

IMPACT OF STORAGE TEMPERATURE AND DURATION ON GERMINATION AND SEEDLING GROWTH OF AFRICAN STAR APPLE (*Chrysophyllum albidum*)

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Abstract

This research was carried out to assess the effect of storage temperature and duration on the germination and seedling growth of *Chrysophyllum albidum*. Completely randomized design with two factors (storage temperature and duration) was used for the experiment. Seeds were divided into three equal parts of 80 seeds each and stored at three different temperatures- Room temperature (25°C), Refrigerator (15°C) and Freezer (0°C). Twenty seeds were collected periodically (1, 2, 3 and 4 weeks) from each storage temperature and sown directly in sharp sand medium for germination with 10 seedlings transplanted into polybags thereafter for the aspect of the study on growth. Another twenty seeds of *C. albidum* were sown in a sharp sand medium immediately after collection (without storage) and 10 seedlings later transplanted into polybags to serve as control. Observation on germination was made daily and terminated after thirty days. Seedling height, leaf number and collar diameter were determined 14 weeks after sowing. Earliest emergence (14 days) was observed in Control and seeds stored at 15°C for 1 and 2 weeks; shortest duration (12 days) was observed in Control and seeds stored at 15°C for 1 week and highest germination percentage (85%) in Control and seeds stored at 15°C for 1 week while latest emergence (31 days) and longest duration (24 days) were observed in 0°C for 1 week and lowest germination percentage (50%) in seeds stored at 25°C for 4 weeks. A significant effect ($p \leq 0.05$) was observed in storage temperature and duration on seedling height; significant effects of storage temperature and non-significant effect of duration on collar diameter and non-significant effect ($p > 0.05$) of storage temperature and duration on leaf production and biomass. The interaction between these effects was not significant ($p > 0.05$). Immediate sowing of seeds of *C. albidum* is recommended; however, storage at 15°C for 1 or 2 weeks should be done when storage becomes inevitable.

Keywords: *Chrysophyllum albidum*, seed storage, temperature, duration, seedling growth

Introduction

Chrysophyllum albidum commonly called African star apple is a forest fruit tree species belonging to the family Sapotaceae and commonly found throughout tropical rain forest and coastal region of West Africa. There have been reports of it occurring naturally in Nigeria, Uganda, Niger Republic, Cameroon, and Côte d'Ivoire (Bada, 1997). The African star apple fruit is a sizable berry with four to five flattened seeds, or occasionally fewer because of seed abortion (Keay, 1989). The popular seasonal fruit is dark yellowish in colour and has semi-circular seeds. It is typically eaten in West Africa with its pericarp (Kadiri *et al.*, 2016). The seeds are usually discarded after the endocarp has been consumed. Ajewole and Adeyeye (1991) reported high lipid content (16.6%) with unsaturated fatty acids accounting for 74% of the oil extracted from the seeds, making it desirable in the context of heart disease risk reduction. However, Essien *et al.* (1995) reported that the lipid content was 3.2% and the unsaturated fatty acid content was 68% (oleic, 29.6%; linoleic, 38.4%). Preliminary investigation found that the seeds of *C. albidum* contain beneficial elements in varying amounts, including crude protein, crude fat, crude fiber, and mineral matter (Ogunleye *et al.*, 2020). It is a great source of nutrient, including vitamins, iron, and flavours for food and raw materials for several manufacturing industries (Adisa, 2000). The fruit has enormous commercial potential, especially after it was reported that its jam may rival raspberry jams and that it could be used to make jellies (Amusa *et al.*, 2003). The fruit has been reported to have very high content of ascorbic acid per 100g of edible fruit or about 100 times that of orange and 10 times that of guava and

cashew (Pearson, 1976). Additionally, the *C. albidum* fruit has 90% anacardic acid, which is used commercially to preserve wood and as a source of resin (Adewusi, 1997; Bada, 1997). To make wine and spirit, the fruits can be fermented and distilled (Orwa et al., 2009). The roots and leaves of the tree, among other parts of the tree, are used medicinally. The bole (trunk) is soft, open-grained, brownish-white, and exceedingly perishable when in contact with the ground. Its wood is good for construction work and the production of tool handles because it is simple to cut and plane, nails well, and takes a fine polish. (Orwa et al., 2009).

