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Evolution of the Merchantability during Storage of Cowpeas (*Vigna unguiculata L Walp)* Bagged Pics Containing a Biopesticide (*Lippia multiflora* Moldenke)

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Authors' contributions

This work was carried out in collaboration between all authors. Author CKK designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors CKK, HMGB, AC, SD, YNK and OC managed the literature searches, analyses of the study performed the structural equation modelling and discuss the conclusion. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was undertaken to evaluate the merchantability of bagged PICS cowpea (*Vigna unguiculata L Walp*) containing a biopesticide (leaves of *Lippia multiflora* Moldenke) during storage.
Study Design: Cowpea grains were collected from April to May 2015 in the Southwest of Côte d'Ivoire and the fresh leaves of *Lippia multiflora* were dried in sunlight for 7 days and chopped before using as biopesticide. The storage bags used were from Purdue Improved Cowpea Storage (PICS) developed by Purdue University for storing cowpeas from Niger.

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Place and Duration of the Study: This study was carried out during June 2015 to February 2016 in the Laboratory of Biochemistry and Food Science, Félix Houphouët-Boigny University, Côte d'Ivoire.

Methodology: For the storage of cowpea seeds, 6 lots (1 control polypropylene bag, 1 control PICS bag, and 4 lots in PICS bags with biopesticide) were used. The 4 lots in PICS bags were filled with different proportions of biopesticides (0.7%, 2.5%, 4.3% and 5% of chopped dried leaves per bag). The filling of the bags (50 kg) was done in stratum, alternating cowpea seeds and leaves of *Lippia multiflora.* Changes in moisture, weight losses and damages caused by insects were then evaluated after 0, 1, 2, 4.5, 7 and 8 months.

Results: Moisture content, weight losses and damages of the control without PICS (polypropylene bags) were respectively 14.67 ± 0.15%, 22.03 ± 0.25% and 43.14 ± 2.79%, respectively at 4.5 months. For the control PICS bag without biopesticide, the values of moisture content, weight losses and damages were 14.10 ± 0.11%, 19.20 ± 1.74% and 37.77 ± 3.27% after 8 months of storage, respectively. The moisture values, weight losses and damages in PICS bags of cowpeas treated with biopesticides were low and less than, 12.10 ± 0.10%, 4.03 ± 0.27% 11.18 ± 0.01%.

Conclusion: Adding *Lipppia multiflora* leaves in PICS bags for storage allows a good preservation and merchantability of cowpeas grains after 8 months.

Keywords: Cowpea; losses and damages; PICS bags; storage; biopesticides.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a legume of Tropical regions of Africa [1]. Cowpea is cultivated for human food and livestock needs [2]. The world production of cowpea is over 5.7 million tons of dry seeds per year, on 7.5 million ha in 2008, with 70% from Africa [3].

Through its role in restoring soil fertility and its compatibility with several plant cultivation associations, cowpea is an essential crop in savanna areas of tropical Africa [4,5]. The leaves, pods and seeds of cowpea are sources of protein, vitamins and minerals [6,7]. Therefore, cowpea is considered as a balanced diet compared to cereals and tubers which are generally low in protein and high in carbohydrates [8,9]. The growing of population correlated with the changes in food habits has favored merchant activities of maize and cowpea [10].

However, the major constraint linked to the processing of cowpea is the difficulty of post harvest preservation of seeds that prevents the farmers to cultivate cowpea in large quantities [11,12]. Therefore, the need for better crop storage is an important step in safeguarding food security [12]. The good practices of crop storage would also guarantee availability of commodities and provide seeds for future campaigns [13,14,15]. It's worth noting that, post-harvest losses and damages are estimated for more than 30% of the production; these losses and damages are mainly due to insects (44%), rodents (30%) and molds and other (26%) [11,16,17]. In the wide range of these pests, cowpea beetles *Bruchidae* including *Callosobruchus maculatus* Fabricius are among the most dangerous because their attacks begin at the field, then spreading to the warehouse where the weevils' population can grow quickly. The damages caused by this beetle are mainly weight loss, nutritional value degradation and reduction of the germination of grains [14,18,19].

