

Effects of packaging materials and storage periods on seed quality and longevity dynamics of *Pericopsis elata* seeds

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Abstract

This study was carried out to determine the effects of packaging materials and storage periods on seed quality and longevity dynamics of an endangered tropical tree species, *Pericopsis elata*. The experimental period was December, 2015 to June, 2016. Seed collection was done at the Bobiri Forest Reserve. The seed storage experiment was set up using 3 x 6 factorial arrangements in Completely Randomized Design with three replications. Six packaging materials (jute, nylon, paper, ziplock bag, airtight bottle and no packaging) and three storage periods (no storage, three months storage and six months storage) were used. Germination percentage, seed vigour, 1000 seed weight, moisture content, carbohydrate, protein and oil contents were assessed before storage, three months and six months after storage. *P. elata* seeds with initial moisture content of 7.5% and packaged in airtight bottle and ziplock bags maintained their moisture contents even as storage periods increased. The initial amounts of seed carbohydrates (1.9%), proteins (37.4%) and oil (31.3%) were maintained in the airtight packaging materials (bottle and ziplock bag) thereby improving seed storability. The seed viability equation predicted that *P. elata* seeds could be stored for 243 years after six months of storage. Seed viability dropped to 164 days after the accelerated aging test was performed on the six-months old stored seeds. The study concluded that the seeds of this important endangered tree can be stored in airtight containers before the next planting season without any loss in seed quality.

Keywords: viability, endangered, vigour, airtight, peroxidation

1. Introduction

Pericopsis elata is from the Fabaceae family, known with the names *afromosia* (DRC, Congo), *assamela* (Cameroon, Côte d'Ivoire) and *kokrodua* (Ghana). Commercially, the tree is known as African teak (with dark heartwood which is red in colour). *Pericopsis elata* (Meeuwen) is characteristic of the semi-deciduous forest (Swaine 1996) and is also an example of a long-lived light-demanding commercial species, together with *Terminalia superba* and *Triplochiton scleroxylon* (Abensperg-Traun 2009). *Pericopsis elata* is expensive due to the high quality of the wood, and its logging started over 50 years ago, mostly in Ghana and Côte d'Ivoire (Dickson et al. 005). In agroforestry systems, *P. elata* serves as an important tree used by cocoa farmers in Ghana to provide shade. *P. elata* seedlings used during field trials, survived despite low initial growth patterns (Anglaere 2005).

Every year, about one million hectares in the tropics are planted with tree seedlings, but only a small percentage is indigenous due to the scanty scientific information on the quality and storage

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potentials of their seeds (Bonner, 1992). Furthermore, seeds of many tropical forest species like *P. elata* quickly lose their viability and vigour before the next planting season due to improper seed handling activities including poor storage containers and conditions (Schmidt 2000). The tree is an endangered species (IUCN 2001) and special attention should be paid to the study of the tree's autecology and relevant information for its sustainability and conservation.

Forests are disappearing nearly one percent a year in sub-Saharan Africa at an alarming rate (FAO, 2003) and high quality tree seeds are needed to support both reforestation and the *in* and *ex-situ* conservation of forest genetic resources (Schmidt 2007). Several studies have indicated the effect of packaging materials on the quality of seeds in terms of germination and viability over a period of time. For instance Bortey *et al.* (2011) indicated that tomato seeds stored in a piece of cloth and tin containers resulted in low percentage germination and vigour compared to seeds stored in glass containers. However, it has been reported that the intensity of quality decreasing of stored seed under different storage techniques differ among plant species and within plant species (Al-Yahya 2001; Guberac *et al.* 2003). Using a sweet corn experiment, Camargo and Carvalho (2008) confirmed the advantages of employing hermetically sealed material to maintain quality of seed, under ambient environments. Moreover, Adebisi *et al.* (2008) included bottles as one of the best materials to store okra seeds. Seeds which are supposed to be planted in the subsequent season need to be dried and stored in moisture barrier materials to control loss of viability and vigour (Justice and Bass 1979). Thus, the choice of material used in storing seed is crucial in ensuring that the quality of seed is maintained during storage. Moreover, since the effect of these storage materials on the quality of seeds of different crops may vary, it is important to investigate and establish the most suitable packaging material for long term conservation of forest genetic resources. This would provide the gene bank managers information on how to maintain the integrity of the seed during storage. It is against this background that this study was conducted to determine the effects of packaging materials and storage periods on seed quality (physiological and biochemical) and to predict the longevity of *Pericopsis elata* seeds

