ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

PLANT BIOLOGY AND BIODIVERSITY MANAGEMENT PROGRAM UNIT

STUDIES ON SEED GERMINATION PHYSIOLOGY, GERMINANT ESTABLISHMENT AND SEEDLING GROWTH PERFORMANCE OF *Ficus sur* Forssk. (MORACEAE)

By

Solomon Getahun

A Thesis Submitted to School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Master of Science in plant biology and biodiversity program unit.

July 2011
Addis Ababa

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Approved by examining board: - Signatures

1. Prof. Legesse Negash (Advisor) _____

2._____ _____

3._____ _____

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Acronyms

EFAP – Ethiopia Forest action program

ISTA – International seed testing agency

GA_3 – Gibberellic acid

KNO_3 – Potassium nitrate

ABA- Abscisic acid

NO – Nitrous oxide

HSD – Highest significance difference

±SE – Plus or minus standard error

ddH_2O – double distilled water

RH – Relative humidity

LAI – Leaf area index

i.e- that is

ca.- Calculated

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ABSTRACT

Ficus sur *is a fig tree that belongs to the genus* Ficus *family Moraceae and order Rosales. It grows at altitudinal ranges 1400-2500 m.a.s.l. Seed production, germination physiology, germinant establishment and seedling growth performance studies were conducted in Addis Ababa University, College of Life Sciences. Seed pretreatments were achieved using 100 µM, 10 µM, 1µM and 0.1µM concentrations of gibberellic acid (GA_3) and potassium nitrate (KNO_3) separately. Plant-derived aqueous smoke extract solutions at relative concentrations of 25%, 50%, 75% and 100% were also used to pretreat seeds and stimulate germination. The control was to treat seeds with double distilled water. Petri dishes were randomly arranged under a light source from one Phillips fluorescent tube set at a height of 50 cm and producing light at a rate of ca. 40 µmol m^{-2} s^{-1}. Temperature and relative humidity of the room were 26.55 ± 2.65^0 C and 44.15 ± 6.25%, respectively. Stereomicroscope examination of seeds was indicated 57.3% of the seeds were damaged and the balance 42.7 % was health. Significant ($P<0.05$) mean germination percentages were achieved from seeds pretreated with 10 µM GA_3, 0.1 µM KNO_3 and 75% aqueous smoke extract solutions. Faster germination initiation, maximum mean germination rate and minimum mean germination time were obtained at 0.1 µM GA3 and 75 % smoke solutions. Germination of seeds sown in pots, germinant establishment and growth performance of seedling were also studied in the glasshouse, where average temperature and relative humidity were 23.55 ± 8^0 C and 72 ± 17 %, respectively. Growth media containing soil mixtures of 4:3:1, 4:3:2, 4:1:3, 2:1:1 and 1:1:1 of red soil, compost and sand, respectively, were used. The growth of seedlings, leaf production and leaf area were significantly ($P<0.003$) better on the 4:3:1soil mixture compared to the control i.e growth medium containing 1:1:1 ratio of red soil, compost, and sand mixture. From these results, it is possible to conclude that GA_3, KNO_3 and plant-derived aqueous smoke extract are suitable pretreatments for attaining maximum germination indexes compared to the control. The results indicate that the species is easy to propagate by seed, seedlings grow quite fast and reach planting size ≈ 50 cm within 5 months.*

Keywords/phrases: *Aqueous smoke*, *Germination, Gibberellic acid, Potassium nitrate, Seedling,* soil mixtures *and Treatment.*

1. Introduction

1.1. Scope of the problem

The disappearance of many plant species due to human activities is depleting the world‟s genetic resources and is putting man‟s heritage of biodiversity under serious threat. Today, most areas across eastern Africa certainly need more trees because people have cut down too many trees without putting effective replanting programs in place (Moir *et al*., 2007).

Conserving biological diversity for tree species is now becoming a priority (Mog‟omba, 2007). Therefore, there is urgent need to preserve genetic diversity including plant resources of known and unknown economical importance (Soladoye *et al.,* 2005). In Ethiopia, it is suggested that conserving, propagating and developing indigenous trees are extremely important (Legesse Negash, 1995; 2010). However, techniques which involve collecting, processing, storing, pretreatment of seeds and risks related to adoption of wrong nursery programme particularly use of unsuitable propagation techniques, are present even in developed countries (Legesse Negash, 1995, 2010; Piotto, 2003; and Piotto and Di Noi, 2003).

In addition to the usual challenges presented by tropical tree seeds which include viability, pretreatment and storage techniques, fig tree reproduction is further complicated by physiological processes, including reduced water potential inside the syconium. If the seeds remain within the syconium for an extended period of time, the microenvironment is extremely hostile to germination, owing to the high concentration of sugars that involve a high osmotic pressure (Piotto and Di Noi, 2003). Clearly, propagation of many indigenous trees species from seeds had been difficult due to lack of precise knowledge on their seed biology and germination physiology (Legesse Negash, 1995; 2010).

Studies on seed germination behavior are integral parts of *ex-situ* conservation that would ensure the restoration of sufficient populations for natural regeneration to happen. However, chemicals that accumulate in the fruit and seed coat during development and remain in the seed after harvest such as various phenols and abscisic acid can act as germination inhibitors (Cirak, 2007).

According to Bewley (1997), there is much more to be learned about the key processes involved in germination, because seeds from different plants have different responses to various environmental and morphological factors of germination. Even though studies on propagation physiology of some indigenous trees of Ethiopia such as *Hagenia abyssinica* (Bruce) J. F. Gmel., *Podocarpus falcatus* (Thunb.) Mirb. *Millettia ferruginea* (Hochst.) Bak., *Acacia abyssinica* Hoch st. ex. Benth., *Olea europaea* L. subsp. *cuspidata* (Wall. ex DC.) Ciffieri, *Cordia africana* Lam *Ficus vasta* Forssk. and others (Legesse Negash, 1995; 2010), *Croton macrostachyus* Hochst. *ex* Del. (Legesse Negash, 2010; Kibebew Wakjira, 2007) were conducted, there was no relevant information on the seed germination physiology, germinant establishment response and seedling growth condition on *F. sur*.

1.2. Problems related to Ficus sur

Ficus sur is characteristic woody species of Montane Forests of Ethiopia (Burger, 1974). *F. sur* grows at altitudinal ranges of 1400-2500 m.a.s.l. and is also widely spread in tropical Africa (Friis, 1989). Fig trees such as *F. sur* are characterized by their mutualistic relationship with typically species-specific pollinator wasps (Nason *et al*., 1996; Machado *et al*., 2001). Consequently, the unusual pollination system and often low population densities would seem to present a number of obstacles to successful pollen transfer and reproduction (Nason *et al*., 1996).

Fig wasps are small pollinators which are less than 2 mm in length and are apparently quite ephemeral as adults, living only for1-3 days under natural conditions (Compton, 1993). Pollinating wasps are generally assumed to be unable to escape once they have entered a fig (Compton *et al*., 1996); they die inside the fruit after pollinating and laying eggs (West, and Herre, 1994). Based on these characteristics, it is not expected fig wasps to be effective at flying long distances in search of receptive female flowering phases of host trees (Nason *et al*., 1996).

In addition to the problems associated with pollinating wasps, diverse communities of non-pollinating or parasitic wasps do also exploit figs (Compton and Hawkins, 1992). For example, in *F. sur* (in addition to pollinating wasps) there are parasitoids such as *Apocrypta guineensis* Grandi, *Sycophaga afflicta* Grandi and *S. cyclostgma* Waterston (Berg and Wiebes, 1992). Compton and Robertson (1988) also reported that Torymidae may also enter the figs; these wasps are strictly seed predators, with adults that do not pollinate and with larvae that develop inside galled ovules. Larvae of the closely related sycophagine wasps also gall the ovules of *F. sur,* but they develop from eggs inserted into ovules from the outside of the fig. These studies were then conducted on *F. sur* with a view to assess morphological integrity of seed, the seed management and seed germination physiology in the laboratory, as well as seed germination experiments and germinant establishment in the glasshouse.

1.3. Objectives

1.3.1. General objective

The general objective of this thesis was to study the morphological integrity of seed in relation to wasps, seed germination physiology, germinant establishment and seedling growth performance of *F. sur* under different treatment conditions.

1.3.2. Specific objectives

The specific objectives of this thesis were to:-

- determine the morphological conditions of *F. sur* seeds;
- determine germination responses of *F. sur* seeds under laboratory and glasshouse conditions using various pretreatments;
- develop effective pretreatments that stimulate seed germination processes; and,
- determine suitable growth media for effective seedling establishment and growth of *F. sur* using different soil mixtures.

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2. Literature Review

2.1. *Ficus sur* Forssk.

2.1.1. Taxonomy and morphological features

Ficus sur is a fig tree that belongs to the genus *Ficus,* family Moraceae and the order *Rosales*, (Barke, 2004; Friis, 1989). There are about 53 genera and over 1,400 species of Moraceae (Legesse Negash, 2010).The synonym for *F. sur* is *Ficus capensis* Thumb. (Igoli *et al.,* 2005) commonly known by broom cluster fig (English) (Hankey, 2003) and Shola in Amharic (Friis, 1989) which is an indigenous tree of Ethiopia (Legesse Negash, 1995; 2010).

Ficus sur is a large, fast growing tree, reaching to 30 m in height, with large and oval green leaves borne on a massive spreading crown (Hankey, 2003). Like other Moraceae, *F. sur* is characterized by milky latex in all parenchymatous tissue. It has also unisexual flowers, and aggregated drupes (Datwyler and Weiblen, 2004).

Ficus sur is a tree with large buttresses and the figs always develop on leafless panicles on the main stem or older branches (Friis, 1989; Legesse Negash, 2010). Although, they can exhibit very different growth forms in different environment, all *Ficus* species are recognized by their inflorescences known as fig (syconium). The fig is a closed receptacle with an opening at the apex. Depending on the species, figs can range in diameter from just a few millimeters up to 20 cm or more (Compton *et al*., 1996). Its inside is lined with numerous uni-ovulate female flowers and male flowers (Jousselin *et al*., 2004). It consists of hundreds or thousands of tiny flowers distributed inside a hollow sphere. These flowers within a monoecious fig, like *F. sur*, vary continuously from longer styled flowers to that of short style. The ovules are close to the fig wall in shorter styled flowers that have long pedicels with ovules close to the fig cavity (Machado *et al*., 2001).

2.1.2. Pollination and seed development

Pollination is one of the most interesting symbiotic interactions between fig wasps and figs of the genus *Ficus* (Otero and Ackerman, 2002). In most plants, pollination is a byproduct of insect visitation. The pollinator interaction occurs via morphological adaptations of flowers that favors pollen deposition on and removal from the pollinator body (Jousselin *et al*., 2003).

It is widely accepted that natural selection acts on flowering plants to improve pollination performance. Many flower traits are thought to have evolved to promote pollinator contact with floral organs during their visit and increase the quantity of pollen deposited (Jousselin *et al*., 2003). Fig species are generally pollinated by species specific pollinator wasps (Noort *et al.,* 1996; Nason *et al*., 1996; Machado *et al*., 2001). However, a variety of insects visits the figs for its copious juice and flesh (Legesse Negash, 2010). The wasps are entirely dependant on fig trees, because their larvae are only found inside the figs, where they feed on some of the female flowers that have been pollinated (Compton *et al*., 1996). The mutualism is obligatory for the plants and their pollinator wasps. Some times, it is thereby centered on an evolutionary conflict; where pollinator females only gaining from the production of wasp progeny while the trees gain by producing both wasps (which carry the trees' pollen to other trees) and seeds (Compton *et al*., 1996). Therefore, there are traits that affect their relationship. For example, *Ficus* species have figs, which enclose male and female flowers, and only connect to the exterior with its ostiole. The ostiole is located at the apex of the fig and protected by a cluster of scales (Otero and Ackerman, 2002). On the other hand, female fig wasps are a highly modified group of insects whose anatomy reflects the adaptations needed to penetrate the ostiole (Noort *et al.*, 1996).

Ficus sur is pollinated by fig wasp species of *ceratosolem* (Compton and Robertson, 1988). For example, both *Ceratosolem silvestrianus* Grandi and *C. flabellatus* Grandi are active pollinator of *F. sur and* co-occurs in Ivory cost, West Africa (Kerdelhue *et al.,* 1997). On the other hand, *Ceratosolem capensis* Grandi pollinates *F. sur* in South Africa and in East Africa (Berg and Wiebes, 1992; Wiebes, 1994; Compton and Robertson, 1988). Pollinating wasps probe their ovipositor down the style to reach the ovule of individual flowers to lay their eggs. Ovules that have been fertilized and did not receive a wasp egg can produce a seed (Machado *et al*., 2001).

Although several hypotheses have been proposed to explain egg deposition patterns of pollinating fig wasps in monoecious figs and why there are always some flowers left for seed production. Some explain that because of the difference in the style length of the female flower; where the seeds are produced in the longer style flower in which the ovipositor of the wasps do not rich (Legesse Negash, 2010; Machado *et al*., 2001).

2.1.3. Ecological and geographical distribution

The genus *Ficus* has more than 750 species of fig trees which are distinguished worldwide (Barke, 2004; Weiblen and Bush, 2002). Most species occur in tropical regions or Afro-tropical fig species (Kerdelhue *et al.,* 1997); but the family is also represented in the subtropics and warm temperate regions (Legesse Negash, 2010). The fig species that show a pan tropical distribution and variety of growth habits widely distributed from North Africa to Western Cape in South Africa (Compton *et al.* 1996; Hankey, 2003), Namibia and East South Africa to Senegal and Ethiopia (Berg and Wiebes, 1992).

Ficus sur is restricted to frost free areas with moderate rainfall (Hankey, 2003). Although, *F.sur* is usually found on riverbanks or in riverine forest; it can also be found in drier savanna woodlands, savanna secondary forests of Africa alongside gallery forests, in sub montane forests, and moist forests. *F. sur* commonly forms part of the hedge in lower slopes where conditions for propagation are better (Gautier, 1996).

2.1.4. Ecological and Economical importance

Indigenous forests are critical in providing clean water, fresh air, fertile soil, food, fiber, fuel, and drugs. Sustainable productivity of ecosystems depends to a large extent on the buffering capacity provided by having rich and healthy indigenous forests (Legesse Negash, 1995). Fig trees are often regarded as a 'keystone' group in tropical forests. This is because of their all-year-round production of figs, often in large quantities, which can potentially maintain vertebrate frugivore populations at times when other fruits are in short supply (Legesse Negash, 2010).

Trees make the soil fertile; since the amount and composition of leaf litter produced largely determines the amount of nutrients to be recycled and the resulting nutrient availability and they also help to prevent desertification (Moir *et al.,* 2007). It can be also regarded as a gigantic photocell which is capable of trapping electromagnetic radiation that comes from the sun (Legesse Negash, 1995).

