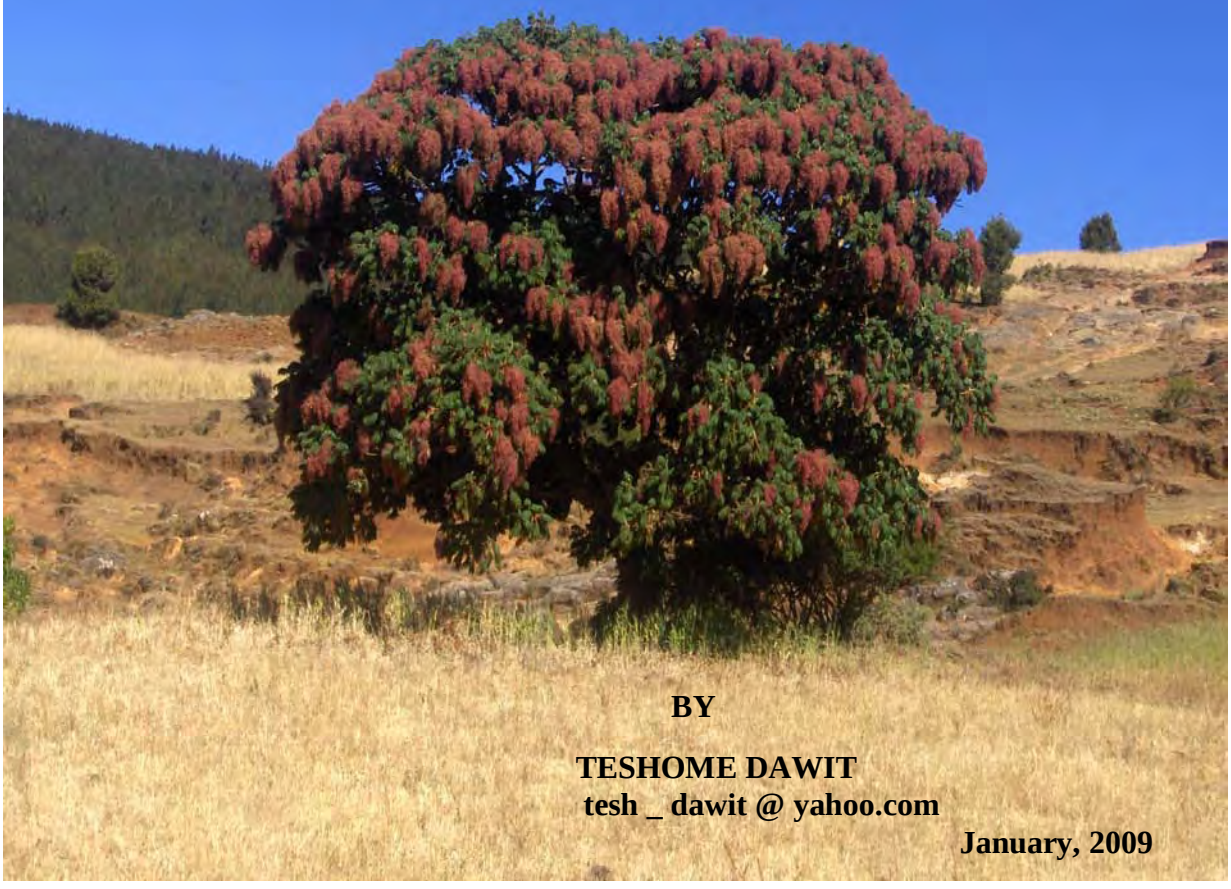
ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

GERMINATION STUDIES AND NURSERY ESTABLISHMENT OF THE THREATENED *Hagenia abyssinica* (BRUCE) J.F.Gmel THROUGH USE OF VARIOUS GERMINATION STIMULANTS



BY

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A thesis Submitted to School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

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ABBREVIATIONS

C	**Control experiment that contained red soil only**
D	**Decomposed cow dung**
DDW	**Double distilled water**
GTP	**Number of germinant transplanted in plastic pots**
H	**Height**
n	**Total number of germinated seeds in Petri dishes and pots**
N	**Total number of seeds in the sample**
P	**Petri dish**
PGG	**Percentage germinant established in the glasshouse**
PSS	**Percentages of seedling survival**
RW	**Root weight**
Sa	**Sand**
So	**Red soil**
SoDSa1	**Potting mix that contained 4, 3, and 2 volumes of red soil, decomposed cow dung and sand respectively**
SoDSa2	**Potting mix that contained 4, 3 and 1 volumes of red soil, decomposed cow dung and sand respectively**
SoDSa3	**Potting mix that contained 4, 1 and 3 volumes of red soil, decomposed cow dung and sand respectively**
SoDSa4	**Potting mix that contained 2, 1 and 1 volumes of red soil, decomposed cow dung and sand respectively**
SoDSa5	**Potting mix that contained 1, 1 and 1 volumes of red soil, decomposed cow dung and sand respectively**
SMK	**Plant derived aqueous smoke extract**
SRR	**Shoot to root ratio**
SSG	**Number of germinant survived in the glasshouse until the 3rd week**
STB	**Seedlings transferred to the nursery bed**
SW	**Shoot weight**

DEDICATION

This thesis is dedicated to those Ethiopians who have contributed more than anyone else in the conservation, establishment and development of Ethiopian indigenous trees. Especially, it is dedicated to Professor Legesse Negash, who influenced my understanding in physiology and propagation biology of *H. abyssinica*.

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First of all I thank the Almighty and everlasting God for the grace and strength He has given me during my study at Addis Ababa University. I would like to express my deepest gratitude to my advisor, Professor Legesse Negash for his generous help in all aspects during my stay in Addis Ababa University. His guidance, constructive comments on the papers and the thesis, positive approach and encouragement throughout my study is highly acknowledged.

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I am greatly indebted to my wife, W/O Roman Deneke, without your commitment, patience, love, encouragement and taking care of our sons, the completion of this study would be impossible. Finally, I would like to acknowledge my brothers, Aklilu Dawit in providing me with Laptop computer, Solomon Dawit in providing me with digital camera to properly carryout this thesis work and other brothers, sisters, relatives, friends and all other individuals who supported me in prayer and who contributed their share for the realization of this study.

ABSTRACT

Studies on the germination physiology of *Hagenia abyssinica* (Bruce) J.F.Gmel were conducted using various levels of germination stimulators or inhibitors. Nursery establishment and growth performance of the resulting germinants were examined using different mixtures of growth media. Seeds were treated using various dilutions of plant-derived aqueous smoke extracts (10, 20, 30, 40 and 50%), different concentrations of GA_3 and KNO_3 (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M) and double distilled water (control). The seeds were germinated under laboratory conditions at 40 µ mol m^{-2} s^{-1}, and at 22^0 C. Also, leaf aqueous extracts of *Eucalyptus globulus* ssp *globulus* at 10, 20, 40, 80 and 100% relative concentrations were applied to determine their effects on *H. abyssinica* seed germination under laboratory conditions. Smoke extract at 40 %, GA_3 at 10^{-4} M and KNO_3 at 10^{-5} M showed higher germination percentage (86, 79.6 and 89.6%, respectively) compared to the control (72%); and the difference was significant at $P \leq 0.05$. Increasing relative concentrations of leaf aqueous extracts of *E. globulus* ssp *globulus* inhibited seed germination and complete failure of seed germination was recorded at 80 and 100% relative concentrations. Growth media containing red soil, decomposed cow dung and sand in the ratio 4:3:2 (SoDSa1), 4:3:1 (SoDSa2), 4:1:3 (SoDSa3) and 2:1:1 (SoDSa4) (19.1 ± 1.35, 20.58 ± 0.62, 19.21 ± 1.5 and 21.9 ± 1.01 cm, respectively) resulted in significantly ($P \leq 0.05$) better growth in height, compared to the control (red soil only) (16.24 ± 0.59). Dry weights of the seedlings grown in the potting treatments SoDSa1, SoDSa3, and SoDSa4 (4.63, 4.30 and 5.53 g) were significantly larger than the control (0.70 g). The MDW (mean dry weight) of the seedlings grown in SoDSa4 was larger compared to the control. From these investigations, it is concluded that pre-treating seeds of *H. abyssinica* with aqueous smoke extract and KNO_3 provided better stimulatory effect for attaining maximum germination percentage. The results also indicate that the seedlings of *H. abyssinica* grew faster and reached planting size within 5 months in a soil mixtures containing red soil, decomposed cow dung and sand in the ratios 2:1:1 and 4:3:2.

Key words/phrases: Allelopathy, Germination, *H. abyssinica*, Seed, Seedling survival.

1. INTRODUCTION

Indigenous forests are critical in providing clean water, fresh air, fertile soil, food, fiber, fuel, and drugs (Legesse Negash, 1995; Thomas and Balakrishinan, 1999). But unwise extraction of the available forest resources coupled with lack of knowledge on the physiology and propagation biology of indigenous trees have resulted in their rapid disappearance (Legesse Negash, 1995).

Hagenia abyssinica (Bruce) J. F. Gmel. (1719) commonly known as *Kosso* in Ethiopia is a monospecific genus belonging to the family *Rosaceae* (Hedberg, 1989). The species is dioecious, grows up to 20 m tall, and multipurpose tree (Legesse Negash, 1995). This tree species was once one of the dominant trees in the upper parts of montane forest belts in Ethiopia as it is in East Africa, but now only scattered trees remain in most areas (Hedberg, 1989). Ethiopia used to contain large stands of *H. abyssinica,* but today the species is sparsely distributed in parts of Bale, Sidamo, Keffa, Harrarghe, Arssi, Gojjam, Wello, Gonder, Tigray, Shewa and Wellega (Legesse Negash, 1995).

Although *H.abyssinica* is protected by law and significant advances have been made towards its propagation and cultivation (EFAP, 1993; Legesse Negash, 1995; Tileye Feyissa *et al*., 2005) tree propagation still continues to decline. It is illegally harvested for its high quality wood and cleared from the land to allow for agricultural expansion. The scarcity of other forest trees due to forest destruction has led to the increased cutting of *H. abyssinica* for making display cabinets, cupboards, veneers, poles, flooring, as well as for fuel wood (Legesse Negash, 1995; Azene Bekelle, 2007). It is also used for truck body construction (Russ, 1944 cited in Legesse Negash, 1995). *H. abyssinica* is one of the most endangered tree species of Ethiopia, because of its great demand for its timber. It is very much needed in the market as there is high demand for the tree's timber, as the tree is characterized by its short bole many trees have to be felled at any one time to get the required volume of timber. Worse still, regeneration in what used to be the natural habitat of the species has become almost impossible as a result of widespread human interference and, probably, as a result of climatic changes (and also perhaps due to

change in the chemical nature of the soil) that have usually accompanied the process of deforestation. Forest fires have also been culprits of this species, although its vulnerability to fire is less pronounced as compared to other tree species (Legesse Negash, 1995).

As the species has separate individuals of male and female trees, and since pollination is effected by wind, the probability for the ovules of the whole female inflorescences to be fertilized by the pollen grains of the male inflorescences may not be one (Legesse Negash, 1995). To achieve successful wind pollination in the tropics, a large number of male and female individuals must grow close to one another. This is because nearly all wind-borne pollen grains fall within hundred meters of the parent plant and the large amount of pollen that are blown by wind reach the vicinity of the ovules only by chance (Raven *et al*, 1986 cited in Legesse Negash, 1995). Hence, if individual plants are widely scattered, the chances that a pollen grain will reach a receptive stigma are very low. Even in cases where individual male and female trees grow close to each other, wind borne pollen grains may find it difficult to reach the stigma because the leaves of evergreen tree species (of other tree species in the community) intercept the pollen grains and consequently, prevent the latter from reaching the female inflorescence (Legesse Negash, 1995).

According to Legesse Negash (1995), other environmental factors such as availability of adequate moisture, prevalence of optimum temperature, as well as absence of severe frost, also important for a successful seed production by the species. Moreover, it requires quite an experience to judge whether or not the individual fruits within a given inflorescence are adequately mature during inflorescence collection, so that the collector is rather sure of getting satisfactory percentage germination. Consequently, in *H. abyssinica*, as in most other tree species, the ability to recognize the maturity level of the inflorescence or the fruit is of prime importance. Given these and other uncertainties, e.g., the geographical and the climatic localities where seeds of the species originate, it is not surprising that percentage seed germination in this tree species is somewhat low and, generally, is rather variable (Legesse Negash, 1995). However, in the face of increasing

threats to the species, and for successful propagation and cultivation of the tree, understanding the germination physiology of the seeds and conditions for the nursery establishment of the seedlings are very critical (Legesse Negash, 1995). Therefore, understanding of the germination physiology of *H. abyssinica* is necessary for successful restoration of the tree through re-afforestation programs.

In the present study, attempts were made to investigate the effects of GA_3 and KNO_3 at different concentration levels and plant-derived smoke extracts at various dilution levels on seed germination of *H. abyssinica*. The study was also conducted to find out the allelopathic effects of *Eucalyptus globulus* ssp *globulus* leaf extracts on germination of *H. abyssinica* seeds.

2. OBJECTIVES OF THE STUDY

2.1. General objective

The objective of this study was to investigate the seed germination behavior, nursery establishment and seedling growth performance of *Hagenia abyssinica* (Bruce) J.F.Gmel. using various pre-treatment methods, soil mixes and allelopathic effect of *Eucalyptus globulus* ssp *globulus* leaf extracts on germination.

2.2. Specific objectives

The specific objectives were to:

1) evaluate the effects of germination stimulants on the germination of *H. abyssinica* seeds;
2) enhance the final germination percentage of seeds of *H. abyssinica* using various dilution levels of cold aqueous smoke (10, 20, 30, 40 and 50 %) and various concentrations (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M) of GA_3 and KNO_3;
3) determine the growth (height and biomass) of seedlings planted in different soil mixes planted in plastic sleeves;
4) determine the survival potential of germinants planted in different soil mixes in the glasshouse;
5) determine allelopathic effect of *Eucalyptus globulus* ssp *globulus* leaf extracts on germination of *H. abyssinica* seeds.

3. LITERATURE REVIEW

3.1. *Hagenia abyssinica*

Synonyms: *Banksia (Bankesia) abyssinica* Bruce (1790), *Brayera anthelmintica* Brayer, *H. abyssinica* var *viridifolia* Hauman, and *H. anthelmintica* (Kunth) Eggleling.

3.1.1. Taxonomy and morphological features

H. abyssinica (Bruce) J. F. Gmel is a monospecific genus belonging to the family *Rosaceae* (Hedberg, 1989; Legesse Negash, 1995). A large number of fruit trees including plums, apples, pears, and loquats are contained in this family (Dale and Greenway, 1961 cited in Legesse Negash, 1995). This family also includes various beautiful roses, blackberries and strawberries (Legesse Negash, 1995). This is a dioecious tree up to 20 m tall with brownish bark peeling in strips, sometimes very thick on old stems. Young branches pubescent with stiff hairs 3-4 mm long. Leaves to 40 cm long with 5-8 narrowly oblong leaflets on each side; Petiole 5-13 cm long, winged (adnate stipules). Inflorescence a conspicuous drooping panicle up to 60 cm long and 30 cm wide. Male flowers are orange to white, female ones reddish at maturity. Fruit small, dry, winged, asymmetric, single seeded, brown syncarp with a single more or less ovoid carpel and fragile pericarp (Hedberg, 1989).

3.1.2. Distribution

H. abyssinica naturally occurs within the undifferentiated afromontane forest (mixed *Podocarpus* forest, *Juniperus-Podocarpus* forest) and dry single dominant afromontane forest *(Juniperus* forest) or forest dominated by *Hagenia (*Friis, 1992). It is often found in association with tree species such as *Scheffleria abyssinica* (A. Rich) Harms, *Scheffleria volkensii* (Harms), *Galiniera saxifrage*, *Rapanea melanophloës* as well as with the mountain bamboo, *Arundinaria alpina* K. Schum. Since the tree is confined to the mountains of tropical Africa, it is characterized as a clear example of an afro-montane

endemisim (Friis, 1992). It is confined to the mountains of Africa with mean annual rainfall ranges between 1,000-1,500 mm/year (Friis, 1992). In drier north of mount Kenya, the *Hagenia* dominated forests are replaced by mixed forest of conifers and broad leaved species in which *H. abyssinica* is codominant (*Hagenio-Juniperion*) (Bussmann, 2001). *H. abyssinica* is geographically distributed in and native to Burundi, Democratic Republic of Congo, Kenya, Malawi, Rwanda, Sudan, Tanzania, Uganda and Zambia. It is an exotic tree species to India (Hedberg, 1989).

