

Full length Research paper

In vitro seed germination biology of *Milicia excelsa* and *Terminalia ivorensis* populations collected in the Benchi Maji zone in southwestern Ethiopia

Mohammed Adefa^{1*} and Yigardu Mulatu²

¹Central Ethiopia Environment and Forest Research Center, EEFRI² Ethiopian Environment and Forest Research Institute (EEFRI)

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Milicia is an excellent source of timber, and we need to find innovative ways to utilize native trees. *Terminalia ivorensis* is an introduced lumber that is difficult to germinate. The main purpose of this experiment is to study the in vitro seed germination physiology of *M. excelsa* and *T. ivorensis*. Therefore, we tested the seed germination physiology of *M. excelsa* and *Terminalia ivorensis* collected in the Benchi district of the Benchi Maji zone in southwestern Ethiopia to examine germination responses at various pre-sowing treatments. A total of 1000 *M. excelsa* seeds were tested with 9 pre-sowing treatments (and controls), 4 iterations each, and 25 seeds per replication at room temperature in a fully randomized design. First sown with disc paper (3 month test period). Meanwhile, *T. ivorensis*. A total of 300 seeds of *ivorensis* were seeded and tested, two pre-sowing treatments (and controls) were repeated 4 times each, and 25 seeds were first sown in sand trays at room temperature (test). Period 3 months). Seed germination analysis of *M. excelsa* was better with sandpaper-rubbed seeds (39%), followed by seeds treated with hot water at 70 ° C for 15 minutes (24%), and seeds soaked in hot water and soaked in 1 g / LNAA. It shows that the germination rate is high. (twenty two%). Analysis of the one-way study shows that the average germination of "sand seeds" is significantly higher than the effect of other pre-sowing treatments at $p < 0.05$. Meanwhile, *T. ivorensis*. Seed germination of *ivorensis* shows a high average germination rate of untreated seeds (62%), followed by "24-hour water-soaked seeds (47%)", but ttest shows significance. Is not ... 0.05 for $p < 0.05$. Overall, *M. excelsa* seed germination capacity. *Excelsa* can be improved by rubbing it with sandpaper and soaking it in hot water or biochemicals such as growth hormone to break the dormancy. In addition, *T. ivorensis*. Other potential pre-sowing techniques can be explored to further explore potential techniques for promoting seed germination of *ivorensis*.

Keywords: *Milicia*, Timber, *Terminalia*, Seeds Germination, Physiology, Pre-sowing, treatments

INTRODUCTION

Ethiopia is becoming dependent on wood products imported, and the demand for sawn wood, paper and ply wood is steadily increasing each year (CSA, 2012). In spite of there are many indigenous timber species in Ethiopia, species used for domestic timber production are limited to 3 to 4 species namely *Eucalyptus*, *Cupressus*, *Pinus* and to some extent *Grevillea*. However, potential timber species including *Milicia excelsa*, restricted within

natural forest ecosystems in south and south-western parts of the country, can be used for production of quality timber (Desalegn *et al.*, 2011). Natural forests in western and south-western Ethiopia are the major sources of livelihood (timber and non-timber forest products) for the communities in the surrounding area (Gole and Fetene, 2007). However, in recent years, conversion of forest ecosystems into agriculture lands (coffee and tea plantations) is becoming increasing. As the result, species including *M. Excelsa* are under high pressure due to random cuttings and deforestation (Amdie, 2007; Besufekad, 2012).

M. excelsa is a deciduous tree with a height ranging from 30- 50m

*Corresponding author's email: mohammed.adefa.seid@gmail.com

and a straight clear bole. The seeds can be stored in airtight containers in a cool dry place or a period of up to 2 years with no significant loss of viability. Under normal conditions, the expected germination rate of mature and healthy seed lot is 45% on average. So, before sowing, seeds can be pretreated using tap water, hot water at 50-60°C, concentrated H₂SO₄ and other techniques. Under nursery conditions, pretreated seeds can be sown in paper and sand, and garden soil, sawdust and poultry substrates with required replications to determine the accurate mean germination percentage (Nzekwe *et al.*, 2013). Similarly, *T. ivorensis* is among the most important of the some 200 species belonging to the genus *Terminalia* (Combretaceae). *T. ivorensis* has bisexual or hermaphroditic flowers (Coates-Palgrave, 1977; Oyewole and Adedamola, 2015). Although male and female flowers are in the same plant, self-pollination do not always produce viable zygotes (Newbegin *et al.*, 1994). Tree seeds always exhibit some degree of dormancy resulting a delay and irregularity of germination seeds in the nursery and even in forest floors (Maiden *et al.*, 1990; Oyewole and Adedamola, 2015). Hence, in order to obtain better germination and quantity of seedlings, it is always necessary to apply pre-sowing treatment techniques (mechanical and physiological). The number of seedlings produced from a given number of seeds is termed as germination rate is the direct measurement of viability of a given seed lot (Guerke, 2005). The seed germination biology of *M. excelsa* is not well studied and the seeds of *T. ivorensis* also germinate with great difficulties. Therefore, the main objective of this experiment is to investigate the seed germination biology of *M. excelsa* and *T. ivorensis* under *in-vitro* laboratory conditions.

