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Original Research Article

EVOLUTION OF OCHRATOXIN A CONTENTS DURING STORAGE OF COWPEA (*Vigna unguiculata L Walp*) BAGGED PICS WITH BIOPESTICIDE (*Lippia multiflora* Moldenke*)* AND ESTIMATION OF DAILY INTAKE IN ADULT IVORIAN

ABSTRACT

Aims: This study aims to test the effectiveness of the cowpea conservation method in PICS bags with a biopesticide on the evolution of ochratoxin A and the estimated daily intake in adult Ivorian

Study Design: Cowpea grains that have undergone any treatment were collected between May and June 2015 in the southwest of Côte d'Ivoire. The fresh leaves of Lippia multiflora were collected and dried in sunlight for 7 days in the center of Cote d'Ivoire. The supply PICS bags was made in Abidjan. All this material was sent to the Laboratory of Biochemistry and Food Sciences, Félix Houphouët-Boigny University, Côte d'Ivoire, to perform the experiment.

Place and Duration of the Study: This study was carried out during June 2015 to February 2016 in the Laboratory of Biochemistry and Food Science, Félix Houphouët-Boigny University, Côte d'Ivoire

Methodology: A central composite design was used for sample constitution. Thus, a control group with polypropylene bags (TSP), a control group in PICS bags without biopesticide (H0) and 4 experimental lots of PICS bags containing different proportions (0.7%, 2.5%, 4.3% and 5% (w / w)) biopesticide respectively noted H1, H2, H3 and H4, have been used. Changes in moisture, the water activity (Aw) and the levels of ochratoxin A (OTA) cowpea grains stored for 8 months were studied

Results: The results reveal the presence of OTA in all samples analyzed. However, only 17.65% of the samples had higher levels of OTA than the reference values set by the European Union. PICS bags contain lower levels of OTA. These levels are even lower when the cowpea is stored in PICS bags containing different concentrations of biopesticide. The estimated daily intake of OTA under the cowpea consumption stored for 8 months were respectively 0.58 ± 0.06 ng / kg body weight/day, 0.66±0.03 ng / kg bw / day , 0.30 ± 0.05 ng / kg body weight / day, 0.30 ± 0.00 ng / kg body weight / day and 0.28 ± 0.00 ng / kg body weight / day for H0 , H1, H2, H3 and H4. These levels are below the maximum permissible reference value set by the WHO.

Conclusion: Storage of cowpeas in PICS bags with the leaves of *Lippia multiflora* appears as a method of effective and inexpensive conservation to ensure the health quality of cowpea.

Keywords: Ochratoxin A, cowpea, PICS bags, storage using biopesticide

1. INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin produced by fungi of the genus Aspergillus and Pencillium mainly *Pencillium verrucossum* in temperate climates and *Aspergillus ochraceus* in warm regions [1] It is transmitted to humans through the usually contaminated food chain toxin [2, 3]. OTA is found mainly in grains (wheat, corn, rice, rye, oats, etc.), offal and meat from animals receiving contaminated food, dried food such as roasted or coffee, cocoa, beans, peas, peanuts and dried fruit [3]. OTA have multiple toxic effects. Indeed, the toxicological studies in laboratory animals showed nephrotoxic, genotoxic, immunosuppressive, teratogenic, neurotoxic and carcinogenic [4-7]. Moreover, the risks that result from human exposure to OTA in foods is considered a major public health problem [8]. Thus, cowpea (*Vigna unguiculata* L. Walp.), legume native to Africa, is grown for its rich nutritional components [9] In West Africa, cowpea is 85% of the area of pulses and 10% of total cultivated land [10]. Sub-Saharan Africa accounts for about 95% of the world's cowpea production.

However, the big problem in the industry is that cowpea storage because of its nutritional richness. Consequently, storage is an important step in preserving the marketability for productions that will be able to speculate. It also represents an important step in preserving hygienic and nutritious qualities for consumers [11]. Indeed, the pest activity creates an environment for mold growth of Aspergillus, Penicillium and Fusarium responsible for the deterioration of the quality of cowpea stocks [12-16].

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Mold, in addition to altering the appearance, smell and taste of grain, also produce ochratoxin A harmful to the health of animals and humans [12, 17].
Also maintaining an ambient humidity levels below the range of mold development is essential to preserve food against fungal colonization. In attics or stores food strike a balance with the ambient relative humidity. General manner, moisture is the presence of water or water vapor in air or in a substance. It represents a parameter for growth, mold and toxinogenesis growth [18].
Legal and regulatory provisions are being taken at the international level by the Codex Alimentarius and most governments to strengthen food safety requirements to protect consumers and the environment from harmful effects of chemical contaminants and mycotoxins, including the ochratoxin A [19, 20].
The methods used as a solution to the storage and preservation of cowpeas, based on the use of pesticides whose residues may cause danger for the consumer and also environmental pollution. Moreover, these products are applied without following good agricultural practices and use [21, 22]. This use can lead to toxicity in the consumer, resistance in pests and also a negative impact on the environment [23]. Thus the need to find an alternative, lower cost, respecting the environment safe for the consumer becomes capital [24, 25]. The current trend is towards the use of aromatic plants containing active molecules insecticidal, insect repellents, fungicides, nematicides and rodenticides [26-33]. These plants, mostly used by people to fight cons diseases remain very low status [24]. These plants biopesticides limit the risk of development of resistance by pests and certain pathogenic microorganisms [31, 34]. Therefore, the study aims to monitor the evolution of ochratoxin A, moisture and water activity during storage of cowpea in PICS bags with leaves of *Lippia multiflora*.

2. MATERIAL AND METHODS

2.1 Experimental site

The experiment was performed at Laboratory of Biochemistry and Food Sciences (LABSA) UFR Biosciences at the University Félix HOUPHOUET-BOIGNY. The different bags were kept in a laboratory storeroom to 27.78 ± 0.19ºC temperature and 75.0 ± 0.99% relative humidity. Wooden pallets were arranged floored as support for PICS bags (Purdue Improved Cowpea Storage).

2.2 Collection of cowpea beans used in the study

Cowpea grains used belong to the local variety "Vya". They were collected from producers of the Loh-Djiboua region (5 ° 50 'North 5 ° 22' West) from April to May 2015, just after harvest. After the shelling, the grains have not undergone any treatment were sent to the laboratory for packaging.

