EFFECTS OF TEMPERATURE VARIATIONS AND PRE-SOWING TREATMENTS ON THE GERMINATION OF *Milicia excelsa*: A CASE STUDY OF SEEDS COLLECTED FROM BENCHI-MAJI ZONE, SOUTH-WESTERN ETHIOPIA

Mohammed Adefa[1,2*], and Yigardu M. Mengesha[2]

[1]Central Ethiopia Environment and Forest Research Center (CEEFRC)
[2]Ethiopian Forestry Development (EFD)

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EFFECTS OF TEMPERATURE VARIATIONS AND PRE-SOWING TREATMENTS ON THE GERMINATION OF *Milicia excelsa*: A CASE STUDY OF SEEDS COLLECTED FROM BENCHI-MAJI ZONE, SOUTH-WESTERN ETHIOPIA. Seeds always exhibit some degree of dormancy, resulting in a delay and irregularity of germination, and the seeds of *Milicia excelsa* often have seed germination difficulty. The main objective of this study was to demonstrate the seed germination variations of *M. excelsa* at room temperature (20-25°C) and incubator (25.6°C). In this study, 400 seeds of *M. excelsa* were tested using three pre-sowing treatments and control. Each treatment was defined as four replications in which 25 seeds per replication were initially sown in a 90 mm diameter petri dish with disc paper in a completely randomized design. The result showed that the mean germination percentage of the control treatment scored about 23.8%, followed by seeded rubbed by hand with a score of 15%. It is observed that room temperature has a significant effect (29%) on the germination of the control treatment compared to the incubator (18.7%) at p=0.05. However, no significant mean germination difference is observed between the effects of room temperature and the incubator in the remaining pre-sowing treatments: Washed with tap water (T1), 2) Washed and soaked in hot water for 15 minutes (T2) and 3) rubbing by hand (T3). However, it can be inferred that room temperature is an ideal temperature that meets plants' physiological seed germination requirement. To understand the importance of temperature and light on the germination of angiosperm, further experiments involving variable ranges of temperature and light intensity can be conducted.

Keywords: Milicia excelsa, seeds, germination, pre-sowing treatments, room-temperature, incubator

PENGARUH VARIASI SUHU TERHADAP PERTUMBUHAN Milicia excelsa*: STUDI KASUS BIJI YANG DIKUMPULKAN DARI ZONA BENCHI-MAJI, ETHIOPIA BARAT SELATAN. Benih selalu menunjukkan beberapa tingkat dormansi, mengakibatkan keterlambatan dan ketidakteraturan perkecambahan, dan benih Milicia excelsa sering mengalami kesulitan perkecambahan benih. Tujuan utama dari penelitian ini adalah untuk mendemonstrasikan variasi perkecambahan benih* M. excelsa *pada suhu ruang (20-25°C) dan inkubator (25,6°C). Pada penelitian ini dilakukan pengujian terhadap 400 benih* M. excelsa *dengan menggunakan tiga perlakuan pra-tabur dan kontrol. Setiap perlakuan terdiri dari empat ulangan dimana 25 benih per ulangan awalnya ditanam dalam cawan petri berdiameter 90 mm dengan kertas cakram dalam rancangan acak lengkap. Hasil penelitian menunjukkan persentase rata-rata perkecambahan pada perlakuan kontrol sebesar 23,8%, diikuti benih gosok dengan tangan dengan skor 15%. Diamati bahwa suhu ruang memiliki pengaruh yang signifikan (29%) pada perkecambahan perlakuan kontrol dibandingkan dengan inkubator (18,7%) pada p=0,05. Namun, tidak ada perbedaan rata-rata perkecambahan yang signifikan yang diamati antara pengaruh suhu kamar dan inkubator pada perlakuan pra-tabur yang tersisa: Dicuci dengan air ledeng (T1), 2) Dicuci dan direndam dalam air panas selama 15 menit (T2) dan 3) menggosok dengan tangan (T3). Namun demikian, dapat disimpulkan bahwa suhu ruang merupakan suhu ideal yang memenuhi syarat fisiologis perkecambahan biji tanaman. Untuk memahami pentingnya suhu dan cahaya pada perkecambahan angiosperma, percobaan lebih lanjut yang melibatkan rentang variabel suhu dan intensitas cahaya dapat dilakukan.*

