Optimization of Post-Harvest Maize Storage using Biopesticides in Granaries in Rural Environment of Côte d'Ivoire

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Abstract: *Lippia multiflora Moldenke (or savannah tea) and Hyptis suaveolens Poit. Benth were tested for their efficacy in protected stored maize cobs and grains against insect damage and mycotoxins contamination in traditional and improved granaries in the center of Côte d'Ivoire. A full factorial design with two levels represented by four parameters affecting maize storage was used. These parameters were: storage time (2 and 6 months), quantity of biopesticides (0 and 5% w/w), storage structures (traditional and improved granary) and form of maize (grains and cobs). Results showed that optimum conditions for maize storage were obtained when maize grain were stored at 6 month with a combination of L. multiflora and H. suaveolens (2.5% w/w each, p<0.05) in traditional and improved granaries. In the planned optimal conditions, the experimental values were 2.21%, 2.16 µg/kg, 287.2 µg/kg, 1.45 µg/kg and 71.33 µg/kg for weight loss, aflatoxin B_1, fuminosin B_1, ochratoxin A and zearalenone respectively in traditional granary and 0.64%, 1.15 µg/kg, 222 µg/kg, 1.8 µg/kg, 50.26 µg/kg for weight losses, aflatoxin B1, fuminosin B1, ochratoxin A and zearalenone respectively in improved granary. These values of weight losses and mycotoxins levels were substantially equal to those predicted.*

Keywords: Maize, weight loss, mycotoxins, *Lippia multiflora*, *Hyptis suaveolens*, optimizing storage methods, experimental design

1. Introduction

Maize, Zea mays L. (Poaceae), makes a substantial contribution to the diets of rural and urban populations [1]. Its cultivation in the region has gradually increased over the years thanks to adoption of better production technologies and improved varieties [2], [3].

In Côte d'Ivoire, maize is cultivated globally by small-scale farmers and is widely grown across the different ecological zones, ranging from the northern savannah to the rain forest belt in the south with a production of 654.738 tons in 2012/2013 for total planted area of 327.800 ha [4], [5]. Maize serves as a major source of food, feed and raw material for agro-allied industries [6].

A recent USAID study highlighted the constraints of the maize sector in West Africa, one of which is postharvest storage [7]. Indeed, after harvest, inadequate infrastructure, and lack of economic means, constrains smallholder farmers to store the maize crop either shelled or unshelled using traditional storage structures and procedures such as rooms of house, cribs, baskets, polypropylene bags, earthen ware and clay granaries [8], [9].

Crops generally kept in these inadequate conditions and structures are subject to insects, rodents and fungi attack and farmers are often obliged to dispose of a significant proportion of the stored grain due to deterioration [10].

The storage fungi, *Aspergillus, Fusarium* and *Penicillium* species, are the major causes of grain deterioration in storage [11]-[14]. Under favourable conditions, some of these storage fungi produce mycotoxins such as aflatoxins, fuminosin, ochratoxin A, zearalenone and déoxynivalenol that result in toxicity hazards to human beings and animals if they are consumed [15], [16].

Control of storage pest is achieved in intensive, large scale production systems with chemical contact pesticides and fumigants [17]. However, application to high concentrations of these synthetic chemicals pesticides to control post-harvest of foodstuffs increases the risk of toxic residues in foodstuffs [18]. Because of the increasing sensitivity of the consumers to this residual pollution and the toxic effects of many synthetic chemicals pesticides, the importance of the use of natural alternate products becomes necessary [19]. In the same way, the restriction imposed by food industry and the organizations of regulation on use of certain synthetic food additives led to renewed interest in the search for alternatives approaches such as biopesticides, in particular those of vegetable origin [20], [21].

The use of aromatic plants, as insecticides and antimicrobic agents has two principal advantages: first they are natural origin which means more safety for the population and the environment and secondly they are considered at the weak risk of development of resistance by insects and pathogenic micro-organisms [22], [23]. Thus, the aim of this study was to optimize, from an experimental design, post-harvest storage of maize in traditional and improved clay granaries in

rural environment by using two leaves of two plants *Lippia multiflora* L and *Hyptis suaveolens*.

2. Material and Methods

2.1 Site Description

Experiments were carried out in the rural farming of Djedou located in the department of Botro (Gbêkê region) (latitude 7°50' North and longitude 5°18'West) in the center of Côte d'Ivoire, 40 km from Bouaké. This region has a humid tropical climate with four seasons, including two rainy seasons from March to June and from September to October with an average annual rainfall ranging between 1200 and 1500 mm. These are intersected by 2 dry seasons ranging from November to February and July to August. The recorded average temperatures range between 23°C- 30°C for 60%-70% of humidity [24].

2.2 Collection of maize used in study

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

2.3 Plant material collection and processing

Plant species *Lippia multiflora* (or savannah tea) and *Hyptis suaveolens* have been selected for their biopesticides properties. These plants are perennials and fragrant shrubs that develop spontaneously from the central to the Northern parts of the country due to the climatic environment [25], [26]. The leaves of *L. multiflora* and *H. suaveolens* were collected from area around Djedou village. After harvest, leaves of *L. multiflora* and *H. suaveolens* were drying at an average temperature of 30 °C for 6-7 days, and kept away from direct sun exposure.

2.4 Implementation of experiment

2.4.1 Characteristics of granaries

A cylindrical clay granary covered with a straw roof side has chosen for the experiment. The latter is commonly used by producers for cereals storage (maize, rice, millet, sorghum, etc.). The granary is built by an expert farmer who carries out work to these free hours after the fieldwork. Thus, the construction can last from 1 to 12 months. To alleviate the difficulty of construction, traditional granary was modified by removing the cylindrical roof which has been replaced by a simple device in its design. Modification related to substitute straw roof with a plastic cover which hermetically covers granaries. The granaries are raised from the ground to prevent moisture and rodent attack. They have a storage capacity between 9 and 12 m^3.

2.4.2 Protocol of maize storage

The experiment was conducted from January to September 2014. It focused on two sites: Djedou village for maize grain storage and Ngodrjenou camp located at 4 km from Djedou

for maize cobs storage. In each site, two improved granaries were tested along with the traditional granaries, all arranged in a randomised block design, and replicated two times.

