Research Article

Optimizing Germination of *Melia volkensii* Gurke after Storage of Seeds and Nuts in Different Storage Conditions

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Abstract

This study was conducted to understand the effect of nature of seed (nut or seed), storage conditions and storage time on the germination of *Melia volkensii* seeds. Two seed lots were stored for germination tests after storage of germination materials for 2, 4 and 6 months. Laboratory experimental setup involved three treatments: 1) some seeds were extracted while others were stored as nuts, 2) Storing the seeds in open and closed containers and 3) storage of the seeds in ambient temperature at 30 °C and cold storage at -20 °C. It was observed that the rate of germination and germination capacity of *M. volkensii* seeds increased with extension of storage time. Germination percentages were analyzed using R and SAS statistical software. Significance of variation for each month was tested using ANOVA at $P \leq 0.05$, and means were separated using fisher's protected least significant difference test. Seed germination after 6 months of storage greatly improved for all storage treatments. The scientific procedure in the present study improved germination from 14 % when seeds were stored for 2 months in open containers at room temperature to 89% when nuts were stored in open containers in room temperature for 6 months. This study is vital in understanding seed storage behavior which is essential in planning and carrying out planting programmes; consequently, enhancing sus-

tainable utilization of *M. volkensii*. Findings on appropriate storage of nuts and seeds of *M. volkensii* will help develop a guideline on establishing a seed stock to ensure seed supply at all times even in times of irregular fruiting.

Keywords: *Melia volkensii*; Germination; Room temperature; Cold storage; Seeds; Nuts and storage containers

1. Introduction

Melia volkensii is a multi-purpose indigenous tree species that has great potential in the arid and semi-arid of East Africa [1, 2]. It is one of the tree species that faces dangerous extinction through unplanned harvesting. However, there have been efforts of conserving the species, where farmers are planting the species on-farm [3]. Ex situ conservation approaches represent the only option for conserving certain highly endangered and rare species [4]. *Melia volkensii* is a prolific seeder, though its mass-multiplication has been constrained by problems in propagation through seed and conventional stem cuttings [5]. Attempts to propagate this species have been constrained by difficulties in seed dormancy and high post-germination mortality [6]. The use of seedlings rather than cuttings should be recommended when promoting the use of this species on dry land farms. Further, storage and handling of extracted *M. volkensii* seeds is not practiced because there is little information on storability of extracted seeds and further research needs to be carried out. Due to difficulties associated with raising seedlings, farmers routinely use wildings that arise from naturally dispersed seed or injured roots [7].

Seeds of plants fall under three different seed storage behavior namely orthodox, recalcitrant and intermediate seeds. The difference between the 3 categories of seeds is based on seed maturation, environmental influences, and evolution. Orthodox seeds are also referred to as drying-tolerant seeds. Their longevity is determined by moisture content; they are able to germinate after stored for many years if stored in hermetic containers. Orthodox seeds can be dried, without injury since their longevity rises with reduction in seed storage moisture content and temperature [8]. Recalcitrant seeds are stored for a short period of time, at a moisture content that is above 20-30 %, since they do not withstand drying. Injury of these seeds could occur at relatively high temperatures.

These seeds are desiccation sensitive and they lose water easily and can initiate germination or deterioration at very high rates. Intermediate seeds fall between the orthodox and recalcitrant seeds. These seeds are favored by moderate drying, but are destroyed by extreme drying [9]. Temporal and spatial effects of water loss are a major environmental factor that explains the difference of desiccation resistance and longevity in seeds from various genetic backgrounds and growth conditions [10].

Melia volkensii can be propagated through three methods namely, vegetative, seeds and wildlings. Use of seeds is the main method of raising *M. volkensii* seedlings [3]. Attempts to raise *Melia volkensii* through stem cuttings and tissue culture have been undertaken by researchers with limited success [11]. Propagation of this tree species using seed and cuttings has given variable results among different researchers which need to be harmonized [12]. Initial seed germination test is carried out immediately after harvest for the hard seeded species and accessions found in some leguminous plants and crop wild relatives can be as low as 45 % and rise after 10-15 years to 95 % or more after storage [13]. The storage and supply of *M. volkensii* seeds is currently based on handling of nuts rather than extracted seeds.

A Melia nut is bulky compared to the enclosed seeds. Good seed storage conditions maintain germ plasm viability. The storage temperature defines the maximum longevity for a seed sample and a stable storage environment is critical to maintain seed viability [14]. Storage standards are intended to ensure that seeds are stored at optimum moisture content.