The capacity of the embryo to germinate is known as seed viability, and it is influenced by a variety of elements including temperature, light, oxygen, water, and species type (Genes and Nyomora, 2018). A key step for the long-term conservation of plant genetic resources is the storage of seeds as *ex situ* germplasm (Pradhan and Badola, 2012). Preservation of quality seed stock in storage for the next season is as important as producing quality seeds every year. According to Adam et al. (2017), seeds are hygroscopic by nature; they absorb and release moisture from and into the air around them until the vapor pressures off the air and seed moisture find balance. This can cause seeds to deteriorate quickly, especially in humid climates. Roberts (1988) also stated that seed moisture content, temperature, and storage periods are among the main factors affecting the relationship between the loss of seed viability during storage and the accumulation of genetic damage in seeds survival. Also, Pradhan and Badola (2008) reported that several variables, including seed type, storage environment temperature, seed moisture content, storage time, and relative humidity, affect how long seeds last when stored. As a result, the storage environment and the loss of seed viability during storage are closely related. (Parmar, 2018; Bhardwaj et al., 2014). Storage potential of seeds is also influenced by the relative humidity of the surrounding atmosphere (Adam et al., 2017).

Increased temperature and moisture content may encourage fungal growth, insect infestation, and increased metabolic activity in the seed, all of which could lead to a decrease in the ability of the seed to germinate (Harrington, 1972). Nasreen et al. (2000) also stated that germination of seeds declines rapidly during storage if temperature is not monitored properly. Bewley and Black (1985) pointed out that seeds stored in unfavourable conditions produce old seeds, which show a range of symptoms, from reduced viability or germinability to more or less full viability but with abnormal development of the seedlings, i.e. inadequate vigour. It is crucial to store seeds in optimum conditions to extend their shelf life since doing so maintains their quality, vigour, and viability by allowing physio-biochemical processes to proceed more slowly (McDonald and Copeland, 2005). However, with the right storage circumstances, seeds may effectively preserve a significant amount of vitality for an extended period of time (Pradhan and Badola, 2012). These methods are particularly important when dealing with endangered species, where the judicious use of seeds as precious genetic material through standardizing effective storage mechanisms is a must to boost species conservation programs (Pradhan and Badola, 2012).

The tree, which is highly prized by farmers and a crucial part of Benin's traditional agroforestry systems, is mostly disregarded by researchers in the area (Ekué et al., 2010). In order to mitigate the human influence on the biological structure of the forest, there is a need for regeneration and adequate silvicultural knowledge of the plant species. It becomes very important to examine how best to raise healthy seedlings for field establishment. This study therefore investigated seed germination and growth characteristics of *C. albidum* under different storage temperature and duration. It provided some information on the silviculture of *C. albidum*, its germination indices and seedling growth performance under different storage temperature and duration. Such information will be useful for the propagation of the species in order to sustain its usefulness to humanity.

Materials and Methods

Experimental Site

The study was conducted in a Plant Nursery domiciled in the Department of Forestry and Wildlife Management of the University of Port Harcourt, Nigeria. The nursery location lies on Latitude 04°53' 38.3"N and Longitude 00.6° 54' 38"E.

Fruit Collection and Processing

Mature fruits of *C. albidum* were harvested from a mother tree in a natural environment. The seeds were depulped manually so as to avoid damaging the seed. The floatation method of seed viability test was employed to detect the viable seeds. Seeds that floated after putting them in a container filled with water was regarded as non-viable and discarded while the seeds that did not float were regarded as viable and used for the experiment.

