

Full Length Research Paper

Seed germination, storability and moisture sorption isotherms of the endangered African rosewood (*Pterocarpus erinaceus*)

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African rosewood (*Pterocarpus erinaceus*) is an endemic, highly exploited and endangered tree species in arid and semi-arid zones of West Africa. Identifying optimum conditions for seed propagation and storage is critical for its conservation. This study assessed the effects of four pretreatments on seed germination and seedling vigour. Varying temperature, duration and packaging on storage deterioration were also evaluated for 12-months. Moisture sorption isotherms at 20, 25 and 30°C were characterized by equilibrating seed samples on series of lithium chloride (LiCl) solutions, generating relative humidity (RH) range of 3-95%. Data were fitted to four mathematical models; Chung Pfof, Halsey, Henderson and Oswin to determine the most suitable for describing the water activity (a_w), equilibrium moisture content (EMC) and temperature relationship. Results indicate that whole fruit sowing reduced germination capacity by more than 50% compared with extracted seeds at 5% probability level. Seeds stored at -10°C recorded the lowest rate of seed deterioration compared with ambient storage, regardless of packaging. Moisture sorption isotherms for rosewood showed a sigmoid (S-shaped) profile characteristic of orthodox seeds, and best described by the Henderson equation. Our findings highlight the need for adopting improved seed extraction technology and orthodox seed storage protocols for *P. erinaceus* conservation.

Key words: seed germination, storability, African rosewood, sorption isotherms.

INTRODUCTION

African rosewood (*Pterocarpus erinaceus* Poir) is a high-valued woody species belonging to the Fabaceae family. The tree is endemic to the Guineo-Sudanian and Sudano-Sahelian zones, where natural stands are under constant pressure due to over-exploitation for its timber (Adjonou et al., 2020). Wood of the species is prized for flooring, ornaments, musical instruments, and furniture,

due to its durability, beauty, and acoustic properties (Antwi-Bosiako et al., 2018). The tree is also used as wood fuel, fodder, in fabric dyeing, and in traditional medicines (Dumenu and Bandoh, 2016; Rabiou et al., 2017). Being heavily exploited in range countries across West Africa, rosewood has been classified as endangered with declining population (IUCN, 2018;

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CITES, 2017). In addition, several range countries have imposed felling and export ban on rosewood timber (Adjonou et al., 2020). These efforts are geared towards ensuring the long-term sustainability of the species. This notwithstanding, Barstow (2018) asserts that smuggling and illegal exploitation of the rosewood continue to surge. Dumenu (2019) also found that while the ban is operative in Ghana, exploitation has rather increased as much as 129%, with the incidence of illegal trade going up by 120% in the past few years.

The situation suggest that trade regulations and conservation designations are inadequate in ensuring sustainability of the species. There is therefore the need to explore techniques for enhancing propagation and natural regeneration for large-scale rosewood plantation and recovery programmes.

Seed germination improvement and storability studies in such highly-demanded but threatened taxa, are important for their conservation. Through such studies, optimum conditions required for successful mass seedling production and improved seed storage protocols will be identified (Bohra et al., 2020). There is a wide range of physical and chemical seed pretreatments devised to improve germination in forest tree seeds (Akpona et al., 2017; Okeyo et al., 2020). These include seed coat removal, piercing, nipping, dewinging (removal of seed/fruit appendages) clipping, and abrasion with sandpaper or chemicals.

According to Bogoeva (2020), moisture sorption isotherms provide information on the optimal conditions of seed storage longevity. Moisture sorption isotherms describe the relationship between relative humidity (RH) or water activity (a_w), and the equilibrium moisture content (EMC) at a constant temperature and pressure (Getahun et al., 2020). It is useful in analysing seed water potential; which indicates the rate of deteriorative reactions in stored products. Isotherms are species-specific, as it is based on seed composition and can be used to estimate to which moisture content seeds can be dried at a given temperature and RH, while maintaining viability (Thomsen and Stubsgaard, 1998). Clearly establishing EMC at various relative humidities and temperatures for seed samples will enable optimum storage conditions to be specified.

