GERMINATION AND STORAGE CHARACTERISTICS OF FIVE INDIGENOUS FRUIT TREE AND TWO SHRUB SEEDS IN MERU SOUTH DISTRICT, KENYA

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not, wholly or in part, been presented for an award of a degree in any other university.

Rithaa Jafford Njeru---------- Date: 10/10/2011

NM11/1584/05

RECOMMENDATION

This thesis has been submitted with our recommendation as University supervisors.

Dr. Moses Karachi _____ Date: 20/10/11

Department of Natural Resources

Egerton University

Dr. Joseph Ahenda _____ Date: 10/10/11

General Manager

Kenya Plant Health Inspectorate Service

P.O Box 49592 -00100

Nairobi

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DEDICATION

To Elosy Gatakaa Njeru and Emelda Gatugi Njeru.

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ABSTRACT

Lack of adequate knowledge on the ecological behaviour of indigenous fruit tree seeds is an impediment to their ex-situ conservation. Seed development in relation to rainfall, seed dormancy, desiccation-tolerance and storage conditions of seven indigenous fruit species; *Myrianthus holstii* Engl., *Dovyalis abyssinica* (A.Rich) Warb, *Tamarindus indica* L., *Vitex payos* (Lour) Merr., *Celtis gomphophylla* Bak., *Rhus natalensis* Krauss and *Berchemia discolor* (Klotzsch) Hemsl were investigated. Seeds at different development stages (mature green and ripe fruits) were subjected to three pre-germination treatments; washing, nipping, nipping then soaking in 500ppm gibberelic acid and the control to test for maturity level and dormancy. The seeds were germinated on 1% agar and in soil as media. To test for desiccation tolerance, seeds from ripe fruits were dried using anhydrous silica gel to various target seed moisture content (TSMC) then germinated on 1 % agar as medium. Seeds at TSMC were also stored for three months at 25° C, 0° C, -10° C and -20° C then tested for germination. Tests were replicated four times. *M*yrianthus *holstii, D. abyssinica* and *R. natalensis* shed their fruits during wet seasons while *T. indica, V. payos, C. gomphophylla* and *B. discolor* shed fruits in the dry season. Germination of seeds from green mature fruits indicated early embryo maturity of all seeds except those of *T. indica,* thus harvesting seeds for propagation can be done prior to fruit ripening. Breaking seed coat by nipping and nipping then soaking in gibberelic acid improved ($P<0.05$) germination of *M. holstii, T. indica V. payos, C. gomphophylla* and *B. discolor* seeds on both media. Desiccation and storage depressed ($P<0.05$) germination of all seeds except those of *T. indica* whose germination increased with loss in seed moisture content (SMC) and lowering storage temperatures. Good Germination (>50 %) of *M. holstii, D. abyssinica, C. gomphophylla* and *R. natalensis* seeds stored at > 15 % SMC and ambient temperature for three months was recorded. *Tamarindus indica, V. payos* and *B. discolor* seeds at 3 % SMC stored at -20° C maintained over 40 % seed germination. This research concludes that *M. holstii, D. abyssinica* and *R. natalensis* seeds fall under recalcitrant category while *T. indica, V. payos* and *B. discolor* seeds are orthodox. *Celtis gomphophylla* seeds are classified as intermediate. Domestication for all the species and *Ex-situ* conservation are recommended for long term seed storage for Orthodox seeds, cryopreservation for Recalcitrant/intermediate and live gene bank for all seeds.

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LIST OF ABBREVIATIONS AND ACRONYMS

AEZ	Agro-Ecological Zone
ANOVA	Analysis of Variance
GPS	Global Positioning System
GA	Gibberelic Acid
IFTs	Indigenous Fruit Trees
IPGRI	International Plant Genetic Resources Institute
ISTA	International Seed Testing Association
LSD	Least Significance Difference
MDGs	Millennium Development Goals
SCR	Seed Coat Ratio
TSMC	Target Seed Moisture Content

CHAPTER ONE
INTRODUCTION

1.1 Background

Plants in form of their fruits, seeds, leaves, flowers, barks and roots are a direct source of the world's staple food. Many edible fruits that grow in wild woodlots in Kenya are nutritionally rich and supplement local diets. The wild fruit trees produce flowers at different times of the year which ensures year round supply of nutrition and provide food security during drought periods (Muok *et al.*, 2001). Fruits of *Adansonia digitata* and *Sclerocarya birrea* are exceptionally rich in vitamin C (Maundu, *et al.*, 1999) while seeds of *Tamarindus indica* contain 63% starch (Morton, 1987).

Wild fruits are a food source that is not normally handled by current agricultural research and extension nor have they featured in forestry research systems, except in the broad context of multipurpose trees (Schreckenberg1 *et al.*, 2006). The limited work that has been carried out on indigenous fruit trees (IFTs) since 1985 has indicated that there is a wealth of information on IFTs, however, research has also indicated an urgent need for identification of some important roles provided by IFTs (Ayling, 1999). There is therefore need to develop and improve existing technologies for conservation, propagation, preservation and processing of indigenous fruits.

Many of these fruits have great potential for alleviating poverty by providing food and nutritional security to local populations. They are collected easily, freely and are available to everyone including the poor. The fruits are also sold in the markets as a source of income to communities especially women (Maundu *et al.*, 1999). If left to grow in crop lands the multipurpose indigenous fruit trees enhance crop production through microclimate modification. New initiatives in agroforestry are seeking to integrate tree species into farming systems and to provide marketable products that will generate income to farmers. One important component of this approach is the domestication of indigenous fruit tree species (Schreckenberg *et al.*, 2006).

As the human population continues to grow, the demand for land required for settlement and intensive agriculture increases at the expense of habitats occupied by undomesticated plant species. This limits *in-situ* conservation of indigenous genetic resources (Maara *et al.*, 2006). Agricultural practices have also concentrated on few conventional exotic fruit species resulting in a neglect of a vast number of potential wild fruit plant species. This implies replacing the once diverse plant life with a narrow range of species to feed an ever-increasing human population. Commercial activities such as charcoal burning and timber harvesting have led to over exploitation of some tree populations to the extent that they can no longer recover through natural regeneration. Such destruction threatens some endemic and rare indigenous fruit tree and shrub species to extinction (Msanga, 1998).

Based on response to desiccation tolerance, plant seeds have been classified into two main categories of seed storage behaviour, orthodox and recalcitrant (Daws *et al.*, 2006). However, information on storage behaviour is available for only about 3% of higher plants (Hong & Ellis, 1996). The success of *ex-situ* conservation depends on the longevity of the seed, the storage conditions used and the ability to regenerate adequate quantities of high quality seeds without genetic change or decline in viability (Eberhart *et al.*, 1991). Seed moisture content and storage temperatures are the most important factors that influence seed storage life (Schmidt, 2000). Optimal storage environment should be observed to maintain viability over a prolonged period of time. Seeds of many of the wild species are sensitive to moisture loss thus exhibiting recalcitrant characteristics. Therefore, storage is only possible for a short period of time which poses a challenge for *ex-situ* conservation through long term seed storage (Prichard *et al.*, 2004).

1.2 Statement of the Problem

Indigenous wild fruits are widely eaten by local people to supplement their nutritional requirements. Nevertheless, cultivation on farm is limited. This is partly due to lack of knowledge on their ecological behaviour, especially seed biology. Their natural habitats for *in-situ* conservation are threatened by changing land uses. Extensive cutting of trees to meet the ever-increasing demand for timber and wood fuel, bush clearance for annual crop farming and settlement leads to reduction of this biodiversity which is a threat to food

security especially during the dry seasons. Conservation of indigenous fruit trees and shrubs with inherent site adaptations is important. However, information regarding their ecological behaviour, seed germination and storage to enhance protocol for propagation and genetic conservation is limited.

1.3 Broad Objective

To investigate physiological and ecological behaviour of seven important indigenous fruit trees seeds

1.4 Specific Objectives

1. To investigate the effect of precipitation during seed shedding on germination of seeds.
2. To investigate the effect of early & late harvesting and dormancy (mature green and ripe fruits) on germination of seeds.
3. To determine the desiccation tolerance of seeds.
4. To determine the effect of storage temperature and moisture content on seed Germination.

1.5 Null hypotheses

Ho_1. Germination of seeds is not affected by precipitation during seed sheddinng.

Ho_2. Seeds from green mature fruits do not germinate

Ho_3 Germination of seeds is not affected by seed dormancy

Ho_4. Seeds are not desiccation tolerant.

Ho_5. Seed germination is not affected by storage temperature and moisture content.

1.6 Justification of the Study

Biodiversity of fruit trees in farmland is essential for direct and indirect food security. With increased climatic instability that has caused frequent agricultural crop failures in the dry lands, the role of indigenous food tree species in providing nutritional supplement to people cannot be ignored. Many countries in the tropics are becoming increasingly aware of the potential of native plant resources as a source of household income, food and nutritional

security and employment particularly for rural poor populations. Kenya has a wide range of well-adapted high value indigenous fruit species that can be utilized for increased agricultural production and economic development if they are exploited.

Domestication of IFTs and their sustainable use could enhance the agricultural sector which is the main source of livelihood. Understanding the ecological behavior, including phenological characteristics, types of dormancy, desiccation tolerance and storage of seeds of IFTs is essential for promoting their propagation, use, *in-situ* and *ex-situ* conservation. This research investigated the effects of seed maturity level, seed dormancy, desiccation and storage temperatures on germination of *Myrianthus holsti* Engl.*i*, *Dovyalis abyssinica* (A.Rich) Warb, *Tamarindus indica* L., *Vitex payos* (Lour) Merr., *Celtis gomphophylla* Bak, *Rhus natalensis* Krauss and *Berchemia discolor* (klotzsch) Hemsl seeds, these are some of the critical factors in propagation and genetic conservation.

1.7 Definition of Terms

Desiccation tolerance: critical moisture content of a seed below which drying kills the embryo.

***Ex-situ* conservation**: It is the process of protecting an endangered plant species or components of biological diversity outside their natural habitat.

Germination: resumption of active growth in an embryo which results in its emergence from the seed and development of those structures essential to normal plant development.

Intermediate seeds: seeds that cannot be dried to moisture content similar to that tolerated by orthodox seeds (< 7.0% M.C.) but are sensitive to low temperatures.

***In-situ* conservation:** It is the process of protecting an endangered plant species in its natural habitat, either by protecting or cleaning up the habitat itself.

Longevity of seed: the period of time that a seed will maintain viability in storage.

Mature green fruits: fruits at full size with essential structures fully developed and at the point of changing coloration for ripening.

Mature ripe fruits: fruits which are fully developed at the stage of being shed naturally.

Moisture content: the amount of water present in a seed and expressed as a percentage of fresh seed weight.

Orthodox: seeds which tolerate drying (maintain viability) to very low moisture content (2-5%) and can be stored for long periods over wide range of environments.

Pre-germination treatment: any modification applied to a seed to hasten germination.

Recalcitrant: seeds which are sensitive to desiccation and chilling, loose viability with drying germinate easily while fresh (> 30% M.C) and have a shot life span hence difficult to store for long periods.

Seed: a fertilized ovule which contains an embryo and nutritive tissue and is enclosed in protective layers of tissues.

Seed development: Period between flowering and seed shedding in a species.

Seed dormancy: a condition under which a viable seed fails to germinate even when supplied with the conditions normally considered suitable for germination.

Seed storage: safe keeping of viable seeds under set conditions from the time of collection until they are required for sowing

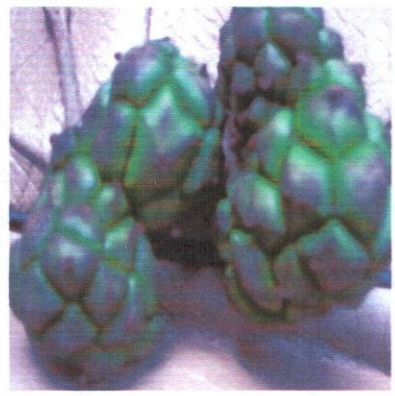
Viability: the ability of a seed to germinate

CHAPTER TWO
LITERATURE REVIEW

2.1 Species Description

2.1.1 Myrianthus holstii Engl.





The tree is commonly known as giant yellow Mulberry (English), Mutuja (Meru), Mutuya (kikuyu) and Saounet (Kipsigis) (ICRAF, 1992). *Myrianthus holstii* belongs to the family *Cecropiaceae* and is a medium- size tree that grows up to 20 m with a short trunk and large branches. It grows in highland forests, especially on forest edges and near watercourses, 900 – 2400 m a.s.l. It requires 1000mm of rainfall and grows in agro climatic zones i – ii. The bark is grey – brown while leaves are very large, 25-60 cm across and compound palmate with 5-7 leaflets. Flowers are in separate sexes; male flowers are on thick branched heads, 4-5 cm across while female flowers are 20-40 on a stalked head which is yellow and are 2 cm in diameter. The fruits are round yellow when ripe with hard sections surrounded by edible pulp. Other uses are fodder (leaves), soil conservation, mulch, shade and firewood (Maundu and Tengäs, 2005).

2.1.2 Dovyalis abyssinica (A. Rich) Warb.

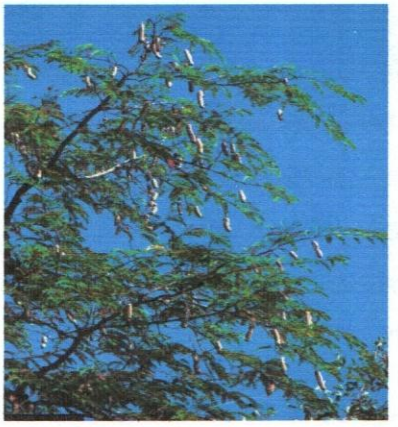




The species is widely known in Kenya as; Mukambura (Meru), Mukambua (kamba), Mwokiot (Kipsigis), Omokorogoinwa (Kisii), Olmorogoi (Maasai), Nakuk (Nandi), Karaturwa (Pokot) and Mbuche (Taveta). *Dovyalis abyssinica* is a spiny

evergreen shrub or tree that has a rounded crown and grows up to 5 m. It belongs to *Flacourtiaceae* family (Maundu and Tengäs, 2005). The species grows in the forest edges, river banks and sometimes in more open woodland. Sometimes it is found as a remnant tree or shrub in coffee plantations. It grows in agro climatic zone ii-iii. The bark is grey with spines of up to 1.5 cm long. Leaves are shiny, dark green, oval, to 5 cm and have unevenly rounded edge with reddish veins. The female flowers are single while male flowers are in clusters. The fruit has orange – yellow pulp when ripe. The fruit is a round berry about 2 cm across surrounded by green calyx. The fruit are edible when raw and the fruit juice is used to flavour porridge. The roots of *D. abyssinica* are also medicinal and the leaves are used for fodder. It is used for live fence and the spines used for beautification.

2.1.3 Tamarindus indica L.

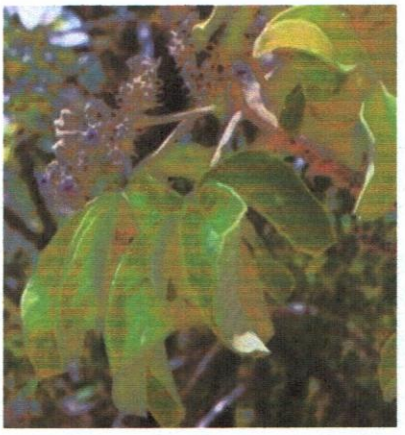


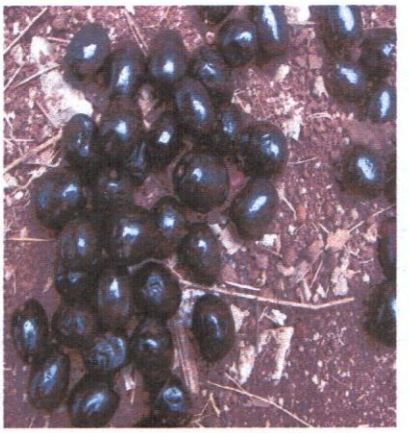


The tree is locally referred to differently by different communities: Tamarind (English), Mkwaju (Giriama), Kithumula (Kamba), Lammaiyat (Kipsigis), Ochwaa (Luo), Oloisojoi (Maasai), Muthithi (Meru), Epeduru (Turkana). *Tamarindus indica* belongs to the family *Fabaceae* (Maundu *et al.,* 1999). It is a large tree, evergreen or deciduous in dry areas and grows up to 30m in agro climatic zone iii-v. The bark is rough, grey – brown and flaky. Leaves are compound on hairy stalks to 5 cm and 10-18 pairs of leaflets. Flowers are small in few flowered heads, buds red, petals gold with red veins. Fruits are pale brown pods when mature, sausage – like and cracked when mature to display sticky brown pulp with 1 -10 angular seeds. The fruits are edible raw and also used for souring porridge. Other uses are timber, furniture, boat building, utensils, poles and firewood, medicinal, fodder, bee forage, shade ornamental, mulch and nitrogen fixing. Seed germination rate is about 90 %. Nick or soak in cold water for 12 h to achieve high germination. The seeds are susceptible to attack

by weevils. Seeds can be stored for long period. Local trade in the fruit is common (Maundu and Tengäs, 2005).

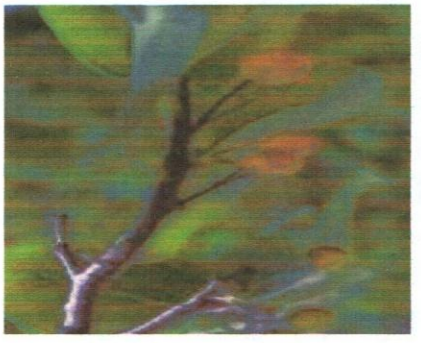
2.1.4 Vitex payos (Lour) Merr.





The species which grows in agro climatic zone iii-iv belongs to the family *Verbenaceae.* It is locally known as; Black plum (English), muburu (Meeru), Mfufu (Swahili), Kimuu (kamba), Mfudu unga (Chonyi). *Vitex payos* is a small deciduous tree that grows up to 8 m high with a low and sparsely, branched and rounded crown. The bark is grey –brown and deeply fissured. Leaves are opposite, each with 5 leaflets, occasionally 3, terminal leaflets 7-20 cm. The leaflets are widest towards the tip. The flowers are small with 5 unequal lobes. They are white, pale blue in colour. The fruits are usually in bunches and are cylindrical to 2.5 cm. The fruits are black when mature (ICRAF, 1992).

2.1.5 Celtis gomphophylla Bak.



Celtis gomphophylla belongs to the family *Ulmaceae.* It is locally known as; Mutoo (Meru), Musaa (Kikuyu), Musa (Luhya) and Nyawend agwata (Luo). *Celtis gomphophylla* grows up to about 12 m, with a spreading crowd. Bark is smooth and pale grey. leaves have 3-haired veins from the base of the oval leaves, outer 2 veins reaching well into the half of the leaf towards the tip. Flowers have sepals but no petals, very small in clusters beside leaves; female flowers above the male flowers on the stalk. Fruits are yellow orange, round and hairy and have hard

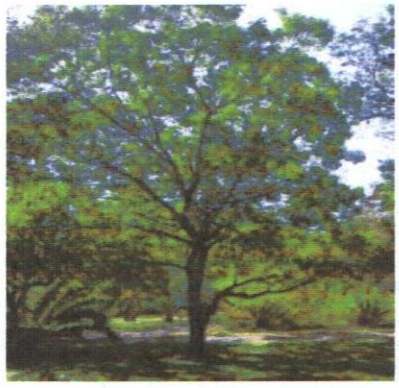
seed inside. The tree does well in agro – ecological zone iii-v and has a wide range of habitats from dry rocky outcrops to riverline areas.