In order to reduce losses and damages of stored cowpea due to pests' activities, promotion of sustainable development and environmental protection, alternative control methods that would be inexpensive, effective and easy to adopt for Third World producers are often recommended. Indeed, the use of chemical insecticides can cause poisoning of consumers, resistance in pests and negative impact on the environment [20]. For this purpose, many natural additives such as native aromatic plants, appear to be effective for cotrolling insects in stored products [21,22]. These plants are natural, which means more safety for the population and the environment. They are also considered at low risk for development of resistance by insects and pathogenic microorganisms [14,23]. These aromatic plants and their derivatives are effective against pests. They are cheaper and guarantee biodiversity [24,25]. Among these insecticides plants, figures *Lippia multiflora,* accessible in all regions of the Ivory Coast and subjected to several works about the biofunctional properties [26,27].

Thus, the objective of this research is to contribute in reducing losses and damages of stored cowpea due to pests' attack by assessing the combined effects of triple bagging (PICS bag) containing *Lippia multiflora* Moldenke leaves as biopesticide.

2. MATERIALS AND METHODS

2.1 Collection, Packaging and Storage of Cowpea Grains

Cowpea grains (beige color variety) were collected from producers of Loh-Djiboua region (5° 50 'North 5° 22' West, Côte d'Ivoire) from April to May 2015, after harvesting. After the shelling step, the grains were packaged and stored in the laboratory at 27.78 ± 0.19ºC and 75.0 ± 0.99% relative humidity. Storage bags used in this study, were made of polypropylene and polyethylene (Purdue Improved Cowpea Storage: PICS) developed by Purdue University for storing cowpeas from Niger. These bags, obtained from suppliers, consisted in triple bagging composed of two polyethylene bags in a polypropylene bag. For the storage of cowpea seeds, 6 lots (1 control polypropylene bag, 1 control PICS bag, and 4 lots in PICS bags) were used. The test lots of bags were filled with different proportions of biopesticides (0.7%, 2.5%, 4.3% and 5% of chopped dried leaves per bag). The cowpea seeds and leaves of *Lippia multiflora* were added alternately as stratum and in small quantities. The leaves of *Lippia multiflora* were deposited at the bottom and the surface of each PICS bag.

2.2 Sampling for Analysis

The sampling was performed for 0, 1, 2, 4.5, 7 and 8 months. Thus, 1 kg of cowpea samples from each PICS bag was collected through the top, the centre and the bottom opening sides. Cowpea samples were analyzed for weight losses, damages and physicochemical properties.

2.3 Determination of Moisture Content

The moisture content was estimated according to AOAC method [28]. For this, 5 g of sample were dried at 105℃ in oven till constant weight. The result was expressed below:

Moisture content (%) = 100-(Wl x 100/Ws)

With Wl, weight lost from samples after drying; Ws, weight of raw samples.

2.4 Damage Assessment and Weight Losses

To assess the damage caused by insects 0.5 kg (about 3500 grains of cowpea) were taken. After visual screening and removal of impurities (insects, powder), the grains was weighed and sorted to separate damaged from healthy grains. Then, both fractions were weighed and counted separately. The percentage damage was estimated using the method described by Harris and Lindblad and Boxall, [29,30]. The assay was performed in triplicate. Damage estimates (D) and weight loss (W) are given by the formulas:

D (%) = (NGA / TG) x 100

NGA = Number of Grains Attacked; TG = Total Grains.

WL (%) = [[(NGA x WHG) - (NHS x WGA)] / (WHG x TG)] x 100

WL = Weight Losses; NGA = Number of Grains Attacked; NHS = Number of Healthy Grains; TG = Total Grains; WGA = Weight of Grain Attacked; WHG = Weight of Healthy Grains.