Kata kunci: Milicia excelsa*, benih, perkecambahan, perlakuan pra-tabur, suhu kamar, inkubator*

* Corresponding author: mohammed.adefa.seid@gmail.com

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I. INTRODUCTION

The efficiency of a viable seed to germinate is determined by a series of factors such as inherent genotypic & physiological traits, temperature, moisture, air/oxygen and light (Tribouillois et al., 2016). Temperature and light are ecological factors of importance in regulating seed germination (Bewley et al., 2014). Temperature is one of the primary factors affecting the percentage and speed of germination. It directly regulates the biochemical reactions and the metabolism involved in the germination process (Probert, 2000). Most species require an appropriate temperature range to achieve maximum germination.

Temperature on seed germination has been studied for several plant species, including many crops (Schopfer et al., 1979; Dorsainvil, 2002; Wilke, Snapp, 2008; Lee, 2009; Jacobsen et al., 2010). Many studies showed that the germination percentage usually increases linearly with temperature up to an optimal temperature, after which the germination percentage decreases sharply (Tolyat et al., 2014; Fallahi et al., 2015; Laghmouchi et al., 2017). Hence, the range of temperatures favourable for seed germination can be described by cardinal (minimum, optimum and maximum) temperatures (Bewley and Black, 1994). Therefore, the ecological requirements of seeds to germinate can be considered an adaptation strategy to guarantee favourable conditions for seedling development and survival in some species (Gresta et al., 2010).

When the temperature increases or decreases, seeds will cease germination and instantly germinate upon being exposed to suitable temperatures. This process is called thermo-inhibition or thermos-dormancy (Huo et al., 2013; Geshnizjani et al., 2018). The seeds of most perennial and annual plants effectively germinate at temperatures ranging from 10°C to 20°C (Washitani and Masuda, 1990).

M. excelsa is a deciduous tree with a height ranging from 30-50m and a straight clear bole. The seeds can be stored in airtight containers in a cool dry place for up to 2 years with no significant loss of viability (Bekele-Tesemma, 2007; Orwa, 2009). *Milicia excelsa* is mostly propagated by seed. The seeds are an orthodox type, small and light brown, about 1.5 mm long and 1.0 mm wide, and with an estimate of 400,000-500,000 seeds per kg (Luemba, 1999; Orwa, 2009). The germination rate of a mature and healthy seed lot *M. excelsa* is about 45% under normal conditions (Nzekwe et al., 2013). Therefore, the main objective of this study was to demonstrate the seed germination variations of *M. excelsa* at room temperature (20-25°C) and incubator (25.6°C).

II. MATERIAL AND METHODS

Seeds were collected from the Bebeka area, located in Benchi district of Benchi-Maji zone, south-western Ethiopia, located in 6.946°N and 35.481°E (Figure 1). Seed collections were made in January and March 2020. The seed germination trial was carried out in the tree seed laboratory of the "Tree Seed Research and Service Unit" of the "Ethiopian Forestry Development (EFD)", Addis Ababa, Ethiopia. The area is currently one of the administration districts of South West Ethiopia Peoples' Region. The area's annual precipitation and temperature pattern is summarized in Figure 2. The climate conditions in Bebeka area is characterized by water stress only during December and January.

After seed collection, the ripened syncarps of *M. excelsa* were extracted by soaking in water for 24 hours, followed by hand squeezing, and seeds were dried to 5.5% moisture content under sunlight. Finally, possible inert materials were cleaned up by the hand method, and the seeds were ready for germination test (Figure 3). Hence, three presowing treatments modified from Nzekwe et al (2013) were employed: 1) Washed with tap water (T1), 2) Washed and soaked in hot water for 15 minutes (T2) and 3) rubbing by hand (T3) as well as a Control. Each treatment had four replications in which 25 seeds were initially sown in each replication. In other words, 100 seeds were sown in 90mm diameter petri dish with disc paper in a completely

