International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2014): 5.611

Optimization of Maize Conservation Methods (*Zea mays* L.) Using Phytopesticides in Polypropylene Bags Stored in Rural Farmer of Cote d'Ivoire

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Abstract: ***The present study aims to improve, from an experimental design, the methods of maize grain conservation in polypropylene bags stored in warehouses in rural environment of Katiola (Central North) and Bondoukou (North-east) of Côte d'Ivoire. The originality of this study lies on the use of phytopesticides (leaves of L. multiflora and H. suaveolens) in maize conservation. Thus, three full factorial designs with two levels represented by four parameters affecting maize conservation were used. The identified parameters were the storage time (2 and 6 months), the nature of phytopesticides (Lippia multiflora and/or Hyptis suaveolens), the quantity of phytopesticides (0 and 5% w/w) and the storage zone (Katiola and Bondoukou). The study determine that the optimal conservation was storage time at 6 months with mixture of L. multiflora and H. suaveolens (2.5% w/w each plant) in Katiola zone. In the planned optimal conditions, the experimental values were 1.53%, 12.1% and 1.10 µg/kg for weight loss, moisture percentage and aflatoxin B1 level respectively in polypropylene bags. These values of weight loss, moisture content and aflatoxin B_1 level were substantially equal to those predicted.***

Keywords: Maize, conservation methods, experimental design, Lippia multiflora, Hyptis suaveolens

1. Introduction

Maize (*Zea mays* L.) is the second cereal most cultivated in Côte d'Ivoire after rice (*Oryza spp*.). In 2013, its production was estimated at 654 738 tons for a total planted area of 327.800 ha [1]. This crop makes a substantial contribution to the diets and incomes of rural populations [2], [3]. It serves also as a major source of food, feed and raw material for agro-allied industries [4].

Cropping problems and post-harvests treatments of maize constitute the main part of the problems encountered by the farmers in rural environment [5], [6]. Several authors estimated that post-harvest losses are relatively high, in range of 20% to 30% because methods used are often inadequate and rudimentary [7], [8].

Johnson *et al.* [3] identified the major insect pests of maize stored in Cote d'Ivoire which are *Sitophilus zeamais*, the maize weevil, *Tribolium castaneum*, *Rhizopertha dominican* and *Catharthus dimidiatus*. The activity of these insects creates a favorable conditions for storage fungi development such as Aspergillus, Penicillium and Fusarium responsible for the deterioration of marketed quality (alteration of appearance, odor and taste of grain) and nutritive values of cereals [9]-[11].
In addition, these fungi also produce mycotoxins (aflatoxins, fuminosines, ochratoxin A, zearalenone and déoxynivalenone) harmful to the health of animals and humans [12].

An alternative to the use of synthetic molecules in the fight against storage pests is the use of phytopesticides, which is part of the millennium directives of environmental [13].

The phytopesticides are aromatic plants containing active molecules for insecticidal, fungicides, bactericides; nematicides properties used on-farm in the fight against pests crop and stocks [14]-[17].

Thus, this work focused on optimization, from an experimental design, the methods of maize conservation in polypropylene bags stored in warehouses in rural environment of Katiola (Central North) and Bondoukou (North-east) of Cote d'Ivoire

2. Material and Methods

2.1 Site description

The study was conducted in the villages of Timbe and Soko respectively located in the departments of Katiola (Hambol region) (8°10'N 5°40'W) and Bondoukou (Gontougou region) (8°30'N 3°20'W) in the Central North and North East of Cote d'Ivoire. The both localities have a humid tropical climate with four (4) seasons, including two (2) rainy seasons from March to July and October to November. These are interspersed with two (2) dry seasons ranging from December to February and August to September. The annual rainfall ranging between 1100 and 1200 mm in Katiola and between 800 and 1400 mm in Bondoukou. The average temperatures recorded in these areas vary between 26.5°C and 33.7°C in Katiola and between 24°C and 29°C in Bondoukou. The recorded average of humidity range between 60%-70% for the both region [18] [19].

2.2 Plant material collection and processing

The biological material consisted of maize grains collected in January 2014 and leaves of plant species *Lippia multiflora*

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(or savannah tea) and *Hyptis suaveolens* collected 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 [20] [21]. After harvest, maize was sun-dried and leaves of *L. multiflora* and *H. suaveolens* were drying at an average temperature kept away from direct sun exposure.

2.3 Implementation of experiment

The implementation of the study was conducted from January to September 2014, with the participation of 2 Informal Groups (IG) of farmers. They are the IG "Sounougou" of Soko in Bondoukou and the IG "Lagnimin" of Timbe in Katiola. These farmers accustomed to preserve their maize grain in polypropylene bags in a corner of the house. Method tested in this study, consisted in adding of phytopesticides (5% w/w) in the polypropylene bags containing maize grains and storing on pallets in warehouses for 8 months. The steps of adding phytopesticides *(Lippia multiflora* and *Hyptis suaveolens*) and deposit bags on pallets constitute the principal modifications made to the method of conservation practiced by these farmers. Leaves of *L. multiflora* and *H. suaveolens* were chopped and the filling of the bags was performed by alternately as maize grains strata and phytopesticides. Thus, polypropylene bags containing 50 kg of maize grain and 5% w/w of *L. multiflora* or *H. suaveolens* or in mixture were stored as described below:

- Treatment 1: 50 kg of maize grain + 2.5 kg of leaves of *L. multiflora*
- Treatment 2: 50 kg of maize grain + 2.5 kg of leaves of *H. suaveolens*
- Treatment 3: 50 kg of maize grain + 1.25 kg of leaves of *L. multiflora* + 1.25 kg of leaves of *H. suaveolens*
- Treatment 4: control (50 kg of maize grain alone)

The treatments were laid out in a randomized complete block design in each zone of study, and each treatment was replicated 3 times.

