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RESEARCH ARTICLE

EVOLUTION OF OCHRATOXIN A, FUMINOSIN B1 AND ZEARALENONE CONTENTS DURING MAIZE (*Zea mays* L.) STORED IN CLAY GRANARIES WITH USE OF BIOPESTICIDES FROM RURAL CONDITIONS AND ESTIMATING OF THE DAILY INTAKE IN THE IVORIAN ADULT

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ABSTRACT

The protective effect of three types of maize stored on the mycotoxins production during storage has been tested. These are the traditional granaries (control) and on the other hand traditional and improved granaries treated with leaves of *Lippia multiflora* and *Hyptis suaveolens* and then cover with polyethylene plastic. Maize stored as cobs and grains are preserved in natural atmosphere in these granaries during 8 months and the contents of moisture, water activity, ochratoxin A (OTA), fumunosin B1 (FB1) and zearalenone (ZEA) were studied and monitoring. Results showed presence of OTA, FB1 and ZEA respectively in 50%, 16.7% and 25% of samples, with rather higher levels than the reference values of the European Union. The levels of OTA, FB1 and ZEA resulted from both maize cobs and grains treated with biopesticides were significantly lower than those recorded with untreated maize of control granaries. For each stage, OTA, FB1 and ZEA levels of maize cobs and grains did not differ whether they are treated in traditional or improved granaries with both plant materials. The estimated daily intakes in OTA, FB1 and ZEA, deriving with consumption of maize stored for 6 months are respectively 13.10±0.40 ng/kg bw/day, 1167±15.05 ng/kg bw/day and 143.73±1.26 ng/kg bw/day from the untreated granaries and 2.00±0.35 ng/kg bw/day, 141±4.10 ng/kg bw/day, 20.0±0.43 ng/kg bw/day from the treated granaries. These levels are lower than the maximal Reference Value tolerated for Toxicity exposure set by the WHO except for OTA daily intake from the untreated granaries. 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.

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INTRODUCTION

Maize (*Zea mays* L.) is the second cereal most cultivated in Côte d'Ivoire after rice (Oryza spp.). Its production increases up to 531,940 tons in 2007 to 661,000 tons in 2013 for a total planted area of 330,000 ha (FAOSTAT 2016). The importance of maize is due to its availability throughout the year (IITA, 2012). Its nutritional advantages (rich in starch, protein, minerals) and economic (crop simple to produce, harvest and store) make it a competitive product that helps to lower the price of basic foodstuffs such as milk and meat in rural farming (Nuss and Tanumihardjo, 2011). Cropping problems and post-harvests treatments of maize constitute the main part of the problems encountered by the farmers in rural environment (Ratnadass 1987; Boone *et al.,* 2008). Several authors estimated that post-harvest losses are relatively high, in range of 20% to 30% because methods used are often inadequate and rudimentary (Noudjou-Wandji, 2007; Gueye *et al.,* 2012). The activity of pests such as lepidopterous and coleopterous creates a favorable conditions for fungi

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development such as *Aspergillus sp., Penicillium sp., and Fusarium sp.* responsible for the deterioration of marketed quality (alteration of appearance, odor and taste of grain) and nutritive values of maize (Fandohan *et al.,* 2005; Kankologo *et al.,* 2009). In general, infestations start at fields and continue throughout the storage duration (Johnson *et al.,* 2012). The contamination of maize by these fungi and their toxic metabolites has been associated with several human and animal diseases including liver and esophageal cancer, particularly in Africa (Rheeder *et al*., 2001). It is not unusual that maize and other commodities are contaminated with two or more molds and mycotoxins at the same time (Domijan *et al.,* 2005; Adetunji *et al.,* 2014). Surveys conducted in Côte d'Ivoire by Sangaré-Tigori *et al.,* 2005 and Kouadio *et al.,* 2014 revealed the co-occurrence of mycotoxins and also high levels of aflatoxins and ochratoxin A in maize and maize flour. 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 ochratoxin A, fuminosin B_1 and zearalenone levels of maize stored in traditional and improved clay granaries in rural conditions of Cote d'Ivoire.

MATERIELS AND METHODS

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).

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.

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.

Experiments implementation

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. Besides, granaries are raised from the ground to prevent moisture and rodent attack. Such systems reveal general storage capacity of 9 m3 to 12 m3 (Photography 1).

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.

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 maize samples from each granary were gathered through the top, the centre and the bottom opening side. Maize samples were then conveyed to laboratory where ochratoxin A, fuminosin B1, zearalenone, moisture and water activity were achieved.

Analytical methods

Determination of moisture

The evolution of the water content of the maize during the conservation was determined by oven method according to the

method described by AOAC (2000). To determine this, 5 g of maize powder were dried at 105 ± 2 °C till a constant weight reached. The water content is expressed according to the percentage of the dry mass. Each test was performed in triplicate.

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.

Ochratoxin A, fuminosin B1 and zearalenone analysis

Chemical reagents (acetonitrile and methanol) and standards (OTA, FB_1 and ZEA) were used for the study. Reagents were purchased from Carlo Erba (Spain) with analytical grade, while standards OTA, FB_1 and ZEA were provided from Sigma (Sigma, St Louis, MO, USA).

Extraction and purification of OTA

The entire sample was crushed in a hammer mill to obtain a homogeneous fine grind. In a Nalgene jar containing 15 g of homogenate, 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 (CE 401/2006).

