In-vitro Seed Germination Biology of Milicia Excelsa Population Collected from Benchi-Maji Zone, South Western Ethiopia

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Abstract

Background: In Ethiopia, species used for timber production are limited to few species. So, it is becoming necessary to find innovative ways of maintaining and improving the genetic quality of species such as *Milicia excelsa* by domesticating them into a wider scale of ecological and social environment. Hence, the main objective of this study was to investigate the seed germination biology and behavior of *Milicia excelsa* under *in-vitro* laboratory conditions so as to promote the domestication and establishment of seed production areas (SPA) *in-situ.*

Methods*:* Seeds collected from Benchi district of Benchi-Maji zone of southwestern Ethiopia were subjected to germination biology in-vitro to determine the germination responses of *M. excelsa* seeds under laboratory conditions. So, a total of 1200 seeds were randomly selected, treated and prepared using four treatments procedures with six replications for paper method and 3 replications for sand method, and tested using paper methods in CRD design. So, 600 seeds were tested using paper and sand methods each where 25 seeds were tested for each replication in paper and 50 seeds for each replication in sand method. The germination process was lasted between 19 March 2020 and 15 May 2020.

Result: The mean germination percentage of seeds of *M. excelsa* was noticed to be lower than 20%. This is below the expected (45%) germination rate of mature and healthy seed lot. Overall, seeds that were not treated using any pre-sowing treatments has shown relatively better germination percentage (19%) followed by seeds rubbed by hand (15%). Analysis of one-way t-test shows that the effects untreated (control –T1) and rubbing by hand treatments (T4) is significant at $p \leq 0.05$ compared to the effects of the other pre-sowing treatments (T2- seeds only washed with normal tap water, and T3- seeds washed and soaked in hot water for 15 minutes) on the germination capability of seeds of *M. excelsa* . However, no significance difference was observed between control (T1) and hand scarification (T2).

Conclusions: The average germination of *M. excelsa* seeds combined for all treatments is lower than 20% which is lower germination when comparing with other similar research reported in other countries. Therefore, it required to include and test other pre-sowing treatments including tissue culture micro-propagation of seeds to maximize the germination of *M. excelsa* seeds helping to get enough seedlings required for the domestication project.

Background

Ethiopia is becoming dependent on wood products imported, and the demand for sawn wood, paper and ply wood is steadily increasing each year [1]. 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, Cupresuss, 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 [2]. 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 [3]. However, in recent years, conversion of forest ecosystems into agriculture lands (coffee and tea plantations) is becoming increasing. As the result, species including *Milicia excelsa* are under high pressure due to random cuttings and deforestation [4, 5].

Milicia excelsa is a deciduous tree with a height ranging from 30–50 m and a straight clear bole. Their seeds are dried to required moisture content (< 10%) either in the sun or under shade. Seed 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. The expected germination rate of mature and healthy seed lot is 45% on average. Before sowing, seeds can be pretreated using tap water, hot water at 50-60^{o}C, concentrated H_2SO_4 and other techniques. Under laboratory and 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 [6]. Therefore, the main objective of this study was to investigate the seed germination biology and behavior of *Milicia excelsa* under *in-vitro* laboratory conditions so as to promote the domestication and establishment of seed production areas (SPA) *in-situ* and *ex-situ*.

Methods

Seeds were collected from around Bebeka area which is located in Benchi district of Benchi-Maji zone, South western Ethiopia, during 2019. 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 procedures to proceed to the germination testing experimentations.

Therefore, 1200 seeds randomly chosen from the population in which 600 seeds were tested using paper discs and the remaining 600 seeds were tested using sand tray method in a Completely Randomized Design (CRD). The total number of treatment were 4 with 9 replications each; 6 replications were tested using paper method (3 replications tested under room temperature and other 3 replications tested in the incubator) where 25 seeds were initially sown; and the other 3 replications were tested under sand method where 50 seeds were initially sown. Germination data was recorded between 19 March 2020 and 15 May 2020.

Result

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

Analysis of one-way t-test shows that the effects untreated (control) and rubbing by hand treatments is significant at $p \leq 0.05$ compared to the effects of the other pre-sowing treatments on the germination capability of seeds of *M. excelsa*. However, no significance difference is observed between control (T1) and hand scarification (T2).

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

Treatments	Mean ($\bar{x}$) percentage germination	SD	SE
Control (untreated)-**T1**	0.19[a]	0.12	0.036
Only washed by tap water-**T2**	0.06[b]	0.075	0.025
Washed and soaked in hot water for 15 minutes-**T3**	0.10[b]	0.066	0.022
Hand scarification/ rubbing-**T4**	0.15[a]	0.062	0.021

Note that values designated with the same alphabet (s) along the mean ($\bar{x}$) vertical column are not significantly different at $p \leq 0.05$.