Ficus sur is one of the gap filling woody species (Taye Jara, 2006). The remaining trees attract animals, mainly birds and bats, because they provide facilities (protection, rest, food) for animals crossing a clearing while moving from one forest stand to another (Guevara *et al.,* 1986). Fig trees may thereby indirectly favor the dispersal of other animal dispersed plant species (Compton *et al*., 1996). The birds of forest ecosystems tend to avoid open spaces and if crossing them, they use the trees as perching sites. The birds and bats attracted are frugivorous. They deposit seeds defecation in their stay. The standing tree may serve as important nuclei of species that establish in an open area by enforcing the secondary succession (Compton *et al.,* 1996).

Information on seed ecology of trees is essential in order to understand community processes like natural regeneration and succession (Demel Teketay and Granstrom, 1997). In a study conducted in Menagesha Suba State Forest of Ethiopia *F. sur* was one of the woody species which naturally regenerated above ground and it was represented in the soil seed bank (Feyera Senbeta and Demel Teketay, 2001). According to Demel Teketay (2005), *F. sur* is one of the plants which succession proceeds if human impact and grazing can be prevented.

In Ethiopia, people use medicinal plants and plant remedies selected over centuries (Tesfaye *et al.,* 2009) and *Ficus* spp. is seldom used in traditional medicine (Gautier, 1996; Faleyimu, 2008). For example, Tesfaye *et al*. (2009) reported that *F. sur* leaves are used as medicinal plant in Konta Special Woreda, Ethiopia. The species is also used as medicine in other African countries such as Nigeria (Faleyimu and Oluwalana, 2008), Ghana (Addo-Fordjour *et al*., 2008) and in Kenya (Muregi *et al*., 2006).

Rural people of Ethiopia are endowed with a deep knowledge concerning the use of wild plants; for example, wild food consumption is still very common in rural areas of Ethiopia, particularly among children. Among the most common wild plant fruits consumed by children are fruits from *Ficus* spp (Guinand and Dechassa Lemessa, 2000). Ripe figs are also collected and eaten by

humans, especially children (Legesse Negash, 2010). The same writer explained that *F. sur* figs are also much liked by birds, bats, as well as domestic and wild animals, including goats, sheep, cattle, monkeys, apes, donkeys.

2. 2. Seeds

Seeds have complex structure that consists of three major components, each with a distinct genotype. The embryo that will become the vegetative plant is diploid, possessing one paternal and one maternal genome equivalent. The endosperm or cotyledon, a structure that provides nourishment for the developing embryo and/or seedling, is triploid with two maternal and one paternal genome equivalents for flowering plants. The testa surrounding the embryo and endosperm or cotyledon is strictly of maternal origin (Ohto *et al.,* 2007).

Seeds are still important starting materials for propagation of many vital tree species (Mog'omba, 2007). New plant generation starts with a seed, which usually contains a fully developed embryo that can survive the period between seed maturation and germination (Bentsink *et al.,* 2007). Propagation from seeds ensures genetic diversity that is maintained by allowing genetic recombination to occur through sexual reproduction. The genetic diversity makes possible the survival and the natural evolution of species in continually changing environmental conditions. The rearrangement of genes leads to the production of individuals that are different from their parents (Piotto and Di Noi, 2003).

2.3. Seed germination

Although germination has surprisingly large number of meanings; in general, the term is applied to seeds, spores, and pollen to indicate when these quiescent structures reinitiate growth (ISTA, 1999). Seed germination is a critical event in the plant life cycle, because, timing of emergence from the protective seed coat is crucial for survival and reproductive success (Nelson *et al*., 2009). Physiologists often consider seed germination to be the period from imbibitions up to the point that embryo growth is initiated and the embryo protrudes through any covering tissues. Seed quality analysts do not consider germination to be complete until the seedling has grown sufficiently to be able to observe and evaluate the root, hypocotyls, and cotyledons (ISTA, 1999). The completion of germination depends on embryo expansion mainly due to cell

elongation driven by water uptake (Bentsink, *et al*., 2007). During seed germination, various stored substrates are reactivated, repaired if damaged, and transformed into new building materials necessary for the initial growth of the embryo (Hadas, 2004).

2. 4. Factors affecting seed germination

Seed germination and seedling establishment, are the most vulnerable life cycle of the plant (Hadas, 2004). Seed viability is required to ensure high germination percentage and plantation (Soyler and Khawar, 2007). But, according to Legesse Negash (1995), propagations of many indigenous tree species from seeds had been difficult due to lack of precise knowledge on their seed biology and germination physiology; because many native plant species have developed survival strategies through evolutionary processes for millions of years. Understanding these strategies in the context of seed physiology is essential for successful plant propagation. Seed germination involves a number of complex cell activities, and both genetic and environmental factors and storage behavior play a key role in modifying tree seed germination (Mog‟omba *et al*., 2007). That is, germination process is controlled by several biological (such as species, seed viability, seed dormancy, and seed size) and environmental (such as moisture availability, temperature, relative humidity, light intensity and duration) factors (Nelson *et al*., 2009; ISTA, 1999).

2.4.1. Seed maturity and Seed dormancy

Investigation of the germination physiology of seeds of several indigenous tree species of Ethiopia has 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). Seed physiological maturities are also important for the planning of collection, since mature seeds have a higher germination rate (Silva *et al.,* 2008). For example, in *Podocarpus falcatus*, fruit commenced when at least 60-70% of them become yellow to get high quality viable seeds (Legesse Negash, 1995).

Seed dormancy refers to a state in which viable seeds fail to germinate when provided with conditions normally favorable to germination i.e. adequate moisture, appropriate temperature regime, a normal atmosphere and in some cases light. For the sake of simplicity, seed dormancy is regarded as the failure of an intact viable seed to complete germination under favorable

conditions. The seeds of some species are prevented from completing germination because the embryo is constrained by its surrounding structures (Bewley, 1997).

Dormancy has evolved as a strategy to avoid germination under conditions where seedling survival is likely to be low (Schmidt, 2000). Hence, timing and location of germination are crucial for the chances of success of the newly produced plant, and accordingly, the temporal and spatial patterns of germination of the seeds of many species are finely tuned to the environmental scenario (Sanchez and Mella, 2004). Therefore, different species have developed a range of seed dormancy strategies that allow dispersal prior to germination, and prevent germination when conditions are unfavorable for establishment (Goodwin *et al.,* 1995).

Although there are several physical and physiological types of dormancy; seed dormancy is divided into two basic types (Evans and Bennet, 2002). These are primary dormancy, which is acquired during seed development or maturation, and secondary dormancy**,** which results from factors after the seed is shed. For instance, seeds that are hydrated and potentially ready to germinate may be exposed to super optimal temperature or salinity conditions and be inhibited from germination, thus becoming "secondarily" dormant. In some cases, secondary dormancy has been related to arise in endogenous ABA content (Srivastava, 2002).

According to Evans and Bennet (2002) primary seed dormancy is more common in nature than secondary dormancy, and can be in the form of exogenous or endogenous dormancy. Exogenous primary dormancy is a condition where essential inputs (e.g. water, light, temperature) are not available to the seed, and germination does not occur. Genetics and environmental factors can also modify the expression of exogenous dormancy, especially for traits such as hard seediness.

Although, not all seeds require exposure to germination stimulating factors in order for germination to occur; for example, seeds within a population may proceed to germinate immediately after dormancy is released as long as water and temperature conditions are adequate; but promotion of germination following dormancy release often requires exposure to specific environmental stimuli (Hilhorst, 2007). Seeds with low vigor are known to respond positively to osmopriming; because DNA repair process can occur at certain water potential to allow metabolism to occur and triggers seed germination (Mog‟omba *et al*., 2007).

2.4.2. Temperature

Seed germination is particularly affected by temperature (Machado Neto *et al.,* 2006; Gulzar *et al.,* 2007). Temperature affects germination capacity and germination rate (Dossatos and Cardoso, 2001). It is usually requiring a thermal periodicity to germinate (Serrano-Bernardo *et al*., 2007) and temperature regulates germination in three ways (Bewley and Black, 1994) by: 1) determining the capacity and percentage of germination, 2) eliminating primary and/or secondary dormancy and 3) inducing secondary dormancy. The optimal germination temperature for most seeds which are not in dormancy is 25 to 30 ^{0}C. Seed germination pattern increases as temperature rises from a minimum (or base) to an "optimum" temperature or temperature range (Machado Neto *et al*., 2006). On the other hand, poor germination is a common phenomenon at suboptimal temperatures (Tzortzakis, 2009; Gulzar *et al*., 2007).

In general, germination responses to temperature are characterized by the three „cardinal temperatures"; the minimum, optimum, and maximum (Allen *et al*., 2007); these can characterize some of the ecological limitations for the geographical distribution of species (Dossatos and Cardoso, 2001). The minimum or base temperature is the lowest temperature at which germination will occur, regardless of how long the seeds are incubated. Similarly, the maximum or ceiling temperature is the highest temperature at which seeds will germinate (Allen *et al*., 2007). The minimum and maximum are the limit temperatures for seed germination, and these characteristics depend on the species, lot, seed dormancy, etc (Machado Neto *et al*., 2006). For example, optimum seed germination and seedling emergence occur at relatively high temperatures (20 – 30° C) for several species such as tomato, eggplant, bean, watermelon, cucumber and melon (Tzortzakis, 2009).

2.4.3. Storage time

In seed, deteriorative changes occur with time (McDonald, 2004). Seeds exhibit variations in storage behavior which varies from orthodox through intermediate to recalcitrant (Berjak and Pammenter, 2004). Gathering, cultivating, selecting and improving wild fruits is important in order to exploit their potential and conserve a valuable genetic pool. This requires understanding of the propagation requirements and seed behavior under storage (Maara *et al*., 2006). Seed

viability under storage conditions is known to vary from species to species and depends on many factors (Mog"omba *et al.,* 2007).

Seeds are classified into recalcitrant and orthodox groups (McDonald, 2004). Orthodox seeds can be dehydrated to low moisture content without drastic effect on germination unlike recalcitrant seeds (McDonald, 2004). Recalcitrant seeds are desiccation intolerant (cannot be dried below approximately 40 percent seed moisture content without damage) and are typically characterized as large seeds with small embryos from tropical trees. Whereas, orthodox seeds, in contrast, are desiccations tolerant this can be dried to 5 percent seed moisture content without damage (Berjak and Pammenter, 2004).

According to Mog"omba *et al.* (2007) seed dormancy is an important factor that can affect germination of each group (orthodox and recalcitrant) at any stage. It is prevalent amongst the orthodox seeds compared to recalcitrant seeds. However, both primary and induced seed dormancy could exist in many tree seeds (Mog"omba *et al*., 2007; McDonald, 2004). Orthodox seeds are characteristic of most agriculturally important crops found worldwide. Orthodox seeds represent most of the seeds found in the world and are among the most agriculturally important species (McDonald, 2004).

2.4.4. Gibberellic acid (GA_3)

Gibberellins were discovered before World War II by Japanese scientists trying to explain the abnormally tall growth and reduced yield of the rice infected with a fungus known as *Gibberella fujikuroi* disease (Taize and Zeiger, 2002). In the 1950s, researchers in England and United States perfected their own methodologies to isolate the active compound from *Gibberella* cultures and named the compound gibberellic acid. Because gibberellic acid application could induce elongation growth in genetic dwarfs of pea and maize, it was surmised that gibberellic acid must also occur in higher plants (Srivastava, 2002).

Gibberellins (GA) is a group of naturally occurring plant hormones and the hormones play a central role in the early germination processes by activating enzyme production and mobilizing storage reserves (Schmidt, 2000; Sarihan *et al*., 2005). According to Sarihan *et al*. (2005), gibberellins are most directly implicated in the control and promotion of germination.

Gibberellins promote growth by increasing plasticity of the cell wall followed by the hydrolysis of starch to sugar which reduces the potential in the cell.

GA_3 promotes germination by accelerating some metabolic processes even in low potential (Sedghi *et al*., 2010); increases the synthesis of hydrolytic enzymes at aleuron layer and by the activity of these enzymes, storage compounds convert to transferable ones (sucrose and glucose) and transfer to embryo (Sedghi *et al.,* 2008). GA_3 was also found effective to allow penetration of oxygen from the surroundings to the embryo and increased germination of seeds (Soyler and Khawar, 2007).

2.4.5. Potassium nitrate (KNO_3)

Potassium nitrate (KNO_3) is the most widely used chemical for promoting germination. Solutions 0.1 to 0.2% KNO_3 are common in routine germination testing and are recommended by the Association of Official Seed Analysts and the International Seed Testing Association for germination tests of many species (Basra, 1994). Several lines of direct investigation indicate that NO is involved in one or more processes that promote the germination of seeds. Although only a few species have been examined in detail; NO promotes the germination of seeds either by reducing seed dormancy or by minimizing the effects of environmental conditions that inhibit germination.

The use of potassium nitrate has been an important seed treatment in seed testing laboratories for many years without a good explanation for its action mechanism (Cetinbas and Koyuncu, 2006). But recently it is known that nitrate is an important nitrogen source for plants and 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, 2002). NO is a main character in plant metabolism that could either be generated endogenously or supplemented by the environment (Jasid *et al.*, 2008).

Nitrogen is used extensively in plant growth and must be present in the plant for cell division. Nitrogen is also vital for protein production in a biological system. Without nitrogen, cell division and protein synthesis stop and the plants eventually die.

Adequate nitrogen increases water use efficiency and absorption of other nutrients by the plant (Hach Company, 1993). Nitrogen is used by plants in two forms, ammonium (NH_4^+) and nitrate (NO_3^-). Ammonium ions are present in soils through decomposition of organic tissue or manure application. Nitrate is the final form of nitrogen decomposition, but can also be supplied by fertilizers, irrigation and precipitation.

2.4.6. Seed response related to fire

Fire is known to stimulate germination in a number of plants (Raizada and Raghubanshi, 2010). In many biodiversity regions, fire events provide an irregular but important opportunity for seedling establishment (Nelson *et al.,* 2009). For example, fire-prone Mediterranean-climate regions are noted for their abundance of plant species whose germination and recruitment are restricted to post fire environments (Keeley and Fotheringham, 1997). For seeds in the soil seed bank it is a common way of limiting readiness to germinate to the post fire period and for many plant species in fire-prone vegetation, the optimum time for germination and establishment is after a fire (Briggs and Morris, 2008). This may be due to the formation of an open space or gap in the local vegetation that occurs following a fire or a reduction in the forest canopy, and is often associated with increased nutrient availability. An increase in soil nitrogen might result in increased NO production and this could be perceived by seeds as a germination cue (Bethke, 2007). Therefore, soil NO_3^-or NO_2 concentrations might function as a ‚gap sensor", allowing seeds to germinate in an environment that is conducive to seedling growth (Bethke, 2007).

According to Minorsky (2002) aerosol smoke and smoke solutions have effects on stimulating seed germination. Smoke extracts have already been used as seed pretreatments for enhancing the conservation of threatened or rare species and used as germination cue (Minorsky, 2002). Soaking seeds in smoked water is the most practical application because large quantities of seed can be quickly and easily treated (Landis, 2000). The slow combustion of dry or green plant material from many sources produces compounds that are water soluble. The active principals are apparently produced around 160°C to 200°C and are volatilized at higher temperatures (Landis, 2000). Although, the identities of the active molecules are unknown, their remarkable effects on seed germination have already found wide application (Minorsky, 2002). On the other hand, according to Raizada and Raghubansh (2010), the nitrogen oxides present in smoke are thought to be the reason for increased permeability of hard seeds, either directly due to oxidation

effects, or after their hydration as acids. Factors such as seed age, light levels, temperature, and hydration levels can also influence the extent of smoke induced germination (Minorsky, 2002). It could be that chemicals present in smoke may also help to enhance water absorption due to enhanced permeability and may lead to greater water absorption and subsequently germination of seeds.