MATERIAL AND METHODS

***Milicia excelsa*:** Seeds were collected from around Bebeka area which is located in Benchi district of Benchi-Maji zone, south western Ethiopia.

The ripen syncarps of *M. excelsa* were extracted by soaking in after for 24 hours followed by hand squeezing; and seeds were dried to 5.5% moisture content using sun light. Finally, seeds were cleaned and purified by hand sorting method. Then, seeds were treated using different pre-sowing techniques for germination test experimentations. So, a total of 1000 seeds were randomly chosen from the population and tested using paper discs method in a completely randomized design. The total 9 pre-sowing treatments (plus control) with 4 replications each where 25 seeds per replication were initially sown (Figure 2 & 3). Germination data was recorded between 19 March 2020 and 15 May 2020.

***Terminalia ivorensis*:** Seeds were collected from around Bebeka area which is located in Benchi district of Benchi-Maji zone, south western Ethiopia. After procurement, the cones were exposed to sun heat for 24h and then the seeds were collected after natural opening. Seeds extracted were further purified to 97% and the moisture content was maintained to <5%. The number of seed/kg was found to be 12155. Hence, the seed samples were divided in tow three equal part of which two parts were treated using (1) seed soaked in water for 24 hours, and (2) seeds soaked for 10 minutes in 80% concentrated H₂SO₄ then abundantly rinsed in running water for 20 minutes, and the third part was maintained as control (Figure 1). The seeds of *T. ivorensis* have a size of greater than 2mm. So, germination trial on sand tray is most recommended following the standards (Ellis *et al.*, 1985; ISTA, 2005; AOSA, 2005). So, a total of 300 pre-treated seed (100 seeds for each treatment divided into 4 replications in which 25 seeds were sown per a tray). Then, proper watering and follow up was done between 19 March 2020 and 15 May 2020). Therefore, data for germinated was recorded for a period of 3 months after sowing.

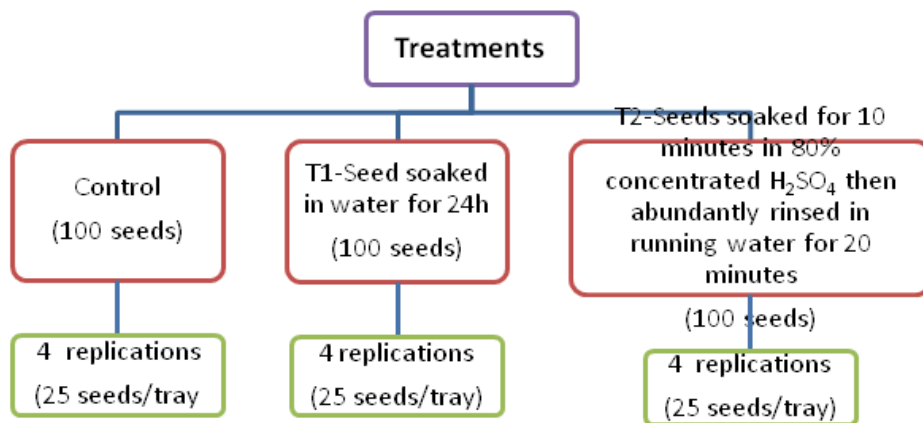


Figure1: Illustration of the sampling and processing of the seeds of *T. ivorensis* before sowing

For both species experimentations, the average percentage germination of each treatment was calculated using the formula:

$$\% \text{ germination} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

RESULT AND DISCUSSION

Germination capability of seeds of *M. excelsa* under different pre-sowing treatments

***Milicia excelsa*:** In this experiment, higher germination percentage was recorded for seeds rubbed by sandpaper (39%), followed by seeds treated by 70°C hot water for 15 minutes (24%) and seeds soaked in hot water and soaked in 1g/L NAA (22%). Analysis of one-way t-test shows that the effects of rubbed by sandpaper is significant at $p \leq 0.05$ compared to the effects of the other pre-sowing treatments. (Figure 2). On the other hand, Mapongmetsem *et al.* (1999) reported that *M. excelsa* tends to have a mean germination of 51.6% for using different pre-sowing treatments.