2.4 Application of full factorial design

Three full factorial designs at 2 levels were carried out according to the model of Faucher [22], to identify the relationship existing between the response functions and independent variables [23], as well as to determine those conditions that optimized post harvest conservation of maize grains. The 4 factors studied were the storage time: 2 and 6 months (X_1), the nature of phytopesticides: *L multiflora* and *H. suavolens* alone or in mixture (X_2), the quantity of phytopesticides: 0 and 5% w/w (X_3), and the storage zones: Bondoukou and Katiola (X_4). Each variable to be optimized was coded at the lower (-1) and higher (+1) levels (Table I). The experimental design led to implementation of 16 trials in each case according to [24]; given a total of 48 trials considering the nature of phytopesticides. The responses studied were weight loss, moisture content and aflatoxin B_1 level.

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

$$\mathbf{Y_n = b_0 + b_1X_1 + b_2X_2 + \ldots. b_kX_k + b_{12}X_1X_2 + \ldots + b_{k\text{-}1k}X_{k\text{-}1}X_k + \ldots + b_{1\ldots k}X_1X_{2\ldots}X_k} \quad \mathbf{(1)}$$

Where $\mathbf{Y_n}$ was the measured response; $\mathbf{b_k}$ the main effect of factor $\mathbf{X_k}$, $\mathbf{b_{k\text{-}1k}}$ the interaction effect between factors $\mathbf{X_k}$ and $\mathbf{X_{k\text{-}1}}$ and $\mathbf{b_0}$ the constant term. Thus, a randomly sample of 3 kg was carried out per bags at 2 and 6 months, in triplicate. Maize samples were then transported to the laboratory where weight loss, moisture and aflatoxin B_1 measurements were made.

Table 1: Independent variables and their coded used for the 2^4 factorial designs

Factors	Independent variable	Code levels	
		Low (-1)	High (+1)
X_1	Storage time (months)	2	6
X_2	Nature of phytopesticides	L. multiflora	H. suaveolens
		L. multiflora	Mixture of L. multiflora and H. suaveolens
		H. suaveolens	Mixture of L. multiflora and H. suaveolens
X_3	Quantity of phytopesticides (%)	0%	5%
X_4	Storage zone	Bondoukou	Katiola

2.5 Analytical methods

2.5.1 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 [25], [26]. 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.

2.5.2 Determination of moisture content

The moisture content was determined by the difference of weight before and after drying the sample in an oven (MEMMERT, Germany) at 105°C until constant weight AOAC [27].

2.5.3 Determination of Aflatoxin B1

Aflatoxin B_1 (AFB_1) was extracted and cleaned up following official method of AOAC [28]. 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

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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 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, moisture content and aflatoxin B1 were determined. For that 16 experiments in each case; given a total of 48 experiments considering the nature of phytopesticides were conducted according the matrix presented in Tables 2, 3 and 4.

The values of regression coefficient determined are given in Table 5. The effect of individual variables and interactions effects was estimated [29].

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, $|coef| > 2\sigma$ [30].

Statistical analysis of data, for the different experimental designs, show that variables such as storage time, quantity of biopesticides and storage zones presented significant effect on weight loss (Table 6). The most important parameter affecting weight loss is the storage time. Also, there is a significant interaction between storage time (X_1) - quantity of biopesticides (X_3), storage time (X_1) – storage zones (X_4) and quantity of biopesticides (X_3) - storage zones (X_4). The predictive equations of weight loss (Y_1), neglecting the non-significant factors, are given by these equations with a satisfactory value R^2 ranging from 0.95 to 0.96.

$$Y_1^a = 10.37 + 7.54\,X_1 - 5.87\,X_3 - 4.8\,X_4 - 4.4\,X_1X_3 - 4.72\,X_1X_4 + 2.5\,X_3X_4 \quad (2)$$

$$Y_1^b = 9.7 + 7.3\,X_1 - 6.2\,X_3 - 4.5\,X_4 - 4.84\,X_1X_3 - 4.47\,X_1X_4 + 2.73\,X_3X_4 \quad (3)$$

$$Y_1^c = 10.16 + 7.36\,X_1 - 6.34\,X_3 - 4.4\,X_4 - 4.76\,X_1X_3 - 4.52\,X_1X_4 + 2.95\,X_3X_4 \quad (4)$$

a: Lippia multiflora ; b : Hyptis suaveolens ; c : mixture of Lippia multiflora and Hyptis suaveolens

The interactions between the various factors influencing weight losses are illustrated in Figure 3. The Weight losses increase significantly, in the control, from 4.27% to 28.22% from the second to sixth months of storage, whereas, in presence of biopesticides, interactions increase slightly from 1.39% to 7.62%. Results show that weight losses are higher in Bondoukou zone.

