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Effectiveness of Hermetic Storage Using PICS Bags and Plastic Jars for Post-Harvest Preservation of *Acacia macrostachya* Seeds

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Abstract

In Burkina Faso, the availability of *Acacia macrostachya* "Zamnè" seeds throughout the year is threatened by the attacks of pests. So, the effectiveness of airtight (hermetic) storage for the preservation of these seeds was evaluated using 20-litre plastic jars and 50 kg PICS bags as hermetic containers with 50 kg polypropylene bags as controls. Seeds of *Acacia macrostachya* (17.5 kg) were stored in each of these storage devices for six months under ambient conditions in the laboratory. The number of storage pest *Bruchidius silaceus* increased significantly in polypropylene bags from 235 to 715 individuals on average/500 g of seeds. But in PICS bags and plastic drums, the number of this pest did not vary significantly (191 and 239 individuals on average/500 g of seeds for plastic jars and PICS bags respectively). In both hermetic devices, we found few individuals of another major storage pest *Caryedon furcatus*. However, polypropylene bags, haboured more pests' in addition high numbers of *C. furcatus* and *Oryzaephilus mercator* which were not found in the hermetic devices. Seeds damage and weight loss increased significantly in polypropylene bags from 7.40% to 20.23% and 0.50% to 3% respectively compared to PICS bags and plastic jars. The germination rate of *A. macrostachya* seeds decreased significantly in PICS bags, plastic jars and polypropylene bags with average percentages of 14%; 11% and 15% respectively compared to the initial average of 27%. PICS bags and plastic jars are therefore effective in preserving the quality of *A. macrostachya* seeds, but the effects of these hermetic devices on seed viability need to be explored further.

Keywords

Airtight Storage, *Acacia macrostachya*, *Bruchidius silaceus*, *Caryedon furcatus*, Seed Quality

1. Introduction

In Burkina Faso, *Acacia macrostachya* Reichenb. ex DC. “Zamnè” is a wild legume whose seeds are a very important non-timber forest product (NTFPs) for rural and urban population. In boiled form, they are used as snacks during parties, customary and religious ceremonies [1]. Due to its high nutritional value, richness in micronutrients, the grain has a potential use in formulations for nutritional improvement and towards achieving food security [2]. Economically, seeds of *Acacia macrostachya* generate significant income for rural populations [3] [4] with the average cost for 1 kg varying between 0.88 and 1.28 USD in the rural areas [5]. Recently, the discovery of peptides and osidic extracts from seeds present another potential of their use in pharmaceutical industry [6]. However, the production and exploitation of the potential of this legume are threatened by insect pests during storage, resulting in a reduction in its quality and commercial value. [7] mentioned that bruchid beetles predominantly infest all Acacia spp. seeds. The Bruchids *Caryedon furcatus* Anton & Delobel (Coleoptera: Chrysomelidae) and *Bruchidius silaceus* Fahr. (Coleoptera: Chrysomelidae) are two major insect pests affecting *A. macrostachya* grains [8] [9]. A recent survey conducted in rural areas in Burkina Faso [5], showed that insect pests cause damages by perforating and reducing germination rate of the grains. The presence of live and dead insects is also observed in storage contributing to reducing seeds quality. Presently, seeds are preserved by treating with heat before storage [5] [10]. However, this method of preservation has some limitations, as it is difficult to apply on a large scale and is not cost-effective. In addition, heat treatment negatively affects seed viability, thereby compromising crop production as the cultivation and domestication of this plant is increasingly encouraged in Burkina Faso. There is, therefore, an urgent need to develop and explore easier, cost-effective preservation techniques that will maintain the integrity of the seeds in terms of aesthetics and viability. Hermetic storage seems to be a promising and much easier way to store and protect seeds. Indeed, several hermetic containers have been successfully tested for storing of leguminous seeds such as triple-bagging technology also named Purdue Improved Crop Storage (PICS)’s bags. This technology has already proven to be effective in storing several crops including cowpea, bambara groundnut, mung bean, pigeonpea and sorghum [11] [12] [13] [14] [15]. To date, in the rural areas, the conservation of seeds in hermetic containers is often done in plastic jars [16]. To our knowledge, no study has been carried out on the hermetic storage of NTFPs, including *A. macrostachya* seeds. Thus, there is a lack of information on the effectiveness of airtight storage of *A. Macrostachya* in plastic jars and PICS bags, which justifies the conduct of this study. Thus, this present study was undertaken firstly to assess the hermetic storage of *A. macrostachya* seeds in plastic jars and PICS bags for controlling insect pests; and secondly to demonstrate the effectiveness of these hermetic storage systems on seeds viability and germination rates.

