Journal of Agriculture and Ecology Research International
11(3): 1-10, 2017; Article no.JAERI.31561
ISSN: 2394-1073

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Efficacy of *Lippia multiflora* (Verbenaceae) and *Hyptis suaveolens* (Lamiaceae) Leaves on Merchant Quality of Stored Maize Grain (*Zea mays* L.) in Côte 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 YK wrote the first draft of the manuscript. Authors OKC and AC managed the analyses of the study. Author DS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAERI/2017/31561
Editor(s):
(1) Monica Rosa Loizzo, Department of Pharmacy, Health Sciences and Nutrition of University of Calabria, Italy.
Reviewers:
(1) Goudoum Augustin, The University of Maroua, Cameroon.
(2) Prisila A. Mkenda, Sokoine University of Agriculture, Tanzania.
Complete Peer review History: http://www.sciencedomain.org/review-history/18578

Original Research Article

Received 13th January 2017
Accepted 21st March 2017
Published 10th April 2017

ABSTRACT

The aim of this study was to monitor the merchant quality of maize grains stored for 9 months in polypropylene bags containing leaves of *Lippia multiflora* and *Hyptis suaveolens*. It was carried out in villages of Timbé and Soko respectively in departments of Katiola (Hambol region, Center-North) and Bondoukou (Gontougo region, Northeast) of Côte d'Ivoire. The parameters determined were

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weight loss, damages, moisture, fat, acidity and peroxide values. The mass losses and damages were determined by methods of Harris, Lindblad and Boxall respectively, while moisture, fat, values of acidity and peroxide were measured according to AOAC's standard methods. The batches treated with the leaves of *L. multiflora* and *H. suaveolens* recorded the best values compared to control batches irrespective of the type of leaf, the study site and the parameter studied. Indeed, the moisture levels of grains varied between 9.00% and 14% for control batches and were less than 13% for the treated batches. Concerning mass losses, the treated batches had rates of less than 15.25% while those of the control batches reached 24.25%. Grain damage was up to 47.40% in the control batches but remained below 32.40% in the treated batches. For the fat content of the grains, the averages decreased from 5.4% to 1.90% for control batches and remained above 2.4% for treated batches. The opposite phenomenon was observed in acidity and peroxide values which were higher in control batches than in treated batches. These results indicate that treatment of maize grains with leaves of *L. multiflora* and *H. suaveolens* makes it possible to inhibit activity of insects and to preserve quality of grains with a remanence of up to 9 months. This inexpensive and easy-to-use treatment should be popularized among farmers.

Keywords: Biopesticide; maize grains; polypropylene bag.

1. INTRODUCTION

Maize 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 [1,2]. Of 22 countries in the world where maize accounts for highest percentage of calories in national diet, 16 are in Africa notably Côte d'Ivoire [3]. Maize is a well-established crop in Côte d'Ivoire [4]. In 2014, annual production was estimated at 680000 tonnes [2]. It is consumed in several forms by both humans and animals. Indeed, fresh maize cobs can be boiled (kaba-bélégué) or simply braised. Maize flour is prepared as cake cooked, boiled or in a more compact form (kabatoh). The fermentation of maize grains produces alcohol that is used in preparation of beverages (tchapalo, beer, whiskey). Whole plant can also be consumed by livestock as forage (fresh or dry) or ensilage [5]. However, various constraints have also been identified in maize production, particularly post-harvest losses.

Food shortages are not only due to inadequate production, but also to post-harvest losses. Indeed post-harvest preservation, particularly of cereals, is currently of concern to producers and consumers in most African countries. The losses are variable according to regions or even countries. Studies have shown that losses due to pests during storage can reach 30% of production [6-8]. Several deterioration agents are responsible for these losses, particularly insects (44%), rodents (30%) and fungi (26%), as described by Huignard [9] and Foua-Bi [10]. This damage not only reduces the weight and germinative power of grains but also degrades their nutritive, merchant and sanitary qualities [11,12]. Maize grains, which are widely consumed throughout the world, do not escape this degradation process as demonstrated by several scientific works [13,14].

