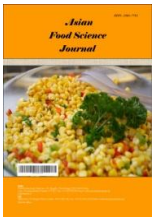

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Evolution of Mycotoxins during Maize Grains Storage in Triple Bags Containing Plants Biopesticides (*Lippia multiflora* and *Hyptis suaveolens*)

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Authors' contributions

This work was carried out in collaboration among all authors. Author YVG designed the study, wrote the protocol, fitted the data and wrote the first draft of the manuscript. Author KKC checked the first draft of the manuscript and achieved the submitted manuscript. Authors NGL, AC and KKC performed the statistical analysis and assisted the experiments implementation. Author GHMB expertized the results interpretations. All authors managed the literature, read and approved the submitted manuscript.

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ABSTRACT

In Côte d'Ivoire, maize plays an essential role as subsistence, commercial and socio-cultural culture. To consume good quality corn, it is necessary to preserve the sanitary quality through a good storage method. The aim of study was to evaluate sanitary quality of stored maize in triple bags containing plants biopesticides. Maize grains were collected in March 2016 in the north of Côte d'Ivoire. The fresh leaves of *Lippia multiflora* and *Hyptis suaveolens* were collected and dried in sunlight for 7 days in the center of Côte d'Ivoire. Triple bags were bought in Abidjan market. All this material was sent to the Laboratory of Biochemistry and Food Sciences, Félix Houphouët-Boigny University, Côte d'Ivoire, to perform the experiment. A central composite design was used for sample constitution. Ten treatments were obtained for the experimentation. The first treatment was conservation of 50 kg of maize grain in a polypropylene bag. The second treatment was conservation of 50 kg of maize grain in a triple bag. The other eight treatments were carried out with PICS bags each containing 50 kg of maize grain and different proportions of chopped leaves *Lippia multiflora* and *Hyptis suaveolens*. Thus, a control group with polypropylene bag (TPPB0), a control group in triple bag without biopesticides (TPB0) and 8 experimental lots of triple bags noted TB1 containing 0.625kg L. multiflora and 0.625kg *H. suaveolens*, TB2 with 0.40 kg of *L. multiflora* and 1.60 kg of *H. suaveolens*, TB3 with 1.60 kg of *L. multiflora* and 0.40 kg of *H. suaveolens*, TB4 with 0.10 kg of *L. multiflora* and 0.40 kg of *H. suaveolens*, TB5 with 0.40 kg of *L. multiflora* and 0.10 kg of *H. suaveolens*, TB6 with 2.5 kg of *L. multiflora* and 2.5 kg of *H. suaveolens*, TB7 with 1.25 kg of *L. multiflora* and TB8 with 1.25 kg of *H. suaveolens* have been used. The contents of moisture, water activity, aflatoxin B1 (AFB1), ochratoxin A (OTA), fuminosin B1 (FB1) and zearalenone (ZEA) were studied. The levels of AFB1, OTA, FB1 and ZEA resulted from maize grains treated with biopesticides were significantly lower than those recorded with untreated maize of control bags. The results show AFB1 levels (from 4.17 ± 0.05 to 5.15 ± 0.06 µg/kg), OTA levels (from to 4.58 ± 0.25 to 6.10 ± 0.01 µg/kg), FB1 levels (from 4.96 ± 0.07 µg/kg to 7.42 ± 0.06 µg/kg) and ZEA levels (from 4.66 ± 0.10 µg/kg to 8.78 ± 0.14 µg/kg). Maize samples stored in triple bagged bags with different proportions of biopesticide were significantly lower than those recorded in the polypropylene woven sample bag (TPPB0) and in the triple bagged control bag (TPB0) during the storage period. Storage of maize grains in triple bags with the leaves of *L. multiflora* and *H. suaveolens* appears as a method of effective and inexpensive conservation to ensure the sanitary quality of maize. This inexpensive and easy-to-use treatment should be popularized among farmers.

Keywords: Mycotoxin; stored maize; biopesticides; triple bagging; sanitary quality.

1. INTRODUCTION

Ranked third in the world after wheat and rice, maize (*Zea mays* L.) is one of the most widely grown cereals in the world. In Côte d'Ivoire, of all food crops, it is the second most cultivated cereal after rice (Oryza spp.), with an annual national production of 760,000 t in 2016 (FAOSTAT, 2016). It plays an essential role as subsistence, commercial and socio-cultural culture [1]. Hence, maize is the staple food of a large segment of the Ivorian populations. Despite the growth in its production and its socio-economic importance, post-harvest losses during storage remain a real challenge for farmers [2]. Indeed, post-harvest losses can reach more than 60% in maize stored in traditional storage structures [3]. These losses are mainly due to insects and molds under inadequate storage conditions. The activity of insect pests creates an environment favorable to the growth of molds of the genus *Aspergillus*, *Penicillium* and *Fusarium* [4,5].

These molds produce mycotoxins responsible for many diseases in humans and animals such as cancer [6]. Maize can be contaminated with two or more mycotoxins. In Côte d'Ivoire, studies revealed mycotoxins co-contamination in maize [7,8]. In response, contemporary control methods consist in the regular use of chemical pesticides [9]. However, pesticides cause environmental pollution, ecological disorders and loss of life due to their intoxication [10]. In Côte d'Ivoire, organochlorine chemical pesticides were found at concentrations ranging from 2 to 59.7 µg / kg in cocoa bean stocks and between 2 and 237 µg / kg in kola nut stocks [11,12]. Thus, it is important to research for developing such alternatives methods of pests and molds control, accessible to farmers and protecting the

environment. Many studies have shown the effectiveness of airtight systems in controlling pests [13,14]. The most used and most practical technology in the peasant environment is triple bagging or PICS bag. This storage method was initiated by the Purdue American University in Niger for the storage of cowpea and for a long period.

