ASSESSMENT OF GERMINATION POTENTIAL AND POPULATION STATUS OF *Melia volkensii* Gürke IN KITUI COUNTY, KENYA

JANE MUKAMI NJEHU (BSc)

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DECLARATION

This thesis is my original work and has not been presented for a degree or other awards in any other University.

Signature........................ Date....3|5|2022........

Jane Mukami Njehu – Reg No: I56/31194/2015

Department of Plant Sciences

SUPERVISORS

We confirm that the work reported in this thesis was carried out by the student under our supervision.

Signature.................................Date....3|6|2022............

Dr. Emily Wabuyele

Department of Plant sciences

Kenyatta University

Signature.................................Date.....3|6|2022............

Dr. Adelaide Mutune

Department of Plant sciences

Kenyatta University

DEDICATION

I dedicate my work to the Almighty God for granting me good health, strength, wisdom and sufficient grace to pursue my studies. I am more grateful to my husband Stephen, mum Josephine and my three children Angel, Wisdom and Prudence for giving me moral support throughout my study period.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
ASALs	Arid and Semi-Arid Lands
DBH	Diameter at breast height
GPS	Global Positioning System
ICRAF	International Centre for Research in Agroforestry
ISTA	International Seed Testing Association
JICA	Japan International Cooperation Agency
KEFRI	Kenya Forestry Research Institute
MC	Moisture Content
NACOSTI	National Commission for Science, Technology and Innovation
NMK	National Museums of Kenya

ABSTRACT

Melia volkensii Gürke is a native tree species that grows in the arid and semi-arid areas of East Africa. There has been overutilization of this tree which may lead to risk of rapid decrease in its community and genetic diversity. Additionally, planning and implementing planting programmes and conservation plans of *M. volkensii* have been hindered by insufficient knowledge on its seed storage behavior. This study sought to develop optimal protocols for seed germination and assess population status of *Melia volkensii* to enhance conservation and supply chain management in Kitui County. *Melia volkensii* fruits were collected from Tiva Forest and the germination experiment was carried out in KEFRI Kitui within nursery conditions. Seed germination experiments were arranged in a completely randomized design with three factors – extraction state, storage container and storage temperature. For extraction, seeds were divided into two lots – extracted seeds and unextracted seed (nut). Two types of storage containers were used namely sealed and unsealed. The storage temperatures were room temperature (30^{o}C) and cold storage (-20^{o}C). Germination tests were done before storage, and later after 2, 4 and 6 months in storage from each of the storage conditions. A hundred seeds and nuts each were sown for each experimental unit. The rate of seedling sprouting was recorded for a duration of 10 days as a percentage of seeds sown. Variation in germination percentages was tested using two-way analysis of variance (ANOVA) at 95 % confidence interval and means separated using fisher's protected least significant difference in R and SAS statistical software. The highest percentage (89 %) was scored in nuts kept in unsealed containers at 30^{o}C at 6 months and the lowest (14 %) in seeds stored in similar conditions for 2 months.The differences were significant. These results indicate that the germination rates of *M. volkensii* seeds increased with increase in period of storage. Additionally, plant population was determined *in situ* across seven transect lines measuring 1050 meters in length. Sampling was carried out in five plots measuring 50 x 50 m established at intervals of 200 meters. Transect 1 and 7 were laid in farmland while the transects between these two were laid in bushland. All tree species represented in each plot were counted. Height and diameter at breast height (DBH) were measured for all *M. volkensii* trees within the plots. *Melia volkensii* seedlings and coppices were counted in 5 x 5 m nested plots within the larger plots. One-way analysis of variance (ANOVA) at 95 % confidence interval was used to test for variance in parameters measured and means separated using Tukey's HSD. There were significant differences in height and DBH means of *M. volkensii* among transects. Transect 2 had the lowest mean height and DBH while the tallest trees were in transects 1, 3, 4, and 6. Transect 1 had highest mean DBH. Means of seedlings were not significantly different among transects. Coppicing was significantly highest in transects 1 and 4. Moreover, relative abundance, species richness and diversity of plant species observed were analyzed using Simpson's index of biodiversity and Shannon-Wiener species diversity index. *Melia volkensii* was the most frequently occurring species with 12.2% (n=67) followed by *Acacia tortilis*10.0 % (n=55). *Calotropis strophela, Commiphora capensis, Maerua crassifolia* were the least represented relative abundance of 0.18 %. Values of indices used varied among transects and did not follow the location of transect implying that diversity was not determined by land disturbance. In conclusion, nuts stored unsealed at room temperature have the highest longevity. Based on diversity and relative abundance of *M. volkensii* and associated tree species in the study area, tree exploitation in Kasaala Location does not cause depletion of *M. volkensii*.

CHAPTER ONE

INTRODUCTION

1.1 Background information

The genus *Melia* belongs to plant family *Meliaceae* (mahogany family) and has less than nine species, most of which are native to Australia and Asia. *Melia volkensii* (Gurke) is the only tree species that is native to East Africa (Mabberley, 1984). *Melia volkensii* is mostly found in regions that lie at an altitude of between 400 and 1600 meters on dry and wooded grasslands. Distribution of the species extends from the dry areas of Somalia to Ethiopia through Tanzania and Kenya (Orwa *et al.*, 2009). This species is known to shed leaves during the dry season and is 6-20 m tall with a diameter of 25 cm at maturity. It is open crowned and laxly branched.

The wood is durable, decay and termite resistant; mostly used in paneling, making frames and rafters. The wood of *M. volkensii* is comparable to Elgon teak or camphor (Maundu and Tengas, 2005) which makes the tree to be over-exploited and thus a great need to replenish it for conservation. *Melia volkensii* does well in soils that drain water properly, for instance the sandy loam. *Melia volkensii* has proved economically and environmentally viable as it cushions against climate change and provides profitable timber. Lateral branches develop from the tree which are usually pruned and the tree develops a bole that is straight. The bark is grey in color and the tree has bright green compound leaves which are 35 cm in length; bearing less than ten deeply lobed leaflets. The flowers are white and small sized while the fruit is a drupe about 4 cm long. The fruit goes through the process of depulping in order to obtain nuts/ hard kernels which enclose the brown black seeds (Kamondo *et al*., 2016).

The economic importance of *M. volkensii* are diverse, for instance it is used for timber, fodder, herbal medicine fuel and making pesticides. Overexploitation of the products of this species has led to depletion of this tree species especially for medicinal use. For instance, dried fruit is used as ruminant feed supplement, leaf extract is applied externally on boils and blisters and it also used for treatment of stomach upsets, eczema, skin rashes and relieving body ache (Joshi, 2007). In the recent past, *M. volkensii* is known as an agroforestry tree which has other desirable properties including coppicing ability (ICRAF, 1992). Researchers, farmers, agricultural extension and education officers have found it to be a tree of high economic value, though it has difficulties in seed germination (Kimondo and Kiamba, 2005). Current dissemination programmes on *M. volkensii* have to utilize plants propagated from stem cuttings and roots, rather than seedlings (Mulatya *et al.,* 2000).

