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Efficacy of *Lippia multiflora* (Verbenaceae) and *Hyptis suaveolens* (Lamiaceae) Leaves on Sanitary Quality during the Storage of Maize Grain (*Zea mays* L.) from Cote D'ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GHMB and PE designed the study, performed the statistical analysis and wrote the protocol. Author KKC wrote the first draft of the manuscript. Authors OKC, YK, AD and KKC managed the analyses of the study. Authors DS and AC managed the literature searches. All authors read and approved the final manuscript

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ABSTRACT

The aim of this study was to monitor the sanitary quality during the storage of maize grains for 9 months in polypropylene bags containing leaves of *Lippia multiflora* and *Hyptis suaveolens*.
It was carried out in the villages of Timbé and Soko respectively in the departments of Katiola and Bondoukou of Cote d'Ivoire in June 2014 to February 2015.

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The parameters determined were water activity, total aflatoxins, aflatoxin B1, ochratoxin A, zearalenone and fuminosin B1. The water activity was measured using a Hygrometer with the McCormick method and the mycotoxin assay (AFS, AFB1, OTA, ZEA, FB1) was performed using a high performance liquid chromatography with a fluorescence detector.
The batches treated with *L. multiflora* and *H. suaveolens* recorded the best values compared to the control independently of the study site and of the parameter studied. *L. multiflora* had a much more marked biopesticidal effect than the leaves of *H. suaveolens* and the mixture of the two leaves. Indeed, the water activities of the grains varied between 0.80 +/- 0.01 and 0.95 +/- 0.01 for the control batches and the treated batches. For total aflatoxins, the treated batches had maximum levels of 65.48 +/- 0.07 µg/kg and 113.93 +/- 1.23 µg/kg respectively in Timbé and Soko in the departments of Katiola and Bondoukou, while those of the control groups reached 335.98 +/- 2.64 µg/kg and 549.74 +/- 2.81 µg/kg at 9 months of storage. For AFB1, OTA, ZEA and FB1, the treated batches had maximum levels of 4.26 +/- 0.01 µg/kg, 4.87 +/- 0.02 µg/kg, 35.16 +/- 0.06 µg/g and 89.26 +/- 0.48 µg/kg respectively at Katiola and 10.26 +/- 0.11 µg/kg, 7.51 +/- 0.08 µg/kg, 89.25 +/- 0.89 µg/kg and 621.26 +/- 4.73 µg/kg in Bondoukou at 7 months of storage. The recommended standards was respectively 5 µg/kg, 5 µg/kg, 200 µg/kg et 2000 µg/kg for AFB1, OTA, ZEA et FB1 in maize grains. The control batches have very high contents exceeding the recommended standards yet at 4 months of storage.
These results indicate that the treatment of maize with leaves of *L. multiflora* and *H. suaveolens* inhibits the activity of insects and molds and allows preserving the quality of the grains with a remanence of up to 7 months. This inexpensive and easy-to-use treatment should be popularized among farmers

Keywords: Zea mays storage; L. multiflora; H. suaveolens; Biopesticides; Côte d'Ivoire.

1. INTRODUCTION

Maize (*Zea mays* L.) is the most widely used basic food crop in sub-Saharan Africa. In addition, more than 300 million people in sub-Saharan Africa depend on maize as source of food and income [1]. Maize is the second cereal most cultivated in Cote d'Ivoire after rice (*Oryza* spp.). Its production increases up to 531.940 tons in 2007 to 680.000 tons in 2014 for a total planted area of 330.000 ha [2]. The importance of maize is due to its availability throughout the year [3]. Its nutritional advantages (rich in starch, protein, minerals) and economic (crop simple to produce, harvest and store) make it a competitive product that helps to lower the price of basic foodstuffs such as milk and meat in rural farming [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]. Maize (*Zea mays* L.) is constantly exposed to the risk of fungi development for having ideal nutrients composition. Moreover, tropical and subtropical climate countries have favorable environmental conditions to the development of the main types of genotoxicant fungi, *Aspergillus, Fusarium* and *Penicillium*. Among the mycotoxins which are found in maize, aflatoxins, zearalenone, ochratoxin A and fuminosin B1 are detached both for the concerning showed by the researchers due to their possible toxicant effect in human beings and animals and for economic reasons [7]. Several authors estimated that post-harvest losses are relatively high, in range of 20% to 30% because methods used are often inadequate and rudimentary [8-9].

Faced with these post-harvest losses, different control methods have been developed. These include chemical control, biological control, use of plant biocidal substances, physical methods and varietal resistance [9-10]. According to Isman [11] and PAN Africa [12], synthetic chemical insecticides are most widely used. The abuse of pesticides to control insects in stored foodstuffs has often resulted in presence of toxic residues on treated products and development of resistance among pests [13]. In developing countries, these disadvantages are added to economic constraints related to the cost and supply of active ingredients [14]. It is important in the face of these problems to look for other alternative methods of control available to farmers, which are cheaper, respectful of environment and guarantee the health of consumers. The current trend is towards the use of aromatic plants containing active molecules insecticidal, insect repellents, fungicides, nematicides and rodenticides [15-18]. These plants, mostly used by people to fight cons diseases remain very low status [19]. These

natural plants limit the risk of development of resistance by pests and certain pathogenic microorganisms [20]. Therefore, this study was initiated to evaluate the efficacy of leaves of two plants (*Lippia multiflora* and *Hyptis suaveolens)* with biopesticide properties on sanitary quality of maize grains stored in polypropylene bags.