2. Materials and methods

2.1. Location of seed collection and seed storage experiment

Bobri Forest Reserve was the selected location for the seed collection activities which took place in December, 2015. This Forest Reserve is in the south-east sub-type of moist semi-deciduous (MSSE) forest in Ghana, making an area of about 5,445 ha (Alder 1993). It is located 35km southeast of the regional capital and 2.5km off the main Kumasi-Accra road at the village of Kubease (Fig.1). It lies on latitude 06^o40'N to 6^o44'N and longitude 01^o15'W to 01^o22'W. The reserve serves as a research site for the Council for Scientific and Industrial Research-Forestry Research Institute of Ghana (CSIR-FORIG). It is a biodiversity conservation site home to many plants and animal species. Deciduous and evergreen species are represented in about equal proportion often with several canopy layers (Hall and Swaine 1981). The climate has a peak rainfall period in June-July, September-October and a marked dry season in November through March. The maximum and minimum temperatures are 36^oC and 24.4^oC.

The seed storage and other laboratory experiments were conducted at the Department of Horticulture, KNUST.

2.2. Experimental design

A 3 X 6 factorial completely randomized design in three replications was used for this experiment. The factors considered were: three storage periods (no storage, three months storage and six months) and six packaging materials [jute (0.9mm thickness), nylon (0.5mm), paper (0.2 mm),

ziplock bag (0.3mm), airtight bottle and no packaging]. The seeds were desiccated to same moisture content of 3.5% using 0.5kg of zeolite beads

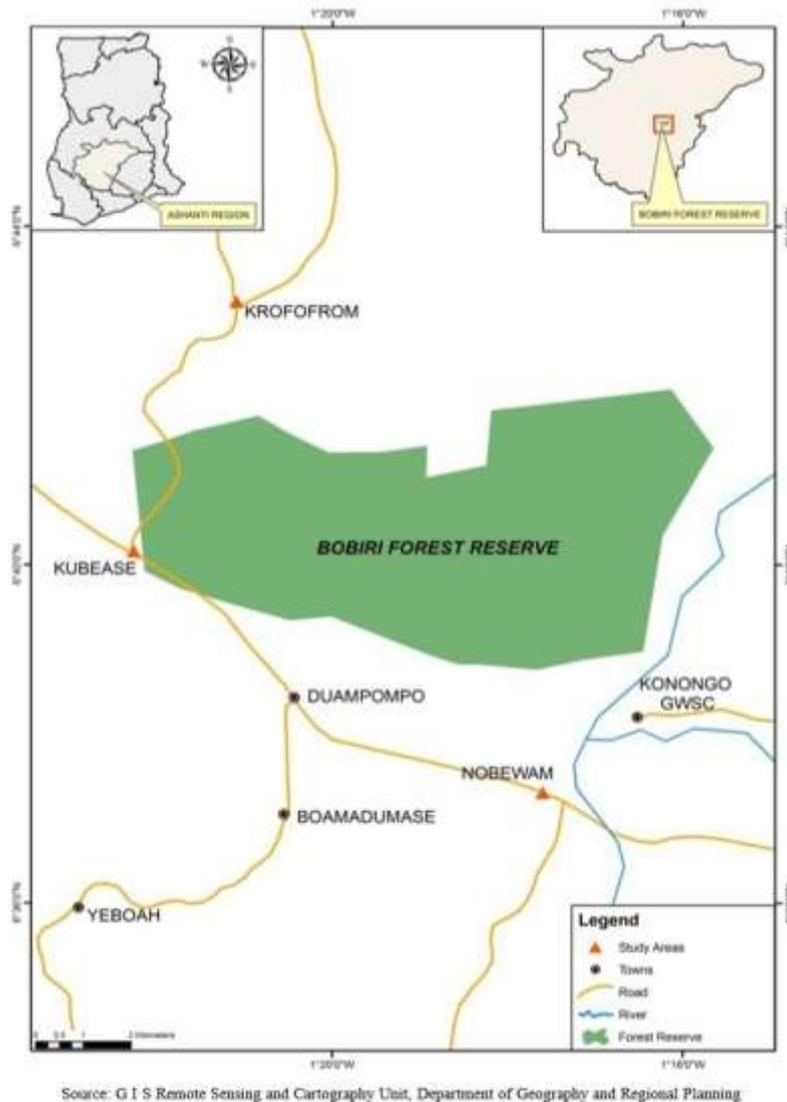


Fig. 1 The map of Bobiri Forest Reserve.