In addition, other studies have shown the insecticidal or repellent activity of certain aromatic plants (Neem, *Lippia multiflora*, *Hyptis suaveolens*) during post-harvest storage of foodstuffs [15,16]. In addition, studies have shown the synergistic effect of the triple bagging system and the leaves of *Lippia multiflora* on the sanitary quality of cowpea grains during storage [17]. However, in Côte d'Ivoire, this study has not yet been repeated on corn. Thus, this study aims to evaluate the sanitary quality of maize grains stored in a triple bagging system in the presence of the leaves of *L. multiflora* and *H. suaveolens*.

2. MATERIALS AND METHODS

2.1 Collection of Maize Grains and Biopesticides Plants Used in the Study

Maize grains and leaves of *L. multiflora* and *H. suaveolens* were collected in March 2016 from producers of Gbêkê region (7°50 North and 5°18 West in center of Côte d'Ivoire). Prior to the storage, maize were sun-dried for 2-3 days before being used for the experiment. While, the *L. multiflora* and *H. suaveolens* leaves were drying at an average temperature of 30 ◦C for 6-7 days, and kept away from direct sun exposure. The dried leaves were chopped into fine particles before being used for the experiment.

2.2 Implementation of Experiment

2.2.1 Using the triple bagging

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

2.2.2 Treatments

The implementation of the study was conducted from March 2016 to September 2017. The storage method is based on the mixture of plants leaves. Method tested in this study, consisted in adding of biopesticides (0-5% w/w) in the polypropylene bags and the triple bagging system containing 50 kg maize grains and storing on pallets in warehouses for 18 months. The filling of the bags was performed by alternately as maize grains strata and biopesticides. The maize grains were stored as follows:

- 1 control batch of 50 kg of maize grain in polypropylene bag without biopesticide (TPPB0);
- 1 control batch of 50 kg of maize grain in triple bagging system without biopesticide (TPB0);
- 1 experimental batch of 50 kg of maize grain in triple bagging system with 2.5% of biopesticides (0.625kg *L. multiflora* and 0.625kg *H. suaveolens*) (TB1)
- 1 experimental batch of 50 kg of maize grain in triple bagging system with 3.99% de biopesticides (0.40 kg *L. multiflora* and 1.60 kg *H. suaveolens*) (TB2)
- 1 experimental batch of 50 kg of maize grain in triple bagging system with 3.99% de biopesticides (1.60 kg *L. multiflora* and 0.40 kg *H. suaveolens*) (TB3)
- 1 experimental batch of 50 kg of maize grain in triple bagging system with 1.01% de biopesticides (0.10 kg *L. multiflora* and 0.40 kg *H. suaveolens*) (TB4)
- 1 experimental batch of 50 kg of maize grain in triple bagging system with 1.01% de biopesticides (0.40 kg *L. multiflora* and 0.10 kg *H. suaveolens*) (TB5)
- 1 experimental batch of 50 kg of maize grain in triple bagging system with 5% de biopesticides (2.5 kg *L. multiflora* and 2.5 kg *H. suaveolens*) (TB6)
- 1 experimental batch of 50 kg of maize grain in triple bagging system with 2.5% de biopesticides (1.25 kg *L. multiflora*) (TB7) ;
- 1 experimental batch of 50 kg of maize grain in triple bagging system with 2.5% de biopesticides (1.25 kg *H. suaveolens*)(TB8).

2.3 Sampling

The sampling was performed at the beginning of the storage (0 month), then 5, 10, 15 and 18 months later, in triplicate. Thus, 2 kg of maize samples from each bag was gathered through the top, the center and the bottom opening sides.

2.3.1 Determination of moisture content and water activity

The moisture content was determined by the difference of weight before and after drying the sample in an oven (MEMMERT, Germany) at 105 ± 2°C until constant weight. The water activity was ascertained using a hygrometer from Hygro Lab Rotronic according to the method of [18]. Thus, a sample of 5 g of maize grains was placed in 10 Aw containers void of any trace of water. After two minutes, value of water activity was directly carried out in the device.

2.3.2 Analysis of aflatoxin B1, ochratoxin A, fuminosin B1 and zearalenone

2.3.2.1 Extraction and purification of aflatoxin B1

Aflatoxin B1 was extracted and purified from maize using the official guidelines of AOAC [19]. To 25 g of maize 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. Aflatoxin B1 was extracted from the out coming filtrate with 3 volumes of 10 mL of chloroform. The extract was collected into a 50 mL flask and processed with rotative evaporator (Buchi Rotavapor 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 Rezist in a chromatographic tube then passed through an immunoaffinity column (column RiDA aflatoxin, Biopharm, Germany).

2.3.2.2 Extraction and purification of ochratoxin A

100 g of the sample of maize was crushed in a hammer mill to obtain a homogeneous fine grind. In a Nalgene jar containing 15 g of grind, 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. 11 mL of filtrate were added 11 ml of saline phosphate buffered (PBS) at pH 7.3. Immunoaffinity columns brand Ochraprep and RBiopharm 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 [20].