2.5. Seedling and germinant establishment

After radical protrusion, seedling establishment takes place, which can be considered a separate process from germination (Bentsink *et al.,* 2007). However, germination includes sequences of complex processes that lead to the initiation of growth in the quiescent embryo in the seeds, seedling development, and emergence from the soil (Hadas, 2004). The processes required careful transplantations of germinant and after care.

2.6. Seedling growth and measurement techniques

Growth in plants is defined as an irreversible increase in volume. The largest component of plant growth is cell expansion driven by turgor pressure. During this processes, cells increase in volume and become highly vacuolated (Taiz and Zeiger, 2002). Seedling growth is affected by conditions both above ground humidity, carbon dioxide, temperature and light as well as below ground water and mineral nutrients. Other organisms, either beneficial or harmful, can also influence plant growth (Jaenicke, 1999). Healthy seedlings need good soil and it should be well drained with a mix of sandy and loamy soil, high in humus and nutrients and slightly acidic (Moir *et al*., 2007). The site preparations with the humus layer increase seedling nitrogen uptake. Moreover, seedling nitrogen uptake was correlated to root growth (Nordborg, 2001). The seedling growth in height, root collar diameter, number of leaves, tap root length and number of primary roots distinctly varied with biomass allocation indicated by the root to shoot ratio. But the growth of root is determined by the physical force of the soil which is highly dependant on both soil moisture and bulk density (Daddow and Warrington, 1983). The bulk density of the soil is in turn determined by the texture of the soil.

Measurements of above-ground biomass could be made through two basic ways: destructive and non-destructive methods. Among the various methods for evaluating forest biomass, the most widely used is the destructive method. However, the methods are not suited to natural

environment, especially if the environment is highly degraded and also with threatened species (Montes *et al.*, 2000). In addition, this method is expensive in terms of time and expended for collecting the data when compared with the non-destructive method.

The other alternative way of measurement of biomass is non-destructive method. Because total harvesting is impractical in forest studies; other methods have been developed to estimate total biomass from non-destructive measurements such as recording the height of selected plants (Vann *et al.,* 1998). The non-destructive measurement technique has the following advantages: 1) it enables to study evolution of individual tree biomass and its components by taking photographs at several year intervals (Montes *et al*., 2000); 2) it can determine the biomass and leaf area of individual trees throughout their growing cycle without disturbing the environment (Montes *et al*., 2000; Vann *et al*., 1998).

3. Materials and Methods

3.1. Study site

Seed germination studies of *F. sur* were conducted in Plant Physiology Laboratory within the plant propagation room of the Plant Biology and biodiversity management Program Unit (Faculty of Life Sciences, Addis Ababa University). Pot germination experiment, germinant establishment and seedling growth performances on different soil mixes were studied in the glasshouse of the same Program Unit using facilities established for research on indigenous trees propagation biology.

3.2. Fig collection and seed extraction

Figs of *F. sur* were collected from an eight-year-old tree found within the nursery of indigenous trees studies center of the Plant Biology and biodiversity management Program Unit (Faculty of Life Sciences, Addis Ababa University).

Mature figs were collected using polyethylene bags and were brought to the Plant Physiology Laboratory. The diameters of the sample figs were measured using Vernier Caliper. In the laboratory, the figs were manually split into halves; they were then spread over a clean plastic sheet covering the laboratory bench (Figure 1). After the fig halves were dried for eight days, ovule sacs with seeds and ovule sacs without seeds (those damaged by pollinator wasps) were examined and counted using Stereomicroscope set at magnification power of 20x.

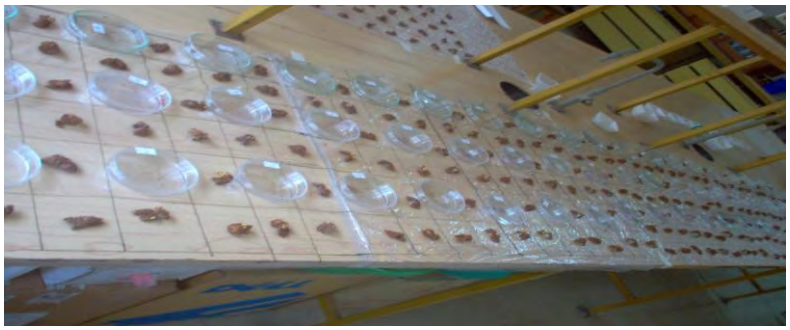


Figure 1.Fig halves drying on the plastic covered laboratory bench.

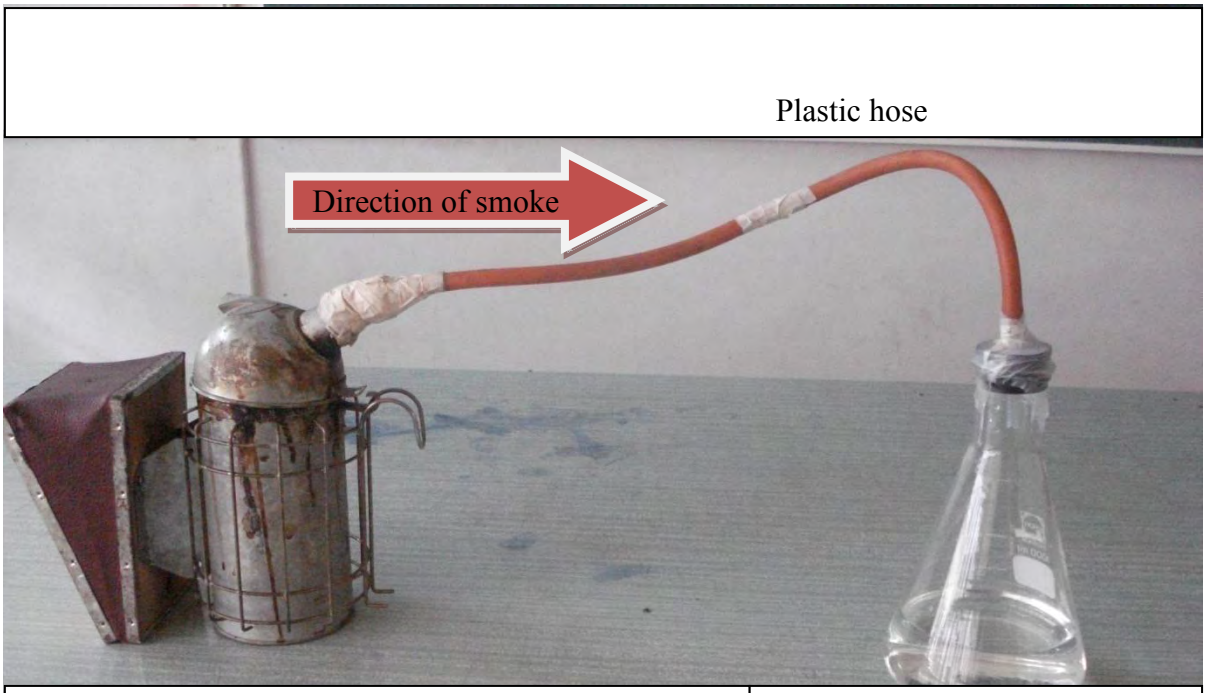
After the fig halves had been dried in air at room temperature for eight days, the seeds were manually extracted and were separated from seed debris that originated from the fig flesh. Seeds were then collected in a plastic Petri dish and stored in the Laboratory. Seeds of *F. sur* have an elliptical shape with brown color and are very small in size. The average weight of 1000 seeds was 0.84 g. Seed germination experiments were conducted after fourteen days of storage at 0^0 C.

3.3. Laboratory studies

3.3.1. Treatment solution preparation

Stock solution of GA_3 was prepared by taking 0.0866 g of the powder hormone $C_{19}H_{22}O_6$ (Sigma Chemical Company, USA). Stock solution of GA_3 at 10^{-3}M was prepared in 250 ml of double distilled water by first dissolving the GA_3 in 20 drops of NaOH. Similarly, stock solution of KNO_3 was prepared by dissolving 0.025 g of KNO_3 in 250 ml double distilled water to yield 10^{-3} M solution. These stock solutions were used to prepare concentrations of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M solutions to be used for the dose-response experiments on seed germination of *F. sur.*

Aqueous smoke extraction was performed by burning 100 g of small branches and leaves of dry plants (such as *F. sur, J. procera* Hochst. ex. Endlicher, and *O. europaea* L. subsp. *cuspidata*) in a 100 mm diameter and 200 mm depth Beekeeper's Smoker (Figure 2). The generated smoke was forced through a plastic hose (as in the direction of the arrow shown in Figure 2) fitted to the mouth of the Smoker by applying pressure on the air-holding and pumping part of the Smoker into a 250 ml Erlenmeyer flask (E-flask) containing 200 ml of double distilled water. The mouth of the E-flask was plugged with a smoke-tight rubber material whose center has been hollowed out to allow the entry of plastic hose to the E-flask. The smoke was forced into the flask for 30 minutes. The resulting smoked water was maintained as a stock solution in a refrigerator at 0°C, and latter used to prepare cold aqueous smoke extract solutions of different dilution levels. The prepared aqueous smoke extract stock solution was used to prepare solutions with relative concentrations of 25%, 50%, 75% and 100% by serially diluting the cold aqueous smoke extract to double distilled water.



Beekeper's smoker filled with smoke producing pieces of wood and leaves.	**E-flask containing double distilled water.**

Figure 2. Arrangements of materials during smoke extraction to produce aqueous smoke stock solution.

The different treatment concentrations of gibberellic acid (GA_3), potassium nitrate (KNO_3), plant derived aqueous smoke extract solutions, and double distilled water and their corresponding concentrations are shown in Table 1.

Table 1. Treatment levels and their corresponding concentrations in μmole and volume by volume (v/v) ratios used for germination studies of *F. sur* seeds under laboratory conditions.

No	Treatments	Levels of concentrations			
		A	B	C	D
1	Gibberellic acid (GA_3)	0.1 μM	1 μM	10 μM	100 μM
2	Potassium nitrate (KNO_3)	0.1 μM	1 μM	10 μM	100 μM
3	Aqueous smoke extract	25 % *	50 % *	75 %*	100 %*
4	Double distilled water (control)	**	**	**	**

Note: In the table, * means volume of treatment stock solution to volume of double distilled water and ** indicates no chemical has been dissolved in the double distilled water.

3.3.2. *F. sur* seed germination

For the Petri dish germination studies, seeds were exposed to pretreatment solutions. Seeds were pretreated (soaked) for 6 hours in 10^{-4},10^{-5}, 10^{-6} and 10^{-7} M of GA_3, 10^{-4},10^{-5}, 10^{-6} and 10^{-7} M of KNO_3, aqueous plant-derived smoke solutions with relative concentrations of 25%, 50%, 75%, and 100% and doubled distilled water (as control).The seed that sank in the bottom of the E-flask were collected and used for the germination experiments. The pretreated seeds were put in 90 X 15 mm diameter by depth plastic Petri dishes. In these experiments, each treatment has had five replicates where by100 seeds were spread per replicate (Petri dish).

A total of seventy five (75) Petri dishes were employed. The Petri dishes were arranged randomly on growth chamber under one Phillips florescent tube set at a height of 50 cm and producing light at a rate of *ca.*40 µmol m^{-2} s^{-1}.

The temperature of the room was regulated by using heater and throughout the germination experiment double distilled water was added as required to moist the filter paper. Relative humidity and temperature of the room were recorded. For the entire experiments; seed germination counts were made every two days starting the first day of germination. Germinated seeds were removed into another Petri dish after recording. Counting was continued until at least 80% of the replication from each treatment shows no new germination for two consecutive counts. A seed were considered germinated at the time when the protrusion of the riadicule occurred. Germination responses of the seeds in the laboratory were expressed in terms of germination percentage, mean germination time taken for 50% seed germination and germination rate.

3.4. Glasshouse studies

Germination experiment and germinant establishment studies were also conducted in the glasshouse of the "Rapid Propagation of Indigenous Trees Program" (Faculty of Life Science, Addis Ababa University). For seedling establishment experiments, seeds were soaked in double distilled water in a 500 ml Beaker for 6 hours. A total of 1200 seeds, which were sunken dawn in the bottom of the Beaker, were planted in pots filled with sand. A total of eight pots (mouth diameter 20 cm, and height 30 cm) were used for germination of the seeds. In each pot, one hundred fifty seeds were planted and the pots were watered twice a day. The resulting young seedlings (often those which produced four leaves) were transplanted into plastic pots containing red soil, compost and sand in ratios of 4:3:1, 4:3:2, 4:1:3, 2:1:1 and 1:1:1respectively.

3.4.1. Pot germination experiments

Pot germination experiments were conducted under glasshouse conditions. The seeds used in this experiment were stored at room temperature for 14 days. A total of 1000 seeds were soaked in double distilled water for 6 hours. For these studies, a total of 20 pots (mouth diameter 20 cm, and depth 18 cm) filled with 1:1:1 red soil, compost and sand mixture. Fifty seeds were planted pre pot. The pots were watered twice a day every morning and late afternoon using tap water.

Germination data collections started as cotyledons of seeds emerged to the surface of the soil and was followed every two days. Data collection was continued until 80% of the pots showed no germination for at least two consecutive counts. For this experiment, germination percentage, time taken for 50% seed germination, and germination rate were analyzed and reported.

3.4.2. Transplantation of germinant and seedling growth responses

The germinant obtained from the laboratory, those pretreated in double distilled water, were transplanted in the plastic sleeves filled with soil and kept in the glasshouse. Continuous follow-up were made to evaluate their survivals. On the other hand, a total of 500 plastic sleeves were used for seedling establishment and growth response experiments. The plastic sleeves filled with red soil, compost and sand in 4:3:1, 4:3:2, 4:1:3, 2:1:1 and 1:1:1 ratio respectively and each ratio had 100 replicates. The plastic bags were moist and hole was made in the center of each plastic bag containing the soil mixtures such that the holes were deep enough to accommodate seedlings‟ root. The polyethylene bags filled with these different soil proportions were arranged in the glasshouse in north to south orientation.

A total of 500 germinants were transplanted randomly to the plastic sleeves arranged in the glasshouse. The transplanted seedlings were maintained in the glasshouse by covering with thin plastic sheets lifted up by using wooden frames of each 50 cm long. After 3 weeks the plastic sheets were removed and the seedlings kept in the glasshouse to compare growth response of the seedling in the glasshouse planted in the different soil mixtures. The seedlings were watered depending on the moisture of the soil using a backpack sprayer twice a day. Growth parameters such as height increment of seedling were measured using “mm” ruler in a three week interval from randomly sampled seedlings per each treatment in a day; starting from the first day of transplanting of the seedling.