Additionally, seed germination is determined partly by storage conditions (Kamondo *et al*., 2016). Unsuitable storage methods regularly result in low germination of seeds and total failure in germination Nasreen, (2000). Domestication of plants is an important aspect which has broadened the understanding of seed storage. Naturally, seeds are categorized in three different ways namely: orthodox, recalcitrant and intermediate seeds (Hong and Ellis, 1996). Orthodox seeds are as well-known as the drying-tolerant seeds. Seed storage behavior of *M. volkensii* is termed as orthodox, and viability is enhanced for many years in airtight containers at a temperature of -20^{o}C. Over a wide variety of storage conditions and temperatures, the lifetime of these materials rises as the amount of moisture and temperature in the environment decreases. This leads to delayed ageing of seeds thus prolonged viability (Liu, *et al.,* 2020). Recalcitrant seeds do not withstand drying well, and as a result, they are not well suited for prolonged storage, even though the necessary level of moisture differs from species to species. Even while intermediate seeds are much more susceptible to drying out and dehydration than recalcitrant seeds, they are far less tolerant of desiccation than orthodox seeds, and they often lose viability much more quickly at low temperatures (Tweddle *et al*., 2003).

It has been recommended that plant species with poor regeneration and consist of low importance value indices be regarded as more important than others in matters of conservation (Maua *et al*., 2020). Natural regeneration of *M. volkensii* enables the plant to survive in an area after a number of events eventually causing change in the composition and the structure of a woodland ecosystem. According to (Nieuwenhuis and Egan 2002), natural regeneration can be defined as establishment of a new forest from the seeds that have been sown, coppice shoots, or root suckers. Coppices arise sporadically on the stump of a tree that was cut previously. Dry land habitats consist of woody plants which have regenerated and continue to exist through alarming events like wind, flooding, fire, storms and anthropogenic activities. When leaves of trees fall and decompose, they produce a favorable habitat for seeds to sprout, consequently increasing the number of seedlings (Midgley and William, 1996).

In semi-arid eco-systems, the flora formation is diverse with plant species interrelating with each other depending on environmental conditions. These conditions include: light, temperature and moisture. Analysis of plant associations increases the knowledge of seed dispersal, environmental heterogeneity and biotic interactions (Woods *et al.*, 2019). Plant species are known to associate randomly when interactions between species are weak. *Melia volkensii* has been reported to occur in Acacia-Commiphora vegetation in ASALs and it is commonly referred to be a natural stands' remnant (Muok, *et al.*, 2010) and (Alados *et al*., 2006). There are positive and negative relationships between the species some of which are common phenomena in plant communities. Relationship between plants explains how interacting plants safeguard each other from stressful conditions for instance; shade of trees and extreme temperature conditions. Plant association among species may be caused by biological relationships or by the varied reactions of species to abiotic stimuli, among other things. Additionally, plant association affects the possible connections among species implying that species can interact only where they co-occur (Johnson, 2018). Nevertheless, there might be elevated associations among species, which means that the apparent lack of other species can have an effect on the link between certain species in certain circumstances (Beth *et al*., 2020; Picket, 1980).

The study of plant population status and association may contribute to development of comprehensive and effective conservation strategies of *M. volkensii* in the arid and semi-arid lands (ASALS) of East Africa. It is therefore significant to comprehend the population status of *M. volkensii* so as to inform if there is high erosion of their population and genetic diversity (Runo *et al*., 2004). Additionally, the study on seed storage of this tree species will inform the best storage conditions for longevity.

Generally proper seed storage improves the survival of plants and the seed have good physiological and physical conditions from the harvesting time until planting time. The study results are anticipated to help stakeholders and policy makers to come up with strategies for optimization of germination and natural regeneration of *M. volkensii*.

1.2 Statement of the problem

Over the years, there has been over-exploitation of *M. volkensii* populations leading to depletion of the genetic diversity of this tree species. Despite its significance as a species that is of high value in the dry areas, *M. volkensii* has proved difficult to propagate adequately for extensive production and dissemination. Effort to boost growing of this tree species have not been spared by propagation challenges (Kyalo, 2004). These include seed dormancy, difficulties in extraction of seeds and inability of *Melia volkensii* seeds to survive the seedling stage. Additionally, there is inadequate knowledge on the appropriate seed storage methods which leads to limited supply of quality seeds.

Moreover, mass production of seedlings has not been achieved due to challenges in the establishment of *M. volkensii* plantations in the arid and semi-arid lands of East Africa. Effort to raise *M. volkensii* through various methods like tissue culture and stem cuttings have been carried out by researchers with little success (Malinda *et al*., 2013). Anthropogenic activities such as clear- cut logging, forest fires, grazing and conversion of forests into farmlands have however greatly affected the populations of *M. volkensii* in the drylands of Kenya. Besides the anthropogenic influence it is not clear how plant species combinations and their biological interactions influence population status and natural regeneration of *M. volkensii* in Kasaala location of Kitui County.

1.3 Justification

The act of storing and supplying *M. volkensii* seeds is presently founded on dealing with nuts rather than seeds that have been processed/extracted. Seeds of *M. volkensii* kept as nuts take up much space making charges of transportation and storage high. One kilogram of *M. volkensii* nuts yields about 200 seeds, it is interesting to note that 4,000 to 4,500 seeds per kilogram of extracted seeds is equivalent to one kilogram of *M. volkensii*. Effective handling and storage of processed/extracted *M. volkensii* seeds is rarely practiced since there is limited information on the best storage methods. However, factors responsible for low germination of *Melia volkensii* after storage are not properly comprehended. Findings from this research will advise on the acceptable practice in handling and storage of *M. volkensii* seeds. Additionally, this study will avail data on post and pre-storage extraction which increases the germination rate of *M. volkensii* and will develop improved protocols to boost propagation of *M. volkensii* (Kamondo *et al*., 2016). According to Nturibi (2010), *M. volkensii* despite its popularity has challenges in germination due to unreliable propagation protocol and poor access of quality seeds. Moreover, optimization of seed storage conditions and assessment of *M. volkensii* population status will help in improving the conservation strategies of *M. volkensii* in the dry lands of Kenya. Findings on appropriate storage of seeds of this tree would assist seed suppliers on coming up with a stock of seeds to guarantee supply of seeds at every time even in times when fruiting is irregular.

1.4 Research Hypotheses

i. The risk of tree depletion has no significant effects on the population status of *Melia volkensii* in Kasaala Location of Kitui County.

ii. Storage conditions of seeds and nuts of *Melia volkensii* have no significant effects on germination.

1.5 Objectives

1.5.1 General objective

To develop optimal protocols for seed germination and assessment of population status of *Melia volkensii* to enhance conservation and supply chain management in Kitui County.

1.5.2 Specific objectives

i. To determine the effect of storage conditions on germination of *M. volkensii* seeds stored as extracted seeds and dried nuts.

ii. To evaluate the population status of *M. volkensii* and associated species under natural conditions in Kasaala Location in Kitui County.

1.6 Significance of the Study

Rise in population, land cultivation, economics and urbanization are the conceivable determinants of deforestation in the countries in East Africa like Kenya, Uganda, Tanzania and Ethiopia (Lemma *et al*., 2021). Seed quality plays an important role in the production of agricultural crops. Comprehending behavior of seed storage is indispensable in planning and conducting programmes of planting which enhance sustainable utilization of plant species. The study will also inform the government organizations and non-governmental organizations (NGOs) to change from using poor methods of seed storage and instead focus on how to assist private organizations to afford best seed storage devices. As a result, there will be affordable quantities and qualities of *M. volkensii* seeds; this will eventually raise the economic revenue for people living in the dry lands of Kenya. Moreover, research on evenness of distribution and the population status of *M. volkensii* will inform on conservation of genes and the environmental diversification of *M. volkensii*. Improved propagation of *M. volkensii* for Kenya's ASALs will place Kenya a notch higher in its efforts to increase its forest cover now standing at 7 % compared to the recommended 10 % (FAO and UNEP, 2020). Findings from this research will help improve the growth of Melia leading to the improvement of propagation and preservation of this tree species. Overall, the study will be a huge milestone in attaining environmental sustainability.