2.3.2.3 Extraction and purification of fuminosin B1

25 g of maize sample were extracted with 50 mL of water blending for 2 min with a hammer mill blender. At five grams of ground maize, 25 mg of NaCl were added and the mixture was shaked on a horizontal mechanical shaker for 120 minutes at 300 rpm, and then centrifuged for 15 minutes at 2500 g. The supernatant was recovered and decreased by 4 mL of hexane. The organic phases were removed by centrifugation for 5 minutes at 2500 g. The aqueous layer was recovered and diluted with 16 mL of phosphate buffered saline (PBS) at pH 7.3, filtered through Whatman N°. 4 filter paper and then applied to a column immunoaffinity Fumoniprep (R Biopharm Rhone Ltd, Glasgow, Scotland) at a flow rate of 1–2 drops/s. The column was washed with 10 mL of the same buffer to 1-2 drops/s for removal of residues. Fumonisin B1 was eluted with 1.5 mL of methanol (HPLC grade) and then 1.5 mL of water. The eluate was collected and evaporated, protected from light in a nitrogen stream. The dry extract was taken up in 200 µL acetonitrile/water (50:50, v/v) and then sonicated for 5 minutes. Then, 50 µL of extract was diluted into 50 µL of a solution of ortho-phthalaldehyde (OPA 40 mg, 1 mL methanol, 5 mL of 0.1 M sodium tetraborate and 50 µL of 2-mercaptoethanol). The resulting sample was packed in a chromatographic tube and the analysis of FB1 was made by HPLC using AFNOR methods [21].

2.3.2.4 Extraction and purification of zearalenone

Twenty-five grams of maize sample were extracted with 50 mL of 125 mL of acetonitrile: water (94:31) blending for 2 min with a hammer mill blender. After filtration through Whatman N° 4 filter paper, 20 mL of the filtrate were diluted with 80 mL of double distilled water. Then, 25 mL of the diluted filtrate was applied to an immunoaffinity column (Easi-Extract® zearalenone, R-Biopharm Rhone Ltd, Glasgow) containing a monoclonal antibody specific for the zearalelone. The column was washed with 10 mL of double distilled water. Zearalenone was eluted

Table 1. Conditions of AFB1, OTA, FB1 and ZEA analysis by HPLC

ITEM	AFB1	OTA	FB1	ZEA
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 excitation : 330 nm	λ excitation : 335 nm	λ excitation : 274 nm
	λ emission : 435 nm	λ emission : 460 nm	λ emission : 440 nm	λ emission : 440 nm
Mobile Phase	Acetonitrile/Water/ Methanol (20/20/60)	Acetonitrile/Water/ Acetic acid (49/49/2)	Acetonitrile/Water (50/50)	Acetonitrile/Water /Methanol (46/46/8)
Inject volume	20 µl	100µl		
Flow rate	1 mL/min			
Column Temperature	40°C			
Rising solvent	Methanol	Acetonitrile		

by applying 1.5 mL of methanol. The eluate was diluted with 1.5 mL of bidistilled water and mixed by vortexing. The resulting sample was packed in a chromatographic tube and the analysis of ZEA was made by HPLC using the method of AOAC and Miraglia and Brera [22,23].

2.3.2.5 Quantification of aflatoxin B1, ochratoxin A, fuminosin B1 and zearalenon

Determination of AFB1, OTA, FB1 and ZEA contents was achieved with high performance liquid chromatography column, using a Shimadzu liquid chromatograph (Kyoto, Japan) fitted with fluorescence detector (Table 1).

2.4 Statistical Analysis

All analyses were performed in triplicate and the full data were statistically treated using SPSS software (version 20.0). It consisted in the repeated measures ANOVA. Means derived from parameters were compared with the Tukey High Significant Difference test at 5% significance level.

3. RESULTS

3.1 Evolution of Moisture Content during Storage

Table 2 shows the evolution of the moisture content of maize grains stored in different batches. With an average of 9.02 ± 0.01% initially (0 months), the moisture content increased significantly (P = .001) during the storage period (Table 2). For the polypropylene bag control batch (TPPB0), the moisture content increases sharply to 16.99 ± 0.02% after 18 months of storage. As for the control group triple bagging system (TPB0), after 18 months of storage, the moisture content is 13.16 ± 0.10%. With regard to the lots stored in bags in triple bagging system with different proportions of biopesticide (TB1, TB2, TB3, TB4, TB5, TB6, TB7 and TB8), the moisture contents are similar after 18 months of storage and have an average value of 12.20 ± 0.05%.

3.2 Evolution of Water Activity

Water activity increases progressively during storage. Control lots differ significantly from the treated batches at 1 month and 5 months respectively for TPPB0 and TPB0. However, there are no significant differences between the experimental batches for water activity and moisture up to five storage months. After 10 months of storage, the water activity is between 0.72 ± 0.00 and 0.90 ± 0.02 for the control and experimental batches. At 15 months, the values for water activity ranged from 0.73 ± 0.01 to 0.94 ± 0.01 for all batches (controls and experimental). After 18 months of storage, the water activity varies between 0.74 ± 0.01 and 0.80 ± 0.02 in the experimental batches while control batches TPPB0 and TPB0 have, respectively, water activity values of 0.96 ± 0.01 and 0.85 ± 0.01 (Table 2).

3.3 Evolution of Aflatoxin B1, Ochratoxin A, Fuminosin B1 and Zearalenone Contents during Storage

3.3.1 Determination of aflatoxin B1 content

Table 2 shows the evolution of the aflatoxin B1 (AFB1) content of maize grains stored in different batches. With an average 0.04 ± 0.00 µg/kg initially, Aflatoxin B1 levels increased gradually during the storage time. Control lots differ significantly from the treated batches at 1 month and 5 months respectively for TPPB0 and TPB0. At 10th month, experimental batches differ significantly from 0.70 ± 0.01 to 1.21 ± 0.01 µg/kg. Indeed, AFB1 content in the experimental batches have a mean between 4.17 ± 0.05 and 5.15 ± 0.06 µg/kg after 18 months of storage. In control batches TPPB0 and TPB0, the AFB1 content are respectively 34.05 ± 0.07 and 7.33 ± 0.05 µg/kg after 18 months of storage (Fig. 1).