2.3. Parameters studied

These seed quality parameters (1000 seed weight, germination percentage and seed vigour) were determined using the rules set out in ISTA (2007). The chemical seed composition (oil, moisture content, protein and carbohydrate) were determined using the rules set out in AOAC (2007).

2.4. Vigour index determination

The vigour index formula proposed by Abdul-Baki and Alderson (1973) was used.

$$\text{Vigour Index} = (\text{Shoot length} + \text{Root length}) \times \text{Germination Percentage}$$

2.5. Measurement of temperature and relative humidity of storage environment

The ambient storage room temperature and relative humidity readings were taken at specified times of 9:00 am, 12:00 pm and 6:00 pm to minimize potential variations and microclimate fluctuations.

Acurite manufactured indoor digital humidity and temperature monitor (00325) was used in taking the readings which is shown in Tab. 3.

2.6. Accelerated ageing test

Accelerated Ageing test was carried out at the end of the sixth month storage using the description proposed by Jianhua and McDonald (1996)

2.7. Using the seed viability equation to predict longevity of stored seeds

At the end of the 6 months storage seeds were aged and their longevity was determined using the seed viability equation (Eq. 1) proposed by Ellis and Roberts (1980).

$$v = K_i - \frac{p}{10^{K_E - C_W \log m - C_H t - C_Q t^2}} \quad \text{Eq. 1}$$

(Ellis and Roberts, 1980)

v = final viability (expressed as %, NEDs or probits) after **p** days storage.

P = storage time (days)

m = % moisture content (fresh weight basis)

t = temperature (°C)

K_i = initial viability of the seed lot at **p** = 0 days (seed lot constant)

C_H and **C_Q** = species-specific temperature constants

K_E and **C_W** = species-specific moisture content constants.

C_H and **C_Q** are species-specific temperature constants. The effect of temperature on seed longevity is similar for all species, at least between -30°C and +90°C, and if individual constants are unknown the "universal" **C_H**, and **C_Q** constants of 0.0329 and 0.000478 may be used. Storage temperature after six months storage =28.78°C and 47°C during the aging period.

2.8. Data analysis

Data collected from the experiment were subjected to analysis of variance using Statistical Software Version 9.0. Tukey's HSD (Honest Significant Difference) was used for mean separation at probability level set at p=0.01.

3. Results

3.1. Initial seed quality characteristics

The initial seed moisture, vigour, vigour index of 1000 seed weight and germination percentage were 7.5%, 23 $\mu\text{S cm}^{-1}\text{g}^{-1}$, 2689.7, 254.7g and 96.3 % respectively (Tab. 1). The initial seed oil, protein and carbohydrate were 31%, 37.4% and 1.9% respectively (Tab. 2)

Tab.1 Initial seed quality characteristics of *P. elata* seeds.

Species	Moisture Content (%)	Vigour ($\mu\text{S cm}^{-1}\text{g}^{-1}$)	Vigour Index	1000 seed weight (g)	Germination (%)
<i>P. elata</i>	7.5	23.0	2689.7	254.7	96.3

Tab.2: The initial proximate composition of *P. elata* seeds.

Species	Oil %	Protein %	Carbohydrate %
<i>P. elata</i>	31.3	37.4	1.9

3.2. Ambient conditions of storage

Relative humidity range between 63.2% and 77.82% whereas the temperature was between 27.9°C and 28.2 °C. The minimum relative humidity was recorded in January, 2016 and the maximum in June, 2016. The minimum temperature was recorded in January, 2016 and the maximum in March, 2016 (Tab.3).

Tab.3 The relative humidity and temperature for the storage environment.

Month	Relative Humidity	Temperature
January	63.2	28.2
February	64.5	29.1
March	75.45	29.45
April	72.3	29.79
May	76.46	28.21
June	77.82	27.9

3.3. Effects of packaging materials and storage periods on carbohydrate content

Significant packaging material and storage period interactions were observed in the percent carbohydrate of *P. elata* seeds (Tab. 4). Seeds in airtight bottle which were not stored produced significantly highest percent carbohydrate, although not different from seeds stored in airtight bottles and ziplock for three and six months of storage periods. The jute packaged seeds stored for six months showed the least carbohydrate content yet not different from those stored in nylon bags, paper bags and the unpackaged.