As for moisture content, which was responsible for various biological phenomena of mycological alteration particular in food, it was significantly influenced by the storage time, the quantity of phytopesticide and the storage zone. However, there were no significant interaction effects between the different factors. The resulting mathematical models with a satisfactory value R^2 ranging from 0.96 to 0.98 are given by these equations below:

$$Y_2^a = 11.8 + 0.64\,X_1 - 0.57\,X_3 - 0.37\,X_4 \quad (5)$$

$$Y_2^b = 11.83 + 0.54\,X_1 - 0.55\,X_3 - 0.54\,X_4 \quad (6)$$

$$Y_2^c = 11.84 + 0.51\,X_1 - 0.58\,X_3 - 0.5\,X_4 \quad (7)$$

a: Lippia multiflora ; b : Hyptis suaveolens ; c : mixture of Lippia multiflora and Hyptis suaveolens

Concerning aflatoxin B_1 (AFB_1) level, it was affected by the storage time, the quantity of biopesticides and the storage zones. The most important parameters affecting aflatoxin B_1 level are the same as in the case of weight losses (factor X_1). Three significant interactions were also observed: storage time (X_1) - quantity of biopesticides (X_3), storage time (X_1) – storage zones (X_4) and quantity of biopesticides (X_3) - storage zones (X_4).

The data showed a good fit, being were statistically acceptable at P=0.05 level and adequate with a satisfactory R^2 value ranging from 0.95 to 0.96. The mathematical models being developed to present the relationships between aflatoxin B_1 and conservation variables are given by these equations:

$$Y_3^a = 32.88 + 15.64\,X_1 - 29.17\,X_3 - 16.7\,X_4 - 14.3\,X_1X_3 - 9.45X_1X_4 + 15.4\,X_3X_4 \quad (8)$$

$$Y_3^b = 32.2 + 17.13\,X_1 - 27.73\,X_3 - 15.3\,X_4 - 15.6\,X_1X_3 - 8\,X_1X_4 + 14\,X_3X_4 \quad (9)$$

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$$Y_3^c = 36.8 + 18.3\ X_1 - 31.96\ X_3 - 14.86\ X_4 - 16.7\ X_1X_3 - 8.1\ X_1X_4 + 13.2\ X_3X_4 \quad (10)$$

a: Lippia multiflora ; b : Hyptis suaveolens ; c : mixture of Lippia multiflora and Hyptis suaveolens

The study of the influence of different parameters on aflatoxin B_1 level was shown in Figure 4. During storage, aflatoxin B_1 level increase significantly in control (without phytopesticides) from 32.1 µg/kg to 92.6 µg/kg in the second to sixth months of storage, whereas in the presence of phytopesticides, it gradually increase from 2.35 µg/kg to 5.07 µg/kg. The interaction between storage time and storage zone shows that aflatoxin B_1 level increases higher in Bondoukou zone than Katiola zone.

4. Discussion

The results of this study show that methods of maize grains conservation with the 2 species plants, *Lippia multiflora* and *Hyptis suaveolens* in polypropylene bags are able to reduce development of pest alteration of maize. Indeed, lower levels of weight loss and aflatoxin B_1 were observed in polypropylene bags with 5% of phytopesticides during storage. The insecticidal and/or repellent activity of the leaves of these plants would be due to the release of bioactive molecules in their essential oils [16]. These results are consistent with the findings of Gueye *et al* [17] 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* [31] also demonstrated the insecticidal activity of powders to 10% w/w 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 [20] 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.

The combination of the 2 plant materials to 2.5% w/w of each did not produce any significant synergistic or additive effect on their repellency compared the use of sheets of single species. Similar observations were made by Ukeh *et al* [31] who reported that the mean number of *Sitophilus. zeamais* population on treated maize cobs stored with *Aframomum melegueta* was not significantly different from the number of population of the same species on treated maize cobs stored with *Zingiber officinale* and on treated maize cobs stored with the mixture of *A. melegueta and Z. officinale.*

The application of the 2 plant materials to protect stored maize from aflatoxin B_1 contamination reduces significantly the production of aflatoxin B_1 compared to controls. Sharma *et al* [32] 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* [33] 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. The result is also in agreement with Shukla *et al* [34] who demonstrated that the essential oil of *Lippia alba* and 2 of its monoterpene aldehyde constituents have antifungal activity and Aflatoxin B_1 inhibition against 17 fungi isolated from 11 edible legume seeds. The essential oil (0.25–1 µL/mL) and its 2 constituents (1 µL/mL) showed remarkable antifungal effects against all the fungal isolates (growth inhibition range 32.1 -100%).