Patterns of germination of seeds in period of time (days)

Analysis of the rate of seed germination in time under different pre-sowing treatments shows that germination started two weeks after sown on sand and paper (N = 300 seeds for each treatment). Untreated seeds (control) attained the maximum seed germination 38th days after sowing and become nearly stationary (i.e. no new germination is observed). However, seeds treated with only tap water (T2) appeared to show the slowest rate of seed germination compared to the other pre-sowing treatment (Fig. 2).

However, seeds rubbed by hand (scarification) appeared to have relatively better rate of germination while untreated seeds (control) showed lower germination rate in paper substrate (Fig. 3). For all treatment types, the maximum possible seeds could be germinated twelve weeks after sown, and remains stationary with no new germination afterward.

On the contrary, seeds washed and soaked in hot water for 15 minutes (T3) is observed to have relatively better rate of germination under sand substrate while seeds washed only by tap water (T2) showed no germination in sand substrate. Similarly, as observed in paper method, the maximum possible seeds could be germinated twelve weeks after sown, and remains stationary with no new germination afterward (Fig. 4).

Discussion

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

In this experiment, the mean germination percentage of seeds of *M. excelsa* is observed to be lower than 20% which is below the expected (45%) germination rate of mature and healthy seed lot. Overall, seeds

that were not treated using any pre-sowing treatments (i.e. control) has shown relatively better germination percentage (19%) followed by seeds rubbed by hand (15%) (Fig. 5).

In this regard, [7] reported that *M. excelsa* tends to have a mean germination of 51.6% where variable germination percentages was recorded using different pre-sowing techniques (i.e. control = 64.6%, hot water soaking = 2.5%, ordinary water soaking = 91%, hand scarification = 100% and 98% [H_2SO_4] treated = 0%). Similarly, [7] also tested the germination of *M. excelsa* using control, cold and hot water treated samples at different temperature sowing in different media combinations (*garden soils, sawdust and poultry droppings*); and the mean germination combined for all treatments were reported to be 46.75% in garden soils and 78.75% in 1:2:1 mixture of garden soil, sawdust and poultry droppings.

Similarly, [8] also tested the germination of the species without any pre-sowing treatments procedure and found that the mean germination was observed to be only 4%. In this study, the reason behind for the observation low percentage of germination as compared to other research reports could be related to possible failure of attaining the right ecological niche, under *in-vitro* laboratory conditions, which is required for proper germination of seeds of *M. excelsa*. Moreover, it is also suggested that the state of viability and health of seeds randomly selected for testing could have possibly affected the germination capability of seeds of the species (Fig. 6).

Effects of growing substrates (paper and sand) on germination capability

It was also attempted to investigate the effects of germination substrates (*i.e. paper and sand*) on the growth of seeds of *M. excelsa*. In this regard, untreated (control) seeds appeared to show no significantly different germination percentage regardless of the sowing substrate, i.e. paper and sand substrates (Table 2). Similarly, [6] also reported the presence of different germination responses under different media substrates.

Table 2
Mean germination distribution between paper and sand methods for control (T1) treatment.

SUBSTRATES	T1 – CONTROL		
	Mean	SD	SE
Paper	0.24^{a*}	0.098	0.04
Sand	0.09^{b}	0.011	0.006

Note that values designated with the different alphabet (s) along the mean vertical column are significantly different at $p \leq 0.05$. The *t-test p* calculated *(pcal) is < 0.05*. Hence, for untreated seeds, it appeared that the mean germination between paper and sand substrates is significantly different at $p \leq 0.05$.

Similarly, for seeds treated with only tap normal water, seeds sown in paper disc showed 8.7% mean germination while totally not germinated under sand substrate. So, the effect of paper disc is quite visible for seeds treated with normal tap water while sand negative effect on the germination capability of seeds only washed by normal tap water. So, sand substrate seems less important for seeds treated with normal tap water (Fig. 7).

However, sand substrate tends to show significance larger effect on the germination of seeds washed and soaked in hot water for 15 minutes (T3). So, it appeared that sand is not important substrate to germinate for seeds treated in hot water for certain period to time compared to the paper disc method (Table 3).

Table 3
Mean germination distribution between Paper and Sand methods for T3. Note that values designated with the different alphabet (s) along the mean vertical column are significantly different at $p \leq 0.05$.

SUBSTRATES	T3- Washed and soaked in hot water for 15 minutes (WS)		
	Mean	SD	SE
Paper	0.08^{b}	0.06	0.02
Sand	0.15^{a*}	0.09	0.03

On the other hand, it is observed that sand and paper appeared to have similar effect on the germination of seeds of *M. excelsa* rubbed and scarified by hand. So, the effects of sand and water substrates on the germination of seeds rubbed and scarified by hand (T4) is not significant at $p \leq 0.05$ (Fig. 8).