Maize (cobs or grains) were on the one hand, intermittently sandwiched with layers of chopped dried leaves of *L. multiflora* and *H. suaveolens* (2.5% w/w of each plant) and on the other hand stored alone without biopesticides (control). The young cooperative of Djedou village has been associated with the different experimental studies. A total of 120 kg of maize (cobs and grains) were used by granary.

2.5 Application of full factorial design

A 2^4 full factorial experimental design was used to identify the relationship existing between the response functions and process variables, as well as to determine those conditions that optimized post harvest storage of maize [27]. The four independent variables or factors studied were the storage: time 2 and 6 months (X_1), quantity of biopesticides: 0 and 5% w/w (X_2), storage structures: traditional and improved granary (X_3) and form of maize: grains and cobs (X_4).

Each variable to be optimized was coded at the lower (-1) and higher (+1) levels (Table 1). The experimental design led to implementation of 16 trials.

The latter, corresponding to the different samples was obtained by combining the lower and higher levels of the different parameters studied, according to the experimental matrix described in Table 2. The coded values of the parameters are replaced by their actual values or states (Table 3) for randomization of the trials.

Sampling was carried out at 2 and 6 months, in triplicate. Thus, a randomly sample of 3 kg from each granary were taken through at top, in centre and on opening side at bottom. Maize samples were then transported to the laboratory where weight loss, aflatoxin B1, fuminosin B1, ochratoxin A and zearalenone measurements were made.

In the full factorial design, the main as well as the interaction effects of various factors are determined by fitting the data into 1er order polynomial equation:

$$Y_n = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{\substack{i=1 \\ i<j}}^{k} b_{ij} X_i X_j \qquad (1)$$

Where Y_n was the measured response, b_o the constant term, b_i and b_{ij} represent the coefficients of the linear and interactive effects, respectively, and X_i and $X_i X_j$ represent the linear and interactive effects of the independent variables (storage time, quantity of biopesticides, storage structures and form of maize), respectively.

Table 1: Experimental values and code levels of independent variables used for the 2^4 factorial designs

Factors	Technological Parameters	Code levels	
		Low (-1)	High (+1)
X_1	Storage time (months)	2	6
X_2	Quantity of biopesticides (%)	0%	5%
X_3	Storage structures	Traditional Granary	Improved Granary
X_4	Form of maize	Grains	Cobs

Table 2: Code of Matrix for full factorial experimental design

Run order	Level of Technological Parameters			
	X_1	X_2	X_3	X_4
1	-1	-1	-1	-1
2	+1	-1	-1	-1
3	-1	+1	-1	-1
4	+1	+1	-1	-1
5	-1	-1	+1	-1
6	+1	-1	+1	-1
7	-1	+1	+1	-1
8	+1	+1	+1	-1
9	-1	-1	-1	+1
10	+1	-1	-1	+1
11	-1	+1	-1	+1
12	+1	+1	-1	+1
13	-1	-1	+1	+1
14	+1	-1	+1	+1
15	-1	+1	+1	+1
16	+1	+1	+1	+1

Table 3: Experimental design of trial according to the matrix of the Full factorial

Run order	Level of Technological Parameters			
	X1	X2	X3	X4
	Storage time (months)	Quantity of biopesticides (%)	Storage structures	Form of maize
1	2	0	TG	Grains
2	6	0	TG	Grains
3	2	5	TG	Grains
4	6	5	TG	Grains
5	2	0	IG	Grains
6	6	0	IG	Grains
7	2	5	IG	Grains
8	6	5	IG	Grains
9	2	0	TG	Cobs
10	6	0	TG	Cobs
11	2	5	TG	Cobs
12	6	5	TG	Cobs
13	2	0	IG	Cobs
14	6	0	IG	Cobs
15	2	5	IG	Cobs
16	6	5	IG	Cobs

TG: traditional granary; IG: improved granary

2.6 Analytical Methods

2.6.1 Assessment of damage and weight loss

To assess the damage caused by insects during storage, samples of 1 kg (approximately 3500 maize kernels) were taken. After sifting and removal of the foreign matters, the grains were weighed and sorted to separate attacked and damaged grains from healthy grains. Then, the two fractions were weighed and counted separately.

The percent grain damage was estimated using the method of counting and weighing of [27], [28]. Assays were performed in duplicate. Thus, the rate of infection is the ratio of grains having at least one hole in the total number of grains. The estimate of the damage (D) and weight loss (W) is given by the formulas:

D (%) = (NGA / NTG) x 100
NGA = Number of grains attacked; NTG = Total Number of grains

W (%) = [[(NGA x PGS) – (NHG x WAG)] / (WHG x NTG)] x 100
NGA = Number of grains attacked; NHG = Number of healthy grains; NTG = Total Number of grains; WAG = Weight of attacked grain; WHG = Weight of healthy grains.

2.6.2 Determination of mycotoxins

Mycotoxins involved in this study were those commonly sought in cereals and pulses in tropical zone. Therefore, in our optimization study of post harvest maize storage in granary, by using full factorial experimental design, aflatoxin B1, fumonisin B1, ochratoxin A and zearalenone were required. Their assay was performed on high performance liquid chromatography (HPLC) with a fluorescence detector.

2.6.2.1 Aflatoxin B1

Aflatoxin B_1 (AFB$_1$) was extracted and cleaned up following official method of AOAC [30]. In a 250 mL erlenmeyer flask containing 25 g of ground maize, 100 mL of methanol-water (v/v, 80: 20) were added. The mixture was homogenized for 2 minutes and then stored at room temperature away from light for 12 hours. The extract obtained was then filtered through Whatman N°. 4 filter paper and 50 mL of the filtrate were added in 40 mL of phosphotungstic acid-zinc sulfate-water (5/15/980, m/m/v) and then kept at a ambient temperature for 15 min. Then the mixture was filtered again on Whatman N°. 4 filter paper in a flask to 500 mL separatory funnel. Aflatoxin was extracted from the filtrate with 3 volumes of 10 mL of chloroform. The extracts were collected into a 50 mL flask and then evaporated to dryness using a rotary evaporator (Buchi Rotavapor R-215) at 40 °C. At the dry extract were added 0.4 mL of hydrochloric acid and 4.6 mL of bidistilled water. The mixture was filtered through filter rezist in a chromatographic tube and then passed through an immunoaffinity column (column RiDA aflatoxin, Biopharm, Germany). The determination of aflatoxin B1 was carried out by HPLC Shimadzu liquid chromatograph (Kyoto, Japan) equipped with a fluorescence detector (λexc 365 nm; λem 435 nm), a column (Shim-pack GVP-ODS 250 mm x 4,6 mm) and a Shim-Pack pre-column (Shim-pack GVP-ODS 10 x 4,6 mm). The operating conditions were as follows: 20 µL of filtrate injection, isocratic mobile phase of methanol/water/acetonitrile (60: 20: 20, v/v/v), at flow rate of 0.5 mL/min. Calibration curves were prepared using standard solutions of aflatoxin B1 (Sigma-Aldrich, St Louis, MO, USA). Detection limits (LOD) of aflatoxin B1 were 6.18 ng/kg, while quantification limits (LOQ) were 6.50 ng/kg. Assays were performed in triplicate.