2.3 Collection and processing of biopesticides

The Laboratory of Biochemistry and Food Science has a scope on the conservation of cereals, pulses and other agricultural products for many years. Biopesticides are a good alternative in the fight against pests and fungus. Thus, *Lippia multiflora* was used in this study for these phytosanitary properties. It is perennial and fragrant shrubs spontaneously encountered in areas of central and northern Côte d'Ivoire [29, 35]. The leaves of L. multiflora were harvested and dried in the sunlight for a week in GBEKE region in May 2015. The dried leaves were chopped into fine particles before use.

2.4 Using the triple bagging

Storage bags used in our study, were made of polypropylene bags and polyethylene bags (Purdue Improved Cowpea Storage: PICS) developed by Purdue University for storing cowpeas from Niger. These bags, obtained from suppliers, are composed of a triple bagging system.

2.5 Retention method for cowpea grains

Storage of cowpea grains was conducted from June 2015 to February 2016, according to a central composite design with 5 levels and 2 variables. The latter has set up 6 lots (1 control polypropylene bag, 1 control with PICS bag, and 4 lots in PICS bags containing different proportions of biopesticides (0.7%, 2.5%, 4.3% and 5% of the mass of bag per sheet). the filling of the bags of 50 kg was made in stratum, alternating cowpea seeds and leaves of biopesticides.

2.5.1 Sampling

The sampling was performed at the beginning of the storage (0 month), then 1; 2; 4,5; 7 and 8 months later, in triplicate. Thus, 1 kg of cowpea samples from each granary was gathered through the top, the

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centre and the bottom opening sides. Cowpea samples were ochratoxine A and physicochemical properties measurements were achieved.

2.5.2 Determination of moisture content

The moisture content was valued according to the method described by [36]. A cowpea sample of 5 g was dried at 105°C into an oven till constant weight. The result was expressed from the equation below:

Moisture content (%) = 100-(Wl x 100/Ws)

With Wl, weight lost from samples after drying; Ws, weight of raw samples.

2.5.3 Determination of water activity

The water activity was measured with a HygroLab Rotronic hygrometer according to indications of McCormick (1995) [37]. Prior to assays, the hygrometer was calibrated with specific water activity salts. Then, samples of 5 g of ground cowpea were put into standard dry empty containers for the Aw analysis. The water activity digital measures were directly displayed by the hygrometer.

2.5.4 Ochratoxin A analysis

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

2.5.4.1 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 [38].

2.5.4.2 OTA determination

Determination of OTA contents was achieved with high performance liquid chromatography column, using a Shimadzu liquid chromatography (Kyoto, Japan) fitted with fluorescence detector. The operating conditions are described in Table 1.

Table 1. Conditions of OTA analysis by HPLC

	OCHRATOXINE A
Pre-column	Shim-pack GVP-ODS 10 x 4.6 mm
Column	Shim-pack GVP-ODS 250 x 4.6 mm
Detector	Fluorescence, λ excitation: 330 nm
	λ emission: 460 nm
Mobile phase	Acetonitrile/Water/Acetic acid (99/99/2)
Inject volume	100µL
Flow rate	1 mL/minute
Column temperature	40°C
Rising solvent	Acetonitrile
Analysis duration	12 minutes

2.5.5 Assessment of ochratoxin A daily intake 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 [39]. To assess ochratoxin A exposure, the mean level of these mycotoxins found in cowpea grains stored at 8 months together with the mean consumption of cowpea and the average body weight of individual adult were used to estimate the daily intake of OTA [40] [41]. According to Langyintuo et al (2003)

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[42], the daily consumption of cowpea is 4.93 g per capita/day. The OTA intake was calculated using formula 1:

EAI = (T x Q)/ bw

With EAI, the estimated of OTA daily intake in pg kg^{-1} of body weight (b.w.) day^{-1}; T, the OTA concentration found in cowpea grains stored (pg/kg); Q, the daily consumption of cowpea 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 mycotoxins fixed by the European Commission [43]. for cowpea "to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs" at level of 2 µg/kg for OTA. Moreover, the estimated intakes were compared to Tolerable Daily Intake (TDI) set at 5 ng/kg bw/day for OTA established by WHO [44] [45].

2.6 Statistical analysis

All analyzes were performed in triplicate and data were statistically processed using the SPSS software (version 20.0). It consisted of an analysis of variance according to two factors: the shelf life and the storage method that is to say, different types of packaging. The comparison of the average values of the measured parameters was performed by one-way ANOVA (STATISTICA Version 7.1) using post hoc test of small statistical difference (LSD). Mean values were considered significantly different at P = 0.05. The significant parameters were emerged and compared using the tukey test with a tolerance of difference less than or equal to 5%. Correlations between parameters were also assessed according to Pearson index then corrected by multiple statistical analysis variances (MSA) using STATISTICA software (version 7.1). The Excel 2007 software was used to build curves of evolution of the parameters over time.

3. RESULTS

3.1 Validation of OTA determination method using HPLC

Using HPLC device, Limit Of Detection (LOD) of ochratoxin A is 5 ng/kg and the Limit Of Quantification (LOQ) is 20 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. The rate of extraction recorded is 86.92±0.39%.

3.2 Evolution settings

Statistical tests indicate a significant change (P <0.01) the content of the parameters evaluated according to storage time and the type of packaging, if the cowpea was in PICS bags or not and whether it was treated or not treaty with the biopesticide (Tables II and III).

3.2.1 Evolution of moisture during storage

Table 2 shows the moisture content of cowpea grains stored in different PICS bags. With an average of 10.03 ± 0.21% at baseline (0 months), the moisture content increased significantly (P < 0.001) during the storage period (Table 3). The higher moisture values were recorded after 4.5 months of storage in the control bag without PICS (14.67 ± 0.15%) and 8 months of storage in the PICS control without biopesticide (14.10 ± 0.11%) (Figure 1). In PICS bags with different proportions of biopesticide moisture contents after 8 months of storage were similar with a average at 12.06 ± 0.11%. Furthermore, the interaction between the type of packaging and the storage period has a significant effect on this parameter as found in Tables 2 and 3.