There is currently little knowledge about seed handling and storage protocols in *P. erinaceus*. Further, thorniness of matured rosewood samara fruits makes it difficult for manual seed extraction during propagation. As such, sowing whole unshelled fruits instead of extracted seeds is a common practice in some seedling nurseries. This study aimed at improving our understanding of seed germination and storage behaviour of the species, and to characterise the sorption isotherms at varying temperatures and RH. Three key research questions are addressed; How does temperature, duration, and packaging material affect the rate of deterioration of *P. erinaceus* seeds in storage? What is the most reliable

description of EMC/RH relation in seeds of the species using mathematical models? And what are the implications of findings on future rosewood conservation and recovery programme?

MATERIALS AND METHODS

Fruits harvesting and sampling

A total of 3000 matured rosewood fruits used in the study, were collected from approximately 27 mother trees within the species distribution range in Ghana. These locations were: Mampong, Kintampo, Kojae and Lawra (ranging from 7° 1'27.42"N, 1°25'47.53"W to 10°39'25.67"N, 2°53'11.34"W). Matured fruits were harvested from the crowns of rosewood trees with a diameter at breast height (dbh) of at least 20 cm. Fruits harvested from all sites were bulked and air-dried for 24 h at 60-70% RH. Seed dimensions were measured, recording the mean values of 20 seeds for each parameter. 1000-seed weight was determined using the mean weight of randomly selected 100 seeds in 3 replicates; expressing results in thousand seed weight.

Pretreatments

A total of 180 rosewood fruits were randomly sampled from the primary composite seedlots for four pre-sowing treatments. These treatments were; sown with whole or intact fruits, dewing fruits, fruits nipped at the distal end, and extracted seeds. This followed a slightly modified treatment technique adopted by Okeyo et al. (2020) when working on *Terminalia brownii*.

Storage experiments

A completely randomised factorial design (3 x 2 x 5) with three storage temperatures (25, 5, and -10°C), two packaging materials (cotton bags and hermetic glass jar), and five storage durations (1, 3, 6, 9 and 12 months) with three replications were used. Seed samples were drawn at various stages of storage duration for electrical conductivity and germination test. Electrolyte leakage was determined by imbibing four replicates of 10 seeds in 50 mL deionised water at 25°C for 2 h. The conductivity of the immersion solution was measured with an electrical conductivity meter (Eutech con. 510). Results were expressed in $\mu\text{Scm}^{-1}\text{g}^{-1}$ (Kundu et al., 2020).

Seed germination and seedling vigour

Treated fruits/seeds from each experimental unit were sown in three replicates of 20 seeds in 4230 m³ plastic germination boxes, filled with sterilised river sand. Seeds were incubated in an automated germination cabinet (LEEC-SL3RH), pre-set to maintain a temperature of 25-30°C with 16:8 h alternating light/dark photoperiod, under 60-70% RH for 36 days. Germination was monitored at a 24-h interval, and seeds were counted as germinated when radicles were at least 1 cm in length. At the final germination count, 10 seedlings were randomly selected from each treatment, and their mean length was determined in cm. Various seed germination indices were calculated according to the following relations:

$$\text{Germination Percentage (GP)} = \text{N/Nt} \times 100$$

where N: Number of total germinated seeds, Nt: Total number of

Table 1. Seed morphological characteristics based on the mean values of 30 seeds.

Seed measurements	Mean \pm SEM
Seed length (mm)	7.74 \pm 0.122
Seed width (mm)	4.71 \pm 0.124
Seed thickness(mm)	2.34 \pm 0.17
1000 seed weight(g)	6180 \pm 2.3
Moisture content in fresh seeds (%)	21.2.2 \pm 0.22
Moisture content in air-dried seeds (%)	14.3 \pm 0.43

seeds incubated.

