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Optimization of Storage Conditions of Cowpea (*Vigna unguiculata* L. Walp) Bagged Pics Containing Biopesticide (*Lippia multiflora)* Leaves by Factorial Design in Cote d'Ivoire

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Authors' contributions

This work was carried out in collaboration between both authors. Author AC designed the study, wrote the protocol and wrote the first draft of the manuscript. Author GHMB reviewed the experimental design and all drafts of the manuscript. Author AC managed the analyses of the study. Author GHMB identified the plants. Author AC performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

This study aims to optimize storage conditions of two cowpea varieties grains in PICS bags containing leaves of *Lippia multiflora*. It was to assess, through a full factorial design, the effect of three factors (variety, biopesticide dose, storage duration) on the merchantability (moisture, water activity, mass loss, damages) and contamination levels of ochratoxin A (OTA), aflatoxin B1 (AFB1)

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and total aflatoxins (AFT) of cowpea grains during 8 months. The grains were collected from producers of the Loh-Djiboua region (5°50 'North 5°2 2' West). After the hulling, the grains have not undergone any treatment were sent to the laboratory for storage. The lots treated with leaves *Lippia multiflora* recorded lower values than those of control groups whatever the parameter studied. Indeed, the damages of grains are 7.60±0.16% and 39.66±1.77% respectively for the treated and control groups. Concerning mycotoxins, treated groups have concentrations of 4.01±0.06 µg/kg and 1.14±0.01 µg/kg respectively for OTA and AFB1. As against untreated groups have concentrations of 22.50±0.87 µg/kg and 8.41±0.48 µg/kg respectively for OTA and AFB1. These results reflect an action of leaves on insect activity and toxigenic molds. The results of full factorial design indicate that action of leaves of *Lippia multiflora* is independent the cowpea variety conserved. Furthermore, the mathematical model derived from this plan allows a prediction of values of parameters studied with Pearson coefficients (R2) equal to 0.99. Thus, the treatment with leaves *Lippia multiflora* of stock of cowpea has a positive impact on conservation of merchant and health quality of grains with a persistence up to 8 months. This inexpensive and easy to use treatment should be vulgarized among farmers.

Keywords: Biopesticide; full factorial design; cowpea; PICS bag.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is an annual herbaceous plant of the family Papilionaceae [1]. In West Africa, it represents 85% of the area of pulses and 10% of total cultivated land [2]. It is grown for its grains but also for its leaves eaten in many areas and fed to cattle in other [3]. Cowpea is a legume whose grains are most consumed in Africa [4]. These grains are, for thousands of people living in the tropics, the main source of protein used in diet [5]. Cowpea offers many benefits to small farmers in terms of food, cash income, animal feed and improving soil fertility [6]. However, not only grains are insufficiently products but they suffer losses in post-harvest treatments. This especially in shops a result of parasitic pressures including insects and fungi.

The storage, after harvest, of cowpea grains is currently a concern for producers and agricultural services in most African countries. The losses are highly variable across regions and even countries. Studies have shown that losses due to pests, during storage, can reach 100% of the production [7]. Several agents of deterioration are responsible for these losses including insects (44%), rodents (30%) and fungi (26%) [8]. These damages, not only, reduce weight and germination of grains but also degrade their market, nutritious and hygienic qualities [9]. They thus lead to the proliferation of producing molds of ochratoxin A and aflatoxins [10]. These mycotoxins are hepatotoxic and nephrotoxic with important carcinogenic effects in rodents. They also have cytotoxic effects, carcinogenic, teratogenic and immunotoxic in humans [11].

Face these post-harvest losses various control methods have been developed. This is among other chemical control, biological control, use of plant biocides, physical methods and varietal resistance [12]. According Isman [13] and PAN Africa [14] synthetic chemical insecticides are the most used. The abusive application of pesticides to control stored product insects has often led to the presence of toxic residues on treated products and led to the emergence of resistant insects [15]. In developing countries, these problems add to the economic constraints related to the cost and supply of active ingredients [16]. It turns out important face these problems, to seek other alternative methods less costly struggle, environmentally friendly and ensuring consumer health.

