in AFB1 and OTA [33]. This observation is made at the two storage sites (Katiola and Bondoukou). These data therefore indicate better preservation of the sanitary quality of stored maize after the addition of leaves of *lippia multiflora, hyptis suaveolens* or the mixing of the two leaves, unlike the storage without any treatment (the control batches).

Indeed, the low levels recorded in the experimental batches after 7 months of storage could be attributed to the insecticidal and / or insect repellent effect of leaves of *lippia multiflora* and *hyptis suaveolens* due to the release of essential substances (bioactive molecules) [34 - 35]; These results are similar to those of Niamketchi et al. [36] in the central region of Côte d'Ivoire. These authors demonstrated the effectiveness of dried leaves of *lippia multiflora* and *hyptis suaveolens* against the development of pests responsible for maize grains alteration in traditional and improved granaries. The results obtained are also in agreement with those of Rose de Lima et al. [37] in Benin, which showed that the essential oils of *pimenta racemosa* and *syzygium aromaticum* would considerably reduce the fungal flora responsible for the production of mycotoxins during the storage of cowpea for a period of 3 months. In addition, studies by Makun et al. [38] demonstrated the inhibitory effect of ethanolic extracts of leaves of *lippia multiflora*, *azadirachta indica* and *blumea perotitiana* on toxigenous cereal molds. The bioactive molecules of *lippia multiflora* consist mainly of oxygenated monoterpenes such as linalool and 1.8 cineole [11, 39]. While monoterpene hydrocarbons, especially sabinene, β-pinene and limonene, predominate in *hyptis suaveolens*. These various mono and sesquiterpene compounds have contributed to the inhibitory activity of antifungal and mycotoxin production (AFB1 and OTA) [40-43]. These antimicrobial agents induce morphological perturbations, rupture of the plasma membrane and alteration of the mitochondrial structure on molds [44]. Tatsadjieu et al. [14] also showed that the essential oil of *lippia rugosa* inhibits the development of *Aspergillus flavus* and limits the production of aflatoxin B1 to an inhibitory concentration of 1 g / L. Sharma et al. [43] showed that the essential oil of *hyptis suaveolens* has inhibitory activity on *Aspergillus flavus, Aspergillus niger* and *Aspergillus ochraceous* producing aflatoxin B1 and ochratoxin A at a concentration of 0.5 g / kg.

The leaves of *hyptis suaveolens* have much more inhibitory and fungicidal effects than *lippia multiflora* and the mixture of the two plants. The composition of the bioactive molecules of *hyptis suaveolens* which differ in quantity and quality with the bioactive molecules of *lippia multiflora* could explain this finding. Therefore, the combination of the different bioactive molecules of the two plants could have low-level antagonistic effects [45], which explain the low level of protection of maize from 8 months on compared to individual plants.

5. CONCLUSION

This work contributes to improving the storage of maize in a farming environment and to the preservation of the health of maize consumers. The results showed that the leaves of the two plants used reduced the risk of exposure to mycotoxins (AFB1 and OTA) over a period of 7 months. Beyond 7 months, the leaves of *hyptis suaveolens* would preserve better the maize grains than the leaves of *lippia multiflora* and the combination of the leaves of these two plants. The technique used is effective and safe for the consumer.