This tree species may be one of the dominant trees in the upper part of the montane forest belt in Ethiopia, as it is in East Africa, but now only scattered trees remain in most areas (Hedberg, 1989). *H. abyssinica* was first described in Ethiopia by James Bruce in 1790. Formerly, the species was once abundant in the semi-humid mountains of Ethiopia with attitudinal range of 2,450 and 3,250 m (Hedberg, 1989). In forest depression, it can descend to 2000 m. Today the species is sparsely distributed in parts of Bale, Sidamo, Keffa, Harrarghe, Arssi, Gojjam, Wello, Gonder, Tigray, Shewa and Wellega (Legesse Negash, 1995).

3.1.3. Ecological and economic importance

H. abyssinica has been playing a number of important roles in the early, as well as in the present economic welfare of the people. Apparently, the larger size of the female inflorescence is ‘meant’ for enhancing its receptive capability for the wind-borne pollen grains of the male inflorescence. Interestingly, both the female and the male flowers produce abundant nectar and pollen, respectively. Consequently, the tree has been useful in traditional apiculture. Accordingly to some unconfirmed reports, honey obtained from *H. abyssinica* may sometimes purge tapeworm. Furthermore, owing to its characteristic branching habit peasants use the branches of the tree for keeping and stabilizing their beehives on them (Legesse Negash, 1995).

The wood of *H. abyssinica* is easy to split into pieces; it has to dry while the tree is still standing either through girdling or through natural death to be used for fuel after some years. The wood is fine-grained, fairly hard and heavy. It appears to have high calorific values and is fragrant during burning (Legesse Negash, 1995). It is also used for many purposes, such as for making display cabinets, cupboards, veneers, poles, flooring as well as for truck body construction (Russ, 1944 cited in Legesse Negash, 1995).

H. abyssinica has extremely valuable biological attributes in fertile soil formation, soil conservation as well as rainwater conservation. The large number of branchlets allows the trees to intercept and reduce the kinetic energy of a stormy rainfall, thus protecting against soil erosion. It also helps to conserve water in the aquifer system of the underlying geological formations. The thick mat formed from the fallen leaves of the tree is important in preventing soil erosion by reducing the direct impact of the drops onto the soil (Legesse Negash, 1995). Moreover, loss of water through surface run-off is very much minimized. The root system of the trees improves drainage and aeration, and aid in retaining soil on sloping land (Fernandes *et al*., 1984). As the proportion of the canopy increases, water infiltration increases and surface run-off decreases resulting in more water in the soil. The result is increased water availability and decreases the effective length of the dry season similar to other indigenous tree species such as *Podocarpus falcatus* (Thumb.) Mirb, *Croton macrostachyus* and *Ficus* species (Legesse Negash, 1995).

Metabiosis is a form of ecological dependence in which one organism must modify the environment before the second is available to live in it (Brown, 1993). Meyer (1966) mentioned that microorganisms in soils depend to a large extent on the litter produced by plants; in turn the plants depend partly upon the mineralization of the nutrients in the plant litter by non-symbiotic microorganisms. The macroorganisms and microorganisms which benefit include plants and the biota of litter and soil. The interaction of a metabiont with other organisms might maintain and prolong the existence of the microhabitat, ecosystem or environment in which the metabiotic population or community exists. For example, litter communities develop under forest trees (metabiont) and the continued existence of the litter ecosystems depends upon inputs (leaves, wood, roots) from the

trees. In turn, the continued existence of the forest depends upon the decomposition of litter and the transformation and mineralization of nutrients by the decomposer community. *H. abyssinica* constantly sheds leaves, provides mulch and helps to build up the organic content of the top soil (Fig.1). Leaf fall also adds nutrients, and improve soil texture. The decomposition of plant residues is influenced by resource quality, decomposer organism and environmental conditions (Swift *et al*., 1979). Moisture and temperature have fundamental effects on plant residue decomposition as they control decomposer activity (Tian *et al*., 1997).

Figure 1. Leaf litter production in *H. abyssinica*. This six year old *H. abyssinica* has a high leaf litter production providing a habitat for many soil microorganisms, hence allowing them to interact with one another.

Bacteria and fungi are ultimately responsible for the biochemical processes in the decomposition of organic residues. Provided that environmental conditions are optimal, leaf production, senescence, abscission and decomposition rate of the tree is high so that the species can produce a great amount of litter per unit time, thus making it an effective nutrient pump (Legesse Negash, 1995). The shade and litter provided by the tree lowers soil temperature, and creates a habitat for micro-organisms, which aid in the break down of organic matter into humus and the fertility of the soil that harbors them (Fernandes *et al*., 1984). This fast litter decomposition rate enriches the soil by releasing nutrients that have been sequestrated in the leaves (Jiregna Gindeba, *et al*., 2004).

H. abyssinica is sometimes left in farmland or derived grassland (Dechasa Jiru, 1990) and may be occasionally planted where it occurs naturally because of its medicinal properties. In the wheat farm where *H. abyssinica* was intercropped, the growth and productivity was high due to high production, accumulation and decomposition of leaf litter. Interviewed farmer in Intoto emphasized the valuable feature of the litter of this species increasing the yield of wheat (Fig. 2). The farmer in this region maintained *H. abyssinica* trees on his farmland in association with annual crops such as wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*).

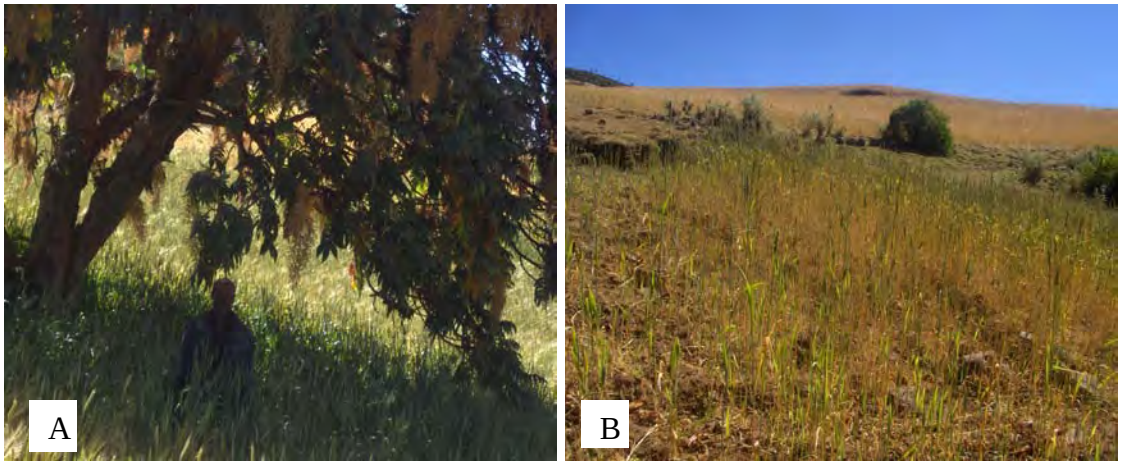

Figure 2. Increased growth and productivity of wheat beneath *H. abyssinica* due to the accumulation and decomposition of leaf litter (A), and a stunted wheat growth in the absence of *H. abyssinica* leaf litter in the same farm (B).

Leomordant, 1972 cited in Berhanu Abegaz *et al*. (1999), claims that it was Godinho, a Portuguese priest who in 1645 described the use of *H. abyssinica* as a vermifuge by Ethiopians. The dried female inflorescence had been widely used for its potent anthelmintic drug under the name "*kousso*", "*kosso*", or "*kusso*", particularly in Africa and the Near East. *Kosso* flowers contain phloroglucinol derivatives similar to those of female *Dryopteris* ferns (Lounasmaa *et al*., 1973 cited in Legesse Negash, 1995; Berhanu Abegaz and Ermias Dagne, 1978). The definite crystalline product from *kosso* appeared to have been the *kosin*, prepared by Messers E. Merk (mentioned in Hems and Todd, 1937) from flowers of this dioecious plant. The extraction process involved treatment of flowers with hot milk of lime and subsequent extraction with alcohol. Kossins exhibit comparable potency as the marketed drugs dichlorophane and niclosomide (Berhanu Abegaz and Ermias Dagne, 1978). Leichsenring (1984), quoted in Hems and Todd (1937), isolated from an ethanol extract of *kosso*, a colorless crystalline compound with no virmicidal property known as *prototoxin*. Interestingly, Leichsenring also managed to isolate an amorphous and a highly toxic substance known as *kosotoxin*.

According to Legesse Negash (1995), the female flowers of *H. abyssinica* in traditional Ethiopia has been used for treating tapeworm. The traditional consumption of raw beef meat commonly leads to contraction of tapeworm (*Taenia saginata* Goeze) which uses cattle as an intermediate host and encysts there. Depending on the variety of the female tree, as well as on the sensitivity of the individual, the amount taken varies depending on the age of the individuals (Pankhrust, 1965; Legesse Negash, 1995). The traditional conoction is usually made from dried female flowers, as well as from other additives. One common additive is the bud of *Croton macrostachyus* Del. The dried inflorescence and the fresh buds are moistened with some water and are pounded using the wooden pestle and mortar. The mixture is 'softened' manually powdered traditional mill. The product is suspended in water and given to the expected person to be drunk. The suspension is bitter and nauseating (Legesse Negash, 1995). In rural areas of Ethiopa, the drug is said to be highly effective and the demand is high (Kloos, 1976; Kloos *et al.*, 1978 cited in Faris Hailu, 1998). However, there are serious side effects if an over dose

of the drug is taken. The side effects range from feeling weak, nauseated and may lead to death in extreme cases (Edemariam Tsega *et al.*, 1978; Low *et al*., 1985).

According to Beentje (1994), the dried female flower heads and the bark infusion serve as a reputed, powerful remedy for intestinal parasites, especially against cestodes. It is also claimed that the bark may be pounded, added to cold water and the liquid is drunk as a remedy for diarrhea and stomach ache in humans (Beentje, 1994). Generally, this is a strong medicine that must not be taken in large quantities; it is also reputed to cause abortions. *H. abyssinica* has been used as an anthelmintic in ruminants (Abebe *et al*, 2000 cited in Githiori, 2004). Githiori (2004) reported that 30 grams of female flowers of *Hagenia abyssinica* immersed in 500 ml of water, shredded in a blender and the preparation was administered orally to individual lambs at a dosage of 1 gram kg^{-1}. Mesfin Tadesse (1986) also pointed out that flowers cure skin disease. Because of these and other similar features, propagation and cultivation of *H. abyssinica* must be considered as one of the priority tasks of indigenous trees restoration.

3.2. Reproduction *of H. abyssinica*

3.2.1. Sexual Reproduction

H. abyssinica is a dioecious species with distinct male and female (Fig.3) trees that are distinguished with certainty only after flowering (Tileye Feyissa, 2006). Flowering and seedling can be observed throughout the year with a break in the months with coldest temperatures. The inflorescences of *H. abyssinica* are quite conspicuous, being readily visible from rather a distance. In taxonomic language, they are said to be drooping panicles (Fig.3). The female inflorescence, a widely accepted and quite potent traditional anti-tapeworm, (Legesse Negash, 1995), may be 60 cm long and 30 cm wide (Hedberg, 1989). The reddish female inflorescence is bulkier than the dull-cream inflorescence of the male. Apparently, the larger size of the female inflorescence is “meant” for enhancing its receptive capability for the wind borne pollen grains of the male inflorescence. Interestingly both the female and the male flowers produce abundant nectar and pollen,

respectively (Legesse Negash, 1995). *H. abyssinica* has separate individuals of male and female tree and pollination is effected by wind. To achieve successful wind pollination in the tropics, a large number of male and female individuals must grow close to one another. This is because nearly all wind-borne pollen grains fall within hundred meters of the parent plant and the large amount of pollen that are blown by wind reach the vicinity of the ovules only by chance (Raven *et al*, 1986 cited in Legesse Negash, 1995). Hence, if individual plants are widely scattered, the chances that a pollen grain will reach a receptive stigma are very low. *H. abyssinica* has fairly small and light seeds. They are borne within a somewhat urn-shaped fruit. Consequently, not all the fruits of any one female inflorescence may produce seeds that have the capacity for germination. This means that successful fertilization in *H. abyssinica* would depend not only on the pollinating factor (wind) but also on such factors as the size and proximity of the two sexes to each other (Legesse Negash, 1995).

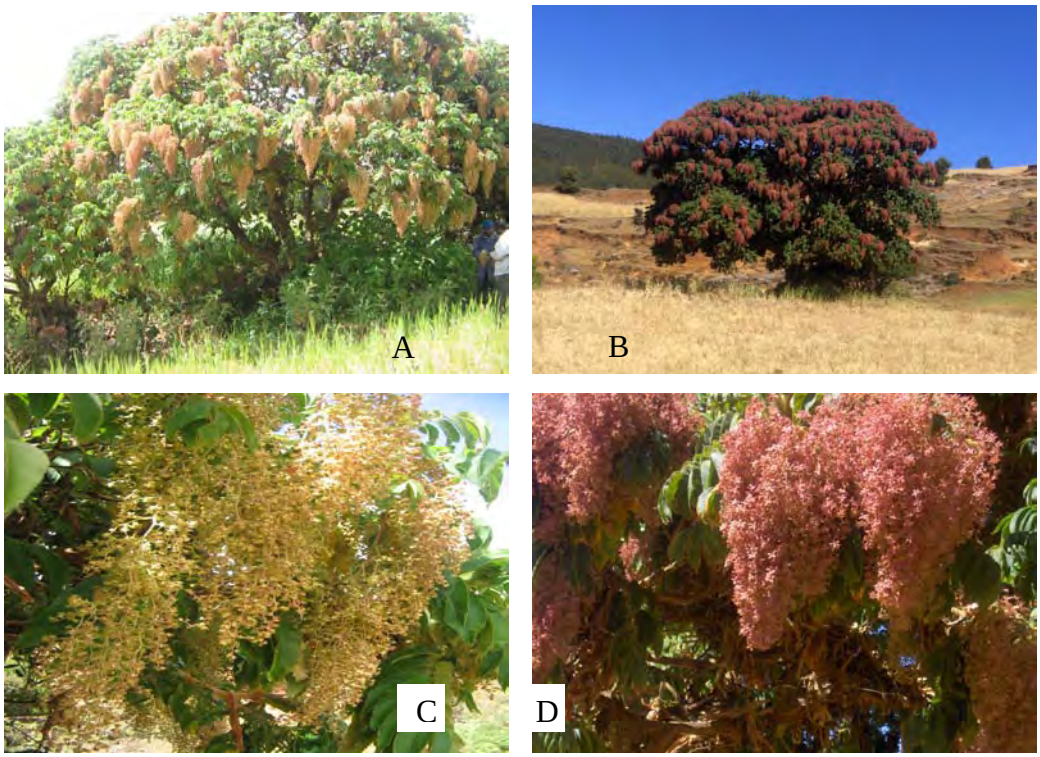

Figure 3. Mature male (A) and female (B) *H. abyssinica* trees from Intoto (north east of Addis Ababa). Male inflorescence (C) orange to white and female inflorescence (D) reddish at maturity of *H. abyssinica*

Seeds of *H. abyssinica germinate* within 10-20 days planting with germination rate of 40-60 % (Hedberg, 1989; Azene Bekele *et al*., 1993; Azene Bekele, 2007).

3.2.2. Micropropagation

Plant tissue culture is the science or art of growing plant cells, tissues or organs on artificial media by isolating them from the mother plant (George, 1993). By using plant tissue culture techniques, complete new plants can be obtained from different explants through direct or indirect morphogenesis and through somatic embryogenesis (Hartmann *et al.*, 2004).

Regardless of its economic and ecological importance, few studies have been carried out on the propagation of *H. abyssinica*. A micropropagation protocol has been developed for *H. abyssinica* using explants from juvenile and mature material origin (Tileye Feyissa, 2006). Using explants of mature tree origin for micropropagation is more advantageous than explants of juvenile origin because the phenotype, and in the case of *H. abyssinica* and other dioecious plants, the sex of the mother plant is known. In *H. abyssinica*, *in vitro* culture allows plant propagation within a relatively short period of time, to obtain clones with known sex and characteristics and to genetically improve the species. The low germination percentage of seeds hampers conventional propagation of *H. abyssinica* (Tileye Feyissa, 2006). Tileye Feyissa (2006) has suggested that this problem can be overcome by *in vitro* propagation if it provides a lot of plantlets within a short time. A protocol for *in vitro* shoot regeneration of *H. abyssinica* has been developed using leaf from *in vitro* shoots of seedling and mature tree origin. Tileye Feyissa *et al*. (2005) have reported that development of micropropagation for *H. abyssinica* using explants from both juvenile and mature material origin has scientific and practical significance in propagation, conservation and improvement of species.