The experiment plans technique provides a rigorous methodology for a specific purpose and this with a minimum test avoiding all fumbling leading to a plethora of inexplicable results. These plans determine the optimum minimizing the number of experiments so the cost leading to measure system responses when we vary certain parameters. Thus, the present study aims to optimize the storage conditions grains of two cowpea varieties in PICS bags with a biopesticide using a full factorial design.

2. MATERIALS AND METHODS

2.1 Plant Material

The biological material was made up of grains of two varieties of cowpea (*Vigna unguiculata L.* Walp) collected from April to May 2015 just after harvest. The grains were collected from

producers of the Loh-Djiboua region (5°50 'North 5°22' West). After the hulling, the grains have not undergone any treatment were sent to the laboratory for storage. The material also included the leaves of *Lippia multiflora* (or savannah tea). This plant was selected because of its biopesticide properties. This is a fragrant shrub that grows wild in central and northern regions of the country because of the climate that prevails [17]. After harvesting, the grains of cowpea were dried in the sun. The 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.

2.2 Storage Method of Cowpea Grains

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. The storage of grain was to add 5% (m/m) of biopesticide (leaves of *L. multiflora*) to the grains of cowpea contained in PICS bags. Thereafter the bags were stored on pallets in the laboratory at room temperature for 8 months. Thus each PICS bag contains 50 kg of cowpea grains and 2.5 kg of leaves of *L. multiflora* except witnesses bags that contained only 50 kg of cowpea grains. At the end of the experiment, samples were taken to determine moisture content, water activity, mass loss, damages of grains, concentrations of ochratoxin A and aflatoxins B1 and total.

2.3 Full Factorial Design (PFC)

The experimental field of study was composed of 3 factors including cowpea variety (X_1), biopesticide concentration (X_2) and storage duration (X_3) (Table 1). The moisture content (Y_1), water activity (Y_2), weight loss (Y_3), damages (Y_4), concentration of ochratoxin A (Y_5), aflatoxin B1 concentration (Y_6) and total aflatoxins concentration (Y_7) of grains were used as responses to evaluate the system. It has been defined 2 levels for each factor: -1 for lower level and +1 for higher level (Table 1). Then the 3 independent variables were combined in the factorial design in $2^3 = 8$ testing (3 number of factors) by combining the 2 levels of 3 factors chosen (Tables 2). Using the results of the responses, the coefficients attached to each effect of main factors were calculated by multiple linear regression method. The choice of influential factors was made by the test of significance of the coefficient. The coefficients whose absolute value is greater than twice the experimental standard deviation (2σe) were selected [18]. To produce the optimum conditions for conservation, a linear function was developed. The general form of the polynomial equation of 1er order is given by equation 1.

$$Yi = \beta 0 + \Sigma \beta iXi + \Sigma \beta ijXiXj \quad (1)$$

Yi was the measured response, β0 the constant term of model, Xi, Xj the independent variables, βi the linear coefficient and βij the interaction coefficient.

2.4 Model Validation

The validation was made by applying the optimal conditions from the factorial design. Each response was assessed through 3 separate runs. Then, the values obtained were compared with those predicted by the model from the factorial design to risk 5%.

2.5 Determination of Moisture Content and Water Activity of Cowpea Grains

The moisture of grains was determined by drying in the oven according to AOAC [19]. A sample of 5 g of grain milling was dried at 105°C to constant mass. The water content was calculated from the mass difference.

The water activity (Aw) was measured using an electronic hygrometer, model HygroLab C V1.0a / 61258306, Rotronic Instrument Corp., Switzerland. A sample of 5 g of milling of cowpea grains was put into standard dry containers for the Aw analysis. The water activity digital measures were directly displayed by the hygrometer.