2. Materials and Methods

Experiments were carried out with naturally infested seeds of *A. macrostachya*.

The seeds were purchased in Saria district, Burkina Faso (latitude: 12˚16W; longitude: 02˚09N) during harvest from farmers who had not yet stored or used insecticides. Empty plastic jars (Manufacturer) of 20 liters capacity (length, width) each and 50 kg PICS bags (manufacturer) were provided by the Central Laboratory of Agricultural Entomology of Kamboinsé. Woven polypropylene bags (50 kg) were used as control. All studies were carried out at the ambient room with an average temperature of 29.13˚C ± 4.53˚C and 20.26% ± 8.80% relative humidity.

Prior to storage, seeds were mixed to make infestation homogeneous. Thereafter, three separate samples of 500 g of seeds were collected randomly to determine their initial infestation levels. Each sample was sieved with a 2.5 mm mesh sieve in order to separate insects from these seeds. Insects from each sample were places in flasks containing 70% (v/v) alcohol. Thereafter, they were subsequently counted by species under a manufacturer binocular microscope to determine their abundance. Insects were identified to species level by taxonomist at the International Institute of Tropical Agriculture (IITA), Benin or in Burkina Faso.

After sieving, three subsamples of 1000 seeds were removed randomly and sorted into undamaged (intact) and damaged (perforated) seeds. The weight of the undamaged grains (a); number of undamaged grains (b); weight of damaged (with holes) grains (c) and number of damaged grains (d) were determined. Percentage of perforated seeds was calculated as $[d/(b + d)] \times 100]$. Percentage weight loss was determined by the same count and weight method using the following formula [17] [18]:

$$\%\text{weightloss} = \left[\left(\left(a \times d\right) - \left(c \times b\right)\right) / \left(a \times \left(d + b\right)\right) \times 100\right]$$

Three subsamples of one hundred seeds randomly collected from each 500 g sample, were allowed to germinate in glass Petri dishes fixed with filter paper. Germination test was carried out for 14 days on a germination table at 35˚C. The percentage of germinated seeds was expressed as proportions that are the amount of germinated seeds of the total seeds × 100 [19].

The maximum quantity of seeds that can be stored in the 20-liter plastic jars is 17.5 kg. In order to standardize the different treatments, 17.5 kg of seeds were stored in PICS bags, plastic jars and control polypropylene bags, and each treatment replicated thrice. The opening of each jar was sealed using a transparent plastic bag before closing hermetically with its lid. The packaging of the seeds in the PICS bags was done following the procedure described in the training manual for technicians on the use of PICS bags [20]. All samples constituted were held on pallets in the Laboratory of the Environment and Forest Department of the Saria Research Station.

After six months of storage, the bags and jars were opened and the same parameters evaluated at the beginning of the study were determined again.

Data analysis: Data were checked for normality using Shapiro-Wilk test. When the data was normally distributed an Analysis of Variance (ANOVA) was used. Where the data was not normally distributed (for example variation in the perforation of seeds due to storage method) a non-parametric statistics Kruskal Wallis ANOVA was used. When the p-value was significant, means comparisons were made using the pairwise.wilcox.test function. Then, a linear analysis of variance was used to test the variation of seed germination, the number of insects per species and the percentage of seed weight losses according to storage methods. All tests were performed using R software version 3.4.3 (2017) at the probability level of 5%.