1.1 The objectives

- Assessing the germination of *M. volkensii* seeds stored either as extracted seeds or dried nuts.
- Evaluating the effect of storage conditions on germination of *M. volkensii* seeds.

1.2 Significance of the study

This study will guide on appropriate practice in storing and handling of *M. volkensii* seeds. Further, this research will provide data on post and pre-storage extraction since storage and distribution of bulky nuts is expensive as they occupy large space. The study will also promote germination of *M. volkensii* and will develop better protocols to facilitate propagation of *M. volkensii*.

2. Materials and Methods

2.1 Study site

High quality mature fruits were collected from Tiva forest in Kitui Central, 194 km East of Nairobi along Kitui-Machakos road [15]. The experiment on germination of *M. volkensii* seeds was set in the nursery beds of KEFRI Kitui, 15km away from Tiva forest. The study area is located at Kitui County at latitude 2 ° 10’ 22’’ South, and longitude 38 ° 0 1’ 0” East and has an altitude between 400 m to 1830 m above sea level [16].The average annual rainfall is between 500-900 mm which has a bi-modal pattern though poorly distributed and erratic. The County is mostly dry and hot with temperatures ranging between 14 °C during the coldest months (July-August) and 34 °C during the hottest months (January-March). This area is conducive for the growth of *Melia volkensii*, a multipurpose, fast growing tree that thrives well in the dry lands of Kenya [3]. This study was conducted in April 2018, a month that is the peak fruit production season for *Melia volkensii* in the study area.

2.2 Experimental design

The collected fruits were de pulped by placing an individual fruit on a piece of stone and hitting with a plank of wood. The nuts were placed in a groove carved out on a wooden plank and underwent cracking using a knife and hammer Kamondo [17]. Seed collection was further improved by cleaning the nuts and drying them in the sun for at least 5 days [7]. The germination materials were divided into 2 lots with seeds being extracted from 1 lot and the other seeds remaining in nuts. Seed lots were later divided into two sub lots referred to as ‘fresh seed’ and ‘dry seed' (fresh seeds were sown immediately after collection, while the dry seeds were sown after drying them for a period of 5 days). A germination test was undertaken at month zero/ initial germination using 400 seeds. The 'dry seed' sub lots were dried to a moisture content of 6 % which was determined by oven drying at 103°C for 24 hours [18]. Germination was determined by standard germination test of 4 replicates each replicate with 100 seeds according to International Seed Testing Association germination protocol, [19].