3.2.2 Evolution of water activity values during storage

Table 2 shows the values of the water activity of cowpea grains stored in different PICS bags. The water activity of cowpea grains in control without PICS (TSP) increases faster in 4.5 months from 0.61 ± 0.01 (0 month) to 0.96 ± 0.01 (4.5 months). This pace is followed by the PICS bag without biopesticide (H0) that in 8 months increased from 0.61 ± 0.01 to 0.92 ± 0.01 (Figure 2). The water activity in 8 months cowpea storage was 0.86 ± 0.01 for H1 and H2. For H3 and H4 the variation is significant, but the average value is 0.70 ± 0.01. The interaction between the type of packaging and the storage time is significant with P = 0,001

3.2.3 Evolution of ochratoxin A content during storage

Table 2 shows the OTA content of cowpea grains stored in different PICS bags. With an average of 1.12 ± 0.01 µg/kg at the start (0 months), the OTA content increased significantly (P = 0.001) with a

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steep slope during the storage period and depending on the type of packaging (Table 2 and Figure 3). The highest OTA values were recorded after 4.5 months of storage in the control bag without PICS (TSP) (23.80 ± 0.40 µg/kg) and 8 months of storage in the control PICS bag without biopesticide (H0) (22.50 ± 0.87 µg/kg) (Figure 3). In PICS bags containing different proportions of biopesticide, the OTA content increased slightly over time (from 0-7 months) (Figure 3), regardless of the amount of biopesticide use, the variation was identical during the same period (Table 2). After 8 months of storage, a difference between the variations was found. Thus, the following values were observed: 9.37 ± 0.42 µg/kg, 4.25 ± 0.69 µg/kg, 4.33 ± 0.06 µg/kg and 4.0 ± 0.01 µg/kg respectively for H1, H2, H3 and H4. Furthermore, the interaction between the type of packaging and the storage period has a significant effect on this parameter as found in Tables 2 and 3.

Table 2. Evolution of the following parameters during storage of cowpea

Parameters	Storage duration	TSP	H0	H1	H2	H3	H4
Mo (%)	0	10,03±0,21^{aA}	10,03±0,21^{aA}	10,03±0,21^{aA}	10,03±0,21^{aA}	10,03±0,21^{aA}	10,03±0,21^{aA}
	1	10,24±0,06^{bA}	10,03±0,06^{aA}	10,07±0,03^{abA}	10,03±0,06^{aA}	10,09±0,10^{abA}	10,00±0,10^{aA}
	2	12,30±0,10^{bB}	10,22±0,02^{aA}	10,17±0,03^{aA}	10,18±0,03^{aA}	10,18±0,03^{aA}	10,17±0,01^{aA}
	4,5	14,67±0,15^{bC}	11,17±0,06^{aB}	10,99±0,01^{aB}	11,05±0,06^{aB}	11,01±0,03^{aB}	10,99±0,01^{aB}
	7	-	12,55±0,11^{bC}	11,63±0,06^{aC}	11,72±0,06^{aC}	11,54±0,04^{aC}	11,56±0,19^{aC}
	8	-	14,10±0,11^{bC}	12,06±0,12^{aD}	12,10±0,10^{aD}	12,06±0,06^{aD}	11,83±0,14^{aC}
Aw	0	0,61±0,01^{aA}	0,61±0,01^{aA}	0,61±0,01^{aA}	0,61±0,01^{aA}	0,61±0,01^{aA}	0,61±0,01^{aA}
	1	0,87±0,01^{bB}	0,62±0,01^{aA}	0,62±0,01^{aAB}	0,61±0,01^{aA}	0,61±0,00^{aA}	0,61±0,01^{aA}
	2	0,90±0,01^{cB}	0,68±0,01^{bB}	0,63±0,00^{aAB}	0,63±0,01^{aAB}	0,62±0,00^{aA}	0,62±0,01^{aAB}
	4,5	0,96±0,01^{cC}	0,73±0,00^{bC}	0,64±0,01^{aB}	0,63±0,01^{aAB}	0,63±0,01^{aAB}	0,63±0,00^{aAB}
	7	-	0,87±0,01^{cD}	0,71±0,01^{bC}	0,67±0,01^{aB}	0,65±0,01^{aB}	0,64±0,01^{aB}
	8	-	0,92±0,00^{dE}	0,86±0,01^{cD}	0,86±0,01^{bC}	0,71±0,01^{abC}	0,70±0,01^{aC}
OTA (µg/kg)	0	1,12±0,01^{aA}	1,12±0,01^{aA}	1,12±0,01^{aA}	1,12±0,01^{aA}	1,12±0,01^{aA}	1,12±0,01^{aB}
	1	7,02±0,19^{bB}	1,09±0,00^{aA}	1,08±0,01^{aA}	1,09±0,00^{aA}	1,11±0,01^{aA}	1,07±0,01^{aA}
	2	10,77±0,44^{bC}	1,60±0,05^{aA}	1,21±0,00^{aA}	1,09±0,01^{aA}	1,09±0,00^{aA}	1,09±0,00^{aAB}
	4,5	23,80±0,40^{cD}	2,94±0,04^{bB}	2,21±0,01^{aA}	2,12±0,01^{aA}	2,10±0,01^{aA}	2,10±0,01^{aB}
	7		15,11±0,08^{dC}	3,45±0,01^{cB}	3,03±0,01^{bB}	2,99±0,01^{bB}	2,00±0,01^{aC}
	8		22,50±0,87^{dD}	9,37±0,42^{cC}	4,25±0,69^{bC}	4,33±0,06^{abC}	4,00±0,01^{aD}

*The mean (± SD) with different lowercase / uppercase letters on the same line / in the same column are different test probability of 5%, **Mo**: moisture, **Aw**: water activity, **OTA**: Ochratoxins A, **TSP** = Control without PICS bag (polypropylene bag); **H0** = Control with PICS bag (no biopesticide); **H1** = PICS bag with 0.7% of biopesticide (w / w); **H2** = PICS bag with 2.5% biopesticide (w / w); **H3** = PICS bag with 4.3% of biopesticide (w / w); **H4** = PICS bag with 5.0% of biopesticide (w / w)*

Table 3. Statistical data for moisture, water activity and Ochratoxins A in cowpea grains according to the type of packaging during the storage period