CHAPTER TWO

LITERATURE REVIEW

2.1 Description of *Melia volkensii*

Melia volkensii, also commonly known as Melia or Mukau (Kamba), belongs to the family Meliaceae. The tree has been reported to have straight and clean boles approximately 4 meters high without whorls and has no forking below 4 meters in height (Esilaba *et al.*, 2011). Trees that belong to Meliaceae family have male and female flowers or only one type of both and are either dioecious or monoecious. In rare occasions, some species of *M. volkensii* have functional hermaphrodite flowers and may be accompanied by male flowers (Styles, 1972). The fruit of *Melia volkensii* is a pulp that contains approximately 10 % crude fat and over 12 % crude protein. The mature leaves contain more than 5 % crude fat and 21 % crude protein (Muok *et al.*, 2010). Melia fruits are depulped to produce nuts which are later cracked for seed extraction. Brown black seeds are found within the hard kernels and they are propagated in a seedbed filled with sterilized river sand. Seedlings of *M. volkensii* develop a carrot-like root which is full of oils and nutrients that allow them to tolerate dry circumstances (Kamondo *et al.*, 2016).

Plate 2.1: ***Melia volkensii*** **seedlings**

2.2 Economic importance of *Melia volkensii*

Over the years *M. volkensii* has been identified as a highly valued commercial tree in the dry lands of Kenya. It is popular in the dry lands because it is able to tolerate drought. It produces high quality timber used for construction, household furniture, fuel wood and apiculture. The tree is used to make bee hives because the timber can be shaped without difficulty and its flowers produce high quality nectar which is converted into honey (Maundu and Tengas, 2005; Muok *et al.* 2010). The wood is long-lasting and could be utilized for interior paneling, as well as for floor tiles, rafters, and frame construction (Luvanda *et al.,* 2015). In addition, its fruits and leaves are used as fodder for goats and cattle. The flowers provide excellent bee forage and the tree also has medicinal properties (Orwa *et al.,* 2009). Additionally, *Melia volkensii* is used for mulching, providing shade and acts as a wind breaker (Tedd, 1997). It is evident that leave extracts of *M. volkensii* are used by some people to control ticks and fleas on goats (Jaoko *et al*., 2020). The tree is also known in herbal preparations where it is being used as an insecticide (Wilps *et al*., 1993). *Melia volkensii* is used in litter accumulation whereby fallen leaves help to improve the soil fertility consequently, increasing the crop yields (Maundu and Tengas, 2005).

2.3 Distribution and ecology of *Melia volkensii*

The growth of *Melia volkensii* is restricted to the dry lands of Kenya, Tanzania, Ethiopia and Somalia (Kimondo and Kiamba, 2005). The tree is commonly found in Machakos, Mwingi, Kitui and Makueni. The tree is also found growing in the bushland and wooded grasslands in Counties like Embu, Isiolo, Taita- Taveta, Samburu and Marsabit (Crossland, *et al.*, 2021). The species is normally found growing in relation with Acacia-Commiphora vegetation (Mutiga, 2021). It emanates in deciduous bushland, sometimes bordering seasonal canals or appearing on rock outcrops (Maundu and Bo, 2005). *Melia volkensii* trees grow well in sandy, soils but prefers sandy-loamy soils with good drainage. The tree can be found growing at altitudes varying from 350 to 1700 meters above sea level, in regions with average yearly rainfall ranging from 300 to 800 millimeters. *Melia volkensii* does not tolerate black cotton soils or areas prone to water logging (Muok *et al.*, 2010).

2.4 Population status and natural regeneration of trees

Comprehension of plant diversity and natural status of woody species is vital in preserving the forests and restoration of degraded areas (Ghanbari *et al.*, 2021). Moreover, natural regeneration can be relied upon to affect the forest stands' dynamics as well as to preserve the genetic features of local populations of trees. It is popular in large scale forest restoration in African regions (Sugimoto *et al.*, 2011). The processes that bring about naturally regenerating forests promote genetic adaptation and sustain local biodiversity and biological interactions. Most of the indigenous tree species undergo natural regeneration, for instance *M. volkensii, Vitellaria paradoxa*, *Vitex keniensis*, *Parkia biglobosa*, *Tilia americana* and *Adansonia digitata* (Ky-Dembele, *et al.*, 2010). *Melia volkensii* sprouts from stumps as well as growing from seeds. In areas where the tree occurs coppicing is also a common mode of regeneration. Coppicing occurs in trees that have been cut from the base, leading to a strong vegetative response and the regeneration of new shoots (Wang *et al*, 2008). Coppice sprouts are largely formed by latent buds that emerge from the trunk of a tree after it has been cut down or otherwise removed. It is evident that trees can grow from stumps which mostly produce a number of stems. The ability to re-sprouting is a common mechanism of *M. volkensii* rejuvenation in dry forests and it should be deliberated as a method of forest rehabilitation and improvement of agroforestry. (Kruger *et al.*, 1997) Therefore, understanding how abiotic and biotic factors influence natural regeneration is significant for studies of ecology (Khaine, 2018).

2.4.1 Biotic and abiotic factors affecting natural regeneration

Natural regeneration of plant communities is interrelated with the differences in conditions of environment. Saplings that grow in old-growth tropical and subtropical forests are more vulnerable to biological and non-biological factors than mature trees that have grown for a long time (Queenborough *et al*., 2009). Majority of the tree species might have specific light, nutrient and topography requirements for regeneration (Jin *et al.*, 2018) although regeneration of species is not very sensitive to the available resources (Dechnik-Vázquez *et al.*, 2016). When there are biotic factors, like the number of adults and seedlings that are of the same species or different species, they can also affect how many trees grow from seed. Tree species go through niche splitting in conjunction with certain abiotic variables in order to facilitate the co-existence and preservation of a diverse range of species. The abiotic factors include water, light, topography and soil nutrients (Chesson *et al.*, 2000).

Barriers that deter plants' regeneration such as steep slopes of the land, inadequate source of seeds, residual trees and poor drainage which lead to mortality of the seedlings that are not able to compete vigorously. Areas with insufficient source of seeds could not be replenished normally and mountainous areas with extremely erodible soils, might not be appropriate to planting or fertilization. The productivity of forest cover can significantly reduce due to accelerated erosion processes. For instance, the organic top soil is subject to displacement through erosion following disturbances found in areas with steep slope (Ross and Dykes, 1996). In some cases, natural regeneration is adequate to re-establish forest cover however, in other cases, seeding directly or seedlings' planting would be required if re-forestation is occurring in a given time (Yang *et al*., 2019).

