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COMPARATIVE EVALUATION OF SIX DIFFERENT STORAGE MATERIALS FOR LONG-TERM PRESERVATION OF HUCKLEBERRY (*Solanum scabrum*) SEEDS

Maurice Njiandoh Mbeboh[1+]
Emmanuel Youmbi[2]
William Armand Mala[3]
Gabriel Ambroise Manga[4]
Amos Funamo Ngwa[5]
Sylvestre Medard Badjel Badjel[6]
Christopher Ngosong[7]

[1]*Department of Plant Biology and Physiology, Faculty of Science, University of Yaounde 1 and Department of Agronomic and Applied Molecular Sciences, Faculty of Agriculture and Veterinary Medicine, University of Buea, Buea, South West Region, Cameroon.*
[1]*Email: ndeselmaurice@gmail.com Tel: (+237)676776456*
[2,3]*Department of Plant Biology and Physiology, Faculty of Science, University of Yaounde 1, Yaounde, Centre Region, Cameroon.*
[2]*Email: youmbi-davidemmanuel@yahoo.fr Tel: (+237)677547311*
[3]*Email: williammala@yahoo.fr Tel: (+237) 699321873*
[4,5,6]*Institute of Agricultural Research for Development (IRAD), Njombe, Littoral Region, Cameroon.*
[4]*Email: mangagabriel@hotmail.com Tel: (+237)699916061*
[5]*Email: ngwa.amos@yahoo.fr Tel: (+237)677567869*
[6]*Email: badjelsylvestre@outlook.fr Tel: (+237)699827375*
[7]*Department of Agronomic and Applied Molecular Sciences, Faculty of Agriculture and Veterinary Medicine, University of Buea, and Leibniz Institute of Vegetable and Ornamental Crops, Großbeeren, Germany.*
[7]*Email: ngosong.christopher@ubuea.cm Tel: (+237)678671071*


(+ Corresponding author)

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ABSTRACT

Smallholder farmers in Cameroon often store seeds from their own-harvest for use in subsequent planting seasons, but they have limited information on appropriate materials for long-term storage of vegetable seeds. Hence, six different materials (sealed aluminium foil sachet, sealed glass bottle, closed plastic cup, sealed paper sachet, sealed polythene sachet, and open-and-seal glass bottle) were evaluated for their effectiveness as storage materials for huckleberry seeds. Factorial ANOVA revealed significant ($P < 0.001$) effect of the different types of storage materials, duration of seed storage, and their interactions on laboratory germination, field emergence, and percentage transplantable see dlings. Significant ($P < 0.05$) variations were observed for laboratory seed germination and field emergence from months 2–5 of preservation across the different storage materials, with the highest performance exhibited by sealed aluminium foil sachet, while glass material exhibited the lowest performance. These performances decreased significantly ($P < 0.05$) for each storage material across months of long-term storage, with the lowest decrease recorded in sealed aluminium foil sachet and highest decrease recorded in glass bottle storage materials. Percentage transplantable seedlings decreased significantly ($P < 0.05$) from month 0–5 for each storage material, with lowest decrease in sealed aluminium foil sachet and highest decrease in open-and-seal glass bottle. Overall, these results demonstrate that sealed aluminium foil sachet is the most effective storage material for long-term preservation of huckleberry seeds under ambient conditions of the study area, which can be adopted by farmers.

Contribution/Originality: This study is one of very few studies that have investigated different storage materials for long-term preservation of huckleberry seeds in Cameroon, and demonstrated that sealed aluminium foil sachet is the most effective under ambient conditions in the study area, which can be adopted by smallholder farmers.