Effects of temperature on germination capability of M. excelsa

Moreover, in paper method, the effect of temperature difference (*room temp. and incubator*) on the germination of seeds was also investigated. It is observed that sowing and growing under room temperature has a significant effect on the germination of control-untreated seeds (29%) compared to their germinating response in incubator (Table 4). The same hold true seeds washed and treated only using normal tap water. However, temperature difference (i.e. sowing and growing in room temp. and incubator) showed no different effect on the germination of seeds treated with hot mater (T3) and rubbed by hand-scarification (T4).

Table 4
Distribution of mean germination of paper method for each treatment under room temperature and incubator. 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 $p \leq 0.05$.

TREATMENTS	MEAN GERMINATION (%)	
	Room temperature (25^oC -30^oC)	Incubator (25^0C)
T1	0.29^a	0.19^b
T2	0.12^c	0.053^d
T3	0.08^e	0.08^e
T4	0.13^f	0.17^f

In this regard, [9] 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, [10] 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.

Conclusion

Milicia excelsa is a deciduous tree ranging from 30–50 m height and a straight clear bole. It is none of the potently timber tree species in Ethiopia naturally 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, seeds of *M. excelsa* collected from Benchi district of Benchi-Maji zone was subjected for germination test under different pre-sowing treatments in seed laboratory of the Central Ethiopia Environment and Forest Research center (CEEFRC). A total of 1200 seeds randomly were tested suing paper and sand methods in a Completely Randomized Design (CRD). It is observed that the average germination of *M. excelsa* seeds combined for all treatments is lower than 20%. This is the quite lower germination when comparing with other similar research reported in other countries. So, it required to include and test other pre-sowing treatments including *in-vitro* micro-propagation of seed germplasm to maximize the germination of *M. excelsa* seeds helping to get enough seedlings required for the domestication project.

Therefore, it is recommended for further studies and experimentation with different treatments such as nicking and immersion in sulphuric acid, to find out if these treatments improve propagation rates. Experimentation using seed tissue culture is also the way forward for achieving better seeds germination capabilities.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

MS has designed and involved in the study work from the beginning to the end. MS also organized, analyzed and wrote up the manuscript. YM has designed the initial project development and involved in this survey work. AA has designed the initial project development along with YM. Similarly, SA, MN and WK have involved in the survey work and partially organized the data.

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References

1. CSA. Statistical Abstracts. Addis Ababa: Central Statistical Agency; 2012.
2. Wood properties of different timber species in Ethiopia
   Desalegn G, Abegaz M, Teketay D, Gezaheghn A. Wood properties of different timber species in Ethiopia, 2010.
3. Gole TW, Fetene M Forests of Sheka: ecological, social, legal and economic dimensions of recent land-use/ land-cover changes-overview and synthesis. In Forests of Sheka: Multidisciplinary case

studies on impacts of land use/ land cover changes, southwest Ethiopia. (Masresha Fetene, Ed.), MELCA Mahiber, 2007. PP 1–20.

4. Amdie Z. Land Use/ Land Cover Dynamics and Vegetation Vulnerability Analysis: a Case Study of Arsi Negele Wereda. MSc thesis, Department of earth science, Addis Ababa University, 2007.
5. Besufekad T. Ensuring sustainable forestry management in Ethiopia from the millennium development goals perspectives. MSc thesis, School of Graduate Studies, Faculty of Law Addis Ababa University, 2012.
6. Nzekwe U, Ubani CS, Ajuziogu GC. Effects of seed pre-treatment and media on seed germination and seedling growth of Iroko, Milicia excelsa (We/w.) C.C. Berg moraceae, Syn. Chlorophora excelsa. Afr J Agric Res. 2013;8(18):2057–62.
7. Mapongmetsem PM, Duguma B, Nkongmeneck BA, Selegny E The effect of various seed pretreatments to improve germination in eight indigenous tree species in the forests of Cameroon. *Annals of Forest Science*, Springer Verlag/EDP Sciences, 1999, 56 (8):679–684.
8. Nyamukuru A, Tabuti JRS, Aduma PR. Propagation and seedling establishment of selected multipurpose woody species of Uganda. International Journal of Biodiversity Science Ecosystem Services Management. 2014;10(4):270–4.
9. Kim HJ, Choi SH, Kim YJ. Effects of Temperature on Seed Germination and Photoperiod on Seedling Growth of Hosta yingeri S.B.Jones. The Japanese Society for Horticultural Science (JSHS). The Horticulture Journal Preview, 2015, P. 1–7.
10. Gairola K, Nautiyal AR, Dwivedi AK. Effect of temperatures and germination media on seed germination of Jatropha curcas Linn. *Advances in Bioresearch*, 2011*2* (*2*): 66–71.