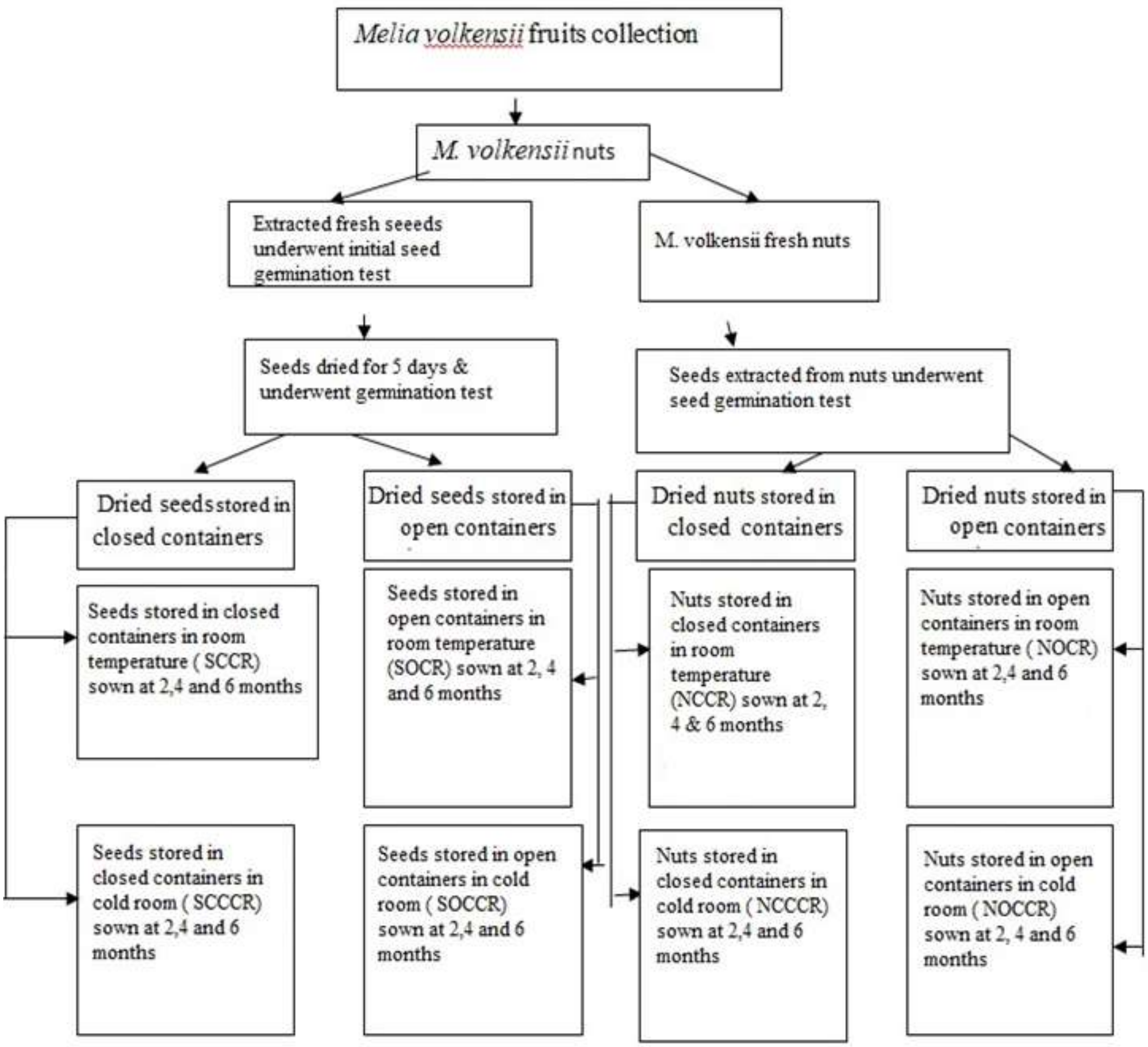

Figure 1: The experimental design diagram showing the steps from *Melia volkensii* fruit collection up to seed storage and germination.

Seeds were germinated in plastic containers using fine, sterilized clean river sand as the germination media [20]. For each germination test, seeds were nipped and disinfected in a solution of 5 grams of ridomil in one litre of water. They were later soaked in cold water for 12 hours. Later they were rinsed with 1 % sodium hypochlorite solution. The seed coat was longitudinally slit using a sterilized razor blade. The seeds were covered with a layer of sand drenched with fungicides/ ridomil [3]. 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 seeds that had germinated.

The remaining seed lots were stored in three different experimental conditions, namely: room temperature (30 °C), and in refrigerator at −20°C, in properly sealed and unsealed plastic containers. The seeds were periodically tested for their germination viability at two-month interval for the next 6 months according to studies on effect of storage conditions (Bharat, 2012) [15]. After the storage period was over, a completely randomized block design consisting of 4 blocks with 25 seeds were sown in the nursery for each treatment in every block. The rate of germination was recorded for a period of 10 days.

2.3 Statistical analysis

Data analysis was performed to test for the assumptions of the two-way ANOVA whereby at 95 % confidence interval, t-test was used to check for germination trend of extracted seeds and nuts stored at different environmental conditions and in different storage containers. The data was subjected to analysis for best model to use by comparing the additive, interactive and blocking models using R statistical software. A two-way ANOVA was conducted to examine the effects of storage specifications and time of storage on transformed germination. Germination means were separated at $P \leq 0.05$ by Fisher's least significant difference procedure test. Tukey test analysis was undertaken to confirm the origin in the main effects and interactions effects picked in the ANOVA.

The test was used to tell which groups were different between storage time and storage conditions. Mean germination time was used as a tool to further improve the practical value of the results through recommendation of storage conditions that would maintain high germination and vigor of the seeds.

3. Results

Melia volkensii stored as seed and nuts whether at cold store or in room temperature in both open and closed containers remained viable when stored for 6 months (Figure 2). In actual fact, seed germination improved with storage for almost all storage specifications expect when nuts were stored in open containers in the cold store for 2 and 4 months respectively. A slight drop in germination at month two and four was observed but the seeds improved in germination at 6 months. This is illustrated in figure 2 below.