2.6 Determining the Damages and Weight Loss of Cowpea Grains

To assess the damage caused by insects during storage, samples of 1 kg (approximately 3500 cowpea grains) 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 Harris and Lindblad [20] and Boxall [21]. Assays were performed in triplicate. 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 equations 2 and 3.

$$\mathrm{D}\ (\%) = \frac{\mathrm{NGA}}{\mathrm{NTG}} \times 100 \qquad (2)$$

NGA = Number of Grains Attacked; NTG = Total Number of Grains

$$\mathrm{W}\ (\%) = \frac{[(\mathrm{NGA} \times \mathrm{WHG}) - (\mathrm{NHG} \times \mathrm{WAG})]}{(\mathrm{WHG} \times \mathrm{NTG})} \times 100 \qquad (3)$$

NGA = Number of Grains Attacked; WHG = Weight of Healthy Grains; NHG = Number of Healthy Grains; WAG = Weight of Grains Attacked.

2.7 Determination of Ochratoxin A and Aflatoxins of Cowpea Grains

2.7.1 Extraction and purification of ochratoxin A

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 methanolbicarbonate 1% (v/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 minutes at 4℃. The supernatant was filtered through 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 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 solvent (methanol/ acetic acid; 98:2; v/v) 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.

2.7.2 Extraction and purification of aflatoxins

In a 250 mL Erlenmeyer flask containing 20 g of cocoa mass, 100 ml of methanol-water (v/v, 8:2) were added. The mixture was homogenized by shaking for 2 minutes and then stored at room temperature in the dark for 12 hours. The homogenate was filtered through filter paper and 50 ml of the filtrate were added 40 ml of zinc sulfate phosphotungstic acid mixture in water (w/w; 50/150 in 1 L of water) then stored at room temperature for 15 minutes. The mixture was filtered through filter paper and aflatoxins were extracted from the filtrate with 3 volumes of 10 ml of chloroform [22]. The extracts were collected and evaporated to dryness using a rotary evaporator at 40℃. A dry extract were added 0.4 mL of hydrochloric acid and 4.6 mL of bidistilled water. The mixture was filtered using a rezist filter in a chromatographic tube and aflatoxin analysis was made by High Performance Liquid Chromatography (HPLC).

2.7.3 Quantification of ochratoxin A and aflatoxins

Ochratoxin A and aflatoxins were detected and quantified by chromatograph HPLC brand Shimadzu coupled to a fluorescence detector in the operating conditions described in Table 3.

2.8 Statistical Analyses

The tests were performed in triplicate and the values in the tables represent the average and standard deviation. The linear coefficients and the 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. Differences were designated significant when p =.05.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Determining the factors effect on the grain moisture

The moisture means determined vary between 10.03 ± 0.16% and 14.13 ± 0.09% for cowpea grains from control groups. By cons, these rates range from 10.03 ± 0.16% to 11.98 ± 0.07% for cowpea grains treated with 5% leaves of of *L. multiflora* (Table 4). In addition, the factors studied X_2 (biopesticide concentration) and X_3 (conservation duration) have a significant linear effect on the moisture content at risk 5%. Linear regression indicates that 99% of variation can be explained by the model used with a Pearson coefficient (R^2) of 0.9994 (Table 5). Also a

significant interaction was observed between X_2 and X_3. Thus equation 4 allows a prediction of moisture rates in cowpea grains during conservation.

$$Y1 = 11{,}523 - 0{,}553X2 + 1{,}488X3 - 0{,}553X2X3 \quad (4)$$

The maximum moisture content of cowpea grains stored in PICS bags with 5% leaves of *L. multiflora* for 8 months is equal to 11.91%. The experimental results of the model validation indicate a moisture of 12.01 ± 0.03%. Statistical analysis indicates that there is no significant difference at risk 5% between prediction (11.91%) and the experimental value (Table 6). These results indicate that optimization model used allows the prediction of moisture of grains during storage following experimental conditions.

3.1.2 Determination of factors effect on the grain water activity

The average values of water activity range from 0.607 ± 0.011 to 0.918 ± 0.011 and 0.607 ± 0.011 to 0.710 ± 0.013 for cowpea grains stored respectively without and with 5% leaves of *L. multiflora* (Table 4). Also factors studied X_2 (biopesticide concentration) and X_3 (conservation duration) have a significant linear effect on grain water activity at risk 5%. Linear regression indicates that 99% of variation can be explained by model used with a Pearson coefficient (R^2) of 0.9990 (Table 5). In addition, a significant interaction was observed between X_2 and X_3. Thus equation 5 for predicting water activity of cowpea grains during conservation is as follows:

$$Y2 = 0{,}709 - 0{,}054X2 + 0{,}101X3 - 0{,}054X2X3 \quad (5)$$

Cowpea beans stored in PICS bags with 5% leaves of *L. multiflora* for 8 months have a maximum water activity equal to 0.702. The water activity of validation of model is 0.697 ± 0.001. Statistical analysis indicates that there is no significant difference at risk 5% between prediction (0.702) and the experimental value (Table 6). These results indicate that optimization model used allows to predict water activity of grains during storage following experimental conditions.