3.3. Seed germination

Seed germination is the process by which dormancy is broken and the growth and further development of the embryo resumes (Thomas *et al*, 1960; Fosket, 1994). Consequently, the seed occupies a strategic position in the life cycle of higher plants (Bewley and Black, 1994). Germination may be triggered by imbibition of water (Koller *et al*., 1962; Fosket, 1994; and VASATIWIKI, 2007). This leads to mobilization of the stored food. In many cases, gibberellins play important signaling role in mobilization of stored food reserves (Fosket, 1994). During this time, energy metabolism resumes, respiration is activated, and the cell cycle may be initiated, while events associated with seed maturation are suppressed (Hilhorst *et al*., 1998). Sometimes, removal of the developing seed from its surrounding fruit tissue is sufficient to permit germination, but germination of the seed cannot occur even under this situation until near maturation (Bewley and Downie, 1996).

For germination to be initiated, three conditions are required (Hartmann *et al*., 2004): (1) the seed must be viable, (2) the seed must be subjected to appropriate environmental conditions: available water, proper temperature regimes, a supply of oxygen and sometimes light, (3) any primary dormancy present within the seed must be overcome. After the seeds have germinated, they require favorable conditions for their establishment. All these factors become available if the seeds have the chance to germinate in their natural habitats or if the original habitat is not seriously disturbed (Legesse Negash, 1995).

3.4. Factors affecting seed germination

Propagation of many indigenous tree species from seeds had been difficult due to lack of precise knowledge on their seed biology and germination physiology (Legesse Negash, 1995). Many plant species have developed survival strategies through evolutionary process for millions of years, understanding these strategies in the context of seed physiology are essential for successful plant propagation. Plants must simultaneously sense a number of environmental conditions and fix the time of seed maturation,

germination, and seedling emergence to particular periods of the year. Plants perform this according to their habitat location and requisite environmental conditions. Some of the factors that affect seed germination are described below.

3.4.1. Degree of seed maturity

Seed set and seed release into the soil occurs at the pick of the dry season and emergence of seedlings under this condition could jeopardize survival. Hence, a structural or physiological adaptive mechanism called dormancy prevents germination during the dry season (Baskin and Baskin, 1989; Legesse Negash, 1995, 2002).

Seed dormancy is a condition where seeds will not germinate even when the environmental conditions (water, temperature, and aeration) are permissive for germination (Hartmann *et al*., 2004). Even a non-dormant or quiescent seed has a unique ability to revert to a dormant state under stressful conditions (Hartmann *et al.*, 2004). Seeds germinate only after the dormancy is overcome or broken either through natural means such as animal gut activities (Manzano *et al*., 2005), wild fire (van Staden *et al*., 2000), rainfall (Hartmann *et al.*, 2004) or through artificial means such as scarification, seed coat cracking, removing chemical inhibitors through leaching by water (Legesse Negash, 1995, 2002; Acquaah, 2002).

Structural dormancy is imposed *via* seed coat, which prohibits the entry of water, air, outward diffusion of possible endogenous germination inhibitors, as well as through mechanical restriction of embryo growth (Hartmann *et al.*, 1997). Species with hard, impervious seed coverings occur in trees such as *P. falcatus, Olea europaea* subsp *cuspidata* (Wall. Ex Dc.) Ciffieri and various *Acacia* species (Legesse Negash, 1995). Seed maturity can also affect seed germination and the emergence of seedlings in many cases. In some species, such as *Pinus* and *Rananculus*, the cones or the fruits are shed before the embryo fully matures. Such physiologically immature seeds must undergo certain enzymatic and biochemical changes to attain maturity (Hartmann *et al.*, 2004). These changes are collectively called after ripening. Immature embryos cannot

germinate. But such seeds can be matured artificially by storing fruits or cones for a certain period of time to allow embryos to mature completely and to germinate (Bewley and Downie, 1996).

Investigation of the germination physiology of seeds of several indigenous tree species of Ethiopia have shown that a certain level of maturity must be reached for the successful germination of the seeds to produce the required amount of seedlings for mass propagation of forest trees (Legesse Negash, 1995). For example, in *P. falcatus*, fruit collection should be commenced when at least 60-70% of them become yellow to get high quality viable seeds. For a given species, it is often the case that either the presence of an impermeable, leathery or hard seed coat or chemical inhibitors within the embryo or within the stored food of the seed or the embryo immaturity prevent seed germination. However, it can sometimes happen that all the four factors combine and prevent seed germination (Hartmann *et al*., 2004). Therefore, the problem associated with seed germination that prevent successful seedling production, depend much on the understanding of the biology of seed dormancy and the maturity condition of the species of interest.

3.4.2. Light

Physiologically mature seeds are either dominant or non-dominant and will germinate once the dormancy is relieved or optimum conditions are provided. Environmental conditions may impose some dormancy condition on seeds and prolonged inhibition of germination may gradually enter a state of secondary dormancy (Karssen, 1995 cited in Ochuodho and Modi, 2007). In light requiring species, *Sisymbrium officinale* and *Arabidopsis*, light (Pfr) plays an important role in the biosynthesis of GA_3 and also increase the sensitivity of seeds to GA_3 (Hilhorst and Karssen, 1988; Baskin and Baskin 1998). These species germinate in darkness when exogenous GA_3 was applied.

Phytochrome appears in two forms, Pr ("red") and Pfr ("far red") which can be reversibly converted to either form by radiation at different wavelengths. Germination is determined by the amount of Pfr relative to the total amount of phytochrome (Mayer and Poljakoff, 1989 cited in Probert, 2000). Phytochrome in the Pr form inhibits germination, whereas Pfr allows proceeding. Dormant seeds have a large quantity of Pr, in non-dormant seeds the phytochrome is mainly in the form of Pfr form. Hilhorst (1990) suggested that induction and breaking of secondary dormancy was phytochrome controlled in light requiring seeds. This was also shown in guava seeds (*Pisidium guajava*), where germination was induced by high P/ FR ratio and alternating temperatures (Sugahara and Takaki, 2004 cited in Ochuodho and Modi, 2007).

Seed germination in many plant species is inhibited by continuous white light and such seeds germinate well in darkness (Bewley and Black, 1994). The seeds of *Cleome gynandra* L. responded negatively to continuous white light during germination at 20^{o} C, when light exposure beyond 12 h drastically reduced germination (Ochuodho and Modi, 2007). Such seeds are referred to as negatively photoblastic (Bewley and Black, 1994; Baskin and Baskin, 1988; Ochuodho and Modi, 2007). Photoinhibition involves the operation of phytochrome A and B, and its effectiveness depends on the duration of exposure and effluence rate (Bewley and Black, 1994). Photoinhibited seeds don't completely recover their germination conditions, except after treatment with GA_3 (Ochuodho and Modi, 2007)

3.4.3. Temperature

Temperature is the most important environmental factor that regulates the timing of the germination, partly due to dormancy and / or release and partly due to climate adaptation (Hartmann *et al*., 2004). Many seeds have minimum, maximum and optimum temperature for germination (Núñex and Calvo, 2000). Though the range of temperature required for seeds to germinate varies depending upon the species, the germination of all seeds is prevented at a very low or very high temperature (Hartmann *et al.*, 2004). Germination occurs over a wide range of temperature, although for each species there is

an optimal temperature and the rate of germination drops off at both above and below that temperature (Srivastava, 2002). According to Hartmann *et al*. (2004), the optimum temperature for non-dormant seeds of most species is between 25 and 30^{o} C, but can be as low as 15^{o} C.

High temperature affects the plant pigment, phytochrome that mediates promotion of germination by light (Karssen and Hilhorst, 1992). Phytochrome occurs either in a stable inactive form (Pr) with an absorption maximum at 660 nm or in an unstable physiologically active form (Pfr) with an absorption maximum of 730 nm. Increased temperature is known to accelerate inactivation of Pfr which may occur either by reversion of Pr or by destruction of the Pfr totally (Hartmann *et al*., 2004). Van der Woude and Toole (1980) demonstrated a direct effect of chilling on phytochrome-controlled lettuce-seed germination. In this study they have shown that a period of 6 h prechilling greatly enhanced the response of seeds to low, normally inhibitory levels of the active form of phytochrome (Pfr) established by far-red radiation.

Temperature also helps to release dormancy of some species. Washitani (1988) demonstrated the effects of temperature on hard-seed coat of *Rhus javanica*, a pioneer tree species of temperate Japan, becomes permeable and the proportion of seeds were capable of germination after exposure to high temperature of about 50 to 75^{o} C for an appropriate length of time. Washitani concluded that, in the wide, dormancy release and viability in seeds of *R. javanica* would occur either as a result of several brief exposure to much higher temperatures during fires. Narrowing and widening of the temperature range for germination could occur by movement in the low or high temperature limit for germination, or both depending on the species (Probert, 2000)

3.4.4. Water and oxygen

For germination to occur, seeds require moisture, and oxygen (Hartmann *et al*, 2004). The uptake of water by seeds, which occurs through imbibition, is the first process that takes place during seed germination. The composition of the seed, the availability of

water in liquid or gases forms in the environment, and the permeability of the seed coat or fruit to water determine the context to which imbibition occurs. Though protein is the chief water imbibing component of the seed, cellulose and pectin also contributes to the swelling of seeds, whereas it was found that starch does not add to swelling (Hartmann *et al*, 2004).

Germination is reduced by stress. For example, germination of lettuce and wheat in saline soil is reduced by decreased in water potential (Berrie, 1984). On the other hand, germination is reduced under water-logged condition. This is because, under water-logged condition, there occurs oxygen stress. Seeds of many species will not germinate at an oxygen level considerably lower than that normally present in the atmosphere (Mayer and Poljakoff-Mayber, 1982). In the laboratory germination tests, seeds of most tree species germinate well with the air available in the germination medium and with exchange through loosely fitting germination containers. Germination will be inhibited by depressed oxygen supply when there is excessive moisture in the medium.

3.4.5. Seed treatments related to fire

Fire is a major environmental selective force that influences plant communities in many parts of the world. Reproductive strategies have evolved as adaptations to the various factors generated by, and /or with, fire (van Staden *et al*., 2000). Many of them have evolved barriers to seed germination that overcome only by fire-related cues (Keeley and Fotheringham, 1998).

Wild fires are natural and widespread features of temperate ecosystems and many plant species have seedling recruitment restricted to habitats created such disturbances. Fire triggered-germination is the result of either heat shock or chemical products of combustion, and species appear to utilize one or the other of these modes. Heat shock stimulated germination is widespread in *Fabaceae*, *Rhamnaceae*, *Convolvulaceae*, *Malvaceae*, *Cistaceae* and *Sterculaceae*, and is found in many ecosystems (Bell *et al*., 1993; Thanos and Rundel, 1995; Brown and van Staden, 1999) while an exhaustive study

of germination characteristic for this taxa is lacking, those that have been studied are described as “hard seeds”, with a prominent waxy cuticle that enforces dormancy by forming a water impermeable barrier. Brief heat shock between 80^{o} to 120^{o} C is sufficient to induce imbibition by loosening cells in localized regions of seed coat such as helium, chalazal cap or possibly denaturing inhibitors to break dormancy (Keeley and Pizzorno, 1986; Keeley, 1987; Bell *et al*., 1993).

Fire provides a number of factors that stimulate germination such as heat, inorganic nutrients, unidentified compounds associated with blackened (charred) wood (Keeley and Pizzorno, 1986) and components of wood smoke (Keeley and Fotheringham, 1997). Smoke has been shown to act as germination cue and as a pretreatment to break seed dormancy of diverse species. The ground breaking news that smoke or smoke derived extracts could have an amazing effect on breaking dormancy and increasing seed germination of many species was first discovered by deLange and Boucher (1990) cited in (Dixon *et al*., 1995; Brown and van Staden, 1997; Keeley and Fotheringham, 1998 and Light *et al.*, 2002) for a South African fynbos shrubs. They found that smoke acted as a cue for breaking dormancy of seeds of a threatened monotypic fynbos species, *Audouinia capitata* (L.), which belongs to the family Bruniaceae. The effects of smoke on seed germination have been demonstrated for many other fynbos species (Brown, 1993), a savannah grass (Baxter and van Staden, 1994), and recently has been reported for California Chaparral annual *Emmenanthe penduliflora* (Keeley and Fotheringham, 1997, 1998).

Dixon *et al.* (1995) examined 94 species from 113 provenances and found 45 species that responded to smoke treatments. Apparently, this indicates that the stimulatory effect of smoke differs markedly within taxa and between seeds of disparate provenances. The stimulatory effect of smoke treatment is related to scarification of the external and subepidermal cuticle thereby increasing the permeability of the semi-permeable subepidermal cuticle (Egerton-Warburton 1998, cited in Mulualem Tigabu *et al*., 2007), its hormone like effects signal further changes leading to germination; and activation of

P^H-dependent growth regulators, such as nitrite, gibberellic acid and potassium cyanide (Keeley and Fotheringham, 1998).

Slow combustion of dry or green plant material from source produce water soluble compounds that stimulate the germination of seeds of many plant species (Baxter and van Staden, 1994; Brown and van Staden, 1997; Light *et al*., 2002). Keeley and Fotheringham (1998), obtained their smoke by burning small branches and leaves of *Adenostoma* in a 150-mm (inside diameter) metal on which a glass funnel of 150 mm (inside diameter) mouth has placed upside down to collect and pass the escaped smoke through a thick 50 cm long, 10-mm inside diameter, rubber hose, which fitted to the funnel end of the glass and fed into a smoke- tight 70-L glass chamber. Observation of water extracts of both charred wood and smoke are equally effective in triggering germination (de Lange and Boucher, 1993, and van Staden *et al*., 1995 cited in Brown and van Staden, 1997) as well as vapors from charred wood or smoke adsorbed onto soil particles (Keeley and Fotheringham, 1997). The chemical signals of smoke influence seed germination not only during the fires and in the immediate post-fire environment, but also the signals lasts for considerable periods after the fire, and can travel to plant communities, long distance away from the fire (van Staden *et al*., 2000). These remarkable effects of smoke on seed germination have found wide application as seed treatments for enhancing the conservation of threatened or rare species, restoration of fire-adapted communities, the horticultural exploitation of desirable plants and in the reclamation of mine spoils and disturbed land (Brown and van Staden, 1997).

Smoke released from burning vegetation contains chemical signal that triggers germination of both fire climax and non-fire climax species, and can break dormancy and improve germination of vegetable crops such as lettuce (Drewes *et al*., 1995 cited in Brown and van Staden, 1997) and Celery (Thomas and van Staden, 1995; Brown and van Staden; 1997, 1999). Seeds of many recalcitrant species were found to break dormancy upon contact with smoke or even with wood ash (Keeley and Fotheringham, 1998). Smoke appears to be almost universal signal to seeds that a fire has occurred and that conditions are suitable for germination.

3.4.6. Gibberellic acid (GA_3)

Gibberellins are naturally occurring plant growth regulators, which may cause a variety of effects including the stimulation of seed germination. Gibberellins include a large range of chemicals that are produced naturally within plants and fungi. Gibberellins were discovered before World War II by Japanese scientists trying to express the abnormally tall growth and reduced yield of rice infected with a fungus known as *Gibberella fujikuroi* (Wineland) Kuhlman (Taize and Zeiger, 1998). An active ingredient was extracted from the fungus and its chemical structure was determined. More than 90 forms of gibberellins have since been found to be an important commercial product (Hartmann, *et al*., 2004).

GA_3 occurs naturally in seeds of many species. Developing seeds and fruits show the highest gibberellin levels. However, there is no evidence that the active gibberellins present in seeds are used for the growth of the seedlings, since the gibberellins decreases to zero in mature seeds. But mature seeds contain GA_{12}-aldehyde, the immediate gibberellin precursor, which is converted into growth-active gibberellins during the early stages of germination (Taize and Zeiger, 1998). Developing seeds are active sites of gibberellin biosynthesis, and studies have found increases in gibberellin levels in seeds during germination (Acquaah, 2002). Work with pea seedlings indicates that gibberellins occur primarily in the young, actively growing buds, leaves and upper internodes. These tissues also appear to be the site of gibberellin synthesis (Coolbaugh, 1985).

Gibberellin application triggers the germination of seeds, in many cases overcoming the need for special or prolonged dormancy breaking conditions such as cold treatment, after-ripening and aging (Aquaah, 2002). Anderson (1985) cited in (Tian *et al*., 2003), applied 1.0 g L^{-1} (0.003 M) of GA_3 solutions to non-hulled and dehulled seeds of gamagrass (*Tripsacum dactyloides* (L) L.) for 24 hours. GA was somewhat effective at breaking dormancy of dehulled seeds, in increasing germination percentage from 40 to 65 % after 30 days of germination. The effect of GA_3 on germination of with their capsule intact was slight, with germination percentage increasing 5 to 8 % when compared to untreated seeds. GA_3 induces embryo growth and stimulates the germination process. GA_3 are well-

documented regulators of germination and associated enzymes with generally having promotive effects (Fincher, 1989). Applied GA_3 also stimulates the production of numerous hydrolases, notably α-amylase, by the aleurone layers of germinating cereal grains. This aspect of GA action has led to use in the brewing industry in the production of malt (Taize and Zeiger, 1998).

