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The effect of storage period on the viability of *Triplochiton scleroxylon* K. Schum. seeds

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ABSTRACT

Triplochiton scleroxylon is a tropical timber with much popularity on the international market. The use of this tree for reforestation programmes has not been very successful partly due to the plant's irregular seed supply and speculations of seed viability loss after long storage periods. Using germination test on nursery beds, this study investigated the optimum storage period for *T. scleroxylon* seeds kept in a sterilized sack under room conditions (25 °C and 65% Relative Humidity) for twelve (12) months. Germination characteristics such as First Day of Emergence (FDE), Mean Emergence Time (MGT), Final Germination Percentage (FGP) and Germination Index (GI) of the seeds were compared. Increasing storage period was associated with high FDG and MGT. Similarly, FGP and GI were lower for seeds stored for long periods (e.g. 16% and 2.6 respectively for seeds stored for 12 months) than those stored for short periods (e.g. 82% and 34.1 respectively for seeds stored for 2 months). The differences between the means of the germination characteristics for the storage periods were statistically significant ($p < 0.05$). FDE and MET had strong positive correlation with storage period ($r = 0.996$ and 0.965, respectively) while FGP and GI had strong negative relationships with storage period ($r = -0.958$ and -0.960, respectively). The low FDG and MGT, and high FGP and GI values obtained for seeds stored for 2 months imply that for maximum germination, seeds of *T. scleroxylon* should not be stored under room conditions for more than 2 months. This information would assist farmers, forest managers and agroforesters intending to store seeds of *T. scleroxylon* for tree planting programmes.

Introduction

Over the past decades, large areas of many tropical forests have been degraded through overexploitation of timber and conversion for agricultural and infrastructural development. According to [25], reforestation, which involves tree planting, is an important process for the re-establishment of tree cover on degraded lands. The Intergovernmental Panel on Climate

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Change IPCC, [20] explained that reforestation is essential for salvaging tropical forests from total extermination by reversing biodiversity loss and mitigating climate change. Cunningham et al. [12] also mentioned that the environmental impacts of deforestation can be lessened by well-designed and intentional tree planting programmes. Realizing the socio-economic and ecological impacts of the loss of forest cover on the globe, the United Nations Environment Programme (UNEP) launched the billion tree campaign in November 2006. UNEP in partnership with Civil Society Organizations (CSOs) and the private sector in all regions of the world sought to plant at least 1 billion trees worldwide in 2007. In April 2016, UNEP reported that about 14.2 billion trees had been planted across the globe [40]. Based on the success of this campaign, many international organizations such as the Wildlife Conservation Society (WCS), the World Wide Fund for Nature – UK (WWF-UK) and BirdLife International (BLI) with the support of Restore UK, an environmentally-based charitable foundation, have introduced a new initiative, which targets the planting of about trillion trees around the world to restore degraded forests.

The success of any reforestation programme depends partly on the regular supply of planting materials such as seeds, seedlings and stem cuttings [25]. Gray and Hamann [18] recommended that seeds from indigenous species should be used for reforestation because they are better adapted to the environment and provide several benefits to the ecosystem. In Sub-Saharan Africa, however, Chechina and Hamann [11] reported that most reforestation programmes have often been done with seeds and other planting materials from exotic tree species due to their availability from international seed banks and the well-established nursery procedures for these species. Tolentino [39] and Yonariza and Singzon [44] also reported that about 80% of all trees planted in tropical reforestation programmes are exotic. According to Cemansky [10], some indigenous species have irregular fruiting cycle, which makes it difficult to rely on them to supply seeds for planting exercises. Pradham and Badola [30] noted that several attempts have been made in the past to collect and store seeds during fruiting periods to ensure their consistent supply and long-term conservation of plant genetic resources. However, there are reports of loss of seed viability of some commercially-important species after long periods of storage [30,33]. Sisman [36] asserted that storing seeds beyond the optimum storage period could reduce their germination potential and seedling establishment. El-Keblawy [14] observed that the germination of *Haloxylon salicornicum* and *Salsola imbricata* seeds was completely inhibited after they were stored in the open field for nine months. Ghasemnezhad and Honermeir [17] also found that the viability of sunflower seeds reduced with longer periods of storage. Sultana et al. [38] similarly observed a reduction in the germination capabilities of okra seeds after long storage periods. The unavailability of reliable data on the effects of storage period on tree seed viability hampers efforts by local farmers, foresters and agroforesters who wish to collect and store seeds of traditional timber species for future reforestation projects. Since seed germination is crucial for the establishment, maintenance and expansion of plant populations and recovery of forests from disturbances [13], research that establishes the optimum period of storage for seeds from local trees in the Tropical African region is required. Thus, this study examined the impact of the period of storage on the viability of seeds from an economically significant tropical timber, *Triplochiton scleroxylon*.