Figures

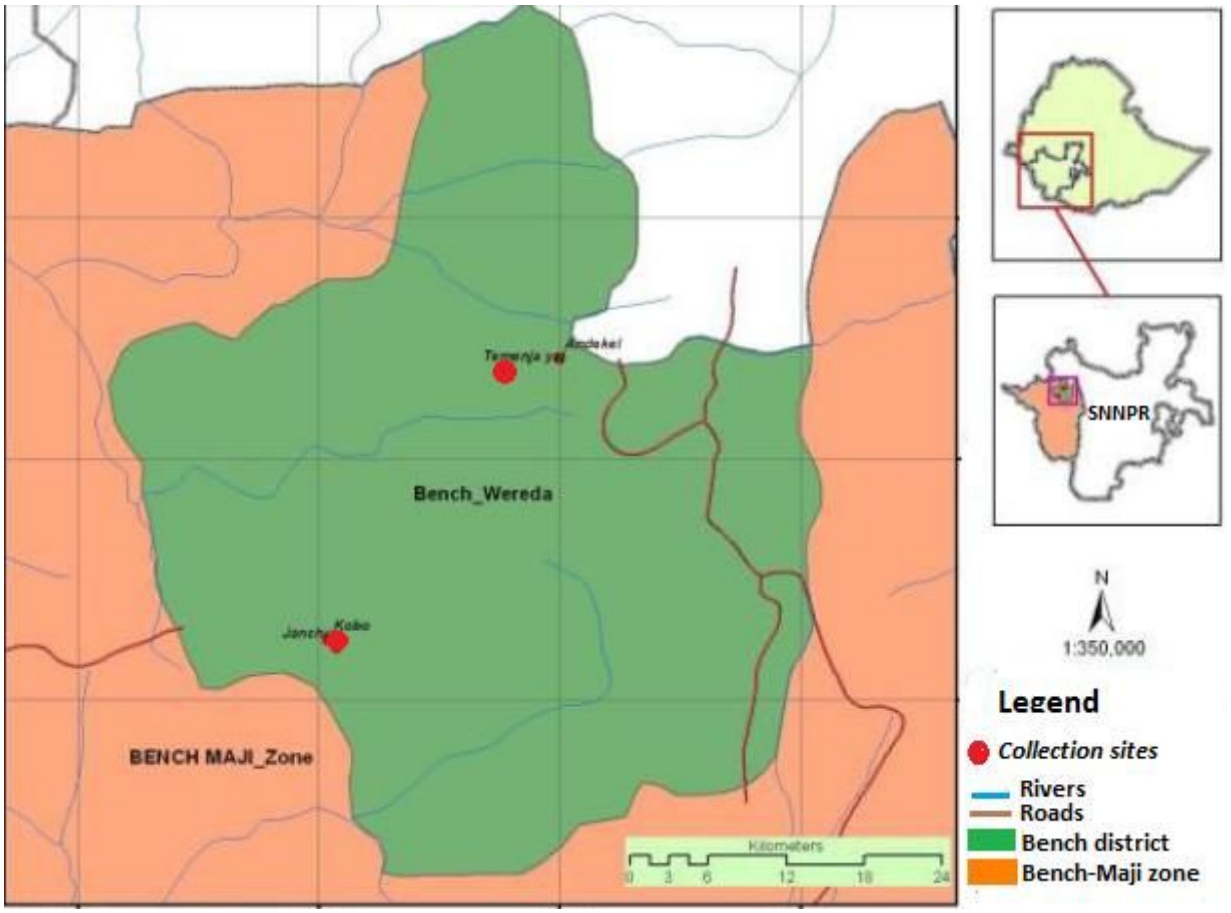

Figure 1

Map of the collection district and sites (Source: own source)