Tab.4 Effect of packaging materials and storage periods on carbohydrate of *P. elata*.

Packaging Materials	Storage Periods			Mean
	0 Month	3 months	6 Months	
Jute	1.21	0.70	0.3	0.74
Nylon	1.22	0.80	0.4	0.81
Paper	1.23	0.75	0.45	0.81
Ziplock	1.23	1.20	1.00	1.17
Airtight bottle	1.25	1.24	1.10	1.20
No Packaging	1.20	0.60	0.35	0.72
Means	1.22	0.90	0.60	-

Tukeys HSD Pack. Material=0.3 Storage Periods=0.2 Pack Mat. x Storage periods=0.7 (0.01):

3.4. Effects of packaging materials and storage periods on *P. elata* seed oil

Significant packaging material and storage period interactions were observed in the percent oil of *P. elata* seeds (Tab.5). Seeds in any of the packaging materials but not stored showed significantly the highest percent oil content. However, the unpackaged seeds stored for six months had the least oil content.

Tab.5 Effects of storage periods and packaging materials on oil content of *P. elata*.

Packaging materials	Storage periods			Mean
	0 Month	3 months	6 Months	
Jute	31.08	26.83	24.83	27.58
Nylon	31.23	27.83	25.33	28.13
Paper	31.33	28.33	25.88	28.50
Ziplock	30.83	29.83	28.33	29.66
Airtight bottle	31.27	30.63	28.83	30.24
No Packaging	31.03	25.83	23.67	26.84
Means	31.13	28.22	26.14	

HSD (1%): Pack. Material= 1.2 Storage Periods=0.7 Pack Mat. x Storage periods=2.6

3.5. Effects of packaging materials and storage periods on protein content

There were significant differences between the storage periods for the protein content of *P. elata* seeds (Tab.6). Significantly highest protein was produced by seeds which were not stored (37.81%), while the least was produced by seeds stored for 6 months (36.36%).

Tab.6 Effect of storage periods on the protein content of *P. elata* seeds.

Storage Periods	Protein (%)
No storage	37.81
3 Months storage	37.32
6 months storage	36.36
HSD (1%)	0.8

3.6. Effects of packaging materials and storage periods on germination percentage

Seeds in airtight bottle without storage recorded the highest germination (96.33%) although not significantly different from seeds stored in airtight bottle (93.33%) and ziplock bags (92.40%) for six months. The least was recorded by unpackaged seeds stored for six months (69.67%) yet similar to seeds stored in jute bag (71.33%), nylon (72.33%) and paper bags (73.30%) for the same period (Tab. 7).

Tab.7 Effect of packaging materials and storage periods on the germination percentage of *P. elata* seeds.

Packaging materials	Storage periods			Mean
	0 Month	3 months	6 Months	
Jute	93.31	81.33	71.33	82.54
Nylon	93.33	83.32	72.32	82.67
Paper	94.32	84.33	73.30	84.23
Ziplock	95.10	93.40	92.40	91.33
Airtight bottle	96.33	95.43	93.33	92.40
No Packaging	92.33	80.31	69.67	80.44
Means	94.18	83.67	73.89	

HSD (1%): Pack. Material=2.5 Storage Periods= 1.5 Pack Mat. x Storage periods= 5.3

3.7. Effects of packaging materials and storage periods on moisture content

Seeds in airtight bottle without storage had significantly the lowest moisture content (3.33%), yet similar to all the other treatment combinations except seeds in jute package stored for six months and unpackaged seeds stored for three and six months. Unpackaged seeds stored for six months had the highest moisture content which was similar to the seeds in jute package stored for six months (Tab. 8).

Tab.8 Effect of packaging materials and storage periods on the moisture content of *P. elata* seeds.

