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Original Article

Validating the Viability of Melia volkensii Seed Stored Extracted and in Nuts for Promoting Nature Based Enterprises and Conservation in Drylands, Kenya

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ABSTRACT

The development and supply of superior germplasm is important for promoting tree planting. Kenya has lost many tree seed sources through deforestation, land degradation, forest encroachment and conversion of agricultural land to housing. Nevertheless, limited access to quality tree seed is a major constraint to sustainable tree production in Kenya and effective storage of seed can ease its availability. *Melia volkensii* tree species is highly valued in the drylands of Kenya for its roles in social-economic, ecological, and environmental protection and conservation. This experiment aims at determining the viability of *Melia* seeds stored extracted and in nuts over one year through evaluation of seed germination. The research investigated the conducive environment that would favour the storage of *Melia* seeds to improve their viability status. The experiment showed that extracted *Melia* seeds stored at room temperature and a temperature of 4 degrees centigrade had higher germination capacities compared to *Melia* seeds stored at a temperature of negative 20 degrees centigrade. The peak average germination speed was 2.11, with a germination value of 3.99. Significant (p-value $\leq$ 0.001) differences were observed in the germination capacities between Melia stored as seeds and in nuts for the first, second, third, fourth, fifth, sixth and seventh germinations (p-value $\leq$ 0.05). From the study, *Melia* stored as seeds at a temperature of 4 degrees centigrade had the highest marginal germination capacity.

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INTRODUCTION

Melia volkensii is a multi-purpose indigenous tree species that has great potential in the Arid and semi-arid lands of East Africa (Mulanda et al., 1987; Indieka et al., 2007). It is highly valued in the drylands of Kenya for its roles in social-economic, ecological, and environmental protection and conservation. It is one of the tree species that faces extinction through unplanned harvesting. However, there have been efforts to conserve the species, where farmers are planting the species on-farm (Muok et al., 2010). *Ex* situ conservation approaches present the only option for conserving certain highly endangered and rare species (Ramsay *et al.*, 2000). *Melia volkensii* (Melia) can be propagated using cuttings (stem and root), seeds and wildlings. The use of seeds is the main method of raising Melia seedlings. It is, therefore, important to have a reliable seed supply system that ensures growers have access to high-quality seeds of Melia. Kenya has lost many tree seed sources through deforestation, land degradation, forest encroachment, and conversion of agricultural land to housing. Limited access to tree seeds of high quality is a major constraint to sustainable tree production in Kenya (Okeyo et al., 2020). For Melia, in particular, the seed supply is constrained by its bulkiness as the current practice is to store and distribute seed in their nuts.

Tree seed is normally stored and supplied as extracted seed to reduce bulk. Extraction denotes the procedure of physically releasing and separating the seeds from their enclosing fruit structure. The main rationale of extraction is to reduce bulk. The seeds typically comprise only 1-5% of the total fruit volume. Bulk reduction helps to reduce the cost of storing and handling. In current practice, it is assumed that seeds of Melia do not maintain viability well when stored as extracted seeds and are therefore normally stored within their endocarp as nuts and extracted just before sowing. The endocarp is a nut that's very hard and contains 1-5 seeds. Melia nuts are bulky compared to extracted seeds. One kilogram of Melia nuts yields about 200 seeds. The number of seeds per kilogram of extracted seeds ranges from 4,000 to 4,500, requiring the handling and storing of about 20 kilograms of nuts (Kamondo et al., 2016).

Storage and handling of extracted seeds in Melia is hardly practised because there's need for more information on the storability of extracted seeds. Seeds fall under three different seed storage behaviours: orthodox, recalcitrant, and intermediate. Orthodox seeds survive considerable desiccation and maintain viability with drying to a moisture content of up to 5%, with longevity in air-dry storage increased predictably by the reduction in seed moisture content and temperature (Hong et al., 1996). The difference between the three categories of seeds is based on seed maturation, environmental influences, and evolution. Temporal and spatial effects of water loss are a major environmental factor explaining the difference in desiccation resistance and longevity in seeds from various genetic backgrounds and growth conditions (Waters, 2015). Work has yet to be done to

