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EFFECTS OF GERMPLASM ACCESSIONS AND PACKAGING MATERIALS ON THE QUALITY CHARACTERISTICS OF ROSELLE SEEDS STORED UNDER AMBIENT CONDITION

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ABSTRACT
Globally, the nutritional and economic importance of the Roselle plant cannot be underestimated. One of the major challenges that come with the conservation of the seeds is the rapid loss of seed quality due to the use of inappropriate packaging materials. This study was conducted to determine the effects of accessions and storage packaging materials on the quality characteristics of the Roselle seeds stored for 12 months. A 12 x 5 factorial arrangement in Completely Randomized Design (CRD) with three replications was used. The first factor was accessions at twelve levels (HS08, HS11, HS19, HS25, HS27, HS32, HS41, HS58, HS59, HS69, HS83, H86). The second factor was storage packaging materials at five levels (paper bag, ziplock bag, pot, plastic bottle and no packaging). The study revealed that storage packaging significantly affected the physiological and biochemical properties of the roselle seeds such that seeds which were packaged in bottles and ziplock bags performed better as compared to the other packaging materials (paper, pot) and the unpackaged seeds. Seeds stored in the bottle and ziplock led to high percentage germination and seed vigour. Seeds of accession HS08 packaged in either bottle and ziplock bag had the highest total phenolic content as well as the highest antioxidant capacity. Pathogenic fungi found on the seeds in the various packaging materials were least in the bottle and ziplock bag. In conclusion, for a long-term conservation of roselle seeds it is imperative to use bottles and ziplock bags.

Keywords: antioxidants, conservation, deterioration, nutrition, orthodox, pathogenic.

INTRODUCTION

Roselle (*Hubiscus isabdarrifa*), is an edible wild plant which is an important source of dietary nutrients and helps with the proper growth, development and functioning of the body. It is an annual shrub of the Malvaceae family whose origin is believed to be tropical Africa or Asia. According to Salami and Afolayan (2021), the younger herbages of roselle are sometimes eaten raw in salads whilst its young shoots, tender calyces and immature fruits are diced for making stews. According to Ansari *et al*. (2013), red calyces of roselle are used to prepare relishing drink known as Zoborodo in Nigeria, Bissap (Senegal) and Karkade (Saudi Arabia), while the green calyces are commonly used in the preparation of stews, soups and sauces. In addition to its use as food, various parts of roselle have been utilized in traditional medicine for the prevention of diseases such as hypertension, diabetics, obesity and cancer (Ashaye, 2013). Roselle seed oil is also used for cooking, producing cosmetics and soap making while the residue is used for feeding livestock and poultry. Countries such as India, Kenya, Russia, Nigeria, Italy, Sudan, Cote D'Ivoire and Southeast Asia have received substantial wealth from the bast fiber produced from Roselle (Mwasiagi *et al*., 2014).

In spite of the many economic uses of the plant, there is rapid loss of viability of Roselle seeds during storage which compromises subsequent production of the crop for sustainable food and nutrition security. In addition, this challenge also poses a very serious threat to germplasm conservation. Long-term conservation of seeds of plant genetic resources is of key importance for food security and preservation of agro-biodiversity (Guzzon *et al.*,2021). For good and quality storage of germplasm, the packaging material used is also considered as one of the most important factors influencing viability and longevity of seeds in such storage (Ali *et al.*, 2018). Certain types of packaging material may accelerate the exchange of energy and mass between the stored seeds and the storage environment leading to deterioration, reduced vigor and loss of viability of the stored seeds (Moreano *et al.*, 2018). Tandoh *et al.* (2017) reported that storage of *Pericopsis elata* seeds in airtight plastic bottles resulted in high viability of the seeds, whilst soybean seeds stored in glass jars also led to enhanced seed germination (Ali *et al.*, 2018). For Roselle however, there is a dearth of information on how different packaging materials affect the quality seed characteristics. The objective of this study therefore was to determine the effects of storage packaging materials and germplasm accessions on the physiological, biochemical and health quality characteristics of Roselle seeds stored for 12 months.

MATERIALS AND METHODS

Source of germplasm accessions

Seeds of twelve accessions of Roselle plant were produced on the research fields at the Department of Horticulture, Kumasi, Ghana between May-November, 2020 and were harvested at the physiological matured stage with moisture content of 10%. The seeds were dried to an equilibrium moisture content of 8% using sun drying prior to the commencement of the storage experiment.

Experimental Design

The experiment was set up in a 12 x 5 factorial arrangement in Completely Randomized Design (CRD) with three replications. The first factor was accessions at twelve levels (HS08, HS11, HS19, HS25, HS27, HS32, HS41, HS58, HS59, HS69, HS83, H86). The second factor was storage packaging materials at five levels (paper bag, ziplock bag, pot, plastic bottle and no packaging).

