Optimization of Storage Methods of Cowpea (*Vigna Unguiculata* L. Walp) Bagged Pics Containing Biopesticide (*Lippia Multiflora*) By Central Composite Experimental Design in COTE D'IVOIRE

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Abstract*— *The dried leaves of Lippia multiflora were tested for the efficiency on the stored cowpea kernels in PICS bags. A central composite design with five levels represented by two factors affecting the beans storage was used for control the evolution of merchantability (weight loss) and health (AFB1, OTA and Aw) quality during the storage. The factors were: storage time (1 to 8 months) and quantity of biopesticides (0 to 5% of the container mass). Results showed that it is possible to assess ideal conditions to keep the cowpea kernel merchantability and health qualities during storage. The quality of the kernels maintained for a concentration in biopesticide greater than or equal to 1,26% during 8 months. In the planned optimal conditions, the experimental values were 3.50±0.50%, 1.48±0.3 µg/kg, 4.54±0.02 µg/kg and 0.71±0.03 for weight losses, aflatoxin B1, ochratoxin A and water activity (Aw) respectively. These values of weight losses, mycotoxins levels and were substantially equal to those predicted by the experimental model.

Keywords*— *Cowpea, PICS bags, biopesticides, central composite design, safety quality.

I. INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp), the first legume of world is an important source of protein and less expensive for African peoples. Cowpea kernels contain essential amino acids. (Smart, 1964; Hignard, 1998; Archana et Jawali, 2007). It is rich in minerals, and vitamins that are essential for optimum health. Thus, cowpea is a high nutritional value of food that could help the Ivorian fight against food shortages. With a global annual production of 3.3 million tons (Kouakou *et al*, 2007), the cowpea are consumed in various forms in West Africa (fried, boiled, mashed, paste and sauce). The others parts of plant are used for animals food (Chapon, 2002). It also contributes to the fertility of soil by fixing nitrogen. Beyond the agronomic and nutritional interest, cowpea has also a socio economic importance (Doumma *et al*., 2011). It is used in funeral rites and various religious ceremonies. (Mukendi *et al*., 2013). Finally, cowpea is a savings in case of necessity (returned school, diseases), hence the importance of postharvest treatments, especially the storage conditions for cowpea quality. Unfortunately, storage is difficult for the farmers because of the pests as weevils. (Agyen-Sampong, 1978; Doumma *et al*., 2011). Environmental conditions and bad practices postharvest cause both damage. Damages start from the field where the larvae of weevil infest cowpea kernel (Huignard, 1995). After harvest, the infested kernels are stored. Studies showed that a rate of 10% of larvae sufficient to degrade 100 % of the harvest in a few months causing weight losses of up to 60 to 70% (Ngamo *et al*., 2007 ; Okunola *et al*., 2007). Furthermore, during their developing larvae remove nitrogen in the form of toxic uric acid that builds up inside the grain making cowpea parasitized undrinkable (Gauthier, 1996). Another aspect inherent in the development of insects is colonization by fungi stocks (Elmer *et al*., 2001; Wen *et al*., 2005). These storage fungi promote quality deterioration by producing mycotoxins such as aflatoxins, ochratoxin A, which are harmful to the health of consumers (Kankolongo *et al*., 2009; Atanda *et al*, 2015.). To face these destructive of stock, producers often use syntheses of pesticides (sometimes prohibited) which misuse the precautionary failure in handling and non-compliance with waiting periods can lead to insect resistance and harmful to environmental and health problems (Kétoh, 1998).

Thus, given the magnitude of the damage caused by the use of these chemicals, the use of biopesticides as an alternative solution has been encouraged in recent decades (Bambara *et al* 2008. Gueye *et al*, 2011; Kayombo *et al*. , 2014). The use of plants for protection is an ancient practice that makes available almost rural food or agricultural production is seasonal as

consumer needs are spread over the entire year. These aromatic plants and their derivatives are an effective fight against pests. They are cheaper and guarantee biodiversity (Regnault-Roger, 2002; Ketoh *et al*., 2005; Isman, 2006; Gueye *et al*., 2011). In these insecticides and/or insectifuge plants, figures *Lippia multiflora* L. *Lippia* is a local plant and accessible in all regions of the Ivory Coast, which was the subject of several works on the biofunctional properties (Tia, 2012; Ekissi *et al*. 2014). Therefore, this work aims to determine the minimum concentration of leaves of L. *multiflora* to sustainably preserve the quality of cowpea beans. This approach is based on a central composite experimental design to optimize post-harvest storage of cowpea beans in PICS bags.