3.4.7. Potassium nitrate (KNO_3)

Nitrate is an important nitrogen source of plants, but also a signal molecule that controls various aspects of plant development. It is assimilated via its reduction by nitrate reductase and other enzymes leading ultimately to the production of amino acids and nitrogen compounds (Taize and Zeiger, 1998). In addition to its role as a nutrient, nitrate was shown to act as a signal molecule that controls numerous aspects of plant development and metabolism (Wang *et al*., 2003).

Nitrate has been known to stimulate germination in a large number of plants (e.g., *Sisymbrium offcinale* L. and *Arabidopsis thaliana* (L.) Heyhn (Hilhorst and Karssen, 1988; Derkx and Karssen, 1993). Nitrate promotes germination possibly by enhancing gibberellin synthesis and this effect is independent of nitrate reduction, suggesting a signaling role of nitrate (Hilhorst and Karssen, 1988). The result of the study by Alboresi *et al*., (2005) supports this hypothesis in that nitrate accumulation in seeds was correlated with a lower requirement of GAs for germination. Based on the detail analysis of the germination response of seeds of *S. officinale* to different exogenous nitrate doses Hilhorst (1990) hypothesized that nitrate receptor can occur in two conformations, a high and a low-affinity state. *Chenopodium album* L. plants, grown under controlled environmental conditions on different level of soil nitrate, produced seeds with proportionally different NO_3- contents (Saini *et al*., 1985). Regardless of the endogenous NO_3^- content, few seeds germinated in water or upon treatment with KNO_3 (Saini *et al*., 1985). Combined application of ethylene and KNO_3 in the dark had a synergistic effect on NO_3^- deficient seeds. The synergism between ethylene and KNO_3 was attributed to the NO_3^- moiety of the nitrate salt. Addition of nitrate, however, masked the interaction

between ethylene and light. Gibberellic acid $_{4+7}$ (GA $_{4+7}$) or red light, each alone or combined with KNO_3, had little effect on germination. When applied together in the dark, ethylene and GA $_{4+7}$ synergistically enhanced the germination of NO_3- deficient seed. The combined effects of the two hormones on *C. album* L. seed were further enhanced by the addition of KNO_3. There was no synergism between ethylene and GA $_{4+7}$ in NO_3^- rich seed. The interactions among GA $_{4+7}$, ethylene and KNO_3 were not affected by light. Saini *et al*., (1985) reported that the sensitivity of C. *album* seeds to ethylene may depend on nitrate availability.

Nitrate has been proposed to stimulant germination by acting as an osmoticum and thus enhance water uptake in the dormant seeds of *A. fatua* (Mclntyre, 1997). But KCL was less effective in stimulating germination than KNO_3 suggesting that the nitrate effect was not purely osmotic (Alboresi *et al*., 2005). Batak *et al*. (2002) reported that exogenous nitrogenous compounds such as nitric oxide were found to stimulate germination in *A. thaliana*. *Emmenanthe penduliflora* Benth showed maximum germination with a concentration of 10 m M KNO_3 under a daily light/dark alternation (Thanos and Rundel, 1995). In a comparative experiment, with various simple inorganic nitrogenous compounds, germination of *E. penduliflora* was again shown to be promoted by nitrates (NH_4NO_3 being slightly more effective than KNO_3) while nitrates and ammonium had a minimal and statistically insignificant effect (Thanose and Rundel, 1995). Promotions by nitrates are obtained in the range of 0-50 m M (optimum around 10-20 m M) while higher concentrations are gradually less efficient and eventually inhibitory when compared with water controls (Karssen and Hilhorst, 1992). Alboresi *et al*. (2005) suggested that nitrate could change the level of ABA of the seed.

3.4.8. Allelochemicals

Allelopathy, a synonym for phytotoxicity, is defined as a direct or indirect harmful effect of one plant on another through the production of chemical compounds that escape into the environment (Rice, 1979). Conceptually, biomolecules (specifically termed allelochemicals) produced by a plant are released into the environment and subsequently

influence the growth and development of neighboring plants. It is important to keep in mind that allelopathy involves the addition of a chemical compound or compounds (secondary metabolites) into the environment, while resource competition involves the removal or reduction of some factors in the environment such as nutrient, water, or light (Wikipedia, 2008). There are hundreds of secondary metabolites in the plant kingdom, and many are known to be phytotoxic (Einhelling, 2002 cited in Wikipedia, 2008). Allelopathic effects of these compounds are often observed to occur early in the life cycle, causing inhibition of seed germination and or seedling growth. The compounds exhibit a wide range of action, from effects on DNA (alkaloids), photosynthetic and mitochondria function (quinines), phytohormone activity, ion uptake, and water balance (phenolics) (Einhelling, 2002 cited in Wikipedia, 2008).

The leaves of *Eucalyptus globulus* releases a number of water soluble allelochemicals (inhibitors) such as chlorogenic, isochlorogenic, ferulic, P-coumaric and caffeic acids and volatile inhibitors, α-pinene, camphene and cineole (Del Moral and Muller,1969, Al-Naib and Al-Monsawi, 1976 cited in Babu and Kandasamy, 1997). According to Rice (1984) phenolics are the most common and widely distributed water soluble chemicals. The escape of these chemicals into the environment occurs through various mechanisms such as leachations, volatilization and microbial decay of dead and fallen parts (Rice, 1984). Reid and Wilson 1985 cited in Babu and Kandasamy, 1997, suggested that *Eucalyptus astringens, E. globulus* and *Eucalyptus sideroxylon* inhibited grass growth through allelopathy. The most potent source of allelochemicals was found to be stem flow, especially *E. globulus* and *E. macrorhynca* (May and Ash, 1990).

4. MATERIALS AND METHODS

4.1. The study site

The germination studies were carried out from October 2007 to July 2008 at Addis Ababa University, Science Faculty, Department of Biology, Plant Tissue Culture and Seed Physiology Laboratory. Studies on germinant establishment, seedling growth and survival was conducted in the glasshouse and on the nursery bed used for indigenous trees research.

4.2. Inflorescence collection and processing

H. abyssinica produces copious amount of seeds, one of the essential requirements for easy propagation of a given species from seed. For this study, mature inflorescences were collected in January, 2008 directly from trees estimated to be between 40-50 years old and naturally growing on Mount Intoto Ca 15 km north east of Addis Ababa (09^0 06.2 N and 038^0 47.3 E) (Fig. 4). The altitude of the area ranges from 2800-2900 m a.s.l. The site was selected on the basis of access to transport, availability of the species in this area and the presence of a few remaining natural stands within the landholdings of the Ethiopian Heritage Trust.

Seeds were collected from all parts of the crown from at least two trees by selecting mature female inflorescence. For inflorescence collection, skilled local tree climbing person was used as assistant. The proximity of the male and female individual trees of the species (Appendix 1) was assessed before making collections of the female inflorescence. The distance between the male and female trees were about in the range of 15 m to 50 m. The male inflorescences are shorter, thinner and lighter (or cream) in color, the female inflorescences are longer, bulkier and reddish in color.

Immediately after collection, the female inflorescences were packed in nylon sacks and transported to the Plant Physiology Laboratory of the Biology Department (AAU) and were kept on the laboratory bench at room temperature for about 2-3 weeks. The inflorescence was avoided from drying in direct sunshine because this would interfere with the viability or germination capacity of the seeds. Then the seeds were separated from the panicles by gently squashing portions of the inflorescence between hands. In this operation, gloves were used. Inflorescences obtained were packed in perforated polyethylene plastic bags and the packages were kept at 4^0 C in the refrigerator and used for different experiments.

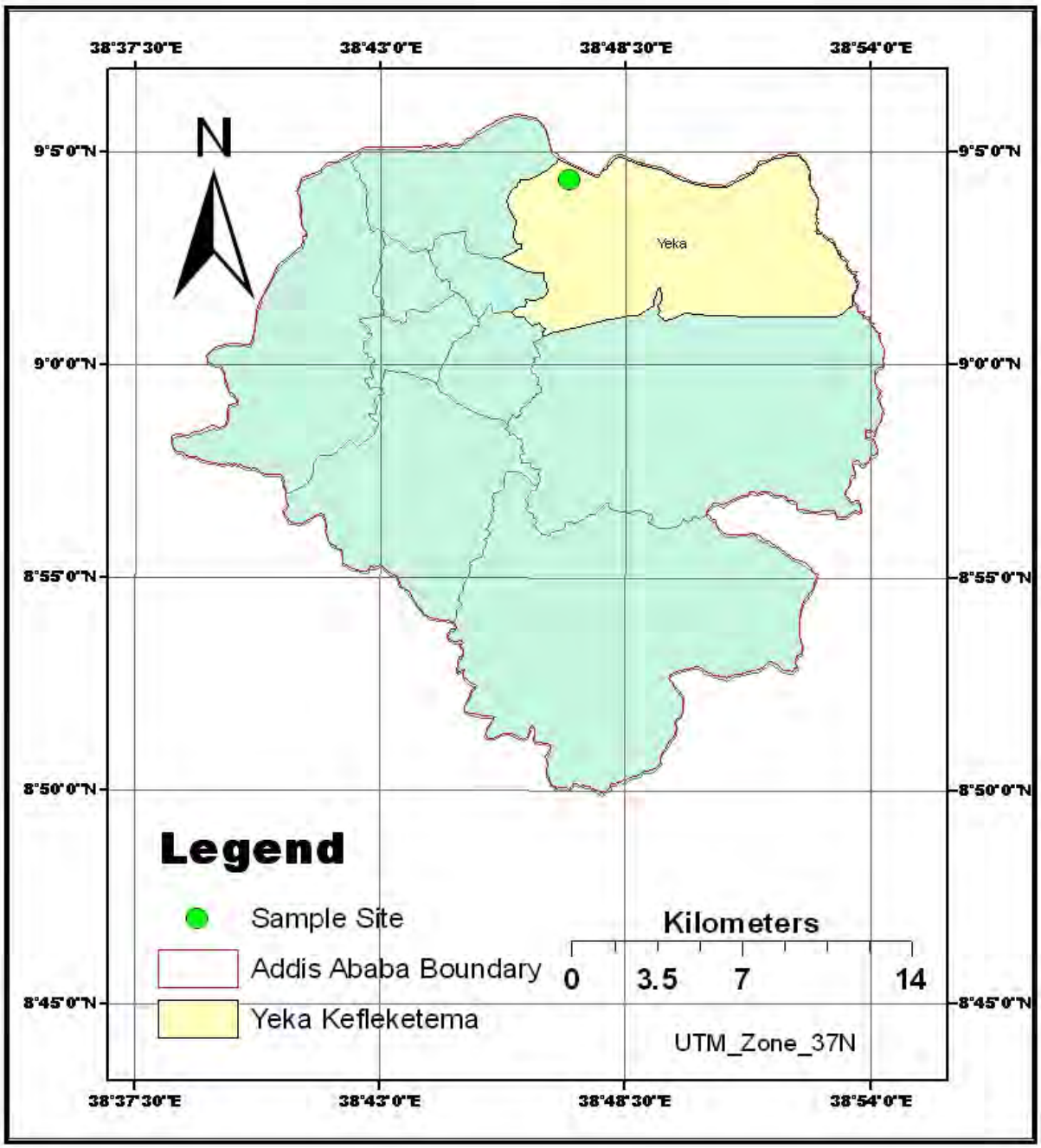

Figure 4. Map of seed collection site, showing the study area.

4.3. Laboratory experiments

Seeds were soaked and aerated for 24 hours in different concentrations of gibberellic acid (GA_3), potassium nitrate (KNO_3), various dilution levels of plant-derived aqueous smoke extracts, and double distilled water (control).

4.3.1. Preparation of aqueous smoke solution

Aqueous smoke solution was produced by combusting twigs of various plants [among which were *Juniperus procera* Hochst. ex Endl. and *Milletia ferruginea* (Hochst.) Baker] in a 100 mm diameter and 200 mm depth beekeeper's smoker. The produced smoke was forced through a plastic hose fitted to the mouth of the smoker by applying pressure on bellow (air holding and pumping part of the smoker) into a 500 ml Erlenmeyer flask containing 250 ml of double distilled water. The mouth of the E-flask was plugged with a smoke tight rubber material whose center has been hollowed to allow the entry of plastic hose to the E-flask. The smoke was forced to bubble into 250 ml of double distilled water for 6 minutes (Fig. 5). The resulting smoke water was maintained as stock water in a refrigerator at 4^{o} C, and the latter used to prepare cold aqueous smoke extract of different dilution levels.

Figure 5. Arrangement of the apparatus used for smoke extraction (After Kebebew Wakjira, 2007)

The active principle(s) in the smoke is (are) reported to be water soluble (Brown and van Staden, 1997). The method of smoke extraction was adapted from Keeley and Fotheringham (1998). The stock solution (100%) was diluted to 10, 20, 30, 40, and 50%, concentrations (v/v) while deionized water served as a control.

4.3.2. Preparation of germination stimulants

Gibberellic acid and potassium nitrate were used to investigate possible stimulation of seed germination of *H. abyssinica*. All the chemicals were "Plant Tissue Culture tested" that had been purchased in powder form from Sigma Chemical Company (ST, Louis, MO, USA).

Stock solution of 10^{-3} M were prepared by dissolving 0.346 gm and 0.101 gm of powders of GA_3 and KNO_3 respectively in 40 drops of 1 N NaOH and 0.101 gm powder of KNO_3 was dissolved in the 10 ml of double distilled water. Dissolved solutions were diluted with distilled water and neutralized with 40 drops of 1 N HCl. The p^H of solutions was adjusted to 6.8-7.5 and the volume was adjusted to 1 L by adding distilled water. Concentrations of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M were prepared by serial dilution from the respective stock solutions and were maintained in a refrigerator at 4^0 C.

4.3.3. Extraction of allelochemicals from leaves of *Eucalyptus globulus* ssp *globulus*

Fresh leaves sample of *E. globulus* ssp *globulus* were collected from 20-30 years old plantation growing at AAU, Science Faculty, near glasshouse.

Samples of fresh leaves with petiole (50 g) were crushed by pestle and mortar, soaked in 500 ml distilled water in 1000 ml conical flask for 24 hours at room temperature and sieved through a 2 mm sieve. Then, the extracts were filtered through Whatman filter paper No. 1. Aqueous extract was obtained as a filtrate of the mixture. This gave 100 % aqueous extract. The extract was considered as a stock solution and a series of solutions with different strengths (10, 20, 40, 80, and 100 %) were prepared by dilution. The seeds were soaked in double distilled water for 24 hours. Then 100 seeds were kept for germination in sterilized Petri dishes lined double with soft paper and moistened with 2-5 ml different concentrations of aqueous extracts. Each treatment has 3 replicates (total number of test seeds: $6 \times 3 \times 100 = 1800$). One treatment was run as a control with double distilled water only. The Petri dishes were maintained under laboratory condition (room temperature, 22^o C). When moisture content of the soft paper declined, 2-5 ml of the respective extract solution was added in the Petri dishes. Numbers of the germinated seeds were counted and, the radicle and shoot length were measured.

4.3.4. Pre-treatments of seeds of *H. abyssinica*

The seeds of *H. abyssinica* were pre-soaked in 250 ml of concentration (dilution) level of test solution in a 500 ml E-flask and were aerated for 24 hours in order to identify the effective pretreatment methods to be used in the experiment. The seeds were washed using tap water. The fruits that sank to the bottom of the E-flask were selected and used for germination experiment. A total of 80 Petri dishes (2 cm high and 9 cm in diameter) overlaid with two layers of soft paper, moistened with the respective test solutions were prepared. The Petri dishes were used for germinating different concentrations (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M) of GA_3, KNO_3 and (10, 20, 30, 40, and 50 %) plant-derived aqueous smoke solution. The corresponding controls were set up using distilled water. There were 5 replicates for each concentration of each germination stimulator solution. There were 50 seeds per Petri dish. The Petri dishes were labeled and placed randomly in the Tissue Culture room. Five ml of the corresponding test solutions were poured onto each Petri dish containing the seeds. The fruits were watered until their lower sides were covered by a thin film of the corresponding solution. The fruits were not over watered. The mean minimum and maximum temperature of the Tissue Culture Laboratory during conducting the experiment were 22.1 ± 0.6 and 27.2 ± 1.3 ^{o}C. The laboratory was with fluorescent lamps at a quantum flux density of Ca 40 μ mol m^{-2} s^{-1}. During subsequent days and upon moisture depletion, germinating seeds were provided with 2-3 ml of the corresponding solution. The numbers of seeds germinated were recorded daily until no more seeds germinated. The number of seeds germinated in each Petri dish using different treatments is shown in Appendix 5. Microphotography of a typical germinant was made using Leica MZ8 stereomicroscopic and Leica Microphotography 28/32 systems (Leica AG, CH-9435 Heerbrugg, and Switzerland). The major observable developmental stages in the germination of *H. abyssinica* seeds is shown in Appendix 3. The germinants were regularly transplanted, following the procedures described by Legesse Negash (1995).