UNDER PEER REVIEW

REFERENCES

1. Cissé M. Caractérisation biochimique et nutritive des grains de variétés de maïs (Zea mays) QPM et ordinaires cultivées en Côte d'Ivoire. Thèse de doctorat en Biochimie Sciences des Aliments, Université Félix Houphouët-Boigny, Abidjan. French. 2013;180.
2. FAOSTAT. FAO statistics division. FAOSTAT. Production. Crops. (Retrieved on March 18, 2015) Available:http://faostat3.fao.org/home/inde x.html
3. IITA. Maize. IITA, Research to nourish Africa. 2012;7.
4. Nuss TE, Tanumihardjo SA. Quality protein maize for Africa: Closing the protein inadequacy gap in vulnerable populations. Advence in Nutrition. 2011;2:217–224.
5. Ratnadass A. Dynamique des populations d'insectes ravageurs des stocks des céréales en milieux villageois d'Afrique tropicale. Thèse Docteur-Ingénieur en Sciences agronomiques. Institut National Agronomique Paris-Grignon. French. 1987; 115.
6. Boone P, Stathacos JDC, Wanzie RL. Sub-regional. Assessment of the maize value chain in West Africa. ATP project, Abt Associates Inc., Bethesda, MD; 2008
7. Noudjou F, Kouninki H, Ngamo LST, Maponmestsem PM, Ngassoum M, Hance T, et al. Effect of site location and collecting period on the chemical composition of Hyptis spicigera Lam. an insecticidal essential oil from north Cameroon. J. Essent. Oil Res. 2007; 19(6):597-601
8. Coulombe, R. A. Aflatoxins. In R.P. Sharma & D.K. Salunkhe (Eds.), Mycotoxins and phytoalexins. 1991; 103–143. Boca Raton: CRC Press.
9. CSHPF (1999). Les mycotoxines dans l'alimentation : évaluation et gestion du risque, éds TEC & DOC, 236 p.
10. SCF (Scientific Committee on Food), (1998). Opinion of the Scientific Committee on food on ochratoxin A (expressed on 17 September 1998). Directorate - Scientific Opinions. European Commission, Brussels, Belgium
11. Tia V. Pouvoir insecticide des huiles essentielles de cinq espèces végétales aromatiques de côte d'ivoire dans la lutte contre les insectes ph ytophages Bemisia tabaci Gen. et Plutella xylostella Lin.: Composition chimique et tests d'efficacité Thèse de doctorat en biochimie sciences des aliments, Université Félix Houphouët Boigny, Abidjan. French. 2012;205.
12. Gueye MT, Goergen G, Ndiaye S, Asieedu EA, Wathelet JP, Lognay G, Seck D. Efficiency of traditional maize storage and control methods in rural grain granaries: A case study from Senegal. Tropicultura. 2013;31:129-136.
13. Alpsoy L. Inhibitory effect of essential oil on aflatoxin activities. African Journal of Biotechnology. 2010; 9:2474-2481.
14. Ekissi AC, Konan AG, Yao-Kouame A, Bonfoh B, Kati-Coulibaly S. Sensory evaluation of green tea from *Lippia multiflora* Moldenke leaves. European Scientific Journal. 2014;10:534-543.
15. Tatsadjieu NL, Dongmo JPM, Ngassoum MB, Etoa F-X et Mbofung CMF. Investigations on the essential oil of Lippia rugosa from Cameroon for its potential use as antifungal agent against Aspergillus flavus Link ex. Fries. Food Control. 2009; 20:161-166.
16. Almiazee MT. The effect of carbon dioxide gas alone or in combinations on the mortality of Tribalium castaneum (HERBST) and T. corfusum du Val (coleoptera, Tenebrionidae). J. Stored Prod. Res. 1971;7:244-252.
17. Kouakou K, Akanvou L, Konan A, Mahyao A, Stratégies paysannes de maintien et de gestion de la biodiversité du maïs (*Zea mays L.*) dans le département de Katiola, Côte d'Ivoire. Journal of Applied Biosciences. 2010;33:2100–2109. French.
18. Ahoussi K, Youann T, Loko S, Adja M, Lasm T, Jourda J, Étude hydrogéochimique des eaux des aquifères de fractures du socle Paléoprotérozoïque du Nord-Est de la Côte d'Ivoire: Cas de la région de Bondoukou. 2012; Afrique Sciences. 2012;8:51-68. French.
19. EC, European Commission. Commission regulation (EC) N° 401/2006 of 28 September of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Union, L70. 2006;14-17
20. AOAC. Official methods of analysis of the association of analytical chemists. 17th Edition. W ashington, DC, USA; 2000.
21. Assidjo E., Sadat A., Akmel C., Akaki D., Elleingand E. et Yao B.. L'analyse des risques : Outils innovant d'amélioration de la sécurité sanitaire des aliments. *Revue Africaine de Science et de Productions Animales.* 2013 ; 11 (6) : 3-13
22. Kroes R., Muller D., Lambe J., Verger P., Visconti A. Assessment of intake from the diet. *Food and*