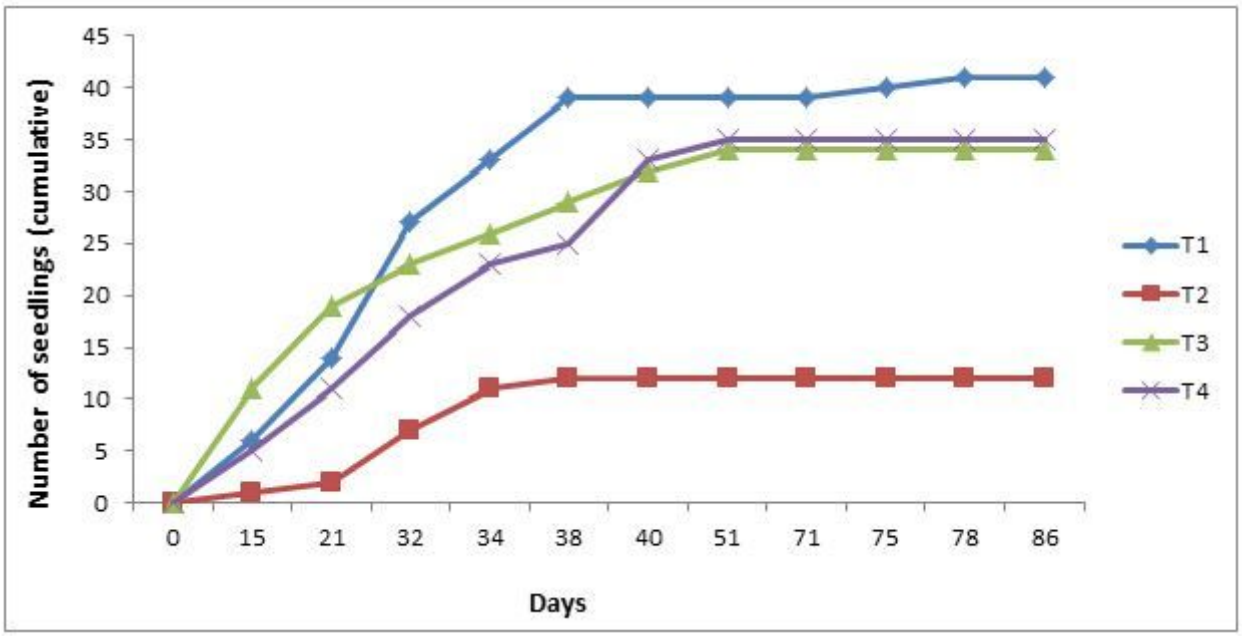

Figure 2

The rate of germination of seeds of M. excelsa over time (days) for the different treatments in paper and sand substrate combined

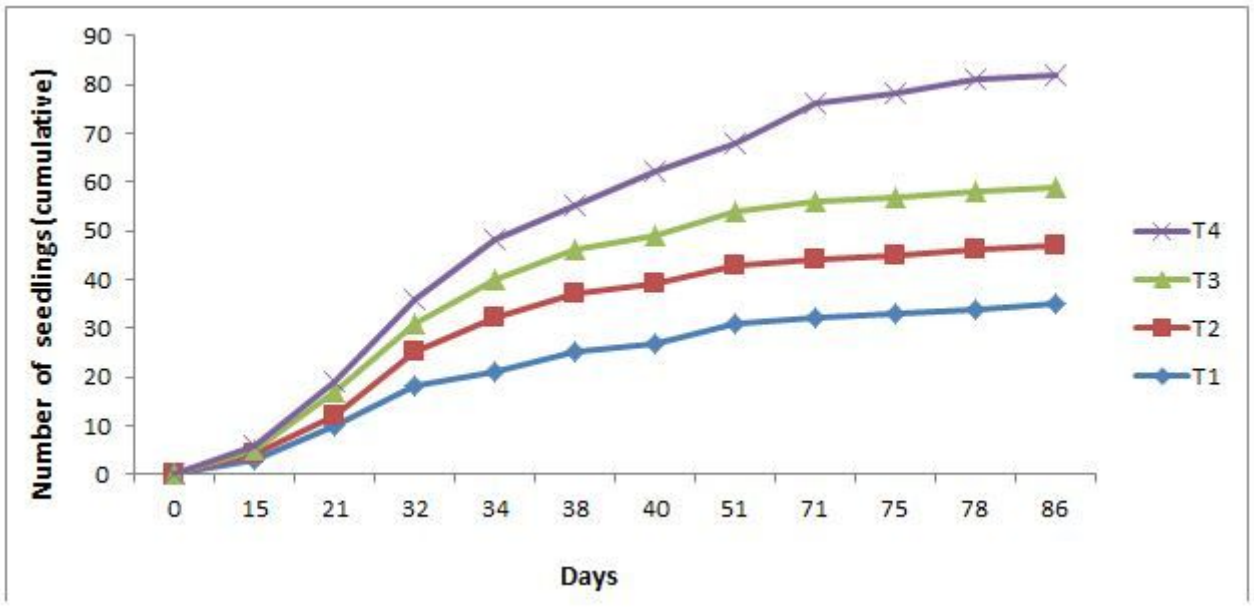

Figure 3

The rate of germination of seeds of M. excelsa over time (days) in paper substrate for the different treatments