2.6.2.2 Fuminosin B1

Fumonisin B1 was assayed by the method described by AFNOR [31]. Thus, 25 g of maize sample was ground in the presence of 50 mL of water. At 5 g 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 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 No. 4 filter paper and then applied to a column immunoaffinity Fumoniprep (A. 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 assay was performed by a Shimadzu high performance liquid brand chromatograph equipped with a fluorescence detector, a C18 reverse phase column (5 ODS-20, 150 x 4.6 mm, Phenomenex). The isocratic mobile phase consisted of an acetonitrile/water/acetic acid (480: 520: 5, v/v/v) at a flow rate of 1 mL / minute. Detection limits (LOD) of fuminosin B1 were 5 ng/kg, while quantification limits (LOQ) were also 4.50 ng/kg. Assays were performed in triplicate.

2.6.2.3 Ochratoxin A

The entire maize 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 then centrifuged at 5000 rpm for 5 min at 4 °C. The supernatant was filtered through Whatman N°. 4 filter paper 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 using two volumes of 1.5 mL of PBS at a flow rate of 5 mL/min. The resulting sample was packed in a chromatographic tube and the analysis of OTA was made by HPLC with a Shimadzu brand fluorescence detector in compliance with the European community regulation [32]. Detection limits (LOD) of ochratoxin A were 0.3 ng/kg, while quantification limits (LOQ) were 2.4 ng/kg. Analyzes were performed in triplicate.

2.6.2.4 Zearalenone

The samples were analyzed according to a modified method of [33]. Thus, 25 g of crushed maize sample were extracted with 125 mL 75% acetonitrile in bidistilled water using a high speed blender for 2 minutes. After filtration on 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, UK) 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. Then 100 µL of the eluate was injected in HPLC chromatograph Shimadzu mark

with a fluorescence detector. The mobile phase consisted of acetonitrile/water/methanol (46: 46: 8 v/v/v) at a flow rate of 1 mL/min. The fluorometer was operated at excitation wavelengths and emission wavelengths respectively of 274 nm and 440 nm. Detection limits (LOD) of zearalenone were 3.75 ng/kg, while quantification limits (LOQ) were 2.50 ng/kg. Analyzes were performed in triplicate

2.7 Statistical analysis

All experiments were done in triplicate and data in tables and figures represent mean values ± standard deviation (n=3). Coefficient and experimental standard deviations were determined by the method of linear regression (MS Excel 2007). Comparison of mean values of measured parameters was performed by a one-way ANOVA (STATISTICA, version 7.1) using post hoc Low Statistical Difference (LSD) test. The mean values were considered significantly different when P=0.05.

3. Results

The full factorial design used was determined the combination of different levels of influential parameters that give the best compounds yields. Weight loss, aflatoxin B1, fuminosin B1, ochratoxin A and zearalenone were determined. For that 16 experiments (2^4) were conducted according the matrix presented in Table 4.

The values of regression coefficient determined are given in Table 5. The effect of individual variables and interactions effects was estimated [34].

Table 5 shows that all variables presented significant effect on maize storage.

Coefficient is known as statistically significant if its absolute value is strictly higher than the double of the experimental standard deviation, |coef|> 2σ [35].

Statistical analysis of data shows that variables such as storage time, quantity of biopesticides and form of maize presented significant effect on weight loss (Table 5). The most important parameter affecting weight loss is the storage time. Also, there is a significant interaction between storage time (X_1) and quantity of biopesticides (X_2). The predictive equation of weight losses (Y_1), neglecting the non-significant factors, is given by equation 1 with a satisfactory value R^2 (R^2 = 0.99).

$$Y_1 = 6.1 + 2.5 X_1 - 5 X_2 - 0.74 X_4 - 2.2 X_1 X_2 \qquad (2)$$

The interactions between the various factors influencing weight loss are illustrated in Figure 3. Weight loss increase significantly in the control granaries from 6.34% to 15.7% from the second to sixth months of storage. Whereas, in presence of biopesticides, interactions increase slightly from 0.85% to 1.37%.

Aflatoxin B_1 level (AFB_1) was affected by storage time and quantity of biopesticides. The most important parameter affecting level of aflatoxin B_1 is the same as in the case of weight losses (factor X_1). Two significant interactions were

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observed: storage time (X_1) - quantity of biopesticides (X_2) and storage time (X_1) – form of maize. The data showed a good fit with equation 2, being were statistically acceptable at $P=.05$ level and adequate with a satisfactory R^2 value ($R^2 = 0.98$). Equation 2 being developed to present the relationships between aflatoxin B_1 and post storage variables.

$$Y_2 = 30 + 22.6\ X_1 - 26\ X_2 - 20.2\ X_1X_2 + 5.5\ X_1X_4 \qquad (3)$$

During storage, aflatoxin B_1 content increase significantly in control granaries (without biopesticides) from 13.03% to 98.62% from the second to sixth months of storage whereas, in granaries with biopesticides, it gradually increase from 1.67% to 6.35% (Figure 4). The interaction between storage time and form of maize shows that aflatoxin B_1 level increases from 92.6% in cobs and 77% in grains.

Fuminosin B_1 level (FB_1) was influenced by storage time, quantity of biopesticides and storage structure. One significant interaction was observed: storage time (X_1) -

quantity of biopesticides (X_2). The mathematical model has a satisfactory value of R^2 ($R^2 = 0.98$)

$$Y_3 = 1091 + 304\ X_1 - 739.2\ X_2 - 176.1\ X_3 - 265.1\ X_1X_2 \qquad (4)$$

Fuminosin B_1 level increases significantly by 20% from the second to sixth month of storage in granaries with combination of *L. multiflora* and *H. suaveolens* (2.5% w/w each). Unlike in control granaries, this level increases significantly by 48% from 1261.04 µg/kg to 2399.29 µg/kg (Figure 5).