1. INTRODUCTION

Indigenous African leafy vegetables such as huckleberry (*Solanum spp.*) are important for food and nutrition security, and various methods of cultivation and processing are known to influence their nutritional value and health-promoting potential (Fontem *et al.*, 2013; Grubben *et al.*, 2014; Pousseu *et al.*, 2014; Odongo *et al.*, 2018). Obtaining viable seeds has emerged as a major challenge for most smallholder farmers, as it has become imperative to use improved seeds to boost productivity (Tscharntke *et al.*, 2012; Bishaw and Atilaw, 2016; Abebe and Alemu, 2017). Seed systems constitute both formal and informal sectors and an integrated system where farmers adopt improved varieties from the formal sector, and informally save seeds from own-harvest for subsequent use (Zewdie and Van Gastel, 2008; Sperling and McGuire, 2010; Meselu, 2019). However, saving seeds over long duration may affect viability that might eventually affect seed establishment, crop growth and productivity (Baoua *et al.*, 2012). Accordingly, various storage materials (e.g. aluminium, paper, and plastic) have been tested for seed preservation across varying durations (Oladiran and Agunbiade, 2010; Narayanan *et al.*, 2012; Kalsa *et al.*, 2019). Furthermore, storage techniques such as the use of hermetic bags, metal silos, inert dust, and botanicals are recommended against storage pest and diseases (Demissie *et al.*, 2008; Gitonga *et al.*, 2013; Guenha *et al.*, 2014; Martin *et al.*, 2015). Various fungal species are associated with vegetable seeds and 19 fungi were recently reported on huckleberry seeds in Cameroon that affect germination (Chowdhury *et al.*, 2005; Tsopmbeng and Fomengia, 2015).

Huckleberry is one of the most cultivated indigenous African leafy vegetables in this study site (Njombe-Cameroon), where farmers commonly use glass bottles to informally store large number of seeds and progressively remove the required quantity at each planting time, which causes rapid loss of seed viability. Notwithstanding, informal seed systems are often the sole source of seeds for neglected and underutilised crop species that are critical for providing majority of essential nutrients to smallholder farming communities (Badstue *et al.*, 2006). By preserving locally adapted varieties through the informal seed system, smallholder farmers are able to improve the existing gene pool or create and maintain diversity, which reduces production risks (Thrupp, 2000; Dorward *et al.*, 2007; Jackson *et al.*, 2007; Nuijten and Van Treuren, 2007; McGuire and Sperling, 2016). However, the longevity, vigour and viability of stored seeds depend on a combination of abiotic (e.g. moisture, temperature, and humidity) and biotic (e.g. pest, pathogens, and damages to nucleic acids and proteins) factors (Ellis *et al.*, 1982; Fujikura and Karssen, 1995; McDonald, 1999; Kiani *et al.*, 2013; Shaban, 2013). Ultimately, the type of storage materials used and duration of seed storage can influence both biotic and abiotic factors, which may eventually reduce the performance of stored seeds. Hence, there is renewed emphasis to transform and strengthen Africa's informal seed systems by incorporating quality germplasm and storage methods that provide additional options to enhance food security (Scoones and Thompson, 2011). Therefore, this study focuses on finding simple and affordable methods of preserving huckleberry seeds by evaluating the effectiveness of six different storage materials as a potential strategy for long-term seed preservation. It was hypothesised that open-and-seal glass bottle storage is less effective, while the sealed aluminium foil sachet is the most effective alternative storage material for preserving huckleberry seeds under ambient conditions of the study area.

2. MATERIALS AND METHODS

2.1. Experimental Site and Setup

This study was conducted at the Institute of Agricultural Research for Development (IRAD) in Njombe, in the Littoral Region of Cameroon. The site is situated at 80m above sea level, and latitude of 4°3–4°8 North and longitude 9°3–9°8 East. It lies at the foot slopes of Kupe Manengumba mountain ridge, comprising fertile sedimentary and volcanic soils. It has an equatorial climate with temperature ranging from 23–35°C with 27.1°C annual mean, and average annual rainfall of 2086 mm, with 72% relative humidity. The rainy season starts from March to November with July to October being the wettest months of the year. Experiments were set up as a two-factorial (e.g. storage materials and duration of storage) design for both laboratory and field trials. Germination

tests were conducted for six different storage materials across five months of this study. After packaging, seeds used for the first test were placed in the respective storage materials, immediately removed without storage and considered as control (Month–0).