4.4. Pot experiments

Pot experiments were conducted in Addis Ababa University within the Science Faculty of the AAU in the glasshouse. Seeds for this study were those collected from Intoto north of Addis Ababa and stored at 4^{o} C for 45 days. Fruits were planted in 10 pots (mouth diameter 20 cm, depth 20 cm) filled with a mixture of red soil, animal dung and sand in the ratio 2:1:1 respectively. In each pot, 100 fruits were planted at about equal distances between each seeds sown by hand. The fruits were covered with the same soil mixture such that they are not buried too much. The seed pots were watered once a day, and dried grass stalks were used to cover the pots conserving moisture. The seed pots were randomly arranged under a wooden bench in the glasshouse. The grass stalks were removed when the seeds germinated and cotyledons emerged from the surface of the soil mixture. But watering of the pots continued until the end of the experiment. The minimum and maximum temperatures in the glasshouse were 18.6 ± 2.1^{o} C and 35.3 ± 4.3^{o} C, respectively, while the corresponding temperatures on the nursery were 16 ± 4^{o} C and 28 ± 3^{o} C. Data on germination responses was collected every 2 days after the commencement of seed germination. Counting continued until the pots showed no new germination for at least 6 consecutive counts in each case. The final germination responses of seeds in glasshouse were expressed in terms of germination percentage.

4.5. Nursery establishment experiments

For the nursery establishment experiment, a total of 6 different soil mixes were used. Of these 6 different soil mixes, 5 were used as treatments and the remaining one was employed as a control for the treatments. Six hundred plastic sleeves (tubes) with a diameter of 10 cm and a length of 15 cm were filled with a mix of red soil, decomposed cow dung and sand in the ratio 4:3:2, 4:1:3, 4:3:1, 2:1:1, and 1:1:1, respectively. The control contained red soil only. The selection of soil, dung and sand mixing were based on the recommendations made by Legesse Negash (1995) for nursery establishment of various indigenous trees of Ethiopia. Each employed treatment as well as the control

contained 100 plastic sleeves of diameter 10 cm and length 15 cm. The plastic sleeves were arranged in a cooler corner of a glasshouse, arranged at random and were watered with tap water. The germinants with well developed radicles were selected from seeds treated with 10^{-4} M GA_3 for transplantation and were kept in a 1000 ml beaker containing tap water until they were transplanted. A hole was made in the center of the soil mixtures of each plastic sleeves such that the hole was deep enough to accommodate the whole length of the radicle. The radicle of the germinant was inserted into the hole leaving the cotyledons just above the surface of the potted soil. The remaining space of the hole around the radicle was covered with a fine soil mixture that was used for filling the plastic sleeves. The plastic pots were watered immediately after transplantation. The transplanted germinants were maintained in the glasshouse by covering them with thin plastic sheet lifted up by using wooden frames of each 40 cm long at intervals of 50 cm for two weeks. The mean minimum and maximum temperatures under the plastic sheets were 15.6 ± 2.1 and 44.2 ± 4.5^{o} C, respectively. The seedlings were watered using sprinkling watering once everyday.

4.5.1. Growth performance of seedlings

After 3 weeks of maintenance in the glasshouse, the seedlings were transferred to nursery bed. Mean minimum and maximum temperatures of the nursery during the study period were 16 ± 4^{o} C and 28 ± 3^{o} C respectively. Growth measurements of the seedlings were carried out in the nursery from March to May, 2008. Seedling heights were recorded for 30 randomly selected seedlings in each soil mix for 10 consecutive weeks at intervals of 2 weeks starting after 3 weeks of transplanting. Seedling survival was counted at the time of height measurements. Finally, 3 seedlings were randomly taken from each treatment in the nursery for biomass determination (after 4 months). The roots of the harvested seedlings were washed thoroughly on a wire sieve (Analysensieb, Weite 1.400 mm, Ser. No. 808145, Germany) to minimize the detachments of the fine roots. The detached parts of the seedlings were tied on the corresponding parts. The shoots and roots of the fresh seedlings were carefully separated by cutting with a dissecting knife. The respective

shoot and roots of each seedling were properly labeled and arranged on the shelves of the oven (Memmert, Beschickung-Loadig mode 100-800, Germany) and dried at 70^{o} C for 24 hours. After the indicated time, the dried shoot and root of each seedling were weighed (g) together (to get total dry weight) and separately (to determine shoot and root weights) by triple beam balance (Florham Park, N.J. 07932, USA).

Finally, the percentages of germinant establishment in the glasshouse (PGG), the percentages of seedling survival on the seedling bed (outside the glasshouse) (PSS) and the shoot to root dry weight ratios (SRR) of the individual harvested seedling were calculated by using equations 1, 2 and 3, respectively. Percentage of germinant establishment in the glasshouse (PGG) refers to the number of germinants (seedlings) survived in the glasshouse until the 3^{rd} week (SSG) divided by the number of germinants transplanted into the plastic pots in the glasshouse (GTP) multiplied by 100.

Hence: $PGG = SSG / GTP \times 100$……..... (1)

Percentage of seedlings survival on the seedling bed (outside the glasshouse) (PSS) also refers to the number of seedlings survived on the nursery bed until the 10^{th} week (SSB) divided by the number of seedlings transferred onto the nursery bed (STB) multiplied by 100.

Hence: $PSS = SSB / STB \times 100$……..... (2)

Shoot to root dry weight ratio (SRR) is the ratio between total shoot dry weight (SW) to that of the root (RW) (Hunt and Burnett, 1973). It was determined by the following formula:

$$SRR = SW/RW \text{……..... (3)}$$

4.6. Statistical analyses

1. Germination percentage (GP) was calculated according to the following formula:

GP (%) = (n/N) × 100, where:

n = total number of germinated seeds per Petri dish;

N = total number of seeds in the sample.

2. The mean germination time (MGT) and germination vigor were determined according to Labouriau and Agudo (1987) as follows:

MGT (days) = $(\sum t_i n_i)/ n$, where:

n_i = percentage seed germinated between two consecutive counts;

t_i = time taken since germination experiment started;

n = total percentage of seeds germinated.

3. Germination vigor (%) = $\sum (G_i/t_i)/ N \times 100$, where:

G_i = number of seeds germinated up to the day under consideration;

t_i = time taken since the first day of incubation;

N = total number of seeds.

Statistical analysis was performed according to the following procedures. The effects of GA_3, KNO_3, plant-derived aqueous smoke solution, and aqueous leaf extract of *E. globulus* ssp *globulus* and distilled water (control) on the germination of *H. abyssinica* seeds and nursery establishment of the seedlings were analyzed by a one-way ANOVA using SPSS for windows version 13.0 with treatments as factors. Duncan's post hoc test ($P \leq 0.05$) was used to determine the homogeneity subsets whenever significant differences existed among mean values of the treatments.

5. RESULTS

5.1. Effects of aqueous smoke, GA_3 and KNO_3 on germination percentage of *H. abyssinica* seeds

Seed germination of *H. abyssinica* began 7-10 days after incubation and almost completed within 18-21 days in all of the treatments including double distilled water (DDW), aqueous smoke extracts, GA_3 and KNO_3. Under seed pre-treatments that employed DDW, aqueous smoke extracts, GA_3 and KNO_3 conditions very few seeds resulted in rotting through fungal attacks. The effects of these various germination stimulators on seed germination percentage of *H. abyssinica* are provided in Figure 6.

Seeds treated with 40% smoke, 10^{-4} M GA_3, and 10^{-5} M KNO_3 (86 ± 2.6, 79.6 ± 2.7 and 89.6 ± 2.9%, respectively) showed significantly ($P \leq 0.05$) higher germination percentage compared to control (72 ± 4.8 %). But 10, 20, 30, 40 and 50% aqueous smoke extracts; 10^{-4}, 10^{-5} and 10^{-6} M GA_3; 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M KNO_3 (76.8 ± 2.9, 81.6 ± 1.6, 82.4 ± 3.3, 86 ± 2.6, 77.6 ± 2.7, 78.2 ± 4.5, 76 ± 1.3, 77.2 ± 0.8, 89.6 ± 2.9, 88 ± 3.5 and 80.4 ± 3.7) showed relatively higher germination percentages than the control (72 ± 4.8%) but differences were statistically significant ($P \leq 0.05$). Seeds treated with 10^{-3} and 10^{-7} M of GA_3, and 10^{-3} M of KNO_3 gave (66 ± 2.8, 68 ± 1.4 and 68.4 ± 7.1%, respectively) showed lower mean germination percentage significantly ($P \leq 0.05$) than the control (DDW).

Mean germination time (MGT) for seeds treated with plant-derived aqueous smoke extracts, GA_3 and KNO_3 were relatively lower than the control (Fig.7) but 20, 30 and 40% aqueous smoke extracts (11.7 ± 2.8, 11.7 ± 2.8 and 11.9 ± 2.9) showed relatively similar MGT values to the control (12.6 ± 2.1). Plant-derived smoke extracts (20, 30 and 40%) (4.5 ± 0.7, 4.5 ± 0.7 and 4.8 ± 0.9, respectively) showed similar stimulatory effect on germination vigor of *H. abyssinica* seeds compared to the control (4.5 ± 0.2) (Fig.8). Plant-derived smoke extracts (10 and 50%) (3.2 ± 0.8 and 3.2 ± 0.8) showed relatively

less effect on the germination vigor of *H. abyssinica* compared to the control (4.5 ± 0.2) (Fig.8). All pretreatments that involved GA_3 and KNO_3 did not show any significant stimulatory or inhibitory effect on the final MGT (Fig.7) and germination vigor (Fig.8) of *H. abyssinica* seeds compared to the control (Figs.7 and 8).

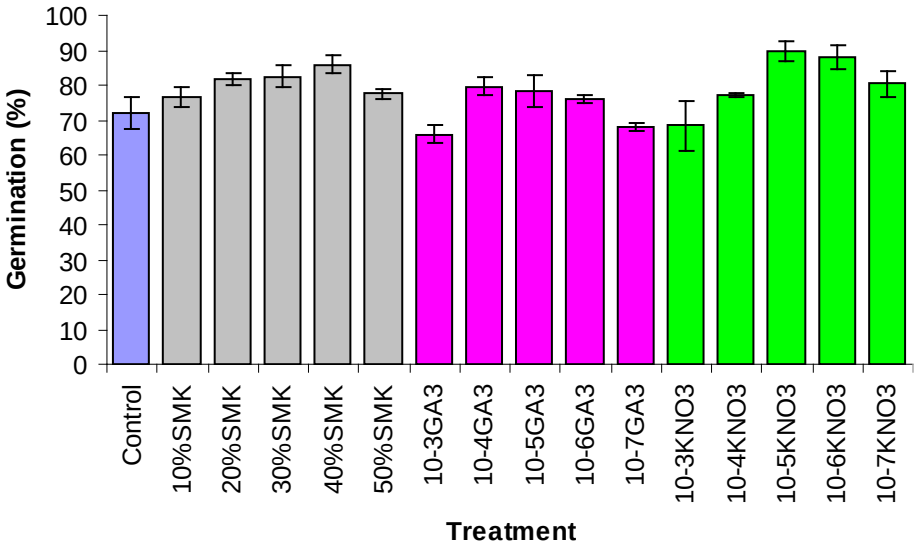

Figure 6. Mean (± SE) percentage germination of *H. abyssinica* seeds treated with control (DDW), different concentration of smoke (SMK) (10, 20, 30, 40 and 50 %), GA_3 (10^{-3} to 10^{-7} M) and KNO_3 (10^{-3} to 10^{-7} M).

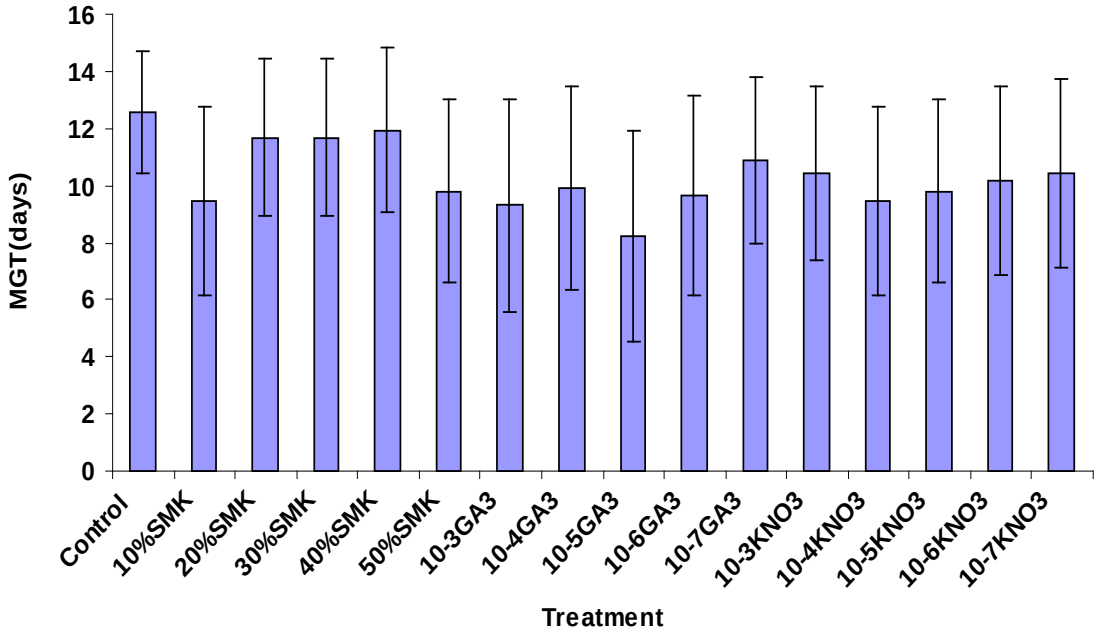

Figure 7. Mean (± SE) germination time of *H. abyssinica* seeds treated with control (DDW), various concentrations of smoke (SMK) (10, 20, 30, 40 and 50%), GA_3 (10^{-3} to 10^{-7} M) and KNO_3 (10^{-3} to 10^{-7} M).

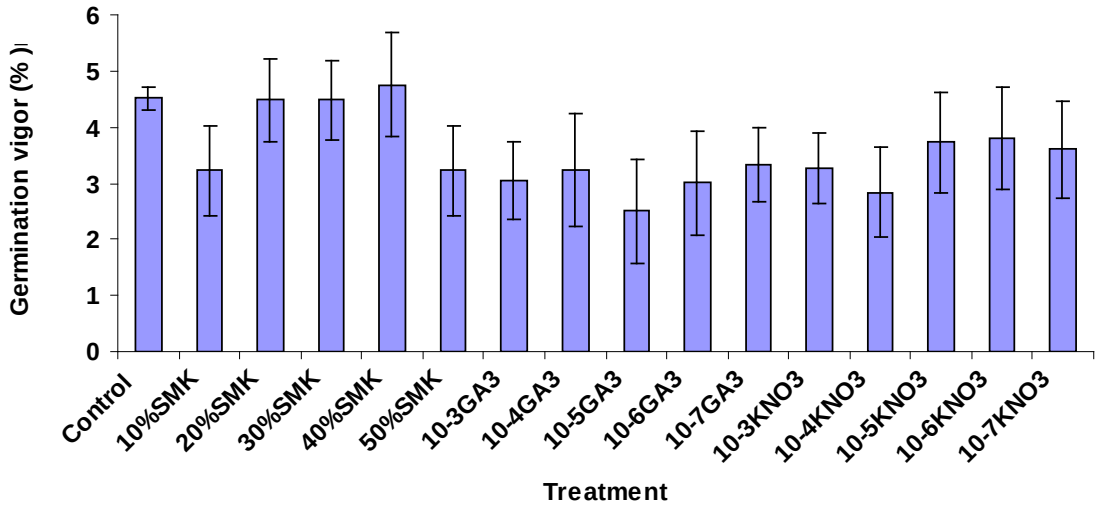

Figure 8. Mean (± SE) germination vigor values of *H. abyssinica* seeds treated with control (DDW), different concentrations of smoke (SMK) (10, 20, 30, 40 and 50%), GA_3 (10^{-3} to 10^{-7} M) and KNO_3 (10^{-3} to 10^{-7} M).