Packaging materials	Storage periods			Mean
	0 month	3 months	6 Months	
Jute	3.53	5.03	6.03	4.94
Nylon	3.63	4.63	5.33	4.84
Paper	3.73	3.83	5.8	4.77
Ziplock	3.38	3.34	3.45	3.35
Airtight bottle	3.33	3.33	3.38	3.32
No Packaging	3.83	6.33	7.17	5.78
Means	3.52	4.42	5.03	

HSD (0.01): Pack. Material=1.2 Storage Periods= 0.7 Pack Mat. x Storage periods= 2.6

3.8. Effects of packaging materials and storage periods on vigour

Unpackaged seeds stored for six months recorded significantly the highest vigour ($34.90 \mu\text{Scm}^{-1}\text{g}^{-1}$), which was not different from seeds packaged in jute bags ($34.80 \mu\text{Scm}^{-1}\text{g}^{-1}$), nylon ($34.85 \mu\text{Scm}^{-1}\text{g}^{-1}$) and paper bags ($34.10 \mu\text{Scm}^{-1}\text{g}^{-1}$). The least vigour was produced by seeds in airtight bottle without storage ($22.85 \mu\text{Scm}^{-1}\text{g}^{-1}$) but similar to seeds packaged in airtight bottle ($23.85 \mu\text{Scm}^{-1}\text{g}^{-1}$) and ziplock bags ($23.85 \mu\text{Scm}^{-1}\text{g}^{-1}$) and stored for six months (Tab. 9).

Tab.9 Effect of packaging materials and storage periods on the vigour of *P. elata* seeds.

Packaging materials	Storage periods			
	0 Month	3 months	6 Months	Mean
Jute	24.85	28.35	34.80	28.69
Nylon	24.45	27.85	34.85	27.72
Paper	24.65	26.81	34.10	27.12
Ziplock	22.90	22.94	23.85	25.18
Airtight bottle	22.85	22.95	23.85	24.72
No Packaging	24.84	29.83	34.90	28.78
Means	24.17	27.367	30.10	

HSD (1%): Pack. Material=0.5 Storage Periods= 0.3 Pack Mat. x Storage periods= 1.1

3.9. Effects of packaging materials and storage periods on vigour index

Seeds in airtight bottle with no storage recorded significantly highest vigour index (2668.30) which was similar to seeds stored for six months in airtight bottle (2665.34) and ziplock bags (2664.35). The least vigour index was recorded by seeds unpackaged and stored for six months which was not different from those stored in jute, nylon and paper bags and stored for the same period (Tab. 10).

Tab.10 Effect of packaging materials and storage periods on the vigour index of *P. elata* seeds.

Packaging materials	Storage periods			
	0 months	3 months	6 Months	Mean
Jute	2604.41	2106.20	1422.32	2042.72
Nylon	2576.20	2158.10	1421.43	2041.30
Paper	2632.14	2184.30	1420.53	2041.31
Ziplock	2665.30	2662.32	2664.35	2180.70
Airtight bottle	2668.35	2663.43	2665.34	2181.73
No Packaging	2604.40	2080.55	1419.72	2042.85
Means	2627.72	2067.12	1473.62	

HSD (1%): Pack. Material=2.5 Storage Periods= 1.5 Pack Mat. x Storage periods=5.3

3.10. Effects of packaging materials and storage periods on 1000 seed weight

Seeds in airtight bottle without storage recorded the highest thousand seed weight which was not different from seeds stored in airtight bottles and ziplock bags for six months and 3 months. The unpackaged seeds stored for six months and three months recorded significantly the lowest thousand seed weight which was similar to seeds stored for six months and 3 months in jute bags, nylon bags and paper bags (Tab. 11).

Tab.11 Effects of packaging materials and storage periods on the thousand seed weight of *P. elata* seeds.

Packaging materials	Storage periods			Mean
	0 months	3 months	6 Months	
Jute	249.33	232.33	235.31	238.67
Nylon	248.31	233.33	237.33	237.67
Paper	249.33	237.33	239.32	238.22
Ziplock	250.30	248.33	247.33	248.34
Airtight bottle	251.33	249.33	243.13	248.62
No Packaging	247.32	235.33	237.10	239.22
Means	259.33	254.33	247.61	

HSD (1%): Pack. Material=5.7 Storage Periods= 4.1 Pack Mat. x Storage periods=6.1

3.11. Estimate of seed longevity after 6 months of storage and accelerated aging of *P. elata* seeds

P. elata seeds stored in airtight bottle after six months had a predicted longevity of 243 years and those not packaged had 5 years. After the six months when the seeds were aged, the longevity greatly dropped to 164 days for the airtight bottle and 20 days for the no packaging (Tab.12).

Tab.12 Estimate of seed longevity after 6 months storage and accelerated aging of *P. elata* seeds.