2.2. Seed Storage

After harvest, seeds of the Ekona variety of huckleberry were extracted from randomly collected fruits at the experimental fields of the Institute of Agricultural Research for development (IRAD) Njombe. Mature fruits that turned purple/black were harvested and put in a permeable bag and fermented for 48hrs in containers filled with tap water. The fermented fruits were hand-crushed, placed in open plastic container filled with tap water and thoroughly stirred. Floating fruit peels and gels were removed by gently tilting the container, and the process was repeated ten times until all fruit peels and gels were completely removed, while valuable seeds remained at the bottom. The clean seeds were wrapped and gently squeezed in cotton cloth, placed on ceiling board and put on a flat surface table in well-ventilated room for 7 days at room temperature (about 27°C). Seed clumps were stirred 2–3 times per day and the dry seeds were handpicked using hand-held lens. The seeds were stored in six different storage materials (sealed aluminium foil sachet, sealed glass bottle, closed plastic cup, sealed paper sachet, sealed polythene sachet, and open-and-seal glass bottle). Each storage material contained six samples, except the open-and-seal glass bottle that had only one sample that was systematically opened and closed at each planting date across the five-month experimental period. One thousand seeds were put in each sample of different storage materials and sealed, and one sample of each storage material was used and discarded after every 30 days, for both laboratory and field tests.

2.3. Laboratory Tests

There were six treatments and three repetitions in the laboratory, and a total of 18 petri dishes (14 cm) were used after every 30 days. Petri dishes were sterilised using bleach (La Croix: National Beverage Corp., USA), one hundred seeds were put in each and 2 ml water added using a syringe. The seed germination was monitored daily across 7 days under laboratory conditions and the procedure repeated after 30 days, giving a total of six trials during a period of 6 months.

2.4. Field Trials

The field experiment was laid out as a randomised complete block design (RCBD) on a 40 m^2 surface area that was cleared and tilled, and partitioned into three replicate blocks, each sub-divided into six treatment plots of 1.3×1 m dimensions. Treatment plots within replicates were separated by 40 cm buffer zone while 50 cm buffer separated replicate blocks from each other. The site was sterilised by spraying with a mixture of 35 g fungicide Penncozeb (Cerexagri Inc., Netherlands) and 25 ml insecticide Dursban (Dow Elanco, USA) dissolved in 11 L water, and allowed for 7 days after sterilisation before sowing. One hundred seeds were sown on four lines in each plot, with 25 seeds per sowing line, and each plot was covered with 250 g of grass mulch for 3 days after sowing. The field site was regularly monitored for weed emergence and manually weeded every seven days using a hoe. The molluscicide Metarex (Villa Ltd, South Africa) was applied at the edges of the experimental field to protect the plants from molluscs (snails, millipedes, and centipedes). The insecticide Cypercal (FMC CORP. USA) was applied in the second and third weeks after planting. The experiment was repeated after every 30 days, giving a total of six experimental trials established during a period of 5 months.

2.5. Data Collection and Analysis

Laboratory seed germination and field emergence were monitored over fifteen days, but seed germination was recorded every 2 days and ended after 7 days, while the number of seed emergence was recorded every 3 days and

ended after 9 days. The number of transplantable seedlings was recorded every 21 days after sowing for plants measuring 6 cm height and above. Data ere recorded as the number of germinated, emerged and transplantable plants per hundred seeds and presented as percentage. All data sets were analyzed using the statistical software package (Statsoft, 2016). Factorial analysis of variance (ANOVA) was performed to determine the influence of storage materials and duration of storage, as well as their interaction on the germination, emergence and transplantable seedlings. Following the observed significances, one-way ANOVA was performed separately for effect of storage materials ($n = 6$) and duration of seed storage (number of months; $n = 6$). Significant data means were compared using Duncan's Multiple Range Test ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Effect of Storage Materials and Duration on Seed Germination