Description of packaging materials

Paper: This was a brown A5 paper bag with dimension of 105 mm x 150 mm. The paper bag had a thickness of 0.065 mm and weighed 70 grams.

Pot: This was a clay pot with dimensions of 35 cm in height and 20 cm in diameter. The pot had a thickness of 6.58 mm and weighed 600 grams.

Ziplock bag: Plain polythene ziplock bag with dimensions of 100 mm x 120 mm and a thickness of 0.055 mm.

Plastic bottle: Plain polypropylene plastic bottle with measurements of 45 cm x 15 cm and an airtight lid. The thickness of the container was 0.508 mm.

Storage procedure

Five hundred (500) grams from the pure seed fraction of each accession was weighed and put into each of the packaging materials. The control was not packaged but kept on wooden table. Each treatment was replicated three times. Packaged seeds were stored for 12 months (November, 2019 to October, 2020) under ambient conditions in a room with a temperature range of 25.22^{0}C- 29.45^{0}C.

Data Collected

Data collected were: Temperature, Relative Humidity of stor age room, Thousand seed weight, germination percentage, Seedling Vigour Index, Seed Vigour, Moisture Content, Crude Fat and Protein, Total Phenolic, Antioxidant Capacity and Seed Fungal Presence and Load. Data was collected at the end of the twelfth month.

Temperature and Relative Humidity of Storage Room

The prevailing temperature and relative humidity readings of the storage room were taken at 09:00 hours, 12:00 hours and 18.00 hours using an indoor digital data logger (The LogTag® UTRID-16).

Thousand Seed Weight

One thousand (1000) seed weight was determined by counting out at random, eight replicates of 100 seeds each from the pure seed fraction. Each replicate was weighed with an analytical balance and the weight recorded. The mean weight of the eight replicates was computed and multiplied by 10 to give the 1000 seed weight for each treatment (ISTA, 2007)

Seed Germination Percentage

Four hundred (400) seeds from the pure seed fraction of each treatment were used to conduct the germination test according to the methods of ISTA (2007).

Seedling Vigour Index

At two weeks after germination, the shoot length and root length of 10 seedlings from each treatment were measured using a metre rule, for each of the three replicates and the average computed. The seedling vigour index was calculated using the formula of Abdul-Baki and Anderson (1973) as follows:

Vigour Index = (Shoot length + Root length) x Germination Percentage

Seed Vigour

The electrical conductivity test was used in determining the vigour of the seeds according to the methods of (ISTA, 2007) and (Milošević *et al*., 2010).

Seed Moisture Content

The Low Constant Temperature Oven method was used to determine the moisture content of the seeds (AOAC, 2007) using this formula and expressed as a percentage (%).

$$\%\ \text{seed moisture content} = \frac{(\text{weight of wet sample} - \text{weight of dry sample}) \times 100}{\text{weight of wet sample}}$$

Seed Crude Fat and Protein Content

The seed crude fat and protein contents from each treatment was determined using the Soxhlet Extraction and Kjeldahl Method respectively, according to the procedures outlined in Jones (1991). The percentage crude fat was computed using the formula;

$$\% \text{ Crude Fat} = \frac{\text{weight of fat}}{\text{weight of sample}} \times 100$$

Seed Total Phenolic Content

Total phenolic content (TPC) of the seeds was determined in accordance with the Folin-Ciocalteau method (Singleton *et al.*, 1999), using garlic acid as a standard. Each treatment sample of 0.5 g weight was dissolved in 100 ml of distilled water in a 100 ml volumetric flask. The mixture was swirled for 30 mins and filtered. Thereafter, 2 ml of the filtrate was pipetted into a test tube. A stock solution of garlic acid was prepared (100 ppm) and dilutions of 5, 10, 20, 40 and 50 ppm were prepared from it. 2ml was taken from each dilution and 1000 µl of 20 % Na_2CO_3 was added to each and filtered. A 20 µl of Folin-Ciocalteau was added to each filtrate and the resultant mixture was incubated for 30 mins at room temperature. The absorbance was measured spectrophotometrically at 760 nm using a UVVIS Spectrophotometer. From the calibration curve, total phenolic content was calculated and expressed as Garlic Acid Equivalent using the formula below.

$$C = c\,\frac{V}{m}$$

where C = total phenolic content mg GAE/g dry extract, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in mL, and m = mass of extract in gram.