2.5 Statistical Analysis

All analyzes were performed in triplicate and data were statistically processed using the SPSS software (version 20.0). The comparison mean values were performed by two-way 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 significant parameters were compared using the Tukey test with a tolerance of 5%. Pearson correlation test was used to assess relationships between the content of moisture, the percentage of weight losses and damages. Then, Multivariate Analyses through Principal Components Analysis (PCA) and Ascending Hierarchical Clusters analysis (AHC) were performed using STATISTICA software (version 7.1).

3. RESULTS

3.1 Evolution of Moisture during Storage

Table 1 shows the moisture content of stored cowpea grains in different PICS bags. With an average of 10.03 ± 0.21% (0 month), the moisture content increased significantly (P <0.001) during the storage period (Table 2). The higher moisture values were recorded after 4.5 months of storage in the control without PICS (14.67 ± 0.15%) and 8 months of storage in the PICS control without biopesticide (14.10 ± 0.11%) (Fig. 1). In PICS bags moisture contents were similar after 8 months with an average of 12.06 ± 0.11%.

3.2 Evolution of Weight Losses

The weight losses before storage (0 month) were 0.28 ± 0.08%. This value rapidly increased at 4.5 months to the value of 22.03 ± 0.25% in the polypropylene control bag. In PICS control bags (without biopesticide), weight loss remained low up to 4.5 months of storage. But the weight loss quickly increased to the value of 19.20 ± 1.74% after 8 months (Table 1 and Fig. 2). In the other lots weight losses were low ranging from 0.28 ± 0.08% to 4.03 ± 0.27% after 8 months of storage (Table 1 and Fig. 2). Changes in weight losses were significant depending on the type of packaging, the storage duration and the interaction between these two parameters (Table 2).

3.3 Evolution of Damages Effects

Before storage (0 month), the registered damages were estimated to 3.25 ± 0.07%. These damages rapidly increased in the control bag without PICS to reach the value of 43.14 ± 2.7% for 4.5 months. From 4.5 to 8 months, an important increase in damage, going from 5.01 ± 0.3 to 37.77 ± 3.27 was noted (Fig. 3). In PICS bags with biopesticide, the damage varied from 3.25 ± 0.7% to 5.81 ± 0.11% after 7 months of storage. .For the batches H1, H2, H3 and H4 the following values 11.18 ± 0.01%, 10.25 ± 0.23%, 8.98 ± 0.16% and 7.31 ± 0.26 were recorded (Table 1 and Fig. 3).

3.4 Correlations between Moisture Content, Weight Losses and Damages

Table 3 highlights the correlation between moisture content, weight losses and damages for the different types of packaging. Indexes of Pearson (r) indicated positive and significant correlations between the three studied parameters for different types of packaging. Thus, moisture content, weight losses and damages were closely correlated during storage of cowpea, with r ranging from 0.83 to 0.98.

3.5 Variability between Types of Packaging, Moisture Content, Weight Losses and Damages

Variability among the studied parameters was structured, first, by a principal component analysis (PCA). These analyzes were performed with the component (or factor) F1 which recorded an intrinsic value greater than 1, according to Kaïser rule (Table 4). Moisture content, weight losses and damages showed negative significant correlations with F1. However, the component F2 (own value 0.21) was associated with F1 for the realization of PCA.