Extraction and purification of fuminosin B1

Twenty-five grams of maize sample were extracted with 50 mL of water blending for 2 min with a hammer mill blender. At five grams of ground maize, 25 mg of NaCl were added and the mixture was shaked on a horizontal mechanical shaker for 120 minutes at 300 rpm, and then centrifuged for 15 minutes at 2500 g. The supernatant was recovered and degreased by 4 mL of hexane. The organic phases were removed by centrifugation for 5 minutes at 2500 g. The aqueous layer was recovered and diluted with 16 mL of phosphate buffered saline (PBS) at pH 7.3, filtered through Whatman N°. 4 filter paper and then applied to a column immunoaffinity Fumoniprep (R Biopharm Rhone Ltd, Glasgow, Scotland) at a flow rate of 1–2 drops/s. The column was washed with 10 mL of the same buffer to 1-2 drops/s for removal of residues. Fumonisin B1 was eluted with 1.5 mL of methanol (HPLC grade) and then 1.5 mL of water. The eluate was collected and evaporated, protected from light in a nitrogen stream. The dry extract was taken up in 200 µL acetonitrile/water (50: 50, v/v) and then sonicated for 5 minutes. Then, 50 µL of extract was diluted into 50 µL of a solution of ortho-phthalaldehyde (OPA 40 mg, 1 mL methanol, 5 mL of 0.1 M sodium tetraborate and 50 µL of 2-mercaptoethanol). The resulting sample was packed in a chromatographic tube and the analysis of FB1 was made by HPLC using AFNOR methods (AFNOR, 2002).

Extraction and purification of Zearalenone

Twenty-five grams of maize sample were extracted with 50 mL of 125 mL of acetonitrile: water (94:31) blending for 2 min with a hammer mill blender. After filtration through Whatman N° 4 filter paper, 20 mL of the filtrate were diluted with 80 mL of double distilled water. Then, 25 mL of the diluted filtrate was applied to an immunoaffinity column (Easi-Extract® zearalenone, R-Biopharm Rhone Ltd, Glasgow) containing a monoclonal antibody specific for the zearalelone. The column was washed with 10 mL of double distilled water. Zearalenone was eluted by applying 1.5 mL of methanol. The eluate was diluted with 1.5 mL of bidistilled water and mixed by vortexing. The resulting sample was packed in a chromatographic tube and the analysis of ZEA was made by HPLC using the method of AOAC, 2000 and Miraglia and Brera, 2000.

OTA, FB1 and ZEN determination

Determination of OTA, FB1 and ZEA contents was achieved with high performance liquid chromatography column, using a Shimadzu liquid chromatograph (Kyoto, Japan) fitted with fluorescence detector. The operating conditions are described in Table I.

Assessement of ochratoxin A, fuminosin B1 and zearalenone daily intakes in adult Ivorian

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 (Assidjo *et al.,* 2013). To assess ochratoxin A, fuminosin B1 and zearalenone exposure, the mean level of these mycotoxins found in maize grains stored at 6 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, FB1 and ZEA (Kroes et al., 2002). According to the National agricultural statistics of Cote d'Ivoire, the daily consumption of maize is 28.4 g per capita/day (Beugre *et al.,* 2014). The OTA, FB1 and ZEA intake was calculated using formula 1:

$$\mathbf{EAI = (T \times Q)/ bw}$$

With EAI, the estimated of OTA, FB1 and ZEA daily intake in ng kg^{-1} of body weight (b.w.) day^{-1}; T, the OTA, FB1 and ZEA concentration found in maize grains stored (µg/kg); Q, the daily consumption of maize grains (g/day); bw, the body weight of the individual adult (70 kg). The estimated intakes were also expressed from the average and maximum levels of

Table I: Conditions of OTA, FB1 and ZEA analysis by HPLC

ITEM	OCHRATOXIN A	FUMONISIN B_1	ZEARALENONE
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: 330 nm,	Fluorescence, λ excitation : 335 nm,	Fluorescence, λ excitation : 274 nm,
	λ emission: 460 nm	λ emission : 440 nm	λ emission : 440 nm
Mobile phase	Acetonitrile/Water/Acetic acid (99/99/2)	Acétonitrile/Water (50/50)	Acétonitrile/Water/Methanol (46/46/8)
Inject volume	100 µL		
Flow rate	1 mL/minute		
Column temperature	40°C		
Rising solvent	Acétonitrile		
Analysis duration	12 minutes	6 minutes	9.5 minutes

mycotoxins fixed by the European Commission (EC 2006; EC 2007 and EC 2010) 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, 2000 µg/kg for the sum of FBs, 200 µg/kg for ZEA. Moreover, the estimated intakes were compared to Tolerable Daily Intake (TDI) set at 5 ng/kg bw/day for OTA, 2000 ng/kg bw/day for the sum of FBs and 500 ng/kg bw/day for ZEA established by WHO (JECFA 1999, JECFA 2001).

Statistical analysis

Statistical analyses were performed using SPSS for Window version 20.0 (SPSS Inc., Chicago, Illinois). Analysis of variance (ANOVA) according to two factors: duration and method of storage and Tukey's HSD test at 5% significance level were used to compare the means of physical parameters and mycotoxins levels detected in each granary system. Pearson correlation test was used to assess relationships between the content of moisture, the water activity and mycotoxins levels. Then, Multivariate Analyses through Principal Components Analysis (PCA) and Ascending Hierarchical Clusters analysis (AHC) were performed using STATISTICA software (version 7.1).

RESULTS

Evolution of the aerothermal parameters

Figure 4 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 30.6±1.97°C. But, a higher temperature of 33.8±3.0°C was noticed in March, while August provided the lowest temperature (27.50±1.10°C). With the relative humidity, general average of 80.5±4.08% was recorded during the study period. The months of January, February and March 2013 (68.7±3.52%, 56.2±5.52% and 70.9±6.0%, respectively) were less humid than the other months among with August recorded the top value of 91.1±5.0%.

Evolution of the Moisture and water activity

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 II and III).

Water activity

Figures 2 and 3 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.

Moisture content

The evolution of the moisture content of the 3 types of granaries during the maize storage is also referred in Figures 2 and 3. With respective means of 9.23±0.06 % and 9.05±0.21 % 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±0.82 % and 13.52±0.68 % 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±0.72 % and 12.74±0.65 %, respectively) and grains (11.85±0.35 % and 11.87±0.48 %, respectively). Besides, the interaction between type and time of storage does not involve any significant effect upon this parameter as shown in previous tables II and III.