Seed Antioxidant capacity

A powdered treatment sample of 0.5 g weight was put into a 15 ml centrifuge tube and 10 ml of distilled water added. The mixture was centrifuged at 10000 rpm for 15 minutes. Thereafter, a mixture comprising 0.2 ml of the centrifuged sample, 0.2 ml distilled water and 6 ml of 0.004% DPPH (1,1-diphenyl-2-picrylhydrazyl) was put in a test tube and shook by hand. The resultant mixture was kept for 30 mins at room temperature in the dark leading to a colour formation. Distilled water was used as a blank. The ability of the treatment sample to scavenge the DDPH was calculated as:

DPPH radical scavenging activity (% inhibition)

$$= 1 - \frac{As}{Ao_i} \times 100$$

Where; A_S =Absorbance of sample; A_o = Absorbance of DPPH solution diluted to same volume of distilled water.

Seed Pathogenic Fungal Presence and Load

The blotter method was used to determine the presence or absence of seed-borne pathogenic fungi in accordance with the procedures of (Mathur and Kongsdal, 2001).

Data analysis

Data collected were subjected to analysis of variance (ANOVA) using Statistix Version 10.0. Tukey's HSD (Honestly Significant Difference) test was used for means separation at probability level of 1%. Pearson's Product Moment correlation and regression analyses were also performed to establish relationships between specified parameters.

RESULTS

Temperature and relative humidity of ambient storage environment

The maximum temperature was recorded in November, 2019 and the minimum in August, 2020. The maximum relative humidity was recorded in June, 2020 and the minimum was recorded in November, 2019 (Table 1).

Table 1: Ambient temperature and relative humidity of storage environment from November, 2019 to October, 2020

	Temperature (^{0}C)		Relative Humidity	
Month and Year	Maximum	Minimum	Maximum	Minimum
November, 2019	29.45	23.9	76.11	65.11
December, 2019	28.3	23.3	77.14	68.14
January, 2020	28.3	23.2	76.85	67.85
February, 2020	28.1	24.1	75.25	66.25
March, 2020	27.1	24.45	80.91	71.91
April, 2020	29.79	24.79	79.25	70.25
May, 2020	28.21	23.21	84.92	75.92
June, 2020	27.93	22.93	86.23	77.23
July, 2020	26.42	21.42	82.33	73.33
August, 2020	25.22	20.22	81.45	72.45
September, 2020	26.92	21.92	81.25	72.25
October, 2020	27.02	22	82.69	73.69
Mean	**27.73**	**22.95**	**80.37**	**71.2**
Average	**25.34**		**75.79**	

Effect of packaging materials on thousand seed weight and moisture content of roselle seeds stored for 12 months

There were no significant ($p \geq 0.05$) packaging materials x roselle accessions interactions for thousand seed weight and for seed moisture content. However, there were significant differences between the packaging materials for thousand seed weight and for seed moisture content (Table 2). Seeds not packaged recorded the heaviest thousand seed weight, significantly greater than those with various packaging materials. The lowest thousand seed weight was recorded in seeds packaged in the bottle (64.31 g) and the ziplock bag (64.33 g). Similarly, seed moisture content was highest in seeds not packaged (9.33%) whiles the least was found in seed packaged in the bottle (8.33%).

Table 2: Effect of packaging materials on thousand seed weight and seed moisture content of roselle seeds stored for 12 months

Packaging Material	Thousand seed weight (g)	Seed moisture Content (%)
No Package	65.33[a*]	9.33[a]
Pot	64.80[b]	8.63[b]
Bottle	64.31[c]	8.33[c]
Ziplock Bag	64.33[c]	8.37[c]
Paper	64.83[b]	8.70[b]
HSD (0.01)	0.453	0.441

*Means with the same alphabets are not significantly ($p \geq 0.05$) different from each other

Effect of packaging materials on germination percentage and vigour of roselle seeds stored for 12 months

There were no significant ($p \geq 0.05$) packaging materials x roselle accessions interactions for seed germination percentage and seed vigour. However, there were significant differences between the packaging materials for seed germination percentage and seed vigour (Table 3). Seeds packaged in the bottle recorded the highest germination percentage (92.33%), significantly greater than those in the other packaging materials. The lowest germination percentage was observed in the seeds not packaged (10.33%). For seed vigour, seeds packaged in the ziplock recorded the least conductivity value representing high vigour (10.33 µS $cm^{-1}g^{-1}$) whiles the seeds not packaged recorded the highest conductivity value representing low vigour (33.33 µS $cm^{-1}g^{-1}$). There were no significant ($p \geq 0.05$) differences between the accessions for germination percentage and seed vigour.