3.1.3 Determining the factors effect on the weight loss of grains

The weight losses range from 0.26 ± 0.08% to 19.21 ± 0.97% for cowpea grains of control group. However, they vary from 0.26 ± 0.08% to 2.27 ± 0.23% for cowpea grains treated with 5% leaves of *L. multiflora* (Table 4). In addition, the factors studied X_2 (biopesticide concentration) and X_3 (conservation duration) have a significant linear effect on the loss of grain mass to risk 5%. Linear regression indicates that 99% of variation can be explained by model used with Pearson coefficient (R^2) of 0.9999 (Table 5). Furthermore, significant interactions were observed between X_1 and X_3 on the one hand and on the other hand between X2 and X3. The weight loss prediction during conservation of cowpea grains is given by equation 6.

$$Y3 = 5{,}501 - 4{,}236X2 + 5{,}231X3 - 0{,}009X1X3 - 4{,}236X2X3 \quad (6)$$

The maximum weight loss of cowpea grains stored in PICS bags with 5% leaves of *L. multiflora* for 8 months is equal to 2.27% for white variety. The experimental results of model validation indicate a weight loss of 2.39 ± 0.02%. Statistical analysis indicates that there is no significant difference at risk 5% between prediction (2.27%) and experimental value (Table 6). These results indicate that optimization model used predicts weight loss of grains during storage following experimental conditions.

3.1.4 Determination of factors effect on damages of grains

Damages of grains means ranged from 3.25 ± 0.07% to 39.66 ± 1.77% and 3.25% ± 0.07 to 7.60 ± 0.16% for cowpea grains stored respectively without and with 5% leaves of *L. multiflora* (Table 4). The factors studied X_2 (biopesticide concentration) and X_3 (conservation duration) have a significant linear effect on damages of grains at risk 5%. Linear regression indicates that 99% of variation can be explained by the model used with a Pearson coefficient (R^2) of 0.9987 (Table 5). In addition, significant interaction was observed between X_2 and X_3. Thus, the prediction equation (7) of damage of cowpea grains during conservation is as follows:

$$Y4 = 13{,}188 - 7{,}815X2 + 9{,}898X3 - 7{,}815X2X3 \quad (7)$$

The cowpea grains stored in PICS bags with 5% leaves of *L. multiflora* for 8 months will have a maximum rate of grain damage 7.46%. The experimental rate of grain damage of model validation is 7.63 ± 0.03%. Statistical analysis indicates that there is no significant difference at risk 5% between prediction (7.46%) and the experimental value (Table 6). These results

indicate that optimization model used predicts the grains damage during storage according to experimental conditions.

3.1.5 Determining the factors effect on concentration of ochratoxin A of grains

The levels of ochratoxin A ranged from 1.11 ± 0.01 µg/kg to 22.50 ± 0.87 µg/kg for cowpea grains from control groups. By cons, they vary from 1.11 ± 0.01 µg / kg to 4.01 ± 0.06 µg / kg for cowpea grains treated with 5% leaves of *L. multiflora* (Table 4). The factors studied X_2 (biopesticide concentration) and X_3 (conservation duration) have a significant linear effect on OTA concentration of grain to risk 5%. Linear regression indicates that 99% of variation can be explained by model used with a Pearson coefficient (R^2) of 0.9974 (Table 5). In addition, significant interaction was observed between X_2 and X_3. The prediction of OTA concentration in cowpea grains during conservation is given by equation 8:

$$Y5 = 7{,}021 - 4{,}462X2 + 5{,}906X3 - 4{,}462X2X3 \quad (8)$$

The maximum concentration of OTA in cowpea grains stored in PICS bags with 5% leaves of *L. multiflora* for 8 months is equal to 4.00 µg/kg. The experimental results for model validation indicate an ochratoxine A concentration of 3.87 ± 0.02 µg/kg. Statistical analysis indicates that there is no significant difference at risk 5% between prediction (4.00 µg/kg) and the experimental value (Table 6). These results indicate that optimization model used predicts concentration of OTA in grain during storage following experimental conditions.

