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EFFECT OF STORAGE TIME AND TEMPERATURE ON GERMINATION ABILITY OF *ESCOECARIA BUSSEI*

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ABSTRACT

This study was conducted in Botany Department Nursery to evaluate the effect of storage time and temperature on germinability of Excoecaria bussei. The orthodox seeds, collected from Dodoma and Singida regions were germinated monthly from September 2013 through June 2014. Each time, seeds were soaked in water for 6 hours to soften then sown in sterile potting media. A Split-Split plot was used to compare temperature [(15°C) and (30°C)] as main plots, populations (Maktupa, Kisalu and Ilola) as Sub plots and storage time (September 2013 through June 2014) as Sub-Sub-plots on percentage seed germination. ANOVA revealed average germination percentages of 20.6±9 for Kisalu, 30.4±10 for Maktupa, and 33.1±7 for Ilola for seeds stored at low temperature (15°C). Seeds stored at 30°C showed respectively 5.52±1, 8.6±3 and 11.6±4germination percentages. Temperature and storage time conferred highly significant difference on germination percentage, while population did not at P >0.05. The interaction effects between population, storage period and storage temperature significantly affected germination percentage at P >0.05. Storage at 15°C can retain seed viability up to 9 months after harvesting but only 3 months at 30°C. If suitable storage conditions are not provided, Excoecaria bussei seeds should be sown immediately for maximum germinability.

Key words: *Escoecaria bussei*, Germinability, Seed Storage, Pawn tree, orthodox seeds

INTRODUCTION

Excoecaria bussei, Pawn broker tree, Pepper-seed or 'Mzenjezenje' in Kiswahili belongs to family Euphorbiacea. It is native to several African countries including Botswana, Kenya, Malawi, Mozambique, Tanzania, Zambia, and Zimbabwe (Smith 1987). It is distributed from Southern Kenya in the north to Botswana in the South, Caprivi, Mozambique, and Zimbabwe. The species thrives at lower altitudes in thicket and riverine fringes (Smith 1987). This plant species is still found in the wild and studies on its oil qualities have shown that it has very high potential use as a source of biodiesel (Moshi 2016). For this reason, it has attracted extensive attention as possibly one of the non-edible, renewable, biodegradable and non-toxic biofuel stock (Moshi 2016). *E. bussei* has been recommended as a good feedstock for biodiesel production due to its numerous advantages as a good yielder and having desired physico-chemical and performance oil characteristics comparable to biodiesel (Moshi 2016). However, information on its propagation and agronomical requirements is still at large and little is known about how it can be multiplied for upscaling and commercialization.

Use of seed is one of the propagation methods that can be used to multiply plants but needs the seeds to be capable of germinating in the first place and therefore the need for knowledge of its methods of

germination. Since most plants produce seeds only once a year, knowledge of methods of seeds preservation for next season's generation was deemed important and so was the determination of how soon the seeds would lose its viability. The goal of this study was therefore to generate knowledge on the longevity and possible germinability of this species from harvest, storage and up to the next harvest so as to increase the production of *E. bussei* seedlings.

Seed viability is the ability of the embryo to germinate and it is affected by a number of factors including temperature, light, oxygen, water and species type. Germinability which is determined by germination percentage is the proportion of seeds that germinate from seeds subjected to the right conditions for growth while the germination rate is the speed with which the seeds germinate and is affected by seed viability, dormancy and environmental effects that impact on the seed and seedling (Zamora 2014). This is given as a percentage of germination over a certain amount of time, for example 90% germination within 20 days. Germination energy is the proportion of seeds in a given sample which germinate within the time of peak germination, generally taken as the highest number of germinations in a 24-hour period (Mrđa et al. 2011). The interest in germination energy is based on a theory that only those seeds which germinate rapidly and vigorously under favourable conditions are likely to produce vigorous seedlings in field conditions, whereas weak seeds or delayed germination is often fatal (Aldhous 1972).