Experimental Design

The experiment was set up in a completely randomized design with two factors (storage temperature and duration). Two hundred and forty randomly selected seeds were used for the three-storage temperatures and four durations (i.e. 20 seeds x 3 temperatures x 4 duration = 240 experimental units). Seeds were divided into three equal parts of 80 seeds each and stored at three different temperatures (25°C-Room temperature, 15°C-Refrigerator and 0°C-Freezer). Seeds stored were placed in plastic containers. *C. albidum* seeds were periodically tested for their germination, viability at weekly interval for 4 weeks. (i.e. 1, 2, 3 and 4 weeks) with a sample of 20 seeds collected from each storage temperature and sown directly in sharp sand medium before transplanting 10 seedlings into polypots with a top soil growth medium. Twenty seeds of *C. albidum* were sown in a sharp sand immediately after collection (without storage) and 10 seedlings later transplanted into polybags which served as control for the treatments. Watering was done daily while weeding was carried out regularly and when required throughout the period of the experiment.

Data Collection

Germination

Observation on germination was made and recorded daily; this was terminated after thirty days (when there was no more germination). Germination percentage, emergence and duration were calculated as stated below.

1. Germination percentage (GP) = $\frac{\text{Total germinated seeds}}{\text{Total seeds sown}} \times \frac{100}{1}$ ----- (1)
2. Germination emergence (GE) = Number of days taken for plumule to emerge after sowing
3. Germination Duration (GD) = Number of days from the first to the last plumule emergence

Seedling Growth

Seedling height, leaf number and collar diameter were determined 14 weeks after sowing. Seedling height was measured from the substrate level to the tip of the youngest leaf using a meter rule; stem collar diameter was measured at the root collar using a digital Vanier calliper, while leaf and branch production were determined by directly counting the number of leaves and branches.

Biomass and Moisture Content

At the end of the experiment, five seedlings per treatment were carefully removed from the polypots and the root system exposed by carefully washing off excess growth media from the roots. Absorbent paper was used for blotting excess moisture from the plants. Seedlings were separated into shoot and root components; separation was at the soil line. The fresh weight of shoot (including the leaves) and root were taken before placing them in a paper bag for drying. The

samples were oven dried at 70°C for three days (72 hrs). Weighing was done using a digital weighing scale calibrated in grams (g) to determine fresh and dry weights and moisture content calculated using the following equation.

$$MC = \frac{FW - DW}{FW} \times 100 \quad (2)$$

Where MC = Moisture Content

FW = Fresh Weight

DW = Dry Weight

Data Analysis

Univariate analysis of variance was performed using General Linear Model in SPSS 16.0 for Windows to determine the effect of storage temperature and duration and their interaction on seed germination and seedling growth. Duncan multiple range test (DMRT) was employed to determine the variation in means.

Results

Effect of storage temperature and duration on the germination of *Chrysophyllum albidum*

Germination Emergence

The germination inception of seeds at different storage temperatures and durations is presented in Figure 1. The number of days which seeds of *C. albidum* took to emerge after sowing varied between 14 and 31 days. Earliest emergence (14 days after sowing) was observed in Control seeds and seeds stored at 15°C for 1 and 2 weeks; followed by seeds stored at 25°C for 1 week (17 days after sowing) while the most delayed emergence was observed in seeds stored at 0°C for 1 week (31 days after sowing). No germination was recorded for seeds stored at 0°C for 2, 3 and 4 weeks.