Triplochiton scleroxylon (Local name: Wawa) is a deciduous forest tree, which grows up to 45 m in height and 1.5 m in diameter. It is abundant in semi-deciduous moist forests and the transition zones between forest and moist savannah in many West African countries including Cameroon, Nigeria, Ghana, Ivory Coast, Sierra Leone and Guinea [19,21,26]. The bole is relatively straight, cylindrical and clear to 24 m of its height. The timber is commercially exploited for building, paneling, carvings, mouldings, blockboard, furniture components and shoe heels [42]. There is a very high demand for the species on the European market. In May 2018 alone, Ghana supplied up to about 1870.411 m^3 of Wawa lumber estimated at €898,395.812 to the European market [16]. Currently, Wawa is rated as an endangered species in some African countries. Despite the high demand, there are few established plantations of the species and it is seldom used for reforestation programmes [34]. Depending on the location of the tree, Wawa may fruit at most once in seven years [9]. Thus, as with most indigenous tropical timbers, the irregularity of fruiting hamper efforts aimed at establishing plantations with Wawa. Bowen et al. [9] also mentioned that the seeds could lose their ability to germinate after storing them for long periods. Even so, information on the optimum period for storing Wawa seeds in order to achieve high germination percentage is scanty. Bowen et al. [9] are the only authors who have worked on the effect of different storage factors (e.g. storage period and temperature) on the viability of *T. scleroxylon* seeds. However, there was no statistical analysis in their work to prove the validity and reproducibility of their data, and for the reliable interpretation of their results. Again, their work did not test the relationship between the storage factors and seed germination ability using correlation analysis. The objective of this study was to re-investigate the effect of storage period on the viability of *T. scleroxylon* seeds kept in a sterilized sack under room conditions (i.e., 25 °C and 65% RH) for twelve (12) months using statistical and correlation analysis. The results would provide information about the optimum storage period, which will ensure maximum germination of Wawa seeds, to Forest managers, Agroforesters and Plantation developers who intend to establish plantations or restore degraded forests with *T. scleroxylon*.

Materials and methods

Study area

The experiment was carried out at the plantation nursery of the University of Energy and Natural Resources, formerly the Faculty of Forest Resources Technology (FFRT) of the Kwame Nkrumah University of Science and Technology (KNUST) (7.34949°N, 2.343501°W). The nursery lies within the high forest zone of Ghana with dry semi-deciduous vegetation. The

Table 1
Influence of seed storage on the viability of *T. scleroxylon* seeds.

Storage period (Months)	First day of emergence (Days)	Mean emergence time (Days	Final germination percentage (%)	Germination index
2	8^{a}	19^{a}	82^{a}	34.1^{a}
4	13^{a}	21.5^{a}	78^{a}	20.3^{b}
6	19^{b}	29.5^{b}	70^{b}	18^{bc}
8	26^{c}	40.5^{c}	56^{c}	16.8^{c}
10	29^{c}	42.4^{c}	24^{d}	6.2^{d}
12	34^{d}	44^{c}	16^{e}	2.6^{e}

Means in the same column with different superscript are significantly different ($p < 0.05$).

annual rainfall is about 1000–1200 mm and has a mean relative humidity of 70%. The topography of the area is fairly flat with sandy loam type of soil and average temperature of 25 °C [24].