II. MATERIAL AND METHOD

2.1 Description site

The experiment was performed at Laboratory of Biochemistry and Food Sciences (LABSA) UFR Biosciences at the University Felix 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.

2.2 Collection of cowpea kernels used in the study

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

2.3 Collection of plants and processing

The laboratory of Biochemistry and Sciences of Food has a field of action on the conservation of the cereals, legumes and other agricultural products since numerous years. The biopesticides represents a very good alternative in struggle against the devastating and mustiness. The plants used in this study are *Lippia multiflora* because of biopesticides properties (Tia, 2012; Ekissi *et al*., 2014). 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. The leaves were collected in Gbeke region in May. After harvest, leaves of L. *multiflora* were drying at an average temperature of 30 ◦C for 6-7 days, and kept away from direct sun exposure. After drying, leaves were chopped into fine particles before use.

2.4 Experiments implementation

2.4.1 Using of PICS bags

Storage bags used, were constituted polypropylene bags and triple bagging (Purdue Improved Cowpea Storage: PICS) coming from Niger.

Initiated by Purdue University in Kenya, PICS bags used for study consisted of two internal layers of polyethylene liners (composed of 80 mm high density) and a third layer made from woven polypropylene. When each layer is tied and closed separately, it creates a hermetically sealed environment for storing harvested grain. These bags were obtained from suppliers.

2.4.2 Protocol of cowpea storage

The experiment was conducted from June 2015 to February 2016. It was implemented using a method of preservation by bagging cowpea kernels in which two control groups and 4 experimental groups were formed. The control groups included one lot containing cowpea kernels polypropylene bag (TSP) and one lot containing cowpea kernels PICS bag (H0). The 4 experimental groups consisted of cowpea kernels PICS bagged in bags with different concentrations (H1: 0.7%; H2: 2.5%; H3: 4.3% and H4: 5%) chopped dried leaves of *Lippia multiflora*. The filling of the bags was made by alternating cowpea kernels and leaves as stratum. The mass of each bag was 50 kg.

2.5 Central composite design application

A five level, two variable central composite designs was applied to find the best combination for keep the health quality of cowpea.

Two independent variables or factors studied were the storage time: from 1 to 8 months (X1), quantity of biopesticides: 0 to 5% w/w (X2) (Table II). The experimental design led to implementation of 11 trials with 4 factorial runs, 4 axial runs (two axial points on each design variable axis at a distance of 1.68 from the design center) and 3 runs at center point. Four experimental responses were determined. It is about the rate of weight loss, of the concentration in aflatoxin B1, and

ochratoxin A and water activity. The coded values of the parameters are replaced by their actual values or states (Table III) for randomization of the trials. Sampling was carried out at 1, 2, 4.5, 7 and 8 months, in triplicate. Thus, a randomly sample of 2.5 kg from each bag was taken through. At the laboratory the rate of weight losses, the concentration in aflatoxin B1 and ochratoxin A as well as the water activity was given In the central composite design, the main as well as the interaction effects of various factors are determined by fitting the data into second order polynomial equation:

$$Y_n = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (1)$$

Where Y_n was the measured response, b_0 is the intercept term, b_1 and b_2 are linear coefficients, b_{12} is the logarithmic coefficient, b_{11} and b_{22} are quadratic coefficients, and X1 and X2 were coded independent variables. (Storage time and quantity of biopesticides).

2.5.1 Assessment of damage and weight loss

To assess the damage caused by insects during storage, 500 g of sample (approximately 3500 cowpea kernels) were taken. After sifting and removal of the foreign matters, the kernels were weighed and sorted to separate attacked and damaged kernels from healthy kernels. Then, the two fractions were weighed and counted separately. The percent kernels damage was estimated using the method of counting and weighing of Harris and Lindblad (1978) and Boxall (1986). Assays were performed in duplicate. Thus, the rate of infestation in the ratio of kernels having at least one hole in the total number of kernels. The estimate of the damage (D) and weight loss (W) is given by the formulas:

$$D\ (\%) = (NKA / TNK) \times 100 \quad (2)$$

With NKA = number of kernels attacked; TNK = total number of kernels

$$P\ (\%) = [(NKA \times WHK) - (NHK \times WAK)] / (WHK \times TNK)] \times 100 \quad (3)$$

With NKA = number of kernels attacked; NHK = Number of healthy kernels; NTG = Total number of kernels; WAK = Weight of attacked kernels; WHG = Weight of healthy kernels.