The results of the experimental analysis shows that the methods of maize grain conservation in polypropylene bags is favored when the variable storage time, nature of phytopesticides, quantity of phytopesticides and storage region were at their highest levels (+1). Thus, the optimum process of post harvest maize storage involves the following parameters:

- Storage time: 6 months
- Nature of phytopesticides: Mixture leaves of *L. multiflora* and *H. suaveolens*
- Quantity of biopesticides: 5%
- Storage zone: Katiola

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Table 1: Experimental results of the use of *L. multiflora* and *H. suaveolens* according to full factorial experimental design

Test set	Independent variables				Experimental responses		
	X_1	X_2	X_3	X_4	Y^a_1	Y^b_2	Y^c_3
1	2 month	A	0 %	Bondoukou	3.86±1.4	11.7±1.2	40.64±7.4
2	6 month	A	0%	Bondoukou	42.4±6.1	13.67±2	132.52±9.8
3	2 month	B	0%	Bondoukou	4.36±1.5	11.7±0.42	51.25±6.5
4	6 month	B	0%	Bondoukou	43.73±8.6	13.8±4.5	152.25±8.1
5	2 month	A	5%	Bondoukou	1.42±1.2	11.2±0.18	2.55±1.1
6	6 month	A	5%	Bondoukou	11.63±2.2	12.5±0.54	6±1.2
7	2 month	B	5%	Bondoukou	1.93±0.1	11±0.22	3.51±1.1
8	6 month	B	5%	Bondoukou	12.2±1.1	11.75±0.58	8±1.5
9	2 month	A	0%	Katiola	3.32±1.4	11.2±0.8	10.37±2.1
10	6 month	A	0%	Katiola	12.85±2.5	12.2±0.78	13±1.6
11	2 month	B	0%	Katiola	5.53±0.6	11.60±1	26.2±2.3
12	6 month	B	0%	Katiola	14.15±2.7	12±1.72	70.3±1.5
13	2 month	A	5%	Katiola	1.1±0.5	10.6±0.1	2.2±0.2
14	6 month	A	5%	Katiola	2.64±1.6	11.2±0.13	3.3±0.1
15	2 month	B	5%	Katiola	1.1±0.2	10.5±0.3	1.13±0.5
16	6 month	B	5%	Katiola	4±1.3	11.51±0.65	3±0.34

A: Lippia multiflora; B: Hyptis suaveolens;
Y1: Weight loss; Y2: Moisture content; Y3: Aflatoxin B1 level; a: Percentage; b: values given on dry matter basis; c: µg/kg

Table 2: Experimental results of the use of L. multiflora and mixture of L. multiflora and H. suaveolens according to full factorial experimental design

Test set	Independent variables				Experimental responses		
	X_1	X_2	X_3	X_4	Y^a_1	Y^b_2	Y^c_3
1	2 month	A	0 %	Bondoukou	3.86±1.4	11.7±1.2	40.64±7.4
2	6 month	A	0%	Bondoukou	42.14±6.1	13.67±2	132.52±9.8
3	2 month	C	0%	Bondoukou	3.45±1	12.5±2.4	42.12±2
4	6 month	C	0%	Bondoukou	42.7±5	14.2±3	141.63±5
5	2 month	A	5%	Bondoukou	1.42±1.2	11.2±0.18	2.55±1.1
6	6 month	A	5%	Bondoukou	11.63±2.2	12.5±0.54	6±1.2
7	2 month	C	5%	Bondoukou	0.95±0.1	11.2±1	4.3±1
8	6 month	C	5%	Bondoukou	7.15±1	12±1	10.25±3
9	2 month	A	0%	Katiola	3.32±1.4	11.2±0.8	10.37±2.1
10	6 month	A	0%	Katiola	12.85±2.5	12.2±0.78	45.9±6
11	2 month	C	0%	Katiola	4.15±1	11.6±1	15.5±2
12	6 month	C	0%	Katiola	13.98±4	12±2	50.8±6
13	2 month	A	5%	Katiola	1.1±0.5	10.6±1.1	2.2±0.2
14	6 month	A	5%	Katiola	2,64±1,6	11.2±0.13	3.3±0.15
15	2 month	C	5%	Katiola	0.87±0.1	10.6±1	3±1
16	6 month	C	5%	Katiola	2.32±1	11±1	4.32±1

A: Lippia multiflora; C: mixture of Lippia multiflora and Hyptis suaveolens
Y1: Weight loss; Y2: Moisture content; Y3: Aflatoxin B1 level; a: Percentage; b: values given on dry matter basis; c: µg/kg

Table 3: Experimental results of the use of H. suaveolens and mixture of L. multiflora and H. suaveolens according to full factorial experimental design