Generally, long-term storability of seeds can be affected by pre-treatment and handling practices that ensure reduction of biochemical activities in relation to moisture content and temperature (Vertucci, 1989). This is consistent with the laboratory germination rate of huckleberry seed that differed significantly ($P < 0.001$) between storage materials, duration of seed storage, and their interaction. Significant ($P < 0.05$) variations of seed germination for the different storage materials were observed from months 2–5 of seed storage Table 1. Generally, the sealed aluminium foil sachet storage material demonstrated highest performance while glass materials had the lowest. Also, seed germination rate for each storage material decreased across months, with 100% germination at month–0 for all storage materials, which decreased significantly at month–5 with the highest in glass bottle storage materials and lowest in sealed aluminium foil sachet Table 1. Considering that all stored seeds received the same post-harvest treatments (Daniels *et al*., 1998; Pearce *et al*., 2001) the observed variations in seed performance are likely influenced by the type of storage materials used and duration of storage (Kandil *et al*., 2013). This may have affected seed viability and vigour by altering moisture or humidity conditions and biochemical activities in the stored seeds (Vertucci, 1989; Mettananda *et al*., 2001; Segnou *et al*., 2012). It has been reported that less/not permeable storage materials likely increased humidity and enhanced seed deterioration (Mbogne *et al*., 2015). Joao Abba and Lovato (1999) also recommended long-term seed storage in waterproof materials, but suggested that constantly opening and closing such materials would render them ineffective. This situation might have influenced the performance of open-and-seal glass bottle storage in this study by altering the moisture and humidity contents in the storage bottles (Ellis and Hong, 2007).

Table-1. Percentage laboratory germination (% ± SD) of huckleberry seeds after seven days for each seed storage material and across 0–5 months of storage.

Seed storage materials	Duration of seed storage (months)					
	0	1	2	3	4	5
Sealed aluminium foil sachet	100.0 ± 0.0aA	100.0 ± 0.0aA	97.7 ± 4.0aA	90.0 ± 1.0aB	80.0 ± 1.0aC	70.0 ± 1.0aD
Sealed glass bottle	100.0 ± 0.0aA	98.7 ± 1.5aA	88.0 ± 7.2bB	78.0 ± 7.2bBC	68.0 ± 7.2bC	48.0 ± 7.2cD
Closed plastic cup	100.0 ± 0.0aA	98.7 ± 2.3aA	95.3 ± 4.2aA	85.3 ± 4.2aB	75.3 ± 4.2aC	55.0 ± 3.6bD
Sealed paper sachet	100.0 ± 0.0aA	96.7 ± 2.1aA	86.0 ± 1.7bB	86.7 ± 2.1aB	76.7 ± 2.1aC	54.7 ± 2.1bD
Sealed polythene sachet	100.0 ± 0.0aA	98.7 ± 2.3aA	96.3 ± 4.0aA	86.3 ± 3.8aB	76.3 ± 3.8aC	55.7 ± 3.2bD
Open-and-seal glass bottle	100.0 ± 0.0aA	97.7 ± 1.5aB	86.7 ± 2.1bC	76.0 ± 1.0bD	66.0 ± 1.0bE	46.0 ± 1.0cF

Values within columns with different lower case letters are significantly different while values within rows with different upper case letters are significantly different ($P < 0.05$).

Although the use of storage materials with same properties (e.g. glass bottles) are expected to exhibit similar performance on stored seeds, constant opening and closure of the open-and-seal glass bottle might have altered storage conditions (e.g. temperature, moisture, and humidity) and increased pest and pathogenic intrusion, which probably caused rapid loss of seed viability as compared to the closed glass bottle. In sum, the aluminium foil sachet storage material demonstrated the highest performance while open-and-seal glass bottle was the lowest, which supports the hypothesis of this study. Likely, the aluminium foil sachet maintained a favourable abiotic environment that limited the development of pathogens within the stored seeds.