Seed collection and storage

Mature fruits were collected from the branches of six randomly selected *T. scleroxylon* trees (age: 30–40 years) located at FFRT using climbing equipment. The fruits were air-dried for three (3) weeks and the seeds mechanically removed. Defect-free seeds were air-dried until their moisture contents were between 5–10%. They were then put into sterilized jute sacks and kept under room conditions (Temperature = 25 °C; Relative Humidity = 65%).

Plot design

A Randomized Complete Block Design (RCBD) was employed for this experiment with six (6) duration of storage as treatments (i.e., 2, 4, 6, 8, 10 and 12 months) and 3 replications for each treatment. Three nursery beds (size = 1×10 m) were constructed and each divided into six sections known as blocks. The position of the block for each treatment was determined by randomization. At the respective dates of the treatment, 150 seeds were taken from storage; 50 seeds each were broadcasted on the blocks on each of the three beds in the morning between 06:00 and 07:00. Each bed was given the same treatment in terms of watering and weed control throughout the experiment. Watering was done twice a day, early in the morning (06:00–07:00) and in the evening (17:00–18:00). Hand weeding and forking of beds were done twice every two weeks.

Data collection, presentation and analysis

The seeds were closely monitored and the following germination characteristics were recorded for each block based on the definitions by Orchard [29], Scott et al. [32], Bench Arnold et al. [7] and Kader [22]:

i. First Day of Emergence (FDE): The day on which the first emergence event was recorded.
ii. Mean Emergence Time (MET) = $\frac{\sum f(x)}{\sum f}$, where f is the number of seeds which emerged on day x.
iii. Final Germination Percentage (FGP): Final number of seeds which germinated expressed as a percentage.
iv. Germination Index (GI): $\frac{n_1}{d_1} + \frac{n_2}{d_2} + \cdots + \frac{n_i}{d_i}$, where n_1 is number of seedlings emerging on day 1 (d_1).

The measure of seed emergence was based on the physical protrusion of the radicle [8,31]. The data were presented in graphs and charts. Analysis of variance (ANOVA) for randomized block design was used to determine the variations among the germination data recorded for the treatments. Fisher's Least Significant Difference (LSD) test was used to determine the treatments that differed from one another. Pearson Product-Moment Correlation Coefficient (r) was used to determine the relationships between the storage periods and the germination parameters at 95% Confidence Level. The Statistical Package for Social Scientists (SPSS) Software (Version 20) was used to generate Scatterplots that described these relationships.

Results

First day of emergence and mean emergence time

It was observed that as storage period increased, the seeds took a long time to emerge from the soil. For example, the First Day of Emergence (FDE) of seeds that were stored for 2 months was 8 days while that for the seeds stored for 12 months was 34 days. The Mean Emergence Time (MET) followed the same trend as the FDE. MET was higher for seeds stored between 8 and 12 months (i.e., 40.5–44 days) than those stored for 2–6 months (i.e., 19–29.5 days). Significant differences ($p < 0.05$) were recorded for the FDE and MET of the seeds for the different storage periods (Table 1).