3.3.2 Determination of ochratoxin A content

With an average 0.04 ± 0.00 µg/kg initially, ochratoxin A (OTA) levels increased gradually during the storage duration. At 1 month and 5 months, there are no significant differences between the experimental batches. While, control lots differ significantly from the treated batches at 1 month and 5 months respectively for TPPB0 and TPB0. As observed for AFB1, at 10th month of storage, Table 2 shows significant increasing of the ochratoxin A levels involving in the experimental batches. There are significant differences for treated lots at 10th month. The experimental batches increased from 4.58 ± 0.25 to 6.10 ± 0.01 µg/kg at 18 months of storage (Fig. 2).

3.3.3 Determination of fuminosin B1 content

Fuminosin B1 (FB1) levels, initially with an average 0.11 ± 0.00 µg/kg, increase progressively during storage. At 1 month and 5 months, there are no significant differences between the experimental batches. While, control lots differ significantly from the treated batches at 1 month and 5 months. At 10th month of storage, there is significant increasing of the FB1 levels involving in the experimental batches (P< .05). At 18 months, the values for fuminosin B1 ranged from 4.96 ± 0.07 to 45.93 ± 0.52 µg/kg for all batches (controls and experimental) (Fig. 3).

3.3.4 Determination of zearalenone content

Zearalenone (ZEA) levels increase progressively during storage, with an initial average 0.24 ± 0.00 µg/kg. In the control batches, ZEA levels increased significantly from 2.37 ± 0.07 µg/kg and 0.24 ± 0.00 µg/kg at 1 month, respectively for TPPB0 and TPB0. At 1 month and 5 months, there are no significant differences between the experimental batches. While, control lots differ significantly from the treated batches at 1 month and 5 months. At 10th month of storage, there are significant differences for treated lots. At 15 months, the ZEA levels in stored maize in experimental batches rise significantly (P< .05). The experimental batches increased from 4.66 ± 0.10 µg/kg to 8.78 ± 0.14 µg/kg at 18 months of storage (Fig. 4).