5.2. Pot experiments

Figure 9 shows the results of germination potential of *H. abyssinica* seeds from Petri dishes in the laboratory and pot experiments in the glasshouse. Mean germination percentage of *H. abyssinica* seeds in 10 pots was 64.1 % (Fig.10). Final germination percentage of glasshouse-germinated seeds was less than those germinated under various treatments DDW (control), Smoke, GA_3 and KNO_3 in Petri dishes in the Laboratory (Fig. 6.). Glasshouse-germinated seeds started germination on day 14 after incubation, reached 64.1%, in between 21 to 23 days. In contrast, seeds incubated in Petri dishes in the Laboratory started germinating 7-10 days after incubation, reaching higher germination percentages as shown in Fig. 6 in different concentrations of DDW, Smoke, GA_3, and KNO_3 in between 18-21 days.

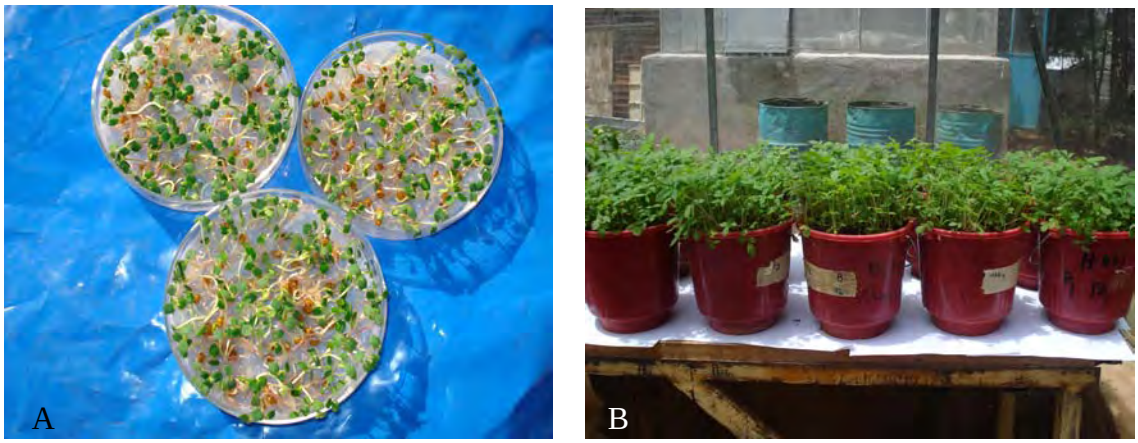

Figure 9. Germinants of *H. abyssinica* seeds in Petri dishes, 22 days after incubation in the laboratory (A), and 2 month old seedlings in the glasshouse (B) were germinated in a soil mixture ratio 2 red soil:1dung:1sand.

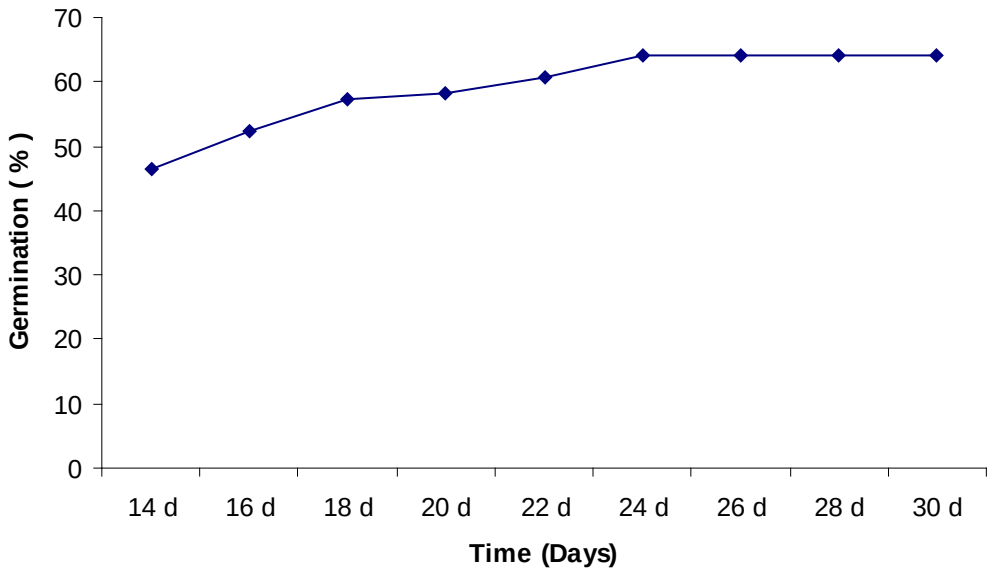

Figure 10. Mean germination percentage of *H. abyssinica* seeds germinated in glasshouse under soil treatment in the ratio of 2 red soil: 1 dung: 1 sand.

5.3. Nursery establishment experiments

5.3.1. Seedling survival

Survival percentages of *H. abyssinica* seedlings in different soil mixtures, 10 weeks after transplantation of germinants, are provided in Table1. Germinant establishment was fairly good in the glasshouse. Seedlings survival was highest (82%) when these were grown in a growth medium of 2:1:1 (red soil, dung and sand, respectively) (Table 1).

Table 1. Percentage of seedling survival 10 weeks after transplanting germinants to polyethylene pots. Seedlings were grown in plastic sleeves and 100 seedlings were transplanted to each of the 6 soil mixes that contained red soil, dung and sand in various ratios.

Treatments	% of survival
Soil: Dung: Sand (4:3:2)	78
Soil: Dung: Sand (4:3:1)	69
Soil: Dung: Sand (4:1:3)	67
Soil: Dung: Sand (2:1:1)	82
Soil: Dung: Sand (1:1:1)	74
Control (soil only)	58

Figure 11. Seedlings of *H. abyssinica* (3 month old) in the nursery under various soil treatments in the ratio of soil: dung: sand (4:3:2, 4:3:1, 4:1:3, 2:1:1, 1:1:1 and control (red soil)

5.3.2. Seedling height

Mean seedling heights of *H. abyssinica* planted in different soil mixes are provided in Figure 12. Mean final heights of the seedlings of the *H. abyssinica* in the 10^{th} week planted in red soil, dung, sand in the ratios of 4:3:2, 4:3:1, 4:1:3, 2:1:1, 1:1:1 and control filled in plastic sleeves were 19.1 ± 1.35, 20.58 ± 0.62**,** 19.1 ± 1.5, 21.9 ± 1.01**,** 18.14 ± 0.82**,** and 16.24 ± 0.59 cm, respectively. These values were significantly ($P \leq 0.05$), higher than the control (16.24 ± 0.59 cm).

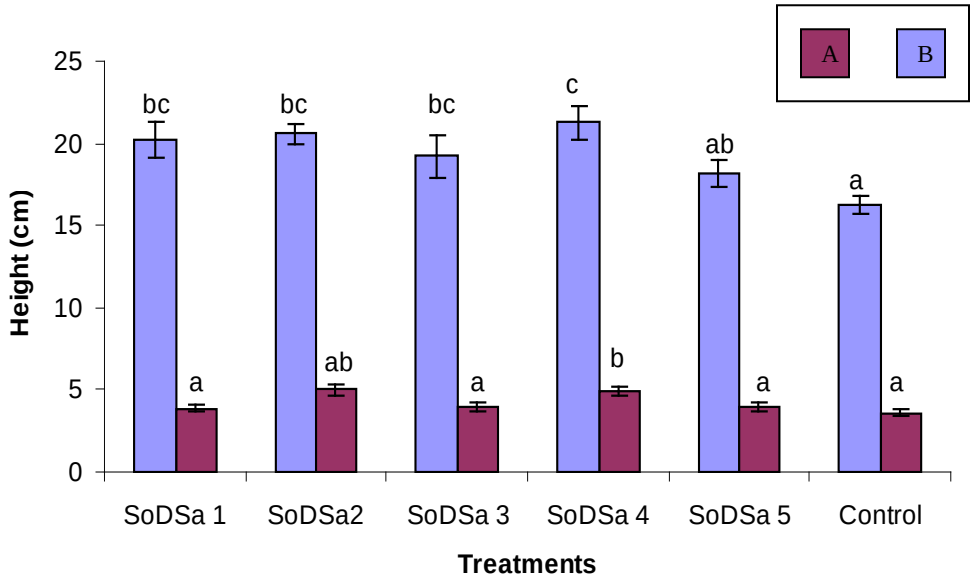

Figure 12. Mean heights (cm) recorded on the 3rd (A), and 10th week (B), for seedlings of *H. abyssinica* grown in different soil mixes. SoDSa1, SoDSa2, SoDSa3, SoDSa4, SoDSa5 and control in the ratios (4:3:2, 4:3:1, 4:1: 3, 2: 1: 1, 1: 1: 1, and control), respectively. Bars show means ± SE and different letters indicate significant mean height differences among treatments (Duncan's post hoc test at $P \leq 0.05$).

On the 3rd week, the seedlings (Fig.12), grown in the soil mixes containing SoDSa4 (4.87 ± 0.27 cm) showed significantly ($P \leq 0.05$) higher mean height than that of the control (3.56 ± 0.21 cm). The rest treatments are not significantly ($P \leq 0.05$) differen tly to that of the control. On the 10th week, the mean height of the seedlings grown in SoDSa1, SoDSa2, SoDSa3, and SoDSa4 (19.1 ± 1.13, 20.58 ± 0.6, 19.21 ± 1.25, and 21.29 ± 1.01 cm) were significantly greater ($P \leq 0.05$) than the control (16.24 ± 0.58).

Figure 13. Fast growing *H. abyssinica* seedlings under nursery conditions (A), achieving a height of 50 cm within 5 months (B).

Seedlings of *H. abyssinica* showed very good growth performances under different soil mixes under nursery conditions. Growth of *H. abyssinica* seedlings were considerably fast (Fig.13.) provided that conditions are suitable, nursery production of seedlings as tall as 50 cm can be achieved within 5 months (Fig.13.). *H. abyssinica* seedlings grow at a faster rate in the diverse soil mixtures due to its inherent ability of producing large number of fine roots and root hairs that enables it to absorb water and nutrients efficiently.

5.3.3. Biomass production

Table 2. Mean dry weight of shoots, roots, total dry weight and shoot-to-root ratios of seedlings of *H. abyssinica* harvested after 4 months from different treatments measured in gram.

Treatment	Shoot dry weight	Root dry weight	Total dry weight	Shoot/ Root
SoDSa1	3.87	0.77	4.63	5.18 ± 0.65^{b}
SoDSa2	1.80	0.23	2.03	8.25 ± 1.46^{c}
SoDSa3	3.70	0.60	4.30	6.15 ± 0.2^{bc}
SoDSa4	4.67	0.87	5.53	5.4 ± 0.17^{b}
SoDSa5	1.03	0.13	1.17	8.17 ± 1.01^{c}
Control	0.43	0.27	0.70	8.17 ± 1.01^{c}

Mean dry shoot and root weights were recorded after 4 month developmental stage of growth for *H. abyssinica* seedling is provided in Table 2. Marked differences were observed in biomass among the seedlings grown on different soil mixes (Table 2). Total dry weight of seedlings grown in SoDSa1, SoDSa3, SoDSa4 (4.63, 4.30 and 5.53 g) were significantly greater than the control (0.70 g).

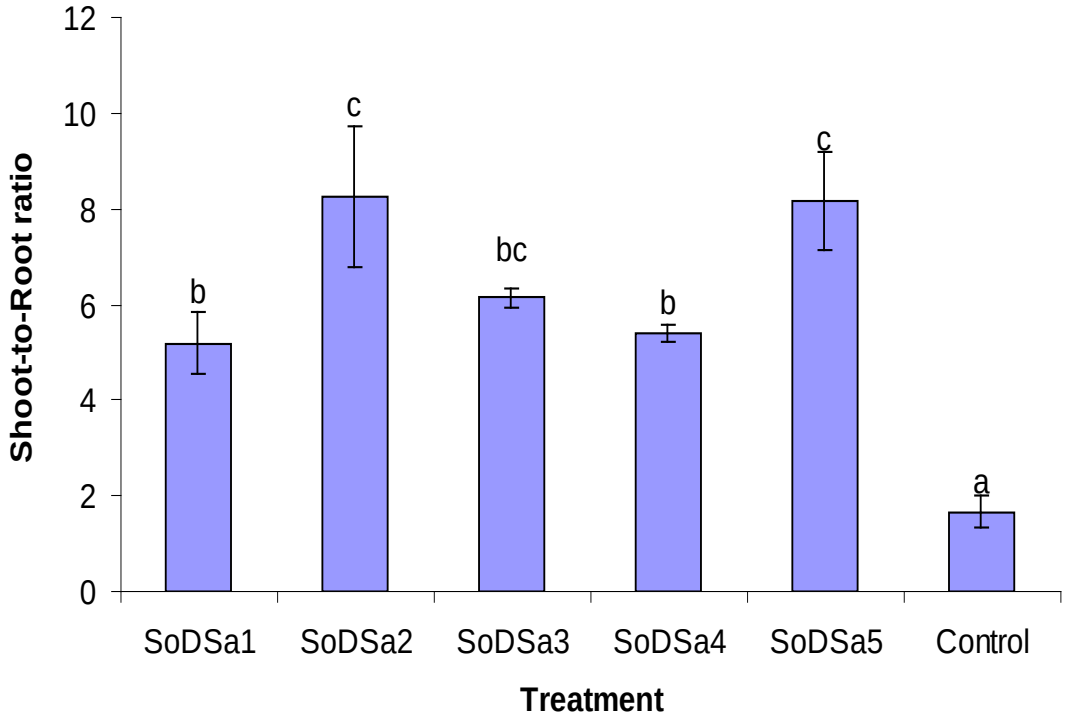

Figure 14. Shoot-to-root dry weight ratios of seedlings of *H. abyssinica* recorded after 4 months.

Shoot-to-root dry weight ratios (SRR) of the seedlings of *H. abyssinica* (Fig.14) grown in all treatments were significantly ($P \leq 0.05$) greater than the control (1.67 ± 0.33). The SRR of seedlings grown in SoDSa2 and SoDSa5 (8.25 ± 1.46 and 8.17 ± 1.01) were significantly ($P \leq 0.05$) larger compared to the rest of the treatments and the control (1.67 ± 0.33).

5.4. Allelopathic effects of *E. globulus* ssp *globulus* on the germination of *H. abyssinica*

Table 3. Seed germination, shoot and radicle growth of *H. abyssinica under* different concentrations of leaf aqueous extracts of *E. globulus* ssp *globulus*. Values are mean ± SE of 6 samples.

Concentration (%)	Germination (%)	Shoot length (cm)	Root length (cm)
Control (%)	71.67 ± 3.53 c	2.01 ± 0.08 b	1.59 ± 0.07 b
10	67.67 ± 3.38 c	1.75 ± 0.05 b	1.56 ± 0.07 b
20	20.67 ± 2.73 b	1.09 ± 0.09 a	1.33 ± 0.08 a
40	11 ± 1.0 a	0.09 ± 0.1 a	1.19 ± 0.08 a
80	No germination	No germination	No germination
100	No germination	No germination	No germination

E. globulus ssp *globulus* leaf aqueous extracts showed differential influence on the germination of *H. abyssinica* seed germination on the control (DDW) (71.67 ± 3.53%). Among the different treatments, fresh leaf aqueous extract at 10% no significant decrease in germination was observed. Treatments at 20 and 40% concentration suppressed the germination and growth of *H. abyssinica* seedlings as compared to the control. Here again at 40% higher suppression in germination was observed and there was a complete failure in germination at 80 and 100% concentration treatments (Table 3). The shoot and radicle in the control was 2.01 ± 0.08 and 1.59 ± 0.07 cm, respectively. No significant reduction in length was observed at 10% concentration treatment as compared to the control. However, a significant reduction in length was observed at 20 and 40% concentration treatment (Table 3) as compared to the control.

6. DISCUSSION

6.1. Effects of smoke extracts, GA_3, and KNO_3 on *H. abyssinica* seed germination

Treatments involving prior imbibitions of the seeds in water indicate that promotion of germination by the smoke solution is highly dependent on the initial period of rapid imbibition. It has been previously shown that direct smoking of seeds and the use of aqueous dilutions of smoke can be used as effective seed pretreatments (Baxter *et al*., 1994; Brown and van Staden, 1999).

Based on the study conducted to see the effects of smoke solution on lettuce seeds, Light *et al.* (2002) reported that no differences were observed between the rate of imbibition for seeds treated with water and those treated with smoke solution, while the solute uptake was faster for seeds treated with smoke solution. Similarly, Keeley and Fotheringham (1998) indicated that the solute uptake of seeds treated with double distilled water was very low, while permeability of semi-permeable membrane increased when seeds were treated with smoke solution for most of the studied California chaparral species. From these two reports, it is possible to learn that smoke affects seed germination responses by changing the permeability of the epidermal cells to the solutes required initiating seed germination.