Longevity of seeds is very variable and depends on many factors; few species exceed 100 years (Ken et al. 1997). In typical soils, the longevity of seeds can range from nearly zero to several hundred years. Some of the oldest still-viable seeds were those of Lotus (*Nelumbo nucifera*) found buried in the soil of a pond; these seeds were estimated by carbon dating to be 1,040 years old (Thomas et al. 2002). The longevity of seeds held under normal storage is mainly determined by seed moisture content and storage temperature, with their life-span increasing predictably with decreasing temperature and moisture content (Ellis et al. 1991). However, there are also wide inherent differences in seed longevity between species. *Ex situ* seed storage is an essential step for the long-term preservation of plant genetic resources. Maintaining seed viability for longer period is very essential to preserve the genetic integrity in stored samples. Simple techniques have been adopted to maintain the seed viability in both domesticated and wild sources (Ellis et al. 1991, Vertucci and Roos 1991). Seed deterioration and loss of viability, is a natural phenomenon occurring during storage (Nasreen et al. 2000, Schmidt et al. 2002) and inappropriate storage medium such as room temperature storage (Hezewijk et al. 1993, Müller et al. 2011) has often resulted in low seed germination. Several factors, including temperature, nature of the seeds, seed moisture content, relative humidity influence the seed longevity during storage (Onyekwelua and Fayose 2007, Pradhan and Badola 2008). Proper storage conditions, may effectively retain substantial viability in seeds over a considerable storage period (Butola and Badola 2004, Chen et al. 2007, Pradhan and Badola 2008).

A long-term storage of seeds, especially under unfavourable conditions, leads to loss of viability. The nature of this physiological damage is variable, e.g. short-term deterioration in the field is different from long term deterioration during storage, which in turn is different from mechanical damage (Mc Donald 1999). All parts of the seed such as the seed coat, which is of maternal origin and acts as a physical and chemical barrier, and the embryo are susceptible to physiological damage. Seeds

are grouped based on their storage characteristics as orthodox and recalcitrant. Orthodox seeds are those that can be dried to moisture contents of 10% or less, in this condition they can successfully be stored at subfreezing temperatures (Robert et al. 1973). Recalcitrant seeds are seeds that do not survive drying and freezing during ex-situ conservation and cannot resist the effects of drying or temperatures less than 10°C; thus, they cannot be stored for long periods like the orthodox seeds because they can lose their viability. Plants that produce recalcitrant seeds include avocado, mango, mangosteen, lychee, cocoa, rubber tree, some horticultural trees, and several plants used in traditional medicine such as species of *Virola* and *Pentaclethra* (Robert et al. 1973).

Seeds of different plant species lose viability at varying degrees. For example, onion seed is very difficult to store while barley seed maintains good germination under a variety of storage conditions (Milošević et al. 1996). Seeds and especially oilseeds including *E. bussei* contain large quantities of carbon stored as carbohydrate, lipid and protein (Bewley and Black 1985). The chemical composition of oilseeds which are rich in lipids causes specific oxidative processes to occur during storage and these are said to cause limited longevity depending on their specific chemical composition (Balešević et al. 2007). Therefore, oils seed storage demands special attention otherwise the oxidative processes may occur too soon that may lead to loss of germination ability and seed viability.

Since *E. bussei* is still in the wild, its germination ability in different environments is not known and so is its methods of preservation. In order to increase their germination ability, the seeds should be immediately available or preserved under good conditions for the future generation. The flowering time for *E. bussei* occurs only once a year in October-November while seed maturity occurs from August to October in different parts of Tanzania. It would be only appropriate if it was known how seeds should be handled (e.g. storage time and storage temperature) for maximum viability and germination rates for successful planning of the amount of seeds to purchase and number of seedling that can be established from a given seed lot. The goal of this study was to evaluate the effect of storage condition on its germination ability.

MATERIALS AND METHODS

Experimental layout

The experiment was set up in the University of Dar es Salaam, Botany Department Nursery area. The experiment was laid in a Split-split plots design, where the Main plots was storage temperatures at 15°C and 30°; Sub plots were assigned to *E.bussei* populations from Maktupa, Kisalu and Ilola while the Sub-sub- plots were assigned to storage time i.e. sampling from seed lot made monthly from September, 2013 through June, 2014.