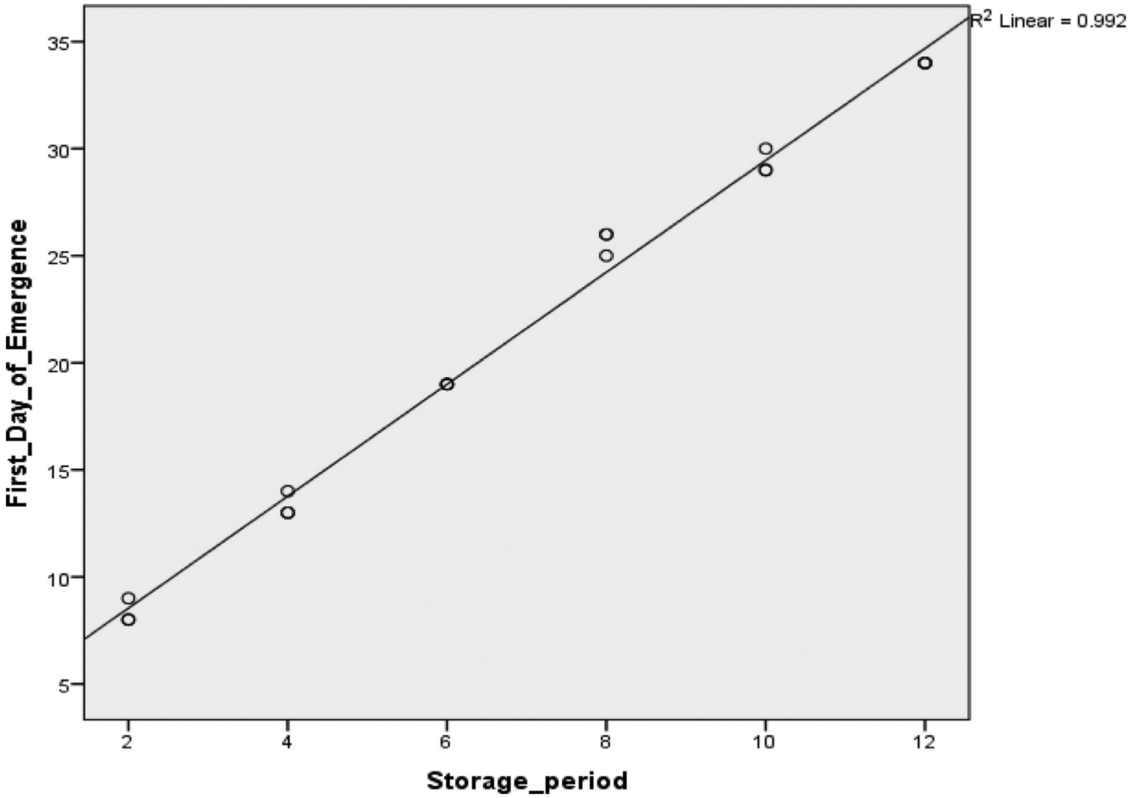

Fig. 1. Correlation between first day of emergence and storage period.

Final germination percentage and germination index

The best Final Germination Percentage (FGP) (82%) was achieved by seeds stored for 2 months, followed by those stored for 4 months (78%) and 6 months (56%). *T. scleroxylon* seeds stored for 10 and 12 months respectively achieved 24% and 16% FGP. Germination index (GI) was highest for seeds stored for 2 months (i.e., 34.1) and lowest for those stored for 12 months (i.e., 2.6). The differences between the FGP and GI recorded for the storage periods were significant ($p < 0.05$) (Table 1).

Linear correlation between storage period and the germination characteristics

The results of the correlation analysis showed that there was a strong positive correlation between First Day of Emergence and storage period ($r = 0.996$), and also between Mean Emergence Time and storage period ($r = 0.965$) (Figs. 1 and 2). A strong negative relationship was found between Final Germination Percentage and storage period ($r = -0.958$), and Germination Index and storage period ($r = -0.960$) (Figs. 3 and 4).

Discussion

First day of emergence and mean emergence time

Seed germination is a critical process in the life cycle of plants [28]. The success of this process principally depends on many factors including how long seeds are stored. The First Day of Emergence (FDE) is the day on which the first emergence event was observed. According to Kader [23], low FDE value indicates a faster initiation of germination and high seed viability. In the present study, high FDE values were obtained for seeds, which were stored for long periods. Mean Emergence Time (MET) is also used to measure the rate and time-spread of germination. Soltani et al. [37] explained that MET is an accurate measure of the time taken for seeds in a particular lot to germinate and has been used to evaluate seed vigour. The lower the MET, the faster a population of seeds has germinated [29]. MET was higher for seeds stored for long periods (e.g. MET for seeds stored for 12 months was 44 days) compared to those stored for short periods (e.g. MET for seeds stored for 2 months was 19 days). Azadi and Younesi [5] observed that MET increases significantly with increasing periods of storage. This was confirmed by the correlation analysis in which a strong positive association ($r = 0.965$) was found between storage period and MET. According to Olosunde et al. [28], prolonged storage periods lead to natural aging and bio-chemical