3. Results

3.1. Development of Insect Pests inside Plastic Jars, Polypropylene and PICS Bags

At the beginning of storage, two primary pests (*Bruchidius silaceus* and *Bruchidius* sp.) and three of their natural enemies (*Entedon omnivorus* Rasplus, *Eupelmus* sp., *Dinarmus magnus* Rohwer and *Anisopteromalus* sp.) were present. However, the seeds were heavily infested with *B. silaceus* among these insect pests (Table 1). After 6-month of storage, the average number of adults of these insect species found in 500 g of seeds stored in PICS bags and plastic jars, remained unchanged. In the polypropylene bags, the number of *B. silaceus* and *Bruchidius* sp. increased significantly from 235 ± 21.66 to 715 ± 104.10 (p = 0.00004858; df = 3; F = 37.066) for *B. silaceus* and from 5 ± 2.65 to 18.67 ± 8.02 (p = 0.00268; df = 3; F = 11.721) for *Bruchidius* sp. (Table 1). A significant appearance of *Caryedon furcatus*, a third primary pest in polypropylene bags, with

Table 1. Number of adult insect pests and parasitoids in 500 g of *A. macrostachya* seeds at the beginning and after six months of storage in plastic jars, PICS bags and polypropylene bags.

Insects species	Mean number of insects per 500 g grains of *A. macrostachya*				
	Initial number	After six (6) months of storage for each storage structure			Probability
		Plastic jars	PICS bags	Polypropylene bags	
Bruchidius silaceus Fahr.	235 ± 21.66^{B}	191 ± 63.22^{B}	239.67 ± 67.34^{B}	715 ± 104.10^{A}	P = 0.00004858; df = 3; F = 37.066
Bruchidius sp.	5 ± 2.65^{B}	0.67 ± 0.58^{B}	1.33 ± 0.58^{B}	18.67 ± 8.02^{A}	P = 0.00268; df = 3; F = 11.721
Caryedon furcatus Anton et Delobel	0.00 ± 0.00^{B}	3.33 ± 2.08^{A,B}	2.33 ± 0.58^{A,B}	7 ± 4.36^{A}	P = 0.04395; df = 3; F = 4.3005
Entedon nr *bruchivorus* Rasplus	1.00 ± 1.00^{A}	0.33 ± 0.33^{A}	0.00 ± 0.00^{A}	1.67 ± 1.15^{A}	P = 0.1375; df = 3; F = 2.4583
Entedon omnivorus Rasplus	1.67 ± 1.20^{A}	0.33 ± 0.33^{A}	0 .00± 0,00^{A}	4.00 ± 3.00^{A}	P = 0.102; df = 3; F = 2.8943
Eupelmus sp.	0.67 ± 0.58^{A}	0.67 ± 0.58^{A}	0.67 ± 0.58^{A}	0.33 ± 0.33^{A}	P = 0.8592; df = 3; F = 0.25
Dinarmus magnus Rohwer	1.00 ± 1.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	1.67 ± 1.53^{A}	P = 0.1433; df = 3; F = 2.4
Anisopteromalus sp.	0.67 ± 0.58^{A}	0.33 ± 0.33^{A}	1.00 ± 1.00^{A}	1.67 ± 1.53^{A}	P = 0.4521; df = 3; F = 0.9722
Dinarmus basalis Rondani	0.00 ± 0.00^{A}	0.67 ± 0.58^{A}	0.00 ± 0.00^{A}	0.33 ± 0.33^{A}	P = 0.2192; df = 3; F = 1.8333
Oryzaephilus mercator Fauvel	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	1.67 ± 1.20^{A}	P = 0.2044; df = 3; F = 1.9231