After two months of data collections of seedlings growing on 5 by 10 cm plastic bags; the seedling were again transplanted into new plastic bag with 20 by 30 cm size containing the some soil proportion. Thirty percent of seedlings from each treatment type were transplanted and kept in the glasshouse. All the transplanted seedlings were used as sample to collect growth performance data of seedlings, the methods were suggested by Legesse Negash (personal communication). Data were collected in fifteen days interval for three months as the length of

shoot was measured by using ruler. The length and maximum width of all leaves were also measured from the sample. The outline of all the leaves from each plant was traced out on a graph paper which had a uniform distribution with area (Bhatt and Chanda, 2003).

3.5. Germination parameters and statistical analyses

Parameters such as germination percentage, mean germination time (MGT), mean germination rate (MGR), were calculated according to Labouriau and Agudo (1987) as follows:-

1. Germination Percentage = (n/N) x 100, where:

n=total number of germinated seeds;

N=total number of seeds in the sample

2. The mean germination time MGT = (Σniti)/n, where:

ni= percentage of seeds germinated between two consecutive counts;

ti= time (in day) taken since germination experiment started;

n= total number of seeds germinated.

3. Mean germination rate MGR= 1/MGT

MGT = mean germination time in days.

4. Germination vigor % (GV) = $\underline{\Sigma\ (G_i/t_i)}$*100/N

Gi= Number of seed germinated up to the day under consideration

Ti= Time taken for total germination

N= Total number of seed used

Statistical analyses were performed according to the following procedures. The effects of GA_3, KNO_3, smoke solutions, and double distilled water on seeds germination were analyzed. Pot experiment in glasshouse on *F. sur* seed germination, germinant establishment and growth of seedlings were also analyzed by a one-way ANOVA using SPSS for windows version 15.0 with treatments as factors. Tukey HSD test was used for the determination of significant differences between mean values of treatments of the laboratory experiments.

The same test was employed for the determination of significant differences between mean values for tests performed in the glasshouse for seedling growth performance experiment under different mixture of soil treatment. Correlation coefficients for the dependant variables of the squares of leaf length and width (L^2 and W^2), sums of length and width (L+W) and the products of length and width (L*W) were calculated for all treatment together. As a measure of fit of the regression equation, the coefficient of determination (R^2) defined as the ratio of the sum of the squares due to regression and the total sum of squares, had been considered (Bhatt and Chanda, 2003). Five percent significant level was used to indicate statistically significant differences between or among treatments. The graphs were generated by using sigma plot version 8.0.

4. Results

4.1. Morphological conditions of *F. sur* seeds

Seed examination using stereomicroscope at a magnification of 20x revealed that 57.3% of the seeds were damaged by wasps. The balance (42.7%) was found to be morphologically sound. These percentage values are more or less similar to those reported for *F.vasta* (Legesse Negash, 2010).

4.2. Seed germination responses of *F. sur*

Effects of GA_3 at concentrations of 0.1, 1, 10 and 100 µM; KNO_3 at concentrations of 0.1, 1, 10 and 100 µM, and aqueous smoke solutions at relative concentrations of 25%, 50%, 75% and 100% on germination of *F. sur* seeds are provided in Figure 3 A, B and C. Significant seed germination occurred between days 3 to 5. Irrespective of the pretreatment chemicals, it was observed that rapid germination response occurred between days 5 and 6. On the other hand, germination response was significantly ($P < 0.05$) different between the control and the treated seeds under average temperature and relative humidity of 26.55 ± 2.65^0 C and 44.15 ±6.25% respectively. Maximum percentage germination ranged from 90 to 93% in the treated seeds, compared to the control which was *ca*. 75%. There were no significant changes in the percentage germination of seeds 10 days after the start of the experiment. No significant final percentage germination was noted among the germination stimulators.

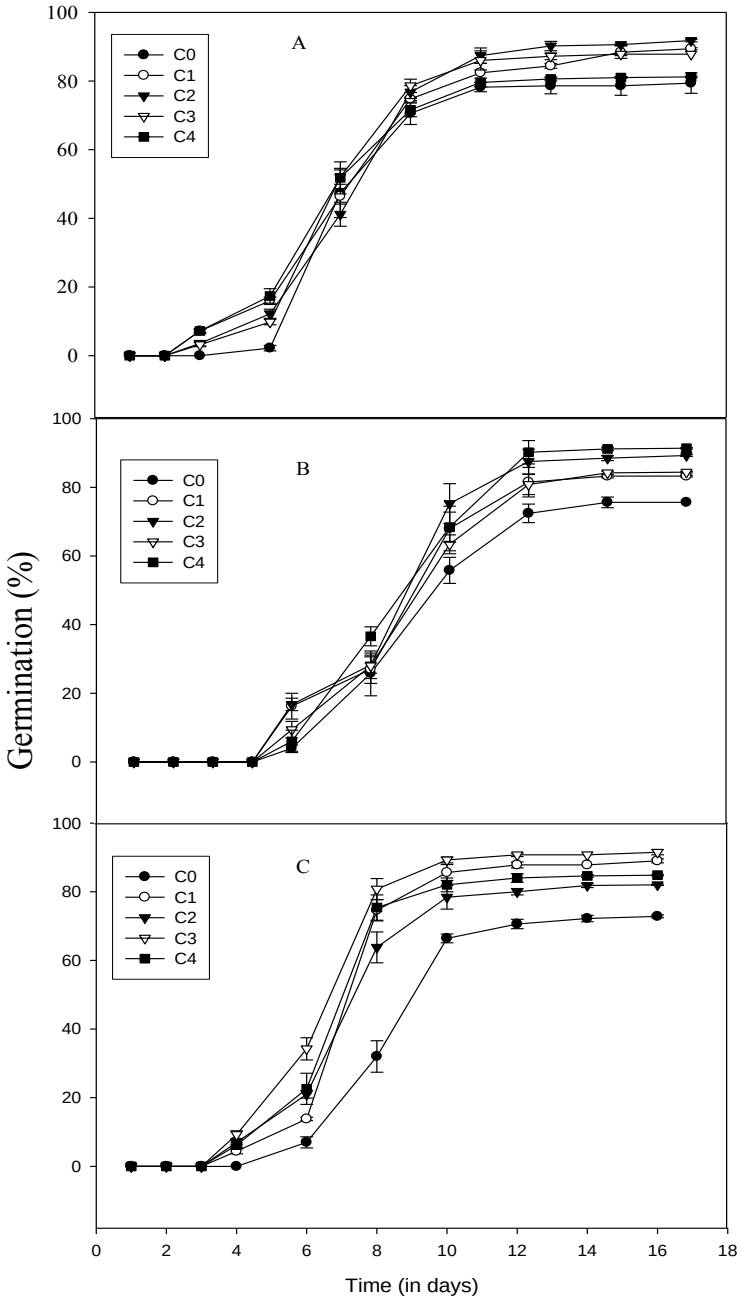


Figure 3. Germination % ± SE of *F. sur* seeds pretreated with C1 = 100, C_2 = 10, C_3 = 1, and C_4 = 0.1 µM of GA_3 (**A**) and KNO_3 (**B**); smoke solutions with C_1=25%, C_2=50%, C_3 =75%, and C_4 =100% relative concentrations (**C**), C0 = double distilled water (control).

The effects of different concentration of germination stimulators on mean germination time are shown in Figures 4. Significant lower mean germination time was obtained in seeds pretreated within 0.1 μM of GA_3 solutions (Figures 4 C) at ($P < 0.05$). Mean germination time decreased with GA_3 concentration. In GA_3 treated seeds with 0.1 μM concentration, 50% seed germination was achieved within seven days after germination started. There were no significant improvements in mean germination time for KNO_3 treated seeds (Figures 4 B).On the other hand, in the case of plant-derived aqueous smoke treated seeds, significantly ($P<0.05$) minimum mean germination time was obtained in seeds pretreated with 75% relative concentration of smoke solution (Figures 4 C).

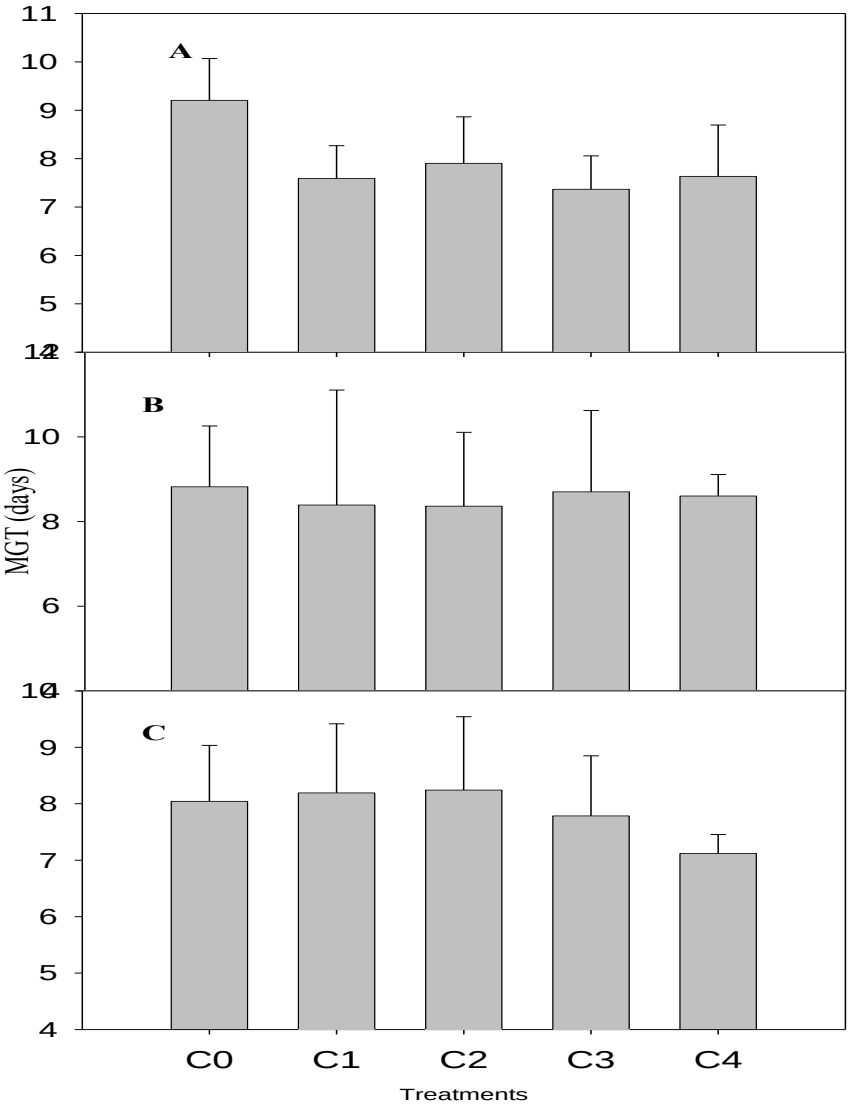


Figure 4. Mean germination time in days of *F. sur* seeds pretreated in aqueous smoke extract solutions (C1=25%, C2=50%, C3=75%, and C4=100%) relative concentrations (**A**); KNO_3 at (C1= 100, C2=10, C3=1 and C4=0.1) µM (**B**); GA_3 at (C1= 100, C2=10, C3=1 and C4=0.1) µM (**C**), C0 = double distilled water (control).

Effects of GA_3 at concentrations of 0.1, 1, 10 and 100 µM; KNO_3 at concentrations of 0.1, 1, 10 and 100 µM and aqueous smoke solutions with relative concentrations of 25%, 50%, 75% and 100% on mean germination rate of *F. sur* seeds are provided in Figure 5. Significant ($P < 0.05$) maximum mean germination rate were obtained in seeds pretreated with GA_3 at 0.1 µM and at 75% relative concentration of plant-derived smoke solution. Highest mean germination rates were obtained in GA_3 and plant-derived aqueous smoke pretreated seeds (Figure 5 A and C). On the other hand, mean germination rates were lower in seeds pretreated with 1 µM and 0.1 µM concentrations of KNO_3 and double distilled water. Potassium nitrate significantly ($P< 0.05$) depressed mean germination rate at concentrations of 1 µM and 0.1 µM compared with similar concentration of GA_3 treatments.

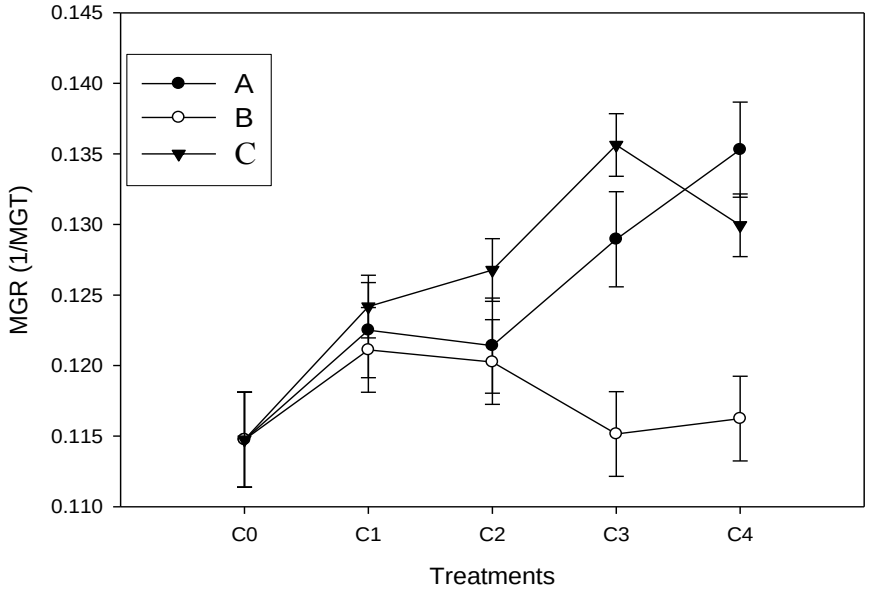


Figure 5. Mean germination rate of *F. sur* seed pretreated in different concentrations of GA_3 with C1 = 100, C2 = 10, C3 = 1 and C4 = 0.1 µM (**A**), KNO_3 with C1 = 100, C2 = 10, C3 = 1 and C4 = 0.1 µM (**B**), aqueous smoke extract solutions with relative concentrations of C1 = 25%, C2 = 50%, C3 = 75%, and C4 = 100% (**C**), C_0 = double distilled water (control).

4.3. Germination studies using pots

Germination of *F. sur* was conducted in the glasshouse using twenty replicate pots. Germination started on the eleventh day of sowing the seeds in the pots. Rapid seed germination was obtained between days 13 and 19 after sowing the seeds in the pots (Figure 6). In this experiment, maximum germination (79.6%) was achieved on the twenty fifth day after the commencement of germination. In the pot germination experiment, 50% of the seeds germinated on the seventeenth day in which the average temperature and relative humidity of the glasshouse were *ca.* 23.55 ± 8^0 C and 72 ± 17%, respectively.