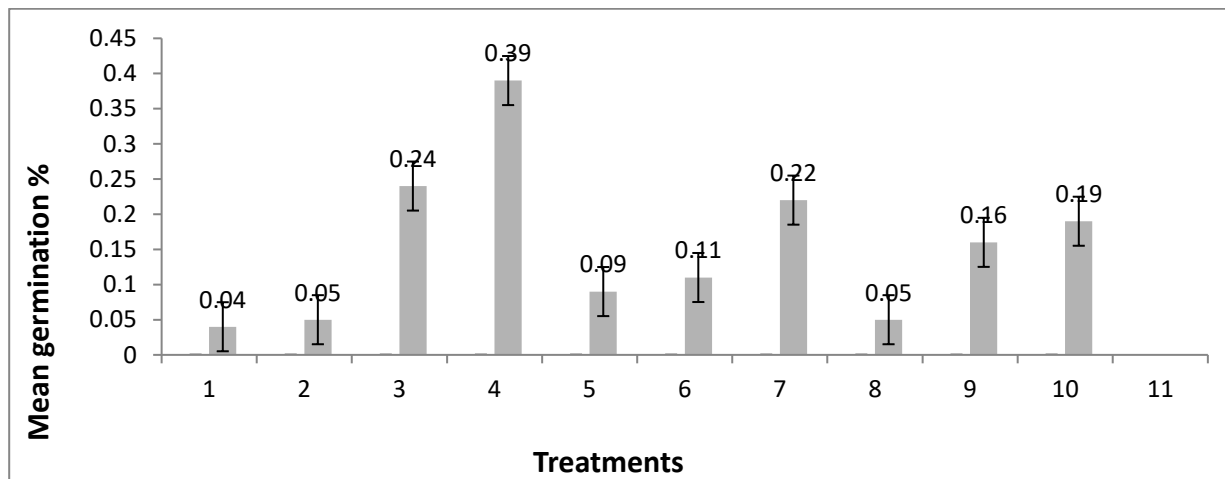


Figure 2: Mean germination percentage of different pre-sowing treatments: 1= Control, 2= Washed and soaked in tap-water for 24hrs, 3= Soaked in 70°C hotwater for 15 minutes, 4= Rubbed by sandpaper, 5= Soaked in 80% H₂SO₄ for 15 minutes, 6= Soaked in hotwater and soaked in 1g/L GA₃, 7= Soaked in hotwater and soaked in 1g/L NAA, 8= Soaked in hotwater and soaked in 1g/L IBA, 9= Soaked in hotwater and soaked in 1g/L BAP, and 10= Hand scarification.

Nyamukuru *et al.* (2014) also tested the germination of the species without any pre-sowing treatments procedure and found that the mean germination was observed to be only .0%. The observation of such variations of seed germination depends on the physiological and genetic quality of seeds of the different population of *M. excelsa* distributed in wider ranges of agro-ecological conditions.

In other words, tree seeds including of *M. excelsa* always exhibit some degree of dormancy resulting a delay and irregularity of germination seeds in the nursery and even in forest floors (Maiden *et al.*, 1990; Oyewole and Adedamola, 2015). Overall, the expected germination rate of mature and healthy seed lot is 45% on average (Nzekwe *et al.*, 2013).

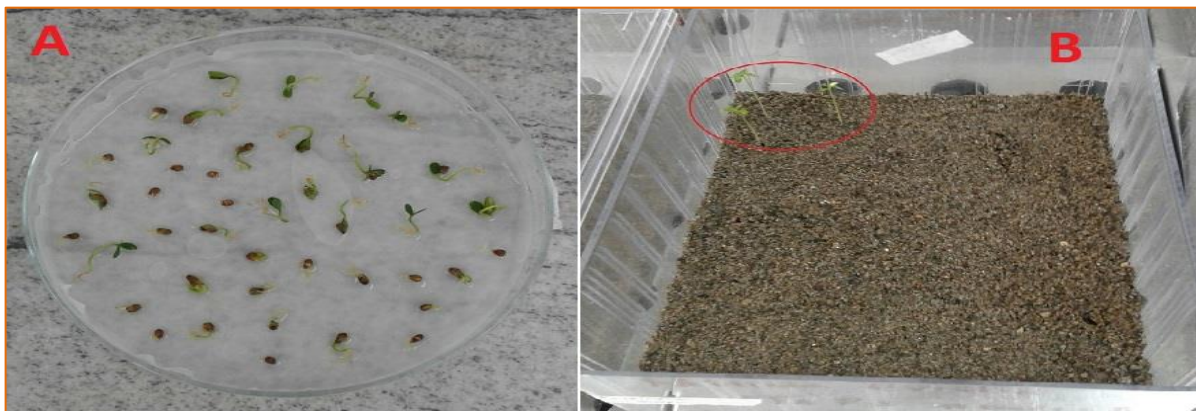


Figure 3: Propagules of *M. excelsa* established on disc paper and transferred to sand tray