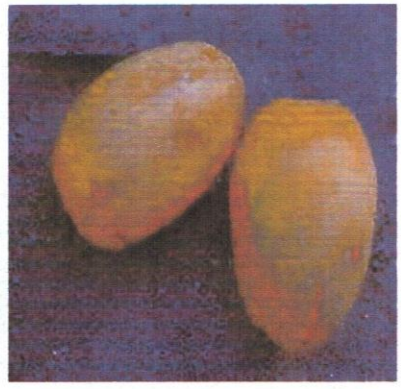
2.1.6 Rhus natalensis Krauss



The plant is widely known in Kenya as: muthigio (Meru), Natal rhus (English) and mtishangwe (Swahili). *Rhus natalensis* is normally a branched shrub that grows up to 8m in height. The shrub is found along the forest edges and besides rivers. This species grows in agroclimatic zone i-iii and up to 2000 m.a.s.l (Maundu and Tengäs, 2005). The bark is grey dotted with breathing pores and leaves are; 3 leaflets with the centre one largest, to 9cm and are usually dark green. The flowers are green – yellow with tiny flowers in loose heads. The fruits are smooth; bean - shaped and red in colour with thin flesh and waxy covering. The fruits are edible. *Rhus natalensis* is also used as medicinal, fodder, dye, toothbrushes and timber (Kokwaro, 1986).

2.1.7 Berchemia discolor (klotzsch) Hemsl





Berchemia discolor grows in agroclimatic zone iv-vi as a riverine vegetation and in bushlands. It is locally known as Bird chery (English), Mkulu (Swahili) and Muthwana (meru). *Berchemia discolor* is a semi-deciduous shrub with grey-black, cracking and scarly bark. The leaves are shiny, dark green and sticky when young. They are oval shaped and yellowish green below. Flowers are small, stalked in loose clusters and are yellow green in colour. The fruits are oval and tapering towards the tip. They are green but turn yellow to reddish - brown when ripe. The fruits have sweet, edible flesh. It's also used for firewood,

charcoal, timber, furniture, poles, tools handles, seasoning, medicine, fodder, bee forage, resin and for dye (ICRAF 1992).

2.2 Flowering and seeding of indigenous fruit trees

Most of the indigenous fruit producing trees flower at different times of the year thus ensuring year round supply of nutrition and provision of food security during drought periods (Muok *et al.*, 2001). Flowering in *Adansonia digitata* occurs before the beginning of the rainy season. However, some species flower during the dry season (Moctar *et al.*, 2006). Subsequently, fruit maturity for different species occurs at different times of the year. Some species shed fruits during the rainy season while others shed during the dry spell.

2.3 Seed maturity and moisture content during shedding

Fruits should normally be harvested when ripe and ready to disperse naturally (Hong and Ellis, 1996). However, monitoring maturity of seeds during their development can often be useful in helping to decide when to harvest. Maturity of fruit for most species coincides with seed dispersal while in others like *Fraxinus spp.*, fruits may form without seeds setting. Some seeds also contain underdeveloped and physiologically immature embryos. In this case, seeds do not germinate at fruit ripening but require ripening period. Seeds may also mature well in advance of fruit ripening. For example, germinable seeds can be obtained from green pods of some *leguminosae*. In teak (*Tectona gradis*) the fruits attain mature size some two months after flowering but it takes additional five months for seeds to mature. In areas with seasonal rains, recalcitrant seeds are adapted to ripen at the start of the rainy season and germinate soon after dispersal from the mother plants.

Sensitivity to seed desiccation is potentially a high-risk regeneration strategy for plants because a prolonged dry spell at the time of seed shedding could result in the death of an entire annual cohort of seeds. This explains the typically large size of desiccation-sensitive seeds, (Tweddle, *et al.*, 2003), and their convex shape, all of which will reduce the likelihood of seed drying (Tompsett, 1992). Desiccation sensitive seeds are therefore shed at high moisture contents (Hong and Ellis, 1998), in a metabolically active state progressing towards germination (Berjak, *et al.*, 1984). They have shorter imbibition period than

desiccation tolerant seeds and thus the speed of germination is generally faster. Their rapid germination may reduce the period which seed desiccation can occur. On the other hand, desiccation-tolerant seeds may form physical defence in the form of a thick seed coat or endocarp, hence, can last for extended periods of time in the soil seed bank.

2.4 Seed characteristics, desiccation and storage

The seeds are the carriers of new plants and hence units of dispersal. They occupy a critical position in the life history of higher plants. Seed response to desiccation is influenced by the seed characteristics such as maturity level, seed coat, shape, size, oil content and moisture content at shedding. Tompsett (1987) reported that in the *Araucariaceae*, the relative level of desiccation tolerance in seeds is associated with seed and/or embryo size, while in *Dipterocarpaceae*, the level of desiccation tolerance is associated with seed size and shape. Desiccation sensitive seeds have a lower surface area to volume ratio than desiccation tolerant species. Hong and Ellis (1998) found that species desiccation sensitive seeds produce large (>1g) and round seeds which are shed at high moisture content. However, in some species, seed size is a poor predictor of seed response to desiccation. Pritchard *et al.*, (1995) found that in *Fabaceae*, there is no relationship between embryo size and the level of desiccation sensitivity. Hong and Ellis (1997) reported that in *Aceraceae*, seed mass is a poor predictor of response to desiccation.

Desiccation tolerant (orthodox) seeds are capable of tolerating desiccation to low moisture contents (generally <7 %) with little effects on viability (Robert, 1973). Desiccation sensitive (recalcitrant) seeds are killed by drying the seeds to water contents as high as 20-30% (Pritchard, 2004). Most seeds are intolerant to desiccation during their early development but they develop ability to survive desiccation overtime although the maximum degreee of desiccation tolerance attained and the rate at which it develops vary substantially among species (Jayanthi, *et. al.*, 1998).

Dry seeds have potential for *ex-situ* genetic conservation through long term storage (Ellis and Hong

, 1995). The success of *ex-situ* conservation depends on the longevity of seed storage, the storage conditions used and the ability to regenerate adequate quantities of high quality seeds without genetic change or decline in viability (Eberhart *et al.*, 1991). Seed moisture

and storage temperatures influence seed storage life (Schmidt, 2000). Understanding the aspects of seed development will help decipher the protocols for seed handling.

2.5 Seed germination

Species with desiccation-sensitive seeds may be able to germinate rapidly when they are shed at high moisture contents and are metabolically active, thus reducing the need for prior imbibition. However, some species with desiccation-tolerant seeds also have seeds that are shed at high moisture contents. Doussi and Thanos (2002) reported that where rainfall is sporadic and unpredictable, it is advantageous for seeds to germinate slowly, at the individual or population level. In either case, the risk of drought- or desiccation-induced mortality when seeds have started to germinate or are at the early seedling stage would be reduced. This explains the slow germination of the desiccation-tolerant species that are shed at high moisture contents. However, for desiccation-sensitive seeds, the overriding concern is for them to germinate rapidly because delayed germination would result in desiccation-induced seed death. Species with desiccation-sensitive seeds are unable to spread the risk associated with unpredictable rainfall after seed is shed. An additional protective adaptation in such species would be a temporal spread of seed-dispersal times, thereby reducing the risk of losing an entire annual cohort of seeds to a dry spell after shedding.

2.6 Seed dormancy

During seed maturation, the embryo may enter a dormant phase in response to desiccation. Seed germination depends on similar environmental conditions as vegetative growth does. Water and oxygen must be available, the temperature must be suitable, and there must be no inhibitory substances present. However, in some cases a viable seed will not germinate even though all the necessary environmental conditions for growth are satisfied (Jérôme *et al.*, 2002). Seed dormancy introduces a temporal delay in the germination process and also maximizes seedling survival by preventing germination under unfavorable conditions. Pre-germination treatments will enable the breaking of all forms of dormancy.

Types of seed dormancy

Two different types of seed dormancy can be distinguished on the basis of the timing of dormancy onset. They are primary dormancy which is exhibited when seeds are released from the plant in a dormant state and secondary dormancy which is exhibited when seeds become dormant if the conditions for germination are unfavorable.

Dormancy can also be categorized on the basis of the cause of dormancy as;

Morphological and physiological dormancy

Endogenous dormancy inherent in the embryo of the seed and is not due to any influence of the seed coat or other surrounding tissues. It is associated with embryo immaturity, presence of germination inhibitors and loss of growth promoters such as Gibberelic acid. (Bewley and Black, 1994).

Physical dormancy

Coat-imposed dormancy occurs by prevention of water uptake by the seed coat. The hard-seededness restricts the penetration of water into the seed (Cock and Robert, 1992 cited by Jérôme, *et al.,* 2002). Mechanical constraint where seed coat is too rigid for the radicle to penetrate such as in nuts is also a form of coat-imposed dormancy. Lowered permeability of seed coat to oxygen also inhibits germination by limiting the oxygen supply to the embryo (Xiaojie *et al.,* 1999). The embryos of such seeds will germinate readily in the presence of water and oxygen once the seed coat and other surrounding tissues are either removed or damaged.

Chemical dormancy

Chemical germination inhibitors in seed covering tissues may prevent germination. However, chemical dormancy is seldom observed alone, but is found in combination with other dormancy types (Bewley 1994). Repeated washing removes the germination inhibiting compounds.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Study site

The seeds were collected from the identified IFT species along Ruguti and Tungu river valleys in Meru South District (Figure 1). This area lies on the slopes of Mt Kenya to the east. The transects traversed four agro- ecological zones. The total population is approximately 212,982 and the total number of households is approximately 46,984 with an average household size of 5 (4.4) persons. Cash crop and mixed subsistence farming is threatening bush land vegetation in the area. The species for this study can mainly be found along the riparian zones and in the bushland.

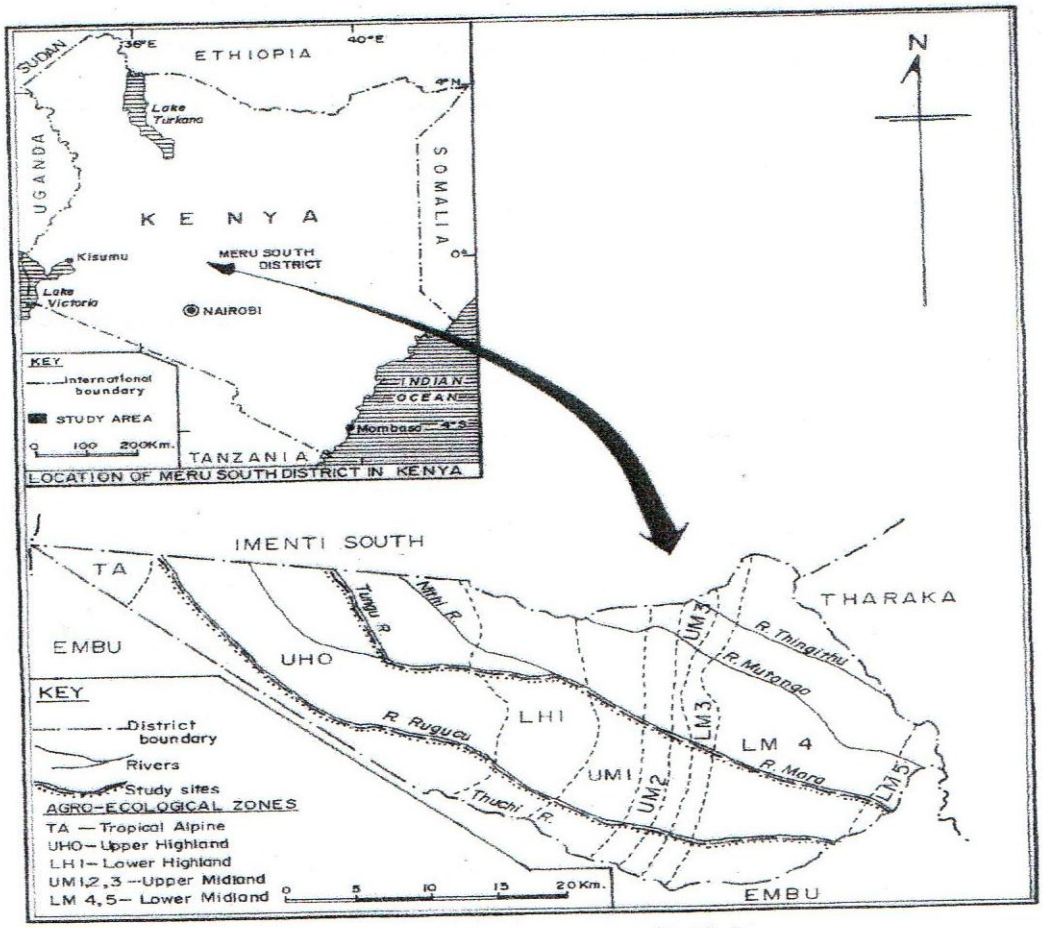


Figure 1: Map of Meru south district showing sites of fruit collection

Source: GOK, 2002

3.2 Description of Zones

Agro- ecological criteria was adopted for zonation (Figure1). However, some zones were combined since there were no physiognomic variations in species distribution. The zones have varying altitude ranges, temperatures and precipitation received. There was no clear delineation between the zones.

3.2.1 Upper zone

This is the high potential upper and lower highland areas (UHO and LH1) with high value cash crops such as Tea and dairy. The mean temperature range is 10 - 18° C and an altitude of 1420 m.a.s.l. Farmers also have relatively high yielding exotic dairy cattle like Friesian and Guernsey. The climate is humid with relatively high rainfall of up to 2000 mm. The zone is just adjacent to the tropical alpine climate in the forest. Farmers have very small farm sizes averaging 0.5 acres, hence cultivation is intensive. The riparian vegetation is cleared for farming and the natural regeneration of ITFs is very low. Fruits such as *Myrianthus holstii* are of significant economic value in the area since they are sold in the markets to supplement income to some families.

3.2.2 Middle zone

Middle zone comprises of the upper midland and part of lower midland area (UM I,ii,iii and LM iv). The mean temperature range is 18 - 21° C and an altitude of 1170 m.a.s.l. Farmers grow tobacco as cash a crop and keep cross and local breeds of cattle. Average farm size is 4 acres. The rainfall is relatively low compared with the upper zone. The riparian vegetation is conserved and the IFTs are different from those found in the upper zone. The IFTs are also common on-farm since they are a major source of income in the area. Fruits of *X. americana, C. spinarum* and *V. payos* are sold in the markets.

3.2.3 Lower zone

This is a low altitude area comprising of lower midland areas (LM4 and LM5). The area receives low rainfall. The mean temperature range is 21 - 27° C and an altitude of 780 m.a.s.l. Dry land crops such as millet, sorghum, cow peas and green grams are grown both for food and as cash crops. Local breeds of cattle and goats are kept in this area. The

farming is mainly extensive and bushes are common. Average farm size is 11 acres. The IFTs especially *Tamarindus indica* is very important in the area; tamarind is sold in large markets in Mombasa and Nairobi.

3.3 Species inventory

An inventory of indigenous fruit tree species was conducted along Rivers Ruguti and Tungu. Global Positioning System (GPS) was used to determine the coordinates for the midpoint of each quadrant of 50 x 50 m² (Table 1). Indigenous fruit tree and shrub species were identified within the 16 quadrants.

Table 1: Zones and sampling points

Zone	**sample sites**	**altitude range**	**rainfall range**	**temperature range**
Upper zone;	Ru1 S00.35518° E037.61189°	1494 -	1400 -	15-
	Ru2 S00.36346° E037.62290°	1340 m a.s. l.	2000 mm	20° C
	Tu1 S00.30767° E037.61433°			
	Tu2 S00.30825° E037.66176°			
	Tu3 S00.31198° E037.69774°.			
Middle zone	Rm1 S00.37749° E037.64166°	1265 -	800 -	18-
	Rm2 S00.39918° E037.68133°	1074 m a.s.l.	1750 mm	24° C
	Tm1 S00.32003° E037.71886°			
	Rm3 S00.40980° E037.70149°			
	Tm2 S00.30004° E037.74477°			
	Tm3 S00.30857° E037.78761°.			
Lower zone	Rl1 S00.40543° E037.73697°	867 -	400 -	21-
	Tl1 S00.33317° E037.83093°	682 m a.s.l.	1200 mm	27° C
	Tl2 S00.33239° E037.86745°			
	Rl2 S00.35027 E037.86935°			
	Tl3 S00.30342° E037.87453°.			

3.4 Phenological observations

The species under study were monitored for natural regeneration, flowering pattern, seeding pattern and seed collection for the experiments. The sampling points were at least 1 km apart.

3.4 Fruit harvesting

Fruits were harvested mature green and when ripe. All the fruits were picked from branches at low heights. However, the tall trees such as *M. holstii*, *T. indica* and *C. gomphophylla* necessitated climbing. The fruits of different species were harvested during different times since fruiting was not synchronous. Fruits were temporarily stored in perforated bags for 2-3 days to allow aeration while preventing fungal infection.

3.6 Seed extraction and sorting

The seeds were extracted at the Kenya Forestry Research Institute laboratories and at the Chuka University College tree nursery section for the laboratory and field germination experiments, respectively. Seeds of *M. holstii, V. payos, D. abyssinica,* and *R. natalensis* were squeezed through fingers. *Tamaindus indica* and *B. discolor* seeds were extracted by soaking fruits for 24 h in cold water while *C. gomphophylla* seeds were extracted through use of pliers to break the hard coat. Cleaning in cold water was done and any deformed, infested, infected or rotten seeds floating were discarded.

3.7 Determination of seed coat ratio

Ten seeds of each species from ripe fruits were dissected and the two components (testa and endosperm) were dried at 103° C for 17 h (ISTA, 1999). Their masses were then determined by use of weighing balance.

3.8 Experimentation

Seeds from mature green fruits were only used for experiments on dormancy, maturity stage, germination media and pretreatment effects on germination. Seeds from ripe fruits were used in similar experiments including determination of desiccation- tolerance and effects of storage temperature on seed longevity.

3.8.1 Research Design

Completely randomized design (CRD) was used. Homogenous media was achieved through sterilization in 1% solution of sodium hypochlorite (NaOHCl) prior to sowing.

3.8.2 Test for effects of maturity stage and seed dormancy

Seeds were subjected to the following pre-germination treatments in the incubator and in the field conditions:

Trt 1 – Seeds washed in cold water in case of chemical dormancy

Trt 2 – Seeds nipped in case of coat imposed dormancy

Trt 3 – Seeds Nipped then dipped in Gibberelic acid in case of embryo dormancy.

Trt 4 – Control (no pre-treatment)

Seeds were germinated in 1% agar solution as the medium in 4 replicates in a germination cabinet set at 25° -30° C in a completely randomized design. 25 seeds were used per replicate. Seeds were sterilized by soaking for 10 minutes in 1% sodium hypochlorite solution before germination. The germination process was monitored and recorded every 2 days (Appendix 2). A seed was considered to have germinated when the protrusion of the radicle was observed. The germinated seeds were discarded after counting. Seed germination was monitored until no further germination occurred.