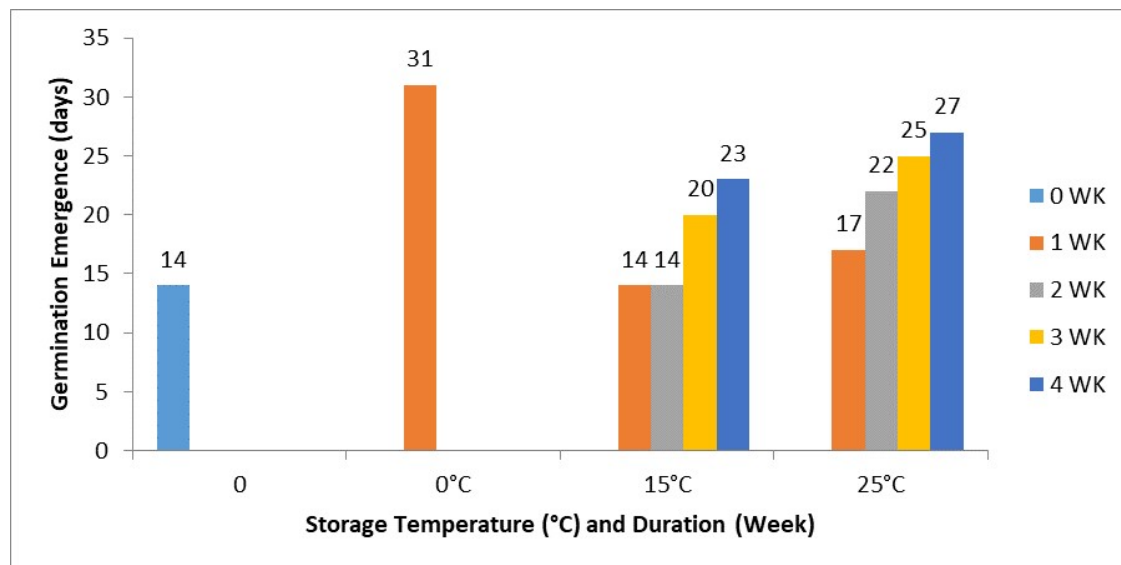


Figure 1: Germination emergence of *C. albidum* under different storage temperatures and durations

Germination Duration

The germination durations of seeds at different storage temperatures and durations are presented in Figure 2. Control seeds (no treatment) and seeds stored at 15°C for 1 week had shortest duration of (12 days), followed by seeds stored at 15°C for 2 weeks with duration of 13 days while the longest duration was observed in seeds stored at 0°C for 1 week (24 days).

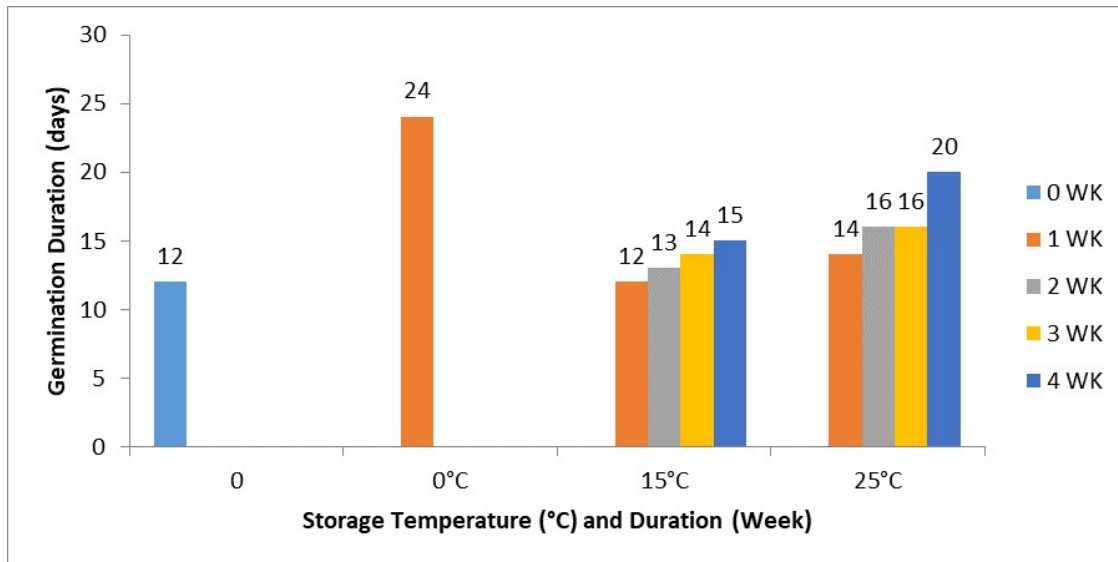


Figure 2: Germination duration of *C. albidum* under different storage temperatures and durations