Within the same line, means values (±standard deviation) followed by the same letters are not significantly different.

an average number of 7 ± 4.36 (p = 0.04395; df = 3; F = 4.3005) was also observed. But there was no significant difference in the number of *C. furcatus* at start and from 500 g of seeds stored in PICS bags and plastic jars (**Table 1**). *Oryzaephilus mercator* Fauvel, a secondary pest also appeared in polypropylene bags after 6-months storage period but with a low infestation level of 1.67 ± 1.20 (p = 0.2044; df = 3; F = 1.9231). In addition, five parasitoids (*Entedon* nr *bruchivorus* Rasplus; *Entedon omnivorus* Rasplus, *Eupelmus* sp., *Dinarmus magnus* Rohwer and *Anisopteromalus* sp.) were found at the beginning and after the storage period.

3.2. Seed Perforation and Weight Loss

At the beginning of storage, rates of seeds perforation and weight loss were 7.40% ± 1.05% and 0.50% ± 0.32% respectively (**Table 2**). At the end of storage, the percentage of seeds with perforation and weight loss in PICS bags and plastic jars remained low at baseline levels (**Table 2**). In contrast, *A. macrostachya* seeds stored in polypropylene bags were riddled with emergence hole. The rate of seeds perforation increased significantly, reaching 20.23% ± 5.10% after sixth months (p = 0.0005718; df = 3; F = 18.649). Likewise, weights of seeds stored in polypropylene bags were 6 times lower than at the beginning of the experiments (**Table 2**).

3.3. Germination of *A. macrostachya* Grains Stored during Six Months in Plastic Jars, Polypropylene and PICS Bags

Prior to storage, the germination rate of seeds was 26.67% ± 3.06% (**Figure 1**). After six months of storage, the viability of seeds stored in all storage containers was affected significantly. Thus, percentage germination of seeds stored in PICS bags, polypropylene bags and Plastic jars dropped to 14% ± 6.56%, 11.33% ± 3.06% and 15.33% ± 4.04% respectively (p = 0.01239; df = 3; F = 7.037).

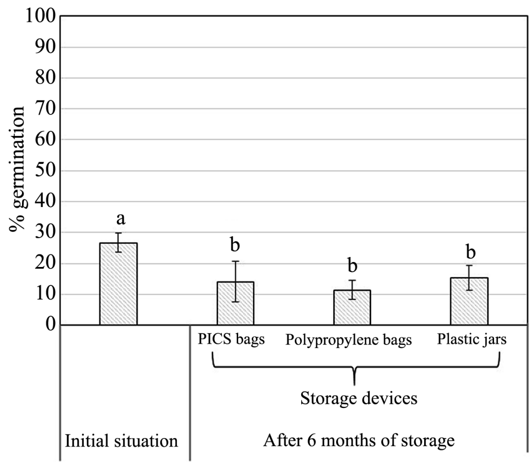

Figure 1. Percentage germination rate (mean ± SE) of *A. macrostachya* seeds at the beginning (start of experiment) and after six months of storage in PICS bags, polypropylene bags and plastic jars. (Means with different alphabetic letters are significantly different according to ANOVA Linear Model test, a = 0.05).

Table 2. Rates of perforation and weight loss of *A. macrostachya* grain at the beginning and after six months of storage in plastic jars, polypropylene and PICS bags.

Storage period and devices	Seeds perforation (%)	Weight loss
Begining of storage	7.40 ± 1.05^B	0.50 ± 0.32^B
Plastic jars	9.53 ± 1.19^B	1.47 ± 0.30^B
PICS bags	6.93 ± 1.92^B	1.11 ± 0.24^B
Polypropylene bags	20.23 ± 5.10^A	3.00 ± 0.69^A
Probabilities	$P = 0.04344$; df = 3; $X^2 = 8.1282$	$P = 0.0005718$; df = 3; $F = 18.649$

Within the same column, means values (±standard deviation) followed by the same letter are not significantly different.