Study influence of different parameters on the level of ochratoxin A shows that it is influenced by storage time, quantity of biopesticides and form of maize. In addition, one significant interaction was observed between storage time (X_1) and quantity of biopesticides (X_2). Equation 4 describe the model of ochratoxin level with a satisfactory value of R^2 ($R^2 = 0.97$)

$$Y_4 = 14.85 + 7\ X_1 - 10\ X_2 + 2.7\ X_4 - 6.14\ X_1X_2 \qquad (5)$$

Table 4: Experimental design (2k, k=4) and corresponding responses

Run order	Independent variables				Experimental responses				
	X_1	X_2	X_3	X_4	Y^a_1	Y^b_2	Y^c_3	Y^d_4	Y^e_5
1	2 month	0%	TG	Grains	8.82±1*	20.26±2	1578.41±25	12.16±1	258.17±5
2	6 month	0%	TG	Grains	16.74±6**	89.26±3**	2875.29±37	32.26±1	354.26±3
3	2 month	5%	TG	Grains	1.23±1	1.26±1	245.25±18	3.52±1	39.26±1
4	6 month	5%	TG	Grains	1.95±1	4.79±1	325.15±12	5.26±1*	51.26±1*
5	2 month	0%	IG	Grains	7.8±1*	18.3±1	1250.16±16	10.5±1	245.45±2
6	6 month	0%	IG	Grains	15.25±2**	78.45±1**	2115.15±23	25.64±2	321.85±5
7	2 month	5%	IG	Grains	0.9±1$^+$	1.03±1	250.21±13	3.28±1	32.16±1
8	6 month	5%	IG	Grains	1.53±1	5.22±1	347.26±10	5±1*	49.26±1*
9	2 month	0%	TG	Cobs	4.94±1	7.25±1*	1257.02±8	13.26±1	219.26±4
10	6 month	0%	TG	Cobs	16.28±3**	124.16±6	3017.26±38	52.26±2	398.17±2
11	2 month	5%	TG	Cobs	0.68±1	2.22±1	387.22±6	4.99±1*	50.22±1*
12	6 month	5%	TG	Cobs	0.89±1$^+$	7.26±1*	451.06±9	6.26±1	75.02±1
13	2 month	0%	IG	Cobs	3.8±1	6.3±1*	958.56±4	10.47±1	208.74±2
14	6 month	0%	IG	Cobs	14.8±1	102.6±5	1689.45±27	41.23±2	369.52±2
15	2 month	5%	IG	Cobs	0.6±1	2.22±1	369.16±5	4.72±1*	55.22±1*
16	6 month	5%	IG	Cobs	1.09±1$^+$	8.13±1*	439.26±6	6.81±1	80.29±3

Data of the same column having the same sign are statistically in the same homogenous group at P=.05
TG: Traditional Granary; IG: Improved granary; Y1: Weight loss; Y2: Aflatoxin B_1 level; Y3: Fuminosin B_1 level; Y4: Ochratoxin A level;
Y5: zearalenone level; a: values given on dry matter basis; b: µg/kg; c: µg/kg; d: µg/kg; e: µg/kg

Table 5: Statstical estimates of coefficient and standard deviation

	Coefficient and standard deviations for each equation									
	Weight loss (Y_1)		Aflatoxin B_1 (Y_2)		Fuminosin B_1 (Y_3)		Ochratoxin A (Y_4)		Zearalenone (Y_5)	
Coefficient	Values	2σ	Values	2σ	Values	2σ	Values	2σ	Values	2σ
b_0	6.1*	0.43	30*	4.96	1091*	102.7	14.85*	2.1	175.5*	8.6
b_1	2.5*		22.6*		304*		7*		37*	
b_2	-5*		-26*		-739.2*		-10*		-121.4*	
b_3	-0.4[ns]		-2.1[ns]		-176.1*		-1.4[ns]		-5.2[ns]	
b_4	-0.7*		2.6[ns]		-20[ns]		2.7*		6.6[ns]	
b_{12}	-2.2*		-20.2*		-265.1*		-6.14*		-27.1*	
b_{13}	-0.04[ns]		-1.8[ns]		-96.1[ns]		-0.8[ns]		-2[ns]	
b_{23}	0.4[ns]		5.5*		24.1[ns]		2.2[ns]		11.75*	
b_{14}	0.3[ns]		2.3[ns]		175.7*		1.4[ns]		5.3[ns]	
b_{24}	0.4[ns]		-1.7[ns]		79.7[ns]		-1.9[ns]		4.55[ns]	
b_{34}	0.05[ns]		-0.6[ns]		-40[ns]		-0.3[ns]		1.58[ns]	

*[ns]: no significant values; *: significant data at $P=.05$.*

Figure 6 shows the effect of interaction between the storage period and quantity of biopesticides on ochratoxin A level. This level increases significantly from 69.35% at control granaries from 11.60% to 37.85% the second to sixth months of storage, but on the other hand in granaries with 5% of biopesticides, it increases slightly 29.16% from 4.13% to 5.83%.

Influence of different parameters on zearalenone level revealed that it depends on storage time and quantity of biopesticides. The most important parameter affecting level of zearalenone is the same as in the case of weight losses, aflatoxin B_1, fuminosin B_1 and ochratoxin A (factor X_1). Two significants interactions were observed: storage time (X_1) - quantity of biopesticides (X_2) and storage time (X_1) – form of maize (X_4). The data showed a good fit with equation 5, being were statistically acceptable at P=.05 level and adequate with a satisfactory R^2 value (R^2 = 0.99). Equation 5 being developed to present the relationships between Zearalenone and post storage variables.

$$Y_5 = 175.5 + 37\ X_1 - 121.4\ X_2 - 27.1\ X_1X_2 + 11.75\ X_1X_4 \qquad (6)$$

Figure 7 shows the effects of interaction between, on the one hand, storage time and quantity of biopesticides and, secondly, storage time and form of maize on zearalenone level. These levels in granaries control increase significantly from 232.90 µg/kg to 360.95 µg/kg in the second to sixth months of storage, whereas in granaries with 5% of biopesticides, they increase significantly from 44.22 µg/kg to 63.96 µg/kg

4. Discussion

The results presented in this study show that the methods of post harvest of maize storage with the two local species plants, *Lippia multiflora* and *Hyptis suaveolens* in traditional and improved granaries are able to reduce development of pest alteration of maize. Indeed, lower levels of weight loss, aflatoxin B_1 of fuminosin B_1, ochratoxin A and zearalenone were observed in granaries with 5% of biopesticides during storage. The combination of the two plants materials to 2.5% w/w of each is effective in comparison with the untreated control maize. Indeed, insecticidal and/or repellent activity of the leaves of these plants would be due to the release of bioactive molecules in their essential oils [22].