Germination Percentage

The germination percentage of seeds at different storage temperatures and durations is presented in Figure 3. Germination percentage varied from 0 to 85%. Control and seeds stored at 15°C for 1 week exhibited highest germination percentage (85%), followed by seeds stored at 15°C for 2 and 3 weeks (75%) and seeds stored at 25°C for 4 weeks (50%) while seeds stored at 0°C for 2, 3 and 4 weeks did not germinate at all (0%).

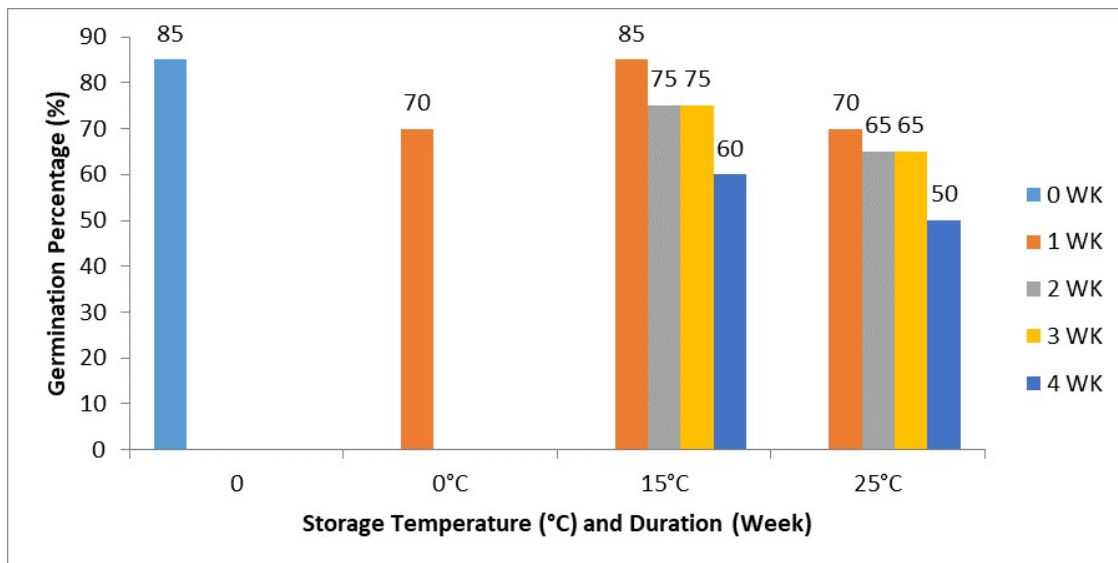


Figure 3: Germination percentage of *C. albidum* under different storage temperature and duration.

Effect of storage temperature and duration on the growth of *Chrysophyllum albidum* seedlings

A two-way ANOVA indicated significant effects ($p \leq 0.05$) of storage temperature and duration on seedling height; significant effects of storage temperature and non-significant effect of duration on collar diameter and non-significant effect ($p > 0.05$) of storage temperature and duration on leaf production and biomass. The interaction between these effects was not significant ($p > 0.05$) (Table 1).