Source of Variation	*df*		*Parameters*		
			Moisture	**Aw**	**OTA**
Types	5	SS	26,21	0,563	1291,73
		F-value	394,75	696,49	2774,60
		P-value	**<0,001**	**<0,001**	**<0,001**
Duration	5	SS	97,55	0,42	1079,31
		F-value	1469,06	517,76	2318,33
		P-value	**<0,001**	**<0,001**	**<0,001**
Types x duration	23	SS	30,78	0,27	1337,41
		F-value	100,75	72,46	624,51
		P-value	**<0,001**	**<.0,001**	**<0,001**
Error	68	SS	0,90	0,01	6,33
Total	102	SS	12543,21	49,25	5131,83

***SS**: sum of squares; **F-value**: value of the statistical test; **P-value**: probability value of the statistical test; df: degree of freedom, Aw: water activity; OTA : ochratoxine A*

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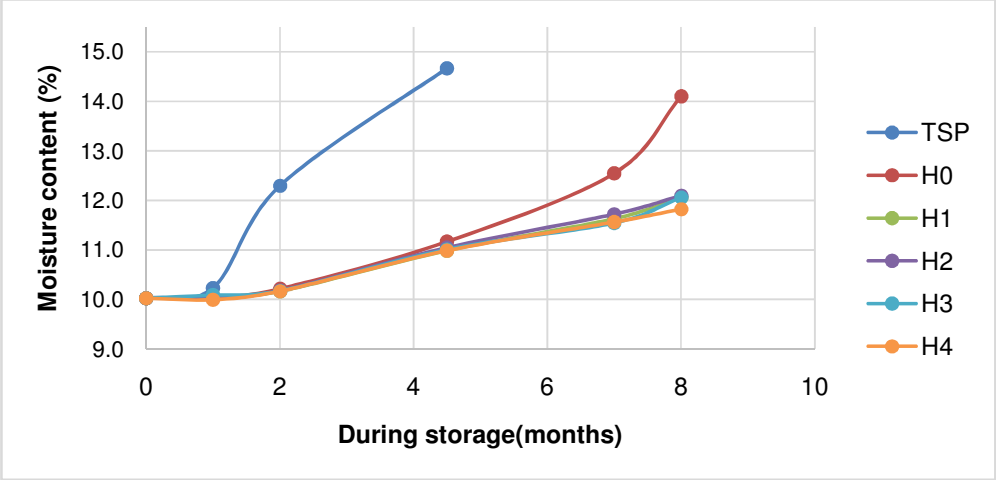

Figure 1: Evolution of moisture content during storage

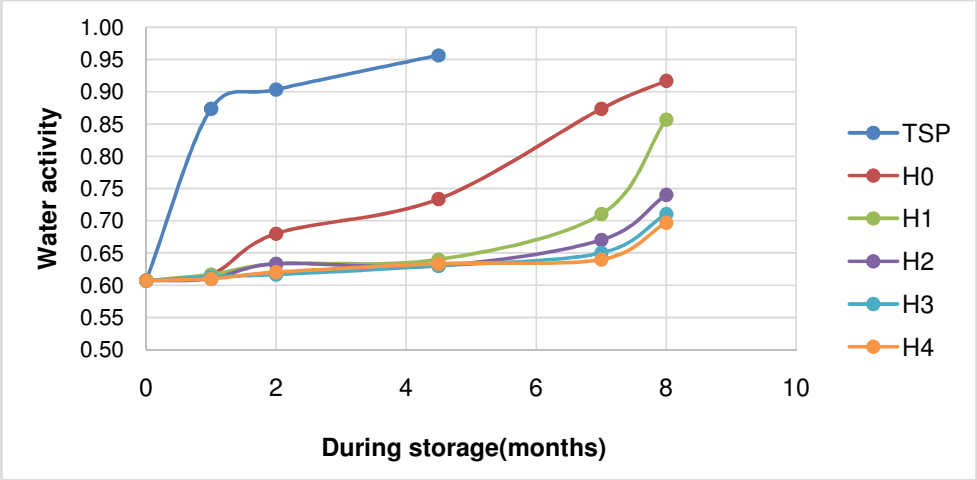

Figure 2 : Evolution of water activity during storage

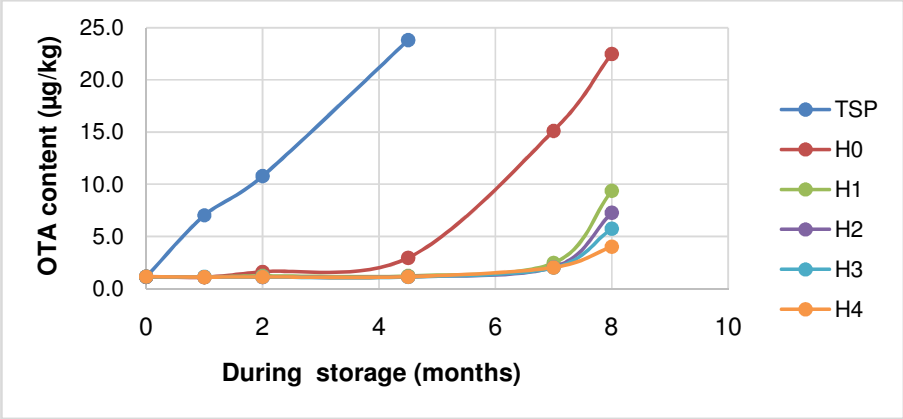

Figure 3: Evolution of Ochratoxins A contents during storage

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H0 : *Control with PICS bag (no biopesticide);* ***H1****: PICS bag with 0.7% of biopesticide (w / w);* ***H2****: PICS bag with 2.5% biopesticide (w / w),* ***H3****: PICS bag with 4.3% of biopesticide (w / w),* ***H4****: PICS bag with 5.0% of biopesticide (w / w)*

3.2.4 Correlations between moisture, water activity and the content of ochratoxin A

Table 4 depicts the correlation between moisture, water activity and the content of ochratoxin A in various types of packaging. The Pearson indexes (r) indicate positive and significant correlations between the three parameters studied for different types of packaging. Thus, moisture, water activity and the content of ochratoxin A are closely correlated during storage of cowpea, with r varying from 0.79 to 0.90. In addition, moisture and water activity change closely (r = 0.79). The ochratoxin A content is directly correlated with the moisture and water activity (respectively r = 0.88 and r = 0.90).