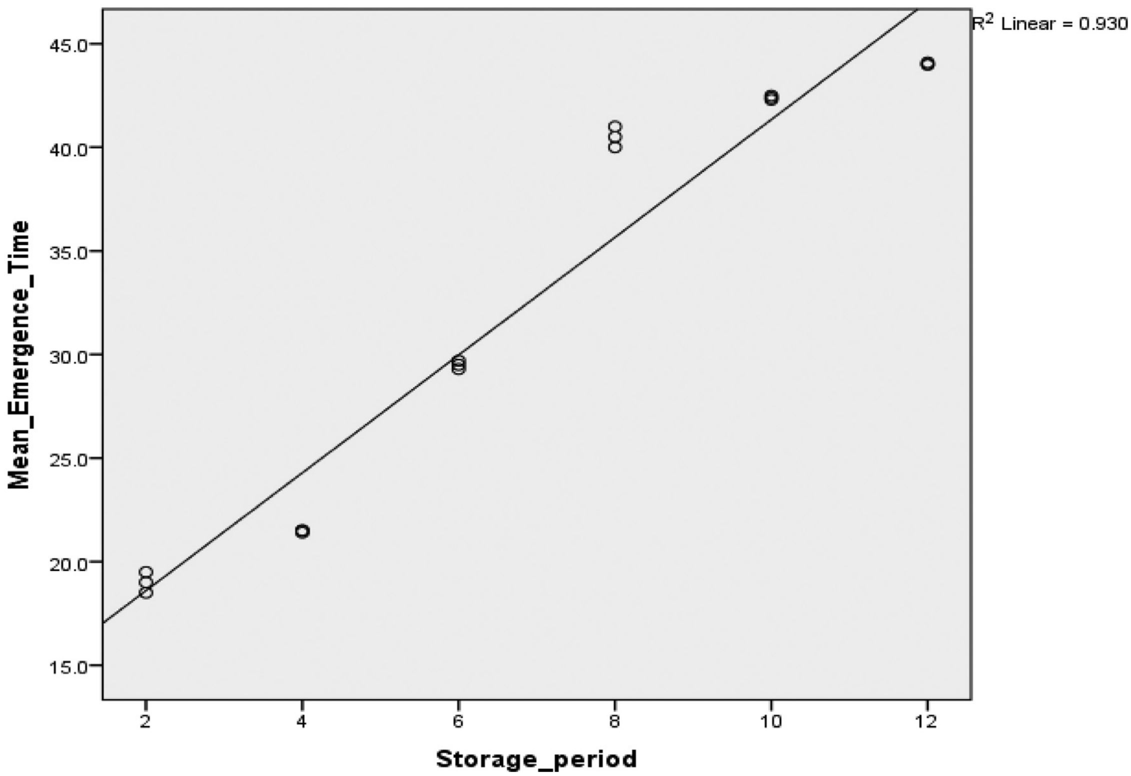

Fig. 2. Correlation between mean germination time and storage period.