2.5.2 Determination of water activity

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

2.5.3 Aflatoxins analysis

2.5.3.1 Extraction and purification of aflatoxins

Chemical reagents (acetonitrile, methanol and chloroform) and standard aflatoxins (AFB1, AFB2, AFG1 and AFG2) were used for the study. Reagents were purchased from Carlo Erba (Spain) with analytical grade, while standard aflatoxins were provided from Sigma (Sigma, St Louis, MO, USA). Biological aflatoxins (B1, B2, G1 and G2) were extracted and purified from cowpea using the official guidelines of AOAC (AOAC, 2005). To 25 g of ground cowpea put in an erlenmeyer flask, 100 mL of 80% methanol aqueous solution were added. The mixture was homogenized, put in darkness at room temperature for 12 h, and then filtered with a Whatman paper (Wathman N°4). Thereafter, 50 mL of the filtrate were added with 40 mL of a mixture deriving from phosphotungstic acid-zinc sulfate-water (5/15/980, w/w/v), and kept at ambient temperature for 15 min before filtration upon Whatman paper. Aflatoxins were extracted from the out coming filtrate with 3 volumes of 10 mL of chloroform. The extracts were collected into a 50 mL flask and processed with rotative evaporator (BuchiRotavapor R-215) at 40°C for evaporation of the chloroform reagent. Finally, 0.4 mL of hydrochloric acid and 4.6 mL of bidistillated water were added to the dry extract, and the solution was filtered through filter resist in a chromatographic tube then passed through an immunoaffinity column (columnRiDAaflatoxin, Biopharm, Germany).

2.5.3.2 Quantification of Aflatoxins

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

v/v/v) and using a gradient programme of 1 mL/min. Assays were performed in triplicate. Validation parameters of the aflatoxins contents analysis, especially Limits of Detection (LOD), Limits of Quantification (LOQ), repeatability and reproducibility traits and percentage of extractions, were valued. Thereafter, the contents of aflatoxins B1, B2, G1 and G2 were estimated, and then the total aflatoxins content was calculated from the sum of the overall aflatoxins. The "table I" presents the HPLC analysis conditions and the results of method validation.

2.5.4 Ochratoxin 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 (CE 401/2006).

2.6 Statistical analysis

All experiments were done in triplicate and data in tables and figures represent mean values ± standard deviation. Multiple linear regression analysis was performed using the Statistica 8 software (Stat Soft, Inc., USA). Experimental data were fitted to the following second-order polynomial model and regression coefficients were obtained.

$$\mathbf{Y_n = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2}$$

Where Y_n is the experimental response; X1 and X2 correspond to the independent variables namely to storage time and quantity of biopesticides respectively. The b_n values represent corresponding regression coefficients.

According to the experimental data, the fitting model represented by equation was constructed and the statistical significance of the model terms was examined by regression analysis and analysis of variance (ANOVA).

III. RESULTS

3.1 Experimental responses obtained using Central Composite Design

A central composite design was used to determine the best conditions of cowpea conservations in PICS bags. The central composite design was developed as presented in the "table IV". This table presents also experimental values of weight loss, aflatoxin B1, ochratoxin A and the water activity.

3.2 Fitting the models

The various values of the determination coefficients R^2 and R^2 fitted for the regression model of the weight losses; aflatoxin B1, ochratoxin A and the water activity were indicated in "table V". These values (respectively of 0,96; 0,97; 0,99 and 0,95 for R2) and of (0,93; 0,93; 0,98 and 0,89 for R2 adjusted) being roughly close to 1 make it possible to say that the second order polynomial models envisaged, defined well the real behavior of the system. Their non-significant lack of fit also showed that these models were good fit. The lack of fitpermitted to justify the adequacy of the model to foresee the variations exactly.

3.3 Effects of the variables on the weight losses percentages

The results of the weights losses obtained, while being based on central composite design, are consigned in "table IV". Multiple regression analysis was carried on the experimental data and the coefficients of the model are evaluated for the significance. The storage time and biopesticide concentration have significant effects (P = 0,001 and P = 0,05).The values of the coefficients for the weight losses are presented in "table V". The final predictive equation of the rate of weight loss (Y1), neglecting the non-significant terms, was given by the equation.

$$Y1 = 1,01 + 1,99X_1 – 0,93X_2 \quad (4)$$

All the linear coefficients (X1 and X2) are significant. The significant terms have a remarkable impact on the merchantability quality (weight loss) of cowpea during the conservation; while the non-significant terms (X_1^2, X_2^2 and the interaction between X_1 and X_2) have a negligible influence. In order to evaluate the effects of the storage time and biopesticide concentration on the weight losses of cowpea during the conservation, "Fig 1" is built starting from the equation above. This figure shows the effects of time and the biopesticide on the rates of weight loss. It indicates that when the variable X_1 is on its higher level and the variable X_2 on its low level, the weight losses increase quickly. However starting from a concentration threshold reached of biopesticide, the increase in the rate of weight loss is inhibited until the eighth month.Beyond this concentration threshold, the increase is always inhibited in a progressive way up to 8 months.