Table 1. Two-Way ANOVA of the Effects of Storage Temperature and Duration on the Growth of *Chrysophyllum Albidum*

	d.f.	Seedling Height (cm)		Collar Diameter (mm)		Leaf Number	
		<i>F cal</i>	<i>P value</i>	<i>F cal</i>	<i>P value</i>	<i>F cal</i>	<i>P value</i>
Storage temperature	2	10.123	.000	3.994	.022	.163	.850
Storage duration	3	6.273	.001	2.041	.114	1.087	.359
Storage temperature x duration	3	1.189	.318	.167	.918	.109	.955

Seedling Height

Control seedlings had better growth in height (14.27 cm) when compared with seedlings from seeds stored at different temperatures and durations although it was not significantly different from seed stored at 15°C for 1 week (14.07 cm). The mean height of *C. albidum* seedling among different temperatures and duration at 14 weeks varied from 11.41 cm at 0°C to 12.92 cm 15°C (12.92 cm) followed by 25°C (11.69 cm) and from 11.07 cm in week 4 to 12.62 cm in week 1. Also, seedling height decreased continuously with increase in storage duration with 1-week storage exhibiting better seedling height while 4 weeks storage displayed worst growth in height (Table 2).

Table 2. Effect of Storage Temperature and Duration on Seedling Height at 14 Weeks after Sowing

Storage Temperature	Duration (Weeks)					Mean
	0	1	2	3	4	
0	14.27±0.51	*	*	*	*	14.27±0.51 ^a
0°C	*	11.41±0.51	*	*	*	11.41±0.51 ^c
15°C	*	14.07±0.51	12.82±0.51	12.66±0.51	12.11±0.51	12.92±0.25 ^b
25°C	*	12.37±0.51	12.21±0.51	12.14±0.51	10.03±0.51	11.69±0.25 ^c
Mean	14.27±0.51 ^a	12.62±0.29 ^b	12.52±0.36 ^b	12.40±0.36 ^b	11.07±0.36 ^c	

^{a,b,c}: Means with the same alphabet do not differ significantly (*p* > 0.05)

Collar Diameter

Highest collar diameter (1.956 mm) was observed in Control when compared with seedlings from seeds stored at different temperatures and durations but was not significantly different (*p* > 0.05) from those of seeds stored at 15°C for 1 week (1.846 mm), 15°C for 2 weeks (1.801 mm) and 25°C for 1 week (1.717 mm). Overall, the mean collar diameter of seedlings among different storage temperatures and durations at 14 weeks varied from 1.597 mm in 0°C to 1.744 mm in 15°C and from 1.578 mm in Week 4 to 1.720 cm in Week 1. Collar diameter was also observed to have decreased continuously with increase in storage duration with Week 1 having the highest value and Week 4, the lowest (Table 3).

Table 3. Effect of Storage Temperature and Duration on Seedling Collar Diameter at 12 Weeks after Sowing

Storage Temperature	Duration (Weeks)					Mean
	0	1	2	3	4	
0	1.956±0.87	*	*	*	*	1.956±0.87 ^a
0°C	*	1.597±0.87	*	*	*	1.597±0.87 ^b
15°C	*	1.846±0.87	1.801±0.87	1.672±0.87	1.655±0.87	1.744±0.04 ^b
25°C	*	1.717±0.87	1.608±0.87	1.599±0.87	1.502±0.87	1.607±0.04 ^b
Mean	1.956±0.87 ^a	1.720±0.05 ^b	1.705±0.06 ^b	1.636±0.06 ^b	1.578±0.06 ^b	

^{a,b,c}: Means with the same alphabet do not differ significantly ($p > 0.05$)

Leaf Production

Mean leaf number of seedlings among different storage temperatures and duration at 14 weeks varied from 2.08 in 25°C to 2.20 in 0°C and Control and from 2.00 in Week 4 to 2.20 in Week 1. Leaf number also decreased continuously with increase in storage duration with seedlings from Control seeds and seeds stored for 1 week, irrespective of temperatures, having highest collar diameter (2.20) and Week 4 (2.00), the lowest (Table 4).