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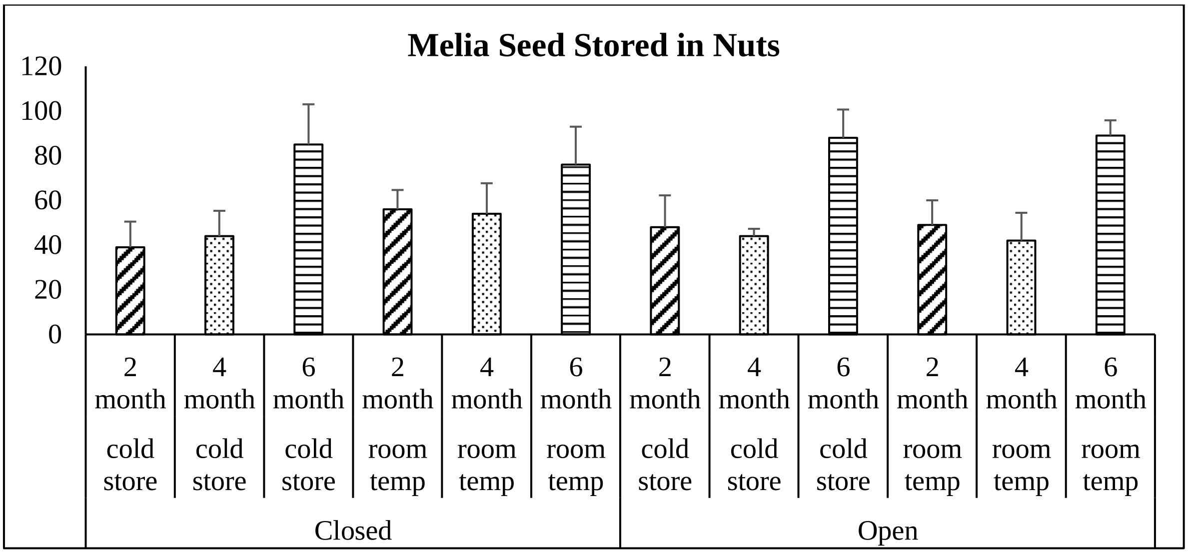
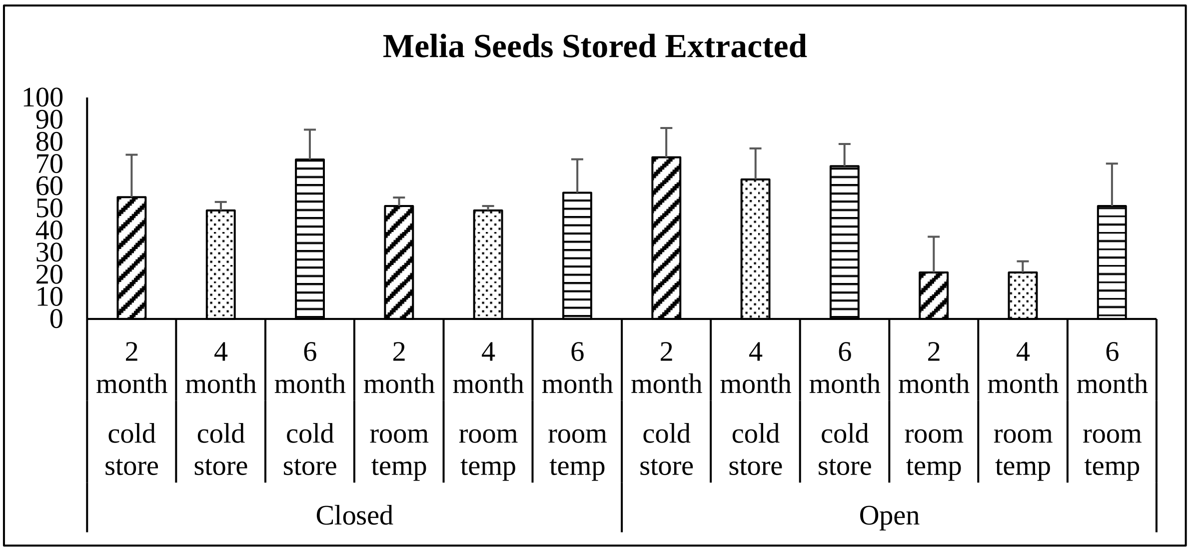
establish whether or not extracted Melia seed is orthodox.

An unpublished report of a preliminary experiment set up in 2018 to determine the viability of Melia seeds at the Kenya Forestry Research Institute (KEFRI) indicated that Melia seeds could be stored for up to 6 months either as extracted seeds or in nuts (Njehu et al., 2021). In general, the germination improved after 3-months storage. The germination at six months (*Figure 1*) was still high and the proposed work is to confirm for how long seed in nuts and extracted can be stored without losing viability. This paper reports on work undertaken to validate the preliminary experiment results while extending the storage period from 6 to 24 months. The results of this experiment will advise on how to manage seed handling and distribution of Melia.

Figure 1: Mean and standard deviation of germination % for all treatment groups





Research Objectives

- To determine whether Melia seed can be stored and distributed as extracted seed as opposed to the current practice of storing and distributing Melia seed as nuts
- Assessing the germination of Melia seeds stored either as extracted seeds or dried nuts.
- Evaluating the effect of storage conditions on germination of Melia seeds.

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Hypotheses

H_0 There is no difference in the viability of Melia seed stored as extracted seeds or in nuts.

H_1: Alternative: There is a difference in the viability of Melia seed stored as extracted seeds or in nuts.

METHODOLOGY

Quality mature fruits with 1/3 of the surface covered with brown patches (*Plate 1*) were collected from the Kibwezi Melia seed orchard at the University of Nairobi farm**,** whose GPS coordinates are 2 degrees 24 minutes South of the Equator, 37 degrees 57 minutes East of Greenwich. Matutre fruits were collected from at least ten clones, with each clone contributing roughly equal amounts (185 kg) from 12 ramets, with each ramet contributing 15 kgs of fruits; fruits were bulked before processing.