Validation of OTA, FB1 and ZEA determination using HPLC

Using HPLC device, Limits Of Detection (LOD) of respective ochratoxin A, fuminosin B1 and zearalenone are 5 ng/kg, 12.5 ng/kg and 2.6 ng/kg, while their Limits Of Quantification (LOQ) are 20 ng/kg, 50.5 ng/kg, 7.43 ng/kg. The mean recoveries fluctuate between 0.26% and 3.75% for the repeatability assays and between 0.89% and 5.67% for reproducibility assays. However, respective rates of extraction recorded for OTA, FB1 and ZEA are 86.92±0.39%, 82.9±2.19% and 93.20±4.21%.

Evolution of ochratoxin A, fuminosin B1 and zearalenone contents

The post-harvest treatments of maize cobs and grains with biopesticides highlight significant reduction of all mycotoxins contents assessed ($P<0.05$) compared to the control granaries untreated samples. All maize samples from the different granaries are ochratoxin A, fuminosin B1 and zearalenone - positive respectively with percentage of 50%, 16.66% and 25% of the maize samples above the maximum residue limit concentration of 5 µg/kg, 2000 µg/kg and 200 µg/kg respectively for OTA, total FBs and ZEA proposed by the regulations of the European Commission (EU Regulation, 2006; 2007 and 2010). The mean contents of OTA, early estimated at 0.32±0.03 µg/kg record a slight growth to 5.83±1.00 µg/kg during 6 months of storage, before increasing up to 22.73±2.43 µg/kg at the 8th month from the biopesticides-treated maize. On the other hand, control granaries involve with rapid increasing of OTA level during the 8 months of storage ranging from 0.32±0.03 µg/kg to 65.25±3.05 µg/kg (Figures 2 and 3). The contents of fuminosin B1 (FB1) of the maize stay at 29.23±4.01 µg/kg or 35.30±2.05 µg/kg at the beginning of storage for cobs and grains respectively. But, this levels also rise significantly ($P<0.05$) up to 548.06±8.06 µg/kg or 715.28±14.28 µg/kg with the traditional granaries, to 555.26±12.07 µg/kg or 801.26±12.04 µg/kg for the improved granaries and reach more considerable values of 3210±21.20 µg/kg or 3451.26±40.97 µg/kg from the control granaries considering the maize grains or cobs, respectively (Figures 2 and 3).

Similarly for zearalenone, the stages of 2, 6 or 8 months of storage provide also higher contents from the untreated maize (219.26±4.17 µg/kg to 258.17±5.00 µg/kg, 354.26±3.10 µg/kg to 398.16±2.06 µg/kg and 415.26±11.17 µg/kg to 451.29±10.00 µg/kg, respectively) than the samples resulting with the biological treatment, stated from 35.71±1.02 µg/kg to 52.72±1.10 µg/kg, 50.26±1.20 µg/kg to 77.66±2.05 µg/kg and 148.77±12.10 µg/kg to 192.27 ±5.52 µg/kg, respectively (Figures 2 and 3).

Correlation between the contents of moisture, water activity and mycotoxins

Tables IV and V show the correlations between water activity, moisture content, OTA, FB1 and ZEA levels in the various technologies of maize storage. The Pearson indexes (r) indicate positive and significant correlations between the five (5) parameters assessed for both maize forms (cobs and grains). Thus, water activity, moisture, OTA, FB1 and ZEA are closely correlated during the storage of the post-harvest maize, r varying from 0.69 to 0.90 for maize cobs and from 0.62 to 0.90 for maize grains. The water activity was directly correlated with the moisture content (r=0.79 and 0.90 for maize grains and cobs respectively). OTA content are directly linked with FB1 content (r=0.95 and 0.92 for maize cobs and grains respectively). ZEA and FB1 contents change tightly (r=0.97 for both maize cobs and grains). Positive significant correlations are also observed between OTA and ZEA contents (r=0.96 and 0.93 for maize cobs and grains respectively).

Variability between storage structures and qualities parameters assessed

Principal Component Analysis (PCA) is performed with the component F1 which record an eigenvalue superior to 1, according to statistical standard of Kaïser (Table VI). The overall parameters display negative significant correlations with F1. Nevertheless, the component F2 (eigenvalue of 0.37) is associated to F1 for fulfillment of the PCA. Figure 5.a shows the correlation circle between the F1-F2 factorial drawing, with 98.02% of the total variance, and the chemicals parameters of the maize stored. The projection of the investigated samples highlights 3 groups of individuals (Figure 5.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 OTA, FB1, ZEA, water activity and moisture content. The second group contains maize samples from the control granaries at 2 months of storage. They are also distinguished by higher levels in OTA, FB1, ZEA, 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, 6 and 8 months, providing slight levels of the parameters mentioned above. The Ascending hierarchical classification (AHC) strengthens the variability resulting from the PCA (Figure 6). At aggregation distance of 20, 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 the control at 2 months of storage inner the third cluster. Those samples also show high levels of OTA, FB1, ZEA, 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, 6 and 8 months of storage, which are lower contents in OTA, FB1, ZEA, water activity and moisture.

Assessment of OTA, FB1 and ZEA intake from maize grains after storage

Table VII shows the OTA, FB1 and ZEA intake estimated from the consumption of maize grains stored for 6 months. The estimated daily intake (DI) in the Ivorian adult consumers are 2±0.35 ng/kg bw/day, 141±4.10 ng/kg bw/day, 20±0.43 ng/kg bw/day for OTA, FB1 and ZEA respectively from the maize grain result from the treated granaries. From the untreated granaries, these intakes are 13.10±0.40 ng/kg bw/day, 1167±15.05 ng/kg bw/day and 143.73±1.26 ng/kg bw/day for OTA, FB1 and ZEA respectively. These traits appear to be largely below the tolerable daily intake set by WHO for respectively OTA (5ng/kg bw/day), FB1 (2000 ng/kg bw/day) and ZEA (500 ng/ kg bw/day) excepting for the OTA intake estimated from the untreated granaries which is 3 times higher than the reference value. OTA, FB1 and ZEA exposures from the untreated granaries are also higher than their maximal concentration acted by European Union. Recorded values represent 84.73%, 44% and 77.44% of the respective maximum quantities of 5 µg/kg for ochratoxin A, 2000 µg/kg for the sum of fuminosins and 200 µg/kg for zearalenone permitted.