Seeds to be used in this study were collected in August 2013 from plant populations in Maktupa, Ilola (Dodoma) and Kisalu villages (Singida) region, Tanzania. *E. bussei* fruits were randomly harvested from selected trees in a population so as to allow equal chances of each plant in the vicinity to be included in the experiment . The seeds were hulled and stored at two different temperatures of (15℃) and (30℃) until the day of the experiment (Ghasemnezhad and Honermeir 2007). Each month for a period of 10 months from September, 2013 through June, 2014 a total of 50 seed batches were drawn from storage and sown in clean washed riverine sand used as potting media. Only 50 seeds were used per batch due to seed shortage. To facilitate germination, hard seed coats were mechanically removed prior to sowing. Dehulled seeds were then soaked in tap water for six hours to allow the

embryo to imbibe water before placing into the sand media as illustrated in Figure 1. Normal irrigation was done as needed to support plants growth and maintain the moisture content.

Figure 1: (a) sowing of *Excoecaria bussei*, (b-c) emergence of shoot (d) seedling plant

Data recording

The numbers of *E. bussei* seeds that germinated and the time the seeds took to germinate were recorded. The numbers of germinated seeds were converted to germination percentage. Analysis of Variance (ANOVA) for the Germination percentage and number of days was then computed to make comparisons between the means for population, storage temperature and storage time treatments (Gomez and Gomez 1999).

RESULTS

Generally, seed germinability was low because no entry germinated beyond 50%. The Analysis of variance (ANOVA) indicated that temperature significantly affected germination percentage ($p<0.01$) but population, storage period or their interactions did not affect germination performance to any significant extent at $p>0.05$ (Table 1).

Table 1: Analysis of variance (ANOVA) of germination performance of *Excoecaria bussei*

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	Computed F	Tabular F	
					5%	1%
Replication	2	551.3	275.76			
Temperature (A)	1	21590.55	21590.55	1071.23***	18.51	98.49
Error (a)	2	40.31	20.155			
Population (B)	2	458.4	229.2	0.0077ns	4.46	8.65
(A x B) Interaction	2	5011.68	2505.84	0.084ns	4.46	8.65
Error (b)	8	238768.5	238768.5			
Storage time (C)	9	31047.08	3449.68	1.67ns	1.95	2.56
(A x C) Interaction	9	19112.26	2123.58	1.67ns	1.95	2.56
(B x C) Interaction	18	3163.64	3449.68	1.05ns	1.65	2.01
(A x B x C) Interaction	18	1625.12	90.28	0.044ns	1.97	2.01
Error (C)	108	2029.58				
Total	179					

***= very highly significant difference and **ns**=not significantly different

As shown in Table 2 the population with the highest mean seed germination percentage was from Ilola (22%) and the lowest were from Kisalu and Maktupa (18%). Temperature of 15ºC gave the highest mean germination (30%) while the storage temperature of 30ºC was associated with the lowest germination (8.5%).

Table 2: Two-Way Table Showing Mean Germination percentage of *E. bussei* populations over the entire experimental period

Populations	Temperature 15°C	Temperature 30°C	Mean
Maktupa	30.4	6.0	18.0
Kisalu	28.0	9.0	18.0
Ilola	33.0	11.0	22.0
Mean	30.0	8.5	19.4

The effect of storage temperature on germination ability shows that it was highly significantly different ($F=1071.23$, $p<0.05$ in (Table 1)). At temperature of 15°C, seeds started with slowest germination percentage (22.9%) but as time increased the germination percentages became higher (56.5%). Highest temperature of 30°C showed highest germination percentage (40.3%) in September immediately after harvest but decreased drastically and in December sampled seeds (Figure 3).