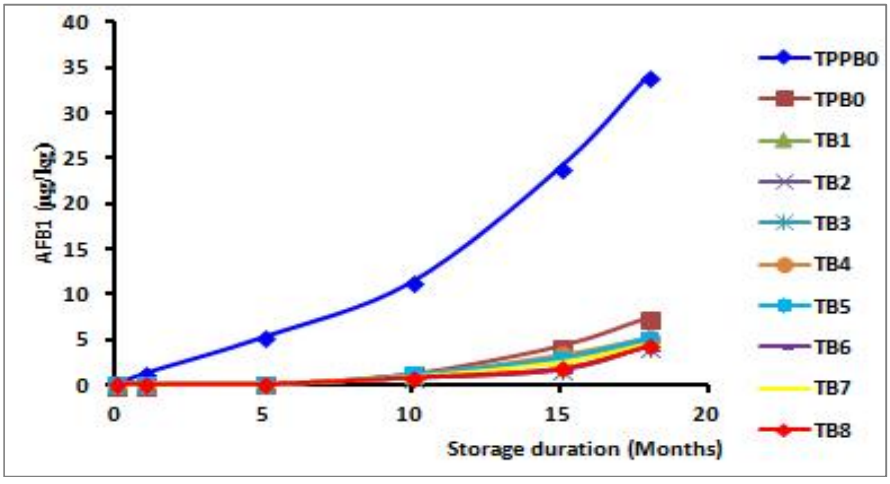

Fig. 1. Evolution of aflatoxin B1 contents in maize stored during 18 months

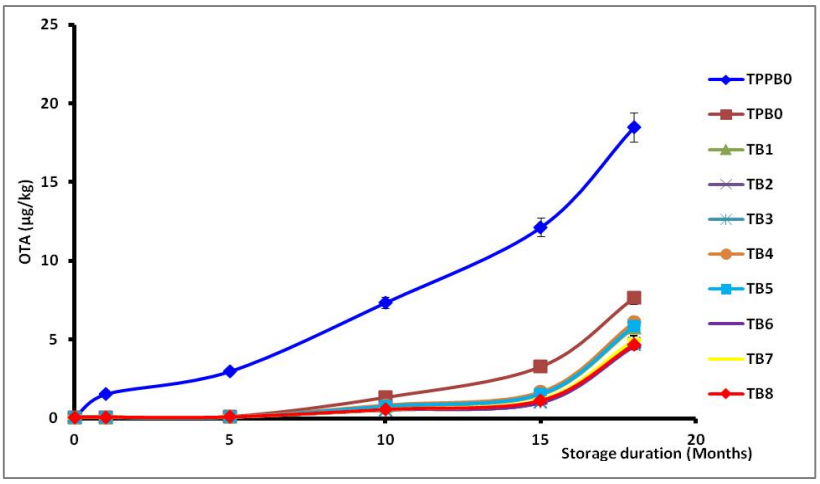

Fig. 2. Evolution of ochratoxin A contents in maize stored during 18 months

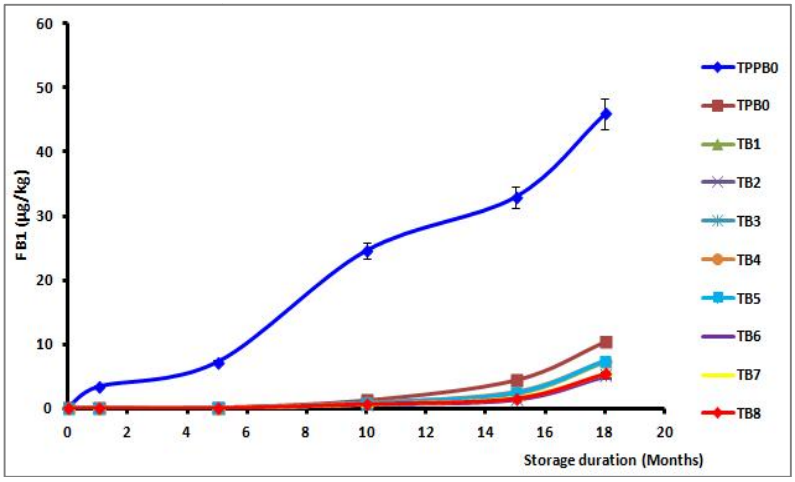

Fig. 3. Evolution of fuminosin B1 contents in maize stored during 18 months

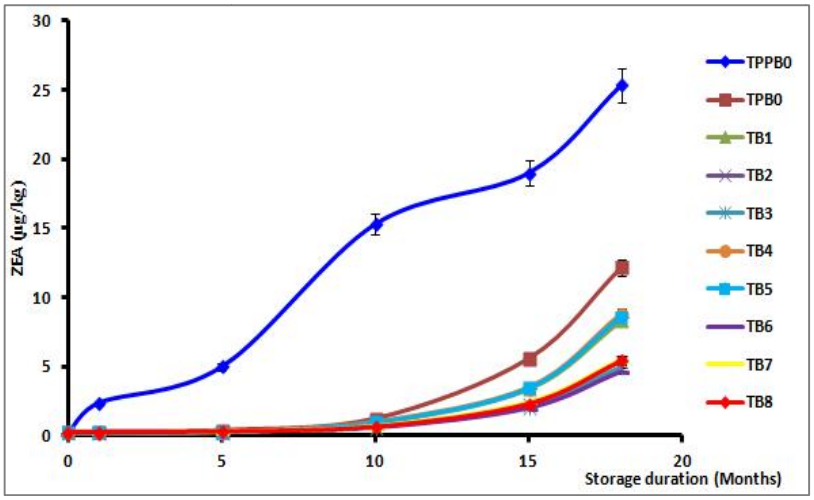

Fig. 4. Evolution of zearalenone contents in maize stored during 18 months

Table 2. Evolution of water activity, moisture, aflatoxin B1, ochratoxin A, fuminosin B1 and zearalenone contents in the stored maize during 18 months