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

Source of	df		Parameters				
Variation			AW	MC	OTA	FB1	ZEA
Types	2	SS	0.002	4.23	5116.28	94354.82	13920.57
		F-value	3.84	6.90	1628.85	3001.68	956.84
		P-value	0.04	0.004	<.001	<.001	<.001
Durations	3	SS	0.04	83.52	9907.10	462995.12	11272.21
		F-value	48.41	90.70	2102.73	1472.18	7747.25
		P-value	<.001	<.001	<.001	<.001	<.001
Types xDurations	6	SS	0.002	1.54	3634.17	173649.35	19471.37
		F-value	1.17	0.84	385.67	5523.13	1338.17
		P-value	0.35	0.56	<.001	<.001	<.001
Error	24	SS	0.006	7.37	37.69	7545.71	349.22
Total	36	SS	27.52	4876.10	30916.34	733045.43	15080.40

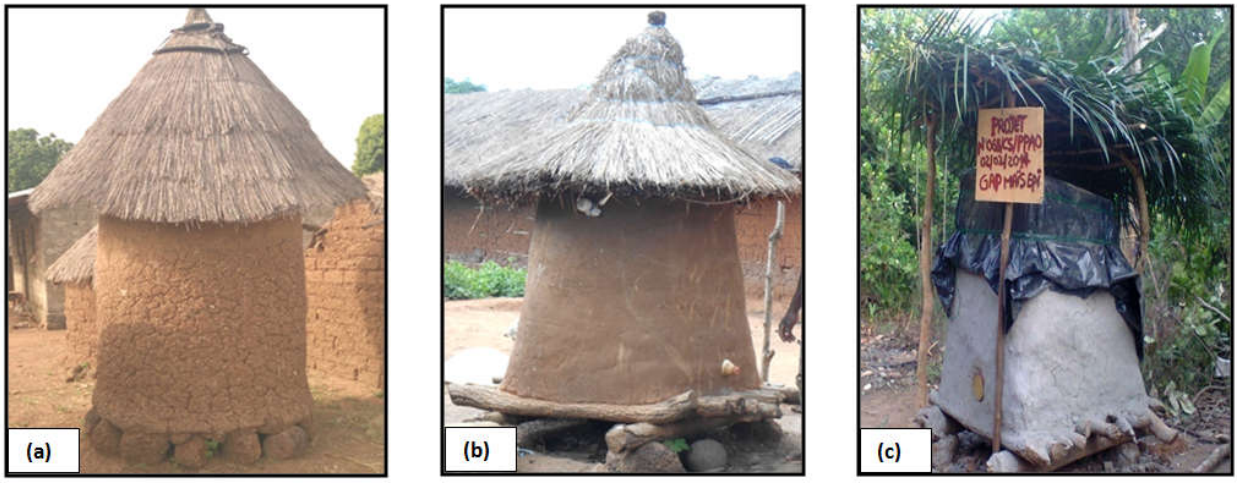
SS, sum of squares; F-value, value of the statistical test; P-value, probability value of the statistical test; df, degree of freedom. MC, moisture content; AW, water activity; OTA, ochratoxin A content; FB1, fuminosin B1 content; ZEA, zearalenone content.

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

Source of	df		Parameters				
Variation			AW	MC	OTA	FB1	ZEA
Types	2	SS	0.004	5.86	2957.21	213087.92	31438.51
		F-value	2.58	11.72	501.13	3750.29	3953.26
		P-value	0.10	<.001	<.001	<.001	<.001
Durations	3	SS	0.04	58.57	4767.55	102988.44	240830
		F-value	17.27	78.13	538.61	1208.28	2018.87
		P-value	<.001	<.001	<.001	<.001	<.001
Types xDurations	6	SS	0.002	6.00	2037.46	926131.44	1116.94
		F-value	0.48	1098	115.10	5433	467.83
		P-value	0.82	0.11	<.001	<.001	<.001
Error	24	SS	0.02	6.00	70.81	618.56	954.32
Total	36	SS	28.20	4527.41	15841.54	660802.32	12514.26

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; OTA, ochratoxin A content; FB1, fuminosin B1 content; ZEA, zearalenone content.

Figure 1. Distribution map of the study zone



Photography 1: Different types of maize storage granaries used for experiments implementation