Table 4: Matrix of Pearson correlation indexes between moisture, water activity and Ochratoxin A during storage

	Moisture	Aw	OTA
Moisture	1,00		
Aw	0,79	1,00	
OTA	0,88	0,90	1,00

The values are significant at P = 0.05 ***; Aw*** *: water activity,* ***OTA*** *: Ochratoxine A*

3.2.5 Variability between types of packaging, moisture, water activity and ochratoxin A levels

Variability among the parameters studied and cowpea storage types was structured, first, by a principal component analysis (PCA). These analyzes were performed with the component (or factor) F1 which recorded an intrinsic value greater than 1, according to Kaïser rule (Table 5). The moisture content, water activity and ochratoxin A show negative significant correlations with F1. However, the component F2 (own value 0.22) is associated with F1 for the realization of PCA.

Figure 4.a shows the factors of the correlation circle of principal components analysis with the studied parameters of the stored cowpea. The first two factors (F1 and F2) respectively have values of 2.71 and 0.22. They express 97.55% of the variability (Table 5). The projection of the characters and individuals is made in the plane formed by the factors 1 and 2 (Figure 4b). There are 4 groups. Group 1 consists of two individuals namely the control without PICS at 4.5 months storage (noted TSP5) and PICS bag control without biopesticide at 8 months of storage (noted H08). The individuals in this group 1 parameters have similar values. Thus, this group is characterized by higher values of ochratoxin A, in moisture and water activity. The second group or individual is a control with PICS at 7 months storage (H07). The characteristics of its parameters differ from other individuals. Its parameters have similar trends to those of TSP5 and H08 but with slightly lower values. The third group is composed of three individuals who are the control without PICS at 1 and 2 months (TSP1, TSP2) and the PICS bag with 0.7% of biopesticide (H18). The values of their parameters are significantly different from other groups. These values are higher than those of group 4 settings and lower than those of group 2. The fourth group contains all samples PICS bags with biopesticide storage every month except H18 and control bag with PICS at 1 to 4, 5 months. This group is characterized by low levels of ochratoxin in moisture content and water activity.

The Ascending hierarchical classification (AHC) established by the "group method with arithmetic average" method (UPGMA) confirms the observed variability in the PCA (Figure 5). Indeed, at the distance of aggregation of 40, the dendrogram indicates four classes observed during storage of cowpea. The first class consists of 2 individuals TSP5 and H08 as the PICS bag control at to 7 months is the second class (H07). The third class consists of 3 individuals (TSP1, TSP2 and H18). These individuals are distinguished separately with relatively high values of the parameters studied. The fourth class includes all PICS bags with biopesticide except H18 and PICS bags control at 1, 2 and 4.5 months. Individuals in the latter group have low values of parameters and substantially similar.

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Table 5: Matrix of eigenvalues of factors resulting from the Principal Components Analysis and correlation with the moisture content, the water activity and the Ochratoxin A levels of the cowpea stored for 8 months

	Fact. 1	Fact. 2	Fact. 3
Eigenvalues	2,71	0,22	0,07
Variance (%)	90,35	7,20	2,45
Cumulative Variance (%)	90,35	97,55	100,00
Moisture	**-0,93**	0,35	0,09
Aw	**-0,94**	-0,30	0,13
OTA	**-0,98**	-0,04	-0,22

Aw *: water activity,* ***OTA*** *: Ochratoxine A*

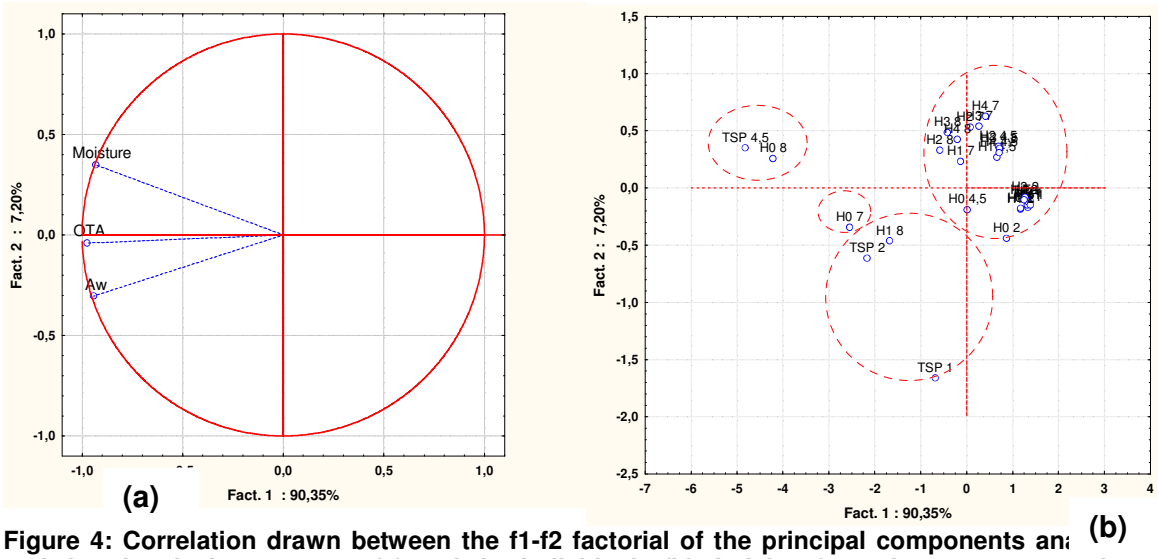

Figure 4: Correlation drawn between the f1-f2 factorial of the principal components and the chemical parameters (a) and the individuals (b) deriving from the cowpea samples studied

TSP (1 to 4,5 months)*, Control without PICS bag (polypropylene bag);* ***H0 (1 to 8 months)****, Control with PICS bag (no biopesticide);* ***H1 (1 to 8 months)****, PICS bag with 0.7% of biopesticide (w / w);* ***H2 (1 to 8 months)****, PICS bag with 2.5% biopesticide (w / w);* ***H3 (1 to 8 months)****, PICS bag with 4.3% of biopesticide (w / w);* ***H4 (1 to 8 months)****, PICS bag with 5.0% of biopesticide (w / w);* ***Aw****, water activity;* ***OTA****, ochratoxine A*

