Changes in Aflatoxins Contents of the Maize (Zea Mays L.) Stored in Clay Granaries with use of Biopesticides from Rural Conditions and Estimation of their Intake

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Abstract— *Maize protection without any risks for human health and environment concerns might be valued on alternative uses of pest control methods that do not only rely on synthetic insecticides. A combination of leaves derived from Lippia multiflora Moldenke and Hyptis suaveolens Poit. Benth were tested for their protective effect on the aflatoxins levels of maize cobs and grains stored in traditional and improved granaries in Côte d'Ivoire. Thus, 4 aflatoxins (B_1, B_2, G_1 and G_2) were determined with high performance liquid chromatography according to the official method of AOAC. Results showed presence of afltatoxins in 58% of samples, and specifically aflatoxin B1 from half the samples, with rather higher levels than the reference values of the European Union. The levels of aflatoxins B_1, B_2, G_1 and G_2 resulted from both maize cobs and grains treated with biopesticides (from 0.06-0.53 µg/kg to 2.18-50.70 µg/kg) were significantly lower than those recorded with untreated maize of control granaries (ranging from 0.06-0.53 µg/kg to 12.48-346.15 µg/kg). In the treated maize, the aflatoxins levels increased slightly during 6 months of storage, while the untreated maize cobs were with significant increasing of the same toxins traits month after month. For each stage, aflatoxins levels of maize cobs and grains did not differ whether they are treated in traditional or improved granaries with both plant materials.*

The estimated risk of exposure in aflatoxins, specifically in total aflatoxins and AFB_1, deriving with intakes of maize stored for 6 months are respectively 114.37±2.2 ng/kg body weight/day and 36.21±0.11 ng/kg body weight/day for the untreated granaries and 7.15±0.04 ng/kg body weight/day and 2.12±0.17 ng/kg body weight/day for the treated granaries. These levels are strongly higher than the maximal Reference Value (0.15 pg/kg body weight/day) tolerated for Toxicity exposure. Therefore, it's necessary to sensitize, on a larger scale, actors of maize path, namely farmers, retailers, processers and consumers about such mycotoxins in maize products for providing health safety to Ivorian populations.

Keywords— stored maize, biopesticides, traditional and improved granaries, intake of aflatoxins, health risk.

I. INTRODUCTION

Maize (*Zea mays* L., Poaceae) has a substantial contribution in the diets of rural and urban populations (Baoua *et al.*, 2014). In Côte d'Ivoire, this crop is generally cultivated by small-scale farmers and widely grows across various ecological zones, from the northern savannah till the rain forest belt in the south (Kouakou *et al*., 2010), with a yield of 654,738 tons in 2012/2013 from 327,800 ha of total planted area (N'da *et al*., 2013). Maize allows diverse dishes such as porridge, couscous or dense paste (tô) eaten with sauce and is totally domestically consumed at the rate of 28.4 per capita (Beugre *et al*., 2014).

A recent USAID study highlighted the maize sector's concerns in Western Africa, one of which is the post-harvest storage (Boone *et al*., 2008). In fact, maize stored in warm and humid conditions and with pests pressure is most prone to infection by toxigenic agents, especially *Aspergillus*, *Fusarium* and *Penicillium*, and therefore to mycotoxins contamination (Sekiyama, 2005).

Aflatoxins are the most current mycotoxins involving with maize contamination (Hell *et al.*, 2002). Among all aflatoxins groups, the aflatoxin B_1 is the most widespread accounting both toxicology and occurrence traits. It is a human carcinogen and has been classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC, 2002). Apart from toxicity hazards to human and animals, aflatoxins cause reduction in nutritional properties, seed viability, grinding quality, sanitary quality and trading value of cereals (Liu *et al.*, 2006). The Food and Agriculture Organization of the United

Nations (FAO) stated that at least 25% of the world's cereal grains are contaminated by mycotoxins, including aflatoxins (FAO, 2004). Such a constraint leads unfortunately to disposal of large amounts of crops stored in inadequate conditions. Maize susceptibility to contamination by toxigenic molds and mycotoxins production has been documented in West African countries such as Côte d'Ivoire, Benin and Nigeria (Sangaré-Tigori *et al.,* 2005; Fandohan *et al.,* 2005; Adetunji *et al.,* 2014).

Thus, proper conditions of maize storage could allow significant improvement in the national farmer's economy by controlling the losses. In fact, the storage technologies have major roles upon the final quality of the resulted grains.

Ensuring optimal efficiency of the storage technologies is highly crucial for the safety of the stored grains and for the consumer's health. Common pests controlling system of stored products is with the application of synthetic contact insecticides (Nukenine *et al.,* 2013) despite many risks on the health of users and consumers and environmental pollution (Regnault-Roger, 2008). Nevertheless, other methods of storage and preservation could be improved for finding alternatives in uses of synthetic pesticides for the post-harvest losses reduction.

The current research deals with statement of maize storage structure that would rely on more efficiency, economical feasibility, environmental safety and could benefit to farmers. The study assesses effects of two local plants, namely *Lippia multiflora* and *Hyptis suaveolens*, deriving with aflatoxins levels of maize stored in traditional and improved clay granaries in rural conditions of Côte d'Ivoire.

II. MATERIAL AND METHOD

2.1 Experimental site

Experiments were carried out in the rural farming community of Djedou village in the department of Botro, Gbèkê region, in the center of Côte d'Ivoire. The village is located at 40 km from Bouaké, with reference points of 7°50' N and 5°18' W. This region has a humid tropical climate with annual rainfall ranging between 1,000 and 1,100 mm in the rainy seasons, temperatures of 21.4°C to 30.6°C and 75% to 80% of relative humidity (CNRA, 2014).

2.2 Collection of the maize used in the study

Maize grains and full maize cobs were bought in January 2014, approximately one month after harvest, from the young cooperative of the Djedou village. Prior to the storage, maize were sun-dried for 2 to 3 days before being used for the experiments.

2.3 Biopesticides collection and processing

Two plant species *Lippia multiflora* and *Hyptis suaveolens* have been selected for their biopesticides properties. Both plants are spontaneous perennial and fragrant shrubs growing from the central to the Northern parts of Cote d'Ivoire (Tia, 2012; Ekissi *et al*., 2014). Leaves of *L. multiflora* and *H. suaveolens* were collected around Djedou village. After harvest, the leaves have been dried out of direct sunlight for 6-7 days.