These results are consistent with the findings of Gueye *et al.* [36] who reported the repellent effect of dried leaves of *Hyptis spicigera* and *Hyptis suaveolens* against maize weevil *Sitophilus zeamais* and *Tribolium castaneum* in traditional granaries over a period of 7 months in Kedougou region Eastern Senegal. Ukeh *et al* [8], [37] also demonstrated the insecticidal activity of powders to 10% w/w and essential oils of *Aframomum melegueta* and *Zingiber officinale* (Zingiberaceae) which significantly reduce the progeny of maize weevil populations in traditional African granaries over a period of about 3 months in Obudu, southeast Nigeria.

These results are consistent with the findings of Tia [25] who reported the insecticidal effects of essential oils of *L. multiflora* and *H. suaveolens* against larval development of *Plutella. xylostella* and *Bemissia tabaci* both herbivorous

insects with lethal dose inducing 50% mortality (LD_{50}) and lethal time inducing 50% mortality (LT_{50}) values of 4.22 µg/L and 7.53 µL/L and 0.22 h and 4.35 h. This author showed that bioactive molecules of *L. multiflora* primarily comprises oxygenated monoterpenes such as linalool and 1,8-cineole whereas those of *H. suaveolens* are dominated by monoterpene hydrocarbon including sabinene, β-pinene and limonene which ones are the major compounds, respectively.

Aflatoxin B_1 (AFB1) and ochratoxin A (OTA) levels to six months storage are superior to the standard that was 5 µg/kg [32]. This increase AFB_1 and OTA levels could be due to a decrease inhibitory activity against *Aspergillus* growth of the plants materials. Similar observations were made by Liu *et al* [38] who have shown that the effectiveness of essential oils based on biopesticides decreases rapidly because of volatile bioactive molecules massively released in the first days after application. However, it should be noted that the presence of these plants materials has significantly decreased the levels of aflatoxin B_1 and ochratoxin A in granaries compared to controls.

Sharma *et al.* [39] showed that the essential oil of *H. suaveolens* has an inhibitory activity on *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus ochraceous* producing mycotoxins such as aflatoxin B_1 and ochratoxin A at level of 500 mg/kg. In addition, study of Tatsadjieu *et al.* [23] also showed that the essential oil of *Lippia rugosa*, a species of the genus Lippia, inhibits the growth of *Aspergillus flavus* and limits the production of aflatoxin B1 to an inhibitory concentration of 1000 mg/L.

Higher fuminosin B_1 levels observed in control granaries reflects the intense insect activity promoting the development of germs. Indeed, Fandohan *et al.* [9] found a significant correlation between the damage caused by lepidopteran beetle infestation and *Fusarium* and contamination of fuminosins B_1 and B_2 in maize stored in Benin. Furthermore, fuminosin B_1 levels in granaries with a combination of *L. multiflora* and *H. suaveolens* 2.5% w/w each are lower than the standard 2000 µg/kg for Fuminosin B_1 and B_2 [40].

As for the other studied mycotoxins, the presence of combination of *L. multiflora* and *H. suaveolens* in granaries limited significantly the increase of zearalenone level compared to the control. Zearalenone levels measured were all below the standard that was set at 200 µg/kg [40].

The results of the experimental analysis shows that post harvest maize storage is favored when the variable storage time, quantity of biopesticides and storage structure were at their highest levels (+1) and when form of maize was variable its low level (-1). Thus, the optimum process of post harvest maize storage involves the following parameters:
- Storage time: 6 months
- Quantity of biopesticides: 5%
- Form of maize: grains
- Storage structure: traditional granary and improved granary

4.1 Validation of 2^4 Full Factorial Design Optimization of post harvest maize storage

The results of the full factorial design were used to determine the optimal conditions for post harvest maize storage. All the models were established with a satisfactory coefficient of determination R^2, ranging from 0.97 to 0.99; which means a close agreement between the experimental results and those predicted by the models. The predictive quality of every model was also tested at the recommended optimum condition. All the responses were replicated three times at the optimum condition, and the results are presented in Table 6.

The arithmetic means of the experimental values were 2.21%, 2.16 µg/kg, 287.2 µg/kg, 1.45 µg/kg and 71.33 µg/kg for weight loss, aflatoxin B_1, fuminosin B_1, ochratoxin A and zearalenone respectively in traditional granary, and 0.64%, 1.15 µg/kg, 222 µg/kg, 1.8 µg/kg and 50.26 µg/kg for weight loss, aflatoxin B_1, fuminosin B_1, ochratoxin A and zearalenone respectively in improved granary.

Experimented data were approaching the predicted values. This indicated that the optimization achieved in the present study was reliable. Deviations between experimental values and the predicted values can be explained by the lack of perfectly fitted models and experimental errors.