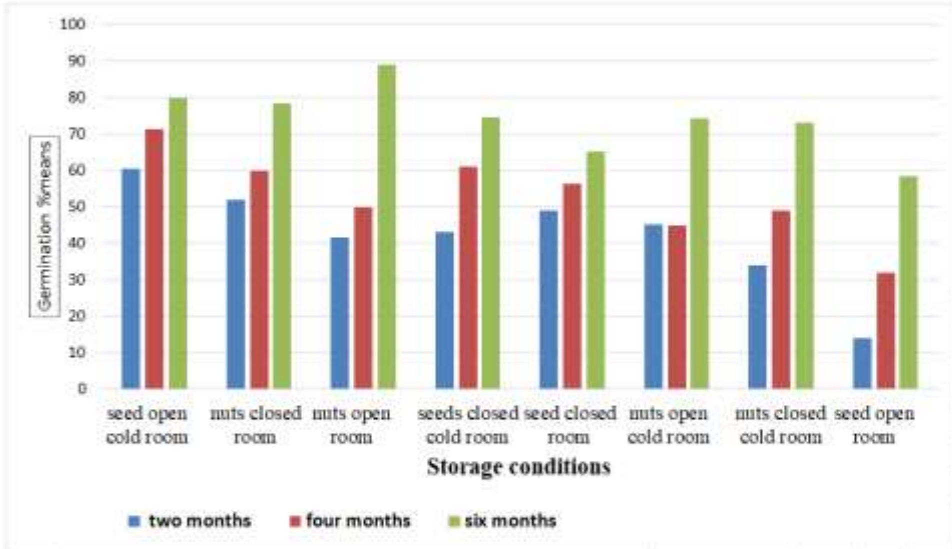

Figure 2: Germination of *Melia volkensii* seeds stored for 0, 2, 4 and 6 months.

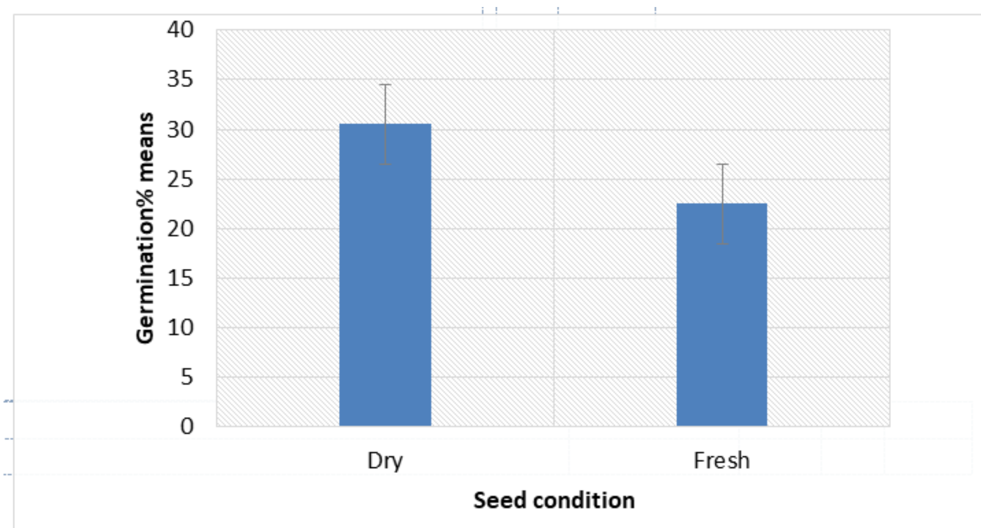

Figure 3: Initial germination percentage of *M. volkensii* of fresh and dried seeds.

At P≤0.05, there was no significant difference between fresh and dried seeds before storage. The dried seeds had a higher mean germination percentage than the fresh seeds (Figure 3). Seed germination after 6 months of storage greatly improved for all storage specifications from the initial germination since when dried seeds were sown they registered 30.5% (Figure 3). Germination increased drastically registering as high as 89.0 % when planting materials were stored in form of nuts in open containers in room temperature for 6 months. The lowest germination when seeds were stored for 6 months in open containers in room temperature had germination of 14% (Figure 2 and Table2). There were no extreme outliers since residuals were normally distributed ($P \leq 0.05$) and there was homogeneity of variances ($P \leq 0.05$). There were significant differences in germination capacity for different treatments, storage time and interaction between treatment and storage time ($p<0.05$) (Table 1).