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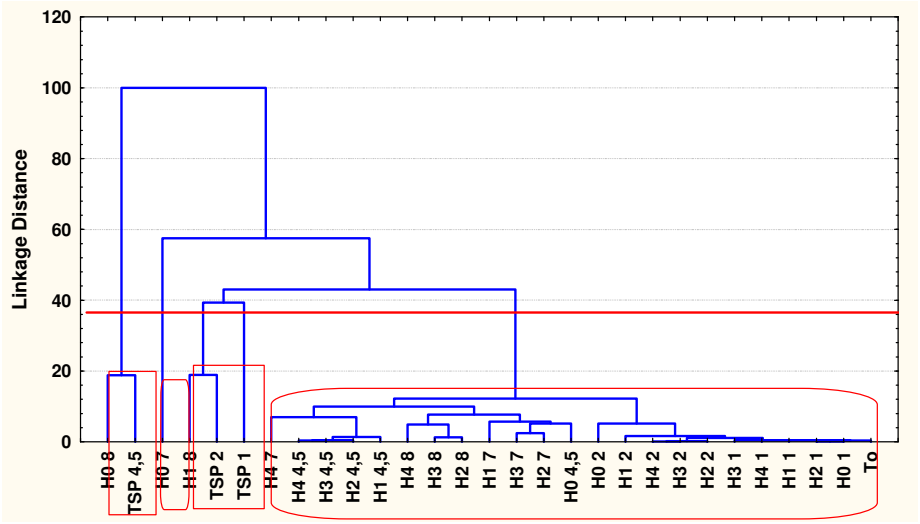

Figure 5: Dendrogram deriving with the ascending hierarchical classification of cowpea samples stored for 8 months according to the parameters assessed

***TSP (1 to 4,5 months)**, Control without PICS bag (polypropylene bag); **H0 (1 to 8 months)**, Control with PICS bag (no biopesticide); **H1 (1 to 8 months)**, PICS bag with 0.7% of biopesticide (w / w); **H2 (1 to 8 months)**, PICS bag with 2.5% biopesticide (w / w); **H3 (1 to 8 months)**, PICS bag with 4.3% of biopesticide (w / w); **H4 (1 to 8 months)**, PICS bag with 5.0% of biopesticide (w / w)*

3.2.6 Assessment of ochratoxin A intake from cowpea grains after storage

Table 6 shows the OTA intake estimated from the consumption of cowpea grains stored for 8 months. The estimated daily intake (DI) in the Ivorian adult consumers for OTA are 1,58±0,06 ng/kg bw/day, 0,66±0,03 ng/kg bw/day, 0,30±0,05 ng/kg bw/day, 0,30±0,00 ng/kg bw/day et 0,28±0,00 ng/kg bw/day respectively H0, H1, H2, H3 and H4 from the cowpea grain result. These traits appear to be largely below the tolerable daily intake set by WHO for respectively OTA (5ng/kg bw/day). Estimates of exposure to ochratoxin A witness without PICS (TSP) have not been calculated because its shelf life could not reach 8 months. At 4.5 months of storage pests have created significant damage such as market and hygienic qualities. So it was removed from storage. Recorded values represent 17.65%, of the maximum quantities of 5 µg/kg for ochratoxin A permitted

Table VI: Ochratoxin A intake estimated from the consumption of cowpea grains from Ivorian adult (intake ng/kg body weight/day)

	AFB1				
	H0	**H1**	**H2**	**H3**	**H4**
Daily Intake (DI) (ng/kg of boby weigth/day)	1,58±0,06	0,66±0,03	0,30±0,05	0,30±0,00	0,28±0,00
Estimated intake to MRL ($AELMR_1$) (µg/kg of cowpea)	2				
Reference value SCF (ng/kg of boby weigth/day)	5				
DI / SCF	0,32	0,13	0,06	0,06	0,06

***$AELMR_1$**: estimated intake for a maximum residue level of Ochratoxin A in cowpea*
***SCF**: Tolerable daily Intake recommended by the Scientific committee on food*
***H0 :** Control with PICS bag (no biopesticide); **H1**: PICS bag with 0.7% of biopesticide (w / w); **H2**: PICS bag with 2.5% biopesticide (w / w), **H3**: PICS bag with 4.3% of biopesticide (w / w), **H4**: PICS bag with 5.0% of biopesticide (w / w)*

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4. DISCUSSION

The results observed in this study show that the post-harvest storage method cowpea in PICS bags with a biopesticide used to limit the evolution of the concentration of ochratoxin A by mycotoxinogene germs. Thus, the values obtained in OTA cowpeas stored in PICS bags with biopesticide are lower and closer than those measured in the control polypropylene bag (TSP) or PICS bag control without biopesticide (H0). The good conservation properties of PICS bag on OTA production were observed over a period of 4.5 months. The measured values remain low and below the normative value set by the European Union which is 5 µg/kg [43]. However, beyond the shelf life (7 and 8 months), OTA levels increase significantly and exceed the normative value.

On storage PICS bag with the leaves of Lippa multiflora, insecticides effects and/or repellents are proven because the concentrations of OTA remain low and below the standard of the 8 months of storage with the exception of storage bag with PICS at 0.7% of biopesticide. These results are consistent with literature data [23]. These results also highlight the inhibitory properties or fungicides of leaves of Lippa multiflora on Aspergillus and Penicillium responsible for OTA production [46-47]. Similar results were also obtained by Gueye et al. (2013) [48] in the region of Kedougou in eastern Senegal. The moisture content of cowpea grains as well as the values of the water activity (Aw) are positively and strongly correlated with the concentrations of OTA (Table III). The water activity is an important parameter in food preservation. The most favorable conditions for development of species Apergillus ochraceus and Penicillium verrucosum producing OTA is a water activity (Aw) greater than 0.88 and temperatures between 6℃ and 47°C [49-50]. The water activity in the polypropylene bag (TSP) and the PICS bag control without biopesticide (H0) could be the basis for increased concentrations of ochratoxin A in them because the values of water activity measured is high; that is to say favor the development of fungal species. For the lot PICS with 2.5% biopesticide, concentrations of ochratoxin A obtained remain below the normative value despite the value of the high water activity (Aw = 0.86) in the eighth month of storage. The water activity is correlated to the moisture content of cowpea grains, increasing the moisture content promote the increase of the concentration ochratoxin A. Therefore, we suggest that for a better storage cowpea in PICS bags biopesticide with a 8 month period will require a minimum concentration of 2.5% biopesticide of incorporation for a satisfactory sanitary quality in relation to OTA.