2.4 Experiments implementation

2.4.1 Granaries main parameters

A cylindrical clay granary covered with a straw roof side was chosen for the experiment. Such convenience is commonly used by farmers to keep their cereal crops (maize, rice, millet, sorghum). The granaries are built by a specialist farmer after the main fieldwork. Such operation runs from 1 to 12 months. To relieve the difficulties encounted, traditional granaries are modified by replacing their cylindrical roof with a simple device in similar design. The straw roof has been substituted with a plastic for hermetical recovering of granaries (Photography 1.b). Besides, granaries are raised from the ground to prevent moisture and rodent attack. Such systems reveal general storage capacity of 9 m^3 to 12 m^3 (Photography 1).

2.4.2 Experimental design

The experiment was carried out using a completely randomned 3x4 factorial design with two forms of maize: cobs and grains. Factors were three types of granaries (control, traditional and improved) and four observation periods (0, 2, 6 and 8 months). The investigation runned from January to September 2014 and the young cooperative of Djedou village was associated. The maize grains storage granaries were built in Djedou village; and the maize cobs storage granaries were located at N'godrjenou camp, 4 km far from Djedou, to facilitate the surveillance and monitoring. Excepted for the control, granaries contained mixtures of chopped dried leaves of *L. multiflora* and *H. suaveolens* at 2.5% w/w of each plant. The required quantities of each plant material were intermittently sandwiched manually in granaries, after 120 kg of maize cobs or grains.

2.5 Sampling

The sampling was performed at the beginning of the storage (0 month), then 2, 6 and 8 months later, in triplicate. Thus, 1 kg of maize samples from each granary was gathered through the top, the centre and the bottom opening sides. Maize samples were then conveyed to laboratory where aflatoxins (B_1, B_2, G_1 and G_2) and physicochemical properties measurements were achieved.

2.5.1 Determination of moisture content

The moisture content was valued according to the method described by AOAC (2000). A maize sample of 5 g was dried at 105°C into an oven till constant weight. The result was expressed from the equation 1 below:

$$\textbf{Moisture content (\%)} = 100-(W_l \times 100/W_s) \quad (1)$$

With W_l, weight lost from samples after drying; W_s, weight of raw samples.

2.5.2 Determination of water activity

The water activity was measured with a HygroLab Rotronic hygrometer according to indications of McCormick (1995). Prior to assays, the hygrometer was calibrated with specific water activity salts. Then, samples of 5 g of ground maize were put into standard dry empty containers for the Aw analysis. The water activity digital measures were directly displayed by the hygrometer.

2.5.3 Aflatoxins analysis

2.5.3.1 Extraction and purification of aflatoxins

Chemical reagents (acetonitrile, methanol and chloroform) and standard aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) were used for the study. Reagents were purchased from Carlo Erba (Spain) with analytical grade, while standard aflatoxins were provided from Sigma (Sigma, St Louis, MO, USA).

Biological aflatoxins (B_1, B_2, G_1 and G_2) were extracted and purified from maize using the official guidelines of AOAC (AOAC, 2005). 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 upon 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 rotative evaporator (Buchi Rotavapor R-215) at 40 °C for evaporation of 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).

2.5.3.2 Quantification of Aflatoxins

Determination of aflatoxins contents was achieved with high performance liquid chromatography column, using a Shimadzu liquid chromatograph (Kyoto, Japan) fitted with fluorescence detector (λexc 365 nm; λem 435 nm) and Shim-pack column and pre-column (Shim-pack GVP-ODS: 250 mm x 4,6 mm, 10 x 4,6 mm, respectively). Twenty (20) µL of the filtrate were injected on the column. Components were eluted with a mobile phase prepared with methanol/water/acetonitrile (60:20:20, v/v/v) and using a gradient programme of 1 mL/min. Assays were performed in triplicate.

Validation parameters of the aflatoxins contents analysis, especially Limits of Detection (LOD), Limits of Quantification (LOQ), repeatability and reproducibility traits and percentage of extractions, were valued. Thereafter, the contents of aflatoxins B_1, B_2, G_1 and G_2 were estimated, and then the total aflatoxins content was calculated from the sum of the overall aflatoxins.

2.6 Assessement of total aflatoxins and aflatoxin B1 exposures from maize daily intake for the adult Ivorian

The mean aflatoxins level in maize grains stored for 6 months, and the mean maize consumption and body weight from Ivorian adult, allowed estimation of the daily exposures to total aflatoxins and aflatoxin B_1 (Kroes *et al.*, 2002; WHO, 2003). According to the National agricultural statistics of Côte d'Ivoire, the daily consumption of maize is 28.4 g per capita/day (Beugre *et al.*, 2014). The aflatoxin exposure or intake was calculated using the formula of the following equation 2:

$$\textbf{EAI} = (T \times Q)/Bw \quad (2)$$

With EAI, the Estimated Aflatoxins exposure from maize daily Intake (ng/kg of Bw/day); T, the aflatoxins contents in maize stored (ng/kg); Q, the daily Intake of maize grains (g/day); Bw, the Body weight of an adult person (70 kg).

The estimated aflatoxins exposures were also expressed in relation with the maximal mean levels of total aflatoxins and AFB_1 reported by the European Union (EU Regulation N° 420/2011) in maize subjected to physical treatment before human

consumption, which are respectively stated to 10µg/kg and 5µg/kg. Moreover, the estimated exposures were compared to the Toxicological Reference Value (TRV) of 0.15 ng/kg bw/day mentioned by the European and International Scientific Committees (SCF, 1994; CSHPF, 1999, JECFA, 1999; 2001). Results were expressed in percentage of total aflatoxins and aflatoxin B_1 related to the TRV.

2.7 Statistical analysis

All analyses were performed in triplicate and the full data were statistically treated using SPSS software (version 20.0). It consisted in Analysis of Variance according to two factors: duration and method of storage. Means derived from parameters were compared with the Tukey High Significant Difference test at 5% significance level. Correlations between parameters were also assessed according to the Pearson index. Then, Multivariate Analyses through Principal Components Analysis (PCA) and Ascending Hierarchical Clusters analysis (AHC) were performed using STATISTICA software (version 7.1).