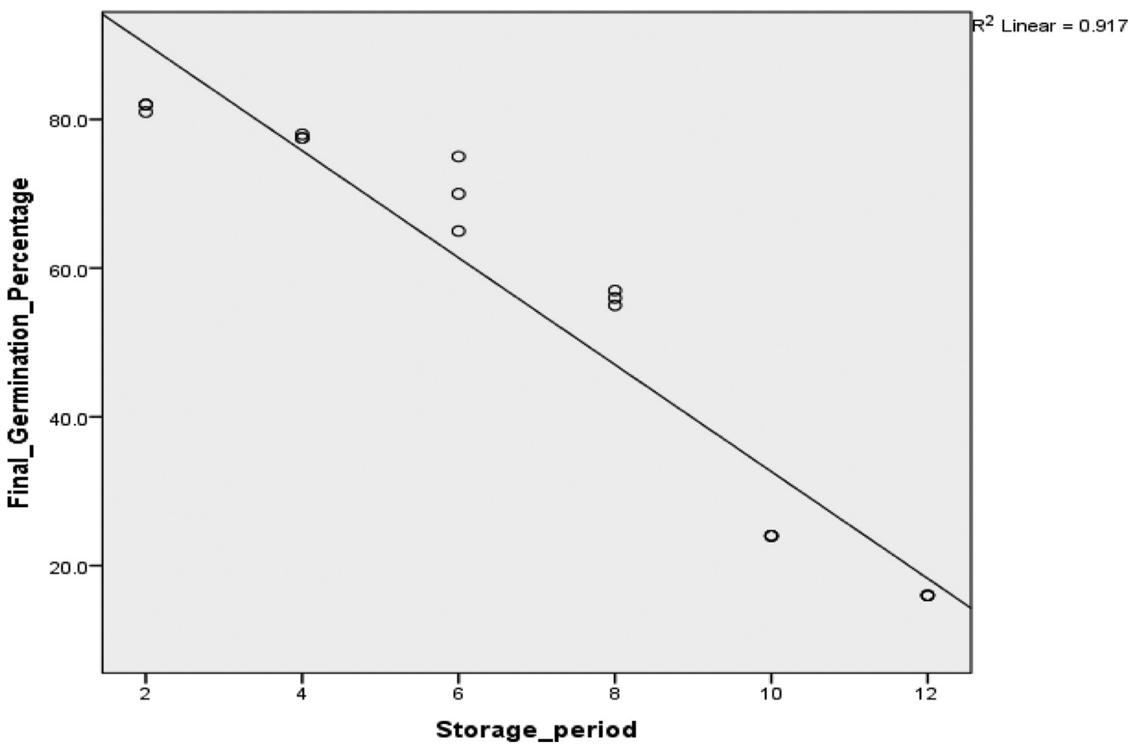

Fig. 3. Correlation between final germination percentage and storage period.

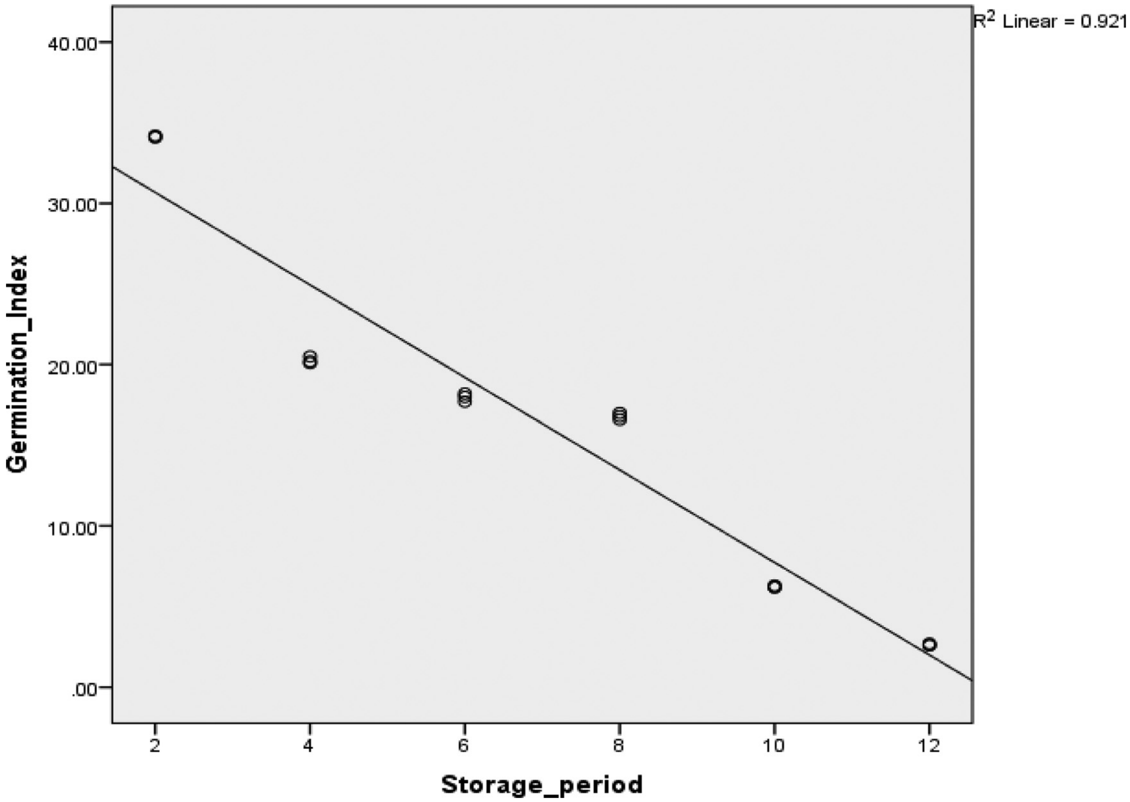

Fig. 4. Correlation between germination index and storage period.