Parameters	Storage duration (Months)	TPPB0	TPB0	TB1	TB2	TB3	TB4	TB5	TB6	TB7	TB8
Aw	0	0.69 ± 0.02^{aA}	0.69 ± 0.02^{aA}	0.69 ± 0.02^{aA}	0.69 ± 0.02^{aA}	0.69 ± 0.02^{aA}	0.69 ± 0.02^{aA}	0.69 ± 0.02^{aA}	0.69 ± 0.02^{aA}	0.69 ± 0.02^{aA}	0.69 ± 0.02^{aA}
	1	0.75 ± 0.02^{bB}	0.72 ± 0.01^{aA}	0.70 ± 0.02^{aA}	0.71 ± 0.01^{aA}	0.70 ± 0.03^{aA}	0.71 ± 0.02^{aA}	0.70 ± 0.01^{aA}	0.70 ± 0.01^{aA}	0.71 ± 0.02^{aA}	0.70 ± 0.01^{aA}
	5	0.88 ± 0.01^{cC}	0.73± 0.01^{aA}	0.72 ± 0.01^{aA}	0.72 ± 0.01^{aA}	0.72 ± 0.01^{aA}	0.73 ± 0.01^{aA}	0.72 ± 0.00^{aA}	0.72 ± 0.00^{aA}	0.72 ± 0.01^{aA}	0.72 ± 0.01^{aA}
	10	0.90 ± 0.02^{dD}	0.76 ± 0.03^{bB}	0.75 ± 0.01^{bB}	0.73 ± 0.01^{aB}	0.72 ± 0.00^{aA}	0.75 ± 0.00^{bB}	0.75 ± 0.00^{bB}	0.73 ± 0.01^{aB}	0.73 ± 0.00^{aB}	0.73 ± 0.01^{aB}
	15	0.94 ± 0.01^{eE}	0.80 ± 0.02^{cC}	0.76 ± 0.00^{bC}	0.73 ± 0.00^{aB}	0.73 ± 0.00^{aB}	0.77 ± 0.01^{bC}	0.76 ± 0.01^{bC}	0.73 ± 0.00^{aB}	0.74 ± 0.01^{aB}	0.73 ± 0.00^{aB}
	18	0.96 ± 0.01^{fF}	0.85 ± 0.01^{dD}	0.79 ± 0.01^{bD}	0.75 ± 0.01^{aC}	0.75 ± 0.01^{aC}	0.80 ± 0.01^{bD}	0.79 ± 0.01^{bD}	0.74 ± 0.01^{aC}	0.76 ± 0.00^{abC}	0.75 ± 0.00^{aB}
Moisture (%)	0	9.02 ± 0.01^{aA}	9.02 ± 0.01^{aA}	9.02 ± 0.01^{aA}	9.02 ± 0.01^{aA}	9.02 ± 0.01^{aA}	9.02 ± 0.01^{aA}	9.02 ± 0.01^{aA}	9.02 ± 0.01^{aA}	9.02 ± 0.01^{aA}	9.02 ± 0.01^{aA}
	1	10.20 ± 0.10^{bB}	9.23 ± 0.06^{aA}	9.10 ± 0.02^{aA}	9.09 ± 0.07^{aA}	9.07 ± 0.04^{aA}	9.17 ± 0.08^{aA}	9.12 ± 0.01^{aA}	9.08 ± 0.03^{aA}	9.12 ± 0.03^{aA}	9.09 ± 0.04^{aA}
	5	14.05 ± 0.07^{cC}	11.37 ± 0.08^{bB}	11.02 ± 0.13^{aB}	10.96 ± 0.06^{aB}	10.96 ± 0.13^{aB}	11.08 ± 0.07^{aB}	10.96 ± 0.06^{aB}	10.92 ± 0.06^{aB}	10.98 ± 0.09^{aB}	10.92 ± 0.07^{aB}
	10	16.67 ± 0.27^{dD}	11.92 ± 0.04^{cC}	11.29 ± 0.03^{bC}	11.11 ± 0.02^{aB}	11.08 ± 0.01^{aB}	11.33 ± 0.08^{bC}	11.29 ± 0.04^{bC}	11.05 ± 0.07^{aB}	11.19 ± 0.05^{abC}	11.15 ± 0.02^{aC}
	15	16.97 ± 0.07^{eE}	12.28 ± 0.06^{cD}	11.85 ± 0.06^{bD}	11.66 ± 0.10^{abC}	11.64 ± 0.04^{abB}	12.14 ± 0.06^{cD}	11.95 ± 0.05^{bD}	11.44 ± 0.05^{aC}	11.78 ± 0.02^{bD}	11.71 ± 0.05^{bD}
	18	16.99 ± 0.02^{eE}	12.76 ± 0.10^{dE}	12.32 ± 0.02^{abC}	12.11 ± 0.10^{aD}	12.07 ± 0.02^{aD}	12.47 ± 0.06^{bE}	12.45 ± 0.18^{bE}	12.07 ± 0.06^{aD}	12.37 ± 0.05^{abE}	12.18 ± 0.03^{aE}
AFB1 (µg/kg)	0	0.04 ± 0.00^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.00^{aA}
	1	1.30 ± 0.01^{bB}	0.04 ± 0.01^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.01^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.01^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.01^{aA}	0.04 ± 0.01^{aA}
	5	5.28 ± 0.05^{cC}	0.05 ± 0.00^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.01^{aA}	0.04 ± 0.01^{aA}	0.04 ± 0.01^{aA}	0.04 ± 0.01^{aA}	0.04 ± 0.00^{aA}	0.04 ± 0.01^{aA}	0.04 ± 0.01^{aA}
	10	11.32 ± 0.60^{dD}	1.25 ± 0.06 ^{bB}	1.21 ± 0.01^{bB}	0.75 ± 0.02^{aB}	0.73 ± 0.03^{aB}	1.15 ± 0.04^{bB}	1.18 ± 0.05^{bB}	0.70 ± 0.01^{aB}	0.82 ± 0.08^{aB}	0.76 ± 0.02^{aB}
	15	23.87 ± 0.12^{eE}	4.25 ± 0.08^{dD}	2.85 ± 0.05^{bC}	1.71 ± 0.02^{aC}	1.59 ± 0.09^{aC}	3.28 ± 0.01^{cC}	2.94 ± 0.04^{bC}	1.58 ± 0.05^{aC}	2.44 ± 0.36^{bC}	1.75 ± 0.03^{aC}
	18	34.05 ± 0.07^{fF}	7.33 ± 0.05^{eE}	5.01 ± 0.00^{cD}	4.22 ± 0.02^{aD}	4.20 ± 0.01^{aD}	5.15 ± 0.06^{cD}	5.07 ± 0.05^{cD}	4.17 ± 0.05^{aD}	4.46 ± 0.11^{aD}	4.30 ± 0.05^{aD}
OTA (µg/kg)	0	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}
	1	1.55 ± 0.01^{bB}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}	0.08 ± 0.00^{aA}
	5	3.00 ± 0.02^{cC}	0.10 ± 0.01^{aA}	0.09 ± 0.00^{aA}	0.09 ± 0.00^{aA}	0.09 ± 0.01^{aA}	0.09 ± 0.00^{aA}	0.09 ± 0.01^{aA}	0.09 ± 0.00^{aA}	0.09 ± 0.00^{aA}	0.09 ± 0.01^{aA}
	10	7.35 ± 0.11^{dD}	1.32 ± 0.05^{bB}	0.75 ± 0.01^{bB}	0.54 ± 0.02^{aB}	0.54 ± 0.00^{aB}	0.85 ± 0.01^{bB}	0.79 ± 0.02^{bB}	0.55 ± 0.03^{aB}	0.57 ± 0.02^{abB}	0.57 ± 0.01^{aB}
	15	12.14 ± 0.06^{eE}	3.27 ± 0.02^{dD}	1.51 ± 0.02^{bC}	1.02 ± 0.03^{aC}	1.01 ± 0.01^{aC}	1.68 ± 0.02^{cC}	1.57 ± 0.02^{bcC}	1.00 ± 0.01^{aC}	1.20 ± 0.01^{bC}	1.13 ± 0.04^{aC}
	18	18.50 ± 0.41^{fF}	7.64 ± 0.25^{fF}	5.75 ± 0.27^{bD}	4.78 ± 0.22^{aD}	4.67 ± 0.03^{aD}	6.10 ± 0.01^{bD}	5.88 ± 0.23^{bD}	4.58 ± 0.25^{aD}	5.01 ± 0.10^{aD}	4.66 ± 0.50^{aD}
FB1 (µg/kg)	0	0.11 ± 0.00^{aA}	0.11 ± 0.00^{aA}	0.11 ± 0.00^{aA}	0.11 ± 0.00^{aA}	0.11 ± 0.00^{aA}	0.11 ± 0.00^{aA}	0.11 ± 0.00^{aA}	0.11 ± 0.00^{aA}	0.11 ± 0.00^{aA}	0.11 ± 0.00^{aA}
	1	3.34 ± 0.16^{bB}	0.13 ± 0.00^{aA}	0.12 ± 0.00^{aA}	0.12 ± 0.00^{aA}	0.12 ± 0.00^{aA}	0.12 ± 0.00^{aA}	0.12 ± 0.00^{aA}	0.12 ± 0.00^{aA}	0.12 ± 0.00^{aA}	0.12 ± 0.00^{aA}
	5	7.21 ± 0.08^{cC}	0.16 ± 0.00^{bB}	0.15 ± 0.01^{aA}	0.15 ± 0.00^{aA}	0.15 ± 0.00^{aA}	0.15 ± 0.00^{aA}	0.15 ± 0.00^{aA}	0.15 ± 0.00^{aA}	0.15 ± 0.00^{aA}	0.15 ± 0.00^{aA}
	10	24.60 ± 0.27^{dD}	1.28 ± 0.04^{cC}	0.93 ± 0.02^{bB}	0.72 ± 0.01^{aB}	0.73 ± 0.03^{aB}	0.94 ± 0.02^{bB}	0.93 ± 0.02^{bB}	0.71 ± 0.01^{aB}	0.75 ± 0.03^{abB}	0.77 ± 0.07^{abB}
	15	32.98 ± 0.14^{eE}	4.43 ± 0.12^{dD}	2.36 ± 0.04^{bcC}	1.59 ± 0.02^{aC}	1.52 ± 0.06^{aC}	2.62 ± 0.04^{cC}	2.51 ± 0.13^{cC}	1.42 ± 0.02^{aC}	1.74 ± 0.04 ^{bC}	1.62 ± 0.02^{bC}
	18	45.93 ± 0.52^{fF}	10.41 ± 0.47^{eE}	7.34 ± 0.11^{cD}	5.20 ± 0.24^{aD}	5.20 ± 0.26^{aD}	7.36 ± 0.02^{cD}	7.42 ± 0.06^{cD}	4.96 ± 0.07^{aD}	5.45± 0.02^{bD}	5.39 ± 0.03^{abD}
ZEA (µg/kg)	0	0.24 ± 0.00^{aA}	0.24 ± 0.00^{aA}	0.24 ± 0.00^{aA}	0.24 ± 0.00^{aA}	0.24 ± 0.00^{aA}	0.24 ± 0.00^{aA}	0.24 ± 0.00^{aA}	0.24 ± 0.00^{aA}	0.24 ± 0.00^{aA}	0.24 ± 0.00^{aA}
	1	2.37 ± 0.07^{bB}	0.26 ± 0.02^{aA}	0.25 ± 0.00^{aA}	0.25 ± 0.00^{aA}	0.25 ± 0.00^{aA}	0.25 ± 0.00^{aA}	0.25 ± 0.00^{aA}	0.25 ± 0.00^{aA}	0.25 ± 0.00^{aA}	0.25 ± 0.00^{aA}
	5	4.99 ± 0.02^{cC}	0.36 ± 0.03 ^{bB}	0.30 ± 0.02^{bB}	0.29 ± 0.02^{aA}	0.29 ± 0.01^{aA}	0.30 ± 0.00^{bB}	0.30 ± 0.00^{bB}	0.30 ± 0.00^{aA}	0.30 ± 0.00^{aA}	0.30 ± 0.01^{aA}