Test set	Independent variables				Experimental responses		
	X_1	X_2	X_3	X_4	Y^a_1	Y^b_2	Y^c_3
1	2 month	B	0 %	Bondoukou	4.36±1	11.7±0.42	51.25±6.5
2	6 month	B	0%	Bondoukou	43.73±4	13.8±4.5	152.25±8.1
3	2 month	C	0%	Bondoukou	3.45±1	12.5±2.4	42.12±2
4	6 month	C	0%	Bondoukou	42.7±5	14.2±3	141.63±5
5	2 month	B	5%	Bondoukou	1.93±0.1	11.2±0.18	3.51±1.1
6	6 month	B	5%	Bondoukou	12.2±1,1	12.5±0.54	8±1.5
7	2 month	C	5%	Bondoukou	0.95±0.1	11±0.22	4.3±1
8	6 month	C	5%	Bondoukou	7.15±1	11.75±0.58	10.25±3
9	2 month	B	0%	Katiola	5.53±0,6	11.60±1	26.2±2.3
10	6 month	B	0%	Katiola	14.15±2.7	12±1.72	70.3±1.5
11	2 month	C	0%	Katiola	4.15±1	11.6±1	15.5±2
12	6 month	C	0%	Katiola	13.98±4	12±2	50.8±6
13	2 month	B	5%	Katiola	1.1±0.2	10.5±0.3	1.13±0.5
14	6 month	B	5%	Katiola	4±1.3	11.51±0.65	3±0.34
15	2 month	C	5%	Katiola	0.87±0.1	10.6±1	3±1
16	6 month	C	5%	Katiola	2.32±1	11±1	4.32±1

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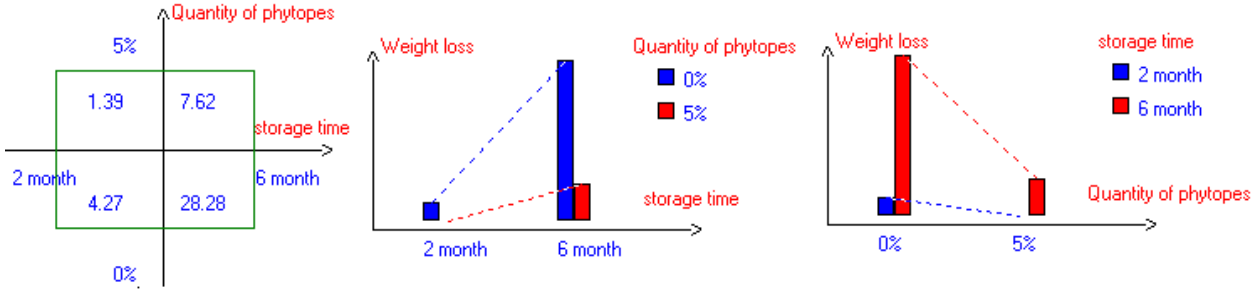
B: Hyptis suaveolens; C: mixture of Lippia multiflora and Hyptis suaveolens
Y1: Weight loss; Y2: Moisture content; Y3: Aflatoxin B1 level; a: Percentage; b: values given on dry matter basis; c: µg/kg

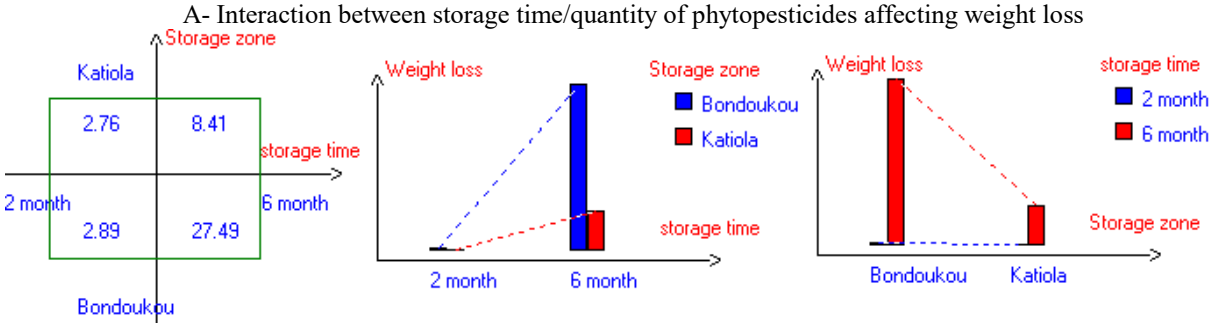
Table 4: Regression coefficients of predicted factorial experimental design of L. multiflora and H. suaveolens alone or in mixture