III. RESULTS

3.1 Evolution of the aerothermal parameters

Figure 2 shows the evolution of the temperature and relative humidity from the experimental site. The mean air temperature during the studies implementation (January to September 2014) was 30.58±1.97 °C. But, a higher temperature of 33.81±3.00 °C was noticed in March, while August provided the lowest temperature (27.50±1.10 °C). With the relative humidity, general average of 80.38±4.08% was recorded during the study period. The months of January, February and March 2013 (68.71±3.52%, 56.21±5.52% and 70.95±6.00%, respectively) were less humid than the other months among with August recorded the top value of 91.12±5.00%.

3.2 Evolution of the Moisture, water activity and aflatoxins parameters

The statistical traits reveal significant changes ($P<0.05$) in the contents of all compounds assessed resulting with both duration and technology of the storage whether the maize was untreated or treated with biopesticides, excepting for moisture content and water activity which haven't accounted any obvious variation from the types of storage (Tables I and II).

3.2.1 Moisture content

The evolution of the moisture content of the 3 types of granaries during the maize storage is referred in Figures 2 and 3. With respective means of 9.23% and 9.05% at the beginning (0 month), the moisture contents increase significantly ($P<0.001$) during the storage period. The highest moisture values are recorded after 8 months of storage in the control granaries with means of 13.82% and 13.52% from maize cobs and grains. These values are superior compared to the moisture deriving with traditional and improved granaries from both maize cobs (12.85% and 12.74%, respectively) and grains (11.85% and 11.87%, respectively). Besides, the interaction between type and time of storage does not involve any significant effect upon this parameter as shown in previous tables I and II.

3.2.2 Water activity

Figures 2 and 3 also show the evolution of the water activity of maize cobs and grains stored in the three types of granaries. The water activity of maize either untreated or treated with biopesticides displays the same trend, and a gradual increase is involved from the storage duration. Indeed, the water activity of 0.83±0.04 at the earlier storage rises up to 0.94±0.03, 0.92±0.08 and 0.90±0.04 eight months later from the maize grains and cobs in respective control, traditional and improved granaries. Overall, there isn't any significant difference between the storage technologies for both maize cobs and grains.

3.2.3 Validation parameters for Aflatoxins assessment using HPLC

Using HPLC device, Limits Of Detection (LOD) of respective aflatoxins B_1, B_2, G_1 and G_2 are 6.18 ng/kg, 0.058 ng/kg, 114.5 ng/kg, 2.64 ng/kg, while their Limits Of Quantification (LOQ) are 6.50 ng/kg, 0.108 ng/kg, 124.9 ng/kg, 2.94 ng/kg. The mean recoveries fluctuate between 0.50% and 3.75% for the repeatability assays and between 0.89% and 4.93% for reproducibility assays. However, for aflatoxins B_1, B_2, G_1 and G_2, respective rates of extraction of 98.92±2.49%, 97.53±1.93% 95.31±0.33% and 97.63±2.10% are recorded.

3.2.4 Aflatoxins Concentrations

During storage, the contents of probed aflatoxins in maize are depicted in Figures 2 and 3. All maize samples studied are aflatoxins-positive. For the overall samples, the aflatoxins contents, ranging between 0.04 and 0.53 µg/kg at the earlier storage, increase all along the storage till 2.18-378.26 µg/kg at the 8^{th} month of storage. Nevertheless, the post harvest treatments of maize cobs and grains with biopesticides highlight significant reduction of the aflatoxins contents ($P<0.05$) compared to the control granaries untreated samples.

The contents of aflatoxin B_1 record a slight growth from 0.28 µg/kg to 5.22 µg/kg during 6 months of storage, before rising suddenly up to 23.25 µg/kg at the 8th month from the biopesticides-treated maize. On the other hand, control granaries involve with rapid increasing of aflatoxin B_1 level during the 8 months of storage (Figures 2 and 3).

Regarding with aflatoxin B_2, means collected from biopesticides samples stay below 1 µg/kg for 6 months of storage, and then reached 0.48 µg/kg to 6.10 µg/kg at the end of the investigation. But the untreated maize allow rapid growth of aflatoxin B_2 once the earlier storage till the 8th month where values of 12.25 µg/kg and 18.92 µg/kg are found for maize grains and cobs, respectively (Figures 2 and 3).

For the aflatoxins G_1, the stages of 2, 6 or 8 months of storage provide higher contents from the untreated maize (10.96 µg/kg to 20.16 µg/kg, 174.26 µg/kg to 183.60 µg/kg and 200.16 µg/kg to 399.25 µg/kg, respectively) than the samples resulting with the biological treatment, stated from 2.02 µg/kg to 3.03 µg/kg, 8.75 µg/kg to 11.26 µg/kg and 39.87 µg/kg to 110.26 µg/kg, respectively (Figures 2 and 3).

The levels of aflatoxins G_2 also differed significantly ($P<0.05$) the control granaries maize (0.05 µg/kg to 19.82 µg/kg) from the treated granaries (0.05 µg/kg to 6.33 µg/kg) as shown in figures 2 and 3.

Accordingly, the 8 months of storage state on significant increasing of the total aflatoxins levels gathering the whole aflatoxins involving with the three technologies investigated. The contents of total aflatoxins of the maize are below 1 µg/kg before storage. But, the levels rise significantly ($P<0.05$) up to 44.93 µg/kg or 76.63 µg/kg with the traditional granaries, to 58.70 µg/kg or 89.47 µg/kg for the improved granaries and reach more considerable values of 346.15 µg/kg or 378.26 µg/kg from the control granaries considering the maize grains or cobs, respectively (Figures 2 and 3).

However, aflatoxins levels do not reveal any significant difference between maize cobs and grains treated with both plant materials in traditional and improved granaries.

In addition, total aflatoxins (in 58% of the maize samples) and aflatoxins B1 (in 50% of the maize samples) are with contents above their maximal level acted by the European Union regulations (EU Regulation N^o 420/2011) and stated at 10 µg/kg and 5 µg/kg, respectively.

3.3 Correlations between moisture content, water activity and aflatoxins levels

Tables V and VI depict the correlations between moisture contents, water activities and aflatoxins levels in the various technologies of maize storage. The Pearson indexes (r) indicate positive and significant correlations between the 7 parameters assessed for both maize forms (cobs and grains). Thus, water activity, moisture, aflatoxins B_1, B_2, G_1, G_2, and total are closely correlated during the storage of the post harvest maize, r varying from 0.66 to 0.99 for maize cobs and from 0.59 to 0.99 for maize grains. So, the water activity and the moisture contents change tightly (r=0.90 and 0.80 for maize cobs and grains, respectively). The aflatoxins B_1 levels are directly correlated with the aflatoxins G_1 levels (r=0.99 for both maize cobs and grains). Positive significant correlations are observed between aflatoxins B_2 and aflatoxins G_2 (r=0.95 and 0.99 for maize cobs and grains respectively) and between total aflatoxins levels and water activity (r=0.68 and 0.61 for maize cobs and grains respectively).

