

Full Length Research Paper

Effect of seed collection times and pretreatment methods on germination of *Terminalia sericea* Burch. ex DC

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Accepted 2 July, 2008

A nursery experiment was conducted to study the effects of seed collection times and pretreatment methods on the germination of *Terminalia sericea* Burch. ex DC. (Combretaceae). *T. sericea* is a multipurpose tree species occurring in the miombo woodlands whose seedling production is hampered by very low seed germination rate. Seeds were subjected to four treatment methods each at four different duration of exposure; soaking in hot water, immersion in concentrated sulphuric acid (95%), nicking and soaking in cold water and fire scorching. First collection was done when 60% of the fruits were deep-green to brown while second collection was done when all fruits were purple-brown to pink-purple. Nicking and soaking in cold water for 12 h gave the highest cumulative germination percentage (51%) for the first collection and appears to be the most feasible and suitable pretreatment method for small scale farmers than use of sulphuric acid. Soaking in hot water for 15 and 20 min and immersion in concentrated sulphuric acid (95%) for 3 and 4 h gave poorest germination (0%). However, in the second collection, use of concentrated sulphuric acid for 2 h gave highest germination (14%) followed by nicking and soaking in cold water for 24 h (12%). Sulphuric acid is expensive, requires proper handling techniques; therefore nicking and soaking in cold water for 12 h is being recommended as a cheaper and less hazardous pretreatment method to improve germination in *T. sericea*.

Key words: Germination, *miombo*, seed collection, seed pretreatment, *Terminalia sericea*.

INTRODUCTION

Terminalia sericea Burch. ex DC. commonly known as Silver terminalia belongs to family Combretaceae and is a common tree or shrub ranging from 6 to 9 m in height. Its range includes *miombo* woodlands of Malawi, Zambia, Mozambique, Tanzania and parts of Botswana, Lesotho, South Africa and Swaziland. It grows in close association with *Brachystegia species*, *Colophospermum species*, *Acacia species*, *Combretum species* and *Commiphora* (Drummond, 1981). Fruiting occurs between January to May and the fruits are normally 2.5 to 3.5 cm x 1.5 to 2.5 cm long, oval, round-tipped and flat with one seed surrounded by two confluent thin wings. The colour ranges

from purple-brown to pink-purple when mature. The species thrives in deep sandy soils in open woodlands, grasslands and edges of dambos or wetlands with moderate rainfall, (Palgrave et al., 1983; Shortec, 1989). In Malawi *T. sericea* dominates the middle altitude zone, 760 - 1300 m above the sea level (masl) and it mainly covers the Phalombe and Kawinga Plains around Lake Chilwa, Vwaza Marsh and Kasungu National Park.

T. sericea is a multipurpose species and its uses include soil conservation, ornamental, medicinal values such as use of roots decoction to cure diarrhoea, relieve colic while hot infusion of outer layers of roots are used to relieve pneumonia (Palgrave et al., 1983). The wood is yellow and hard providing useful durable items such as carvings, fencing posts, charcoal, fuel wood, building material and tool handles (Shortec, 1989; Kitchin and Pullinger, 1982). Rapid increase in population growth and

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agricultural activities in Malawi has resulted in the disappearance of most tree species including *T. sericea*. Seed collection needs to be done at the time that would optimize seed viability and longevity in storage (McCormark, 2004). Seed maturation time is therefore an important factor to consider during collection as harvesting too early may result in losses due to incomplete development while delayed collection may result in reduced viability due to exposure to others factors such as hardening of seed coat, insect-pest and disease damage (Hossain et al., 2005).

Several basic methods are used to overcome seed-coat dormancy in many species. The mechanism for breaking dormancy varies from species to species and most often involves drying, exposure to high or low temperature, exposure to light, leaching of chemical inhibitors through soaking in cold or warm water, mechanical scarification such as nicking and chipping and acid scarification among other methods (I.S.T.A., 2003). Because of its importance in providing many uses and services, *T. sericea* has attracted a lot of attention in the miombo ecozone. Efforts aimed at planting this species have been unsuccessful due to the low germination rate under natural conditions (IITO, 2003). Environmental conditions during seed formation and maturation have a remarkable effect on seed germination and dormancy (Perez-Garcia, 1997). There has been little experimental research dealing with the effect of seed collection times and relevant pretreatment methods on the germination of *T. sericea*. The purpose of this study was to determine the effect of time of seed collection and seed pretreatment methods on viability and germination of *T. sericea*. An understanding of these factors is crucial for successful regeneration and recruitment of this long-lived multi-purpose tree species.