Table 6: Experimental data for verification of the models predicted at optimal condition

Optimal condition		TG		IG	
		Pred	Exp	Pred	Exp
X_1= 6 month	Weight loss (%)	2.21[*]	3.57±1[*]	0.64[**]	0.66±0[**]
X_2= biopest 5%	AFB_1 (µg/kg)	2.16[*]	2.76±1[*]	1.15[**]	0.90±1[**]
X_3= IG and TG	FB_1 (µg/kg)	287.7[*]	332.5±12[*]	222[**]	214.8±4[**]
X_4= Maize grains	OTA (µg/kg)	1.45[*]	1.63±1[*]	1.8[**]	1.3±1[**]
	ZEN (µg/kg)	71.33[*]	72.6±7[*]	50.26[**]	52.35±1[**]

Data of the same line having the same sign are statistically in the same homogenous group at P=.05
Pred= predicted values, Exp= experimental values
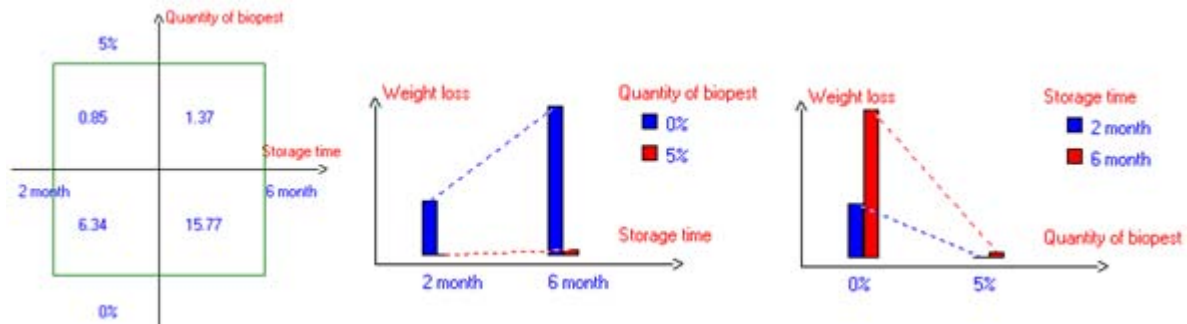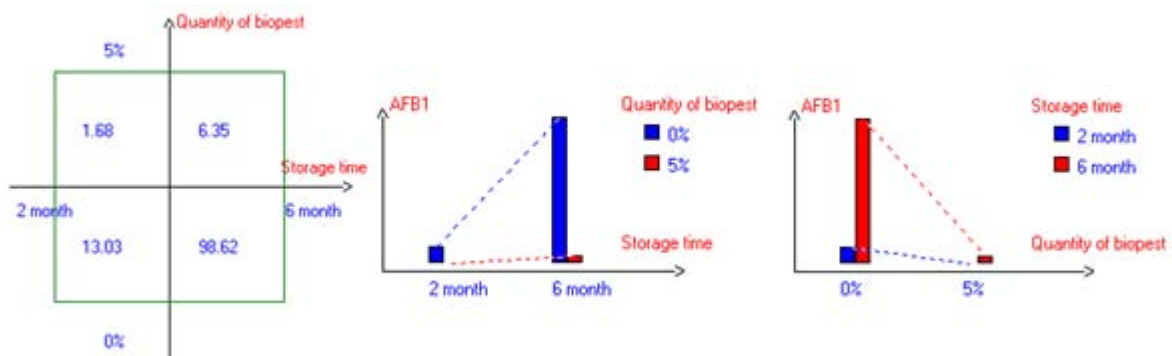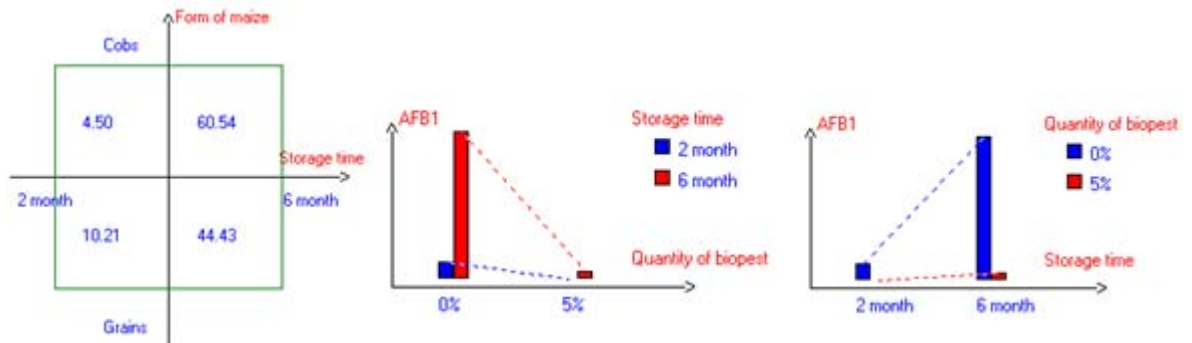TG: Traditional granary; IG: Improved granary

Figure 3: Interaction between storage time and quantity of biopesticides affecting weight loss

A- Interaction storage time/quantity of biopesticides affecting aflatoxin B_1 level

B- Interaction storage time/quantity of biopesticides affecting aflatoxin B_1 level

Figure 4: Interaction between factors affecting aflatoxin B_1 level
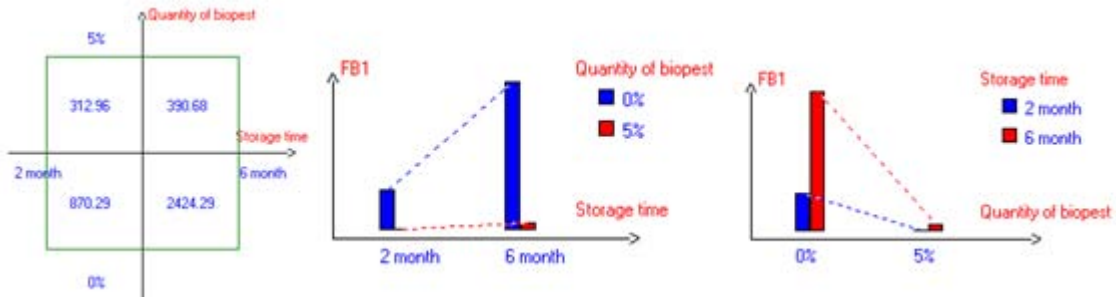