The collected fruits were de-pulped using a mallet and wooden plank. The nuts were cleaned with running water and dried in the sun for at least five days. Seeds were extracted by placing the nuts in a groove carved out on a wooden plank and cracking using a knife and hammer (Kamondo et al., 2016) (See *Plate 2*). A germination test was done on extracted seeds without subjecting them to storage to obtain germination at month zero (initial germination). The initial and subsequent germination trials were set at KEFRI nursery and at a Melia tree grower's farm (Kituku's Farm) in Kibwezi Sub-County upon subjecting the seed to requisite pre-sowing treatment. Initial germination was determined by a standard germination test of 4 replicates and each replicate with 100 seeds according to the International Seed Testing Association germination protocol (Soares et al., 2016).

In undertaking the germination test, seeds were nipped and disinfected in a solution of 5 grams of ridomil in one litre of water. They were subsequently soaked in cold water for 12 hours. Next, they were rinsed with 1% sodium hypochlorite solution. The seed coat was longitudinally slit using a sterilized razor blade. Seeds were germinated in plastic containers using fine, sterilized, clean river sand as the germination media (Dolor, 2009). The seeds were covered with a layer of sand drenched with fungicides/ ridomil (Muok et al., 2010). The number of seedlings germinated was recorded daily for a period of 10 days. Once scored, the germinated seeds were removed with a pair of forceps to ensure the daily counts were for germinated seeds.

The dried nuts were divided into two lots, with seeds extracted from one lot and the other remaining in nuts. Extracted seeds were dried to a moisture content of less than 10% moisture content. Seeds and nuts were stored in three different experimental conditions: room temperature (average 26 °C), in a cold store set at 4 °C and in a refrigerator at negative 20 degrees centigrade in sealed and unsealed plastic containers. The seed stored extracted and seed extracted from the nuts at the period of testing were tested for germination at one-month intervals for the next seven months, according to studies on the effect of storage conditions (Probert et al., 2007; Bharat, 2012; Okeyo et al., 2020). The germination at KEFRI Kibwezi and Kituku's Farm nurseries used a completely randomized block design consisting of 4 blocks with 25 seeds for each treatment in every block. Germination was recorded daily for a period of 10 days.

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Plate 1: Mature Melia fruits

Plate 2: Extracted Melia seeds and nuts

The data collected during the experiment included the number of germinated seed and the number of days taken to germinate. The germination capacity was calculated as a percentage using the number of seeds sown and the total number of seeds germinated. Data collection, visualizations, and analyses, i.e., coefficient of variation, germination value/ speed, means and variance of germination, were done using Excel and R software.

Objective	Analysis
Initial seed germination	Number of seed germinated
Germination re-tests during storage	• Germination value and speed • Co-efficient of variation during germination • Mean Daily Germination (MDG) • Germination capacity/ viability • Means and variance of germination

RESULTS

Germination Capacity

The experiment showed that the majority of the *Melia* seeds stored extracted at room temperature of 26 degrees centigrade ± 2 degrees centigrade, the temperature of 4 degrees centigrade and cold storage at negative 20 degrees centigrade germinated after seven days. Whereas, only a few *Melia* seeds stored in nuts germinated in KEFRI Kibwezi site. In the Kituku site, the majority of the *Melia* seeds stored as extracted seed germinated after seven days, and a slightly better germination capacity for *Melia* seeds stored in nuts as compared to the KEFRI site. The viability of Melia seeds regardless of storage form generally tends to decrease with an increase in storage time in both KEFRI and Kituku sites (*Table 1*, *Figure 2*

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Table 1: Descriptive Statistics

Main treatment	Experiment Site		Initial germ % 28/5/2020	First germ % 9/7/2020	Second germ % 6/8/2020	Third germ % 11/9/2020	Fourth germ % 13/11/2020	Fifth germ % 1/2/2021	Sixth germ % 15/03/2021	Seventh germ % 12/10/2021
Melia stored seeds	KEFRI/ KBZ	Mean	81.25	34.49	33.49	8.33	25.73	31.75	18.8	28.18
		Std. Err of Mean	1.584	3.34	1.94	1.52	2.03	2.64	1.9	3.26
	Kituku	Mean	84.33	34.27	27.28	38.75	14.2	26.9	40.93	29.79
		Std. Err of Mean	.945	1.29	1.21	4.3	1.71	3.33	4.47	3.91
Melia stored nuts	KEFRI/ KBZ	Mean	81.25	5.19	20.61	5.08	33.57	20.49	9.27	24.71
		Std. Err of Mean	1.584	.92	2.05	.96	1.83	2.15	1.03	2.7
	Kituku	Mean	85.75	40.24	22.15	11.25	18.96	10.53	10.31	17.09
		Std. Err of Mean	.728	2.82	2.68	1.87	1.95	2.17	3.99	2.22
Total	KEFRI/ KBZ	Mean	81.25	19.84	27.05	6.71	29.65	26.63	14.04	26.29
		Std. Err of Mean	1.11	2.74	1.68	.92	1.47	1.92	1.28	2.08
	Kituku	Mean	85.04	37.26	24.71	25	16.58	19.46	25.62	22.86
		Std. Err of Mean	.599	1.59	1.5	3.07	1.33	2.39	3.71	2.33