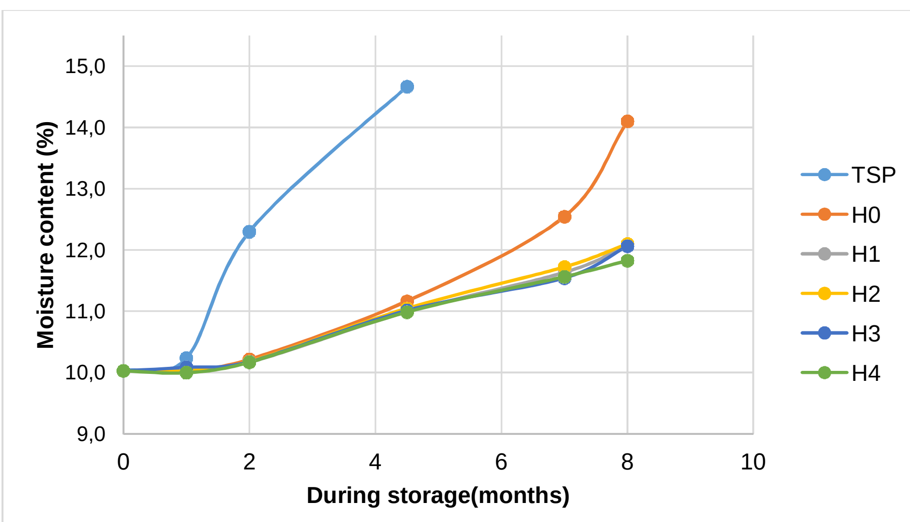


Fig. 1. Evolution of moisture content during storage of cowpea seeds

Table 1. Evolution of weight losses, damages and moisture content during storage of cowpea seeds

Parameters	Storage during (months)	TSP	H0	H1	H2	H3	H4
Weight losses (%)	0	0.28±0.08^{aA}	0,28±0,08^{aA}	0.28±0.08^{aA}	0.28±0.08^{aA}	0.28±0.08^{aA}	0.28±0.08^{aA}
	1	7.60±0.17^{bB}	0.41±0.00^{aA}	0.45±0.02^{aAB}	0.28±0.05^{aAB}	0.28±0.05^{aA}	0.26±0.05^{aA}
	2	13.03±0.22^{cC}	0.58±0.02^{bA}	0.25±0.03^{aA}	0.24±0.03^{aA}	0.22±0.01^{aA}	0.25±0.03^{aA}
	4.5	22.03±0.25^{cD}	2.00±0.18^{bA}	0.90±0.05^{aB}	0.80±0.07^{aB}	0.57±0.04^{aA}	0.50±0.07^{aB}
	7	-	12.08±0.29^{dB}	2.71±0.24^{cC}	2.14±0.11^{bC}	1.40±0.21^{aB}	1.33±0.03^{aC}
	8	-	19.20±1.74^{bC}	4.03±0.27^{aD}	3.23±0.11^{aD}	2.31±0.23^{aC}	2.25±0.03^{aD}
Damages (%)	0	3.25±0.07^{aA}	3.25±0.07^{aA}	3.25±0.07^{aA}	3.25±0.07^{aA}	3.25±0.07^{aA}	3.25±0.07^{aA}
	1	10.77±0.07^{bB}	3.38±0.03^{aA}	3.36±0.03^{aA}	3.37±0.03^{aA}	3.37±0.03^{aA}	3.38±0.03^{aA}
	2	35.15±1.03^{bC}	3.74±0.09^{aA}	3.35±0.20^{aA}	3.37±0.19^{aA}	3.40±0.17^{aA}	3.35±0.20^{aA}
	4.5	43.14±2.79^{bD}	5.01±0.03^{aA}	3.80±0.16^{aB}	3.60±0.12^{aA}	3.57±0.14^{aA}	3.60±0.12^{aA}
	7	-	33.18±2.81^{bB}	5.81±0.11^{aC}	5.25±0.08^{aB}	4.50±0.08^{aB}	4.43±0.18^{aB}
	8	-	37.77±3.27^{bB}	11.18±0.01^{aD}	10.25±0.23^{aC}	8.98±0.16^{aC}	7.31±0.26^{aC}
Moisture (%)	0	10.03±0.21^{aA}	10.03±0.21^{aA}	10.03±0.21^{aA}	10.03±0.21^{aA}	10.03±0.21^{aA}	10.03±0.21^{aA}
	1	10.24±0.06^{bA}	10.03±0.06^{aA}	10.07±0.03^{abA}	10.03±0.06^{aA}	10.09±0.10^{abA}	10.00±0.10^{aA}
	2	12.30±0.10^{bB}	10.22±0.02^{aA}	10.17±0.03^{aA}	10.18±0.03^{aA}	10.18±0.03^{aA}	10.17±0.01^{aA}
	4.5	14.67±0.15^{bC}	11.17±0.06^{aB}	10.99±0.01^{aB}	11.05±0.06^{aB}	11.01±0.03^{aB}	10.99±0.01^{aB}
	7	-	12.55±0.11^{bC}	11.63±0.06^{aC}	11.72±0.06^{aC}	11.54±0.04^{aC}	11.56±0.19^{aC}
	8	-	14.10±0.11^{bC}	12.06±0.12^{aD}	12.10±0.10^{aD}	12.06±0.06^{aD}	11.83±0.14^{aC}