3.4 Influence of the variables on water activity

The multiple regression analysis, executed on the experimental data, permitted to value the coefficients of the model. These coefficients are valued to know the significant effects.

$$Y4 = 0,63 + 0,06X_1 - 0,05X_2 \quad (5)$$

All the linear terms (X1 and X2) are significant. The significant terms have a remarkable effect on the water activity during the conservation. The storage time and the concentration of the biopesticide have a significant influence (P = 0,001 or P = 0,05) on the water content. The quadratic terms X_1^2, X_2^2 and the interaction between the 2 variables (X_1 and X_2) study shows a non-significant influence. "Fig 4" indicates the effects of the storage time and the biopesticide concentration on the water activity. Increase in the storage time entails an increase in the water activity and biopesticide concentration entails a negative effect on the increase of the water activity during the conservation.

3.5 Effects of the variables on the aflatoxin B1 contents

The results show that the aflatoxin B1 (AFB1) contents obtained starting from the various combinations of conservation after 4,5 months are of 1,17±0,03 µg/kg for the PICS bag without biopesticide and of 0,07±0,00 µg/kg in the PICS bags with 5% of biopesticide.By applying a multiple regression analysis, the relations examined between the independent variables and the aflatoxin contents of were expressed in the equation below.

$$Y2 = 0,57 + 0,72X_1 – 0,70X_2 \quad (6)$$

The linear terms (X_1 and X_2) are significant. These significant terms have a remarkable impact on the aflatoxin B1 contents during storage. The effect of the storage time and the biopesticide concentration are significant (P = 0,001).On the other hand the quadratic terms (X_1^2 and X_2^2) and the interaction between (X_1 and X_2) are not significant and have a negligible influence on the aflatoxin B1 contents. The surface plot in "Fig 2" shows the effect of the time and biopesticide concentration on the aflatoxin B1 contents. The aflatoxin B1 contents increase significantly in time during the conservation (P = 0,001).However, the negative effect of the variable X_2, starting from a certain concentration threshold, inhibits to a significant degree the aflatoxin B1 concentrations (P = 0,001).

3.6 Effects of the variables on the ochratoxin A contents

The results show that the contents of ochratoxin A obtained, starting from the various combinations after 4,5 months of conservation, are 2,94±0,04 µg/kg in the PICS bags without biopesticide and of 1,1±0,01 µg/kg in the PICS bags with 5% of biopesticide. The multiple analysis regression of the relations examined between the independent variables and the ochratoxin A contents are expressed in the equation below.

$$Y3 = 1,97 + 2,68X_1 – 1,12X_2 \quad (7)$$

The linear coefficients (X_1 and X_2) are significant. The significant terms have a remarkable impact on the ochratoxin A contents during the conservation. The storage time and biopesticide concentration effects are significant (P = 0,001), on the ochratoxin A content. On the other hand the quadratic terms (X_1^2 and X_2^2) and the interaction between (X_1 and X_2) are not significant and have a negligible influence on the ochratoxin A content. The surface plot in "Fig 3" shows storage time and biopesticide concentration effect on the ochratoxin A content. The increase in the storage time entrain a significant increase in the ochratoxin A content (P = 0,001).On the other hand a concentration threshold of biopesticide has a negative effect on the increase of ochratoxin A content (P = 0,001).

TABLE 1
OPERATING CONDITIONS OF THE AFLATOXIN B1 AND THE OCHRATOXIN A PROPORTIONING BY HPLC AND RESULTS OF THE VALIDATION METHOD.