Storage conditions			**Months in storage**		
Extraction	Temp	Container	2	4	6
Seeds	Room	Open	14.0 ± 1.37^{f}	32.0 ± 2.7^{e}	58.5 ± 4.1^{bc}
		Closed	49.0 ± 0.58^{bc}	56.5 ± 0.36^{bc}	65.1 ± 1.8^{c}
	Cold	Open	60.58 ± 2.4^{a}	71.5 ± 1.9^{a}	79.8 ± 3.7^{ab}
		Closed	43 ± 9.6^{d}	61.0 ± 2.3^{b}	74.3 ± 3.1^{ab}
Nuts	Room	Open	41.7 ± 2.6^{d}	50.0 ± 3.1^{cd}	89.0 ± 3.2^{a}
		Closed	51.8 ± 3.2^{b}	60.0 ± 2.7^{b}	78.8 ± 3.5^{ab}
	Cold	Open	45.1 ± 3.3^{bcd}	45 ± 1.0^{d}	74.7 ± 5.1^{ab}
		Closed	34.0 ± 1.8^{e}	49.0 ± 2.3^{cd}	73.4 ± 5.2^{ab}
P value			<0.0001	<0.0001	<0.0001

Table 1: Mean germination capacity (%) of *Melia volkensii* seeds stored for 0, 2, 4 and 6 months.

From the above table, for seeds stored in open containers under room temperature, there was a significant difference in germination between the second and fourth month ($p<0.05$). Additionally, there was no significance difference in germination for seeds stored in open and closed containers in cold room between the second and fourth month ($p>0.05$). Seeds stored in closed containers in cold room had a significant difference between the second and the fourth month ($p<0.05$). Nuts stored in the open containers in room temperature had significance difference between the second and the sixth month ($p<0.05$). However, nuts stored in closed containers in room temperature had no significance difference ($P>0.05$).

From table 2, both treatment group and time stored indicate that the two groups are statistically significant ($p<0.05$). These results indicate clearly that storing the materials as seeds or as nuts and the time stored have no significance difference on germination.

Source of variation	Type III SS	Mean square	F value	P value
Block	1800.02778	600.00926	143.52	<.0001
Storage-condition	27486.77778	3926.68254	939.27	<.0001
Time _stored	49954.77778	24977.38889	5974.66	<.0001
Block*storage_cond	6720.97222	320.04630	76.56	<.0001
Time_stored*block	2525.22222	420.87037	100.67	<.0001
Time_stor*storg_cond	7884.72222	563.19444	134.72	<.0001
Time _s*block*storg_cond	12303.94444	292.95106	70.07	<.0001

Table 2: Analysis of variance of germination for *Melia volkensii* seeds stored under different storage conditions (0, 2, 4 and 6 months).

t- grouping	Mean	N	Time-stored
A	74.1250	96	6
B	53.1250	96	4
C	42.4167	96	2

Table 3: Comparison of germination means for different storage period (2nd, 4th and 6th months).

From the information in table 3 above, there was significance difference in the germination means amongst the 2nd, 4th and 6th months (P<0.05). Consequently, an analysis of simple main effects for treatment and storage time was performed. There was a significant difference in the germination means between the second and the sixth month (Tukey test, P<0.05). However, there was no significant difference in the mean germination between the fourth and the sixth month (Tukey test, P>0.05). There was a significant difference of germination score among all pairwise comparison among the 3 storage periods except for nuts that were stored in open containers in room temperature and seeds that were stored in closed containers in cold room (P<0.05). There was no significant difference in germination score among all pairwise comparison except for seeds that were stored in closed containers in room temperature and nuts that were stored in open containers in room temperature (p>0.05) (Figure 2).

4. Discussion

There are factors that influence the status of seed during storage. Such factors include storage temperature, nature of seed (nuts or seed) and storage time. The extracted seeds and those enclosed in nuts and stored for a period of two, four and six months significantly improved under all storage conditions from the initial germination of 30.5% to 89%. *Melia volkensii* seeds stored in open containers under cold storage recorded a higher mean germination percentage as compared to seeds stored in closed containers under cold storage. These results are akin to findings according to a *Melia volkensii* growing guide in Kenya's dryland regions (2018). From the study, it is evident that storing extracted seeds under air-tight conditions damages the seeds, leading to lower germination ability. The nuts stored for a period of 2, 4 and 6 months in open containers under room temperature in open and in closed containers had a higher mean germination percentages as compared to seeds stored under similar storage conditions. This trend was probably ascribed to the stony layer covering *M. volkensii* seeds, enabling them to preserve the right moisture content, light and temperature for a longer time.