Table 4. Effect of Storage Temperature and Duration on Leaf Production at 14 Weeks after Sowing

Storage Temperature	Duration (Weeks)					Mean
	0	1	2	3	4	
0	2.20±0.12	*	*	*	*	2.20±0.12 ^a
0°C	*	2.20±0.12	*	*	*	2.20±0.12 ^a
15°C	*	2.20±0.12	2.20±0.12	2.10±0.12	2.00±0.12	2.13±0.06 ^a
25°C	*	2.20±0.12	2.10±0.12	2.00±0.12	2.00±0.12	2.08±0.06 ^a
Mean	2.20±0.12 ^a	2.20±0.07 ^a	2.15±0.09 ^a	2.05±0.09 ^a	2.00±0.09 ^a	

^{a,b,c}: Means with the same alphabet do not differ significantly ($p > 0.05$)

Effect of Storage Temperature and Duration on Biomass Accumulation of *Chrysophyllum Albidum*

A two-way ANOVA indicated non-significant effects ($p > 0.05$) of storage temperatures and durations and their interaction on seedlings fresh weight, dry weight and moisture content (Table 5). Highest plant fresh weight was found in the Control (0.860g) followed by 15°C at Week 1

(0.844g) and 25°C at Week 1 (0.794g) while 25°C at Week 4 had the lowest fresh weight (0.636g). Also, higher dry weight was observed in the Control and 15°C at Week 1 (0.318g) and lowest in 25°C at Week 4 (0.242g). Moisture content was highest in the Control (0.542g) followed by 15°C at Week 1 (0.526g) and 25°C at Week 1 (0.502g) while 25°C at Week 4 had the lowest fresh weight (0.394g). (Table 6)

Table 5. Two-Way ANOVA of the Effects of Storage Temperature and Duration on Biomass Accumulation of *Chrysophyllum Albidum*

	d.f.	Fresh Weight (g)		Dry Weight (g)		Moisture Content	
		F cal	P value	F cal	P value	F cal	P value
Storage temperature	2	2.026	.145	2.971	.063	.584	.562
Storage duration	3	1.930	.140	2.049	.122	.655	.585
Storage temperature x duration	3	.303	.823	.111	.953	.189	.904

Table 6. Effect of Storage Temperature and Duration on Moisture Content of *Chrysophyllum Albidum* Seedlings

Storage Temperature	Duration (Weeks)					Mean
	0	1	2	3	4	
Plant Fresh Weight (g)						
0	0.86±0.05	*	*	*	*	0.86±0.05 ^a
0°C	*	0.78±0.05	*	*	*	0.78±0.05 ^{ab}
15°C	*	0.84±0.05	0.79±0.05	0.77±0.05	0.76±0.05	0.79±0.03 ^{ab}
25°C	*	0.79±0.05	0.74±0.05	0.73±0.05	0.64±0.05	0.72±0.03 ^b
Mean	0.86±0.05 ^a	0.81±0.03 ^{ab}	0.76±0.04 ^{ab}	0.75±0.04 ^{ab}	0.70±0.04 ^b	
Plant Dry Weight (g)						
0	0.32±0.02	*	*	*	*	0.32±0.02 ^a
0°C	*	0.27±0.02	*	*	*	0.27±0.02 ^a
15°C	*	0.32±0.02	0.29±0.02	0.28±0.02	0.28±0.02	0.29±0.01 ^{ab}
25°C	*	0.29±0.02	0.27±0.02	0.25±0.02	0.24±0.02	0.27±0.01 ^b
Mean	0.32±0.02 ^a	0.29±0.01 ^{ab}	0.28±0.01 ^{ab}	0.27±0.01 ^b	0.26±0.01 ^b	
Moisture Content (g)						
0	0.54±0.05	*	*	*	*	0.54±0.05 ^a
0°C	*	0.51±0.05	*	*	*	0.51±0.05 ^a
15°C	*	0.53±0.05	0.50±0.05	0.49±0.05	0.48±0.05	0.50±0.03 ^a
25°C	*	0.50±0.05	0.46±0.05	0.47±0.05	0.39±0.05	0.46±0.03 ^a
Mean	0.54±0.05 ^a	0.51±0.03 ^a	0.48±0.04 ^a	0.48±0.04 ^a	0.44±0.04 ^a	