	Aflatoxin B1 (AFB1)	Ochratoxin A (OTA)
pre-column	Shim-pack GVP-ODS 10 x 4.6 mm	
Column	Shim-pack GVP-ODS, 250 mm x 4.6 mm	
Detector	Fluorescence, λ excitation : 365 nm, λ emission : 435 nm	Fluorescence, λ excitation: 330 nm, λ emission: 460 nm
Mobile phase	methanol/water/acetonitrile (60:20:20, v/v/v)	Acetic Acid/water/ acetonitrile (2/99/99, v/v/v)
Volume injected	20 µL	100 µl
Debit	1 mL/minute	
Column temprature	40°C	
Rinsing solvent	Methanol	Acetonitrile
Analysis time	15 minutes	9 minutes
Limits of detection (LOD)	6,18±1,23 ng/kg	0,050±0,002 µg/kg
Limits of quantification(LOQ)	6,50±0,25 ng/kg	0,201±0,008 µg/kg
Repeatability	2,08±0,24%	0,26±0,07%
Reproducibility	3,20±0,45%	5,67±0,12%
Extraction yields	98,92±3,76%	86±2,15%

TABLE 2
INDEPENDENT VARIABLES AND THEIR CODED AND ACTUAL VALUES USED

Independent Variable	Symbol	Coded level				
		-1,41	-1	0	1	1,41
Storage Time (month)	X_1	1	2	4,5	7	8
Concentration of Biopesticides (%)	X_2	0	0,7	2,5	4,3	5

TABLE 3
ESTABLISHMENT OF EXPERIMENTAL TABLE TESTS OF THE CCP

Run Order	Factors	
N°	**X_1 (Month)**	**X_2 (%)**
1	2	0,7
2	7	0,7
3	2	4,3
4	7	4,3
5	1	2,5
6	8	2,5
7	4,5	0
8	4,5	5
9	4,5	2,5
10	4,5	2,5
11	4,5	2,5

TABLE 4

RESPONSE SURFACE CENTRAL COMPOSITE DESIGN AND EXPERIMENTAL RESULTS.

Run order	Independents Variables		Experimental responses			
	X_1 (month)	X_2 (%)	Y1 (%)	Y2 (µg/kg)	Y3 (µg/kg)	Y4
1	-1(2)	-1(0,7)	0,47±0,03	0,46±0,00	1,28±0,00	0,64±0,00
2	1(7)	-1(0,7)	2,63±0,24	1,25±0,01	3,87±0,01	0,71±0,01
3	-1(2)	1(4,3)	0,07±0,01	0,019±0,00	0,39±0,00	0,60±0,00
4	1(7)	1(4,3)	1,40±0,21	0,46±0,01	2,89±0,01	0,65±0,01
5	-1,41(1)	0(2,5)	0,08±0,00	0,13±0,00	0,29±0,00	0,61±0,01
6	1,41(8)	0(2,5)	3,23±0,11	1,31±0,05	4,25±0,69	0,71±0,01
7	0(4,5)	-1,41(0)	2,00±0,18	1,17±0,03	2,94±0,04	0,70±0,00
8	0(4,5)	1,41(5)	0,50±0,07	0,07±0,00	1,10±0,01	0,63±0,00
9	0(4,5)	0(2,5)	0,90±0,07	0,55±0,01	1,98±0,01	0,62±0,01
10	0(4,5)	0(2,5)	1,15±0,07	0,58±0,01	2,08±0,01	0,65±0,01
11	0(4,5)	0(2,5)	0,98±0,07	0,57±0,01	1,86±0,01	0,63±0,01

Y1 (%): Weight losses percentage ; Y2 : Aflatoxin B1 content ; Y3 : Ochratoxin content ; Y4 : Water activity

TABLE 5

REGRESSION COEFFICIENTS OF PREDICTED QUADRATIC POLYNOMIAL MODELS FOR WEIGHT LOSSES, AFLATOXIN B1, OCHRATOXIN A AND WATER ACTIVITY.

Coefficients	Coefficients estimated			
	Weight losses (Y1)	Aflatoxin B1 (Y2)	Ochratoxin A (Y3)	Water activity (Y4)
b_0	$1,01^{**}$	$0,57^{***}$	$1,97^{***}$	$0,63^{***}$
Linear				
b_1	$1,99^{***}$	$0,72^{***}$	$2,68^{***}$	$0,06^{***}$
b_2	$-0,93^{**}$	$-0,70^{***}$	$-1,12^{***}$	$-0,05^{**}$
Quadratic				
b_{11}	$0,49^{ns.}$	$0,09^{ns.}$	$0,28^{ns.}$	$0,02^{ns.}$
b_{22}	$0,08^{ns.}$	$-0,01^{ns.}$	$0,03^{ns.}$	$0,02^{ns.}$
Cross products				
b_{12}	$-0,41^{ns.}$	$-0,17^{ns.}$	$-0,04^{ns.}$	$-0,01^{ns.}$
R^2	0,96	0,97	0,99	0,95
R^2 adj	0,93	0,93	0,98	0,89
Lack of fit (P-value)	$0,13^{ns.}$	$0,01^{*}$	$0,26^{ns.}$	$0,71^{ns.}$