2. MATERIALS 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 Northeast of Cote d'Ivoire. Both localities have a humid tropical climate with 4 seasons, including 2 rainy seasons from March to July and October to November. These are interspersed with 2 dry seasons ranging from December to February and August to September. The annual rainfall ranges 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, while the average humidity ranged between 60%-70% in both region [21-22].

2.2 Plant Material Collection and Processing

The biological material consisted of maize grains (Hybrid variety) collected in January 2014 (from the cooperatives of Timbe and Soko) and leaves of plant species *Lippia multiflora* (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 conditions [15,18]. Approximately one month after harvest, maize was sun-dried and leaves of *L. multiflora* and *H. suaveolens* were dried under shade and chopped.

2.3 Treatments

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 9 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 preservation practiced by these farmers. 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 *H. suaveolens* (A) or *L. multiflora* (B) or in mixture (A+B) were stored as described below:

Treatment 1: 50 kg of maize grain + 2.5 kg of leaves of *H. suaveolens* (A);
Treatment 2: 50 kg of maize grain + 2.5 kg of leaves of *L. multiflora* (B);
Treatment 3: 50 kg of maize grain + 1.25 kg of leaves of *L. multiflora* + 1.25 kg of leaves of *H. suaveolens* (A+B);
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. Each month samples were taken for analysis.

2.4 Determination of Water Activity

The water activity was measured with a HygroLab Rotronic hygrometer according to indications of McCormick [23]. Prior to assays, the hygrometer was calibrated with specific water activity salts. Then, samples of 5 g of ground maize were put into standard dry empty containers for the Aw analysis. The water activity digital measures were directly displayed by the hygrometer.

2.5 Aflotaxins, Ochratoxin A, Fuminosin B1 and Zearalenone Analysis

Chemical reagents (acetonitrile, methanol and chloroform) and standards (ochratoxin A (OTA), aflatoxins (AFs), fuminosin B1 (FB1) and zearalenone (ZEA)) were used for the study. Reagents were purchased from Carlo Erba (Spain) with analytical grade, while standards OTA, AFs, FB_1 and ZEA were provided from Sigma (Sigma, St Louis, MO, USA).

2.5.1 Extraction and purification of OTA

100 g of the sample 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. To 11 mL of filtrate were added 11 ml of saline phosphate buffered (PBS) at pH 7.3. Immunoaffinity columns brand Ochraprep and R-Biopharm were conditioned with 10 mL of PBS. Purification of 20 ml of the mixture was made on immunoaffinity columns and OTA extraction was performed with two volumes of 1.5 mL of PBS at a flow rate of 5 mL/minute. The resulting sample was packed in a chromatographic tube and the analysis of OTA was made by HPLC using the European community regulation [24].

2.5.2 Extraction and purification of aflatoxins

Biological aflatoxins (B1, B2, G1 and G2) were extracted and purified from maize using the official guidelines of AOAC [25]. To 25 g of ground 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 using Whatman paper. Aflatoxins were extracted from the outcoming filtrate with 3 volumes of 10 mL of chloroform. The extracts were collected into a 50 mL flask and processed with rotatory evaporator (Buchi Rotavapor R-215) at 40°C to evaporate 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).

2.5.3 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 [26].

2.5.4 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 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 [25,27].

2.6 Aflatoxins, OTA, FB1 and ZEA Determination

Determination of AFs, 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).

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

	Aflatoxin B1	Ochratoxin A	Fuminosin B1	Zéaralenone
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,	Fluorescence, λ excitation: 330 nm,	Fluorescence, λ excitation: 330 nm,	Fluorescence, λ excitation: 330 nm,
	λ émission : 435 nm	λ émission: 460 nm	λ émission: 460 nm	λ émission: 460 nm
Phase mobile	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	100 µl	100 µl	100 µl
Flow rate	1 mL/minute			
Column temperature	40°C			
Rising solvent	Methanol	Acetonitrile		
Analysis duration	15 minutes	12 minutes	6 minutes	9,5 minutes

2.7 Statistical Analysis

All analyzes were performed in triplicate and data were statistically processed using the SPSS software (version 20.0). It consisted to an analysis of variance according to two factors: the effect of different biopesticides and the storage duration. The comparison of the average values of the measured parameters was performed by ANOVA (STATISTICA Version 7.1) using post hoc test of small statistical difference (LSD). Mean values were considered significantly different at P = 0.05. The Excel 2007 software was used to build curves of evolution of the parameters over time.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Water activity

The water activity of maize grains increased during storage regardless of study site and grain batches (Fig. 1; Fig 2). For control batches, these values varied between 0.80 +/- 0.01 and 0.94 +/- 0.01 in Katiola and between 0.80 +/- 0.01 and 0.95 +/- 0.01 in Bondoukou. Concerning the batches tested, the water activity increases progressively. All values of water activity (Aw) are high and all above 0.88 at 9 months of storage. These Aw values in the experimental batches do not really differ from those of the control batches in the two storage zones.