Parameters	Storage duration (Months)	TPPB0	TPB0	TB1	TB2	TB3	TB4	TB5	TB6	TB7	TB8
	10	15.30 ± 0.03^{dD}	1.21± 0.01 ^{bC}	0.95 ± 0.05^{bC}	0.63 ± 0.03^{aB}	0.62 ± 0.03^{aB}	1.04 ± 0.05^{bC}	0.98 ± 0.01^{bC}	0.59 ± 0.07^{aB}	0.67 ± 0.02^{aB}	0.64 ± 0.04^{aB}
	15	18.99 ± 0.04^{eE}	5.58 ± 0.22^{dD}	3.40 ± 0.16^{bD}	2.26 ± 0.06^{aC}	2.10 ± 0.01^{aC}	3.52 ± 0.06^{bD}	3.44 ± 0.05^{bD}	2.01 ± 0.01^{aC}	2.39 ± 0.04^{abC}	2.32 ± 0.02^{aC}
	18	25.31 ± 0.07^{fF}	12.16 ± 0.20^{eE}	8.32 ± 0.08^{cE}	5.34 ± 0.04^{aD}	4.93 ± 0.05^{aD}	8.78 ± 0.14^{cE}	8.56 ± 0.16^{cE}	4.66 ± 0.10^{aD}	5.45 ± 0.07^{abD}	5.44 ± 0.02^{aD}

The mean (± SD) with different lowercase / uppercase letters on the same line / in the same column are different test probability of 5%, TPPB0 = Control with polypropylene bag; TPB0 = Control with PICS bag (no biopesticide); TB1 = PICS bag with 2.5% of biopesticide (0.625kg L. multiflora and 0.625kg H. suaveolens) (w / w); TB2 = PICS bag with 3.99% biopesticide (0.40 kg L. multiflora and 1.60 kg H. suaveolens) (w / w); TB3 = PICS bag with 3.99% of biopesticide (1.60 kg L. multiflora and 0.40 kg H. suaveolens) (w / w); TB4 = PICS bag with 1.01% of biopesticide (w / w) (0.10 kg L. multiflora and 0.40 kg H. suaveolens) ; TB5= PICS bag with 1.01% de biopesticide (0.40 kg L. multiflora and 0.10 kg H. suaveolens ; TB6= PICS bag with 5% de biopesticide (2.5 kg L. multiflora et de 2.5 kg H. suaveolens) ; TB7= PICS bag with avec 2.5% de biopesticide (1.25kg L. multiflora); TB8= PICS bag with 2,5% de biopesticide (1.25kg H. suaveolens)