Coefficient and standard deviations for each equation																		
	L. multiflora and H. suaveolens						*L. multiflora and mixture*						*H. suaveolens and mixture*					
Co-efficients	*Weight loss (Y1)*		*Moisture (Y2)*		*Aflatoxin B1 (Y3)*		*Weight loss (Y1)*		*Moisture (Y2)*		*Aflatoxin B1 (Y3)*		*Weight loss (Y1)*		*Moisture (Y2)*		*Aflatoxin B1 (Y3)*	
	Values	*2σ*	*Values*	*2σ*	*Values*	*2σ*	*Values*	*2σ*	*Values*	*2σ*	*Values*	*2σ*	*Values*	*2σ*	*Values*	*2σ*	*Values*	*2σ*
b_0	10.37*	2.44	11.77*	0.16	32.88*	9.14	9.7*	2.54	11.83*	0.23	32.2*	6.4	10.16*	2.73	11.84*	0.17	36.8*	6.41
b_1	7.54*		0.64*		15.64*		7.3*		0.51*		17.13*		7.36*		0.5*		18.3*	
b_2	0.5 ^{ns}		-0.01 ^{ns}		6.57 ^{ns}		-0.21 ^{ns}		0.06 ^{ns}		1.8 ^{ns}		-0.71 ^{ns}		-0.01 ^{ns}		-2.82 ^{ns}	
b_3	-5.87*		-0.57*		-29.17*		-6.2*		-0.55*		-27.73*		-6.34*		-0.58*		-31.96*	
b_4	-4.8*		-0.37*		-16.71*		-4.5*		-0.54*		-15.3*		-4.4*		-0.5*		-14.86*	
b_{12}	0.1 ^{ns}		-0.03 ^{ns}		3.28 ^{ns}		-0.2 ^{ns}		-0.1 ^{ns}		0.64 ^{ns}		-0.28 ^{ns}		-0.1 ^{ns}		-0.53 ^{ns}	
b_{13}	-4.43*		-0.11 ^{ns}		-14.3*		-4.84*		-0.13 ^{ns}		-15.7*		-4.76*		-0.07 ^{ns}		-16.7*	
b_{14}	-4.72*		-0.12 ^{ns}		-9.46 *		-4.47*		-0.21 ^{ns}		-8*		-4.52*		-0.12 ^{ns}		-8.1 *	
b_{23}	-0.2 ^{ns}		-0.15 ^{ns}		-6.38 ^{ns}		-0.47 ^{ns}		-0.13 ^{ns}		-0.81 ^{ns}		-0.3 ^{ns}		-0.16 ^{ns}		3.42 ^{ns}	
b_{24}	0.1 ^{ns}		0.13 ^{ns}		2.41 ^{ns}		0.4 ^{ns}		0.05 ^{ns}		-0.3 ^{ns}		0.3 ^{ns}		0.04 ^{ns}		-0.74 ^{ns}	
b_{34}	2.5 *		-0.01 ^{ns}		15.41*		2.73 *		-0.1 ^{ns}		14*		2.95 *		-0.13 ^{ns}		13.2*	

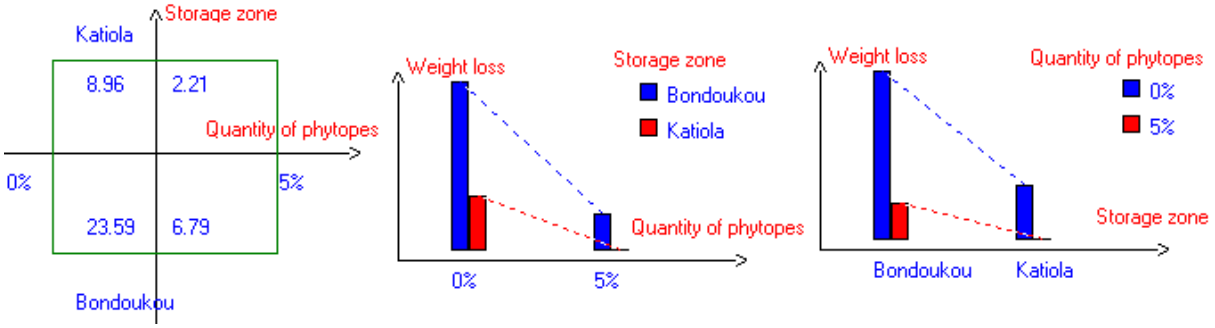
*^{ns}: no significant values; *:significant data at P=.05.*



A- Interaction between storage time/quantity of phytopesticides affecting weight loss



B- Interaction between storage time /storage zone affecting weight loss



C-Interaction between quantity of phytopesticides/ storage zone affecting weight loss