Key: Germ % - germination percentage

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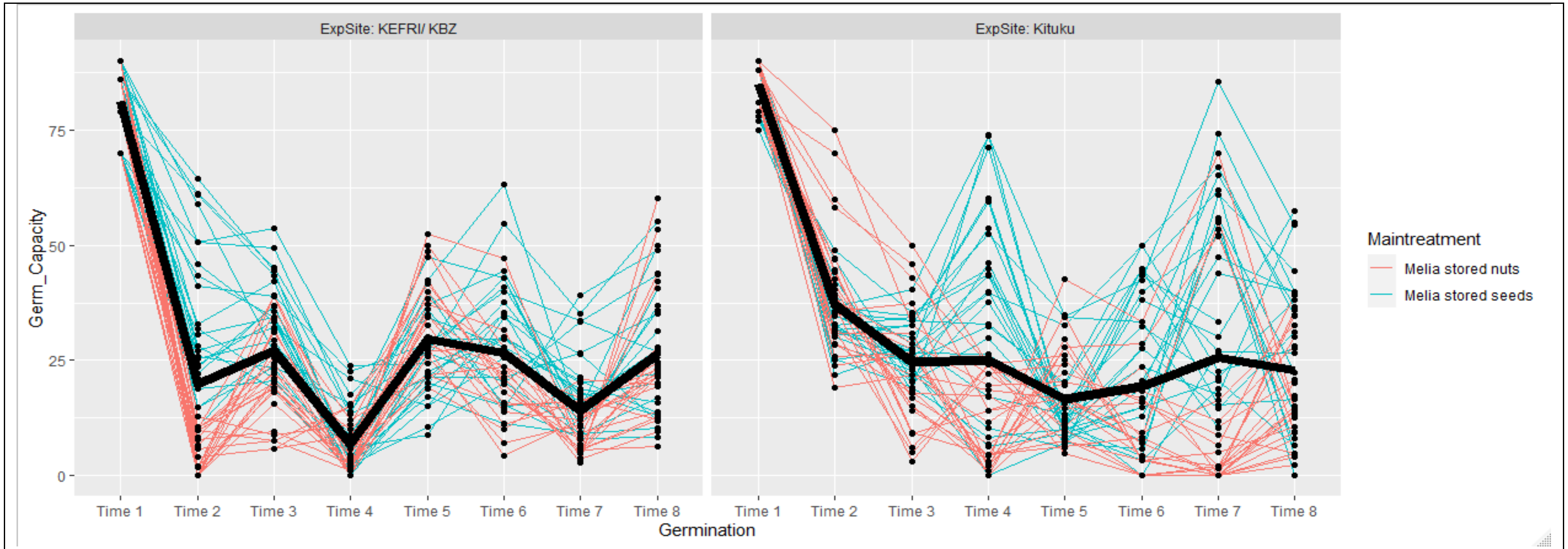
Figure 2: Longitudinal plots showing germination capacities for Melia stored as seeds and in nuts at different germination periods



Legend: Time 1: Initial germination on 28/5/2020; Time 2: First germination on 9/7/2020; Time 3: Second germination on 6/8/2020; Time 4: Third germination on 11/9/2020; Time 5: Fourth germination on 13/11/2020; Time 6: Fifth germination on 1/2/2021; Time 7: Sixth germination on 15/03/2021; Time 8: Seventh germination on 12/10/2021

Germination Value

Germination Value (GV) was derived using a formula proposed by Czabator (1962);

$GV = MDG * PV$ where MDG – mean daily germination and PV – peak value. MDG was calculated by dividing the germination capacity (%) by the number of days to the end of the experiment. The average germination speed peaked during the fourth [13/11/2020] and fifth [1/2/2021] germination periods (*Table 2*).

$$GV = 1.89 * 2.11 = 3.99$$

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Table 2: Average germination speed

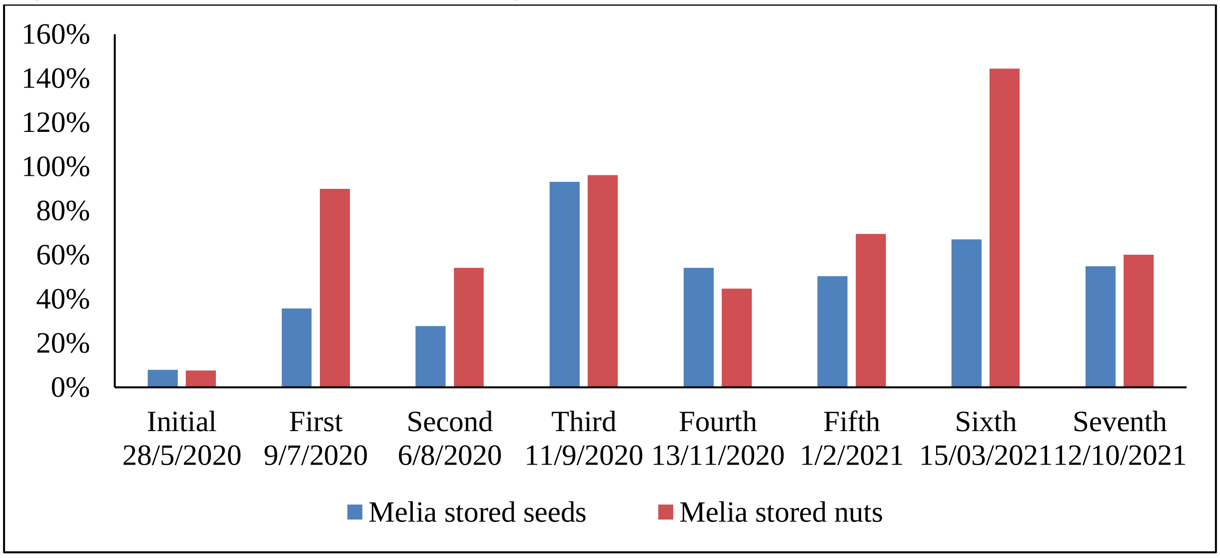
Germination period	Average Germination Speed
First	1.94
Second	1.99
Third	1.44
Fourth	2.1
Fifth	2.11
Sixth	1.53
Seventh	1.89