3.2. Storage Materials and Duration Affect Seedling Emergence and Performance

The seedling emergence rate of huckleberry differed significantly ($P < 0.001$) between storage materials, duration of seed storage, and their interaction. Significant ($P < 0.05$) variations in seedling emergence were observed for the different storage materials at months 2–5 of seed storage Table 2. A similar trend was observed for field seed emergence and laboratory seed germination, as the sealed aluminium foil sachet storage material demonstrated highest performance while the open-and-seal glass bottle was the lowest. Also, seedling emergence for each storage material decreased significantly ($P < 0.05$) from month 0–5, with the lowest decrease in sealed aluminium foil sachet and highest decrease in open-and-seal glass bottle storage materials Table 2. Transplantable huckleberry seedlings differed significantly ($P < 0.001$) between storage materials, duration of seed storage, and their interaction. Significant ($P < 0.05$) variations in transplantable seedlings were observed for the different storage materials from months 2–5 of seed storage Table 3. Sealed aluminium foil sachet storage demonstrated highest performance while open-and-seal glass bottle was the lowest. Transplantable seedlings decreased significantly ($P < 0.05$) for each storage material from months 0–5, with the lowest decrease in sealed aluminium foil sachet and highest decrease in open-and-seal glass bottle storage materials Table 3. The decrease in germination and emergence at six months storage could be related to senescence that occurs with the loss of physiological efficiency that can be retarded by appropriate storage materials but might not be completely avoided (Segnou *et al*., 2012). Aluminium foil storage had the highest germination and emergence rates above 70% across all the investigated months, which is consistent with the standard germination rate set by the International Seed Testing Association for leafy vegetable seeds. Contrastingly, all the other storage materials had less than 70% germination rate with glass materials demonstrating the lowest performances. Moreover, the open-and-seal glass bottle storage demonstrated the lowest seed germination and emergence rates over time, while both glass bottle storage methods showed a similar trend for seed germination and emergence after five months of storage. Frequent opening of the open-and-seal storage bottle probably caused the observed difference with sealed glass bottles, since materials of the same properties are expected to exhibit similar performance after long-term storage under the same environmental conditions (Joao Abba and Lovato, 1999; Ellis and Hong, 2007). The variations between laboratory germination and field emergence rates are probably due to biotic and abiotic factors in the field compared to controlled laboratory conditions. While results of laboratory germination tests are often invariably higher, they are lower under field conditions. This discrepancy could be due to confounding abiotic (e.g. soil moisture variability, unsatisfactory temperatures, and humidity, insufficient light, evaporation, etc.) and biotic (e.g. predation, pathogens, and damages at nucleic acids and protein levels) factors (Ellis *et al*., 1982; Fujikura and Karssen, 1995; McDonald, 1999; Kiani *et al*., 2013; Shaban, 2013). The variations in germination and emergence rates between different storage materials are consistent with the induced changes in storage environment that affect the internal biochemistry, viability and vigour of seeds after long-term storage. Meanwhile, the slight difference between seedling emergence rate and the percentage of transplantable seedling reflects the vigorous nature of the germinated seedlings for all storage materials. In sum, a combination of type of storage material and duration of storage may have caused seed deterioration due to lipid peroxidation that could lead to subsequent loss of seed viability and vigour (Shelar *et al*., 2008; Tatić *et al*., 2012). The use of improper materials for long-term seed storage may cause fungal development,

which might reduce seed viability and vigour by consuming valuable food reserves in the endosperm that are required by the embryo during germination (Devi *et al.*, 2005). Overall, confounding biotic factors such as storage pests and diseases (Jamadar *et al.*, 2001; Hamim *et al.*, 2014; Tsopmbeng and Fomengia, 2015) and abiotic factors such as varying environmental conditions (Ellis and Hong, 2007; Mbogne *et al.*, 2015) surrounding the stored huckleberry seeds may have affected their viability and vigour, which influenced the seed performance.