MATERIALS AND METHODS

Seed collection and preparation

T. sericea seeds were collected when the colour of most fruits (about 60%) was still deep green just before turning to brown at Liwonde Forest Reserve (35° 12' S, 15° 12' E) in Malawi. Two collections were carried out at an interval of 6 weeks; 18th June and 1st August 2007, respectively. The fruits were collected from randomly selected fruiting branches. After seed extraction and cleaning, the moisture content was determined by oven drying at 103°C for 17 h (ISTA, 2003). Aborted and predated seeds were discarded and the intact plump seeds were surface sterilized with 1% sodium hypochlorite prior to any experimental usage.

Experimental design and treatments

The experiment on seed pretreatment were conducted at Forestry Research Institute of Malawi (FRIM) Zomba nursery located at 35° 19' S, 15° 26' E and 1,100 masl, Malawi. The two seed collection times were sown and monitored at different times, at each sowing there were 17 sub-treatments from four main treatments with each treatment at four different levels of time of exposure to treatment method: soaking in hot water, immersion in concentrated sulphuric

acid, nicking and fire scorching. The control experiment consisted of seeds that were left intact (without soaking, immersion in sulphuric acid, nicking or fire scorching). Each sub-treatment had four replicates with a total of 100 seeds for each subtreatment and 25 seeds per replicate. The experiments were laid out in a randomized complete block design.

Hot water treatment

Water was boiled to approximately 100°C and the heat source removed, then the seeds on a wire mesh were soaked into the hot water. The seeds were left to soak for different periods in hot water after which they were removed. The soaking periods were divided into four subtreatments namely; soaking for 5 min, soaking for 10 min, soaking for 15 min and soaking for 20 min.

Sulphuric acid immersion

Concentrated sulphuric acid (95%) was added separately to the container each containing 100 seeds and left to soak for different periods. After immersion the solution was drained off and seeds repeatedly rinsed in running tap water until considered safe to handle. The periods applied were as follows: immersion for 1 h, immersion for 2 h, immersion for 3 h and immersion for 4 h.

Nicking

From the seed lot, 100 seeds were mechanically nicked using secateur. The seeds were then soaked in cold water for different periods as follows: no nicking and soaking; nicking and soaking for 6 h; nicking and soaking for 12 h; nicking and soaking for 24 h.

Fire scorching using hot wire treatment

This treatment involved scorching 100 seeds separately using hot wire. The wire was heat to red hot and then pierced onto the seed surface on two sides apart from the side of the micropyle. Sub treatments in this pretreatment method included; fire scorching and no soaking; fire scorching and soaking for 6 h, fire scorching and soaking for 12 h; fire scorching and soaking for 24 h.

Germination experiment

Germination blocks of 60 x 30 cm were prepared at the nursery and filled with soils from miombo woodland. The germination beds were sterilized before the seeds and treated seeds were placed on the surface and covered with a thin layer of river sand. After sowing at the nursery watering was done accordingly to keep the beds with adequate moisture. Germination was defined as the emergence of the radicle from the seed coat. Germination was recorded every day and allowed to proceed for 56 days. Germination capacity was assessed by rapid staining method where ungerminated seeds were cleaned with water and immersed in 1% tetrazolium chloride for 8 h at 25°C. Pink embryos were scored as alive and germination was expressed as percentage of viable seeds.

Data analysis

Daily germination percentages were summed up to obtain cumulative germination percentage for each treatment. Results of the germination studies were subjected to an analysis of variance (ANOVA) using MINITAB 14.0 (2000) following arcsine transform-

Table 1. Overall mean germination of *T. sericea* seeds for the two collection times eight weeks after sowing.