Co-efficient of Variation

Overall, Melia seed stored extracted had a lower co-efficient (CoV) variation, 67.8%, compared to Melia seed stored in nuts, 98.8%. Across the germination periods, the viability of Melia seed stored in nuts had a higher CoV as compared to Melia stored as extracted seeds (Figure 3).

$$coefficient\ of\ variation = \frac{\sigma}{\mu} * 100 \quad where;\ \sigma - Standard\ deviation, \mu - mean$$

Figure 3: Coefficient of variation across germination periods



Analysis of Variance

We restructured the data and applied log transformation before testing for equality of mean germination capacities using Analysis of Variance (ANOVA test). Levene's test was used to test the homoscedasticity assumption. Levene's test had a *p*-value ≤ 0.000; thus, the differences in the germination capacities were unlikely to have emanated from sampling a population with equal variance. We conclude by rejecting the null hypothesis and noting that there was a difference between the variances in the population.

Table 3: Levene's Test of Equality of Error Variances [a, b]

		Levene Statistic	df1	df2	Sig.
Germination capacity	Based on Mean	4.829	11	711	.000
	Based on Median	4.448	11	711	.000

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	Based on the Median and with adjusted df	4.448	11	634.397	.000
	Based on trimmed mean	4.718	11	711	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a. Dependent variable: Germination capacity
*b. Design: Intercept + Main treatment + Sub treatment + Treatment + Main treatment * Sub treatment + Main treatment * Treatment + Sub treatment * Treatment + Main treatment * Sub treatment * Treatment*

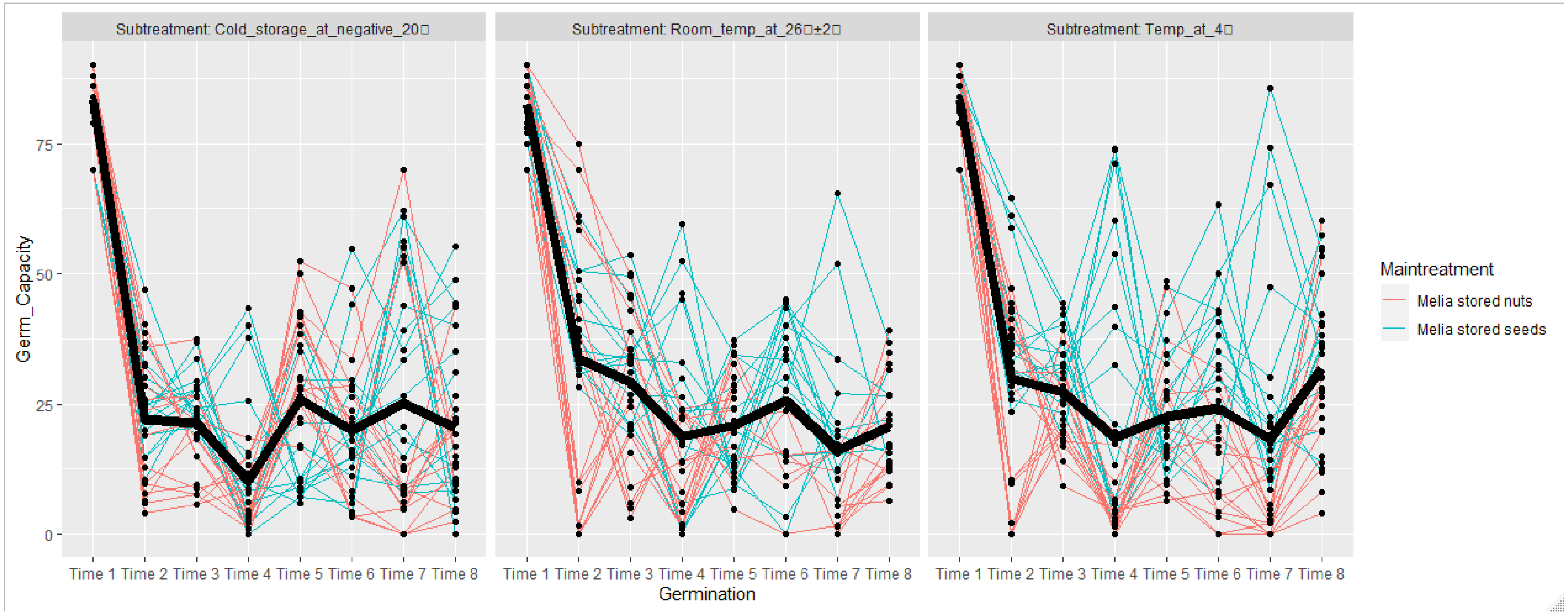
An Analysis of variance indicated that there were significant (p-value ≤ 0.001) differences in the germination capacities among Melia stored as extracted seeds and in nuts [main treatment]. No significant (p-value = 0.159) differences were observed in the viability for seeds extracted. and seed in nuts stored under room temperature, cold storage, and a temperature of 4 ^{0}C [sub-treatment]. Similarly, no significant (p-value = 0.725) differences were observed in germination capacities for seeds extracted and seed in nuts exposed to different treatments (open and closed germination). Furthermore, there were no significant differences in germination capacities for seed stored extracted and seed stored in nuts when exposed to a combination of treatments [main treatment * Sub-treatment, p-value = 0.174; Main treatment * Treatment, p-value = 0.191; Sub treatment * Treatment, p-value = 0.264; Main treatment * Sub treatment * Treatment, p-value = 0.058] (*Table 4*).