Figure 5: Interaction between storage time and quantity of biopesticides affecting fuminosin B_1 level
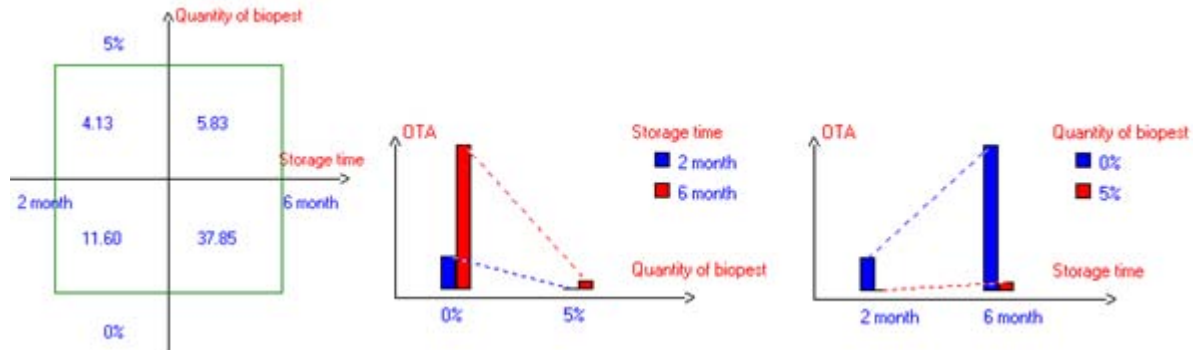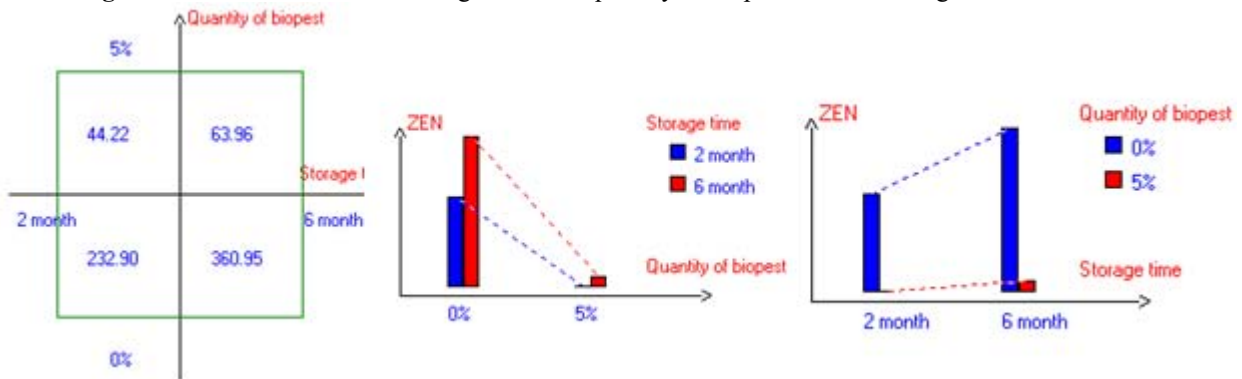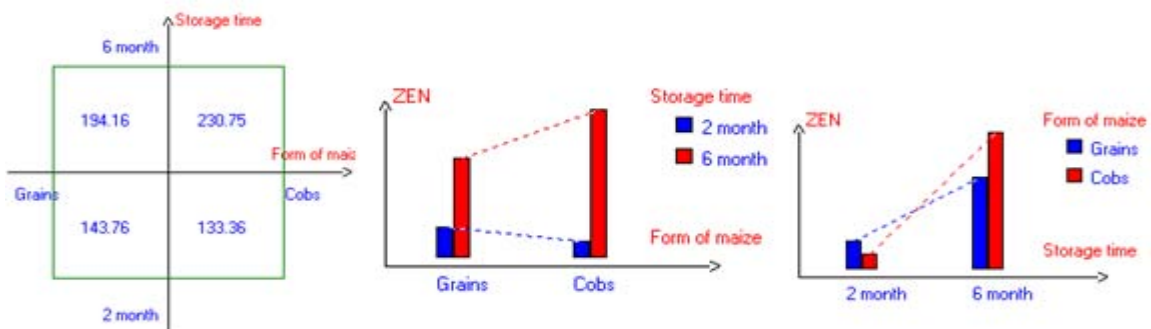

Figure 6: Interaction between storage time and quantity of biopesticides affecting ochratoxin A level

C- Interaction storage time/quantity of biopesticides affecting zearalenone level

D- Interaction storage time/form of maize affecting zearalenone level

Figure 7: Interaction between factors affecting zearalenone level

5. Conclusion

The results of this study indicate that *L. multiflora* and *H. suaveolens* leaves are effective for post-harvest maize storage in granaries against the insect pests and fungal contamination. The use of experimental design showed that it is possible to store maize over a period of six months without altering the market and healthy qualities of the grain. Moreover, post harvest maize storage developed that uses biopesticides in a traditional and improved granaries has been validated by an experimental design. This technique is inexpensive, easily carried and fits into the millennium guidelines of respect for the environment.
However, the study needs further investigation for a better control of the storage conditions to ensure the market qualities, nutritious and hygienic maize after storage.

6. Acknowledgements

References

[1] FAOSTAT. FAO Statistics Division. FAOSTAT. Production. Crops, 2011. Retrieved on August 12, 2015 from: http://faostat3.fao.org/home/index.html.

[2] M. Smale, D. Byerlee, T. Jayne. Maize revolutions in Sub-Saharan Africa. In: Policy Research Working Paper Series 5659. The World Bank, 2011. Retrieved 22/08/2015, from. http://www-wds.worldbank.org/servlet/WDSContentServer/WDSP/

[3] I. Baoua, A. Laouali, B. Ousmane, D. Baributsa and L. Murdock. PICS bags for post-harvest storage of maize grain in West Africa. Journal of Stored Products Research. 2014;58:20-28.

[4] K. Kouakou, L. Akanvou, A. Konan and A. Mahyao. Stratégies paysannes de maintien et de gestion de la biodiversité du maïs (Zea mays L.) dans le département de Katiola, Côte d'Ivoire. Journal of Applied Biosciences. 2010;33:2100 – 2109

[5] A. N'da, L. Akanvou and K. Kouakou. Gestion locale de la diversité variétale du maïs (*Zea mays* L.) violet par les Tagouana au Centre-Nord de la Côte d'Ivoire. International Journal of Biological and Chemical Sciences. 2013;7(5): 2058-2068

[6] K. Deffan., L. Akanvou, R. Akanvou, G. Nemlin and P. Kouamé. Evaluation morphologique et nutritionnelle de varieties locales et améliorées de maïs (Zea mays L.) produites en Côte d'Ivoire. Afrique Sciences. 2015;11(3): 181-196.

[7] P. Boone, J. Stathacos and R. Wanzie. Sub-regional. Assessment of the Maize Value Chain in West Africa. ATP Project, Abt Associates Inc., Bethesda, MD. 2008.