Table-2. Percentage field emergence (% ± SD) of huckleberry seedlings at nine days after nursery planting for each seed storage material and across 0–5 months of storage.

Seed storage materials	Duration of seed storage (months)					
	0	1	2	3	4	5
Sealed aluminium foil sachet	100.0 ± 0.0aA	95.3 ± 1.3aB	90.7 ± 1.8aC	85.7 ± 1.8aD	80.3 ± 1.3aE	70.3 ± 1.3aF
Sealed glass bottle	100.0 ± 0.0aA	88.3 ± 1.3abB	78.7 ± 1.8bC	71.3 ± 1.3cD	61.3 ± 1.3bcE	49.0 ± 0.9eF
Closed plastic cup	98.0 ± 0.0aA	91.3 ± 1.0aB	81.0 ± 0.9bC	74.0 ± 0.9bcD	64.0 ± 0.9bcE	51.3 ± 1.8dF
Sealed paper sachet	100.0 ± 0.0aA	90.0 ± 4.3abB	84.7 ± 3.9abC	77.7 ± 3.9bD	66.3 ± 3.0bE	56.3 ± 3.0bF
Sealed polythene sachet	100.0 ± 0.0aA	82.7 ± 5.8bB	78.3 ± 4.8bC	72.0 ± 3.8bcD	62.0 ± 3.8bcE	53.3 ± 1.8cF
Open-and-seal glass bottle	99.0 ± 0.0aA	91.0 ± 2.3aB	79.7 ± 1.8bC	72.3 ± 1.3bcD	60.7 ± 1.0cE	40.3 ± 1.3fF

Values within columns with different lower case letters are significantly different while values within rows with different upper case letters are significantly different ($P < 0.05$).

Table-3. Percentage transplantable (% ± SD) huckleberry seedlings after 21 days in the nursery for each seed storage material and across 0–5 months of storage.

Seed storage materials	Duration of seed storage (months)					
	0	1	2	3	4	5
Sealed aluminium foil sachet	95.0 ± 0.0aA	87.7 ± 2.5aB	85.7 ± 2.1aA	80.7 ± 2.1aC	75.3 ± 1.5aD	65.7 ± 2.1aE
Sealed glass bottle	95.0 ± 0.0aA	79.7 ± 2.1aB	70.7 ± 2.1bC	64.3 ± 1.5cD	54.3 ± 1.5cE	42.0 ± 1.0dF
Closed plastic cup	93.0 ± 0.0aA	81.0 ± 4.0aB	72.3 ± 2.1bC	67.3 ± 0.6bcD	57.3 ± 0.6bcE	43.7 ± 1.5cdF
Sealed paper sachet	95.0 ± 0.0aA	77.0 ± 5.3aB	72.3 ± 4.0bBC	70.0 ± 3.6bC	59.0 ± 3.0bD	49.3 ± 3.5bE
Sealed polythene sachet	95.0 ± 0.0aA	76.3 ± 6.5aB	71.3 ± 3.5bB	65.0 ± 3.0cC	55.7 ± 3.2bcD	46.3 ± 1.2bcE
Open-and-seal glass bottle	94.0 ± 0.0aA	80.0 ± 3.0aB	70.0 ± 2.0bC	64.0 ± 1.0cD	53.3 ± 1.5cE	33.3 ± 1.5eF

Values within columns with different lower case letters are significantly different while values within rows with different upper case letters are significantly different ($P < 0.05$).

4. CONCLUSION

Overall, sealed aluminium foil sachet is the most effective material for long-term storage of huckleberry seeds under ambient conditions of the study area, which allows seeds to be kept for longer duration. In the absence of viable seed systems, it can be recommended for local smallholder farmers to use aluminium foil sachets for best quality preservation of huckleberry seeds over six months that meets their specific needs. This is commensurate with the renewed emphasis to transform and strengthen the informal seed sector in Africa within a uniquely African Green Revolution that provides additional options for farmers to mitigate crop production shocks and enhance productivity, in order to ensure food and nutrition security.

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