EFFECT OF TEMPERATURE AND STORAGE DURATION ON VIABILITY OF EAST AFRICAN SANDALWOOD

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ABSTRACT

East African Sandalwood *(Osyris lanceolata)* is highly valued in the manufacturing of perfumery and medicinal products, and there is need for its domestication. Seed storage behavior was determined as the information is important in planning and implementing the species planting programmes and conservation strategies. Fresh seeds and those dried to a moisture content of 7% were placed in airtight plastic vials and stored at a constant temperature of 20^0 C, ambient temperature and in a cold room set at -20^0 C, respectively. At 0, 3, 9, 12 and 24 months of storage, seeds were subjected to a germination test. At month zero, dried seeds had scored better in mean parameter values for germination capacity (G), mean germination time (MT) and germination value (GV) than fresh seeds. Germination capacity of seed stored fresh dropped rapidly by the third month in all the storage environments from 69% to mean less than 16% making it inconsequential to test for storability. Germination capacity of dried seed dropped gradually in all the storage environments.. Dried seed stored at ambient and constant temperatures registered G of over 70% in 3 months and over 60 % at 3 to 9 months. By 24 months, the G dropped drastically to 2% for seed stored at ambient and constant temperatures and to 29% for seed stored in cold room. There was significant difference in G, GV and MT ($p<0.01$) depending on the period of storage. The results indicate that sandalwood seed is neither a classical recalcitrant nor orthodox and may be classified as having intermediate seed storage behaviour but withstanding drying to low moisture content.

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INTRODUCTION

East African Sandalwood (*Osyris lanceolata* Hochst. and Steud.) is in the family *santalaceae* and is a dioecious evergreen tree that grows up to 6 meters in height. The species has a wide ecological distribution in eastern and southern Africa. It grows in diverse locations including rocky sites, along dry forest margins, evergreen bushlands, grasslands and thickets at an altitude range of 900 to 2550 m above sea level (Beentje, 1994). The species grows naturally as a parasite through root attachments on other trees such as *Dodonea viscosa*, *Searsia natalensis*, and *Carissa spinarum (*Mwang'ingo *et al*., 2005). Due to overexploitation to meet international demand for its perfumery and medicinal products, *O. lanceolata* (Osyris) has been listed as an endangered species in Eastern Africa and its populations in the region are protected under the Convention on International Trade in Endangered Species (CITES) Appendix II (USF&WS, 2013).

The method for propagating sandalwoodis through seed has been developed to help in domestication and mass planting of the species, which will subsequently ease pressure on the few remaining wild populations (Kamondo *et al*., 2012). However, information on how long seeds of the tree can remain viable after collection is lacking.

MATERIALS AND METHODS

Ripe fruits were hand-picked from wild population of sandalwood in Kitui County, Kenya and subjected to flotation where floating fruits were discarded. The fruits that sank were depulped and the seed subjected to floatation where any floating seeds were discarded. The sinkers were divided into two sublots referred to hereafter as 'fresh seed' and 'dry seed'. A germination test was undertaken on the fresh seed to assess initial germination. The dry seed sublot was dried for three days to a moisture content of 7% at the Genebank of Kenya air-lock and a sample drawn to determine the initial germination. Both germination tests were based on 4 replicates of 25 seeds each. A germination test was conducted on sand in 4 germination trays with each tray as a replicate, and placed in a non-mist propagator with each tray holding 25 seeds. A seed was considered to have germinated immediately a germinant emerged from the sand. The number of germinated seedlings was scored daily for a period of 80 days.

Fresh and dry seed sublots were packaged into airtight plastic vials and stored at;

- ambient conditions on the cupboard shelf
- dry-room temperatureregulated at a constant temperature of 20 ^{0}C
- cold room set at -20 ^{0}C.

At 3, 9, 12 and 24 months of storage, seeds from both sublots were subjected to a germination test using same testing environments as for the previous germination except that the number of seeds used were reduced to 75 due to limitation of seed quintiles, and replicated in 3 germination trays with each tray holding 25 seeds. The number of seedlings germinated was scored daily from the day of initial germination for a period of 80 days.

Data analysis

The benchmark germination curves were superimposed on germination curves of stored seeds to graphically show the effect of time and environment. The resultant germination curves guided the identification of datasets that required further analysis for statistical significance. The germination processes were compared for statistical significance using parameters associated with seed germination. Germination capacity (G), mean germination time (MT), and germination value (GV) and their means and variances were used to test for statistical significance. The template published by Marli *et al*. (2009), was used to ensure correct calculations of mean germination time (MT). The germination process parameters for testing significance were calculated and analyzed as follows:

Germination capacity

Germination capacity is the proportion of a seed sample that has germinated normally in a specified test period, usually expressed as a percentage. The differences in germination capacity among the treatment means were tested through factorial analysis on the arcsine transformed germination percentages. When comparing seedlots, high germination capacity is indicative of higher quality seedlots (Matthews *et al*. 2011, Marli and Santana, 2006).