Daily consumption of cowpea and its derivatives in Cote d'Ivoire is 4.93 grams per person [42]. Whereas the average weight of the adult population is 70 kg and the average concentrations of OTA after 8 months of cowpea storage, Acceptable Daily Intake (ADI) of each type of packaging is below the reference value. If the daily consumption of cowpeas in Côte d'Ivoire increases relative to a change in diet in rural areas, the daily dose of exposure to ochratoxin A could increase above the reference value. Right now a greater awareness should be conducted at the place of actor's cowpea to avoid toxigenic effects of OTA on consumers.

5. CONCLUSION

The study of the evolution of Ochratoxin A content during storage of cowpea in PICS bags with or without biopesticides has demonstrated the effectiveness of PICS bag and leaves of Lippia multiflora during the cowpea storage. We have seen a deterioration of the health quality of cowpea quickly when stored in polypropylene bag (shelf life of less than 4.5 months). This quality degradation results in a significant increase in moisture, the water activity values and concentrations of ochratoxin A. The triple bagging technique allows to extend the shelf life of cowpea grains up to 6 months. Using leaves of Lippia multiflora has potentially extended the sanitary quality of cowpea during the 8 months of storage. The inhibitory properties, fungicides, insecticides and repellents of leaves of Lippia multiflora were found. Therefore, the technology developed in this study could be an alternative to the use of synthetic pesticides in the storage and conservation of cereals and pulses. It is inexpensive, easy to perform and enter the Millennium guidelines for environmental protection.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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REFERENCES

1. Gnonlonfin GJ, Hell K, Adjovi Y, Fandohan P, Koudande DO, Mensah GA et al. A review on alfatoxin contamination and its applications in the developping world: a sub-saharian African perspective, Critical Review in Food Science and Nutrition. 2013 ; 53:349-365.
2. Bâcha H, Maaroufi K, Achour A, Hammami M, Ellouz F, Creppy EE. Ochratoxines et ochratoxicoses humaines en Tunisie. In: Creppy EE, Castegnaro M, Dirheimer G, editors. Human ochratoxicosis and its pathologies, 231. Colloque Inserm/John Libbey Eurotext; 1993. p. 111-21.
3. Yves Thé, Pierre Manda, Eric Elleingand, Felix Houphouët Yapi, Francis Adou Yapo, Jean David N'Guessan et al. Rôle de l'ochratoxine A dans le développement des tumeurs de la vessie chez les patients ivoiriens. Société Française de Toxicologie Analytique. Publié par Elsevier Masson SAS. 2015 ; 2352-0078/© 2014. French
4. Delage N, d'Harlingue A, Colonna B, Ceccaldi, Bompeix G. Occurrence of mycotoxins in fruit juices and wines. Food control. 2003 ; 14 : 225-227.
5. Da Rocha Rosa CA, Palacios V, Combina M, Fraga ME, De Oliveira Rekson A, Magnoli CE et al. Potential ochratoxin A producers from wine grapes in Argentina and Brazil. Food and Additives Contaminants. 2002 ;19:408-414.
6. Shephard GS, Fabiani A, Stockenstrom S, Mshicileli N, Sewram V. Quantitation of ochratoxin A in south African wines. Journal of Agriculture and Food Chemistry. 2003;51:1102-1106.
7. Otteneder H, Majerus P. Occurrence of ochratoxin A in wines : influence of the type of wine and its geographical origin. Food and Additives Contaminants. 2000;17:793-798.
8. Damien Dagbedji Toffa. Étude de la contamination de certains aliments d'origine végétale de la République du Niger les moisissures toxinogènes. Thèse de doctorat en Mycologie-Environnement, université mohammed v faculté des sciences rabat ; Juin 2015. French.
9. Tengo NS. Technique de conservation des légumineuses et sécurisation de la production des paysans: cas du niébé dans le département du Diamare. Mémoire de Master, Institut Supérieur de Sahel, Université de Maroua. 2011 ;142 pages. French.
10. Alene AD, Coulibaly O et Abdoulaye T. The world cowpea and soybean economies: Facts, trends, and outlook. Lilongwe, Malawi: Institut International d'agriculture tropicale. 2012.
11. D.P. Folefack *et al,* 2013. Vulgarisation de la méthode du triple ensachage pour le stockage amélioré du niébé en zone sahélienne du Nord Cameroun: Enjeux et perceptions paysannes. TROPICULTURA. 2013**;**31(3): 170-178. French.
12. K Hell, K Cardwell, M Setamou and HM Poehling. The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, West Africa. Journal of Stored Products Research. 2000;36: 365-382.
13. B Dubale, A Solomon, B Geremew, R Sethumadhava and S Waktole. Mycoflora of grain maize (Zea mays L.) stored in traditional storage containers (Gombisa and sacks) in selected woredas of Jimma zone, Ethiopia. African Journal of Food, Agriculture, Nutrition and Development. 2014; 14: 8676-8694.
14. P Fandohan, B Gnonlonfin, K Hell, WF Marasas and MJ Wingfield. Impact of indigenous storage systems and insect infestation on the contamination of maize with fumonisins. African Journal of Biotechnology. 2005;5(7):546-552.
15. Amadi and D Adeniyi. Mycotoxin production by fungi isolated from stored grains. African Journal of Biotechnology. 2009;8(7):1219-1221.
16. M Kankolongo, K Hell and I Nawa. Assessment for fungal, mycotoxin and insect spoilage in maize stored for human consumption in Zambia. Journal of Sciences Food and Agriculture. 2009; 10 p
17. Udoh J, Cardwell K, Ikotun T. Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. Journal of Stored Products Research. 2000 ; 36:187-201
18. DD Toffa, N Mahnine, L Ouaffak, A El Abibi, FZ El Alaoui, A Zinedine. First survey on the presence of ochratoxin A and fungi in raw cereals peanut available in the Republic of Niger. Food control. 2013;32:558-562.
19. FAO. Réglementations relatives aux mycotoxines dans l'alimentation humaine et animale, à l'échelle mondiale en 2003. Étude FAO Alimentation et Nutrition. 2003 ; N° 81, 188 p.French.
20. Commission Européenne. Règlement (CE) No 123/2005 de la commission du 26 janvier 2005 modifiant le règlement (CE) No 466/2001 en ce qui concerne l'ochratoxine A. Journal officiel de l'Union européenne. 2005 ;L 25, pp. 3-5. French.