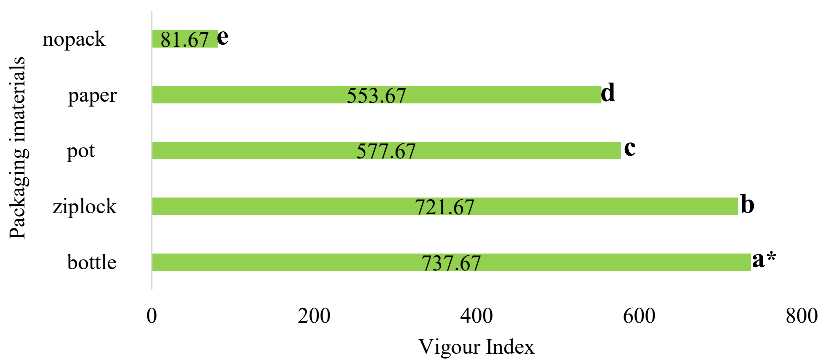
Table 3: Effect of packaging materials on germination percentage and seedling vigour of roselle seeds stored for 12 months

Packaging Material	Seed Germination Percentage (%)	Conductivity (Seed Vigour) (µS $cm^{-1}g^{-1}$)
No Package	10.33[e*]	33.33[a]
Pot	72.30[c]	14.33[c]
Bottle	92.33[a]	11.33[d]
Ziplock Bag	90.33[b]	10.33[e]
Paper	69.31[d]	27.33[b]
HSD (0.01)	1.388	2.234

*Means with ithe same alphabets are not significantly ($p \geq 0.05$) different from each other

Effect of packaging materials on seedling vigour index of roselle seeds stored for 12 months

There were no significant (p≥0.05) packaging materials x roselle accessions interactions for seedling vigour index. However, there were significant differences between the packaging materials for seedling vigour index (Figure 1). Seeds packaged in the bottle recorded the highest seedling vigour index (737.67), significantly greater than those in the other packaging materials. The lowest seedling vigour index was observed in the seeds not packaged (81.60). There were no significant (p≥0.05) differences between the accessions for seedling vigour index.



**Bars having different letters are significantly different (P<0.05) from each other.*

Figure 1: Effect of packaging materials on seedling vigour index of roselle seeds at 12 months of storage

Effect of accessions on carbohydrate, protein and fat content of roselle seeds stored for 12 months

There were no significant (p≥0.05) packaging materials x accessions interactions for carbohydrate, proteins and fat contents of roselle seed. However, there were significant differences between the accessions for seed carbohydrate, seed proteins and seed fat (Table 4). Seeds of accessions HS58 recorded the highest seed carbohydrate content (34.48%), significantly different from all the other treatments, yet similar to that of accession HS19. The least carbohydrate content was found in seeds of accessions HS27, HS32, HS59, HS69 and HS83. For seed proteins, seeds of accessions HS19, HS32, HS59 and HS86 recorded the highest protein content (28.46%), significantly different from the other treatments. Seed of accessions HS08, HS25, HS41 and HS69 recorded the least seed proteins. For fat content, seeds of accession HS19 recorded the highest fat content significantly different from accessions HS25, HS59, HS83 and HS86, yet similar to the seven other accessions (Table 4).

Table 4: Effect of accessions on carbohydrate, protein and fat contents of roselle seeds stored for 12 months

Accessions	Carbohydrate (%)	Protein (%)	Fat (%)
HS08	32.47^{c*}	26.46^{c}	21.23^{ab}
HS11	33.47 i^{b}	27.46^{b}	21.43^{ab}
HS19	34.47^{a}	28.46^{a}	21.90^{a}
HS25	32.47^{c}	26.46^{c}	21.13^{b}
HS27	32.46^{c}	27.46^{b}	21.23^{ab}
HS32	32.46^{c}	28.46^{a}	21.23^{ab}
HS41	33.47^{b}	26.46^{c}	21.43^{ab}
HS58	34.48^{a}	27.46^{b}	21.53^{ab}
HS59	32.46^{c}	28.46^{a}	21.13^{b}
HS69	32.46^{c}	26.46^{c}	21.23^{ab}
HS83	32.46^{c}	27.46^{b}	21.03^{b}
HS86	33.27^{bc}	28.46^{a}	20.83^{c}
HSD (0.01)	0.809	0.809	0.748

*Means withithe same alphabetsiare not significantly ($p \geq 0.05$) different from each other

Effect of packaging materials on carbohydrate, protein and fat content of roselle seeds stored for 12 months

There were no significant ($p \geq 0.05$) packaging materials x accessions interactions for seed carbohydrate, proteins and fat contents. However, there were significant differences between the packaging materials for carbohydrate, proteins and fat (Table 5). Seeds packaged in the bottle recorded the highest seed carbohydrate content (36.25%), significantly greater than those in the other packaging materials. The lowest seed carbohydrate content (27.25%), was observed in the seeds not packaged. For seed proteins, seeds which were packaged in the bottle recorded the highest protein content ((30.67%), significantly different from the other treatments. The lowest seed protein content (23.67%) was observed in the seeds not packaged. For fat content, seeds stored in the bottle produced the highest fat content, significantly different from those in the paper and no packaging treatments, yet similar to those in the pot and ziplock packaging (Table 5).