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 [8,15]. According to Isman [16] and PAN Africa [17], 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 [18]. In developing countries, these disadvantages are added to economic constraints related to the cost and supply of active ingredients [19]. 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. Therefore, this study was initiated to evaluate the efficacy of leaves of 2 plants (*Lippia multiflora* and *Hyptis suaveolens)* with biopesticide properties on merchant 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 North East of Cote d'Ivoire. 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 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℃ and 33.7℃ in Katiola and between 24℃ and 29℃ in Bondoukou, while the average humidity ranged between 60%-70% in both region [20,21].

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 [22,23]. 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 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℃ until constant weight [24].

2.5 Assessment of Damage and Weight Loses

To assess the damage caused by insects during storage, samples of 1 kg (approximately 3500 maize grains) were taken. After sifting and removal of the foreign matters, the grains were weighed and sorted to separate attacked and damaged grains from healthy grains. Then, the two fractions were weighed and counted separately. The percent grain damage was estimated using the method of counting and weighing of Harris and Lindblad [25] and Boxall [26]. Assays were performed in triplicate. Thus, the rate of infection is the ratio of grains having at least one hole in the total number of grains. The estimate of the damage (D) and weight loss (W) is given by the formulas 1 and 2 respectively.

$$\mathrm{D}\ (\%) = \frac{\mathrm{NGA}}{\mathrm{NTG}} \times 100 \qquad (1)$$

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

$$\mathrm{W}\ (\%) = \frac{[(\mathrm{NGA} \times \mathrm{WHG}) - (\mathrm{NHG} \times \mathrm{WAG})]}{(\mathrm{WHG} \times \mathrm{NTG})} \times 100 \qquad (2)$$

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

2.6 Extraction and Characterization of Fat

The determination of free fatty acids content involved extraction of the maize fat to the soxlhet hexane during 8 h [27]. Then, the fat acidity value was determined by titrating the diethyl ether/ethanolic solution of maize oil with an ethanolic solution of sodium hydroxide using phenolphthalein indicator [27]. Finally, the rate in free fatty acids (FFA) was expressed as the percentage of oleic acid per gram of maize fat content, calculated according to the formula 3.

$$\text{FFA} = \frac{\text{Volume in ml of KOH} \times \text{NKOH} \times 282}{10 \times \text{Maize fat mass}} \quad (3)$$

Molar Mass of the oleic acid = 282 g/mol.

The peroxide value was determined by titrating chloroform/glacial acetic acid/potassium iodide solution of maize oil with an aqueous solution of sodium thiosulphate using starch as indicator [27].

2.7 Statistical Analysis

All analyses were performed in triplicate and the means, projection of samples and the phylogenetic tree of ascending hierarchical classification were statistically treated using STATISTICA 7 software (version 7.1). The change of the parameters of the grain merketability was evaluated through graphics made from Excel 2007 software.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Moisture content

The moisture content of maize grains increased during storage regardless of study site and grain batches (Fig. 1). For control batches, these rates varied between 9.5% and 12.5% in Katiola and between 9.00% and 14.00% in Bondoukou. Concerning the batches tested, the rates were less than 12% and 13% respectively in Katiola and Bondoukou. The low rates were obtained in the maize treated with the leaves A followed by the mixture of leaves (A + B), whatever the study site and storage duration.

3.1.2 Damage and weight loss during storage

Grain weight losses increased during storage regardless of the study site and grains batches (Fig. 2). The control batches had weight losses varying between 0.72% and 10.72% in Katiola and between 0.25% and 24.25% in Bondoukou, while the losses in the treated grain remained below 5.72% and 15.25% respectively at Katiola and Bondoukou. Moreover, for the batches tested, weight losses were identical during the first 4 months of storage, regardless of study site and leaves used. Contrariwise, low losses are observed with leaves A and mixture (A + B) from the fifth month of storage, whatever the study site.