The mean (± SD) with different lowercase / uppercase letters on the same line / in the same column are different test probability of 5%, TSP = Control without PICS bag (polypropylene bag); H0 = Control with PICS bag (no biopesticide); H1 = PICS bag with 0.7% of biopesticide (w / w); H2 = PICS bag with 2.5% biopesticide (w / w); H3 = PICS bag with 4.3% of biopesticide (w / w); H4 = PICS bag with 5.0% of biopesticide (w / w)

Table 2. Statistical data for moisture, weight losses and damages in cowpea grains according to the type of packaging during the storage period

Source of variation	df		Parameters		
			Moisture	Weight losses	Damages
Types	5	SS	0.11	132	136.86
		F-value	4.16	4395.86	17594.52
		P-value	**=0.05**	**<0.001**	**<0.001**
Duration	0	SS	0.00	0.00	0.00
		F-value	0.00	0.00	0.00
		P-value	**<0.001**	**<0.001**	**<0.001**
Types x duration	0	SS	0.00	0.00	0.00
		F-value	0.00	0.00	0.00
		P-value	**<0.001**	**<.0.001**	**<0.001**
Error	12	SS	0.06	0.07	0.02
Total	18	SS	1827.47	175.20	518.24

SS: Sum of squares; F-value: value of the statistical test; P-value: Probability value of the statistical test; df: degree of freedom