2.5 Plant species associations in nature and its relationship to the environment

Plant species association can be defined as the relationship of various species found in an environment. It is also described as a motionless organic link created by the interrelationship of species. However, niche of species regulates how species relate with the environment surrounding them; by having different niches species are able to coexist (Albrecht *et al*., 2001). Species diversity is defined as the number and relative abundance of species found in a given biological organization (Whittaker *et al.,* 2001).

In plant communities, positive and negative relationships amongst species are very common phenomenon. The concept of plant associations informs on the status of conditions of environment like light, temperatures as well as humidity (Dale, 1999). The relationship among species appears from biological associations or diverse reaction of species to abiotic factors and on the other hand, it affects the possible association among species. This implies that species can interact only where they coexist in the same environment (Kershaw and Looney, 1985**).** Species association in a virgin forest provides sufficient information on how to manage forests. For instance, *M. volkensii* and *Cassuarina equisetifolia* improved fertility of soil through augmented nitrogen and phosphorous which was credited to fixation of nitrogen by *C. equisetifolia* through Frankia bacteria and nutrient recycling by *M. volkensii (*Mwadalu *et al.,* 2021). Additionally, species relationship between dominant species of trees like saplings and mature trees was examined in a National Nature Reserve in China. The findings indicated that species relationship showed a remarkable net positive relationship, showing a compelling equilibrium of a steady formation and species composition in old-growth forest (Ziwei *et al*., 2019).

The growth rate of native forests and other crops decelerates as intraspecific competition becomes higher making it a negatively density dependent process. As a result, plant characteristics linked to resource use end up being significant determinants of aggressive relations to community of plants (Duffy, 2006). Previous study on factors affecting multipurpose trees and shrubs utilization in ASALS in Kenya showed that there was an important association amongst the frequencies of woody tree species and the inclusive ultra violet radiation index (Kisangau, 2021).

2.6 Seed storage conditions as determinants of longevity

Seed germination is defined as hydration of the seed leading to emergence of the embryonic radicle and shoot (Bewley *et al.,* 2013). Maintaining seed viability over extended periods in storage is necessary for preserving the genetic integrity of plants. Successful seed storage conditions help in preserving the genetic integrity of *Melia volkensii* for long periods. Seed longevity in storage exemplified by initial viability, freedom from fungal and insect attack, seed maturity, physiological deterioration, and mechanical damage is determined by factors like moisture content of seeds, nature of seeds, temperature and relative humidity (Rajjou *et al.*, 2008). On the basis of storage behavior, three kinds of seed are recognized which are orthodox, recalcitrant and intermediate seeds (Berjak and Pammenter, 1997). Recalcitrant seeds are sensitive to chilling, are highly hydrated and they cannot withstand intensive desiccation **(**Bustam, *et al.,* 2021). *Melia volkensii* seed behavior is orthodox thus can retain its viability for several years under appropriate storage conditions (Orwa *et al*., 2009).

2.6.1 Seed moisture content

Seed moisture is the most crucial component in sustaining germination rate during storage, because it is the principal factor influencing all cellular processes (Bonner *et al*., 2008). The metabolism rates of both orthodox and recalcitrant seeds could be decreased by maintaining the seedlings in a dry condition at cold temperatures. True orthodox seeds maintained at low moisture content of between 5-10 % can be stored comfortably at nearly any temperature compared to recalcitrant seeds. Therefore, seed moisture content at the time of storage determines how low the temperature can be set for seed storage. Orthodox seeds can be exposed to sub-zero temperatures, while recalcitrant seeds cannot. Orthodox seeds can be stored for a few years at temperatures as low as -20°C (Ellis *et al.*, 1992) and (Ellis and Hong, 2006).

2.6.2 Seed storage temperature and relative humidity

The ideal storage temperature for seeds is approximately 14^{o}C with a relative humidity of slightly less than 40 %. Most refrigerators have high relative humidity and hold a temperature of approximately 14.2^{o}C (Bharat *et al.,* 2012). Seeds stored in a refrigerator should be kept in containers that are tightly closed to keep the humidity levels low. Orthodox seeds will sustain survivability better when hardened to low content of moisture of roughly 4¬8 percent and then preserved in an environment in which humidity is regulated, than when preserved in equilibrium with ambient air humidity (Zhang *et al.,* 2021). Cool condition is mostly favorable for storage of orthodox seeds. Long-term conservation of gene resources of orthodox agricultural seeds is at a temperature of less than -20^{o} C and a moisture content of approximately -6^{o}C to 16^{o}C. Previous study shows that half-life is doubled for seeds dried to equilibrium and thereafter to approximately -20^{o}C in waterproof containers under long-term gene bank (Ellis, 1996).

2.6.3 Factors responsible for germination of *Melia volkensii*

The longevity of orthodox seeds, also referred to as drying-tolerant seeds is thus determined by content of moisture (MC) and temperature. Previous research indicates that factors responsible for poor germination of *Melia volkensii* during storage have been attributed to both physiological and physical factors (Milimo and Hellum, 1989). It is documented that farmers have used various traditional technologies to break seed dormancy to enhance propagation of *Melia volkensii*. These methods include: scarification procedure which is done by burning of the nuts. Nuts that are regurgitated by goats are usually used by farmers to raise seedlings (Mwamburi, 2005). Additionally, there are other methods that are used by farmers for instance splitting or cracking of the nuts and sowing of them in troughs (Kidundo 1997).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