Table 5: Effect of packaging materials on carbohydrate, protein and fat contents of roselle seeds stored for 12 months

Packaging materials	Carbohydrate (%)	Protein (%)	Fat (%)
No Package	27.25^{d*}	23.67^{d}	19.84^{c}
Pot	35.17^{b}	28.67^{b}	21.85^{a}
Bottle	36.25^{a}	30.67^{a}	22.01^{a}
Ziplock iBag	35.25^{b}	28.67^{b}	21.85^{a}
Paper	31.25^{c}	25.67^{c}	20.85^{b}
HSD (0.01)	0.809	0.453	0.423

*Means with the same alphabets are not significantly (p≥0.05) different from each other

Effects of packaging materials and accessions on total phenolic content of roselle seeds stored for twelve months.

There were significant packaging materials x accessions interactions ($p<0.01$) for total phenolic content of roselle seeds (Table 6). Seeds of accession HS08 stored in the bottle recorded the highest total phenolic content (200.30 GAEs/100mg), which was similar to the same accession HS08 stored in ziplock material. The lowest total phenolic content was recorded in accession HS41 which was not packaged. Among the accessions, the highest total phenolic content was produced by accession HS08 (186.93 GAEs/100mg) and the least by accession HS41 (114.47 GAEs/100mg) although similar to accession HS86 (126.33 GAEs/100mg). Among the packaging materials, seeds stored in the bottle produced the highest total phenolic content (171.92 GAEs/100mg) which was similar to seeds stored in ziplock bag (171.90 GAEs/100mg). The least was produced by seeds which were not packaged (124.25 GAEs/100mg) (Table 6.).

Table 6: Effect of packaging materials and accessions on Total Phenolic Content (GAEs/100g) of roselle seeds stored for 12 months

Total Phenolic Content (GAEs/100g)						
	Packaging materials					
Accessions	**No package**	**Paper**	**Bottle**	**Pot**	**Ziplock**	**Means**
HS08	173.33^{efg*}	180.33^{cde}	200.33^{a}	180.33^{cde}	200.33^{a}	186.93^{a}
HS11	129.33^{opq}	159.33^{hi}	179.33^{de}	159.33^{hi}	179.33^{de}	161.33^{e}
HS19	119.33^{rs}	149.33^{jk}	169.33^{fg}	154.33^{jk}	169.33^{fg}	151.33^{g}
HS25	124.33^{qr}	154.33^{ij}	174.33d^{e}	149.33^{ij}	174.33^{de}	156.33^{f}
HS27	99.33^{t}	127.67^{pgr}	129.33^{jk}	149.33^{opq}	149.33^{jk}	131.00^{i}
HS32	114.33^{s}	144.33^{kl}	164.33^{gh}	144.33^{kl}	164.33^{gh}	146.33h
HS41	84.33^{u}	114.33^{s}	129.33^{opq}	114.33^{s}	130.00^{nopq}	114.47^{k}
HS58	139.33^{lmn}	169.33^{fg}	189.33^{bc}	169.33^{fg}	189.30^{bc}	171.33^{bc}
HS59	134.33^{lmnop}	164.33^{gh}	187.33^{bcd}	164.33^{gh}	184.33^{bcd}	166.33^{d}
HS69	137.33^{lmno}	167.33^{fgh}	184.33^{bcd}	167.33^{fgh}	187.33^{bcd}	169.33^{cd}
HS83	141.33^{klm}	171.33^{efg}	191.33^{ab}	171.33^{efg}	191.33^{ab}	173.33^{b}
HS86	94.33^{t}	124.33^{qr}	144.33^{kl}	124.33^{qr}	144.33^{kl}	126.33^{j}
Means	124.25^{c}	152.19^{b}	171.92^{a}	152.33^{b}	171.90^{a}	
HSD (0.01) Accessions=3.535, Pack Mat=1.978, Accessions*Pack Mat=9.548						

*Means with the same alphabets are not significantly ($p \geq 0.05$) different from each other

Effect of packaging materials and accessions on total antioxidant capacity (%) of roselle seeds stored for twelve months.