Table 3. Statistical data for moisture, water activity, aflatoxin B1, ochratoxin A, fuminosin B1 and zearalenone in maize grains according to the type of packaging during the storage period

Source of Variation		Parameters					
		Moisture	Aw	AFB1	OTA	FB1	ZEA
Types	df	9	9	9	9	9	9
	SS	171.43	0,24	2063.28	557.59	4921.60	1448.78
	F-value	3590.54	180.77	13268.84	5945.53	13709.41	71734.65
	P-value	**< .001**	**< .001**	**< .001**	**< .001**	**< .001**	**< .001**
Error Types	df	20	20	20	20	20	20
	SS	0.11	0.00	0.35	0.20	0.80	0.05
Duration (months)	df	3.54	2.37	1.33	1.06	1.65	1.84
	SS	382.63	0.20	1442.79	981.61	2385.85	1703.49
	F-value	16985.88	257.44	10856.12	15101.48	21722.98	83752.59
	P-value	**< .001**	**< .001**	**< 0.001**	**< .001**	**< .001**	**< .001**
Error Duration	df	70.88	47.37	26.56	1.30	32.92	36.83
	SS	0.45	0.01	2.65	21.20	2.20	0.41
Type x Storage duration	df	31.90	21.32	11.95	9.54	14.81	16.57
	SS	70.78	0.01	1883.70	394.01	3613.81	963.96
	F-value	349.12	14.62	1574.86	673.52	3655.93	5265.94
	P-value	**< .001**	**< .001**	**< .001**	**< .001**	**< .001**	**< .001**

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

4. DISCUSSION

The results observed in this study showed that triple bags in presence of plants *Lippia multiflora* and / or *Hyptis suaveolens* slow down the evolution of the concentrations of aflatoxin B1 (AFB1), ochratoxin A (OTA), fuminosin B1 (FB1) and zearalenone (ZEA) by inhibiting the development of mycotoxinogenic germs. Indeed, the concentrations of AFB1, OTA, FB1 and ZEA in the experimental batches in presence of plants are lower than the control batches in the polypropylene bag without biopesticides (TPPB0) and in the triple bagging without biopesticides (TPB0). These results are in agreement with those of the work of [16]. These authors have shown that the plants *L. multiflora* and *H. suaveolens* have the capacity to slow down the evolution of mycotoxins (OTA, FB1, ZEA) in stored maize in granaries to their antifungal activity.

Indeed, the studies of [24] also showed the antifungal activity of *Lippia multiflora* Moldenke, *Boscia senegalensis* (Pers.) Lam and *Ziziphus mucronata* Willd. against *Puccinia arachidis* Speg, the fungus responsible for peanut rust. In addition, the combination of plants and the triple bagging system would inhibit the metabolism of insects and molds. They can also be explained by both the reduction of oxygen (O2) and the insecticidal and fungicidal activity of plants [25,26]. This antifungal activity is due to the presence of mono and sesquiterpene compounds in these plants [27]. The results of our work are also in agreement with those of [17]. The authors observed a slight change in OTA concentrations in cowpeas stored in triple bags with the addition of *L. multiflora* in different proportions.

Previous studies on the risk of aflatoxin contamination before and after the maize harvest in West Africa showed that rural Africans are regularly exposed to a level of aflatoxins which could cause serious health problems long-term [28]. The results of the present study on AF concentrations in the experimental batches have shown that this study could reduce the risk of contamination. Compared to the standard set by the European Union on OTA (5 µg / kg), the OTA concentrations in the treated batches are all lower until the 15th month of storage. While in the 18th month of storage, the level of OTA in the treated batches increased significantly and exceeded the standard with the exception of batches TB2, TB3, TB6 and TB8.

The concentrations of FB1 and ZEA in treated batches are lower than the standards fixed by the European Union, of 2000 and 500 µg / kg respectively until the 18th month of storage. A study on the contamination of mycotoxins in cereals and peanuts in Côte d'Ivoire revealed that all the corn samples were contaminated with zearalenone up to a concentration of 50 µg / kg [8]. In addition, water activity is an important parameter in the preservation of food. For the prevention of fungal development, the corn kernels must have an Aw of less than 0.70 being the accepted limit value [29]. According to these authors, maize grains should have moisture content below 14% to prevent the growth of *A. flavus* and aflatoxin accumulation in corn. Thus, in the present study, the slowdown in mycotoxins observed in the experimental batches could be explained by a slower evolution of the Aw in these batches. However, the water activity observed in the corn kernels stored in the triple bagging system in the presence of plants, after the storage time, is higher than the limit values recommended by these authors. Despite the high level of water activity observed during storage, the development of mycotoxins is slowed down by the effect of biopesticides and the humidity level of the experimental batches which is lower than the recommended humidity level. These results are in agreement with those of [30]. The co-contamination observed in our study was revealed in previous studies in Côte d'Ivoire [8,9].

5. CONCLUSION

This study contributes to improving corn storage and preserving the health of corn consumers. The results showed that the leaves of *L. multiflora* and *H. suaveolens* in the triple bagging system reduce the level of mycotoxins contamination. The storage technology used is inexpensive, easily applicable and environmentally friendly. However, for greater efficiency, it would be important to encourage good post-harvest practices.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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