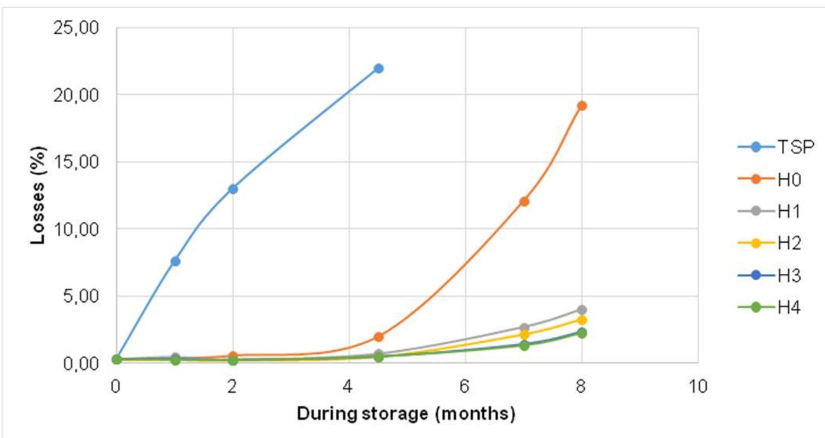


Fig. 2. Evolution of weight losses during storage of cowpea seeds

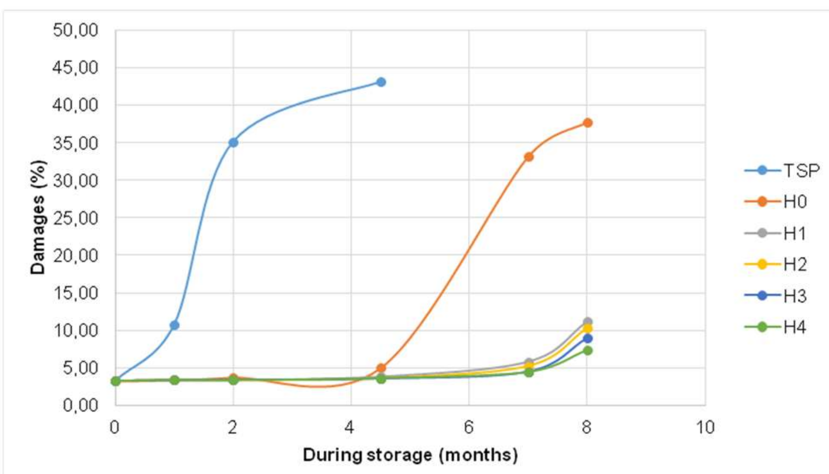


Fig. 3. Evolution of damages during storage of cowpea seeds

TSP = Control without PICS bag (polypropylene bag); H0 = Control with PICS bag (no biopesticide); H1 = PICS bag with 0.7% of biopesticide (w / w); H2 = PICS bag with 2.5% biopesticide (w / w); H3 = PICS bag with 4.3% of biopesticide (w / w); H4 = PICS bag with 5.0% of biopesticide (w / w)

Table 3. Matrix of Pearson correlation indexes between weight losses, damages and moisture content during storage of cowzpea seeds

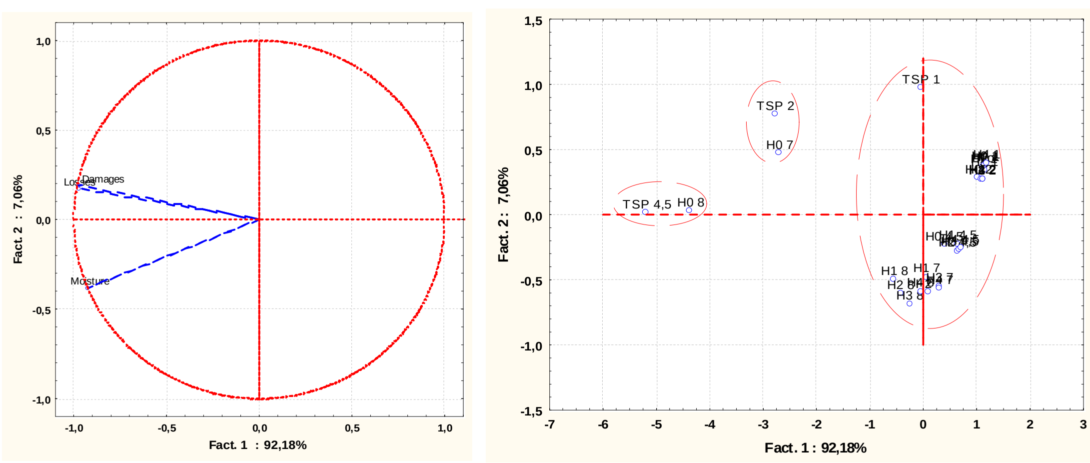
	Weight losses	Damages	Moisture
Weight losses	1		
Damages	0.98	1	
Moisture	0.84	0.83	1

Fig. 4a shows the factors of the correlation circle of PCA to other parameters of the stored cowpea. The first two factors (F1 and F2) had values of 2.76 and 0.21 respectively. These values expressed 99.24% of the variability (Table 3). The projection divided individuals into 3 groups. Group 1 consisted of two individuals namely the control without PICS at 4.5 months storage (TSP5) and PICS bag control without biopesticide at 8 months of storage (H08). This group is characterized by the highest values of weight losses, damages and moisture. The second group is composed of two individuals, which are the control without PICS at 2 months of storage (TSP2) and control with PICS at 7 months of storage (H07). The characteristics of their parameters differ from other individuals. The third group contains all samples PICS bags with biopesticide storage at every month, controls bag with PICS of 1 to 4.5 months and the control without PICS at 1 month of storage. This group is characterized by low values of weight losses, damages and moisture.

The dendrogram based on Ascending Hierarchical Classification (AHC) indicated three classes observed during storage of cowpea (Fig. 5). The first class consists of 2 individuals TSP5 and H08. The control without PICS at 2 months (TSP2) and PICS bag control at 7 months (H07) constituted the second class. Group 1 had the highest values followed by the group 2. The third group included all PICS bags with biopestide, the PICS control bags at 1, 2 and 4.5 months and the control without PICS at 1 month. Individuals in the latter group had low values of parameters.