Figure 1: Interaction between factors affecting weight loss

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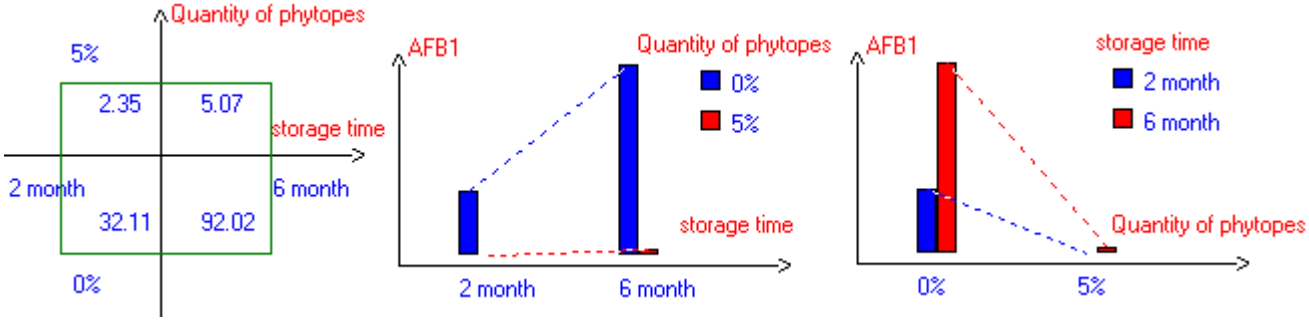
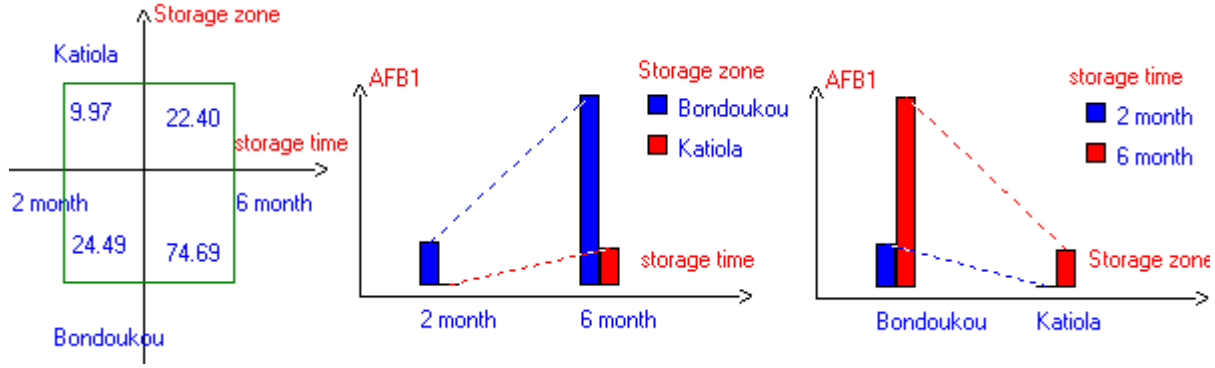
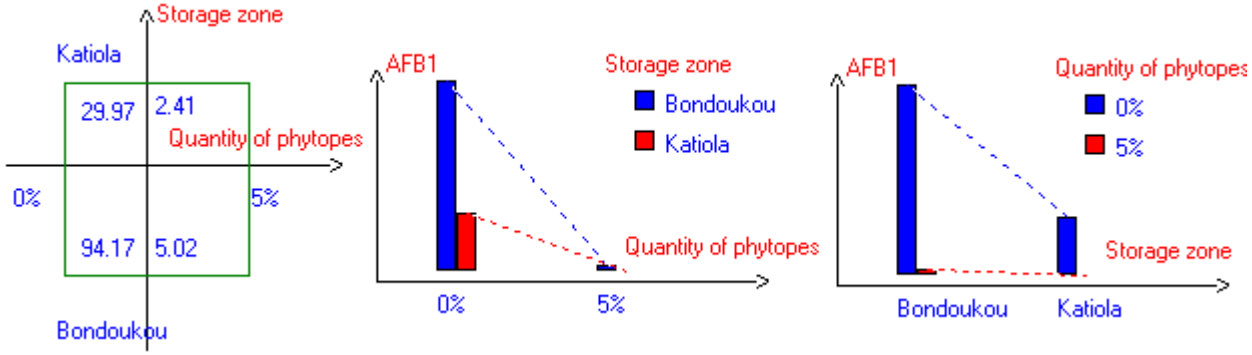


A- Interaction between storage time/quantity of phytopesticides affecting aflatoxin B1 level



B-Interaction between storage time /storage zone affecting aflatoxin B1 level



C-Interaction between quantity of phytopesticides/ storage zone affecting aflatoxin B1 level

Figure 2: Interaction between factors affecting aflatoxin B_1 level

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 0.95 to 0.98; 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 3 times at the optimum condition, and the results are presented in Table VIII. The arithmetic means of the experimental values were 1.53%, 12.2% and 1.1 µg/kg for weight loss, moisture content and aflatoxin B_1 level respectively.

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

Optimal Condition		*Experimental Values*	*Predicted Values*
X_1= 6 Month	Weight loss (%)	1.53± 0.5 [a]	1.2 [a]
X_2= Mixture of L. multiflora and H. suaveolens	Moisture content (%)	12.2± 2.3 [b]	11.80 [b]
X_3= Phytopest 5%	AFB_1 (µg/kg)	1.1± 0.1 [c]	0.85 [c]
X_4= Maize grains			

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

5. Conclusion

The results of this study indicate that *L. multiflora* and *H. suaveolens* leaves are effective for post-harvest of maize grain conservation in polypropylene bags stored in warehouses in rural environment. These phytopesticides can fight effectively against storage pests and fungal contamination. The experimental design has identified

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optimal conditions of maize grains conservation in polypropylene bags: it is 6 month storage period, use of 5% of dried leaves of *L. multiflora* and *H. suaveolens* alone or in admixture.

The technology used is inexpensive, easily carried and fits into the millennium guidelines of respect for the environment. However, this study should be continued with other types of packaging for better control of storage conditions to ensure the market qualities, nutritive and hygienic maize grain after storage.

6. Acknowledgements

We thank Dr. Angniman Ackah Pierre, Executive Director of Interprofessional Fund for Agricultural Research and Advisory services (FIRCA), Coordinator of the West Africa Agricultural Productivity Program (WAAPP/PPAAO) in Côte d'Ivoire, who kindly entrusted the implementation of this project to the Laboratory of Biochemistry and Food Sciences (LABSA) of Félix Houphoüet-Boigny University. We also extend our thanks to the authorities and farmers of Soko and Timbé village for their warm welcome.