Terminalia ivorensis: Analysis of the germination records shows that seeds that were not treated (i.e. control) show better germination compared to the seeds that were treated using different pre-sowing treatments. Accordingly, the mean germination percentage of untreated seed is found to 62% followed by seeds soaked for 24 hours (47%). However, seeds treated with 80% [H₂SO₄] for 10 minutes then abundantly rinsed in running water for 20 minutes shows no germination at all (Figure 4 & 5). So, both the 62% and 47% germination rates recorded are fairly accepted ones considering the low germination capacity of *T. ivorensis* in laboratory

conditions. However, other possible pre-sowing treatment techniques can be attempted to further increase the seed germination of *T. ivorensis*. On the other hand, under nursery conditions the seed germination test for *T. ivorensis* conducted by Ibe *et al* (2015) using three growing media (sawdust, sterilized river sand and garden topsoil) show that the highest possible germination percentage recorded was 9.44% using garden topsoil growing media. Normally, the seed of *T. ivorensis* has great difficulty of germination because of the presence of hard seed coat; most probably because this is thick and lignified one.

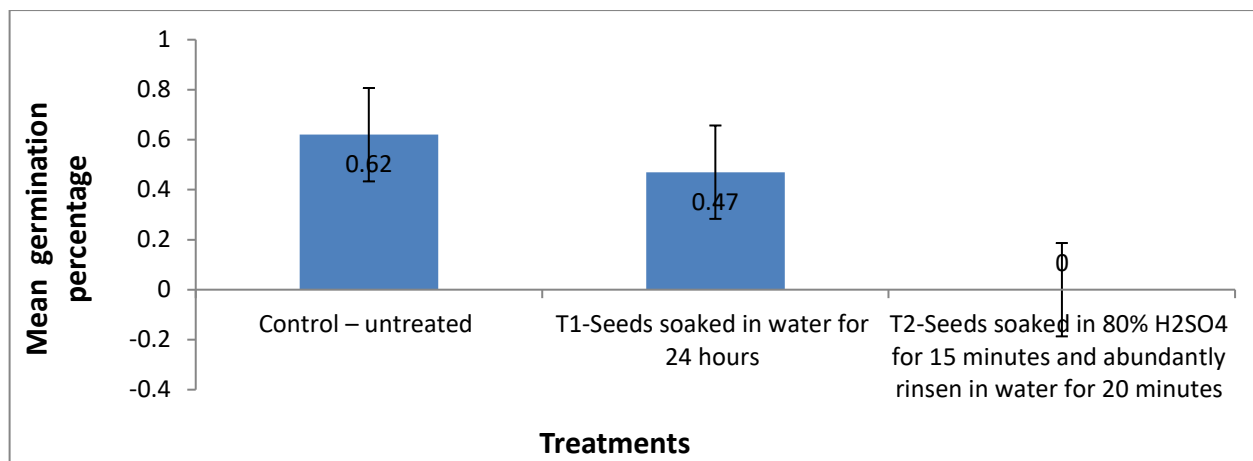


Figure 4: Mean germination percentages of the different treatments in sand substrate after six months observation



Figure 5: In-vitro seed germination trail of *T. ivorensis* on sand tray

CONCLUSION AND RECOMMENDATION

Milicia excelsa is an important potential timber trees which is indigenous to Ethiopia. The species is mainly distributed across south and south western part of Ethiopia. In order to promote its domestication, a study of the seeds germination biology is most important task to

be accomplished. Therefore, in this study the seeds of *M. excelsa* were tested in order to understand the germination physiological response in different pre-sowing treatments. So, seeds scarification using sandpaper is observed to be satisfactory (39%) considering the average germination of seeds of *M. excelsa* in natural conditions (45%). *Terminalia ivorensis*

is an exotic timber tree to Ethiopia. The seeds of *T. ivorensis* germinate with great difficulty which is most probably caused by the thick and lignified seed coat. In this experiment of seed germination test for *T. ivorensis*, untreated seeds performed better germination compared to seeds soaked and treated using water and H₂SO₄. Therefore, the 62% mean germination recorded from untreated seeds and even and the 47% germination recorded from seeds soaked using water for 24hrs are good germination outputs considering the low germination capacity of *T. ivorensis* in laboratory and natural conditions. To sum up, it is also suggested that further studies and experimentation using other pre-sowing treatments such as growth hormones and seed tissue culture may help improve germination rates of both species.

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Declaration of Competing Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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