3.4 Variability between storage technologies, moisture content, water activity and aflatoxins levels

Principal Component Analysis (PCA) is performed with the component F1 which record an eigenvalue superior to 1, according to statistical standard of Kaïser (table VII). The overall parameters display negative significant correlations with F1. Nevertheless, the component F2 (eigenvalue of 0.36) is associated to F1 for fulfillment of the PCA. Figure 3.a shows the correlation circle between the F1-F2 factorial drawing, with 99.85% of the total variance, and the chemicals parameters of the maize stored. The projection of the investigated samples highlights 3 groups of individuals (Figure 3.b). The Group 1 consists mainly in samples from control granaries at 6 and 8 months of storage which are close to the negative correlated traits of F1. Individuals from this group exhibit highest levels of aflatoxins, water activity and moisture content. The second group contains maize samples from the treated granaries (traditional and improved) at the 8th month of storage. They are distinguished by higher levels in aflatoxins, water activity and moisture content than individuals of the third group which is drawn by the samples from treated granaries (traditional and improved) at 2 and 6 months and the control granaries at 2 months of storage, providing slight levels of the parameters mentioned above.

The Ascending hierarchical classification (AHC) strengthens the variability resulting from the PCA (Figure 4). At aggregation distance of 18, the dendrogram shows four clusters of the maize samples. The first cluster is the control granaries at 8 months, while the untreated granaries at 6 months of maize storage consist in the cluster 2: both maize samples are provided in highest values of the parameters assessed. The maize samples deriving from traditional and improved treated granaries at the 8th month of storage inner the third cluster. Those samples also show high levels of aflatoxins B_1, B_2, G_1, G_2, water activity and moisture contents, but remain lower than samples of the clusters 1 and 2.

The fourth cluster includes maize samples from the treated granaries at 2 and 6 months and the control at 2 months of storage, which are lower contents in aflatoxins B_1, B_2, G_1 and G_2, water activity and moisture content.

3.5 Assessment of aflatoxins intake from maize grains after storage

Table VIII shows the aflatoxin B_1 and total aflatoxins intakes estimated from the consumption of maize grains stored for 6 months. Accounting a daily consumption of maize of 28.4 g per capita/day in Côte d'Ivoire and a mean weight of 70 kg from the adult population, the estimated aflatoxins intakes are 114.37±2.2 ng/kg body weight/day and 36.21±0.11 ng/kg body weight/day for the untreated granaries and 7.15±0.04 ng/kg body weight/day and 2.12±0.17 ng/kg body weight/day for the treated granaries for the total aflatoxins and aflatoxin B_1, respectively.

Compared with the Toxicity Reference Value (0.15 ng/kg body weight/day), the exposures are at least for the untreated granaries 241 and 762 times higher for aflatoxin B1 and total aflatoxins, respectively. For the treated granaries, the exposures are at least 14 and 48 times higher for aflatoxin B1 and total aflatoxins, respectively.

Aflatoxin B_1 and total aflatoxins exposures are also higher than their maximal concentration acted by European Union. Recorded values represent 104.36% and 176.17% of the respective maximum quantities of 5 µg/kg for Aflatoxin B_1 and 10 µg/kg for total aflatoxins permitted.

PHOTOGRAPHY 1: DIFFERENT TYPES OF MAIZE STORAGE GRANARIES USED FOR EXPERIMENTS IMPLEMENTATION.

(A) CONTROL GRANARY; (B) TRADITIONAL GRANARY; (C) IMPROVED GRANARY.

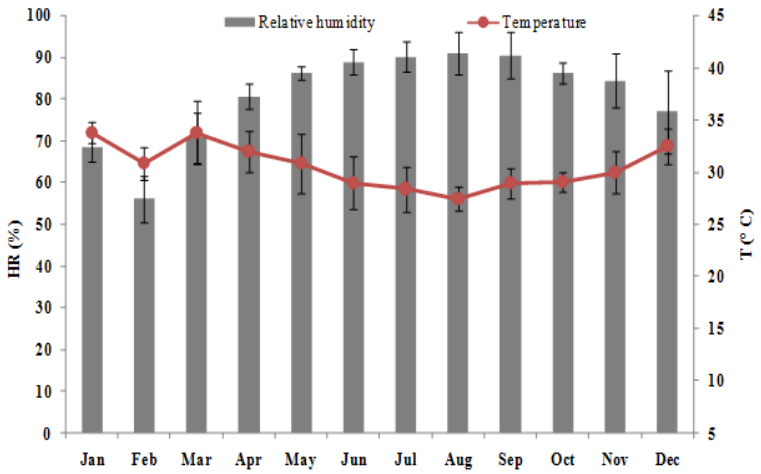

FIGURE 1: MAIN CHANGES IN AMBIENT TEMPERATURE AND RELATIVE HUMIDITY OF STUDY SITE

Table I: Statistical data for water activity and contents of moisture and aflatoxins in maize cobs under different storage conditions

Source of Variation	Df	Statistical trait	Parameters						
			MC	AW	AFB_1	AFB_2	AFG_1	AFG_2	Total Aflatoxins
		SS	4.23	0.01	18245.88	161.40	66300.05	340.69	349707.97
Types	2	F-value	6.90	3.84	1293.80	413.60	5435.75	1206.31	4119.30
		P-value	0.004	0.36	<.001	<.001	<.001	<.001	<.001
		SS	83.52	0.13	20936.91	191.70	79424.62	647.92	620407.73
Durations	3	F-value	90.70	48.41	1484.62	491.24	6511.80	1529.42	4871.97
		P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001
		SS	1.54	0.02	5537.70	48.22	20820.10	304.40	323957.01
Types x Durations	6	F-value	0.84	1.17	392.66	123.55	1707	359.22	1272
		P-value	0.56	0.35	<.001	<.001	<.001	<.001	<.001
Error	24	SS	7.37	0.006	338.46	9.37	292.73	3.39	1018.74
Total	36	SS	4876.10	27.52	1875.37	1717.10	69474.48	1857.15	1822284.77

SS, sum of squares; F-value, value of the statistical test; P-value, probability value of the statistical test; df, degree of freedom. MC, moisture content AW, water activity content ; AFB_1, aflatoxin B_1 contents; AFB_2, aflatoxin B_2 contents; AFG_1, aflatoxin G_1 contents; AFG_2, aflatoxin G_2 contents; total aflatoxins,.aflatoxins $B_1+B_2+G_1+G_2$.