3.1.2 Validation of AFB1, OTA, FB1 and ZEA determination using HPLC

Using HPLC device, Limits Of Detection (LOD) of respective aflatoxin B1, ochratoxin A, fuminosin B1 and zearalenone are 6.18 10^{-3} µg/kg, 5.00 10^{-3} µg/kg, 12.50 10^{-3} µg/kg and 2.60 10^{-3} µg/kg, while their Limits Of Quantification (LOQ) are 6.50 10^{-3} µg/kg, 20.00 10^{-3} µg/kg, 50.50 10^{-3} µg/kg, 7.43 10^{-3} µg/kg. The mean recoveries fluctuate between 0.26% and 3.75% for the repeatability assays and between 0.89% and 5.67% for reproducibility assays. However, respective rates of extraction recorded for AFB1, OTA, FB1 and ZEA are 97.53%, 86.92±0.39%, 82.9±2.19% and 93.20±4.21%

3.1.3 Evolution of aflatoxins, ochratoxin A, fuminosin B1 and zearalenone contents

The different batches involved are initially all contaminated by mycotoxins, namely AFB1, AFT, OTA, FB1 and ZEA. The lots from Bondoukou are more contaminated than the lots in Katiola.

3.1.3.1 Aflatoxin B1

The evolution of the AFB1 content is different according to the treatments applied to the storage of maize grains. In the control batches, the AFB1 content increased significantly from 0.10 +/- 0.01 µg/kg and 2.00 +/- 0.01 µg/kg at 1 month, respectively, to 111.25 +/- 1.25 µg/kg and

187.25 +/- 1.65 µg/kg at 9 months respectively for samples from Katiola and Bondoukou. For the experimental batches, this evolution is very low. Up to 7 months of storage, the AFB1 content are respectively approximately 3.30 +/- 0.01 µg/kg and 8.00 +/- 0.02 µg/kg for samples from Katiola and Bondoukou whatever the type of treatment applied. At 9 months of storage, we find that the AFB1 content in batch B is the lowest, followed by batch A and finally batch A + B. These AFB1 content are much lower than those of the control batches (Fig. 3; Fig.4).

3.1.3.2 Total Aflatoxins

The change in AFT content differs according to the different batches of maize grains. In the control batches, AFT content increased significantly from 0.40 +/- 0.00 µg/kg and 6.00 +/- 0.01 µg/kg at 1 month to 335.98 +/- 2.64 µg/kg and 549.74 +/- 2.81 µg/kg at 9 months respectively for samples from Katiola and Bondoukou. For the experimental batches, this evolution is very low. Up to 7 months of storage, the AFT content are approximately 11.27 +/- 0.08 µg/kg and 23.79 +/- 0.13 µg/kg respectively for samples from Katiola and Bondoukou whatever the type of applied treatment. At 9 months of storage, we find that the AFT content in batch B is lowest, followed by batch A and finally batch A + B. These AFT content are much lower than those of the control batches (Fig. 5; Fig.6).

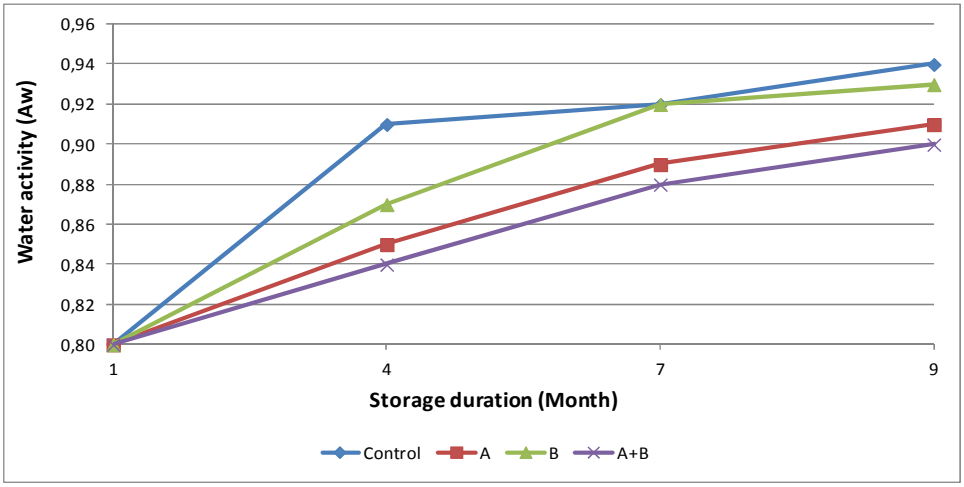

Fig. 1. Change in water activity of maize grains during storage at Katiola
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

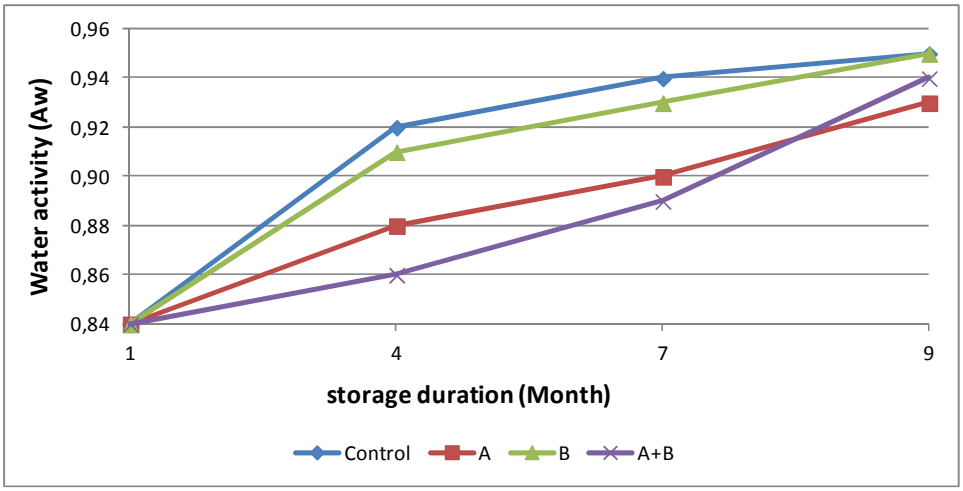

Fig. 2. Change in water activity of maize grains during storage at Bondoukou
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