****Significant at P = 0,05 ; ***Significant at P = 0,001 ; ns : no significant ; R2 : Regression Coefficient, P : probability**

TABLE 6
PREDICTED AND EXPERIMENTAL VALUES OF RESPONSES UNDER IDEAL CONSERVATION CONDITIONS

Responses	Ideal Conditions	
	Predicted value	Obtained value
Weight losses (%)	3,46 [a]	3,50±0,5 [a]
AFB1 (µg/kg)	1,50 [a]	1,48±0,3 [a]
OTA (µg/kg)	4,56 [a]	4,54±0,02 [a]
Aw	0,73 [a]	0,71±0,03 [a]

Data of the same line having the same sign are statistically in the same homogenous group at P=.05.

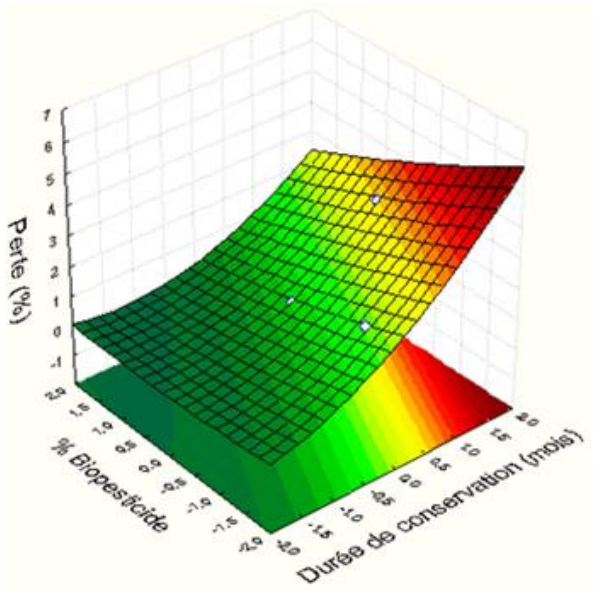

FIGURE 1: EFFECTS OF STORAGE TIME AND BIOPESTICIDE ON THE WEIGHT LOSSES

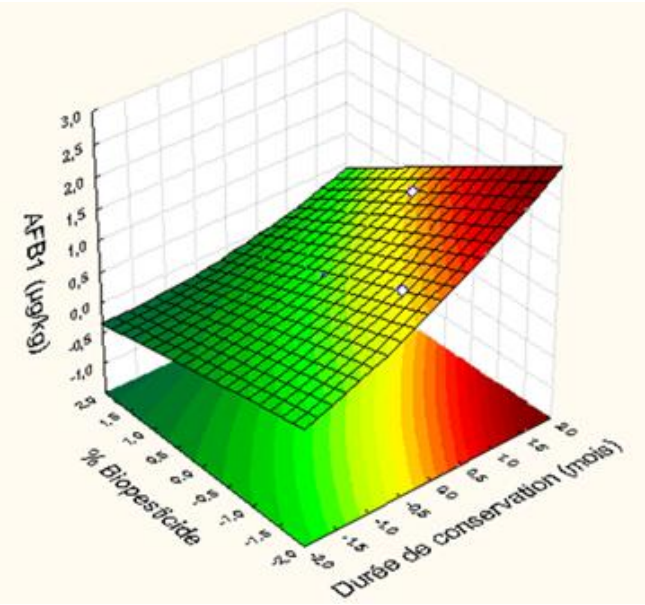

FIGURE 2: EFFECTS OF STORAGE TIME AND BIOPESTICIDE ON AFB1 CONTENT

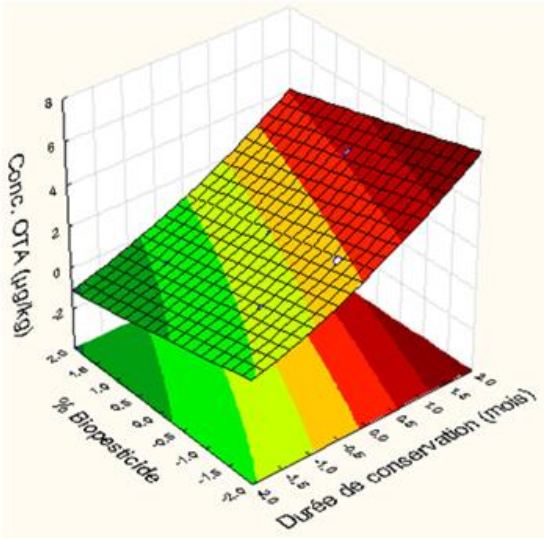

FIGURE 3: EFFECTS OF STORAGE TIME AND BIOPESTICIDE ON OTA CONTENT

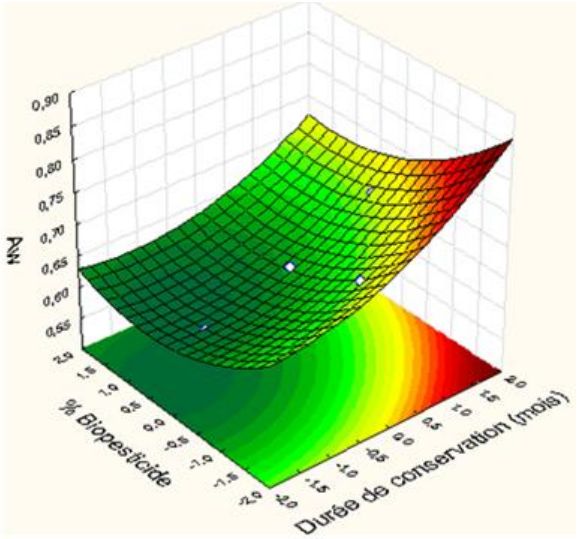

FIGURE 4: EFFECTS OF STORAGE TIME AND BIOPESTICIDE ON WATER ACTIVITY

TABLE 7
EXPERIMENTAL VALUES UNDER IDEAL CONDITIONS AND CONTROL BAG WITHOUT PICS

Responses	Ideal conditions		Without biopesticide
	Obtained values	Predicted values	TSP (4,5 month)
Weight losses (%)	3,50±0,5[b]	3,46[b]	22,03±0,25[a]
AFB1 (µg/kg)	1,48±0,3[b]	1,50[b]	9,21±0,03[a]
OTA (µg/kg)	4,54±0,02[b]	4,56[b]	23,80±0,53[a]
Aw	0,71±0,03[b]	0,73[b]	0,96±0,02[a]

The values on the same line presenting the same signs are statistically in the same homogeneous group with P = 0,05

TABLE 8
EXPERIMENTAL VALUES UNDER IDEAL CONDITIONS AND PICS BAG WITHOUT BIOPESTICIDE