Table II: Statistical data for water activity and contents of moisture and aflatoxins in maize grains under different storage conditions

Source of Variation	Df	Statistical trait	Parameters						
			MC	AW	AFB_1	AFB_2	AFG_1	AFG_2	Total Aflatoxins
		SS	5.86	0.002	10276.04	105.94	31745.47	103.49	90051
Types	2	F-value	11.72	2.58	3638.96	685.02	1650.77	1353.73	13958.47
		P-value	<.001	0.096	<.001	<.001	<.001	<.001	<.001
		SS	58.57	0.01	5606.94	57.98	17897.74	63.68	50244.54
Durations	3	F-value	78.13	17.27	1985.54	374.91	933.13	832.91	7788.47
		P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001
		SS	6.00	0.00	2364.27	24.23	6906.93	23.28	19863.86
Types x Durations	6	F-value	1098	0.48	837.24	156.70	3602.14	304.47	3079.02
		P-value	0.11	0.82	<.001	<.001	<.001	<.001	<.001
Error	24	SS	6.00	0.02	67.77	3.71	46.10	1.84	154.83
Total	36	SS	4527.41	28.20	7232.67	759.47	228956	777.51	643155.32

SS, sum of squares; F-value, value of the statistical test; P-value, probability value of the statistical test; df, degree of freedom. MC, moisture content AW, water activity content ; AFB_1, aflatoxin B_1 contents; AFB_2, aflatoxin B_2 contents; AFG_1, aflatoxin G_1 contents; AFG_2, aflatoxin G_2 contents; total aflatoxins,.aflatoxins $B_1+B_2+G_1+G_2$.

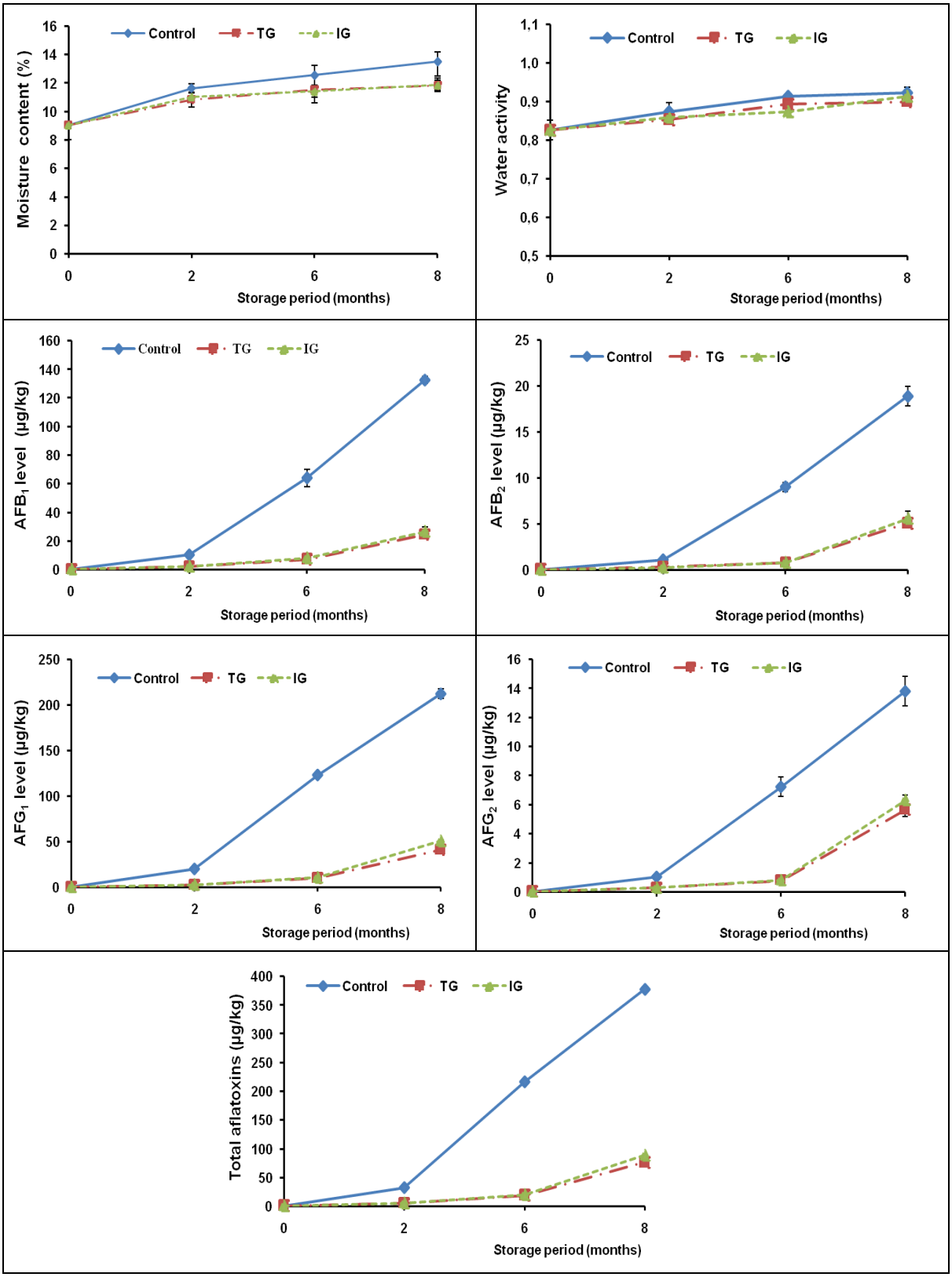

FIGURE 2: EVOLUTION OF MOISTURE CONTENT, WATER ACTIVITY AND AFLATOXINS LEVELS OF MAIZE COBS ACCORDING TO THE STORAGE CONDITIONS (ON DRY WEIGHT BASIS)