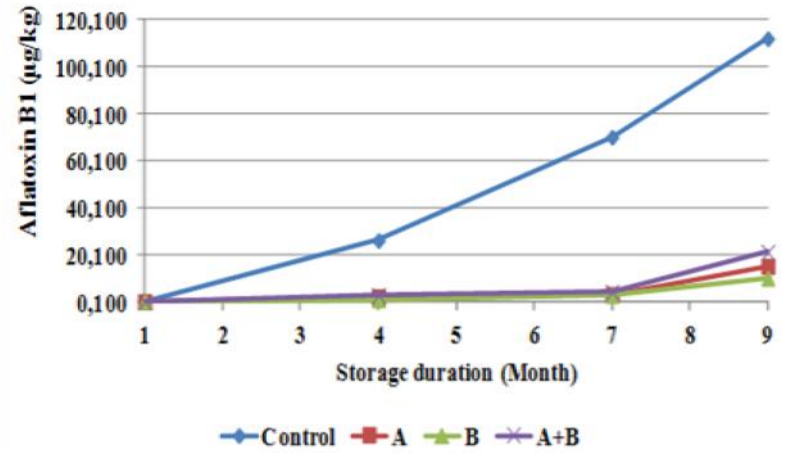

Fig. 3. Change in aflatoxin B1 of maize grains during storage at Katiola
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

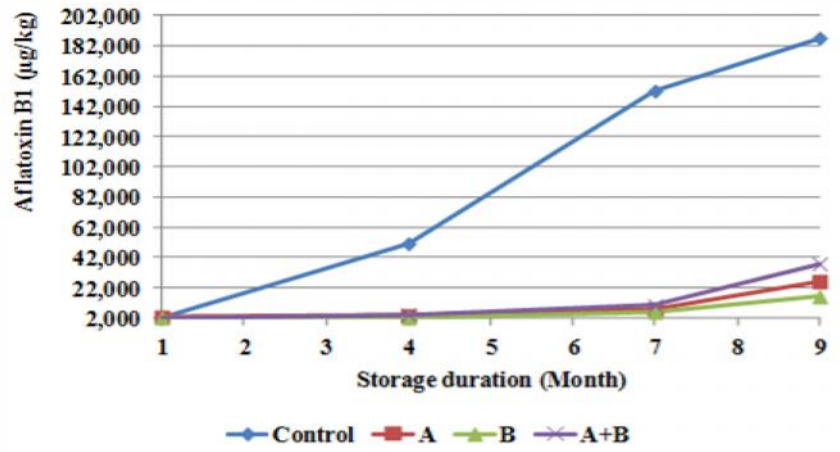

Fig. 4. Change in aflatoxin B1 of maize grains during storage at Bondoukou
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

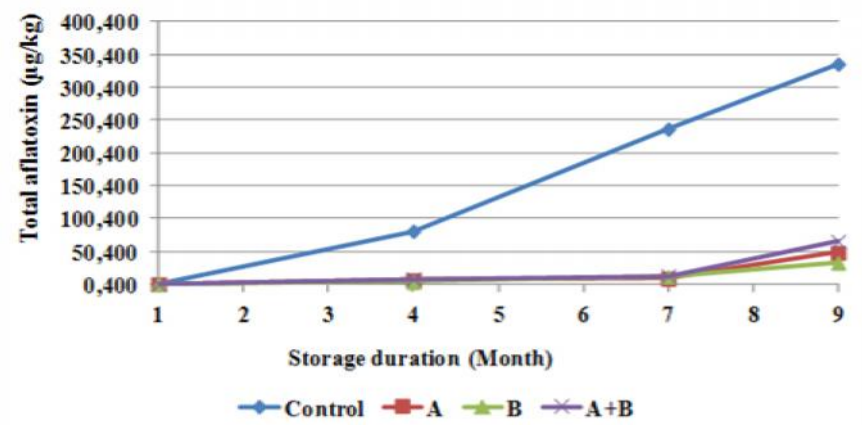

Fig. 5. Change in Total Aflatoxin of maize grains during storage at Katiola
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