changes in seed, which causes them to deteriorate and reduce their energy to germinate. Thus, seeds under storage lose their viability with time, which reduces the vigor with which their radicals penetrate through the endosperm and seed coat, and physically protrude or germinate. Verma and Tomer [41] and Yilmaz and Aksoy [43] made similar observations when they studied the germination characteristics of *Brassica campestris* and *Rumex scutatus* seeds respectively. The variations in the FDE and MET of *T. scleroxylon* seeds could be explained by the loss of viability due to aging associated with the different storage periods. Thus, *T. scleroxylon* seeds would achieve good germination results when they are stored for shorter periods than longer periods.

Final germination percentage and germination index

The duration of seed storage is important in plant gene bank management because it influences the Final Germination percentage (FGP) and Germination index (GI) of seeds, and the health and vigour of plant stands [28]. GI gives an indication of both the germination percentage and germination speed. Bench et al. [7] explained that high FGP and GI values denote high vigour and germination ability of a seed population. In an experiment to determine the effects of storage on the germination characteristics and enzyme activity of sorghum seeds, Azadi and Younesi [5] observed that longer duration of storage reduced the ability of the seeds to germinate than shorter durations. They found that seeds that were not stored (i.e., control; 0 day of storage) but directly sown attained over 90% FGP compared to the 25–50% FGP achieved by those stored for 180 days. After eleven-year storage period, the FGP of *Periploca angustifolia* Labill. reduced to 31% [1]. Singh [35] found that the FGP of *Pinus halepensis* seeds declined to as low as 18% after storing them under ambient/room conditions for 150 days. Arya and Arya [4] obtained similar results for the seeds of *Albizia lebbek*, *Casisia stomea* and *Prosopis specigera*. Acherkouk et al. [2] also reported differences in the germination rates of *Anthyllis cytisoïdes L.* seeds collected and stored for many years. Seeds that had been stored for 9 years (2006–2015) recorded 12.7% germination while those stored for 6 years (2009–2015) had 20.57% germination rate. Bowen et al. [9] tested the effects of different storage factors that are operative during the storage of *T. scleroxylon* seeds and concluded that at 20 °C, the seeds completely lose their ability to germinate after 20 days of storage. High GI (6.01) was recorded for *Zea mays L.* seeds, which were stored for 3 months compared to those stored for 42 months (1.01) by Garoma et al. [15]. Our results conform to the observations made by the authors mentioned earlier. High FGP and GI were recorded for seeds stored for shorter periods than those stored for longer periods. For example, the FGP and GI for seeds stored for 2 months were 82% and 34.1 respectively while those for seeds stored for 12 months were 16% and 2.6 respectively. This was further confirmed by the strong negative correlations recorded between FGP and storage period, and also between GI and storage period. Akhtar et al. [3] observed that decreasing FGP and GI was related to chromosomal aberrations, which occur under long storage periods. Longer periods of storage denature the protein content, reduce α-amylase activity and carbohydrate contents of seeds [6,27], which reduces their viability. This could explain the low FGP and GI recorded for *T. scleroxylon* seeds stored for long periods (10–12 months).

Conclusion

In this study, the effect of storage duration on the viability or germination capacity of *T. scleroxylon* seeds stored under room conditions for 12 months was investigated. The following conclusions can be made:

1. *T. scleroxylon* seeds tend to lose their viability with long periods of storage.
2. For maximum germination, the optimum storage period for *T. sceroxylon* seeds under room conditions is 2 months or less.

Declaration of Competing Interest

None.

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