[8] D. Ukeh, S. Umoetok, A. Bowman, A. Luntz, J. Pickett and M. Birkett. Alligator pepper, *Aframomum melegueta*, and ginger, *Zingiber officinale*, reduce stored maize infestation by the maize weevil, *Sitophilus zeamais* in traditional African granaries. Crop Protection. 2012; 32:99-103.

[9] P. Fandohan, B. Gnonlonfin, K. Hell, W. F. Marasas and M. J. 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.

[10] M. Dejenea, J. Yuen, R. Sigvald. Effects of storage methods, storage time and different agro-ecological zones on chemical components of stored sorghum grain in Hararghe, Ethiopia. Journal of Stored Products Research. 2006;42:445–456

[11] K. Hell, K. Cardwell, M. Setamou and H.-M. 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.

[12] Amadi and D. Adeniyi. Mycotoxin production by fungi isolated from stored grains. African Journal of Biotechnology. 2009;8(7): 1219-1221.

[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] A. Di Domenico, D. Christ, E. Hashimoto, C. Busso and S. Coelho. Evaluation of quality attributes and the incidence of Fusarium sp. and Aspergillus sp. in different types of maize storage. Journal of Stored Products Research. 2015; 61: 59-64.

[15] S. Atanda, P. Pessu, S. Agoda, I. Isong, O. Adekalu, M. Echendu and T. Falade. Fungi and mycotoxins in stored foods. African Journal of Microbiology Research. 5 (25): 4373-4382.

[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] O.Atanda, I. Akpan and F.Oluwafemi. The potential of some spice essential oils in the control of A. parasiticus CFR 223 and aflatoxin production. Food Control. 2007; 18: 601–607.

[18] E. Adjou and M. Soumanou. Efficacité des extraits de plantes dans la lutte contre les moisissures toxinogènes isolées de l'arachide en post-récolte au Benin. Journal of Applied Biosciences. 2013 ; 70: 5555- 5566

[19] S. Bankole and O. Mabekoje. Mycoflora and occurrence of aflatoxin B1 in dried yam chips from markets in Ogun and Oyo States, Nigeria. Mycopathologia. 2004; 157: 111-115.

[20] M. Isman. Botanical insecticides, deterrents, and repellents in modem agriculture and an increasing1y regu1ated world. Annual Review of Entomology. 2006; 51: 45-66.

[21] L. Alpsoy. Inhibitory effect of essential oil on aflatoxin activities. African Journal of Biotechnology. 2010; 9: 2474-2481

1735

[22] T. Ngamo, M. Ngassoum and F. Malaisse. Use of essential oil of aromatic plants as protectant of grains during storage. Agricultural Journal. 2007 ; 2: 204-209.

[23] N. Tatsadjieu, J. Dongmo, M. Ngassoum, F.-X Etoa. and 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.

[24] A.Kouassi, A. Mamadou, K. Ahoussi and J. Biemi. Simulation de la conductivité électrique des eaux souterraines en relation avec leurs propriétés géologiques: cas de la Côte d'Ivoire. Revue Ivoirienne des Sciences et Technologie. 2013;21: 138-166.

[25] V. 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. 2012, Abidjan, 205 p

[26] A. Ekissi, A. Konan, A. Yao-Kouame, B. Bonfoh and S. Kati-Coulibaly. Sensory evaluation of green tea from Lippia multiflora Moldenke leaves. European Scientific Journal. 2014;10: 534-543

[27] N. Aboua, B. Yao, S. Gueu, A. Trokourey. Optimization by experimental design of activated carbons preparation and their use for lead and chromium ions sorption. Res. J. Agric. Biol. Sci. 2010; 6: 665-670.

[28] Harris K. L. et Lindblad C. J. (1978). Post-harvest grain loss assessment methods- American Association of Agricultural Chemists, St Paul, Minnesota, 193 p.

[29] R. Boxall. A critical review of the methodology for assessing farm level grain losses after harvest. Report of the Tropical development and Research Institute. 1986; G191:139 p.

[30] AOAC. Aflatoxins in corn, raw peanuts and peanut butter: immunoaffinity column (aflatest) method. AOAC International. 2005.

[31] Association Française de Normalisation (AFNOR) NF EN 13585. V03-124. Bailly J.D. *et al*., 2005, Rev. Med. Vet., (156), 547-554.

[32] Commission Européenne. Règlement (CE) N° 401/2006 de la commission du 23 février 2006 portant fixation des modes de prélèvement d'échantillons et des méthodes d'analyse pour le contrôle officiel des teneurs en mycotoxines des denrées alimentaires. Journal Officiel de l'Union Européenne L70/12. 2006.

[33] M. Miraglia and C. Brera. Mycotoxins in Grains and Related Products. In "Food Analysis by HPLC". Leo M.L Nollet 2nd eds. Marcel Dekker, Inc. New York. 2000; Chapter n° 12. pp: 493-522.

[34] M. Feinberg. La validation des méthodes d'analyse: approche chimiométrique de l'assurance qualité au laboratoire, Paris:Masson. French. 1996.

[35] E. Assidjo, B. Yao, E. Akou and G. Ado. Optimisation of the treatment conditions of cocoa butter in order to reduce non-quality. Journal of chemom. 2005; 19: 543-548.

[36] M. Gueye, G. Goergen, S. Ndiaye, E. Asieedu, J-P Wathelet, G. Lognay and D. Seck. Efficiency of Traditional Maize Storage and Control Methods in Rural Grain Granaries: a Case Study from Senegal. Tropicultura. 2013; 31: 129-136.

[37] D. Ukeh, M. Birkett, J. Pickett, A. Bowman and A. Luntz. Repellent activity of alligator pepper, *Aframomum melegueta*, and ginger, *Zingiber officinale*, against the maize weevil, *Sitophilus zeamais*. Phytochemistry. 2009;70:751–758.

[38] Z. Liu and S. Ho. Bioactivity of the essential oil extracted from *Evodia rutaecarpa* Hook f. and Thomas against the grain storage insects, *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst). Journal of Stored Product Research. 1999;35: 317-328.

[39] N. Sharma, U. Verma and A. Tripathi. 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.

[40] Commission Européenne. Règlement (CE) N° 1881/2006 de la commission du 19 décembre 2006 portant fixation des teneurs maximales pour certains contaminants dans les denrées alimentaires. Journal Officiel de l'Union Eupéenne.

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