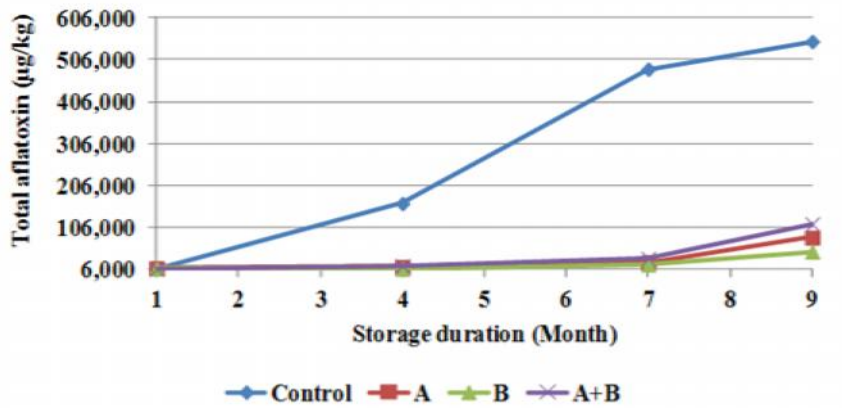

Fig. 6. Change in Total Aflatoxin of maize grains during storage at Bondoukou
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

3.1.3.3 Ochratoxin A

The evolution of the OTA content differs according to the different lots of maize grains. In the control batches, OTA levels increased significantly from 0.80 +/- 0.01 µg/kg and 1.90 +/- 0.01 µg/kg at 1 month to 112.25 +/- 1.05 µg/kg and 142.25 +/- 1.31 µg/kg at 9 months respectively for samples from Katiola and Bondoukou. For the experimental batches, this evolution remains very low. Up to 7 months of storage, OTA content are approximately 11.27 +/- 0.06 µg/kg and 23.79 +/- 0.22 µg/kg respectively for samples from Katiola and Bondoukou. At 9 months of storage, we find that the OTA content in batch B is lowest, followed by batch A and finally batch A + B. These OTA content are much lower than those of the control batches (Fig. 7; Fig. 8).

3.1.3.4 Zearalenon

The evolution of the ZEA content differs according to the different lots of maize grains. In the control batches, ZEA content increased significantly from 8.10+/- 0.03 µg/kg and 35.00 +/- 0.47 µg/kg at 1 month to 512.25 +/- 2.00 µg/kg and 558.28 +/- 2.04 µg/kg at 9 months, respectively for samples from Katiola and Bondoukou. For the experimental batches, this evolution remains very low. Up to 7 months of storage, ZEA content are approximately 26.53 +/- 0.09 µg/kg and 69.77 +/- 0.10 µg/kg respectively for samples from Katiola and Bondoukou. At 9 months of storage, we find that the ZEA content in lot B is the lowest, followed by lot A and finally lot A + B. These ZEA levels are much lower than those of the control batches (Fig. 9; Fig. 10).

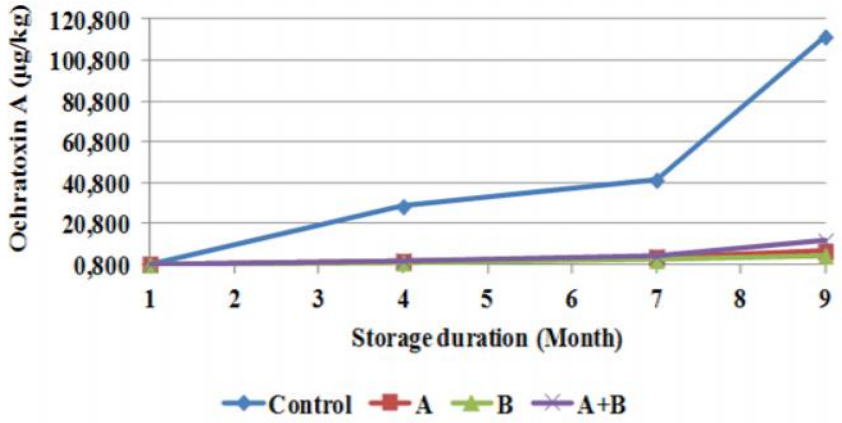

Fig. 7. Change in Ochratoxin a of maize grains during storage at Katiola
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

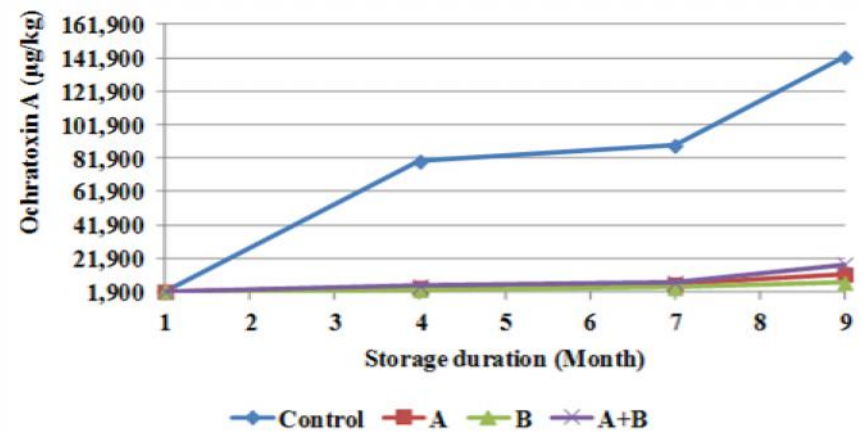

Fig. 8. Change in Ochratoxin a of maize grains during storage at Bondoukou
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