Germination Index (GI) = $\sum Ni/Di$

Where:

Mean germination time (MGT) = $\sum Ni Di / \sum Ni$

where, Ni: Number of seed germinated on ith day, Di: Number of days after start of incubation until ith day. Seedling vigour index (SVI) = Germination percentage \times seedling length.

Moisture sorption isotherms

Seed samples were equilibrated over series of lithium chloride (LiCl) salt solution conditioned to generate constant RH environments ranging from 3-95%. The solutions were prepared by dissolving specified weights of LiCl granules (5-90 g) in 100 mL distilled water, at 20, 25 and 30°C for 24 h (MSBP, 2002). RH values for the solutions at the various temperatures were measured using a portable hygrometer (*Tinnytag*) with temperature/RH probe. Water activity (a_w) values of each salt solutions were equal to the relative humidity decimal ($a_w = RH/100$) following Menkov (2000) and Arslan-Tontul (2020). Seeds samples placed in small open dishes were suspended above the LiCl solution in the glass jar using a wire mesh. Weights of samples were monitored daily for weight loss/gain. Equilibrium was acknowledged when three consecutive weight measurements showed a difference of less than 0.0001 g. Equilibrium moisture content (EMC) for seeds that had equilibrated was determined gravimetrically by the oven-dry method (ISTA, 2015). Moisture sorption isotherms were obtained by plotting EMC values the various temperatures against the corresponding a_w (Merrit et al., 2003; Arslan-Tontul, 2020).

Data analysis

Results of germination treatment were analysed using the *GerminR* package in R statistical software (Lozano-Isla et al., 2019; R Core Team, 2018). Treatments differences and interactions were assessed with analysis of variance at 5% probability. A post-hoc LSD test was performed to compare treatment means. Further, four well-known models (Equations 4 to 7) were used for analysing moisture sorption experimental data. These were; Chung Pfof, Halsey, Oswin and Henderson equations (ASAE 2000). These models were expressed mathematically as:

$$\text{Chung Pfof, } a_w = \exp \left[\frac{-A}{t+B} \exp(-cM) \right] \quad (1)$$

$$\text{Halsey, } a_w = \exp \left[\frac{-\exp(A+Bt)}{M^C} \right] \quad (2)$$

$$\text{Henderson } a_w = 1 - \exp[-A(t+B)M^C] \quad (3)$$

$$\text{Oswin } a_w = \frac{1}{(A+Bt/M)^{C+1}} \quad (4)$$

where; a_w is the water activity (decimal), M is the moisture content (%), A, B, C are model coefficients, and t is the temperature (°C). A non-linear regression was used to fit the four models into the moisture sorption experimental data. The suitability of the model was compared using their mean relative percentage error (P%), standard error of moisture (SEM) and the randomness of residual plots. The model that met the commonly accepted standards (lowest P and SEM with more randomized residuals) was selected as the best-fit (Arslan-Tontul, 2020).

RESULTS

Table 1 presents the summary of the mean values of measured seed dimensions and the standard error of their means. Seed length ranged from 7.21- 8.41 mm. Seed sizes and shapes showed some variations. The highest and lowest recorded values for seed width were 5.62 and 2.51 mm respectively.

Pretreatment

Results showed significant differences ($F_{(7,0864)}$, $P = 0.0053$, ($F_{(12,0732)}$, $P = 0.0024$) in Final germination percentage (GP) and Seedling vigour index (SVI) of extracted seeds and whole fruits sowing (Table 2). Extracted seeds recorded the highest final germination of 94.2% compared with the lowest (44%) in whole fruits sowing. SVI of extracted *P. erinaceus* seeds was about four times greater than that of whole fruits sowing. Figure 1 presents the graphical summary of cumulative germination for all four pretreatments during the 36-days incubation period.