TG, traditional granary; IG, improved granary

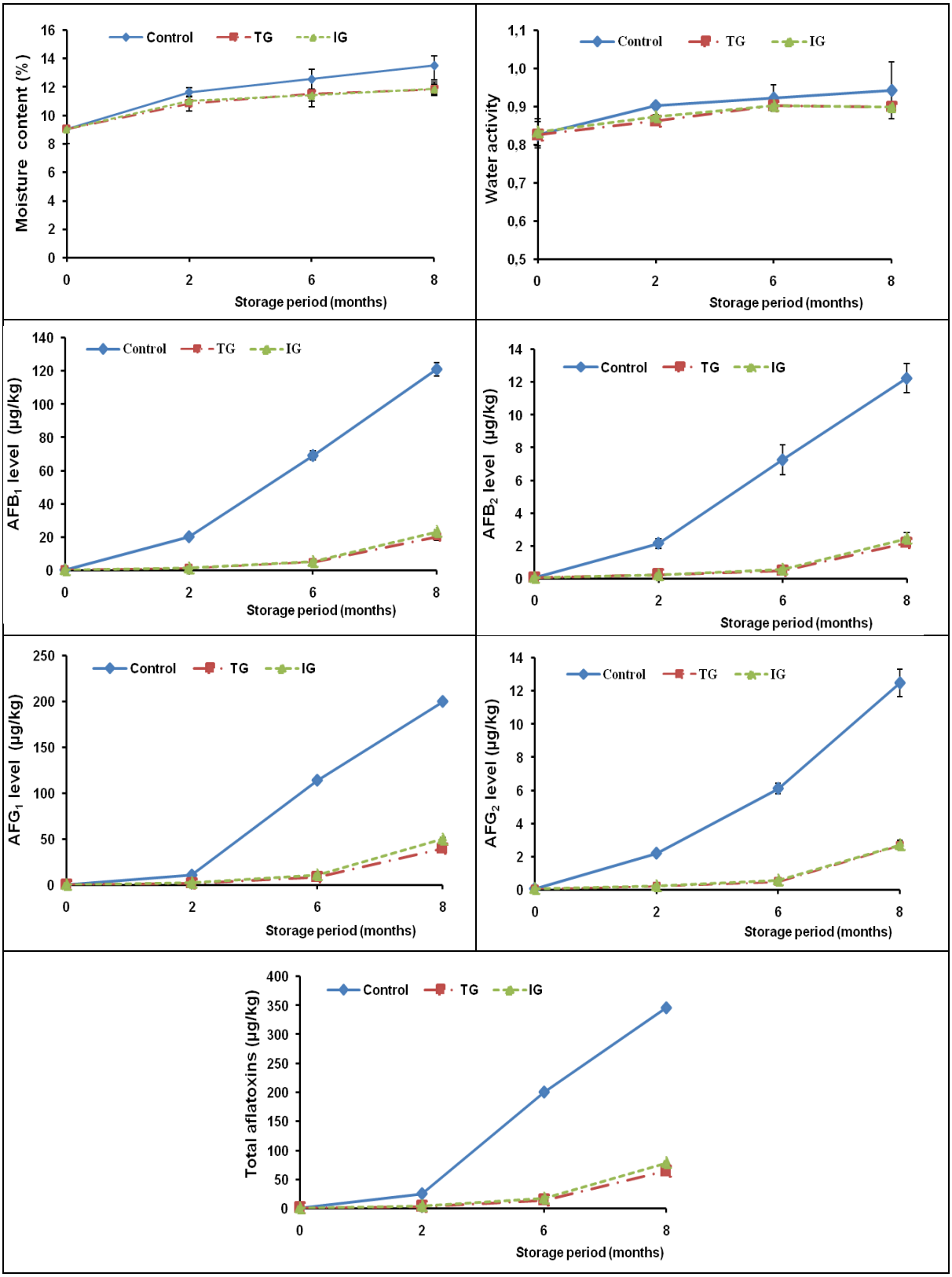

FIGURE 3: EVOLUTION OF MOISTURE CONTENT, WATER ACTIVITY AND AFLATOXINS LEVELS OF MAIZE GRAINS ACCORDING TO THE STORAGE CONDITIONS (ON DRY WEIGHT BASIS)

TG, traditional granary; IG, improved granary

TABLE III

MATRIX OF PEARSON CORRELATION INDEXES BETWEEN MOISTURE, WATER ACTIVITY AND AFLATOXINS LEVELS OF MAIZE COBS;

	AW	MC	AFB_1	AFB_2	AFG_1	AFG_2	TAF
AW	1						
MC	0.90	1					
AFB_1	0.66	0.65	1				
AFB_2	0.70	0.68	0.96	1			
AFG_1	0.68	0.66	0.99	0.98	1		
AFG_2	0.75	0.72	0.95	0.95	0.96	1	
TAC	0.68	0.66	0.99	0.97	0.99	0.96	1

The values are significant at P=0.05; AW, water activity content; MC, moisture content ; AFB_1, AFB_2, AFG_1, AFG_2, TAF: respective contents of aflatoxins B_1, B_2, G_1, G_2 and total aflatoxins.

TABLE IV

MATRIX OF PEARSON CORRELATION INDEXES BETWEEN MOISTURE, WATER ACTIVITY AND AFLATOXINS LEVELS OF MAIZE GRAINS.

	AW	MC	AFB_1	AFB_2	AFG_1	AFG_2	TAF
AW	1						
MC	0.80	1					
AFB_1	0.62	0.71	1				
AFB_2	0.64	0.72	0.99	1			
AFG_1	0.59	0.70	0.99	0.99	1		
AFG_2	0.59	0.72	0.99	0.99	0.99	1	
TAC	0.61	0.71	0.99	0.99	0.99	0.99	1

The values are significant at P=0.05; AW, water activity content; MC, moisture content ; AFB_1, AFB_2, AFG_1, AFG_2, TAF: respective contents of aflatoxins B_1, B_2, G_1, G_2 and total aflatoxins.

TABLE VII

MATRIX OF EIGENVALUES OF FACTORS RESULTING FROM THE PRINCIPAL COMPONENTS ANALYSIS, AND CORRELATION WITH THE MOISTURE CONTENT, THE WATER ACTIVITY AND THE AFLATOXINS LEVELS OF THE MAIZE STORED FOR 8 MONTHS.