Packaging Materials	After 6 months of storage	After accelerated aging
	Longevity (years)	Longevity (Days)
Jute	11	31
Nylon	21	49
Paper	38	64
Ziplock	200	155
Airtight bottle	243	164
No packaging	5	20

4. Discussion

Seeds stored in airtight and ziplock bags for three and six months periods had the highest seed quality but those which were not packaged but stored for 3 months and six months had reduced seed quality. The other porous materials like jute, nylon and paper also lost their quality considerably in storage. Additionally, an increase in storage periods under relatively high temperature and humidity also caused a reduction in vigour and viability of the stored *P. elata* seeds. These observations could be due to the rapid exchange of gases between the seeds and their ambient environment under high relative humidity for the storage experiment, resulting in reabsorption of moisture by the seeds due to their hygroscopic nature thereby enhancing metabolic activities and oxidation processes. These metabolic activities and oxidation processes eventually depleted the essential food reserves in the seed leading to gradual loss of vigour and viability. According to Tonin and Perez (2006), the type of packaging at the time of seed storage becomes extremely relevant on the quality indicators, when the packaging can

minimize the rate of seed spoilage, and continue to regulate the initial water content of seeds in storage, preventing the speed at which seeds respire. Furthermore, in the process of seed deterioration, the increase on peroxidation of lipids results in damages to the cell membrane, and consequent generation of toxic by-products (Schwember and Bradford 2010). There was therefore the need to use moisture proof containers like the airtight bottle and the ziplock material which served as barrier against gaseous exchange and maintaining the quality of the seeds within the period of storage. The results of the current study also supports the findings of Schmidt (2000) mentioned that seeds of tropical trees, stored into a low oxygen levels, reduce the rate at which their seeds deteriorate and age. Sastry et al. (2007) also reported that seeds with low moisture content reduced respiration and deterioration and thereby enhanced the quality of stored seeds. Seeds stored in airtight bottle and ziplock bags for three months and six months periods had the highest carbohydrate, oil and protein contents but those which were not packaged but stored for three months and six months had reduced amounts. As the storage periods increased, the loss of carbohydrates, proteins and oils also reduced rapidly. Such observations could be attributed to the hydrolysis of available carbohydrates into sugars, peroxidation of seed oil and possible denaturation of storage proteins as storage duration increased. According to Bemal-Lugo and Leopold (1992), carbohydrate hydrolysis into sucrose reduced vigour and germinability of stored seeds over time. Nagel and Börner (2010) reported that lipid peroxidation could be non-enzymatic (auto peroxidation) or enzymatic (lipooxygenase) and both mechanisms lead to aging in seed. Murthy and Sun (2000) also indicated that lipid peroxidation could give rise to secondary products that could denature proteins and DNA in a non-enzymatic manner by means of the Maillard and Amadori reactions. Furthermore, Mutters (2003) reported that chemical constituents greatly change in storage especially within 3-4 months at very high temperatures. Results of this study agrees with previous findings that the consequence of peroxidation of polyunsaturated fatty acids of seed membranes was the destabilization of the membranes, which lead to uncontrolled leakage of solutes (Priestley et al., 1980). The results of this study showed that seed vigour significantly decreased after increasing the storage periods which eventually caused seed deterioration. *P. elata* can be stored for longer periods in airtight containers and that the accelerated aging conditions fastened the deterioration of the seeds, hence reducing their viability drastically. This suggests that under harsh conditions when seeds are aged, seed moisture increases under high temperature and relative humidity which promotes protein degradation, sugar hydrolysis, rise in metabolic and oxidation reactions. Murthy et al. (2002) opined that temperature and moisture content are the most important factors affecting the rate of seed deterioration and that a wide range of seed water contents and storage temperatures was used to determine the relationship between advances in seed aging and several possible primary biochemical deterioration processes, including lipid peroxidation and sugar hydrolysis.

5. Conclusion

Results obtained from this study has shown that airtight bottle and ziplock bag used for storage of *P. elata* seeds improved viability of seeds stored for three and six months as compared to jute, paper, nylon and no packaging materials. Airtight bottle and ziplock bag which are non-porous materials maintained seed moisture, vigour, v-gour index, protein, carbohydrate and oil contents. Seed deterioration was minimized in these packaging materials as compared to the others (jute, nylon, paper and the unpackaged seeds) however temperature and relative humidity were high and fluctuated under ambient storage conditions which ultimately contributed to reducing seed quality and longevity. After the sixth month storage, longevity (years) of *P. elata* was 243 years when stored in airtight bottles. When seeds were aged artificially, their longevity hugely reduced to 164 days.

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