Germination value

Germination value is a composite value that combines both germination speed, which was obtained by dividing cumulative germination percentage by number of days or specified time interval, and total germination. The germination value was calculated according to the formula published by Diavanshir and Porubeik (1976) as follows:

$$GV = (\sum DGs/N) \times GP/10$$

Where:

GV is germination value,

GP is germination percentage at the end of the test period

DGs is Daily germination speed obtained by dividing the cumulative germination percentage by the number of days since sowing

N is the total number of daily counts, starting from the date of first germination

10 is a constant.

In each germination test, germination values were calculated for each day commencing on the day of germination to the end of the testing period of 80 days. The maximum germination value was taken as the true germination value for the respective germination test as guided by Diavanshir and Porubeik (1976), and was used to make comparisions of treatment effects. High germination value denotes higher quality seed.

Mean germination time

The mean germination time is a measurement of the average length of time required for maximum germination of a seedlot (Marli and Santana, 2006), and was expressed in days corresponding to the same units of time used in counting germination. The mean germination time $\bar{t}$ was calculated according to Marli *et al*., (2009), with the expression:

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

Where t_i: time from the start of the experiment to the i^{th} observation; n_i: number of seeds germinated in the i^{th} time, and k: last time of germination (Marli *et al*., 2009). When comparing two seedlots, the seedlot with lower mean germination time is indicative of higher quality seed.

RESULTS

Benchmark germination results

Fresh seed germinated from day 28 reaching peak germination of 69% on day 61, while dry seed germinated from day 21 reaching peak germination of 85% on day 51 (Figure 1). Germination for dry seedsshowed better mean values for germination rate (G), mean germination time (MT) and germination value than fresh seeds (Table I).

The data for germination rate (G), mean germination time (MT) and germination value were subjected to analyses of variance to test for differences in the seed categories (Table I).

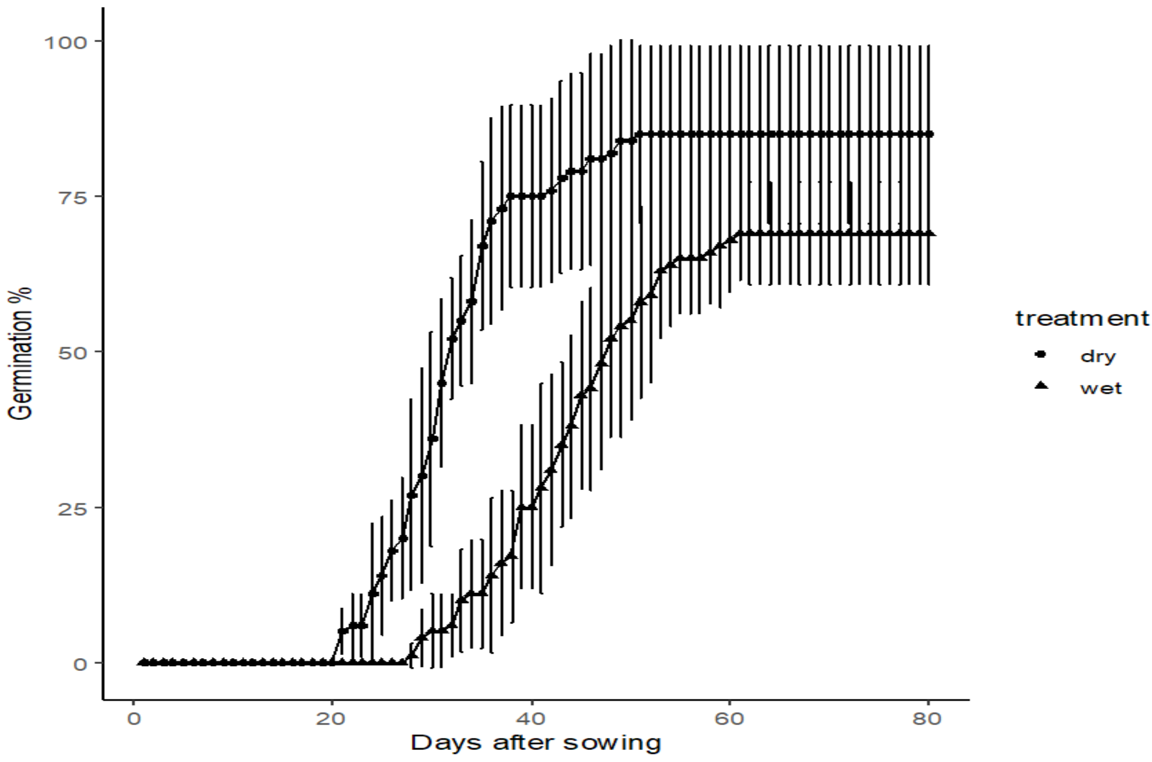

Figure 1. Germination trends for fresh and dry *Osyris lanceolata* seeds at zero month

There were significant differences ($p<0.05$) in germination value between fresh seed and dried seed. Similarly, there were significant differences between fresh and dried seed in mean germination time. However, there were no significant differences between fresh and dried seed in germination rate.

stored in the cold room at three months, which registered a germination of 16% (Table III). As germination capacity of seed stored fresh in any of the environment was poor, the germination process of these seeds was not subjected to any further scrutiny.