Factors	F1	F2	F3	F4	F5	F6	F7
Eigenvalues	6.63	0.36	0.009	0.0006	0.0002	0.00009	0.0000
Variances (%)	94.66	5.17	0.13	0.0089	0.0028	0.00012	0.0000
Cumulative variance (%)	94.66	99.85	99.98	99.99	100	100	100
AW	-0,91	0,40	-0,06	0,00	0,00	0,00	0,00
MC	-0,94	0,33	0,07	0,00	0,00	0,00	0,00
AFB_1	-0,99	-0,14	0,00	-0,01	0,01	0,00	0,00
AFB_2	-0,99	-0,14	-0,01	-0,01	-0,01	0,00	0,00
AFG_1	-0,99	-0,13	0,00	0,00	0,00	0,00	0,00
AFG_2	-0,99	-0,13	0,00	0,02	0,00	0,00	0,00
TAF	-0,99	-0,14	0,00	0,00	0,00	0,00	0,00

Values of significant correlations in bold at P = 0.05; AW, water activity content; MC, moisture content ; AFB_1, AFB_2, AFG_1, AFG_2, TAF: respective contents of aflatoxins B_1, B_2, G_1, G_2 and total aflatoxins.

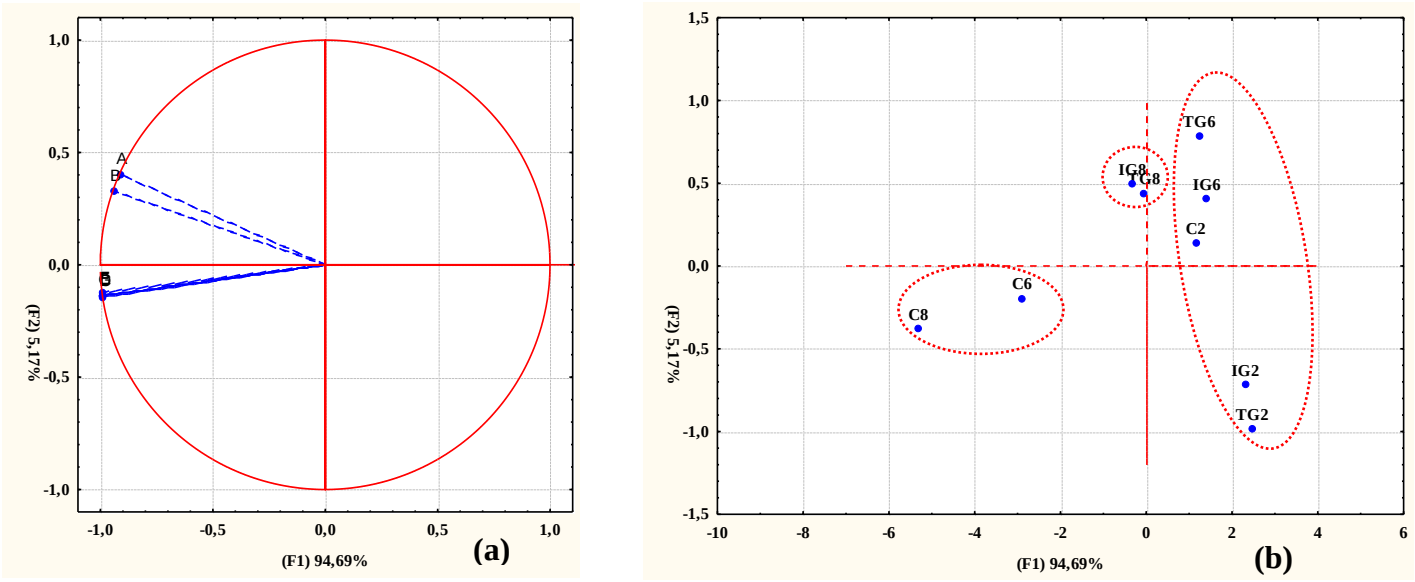

FIGURE 4: CORRELATION DRAWN BETWEEN THE F1-F2 FACTORIAL OF THE PRINCIPAL COMPONENTS ANALYSIS AND THE CHEMICAL PARAMETERS (A) AND THE INDIVIDUALS (B) DERIVING FROM THE MAIZE SAMPLES STUDIED

A, water activity content; B, moisture content; C, D, E, F, G: respective contents of aflatoxins B_1, B_2, G_1, G_2 and total aflatoxins. C_2, TG_2, IG_2: control, traditional and improved granaries at 2 months of storage; C_6, TG_6, IG_6: control, traditional and improved granaries at 6 months of storage; C_8, TG_8, IG_8: control, traditional and improved granaries at 8 months of storage.

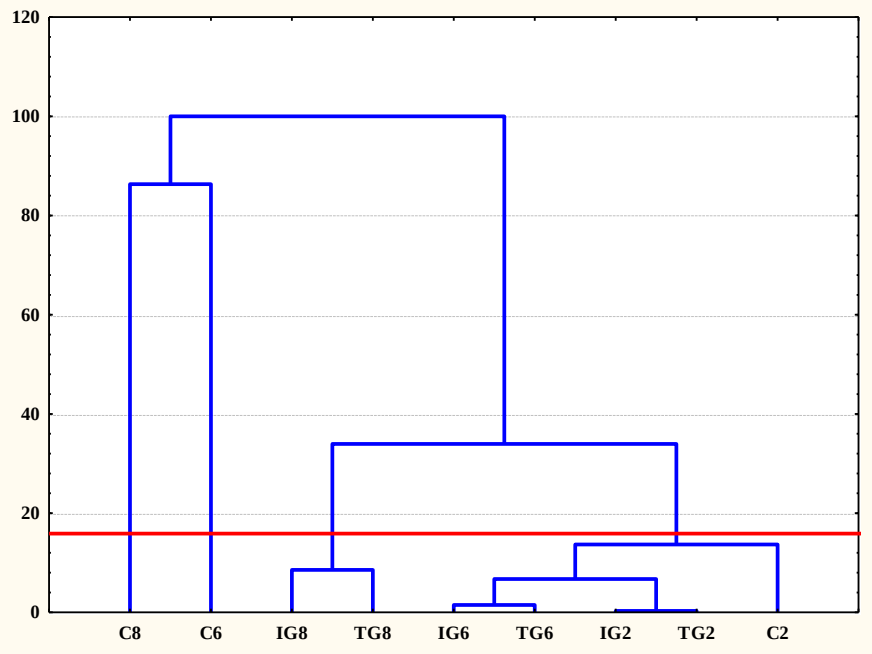

FIGURE 5 : DENDROGRAM DERIVING WITH THE ASCENDING HIERARCHICAL CLASSIFICATION OF MAIZE SAMPLES STORED FOR 8 MONTHS ACCORDING TO THE PARAMETERS ASSESSED.