References

[1] N'da, L. Akanvou, 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

[2] R Blassonny. Ivoire grain, http://www.ivoiregion.net/index.php/lanouvelle/ 726 germinationle-mais-veut-damer-le-pion-au-coton (2013)

[3] F. Johnson, K. N'Zi, Seri-Kouassi, K. Foua-Bi. Aperçu des problèmes de stockage et incidences des insectes sur la conservation du riz et du maïs en milieux paysans : cas de la région de Bouaflé – Côte d'Ivoire. European Journal of Scientific Research, 2012 ; 83 :349-363.

[4] K. Deffan., L. Akanvou, R. Akanvou, G. Nemlin, 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.

[5] Ratnadass. Dynamique des populations d'insectes ravageurs des stocks des céréales en milieux villageois d'Afrique tropicale. , 1987 ; Thèse Docteur-Ingénieur en Sciences agronomiques. Institut National Agronomique Paris-Grignon, 115 p

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

[7] F. Noudjou-Wandji. Utilisation des huiles essentielles pour la protection des grains contre les insectes ravageurs au Nord du Cameroun. 2007 ; thèse de doctorat en sciences agronomiques et ingénierie biologique, Université de Gembloux, Belgique, 206p

[8] M. Gueye, D. Seck, J-P. Wathelet, G. Lognay. Typologie des systèmes de stockage et de conservation du maïs dans l'est et le sud du Sénégal. Biotechnol. Agron. Soc. Environ., 2012; 16: 49-58.

[9] K. Cardwell., J. Kling, B. Maziya-Dixon, N. Bosque-Pérez. Relationships among *Fusarium verticillioides* and *Aspergillus flavus* ear rot pathogens, insects and grain quality in four maize genotypes in the low land tropics of Africa. 2000; Phytopathology 90: 276–284.

[10] P. Fandohan, B. Gnonlonfin, K. Hell, W. F. Marasas, 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.

[11] M. Kankolongo, K. Hell, 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.

[12] K. Hell, K. Cardwell, M. Setamou, 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.

[13] M. Isman. Botanica1 insecticides, deterrents, and repellents in modem agriculture and an increasing1y regu1ated world. 2006; Annual Review of Entomology, 51: 45-66.

[14] D. Kossou, P. Atachi, T. Zannou, S. Bougourou. Evaluation de l'activité insecticide de deux plantes *Hyptis suaveolens* (Linn) et *Khaya senegalensis* (A. Juss) sur les insectes ravageurs du niébé (*Vigna unguiculata* L. Walp.). 2007 ; Sciences & Nature 4: 17-26.

[15] L. Tapondjou, C. Adler, H. Bouda, D. Fontem. Efficacy of powder and essential oil firom *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. 2002; Journal of Stored Products Research. 38: 395-402.

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

[17] 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.

[18] K. Kouakou, L. Akanvou, A. Konan, 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

[19] K. Ahoussi, T. Youann, S. Loko, M. Adja, T. Lasm et J. Jourda. Étude hydrogéochimique des eaux des aquifères de fractures du socle Paléoprotérozoïque du Nord-Est de la Côte d'Ivoire : Cas de la région de Bondoukou. 2012 ; Afrique Sciences, 8: 51-68.

[20] 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

[21] Ekissi, A. Konan, A. Yao-Kouame, B. Bonfoh, S. Kati-Coulibaly. Sensory evaluation of green tea from Lippia

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multiflora Moldenke leaves. European Scientific Journal. 2014;10: 534-543

[22] J. Faucher. Les plans d'expériences pour le réglage de commandes à base de logique floue. 2006 ; Doctorat. Institut National Polytechnique de Toulouse, France. 160 pp.

[23] 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.

[24] L. Niamketchi, H. G. Biego, O. Chatigre, D. Amané, E. Koffi, A. Adima. Optimization of Post-Harvest Maize Storage using Biopesticides in Granaries in Rural Environment of Côte d'Ivoire. 2015; International Journal of Science and Research, 4 (9) : 1727-1736

[25] K. Harris and C. Lindblad. Post-harvest grain loss assessment methods - American Association of Agricultural Chemists. 1978, St Paul, Minnesota, 193 p.

[26] 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.

[27] AOAC. Official methods of analysis (13th ed.). Association of Official Analytical Chemists. Arlington, VA, USA. 1990.

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

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

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

[31] 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.

[32] N. Sharma, U. Verma, 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.

[33] R. Shukla, A. Kumar, P. Singh, K. Dubey. Efficacy of *Lippia alba* (Mill.) N.E. Brown essential oil and its monoterpenes aldehyde constituents against fungi isolated from some edible legume seeds and aflatoxin B1 production. 2009; International Journal of Food Microbiology 135, 165–170.

[34] N. Tatsadjieu, J. Dongmo, M. Ngassoum, F.-X Etoa, C. Mbofung. Investigations on the essential oil of Lippia rugosa from Cameroon for its potential use as antifungal agent against Aspergillus flavus Link ex. Fries. Food Control. 2009; 20: 161-166.

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