Treatment	Mean germination % (1 st collection)	Mean germination % (2 nd collection)
Control	5.25 ± 2.45 ^{cd}	0.50 ± 0.58 ^{ab}
Hot water soaking for 5 min	1.25 ± 0.50 ^{ab}	0.75 ± 0.50 ^{abc}
Hot water soaking for 10 min	0.75 ± 0.96 ^a	2.00 ± 0.82 ^{cde}
Hot water soaking for 15 min	0.00 ± 0.00 ^a	0.75 ± 0.96 ^{abc}
Hot water soaking for 20 min	0.00 ± 0.00 ^a	1.25 ± 1.90 ^{abcd}
H ₂ SO ₄ soaking for 1 h	2.75 ± 0.50 ^{ab}	1.75 ± 1.71 ^{bcd}
H ₂ SO ₄ soaking for 2 h	1.50 ± 2.38 ^{ab}	3.50 ± 1.73 ^f
H ₂ SO ₄ soaking for 3 h	0.50 ± 0.58 ^a	0.00 ± 0.00 ^a
H ₂ SO ₄ soaking for 4 h	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Nicking with no soaking	8.25 ± 2.99 ^{de}	1.00 ± 1.41 ^{abcd}
Nicking and soaking for 6 h	9.00 ± 3.74 ^{ef}	2.00 ± 1.15 ^{cde}
Nicking and soaking for 12 h	12.75 ± 3.31 ^g	2.25 ± 0.96 ^{def}
Nicking and soaking for 24 h	9.75 ± 4.86 ^{efg}	3.00 ± 1.15 ^{ef}
Fire and no soaking	4.50 ± 1.29 ^{bc}	0.75 ± 0.50 ^{abc}
Fire and soaking for 6 h	5.25 ± 2.06 ^{cd}	0.75 ± 0.96 ^{abc}
Fire and soaking for 12 h	8.00 ± 5.48 ^{cde}	0.00 ± 0.00 ^a
Fire and soaking for 24 h	8.50 ± 2.38 ^{de}	0.00 ± 0.00 ^a

Values in the same column followed by same letter do not significantly ($P < 0.05$) differ.

Table 2. Analysis of variance table for cumulative germination of *Terminalia sericea* for first seed collection.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Subtreatments	16	17545	1097	9.95 ^{**}
Replicates	3	108	36	0.33 ^{ns}
Error	48	5288	110	
Total	67	22981		

^{**}Significant at $P \leq 0.01$.

^{ns}Not significant at $P \leq 0.05$.

tion of all percent germination data. Differences between treatment means were separated using the Least Significant Difference Test (LSD) at the 0.05 level. Arcsine-transformed means and standard errors were back transformed for graphic presentation.

RESULTS

Seed germination percentage

The moisture content of the seeds was 17 and 9% for first and second collection times, respectively. Although there were significant differences ($p \leq 0.05$) between pretreatments, these were only due to the hot water and the sulphuric acid pre-treatments in the first and second collection, respectively (Table 1). In the first collection, nicking and soaking in cold water gave the highest germination percentage and specifically nicking and soaking for 12 h with 12.75% mean germination. Fire scorching and soaking ranked second among the treatments with 8.50% for scorching and soaking for 24 h. The