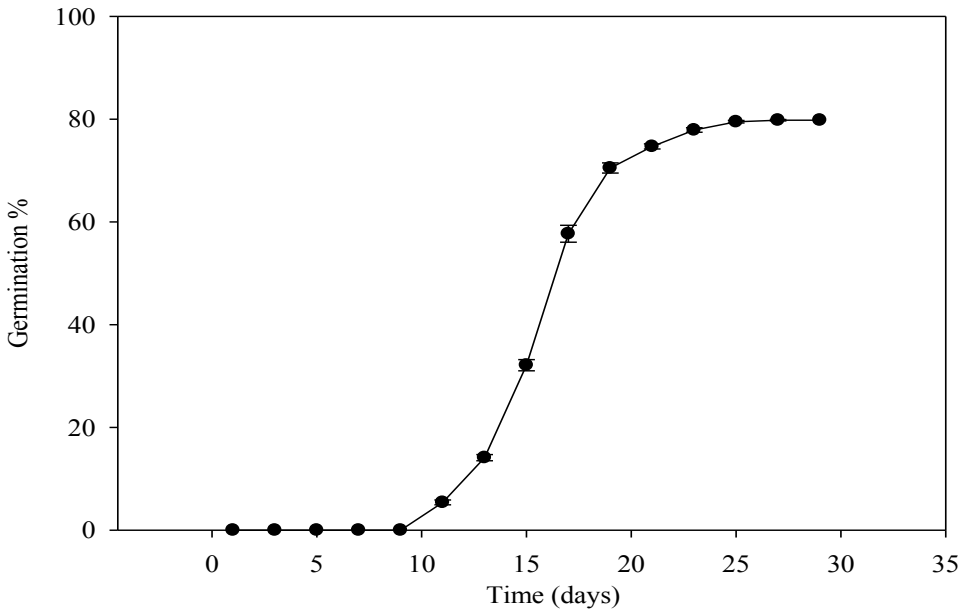


Figure 6. Germination pattern of *F. sur* seeds in pots containing mixtures of red soil, compost and sand in a 1:1:1 ratio and under glasshouse conditions.

4.4. Germination vigor of *F. sur* seeds

Germination vigor percentage of both glasshouse and laboratory sown seeds were computed and the results showed that laboratory germinated seeds were much higher than that of the glasshouse germinated seeds under the same pretreatment i.e. double distilled water. In the laboratory, most seeds completed their germination after 15 days compared to 29 days in the glasshouse.

The germination vigor percentage between different treatment groups under laboratory condition has significant difference at ($P < 0.05$). Maximum germination vigor value was obtained in GA_3 and KNO_3 treated seeds at 0.1 µM and in seeds pretreated within smoke solution at 75% relative concentration (Figure 7).

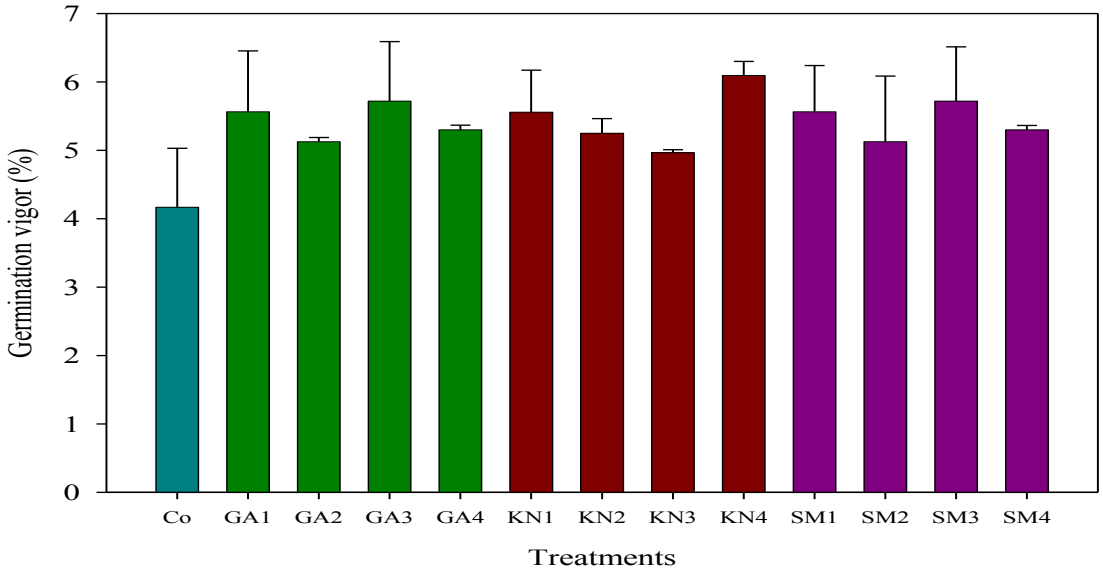


Figure 7. Germination vigor of *F. sur* seed pretreated in different treatment groups. Where C0 =double distilled water, GA stands for concentrations of GA_3 of (GA1-GA4) = (100, 10, 1, 0.1) µM of GA_3, KN for concentrations of KNO_3 in (KN1-KN4) = (100, 10,1and 0.1) µM of KNO_3 and SM stands for relative concentrations of smoke in which (SM1-SM4) = (25% -100%) smoke solutions.

4.5. Germinant establishment and seedling growth responses

After two month follow-up, *ca.* 85% of the transplanted germinant from laboratory into plastic sleeves filled with soil were established into seedling under glasshouse condition. On the other hand, the survival and establishment of seedlings, germinated in pots in the glasshouse, were 100% in all the soil mixtures.

Seedlings that were planted on different soil mixtures have shown differences in their mean height growth. In the first fifteen days there was significant ($P < 0.05$) growth difference in the height of seedlings planted on soil mixture of 4:1:3 red soil, compost and sand with mean height of 9.1 ± 0.43 cm (Table 2). These were not continued, because mean height growth of seedlings was similar after a month starting from the date of transplanting. However, the growth of *F. sur* seedlings on plastic pots filled with 4:3:1 red soil, compost and sand mixture, respectively showed significant growth in height on the 45^{th} day from the control, with mean height of 19.8 ± 2.02 cm. Finally, seedlings planted on soil mixture ratio of 4:3:1 red soil, compost and sand, respectively, showed significantly ($P < 0.003$) better growth in height (49.7 ± 5.17 cm) than those growing on the control group with mean height (33.7 ± 4.4 cm). On the other hand, seedling planted on soil mixture of 4:1:3 red soil, compost and sand respectively reduced the growth of seedlings in height from the control on the 90^{th} day.

Table 2. Mean height (cm) increments of glasshouse-grown *F. sur* seedlings. Plants were grown in plastic sleeves filled with red soil, compost, and sand in various proportions given below. The control was a soil mixture containing a 1:1:1 ratio of red soil, compost and sand soil. Numbers in parenthesis are standard deviations, where **n** = 30 measurements per treatment or sample size. Within a column, means followed by different letters are significantly different and mean value with similar alphabet do not show significance difference.

	Mean growth (days)					
Treatment soil ratio	15	30	45	60	75	90
Red:compost:sand (4:3:1)	8.3^{ab}	12.5^{a}	19.8^{a}	22.4^{a}	35.7^{a}	49.7^{a}
	(±0.63)	(±1.36)	(±2.02)	(±1.92)	(±3.2)	(±5.17)
Red:compost:sand (4:3:2)	7.9^{b}	12.1^{a}	17.9^{ab}	19.2^{c}	26.8^{b}	34.3^{b}
	(±0.55)	(±0.94)	(±1)	(±1.7)	(±2.4)	(±6.3)
Red:compost:sand (4:1:3)	9.1^{a}	12.7^{a}	18.8^{ab}	20.1^{bc}	23.3^{bc}	27.3^{c}
	(±0.43)	(±1.14)	(±1.25)	(±1.4)	(±2.9)	(±5.2)
Red:compost:sand (2:1:1)	8.6^{ab}	11.6^{a}	17.3^{ab}	18.8^{c}	24.4^{c}	31.5^{bc}
	(±0.54)	(±0.96)	(±1.51)	(±1.76)	(±2.9)	(±5.2)
Red:compost:sand (1:1:1)	8.7^{ab}	12.4^{a}	18.4^{ab}	21.8^{ab}	24.9^{bc}	33.7^{b}
	(±0.53)	(±0.81)	(±1.03)	(±1.4)	(±2.9)	(±4.4)

Leaf number produced in the different soil mixture and leaf area indexes are given in Figures 8 and 9, respectively. The growth responses in these parameters were significant at ($P < 0.003$). Unlike in the case of growth in height, differences in the production of number of leaves were observed on the first day of measurement almost in most of the treatments (Figure 8). They were best in soil mixture proportions of 4:3:1, 4:3:2 when compared with the control group. Whereas, in soil mixture proportion 4:1:3 red soil, compost and sand, respectively, growth of seedlings in terms of number of leaf production and leaf area were reduced.

In addition to the above differences, from the visual observation, in the development of seedling the leaves looked like yellowish in color after seventh week starting from the time of transplantation on the plastic sleeves of 5 by10 cm size. This was serious in the soil mixture of 4:1:3 red soil, compost and sand respectively; but in the soil mixture of 4:3:1 and others were darker green.

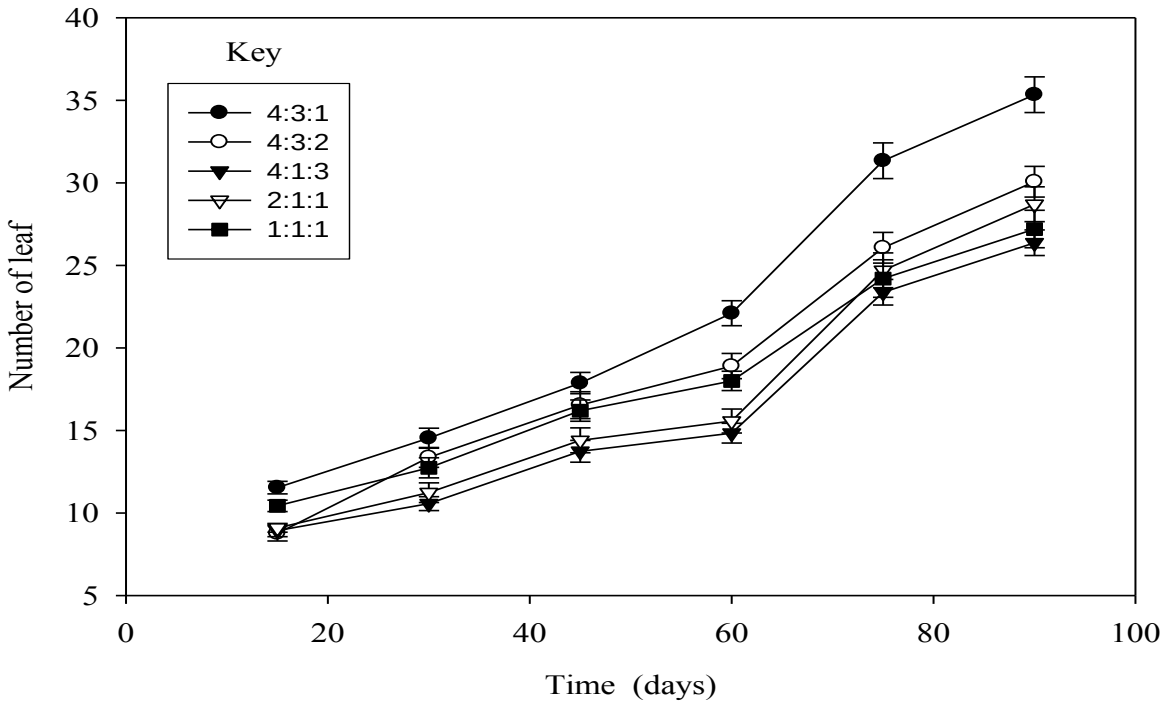


Figure 8. Effect of different soil mixtures proportion (4:3:1, 4:3:2, 4:1:3, 2:1:1 and 1:1:1 ratio of red, compost and soil respectively as indicated on the key) on the number of leaf produced by *F. sur* seedling.

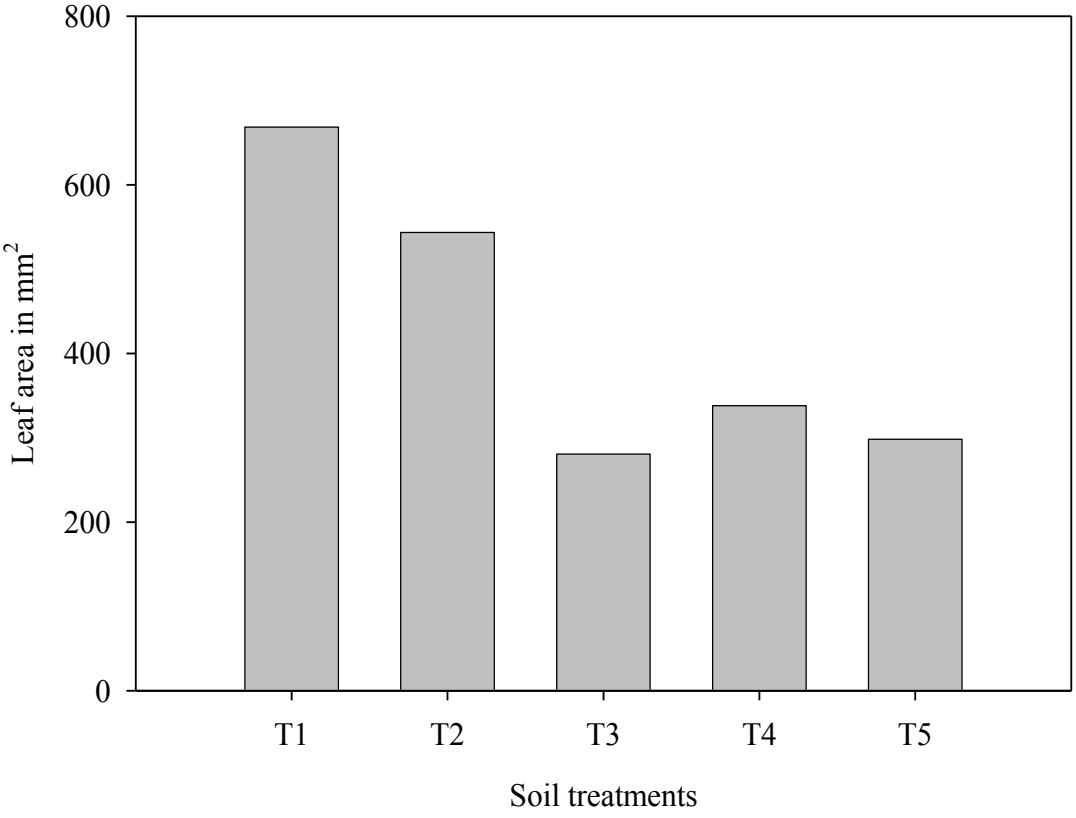


Figure 9. Effects of different soil mixture treatments on mean leaf area in mm^2 of *F. sur* seedling. Where T_1 = 4:3:1, T_2 = 4:3:2, T_3 = 4:1:3, T_4 = 2:1:1, and T_5 = 1:1:1 ratio of red soil, compost and sand, respectively.