(a) Control granary; (b) Traditional granary; (c) Improved granary

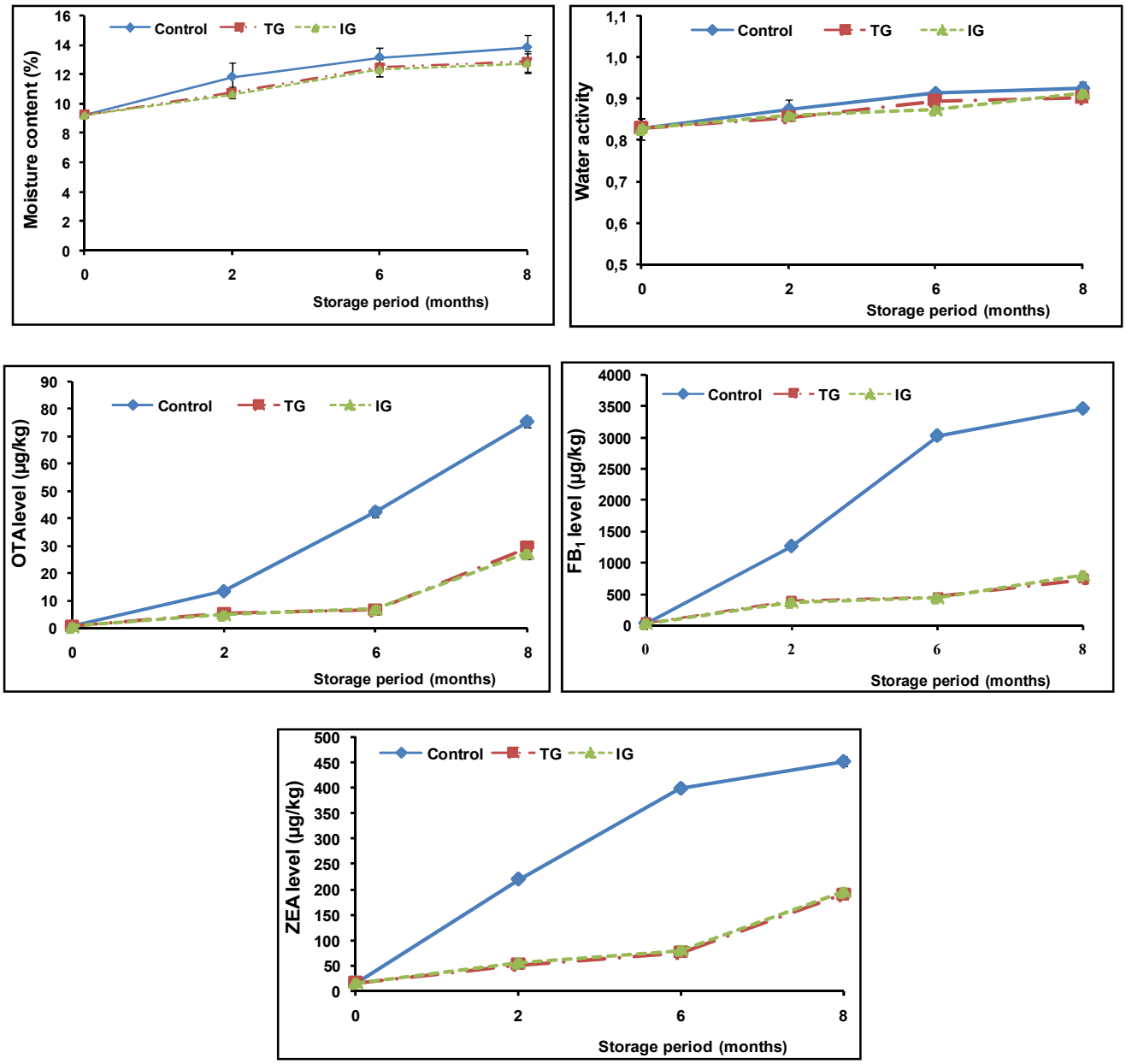


Figure 2. Evolution of moisture, water activity and ochratoxin A contents of maize cobs according to the storage conditions