Highest levels of germination of smoke- treated were also reported previously for many plant species of Australia (Bell *et al*., 1993; Dixon *et al*., 1995; Roche *et al.*, 1997) and South Africa (Brown *et al*.; 1993, 1994). The active component of charate was found to be water soluble (Keeley & Nitzberg, 1984) and was postulated as the triggering factor for seed germination induction (Keeley & Pozzorno, 1986). However, several reports (e.g., Baxter and van Staden, 1994; Keeley and Fotheringham, 1998; Light *et al*., 2002) indicated that little is known regarding the exact physiological mechanisms for smoke

related responses, and which active component(s) in smoke stimulate seed germination and how.

In the present study, pre-treatments involved various dilutions of smoke extracts improved germination percentage, but there was no significant difference among the various dilutions of smoke extracts. From the homogeneity test it was found that seed germination at 10 and 50% aqueous smoke extract concentration was not significantly ($P \leq 0.05$) different from the control; whereas at 20, 30 and 40% dilutions seed germination were significantly ($P \leq 0.05$) different from the control. Maximum germination percentages were observed at these dilutions but highest germination percentage was observed at 40% concentration.

From the present study, induction of germination by pre-treatments that involved all dilutions of aqueous smoke extracts was comparable to the results obtained from seeds pre-treated with double distilled water. Possibly, certain ions in smoke might have masked the effects of fluctuating temperature to improve seed germination. But the fact that pre-treatments that involved aqueous extracts significantly increased seed germination of *H. abyssinica*. This indicates that smoke pre-treatment have additive effects on the stimulation of seed germination. Various metabolic activities and the increment in germination percentage due to smoke mediation in *H. abyssinica* is a matter that needs further investigation.

GA_3 is important in the promotion and maintenance of germination. Groot *et al*., (1998) concluded that the key role of GA_3 in seed germination is to induce the synthesis of endosperm cell wall degrading enzymes to hydrolyze the layers surrounding the radicle until they no longer act as a physical barrier to radicle emergence. The effect of GA_3 on germination of seeds varies with the concentration applied and the plant species (Taiz and Zeiger, 1998).

In this study, seeds of *H. abyssinica* treated with GA_3 at 10^{-4} to 10^{-6} M showed slightly better germination percentage compared to the control, whereas at 10^{-4} M germination

percentage was higher than the control. This result is in agreement with the result reported by Legesse Negash (1993) on seeds of *Olea europaea* ssp *cuspidata* and seeds of *Prunus africana* (Legesse Negash, 2004) treated with 10^{-4} M of GA_3 resulted in maximum germination percentage. Similarly, aleuronic layer of barley seed is a uniform tissue consisting of one cell type responds to GA_3 by producing a wide spectrum of hydrolytic enzymes. When isolated aleurone layers are incubated in optimal concentrations (10^{-5} to 10^{-4} M) containing 10-20 mM $Ca^{2+,}$ numerous hydrolytic enzymes are produced (Mozer, 1980 cited in Russel and Macmillan, 1984). The effect of 10^{-4} M of GA_3 on seeds of *H. abyssinica* increased germination percentage than the control but not significantly. Similarly Legesse Negash (1992, 2003) reported that the effect of GA_3 was not increased significantly the final germination percentage of *Podocarpus falcatus.* This absence of significant response to the application of GA_3 on seeds of *P. falcatus* might indicate that germination of *P. falcatus* seeds is not initiated by the synthesis of hydrolytic enzymes in cereals enhance seed germination (Russel and Macmillan, 1984). Most likely, the stimulatory effect of GA_3 on seed germination of *H. abyssinica* could be due to the weakening of those structures through enzymatic degradation. The failure of a small portion of the seeds to respond to GA_3 could be due to insensitivity or the presence of other factors limiting the process leading to germination.

KNO_3 was also observed to enhance germination in some species such as *Sisymbrium officinale, Arabidopsis thaliana* (Hilhorst and Karssen, 1988; Derkx and Karssen, 1993) and *Emmenanthe penduliflora* (Keeley and Fotheringham, 1997). Thanos and Rundel (1995) indicated that KNO_3 becomes an effective germination promoting agent when combined with light and appropriate temperature. KNO_3 solution was used to stimulate germination on seeds of *Podocarpus falcatus* (Legesse Negash, 1992) and seeds of *Angelica glauca* (Butola and Badola, 2004).

In this study, seed germination percentage of *H. abyssinica* treated with KNO_3 was decreased at 10^{-3} M compared to the control. Germination percentage at 10^{-5} M and 10^{-6} M concentrations were significantly ($P \leq 0.05$) higher than the control and the maximum germination percentage was at 10^{-5} M. This result is in agreement with the result reported

by Thanos and Rundel (1995) on seeds of *Emmenanthe penduliflora* treated with 10 mM KNO_3 under daily light/dark alteration resulted in maximum promotion of germination percentage. Promotion by nitrates is obtained in the range 0-50 mM (optimum around 10-20 mM) while higher concentrations gradually was less efficient and eventually inhibitory when compared with water controls (Karssen and Hilhorst, 1992). This is due to that KNO_3 stimulates seed germination since the reduction of nitrate in the cytosol requires NAD(P)H and the synthesis and stabilization of nitrate reductase is also affected in the presence of nitrate (Beevers and Hageman, 1969 cited in Legesse Negash, 1992). A report by Legesse Negash (1992) also showed the effectiveness of KNO_3 at (0.5 %) to stimulate seed germination of *Podocarpus falcatus.*

6.2. Pot experiments

The fairly better final germination percentage of *H. abyssinica* seeds (64.1%) in the glasshouse may possibly be due to the high moisture of the pots combined with the warmth of the glasshouse. Legesse Negash (2004) indicated that favorable moisture speeded up germination of various indigenous trees of Ethiopia. Narrow temperature range facilitates favorable conditions for the soil microorganisms that speed up the breakdown of seed coats to speed up seed germination (Bell *et al.,* 1993). Bell *et al*. (1993) studied 43 western Australian species and showed that most of the species they studied germinated with increased rate and vigor at lower and less fluctuating temperatures than at more fluctuating higher temperatures. The present study has shown that fluctuations of day and night temperatures increased seed germination under glasshouse conditions. Consequently, increased rate and final germination percentage of pot-germination of seeds inside the glasshouse might indicate the importance of more fluctuating temperatures and high moisture, though factors other than these can possibly exist.

6.3. Nursery establishment conditions

From the present studies, it has been observed that seedlings have survival rate of 100% in the nursery. *H. abyssinica* is known to grow under cooler and highland ecological conditions. Legesse Negash (2002) underlined that an essential and first step in the domestication of plants should be to have knowledge about their propagation requirements and rate of growth both at the nursery and under field conditions.

6.3.1. Seedling survival

Seedling survival and growth was affected by several factors such as temperature (Criddle *et al*., 2005), light (Mason *et al.,* 2004) and nutrient availability (Tanner *et al.*, 1998). In this study, seedling survival of *H. abyssinica* was investigated using different soil mixes. Differences were observed on seedling survival in different soil mixes under the glasshouse condition. The highest seedling survival percentage was recorded in SoDSa4 (2:1:1) 82% but in the control (red soil only) it was 58% which is much lower than the number of survivals in other soil mixes that contain animal dung. This indicates that the difference in percent survival might possibly be due to the provision of decomposed cow dung. Seedling survival was 100% in various soil mixes in the nursery conditions, i.e., no seedling died after it has been transferred from the glasshouse to the nursery condition. Therefore, it can be concluded that survival of *H. abyssinica* seedlings in the nursery is the function of decomposed cow dung which is rich in plant nutrients (Meludu, 2005) and biomass allocation.

6.3.2. Growth performance of seedlings

6.3.2.1. Seedling height

Seedlings of the same species may have different seedling heights in different environmental factors (Agren and Ingestad, 1987). In this study seedling growth of *H. abyssinica* was investigated. Significant ($P \leq 0.05$) height differences were observed on seedlings that grew in different soil mixtures (SoDSa1, SoDSa2, SoDSa3, SoDSa4, SoDSa5) and the control treatments. Seedlings in the control treatments were the shortest compared to seedlings of other soil mixes. The final seedling height was highest in SoDSa4 and the highest was significantly ($P \leq 0.05$) different compared to the control. This result showed that the growth of seedling of *H. abyssinica* is enhanced by the provision of decomposed cow dung. This difference might be due to nutrient availability difference between animal dung and the soil. Meledu (2005) reported that animal dung is rich in plant nutrients (especially nitrogen and phosphorus), which can increase crop production better than artificial fertilizers.

6.3.2.2. Seedling biomass

A standard assumption in plant ecology is that plants respond to their environment in such a way as to optimize their resource use (Bloom *et al*., 1985 cited in Agren and Franklin, 2003). One expression of such optimization is the allocation between shoot and root in response to nutrient availability. In general, when nutrient availability increases, plants allocate relatively less to their roots, which is consistent with a resource optimization hypothesis as increasing nutrient availability means that less effort is required to acquire this resource (Agren and Franklin, 2003).

In this study, marked differences were obtained in mean dry weight among the seedlings grown on different soil mixes. Seedlings grown in SoDSa1, SoDSa3 and SoDSa4 showed greater dry weight compared to those grown in the soil only. From these results it is

possible to say that the proportion of decomposed cow dung is influential to enhance biomass since it is rich in plant nutrients (Meledu, 2005). The investigation of Agren and Franklin (2003) is in agreement with this result, in that the availability of enough nitrogen and other nutrients increased the biomass of the plant significantly.

The study revealed differential allocation of biomass to shoots and roots, which affected growth and survival of plants. Shoot to root ratio indicates the relative proportion of growth allocated to shoots versus roots in a given condition. In this study, inclusion of decomposed cow dung and sand in the red soil increased biomass allocation to shoots more than roots. This result indicates the growth and distribution of dry matter in seedlings of *H. abyssinica*. The soil mixes in the ratio SoDSa4 (2:1:1) and SoDSa1 (4:3:2) in these result showed better growth and distribution of dry matter in seedlings of *H. abyssinica.* Though, the potting mixes SoDSa2 (4:3:1) and SoDSa5 (1:1:1) resulted in unbalanced biomass allocation. These potting mixes increased biomass allocation to shoots than roots; this may be due to a decline in carbohydrate which will initiate more shoot production (Coder, 1998). The result is a greater allocation of carbohydrate to shoot production and less to roots.

Shoot-root-ratios of *H. abyssinica* seedling were much lower in control treatment, compared to shoot-to-root ratios of soil mixes containing animal dung. This showed that shoot growth is more favored than root growth in pots having animal dung and the reverse is true for control treatment. Thus, the data observed in the studied species was consistent with the hypothesis that some plants do exhibit an optimizing maximum relative growth rates under nitrogen (Agren and Franklin, 2003). This means that when the nutrient availability increases, plant allocates less biomass to their roots. This result is in agreement with Thornely (1972) model, which suggests that a decrease in C substrate acquisition would result in an increase in S: R ratio while a decrease in N-substrate acquisition would cause S: R ratio to decrease.

6.4. Allelopathic effects of *E. globulus* ssp *globulus* on the germination of *H. abyssinica* seeds

Eucalyptus leaf leachates (leaf extract) had the strongest allelopathic effect on seed germination. Tefera (2002) also found that the inhibitory allelopathic impact of leaf extract was more powerful than of other vegetative parts. Leaf extracts, decaying leaves, and soil collected under *Eucalyptus* canopies inhibited germination and early growth of associated plant species (Rice, 1979). The chemical constituents of *Eucalyptus* foliage have been the subject of many studies. All species of *Eucalyptus* contain terpenoids and phenolic compounds in their foliage. The presence of allelochemicals in the *Eucalyptus* plant has also been confirmed from the studies conducted on *E. camaldulensis* (Del Moral and Muller, 1970) and *E. citrodora* (Nishimura *et al*., 1984). Allelopathy can be separated from other mechanism of plant interference in that the detrimental effect is exerted through the release of chemicals (Putnam and Duke, 1978). Generally these chemical inhibitors belong to a group of materials known as secondary plant compounds. Discharge of these secondary substances into the environment occurs by leaching of water soluble toxins from above-ground plant parts in response to the action of rain, fog or dew (Tukey, 1966 cited in Babu and Kandasamy, 1997).

The study demonstrated that leaf aqueous extracts of *E. globulus* ssp *globulus* exhibit significant inhibitory effects on seed germination of *H. abyssinica*. Among the treatments, 40, 80 and 100% aqueous leaf extracts had the strongest inhibitory effect on germination. The 80 and 100% leaf aqueous extract of *E. globulus* ssp *globulus* resulted in complete failure of seed germination in *H. abyssinica.* This could occur only when some allelochemicals present in the leaf extract prevent growth of embryo, or caused the death.

Generally, the possible mechanisms suggested for the reduction in seedling growth are the inhibiting effect on the organelle membrane which produces adenosine triphosphate (ATP), resulting in lower ATP production due to the displacement of the mitochondria

and photosynthetic electron flow (Morohashi and Suguimoto, 1988 cited in Hegab *et al*., 2008). The inhibitory effect of *E. globulus* ssp *globulus* on seed germination is due to the presence of growth inhibitors (allelochemicals) in the extracts. The study indicated that aqueous extracts of fresh leaves of *E. globulus* ssp *globulus* has allelochemicals with significant effect on seed germination of *H. abyssinica.*

7. CONCLUSIONS AND RECOMMENDATIONS

H. abyssinica propagates effectively by means of seeds, which is practically and economically advantageous. The present study reports the germination responses of *H. abyssinica*, which had not previously been clearly elucidated. This study showed significantly ($P \leq 0.05$) higher germination percentage of seeds treated in plant-derived aqueous smoke extracts (10, 20, 30, 40 and 50%), GA_3 (10^{-4}, 10^{-5} and 10^{-6} M) and KNO_3 (10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M). But seeds treated with 40 % aqueous smoke extract, 10^{-4} M GA_3 and 10^{-5} M KNO_3 showed significantly ($P \leq 0.05$) higher germination percentage compared to the control. It appears that the active compound(s) in smoke act as a major overriding cue for germination in *H. abyssinica*. Generally, the germination level in all treatments including the control showed an increased germination rate compared to the results of some literatures which suggested that the germination rate for *H. abyssinica* was 40-60% (Hedberg, 1989; and Azene Bekelle *et al.*, 1993, 2007). Transplanted germinants into the potting media prepared from the mixes of red soil, decomposed cow dung and sand in the ratios 4:3:2, 4:3:1, 4:1:3, 2:1:1 and 1:1:1 have significantly ($P \leq 0.05$) improved the growth in height of the seedlings compared to the control.

Provided that growing conditions are suitable, seedlings of planting size can be obtained within 5 months in pots containing mixes of red soil, decomposed cow dung and sand in ratios 4:3:2 and 2:1:1. This rapid growth of seedlings under nursery conditions is economical as it would avoid keeping seedlings on the nursery for extended period of time. Based on the above conclusions, the following recommendations are proposed:

1. *H. abyssinica* could be one of the most important indigenous trees of Ethiopia for productivity, accumulation and decomposition of leaf litter. The tree has a lot of biological attributes including its ability in fertile soil formation, soil conservation as well as rainwater conservation.

2. Application of decomposed cow dung to the soil mix enhances seedling growth of *H. abyssinica.*
3. From the present study, the germination response of seeds of *H. abyssinica* to aqueous smoke extracts and KNO_3 pre-treatment was very high. Aqueous smoke extract and KNO_3 pre-treatment can be used as an important germination cue. Seed pre-treatment that involved plant-derived smoke extracts resulted in significant increase in the final germination percentage of *H. abyssinica*. Smoke might therefore have the same effect on other responding species. However, smoke enhanced seed germination of *H. abyssinica* should be the subject of future study as little is known regarding the physiological mechanism of this response (Baxter and van Staden, 1994; Keeley and Pizzorno, 1986; Light *et al.,* 2002).