UNDER PEER REVIEW

21. Camara A. Lutte contre Sitophylus oryzae L. (Coleoptera : Curculionidae) et *Tribolium castaneum* Herbst (Coleoptera : Tenebrionidae) dans les stocks de riz par la technique d'étuvage traditionnelle pratiquée en basse-Guinée et l'utilisation des huiles essentielles végétales. Thèse, Université du Québec à Montréal, Canada ; 2009. 173 p. French
22. Toumnou AL, Seck D, Namkosserena S, Cisse N, Kandioura N, Sembene M. Utilisation des plantes indigènes à effet insecticide pour la protection des denrées stockées contre des insectes ravageurs à Boukoko (Centrafrique). *International* Journal of Biological and Chemical Sciences. 2012 ;6:1040-1050. French
23. Ngamo TSL and Hance T Diversité des ravageurs des denrées et méthodes alternatives de lutte en milieu tropical. *Tropicultura.* 2007;25:215-220. French.
24. L Niamketchi, HG Biego, O Chatigre, D Amané, E Koffi, A 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.
25. M Isman. Botanical insecticides, deterrents, and repellents in modem agriculture and an increasingly regulated world. Annual Review of Entomology. 2006;51:45-66.
26. Tchoumbougnang F, Jazet Dogmo PM., Sameza ML, Mbanjo EGN, Fotso GBT, Zollo APH et al. Activité larvicide sur *Anopheles gambiae* Giles et composition chimique des huiles essentielles extraites de quatre plantes cultivées au Cameroun. Biotechnologie Agronomie Société Environnement. 2009 ;13(1):77-84. French.
27. .Mawussi G. Bilan environnemental de l'utilisation de pesticides organochlorés dans les cultures de coton, café et cacao au Togo et recherche d'alternative par l'évaluation du pouvoir insecticide d'extraits de plantes locales contre le scolyte du café (*Hypothenmus hampei* Ferrari). Thèse de Doctorat de l'Université de Toulouse, France ; 2008. 204 p. French.
28. Tia. Pouvoir insecticide des huiles essentielles de cinq espèces végétales aromatiques de côte d'ivoire dans la lutte contre les insectes phytophages 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. 2012 ; 205 p. French.
29. Keita SM, Umoetock SB et Smith JG. The insecticidal activity of petroleum ether extract of *Hyptis suaveolens* Poit (Labiatae) seeds on *Plutella xylostella* (L) (Lepidoptera: Yponomeutidae). Agricultural Journal. 2006;1:11-13.
30. Tapondjou LA, Adler C, Bouda H, Fontem DA. Efficacy of powder and essential oil firom *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. Journal of Stored Products Research. 2002 ;38:395-402.
31. T Ngamo, M Ngassoum, F Malaisse. Use of essential oil of aromatic plants as protectant of grains during storage. Agricultural Journal. 2007;2:204-209
32. 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.
33. L Alpsoy. Inhibitory effect of essential oil on aflatoxin activities. African Journal of Biotechnology. 2010;9:2474-2481
34. N Tatsadjieu, J Dongmo, M Ngassoum, FX Etoa, C Mbofung. 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
35. 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
36. AOAC. Preparation of standards for mycotoxins. AOAC International Official Methods of analysis. Natural Toxins. 2000; 49:4-5.
37. Mc Cormick. Determination of water activity. Mc Cormick and Company, Inc. Manual of technical methods and procedures. Baltimore, USA;1995.
38. Commission Européenne. Règlement (CE) No401/2006 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 Européenne. 2006 ;L70/12. French.
39. International R Kroes, D Muller, J Lambe, P Verger, A Visconti, "Assessment of intake from the diet" Food and Chemical Toxicology. 2002; vol.40, p.327-385,
40. OMS (Organisation Mondiale de la Santé), "Régime alimentaire, nutrition et prévention des maladies chroniques", Rapport d'une consultation OMS/FAO d'experts, Genève, OMS, Série de rapport technique. 2003 ; n° 916, p189. French.

UNDER PEER REVIEW

41. AS Langyintuoa, J Lowenberg-DeBoer, M Faye, D Lambert, G Ibro, B Moussa et al. Cowpea supply and demand in West and Central Africa Field Crops Research. 2003;82:215-231.
42. COMMISSION REGULATION (EU) amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. April 2011;No 420/2011
43. 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, p69-77.
44. JECFA, (Joint FAO/WHO Expert Committee on Food Additives and Contaminants), “Safety evaluation of certain mycotoxins in food”,WHO Food Additif. 2001; Series; 47.
45. Sharma N, Verma UK, 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; 8-13th August, pp 99-116
46. GOLY Cyrille, Yaya SORO, Brice KASSI, Adjehi DADIÉ, Siaka SORO, Marcellin DJE. Antifungal activities of the essential oil extracted from the tea of savanna (Lippia multiflora) in Côte d’Ivoire. Int. J. Biol. Chem. Sci. 2015;9(1):24-34.
47. Guèye MT, Seck D, Wathelet JP, Lognay G. Typologie des systèmes de stockage et de conservation du maïs dans l’est et le sud du Sénégal. Biotechnol. Agron. Soc. Environ. 2012;16:49-58.
48. André EL KHOURY. Champignons Mycotoxinogènes et Ochratoxine A (OTA) et Aflatoxine B1 (AFB1) dans les vignobles libanais : Occurrence et Origine. Thèse présentée pour obtenir le titre de docteur de l’institut national polytechnique de toulouse ; Juillet 2007. French.
49. Schuster E, Dunn-Coleman N, Frisvad JC, Van Dijck PW. On the safety of Aspergillus niger a review. Applied Microbiology and Biotechnology. 2002;59:426–435