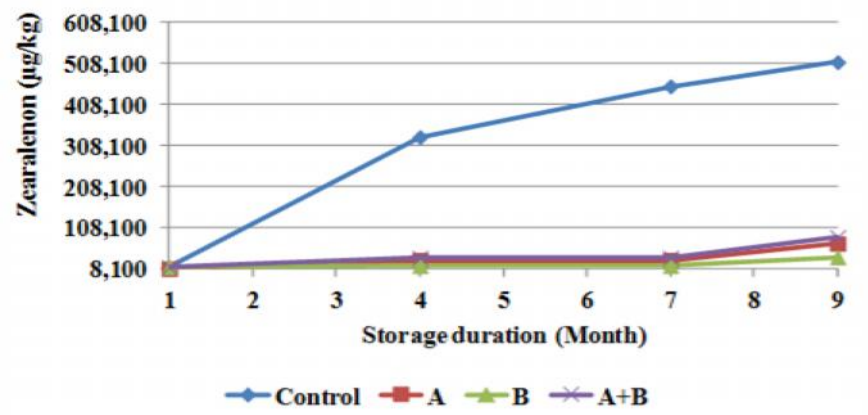

Fig. 9. Change in Zearalelon of maize grains during storage at Katiola
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

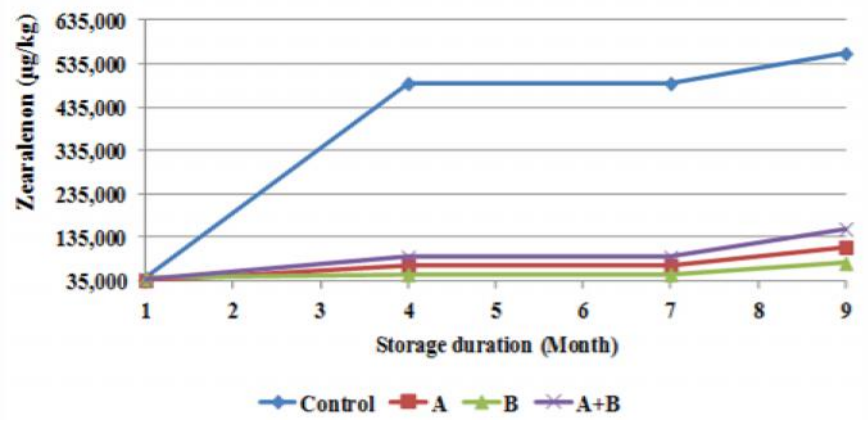

Fig. 10. Change in Zearalelon of maize grains during storage at Bondoukou
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

3.1.3.5 Fuminosine B1

The change in FB1 content differs according to the different batches of maize grains. In the control batches, FB1 content increased significantly from 8.10 +/- 0.06 µg/kg and 35.0 +/- 0.50 µg/kg at 1 month to 512.25 +/- 1.87 µg/kg and 558.28+/- 2.00 µg/kg at 9 months respectively for samples from Katiola and Bondoukou. For the experimental batches from Katiola, this evolution remains very low over 9 months of storage. This FB1 content mean increase from 35.00 +/- 0.31 µg/kg in the first month to 89.22 +/- 0.65 µg/kg in the 9th month. For the experimental batches of Bondoukou, the evolution is observed up to 9 months with FB1 contents respectively 782.,28 +/- 2.72 µg/kg, 521.25 +/- 1.55 µg/kg and 879.25 +/- 2.90 µg/kg for lot A, lot B and lot A + B. These levels of FB1 are much lower than those of the control batch (Fig. 11; Fig. 12).

3.1.4 Remaining effect of leaves on grain quality

The sample projection shows two types of class. The class of samples with the highest content of mycotoxins studied (AFB1, AFT, OTA, ZEA and FB1) and the class of samples with low content of mycotoxins studied. The phylogenetic tree of ascending hierarchical classification indicates that all control batches of Katiola and Bondoukou have high content of mycotoxins studied at 4 months whereas the batches treated with the leaves with biopesticide effect have low content of mycotoxins studied all along the storage (Fig. 13; Fig. 14).

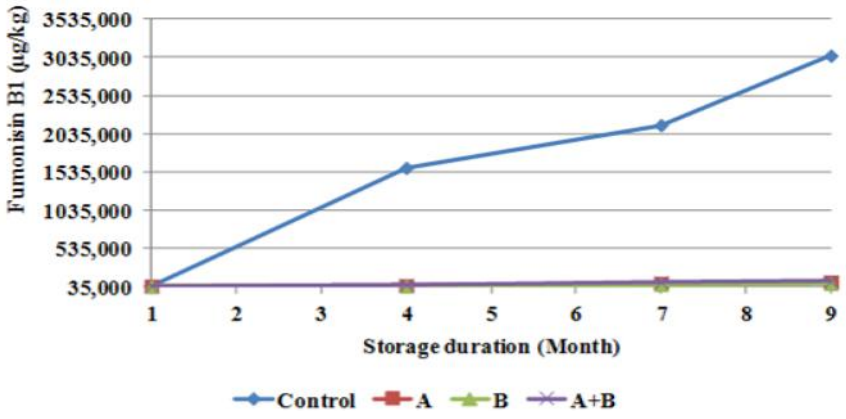

Fig. 11. Change in Fuminosin B1 of maize grains during storage at Katiola
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