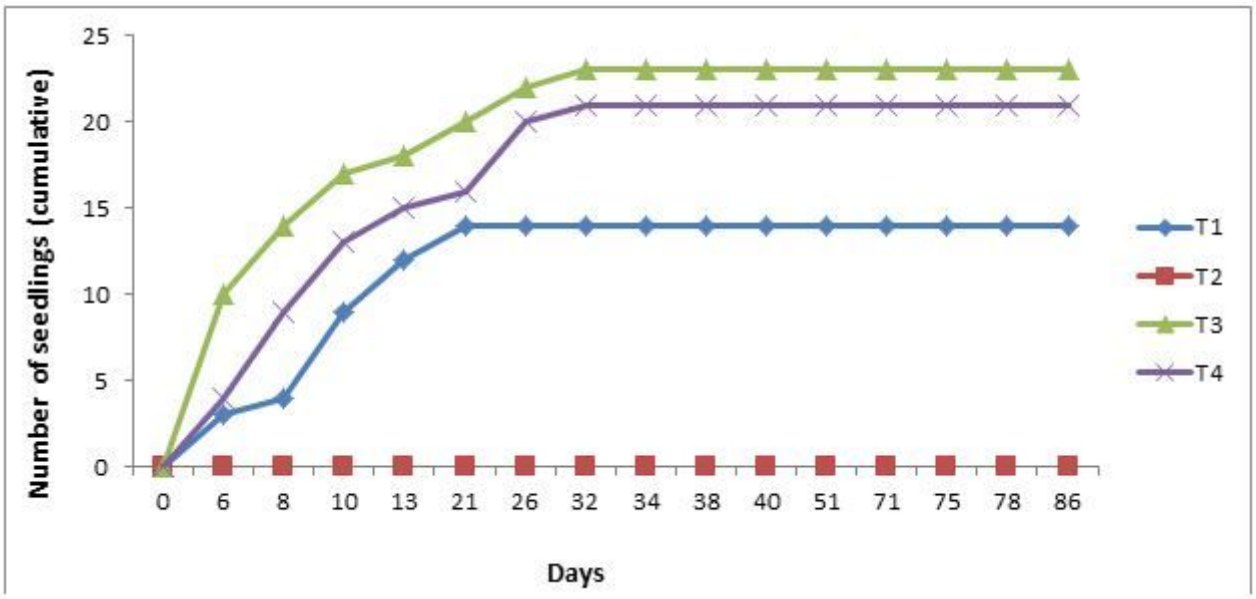

Figure 4

The rate of germination of seeds of M. excelsa over time (days) in sand substrate for the different treatments