Responses	Ideal conditions		Without biopesticide
	Obtained values	Predicted values	PICS (8 month)
Weight losses (%)	3,50±0,5[b]	3,46[b]	19,20±1,74[a]
AFB1 (µg/kg)	1,48±0,3[b]	1,50[b]	8,41±0,62[a]
OTA (µg/kg)	4,54±0,02[b]	4,56[b]	22,50±1,15[a]
Aw	0,71±0,03[b]	0,73[b]	0,92±0,01[a]

The values on the same line presenting the same signs are statistically in the same homogeneous group with P = 0,05

TABLE 9
OBTAINED VALUES AFTER 4,5 MONTH OF CONSERVATION IN CONTROL WITHOUT PICS AND PICS CONTROL WITHOUT BIOPESTICIDE.

Responses	Without biopesticide	
	TSP (4,5 month)	PICS (4,5 month)
Weight losses (%)	22,03±0,25[a]	2,00±0,18[b]
AFB1 (µg/kg)	9,21±0,03 [a]	1,17±0,03[b]
OTA (µg/kg)	23,80±0,53[a]	2,94±0,04[b]
Aw	0,96±0,02[a]	0,70±0,00[b]

The values on the same line presenting the same signs are statistically in the same homogeneous group with P = 0,05

IV. DISCUSSION

The results observed in this study show that the conservation of cowpea kernels in PICS bags in the presence of *Lippia multiflora* leaves is effective against the development of pests responsible for the alteration of the health and marketability of grains. Indeed small weight loss percentages and water activity acceptable are observed in the presence of biopesticide. Furthermore, inhibition of aflatoxin B1 and ochratoxin A contents was observed from a threshold concentration of biopesticides. A minimum concentration of 1.26% leaves of Lippia multiflora is sufficient to guarantee the quality of cowpea kernels over 8 months.