3.8.3 Desiccation - Tolerance

Initial moisture content guided the determination of various target moisture contents (TMC) according to IPGRI protocol (Hong and Ellis 1996, Appendix 3). Target Seed Moisture Content for the species understudy varied in relation to their initial moisture content (Table 2)

Table 2: Desiccation levels

	Species						
	M. holstii	*T. indica*	*V. payos*	*C. gomphophylla*	*D. abyssinica*	*R. natalensis*	*B. discolor*
Moisture Content (%)	25%,	15%,	20%,	20%,	30%	30%	20%,
	20%,	12%,	15%,	15%	25%	25%	15%
	15%,	9%	9%,	9%,	20%	20%	9%,
	12%	6%	6%	6%,	15%	15%	6%,
	9%,	3%	3%,	3%.	10%	10%	3%.
	6%				5%	5%	

Anhydrous silica gel was used to desiccate the seeds (Hong and Ellis, 1998). The following formula was used to determine when the sample seeds had attained the targeted moisture content:

Weight of seed (g) at TMC = (100-Initial MC %) x initial Weight of seed

(100-TMC %)

The TMC was expressed on the wet basis (%w.b) and reported to the nearest 0.1% (Hong and Ellis, 1996). Once the TMC was achieved, germination tests were carried out.

3.8.4 Effects of storage conditions

Seeds at each target moisture content were stored in air tight plastic containers at the following temperatures: 25° C, 0° C, -10° C and -20° C. After three months of storage, germination test was carried out in KEFRI laboratory at room temperature (18 - 25° C).

3.9 Statistical analysis

Data was analyzed using Wink's Statistical Data Analysis (Elliot, 2007). ANOVA was conducted on percentage germination to determine the difference between the pre-germination treatments, desiccation levels and storage temperatures. Least significance difference (LSD) at $P<0.05$ was used to test for significant differences among treatment means.

CHAPTER FOUR

RESULTS

4.1 Relationship between precipitation and seed shedding

4.1.1 Rainfall received and distribution

The total annual rainfall received was 1330mm in the upper zone, 1210 mm in the middle zone and 897 mm in the lower zone. On average, the highest amount of rainfall (271.43 mm) was received in April. Rainfall was distributed in all the months except in September (Figure 2)

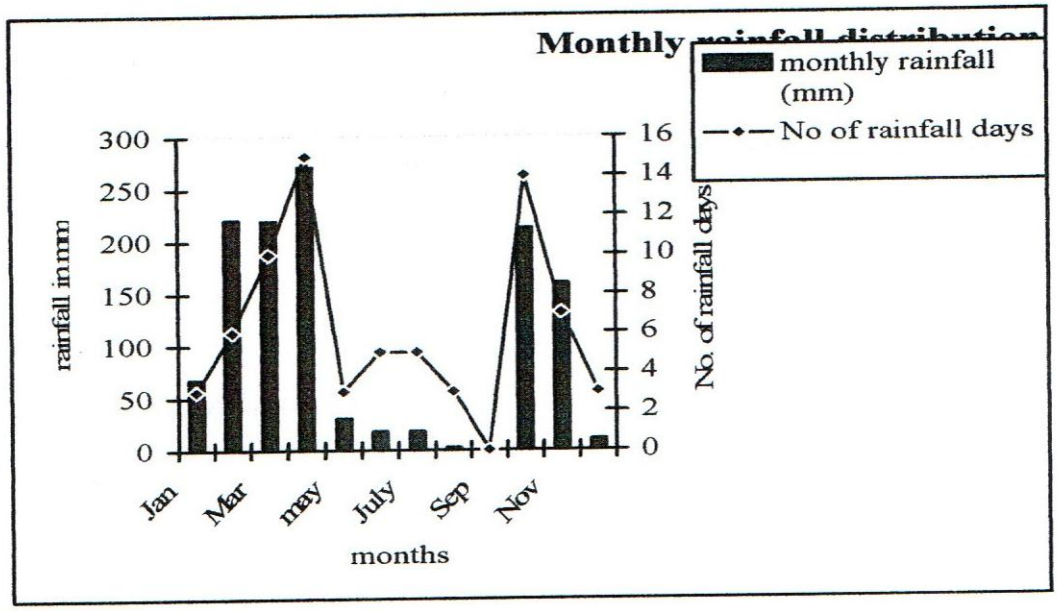


Figure 2: Rainfall received and duration

4.1.2 Species distribution along the transects

Nineteen fruit tree and shrub species were identified along the transects. Forty two percent of the species observed had few saplings (<5) as understorey component while 36.84% had no saplings. Twenty one percent had >5 saplings (Table 3). *Myrianthus holstii* and *B. discolor* were only found in the upper and lower zones respectively while *T. indica* and *C. gomphophylla* were absent in the upper zone but grew in the middle and lower zones (Table 4).

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Table 3: Natural regeneration of fruit tree/shrub species

Number of saplings	Species (%)
0	36.84
≤ 5	42.10
≥ 5	21.05

0. - No saplings observed, (≤ 5) - Few saplings observed and (>5) - Many saplings observed

Table 4: Regeneration of species under study.

Species	Zone					
	Upper		Middle		Lower	
	Mature Tree	Understorey	Mature Tree	Understorey	Mature Tree	Understorey
Myrianthus holstii	3	0	0	0	0	0
Dovyalis abyssinica	3	1	1	1	0	0
Tamarindus indica	0	0	5	3	5	3
Vitex payos	1	0	5	1	4	1
Celtis gomphophylla	0	0	4	3	2	0
Rhus natalensis	5	1	4	1	0	0
Berchemia discolor	0	0	0	0	4	3

0. - No saplings, 1. - ≤ 5 saplings and 3. - >5 saplings observed

4.1.3 Fruit (seed) development

Flowering and seeding occurred at different times of the year. Flowering was rated as fair or good. *Myrianthus holstii, D. abyssinica, C. gomphophylla* and *B. discolor* seeding rate was lower than their flowering rate while seeding and flowering rates of *V.payos T. indica* and *R. natalensis* were similar (Table 5). Seed shedding by *M. holstii, D. abyssinica* and *R. natalensis* coincided with onset of the rainfall season while other species shed their seeds during a dry spell (Figure 3).

Table 5: Species Flowering and seeding

Species	Flowering		Seeding	
	Period	rating (%)	Period	rating (%)
M. holstii	December- January	2	March – April	1
D. abysinica	August – September	3	November – December	1
T. indica	February- March	2	August –October	2
V. payos	November- Dec.	3	May – June	3
C. gomphophylla	August – October	3	December - January	2
R. natalensis	December - March	3	April – July	3
B. discolor	October - Dec	3	February – May	2

Rating; 1. <40 % - Poor, 2 -40-60 % - Fair and 3. >60 %-Good

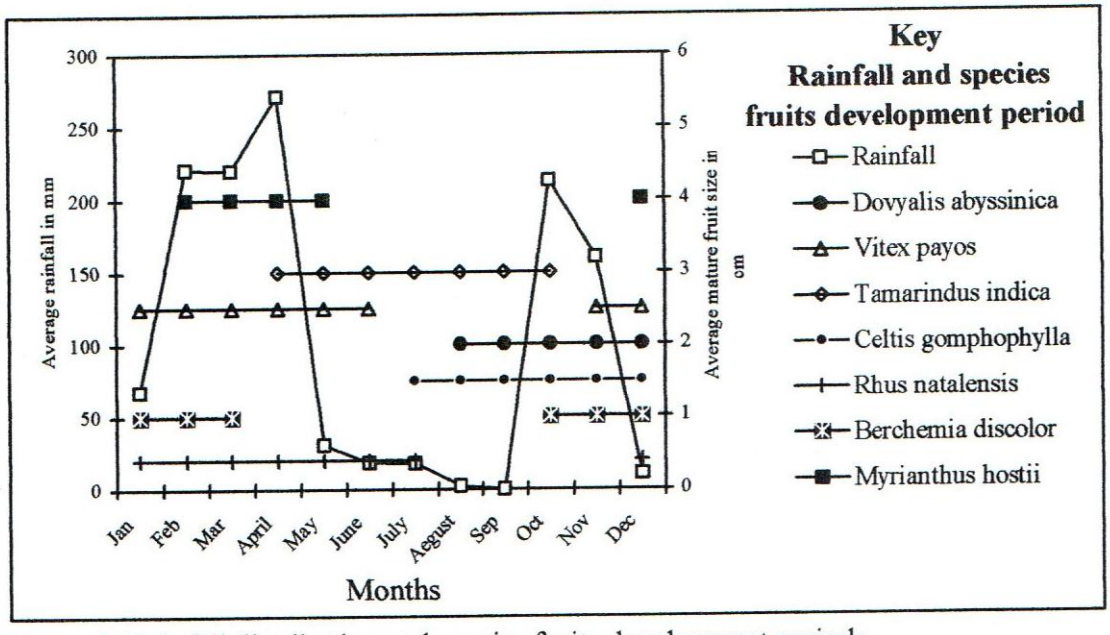


Figure 3: Rainfall distribution and species fruits development periods

4.1.4 Seed coat ratio

The ratio of the testa mass to the total mass of the seed (Seed coat ratio) was determined as *M. holstii*, *D. abyssinica*, *T. indica*, *C. gomphophylla* and *R. natalensis* had SCR ≤ 0.5 while that of *V. payos*, and *B. discolor* was ≥ 0.5 (Table 6).

Table 6. Relationship between components of seed morphology

Seed Component	weight (g)						
	M. holstii	*D. abyssinica*	*T. indica*	*V. payos*	*C. gomphophylla*	*R. natalensis*	*B. discolor*
Fruit	1.25±0.35	2.17±0.41	1.36±0.19	1.69±0.32	2.53±0.50	0.90±.52	2.31±0.42
Seed mass	0.56± 0.17	0.03± 0.015	0.71± 0.21	0.95± 0.21	0.06± 0.013	0.25±0.12	0.46±0.24
Seed testa	0.09± 0.03	0.01±0.007	0.24±0.07	0.67±0.15	0.04±0.009	0.11±0.04	0.31±0.16
endosperm and embryo	0.46± 0.14	0.02± .008	0.48±0.14	0.33±0.06	0.02±0.004	0.14±0.08	0.16±0.08
SCR	0.17	0.33	0.33	0.71	0.67	0.44	0.67

4.2 Effects of maturity stage, dormancy and medium on seed germination

4.2.1 Myrianthus holstii

Germination of seeds on agar medium started from day 4 and ceased between days 12 - 18. Nipped seeds started germinating at day 4 while washed and the control seeds commenced germination at day 6 for seeds from both Green mature and ripe fruits (Figure 4 and 5). Highest germination was recorded between days 6 - 8 for nipped seeds and 10-14 for washed and the control seeds. Germination in soil medium started on day 10. Maximum germination was recorded between days 12-14 for all the treatments (Figure 6).

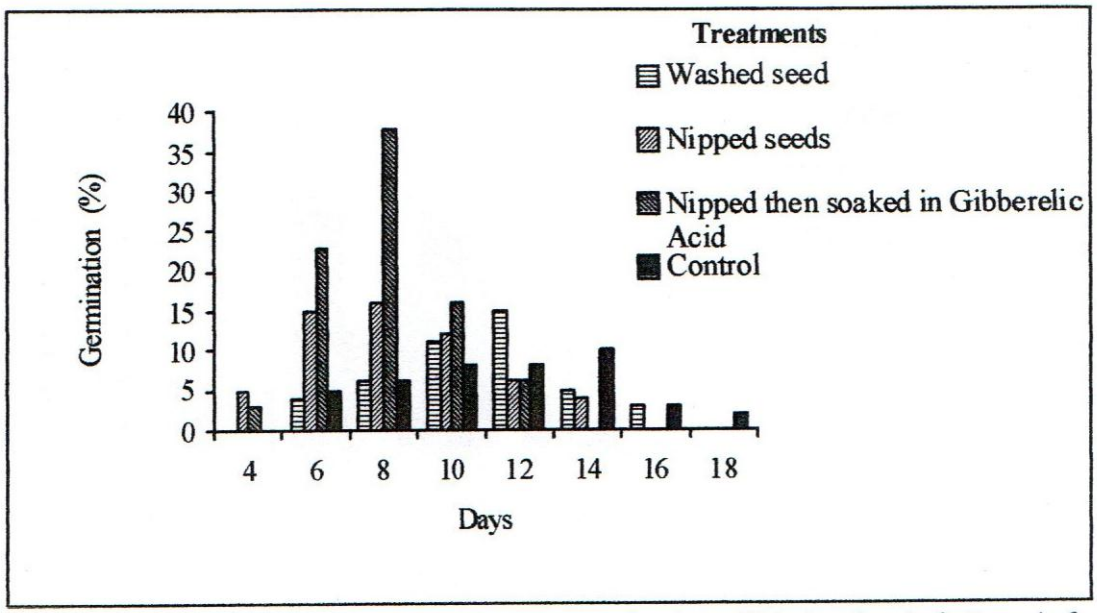


Figure 4. Effects of pre-treatments on time to germination of *Myrianthus holstii* seeds from mature green fruits in agar as medium

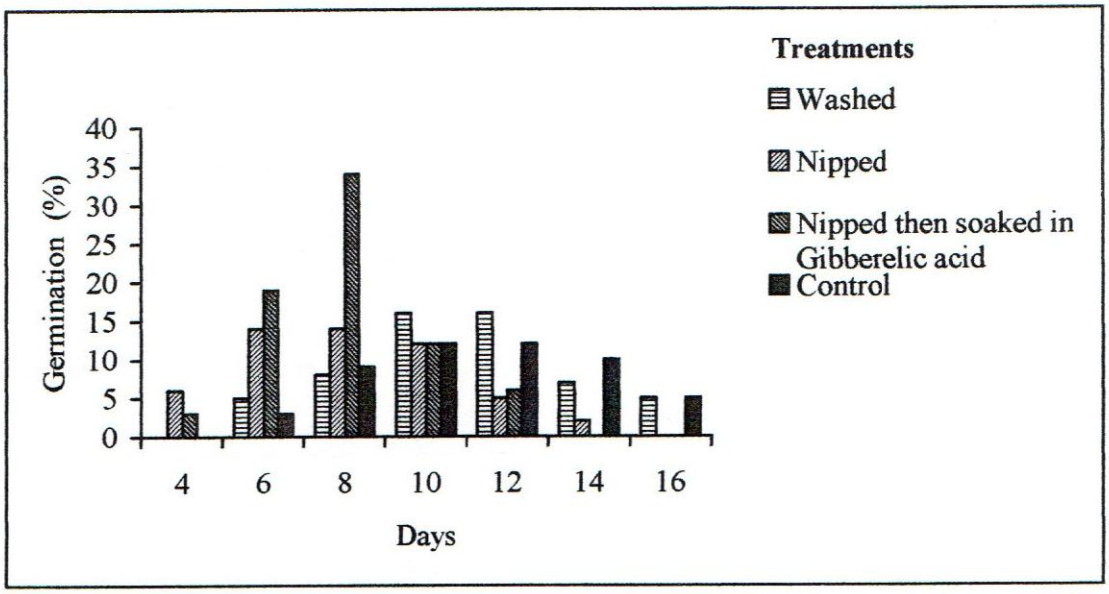


Figure 5. Effects of pre-treatments on time to germination of *Myrianthus holstii* seeds from ripe fruits in agar as medium

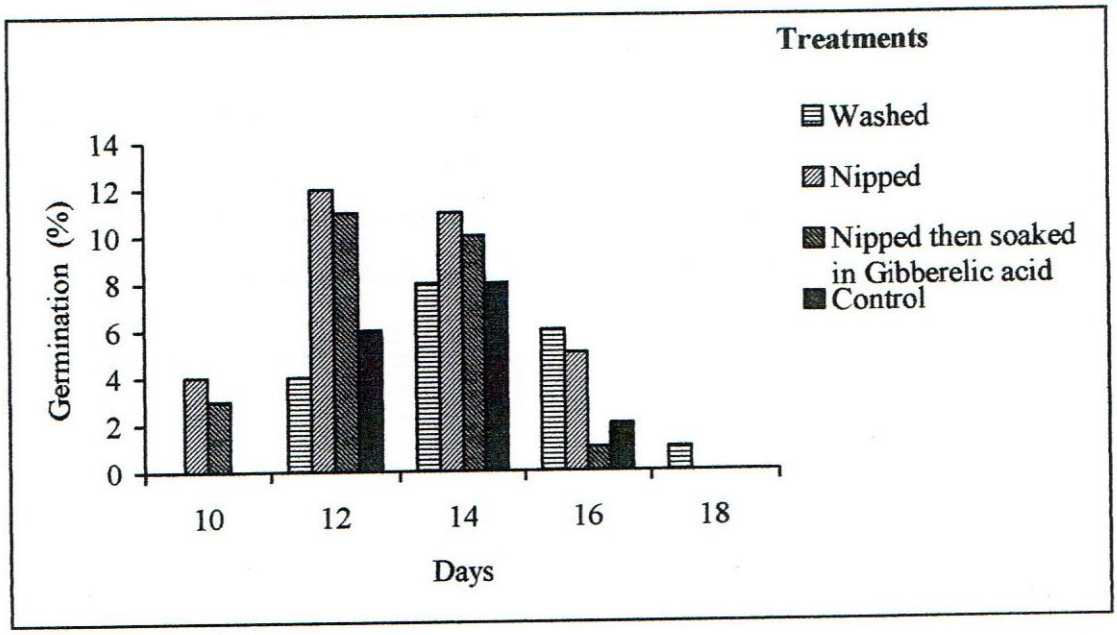


Figure 6. Effects of pre-treatments on time to germination of *Myrianthus holstii* seeds from ripe fruits in soil asmedium

Overall germination was superior (P <0.05) in agar as medium (Table 7). Seed germination also decreased (P<0.05) with seed maturity. Nipping and nipping then dipping in gibberelic acid improved (P <0.05) germination of seeds from mature green fruits.

Table 7: Effects of pre-treatments, seed source and germination medium on germination of *Myrianthus holstii* seeds

Pre-treatments	Maturity stage		medium	
		Agar	agar	Soil
1	G	38		
	R		51	17
2	G	57		
	R		42	26
3	G	76		
	R		58	24
4	G	41		
	R		47	18
LSD (P<0.05) within		2.45	2.34	2.12
LSD (P<0.05) between			17.15	

1. Seeds washed in tap water, 2. Seeds nipped, 3. Seeds nipped then soaked in Gibberelic acid, 4. Control; G, seeds from mature green fruits and R, seeds from ripe fruits.

4.2.2. Dovyalis abyssinica

Germination was recorded from day 4 and was maximum at day 6 for all the treatments on agar medium (Figures 7 and 8). Washed and the control seeds completed germination 2 days after other treatments. Germination on soil medium commenced on day 6 and was maximum at day 8 for all the treatments. It was completed at day 12 (Figure 9).