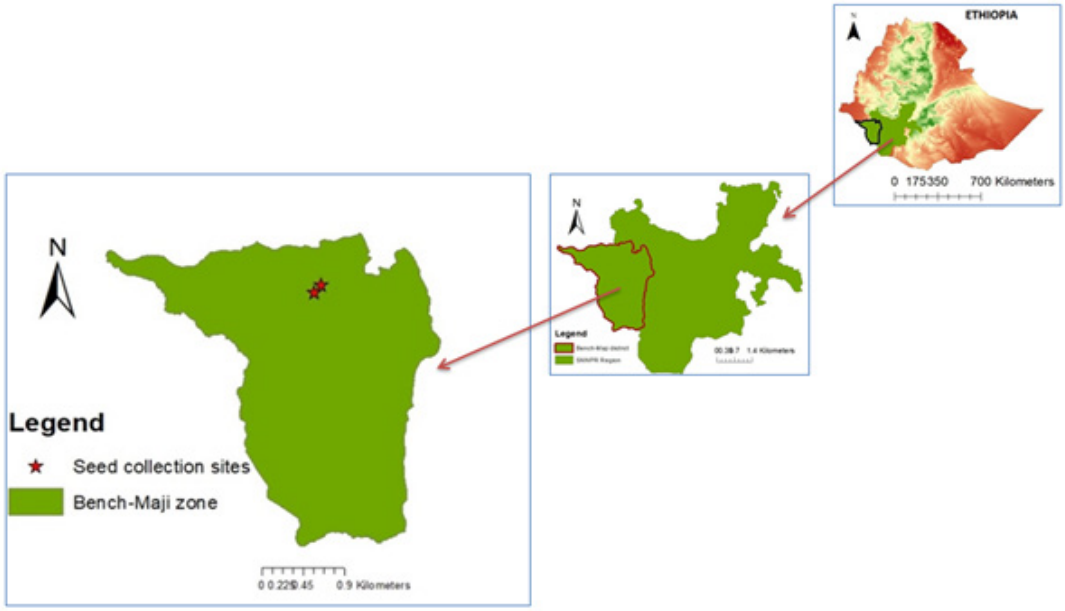

Figure 1. Map of the study area and seed collection sites

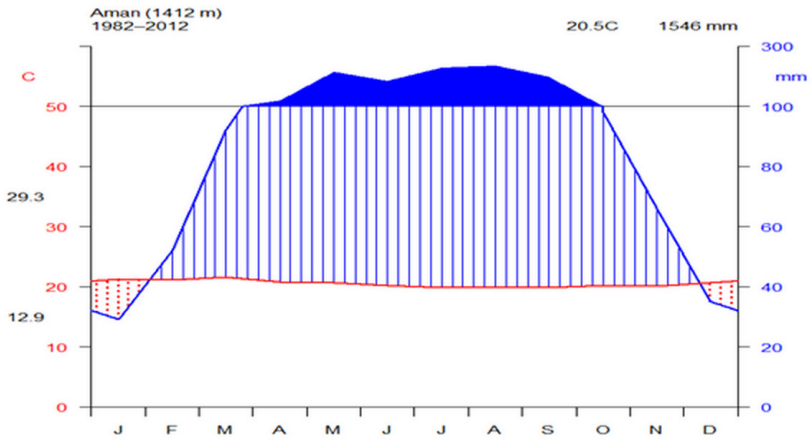

Figure 2. Walter climate diagram representing the climatic conditions of Mizan-Aman, the area of the seed collections

randomized design. The germination duration lasted for 3 months, where initial germination recording was taken starting from second weeks after sowing and every day for 3 months. The effect of temperatures on the germination of *M. excelsa* was determined by putting equal sets of replications from each treatment under room temperate (20-25°C) and in the incubator (25.6°C). The effect of temperature variations on the growth of *M. excelsa* under the different presowing treatments was analysed and determined independently using independent one-way t-test.