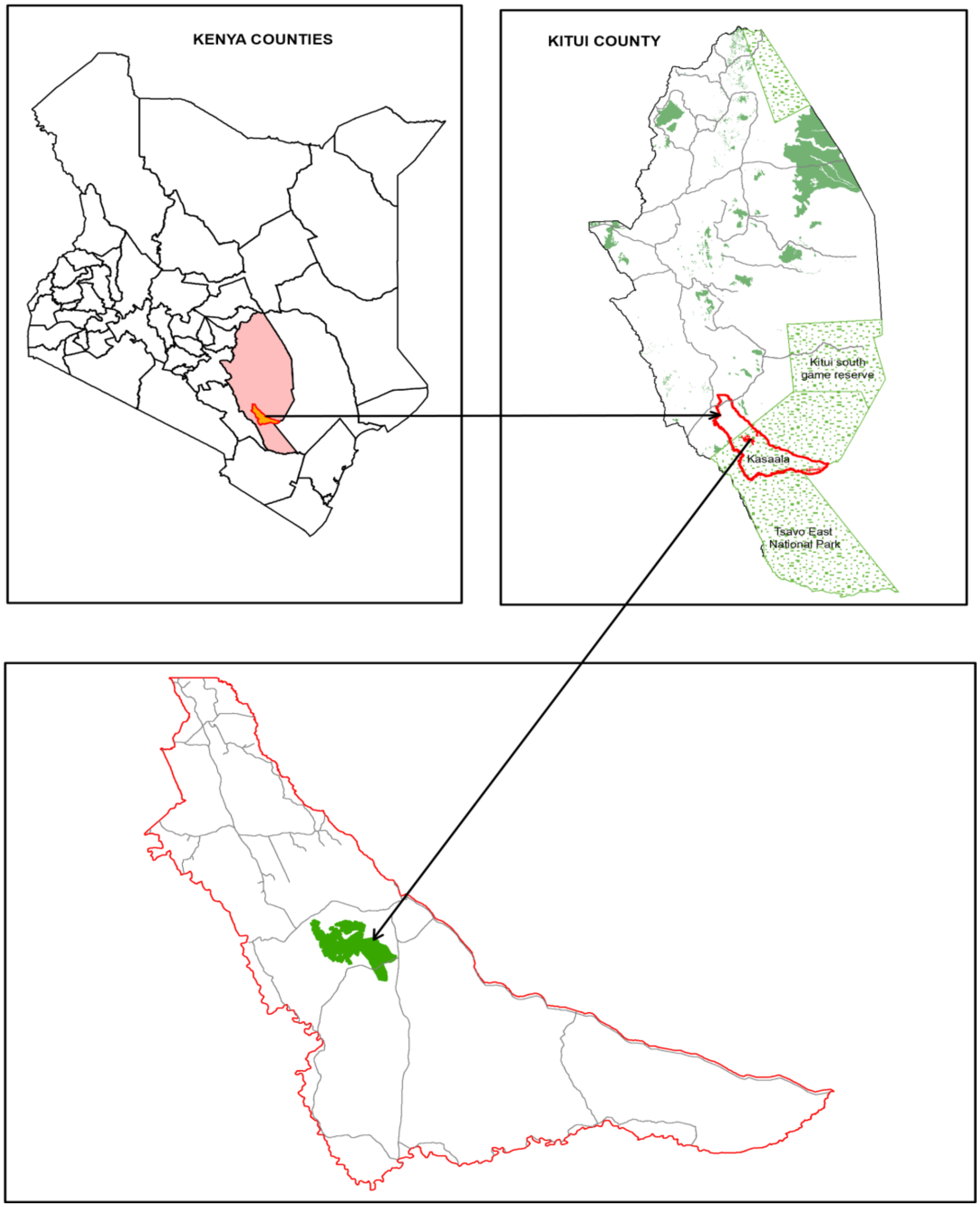
Field study was undertaken in two sites of Kitui County, namely Kasaala and Tiva forests. Kasaala is a Location in Ikutha Sub-County of Kitui South constituency about 440 km from Kenya's capital city Nairobi, and approximately 21 km from Tsavo East National Park (Figure 3.1). Kasaala is known to host *Melia volkensii* trees that naturally occur unlike in Tiva forest where the trees are planted. Natural regeneration of *M. volkensii* is likely to occur in Kasaala because most land area is undisturbed. Tiva forest is 194 km East of Nairobi along Kitui-Machakos road (Figure 3.1). The experiment on germination of *M. volkensii* seeds was established in the nursery beds of KEFRI Kitui, 15 km away from Tiva Forest.

The soils of the study area consist of metamorphic parent material and the dominant soil groups are alfisols, ultisols, oxisols, and lithic soils. These soils are highly erodible and are of low fertility. The ultisols and alfisols are also susceptible to capping, which increases runoff and makes the clay soils hard to cultivate by the end of the dry season (Barber *et al*., 1981). Approximately, less than 20 % of Kitui County has well-drained, deep, friable red and brown clays of good fertility. More than 60 % of the region has relatively shallow, sticky, red, black, and brown clays of variable fertility (Jaetzold and Schmidt, 1983). The study area, Kitui County, shares a similar geographical and vegetation structure with the Tsavo East National park. This environment favors the growth of the naturally growing *M. volkensii* being adapted to the dry weather conditions (Gachathi, 1994).

Kitui County is among the semi-arid Counties in Kenya that is faced with serious water scarcity. The numerous droughts encountered have resulted to a decline in water supplies which has likely contributed to the drying up of most of the seasonal rivers. The County is mostly dry and hot with temperatures ranging 14°C during the coldest months (July-August) and 34°C between January and March which are known to be the hotter months. The County receives between 500 mm and 1050 mm of rainfall annually, with an average rainfall of 900 mm a year (Gladys, 2017). The study area has an elevation between 400 m to 1830 m above sea level (Jaetzold, 2006) and the most widespread vegetation type in Kitui, is a thicket and bushland, especially Acacia-Commiphora associations (Ndegwa *et al*., 2016).

3.1.1 Population and livelihood activities in Kitui County

Agriculture contributes more than 80 % of the income generated by the remote population and directly hires more than 35 % of the population in Kitui County. More than 80 % of the population is engaged in farming activities especially in the production of crops such as cereals, horticultural and industrial crops (Yohannis, 2019). The total annual average of cereal production in the County is 80,680 metric tonnes (Mt), valued at KSh 4.24 billion and industrial crops (sunflowers, cotton and sisal are valued at KSh 29.04 million for an average production of 771 Mt. The average production of horticultural crops is approximately 36,950 Mt; at a cost of nine hundred and ninety million (Mbiti and Misango, 2021). Rearing livestock is also a major economic activity in Kitui County (Kivunzya, 2019). Households relies on livestock for food security and for economic production. The major components of livestock in Kitui County include indigenous goats, cows, fowl, and donkeys. The farmers prefer these animals because they are resilient to the hot and dry climatic conditions that occur during drought. Of the total farming households, more than 40 % rear indigenous cattle and approximately 70 % raise chicken and goats (Kivunzya, *et al.*, 2018). More than 40 % of households raise donkeys which are used as a means of transport especially when fetching water across long distances during dry seasons. Donkeys are sometimes included in dowry negotiations (Mbiti and Misango 2021). Annual production of honey in the County is 960 tonnes with 14 % of households engaged in the enterprise (Warui *et al.*, 2018; Musinguzi, *et al.*, 2018).



Legend

Roads

Tiva forest

Scale 1:500,000

Figure 3.1: Map showing Tiva Forest within Kasaala Location in Kitui County

3.2 Research design

3.2.1 Establishment of sampling plots for study of population status of *M. volkensii* and associated species in Kasaala location

Plant sampling was done using a stratified design; this is a sampling method which encompasses the population division into minor sub-groups called strata. The entire population of trees was distributed into two different sites namely bushland and farmland. A bushland can be defined as an area covered with shrubs and trees or any other natural vegetation while a farmland is a land used for farming (Sisay *et al.,* 2021**)**. The bushland in the study area consisted of a secondary vegetation of Acacia- Commiphora while the farmland was used for production of sorghum, cowpeas, maize and pigeon peas which were intercropped with *M. volkensii*. Broad vegetation formations of the study area consist of natural and manmade features. The natural features include seasonal rivers and valleys, while the man-made features include dams, urban settlements and roads. Seven transects were established to cover the whole forest using guidelines by Plumptre (1994). Transects 1 and 7 were laid on the disturbed sites where farming, grazing and cutting down of trees was rampant in search of timber and fuel. Transects 2,3,4,5 and 6 were laid on the bushland where natural regeneration was taking place (figure 3.2). Along each transect, 5 plots were established by using a geographic coordinate system to locate latitude and longitude of the study area. The number of trees, coppices and seedlings that were found along the length of the line and inside the plots were counted and recorded in data sheets.

3.2.2 Sampling of *M. volkensii* and associated tree species

Fifty by fifty-meter plots were established across transects measuring 1050 meters. Five plots were put in every transect at a range of 200 meters space between each plot (Manning *et al.,* 2020). All the trees of *M. volkensii* and associated tree species were sampled within the plots. Diameter at breast height (DBH) and height of *M. volkensii* trees were determined in each plot. A diameter tape was used to determine DBH at 1.5 meters from the ground. Suunto clinometer was adopted in measuring the tree height from the base of the tree and a linear tape was used to measure 20 meters. A nail was fixed on the tree's bark where the linear tape was hooked and placed horizontally from the eye to the tree. The linear tape and the clinometer were held by both hands and the bottom measurement of the clinometer was subtracted from the top reading. Additionally, coppices and mature trees were sampled within a fifty by fifty meters plot, while survival counts of seedlings was done within a 5 by 5 sub-plot nested within and at the corners of the 50 by 50 m plot.