Table 4: Analysis of Variance

Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**	**Partial Eta Squared**
Corrected Model	9.714[a]	11	.883	5.429	.000	.077
Intercept	1293.199	1	1293.199	7950.227	.000	.918
Main treatment	6.718	1	6.718	41.303	.000	.055
Sub treatment	.600	2	.300	1.845	.159	.005
Treatment	.020	1	.020	.124	.725	.000
Main treatment * Sub treatment	.570	2	.285	1.753	.174	.005
Main treatment * Treatment	.278	1	.278	1.711	.191	.002
Sub-treatment * Treatment	.434	2	.217	1.333	.264	.004
Main treatment * Sub treatment * Treatment	.933	2	.466	2.867	.058	.008
Error	115.653	711	.163			
Total	1430.818	723				
Corrected Total	125.367	722				
R Squared = .077 (Adjusted R Squared = .063)						
Dependent Variable: Germination capacity						

From the experiment, no significant differences were observed in the germination capacities between different sub-treatments. Melia stored as seeds at a temperature of 4 ^oC had the highest germination capacity achieved during the sixth germination. Whereas seed in nuts stored in cold temperatures of negative 20 degrees centigrade had the highest germination capacities, the highest achieved during the sixth germination (*Figure 4*).

Figure 4: Average germination (%) capacity for extracted seeds and seed in nuts stored under different conditions (Cold storage, room temperature [26 ºC] and at 4 ºC).



Legend: Time 1: Initial germination on 28/5/2020, Time 2: First germination on 9/7/2020, Time 3: Second germination on 6/8/2020, Time 4: Third germination on 11/9/2020, Time 5: Fourth germination on 13/11/2020, Time 6: Fifth germination on 1/2/2021, Time 7: Sixth germination on 15/03/2021, Time 8: Seventh germination on 12/10/2021

The germination speeds were high during the initial germination, with an average of 10.1875. After storage, Melia seeds stored at a temperature of 4 ºC had the highest average germination speeds (*Figure 5*).

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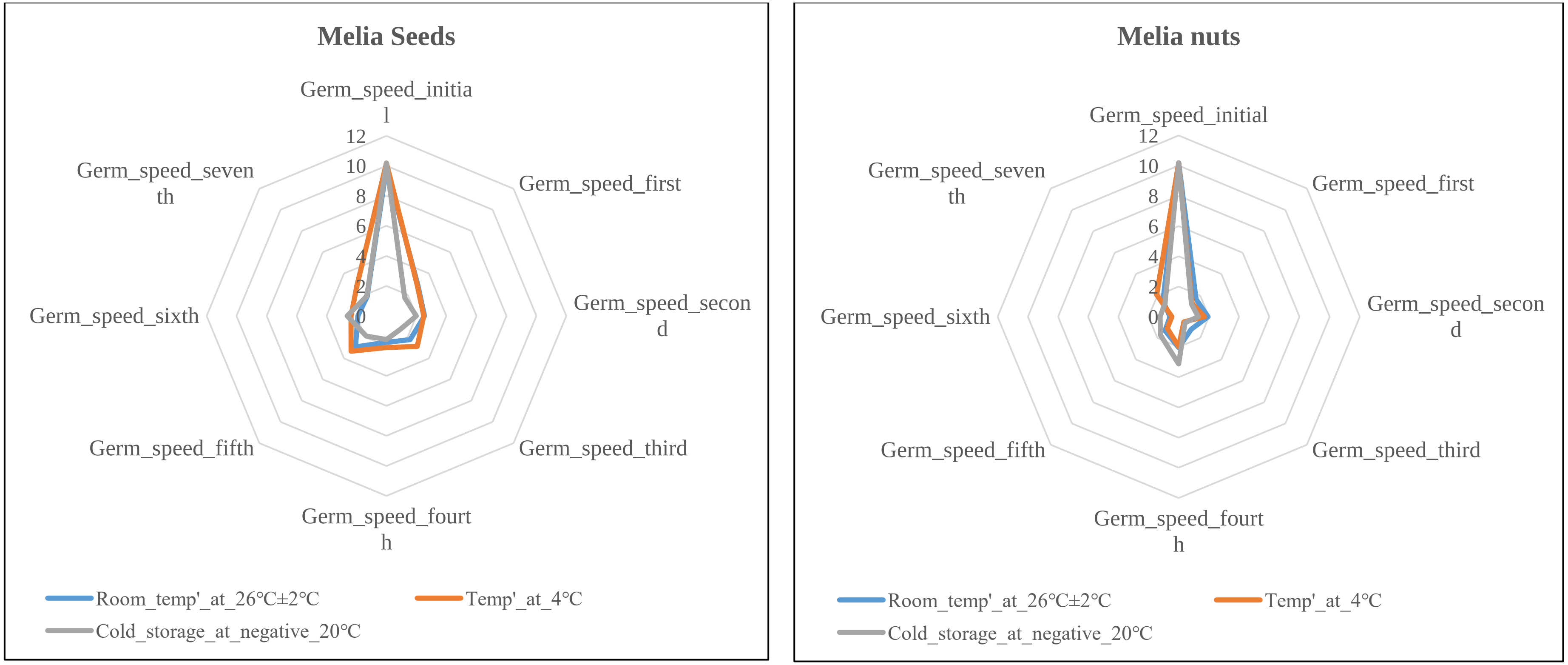
Figure 5: Average germination speeds for Melia seed stored extracted and in nuts at different temperatures