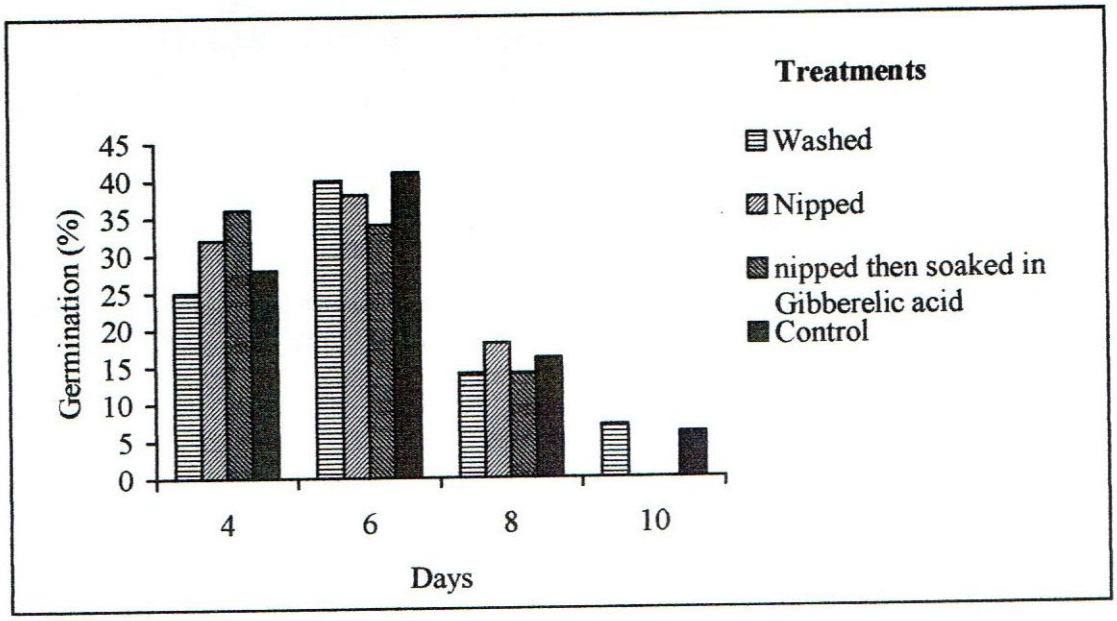


Figure 7. Effects of pre-treatments on time to germination of *Dovyalis abyssinica* seeds from Mature Green fruits in agar asmedium

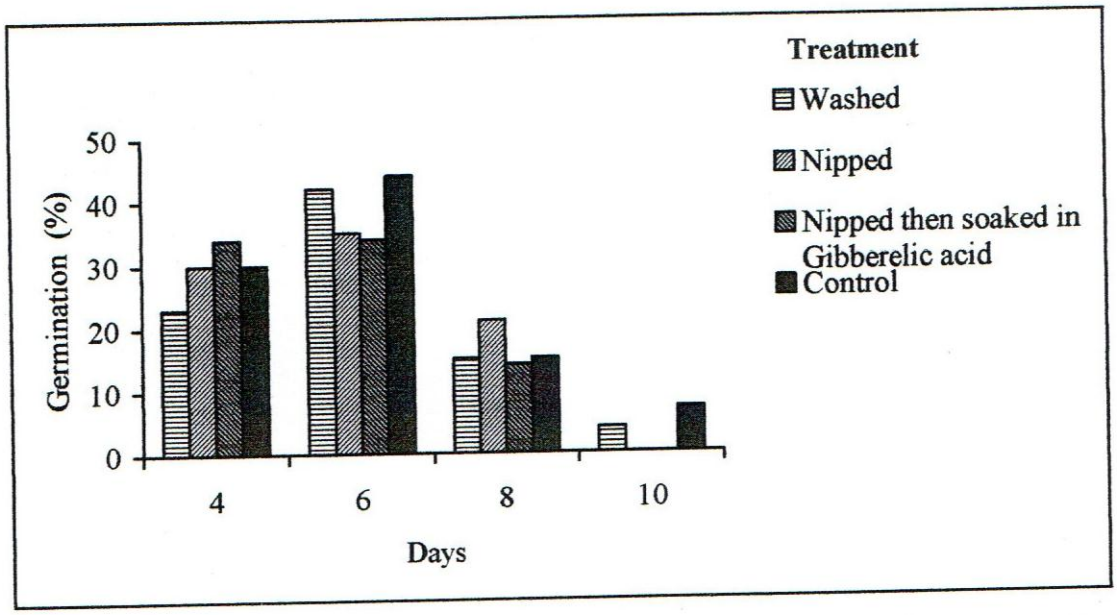


Figure 8. Effects of pre-treatments on time to germination of *Dovyalis abyssinica* seeds from ripe fruits in agar as medium

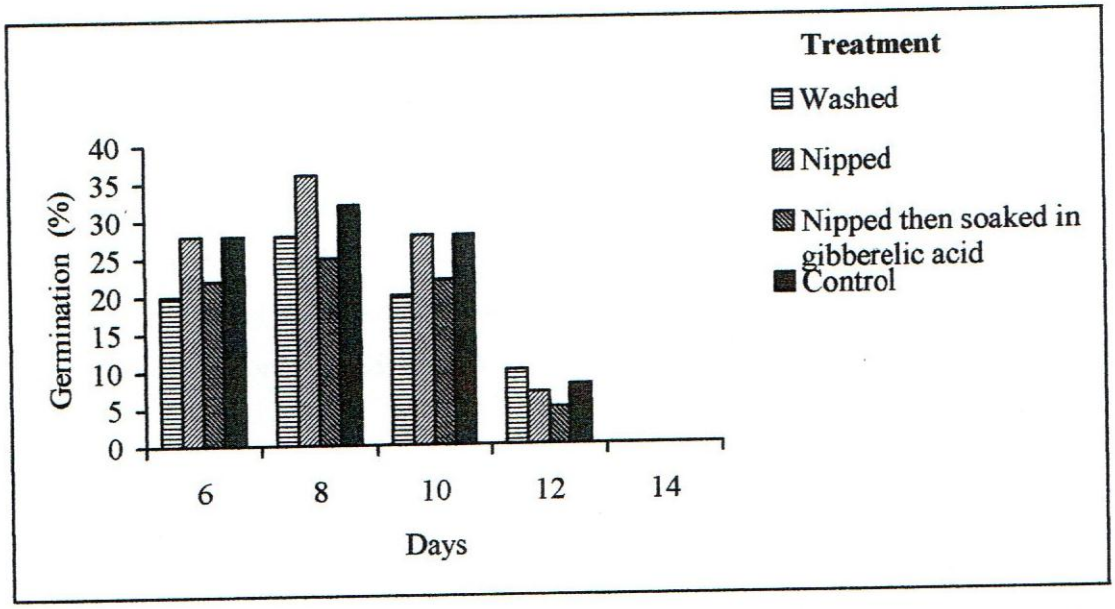


Figure 9. Effects of pre-treatments on time to germination of *Dovyalis abyssinica* seeds from ripe fruits in soil as medium

Overall, germination was lower ($P < 0.05$) in soil as the medium. Pre-treatments also tended to depress germination. There was no seed maturity effects on germination (Table 8).

Table 8: Effects of pre-treatments, seed source and germination medium on germination of *Dovyalis abyssinica* seeds.

Pre-treatments	Maturity stage		medium	
		Agar	agar	Soil
1	G	86		
	R		84	68
2	G	88		
	R		86	58
3	G	84		
	R		82	62
4	G	90		
	R		96	71
LSD (P<0.05) within		3.9	2.27	3.23
LSD (P<0.05) between			8.24	

1. Seeds washed in tap water, 2. Seeds nipped, 3. Seeds nipped then soaked in Gibberelic acid, 4. Control; G, seeds from green mature fruits and R, seeds from ripe fruits.

4.2.3 Tamarindus indica

Germination of seeds from green mature fruits in agar for nipped and nipped then soaked in gibberelic acid seeds started at day 6 while other treatments started germinating at day 12 for the seed from green mature fruits (Figure 10). Seeds that were nipped then soaked in gibberelic acid had the highest germination at day 8 while those nipped had the highest germination at day 10. Maximum germination for washed and the control seeds was between days 14 and 16. Treatments with nipped seeds completed germination on day 16 while washed and control seeds completed germination at day 24 for the treatments in agar medium

Seeds from mature ripe fruits in agar commenced germination at day 10 while control seeds started germination at day 14. Germination of all seeds was complete at day 28. Germination was highest at day 12 for nipped then soaked in gibberelic acid, day 14 for nipped, day 18 for washed and day 22 for control seeds (Figure11). Germination of seeds in soil started at day 12 and was completed by day 22; highest germination was recorded between days 14 and 16 for all treatments (Figure 12).

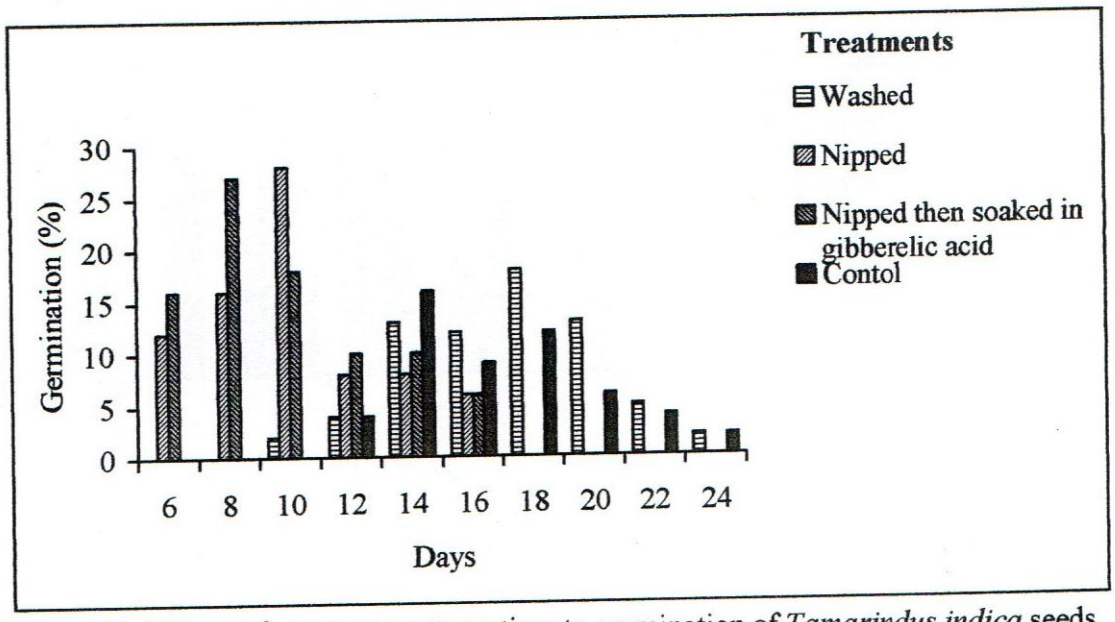


Figure 10. Effects of pre-treatments on time to germination of *Tamarindus indica* seeds from mature green fruits in agar as medium

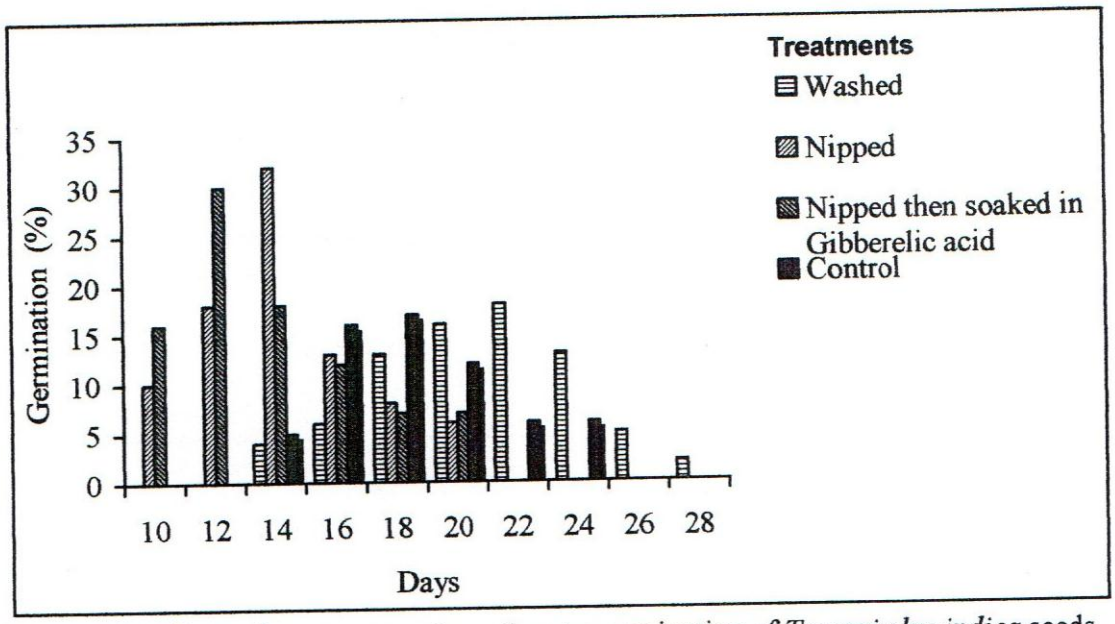


Figure 11. Effects of pre-treatments on time to germination of *Tamarindus indica* seeds from ripe fruits in agar as medium

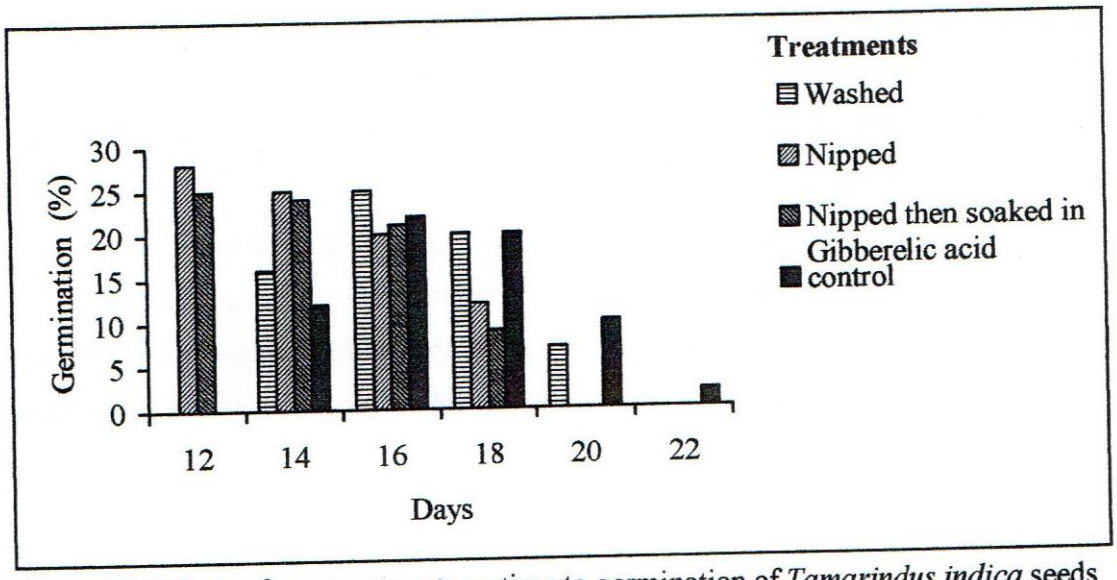


Figure 12. Effects of pre-treatments on time to germination of *Tamarindus indica* seeds from ripe fruits in soil as medium

Germination of *T. Indica* seed was generally similar in both media. Pre-treatment generally improved seed germination ($P<0.05$) which was most marked in the nipping and nipping then soaking in gibberelic acid treatments. There was no seed maturity effects on seed germination (Table 9).

Table 9: Effects of pre-treatments, seed source and germination medium on germination of *Tamarindus indica* seeds.

Pre-treatments	Maturity stage	medium		
		Agar	agar	Soil
1	G	63		
	R		71	68
2	G	68		
	R		87	85
3	G	87		
	R		90	82
4	G	59		
	R		68	64
LSD ($P<0.05$) within		3.12	4.33	3.38
LSD ($P<0.05$) between			18.47	

1. Seeds washed in tap water, 2. Seeds nipped, 3. Seeds nipped then soaked in Gibberelic acid, 4. Control; G, seeds from green mature fruits and R, seeds from ripe fruits.

4.2.4 Vitex Payos

Germination in agar was recorded from day 6 for seeds that were nipped or nipped then soaked in Gibberelic acid and day 8 for washed and control seeds from both Green mature and mature ripe fruits (Figures 13 and 14). Highest germination was recorded on day 8 for nipped then soaked in gibberelic acid seeds, day 10 for nipped seeds and days 12 and 14 for washed and control seeds. Germination in soil was recorded from day 14 for nipped seeds and day 14 for washed and control seeds. Highest germination for treatments with nipped seeds was at day 16 and at day 20 for washed and control seeds (Figure 15).

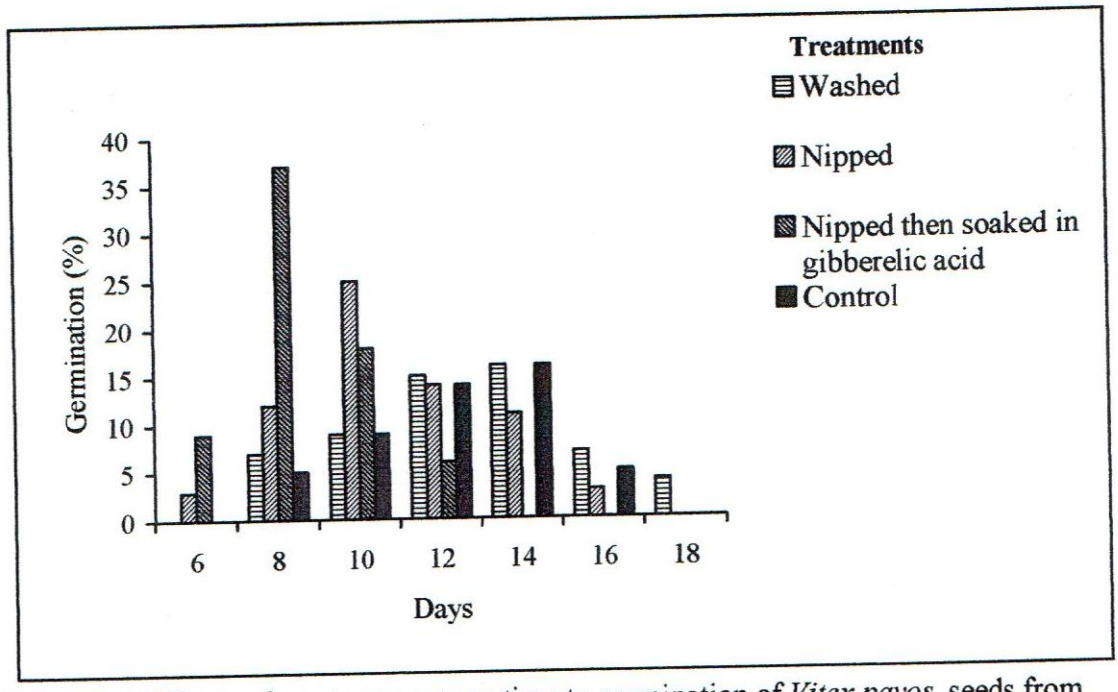


Figure 13. Effects of pre-treatments on time to germination of *Vitex payos* seeds from Mature Green fruits in agar amedium

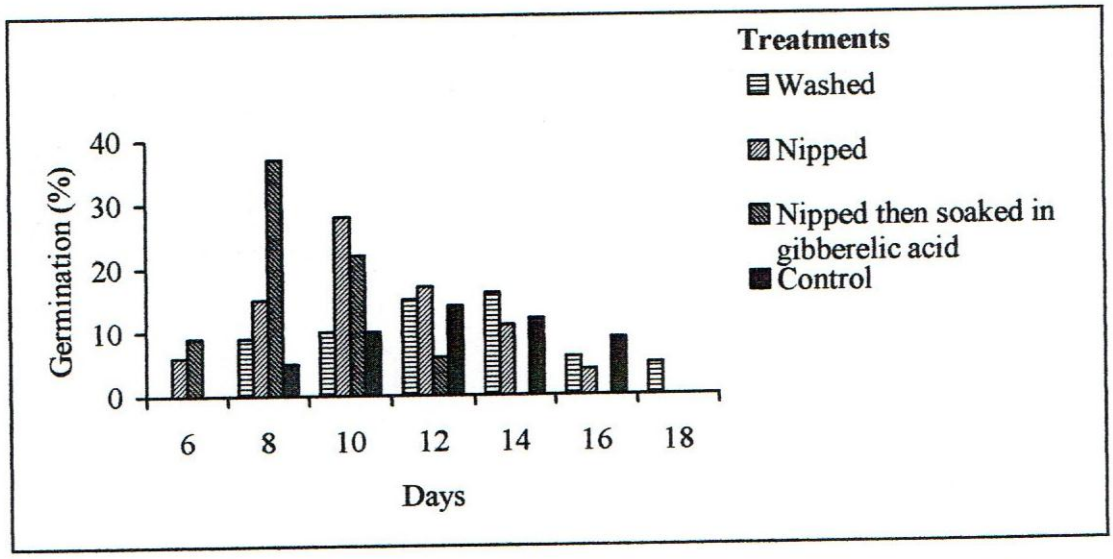


Figure 14. Effects of pre-treatments on time to germination of *Vitex payos* seeds from ripe fruits in agar as medium.