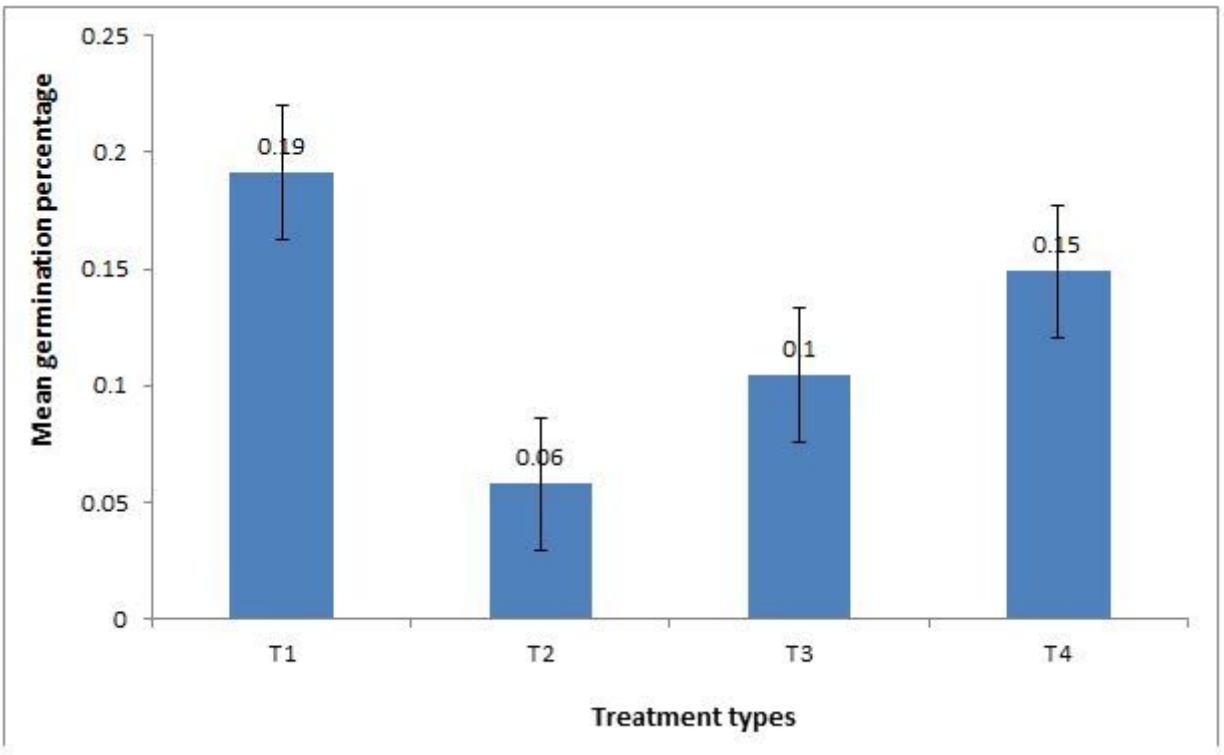

Figure 5

Mean germination distribution for T1, T2, T3 and T4. The probability (p-value) calculated using a one pair t-test: pt1t2=0.0001, pt1t3=0.06, pt1t4=0.14, pt2t3=0.11, pt2t4=0.004 and pt3t4=0.10.