Fig. 3 shows that damage to maize grain increased during storage regardless of the study site and grains batches. For the control batches, the damage varied between 1.94% and 36.94% in Katiola and between 2.40% and 47.40% in Bondoukou. On the other hand, these losses remained below 11.94% and 32.40% respectively for Katiola and Bondoukou for the batches tested. The effects of leaves (A, B, A + B) on damage were relatively similar regardless of study site and storage duration.

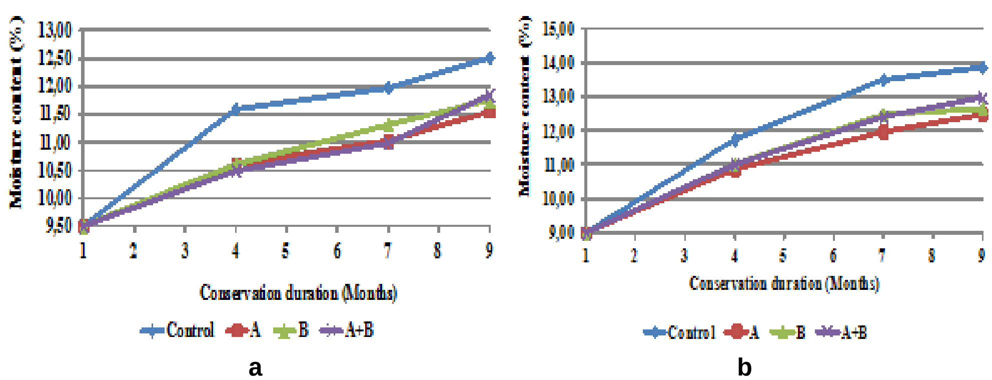
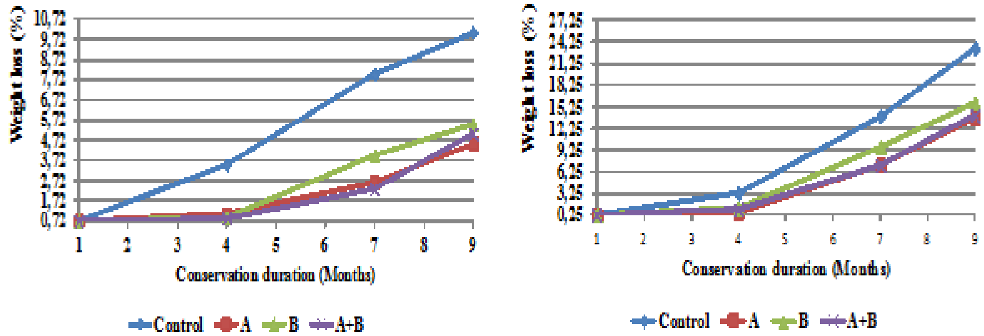


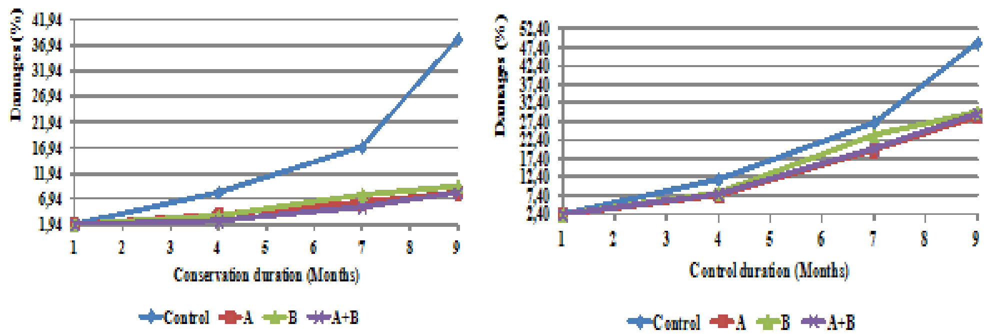
Fig. 1. Change in moisture content of maize grains during storage at Katiola (a) and Bondoukou (b)