The total germination percentage (TGP) of each treatment was calculated using the formula: TPG = (Number of seeds germinated/ Number seeds initially sown)*100. Hence, the mean germination percentage (MGP) of each treatment was calculated through dividing the TGP by the number of replications (n) under each treatment. Furthermore, Following Ellis and Roberts (1981) the mean germination time (MGT) of each treatment was calculated using the formula: MGT = $\sum$ (n × d) / N; Where "n" = number of seeds germinated on each day, "d" = number of days from the

beginning of the test, and "N" = total number of seeds germinated at the termination of the experiment.

III. RESULT AND DISCUSSION

The mean germination percentage of the control treatment scored about 23.8% followed by seeded rubbed by hand (T3) with a score of 15% (Table 1 and Figure 4). Under normal conditions, the germination rate of a mature and healthy seed lot *M. excelsa* is about 45% (Nzekwe et al., 2013). This proves that the germination performance of seed across a period is depended on the genetic structure and locations from the seeds were collected so that the environmental conditions experienced by mother plants determine germination patterns (Sales et al., 2013). Similarly, Mapongmetsem et al. (1999) indicated that *M. excelsa* tends to have a mean germination of 51.6% for using different pre-sowing treatments. The highest TGP was observed for untreated seeds (i.e. 95.2%) which were the combined observation of the four replications (Table 1), followed by seeds rubbed by hand (i.e. 60%).

Form Table 1 note that the values designated with the same alphabet (s) along the mean ($\bar{x}$) vertical column are not significantly different at p=0.05, and vice versa.

The longest mean germination time (MGT) of 49 days was recorded by T3, followed by the Control with 37 days of MGT; and the shortest MGT was observed by T1(Figure 5).

Seeds of *Milicia excelsa*

Figure 3. Seed of *M.excelsa* collected and processed for germination test

Table 1. Summary of TGP, MGP, standard deviation (SD) and standard error (SE) calculated for the four treatment types.

Treatments	TGP	MGP	SD	SE
Control (untreated)	95.2	23.8[a]	0.12	0.036
Washed by tap water-T1	34	8.5[b]	0.075	0.025
Washed and soaked in hot water for 15 minutes-T2	32	8[b]	0.066	0.022
Rubbing by hand -T3	60	15[a]	0.062	0.021

Remarks:
The *p*-value calculated using an independent pairwise t-test: *p*(Control Vs T1) = 0.0001, *p*(Control Vs T2) = 0.06, *p*(Control Vs T3) = *0.14*, *p*(T1 Vs T2) =*0.11*, *p*(T1 Vs T3) = 0.004 and *p*(T2 Vs T3) = 0.01.

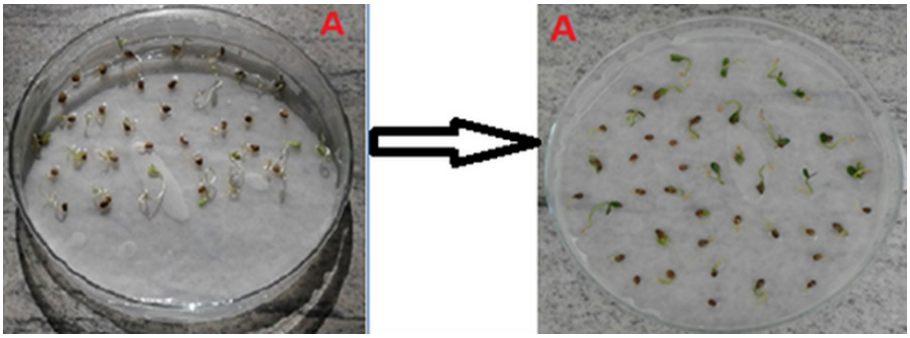

Figure 4. Germinated seeds isolated, after data records, from their respective petri-dishes to transplantation into polythene bag