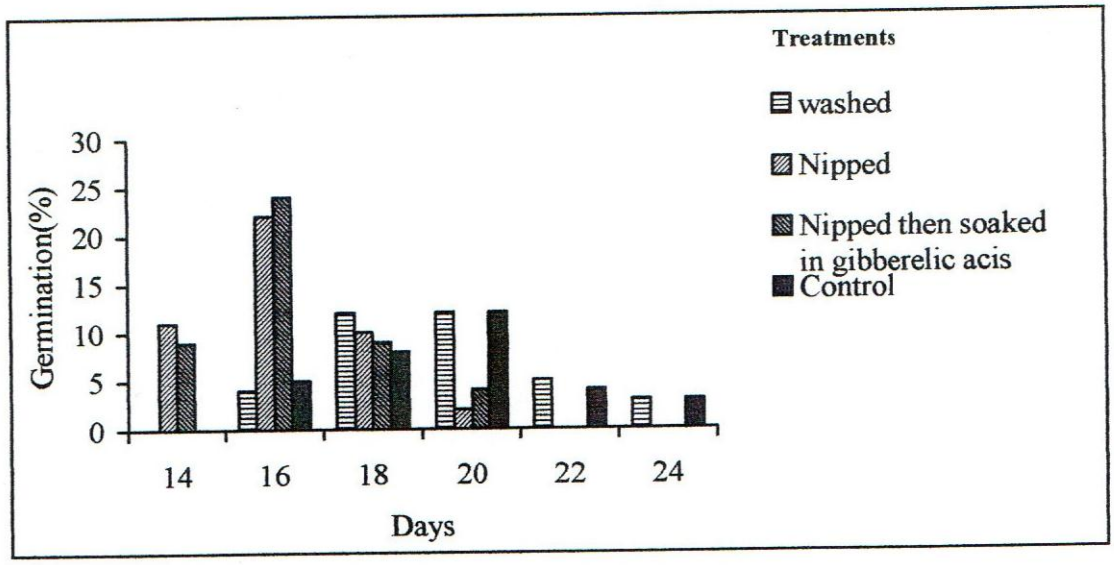


Figure 15. Effects of pre-treatments on time to germination of *Vitex payos* seeds from ripe fruits in soil amedium

Germination was lower in soil as the germination medium. Nipping or nipping and soaking in gibberelic acid improved ($P<0.05$) germination (Table 10). There was no seed maturity effects on germination in this species.

Table 10: Effects of pre-treatments, seed source and germination medium on germination of *Vitex payos* seeds.

Treatments	Maturity stage		medium	
		Agar	agar	Soil
1	G	50		
	R		54	34
2	G	68		
	R		77	45
3	G	70		
	R		74	46
4	G	49		
	R		50	32
LSD (P<0.05) within		3.41	3.58	3.69
LSD (P<0.05) between			17.73	

1. Seeds washed in tap water, 2. Seeds nipped, 3. Seeds nipped then soaked in Gibberelic acid, 4. Control; G, seeds from green mature fruits and R, seeds from ripe fruits.

4.2.5 Celtis gomphophylla

Germination in agar for seeds from green mature and mature ripe fruits commenced from day 4 and 6, respectively (Figures 16 and 17), and was completed at day 16. Germination was highest at day 8 – 12. Germination started at day 14 in soil and was complete on day 22. Highest germination was at day 16 and 18. Nipped seeds commenced germination 2 days earlier than other treatments (Figure 18).

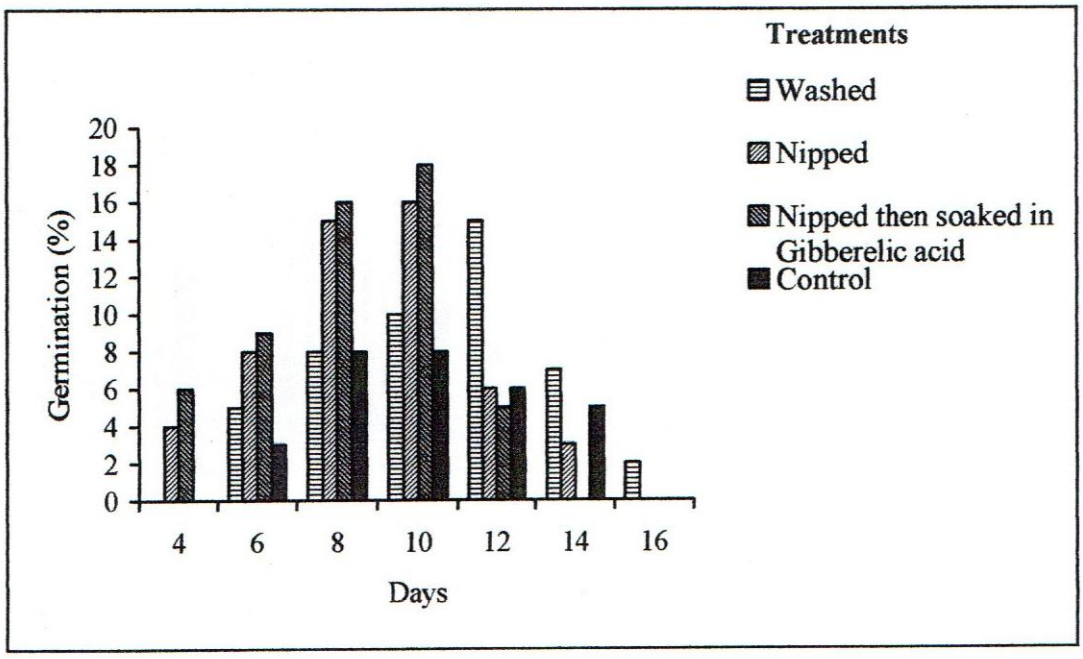


Figure 16. Effects of pre-treatments on time to germination of *Celtis gomphophylla* seeds from Mature Green fruits in agar as medium

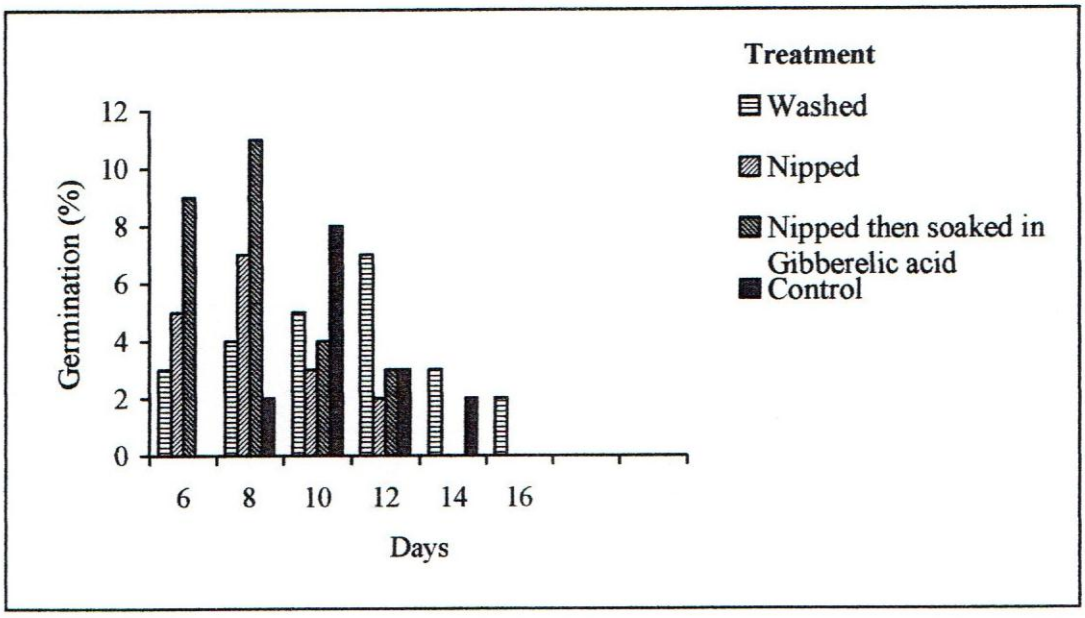


Figure 17. Effects of pre-treatments on time to germination of *Celtis gomphophylla* seeds from ripe fruits in agar a medium

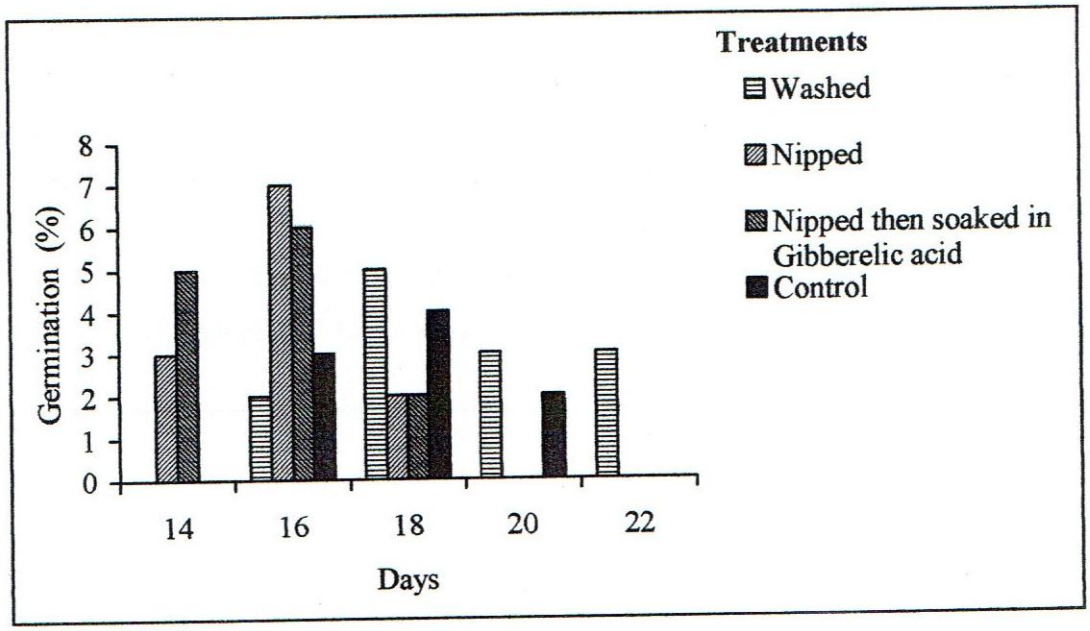


Figure 18. Effects of pre-treatments on time to germination of *Celtis gomphophylla* seeds from ripe fruits on soil as medium

Overall, germination was lower ($P<0.05$) in soil. Germination also decreased ($P<0.05$) with seed maturity. Nipping seeds from ripe fruits depressed germination in agar (Table 11).

Table 11: Effects of pre-treatments, seed source and germination medium on germination of *Celtis gomphophylla* seeds.

Treatments	Maturity stage	medium		
		Agar	agar	Soil
1	G	47		
	R		24	13
2	G	44		
	R		17	12
3	G	52		
	R		26	16
4	G	30		
	R		15	9
LSD ($P<0.05$) within		3.63	2.46	1.78
LSD ($P<0.05$) between			10.35	

1. Seeds washed in tap water, 2. Seeds nipped, 3. Seeds nipped then soaked in Gibberelic acid, 4. Control; G, seeds from green mature fruits and R, seeds from ripe fruits.

4.2.6 Rhus natalensis

Germination was recorded from day 4 and was highest in day 6 for all the treatments on agar (Figures 19 and 20). Germination in soil commenced in day 4 and was highest at day 6 for nipped and nipped then soaked in gibberelic acid seeds, day 8 for washed seeds and day 10 for the control treatment. Germination was complete at day 16 and 12 for control and washed seeds respectively. Other treatments completed germination at day 10. (Figure 21).

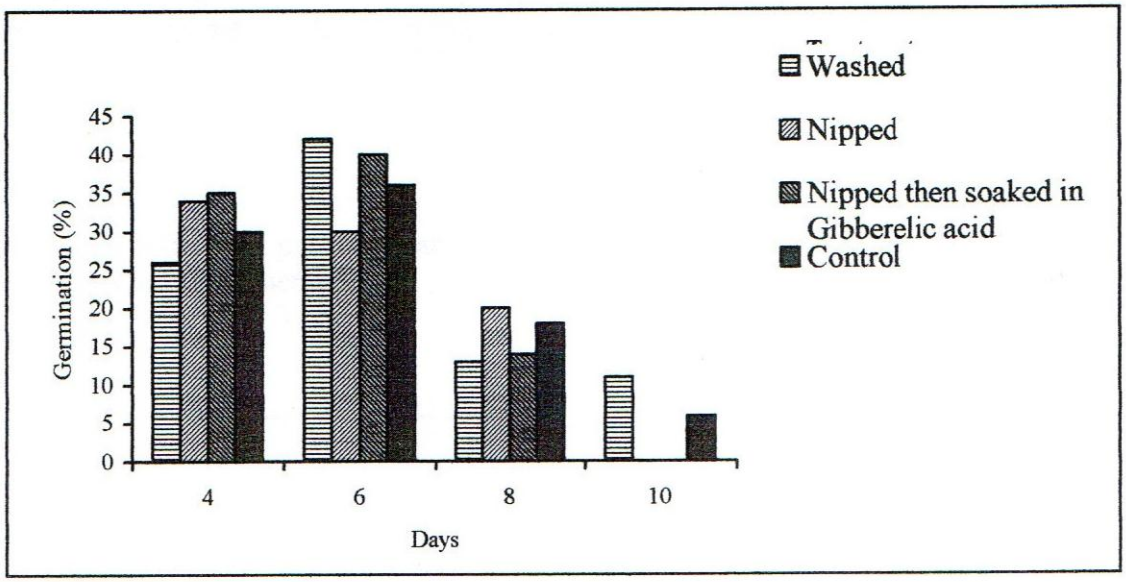


Figure 19. Effects of pre-treatments on time to germination of *Rhus natalensis* seeds from Mature Green fruits in agar as medium

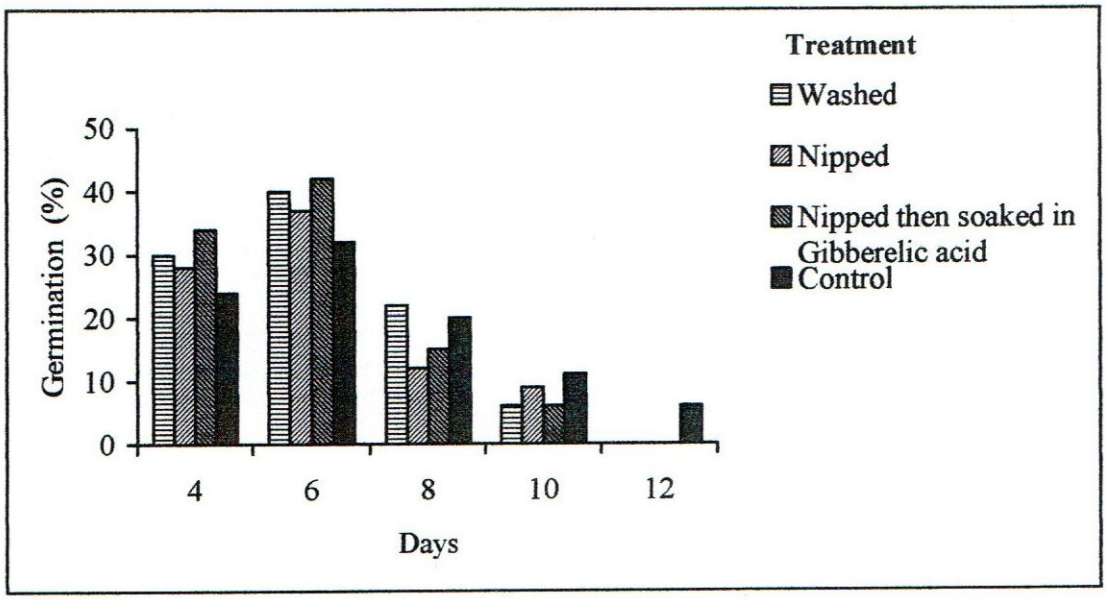


Figure 20. Effects of pre-treatment on time to germination of *Rhus natalensis* seeds from ripe fruits in agar as medium

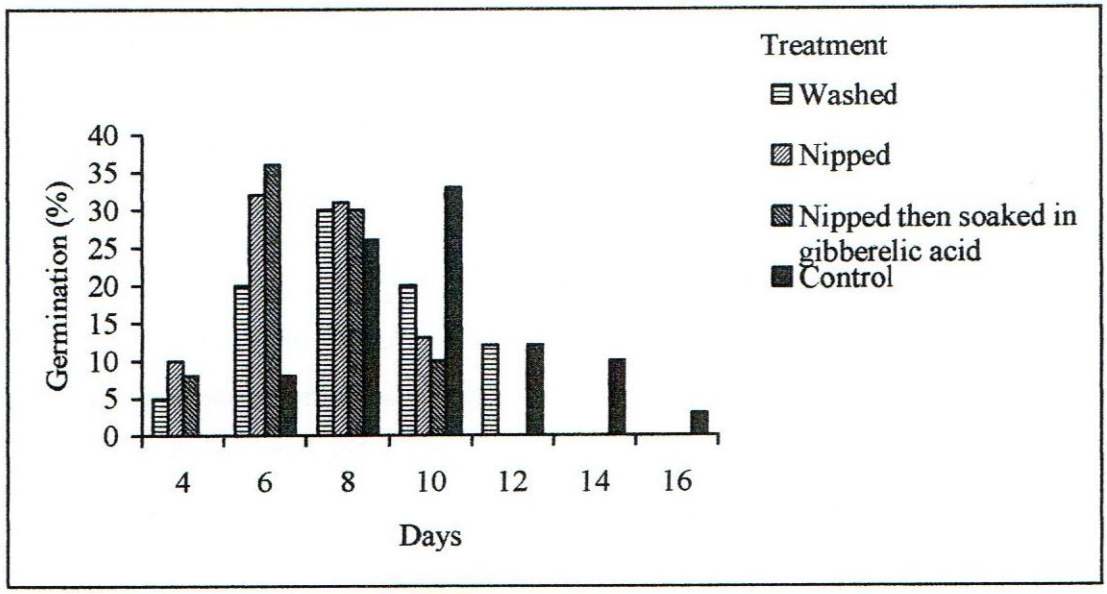


Figure 21. Effects of pre-treatments on time to germination of *Rhus natalensis* seeds from ripe fruits in soil as medium

Germination was similar ($P<0.05$) in all the media. Pre-treatment and seed maturity also had no effects on germination (Table 12).

Table 12: Effects of pre-treatments, seed source and germination medium on germination of *Rhus natalensis* seeds.