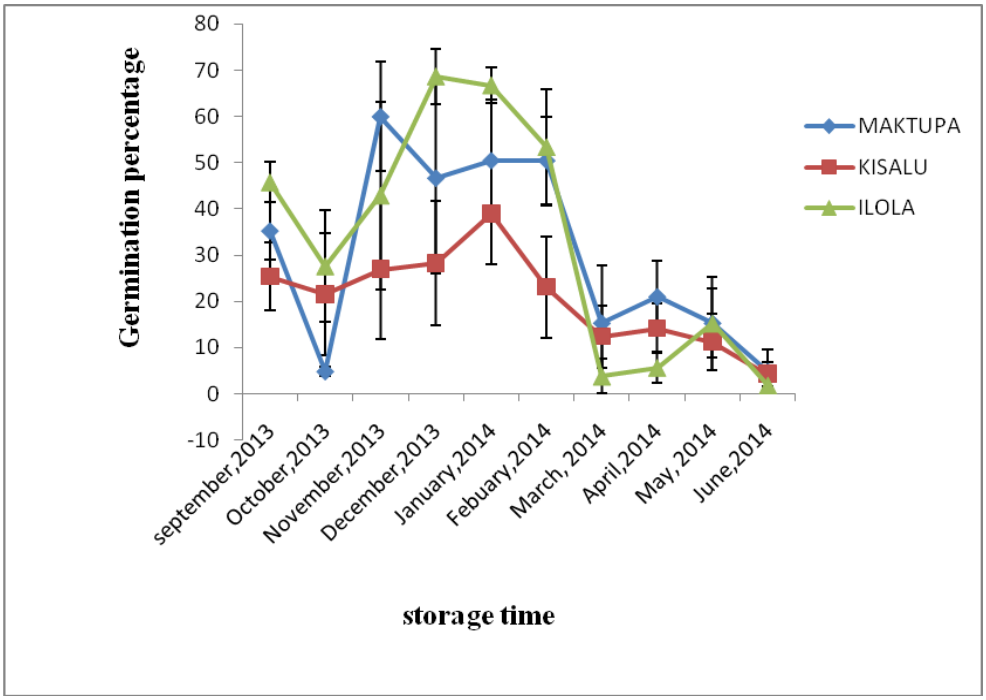

Figure 2: **Mean** Monthly Germination Percentage of *E. bussei* at temperature (15°C)

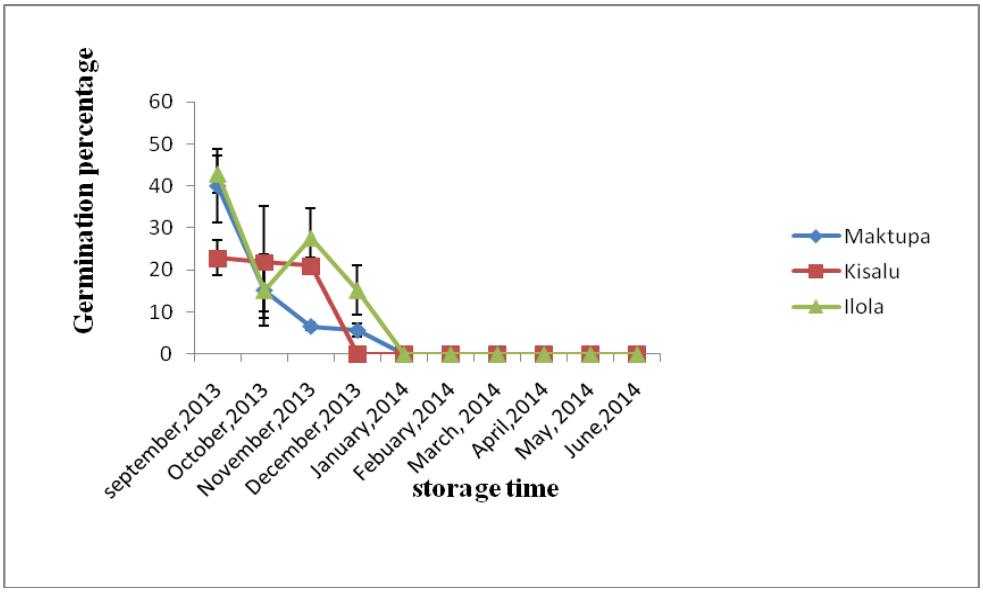

Figure 3: Mean Monthly Germination Percentage of *E. bussei* at temperature (30°C)