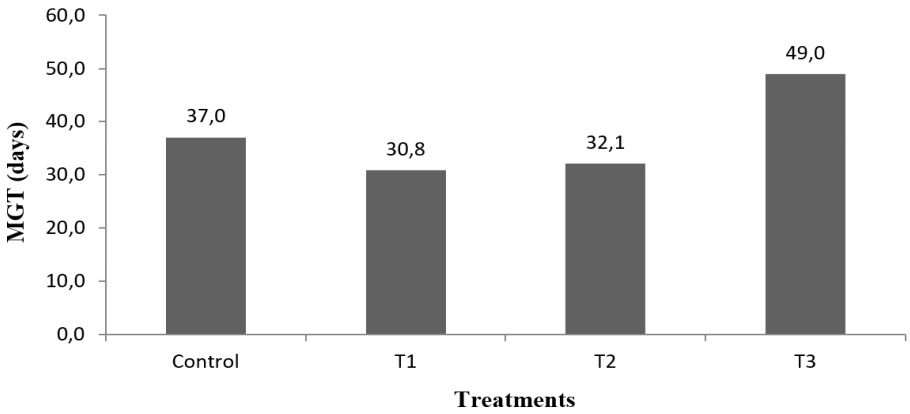

Figure 5. Mean germination time (MGT) required by the different seed treatments

Table 2. Distribution of MGP for each treatment under room temperature and incubator.

TREATMENTS	MEAN GERMINATION (%)		T-test p-value
	Room temperature (20-25°C)	Incubator (25.6°C)	
Control	29[a]	18.7[b]	0.007
T1	12[c]	5[c]	0.21
T2	8[d]	8[d]	0.5
T3	13[e]	17[e]	0.11

Remarks:

For each treatment, values designated with the similar alphabet (s) along the horizontal rows are not significantly different, while values with different alphabet (s) along the horizontal rows are significantly at p=0.05

This indicated that seeds rubbed by hand (T3) were slowly germinated in time, while seeds treated with only normal tap water (T1), were germinated faster in time. This finding has great implications when it comes to selection of appropriate seed treatment technique and nursery management and conservation of the *M. excelsa*.

The effect of temperature difference (room temperature and incubator) on the germination of seeds was also investigated (Table 2). It is observed that sowing and growing under

room temperature significantly affecting the germination of the control treatment (29%) compared to its counterpart i.e. in the incubator with 18.7% (Table 2). However, temperature difference (i.e. sowing and growing in room temperature and incubator) showed no significant difference on the germination of seeds soaked and treated with only normal tap water (T1), hot water (T2) and rubbed by hand (T3).

In this study, except in treatment 3, the room temperature is observed to favour the germination of the seeds of *M. excelsa*. The effect of room temperature (20-25°C) in the control treatment is significantly larger compared to the effect of the incubator (25.6°C) at $p < 0.05$. This might be because room temperature is more ideal to the niche of the species and meets because room temperature is more ideal to the niche of the species and meet one of the microclimate required for seed germination. Moreover, the temperature ranging from 20-25°C for the room temperature itself could favour seed physiology and germination ability.

In this regard, Heidari et al (2014) also indicated that the temperature had a significant effect on the maximum germination percentage, germination uniformity, germination rate of seeds. Kim et al (2016) also reported that the incubation temperature and photoperiod significantly affect the germination responses of species such as *Hosta yingeri*; and the optimum temperature for germination was reported to be around 30°C considering the final germination percentage. Similarly, Gairola et al (2011) also reported the effect of temperature and media on seed germination of *Jatropha curcas* in which the optimum temperature for germination was recorded at around 30°C.

IV. CONCLUSION

This study tried to test the germination biology of seeds of *M. excelsa* collected from Aman-Bebeka area located in Benchi district of Benchi-Maji zone, south-western Ethiopia. The study was meant to test the seed germination capability of *M. excelsa* using different pre-sowing treatments and temperature variation. In this study, room temperatures (20-25°C) generally showed relatively promotes the seed germination ability of *M. excelsa* compared to the seed germination in an incubator in defined temperature (25.6°C) Overall, it is observed that the species has a germination difficulty because of mechanical and physiological reasons. Hence, breaking the seed dormancy through different mechanical and biochemical ways is the most important option to increase the seed germination capability of tree seeds to promote forestry. Finally, it is recommended that further studies and experimentation incorporating other pre-sowing treatments and seed tissue culture may help improve germination rates of the species.

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