Treatments	Maturity stage		medium	
		Agar	agar	Soil
1	G	92		
	R		95	90
2	G	84		
	R		90	86
3	G	89		
	R		92	84
4	G	90		
	R		88	92
LSD (P<0.05) within		2.07	2.07	2.04
LSD (P<0.05) between			5.37	

1. Seeds washed in tap water, 2. Seeds nipped, 3. Seeds nipped then soaked in Gibberelic acid, 4. Control; G, seeds from green mature fruits and R, seeds from ripe fruits.

4.2.7 Berchemia discolor

Germination of seeds from green fruits was recorded from day 4 and was highest at day 6 for nipped and nipped then soaked in gibberelic acid and at day 10 for washed and the control treatments. Seeds from ripe fruits commenced germination at day 4 and completed in day 14. Germination was highest at days 8 and 10 for washed and the control treatments respectively. Other treatments completed germination by day 8 (Figures 22 and 23). Germination in soil commenced at day 6; at was highest at day 8 for nipped and nipped then soaked in gibberelic acid and at day 12 for the washed and control treatments. Germination was completed on day 16 for control and washed treatments. Other treatments completed germination on day 12. (Figure 24).

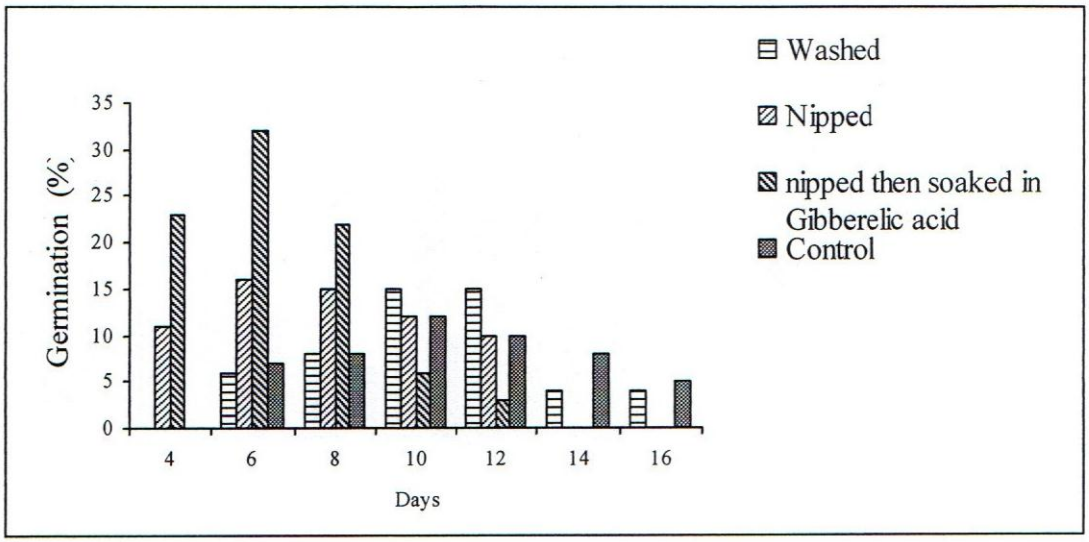


Figure 22. Effects of pre-treatments on time to germination of *Berchemia discolor* seeds from Mature Green fruits in agar as medium

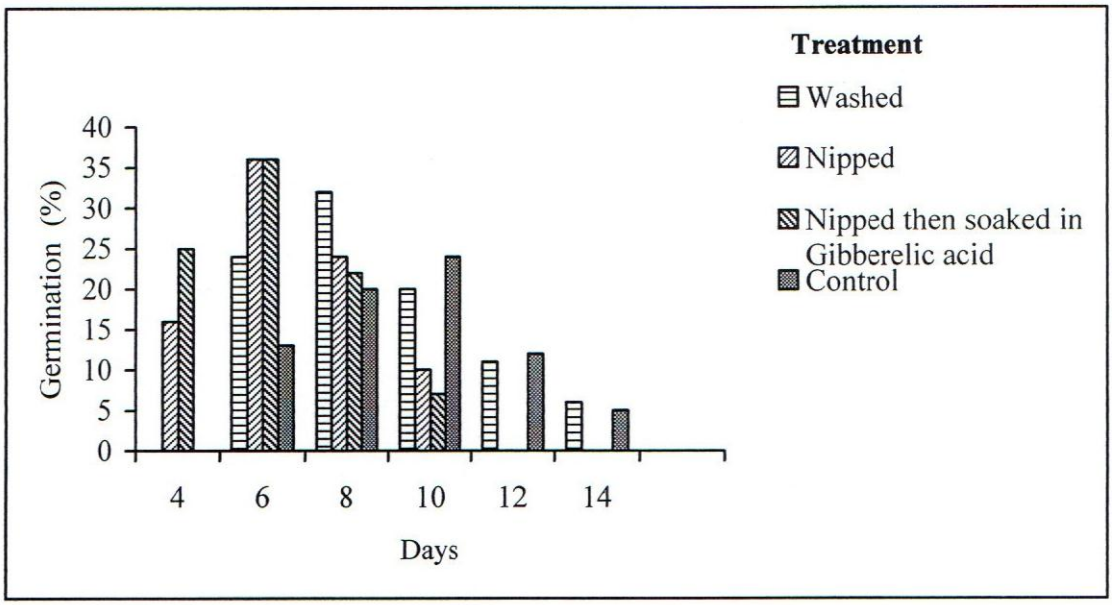


Figure 23. Effects of pre-treatments on time to germination of *Berchemia discolor* seeds from ripe fruits in agar as medium

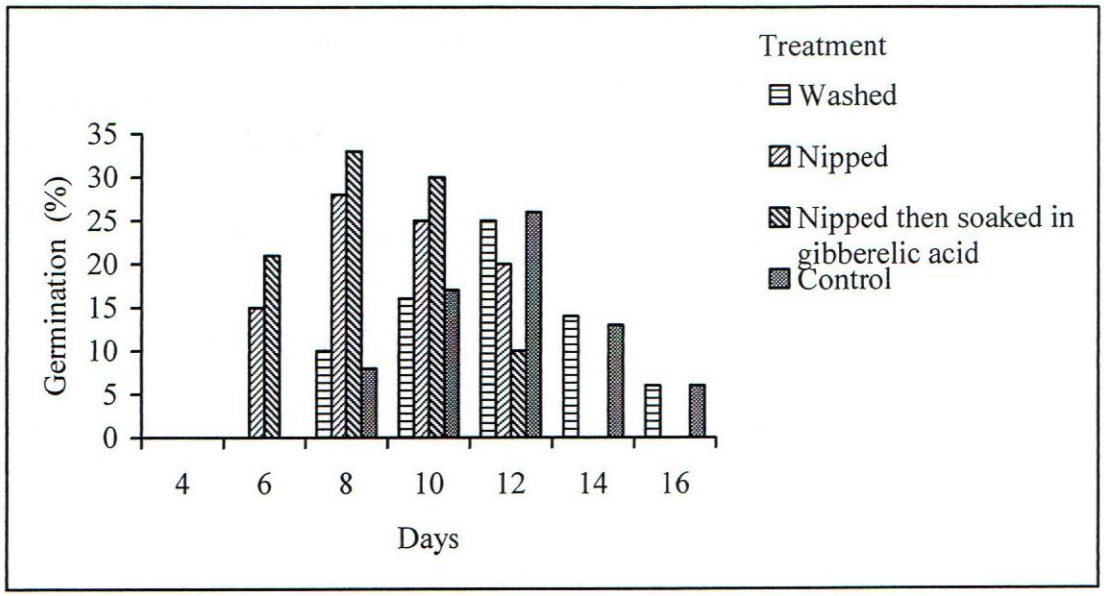


Figure 24. Effects of pre-treatments on time to germination of *Berchemia discolor* seeds from ripe fruits in soil as medium

Pre-treatment generally improved ($P < 0.05$) germination of seeds in all media (Table 13). Germination was also higher for seeds from ripe fruits compared with those from green fruits. Similarly, there were no effects of media on germination

Table 13: Effects of pre-treatments, seed source and germination medium on germination of *Berchemia discolor* seeds.

Pre-treatments	Maturity stage		medium	
		Agar	agar	Soil
1	G	58		
	R		78	71
2	G	64		
	R		85	82
3	G	86		
	R		90	88
4	G	56		
	R		74	68
LSD (P<0.05) within		3.74	2.56	2.70
LSD (P<0.05) between			16.71	

1. Seeds washed in tap water, 2. Seeds nipped, 3. Seeds nipped then soaked in Gibberelic acid, 4. Control; G, seeds from green mature fruits and R, seeds from ripe fruits.

4.3 Desiccation Tolerance

4.3.1 Myrianthus holstii

Seeds at 27.8% moisture content started germination at day 6 while those at moisture content of 19.6% and 15.3% germinated at day 8 and those at moisture content 10.9%, 8.5% and 6.2% started at day 10 (Figure. 25). Highest germination was recorded between days 10 and 14 for all the treatments.

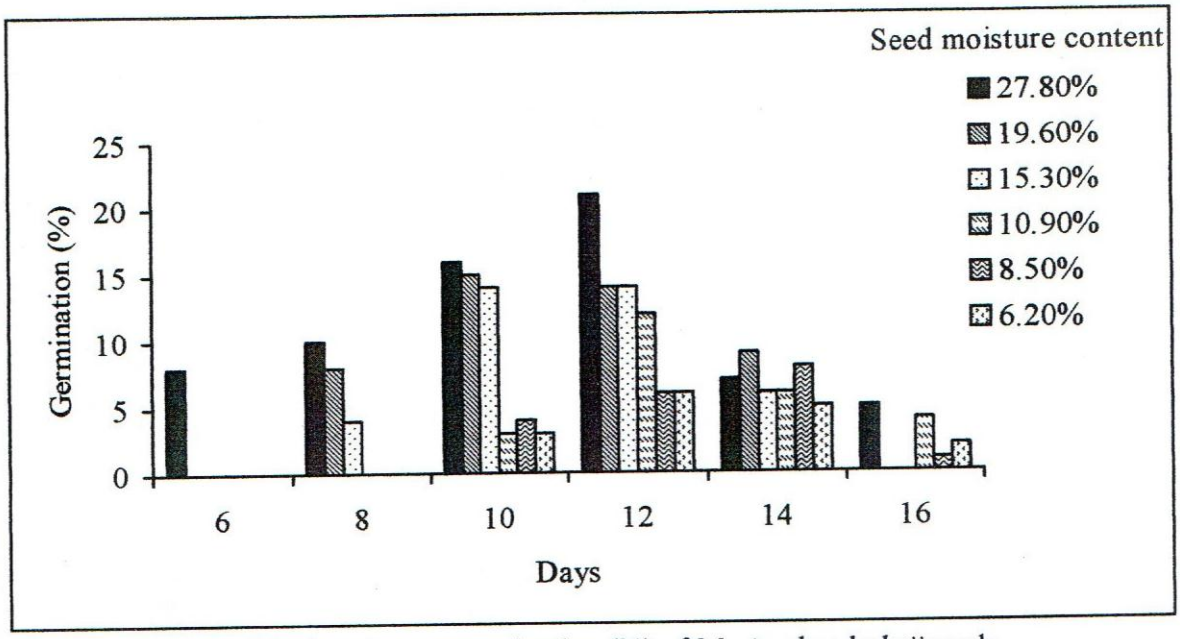


Figure 25. Effects of desiccation on germination (%) of *Myrianthus holstii* seeds.

Drying depressed ($P < 0.05$) germination. Drying seeds to between 10.9 % - 6.2 % moisture content had similar germination percentage which was significantly lower ($P < 0.05$) than those dried to between 19.6 % and 15.3 %. Highest germination was recorded for seeds at the initial (27.8 %) moisture content (Table 14).

Table 14: Effects of desiccation on germination of *Myrianthus holstii* seeds

TSMC	Attained SMC (%)	Germination (%)
27.8[a]	27.8	67
20,	19.6	45
15,	15.3	39
12,	10.9	27
9,	8.5	19
6	6.2	16
LSD (P<0.05)		2.66

[a] Initial seed moisture content

4.3.2 Dovyalis abyssinica

Germination started at day 3 for seeds dried to moisture content 32.4%-19.1% and day 6 for seeds dried between 15.8% and 5.2% (Figure 26). Highest germination was recorded between day 6 and 8 for all treatments and germination was complete at day 12.

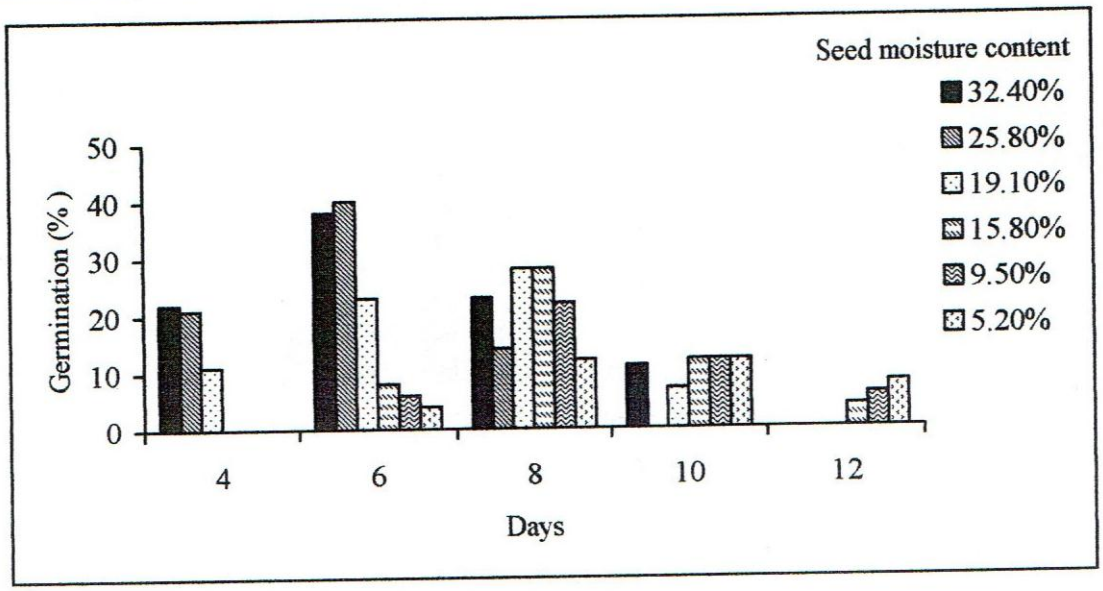


Figure 26. Effects of desiccation on germination (%) of *Dovyalis abyssinica* seeds.

Germination declined ($P < 0.05$) with loss of seed moisture content Seeds dried to 5.2% seed moisture content had the lowest germination percentage (Table 15).

Table 15: Effects of desiccation on germination of *Dovyalis abyssinica* seed

TSMC	Attained SMC (%)	Germination (%)
32.4[a],	32.4	94
25,	25.8	76
20,	19.1	69
15,	15.8	52
10,	9. 5	46
5	5.2	26
LSD (P<0.05)		3.32

[a] Initial seed moisture content

4.3.3. Tarmarindus indica

Seeds at 18.7% and 14.8% moisture contents started germination at day 14 while in other treatments germination commenced on day 16 and was complete at day 24. (Figure 27). Highest germination was recorded between days 18 – 20 for all the treatments.

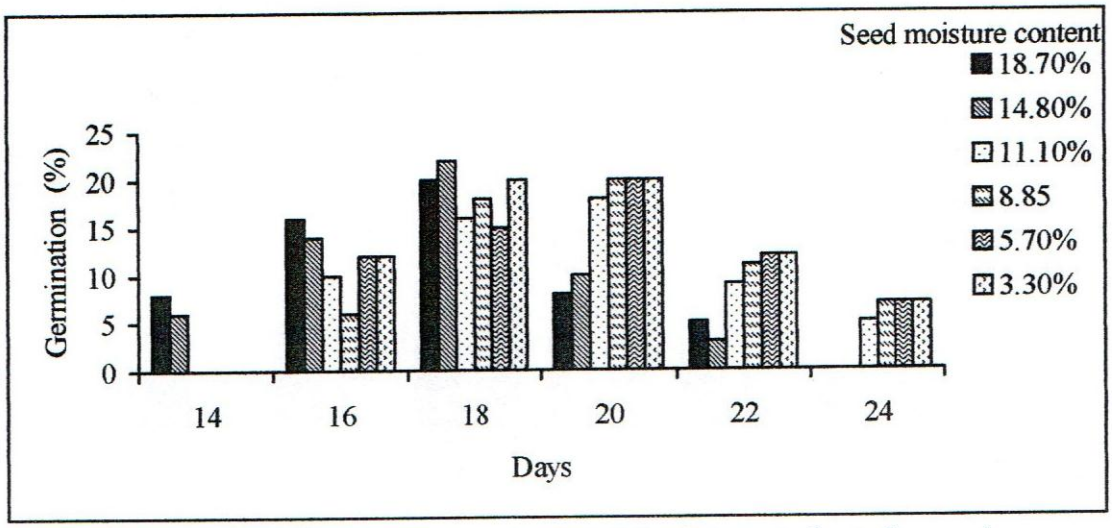


Figure 27. Effects of desiccation on germination (%) of *Tamarindus indica* seeds

Drying seeds improved ($P < 0.05$) germination of *T. indica* seeds (Table 16).

Table 16: Effects of desiccation on germination of *Tarmarindus indica* seed

TSMC	Attained SMC (%)	Germination (%)
18.7[a],	18.7	53
12,	12.4	58
9,	8.8	62
6	5. 7	66
3	3.3	71
LSD (P<0.05)		3.42

[a] Initial seed moisture content

4.3.4 Vitex payos

Germination of seeds at 22.4% and 16.2% moisture content started at day 14 while in other treatments, germination commenced at day 16 and was complete at day 24. (Figure 28). Highest germination was recorded between days 18 and 20 for all the treatments.

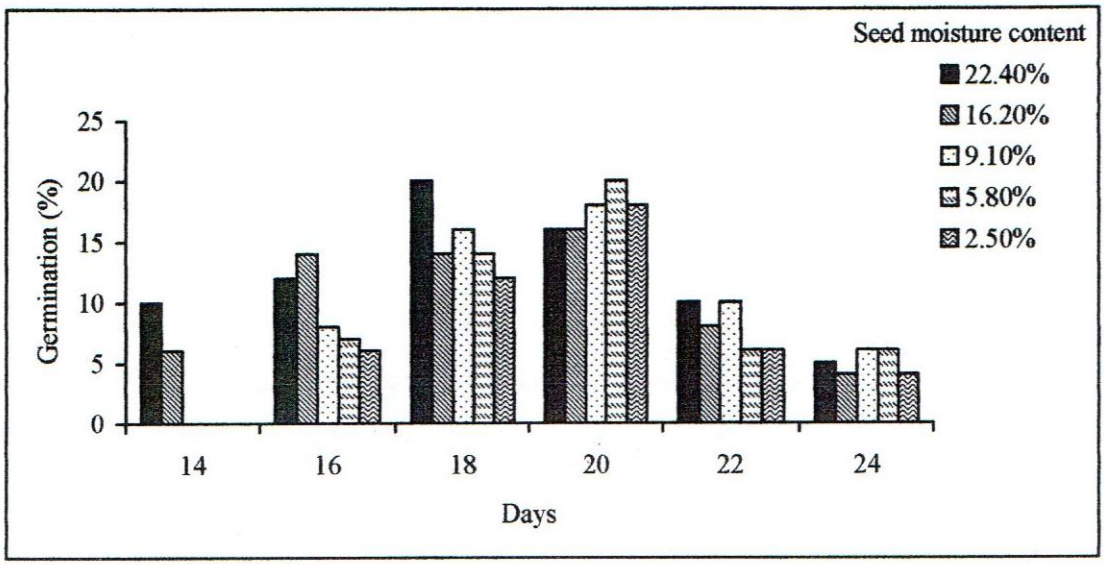


Figure 28. Effects of desiccation on germination (%) of *Vitex payos* seeds.