3.1.6 Determination of factors effect on aflatoxin B1 concentrations of grains

The average concentrations of aflatoxin B1 ranged from 0.15 ± 0.01 µg/kg to 8.41 ± 0.48 µg/kg and 0.15 ± 0.01 µg/kg to 1.14 ± 0.01 µg/kg cowpea grains stored respectively without and with 5% leaves of *L. multiflora* (Table 4). The factors studied X_2 (biopesticide concentration) and X_3 (conservation duration) have a significant linear effect on AFB1 concentration of grains to risk 5%. Linear regression indicates that 99% of variation can be explained by the model used with Pearson coefficient (R^2) of 0.9992 (Table 5). In addition, significant interaction was observed between X_2 and X_3. Thus equation (9) for predicting AFB1 concentration during conservation of cowpea grains is as follows:

$$Y6 = 2{,}426 - 1{,}785X2 + 2{,}271X3 - 1{,}785X2X3 \quad (9)$$

The Cowpea grains stored in PICS bags with 5% leaves of *L. multiflora* for 8 months will have a maximum AFB1 concentration of 1.13 µg/kg. The experimental AFB1 concentration of grain for model validation was 0.99 ± 0.01 µg/kg. Statistical analysis indicates that there is no significant difference at risk 5% between prediction (1.13 µg/kg) and the experimental value (Table 6). These results indicate that optimization model used predicts concentration of AFB1 in grain during storage following experimental conditions.

3.1.7 Determining the factors effect on concentrations of total aflatoxins of grains

The mean levels of total aflatoxins ranged from 2.29 ± 0.05 µg/kg to 98.02 ± 0.43 µg/kg for cowpea grains control groups and 2.29 ± 0.05 µg/kg to 16.62 ± 0.84 µg/kg for cowpea grains treated with 5% leaves of *L. multiflora* (Table 4). The factors studied X_2 (biopesticide concentration) and X_3 (conservation duration) have a significant linear effect on the AFT concentration of grains to risk 5%. Linear regression indicates that 99% of variation can be explained by the model used with Pearson coefficient (R^2) of 0.9998 (Table 5). In addition, significant interaction was observed between X_2 and X_3. The prediction of AFT concentration in cowpea grains during conservation is given by equation 10:

$$Y7 = 29{,}626 - 20{,}454X2 + 27{,}330X3 - 20{,}454X2X3 \quad (10)$$

The maximum of AFT concentration in cowpea grains stored in PICS bags with 5% leaves of *L. multiflora* for 8 months is equal to 16.05 µg/kg. The experimental results of model validation indicate AFT concentration of 15.99 ± 0.03 µg/kg. Statistical analysis indicates that there is no significant difference at risk 5% between prediction (16.05 µg/kg) and the experimental value (Table 6). These results indicate that optimization model used predicts concentration of AFT in grain during storage following experimental conditions.

3.2 Discussion

The results indicate that cowpea grains stored in PICS bags with 5% leaves of *Lippia multiflora* are better preserved than those of control groups. Indeed, it is observed a decrease in humidity,

water activity, weight loss, grain damage, OTA concentrations, AFB1 and AFT in batches treated with 5% leaves of *L. multiflora* during conservation. This reflects an improvement in merchantability and sanitary quality of said cowpea grains. These results reflect efficacy of leaves of *L. multiflora* in conservation of cowpea grains. This efficiency is due to the bioactive molecules present in essential oil from this plant [23]. Some authors in Burkina Faso analyzed the chemical composition of essential oil from leaves of *L. multiflora* and the main compounds are of type thymol, p-cymene and acetate thymyle [24].