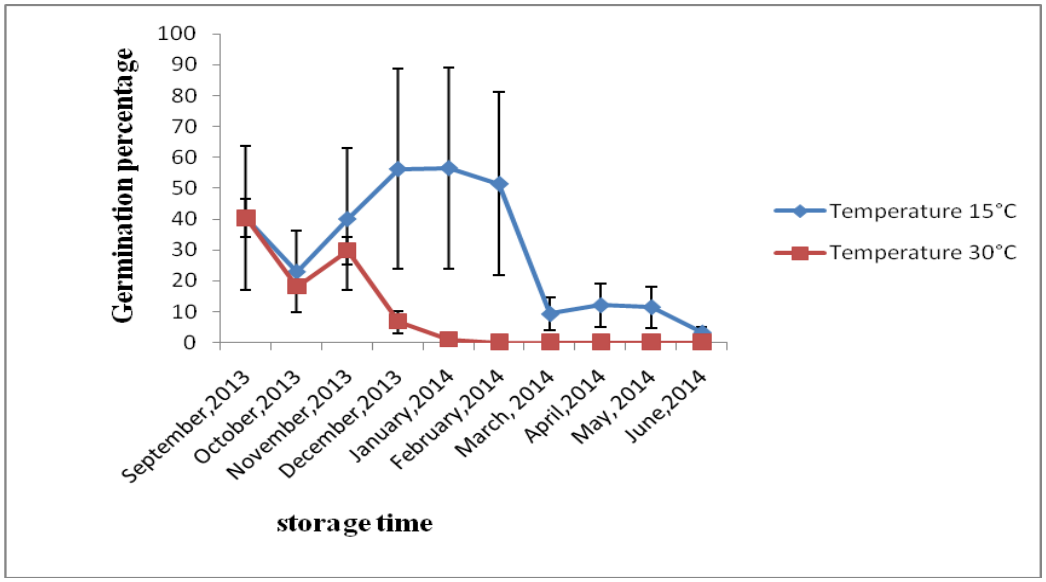

Figure 4: Effect of storage temperature on germination percentage

The effect of storage period from September 2013 to June 2014 showed not to be statistically different on the germination percentage (F= 1.67, p >0.05). Storage period with the highest germination percentage was for the seed batch sown in January, 2014 (56.5%) at temperature (15°C) and September 2013 (40.32%) at (30°C) temperature. The lowest germination percentage was for the seed batch sown in February, March, April, May and June which did not germinate at all at 30°C temperature and in seed batch sown in June (3.17%) at 15°C temperature (Figure 3).

The interaction effect between storage temperature and population on the germination percentage showed no statistically significant differences (F=0.084, p>0.05) (Table 1). The interaction effects between storage time and storage temperature showed no statistically significant differences on the germination percentage either (F=1.05, p>0.05). The interaction between population and storage period showed no statistically different effect on the germination ability (F= 0.09, p>0.05 ANOVA Table). However, the interaction effects between population, storage temperature and storage period showed highly significant difference (where the F= 0.044, p < 0.05 in ANOVA Table). As storage time increased the seed germination ability declined. Although it was short of statistical significance, the seed germination at higher temperature (30°C) declined more than seed germination at (15°C).

DISCUSSION

Based on the analysed results, storage temperature showed the most significant effect on *E. bussei* seed germinability. Seeds stored at high temperature (30°C) resulted in lowest germination ability than those stored at low temperature (15°C) observed during the study, differences that were also statistically significant. Seeds stored at (15°C) stopped germinating in June, 2014 while those stored at (30°C) stopped germinating already in January 2014. Possibly, high temperature resulted in accelerated metabolic activities including

premature enzymatic oxidation and denaturation of proteins, which lowered enzymatic activity of seeds prior to the intended germination. In their studies on maize, soybean and sunflower oil seeds (Šimić et al. 2005 reported that storage temperature at 25°C showed lower germination of 60 % associated also with higher seed quality losses than in those stored at lower temperature (12°C) which showed germination of 75%. High storage temperature accelerates seed deterioration, causing seed quality losses and therefore lower germination percentage of seed.