Voucher specimens of *M. volkensii* and associated tree species were collected and identified in the field using identification guides (reference books, plant identification manuals and identification keys to plants). The voucher specimens were prepared by pressing the fresh materials on herbarium press boards and later drying the specimens using a plant drier (Miller, 1999). They were mounted on labelled herbarium sheets and deposited in the Kenya Forestry Research Institute local herbarium at Muguga. The specimens that could not be easily identified in the field were taken to the herbarium of the National Museums of Kenya for identification.

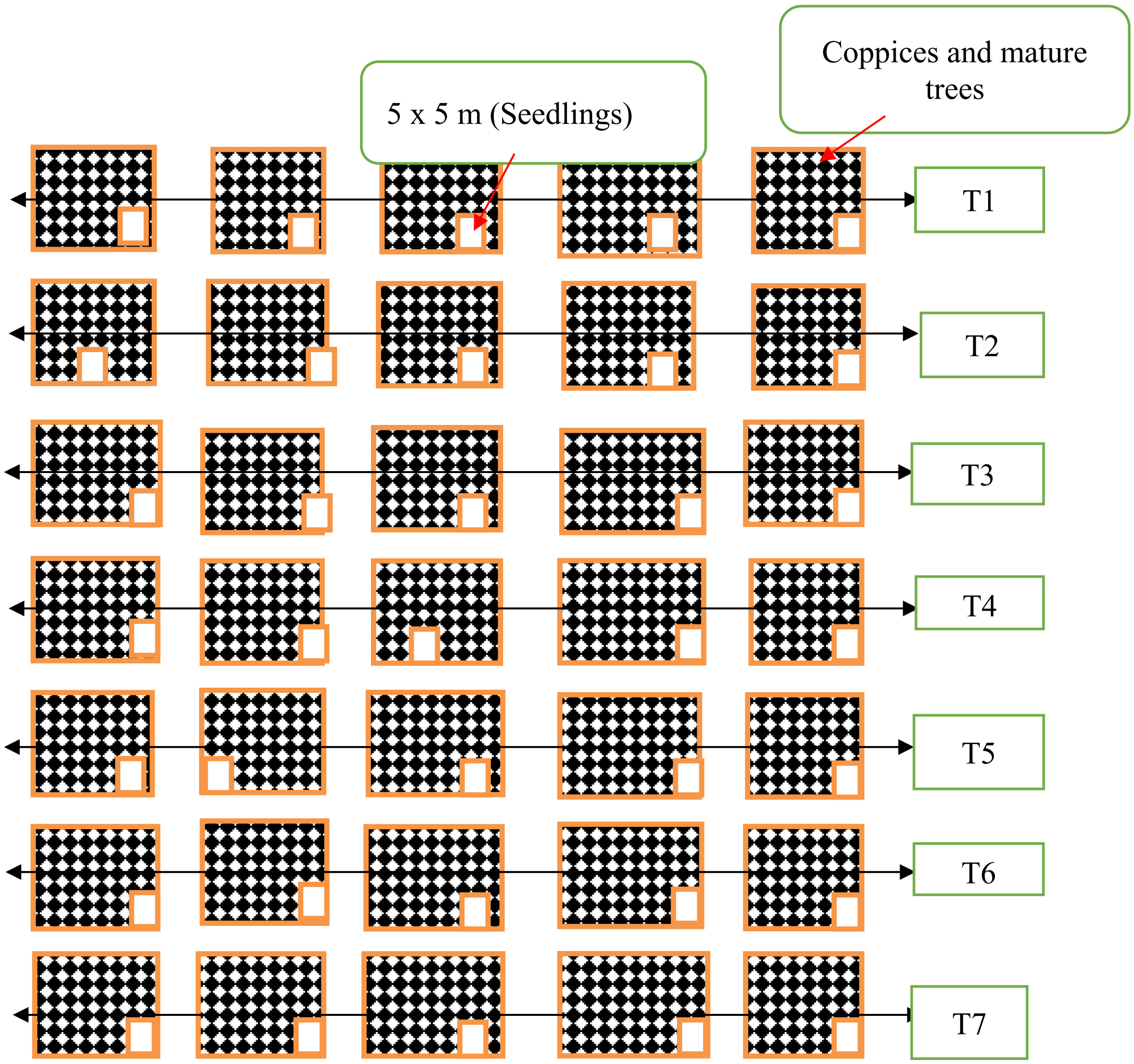


Figure 3.2: Diagram (not drawn to scale) showing line transects established in Kasaala Location

3.3 Germination and viability experiments

Mature ripe fruits of *M. volkensii* were collected from Tiva forest and depulped. *Melia volkensii* fruits were depulped by removing the outer fleshy part using a plank of wood to while placing them on stone. After depulping the fruits, the nuts obtained were dried and were put on a groove carved out on a wooden plank and cracked open using a knife and hammer (Kamondo *et al*., 2016). The germination materials were divided into two lots the first lot being *M. volkensii* nuts and the second lot extracted seeds. The nuts were cleaned in water and sun-dried for 5 days in preparation for seed extraction. The dry seed sub lots were also sun-dried for three days to a moisture content (MC) of 6 % that was determined by oven drying at 103°C for 24 hours (Ellias *et al.,* 1990). The moisture content was calculated by subtracting the dry weight of seeds from the initial/wet weight of seeds multiplied by one hundred (100).