Seeds dried to 2.5% moisture content had the lowest ($P < 0.05$) germination. germination was similar for seeds dried between 22.4 % and 5.8 % seed moisture contents (Table 17)

Table 17: Effects of desiccation on germination of *Vitex payos* seed

TSMC	Attained SMC (%)	Germination (%)
22.4[a],	22.4	73
15,	16.2	62
9,	9.1	58
6,	5.8	55
3	2. 5	46
LSD (P<0.05)		4.5

[a] Initial seed moisture content

4.3.5 Celtis gomphophylla

Seeds at 23.1% and 14.7% moisture contents commenced germination at day 12 while seeds at 8.8% - 3.1 % moisture content started germination at day 14 and was complete at day 22 (Figure 29). Highest germination was recorded between days 16 – 18 for all the treatments.

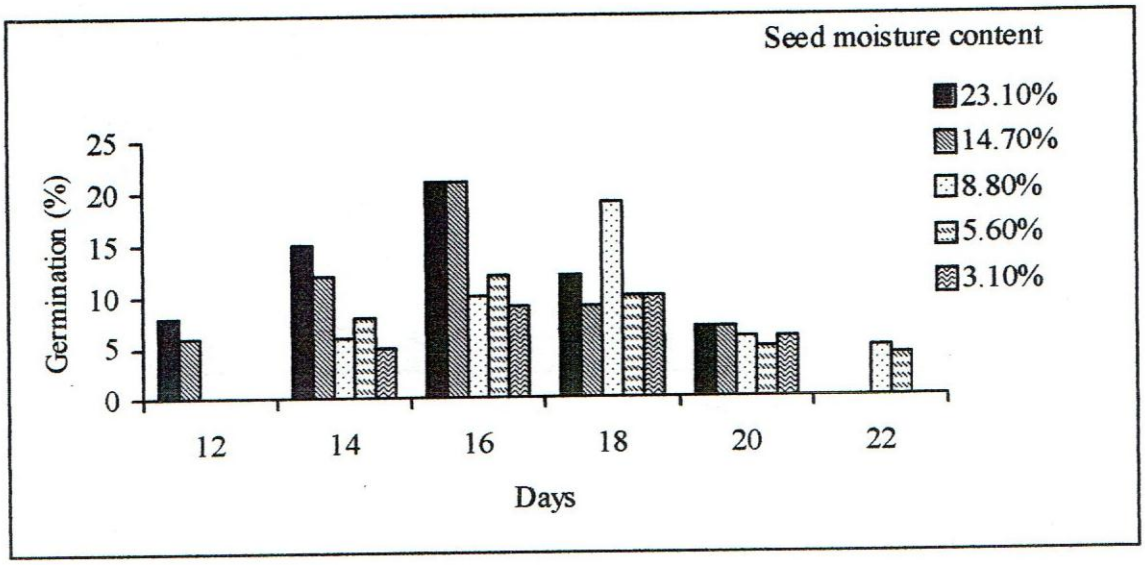


Figure 29. Effects of desiccation on germination (%) of *Celtis gomphophylla* seeds.

Germination declined ($P < 0.05$) with loss of seed moisture content. Seeds dried to 3.1% seed moisture content had the lowest germination (Table 18).

Table 18: Effects of desiccation on germination of *Celtis gomphophylla* seed

TSMC	Attained SMC (%)	Germination (%)
23.1[a],	23.1	63
15,	14.7	55
9,	8.8	46
6,	5.6	39
3	3. 1	30
LSD		3.65

[a] Initial seed moisture content

4.3.6 Rhus natalensis

Germination started at day 3 except for seeds dried below 10% moisture content which commenced germination at day 6. Highest germination was recorded between days 6 and 8 for all treatments and was complete at day 12 (Figure 30).

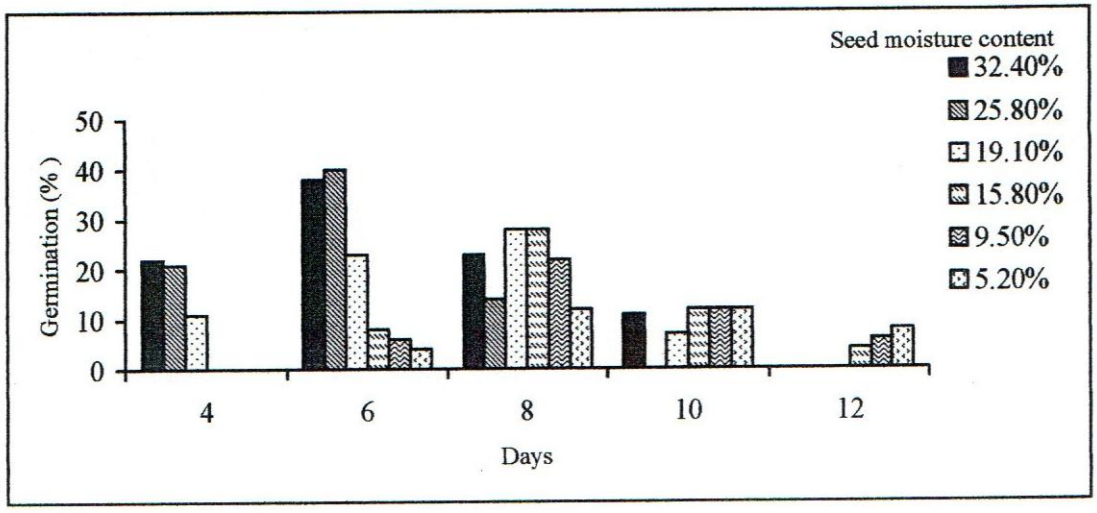


Figure 30. Effects of desiccation on germination (%) of *Rhus natalensis* seeds.

Germination declined significantly ($P < 0.05$) with drying; it was higher on seeds at initial moisture content of 33.6 %. High germination (>70 %) was recorded for seeds with seed moisture content >20 % (Table 19)

Table 19: Effects of desiccation on germination of *Rhus natalensis* seed

TSMC	Attained SMC (%)	Germination (%)
33.6[a],	33.6	92
25,	26.2	95
20,	20.4	78
15,	15.3	40
10,	11.0	28
5	5.9	15
LSD (P<0.05)		3.42

[a] Initial seed moisture content

4.3.7 Berchemia discolor

Germination started at day 6 except for seeds dried below 10% moisture content which commenced germination at day 8. Highest germination was recorded between days 8 and 10 for all treatments and was complete at day 14 (Figure 31).

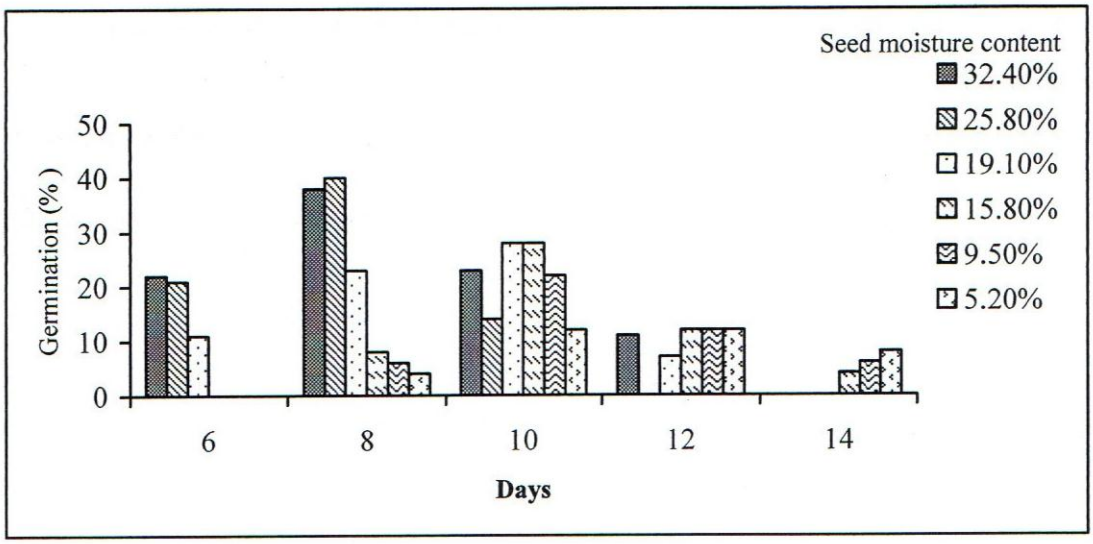


Figure 31. Effects of desiccation on germination (%) of *Berchemia discolor* seeds.

Germination declined ($P < 0.05$) with loss of seed moisture content. High germination (>70%) was recorded for seeds at >20 seed moisture content % (Table 20).

Table 20: Effects of desiccation on germination of *Berchemia discolor* seed

TSMC	Attained SMC (%)	Germination (%)
24.8[a],	24.8	88
15,	15.6	76
9,	8.9	70
6,	6.2	54
3,	3.3	38
LSD (P<0.05)		3.66

[a] Initial seed moisture content

4.4 Effects of storage temperature and moisture content on seed longevity

4.4.1 Myrianthus holstii

Seed germination varied in response to storage temperatures and moisture content (Table 21). Seeds stored at 25° C had the highest germination percentage while those at -10° C had lowest germination percentage. There was no germination recorded on seeds stored at -20° C. Seeds at 19.6% moisture content stored at 25°C had the highest germination percentage.

Table 21: Effects of storage temperature and Moisture content on longevity of *Myrianthus holstii* seeds

Storage Temperature (°C)	Seed moisture content (%)					
	27.8	19.6	15.3	10.9	8.5	6.2
25	64	72	68	32	24	16
0	24	32	20	24	16	8
-10	12	16	12	8	8	4
-20	0	0	0	0	0	0

4.4.2 Dovyalis abyssinica

Lowering storage temperature and seed moisture content depressed germination (Table 22). Storage temperatures at 25° C had the highest germination percentage. No germination was recorded on seeds stored at -10° C and -20° C for seed at <10 % moisture content. Seeds at 26% moisture content had highest germination percentage after storage.

Table 22: Effects of storage temperature and moisture content on longevity of *Dovyalis abyssinica* seeds

Storage Temperature (°C)	Seed moisture content (%)					
	32.4	25.8	19.1	15.8	9.5	5.0
25	56	60	48	44	20	16
0	24	28	24	24	12	8
-10	12	16	16	12	0	0
-20	0	0	0	0	0	0

4.4.3 Tamarindus indica

Overall germination was good (Table 23). Seeds at <12 % moisture content and stored at room temperature had over 50 % germination. Germination was lowest on seeds stored at initial moisture content.

Table 23: Effects of storage temperature and moisture content on longevity of *Tamarindus indica* seed

Storage Temperature (°C)	Seed moisture content (%)				
	18.7	12.4	8.8	5.7	3.3
25	56	36	54	64	72
0	36	44	36	40	56
-10	28	32	32	44	48
-20	20	24	40	48	60

4.4.4 Vitex payos

Seed germination varied in response to storage temperature and moisture content (Table 24). Over 70% germination was recorded on seeds at < 17 % and >6 % moisture content stored at room temperature. Seeds stored at temperature >0°C had over 60 % germination. Seeds at initial moisture content and those dried to 2.5 % moisture content had low germination rate.

Table 24: Effects of storage temperature and moisture content on longevity of *Vitex payos* seeds

Storage Temperature (°C)	Seed moisture content (%)				
	22.4	16.6	9.1	5.8	2.5
25	52	72	76	72	48
0	36	58	58	84	56
-10	28	36	36	62	44
-20	8	28	36	60	40

4.4.5 Celtis gomphophylla

Desiccation and low storage temperature reduced seed germination (Table 25)

Table 25: Effects of storage temperature and moisture content on longevity of *Celtis gomphophylla* seeds

Storage Temperature (°C)	Seed moisture content (%)				
	23.1	14.7	8.8	5.6	3.1
25	60	52	44	28	24
0	36	38	52	20	16
-10	8	12	16	16	12
-20	0	0	12	8	4

4.4.6 Rhus natalensis

Drying and lowering storage temperature depressed seed germination (Table 26). Seeds stored at 25° C with >15.3 % seed moisture content had good germination (>60%). Germination of seeds stored at -10° C or lower had <20 % germination percentage irrespective of SMC while those stored at -20°C did not germinate.

Table 26: Effects of storage temperature and moisture content on longevity of *Rhus natalensis* seeds

Storage Temperature (°C)	Seed moisture content (%)					
	33.6	26.2	20.4	15.3	11.0	5.9
25	64	76	88	71	58	56
0	52	60	64	32	44	36
-10	20	28	16	16	32	16
-20	0	0	0	0	12	0

4.4.7 Berchemia discolor

Germination was >60% for seeds at >6% and <15% seed moisture content that had been stored at room temperature (Table 27). Seeds at < 16% moisture content that were stored at -20°C recorded over 40% germination.

Table 27: Effects of storage temperature and moisture content on longevity of *Berchemia discolor* seeds

Storage Temperature (°C)	Seed moisture content (%)				
	24.8	15.6	8.9	6.2	3.3
25	68	84	82	68	44
0	32	68	78	56	38
-10	24	60	62	52	40
-20	12	44	32	40	48

CHAPTER FIVE
DISCUSSION

5.1 Species distribution and inventory

The fruit trees were well distributed along the three agro climatic zones, although diversity increased with aridity. The lower rainfall (897 mm) zone had higher (26.3%) diversity of the fruit tree species compared to the higher rainfall (1330 mm) zone (21.05 %). Middle zone received 1210 mm in 1 year. *Myrianthus holstii, D. abyssinica* and *R. natalensis* which are desiccation - sensitive were found on the Agro climatic zones I and ii (upper zone) while *C. gomphophylla, V. payos, T. indica* and *B. discolor* which are desiccation – tolerant were found in agro climatic zone iii and iv (lower Zone). This concurs with Tweddle *et al.,* (2003) who reported that desiccation - sensitive species decrease in abundance with increasing aridity. The middle zone was heavily cultivated which resulted in low species diversity.

No saplings were observed for 37% of the fruit tree species while 42.1% of the trees had <5 saplings recorded (50 m x 50 m quadrant); an indication that the species are over exploited or that the habitats were disturbed to the extent that natural regeneration was hampered. Also, the fruits were harvested long before they had ripened and naturally shed.

5.2 Effects of precipitation on fruit development

Ripening and shedding of *M. holstii, D. abyssinica* and *R. natalensis* coincided with the wet seasons. This is a characteristic of recalcitrant species. Prichard *et al.,* (2004) reported that; the time for seed dispersal for desiccation - sensitive species coincides with the wettest months of the year. Further, desiccation sensitive seeds of African dryland trees were typically shed when rainfall exceeds approximately 60 mm which would allow immediate germination.

Tamarindus indica, V. payos, C. gomphophylla and *B. discolor* fruits were ripe for shedding during the dry spells; signifying they could be orthodox. Berjak *et al.,* (1989) reported that orthodox seeds undergo a period of drying during their maturation and are shed at low seed water content which is in equilibrium with the prevailing relative humidity. However,

Orthodox seeds can withstand dehydration to around 5 % percent even when maturation drying is not completed prior to shedding.

5.3 Seed Coat Ratio

Low (<0.5) seed coat ratio of *M. holstii, D. abyssinica* and *R. natalensis* implies that higher mass of the endosperm and embryo compared with the testa which allows for immediate germination on seed maturity. This is a characteristic of recalcitrant seeds. These results agree with Daws *et al.* (2006) and Pritchard *et al.* (2004) who reported that dessication sensitive seeds have thin seed coat (Low SCR), are shed at high water content and are frequent in wet habitats. Seed hardness for these seeds protects the embryo from pests infestation. *Tamarindus indica* seeds had low SCR thus preserving energy in endosperm for maturation and storage.

Vitex payos, C. gomphophylla and *B. discolor* seeds were found to have high (>0.7) SCR implying that energy to defense is invested in the testa for seed protection. This enhances seed survival during subsequent seasons due to reduction in moisture loss in agreement with Daw *et al.* (2006) who reported that on average SCR is lower in desiccation sensitive than tolerant species. These seeds were collected from harsh conditions with low rainfall and relatively high temperatures (>24° C).

5.4 Effects of maturity stage and dormancy

High germination percentage by seeds that were nipped then soaked in gibberelic acid and those that were only nipped indicates that breaking the hard seed coat improved seed germination of *T. indica, M. holstii, V. payos* and *C. gomphophylla.* Improved germination of *T. indica* and *M. holstii* seeds from mature green fruits that were nipped then soaked in gibberelic acid suggests existence of embryo seed dormancy. Good germination (>70 %) of seeds from green fruits of *M. holstii, D. abyssinica.* and *R.natalensis* indicates that seed maturity occurs before ripening. This agrees with Berjak *et al.* (1984) who reported that most recalcitrant seeds mature in their fruits. They are usually covered with fleshy or juicy layers and an impermeable testa. In recalcitrant seeds the embryo starts to develop early and

growth hormones are mobilized and translocated to the meristems ready for germination (Poulsen, 1995). Low germination of seeds from green fruits of *T. indica, V. Payos, C. gomphophylla* and *B.discolor* in all treatments other than the nipped then soaked in gibberelic acid shows that embryo matures on or after ripening.

Myrianthus holstii, D. abyssinica and *R. natalensis* can be harvested while the fruits are green mature and seeds extracted for propagation as long as there is sufficient moisture for the survival of the seedlings. Timely *in-situ* conservation would control the competition for ripe fruits hence ensuring continuity of these endangered species. *Tamarindus indica, V. Payos, C. gomphophylla* and *B. discolor* seeds mature on or after ripening hence harvesting can be done on ripe fruits. Subsequently, ex-*situ* conservation is possible for these species.

5.5 Effects of desiccation

Drying *M. holstii, D. abyssinica* and *R. natalensis* seeds declined germination rapidly. Germination of *M. holstii* seeds decreased from 67 % at the initial (27.8%MC) moisture content to 16 % at 6.2 % moisture content while drying *D. abyssinica* seeds from 32.4 % to 5.2 % seed moisture content reduced germination from 94 % to 26 % respectively. Germination of *R. natalensis* seeds declined from 92 % on seeds at initial moisture content to 15 % on seeds at 5.9 % moisture content thus showing seeds of these species are desiccation - sensitive. Seeds at >19% seed moisture content had similar germination percentage.This gradual decrease in germination with decrease in SMC concurs with Hong and Ellis (1996) who reported that as fresh recalcitrant seeds loose moisture, initial viability loss is small, but a rapid decline follows after the lowest safe moisture content (LSMC) is surpassed. Further, they reported that the critical moisture content for some tropical recalcitrant species could be as low as between 15 % - 12 %. Also, Varghese and Naithans, (2001) observed similar lowest safe moisture content for *Azandirachta indica* (10.9 %).