C_2, TG_2, IG_2: control, traditional and improved granaries at 2 months of storage; C_6, TG_6, IG_6: control, traditional and improved granaries at 6 months of storage; C_8, TG_8, IG_8: control, traditional and improved granaries at 8 months of storage.

TABLE VIII
AFLATOXINS INTAKE ESTIMATED FROM THE CONSUMPTION OF MAIZE GRAINS FROM IVORIAN ADULT (INTAKE NG/KG BODY WEIGHT/DAY)

	AFB_1		Total aflatoxins	
	C	IG	C	IG
Estimated intake (EI)	36.21±0.11	2.18±0.17	114.37±2.21	7.15±0.04
Toxicity Reference Value (TRV)	0.15			
EI/TRV	241	14	762	48
Estimated intake to MRL ($AELMR_1$)	2		4.1	

$AELMR_1$: estimated intake for a maximum residue level of aflatoxin B1 and total aflatoxin in maize.
C, control granary; IG, improved granary

IV. DISCUSSION

The resumption and increase of water activities and moisture contents in granaries storage systems for both maize cobs and grains could be related to the air relative humidity, mean of which is around 70%-80% (CNRA, 2014). In fact, few increasing in the relative air humidity above 70% involves with great rising of the moisture content of the stored grains (Di Domenico *et al*., 2015). At the end of storage, both maize cobs and grains presented moisture contents above the limit of 13% recommended for maize safe storage (Mohale *et al.*, 2013). But high water activities recorded in granaries storage systems are more susceptible to spoilage, fungal contamination and rapid aflatoxin production (Schwartzbord *et al.*, 2015).

Generally, since plant products are bioactive against specific pest species, environmentally biodegradable, non-toxic to natural enemies and potentially suitable for use in integrated pest management programs (Isman, 2006), they could be exploited as safer stored-product pest control agents. The study highlighted the effective action of two local plant species, *Lippia multiflora* and *Hyptis suaveolens* against pest alteration. Combinations of 2.5% (w/w) of each plant material enhance reduction of pest in stored maize comparing with the control untreated maize. Indeed, the aflatoxins levels of the treated maize cobs and grains recorded slight increasing during 6 months of storage, when the untreated maize already allowed great pest production. Thus, maize cobs and grains have been significantly protected by such treatments from pest infestation up to 6 months in traditional and improved storage granaries. The biological effect of both plants could result from the release of bioactive molecules involved with the plants leaves oils (N'gamo *et al*., 2007). The combination of plant materials did also produce significant synergistic or additive effect on inhibition activity against pest growth. Our results corroborate the works of Shukla *et al*. (2009). These authors mentioned the antifungal and aflatoxin B_1 inhibition activities of oil from *Lippia alba* and two of its monoterpene aldehyde components against seventeen fungi isolated from eleven edible legume seeds. With respective concentrations of 0.25–1 µL/mL and 1 µL/mL this plant oil and components showed remarkable antifungal effects ranging between 32.1% and 100% of growth inhibition. This study also agree with Sharma *et al.* (2004) who showed the inhibition action of 50 mg/kg of the oil deriving with *H. suaveolens* on aflatoxin B_1 and ochratoxin producing fungi, specifically *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus ochraceous*. In addition, study of Tatsadjieu *et al*. (2009) revealed the positive effect of 1,000 mg/L of the plant oil from *Lippia rugosa* for the growth inhibition of *Aspergillus flavus* and the limitation of aflatoxin B_1 production. Moreover, the current experiments are consistent with Tia (2012) who reported the insecticidal effects of plants oils of *L. multiflora* and *H. suaveolens* against larval development of herbivorous insects *Plutella. xylostella* and *Bemissia tabaci.* For 50% mortality, the respective lethal dose (LD_{50}) and time (LT_{50}) are 4.22 µg/L and 7.53 µL/L and 0.22 h and 4.35 h against both enemies. According to this author, the main bioactive molecules of *L. multiflora* are oxygenated monoterpens such as linalol and 1,8-cineole; whereas monoterpen hydrocarbons particularly sabinene, β-pinene and limonene predominate from the *H. suaveolens* (Tia, 2012). However, the great rising in levels of aflatoxins beyond 6 months of the treated storage could be due to a decrease repellent activity of the plants materials. Similar observations were made by Liu *et al.* (1999) who explained the rapid drooping in the effectiveness of plants oil-basis biopesticides by massive releases of the volatile bioactive molecules in the first days after application.

The data from various maize parameters state on a better aflatoxins levels of the maize stored after adding combination of *L. multiflora* and *H. suaveolens* than the storage without any treatment. Granaries treated with biopesticides at 2 and 6 months are similar to those obtained at 2 months in the untreated granaries. In addition, this attempt shows that the protective

property of the combination of both local plants used is more effective at 6 months of maize storage than at 8 months of storage; agreeing with previous report accounting the changes in the nutritive compounds of maize stored in granaries with biopesticides (Niamketchi *et al.*, 2016)

Compared to the Toxicological Reference Value of 0.15 ng/kg body weight/day (SCF, 1994; CSHPF, 1999, JECFA, 1999; 2001), the intakes values estimated from total aflatoxins and aflatoxins B_1 are higher than the reference value. Such a concern may involve in significant risk to the health of Ivorian populations caused by the chronic exposure to aflatoxins in maize-basis food diets. This result was similar to the studies carried out by Sangaré-Tigori *et al.*, 2006 who reported 100% of contaminated maize samples from Côte d'Ivoire with average levels of 41.5 µg/kg and determinate a daily intake of 99 ng/kg bw/day.

V. CONCLUSION

This attempt suggests a better storage of maize cobs and grains treated with biopesticides over duration of six months. Beyond that period the sanitary quality of the maize is not acceptable because of a high risk of aflatoxins exposure. Hence, leaves of *Lippia multiflora* and *Hyptis suaveolens* could be potentially applied in food preservation, as alternatives to chemical pesticides in order to improve the self-life of staple foods, especially cereals.

The technology is inexpensive, easily carried and fits with the millennium guidelines of environment suitability. However, the study needs further investigation to preserve the quality, and ensure healthy and nutritional value of the maize after storage. Therefore, in view of the toxicity of aflatoxins, it is imperative to foster best practices of harvesting, drying and storage of the maize grains in order to provide health safety to populations.

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