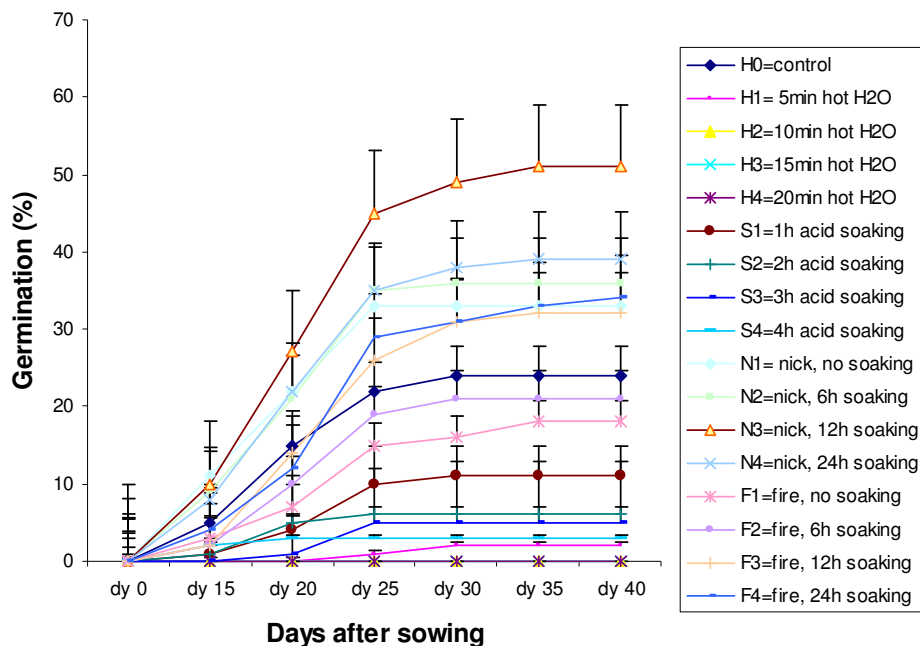
least mean germination (0.50%) was obtained when seed was soaked in hot water (Table 1). During the second collection, a relatively good number of seeds had germinated under the nicking and soaking treatments. However, on the overall it was observed that soaking in sulphuric acid for 2 h yielded a relatively higher (3.50%) mean germination but did not differ significantly ($P \leq 0.05$) from nicking and soaking for 24 h with mean germination of 3.0%.

Cumulative germination

The cumulative germination percentage was significantly different ($P \leq 0.01$) between the different seed pretreatment methods. Nicking and soaking in water for 12 h had the highest cumulative germination (51%) while soaking in sulphuric acid had the lowest cumulative germination for the first seed collection (Table 2, Figure 1). Significant differences ($P \leq 0.05$ to 0.001) were found for most of the pretreatments assessed for the second seed collection

Table 3. Analysis of variance table for cumulative germination of *Terminalia sericea* for second seed collection.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Subtreatments	16	1172.2	73.3	4.42**
Replicates	3	89.2	29.7	1.80 ^{ns}
Error	48	794.8	16.6	
Total	67	2056.2		

Significant at $P \leq 0.01$.^{ns}Not significant at $P \leq 0.05$.Figure 1.** Cumulative seed germination percentage of *T. sericea* during first seed collection

time (Table 3). During this period, germination started within the first 14 days after sowing (Figure 2), but unlike in the first collection cumulative germination increased at an increasing rate up to 35 days after sowing. However, germination rate was so low such that the highest cumulative germination percentage (14%) was recorded after 40 days for seeds immersed in sulphuric acid for 2 h followed by nicking and soaking for 24 h (12%). The control experiment was amongst the treatments with lowest cumulative germination percentages. No assessment was done on seedling development but the observations of the results, however, indicate that treatments did not strongly affect seedling development.

DISCUSSION

The hard testa of many dry woodland species has evolved to withstand unfavourable conditions such as heat caused by sunlight, the strong teeth of dispersing animals, severe drought and mechanical damage (Doran

et al., 1983). *Terminalia species* present difficulties in germination in many parts of the world if sown untreated. In general, the first seed collection time suggests that nicking and soaking in cold water for 12 h as the best pretreatment method. Nicking is known to break physical dormancy of seeds with hard coats which inhibit water uptake and gas exchange as in *Acacia* and *Terminalia species* (Doran et al., 1983; Tietema et al., 1992; Hossain et al., 2005). Nicking allows entrance of water and air into the seed. Soaking in water dissolves and leaches out the chemicals causing dormancy. The water then moves phenolic compounds which are soluble in water from embryo to aleurone layer of the endosperm (Deghan et al., 2003). The results of the present study are also supported by the findings of Nainar et al. (1999) who showed that among the seed pre-treatments including mechanical scarification, hot water treatment and sulphuric acid treatment; mechanical scarification gave the highest germination percentage (60%) in *Terminalia chebula*.