Regression analyses were conducted for parameters, such as leaf length* width, width square, length square, and the sum of length and width, to test which parameters fits with the actual leaf area. From the results of linear regression analyses to estimate leaf area index of *F.sur* at seedling level width square (W^2) with some factor b = 0.07, r^2 = 0.97 and P < 0.005 was best fit (Figure 10).

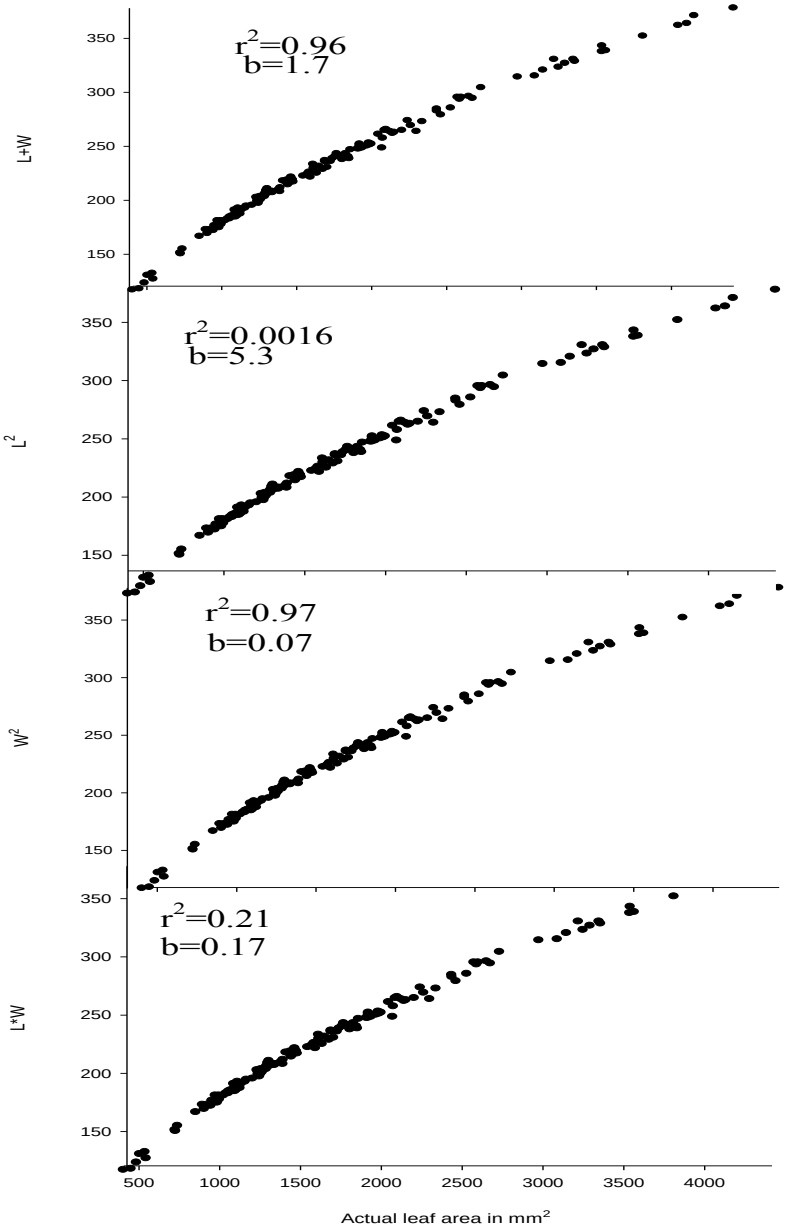


Figure 10. Actual leaf area Vas estimated leaf area of *F. sur* using factor b (sum of leaf length and width or L+W, length square or L^2, width square or W^2, product of length and width or L*W). Where „W" means width at the maximum size, and „L" means length through the midrib, r = regression coefficient, b= factor.

5. Discussion

5.1. Effect of wasps on the morphological conditions and number of seeds produced

Wasps are important to pollinate the flower and to facilitate the maturity of the figs (Weiblen and Bush, 2002). As the female pollinator wasps enter in to the fig through the ostiole, (in the case of F. sur species of Ceratosolem silvestrianus Grandi, C. flabellatus Grandi, Ceratosolem capensis Grandi), they also lay their eggs within the ovule of the fig flowers (Compton and Robertson, 1988). In addition to these pollinator species, there are remarkable diversity of wasps that have direct effects on quality and quantity of fig seeds (Compton et al., 1996; Machado et al., 2001). Large number of seeds damaged in the fig, was ca.57.3% and the ovule sacs contained larvae and pupa of insects. Some of them were empty because the insects consumed their contents and completed their life cycle. These observations support the notion that interaction between the wasps and the host plant in Ficus ranges from mutualism to parasitism (Berg and Wiebes, 1992; Compton and Hawkins, 1992; Weiblen and Bush, 2002).

5.2. Effect of GA_3, KNO_3 and aqueous smoke solutions on germination of *F.sur* seed

Seed germination is obviously a complex phenomenon which involves finely regulated catabolic and anabolic processes where hormones and chemical ions play a key role (Ali *et al.,* 2005). The process starts as the seed imbibes. Hydration of seeds not only restores metabolic activity of quiescent embryos, it also activates the embryonic machinery such that it can receive signals that are involved in breaking certain types of seed dormancy (Srivastova, 2002). During imbibitions, hydrophilic groups (-NH_3, -OH and/or –COOH) of proteins and carbohydrates, located in the seed coat attract the dipolar water molecules to form hydrated shell around these macromolecules. This facilitates not only the swelling of the seed coat, but also makes it more permeable to oxygen and water (Ali *et al.,* 2005).

In these germination studies, different concentration of GA_3, KNO_3 and plant-derived aqueous smoke extract solutions were employed. These pretreatment groups caused variations in seed germination response. It was obtained that soaked seeds in growth promoters like GA_3 and

smoke extract solutions significantly ($P < 0.05$) affected total germination percentage, reduced the time required for initiation of seed germination and time required for 50% germination for *F. sur* seeds when compared with seeds soaked in double distilled water.

According to many reports, pretreatments of GA_3 with different concentrations was capable to initiate seed germinations in many species of plants such as *Plantgo lanceolata* L. (Sarihan *et al*. 2005), *Beta vulgaris* L. (Jamil and Rha, 2007), and tree species such as *Argania spinosa* L. (Al-menaie, 2007). Singh *et al*. (2010), Sedghi *et al*. (2010), Sedghi *et al.* (2008), Schmidt (2000) and Sarihan *et al*. (2005) also reported the role of GA_3 on seed germination.

Soaking of *F. sur* seeds in GA_3 pretreatment solutions were effective in initiation of germination and reduced the time required to achieve 50% seed germination faster than the seeds soaked in double distilled water and it improved the total germination percentage. This may indicate that these treatments were effective in inducing metabolic activity in the embryo required for the initiation of germination processes in *F. sur* seed. The effect of these treatments could be explained by more rapid water uptake of the treated seeds than the control. Similarly, GA_3 pretreatments were reported that it increases water uptake in seeds of *Beta vulgaris* L. (Jamil and Rha, 2007) and these might facilitate the entrance of oxygen (Soyler and Khawar, 2007).

GA_3 is most directly implicated in the control and promotion of germination (Sarihan *et al*., 2005). It promotes growth by increasing plasticity of the cell wall followed by the hydrolysis of starch to sugar which reduces the potential in the cell; but its effect is dose dependent. According to Srivastova (2002), effects of GA_3 on seed germination were dependant on concentration difference. Therefore, various concentrations of GA_3 solutions that affected different parameters of germination of seeds, and its effect also was species dependant. For example, *Hypericum orientale* L. and *H. perfoliayum* L. showed significant ($P < 0.01$) higher germination rate at concentration of 100 mg/l; on the other hand, *H. pruinatum* Boiss. and Bal. maximum germination was reported with 150 mg/l GA_3 treated seeds. Similarly, in these studies, the effect of GA_3 pretreatments showed dose dependent effects on *F. sur* seeds; where, significant ($P < 0.05$) maximum mean germination percentage were obtained in seeds pretreated within 10 µM GA_3 (Figure 3A).

This result was more or less similar with Singh *et al.* (2010), where 10 µM GA_3 concentrations was effective to achieve maximum germination in *Rhododendron niveum* Hook f. Similarly in this study, GA_3 at different concentrations initiated germination of *F.sur* seeds better than the control group. This might be due to the effect of GA_3 on the embryo of the seed to rapture and open the seed coat either by increasing metabolic activity in the embryo or by increasing the water uptake of the seed coat during imbibitions and increased the elasticity of seed coat to permit the release of the radicle.

Nitrogen-containing compounds reduce seed dormancy across a wide range of species and have been known to stimulate germination of seeds (Derex and Karssen, 1993). For example, different doses of KNO_3 with 1000 ppm, 2000 ppm and 4000 ppm improved germination percentage of *Plantago lanceolata* L. over the control (double distilled water treated seeds) (Sarihan *et al*., 2005). In this connection, it is important to mention that nitric oxide (NO) is a reactive, gaseous free radical which also functions as a potent signaling molecule in plants (Bethke *et al.,* 2007). It promotes the germination of seeds, either by reducing seed dormancy or by minimizing the effects of environmental conditions that inhibit germination.

Germination of seeds pretreated with 0.1 µM KNO_3 solution were ($P < 0.05$) better than the seeds of *F. sur* pretreated in double distilled water (Figure 3 B). This may be due to the effects of nitrate from KNO_3; because, nitrate is an important nitrogen source for plants. On the other hand, all the four level of concentration of KNO_3 were not active enough to reduce the mean germination time required for *F. sur* seeds germination and fails to initiate significant early germination when compared to the control. Similar result was also reported that in both KNO_3 treated and untreated seeds of *Trichilia emetica* L. it took nearly the same period to start and complete germination (Msanga and Maghembe, 1992). These might be due to the adverse effects related to concentration levels of KNO_3 that results into high osmotic pressure created by the chemical in the substrate. It has been reported that high concentration of solutes increase osmotic pressure of the germination solutions and makes imbibitions difficult (Msanga and Maghembe, 1992). KNO_3 solution was able to achieve maximum germination percentage with 0.1 µM concentration. Nitrate stimulates seed germination when it is at low doses (Allen *et al*., 2007). It was also explained as biphasic germination response to nitrate indicated that a high-

affinity response occurred at 1mM nitrate whereas a low-affinity response occurred at 10 mM nitrate. Therefore, from this result it is possible to learn that the effect of KNO_3 on the seed germination physiology depending on the concentration of the chemical and is effective at its small amount in a solution.

In the case of smoke treated seeds, it was obtained that significant ($P < 0.05$) germination initiation, maximum mean germination percentage and smaller mean germination time than the control for *F. sur* seeds. It was also reported that the use of smoke water has applications in the rehabilitation of disturbed areas, horticultural industries, and ecological management, and crop production, particularly organic farming systems. Especially, such applications are primarily used for species that are difficult to germinate under normal circumstances (Adkin and Peters, 2001).

Smoke water has the ability to overcome dormancy in many number of species that are classified as weeds of arable land of Europe. The smoke can promote or inhibit germination depending on the concentration. The effective concentration range of smoke water may depend on the solution preparation procedures (Adkins and Peters, 2001). For example, highly concentrated smoke water solutions may inhibit germination of some species, so growers would need to experiment with different dilutions to get the best effect (Londis, 2000). Comparing with the control, 75 % relative concentration of smoke solutions improved germination percentage. It was also significantly ($P < 0.003$) effective in reducing the mean germination time of this species better than the control group. The results of smoke treatments for this species were similar with the results of Kibebew Wakijira (2007) on *Croton machrostachyus.* Thus, the significant effect of smoke solutions on germination of *F. sur* seed may be due to the presence of germination stimulating water soluble substance produced during combustion of the plant material.

Even though, the identity of the compounds of smoke responsible for dormancy release remains unknown (Adkins and Peters, 2001), some suggested that, there are many water soluble and volatile substance in the smoke. For example, nitrogen oxide (Keeley and Fotheringham, 2000), and NO*x*, a butenolide, or both in smoke may induced dormancy release of seeds in many species of plants (Bethke *et al*., 2007).

It was explained that NO promotes the germination of seeds, either by reducing seed dormancy or might be by minimizing the effects of environmental conditions that inhibit germination. The treatment has much ecological use in the reestablishment of degraded land; because, the technique is easy to use and fires have the capacity to produce large amounts of nitrogen oxides, including NO_2 and NO, and these compounds can effectively reduce dormancy of many species. Bethke *et al*. (2007) also reported that inorganic forms of nitrogen such as nitrate, nitrite, and ammonium are often potent promoters of germination.

In these germination studies, hundred percent seed germination was not achieved in all the entire experiments. Germination assays are a measure of the integration of many events that happened in the history of seeds on the mother plant and the various environmental factors encountered during seed storage and germination (Bentsink *et al*., 2007). Therefore, the failure to achieve hundred percent seed germination might be due to after ripening problem (i.e. the time required for seeds to dry after harvest).

Seeds of *F. sur* were used for germination in a few days (14 days). This might be a factor that affects total seed germination. Because freshly harvested seeds of many plants do not germinate or show uneven germination under optimal incubation conditions (Srivastova, 2002). The same author reported that seed germination is controlled by the alternative balanced concentration between ABA and GA_3 in the seed. Therefore, it might be due to an interaction between these endogenous plant hormones and water potential difference between the seed and its environment (Srivastova, 2002).

5.3. Pot germination experiments

Although all the seeds sowed on pots in the glasshouse did not germinate, more than fifty percent seed germination was achieved in this experiment. Some of the seeds did not germinate may be due to the mechanism developed by the species to avoid hazardous conditions which is not suitable for the establishment of seedling from the germinant (Legess Negash, 1995; 2010). Extensive surveys of germination of fresh seeds sown under suitable conditions indicated that rapid germination is the most common response in tropical rain forests although delayed germination is fairly common (Garwood, 1989).

Because, chemicals that accumulate in the fruit and seed coat during development and remain in the seed after harvest such as various phenols, coumarin and abscissic acid can act as germination inhibitors.

Germination rate of seeds were also uniform almost in all the pots; this may be due to similar environmental conditions in the glasshouse. Similarly, it was suggested that favorable moisture conditions and less variable day and night temperatures speeded up germination of seeds of various indigenous trees of Ethiopia (Legesse Negash, 2004). For example, better final percentage and faster rate of germination of *Croton macrostachyus* seeds were reported. It was explained that it was due to, narrow temperature ranges and the high relative humidity of the glasshouse (Kibebew Wokijira, 2007). Therefore, this result may be due to these environmental conditions of the glasshouse.

5.4. Transplanting and establishment experiment under glasshouse

In these experiments, survival of seedlings transplanted from the glasshouse germinant was 100%. This may be due to the appropriate method of germination medium and transplanting technique, duration or time of transplanting, appropriate care taken after transplanting of seedling such as watering, weeding, exposure to light and shade and suitable environmental condition in the glasshouse. Successful establishment of seedlings depends on planting, stock quality (Neves and Paiva, 2003) and environmental conditions of the planting site. Physiological characteristics of seedlings may determine their ability to overcome the transplanting stress (Raftoyannis *et al*., 2003).