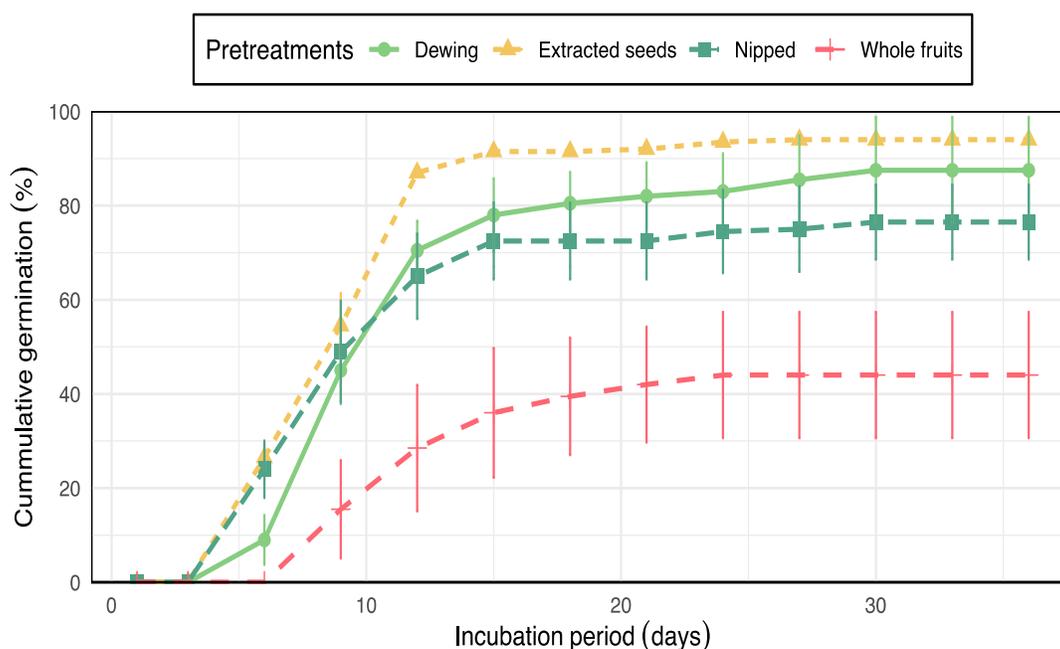
Seed storage

Summary of *P. erinaceus* seed germination at varying

Table 2. Mean values of seed germination indices for pretreatments.

Treatment	GP (%)	MGT (days)	SI	SVI
Whole fruits	44.2 ± 0.24 ^a	13. ±0.2	0.331	230.4 ^a
Extracted seeds	94.2 ±1.32 ^b	9.3 ±0.2	0.418	882.94 ^b
Dewinged	87.5 ±1.02 ^c	11.5 ±0.3	0.294	562.51 ^c
Nipped Fruits	76.5 ± 0.2 ^c	10.3 ±0.2	0.264	634.42 ^c
C.V (%)	9.4	6.3		21.2

GP- Final germination percent, MGT- Mean germination time, SI- Synchrony index of germination, SVI- seedling vigour index. Figures with the same letters are not statistically significant at the 5% probability level.

**Figure 1.** Cumulative germination of pretreatments for the of 36 days incubation.

storage temperature (T), duration (D), and types of packaging (P) is shown in Figure 2. Results indicated a general decline in germination rate (a measure of deterioration) with increasing storage duration across all treatment combinations. The highest rate of deterioration was recorded in rosewood seedlots stored in cotton bags under ambient conditions (25°C/CB), where germination declined by more than 50% after 12 months of storage duration (from 87% in month 1 to 31% in month 12).

This treatment also recorded the highest mean electrical conductivity of 140 $\mu\text{Scm}^{-1}\text{g}^{-1}$. Storage at -10°C in a hermetic glass jar (-10°C/GL) recorded the lowest rate of deterioration after 12 months of storage (from 92% in month 1 to 68% in month 12). Accordingly, this storage condition recorded the mean least electrical conductivity of 65 $\mu\text{Scm}^{-1}\text{g}^{-1}$. Results of the analysis of variance summary (Table 3) in the generalized linear model indicate that temperature (T) and storage duration (D),

had a significant effect on rosewood germination ($p < 0.0001$, $\alpha = 0.05$) regardless of the type of packaging ($p = 0.10290$).