Drying *T. indica* seeds improved germination. Highest germination (71 %) was recorded at 3.1 % seed moisture content. The observed gradual increase in germination with drying can be attributed to maturation drying. Desiccating depressed germination of *V. payos* and *B. discolor* seeds. However, they maintained >40 % seed germination at the lowest attained

seed moisture content thus indicating that seeds of these species are desiccation tolerant. Drying *V. payos* seeds from 22.4 % to 5.8 % SMC depressed germination by 18 % units. Further drying to 2.5 % SMC depressed germination by only 9 % units which was not significantly ($P<0.05$) different with germination at 5.8 % SMC. Drying *B. discolor* from 24.8 % to 6.2 % SMC depressed germination by 34 % units. Further drying to 3.3 % seed moisture content depressed germination by only 16% units. This concurs with Maara *et., al.* (2006) who recorded 11 % units decline in germination of *Ximenia americana* seeds on desiccation from 22.5 % to 3.8 % SMC. Fletcher and Prichard (2000) also recorded 14 % germination decline on desiccating *X. americana* from 12.5 % to 3.2 % SMC. Berjak *et., al.* (1984) reported that unless debilitated by zero-tolerant storage fungi, orthodox seeds should maintain high vigour and viability at least from harvest until the next growing season for many decades at -18 °C (IPGR 1976). Orthodox seeds can withstand dehydration to about 5 % moisture content even when maturation drying is not completed prior to shedding. Germination of *C. gomphophylla* seeds declined from 63 % at 23.1 % SMC to 30 % at 3.1 % SMC. Seed dried to >9 % SMC maintained >50 % germination thus indicating the seeds of this species can be classified as intermediate.

5.6 Effects of storage temperature and moisture content on seed longevity

Low and sometimes lack of germination on seeds stored at < 0° C indicates that *M. holstii, D. abyssinica* and *R. natalensis* seeds are recalcitrant and can only be stored for a short period at ambient temperature and at 19 % to 25 % SMC without loss of viability. The loss in viability with decreasing storage temperatures and moisture content concurs with Schmidt (2000) who reported that recalcitrant seeds have a short longevity and rapidly loose viability under storage. *Celtis gomphophylla* Seeds maintained viability with decreasing seed moisture content. Germination declined with reducing storage temperatures at 25° C, 0° C, -10° C and -20° C. Seeds of this species can be stored at >0° C and at moisture content range of 14 % - 6 % thus they can be classified as in the intermediate category.

Tamarindus indica, V. payos and *B. discolor* seeds germinated after 3 months of storage at 25° C, 0° C, -10° C and -20° C temperatures. Germination was good (>50 %) for seeds at <16 % and >6 % SMC stored at < 0° C. The seeds stored at -20° C had >35 % germination

indicating that seeds can be classified as orthodox and can be stored for long periods when dried to <10 % to > 3 % seed moisture content. Higher germination percentage for *T. indica* after 3 months storage under ambient temperature compared with germination at initial seed moisture content agrees with the findings by Dudley *et al.* (2001) who reported a fivefold increase in the germination rate of *kigelia africana* seeds after three months of storage. This was partly attributed to an increase in maturity of the seeds during storage and to priming effects. Most orthodox seeds maintain high viability under ambient temperatures, at least from harvest to subsequent sowing season, although storing them at low temperatures is preferred in order to reduce ageing and prevent microbial infection and fungal activity. Jayanthi *et al.,* (1998) recommended temperatures of -20° C for storage of orthodox seeds as a means of minimizing fungal attack. Similarly, when seed moisture content is below 14 %, no ice crystals form from within cells upon freezing such that storage of dry seeds at sub-zero temperatures should improve longevity provided freezing occurs in a dry atmosphere.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

- Maturity of *M. holstii* seeds occurs prior to fruit ripening and shedding. The seeds also exhibited hard seed coat. Rainfall during seed shedding is critical as it enhances breaking of hard seed coat. Seeds lost viability on storing hence this mode of conserving seeds is not recommended. Seeds were also desiccation sensitive. Therefore, they should be processed and sown when fresh. Seeds can, however, be temporally stored for a short duration at room temperature after shedding. *Myrianthus holstii* seeds are therefore recalcitrant and can be conserved *in-situ* in protected areas.

- Seeds of *D. abyssinica* matured before fruits were ripe. Seed shedding coincided with the rainfall period. Pre-germination treatment did not have any significant effects on germination, and that drying seeds depressed germination. Storage for three months also depressed germination portraying that the seeds are recalcitrant, Hence, seeds can only be temporally stored for a short duration at room temperature after shedding. For good germination, storage should be at $\geq$ 25 % seed moisture content. *Dovyalis abyscinica* can be conserved *in-situ* in protected areas.

- Shedding of *T. indica* seeds coincided with the dry spell. Breaking hard seededness improved germination. Drying seeds to 3.3 % moisture content significantly improved germination; hence the seeds are desiccation tolerant. The seeds can be stored at -20°C and at 3% moisture content for a long period. The research shows that *T. indica* seeds are orthodox and hence *ex-situ* conservation in genebanks is possible.

- *Vitex payos* seeds were mature before fruits were ripe. Shedding occurred during the rainy season. Breaking the seed coat by nipping improved germination signifying the presence of seed coat imposed dormancy. Seeds maintained viability at 3.5 % moisture content. Seeds can also be well stored at temperatures between 0° C and -

10° C and between 22.4% to 5.8 % seed moisture content. The results show that *V. payos* seeds are orthodox and hence *ex-situ* conservation in gene bank is possible.

- Seeds of *C. gomphophylla* were mature before fruit rippening. Seed shedding was during the dry season. The pre-germination treatments did not have any significant effects on germination. The seeds germinated well at a wide range of seed moisture contents and therefore it can be concluded that the species is desiccation tolerance. Seeds can be stored at shedding seed moisture content (23.1 %) at temperatures above 0° C. Based on the results, *C. gomphophylla* seeds fall under intermediate category of seeds and both *ex-situ* and *in-situ* approaches of conservation can be applied.

- Seeds of *R. natalensis* matured before fruits were ripe. Seed shedding coincided with the rainfall period. Pre-germination treatment did not have any significant effects on germination, and that drying seeds depressed germination. Storage for three months also depressed germination portraying that the seeds are recalcitrant hence seeds can only be temporally stored for a short duration at room temperature after shedding. For good germination, storage should be at $\geq$ 20 % moisture content. *R.natalensis* can be conserved *in-situ* in protected areas.

- *Berchemia discolor* seeds mature before fruits are ripe. Shedding occurred during the dry season. Breaking the seed coat by nipping improved germination signifying the presence of seed coat imposed dormancy. Seeds germinated well (>40%) at 3.3% moisture content and can be well stored at temperatures between 0° C and -20° C and between 15% to 6 % seed moisture content. The results show that *V. payos* seeds are orthodox and hence *ex-situ* conservation in gene bank is possible.

6.2 RECOMMENDATIONS

- Mature green and ripe fruits of *M. holstii, D. abyssinica, V. payos, C. gomphophylla, R. natalensis* and *B. discolor* can be used as source of seed for propagation but only seeds from ripe fruits of *T. indica* results in reasonable germination.

- Seeds that mature before fruits are ripe can be used to advantage for propagation before fruits are harvested for human consumption.

- Long term seed storage for Orthodox seeds, cryopreservation for Recalcitrant/intermediate and live gene bank for all fruit trees.

- Domestication on farm of the fruit tree species understudy.

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APPENDICES

Appendix 1: Meru South District fact sheet.

The district fact sheet shows a broad range of information about the district at a glance. It captures factual information like the district area, topography and climate, demographic and population profile and various socio-economic indicators.

Area	
Total area	1,092.9 km^2
Arable area	550 km^2
Non arable land	177.6 km^2
Water mass	N/A
Gazetted forest	360 km^2
Urban area	5.3 km^2
Topography and climate	
Altitude	400-5200 M.A.S.L
Rainfall range (annual average)	500 -2200 MM
Temperature range	14°-27°
Temperature average	20.5°
Demographic and population profile (1999)	
Population size	212,982
Population structure: Males	103,900
Females	109,082
Female/male sex ratio	100:95
Total No. of youthful pop. (15-25)	49,277
Population of primary school going age	47,167
Population of secondary school going age	21,345
Total labour force	117,457
Dependency ratio	100:81
Population growth rate	1.2%
Socio-economic indicators	

Total number of households	46,984
Average household size	4.4
Number of female headed households	8,870 (18.3%)
Number of disabled	2,000
No. of children needing special protection	144
No. and % age living in absolute poverty (rural)	21,911 (45.04%)
No. and % age living in absolute poverty (urban)	4,296 (57 %)
Contribution of National poverty	1.59%
Sectoral contribution to household income	
Agriculture	45%
Rural self employment	12%
Wage employment	38.5%
Urban Self Employment	5%
Number of Unemployed	89, 544
Agriculture	
Average farm size low potentia	l4 ha
High potentia	l0.7 ha
Main food crops produced; Maize, Beans, Millet, Sorghum, Pigeon Peas	
Main Cash crops	Tea, Coffee and Cotton
Total acreage under food crops	30, 000 ha
Total acreage under cash crops	10, 538 ha

Source: Meru South District Development plan, 2002-2008

Appendix 2. Germination test results sheet.

Experiment location.....Medium.....
Method.....Temperatures.....
Test No.....Batch No.....
Species.....Provenance.....
Storage Duration.....Storage Temperatures.....
.Aspect of seeds .....pre-treatment .....
Date test started.....Date Closed.....

Date	Replicate 1	Replicate 2	Replicate 3	Replicate 4
Total No. of germinated seeds				
Non-Germinated: R- Rotten D- Dead E- Empty I- Insect				

Remarks.....
.....
.....
.....

Appendix 3. Desiccation and storage protocol

Initial mc (before processing) (%)	Target mc (%)
≤ 10	9, 6, 3
11-15	12, 9, 6, 3
16-20	15, 12, 9, 6, 3
21-25	20, 15, 9, 6, 3
26-30	25, 20, 15, 12, 9, 6
31-35	30, 25, 20, 15, 10, 5
36-40	35, 30, 25, 20, 10, 5
41-45	40, 35, 30, 20, 10, 5
46-50	45, 40, 35, 25, 15, 8
51-55	50, 45, 40, 35, 25, 10
56-60	55, 50, 45, 35, 25, 10
>60	60, 50, 40, 30, 20, 10

Source: Hong and Ellis, (1996)

Appendix 4: Species distribution

Nineteen indigenous fruit tree species were observed along the transects. 42.1% of the species had few saplings (<5) underneath while 36.84% had no saplings observed. 21.05% had many saplings (>5) underneath (Table 1)

Natural Regeneration

Species	Sapling score		
Myriathus holstii,	0		
Vangueria madagascariensis,	1		
Dovyalis abyssinica	1		
Olea africana	0		
Rubus vulkensii,	1		
Synsepalum brevites	1		
Rhus natalensis	1		
Carissa spinarum	0		
Vitex payos	3		
Tamarindus indica	1		
Adansonia digitata	0		
Berchemia discolor	3		
Hyphaene compressa	0		
Olea capensis	0		
Psidium guajava	3		
Celtis gomphophylla,	3		
Ximenia Americana	1		
Oncoba spinosa	0		
	0	1	3
Percentage scores	36.84	42.10	21.05

0. - No saplings observed, 1. - Few saplings (<5) observed and 3. - Many saplings (≥5) observed

Appendix 5: Rainfall received

Upper Zone –Kirege

	J	F	M	A	M	J	J	A	S	O	N	D
Mm	66.9	265	329	297.2	2.2	19	19	3.4	0	162.5	160	6
Days	3	7	17	16	2	5	5	4	0	17	8	3

Middle Zone –Kanwa

	J	F	M	A	M	J	J	A	S	O	N	D
Mm	66.9	176.9	255	258.5	0.4	17.5	17	0	0	309.2	199.9	8.6
Days	3	7	8	17	1	5	5	0	0	13	7	3

Lower Zone –Weru

	J	F	M	A	M	J	J	A	S	O	N	D
Mm	65.7	176.9	75.5	278	0.6	6.3	17	0.8	0	165.8	118.7	16
Days	3	7	5	10	1	2	5	1	0	11	6	2

Appendix 6: ANOVA Tables

(i) Myrianthus holstii

Seeds from Green mature fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	259.	15			
Pre-treatment	228.5	3	76.17	29.97	<.001
Error	30.5	12	2.54		

Seeds from mature ripe fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	63.44	15			
Pre-treatment	35.69	3	11.9	5.14	0.0162
Error	27.75	12	2.31		

seeds from mature ripe fruits on soil

Source	S.S.	DF	MS	F	Appx P
Total	37.44	15			
Pre-treatment	14.69	3	4.9	2.58	0.1019
Error	22.75	12	1.9		

Seeds from both green mature and mature ripe fruits

Source	S.S.	DF	MS	F	Appx P
Total	3640.92	11			
Germination medium	2606.17	2	1303.08	11.33	0.0035
Error	1034.75	9	114.97		

Seeds from mature ripe fruits

Source	S.S.	DF	MS	F	Appx P
Total	390.44	15			
Storage temperature	355.69	3	118.56	40.94	<.001
Error	34.75	12	2.9		

Seeds from mature ripe fruits

Source	S.S.	DF	MS	F	Appx P
Total	10527.33	23			
TSMC	1579.33	5	315.87	64	0.6755
Error	8948.	18	497.11		

(ii) *Dovyalis abyssinica*

Seeds from green mature fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	85.	15			
Pre-treatme	5.	3	1.67	.25	0.8598
Error	80.	12	6.67		

Seeds from mature ripe fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	55.	15			
Pre-treatment	29.	3	9.67	4.46	0.0252
Error	26.	12	2.17		

Seeds from mature ripe fruits on soil

Source	S.S.	DF	MS	F	Appx P
Total	78.44	15			
Pre-treatment	25.69	3	8.56	1.95	0.1758
Error	52.75	12	4.4		

Seeds from mature ripe fruits

Source	S.S.	DF	MS	F	Appx P
Total	1558.92	11			
Germination medium	1320.17	2	660.08	24.88	<.001
Error	238.75	9	26.53		

Seeds from mature ripe fruits

Source	S.S.	DF	MS	F	Appx P
Total	7802.	23			
Storage temperature	5495.33	3	1831.78	15.88	<.001
Error	2306.67	20	115.33		

Seeds from mature ripe fruits

Source	S.S.	DF	MS	F	Appx P
Total	7802.	23			
TSMC	1406.	5	281.2	.79	0.5696
Error	6396.	18	355.33		

((iii) Tamarindus indica

Seeds from Green mature fruit on agar

Source	S.S.	DF	MS	F	Appx P
Total	176.94	15			
Pre-treatment	127.69	3	42.56	10.37	0.0012
Error	49.25	12	4.1		

Seeds from mature ripe fruit on agar

Source	S.S.	DF	MS	F	Appx P
Total	177.75	15			
Pre-treatment	83.25	3	27.75	3.52	0.0488
Error	94.5	12	7.88		

Seeds from mature ripe fruit on soil

Source	S.S.	DF	MS	F	Appx P
Total	146.44	15			
Pre-treatment	88.69	3	29.56	6.14	0.009
Error	57.75	12	4.81		

Seeds from mature ripe fruit

Source	S.S.	DF	MS	F	Appx P
Total	1305.67	11			
Germination medium	106.17	2	53.08	.4	0.6827
Error	1199.5	9	133.28		

Seeds from mature ripe fruit

Source	S.S.	DF	MS	F	Appx P
Total	3599.	19			
Storage temperature	1192.6	3	397.53	2.64	0.0847
Error	2406.4	16	150.4		

Seeds from mature ripe fruit

Source	S.S.	DF	MS	F	Appx P
Total	3599.	19			
TSMC	1768.	4	442.	3.62	0.0295
Error	1831.	15	122.07		

(iv) Vitex payos

Seeds from green mature fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	154.44	15			
Pre-treatment	95.69	3	31.9	6.51	0.0073
Error	58.75	12	4.9		

Seeds from mature ripe fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	273.44	15			
Pre-treatment	208.69	3	69.56	12.89	<.001
Error	64.75	12	5.4		

Seeds from green mature fruits on soil

Source	S.S.	DF	MS	F	Appx P
Total	122.94	15			
Pre-treatment	54.19	3	18.06	3.15	0.0646
Error	68.75	12	5.73		

Seeds from green mature fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	2466.92	11			
Germination medium	1360.67	2	680.33	5.53	0.0271
Error	1106.25	9	122.92		

Seeds from green mature fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	6770.2	19			
Storage temperature	2867.8	3	955.93	3.92	0.0284
Error	3902.4	16	243.9		

Seeds from green mature fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	7119.	19			
TSMC	3186.	4	796.5	3.04	0.0509
Error	3933.	15	262.2		

(v) *Celtis gomphophylla*

Seeds from green mature fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	133.	15			
Pre-treatment	66.5	3	22.17	4.	0.0346
Error	66.5	12	5.54		

Seeds from mature ripe fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	51.75	15			
Pre-treatment	21.25	3	7.08	2.79	0.0862
Error	30.5	12	2.54		

Seeds from mature ripe fruits on soil

Source	S.S.	DF	MS	F	Appx P
Total	18.94	15			
Pre-treatment	2.69	3	.9	.66	0.5914
Error	16.25	12	1.35		

Seeds from green mature ripe fruits

Source	S.S.	DF	MS	F	Appx P
Total	2412.92	11			
Germination medium	2036.17	2	1018.08	24.32	<.001
Error	376.75	9	41.86		

Seeds from mature ripe fruits

Source	S.S.	DF	MS	F	Appx P
Total	6299.8	19			
storage temperature	4347.8	3	1449.27	11.88	.001
Error	1952.	16	122.		

Seeds from green mature fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	6299.8	19			
TSMC	740.8	4	185.2	.5	0.7364
Error	5559.	15	370.6		

(vi) *Rhus natalensis*

Seeds from green mature fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	30.44	15			
Pre-treatment	8.69	3	2.9	1.6	0.2416
Error	21.75	12	1.81		

Seeds from mature ripe fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	28.44	15			
Pre-treatment	6.69	3	2.23	1.23	0.3416
Error	21.75	12	1.81		

Seeds from mature ripe fruits on soil

Source	S.S.	DF	MS	F	Appx P
Total	27.75	15			
Pre-treatment	6.75	3	2.25	1.29	0.3239
Error	21.	12	1.75		

Seeds from mature ripe fruits

Source	S.S.	DF	MS	F	Appx P
Total	124.67	11			
Germination Medium	23.17	2	11.58	1.03	0.3965
Error	101.5	9	11.28		

Seeds from mature ripe fruits

Source	S.S.	DF	MS	F	Appx P
Total	17270.5	23			
Storage temperature	15341.83	3	5113.94	53.03	<.001
Error	1928.67	20	96.43		

Seeds from mature ripe fruits

Source	S.S.	DF	MS	F	Appx P
Total	17270.5	23			
TSMC	647.5	5	129.5	.14	0.9805
Error	16623.	18	923.5		

(vii) *Berchemia disclor*

Seeds from Green mature fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	212.	15			
Pre-treatment	142.	3	47.33	8.11	0.0032
Error	70.	12	5.83		

Seeds from mature ripe fruits on agar

Source	S.S.	DF	MS	F	Appx P
Total	69.75	15			
Pre-treatment	36.75	3	12.25	4.45	0.0253
Error ..	33.	12	2.75		

Seeds from mature ripe fruits on soil

Source	S.S.	DF	MS	F	Appx P
Total	99.75	15			
Pre-treatment	62.75	3	20.92	6.78	0.0063
Error	37.	12	3.08		

Seeds from mature fruits

Source	S.S.	DF	MS	F	Appx P
Total	1510.	11			
Germination medium	526.5	2	263.25	2.41	0.1452
Error	983.5	9	109.28		

Seeds from mature fruits

Source	S.S.	DF	MS	F	Appx P
Total	7356.8	19			
Storage temperature	3012.8	3	1004.27	3.7	0.0339
Error	4344.	16	271.5		

Seeds from mature fruits

Source	S.S.	DF	MS	F	Appx P
Total	7356.8	19			
TSMC	2774.8	4	693.7	2.27	0.1099
Error	4582.	15	305.47		

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