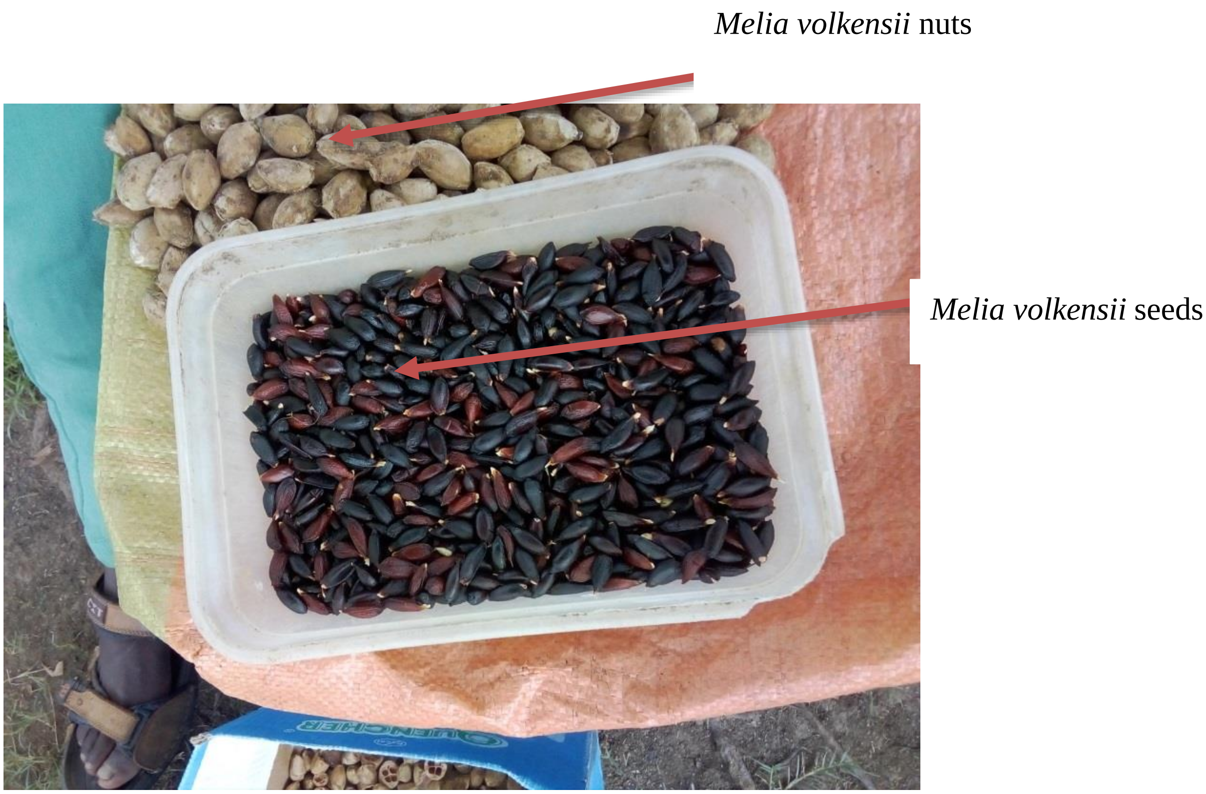


Plate 3.1: Dried nuts and extracted seeds of *Melia volkensii*

Four hundred dry seeds were used for initial germination in 4 replicates of 100 seeds each as per International Seed Testing Association germinationn protocol (ISTA, 2012). During test for germination, seeds were compressed and decontaminated in a solution of 5 grams of a fungicide Ridomil Gold fungicide (Metalaxyl-M and S-isomer) in 1 litre of distilled water. The seeds were later soaked in cold water (4^{o}C) for 12 hours and finally rinsed with 1 % sodium hypochlorite solution. The coat of seeds was split longitudinally utilizing a razor blade that was decontaminated. Seeds were germinated in plastic containers using fine sterilized clean river sand as the substrate (Dolor, 2009). The seeds were covered with a layer of sand drenched with fungicide (Muok *et al.*, 2010). The number of germinated seedlings was daily recorded for a period of 10 days. Once scored, the sprouted seedlings were retrieved using a pair of tweezers to confirm the daily counts were for newly propagules.

In order to test the storage conditions' effect on germination of *M. volkensii*, seeds and nuts were stored under different conditions and tested for germination at an interval of 2 months after storage. The experimental conditions were room temperature (30^{o}C) and in refrigerator at -20°C; under each temperature condition seeds and nuts were stored in sealed or plastic containers that are uncapped. The germination materials were tested for their germination at a 2-month interval for the next 6 months (Bharat, 2012; Probert, 2007). Each germination test consisted 4 replicates each with 25 seeds or nuts. Thus, the experimental design was three factorials arranged in a completely randomized design (figure 3.3). Germination was recorded for a period of 10 days.

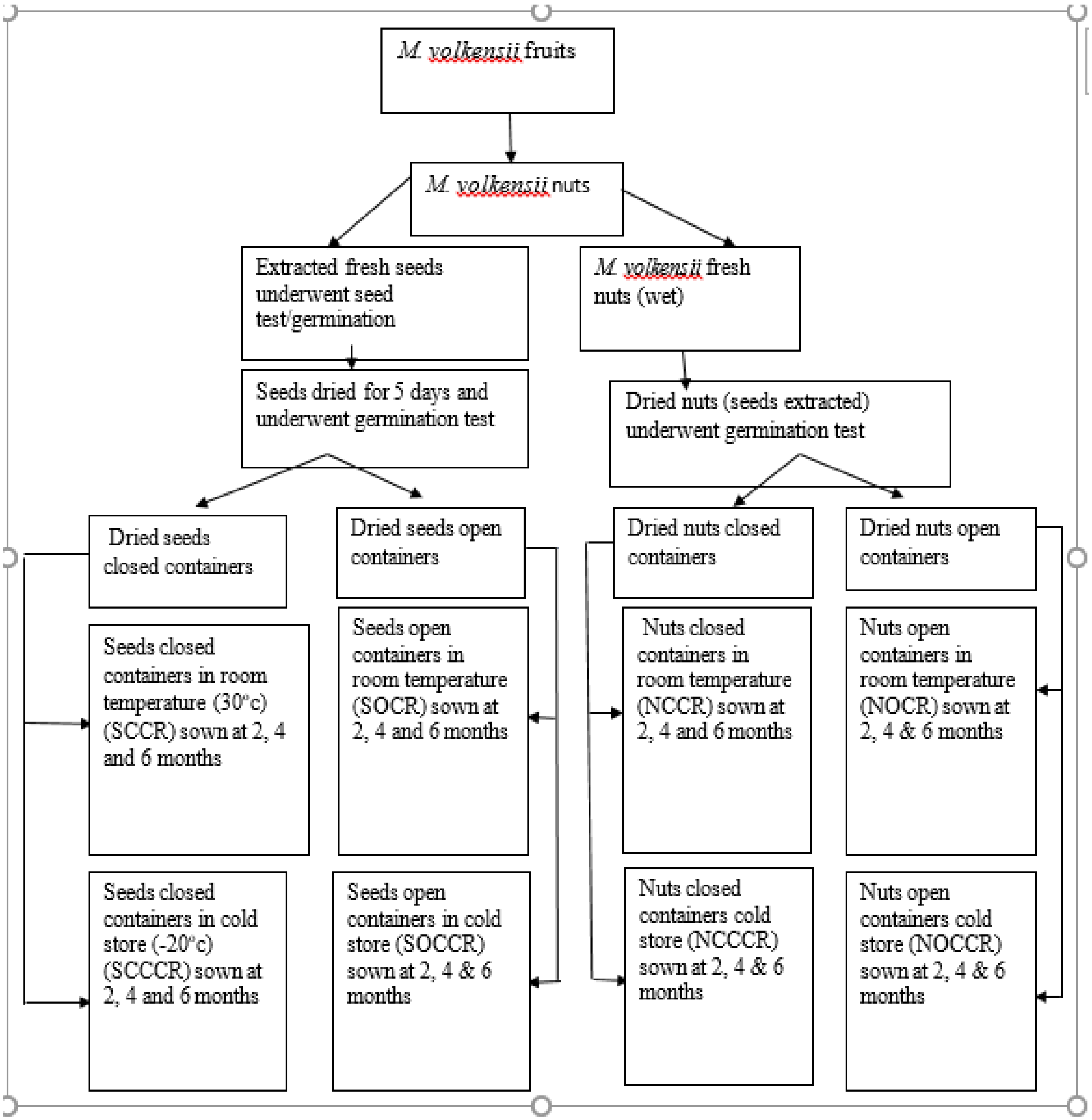


Figure 3.3: Flow diagram showing storage of *Melia volkensii* seeds and nuts

Plate 3.2: Collection of *Melia volkensii* fruits from Tiva forest

Plate 3.3: Seed extraction and commencement of *M. volkensii* germination tests

Plate 3.4: *Melia volkensii* fruits

3.4 Data Analysis

Data collected were entered into Microsoft Excel spreadsheets (2019) for subsequent statistical procedures. Statistical Analysis Software (SAS), version 9.4 was used to analyze germination, while R software version 2.1.3 was used to analyze the population status of *M. volkensii.*