Table 4 shows the obtained results of the EMC on wet basis and its related water activity at the various experimental temperature regimes ($t = 20, 25$ and 30°C). Water activity of the series of LiCl salt concentrations were obtained by dividing the generated RH of the solution by 100 ($aw = \text{RH}/100$) at constant temperature. Results indicate that the EMC values of rosewood ranged between 27 and 2.13% over the whole experimental period. EMC declined with increasing temperature at constant aw .

Figure 3 compares the moisture sorption isotherms at $t = 20, 25$ and 30°C , respectively. Generally, at all experimental temperature regimes, the moisture release curve is characteristic of the sigmoid (S-shaped profile), as found by Bogoeva (2020), Asomaning (2011) and

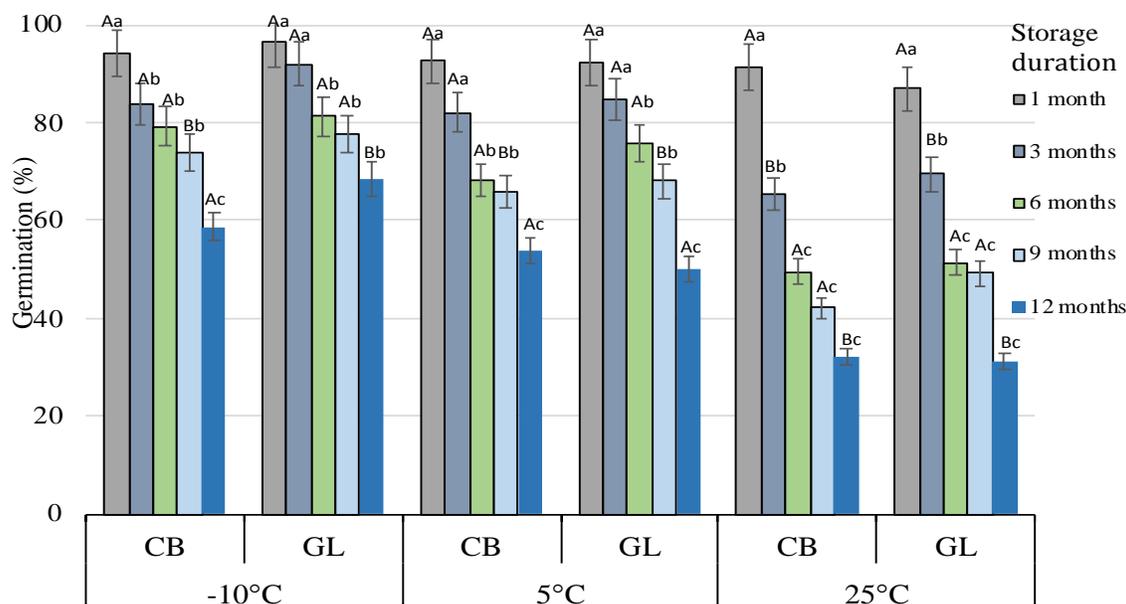


Figure 2. Seed germination under different storage temperatures packaging and duration. Bars with the same letters are not significant at the 5% probability level.

Table 3. Generalised linear models of the experimental seed storage conditions of *P. erinaceus*.

Source variation	Electrical conductivity		Germination	
	F-value	P-value	F-value	P-value
T	25.2219	<0.0001 ^a	59.4105	<0.0001 ^a
D	337.2531	<0.0001 ^a	179.1796	<0.0001 ^a
P	25.6181	<0.0001 ^a	2.7203	0.10290
T x D	2.5269	0.1157	5.1392	0.00262 ^a
T x P	0.5025	0.4804	2.6673	0.10626
D x P	2.2259	0.13952	1.2982	0.25787
T x D x P	7.8177	0.006	0.6440	0.42459

T- temperature, D-duration, P- packaging residual standard error (RSE) is 6.588, adjusted R² is 0.7328 for Electrical conductivity model residual standard error (RSE) is 15.34, adjusted R² is 0.8158 Values with the same letters were not significant at the 5% probability.