There were significant packaging materials x accessions interactions ($p<0.01$) for total antioxidant capacity of roselle seeds (Table 7). Seeds of accession HS08 packaged bottle produced the highest total antioxidant capacity (100.17%) although similar to HS08 stored in ziplock (100.17%), significantly different from the other treatment combinations. The lowest seed total antioxidant capacity was produced by accession HS41 which was not packaged but similar to accessions HS86. Among the accessions, highest total phenolic content was produced by accession HS08 (93.47%) and the least by accession HS41 (57.23%) which was similar to accession HS86 (63.17%). Among the packaging materials, seeds stored in the bottle produced the highest total antioxidant capacity (85.96%) which was similar to seeds stored in ziplock bag (85.91%). The least (62.13%) was produced by seeds which were not packaged (Table 7).

Table 7: Effect of packaging materials and accessions on total antioxidant capacity (%) of roselle seeds stored for twelve months

Total antioxidant capacity (%)						
			Packaging materials			
Accessions	No package	Paper	Bottle	Pot	Ziplock	Means
HS08	86.67^{efg}	90.17^{cde}	100.17^{a}	90.17^{cde}	100.17^{a}	93.47^{a}
HS11	64.67^{opq}	79.67^{hi}	89.67^{de}	79.67^{hi}	89.67^{de}	80.67^{e}
HS19	59.67^{rs}	74.67^{jk}	84.67^{fg}	74.67^{jk}	84.67^{fg}	75.67^{g}
HS25	77.17^{qr}	77.17^{de}	87.17^{ef}	72.17^{kl}	87.17^{de}	78.17^{f}
HS27	49.67^{t}	63.83^{pqr}	74.67^{jk}	64.67^{opq}	74.67^{jk}	65.50^{i}
HS32	57.17^{s}	72.17^{kl}	82.17^{gh}	57.17^{kl}	82.17^{gh}	73.17^{h}
HS41	42.17^{u}	57.17^{s}	64.67^{opq}	57.17^{s}	65.00^{nopq}	57.23^{k}
HS58	69.67^{lmn}	84.67^{fg}	94.67^{bc}	84.67^{fg}	92.17^{bc}	85.67^{bc}
HS59	67.17^{qr}	82.17^{gh}	92.17^{bcd}	82.17^{gh}	93.67^{bcd}	83.17^{d}
HS69	68.67^{lmno}	83.67^{fgh}	93.67^{bcd}	83.67^{fgh}	93.67^{bcd}	84.67^{cd}
HS83	70.67^{klm}	85.67^{efg}	95.67^{ab}	85.67^{efg}	95.67^{ab}	86.67^{b}
HS86	47.17^{t}	62.17^{qr}	72.17^{kl}	62.17^{qr}	72.17^{kl}	63.17^{j}
Means	62.13^{c}	76.10^{b}	85.96^{a}	76.17^{b}	85.91^{a}	
HSD (0.01) Accessions=1.767, Pack Mat=0.988, Accessions*Pack Mat=4.77						

*Means with the same alphabets are not significantly (p≥0.05) different from each other

Effect of packaging materials and accessions on fungal presence and percent load in roselle seeds stored for 12 months

The pathogenic fungi found on roselle seed stored for 12 months were *Aspergillus niger*, *Curvularia geniculata, Phoma lingam, Fusarium monilliforme* and *Botrydiplodia theobrome.* There were no significant (p≥0.05) packaging materials x accessions interactions for the percent load of the identified pathogenic fungal species. However, there were significant differences between the packaging materials for these identified fungi (Figures 2, 3, 4, 5 and 6). The bottle and ziplock packaging materials recorded the lowest percentage loads of these pathogenic fungi. The highest fungal percentage loads were found among the paper packaging and no packaging (Figures 2-6).

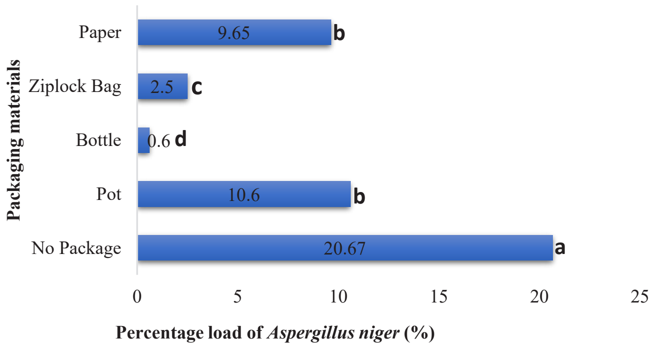


Figure 2: Effect of different packaging materials on percentage load of *Aspergillus niger* on roselle seeds stored for 12 months