Table 4. Matrix of eigenvalues of factors resulting from the principal components analysis and correlation with the moisture content, the weight losses and the damages levels of the stored cowpea seeds (8 months)

Item	Fact. 1	Fact. 2	Fact. 3
Eigenvalues	2.76	0.21	0.02
Variance (%)	92.18	7.06	0.76
Cumulative variance (%)	92.18	99.24	100
Losses	-0.98	0.17	0.11
Damages	-0.98	0.19	-0.1
Moisture	-0.92	-0.38	0



(a) **(b)**

Fig. 4. Correlation between the f1-f2 factorial of the principal components analysis deriving from the stored cowpea seeds

TSP (1 to 4,5 months), Control without PICS bag (polypropylene bag); H0 (1 to 8 months), Control with PICS bag (no biopesticide); H1 (1 to 8 months), PICS bag with 0.7% of biopesticide (w / w); H2 (1 to 8 months), PICS bag with 2.5% biopesticide (w / w); H3 (1 to 8 months), PICS bag with 4.3% of biopesticide (w / w); H4 (1 to 8 months), PICS bag with 5.0% of biopesticide (w / w)

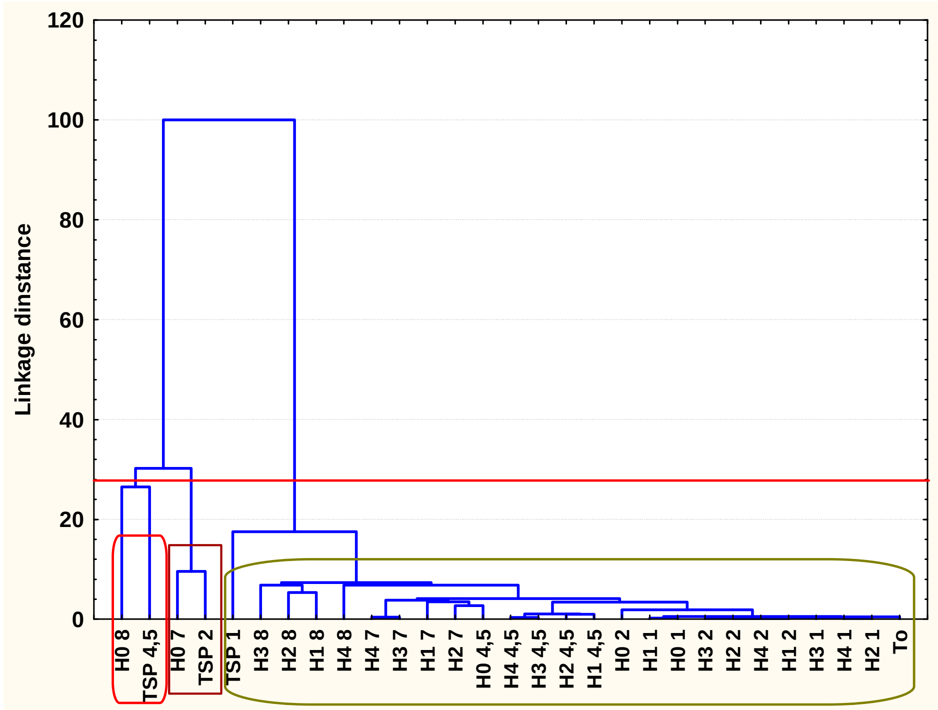


Fig. 5. Dendrogram deriving from the ascending hierarchical classification (AHC) of cowpea samples stored for 8 months

TSP (1 to 4,5 months), Control without PICS bag (polypropylene bag); H0 (1 to 8 months), Control with PICS bag (no biopesticide); H1 (1 to 8 months), PICS bag with 0.7% of biopesticide (w / w); H2 (1 to 8 months), PICS bag with 2.5% biopesticide (w / w); H3 (1 to 8 months), PICS bag with 4.3% of biopesticide (w / w); H4 (1 to 8 months), PICS bag with 5.0% of biopesticide (w / w)