Menkov (2000). Table 5 shows the calculated results of coefficients of the three-parameter models and their corresponding mean relative percentage error (P%), standard error of moisture (SEM) and nature of the residual plots are presented in Figure 3.

DISCUSSION

Results for germination experiment showed that sowing of whole samara fruit reduced germination capacity ($p < 0.001$) by more than 50% compared with extracted seeds. This suggests that when propagating *P. erinaceus* with the whole samara fruits, physical dormancy imposed

by the fruit pericarp is substantial. Fruit pericarps and other extensions in some tropical tree's species have been identified as a barrier to seed germination, by restricting moisture and oxygen availability (Bewley, 1994). Okeyo et al. (2020) reported similar findings in *T. brownii*, where extracted seeds recorded the highest germination of more than 70% with whole samara fruits failing to germinate after 60 days of incubation. Thus, the removal of the pericarp eliminated germination restrictions and enhanced imbibition and oxygen uptake. Seed could therefore germinate with reduced mean germination time (from 13 days in whole fruit to 9 days in extracted seeds). Further, extracted seeds showed improved seedling vigour. De-winged and nipped fruits pretreatments

Table 4. Equilibrium moisture content M (%) of rosewood seeds at different water activities (a_w) and temperatures t (°C). SG represents equilibration with silica gel, sd is the standard deviation based on three replications.

LiCl conc. (g/100 mL)	20°C			25°C			30°C		
	a_w	EMC	SD	a_w	EMC	SD	a_w	EMC	SD
90	0.042	4.26	0.12	0.032	8.4	0.01	0.031	2.14	0.03
80	0.057	7.01	0.13	0.051	6.3	0.13	0.055	6.2	0.04
70	0.194	10.1	0.02	0.182	8.5	0.36	0.722	8.0	0.13
60	0.226	12.2	0.23	0.210	16.2	0.02	0.221	9.0	0.17
50	0.271	16.4	0.12	0.27	14.2	0.41	0.274	11.2	0.04
40	0.426	14.5	0.02	0.414	17.6	0.68	0.426	16.3	0.16
30	0.454	16.2	0.03	0.451	19.4	0.05	0.47	13.1	0.02
20	0.601	19.3	0.14	0.602	22.1	0.07	0.59	14.3	0.03
10	0.655	17.2	0.26	0.712	23.2	0.21	0.810	16.2	0.41
5	0.801	21.6	0.31	0.926	27.3	0.32	0.92	19.42	0.30
SG	0.011	3.6	0.02	0.031	3.2	0.02	0.02	2.13	0.07