The results of the water activity of cowpea kernels show a perfect mastery of this parameter is important in preventing the proliferation of weevils and fungi, responsible for the impairment of quality of cowpea. The values obtained for the polypropylene bag control group remains very high after 4.5 months of storage (9,21±0,03 µg/kg and 23.8 ± 0.53 µg/kg for aflatoxin B1 and ochratoxin A). These values are much higher than the normative values set for 2 toxic substances (2 µg/kg and 5 µg/kg respectively) by the European Commission (EU Regulations No 165 / 2010 and No 420/2011). After 4.5 months of storage, the results for cowpea kernels in PICS bags (with or without biopesticides) guard their commercial and sanitary qualities. The measurement of weight losses of aflatoxin B1 and ochratoxin A change very little. From the seventh month of storage, the values obtained for the batch of cowpeas stored in PICS bags without biopesticide, remain high compared to those obtained for cowpeas in PICS bags with biopesticide. These results reflect the usefulness of the PICS bags and also the efficiency of leaves Lippia multiflora for cowpea conservation. This efficiency translates into insecticidal and / or repellent of the leaves of the plant that would be due to the release of bioactive molecules in their essential oils (N'gamo et al., 2007). Our results are similar to the work done by Niamketchi et al. (2015) in the center of the Ivory Coast region. These authors demonstrated the effectiveness of dried leaves of Lippia multiflora and Hyptis suaveolens against the development of pests responsible for the alteration of the grains in the traditional and improved granaries. Our results are also in agreement with

those of Rose of Lima et al. (2014) work in Benin, which showed that the essential oils of Pimenta racemosa and Syzygium aromaticum reduced significantly the fungal flora responsible for the production of mycotoxins during cowpea conservation over a period of 3 months. In addition to studies by Makun et al. (2012) have demonstrated the inhibitory effect of ethanol extracts of leaves of Lippia multiflora of Azadirachta indica and Blumeaperotitiana on cowpea toxigenic molds. The bioactive molecules of L. multiflora primarily comprises oxygenated monoterpenes such as linalool and 1,8 cineole (Tia, 2012). These antimicrobial agents cause at the many mold damage such as morphological disruption, disruption of the plasma membrane and impaired mitochondrial structure (Billerbeck, 2001).

After eight months of storage, in PICS bags without biopesticides, the concentrations of aflatoxin B1 (8.41 ± 0.62 µg/kg) and ochratoxin A (22.5 ± 1.15 µg/kg) are superior to normative values (EU Regulations No 165/2010 and No 420/2011). By cons, in cowpea lots PICS bags with biopesticide, the aflatoxin B1 and ochratoxin A values increase only slightly remaining substandard. However it should be noted that a minimum concentration required for optimum efficiency. Tatsadjieu et al. (2009) showed that the essential oil of *Lippia rugosa*, a species of the genus Lippia, inhibits the growth of *Aspergillus flavus* and limit the production of aflatoxin B1 at concentration of 1000 mg/L.

The results of the experimental analysis show that conservation is favored when the cowpea variable storage time is at its highest level (+1) and when the encoded value of the variable amount of biopesticides is (-0.70). Thus, the ideal process of cowpea grain retention involves the following parameters:

• Storage time: 8 months

• Quantity of biopesticides for storage: 1.26%

4.1 Verification and experimental validation

By using Statistica 8.0 software desirability function, the ideal conditions of cowpea kernels conservations were envisaged, with 1,26% of biopesticide for 8 months. Higher possible values of weight loss and health quality (AFB1, OTA, and Aw) were determined in table VI. Experimented data were approaching the predicted values in the optimal conditions mentioned above (table VI). This means that there is a high degree suitable between the values observed in the experiment and those predicted by the regression model.

For all the parameters of commercial and sanitary qualities, the experimentally obtained values are significantly lower than those obtained in the control bag without PICS (TSP) after 4.5 months of storage (Table VII). Also under these same conditions, the experimentally obtained values remain always much lower than those obtained in the control bag PICS without biopesticide after 8 months of storage (Table VIII). Furthermore, obtained values for all parameters in the control bag without PICS remain lower than PICS bag after 4.5 months (Table IX).

V. CONCLUSION

The results of our study confirm the importance of PICS bags for cowpea kernels conservation. This container extends the storage time of grains while commercial and health qualities. This study also shows that the addition of *Lippia multiflora* leaves, as biopesticides, extends more storage time of cowpea in Côte d'Ivoire. Thus, this biopesticides can fight effectively against insect pests and fungal contamination. This study allowed determining the ideal conditions of storage from central composite design. Optimal storage conditions of cowpea obtained in our study were 1.26% as the minimum concentration of L. multiflora leaves for a period of 8 months. The method developed in this study from a biopesticide in PICS bags is inexpensive and promising for Ivorian producers. However, this study should be deepened in order to preserve the nutritional quality after conservation.

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