Fire scorching and soaking in water as well as control

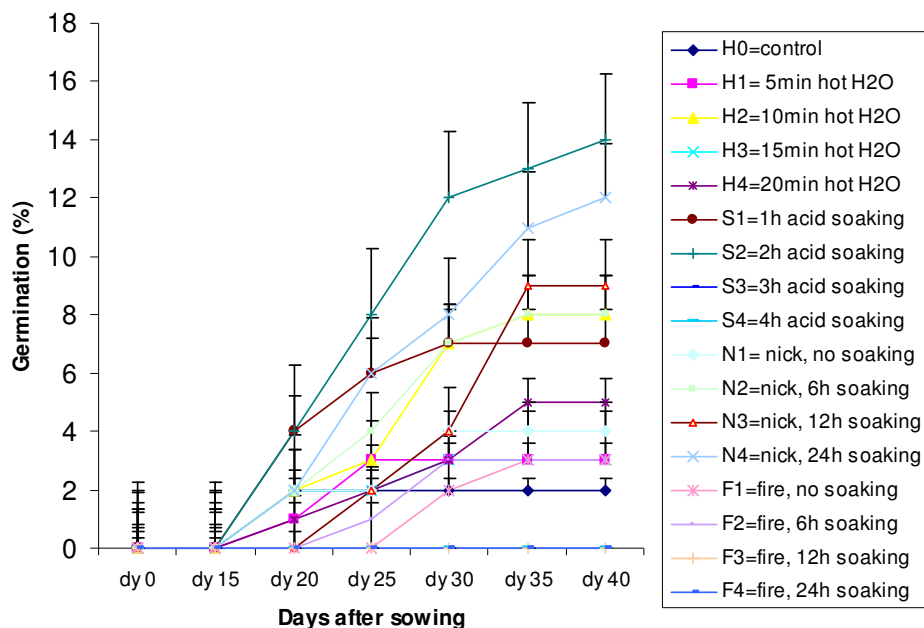


Figure 2. Cumulative germination percent of *T. sericea* for second collection time.

ranked second giving higher germination percentage ranging from 18 to 34% of the untransformed data suggesting occurrence of a moderate seed coat-imposed dormancy than physiological dormancy (data not shown). The findings from this study confirm earlier reports of occurrence of moderate seed-coat dormancy in *Tylosema esculentums* whereby manual scarification through nicking yielded better germination results (Travlos et al., 2006). The low germination percentage associated with soaking in hot water and immersion in sulphuric acid during the first collection could be attributed to damage of seed that had high moisture content of 17%. The high moisture content might have allowed sulphuric acid and hot water to easily penetrate the coat and damage the embryo. These results are in disagreement with Corbineau and Côme (1993) who recommends the use of sulphuric acid to breaking seed dormancy of *Terminalia ivorensis*. However, their study reported that gibberellic acid was added after sulphuric acid treatment to improve the germination rate from 20 to 56%. Notwithstanding, hot water treatments as well as sulphuric acid have effectively been used to break physical dormancy in many plant species with hard seed coat (Laurent and Chamshama, 1987; Ngulube and Chipompha, 1983). The effectiveness of hot water treatment and fire scorching depend on the length of time and depth of the wire into the seed respectively as these influence the total amount of heat the seeds are exposed to over the soaking period (Ngulube, 1988). The two methods therefore require very careful calibration.

In the second collection time, treatment with sulphuric acid specifically for 2 h without soaking in water prior to sowing gave relatively higher germination percentages