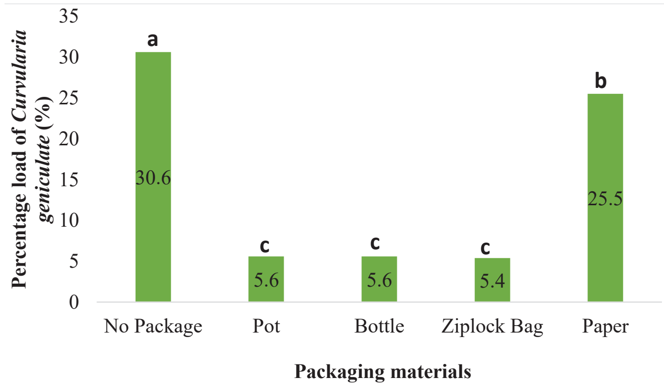


Figure 3: Effect of different packaging materials on percentage load of *Curvularia geniculate* on roselle seeds storedifor 12 months

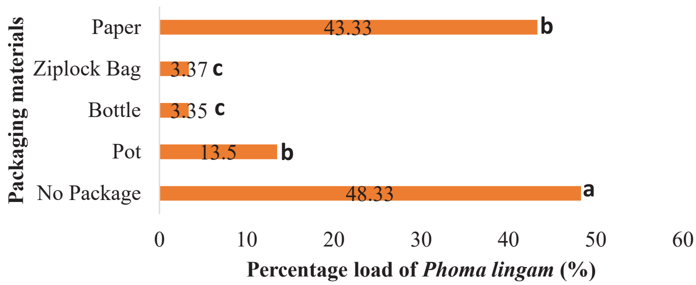


Figure 4: Effect of different packaging materials on percentage load of *Phoma lingam* on roselle seeds stored for 12 months

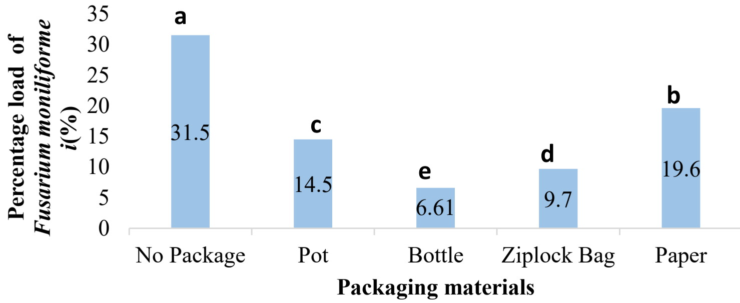


Figure 5: Effect of different packaging materials on percentage load of *Fusarium monilliforme* on roselle seeds stored for 12 months

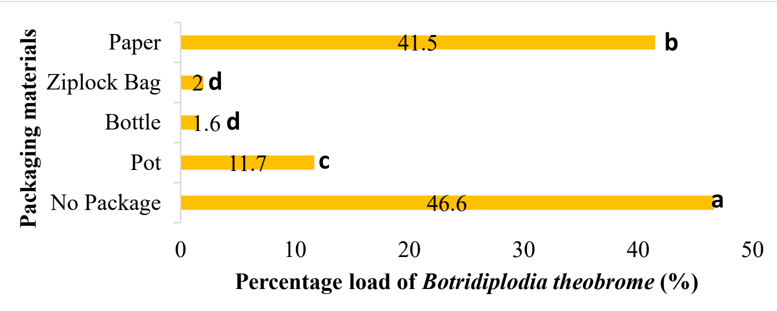


Figure 6: Effect of different packaging materials on percentage load of *Botrtidiplodia theobrome* on roselle seeds stored for 12 months

Correlations among the seed physiological and biochemical parameters

There were significant positive correlations among seed germination and the following parameters: seed carbohydrate content (r= 0.91), seed crude fat content (r= 0.79), seed protein content (r= 0.84), seed vigour (r= 0.88), seed total phenolic content (r=0.63) and seed total antioxidant capacity (r=0.74). There were also significant positive correlations among seed vigour and seed carbohydrate content (r= 0.94) and with seed protein content (r= 0.90). Additionally, there was a significant positive correlation among seed total phenolic content and seed total antioxidant capacity (r=0.98). There were however, negative significant correlations among seed germination and seed moisture content (r= - 0.98) as well as among seed vigour and seed crude fat content (r= - 0.77) (Table 8).