For Melia seed stored extracted and in nuts, there were no significant differences in germination capacities for seeds stored in open and closed containers under different temperatures. *Figure 6* shows that seeds stored in closed containers at 4 °C had the highest viability, whereas those stored in cold storage had the least viability. *Figure 7* shows that nuts stored in open and closed containers at room temperature had both the highest and lowest germination viability, respectively. Comparing *Figures 6* and *7*, Melia stored as seeds had a better viability index as compared to Melia seed stored as nuts.

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Figure 6: Marginal mean viability for Melia seed stored extracted

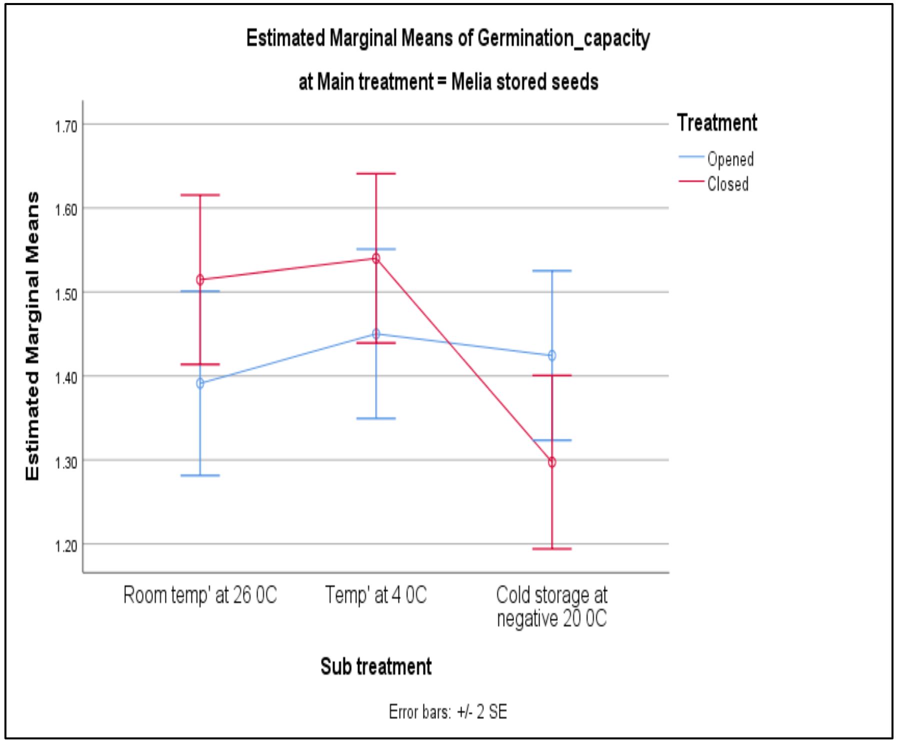
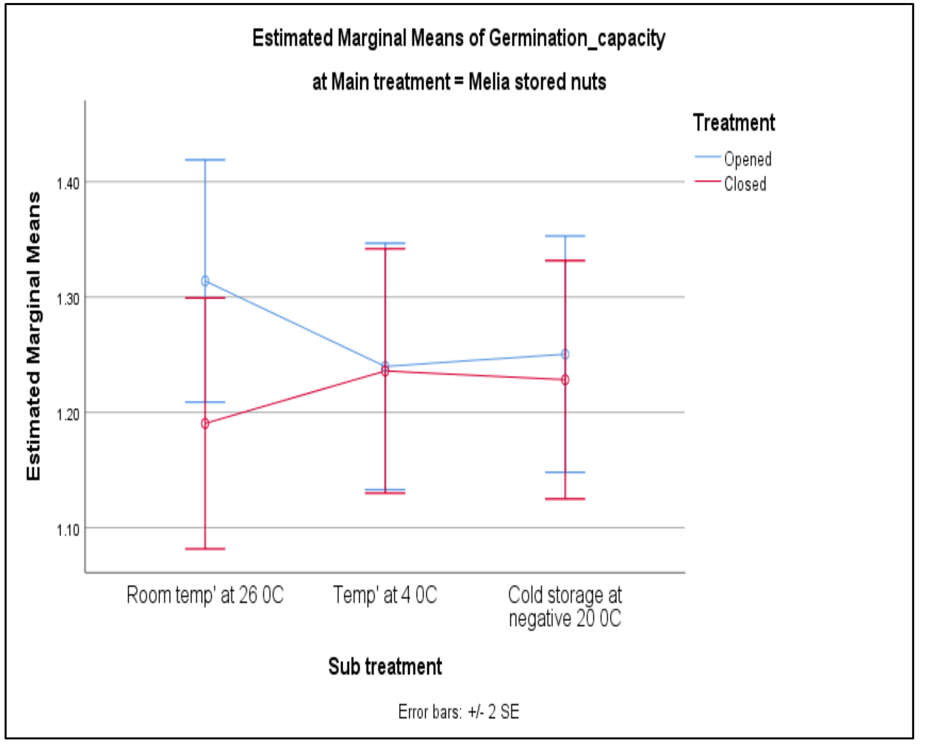