Based on this study, the population, which showed highest germination percentage was from Ilola (68.57%) collected in December and the lowest germination percentage was shown in June, 2014 (1.91%) for Ilola population at temperature (15°C). Temperature of 30°C showed highest germination percentage in the population from Maktupa (40.32%) for seeds sown in November and September while the lowest germination percentage (0%) was shown in populations from Maktupa and Ilola sown in January, 2014 through June, 2014. The seeds that did not germinate indicate that they had lost viability due to long period of being stored at higher temperature that were possibly not favorable. Analysis of Variance showed that, there was no significant difference in germination percentages between populations possibly because Maktupa and Ilola population were sampled in Dodoma region and Kisalu population was sampled from Singida region, which are more or less found in the same agro-ecological zones (FAO 1996).

Interaction between storage temperature and population showed that, there was no statistically significant difference in seed germination ability possibly because the seeds population, which was similar confounded the results.

The effect of storage period from September 2013 to June 2014 can be inferred that, there is significant difference of storage time on seed germination of *E. bussei* seeds. In batch sown in September to November the germination percentage could have been low, because the seeds were harvested shortly from the field and sown therefore their embryo could have still been immature. Storage period with the highest germination percentage was for batch sown in January (56.6%) at temperature (15°C) and November 2013 (40.32%) at temperature (30°C). The lowest germination percentage was from seed batches sown from February to June 2014, which did not germinate at all at temperature (30°C); as the time of storage progressed, the seeds lost viability. Other researchers, Ahmadkhan et al. 2000 and Morello et al. 2004 proved that, the development of rancidity due to oxygen dependent deterioration of lipids in vegetable oils has been recognized as the predominant cause of oil deterioration and germination reduction during storage. Suriyong (2007) also reported that the ageing process naturally affects the quality of seeds during storage at various conditions; particularly the oil content, which is sensitive to deterioration as a result of the oxidation processes a reaction between unsaturated fatty acids and oxygen.

The increase of storage period resulted in decline in ability of seeds to germinate. As reported by (Walter et al. 2005) the length of storage time is strongly influenced by environmental and genetic factors such as storage temperature and seed moisture content. When seeds deteriorate during storage, they lose vigor, become more sensitive to stress during germination and ultimately become unable to germinate as reported by Rajjou and Debeaujon (2008).

The interaction effects between storage temperature and storage period showed statistically significant difference. This

could have been due to the possibility that, the seeds stored at temperature (30°C) had higher metabolic activity which resulted to lowered germination ability while the seeds stored at low temperature (15°C) maintained relatively higher germination ability due to lowered metabolic activity of seeds. Seed batches sown from February, 2014 to June, 2014 did not germinate at temperature of 30° C. As storage period increased the seeds lost their viability faster possibly due to oil deterioration of seeds which hindered the seeds from germinating well.

For six months, *Excoecaria bussei* seed showed highly significant difference in germinability but as the storage time increased the *E. bussei* seed showed no significant difference in germinability because the mean germination percentage was the same. Neg and Anderson (2005) also showed that storage time and storage temperature had significant effect on free fatty acid content in Quinoa seed oil. During storage, products especially stored oils compositions can be influenced by several storage conditions (Azhari et al. 2008).

CONCLUSION

When all factors (storage temperature, storage period and populations) were considered together they influenced germinability by decreasing *Excoecaria bussei* germination ability. Temperature and storage duration were the most important factors which affected seeds germinability. Storage temperature highly affected the seed germination percentage, which also declined with increasing storage time. Storing seeds at 15°C can retain seed viability for longer period of time of up to 9 months after harvesting but only for 3 months when stored at temperature at 30°C. If suitable storage conditions are not provided, then *Excoecaria bussei* seeds should be sown immediately for maximum germination percentages. Since harvesting was done only once at the end of August, 2013, further studies are needed to assess the reproductive physiology of *E. bussei* so as to ascertain the best time of harvesting seeds for optimal germinability.

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