In addition, the seedling transplanted was covered by polyethylene plastic sheets because young seedlings should be protected from intense radiation, temperature, wind and soil water evaporation (Neves, and Paiva, 2003). The seedlings were watered twice a day using Backpacked sprinkler in the morning and in late afternoon. Distribution and the physiological condition of the seedling root system can have a strong influence on the survival and early growth of seedlings transplanted to field environments (Neves and Paiva, 2003). Therefore, this achievement may be due to the potential of the roots of *F. sur* to adapt and grow in to different soil types. This has, on the other hand, sound ecological importance that the species can establish its seedling in those soil mixtures.

The germinant from the laboratory experiment were transplanted into polyethylene bags of size 5 by 10 cm diameter and height respectively. The polyethylene bags or sleeves were filled with 2:1:1 ratio of soil mixture of red soil, compost and sand respectively; suggested by Legesse Negash (2010) for *Ficus vasta*. The survival of seedling was evaluated by counting the well established seedlings after two months. From the result obtained *ca.* 85% of the transplanted germinant were established and grew into seedlings.

This failure (not to achieve 100% establishment) might be due to both biotic and abiotic factors that affect the successful growth and establishment of plants in the soil. For example, Legesse Negash (2010) explained that transplanting of Petri dish germinated seeds into the field has difficulty in that the riadicel or root hairs penetrate into the soft or filter paper used on the Petri dish. Therefore, this may be the reason why 100% germinant establishment was not achieved. Hence, the root hairs might be damaged during the transplanting process and may be due to the stress that the germinant faces in the new environmental conditions. In addition to this, since the size of the seed of *F. sur* is very small, it may not be able to support or nourish the germinant until it withstands the unfavorable condition outside the Laboratory.

5.5. Growth performance of seedlings on different soil mixes

The effects of soil compaction on plant growth are a complex interaction between many soil and plant properties (Daddow and Warrington, 1983). Poor growth difference of the seedlings on these soil mixtures in the first eight weeks supports the idea that appropriate moisture and sufficient air are more important for growth performances in the field at the early growth stages than the nutrient levels (Elberse *et al*., 2003). Soil texture is one of the most important factors affecting moisture availability. In addition to these, restricted root penetration and elongation reduce the volume of soil that can be exploited by a plant for essential nutrients and water, which can cause a reduction in total growth (Daddow and Warrington, 1983).

The growth performance of seedling in the glasshouse was effective in height, production of leaf, leaf area increment or growth starting from the second data collection. The fast growth in leaf area and production of leaf were an indication that the species has ecological importance. Because, leaf area index is an important structural parameter for quantifying the energy and mass exchange characteristics of terrestrial ecosystems such as photosynthesis, respiration, transpiration, carbon and nutrient cycle, and rainfall interception (Gong, *et al*., 2003). Because leaf area index is a critical variable in models that attempt to simulate carbon, nutrient, water, and energy flux for forest ecosystems; statistical analyses were conducted on its different parameters, such as regression analyses between the actual leaf area and estimated leaf area from linear measurement taken on the surface of the sampled leaf. This is very crucial to develop methods to estimate the leaf area of the species in relatively minimum time requirement and better accuracy.

To test the effect of growth medium on the productivity of this species were considered. Therefore, in all the parameters considered for leaf and the growth in height at the seedling level on soil proportions mixture 4:3:1and 4:3:2 significantly improved the increment of the biomass productivity for this species. These results may be due to the effect of the compost that provides mineral nutrients for the growth of *F. sur* seedling. The better seedling growth on equal proportion mixture of soil than soil mixture of 4:1:3, match with the results of Kibebew Wakijira on *C. macrostachyus,* which may be due to the better nutrient availability, air circulation and moisture holding capacity of the soil mixture in a 1:1:1 ratio.

6. Conclusions and Recommendations

6.1. Conclusions

The study found that the majority of the seeds were consumed by the very same wasps that were essential for the embryonic development of the seeds and/or parasitic. Germination responses of *F. sur* seeds to different concentrations of GA_3, KNO_3 and plant-derived aqueous smoke extract solutions showed that these germination stimulants increased percentage germination. Mean germination time could also be reduced by applying GA_3 and plant-derived aqueous smoke solution at different concentrations. Overall, the study reveled that *F. sur* does not have germination related problems. The biomass increment was observed in seedlings planted in soil mixture of 4:3:1 red soil, compost, and sand, respectively. From the result, soil mixture relatively with more compost and relatively high water holding capacity is effective to increase seedling growth of *F. sur*.

6.2. Recommendations

Collection of fig and seed management: *F. sur* figs should be harvested when they are mature and brownish-yellow in color and should be dried under shade at room temperature for a week. The seeds can be stored at 0^0 C which can latter give successful germination under favorable conditions.

Pretreatment of seeds: For faster germination initiation and better germination percentage can be achieved through one of the chemical pretreatments such as GA_3 at 10 μM and aqueous smoke extract solution at 75 % relative concentration and KNO_3 at 0.1 μM.

Establishments of germinants in potted soil: Transplanting germinates from Petri dishes to potted soils requires skill and patience as the germinates are quite tiny. Once the germinants are successfully transplanted, and provided that these are protected from desiccation by both regular watering and thin polyethylene plastic cover, germinant establishment success is often 100%, and subsequent growth rate is rapid.

Growth performance of seedling of F. sur*:* Soil mixture proportion 4:3:1 of red soil, compost and sand, respectively, resulted in better growth performance and is therefore recommended for *F. sur* seedling establishment and growth.

7. References

Addo-Fordjour, P., Anning, A. K., Belford, E. J. D. and Akonnor, D. (2008). Diversity and conservation of medicinal plants in the Bomaa community of the Brong Ahafo region, Ghana. *Journal of Medicinal Plants Research* **2**:226-233.

Ali, B., Hayat, S. and Ahmad, A. (2005). Response of germinating seeds of *cicer arietinum* to 28-homobrassinolide and/or potassium. *Gen. Appl. Plant physiology,* **31**: 55-63.

Adkins ,S. W., Peters. N.C.B. (2001). Smoke derived from burnt vegetation stimulates germination of arable weeds. *Seed Science Research* **11**: 213-222.

Allen, P. S., Benech-Arnold, R. L. Batlla, D. and Bradford, K. J. (2007). Modeling of seed dormancy: In Seed development, dormancy, and germination, pp, 72-106, (Bradford, K. and Nonogaki, H. eds.) Blackwell Publishing Ltd, USA.

Al-Menaie, H., Bhat, N.R., El-Nil, M. A., Al-Doser, Al-Shatti, A.A., Gamalin, P. and Suresh, N. (2007). Seed germination of Argan (Argania spinosa L.) *American-Eurasian Journal of Scientific Research* **2:** 01-04

Barke, J. (2004). Effects of the non pollinating fig wasps *Ceratosolen galili* and *Sycophaga syco mori* on C*eratosolen arebicus* the pollinator of *Ficus sycomorus* Pritoria, South Africa.

Basra, A. S. (1994). Stress-Induced Gene Expression in Plants. Harwood Academic Publishers Chur, Switzerland

Bentsink, L., Soppe, W. and Koornneef, M. (2007). Genetic aspects of seed dormancy: In Seed development, dormancy, and germination, pp, 72-106, (Bradford, K. and Nonogaki, H. eds.), Blackwell Publishing Ltd, USA.

Berg, C. C. and Wiebes J. T. (1992). African fig trees and fig wasps. North-Holand, ISPN 0-444-85741-9, pp. 77.

Berjak, P. and Pammenter, N. W. (2004). Recalcitrant seeds. **In**: Handbook of seed physiology: applications to agriculture, pp.305-335, (Roberto. L. Benech-Arnold, and Sanchez, R. A. eds.), Food Products Press and the Haworth Reference Press imprints of The Haworth Press, Inc., 10 Alice Street, Binghamton, NY 13904-1580.

Bethke, P. C., Libourel, I. G. L., and Jones, R. L. (2007). Nitric oxide in seed dormancy germinat ion. **In**: Seed Development, Dormancy and Germination, pp.153-175, (Bradford, K. J. and Nonogaki eds.), Blackwell Publishing Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK, and ISBN-10:1-4051-3983-8.

Bewley, J. D. (1997). Seed germination and dormancy. *The plant Cell* **9**:1055-1066.

Bewley, J.D. and Black, M. (1994). Seeds: Physiology of development and germination.2nd ed. Plenum Press, NY. PP.445.

Bewley, J., D., Black, M. (1985). Seed physiology of development and germination. Plenum Publishing Corporation, New York.

Briggs, C. L. and Morris, E. C.(2008). Seed-coat Dormancy in *Grevillea linearifolia*: Little Change in Permeability to an Apo plastic Tracer after Treatment with Smoke and Heat Annals *of Botany* **101**: 623–632.

Burger, W. C. (1974). Flowering Periodicity at Four Altitudinal Levels in Eastern Ethiopia: *Biotropica*, **6**:38-42.

Cetinbas, M. and Koyuncu, F. (2006). Improving germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea *Hort. Sci.* **33**:119–123.

Cirak C., (2007). Seed germination protocols for *Ex situ* conservation of some *Hypericum* species from Turkey. *Am. J. Plant Physiol*., 2: 287-294.

Compton, S. G. and Robertson, H. G. (1988). Complex interactions between mutualisms: ants tending homopterans protect fig seeds and pollinators. *Ecology* **69**:1302-1305.

Compton, S. G. (1993). One way to be a fig. Afr. Entomol. **1**: 151-158.

Compton, S. G., Wiebes, J. T. and Berg, C. C. (1996). The Biology of Fig Trees and Their Associated Animals. *Journal of Biogeography* **23**: 405-407.

Compton, S. G. & Hawkins, B. A. (1992). Determinants of species richness in southern African fig wasp assemblages. *Oecologia B*, *erl.* **91**: 68-74.

Daddow, R. L. and Warrington, G. E. (1983). Growth-limiting soil bulk densities as influenced by soil texture. Watershed systems development group, USDA forest service, Fort Collins, Colorado 80524.

Datwyler, S. L. and Weiblen, G. D. (2004). On the origin of the fig: phylogenetic relationships of moraceae from *ndh*f sequences. *Amer. J. bot.* **91**:767- *777*.

Demel Teketay (2005). Seed and regeneration ecology in dry Afromontane forests of Ethiopia: II. Forest disturbances and succession. *Tropical Ecology* **46**: 45-64.

Demel Teketay and Granstrom, A. (1997). Seed viability of Afromontane tree species in forest soils. *Journal of Tropical Ecology* **13**:81-95.

Derex, M. P. M. and Karssen, C. M. (1993). Variability in light, gibberellins and nitrate requirement of Arabidopsis thaliana seeds due to harvest time and conditions of dry storage. *Journal of plant physiology* **141**:574-582.

Dossantos, D., and Cardoso, V. (2001). Thermal-biological aspects on the seed germination of *Cucumis aniguria* L.: influence of the seed coat. Revta brasil. Bot., 24:435-440.

EFAP (1994). The challenge for development. Ethiopian Forest Action program (EFPA), Addis Ababa, Ethiopia.

Elberse, I.A., Turin, J.H., Wackers, F.L., van Damme, J.M. and van Tienderen, P.H. (2003). The relation between relative growth rate and susceptibility to aphids in wild barley under different nutrient levels. *Oecologia* **137**: 564-571.

Evans, A. F. and Bennett, M. A. (2002). Seed Dormancy Mechanisms in Vegetable Crop Specie. Ohi State University, Columbus, OH 43210, USA.

Faleyimu, O.I. and Oluwalana, S. A. (2008). Medicinal value of forest plant seeds in Ogun state, Nigeria *World Journal of Biological Research,* www.interscholar.org/wjbr accessed in 11/08/2010.

Feyera Senbeta and Demel Teketay (2001). Regeneration of indigenous woody species under the canopies of tree plantations in Central Ethiopia. *Tropical Ecology* **42**: 175-185.

Friis, I. (1989). Moraceae: **In** Flora of Ethiopia. pp 271-286. (Hedberge, I. and Edward, S., eds.), *Pittosporaceae* to *Araliaceous*. Addis Ababa University and Asmera, Ethiopia; Uppsala, Sweden, The National Herbarium, Vol. 3.

Garwood, N. C. (1989). Tropical soil seed banks: a review. pp. 149-209. *In*: M.A. Leck, V.T. Parker & R.L. Simpson (eds.) *Ecology of Soil Seed Banks*. Academic Press, San Diego & California.

Gautier, D. (1996). *Ficus (Moraceae*) as part of Agrarian systems in the Bamileke region (Cameroon). *Econ. Bot.* **50**:318-326.

Goodwin, J. R., Doescher, P. S. & Eddleman, L. E. (1995). After ripening in *Festuca idahoensis* seeds: adaptive dormancy and implications for restoration. *Restor. Ecol.* **3**:137–142.

Guevara, S., Purara, S. E., and Maarel, E. (1989). The role of remnant forest trees in tropical secondary succession. *vegetario*. **66**:77-84.

Guinand, Y. and Dechassa Lemessa (2000). Wild-food Plants in Southern Ethiopia: *Reflections on the role of 'famine-foods' at a time of drought.* UN-Emergencies Unit for Ethiopia, Addis Ababa.

Gulzar, S., M. A., Khan and Liu X. (2007). Seed Germination Strategies of *Desmostachy bipinn ata*: A Fodder Crop for Saline Soils. *Rangeland Ecol. Manage.* **60**:401-407.

Hach Company (1993). Soil and irrigation water interpretation manual. Printed in U.S.A. 5-15-93-2ED, rb rev1 10/01.

Hadas, A. (2004). Seed bed preparation the soil physical and environment of germinating seeds: **In** Handbook of seed physiology : applications to agriculture, pp3-49.(Roberto, L. Benech-Arnold, and Sanchez, R. A. eds) Food Products Press and The Haworth Reference Press, imprints of The Haworth Press, Inc., 10 Alice Street, Binghamton, NY 13904-1580.

Hankey, A, (2003). *Ficus sur* Forssk Witwatersand National Botanical Garden www.plantzafrica .com. Accessed 4/8/2010.

Hilhorst, H.W. M. (2007). Definitions and hypotheses of seed dormancy: In Seed development, dormancy, and germination, pp.80. (Bradford, K and Nonogaki, H. eds.), Annual plant reviews Blackwell Publishing Ltd.

Igoli, J. O., Ogaji, O. G., Tor-Anyiin, T. A. and Igoli, N. P. (2005).Traditional medicine practice amongst the Igede people of Nigeria. PartII *Afr. J. Trad.* **2**: 134 – 152.

ISTA (1999). International rules for seed testing. *Seed Science and Technology* 27 Supplement.

Jaenicke, H. (1999). Good Tree Nursery Practices: Practical Guidelines for Research Nurseries. Majestic Printing Works Nairobi, Kenya PP. 91.