(14%, Figure 2). Hardening and sturdiness of the seed may have a bearing on this outcome. It was difficult for the acid to easily penetrate through the hard coat as such only managed to disintegrate the seed coat cellulose. This supports the findings of Corbineau and Côme (1993) who showed that the germination percentage in *Terminalia ivorensis* was improved for well-dried seed (5 - 9% moisture content). The acid treatment has also been reported as an efficient method to increase, accelerate seed germination of species with hard impermeable seed coat (McDonald and Omoruyi, 2000). However, time of exposure is critical and needs to be quantified for each species since seeds exposed for a long period can be damaged easily (Schmidt, 2000). This agrees with the current study where soaking in sulphuric acid for 3 and 4 h gave 0% germination. This fact was also confirmed by McDonald et al. (2002) on *Tamarindus indica* and *Prosopis africana* where immersion of seed in sulphuric acid for more than 60 min increased the number of damaged seeds hence tremendously reducing germination percentage. The sulphuric acid treatment requires resistant containers and presents a risk to both the operator and seeds. Furthermore, sulphuric acid is expensive for small scale growers such that its use and applicability to rural areas where these trees are planted may be limited. However, sulphuric acid did not improve germination compared to nicking, which agrees with studies done in *Sclerocarya birrea* where nicking and soaking improved germination to over 75% (Akinnifesi et al., 2007).

Testing seed viability with 1% tetrazolium chloride showed that a higher percentage of seeds were dead. This is attributed to seed abortion where over 50% of the

collected fruits had shriveled seeds. Collection of immature *T. sericea* seed results in seed shrinkage and delayed seed collection may result in high seed physical dormancy. Thus timely collection of the seed after proper phenology studies would reduce possibilities of low seed germination (Laurent and Chamshama, 1987). There is generally limited knowledge on flowering and fruiting habits of most indigenous species of miombo woodland including *T. sericea* (Maghembe et al., 1992) hence there is great need to develop maturity indices of fruit or seeds to facilitate collection of mature seeds that can improve germination rate. The difference in two collection times generally is due to differences in hardness of their seed coats. Seed coat impermeability is due to structural and chemical changes that take place during late stages of seed maturation. Loss of water and the subsequent shrinkage of cells result in closer packing of cells and even in cell collapse and lumen obliteration in the testa (Kigel and Galili, 1995). Apart from hard seed coat impermeability, there might also be embryo dormancy causing the poor germination. The site where the seed used in the study was collected has persistent higher temperatures which affects hardness of seed coat and embryo dormancy. Overcoming dormancy through softening of the testa to improve water uptake are therefore, crucial in the life cycle of hard seeded species.

Conclusion

The study has revealed that germination of *T. sericea* can be improved by collecting the fruits while they are still deep green to slightly brownish with about 17% moisture content and this is estimated at 26 weeks from the onset of flowering. Nicking and soaking in cold water for 12 h is the overall best pretreatment method when the seed is collected before the seed coat has developed very hard covering. Despite its effectiveness, nicking is laborious and its applicability is only limited to small samples. On the other hand, a rotating drum that brings seeds into contact with sharp gravel has in some species been used and can be recommended for bulky seeds (Doran et al., 1983). Soaking in concentrated sulphuric acid for 2 h proved to improve germination of fully matured, dry and brown coloured fruits with as low as 9% moisture content. There is need for biological studies that may lead to development of maturity indices of *T. sericea* fruit seed that may facilitate collection of seed that can provide optimal germination rates

ACKNOWLEDGEMENTS

The authors would like to thank the staff of Forest Research Institute of Malawi for providing facilities for the research activities. We are grateful to S. Makawa, Maganizo Namoto and Violet Msukwa for their involvement in the execution of field work. The Millennium Seed

Bank Project, Royal Botanical Garden at Kew in England and Dr Paul Smith are acknowledged for provision of financial support to enable Michael Likoswe complete his studies and conduct this research as a partial fulfillment of his B.Sc. degree in Forestry.