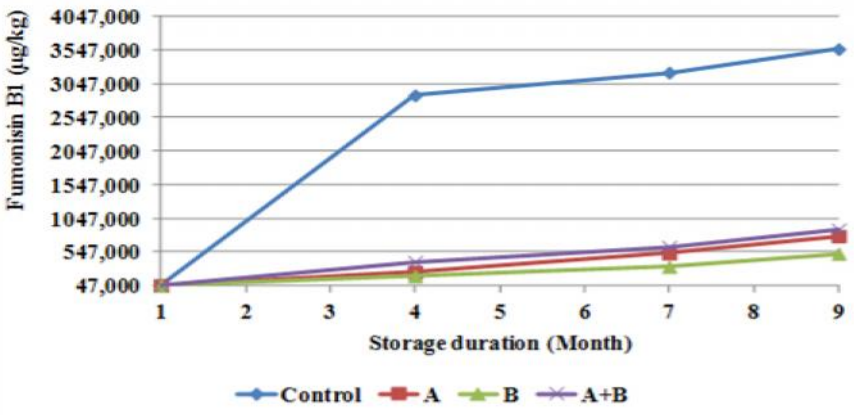

Fig. 12. Change in Fuminosin B1 of maize grains during storage at Bondoukou
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

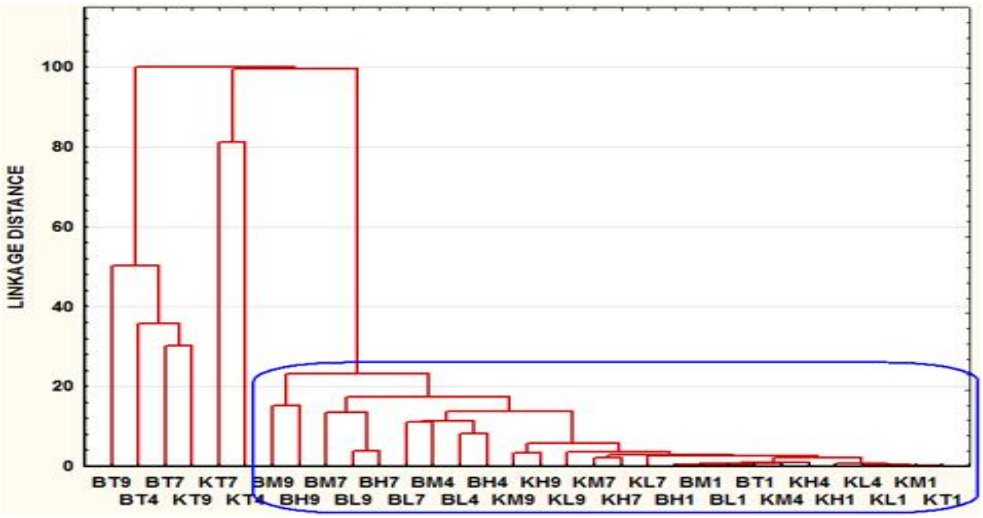

Fig. 13. Phylogenetic tree of ascending hierarchical classification relating to sanitary quality of maize grains samples treated with *H. suaveolens* and *L. multiflora* in Bondoukou and Katiola

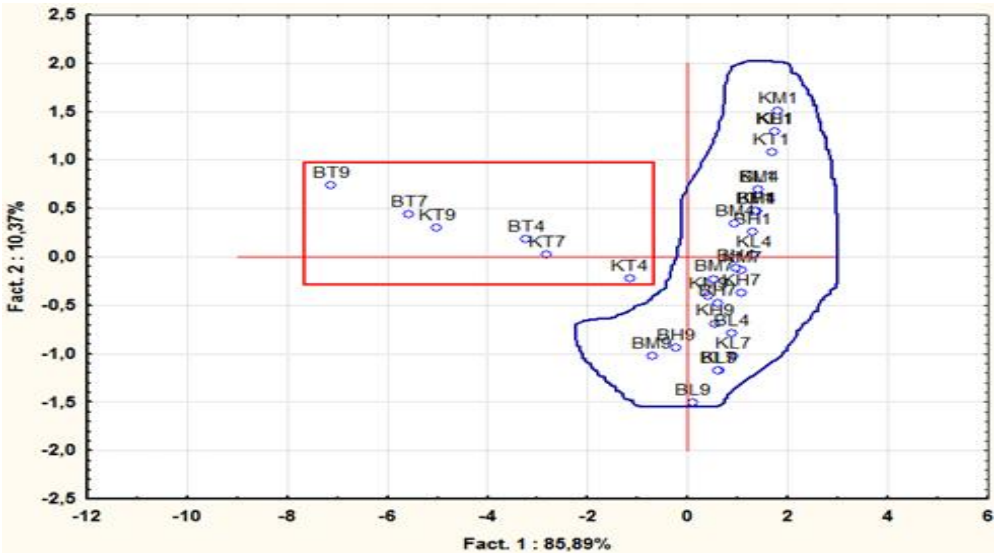

Fig. 14. Projection of samples relating to sanitary quality of maize grains samples treated with *H. suaveolens* and *L. multiflora* in Bondoukou and Katiola

BT: Sample control of Bondoukou; BL: Sample of Bondoukou with lippia multiflora; BH: Sample of Bondoukou with hyptis suaveolence; BM: Sample of Bondoukou with the mixt of lippia multiflora and hyptis suaveolence; KT: Sample control of Katiola; KL: Sample of Katiola with lippia multiflora; KH: Sample of Katiola with hyptis suaveolence; KM: Sample of Katiola with the mixt of lippia multiflora and hyptis suaveolence;. 1; 4; 7; 9: Months of samples storage.