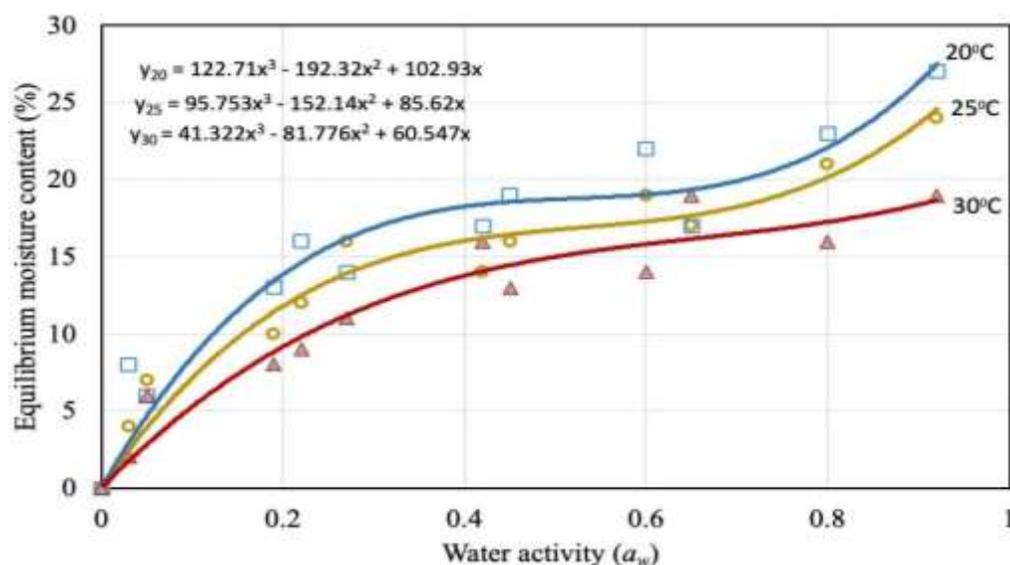


Figure 3. Moisture sorption isotherms of *P. erinaceus* seeds at 20, 25 and 30°C, respectively.

showed intermediate germination as a result of improved pericarp permeability that promoted moisture and oxygen uptake by the seed (Mewded et al., 2018). Reduced germination in whole fruit sowing could also be due to seed-borne pathogenic fungi carried by the pericarp. Some studies have isolated several seed-borne fungi genera such as *Fusarium*, *Colletotrichum*, and *Alternaria* associated from fruit pericarps (Nandi et al., 2017).

Analysis of storage experimental data shows that seedlots under the different treatments retained their germination capacity and viability throughout the 12-months experimental duration. This is confirmed by Johnson et al. (2019), who found that *P. erinaceus* seeds preserved their germination capacity regardless of storage conditions and phytogeographical provenance for at least

a year. However, there was a general decline in germinability with increasing duration across all treatments. Seeds stored in cotton bags under ambient conditions (25°C/CB) had accelerated deterioration compared with all other storage treatments. The higher electrical conductivity and low germination capacity after 12 months for this treatment was an indication of weakening seed coat integrity. Some authors are of the view that seedlots kept under ambient conditions are often subjected to fluctuating temperatures of 22°C minimum and 25°C maximum. This wider thermal and RH range likely reduces the ability of seeds to reach a hygroscopic balance over a sustained period, thereby increasing the rate of deterioration. Packaging effect was not significant compared with temperature, duration and