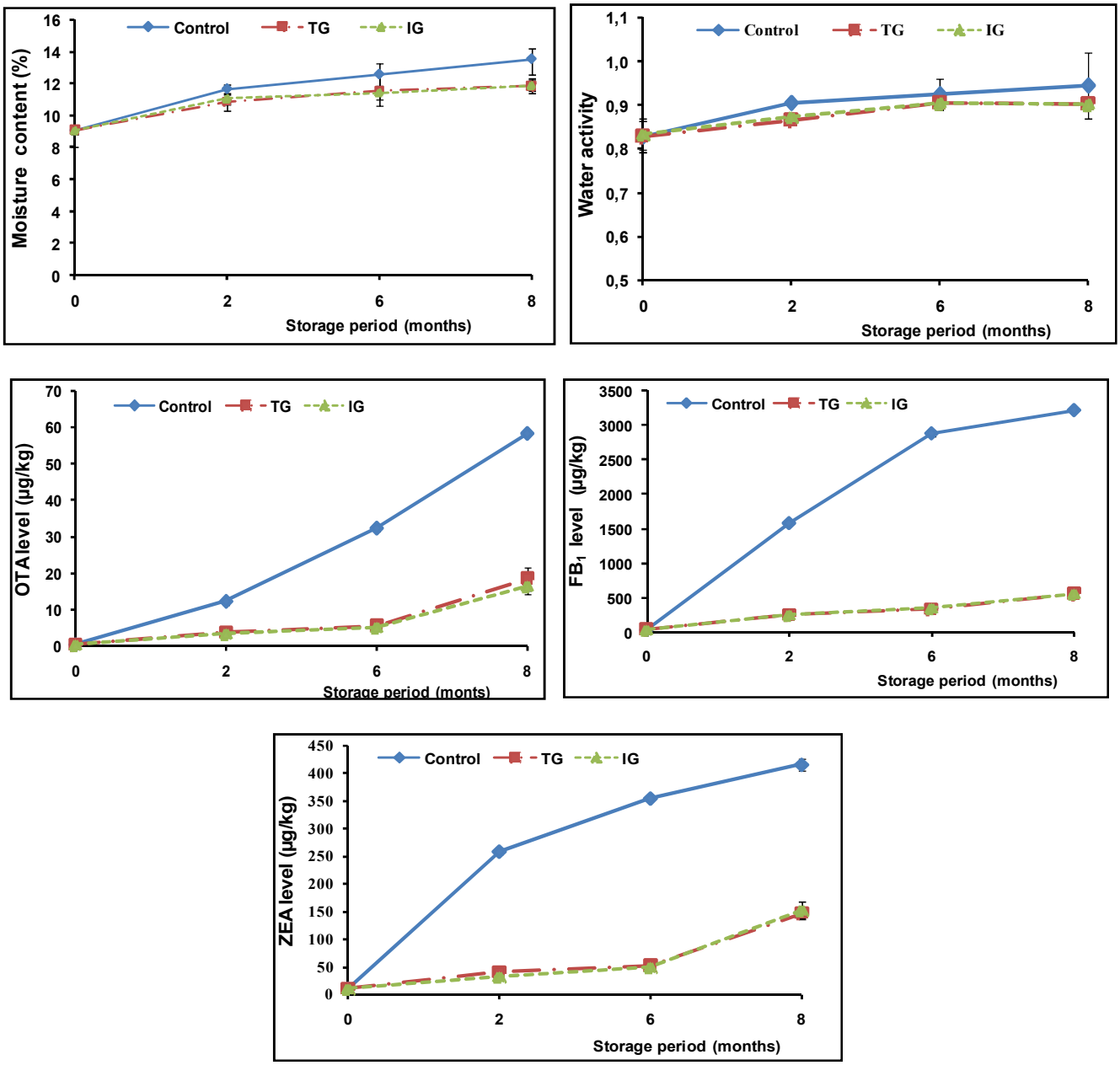


Figure 3. Evolution of moisture, water activity and ochratoxin A contents of maize grains according to the storage conditions

Table IV: Matrix of correlations between physicochemical and mycotoxins levels characters of maize cobs

	AW	MC	OTA	FB1	ZEA
AW	**1**				
MC	**0.90****	**1**			
OTA	**0.76****	**0.74****	**1**		
FB1	**0.69****	**0.70****	**0.95****	**1**	
ZEA	**0.77****	**0.77****	**0.96****	**0.97****	**1**

*·**= significant at P< 0.05 and 0.01, respectively; MC, moisture content; AW, water activity; OTA, ochratoxin A content; FB1, fuminosin B1 content; ZEA, zearalenone content.

Table V: Matrix of correlations between physicochemical and mycotoxins levels characters of maize grains

	AW	MC	OTA	FB1	ZEA
AW	**1**				
MC	**0.79****	**1**			
OTA	**0.62****	**0.76****	**1**		
FB1	**0.63****	**0.74****	**0.92****	**1**	
ZEA	**0.66****	**0.78****	**0.93****	**0.97****	**1**

*·**= significant at P< 0.05 and 0.01, respectively; MC, moisture content; AW, water activity; OTA, ochratoxin A content; FB1, fuminosin B1 content; ZEA, zearalenone content.

Table VI: Eigenvalues and correlation matrices factors of principal components analysis with physicochemical and mycotoxins levels of maize stored studied

Factors	F1	F2	F3	F4	F5
Eigenvalues (%)	4,53	0,37	0,07	0,02	0,01
Variance (%)	90,70	7,33	1,35	0,47	0,15
Cumul. var (%)	90,70	98,02	99,37	99,85	100,00
Aw	-0,93	0,35	0,07	0,00	0,06
Moisture	-0,95	0,29	0,00	-0,03	-0,06
FB1	-0,94	-0,33	0,07	-0,10	0,01
OTA	-0,97	-0,08	-0,22	0,02	0,02
ZEA	-0,96	-0,22	0,09	0,11	-0,01

Table VII: OTA, FB1 and ZEA intake estimated from the consumption of maize grains from Ivorian adult (Intake ng/kg body weight/day)

	OTA		FB_1		ZEA	
	C	**IG**	**C**	**IG**	**C**	**IG**
Daily intake (DI)	13.10±0.40	2±0.35	1167±15.05	141±0.35	143.73±1.26	20±0.43
Estimated intake to MRL ($AELMR_1$)	2		811		81	
Reference value SCF	5		2000		500	
DI/SCF	2.62	0.41	0.58	0.0705	0.30	0.04

$AELMR_1$: estimated intake for a maximum residue level of OTA, FB1 and ZEA in maize;
SCF: Tolerable daily Intake recommended by the Scientific committee on food;
C, control granary; **IG**, improved granary

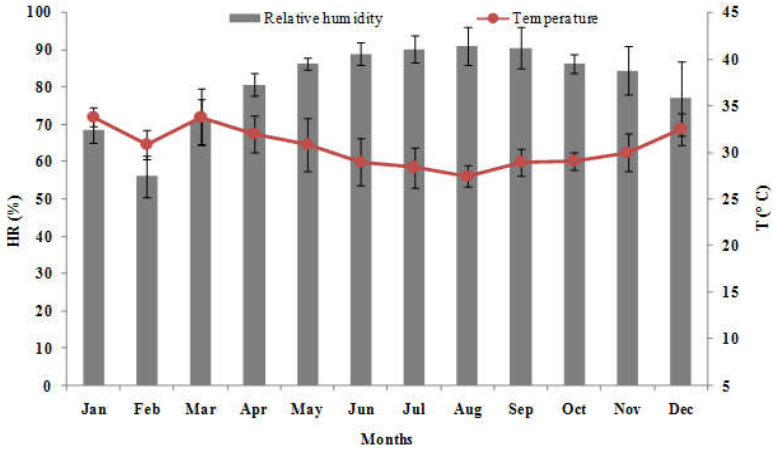
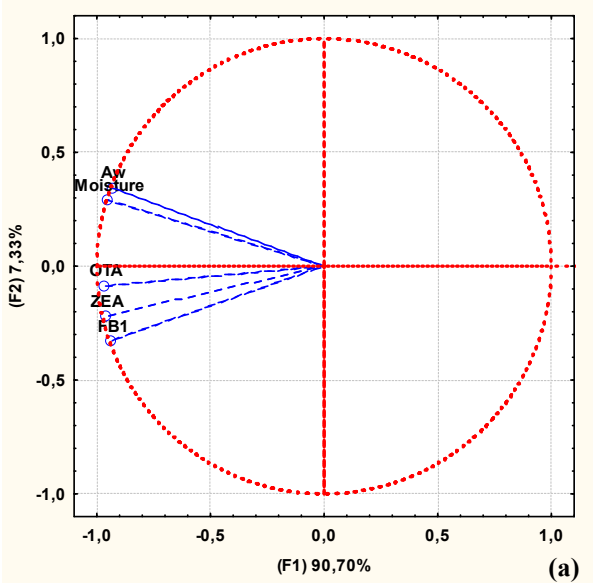