Jamil, M. and Rha, E. S. (2007). Gibberellic acid (GA_3) enhances seed water uptake, germination and early seedling growth in sugar beet under salt stress. *Pak. J. Biol. Sci*.**10**:654-658.

Jasid, S., Simontacchi, M. and Puntarulo, S. (2008). Exposure to nitric oxide protects against oxidative damage but increases the labile iron pool in sorghum embryonic axes *Journal of Experimental Botany* http://jxb.oxfordjournals.org accessed in 10/8/2010.

Jousselin, E., Hossaert

McKey, M., Herre, E. A. and Kjellberg, F. (2003). Why Do Fig Wasps Actively Pollinate Monoecious Figs? *Oecologia* **134**: 381-387.

Jousselin, E., Kjellberg, F. and Herre, E. A. (2003). Flower Specialization in Passively Pollinated Monoecious Fig: A Question of Style and Stigma? *International Journal of Plant Sciences* **165**:587-593.

Kibebew Wakjira (2007). Seed Germination Physiology and Nursery Establishment of *Croton macrostachyus* Hocht. Ex Del. MSc. Thesis (Unpublished), Addis Ababa, Ethiopia.

Keeley, J. E. and Fotheringham, C. J. (1997).Trace Gas Emissions and Smoke-Induced Seed Germination *Science* **276**: 1248-1249.

Kerdelhue, C., Hochberg, M. E. and Rasplus, J. Y. (1997). Active Pollination of *Ficus sur* by Two Sympatric Fig Wasp Species in West Africa *Biotropica,* **29**:69-75.

Khan, M. A., Irwin, A., Gul, U. and Gul, B. (2003). Alleviation of salinity-enforced seed dormancy in atriplex prostrate. *Pak. J. Bot*., **35**: 906-912.

Kusamba Chifundera (2001). Contribution to the inventory of medicinal plants from the Bushi area, South Kivu Province, Democratic Republic of Congo Fitoterapia **72**:351-368.

Labouriau, L. G. and Agudo, M. (1987). The physiology of seed germination in *Salvia hispanica* L. *Anais de Acadamia de Ciencias* **59**:37-56.

Lachaise, D. (1977). Niche separation of Africa Lissocephala within the Ficus Drosophiid Communities. *Oecologia* **31**:201-214.

Landis, T. D. (2000).Where there is smoke there‟s germination? *Native plants Journal* **1**:25- 29.

Legesse Negash (1990). Ethiopia's Indigenous Forest Species and the Pervasive Effects of deforestation. *SINET newsletter, Vol.**14**,* No.

Legesse Negash (2004). Rapid seed-based propagation method for the threatened African Cherry (*Prunus africana*). *New Forests* **27**: 215-227.

Legesse Negash, (1995). Indigenous trees of Ethiopia: Biology, Use and Propagation Technique Printed by SLU Reprocentcentralen Umea, Sweden. ISBN 91-7191-105- 7, pp. 285.

Legesse Negash, (2010). A selection of Ethiopian‟s Indigenous trees Biology, uses and Propagation techniques, Addis Ababa University press, Addis Ababa, Ethiopia, pp.386.

Leubner-Metzger, G. (2003). Brassinosteroids promote seed germination. **In**: *Brassinosteroids,* PP.119-128, (Haya, S. and Ahmad, A. eds.), Kluwer Academic Publisher, Printed in the Netherlands.

Maara, N. T., Karachi, M. and Ahenda, J. O. (2006). Effects of germination treatment desication and storage temperature on germination of C*arissa edulis, is* and *menia Americana* seeds. *Journal of Tropical Forest Science* **18**:124–129.

Machado, C. A., Jousselin, E., Kjellberg, F., Compton, S. G. and Herre, E. A. (2001). Relationshi Historical Biogeography and Character Evolution of Fig-Pollinating Wasps. *Biological Sciences* **268**: 685-694.

Machado Neto, N. B., Prioli, M. R., Gatti, A. B. and Cardoso, V. J. M. (2006).Temperature effec ts on seed germination in races of common beans (Phaseolus vulgaris L.). *Acta Sci. Agron.***28**:155-164.

Msanga, H. P. and Maghembe, J. A. (1992). Germination of woodland mahogany *(a* following manual seed coat scarification and potassium nitrate treatments. *Journal of Tropica Forest Science* **5**: 518-527.

McDonald, M. B. (2004). Orthodox seed Deterioration and its repair: **In** Handbook o seed physio logy applications to agriculture, pp.271-304, (Roberto. L., Benech-Arnold, and Sanchez, R. A. eds.), Food Products Press and the Haworth Reference Press imprints of the Haworth Press, Inc., 10 Alice Street, Binghamton, NY 13904-1580.

Merritt, D. J., Kristiansen, M., Flematti, G. R., Turner, S. R. Ghisalberti, E. L., Trengove R. D. and Dixon, K.W. (2006). Effects of a butenolide present in smoke on light mediated germ inate on of Australian Asteraceae. *Seed Science Research* **16**: 29–35.

Minorsky, P. V. (2002). The Hot and the Classic: smoke-induced germination. *Plant Physiology* **128**: 1167–1168.

Moir, K., Vandenbosch, T. and Scull-Carvalho, S. (2007). Growing trees and Gardens for life; Practical Tips for healthy tree nurseries and home gardens, Jacaranda Designs Ltd, ISBN 9966-956-54-9Nairobi, Kenya pp.85.

Montes,N., Gauquelin,T., Badri, W., Bertaudiere, V., and Zaoii, E. (2000). A non-destructive methods for estimating above ground forest biomass in threatened woodlands. *Forest Ecology and Management* **130**:37-46.

Mog‟omba, S. A. Du Toit, E. S. and Akinnifesi, F.K. (2007). Germination Characteristics of Tree Seeds: Spotlight on Southern African Tree Species. *Tree and Forestry Science and Biotechnology*, South Africa, pp. 1-8.

Muregi, F. W., Ishih, A., Miyase, T., Suzuki, T., Kino, H., Amano, T., Mkoji, G. M. and rada, M. **(**2007). Ant malarial activity of metabolic extracts from plants used in Kenyan ethno medicine and their interactions with chloroquine (CQ) against a CQ-tolerant rodent parasite, in mice. *Journal of Ethno pharmacology* **111**:190-195.

Nason, J. D., Herre, E. A. and Hamrick, J. L. (1996). Paternity analysis of the breeding structure of the strangler fig populations: evidence for substantial longer distance wasp dispersal. *Journal of Biogeography* **23**: 501-512.

Nelson, D. C., Riseborough, J., Flematti, G. R., Stevens, J., Ghisalberti, E. L., Dixon, K. W. and Smith, S. M. (2009). Karrikins Discovered in Smoke Trigger Arabidopsis Seed Germination by a Mechanism Requiring Gibberellic Acid Synthesis and Light. *Plant Physiology* **149**:863–873.

Neves, I. and Paiva, V. (2003). Nursery production – a responsible contribution for sustainable forest management. **In**: Nursery production and stand establishment of broad-leaves to promote sustainable forest management, pp101, (APAT ed.), TCF, Rome, Italy.

Noort, S. and Compton, S. G. (1996). Convergent evolution of agaonine and sycoecine (Agaonidae, Chalcidoidea) head shape in response to the constraints of host fig morphology– J. Biogeogr. 23: 415–424.

Nordborg, F. (2001). Effects of Site Preparation on Soil Properties and on Growth, Damage and Nitrogen Uptake in Planted Seedlings. Swedish University of Agricultural Sciences, Doctoral thesis.

Ohto, M., Stone, S. L. and Harada, J. J. (2007). Genetic control of seed development and seed mass. **In**: Seed development, dormancy, and germination, pp. 17, (Bradford, K. and Nonogaki, H. eds.), Blackwell Publishing Ltd, USA.

Otero, J. T. and Ackerman, J. D. (2002). Flower Style Length and Seed Production in Two Species of *Ficus* (Moraceae) in Puerto Rico. *Caribbean Journal of Science* **38**: 249-251.

Piotto, B. (2003). Propagation of Mediterranean trees and shrubs by seed: **In** Nursery production and stand establishment of broad-leaves to promote sustainable forest management, pp101, (APAT ed.), TCF, Rome, Italy.

Piotto, B. and Di Noi, A. (2003). Seed propagation of Mediterranean trees and shrubs. Agency for the protection of the environment and for technical services Via Vitaliano Brancati, 48 - 00144 Roma – Italy.

Raftoyannis, Y., Radoglou, K. and Halivopoulos, G. (2003). Comparative study of physiological response of broad leaved species to transplanting stress. **In**: Nursery production and stand establishment of broad-leaves to promote sustainable forest management, pp101, (APAT ed.) TCF, Rome, Italy.

Raizada, P. and Raghubansh, A. S. (2010). Seed germination behavior of *Lantana camara* in res ponse to smoke. *Tropical Ecology* **51**: 347-352.

Sakai, S. (2001). Thrips pollination of Androdioecious *Castilla elastica* (*Moraceae*) in a seasonal tropical forest. *Amer. J. Bot.* **88**: 1527–1534.

Sanchez, R. A. and Mella, R. A. (2004). The exit from dormancy and the indication of germinati on: physiological and molecular Aspects. **In**: Handbook of seed Physiology: applications to agriculture, pp.221-245, (Roberto. L., Benech-Arnold, and Sanchez, R. A. eds.), Food Products Press and The Haworth Reference Press, imprints of The Haworth Press, Inc., 10 Alice Street, Binghamton, NY 13904-1580.

Sarihan, E. O., Ipek, A., Khawar, K. M., Atak, M. and Gurbuz, B. (2005). Role of GA_3 and KNO_3 in improving the frequency of seed germination in *Plantago lanceolata* L. *Pak. J.Bot*. 37: 883-887.

Sawar, G. (2000). Uses of compost for crop production in Pakistan, Okologie und Umweltsicher u ng, Pakistan, pp. 1-195.

Schmidt, L. (2000). „Guide to Handling of Tropical and Subtropical Forest Seed" Danida Forest Seed center pp.1-40.

Sedghi, M., Gholipouri, A. and Sharifi, R. S. (2008). γ Tocophero accumulation and floral differ entiation of medicinal pumpkin (*Cucurbita pepo* L.) in response to plant growth regulators. *Not. Bot. Hort. Agrobot. Cluj*. **36**:80-84.

Sedghi, M., Nemati, A. and Esmaielpour, B. (2010). Effect of seed priming on germination and seedling growth of etnomedicinal plants under salinity. *Emir. J. Food Agric*. 22: 130-139.

Serrano-Bernardo, F., Rosua, J. L. and Dlaz-Migue, M. (2007). Light and temperature effects on seed germination of four native species of Mediterranean high mountains (Spain*). International Journal of Experimental Botany* **76**: 27 -38.

Sileshi, G., Akinnifesi, F. K., Mkonda, A. and Ajayi, O. C. (2007). Effect of growth media and fertilizer application on biomass allocation and survival of *Uapaca kirkiana* Muell Arg se edling *Scientific Research and Essay* **2**: 408-415.

Silva, R., Wallenstein, N., Riedel, C. (2008). Seismic swarm activity during 2003-2004 in the seismogenic Fogo-Congro area (Sao Miguel, Azores): relocation and focal mechanisms (Poster). IAVCEI (International Association of Volcanology and Chemistry of the Earth's Interior) General Assembly, August 17 – 22.

Singh K.K., Gurung, B., Rai, L.K. and Nepal, L.H. (2010).The Influence of Temperature, Light and Pre treatment on the Seed Germination of Critically Endangered Sikkim Himalayan Rhododendron (*R. niveum* Hook f.). *J. Amer. Sci.* **6:**1-8.

Soladoye, M. O., Sonibare, M. A., Nadi, A. O. and Alabi ,D. A. (2005). Indigenous Angiosperm biodiversity of Olabisi Onabanjo University permanent site.

Soyler, D. and Khawar, K. M. (2007). Seed germination of caper (*Capparis ovata* var. *Herbacea*) Using α Naphthalene Acetic Acid and Gibberellic Acid. *International Journal of Agriculture* and *Biology* 1560–8530, 09–1–35–37 http://www.fspublishers.org.

Spelman, K. (2009). "Silver Bullet" Drugs vs. Traditional Herbal Remedies: Perspectives on Malaria ***www.herbalgram.org***, *accessed* in 28/7/2010.

Srivastava, L. M. (2002). Plant growth and development, Hormones and environment. Elsevier science, California, USA, Pp 757.

Taiz, L. and Zeiger, E. (2002). Plant Physiology. Third Edition. Sinaure Associates, Inc. Publishe rs, Sunderland, Massachusetts, pp. 690, ISBN: 0878938230.

Taye Jara (2006). Canopy gap regeneration and dynamics in the afromontane Forest of Bale Mountains. A Thesis Presented to the School of Graduate Studies Addis Ababa University, Ethiopia.

Tesfaye Hailemariam Bekalo, Sebsebe Demissew Woodmatas and Zemede Asfaw Woldemariam (2009). An ethno botanical study of medicinal plants used by local people in the lowlands of Konta Special Woreda, southern nations, nationalities and peoples regional state, Ethiopia *Journal of Ethnobiology and Ethnomedicine* 5:26, *http://www.ethnobiomed.com*.

Tzortzakis, N. G. (2009). Effect of pre-sowing treatment on seed germination and seedling vigor in endive and chicory *Hort. Sci*. **36**: 117–125.

Vann, D., Palmiotto, P., and Strimbeck, G., (1998). Allometric equations for two South American conifers: Test of a non-destructive method. Forest Ecology and Management **106**:55-71.

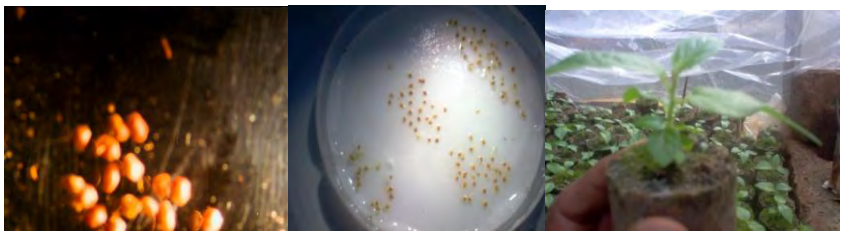
Weiblen, G. D. (2000). How to be a fig wasp. *Annu. Rev. Entomol*. **47**:299-330.

Weiblen, G, D. and Bush, G. L. (2002). Speciation in fig pollinators and parasites, short commun ication. *Molecular Ecology* **11**:1573-1578.

West, S. A. and Herre, E. A. (1994).The Ecology of the New World Fig-Parasitizing Wasps Idarnes and Implications for the Evolution of the Fig-Pollinator Mutualism. *Biological Sciences* **258**: 67-72.

8. Appendies

Appendix 1. Mature fig collection from eight-year-old *F.sur* tree.



A B C

Appendix 2. A) Seeds B) Germinants C) Seedlings

DECLARATION

I, the undersigned, declare that this is my original work which has not been presented for a degree in any other University and that all sources of materials used for the thesis have been fully acknowledged.

Solomon Getahun

July 2011

Addis Ababa Ethiopia