REFERENCES

- Akinnifesi F, Sileshi G, Mkonda A, Ajayi OC, Mhango J, Chilanga T (2007). Germplasm supply, propagation and nursery management of miombo fruit trees. In: Akinnifesi FK, Sileshi G, Ajayi O, Tchoundjeu Z, Matakale P (eds). Indigenous fruit trees in the tropics: domestication, utilization and commercialization. CABI Publishing, UK, p. 349.
- Corbinau F, Côme D (1993). Improvement of Germination of *Terminalia ivorensis* Seeds. For. Genet. Res. Info. no. 21.
- Deghan B, Norcini JG, Kabat SM, Pérez HE (2003). Effect of Seed Scarification and Gibberellic Acid Treatment on Seedling Emergence of Sky-Blue Lupine (*Lupinus diffusus*). J. Environ. Hortic. 21(2): 64-67.
- Drummond RB (1981). Common trees of the central water shade woodlands of Zimbabwe: National Herbarium and Botanic Gardens, Department of Research and Specialist Services, Salisbury, pp. 166-167.
- Doran JC, Turnbull JW, Boland DJ, Gum VB (1983). Hand book on seed of dry zone *Acacias*. FAO Rome, pp. 58-63.
- Hossain MA, Arefin MK, Khan BM, Rahman MA (2005). Effects of seed treatments on Germination and seedling growth attributes of Horitaki (*Terminalia chebula* Retz.) in the nursery. Res. J. Agric. Biol. Sci. 2: 135-141.
- International Seed Testing Association (ISTA) (2003). International Rules for Seed Testing. ISTA, Bassersdorf, Ch-Switzerland.
- International Tropical Timber Organisation (IITO) (2003). Forestry Research News: Indicators and Tools for Restoration & Sustainable Management. Issue 3: 3-8.
- Kigel J, Galili G (1995). Seed Development and Germination. Marcel Dekker, Inc, New York, pp. 664-666.
- Kitchin AM, Pullinger JS (1982). Trees of Malawi with some shrubs and climbers. Blantyre Print and Publishing, Blantyre, Malawi, pp 58.
- Laurent N, Chamshama SAO (1987). Studies on the germination of *Erythrina abyssinica* and *Juniperus procera*. Int. Tree Crops J. 4: 289-291.
- Maghembe JA, Kwesiga F, Ngulube M, Prince H (1992). Domestication Potential of Indigenous Fruit Trees of the Miombo Woodlands of Southern Africa.
- McCormark JH (2004). Principles and practices of seed harvesting, processing, and storage: an organic seed production manual for seed growers, Creative Commons, Stanford, California 94305, USA, p. 28.
- McDonald I, Omonhinmin AC, Ogboghodo IA (2002). Germination ecology of two species *Tamarindus indica* and *Prosopis africana*. Seed Technol. 24: 103-107.
- McDonald I, Omoruyi O (2003). Effect of seed pre-treatment on germination of two surface types of *Dialium guianense*. Seed Technol. 25: 41-44.
- MINITAB (2000). Release 14, Minitab Inc. 3081 Enterprise Drive, State College, PA, USA.
- Nainar P, Sundharaiya K, Ponnuswamy V (1999). Germination studies in *kadukkai* (*Terminalia chebula*). South Ind. Hortic. 47: 1-6.
- Ngulube MR, Chipompha NWS (1987). Effect of seed pretreatment on the germination of some hardwood species for dry zone afforestation in Malawi. In: Kamra SK, Ayling RD (Eds.) Proceedings of the International symposium on Forest Seed Problems in Africa. Harare, Zimbabwe, pp. 216-224.
- Ngulube MR (1988). Effect of seed pretreatment on the germination of teak (*Tectona grandis* Linn. F) in the nursery. J. Trop. For. 4: 143-146.
- Palgrave KC, Drummond BR, Moll JE (1983). Trees of Southern Africa. Second edition, C. Struik publishers (Pty) Limited, Cape Town, South Africa, p. 685.
- Perez-Garcia F (1997). Germination of *Cistus ladanifer* seeds in relation

- to parent material. *Plant Ecol.* 133: 57-62.
- Schmidt L (2000). Dormancy and pretreatment. *Guide to handling of tropical and subtropical forest seeds.* Danida Forest Centre, pp. 303.
- Shortec C (1989). *An introduction to the common trees of Malawi.* Wildlife society of Malawi, Malawi, p. 74.
- Tietema C, Merkesdkh E, Shortem J (1992). *Seed germination of Indigenous trees in Botswana.* FAM Garborone, Botswana, Acts Press, Nairobi and Biomass users network, Kenya, pp. 48-49.
- Travlos I, Economou G, Karamanos (2006). Germination and emergence of hard seed coated *Tylosema esculentum* (Burch) A. Schreib in response to different pre-sowing seed treatments. *J. Arid Environ.* 68(3): 501- 507.