A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora



a **b**

Fig. 2. Change in weight loss of maize grains during storage at Katiola (a) and Bondoukou (b)
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora



a **b**

Fig. 3. Change of damages of the maize grains during storage at Katiola (a) and Bondoukou (b)
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora

3.1.3 Fat content, acid content and peroxide content during storage

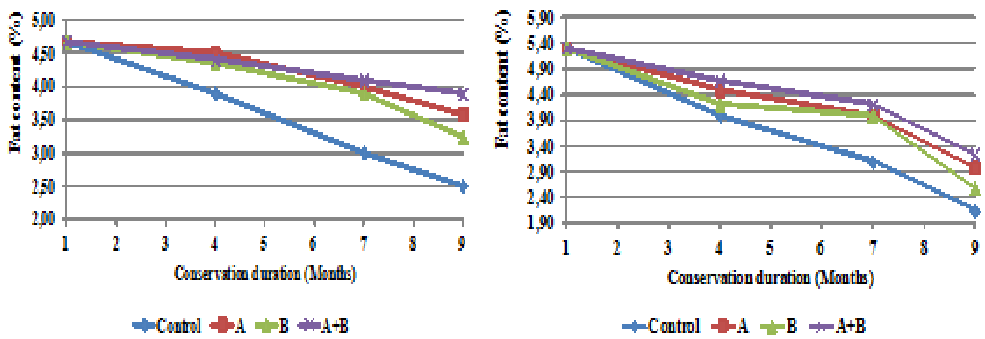
Fat content decreased during storage regardless of study site and grains batches (Fig. 4). The control batches had fat contents ranging between 4.50% and 2.50% in Katiola and between 5.40% and 1.90% in Bondoukou, while the treated grains had levels greater than 3% and 2.40% respectively at Katiola and Bondoukou. The high fat content was obtained in the grains treated with mixture of leaves of *H. suaveolens* and *L. multiflora* followed by leaves of *H. suaveolens* alone regardless of study site and storage duration.

Fig. 5 shows that acidity of maize grain increased during storage regardless of the study site and grains batches. It was greater than 6.50% in Katiola and 7.49% in Bondoukou for the control batches. However, for the treated batches, this acidity was less than 6.50% whatever study site and leaves used. The low acid value was obtained with mixture of leaves *H. suaveolens* and *L. multiflora* followed by leaves of *H. suaveolens* alone regardless of study site and storage period.

Peroxide index for control batches varied between 2.5 meq O2 / kg and 7.0 meq O2 / kg in Katiola and between 2.8 meq O2 / kg and 7.8 meq O2 / kg in Bondoukou. In the treated batches, it was less than 6.5 meq O2 / kg and 6.8 meq O2 / kg respectively in Katiola and Bondoukou. The smallest indices of peroxide were obtained in the grains treated with the mixture of leaves *H. suaveolens* and *L. multiflora* followed by leaves of *H. suaveolens* alone whatever study site and duration of storage (Fig. 6).

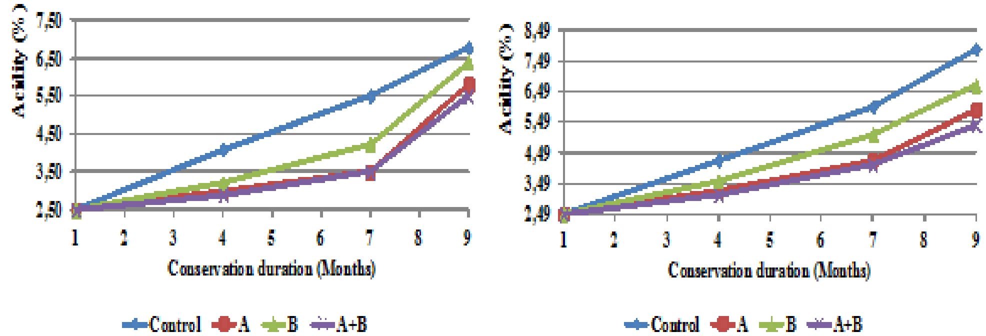
3.1.4 Remaining effect of leaves on grain quality