Figure 7: Marginal mean viability for Melia seed stored in nuts



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DISCUSSION

Germination capacity of Melia seed stored extracted and in nuts generally tended to decrease with increased storage time in both KEFRI and Kituku sites regardless of storage conditions. However, the decrease is not drastic to qualify the seed as recalcitrant. Melia stored as seeds had the best average germination speeds across all germination periods except for the sixth. The significance of germination speed in Melia is important on one hand due to the susceptibility of the seed to fungal attack. However, since the germination period of Melia is completed over a short period of time, the speed of germination alone might not be a very important criterion on making a decision on whether to store seed as extracted or in nuts. Figure 6 shows that seeds stored in closed containers at 4 degrees centigrade had the highest viability, whereas seeds stored in closed containers at cold storage had the least viability. Figure 7 shows that seed stored in nuts stored in open and closed containers at room temperature had both the highest and lowest germination viability, respectively. Comparing Figures 6 and 7, Melia stored as seeds had a better viability index as compared to Melia seed stored in nuts. The best viability was achieved when Melia was stored as seeds at a temperature of 4 degrees centigrade.

The seed germination curves plotted are not smooth, with seeds registering higher germination capacities in subsequent months than in previous ones without a smooth incline or decline. This characteristic had also been reported in the preliminary study and explained by the possibility of the existence of post-harvest ripening in Melia. The work undertaken in this trial note that the fluctuation is too erratic to be attributed to post-harvest ripening only. Rather we suggest that Melia seed is also very sensitive in the application of its elaborate pre-sowing treatment as well as the weather conditions prevailing during the planting. The ideal temperature for the sowing of Melia is prescribed as around 30 degrees centigrade. Granted that the trials were set in open nurseries, it is plausible that the prevailing temperature played a big role in resultant germination.

The current results indicate that *Melia* seed can be stored in closed containers at room temperature (26 ± 2 degrees centigrade). Maintenance of seed viability with drying to a moisture content of less than 10% is characteristic of orthodox seed (McDonald, 2004). Such seed is expected to have their viability preserved much better in cold storage, consistent with the storage behaviour of orthodox seed (Hong et al., 1996). However, the results of the experiment do not tally with this expectation. It is plausible that Melia seed has intermediate or orthodox storage behaviour. Intermediate seeds generally tolerate desiccation to moisture contents in equilibrium with about 40-50% relative humidity. i.e., about 10% moisture content to 7%. Another feature of intermediate seeds of tropical origin is the fact that the longevity of dry seeds (7-10% moisture content) is reduced with a reduction in storage temperature below about 10 °C (Ellis *et al.*, 1990; Okeyo et al., 2020)

CONCLUSIONS/ WIDER IMPLICATIONS OF FINDINGS

From the experiment, we reject the null hypothesis and conclude that there is a difference in the viability of Melia stored as extracted seeds or in nuts.

The study observed that in both KEFRI/KBZ and Kituku sites, *Melia* seed stored extracted had higher germination capacities than seed stored in nuts. *Melia* seeds stored at a temperature of 26 ± 2 degrees centigrade (room temperature) had higher germination capacities as compared to *Melia* seeds stored at cold storage negative 20 degrees centigrade. Notably, there were no significant differences in the mean germination capacities between *Melia* stored in open or closed containers.

Thus, it can be concluded that the ideal storage conditions to ensure optimum germination capacity for the seeds is that: *Melia* should be stored in the form of seeds at room temperature

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(26 ± 2 degrees centigrade) in closed containers. This would reduce the storage bulk, as storing seeds would be favoured over storing nuts.

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“The views expressed in this information product are those of the author(s) and do not necessarily reflect the views or policies of KEFRI”.

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