4. Discussion

Our results have shown that plastic jars and PICS bags can be used to effectively store *A. macrostachya* seed grains showing no differences in their efficiencies in comparison to the control. Hermetic storage also showed promising results in the preservation of seeds aesthetics, protecting seeds from stored pests and weight losses but not seeds viability.

[21] noted that most Bruchinae species are mostly associated with legumes, particularly those belonging to Fabaceae family. In the case of *A. macrostachya* seeds, the significant abundance of *Bruchidius silaceus* and *C. furcatus* in polypropylene bags after six months of storage compared to the beginning of storage confirms their status as seeds pests, as reported by [8] [9] [22]. The higher abundance of *B. silaceus* at the beginning of storage compared to *C. furcatus* could be explained by the harvest period which usually occurs in the last fortnight of November. A similar observation was made by [23] on the seeds of *Acacia raddiana*. The seeds of this legume harvested between November and March were heavily infested by *Bruchidius aurivillii* than by *Caryedon longispinosus*. However, when seeds were harvested between April and May, insects were less abundant, displaying an opposite trend [23]. Our results showed that, proper storage of *A. macrostachya* seeds under hermetic conditions in PICS bags and plastic jars prevents the deterioration of seeds (reduced weight loss and seed perforation) by bruchids. The effectiveness of such hermetic containers has been demonstrated by [13] on Bambara groundnut and [11] on cowpea. According to [24], hermetic storage inhibits the development of insects thus preventing damages such as weight loss. Indeed, in airtight storage structures, after a certain time, the oxygen which is present gets used up by the pests themselves and by the respiration of seeds [25]. When the oxygen level becomes sufficiently low, the insects die due to asphyxia [26]. However, the biological activity of the seeds releases metabolic water and CO_2 according to the equation $C_6H_{12}O_6 + 6O_2$ g $6H_2O + 6CO_2$ + energy [27] [28]. Water from oxidation of carbohydrates is the major source of water for those insects that live in extremely dry environment [27]. The respiratory activity of the seeds is limited in hermetic containers, this reduces the quantity of water released from metabolism by these seeds. Thus,

these insects eventually die by desiccation [27]. The proliferation of different primary pests and the appearance of *C. furcatus* in polypropylene bags after six months storage, resulted in a significant increase in the percentage of weight loss and the rate of perforated seeds, by six and three folds respectively. Because of their porosity, polypropylene bags allow the renewal of air they contain via circulation and exchange with the environment, thus promoting the development and survival of insects [29].

The average germination rate of 27% obtained at the beginning of the experiment is relatively low. This could be explained by the high level of seed infestation at the beginning of storage. [30] reported that the germination ability of *Acacia gerrardii* was reduced significantly by bruchid larva infestation. Thus, the germination rate of *A. gerrardii* healthy seeds was 29.6%, whereas it drops to 7.2% when these seeds are infested. Previously, [31] showed that the germination rate of *Acacia macrostachya* seeds from Laba and Tiogo (two localities in Central West Burkina Faso), was respectively 96% and 95% when they were not infested with insect larvae; these rates dropped to 30% and 27% respectively upon infestation with two insect larvae. An average of 7.40% of seeds was also perforated, which could have a negative impact on their germination. A similar study carried out on *Acacia tortilis* showed that the germination rate was null when the seeds had at least one emergence hole [32]. After six months of storage, the germination rate dropped significantly in polypropylene bags as well as in plastic jars and PICS bags. Earlier on [14] showed the germination rate of mung beans and pigeonpea seeds dropped after six months of storage in PICS bags, but contrary to our results, this drop was marginal. The loss of seeds viability could be explained by an increase on temperature and humidity inside these hermetic structures for a long time [33] [34]. Perhaps, reduced storage period could be an option to preserve seed viability as efforts of domestication and nursery development are on the way pending exploration of other storage methods.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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