The decline in insect activity on cowpea grains in our study may be related to chemical composition of essential oil and possible synergistic effects between components [25]. Biego and Chatigre [26] have also mentioned the decrease in weight loss and damage of insect in their conservation study of maize grains in polypropylene bags with leaves of *L. multiflora*. Other authors have mentioned this action on insects in conservation of cowpea grains from powders [27,28] and essential oils [29] from other plants.

Table 1. Definition of the experimental field of factorial design

Main factors	Levels	
	Lower (-1)	Superior (+ 1)
Variety of cowpea grains : X_1	White	Red
biopesticide Concentration (%) : X_2	0	5
Conservation duration (month) : X_3	0	8

Table 2. Yates matrix and experimental plan associated with factorial design

Test number	X_1	X_2	X_3
	Variety	Concentration (%)	Duration (Month)
1	1 (Red)	1 (5)	1 (8)
2	-1 (White)	1 (5)	1 (8)
3	-1 (White)	-1 (0)	1 (8)
4	1 (Red)	-1 (0)	-1 (0)
5	-1 (White)	1 (5)	-1 (0)
6	1 (Red)	-1 (0)	1 (8)
7	1 (Red)	1 (5)	-1 (0)
8	-1 (White)	-1 (0)	-1 (0)
Level -1	**White**	**0**	**0**
Level +1	**Red**	**5**	**8**

Table 3. Operating conditions of HPLC determination of ochratoxin A and aflatoxins

Designation	Ochratoxin A	Aflatoxins
Pre column	Shim-pack GVP-ODS 10 x 4.6 mm	
Column	Shim-pack GVP-ODS, 250 mm x 4.6 mm	
Detector (Fluorescence)	λ excitation : 330 nm, λ emission : 460 nm	λ excitation : 365 nm, λ emission: 435 nm
Mobile phase	Acetonitrile/Water /Acetic acid (99/99/2; v/v/v)	Methanol/Water/Acetonitrile (6/2/2; v/v/v)
Volume injected	100 µl	20 µl
Flow rate	1 mL/minute	
Column temperature	40℃	
Rinsing solvent	Acetonitrile	Methanol
Duration of analysis	12 minutes	13 minutes
Detection limit (LD)	0,06 µg/kg	5,18 ng/kg
Limit of quantitation (LQ)	0,25 µg/kg	32 ng/kg
Extraction yield	87±0,23%	80±0,6%

Table 4. Results from the full factorial design testing (PFC)

Test number	Levels of technological parameters			Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7
	X_1	X_2	X_3							
	Variety	Biopesticide dose (%)	Duration (Month)							
1	1	1	1	11,83±0,10	0,697±0,009	2,25±0,03	7,31±0,26	4,00±0,01	1,14±0,01	16,62±0,84
2	-1	1	1	11,98±0,07	0,710±0,013	2,27±0,23	7,60±0,16	4,01±0,06	1,12±0,01	15,48±0,18
3	-1	-1	1	14,13±0,09	0,918±0,011	19,21±0,97	39,66±1 ,77	21,20±0,42	8,12±0,04	98,02±0,43
4	1	-1	-1	10,03±0,16	0,607±0,011	0,28±0,06	3,25±0,0 7	1,12±0,01	0,16±0,01	2,30±0,01
5	-1	1	-1	10,04±0,08	0,609±0,009	0,26±0,08	3,33±0,0 3	1,11±0,01	0,15±0,01	2,29±0,05
6	1	-1	1	14,10±0,07	0,917±0,004	19,20±1,74	37,77±2, 07	22,50±0,87	8,41±0,48	97,71±0,55
7	1	1	-1	10,03±0,16	0,607±0,011	0,28±0,06	3,25±0,07	1,12±0,01	0,16±0,01	2,30±0,01
8	-1	-1	-1	10,04±0,08	0,609±0,009	0,26±0,08	3,33±0, 03	1,110±0,01	0,15±0,01	2,29±0,05

Y_1 : Moisture (%) ; Y_2 : Water activity ; Y_3 : Weight loss (%) ; Y_4 : Damages of the grains (%); Y_5 : Ochratoxin A content (µg/kg); Y_6 : Aflatoxin B_1 content (µg/kg); Y_7 : Content of total aflatoxins (µg/kg).