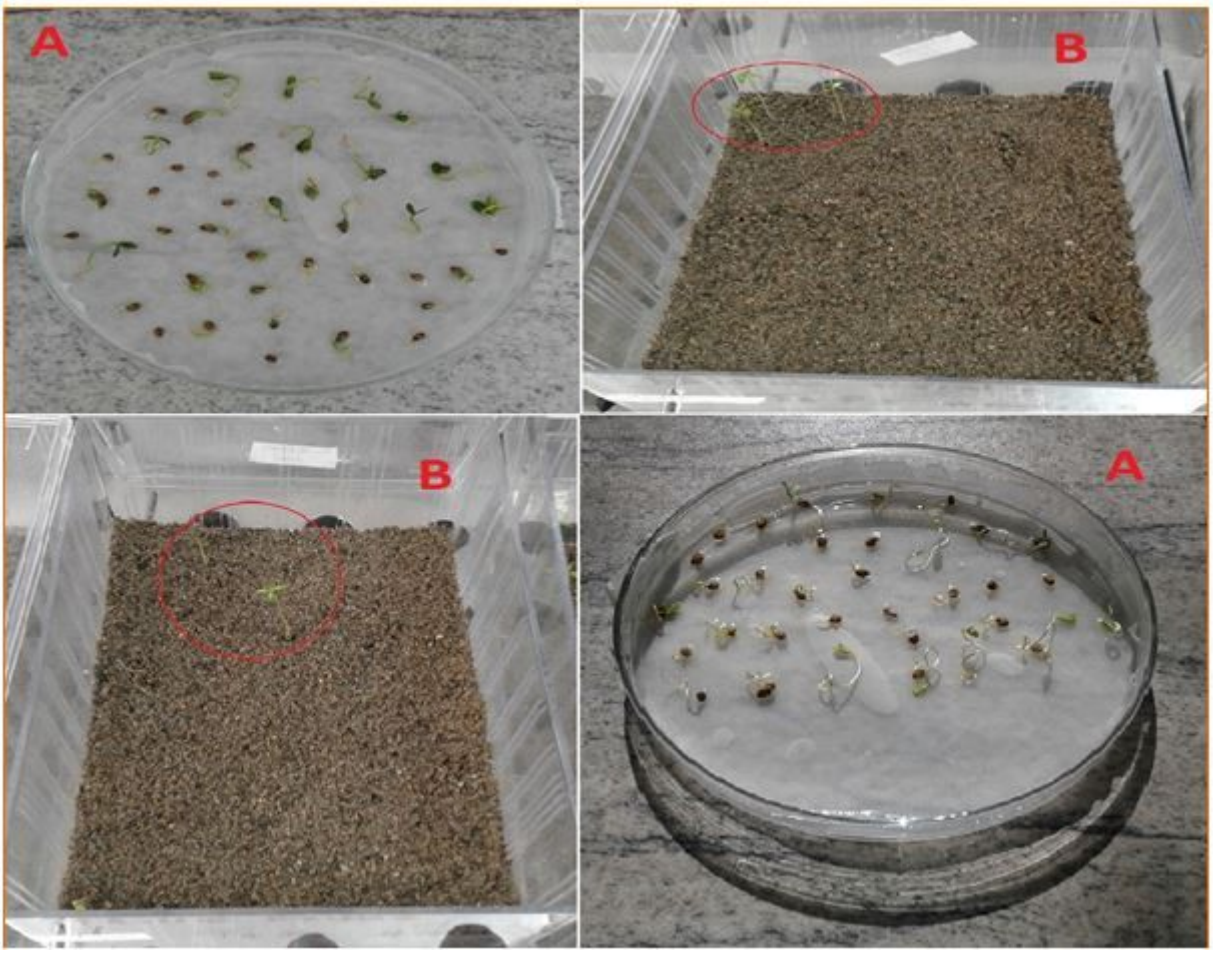

Figure 6

Seedlings of M. excelsa established on paper and sand substrates

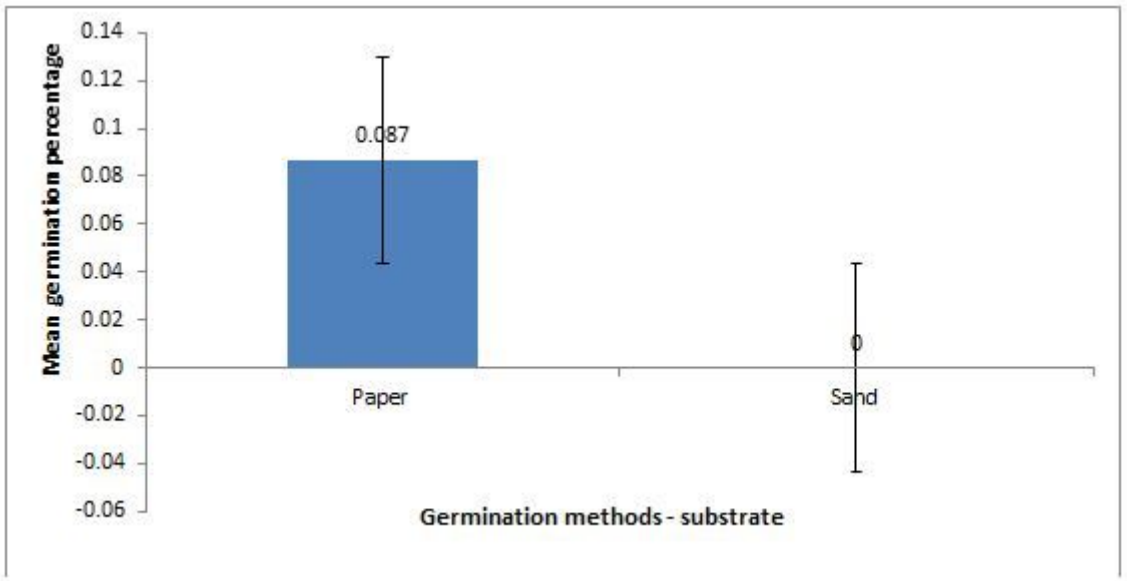

Figure 7

Mean germination distribution between Paper and Sand methods for T2.

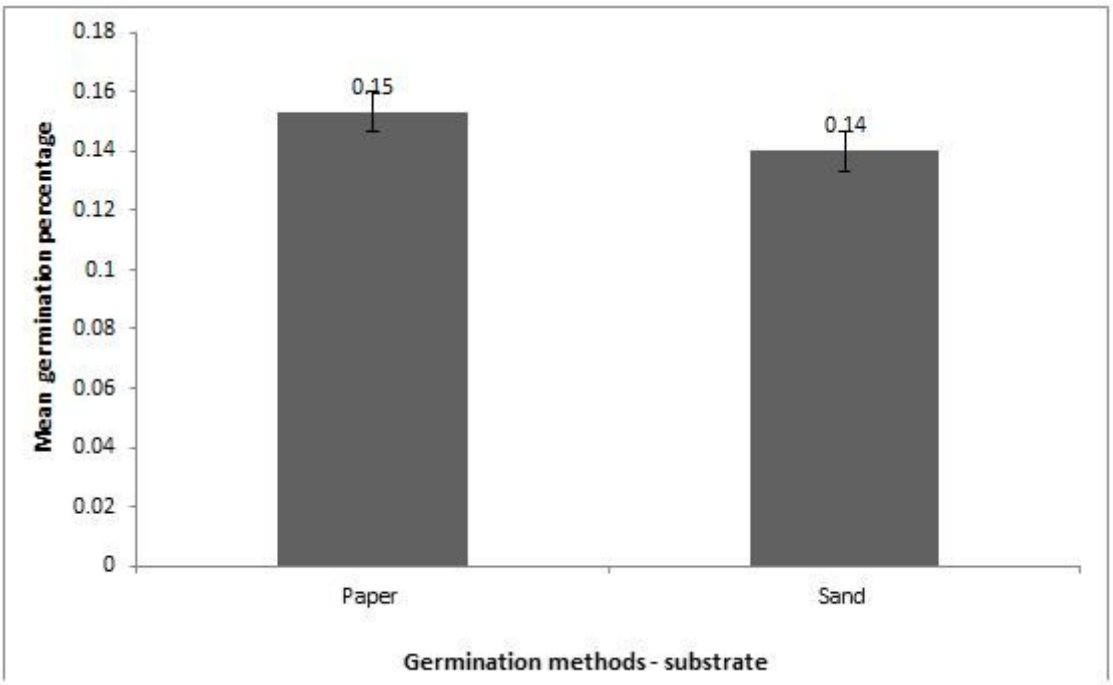

Figure 8

Mean germination distribution between Paper and Sand methods for T4.