TABLE I- ANALYSIS OF VARIANCE (ANOVA) FOR GV AND MT FOR *OSYRIS LANCEOLATA* SEED SUBLOTS AT ZERO MONTHS

	GV			MT		
Source of variation	DF	MS	F	DF	MS	F
Rep	3	13.3	2.4	3	3.86	0.27
Seed categories	1	79.1	14.5*	1	260.3	18.2*
Residual	3	5.4		3	14.27	

Note: * significant at 5% level.

The data for the two significant variables were further subjected to Least Significant Difference (LSD) test for means separation (Table II).

Germination of seed stored dry dropped over time in all the storage environments from the benchmark germination of 85%. Seed stored in the cold room

TABLE II- TREATMENTS MEAN SEPARATIONS FOR GERMINATION VALUE AND MEAN GERMINATION TIME

Seed category	Germination Time	Germination Value
Fresh	43.45a	6.40b
Dry	32.03b	12.69a
Mean	37.74	9.54
$LSD_{(0.05)}$	8.52	5.24
CV%	10.03	24.4

After mean separation test for germination time, dry seed category had a significantly shorter time (32.03) compared to fresh seed category. The dry seed category had significantly higher germination value (12.69) compared to the fresh seed category.

Germination trends in seed stored fresh and dried

Germination of seed stored fresh dropped rapidly over time in all the storage environments registering germination lower than 10%, except for seed

registered the highest drop at each trial period. Whereas seed stored at ambient and dry room conditions registered germination of over 70% in 3 months and over 60% at 3 to 9 months, seeds stored in the cold room registered germination of 60% and over 56% for the same periods. By 24 months, the germination capacity dropped drastically to around 21% for seed stored at ambient and dry room conditions and 29% for seed stored in cold room (Table IV).

TABLE III- MEAN GERMINATION CAPACITY (%) OF FRESH *OSYRIS LANCELOTA* SEEDS STORED AT AMBIENT, DRY ROOM AND IN COLD ROOM CONDITIONS FOR 0, 3, 9, 12 AND 24 MONTHS

Storage environment	0 months	3 months	9 months	12 months	24 months
Ambient	69	5.3	0	0	0
Dry room	69	2.7	0	0	0
Cold room	69	16.0	1.3	1.3	0

TABLE IV- MEAN GERMINATION CAPACITY (%) OF DRY SEEDS STORED AT AMBIENT, DRY ROOM AND IN COLD ROOM CONDITIONS FOR 0, 3, 9, 12 AND 24 MONTHS

Storage environment	0 months	3 months	9 months	12 month	24 month
Ambient	85	76	69	65	21
Dry room	85	7	68	68	21
Cold room	85	60	56	51	29

At 3 months, seed stored at ambient and dry room conditions had similar mean germination time of about 45 days, while seed stored at the cold room had a lower mean germination time of 35 days. At 9 and 12 months, the range in mean germination time narrowed among the seedlots to no more than 5 days with the highest MT being about 33 days at dry room for 12 months and lowest being about 29 days at ambient temperature for 9 months. At 3 and 12 months, the highest germination value was for seeds stored at the cold room while, at 9 months, seeds stored at ambient conditions had highest GV. Seeds stored at dry-room temperature had the lowest GV for all the storage periods. There were significant differences in germination capacity for different storage time ($p<0.01$) (Table V). When mean separation was undertaken, it was established that the difference in germination was between the germination at 24 months and the germination at the rest of germination time (Table VI). There were significant differences in germination value for different storage time. There were also differences in storage environment ($p<0.01$). The storage environment marginally affected GV at different storage times ($p=0.05$) (Table VII). Further analysis showed that the differences in GV was due to storage time and also due to storage environment were mainly as a result of differences between 24 months and the rest of the storage times (Table VIII).