Table 5. Estimated coefficients and the experimental standard deviation of the results from the full factorial design

Coefficients	Moisture		Water activity		Weight loss		Dommages of the grains		OTA content		AFB1 content		AFT content	
	Values	2σe	Values	2σe	Values	2σe	Values	2σe	Values	2σe	Values	2σe	Values	2σe
β0	11,523*		0,709*		5,501*		13,188*		7,021*		2,426*		29,626*	
β1	-0,025		-0,002		0,001		-0,293		0,163		0,041		0,104	
β2	-0,553*		-0,054*		-4,236*		-7,815*		-4,462*		-1,785*		-20,454*	
β3	1,488*	0,03	0,101*	0,003	5,231*	0,002	9,898*	0,4	5,906*	0,328	2,271*	0,069	27,330*	0,363
β12	-0,015		-0,001		-0,001		0,2		-0,164		-0,034		0,181	
β13	-0,02		-0,001		-0,009*		-0,252		0,158		0,036		0,102	
β23	-0,553*		-0,054*		-4,236*		-7,815*		-4,462*		-1,785*		-20,454*	
R^2	0,9994		0,999		0,9999		0,9987		0,9974		0,9992		0,9998	

** Significant values, coefficients whose absolute value is greater than twice the experimental standard deviation (2σe) are statistically significant, |Coef|>2σe [35], [36].*
***R^2** Pearson coefficient*

Table 6. Experimental values obtained with optimal conditions for checking the model resulting from the full factorial design

Optimal conditions	Moisture (%) Y1		Water activity Y2		Weight loss (%) Y3		Domages of grains (%) Y4		Ochratoxin A (µg/kg) Y5		Aflatoxin B1 (µg/kg) Y6		Total of aflatoxins (µg/kg) Y7	
	Exp	Pred	Exp	Pred	Exp	Pred	Exp	Pred	Exp	Pred	Exp	Pred	Exp	Pred
X1 = White X2 = 5% X3 = 8 months	12,01±0,03*	11,91*	0,697±0,001**	0,702**	2,39±0,02*	2,27*	7,63±0,03**	7,46**	3,87±0,02*	4	0,99±0,01**	1,13**	15,99±0,03*	16,05*

Exp = Experimental; Pred = Prediction

Values in the same column with the same sign are not significantly different to risk 5%.

The action of leaves of *L. multiflora* on fungal organisms resulting in the decrease in production of mycotoxins (OTA AFB1 and AFT) could also be explained by presence of essential oils. The mode of action of essential oils on microorganisms depends in particular on their hydrophobicity allowing them to penetrate the phospholipid bilayer of cell membrane [30]. The inhibition of decarboxylation of amino acids and synthesis of DNA, RNA, proteins and polysaccharides has been reported [31]. Indeed, thymol can adhere to microorganisms by attaching to proteins and lipopolysaccharides parietal through their functional groups thus reaching the more vulnerable inner membrane [32]. This activity on mould was also mentioned by Biego and Chatigre [26] in their study on conservation of maize grain by lower production of aflatoxin B1. Other authors investigated antifungal activity of some essential oils from other plants on toxinogenic moulds isolated from peanut [33] and *Aspergillus ochraceus* toxinogenic *In vitro* [34].

The results of full factorial design indicate that grain conservation method in PICS bags with 5% leaves of *L. multiflora* is applicable to cowpea grains. This method is also independent of cowpea variety for all parameters studied. However, an interaction was observed between variety and the conservation duration for the weight loss of grains. The mathematical model resulting from full factorial design allows prediction of different parameters studied with Pearson coefficients at least equal to 0.9974. In addition, validation of that model revealed no significant difference between predicted values and those obtained under optimum conditions of conservation of cowpea grains.

4. CONCLUSION

The leaves of *L. multiflora* have insecticidal properties, antifungal and improve the merchantability and the health quality of cowpea grains during storage. The afterglow is at least 8 months and efficiency is independent of variety of stored cowpea grains. They could be an effective alternative in conservation of cowpeas replacing the synthetic antifungals that are not without consequences on health of consumer. This treatment should be popularized from farmers because it is inexpensive and easy to use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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