The samples projection shows 2 types of samples, particularly those with high moisture content, weight losses, damages, acid content and peroxide content and those with a high fat content. The phylogenetic tree of ascending hierarchical classification indicates that treated samples from Katiola show high fat content regardless of leaves used and storage duration. While those from Bondoukou have high levels of moisture, weight loss, damage, acidity and peroxide index, regardless of leaves used and storage duration (Fig. 7).



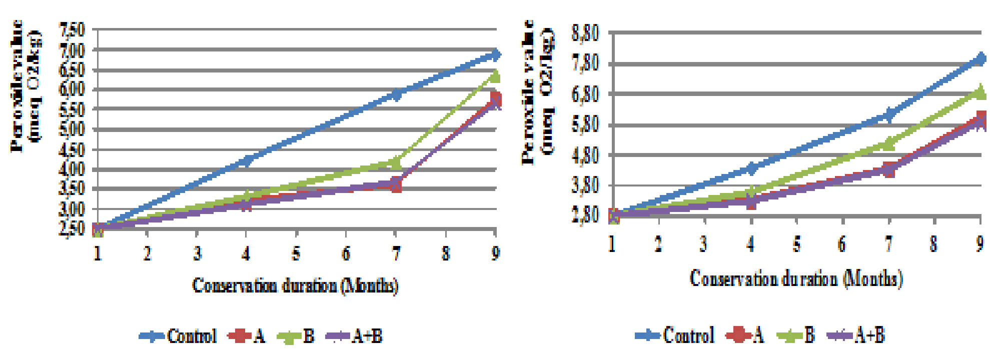
a b

Fig. 4. Change of fat content of maize grains during storage at Katiola (a) and Bondoukou (b)
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora



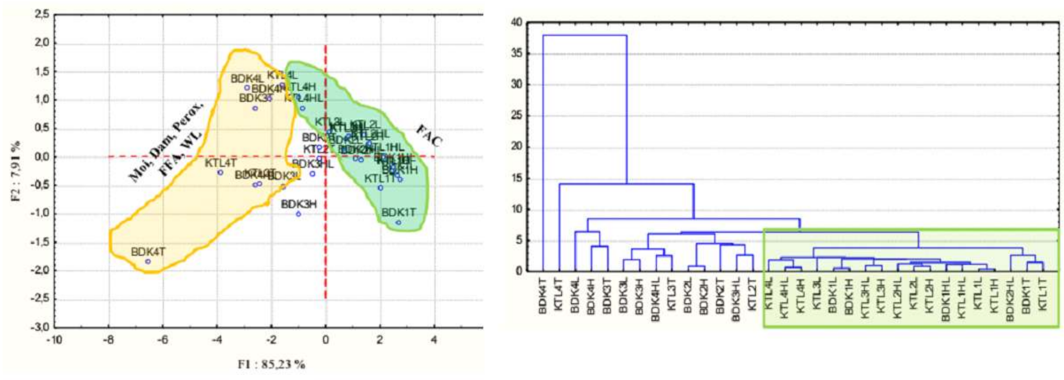
a b

Fig. 5. Change of acidity of maize grains during storage at Katiola (a) and Bondoukou (b)
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora



a b

Fig. 6. Change of peroxide value of the maize grains during storage at Katiola (a) and Bondoukou (b)
A: H. suaveoens; B: L. multiflora and A+B: H. suaveoens + L. multiflora



a b

Fig. 7. Projection of samples (a) and phylogenetic tree of ascending hierarchical classification (b) relating to merchant quality of maize grains samples treated with *H. suaveolens* and *L. multiflora* in Bondoukou and Katiola