TABLE V - ANALYSIS OF VARIANCE OF GERMINATION CAPACITY FOR DRIED *OSYRIS LANCEOLATA* SEED STORED AT AMBIENT, DRY ROOM AND IN COLD ROOM CONDITIONS FOR 0, 3, 9, 12 AND 24 MONTHS

Source of variation	DF	MS	F pr.
Block stratum	2	268.44	
Storage time (ST)	3	3925.93	<.001
Storage Environment (SE)	2	312.44	0.017
ST*SE	6	131.70	0.097
Residual	22	63.35	

TABLE VI- MEAN SEPARATION OF GERMINATION CAPACITY OF DRIED OSYRIS LANCEOLATA SEED STORED FOR 3, 9, 12, AND 24 MONTHS.

Storage time in months	N	Subset	
		1	2
24	9	24.000	
12	9		61.333
9	9		64.444
3	9		69.777

TABLE VII- ANALYSIS OF VARIANCE OF GV FOR DRIED OSYRIS LANCEOLATA SEED STORED AT AMBIENT, DRY ROOM AND IN COLD ROOM CONDITIONS FOR 0, 3, 9, 12 AND 24 MONTHS

Source of variation	DF	MS	F
Block stratum	2	0.3249	2.17**
Storage time (ST)	3	5.2056	34.84**
Storage Environment (SE)	2	1.4788	9.90**
ST*SE	6	0.3698	2.47*
Residual	22	0.1494	

TABLE VIII- MEAN SEPARATION ACCORDING TO SIGNIFICANCE IN EFFECT OF STORAGE TIME ON GV USING TUKEY’S B TEST

Storage time (Months)	N	Subset	
		1	2
24.0	9	0.23438	
12.0	9		1.72818
3.0	9		1.73012
9.0	9		1.80319

TABLE IX - ANOVA OF MT FOR DRIED OSYRIS LANCEOLATA SEED STORED AT AMBIENT, DRY ROOM AND IN COLD ROOM CONDITIONS FOR 0, 3, 9, 12 AND 24 MONTHS

Source of variation	DF	MS	F
Block stratum	2	23.391	3.08
Storage time (ST)	3	277.469	36.58**
Storage Environment (SE)	2	49.479	6.52**
ST*SE	6	34.600	4.56*
Residual	22	7.586	

Results also indicated highly significant differences in MT for different storage time and storage environment ($p<0.01$) (Table IX). There was interaction between storage time and environment implying storage environment influenced MT at different storage times.

DISCUSSION

The floatation process subjected to ripe fruits and extracted seed followed the recommended protocol for producing best grade Sandalwood seed of high viability (Kamondo *et al*., 2012). Results indicated that germination capacity and germination value of dry sandalwood seed was higher than that of fresh seed and that the seed could withstand drying to moisture content of 7% Maintenance of viability of seed with drying to moisture content of less than 10% is characteristic of orthodox seed (McDonald, 2004). The storage experiment established that fresh sandalwood seeds, when stored rapidly lose viability. This result is inconsistent with Mwang'ingo *et al*. (2004), who reported a limit of 20 % moisture content for maintenance of seed viability for the species during seed storage, suggesting the seed to be recalcitrant. Recalcitrant seeds are sensitive to desiccation and freezing (Berjak and Pammenter, 2004; McDonald, 2004). Death of recalcitrant seeds due to loss of moisture is mainly attributed to the loss of membrane integrity and nuclear disintegration (Chin, 1995)

Dried sandalwoods seeds maintained good germination rates, mean germination time and germination value for the first year, even when stored in subzero temperature, but the seed viability plummeted when tested after two years. Orthodox seed are expected to maintain viability in storage for a long time, with true orthodox seeds known to withstand sub-freezing temperatures for long periods, up to several decades when dried to 10 % or less moisture content (Bonner 1990, Mng'omba *et al*., 2007). The results therefore indicated that Sandalwood is not a true orthodox seed.

Based on our findings, we classify *Osyris lanceolata* seed as intermediate seed that benefit from drying to low temperatures and storing in either cold or ambient temperatures. Although we classify sandalwood seed as intermediate, it is noted that the behaviour of the seed is not fully in conformity with the definition of this category of seed, as it withstand drying to the same levels as that used in storing of orthodox seed. According to Andrade *et al*. (2003), intermediate seeds are relatively desiccation-tolerant, but will not withstand removal of water to levels as low as orthodox seeds. These species, particularly if they are of tropical origin, may also be chilling-sensitive, even in the desiccated state (Hong and Ellis, 1996; 1998).

CONCLUSIONS AND RECOMMENDATIONS

Results showed that dried sandalwood seed tolerate storage in ambient temperature. The information provided on seed viability of Sandalwood, is important in planning and implementing planting programmes of the species, Since the species tolerate storage in ambient temperature, it makes it easy to store without requiring sophisticated storage facilities. However, the limited time of seed storage of about one year indicated that the seed planting programmes cannot be based on storing of large quantities of seed for long periods of time. Seed stored for more than one year would result to low viability. The results also show that sandalwood is not amenable to long-term seed conservation in cold rooms.

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