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9. APPENDICES

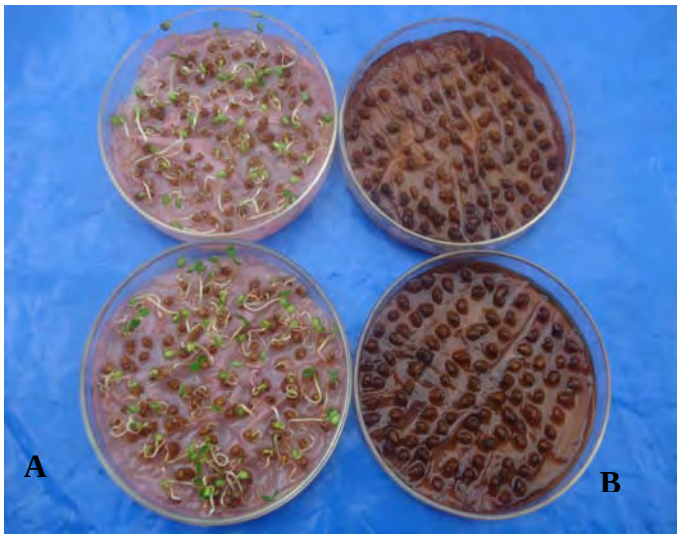
I II

III IV

Appendix 1. The relative positions of the male and female (I, II, III, and IV) trees of *H. abyssinica* from which the inflorescences were collected. The trees were ca 20-40 m apart.

Appendix 2. Flowers at the bud stage and open florets of *H. abyssinica.* Male (A) and female (B) flower buds; male (x 6.3) (C) and female(x 6.3) (D) florets.

Appendix 3. Major observable developmental stages during the germination of *H. abyssinica* seeds. Seeds of matured fruits after their fruit walls removed (A); Radicle emergence (x 8) (B); After the germinant has freed itself from the floral and the fruit parts (C); Fully developed cotyledons and appearance of the first true compound leaf (D).



Appendix 4. Allelopathic effect of *E. globulus* ssp *globulus* on the germination of *H. abyssinica* seeds. Germinating seeds in Petri dish treated with DDW (A) and a complete failure of seed germination under 80 and 100 % leaf aqueous extracts of *E. globulus* ssp *globulus* (B).

Appendix 5. Number of seeds of *H. abyssinica* germinated in each Petri dish using different treatments. (P = Petri dish)

Stimulator	Concentration (M)	P_1	P_2	P_3	P_4	P_5	%
DDW(Control)	DDW	40	28	40	39	33	72.0
Smoke	10%	41	41	38	43	41	81.6
	20%	44	41	43	43	35	82.4
	30%	41	42	39	43	41	82.4
	40%	48	42	41	43	41	86.0
	50%	36	39	39	40	40	77.6
GA_3	10^{-3}M	37	34	34	29	31	66.0
	10^{-4}M	39	43	43	37	37	79.6
	10^{-5}M	41	44	43	31	38	78.8
	10^{-6}M	39	39	36	37	39	76.0
	10^{-7}M	32	34	35	36	33	68.0
KNO_3	10^{-3}M	46	34	37	27	27	68.4
	10^{-4}M	38	38	39	40	38	77.2
	10^{-5}M	41	44	47	43	49	89.6
	10^{-6}M	44	47	48	43	38	88.0
	10^{-7}M	43	37	35	42	45	80.8

Appendix 6. Height of seedlings of *H. abyssinica* grown in different soil mixes of red soil, decomposed cow dung and sand in various ratios (4:3:2, 4:3:1, 4:1:3, 2:1:1, 1:1:1 and control). H = Height

Soil : Dung: Sand (4:3:2)

H_1	H_2	H_3	H_4	H_5
5.0	4.5	4.0	5.0	6.1
3.9	2.5	5.0	5.0	4.2
3.2	3.5	3.0	4.8	4.5
4.0	2.5	4.0	5.0	3.0
3.5	2.0	3.2	5.0	4.5
3.0	3.0	4.6	2.6	1.8
6.3	9.2	7.2	4.3	9.2
7.3	9.0	7.2	10.0	8.5
6.3	8.5	9.0	11.0	12.0
3.0	5.0	4.0	7.2	8.0
5.0	6.0	4.3	3.0	5.2
10.0	8.3	10.0	5.0	4.0
13.0	14.0	12.5	14.8	13.0
14.5	9.5	14.4	14.5	13.0
15.0	15.0	14.0	14.0	10.0
6.8	9.0	8.2	8.0	7.0
8.0	9.5	8.5	8.8	7.6
8.0	8.5	6.5	9.0	10.0
22.3	22.3	18.5	22.2	21.8
22.0	20.5	19.5	23.0	18.0
9.0	10.0	9.0	7.0	10.0
20.0	15.0	18.5	19.0	16.5
9.8	10.5	8.0	11.0	13.0
13.0	11.5	14.0	13.0	13.0
26.2	25.7	25.4	30.5	26.9
25.7	26.0	24.7	26.7	26.8
11.2	9.2	11.7	11.7	12.2
20.0	17.0	19.2	21.2	18.7
12.2	11.7	13.2	14.2	11.7
19.2	20.7	19.7	14.7	18.7

Soil : Dung: Sand (4:3:1)

H_1	H_2	H_3	H_4	H_5
4.0	2.5	5.2	5.0	4.0
5.0	4.5	4.5	5.4	4.0
5.0	6.0	6.2	4.8	4.5
6.0	5.0	6.2	5.8	5.2
1.0	1.0	1.5	1.5	1.0
2.0	7.0	6.0	4.8	3.8
6.2	7.1	9.0	5.0	2.4
7.0	5.0	7.3	7.5	7.0
7.0	12.3	10.5	11.0	12.2
7.0	12.0	10.0	12.2	12.0
4.8	4.5	4.0	5.2	9.0
7.0	7.0	7.8	16.0	16.2
10.5	10.4	11.0	13.0	8.5
10.0	12.0	10.0	11.0	10.5
8.8	15.0	15.5	14.0	15.0
12.5	16.0	15.0	16.5	13.0
6.5	11.0	11.0	10.0	4.5
14.0	15.5	15.5	12.0	9.0
12.0	13.5	20.0	17.0	13.0
13.0	12.0	16.0	19.0	18.5
16.0	11.0	16.0	20.0	17.0
19.0	20.5	21.5	21.0	19.2
19.0	11.0	19.5	21.0	20.0
14.5	19.0	16.5	11.0	10.0
24.2	22.7	26.7	17.7	17.0
18.2	20.7	26.0	26.7	22.7
16.7	20.7	18.7	17.4	16.2
23.2	21.2	22.0	21.2	21.7
20.2	22.2	18.4	21.7	21.2
24.7	17.2	21.7	14.2	14.1

Soil :Dung :Sand (4:1:3)

H_1	H_2	H_3	H_4	H_5
5.2	4.2	4.0	5.0	4.8
5.0	4.8	3.5	5.0	5.5
3.0	3.1	2.8	6.0	2.6
4.0	4.8	4.0	3.8	3.5
3.8	5.5	5.8	7.0	5.5
1.0	2.0	1.0	1.0	1.5
8.0	7.0	9.3	8.0	6.0
7.0	7.4	9.3	10.7	4.3
5.0	6.0	5.0	8.4	10.5
4.0	5.0	4.3	6.0	5.4
6.0	10.2	11.0	12.4	11.0
13.0	10.4	14.0	11.0	11.5
14.0	14.5	13.0	13.5	12.0
10.5	9.0	11.0	9.2	10.0
8.8	8.0	7.5	7.8	6.0
12.5	17.0	19.0	17.5	15.0
7.0	7.2	8.0	5.0	6.0
19.0	16.0	18.0	19.0	20.0
19.2	24.0	19.5	21.5	18.5
9.5	9.4	16.0	16.0	8.0
12.0	13.5	13.0	17.0	9.5
6.0	8.0	7.0	10.0	8.5
21.2	24.0	24.8	23.5	25.0
23.2	23.6	24.7	21.7	22.9
23.0	24.2	20.7	24.2	22.0
16.2	10.2	10.2	16.7	16.0
15.2	14.7	18.2	17.0	20.7
11.2	11.7	10.2	6.7	7.2
29.2	30.9	27.2	27.9	28.7

Soil: Dung: Sand (2:1 :1)

H_1	H_2	H_3	H_4	H_5
5.0	4.5	5.1	6.0	7.0
6.0	5.4	5.5	6.0	6.5
5.4	6.8	4.5	7.0	2.0
4.0	4.5	3.5	6.2	7.0
3.0	4.5	3.0	5.8	3.9
3.0	2.0	5.0	5.4	2.8
7.2	10.0	10.2	11.3	6.3
11.0	10.4	5.3	10.0	13.0
6.0	16.0	11.0	12.6	4.0
8.0	10.0	11.0	9.0	11.3
9.0	8.3	10.0	11.0	9.4
16.0	15.8	14.0	13.5	12.5
17.0	16.0	14.0	15.5	17.0
16.0	18.0	13.5	15.0	18.0
13.0	9.0	11.0	17.0	12.5
11.0	12.0	11.0	9.0	12.0
9.0	9.0	7.0	6.5	9.8
24.5	21.5	21.5	29.5	23.0
23.5	22.0	20.5	19.5	22.4
18.0	22.0	16.0	20.0	19.0
13.0	22.5	19.5	23.5	21.0
9.5	8.5	9.8	11.0	14.0
17.0	14.0	18.5	18.0	16.0
27.7	25.8	27.7	26.7	26.7
24.8	30.6	25.2	26.2	28.7
20.7	22.7	17.2	17.2	17.7
13.8	23.2	26.2	22.7	25.2
14.7	10.7	10.2	15.2	14.7
18.2	16.7	20.7	20.2	20.7

Soil: Dung: Sand (1:1:1)

H_1	H_2	H_3	H_4	H_5
5.0	5.1	3.8	3.2	4.3
4.0	4.5	6.0	5.5	5.8
2.5	3.0	1.5	1.2	2.2
4.0	2.5	4.8	5.0	6.0
6.0	4.0	3.0	1.8	4.0
4.5	2.5	6.0	3.0	4.2
7.1	6.0	5.5	4.2	7.2
7.5	8.0	5.5	5.0	8.0
5.0	2.2	3.0	4.0	3.3
6.0	7.0	7.4	9.0	8.5
5.4	4.2	8.5	10.3	5.0
8.0	8.0	10.2	11.0	9.5
10.0	10.8	8.0	7.5	12.0
9.0	10.2	9.0	13.0	10.0
9.5	8.0	4.5	6.0	6.8
7.8	9.0	8.5	11.0	10.8
7.0	7.5	12.0	9.0	11.0
9.2	9.5	12.0	12.2	13.5
17.8	15.0	13.0	21.0	21.5
18.0	11.0	16.5	15.8	17.8
14.0	12.0	13.0	13.0	11.0
9.0	10.0	12.0	10.5	6.0
15.0	18.0	17.0	19.0	20.0
7.5	16.0	12.0	13.0	18.0
22.2	22.7	25.5	20.0	23.7
19.2	20.6	22.7	24.2	17.7
14.7	17.7	14.2	15.2	15.7
12.7	11.7	15.4	8.0	12.9
16.2	22.0	17.0	18.7	19.2
12.0	15.2	24.2	19.3	23.7

Soil only (Control)

H_1	H_2	H_3	H_4	H_5
5.0	3.5	4.4	4.5	4.0
3.2	1.2	2.2	2.5	4.8
3.5	4.8	3.0	5.0	3.0
3.5	4.5	6.0	3.2	4.2
4.0	2.8	4.2	3.5	4.0
1.8	1.2	3.5	1.5	4.5
7.0	8.0	5.0	8.5	6.0
6.0	4.4	4.0	9.4	9.0
5.0	5.5	6.0	6.0	4.0
10.0	12.0	9.0	10.0	10.4
6.5	5.0	7.0	6.0	3.0
4.3	10.4	6.0	9.4	3.0
8.0	6.5	9.0	9.5	9.8
7.2	9.0	13.0	11.0	8.8
10.0	6.2	7.0	8.0	9.0
13.0	14.5	12.0	14.8	10.0
8.0	6.6	7.0	8.0	6.8
7.8	8.9	10.0	9.0	8.0
14.0	10.5	14.5	17.5	13.5
13.0	10.0	16.0	16.5	16.0
15.0	15.5	16.0	21.0	19.0
9.0	10.0	13.0	10.0	9.5
12.0	12.0	9.8	11.0	8.0
8.5	12.0	11.5	15.0	12.0
19.9	18.3	17.2	15.1	16.2
19.2	19.2	20.2	20.1	13.7
17.0	16.2	17.7	21.2	16.2
13.2	15.2	15.2	13.2	17.2
13.4	13.2	11.0	12.7	9.0
11.2	18.7	21.7	18.7	16.2

Appendix 7. Mean germination percentage and Standard Error (SE) for seedlings of *H. abyssinica* in different stimulants (plant derived smoke extracts, GA_3 and KNO_3).

Treatment	Mean germination % (± SE)
Control	72.0 ± 4.8
10%SMK	76.8 ± 2.9
20%SMK	81.6 ± 1.6
30%SMK	82.4 ± 3.2
40%SMK	86.0 ± 2.6
50%SMK	77.6 ± 1.5
10-3GA3	66.0 ± 2.8
10-4GA3	79.6 ± 2.7
10-5GA3	78.2 ± 4.5
10-6GA3	76.0 ± 1.3
10-7GA3	68.0 ± 1.4
10-3KNO3	68.4 ± 7.1
10-4KNO3	77.2 ± 0.8
10-5KNO3	89.6 ± 2.9
10-6KNO3	88.0 ± 3.5
10-7KNO3	80.4 ± 3.7

Appendix 8. Mean monthly rainfall (A), minimum temperatures (B) and maximum temperatures (C) recorded from 1997-2006 at the Geological Survey of Addis Ababa University.

Source: National Meteorological Service Agency: Data from Geological Survey of Addis Ababa University (1997-2006).

A

	Monthly mean rainfall (mm)												
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Average
1997	39.2	0	25	51.3	38.5	104	273	194	114	62	50	1.5	79.37
1998	55.2	21.0	49	48.5	154	124	285	260	214	117	0	0	110.64
1999	2.9	0.3	29	16.3	23.8	120	269	305	88	55	0	0	75.78
2000	0	0	2.4	49.9	110	145	245	306	251	46	21	0	97.99
2001	0	12	211	25	168	214	428	246	132	15	0	0	120.85
2002	14.7	21	90	56.5	63.1	173	255	216	109	0.2	0	17	84.54
2003	10.0	34	63	99.3	20.2	152	292	233	214	0.8	2	55	97.86
2004	24.8	20	50	140	30.1	142	249	269	164	77	0	0	97.04
2005	45.9	52	83	161	150	180	246	315	183	29	4	0	120.71
2006	0.7	11	124	71.9	74.6	145	356	244	239	54	4	0	110.43
Average	19.3	17	73	72	83.3	150	290	259	171	46	8	7.3	99.52

B

Year	Monthly mean minimum temperatures (T^{o} C)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Average
1997	10.2	7.9	11.7	12	12	12	11	11	11	11	10	8.1	10.62
1998	10.6	12	12.7	13	13	12	12	12	11	11	7.7	5.8	11.05
1999	8.3	9.6	11.4	12	12	11	11	11	11	9.4	7.2	7.2	10.03
2000	7.4	8.1	10.2	12	12	11	11	11	11	12	12	8.6	10.55
2001	8.4	9.1	11.6	12	12	11	11	12	11	10	7.8	8.3	10.24
2002	8.6	10	11.8	12	13	11	11	13	15	13	8.6	10	11.48
2003	9.7	11	11.3	12	13	12	12	12	11	9.3	8.4	7.7	10.8
2004	10.3	9.3	10.3	12	12	11	11	15	11	8.9	7.8	8.1	10.6
2005	8.3	9.2	11.8	13	13	15	11	11	11	9.4	8.1	6.3	10.6
2006	8.6	11	11.5	12	12	11	11	11	10	10	8.4	8.6	10.4
Average	9.04	9.7	11.4	12	12	12	11	12	11	10	8.6	7.9	10.64

C

Year	Monthly mean maximum temperatures (T^{o} C)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Average
1997	24.2	26	26.4	24	27	25	22	21	24	23	23	24	24.03
1998	23.7	26	25.4	27	25	25	21	21	22	22	23	23	23.72
1999	24.4	27	25.2	28	26	25	20	22	23	22	23	23	23.91
2000	25	26	27.3	26	25	23	22	20	22	22	23	24	23.72
2001	24.4	26	23.7	25	25	23	22	21	23	24	24	24	23.65
2002	24	26	25	26	26	24	23	21	22	24	24	24	24.13
2003	24.4	26	25.3	25	27	24	21	21	22	24	23	23	23.71
2004	24.5	25	25.7	24	26	23	21	21	22	23	24	24	23.47
2005	23.8	27	24.9	26	24	24	21	21	22	23	23	24	23.64
2006	24.7	26	25.4	24	25	24	22	21	22	24	24	24	23.7
Average	24.3	26	25.4	25	26	24	21	21	22	23	23	24	23.77

Declaration

I, the under signed, declare that this thesis is my own original work, has not been presented for a degree in any university and that all sources of materials used for the thesis have been duly acknowledged.

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Date: January, 2009

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