Moi: Moisture; WL: Weight Loss; Dam: Damages; FAC: Fat Content; FFA: Acidity value; Perox: Peroxide value; BDK: Bondoukou; KTL: Katiola; 1, 2, 3, 4 : Number of samples; T: Control; H: Hyptis; L: Lippia; HL: Hyptis + Lippia In orange, samples with high values of WL, Dam, Moi, Perox and FFA. In green, samples with high values of FAC

3.2 Discussion

L. multiflora, *H. suaveolens* leaves used in this study positively influenced the merchant quality of stored maize grains including moisture, weight loss, damage to grain, fat content, acidity and peroxide index. The results indicate a decrease in the moisture content of grains relative to control batches. The action of leaves would have affected the water absorption capacity of grains. Indeed, grains have the ability to absorb moisture from the surrounding air. This exchange is function of degree of humidity of the air, its temperature and the physical state of grains. This property determines the water content of the batch and plays a role in the long-term preservation of grains [28]. The decrease in moisture could be explained by the decrease in the activity of pests on the grains tested or even their good physical state. Biego and Chatigre [14] also showed the significant effect of the amount of leaves of *L. multiflora*, *H. suaveolens* and the blending of these two species (biopesticides) on maize grains moisture. However, this result is contrary to that of Aringbangba [29] who did not observe any effect on the water absorption capacity of treated grains after the use of extracts and powder of *Eugenia aromatica*.

There was a decrease in weight losses and damage on the grains compared to the control batches. This would reflect a decrease in the degrading activity of insects from stocks on the grains. These results could be explained by the insecticidal or repulsive properties of leaves of *L. multiflora* and *H. suaveolens* on insect pests of maize grains which also corroborate the findings by Niamketchi et al. [30]. These authors observed a decrease in the insect activity on maize stored in granaries in presence of a mixture of leaves of *L. multiflora* and *H. suaveolens*. The repulsive properties of dried leaves of *L. geminata* on *Sitotroga cerealella* for stored rice were shown by Prakash and Rao [31] in India. Gueye et al. [32] showed the repellent or insecticidal properties of dried leaves of *H. spicigera* and *H. suaveolens* on the stock insects for maize stored in traditional granaries. Some authors have linked these insecticidal or repellent properties to the composition of the essential oil derived from these plants [33,34].

Concerning the fat content of seeds, it decreased more rapidly in the control batches than the treated batches. The opposite phenomenon was observed with the acidity and the peroxide index of the oil from the seeds. These phenomena could be explained on one hand by the increase in moisture content and on the other hand by the increase in insect activity (weight loss and grain damage). The increase in moisture would lead to the chemical hydrolysis of triacylglycerols by the action of water which contributes to the increase of grains acidity. Moreover, this high moisture could catalyze non-enzymatic oxidation of fatty acids and favor the production of peroxides [35-37]. Furthermore, the increase in grains damage (insect activity) could lead to the loss of part of germs and contribute to the reduction of the fat content. Hoopen and Maiga [38] indicated that the germ contains 35% to 40% of the oil contained in the maize grains.

The remanence of leaf effect on the merchant quality of maize grains varied according to the study site but was identical within the site regardless of leaf type. This result is similar to those of Biego and Chatigre [14] and Niamketchi et al. [30] who found no statistically significant differences between leaves effect of species studied on the parameters of the merchant and sanitary quality of maize grains during storage. These authors also identified the study site as a factor significantly influencing the pesticidal properties of the leaves.

4. CONCLUSION

The leaves of *L. multiflora* and *H. suaveolens* possess biopesticidal properties which preserve the merchant quality of maize grains during storage in polypropylene bags. The remanence could reach 9 months and the efficiency was marked on all parameters studied (moisture, weight loss, damages of grains, fat content, index of acidity and peroxide). They could constitute an effective alternative for the storage of corn kernels as a replacement for synthetic pesticides which have consequences for the health of the consumer. These results should be popularized among maize producers because they are effective, inexpensive and easy to use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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