^{a,b,c}: Means with the same alphabet for each evaluated attribute do not differ significantly ($p > 0.05$);

Discussion

Seed germination is a crucial stage in plant development and can be considered as a determinant for plant productivity (Ali and Elozeiri, 2017). Keeping seeds viable for a longer period of time is crucial to maintaining the genetic integrity of stored samples (Genes and Nyomora 2018). From this study, storage temperatures and durations had a great influence on the germination of seed. Seeds sown immediately after processing (Control) had shorter emergence and duration and

highest germination percentage. Germination emergence, duration and percentage were found to decrease with an increase in storage duration. This could be due to bio-chemical changes and natural aging which deteriorates the seed and reduces energy to germinate (Adam *et al.*, 2017). Reduced germination may be as a result of biochemical manifestation, membrane degradation, decrease in enzyme activity or changes in chemical constituents of the cell (Mubvuma *et al.*, 2013). Zheng and Ma (2014) reported that germination percentage reduced with increase in the number of incubation days in seeds of *Bombax ceiba*. According to Roberts (1972) and Villiers (1973), some seeds could be stored for a long term while others are very sensitive to storage and are therefore required to be sown immediately the mature seeds have been harvested to prevent physiological aging. Also, under different storage temperatures, germination varied with seeds stored at 15°C displaying better germination when compared with those stored at 25°C and 0°C (which had lowest emergence and duration). This finding agrees with that of Ellis *et al.* (1991) that optimum germination in *Carica papaya* occurred in seeds stored at 15°C when compared with those stored at cooler or hotter temperatures. Genes and Nyomora (2018) noted that seeds stored at high temperature (30°C) resulted in lowest germination ability than those stored at a lower temperature (15°C). On the contrary, it was observed that *Swertia chirayita* seeds stored at 4°C produced low mean germination time and higher germination percentage when compared to those at lower temperatures (Pradhan and Badola, 2012). According to Adelani *et al.* (2017), decrease in the germination percentage of *C. albidum* seeds with increased period of storage shows that they are recalcitrant in nature. Anandalakshmi *et al.* (2005) noted that recalcitrant seeds are shed at relatively high moisture contents and are sensitive to desiccation. The inability of seeds stored at 0°C to germinate after the first week of storage is an indication that freezing temperatures are not suitable for the storage of *C. albidum*.

The storage condition of the seed prior to germination is a determining factor in plant development and plant productivity (Ali and Elozeiri, 2017). Higher seedling height and collar diameter observed in seedlings from Control seeds and seeds stored at 15°C was expected as germination performance in both conditions was favourably comparable. According to Ali and Elozeiri (2017), seed germination significantly plays a major role in seedling growth of species. Germination, growth rate and gestation period are some of the unique features of the plants under cultivation (Iroko *et al.*, 2013). Also, seedling growth was observed to have reduced with increase in the duration of storage. This also agrees with the report of Zhang *et al.* (2015) that seedling growth of *Litchi chinensis* reduced with increase in storage duration but disagrees with that of Silveira *et al.* (2014) who noted that seedlings of *Moringa oleifera* had higher seedling growth performance with increase in storage duration. The physiological peculiarities and responses of seeds of different species to storage conditions and durations, may be responsible for the observed difference.

In addition, the higher biomass and moisture content observed in 15°C when compared with other storage temperatures is an indication that it is the most suitable storage temperature for *C. albidum*.

Conclusion

This study revealed that seeds of *C. albidum* are best sown immediately after extraction since the seeds are recalcitrant and can lose viability with storage. However, if the seeds need to be stored, 15°C is the most suitable storage temperature since it gave better germination and seedling growth performance than the other storage temperatures.

Recommendation

It is recommended therefore that farmers and foresters may store the seeds of *C. albidum* at 15°C so as to achieve better germination and growth results when immediate sowing is not possible. Storage of *C. albidum* seeds at freezing temperature (0°C) should be avoided as seeds lost viability beyond one week of storage.

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