Samples surrounded in red have the highest values of mycotoxins and those surrounded in blue have low values

3.2 Discussion

L. multiflora, *H. suaveolens* leaves used in this study positively influenced the sanitary quality of stored maize grains including total aflatoxins, aflatoxin B1, ochratoxin A, zearalenon and fuminosin B1 [28].

All maize samples from the different lots (untreated or treated with biopesticides) were

found to be mycotoxin-positive, particularly aflatoxin B1, ochratoxin A, fuminosin B1 and zearalenone. The results indicate a slight change in the levels of Aflatoxin B1, Total Aflatoxins, Ochratoxin A, Zearalélone and Fuminosine B1 in the experimental batches compared to the different control batches during storage. This observation is made on the two storage sites (Katiola and Bondoukou). These data therefore indicate a better preservation of the sanitary quality of stored maize after addition of *L. multiflora* leaves, *H. suaveolens* or the mixture of the two leaves than storage without any treatment that is to say the control batches. The AFB1, AFT, OTA, FB1 and ZEA levels of processed maize grain showed a slight increase during 9 months of storage, when untreated corn saw very high levels of mycotoxins at the two storage sites.

The water activity in the different batches of the two production zones is initially greater than 0.65. Beyond this value, there are growths of molds responsible for the production of mycotoxins, namely aflatoxins, ochratoxin A, zearalenone and fuminosin B1. Despite this high value of water activity, growth of mold was slowed down by the biopesticide effect of the leaves of *Lippia multiflora* and *Hyptis suavelons*. Thus demonstrating the efficacy of these plants for biopesticidal properties. These findings are similar to the work of Konan et al. and Niamketchi et al. [29-30] which demonstrated the biopesticidal effect of the leaves of these two plants on insect pests and molds during storage of cowpea in a triple bagging system and storage of maize in the traditional improved granaries.

Indeed, the low levels recorded in the experimental batches after 9 months of storage could be attributed to the insecticidal and / or insect repellent effect of the leaves of *Lippia multiflora* and *Hyptis suaveolens* due to the release of essential substances (bioactive molecules) [31-32]; These results are similar to those of Niamketchi et al. [19] in the central region of Cote d'Ivoire. These authors showed the effectiveness of dried leaves of *Lippia multiflora* and *Hyptis suaveolens* against the development of pests responsible for corn grain alteration in traditional and improved granaries. The results obtained are also in agreement with those of Rose de Lima et al. [33] in Benin, which showed that essential oils of *Pimenta racemosa* and *Syzygium aromaticum* would considerably reduce the fungal flora responsible for the production of mycotoxins during cowpea storage for a period of 3 months. In addition, studies by Makun et al. [34] demonstrated the inhibitory effect of ethanolic extracts of leaves of *Lippia multiflora*, *Azadirachta indica* and *Blumea perotitiana* on toxigenic molds of cereals. The bioactive molecules of *Lippia multiflora* consist mainly of oxygenated monoterpenes such as linalool and 1,8 cineole [15,35]. While monoterpene hydrocarbons, in particular sabinene, β-pinene and limonene, predominate in *H. suaveolens*. These various mono and sesquiterpene compounds contributed to the inhibitory activity of antifungal and mycotoxin production (AFB1, AFT, OTA, FB1 and ZEA) [36-39]. These antimicrobial agents induce morphological perturbations, rupture of the plasma membrane and alteration of mitochondrial structure on molds [40]. Tatsadjieu et al. [20] also showed that *Lippia rugosa* essential oil inhibits the development of *Aspergillus flavus* and limits aflatoxin B1 production to an inhibitory concentration of 1 g/L. Sharma et al. [39] showed that the essential oil of *H. suaveolens* has inhibitory activity on *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus ochraceous* producing aflatoxin B1 and ochratoxin A at a concentration of 0.5 g/kg.

According to the data obtained, the leaves of *Lippia multiflora* have a much more fungicidal effect on molds than leaves of *Hyptis suavelons*. Mixing the leaves of both plants would produce a less fungicidal effect compared to the two plants taken individually. Mixing the biopesticidal bioactive molecules of both plants could produce antagonistic effects on mold reduction. The results are consistent with those of Jonhson et al. [41], which examine the effect of 7 biopesticide plants on the conservation of CI cereals.

It should be noted that the batches of Bondoukou storage site are much contaminated by mold and insects as the batches of Katiola site but the effect of the leaves of the biopesticidal plants studied are identical on these two sites. This result is similar to that of Biego and Chatigre [42], of Niamketchi et al. [30] and of Ezoua et al. [28] who found no statistically significant difference between the effect of the leaves of the species studied on the parameters of the market quality and the sanitary quality of the corn kernels during storage.

4. CONCLUSION

The leaves of *Lippia multiflora* and *Hyptis suaveolens* possess biopesticide properties

which preserve the sanitary quality of maize grains when stored in polypropylene bags. The remanence could reach 7 months and the efficacy was marked on all the studied parameters (AFB1, AFT, OTA, FB1, ZEA). They could be an effective alternative for the storage of maize grains as a substitute for synthetic pesticides that have consequences for the health of the consumer. These results should be popularized among maize producers because they are efficient, inexpensive and easy to use.

For more efficacy of the leaves of the two plants used on mycotoxins, consideration should be given to the use of extracted essential oils derived from the leaves of these plants and also to identify bioactive molecules with a biopesticidal effect

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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