Figure 4. Average variation in air temperature and relative humidity of the study site



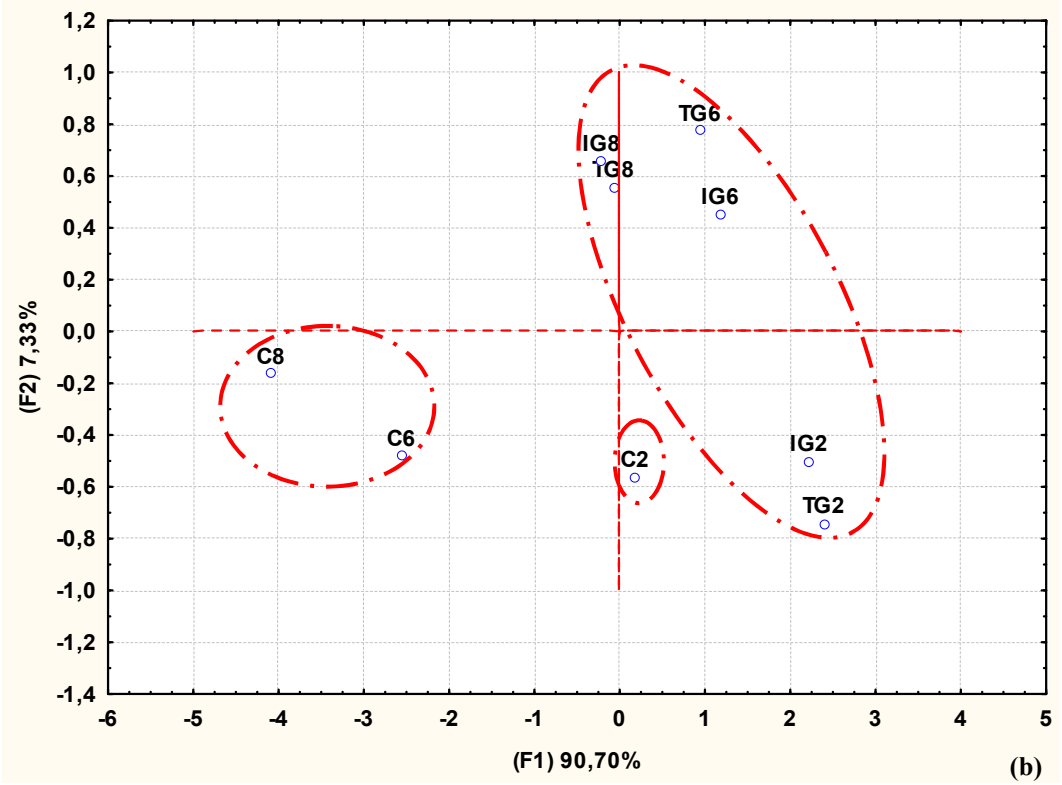


Figure 5. Correlation drawn between the F1-F2 factorial of the principal components analysis and the chemical parameters (a) and the individuals (b) deriving from the maize samples studied

MC, moisture content; AW, water activity; OTA, ochratoxin A content; FB1, fuminosin B1 content; ZEA, zearalenone content
C2, TG2, IG2: control, traditional and improved granaries at 2 months of storage; C6, TG6, IG6: control, traditional and improved granaries at 6 months of storage; C8, TG8, IG8: control, traditional and improved granaries at 8 months of storage

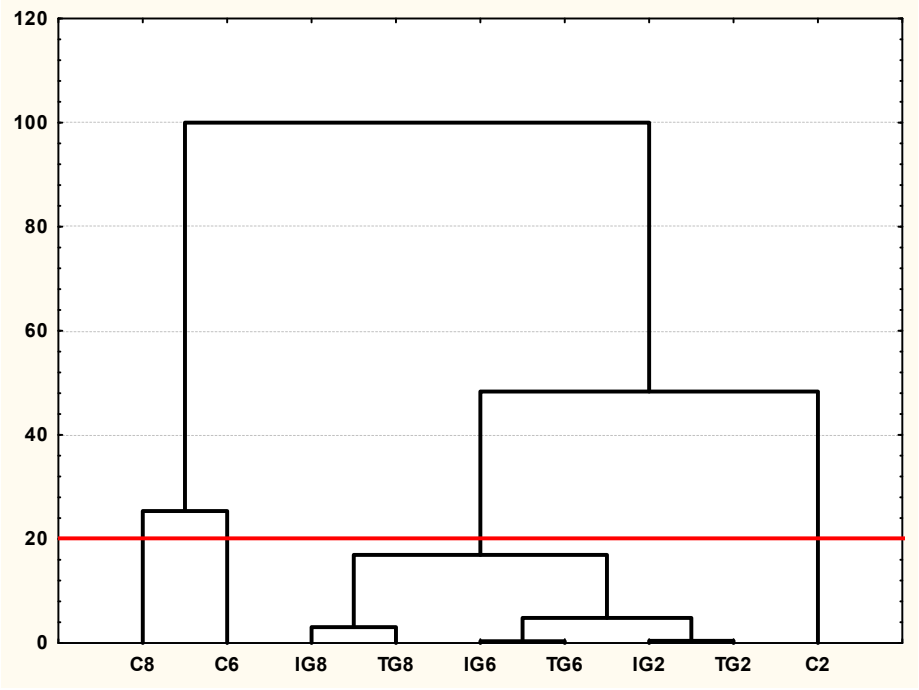


Figure 6. Dendrogram deriving with the Ascending Hierarchical Classification of maize samples stored for 8 months according to the parameters assessed

C2, TG2, IG2: control, traditional and improved granaries at 2 months of storage; C6, TG6, IG6: control, traditional and improved granaries at 6 months of storage; C8, TG8, IG8: control, traditional and improved granaries at 8 months of storage.