4. DISCUSSION

The results of this study showed that the post-harvest storage of cowpea in PICS bags containing a biopesticide was able to reduce weight losses, damages by insect pests and also the moisture content. The reducing of weight losses and damages by PICS bags may be due to the fact that the PICS bags are sealed with a vacuum which would prevent the proliferation of insects and slowing the progression of the studied parameters [31,32]. After 7 and 8 months, the weight losses, damages and moisture content increased significantly in the PICS bag without biopesticide. The weight losses, damages and the moisture in the PICS bags containing leaves of *Lippia multiflora* were low due to the inhibitory effect of leaves of *Lippa multiflora* on pest insects and fungi as highlighted by Goly et al. [33,34]. Our results were also in agreement with those of Rose de Lima et al. [35] which showed that the essential oils of *Pimenta racemosa* and *Syzygium aromaticum* reduced significantly the fungal flora responsible for the production of mycotoxins during cowpea preservation over a period of 3 months. In addition, the studies of Makun et al. [36] have demonstrated the inhibitory effect of ethanol extracts of leaves of *Lippia multiflora, Azadirachta indica* and *Blumea perotitiana* on cowpea toxigenic molds. The bioactive molecules of *L. multiflora* primarily comprises oxygenated monoterpenes such as linalool and 1,8 cineole [26]. These antimicrobial agents cause mold damages such as morphological disruption, disruption of the plasma membrane and impaired mitochondrial structure [37].

The conditions of high temperature and humidity of the storage environment affect the moisture of the grains during storage and therefore the growth of insects and microorganisms in cowpea during storage [38,39,40]. The fluctuation of the temperature and humidity in the storage bags greatly affects the quality of stored grain as the loss of nutritional value, weight losses and damages caused by insects, etc. [38,41,42].

The moisture content of cowpea grains is positively correlated with the weight losses values and damages (Tables 3 and 4). The increase of moisture content may be due to the respiration phenomenon during storage [43]. Previous studies have also showed an increase in the moisture content of the grains during storage because of the biological activities of insects and fungi [44-46]. The increase of moisture content could also be due to the changes of relative and temperature area during the storage period [47,48]. The single bag polypropylene control which is permeable to air and water depicted high values in the studied parameters leading therefore for destocking grains after 4 and 5 months. Indeed, the grains of this bag lost all the nutritional, sanitary and market qualities. Contrary to this, PICS bags being impermeable to air and water were able to maintain low values in weight losses, damages and moisture after 4.5 months. Indeed, the air tightness of triple bagging has kept the weight losses and damages to low values and insect pests in a dormant state for 4.5 months. PICS bags with biopesticide were able to maintain low values in weight losses, damages and moisture up to 8 months due to the insecticide effect *Lippia multiflora* leaves [32]. Thus, the leaves of *Lippia mutiflora* may have inhibitory and insecticide effects, extending dormant larvae contained in cowpea grains where low values were observed up to 8 months of storage.

The duration of storage has increased the fungal infestation throughout the experience particularly in the control bag (without PICS) s and to a lesser extent in the PICS bag control without biopesticide. This increase in the percentage of fungi and insect pests could be attributed to the presence of higher moisture content of the grains. This findings were similar to those of Paraginski et al. [49] and Aktaruzzaman et al. [50] for grains stored in inadequate conditions.

5. CONCLUSION

The study based on the evolution of losses and damages during storage of cowpea in PICS bags with or without biopesticides has demonstrated the effectiveness of PICS bag and leaves of *Lippia multiflora* for the preservation of cowpea seeds. Triple bagging technique allows to extend the shelf life of cowpea grains up to 6 months and using leaves of *Lippia multiflora* as biopesticide has potentially extended the merchantability of cowpea during 8 months of storage. Optimal storage conditions of cowpea obtained in our study were 0.7% as the minimum concentration of *L. multiflora* leaves for a period of 8 months. Therefore, PICS bags with biopesticide could be an inexpensive and environmental alternative to the usage of synthetic pesticides for the storage and preservation of cereals and pulses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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