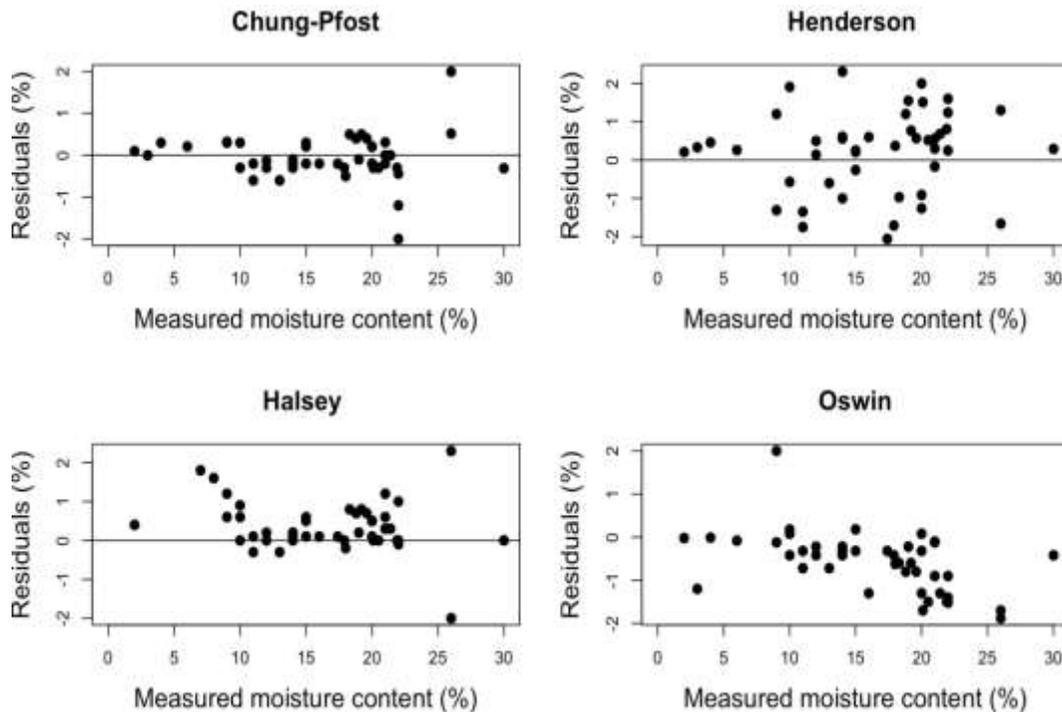


Figure 4. Residual plot distribution of the four sorption models.

their interaction.

Generally, storage at -10°C recorded the lowest rate of seed deterioration (from 92 to 68% in 12 months) and is therefore considered to be amongst the optimum conditions for long-term seed conservation of *P. erinaceus*.

The moisture sorption isotherm indicated that at constant a_w or RH (decimal), EMC of the species reduced with increasing temperature. The general opinion is that the EMC values decrease with increasing sorption temperatures due to breaking away of water molecules from their sorption sites easily at high energy levels (Samapundo et al., 2007). Three distinct regions observed on all moisture sorption curves show the strength of water binding to seed tissues, and the nature of the binding site (Baldet et al., 2008). The steep portions of the slope at low RH indicate water molecules tightly bound to ionic sites in seed tissues, creating hydrophilic surfaces that strongly resist dehydration (Moravec et al., 2008). The relatively flat middle portion of the curve represents the interaction of water with less hydrophilic surfaces which subsequently become saturated with water. After this point, multilayer molecular sorption sites are formed at the latter part of equilibration which is indicated by the curve increasing sharply (Moravec et al., 2008). The reverse sigmoidal shape of the isotherms curve has been reported for other tropical forest tree species such as *Terminalia superba*, *Entandrophragma angolenses* and *Khaya anthotheca*

(Asomaning et al., 2011). This shape is characteristic of tropical tree seeds with orthodox storage classification (Copeland and McDonald, 1996).

The graphical analysis of residual distributions presented in Figure 4 for the Sorption models confirm the suitability of the Henderson model (Equation 6) for describing the moisture sorption process. The model meets the commonly accepted standards of lowest P (9.07%) and SEM (0.047), with a highly randomized residual plot in Table 3 and Figure 4. On the basis of obtained results, we offer (or recommend) the Modified Henderson equation as ensuring good fitness in expressing the sorption isotherm of *P. erinaceus* seeds.

CONCLUSIONS AND RECOMMENDATIONS

Our findings suggest that seed extraction of rosewood improves germination and seedling vigour, compared with whole fruit sowing. For mass propagation of the species towards recovery, appropriate investment in simple seed extraction technology is crucial in ensuring the availability of good quality seeds. Storability in *P. erinaceus* seeds is affected by temperature and duration regardless of packaging technology. Storing seeds at -10°C slows deterioration rate by more than 50% compared with storage in ambient conditions. Thus, cool-dry environment is therefore recommended for long term *ex-situ* seed conservation. The moisture sorption isotherm

Table 5. Parameters of the fitted sorption models to the experimental data of rosewood seeds.

Model parameter	Sorption models			
	Chung Pfast	Halsey	Henderson	Oswin
A	413.732	3.84723	0.00021	9.81526
B	0.26953	-0.01926	2.06714	-0.10254
C	57.2314	1.916351	0.18073	0.29461
P (%)	13.67	14.48	9.07	11.26
SEM	1.25	1.03	0.047	1.22
Residual plot	Patterned	Patterned	Random	Patterned

shows that *P. erinaceus* exhibit orthodox storage physiology. On the basis of obtained results, we suggest that the Henderson model most adequately describes the sorption isotherm of *P. erinaceus* seeds. We recommend the adoption of protocols for handling orthodox tree seeds as applicable. This information will be useful for the design of rosewood seed drying equipment, modelling, the storage process and predicting storage longevity towards *ex-situ* conservation to *P. erinaceus*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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