UNDER PEER REVIEW

Chemical Toxicology, 2002 ; 40 : 327-385

23. Beugre G. A., Yapo B. M.,Blei S. H., Gnakri D. Effect of Fermentation Time on the Physico-ChemicalProperties of Maize Flour. *International Journal of Research Studies in Biosciences,* 2014 ;2: 30-38.
24. CE. Commission Européenne : Règlement (CE) No 401/2006 de la commission du 23 février 2006 portant fixation des modes de prélèvement d'échantillons et des méthodes d'analyse pour le contrôle officiel des teneurs en mycotoxines des denrées alimentaires. *Journal Officiel de l'Union Eupéenne.* 2006 : L70/12, pp. 3-25.
25. CE Commission Européenne : Règlement N° 1126/2007 du 28 Septembre 2007 modifiant le règlement N° 1881/2006 portant fixation de teneurs maximales pour certains contaminants dans les denrées alimentaires en ce qui concerne les toxines du Fusarium dans le maïs et les sous produits. *Journal officiel de l'Union européenne*, 2007 ; L255, 16-17.
26. CE Commission Européenne : Règlement N° 165/2010, du 26 Février 2010 modifiant le règlement N° 1881/2006 portant fixation de teneurs maximales pour certains contaminants dans les denrées alimentaires en ce qui concerne les aflatoxines. *Journal officiel de l'Union européenne*, 2010 ; L50, 11-12.
27. JECFA, Joint FAO/WHO Expert Committee on Food Additives and Contaminants Evaluation of certain food additives and contaminants, Fourty-nine report, WHO Technical Report. Geneva 1999; Series N°884, pp. 69-77.
28. JECFA, Joint FAO/WHO Expert Committee on Food Additives and Contaminants Safety evaluation of certain mycotoxins in food. WHO Food Additives Series 47, FAO food and Nutrition; Geneva, 2001; 74 p.
29. Association scientifique et technique de l'eau et de l'environnement (ASTEE) Guide pour l'évaluation du risque sanitaire dans l'étude d'impact d'une UIOM. 2003 ; 60 p.
30. Wagacha J. M. et Muthomi J. W. Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbioogy*, 2008 ; 124: 1-12
31. IARC, International Agency for Research on Cancer. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. In. *Monographs on the evaluation of carcinogenic risks to humans*, 2002; 82: 171-74.
32. Clark H. A. et Snedeker S. M. Ochratoxin A: Its cancer risk and potential for exposure. *Journal of Toxicology and Environmental Health*, 2006; 9: 265–296.
33. Ezoua Pierre, Adama Coulibaly, Ysidor Konan, Daouda Sidibe, Olivier Kouame Chatigre, Godi Henri Marius Biego. Efficacy of Lippia multiflora (Verbenaceae) and Hyptis suaveolens (Lamiaceae) leaves on merchant quality of stored maize grain (Zea mays L.) in Côte d'ivoire. Journal of Agriculture and Ecology Research International. 2017;11(3):1-10
34. Ngamo T, M Ngassoum, FMalaisse. Use of essential oil of aromatic plants as protectant of grains during storage. Agricultural Journal. 2007;2:204-209.
35. Konan K. Constant, Coulibaly Adama, Sidibe Daouda, Chatigre Olivier, Biego Godi Henri Marius. Evolution of aflatoxins levels during storage of Cowpeas (Vigna unguiculata L Walp) Bagged pics containing Lippia multiflora moldenke leaves and ivorian exposure risk. International Journal of Science and Research. 2016;5(7):678-691.
36. Niamketchi Léonce, Henri Godi Biego, Olivier Chatigre, Didier Amané, Emmanuel Koffi, Augustin Adima Optimization of post-harvest maize storage using biopesticides in granaries in rural environment of Côte d'Ivoire. International Journal of Science and Research. 2015; 4(9):1727-1736
37. Rose de Lima FH, Euloge SA, Edwige DA, Dominique CKS, Mohamed MS. Caractéristiques biochimique et sensorielle du niébé (Vigna unguiculata) conservé au moyen des huiles essentielles extraites de plantes de la famille des Myrtaceae. International Journal of Innovation and Applied Studies. 2014;9:428-437
38. Makun HA, Anjorin ST, Abidoye AS, Rufai AR et Kabiru YA. Incidence and botanical control of seed-borne fungi of cowpea in Niger state, Nigeria. Journal of Agricultural and Biological Science. 2012;8:654-658.
39. Kanko C, Koukoua G, N'Guessan YT, Fournier J, Pradère JP et Toupet L. Contribution à l'étude phytochimique de Lippia multiflora (Verbenaceae). Comptes Rendus. Chimie, French. 2004;7:1029–1032.
40. Rao VK, Girisham S, Reddy SM. Inhibitory effect of essential oils on growth and ochratoxin a production by Penicillium species. Research Journal of Microbiology. 2015;10:222-229.
41. Passone M, Girardi N, Etcheverry M. Evaluation of the control ability of five essential oils against Aspergillus section Nigri growth and ochratoxin A accumulation in peanut meal extract

UNDER PEER REVIEW

agar conditioned at different water activities levels. International Journal of Food Microbiology. 2012;159:198–206.

42. Fandohan P, Gnonlonfin B, Hell K, Marasas WFO, Wingfield MJ. Impact of indigenous storage systems and insect infestation on the contamination of maize with fumonisins. African Journal of Biotechnology. 2005;5:546-552.

43. Sharma N, Verma UK et Tripathi A. Bioactivity of essential oil from Hyptis suaveolens against mycoflora. Proceedings of an international conference on controlled atmosphere and fumigation in stored products. Gold-Coast Australia. 2004;99-116

44. De Billerbeck VG, Roques CG, Bessière JM, Fonvieille JL et Dargent R. Effects of Cymbopognon nardus (L.) W. Watson essential oil on the growth and morphogenesis of Aspergillus niger. Canadian Journal of Microbiology. 2001;47:9-17

45. Ezoua Pierre, Konan K. Constant, Amane Didier, Adama Coulibaly, Ysidor Konan, Daouda Sidibe, Olivier Kouame Chatigre and Godi Henri Marius Biego Efficacy of Lippia multiflora (Verbenaceae) and Hyptis suaveolens (Lamiaceae) Leaves on Sanitary Quality during the Storage of Maize Grain (Zea mays L.) from Cote D'ivoire. Asian Journal of Biotechnology and Bioresource Technology. 2017; 1(2): 1-15.