These results are also in agreement with the findings of Jaoko (2020) [21] that *M. volkensii* seed extraction possess a high oil content, which comes from health delicate unsaturated fats. Upon exposure to heat, light, and oxygen, the seeds decompose, becoming rancid. In response, the seeds lose viability and thus have a short shelf life that leads to a lower rate of germination. In my study, *M. volkensii* nuts that are protected by the outer hardcover exhibited prolonged viability. This is probably because the stony layer that covers the *M. volkensii* seeds protects the seeds from adverse conditions for instance very high temperatures and humidity. The findings confirmed that nuts are free from damage caused by pests, mold and excess external moisture; hard shell provides greater protection against microbial infection. Considering that, *M. volkensii* thrives in arid and semi-arid habitats, the pulp and the hard seed coat enables this tree species to remain viable until the conditions are right for germination.

Seeds stored in open containers under cold room temperature had a significance difference and a higher mean germination percentage as compared to seeds stored in open containers under room temperature. This can be attributed to the ability of a cold room to store seeds at the correct temperature while controlling the moisture level unlike the case of ambient temperature. The results agree with the findings by Division of Agriculture and Natural Resources (2020) that seeds should be dried and stored at cold temperatures for long-term preservation.

Cryopreservation, or freezing in liquid nitrogen at -180°C can also be used for extremely long-term storage. The results are entirely in line with what Vasques et al., (2014) [22, 23] observed in seeds of three Mediterranean shrub species stored at a low temperature. The stored seeds in that study maintained a high viability and vigor for a couple of years but ambient temperature storage led to a marked decline after one year ending in almost complete mortality after 4 years of shelf storage. By contrast, Croft et al., (2012) [24] stated that very cold storage for instance refrigerators consists of quite high relative humidity which can damage seeds due to high moisture content, therefore according to their study, storing seeds in cold storage is not fit for seed viability.

Nonetheless, seeds stored at room temperature in sealed containers for 2, 4, and 6 months showed a higher germination percentage than those kept in open containers but the same temperature conditions. This is probably due to lack of a barrier in the open containers (between the seed and the outside atmosphere), thus allowing movement of oxygen and excessive water leading to irreversible seed deterioration. The high germination agrees with findings of Islam, (2018) [25, 26] that sealed containers had good performance pertaining germination capacity. This is akin to Alemayehu (2020) [27] findings that agricultural seeds stored in ambient conditions in open bins often result in short storage life and seed deterioration in hot, humid regions.

Open environments also expose the seeds to pest attacks, especially fungal pathogens. This leads to lower germination percentages compared to seeds stored in sealed containers. These findings concur well with the study of Tshisola (2014) [28] that air contains 21 % oxygen, which reacts with essential materials in the plant seeds. This reaction with oxygen reduces the quality and germination capacity of seeds.

Sealed or hermetic storage systems are a very effective means of controlling seed moisture and insect activity, especially in tropical conditions [29]. According to Mattana, (2019) [30], reducing the seed moisture content up to 10 % after drying the seeds, they attain moderate seed viability under different storage conditions.The study is also in agreement with the observation by Fenolossa (2020) [31], stating that viability and germination tested in two distinct populations of *Carissa edulis* showed germination of 70–90% for seeds that had reduced moisture content (< 13%). In contrast, germination percentage reduced to 20% in Population two, which had higher moisture content. Almost similar results were observed by (Himstedt, 2002) [32] who stated that Macadamia kernels rapidly develop rancidity when stored at room temperature at higher moisture content.

5. Conclusion and Recommendation

From this study it was evident that the initial germination had a germination of 30.5 %. However, seed germination improved with storage for almost all storage specifications from registering as low as 14 % (seeds were stored in open containers in room temperature) to as high as 89% (nuts stored in open containers in room temperature).

The study reveals that the three different treatments that were used in the laboratory experimental setup improved in germination capacity regardless of the storage conditions. The study is vital and applicable when seeds are to be preserved for long term in gene banks or exported to far-off places without degradation which occurs between the field and the final destination.

Additionally, the study concludes that *M. volkensii* are orthodox seeds whose viability was maintained when seeds had been dried to a moisture content of 6 % (by oven drying at 103°C for 24 hours).

The study recommends that *M. volkensii* seeds be stored and distributed as extracted seeds as opposed to current practice of storing and distributing them when enclosed in nuts. Seed extraction will help in reducing seed bulkiness; consequently, reduced cost as seeds will occupy small space unlike the nuts since one kilogram of *M. volkensii* nuts yields about 200 seeds.

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