Table 8: Correlations among the physiological and biochemical seed parameters

Correlation variables	Correlation coefficient (r)	Prob. level (P= 0.01)
Germination and carbohydrate content	0.91	0.000
Germination and crude fat content	0.79	0.001
Germination and protein content	0.84	0.001
Germination and vigour	0.88	0.000
Germination and moisture content	-0.98	0.002
Germination and total phenolic content	0.63	0.000
Germination and total antioxidant capacity	0.74	0.001
Seed vigour and carbohydrate content	0.94	0.001
Seed vigour and crude fat content	-0.77	0.000
Seed vigour and protein content	0.90	0.000
Total phenolic content and Total antioxidant capacity	0.98	0.000

Regressions between seed germination and seed physiological and biochemical parameters

Seed germination percentage was adversely affected by seed moisture content such that 95% of the variation in the percentage seed germination was explained by the seed moisture content (Equation 1). On the other hand, seed germination percentage was positively influenced by the carbohydrate and fat contents of the seed such that 83% of the variations in seed germination percentages were explained by the seed carbohydrate and fat contents, respectively (Equations 2 and 3). Additionally, seed vigour was also positively influencediby the seed proteins such that 82% of the variation in the seed vigour was explained by the seed proteins (Equation 4).

$Y_{(Germination)} = 748.816 - 81.3977_{(Moisture\ content\ )}$ ....Equation 1

$R^2 = 0.95$, $p < 0.0000$

$Y_{(Germination)} = -192.639 + 7.77696\ _{(Carbohydrate)}$ ....Equation 2

$R^2 = 0.83$, $p < 0.0000$

$Y_{(Germination)} = -588.319 + 31.308\ _{(Fat)}$ ....Equation 3

$R^2 = 0.83$, $p < 0.0000$

$Y_{(Vigour)} = -108.590 + 3.22266\ \ _{(Protein)}$ ....Equation 4

$R^2 = 0.82$, $p < 0.0000$

DISCUSSION

The viability of orthodox seeds can be maintained at reduced moisture content with subsequent drying to enhance their longevity (Ranganathan and Groot, 2023). Orthodox seeds not only tolerate desiccation to low moisture values, but their longevity increases systematically with the decrease in temperature iand moisture content. Many of such seeds are expected to survive for a long period of time under genebank storage conditions (Walters and Pence, 2021). In the present study, a similar observation was also made where seeds stored in the bottle and ziplock packaging materials led to a reduction in the moisture content of the seeds with a subsequent reflection in the high percentage seed germination and seed vigour after 12 months of storage. Kehinde *et al*. (2022) similarly indicated that plastic containers and bottles that can afford water proof and airtight conditions were the best ior seed storage of tomato seeds since they increased the barrier properties that stopped the exchange of oxygen between the seeds and the storage environment. Dadlani *et al.* (2023) also stressed that seeds stored in such water proof and airtight containers ensured the maintenance of low seed moisture content thereby preventing seed deterioration and enhancing seed viability. Furthermore, the use of the bottles and ziplock bags also ensured that the seed carbohydrate, fat and protein contents were maintained at the highest levels. These positive biochemical observations could be due to the nature of these packaging materials that prevented the hydrolysis of available carbohydrates (Rodríguez-España *et al*., 2022), the peroxidation of seed oil and the denaturation of the storage proteins from taking place (Nagel and Börner 2010).

As regards the phenolic content and antioxidant capacity of the seeds, there were variations in the interactions between the accessions and the packaging materials such that seeds of accession HS08 packaged in either bottle and ziplock bag recorded the highest total phenolic content as well as ithe highest antioxidant capacity. Mhamdi *et al*. (2010), explained that the possession of quality phenolics and its related antioxidants in seeds was essential for the maintenance of viability of the seeds after storage. Further, phenolics that have the antioxidant capability because of redox properties, could neutralize the highly reactive oxygen species which are responsible for seed deterioration (Dumanović *et al*., 2021). The positive relationship between phenolic content and antioxidant capacity was also confirmed in this present study by the very high correlation observed between the two parameters. The results of the current study is in agreement with findings of Phewphong *et al.* (2023) who reported that roselle seeds have high levels of total phenolic compounds as well as bioactive components and DPPH radical scavenging capacity.

CONCLUSION

Storage packaging significantly affected the physiological and biochemical properties of the roselle seeds such that seeds which were packaged in bottles and ziplock bags performed better as compared to the other packaging materials (paper, pot) and the unpackaged seeds. Seeds stored in the bottle and ziplock led to a reduction in the moisture content of the seeds which subsequently reflected in the high percentage germination, high seed vigour and least pathogenic fungi loads. Also, seeds of accession HS08 packaged in either bottle and ziplock bag had the highest total phenolic content as well as the highest antioxidant capacity.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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