DISCUSSION

This study was carried out to strengthen ability of poor rural small-holder farmers in improving their crops productivity and incomes with low-cost, sustainable and environmental suitability. The increase in the water content of maize cobs and grains stored in the granaries may be explained by moisture resumption, due to the maize being highly hygroscopic. However, both maize cobs and grains in the treated granaries cover with the plastic polyethylene film presented moisture contents above the limit of 13% recommended for maize safe storage (Mohale *et al.,* 2013). This result demonstrates that the plastic polyethylene film slow down moisture resumption by maize. The plastic served as a barrier against the exchange of maize cobs and grains with ambient air, preventing moisture resumption and providing good conservation material, despite the unfavorable climatic conditions (Asiedu, 1991). Granary also appeared to be a good storing media for maize. Probable reason that can be explained based on the materials with which this storage was made up off. This property impedes the worsening of the stored grains. These results are consistent with the findings of Raza *et al.* (2010) who reported that earthen pots from different types of containers commonly used in Pakistan for wheat storage are moderately sealed or less affected by high temperature and humid environment. During the 8 month of study, the average air temperature was 30.583 ± 1.97 °C. While this temperature is appropriate for maize cobs and grains storage, it also promotes fungi development. The mean development thresholds of fungi range between 10 °C and 40 °C (Atanda et al., 2011). Moreover, to prevent fungi development, maize grains must have a water activity (a_w) less than 0.65, which is the accepted standard value (Pitt and Hocking, 1985). Along the storage, the mean water activity recorded in the different granaries storage systems ranging from 0.83 to 0.91. These high values appear to be more susceptible to spoilage, fungal contamination and rapid mycotoxins production (Bluma *et al.,* 2008). Since, all maize samples from the tested granaries (untreated or treated with biopesticides) were found to be ochratoxin A, fuminosin B1 and zearalenone positive with an increase levels over the storage period. But, the data from various maize mycotoxins contents (OTA, FB1 and ZEA) state a better hygienic preservation of the maize stored after adding combination of *L. multiflora* and *H. suaveolens* and cover the granaries with the plastic polyethylene film than the storage without any treatment. Indeed, the OTA, FB1 and ZEA levels of the treated maize cobs and grains recorded slight increasing during 6 months storage, when the untreated maize already allowed great mycotoxins production. Thus, maize cobs and grain were significantly protected by these treatments from pest and fungi infestation up to 6 months in traditional and improved storage granaries. Combinations of 2 plants and the use of the plastic polyethylene film as barrier against the exchange of maize cobs and grains with ambient air enhance reduction of insect and mycotoxins in stored maize comparing with the control untreated maize. These results agree with the works of Fandohan *et al.,* 2004 who observed a reduction of fuminosin production when maize treated with different concentration of essential oils was stored in closed conditions than in open storage conditions. These authors noticed also that essential oils consist of volatile compounds that are more likely to diffuse rapidly in air when the storage container is open. According to Kanko (2004) and Tia (2012), the main bioactive molecules of *L. multiflora* are oxygenated monoterpenes such as (E)-Nerolidol, linalol and 1,8-cineole; whereas monoterpene hydrocarbons particularly sabinene, β-pinene and limonene predominate from the *H. suaveolens*. This combination of different mono and sesquiterpene compounds contributed to the antifungal and mycotoxins (OTA, FB1 and ZEA) production inhibitory activity (Rao *et al.,* 2015; Passone *et al.,* 2012; Fandohan *et al.,* 2004).

The coexistence of OTA, FB1 and ZEA in this study should be taken into consideration as claimed by the IARC (2002). This is particularly important in regard to possible synergism and additive effects of these mycotoxins. Dietary exposure to these mycotoxins can cause various adverse health effects in humans, including nephrotoxic, teratogenic immunotoxic effect, esophageal cancer and hormonal disruption (Chelule *et al.,* 2001; Sangaré-Tigori *et al.,* 2005 Kouadio *et al.,* 2014). Such co-contamination has been previously observed in maize samples and with other food samples such as peanut and millet (Kouadio *et al.,* 2014; Sangaré-Tigori et al., 2006). Therefore, in view of the toxicity of OTA, FB1 and ZEA, a great deal of effort must be make to eliminate or reduce OTA, FB1 and ZEA content in foods and feedstuffs of the maize by foster best practices of harvesting, drying and storage in order to provide safety to Ivorian people health. Daily consumption of maize and its derivatives in Côte d'Ivoire is 28.4 grams per person (Beugré et al., 204). Considering that the average weight of adult population is 70 kg and the average concentration of OTA, FB1 and ZEA, after 6 months storage, the Tolerable Daily Intake (TDI) of the treated granaries are lower than the untreated and stay below the reference value. In contrast, Sangaré-Tigori *et al.,* 2006 found TDI of 7.8 ng/kg bw/day, 3,900 ng/kg bw/day and 1,560 ng/kg bw/day respectively for OTA, FB1 and ZEA in samples from Côte d'Ivoire above the tolerable daily intake set by WHO (JECFA, 1999; JECFA, 2001).

Conclusion

From the various technologies, the study showed that there is no difference between maize cobs and grains stored in granaries treated with biopesticides and cover with a plastic polyethylene films. Data from the parameters suggests a better storage of maize cobs and grains treated with biopesticides over duration of six months. This study has demonstrated that *L. multiflora* and *H. suaveolens* can also serve as alternative means to reduce the pest and fungi infestation. These plants are a common weed that is found growing in most wastelands as such could be easily obtained by farmers and used for pest management. This storage technique is inexpensive, easily carried and fits into 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.

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