3.4.1 Population status of *M. volkensii* and diversity of associated tree species

One-way analysis of variance (ANOVA) at 5 % significance level was used to test whether there were any statistically significant differences between the means of diameter at breast height (DBH) and height of the trees in the sampled plots. The statistical test was also used to determine if the means of mature trees, coppices and seedlings were different. Tukey honestly significant difference (HSD test) was utilised to separate means for all the parameters. Data obtained from counting trees in Kasaala forest were used to derive total density of plant species, species richness, relative abundance, and diversity of plants in the study area. Relative species abundance was used to compute the occurrence of each species in comparison with the other species recorded. Shannon wiener diversity index was used to estimate the tree species richness and evenness of individual distribution in the tree species. Additionally, Simpson's diversity index was instrumental in estimating diversity of trees and estimating the probability of two individual trees that belonged to different species (Spellerberg, 2003).

3.4.2 Formulae used for determining tree parameters were as follows:

i) Simpson's diversity index was computed as follows:

$$D=\sum_{i=1}^{R}\frac{ni(ni-1)}{N(N-1)}$$

Where N = the total number of organisms of a particular species, while n = the total number of organisms of all species while R symbolizes richness which is a measure of how many different types the dataset of interest contains.

ii) Shannon-Wiener was computed as follows:

$$H'=\frac{N\ln N-\sum(ni\ln ni)}{N}$$

a) Where *n*i is the number of individuals in species *i*, and *N* is the total number of species in the" sample.
b) Species richness = number of species per specified number of individuals.

Since the datasets were small, the number of coppices, seedlings and mature trees were counted manually.

c) Species density = number of species per unit area. Species density per m^2 is usually used to measure species richness.

d) Relative species abundance = $\frac{\text{Number of plant species from one group}}{\text{Total number of species from all groups}}$

e) Plant diversity= (ni/N). N is total number of individuals in S species, while ni is the number of individuals in the ith species.

3.4.3 Germination tests

Germination data for stored nuts and extracted seeds were compared using the t test at 5 % level of significance using a two-way ANOVA to analyze the effects of seed storage treatments and seed storage time. The treatments used were seeds stored either as extracted seeds or nuts at a temperature of 30^o C and -20^oC in closed and open containers. In this study seed storage time was 2, 4 and 6 months. At $P < 0.05$, germination means were separated using Fisher's least significant difference test. At confidence interval of 95 %, the germination trend of extracted seeds and nuts that were stored at different environmental conditions and in different containers was tested using a t test.

3.5 NACOSTI research license

The license is an ethical approval to conduct research on optimization of germination and assessment of population status of *Melia volkensii* Gürke under natural conditions in Kitui County, Kenya for a period ending: 20/April/2022 (Appendix 10).

CHAPTER FOUR

RESULTS

4.1 Population status of *M. volkensii* in study area (Kasaala Location)

4.1.1 Diversity and relative abundance of tree species in Kasaala Location

In this study, a total of 20 families were represented in the sampled plots. These included Fabaceae (9 species), Euphorbiaceae (3 species), Bursereaceae (5 species) and Tiliaceae (6 species). There were 67 plants of which *M. volkensii* represented 12.2 % relative abundance and *Acacia tortilis* accounted for 10.0 %. *Adansonia digitata* represented a relative abundance of 9.5 % while *Commiphora baluensis* accounted for 8.5 % (Table 4.1).

Table 4.1: Diversity of *M. volkensii* and associated tree species in Kasaaala Location

Family name	Species name	Number of trees	Relative abundance
Acanthaceae	*Duosperma cooperi*	4	0.73
Aizoaceae	*Delosperma cooperi*	2	0.36
Anacardiaceae	*Lannea schimperi*	15	2.73
Asclepiadaceae	*Calotropis strophela*	1	0.18
Burseraceae	*Commiphora africana*	11	2.00
	Commiphora baluensis	8	8.46
	Commiphora capensis	1	0.18
	Commiphora edulis	17	3.10
	Commiphora eminii	7	1.28
Sterculiaceae/Malvaceae	*Sterculia rhynchorcarpa*	14	2.55
Capparaceae	*Boscia angustifolia*	12	2.19
	Maerua crassifolia	1	0.18
	Maerua decumbens	7	1.28
	Maerua holstii	3	0.55

	Thylachium thomasii	2	0.36
Rubiaceae	*Tennantia sennii*	7	1.28
Combretaceae	*Combretum aculeatum*	17	3.10
	Terminalia brownii	2	0.36
	Terminalia prunioides	7	1.28
Euphorbiaceae	*Euphorbia ingens*	1	0.18
	Euphorbia tirucalli	1	0.18
	Eurphobia scheffleri	1	0.18
Fabaceae	*Albizia anthelmintica*	13	2.37
	Bauhinia coriacea	1	0.18
	Bauhinia natalensis	15	2.73
	Cassia abbreviata	17	3.10
	Dalbergia melanoxylon	1	0.18
	Delonix alata	15	2.73
	Erythrina melanacantha	8	1.46
	Tamarindus indica	8	1.46
	Tephrosia villosa	2	0.36
Malvaceae	*Adansonia digitata*	52	9.47
Tiliceae	*Grewia africana*	2	0.36
	Grewia bicolor	2	0.36
	Grewia calymmatosepala	12	2.19
	Grewia flava	4	0.73
	Grewia tephrodermis	32	5.28
	Grewia villosa	18	3.28
Meliaceae	*Melia volkensii*	67	12.20
Mimosaceae	*Acacia busciae*	3	0.55
	Acacia mellifera	7	1.28
	Acacia nilotica	29	5.28
	Acacia senegal	15	2.73
	Acacia tortilis	55	10.02

Portulacaceae	*Calyptrotheca taitensis*	4	0.73
Rhamnaceae	*Berchemia discolor*	9	1.64
Solanaceae	*Solanum campylacanthum*	4	0.73
	Solanum taitensis	2	0.36
Zygophyllaceae/ Balanitaceae	*Balanites aegyptiaca*	12	2.19
Phyllanthaceae	*Bridelia taitensis*	2	0.36
Grand total		**552**	**100 %**

4.1.2. Mean number of *Melia volkensii* trees in the study area (Kasaala Location)

There were significant differences in the number of *M. volkensii* trees that were found in the transects ($P < 0.05$) [table 4.2]. The highest number of *M. volkensii* trees were recorded in transect 2 while the lowest values were obtained in transects 1, 5 and 6 ($P < 0.05$). The differences were not however consistent with where transects were laid meaning that disturbance of land did not influence the number of trees regenerating.

Table 4.2: Mean number of *Melia volkensii* trees in Kasaala Location

Transect number	Mean number of *M. volkensii* trees
1.	1.20 ± 0.84^{b}
2.	4.80 ± 1.40^{a}
3.	2.40 ± 1.12^{ab}
4.	2.20 ± 0.86^{ab}
5.	0.60 ± 0.25^{b}
6.	0.80 ± 0.37^{b}
7.	1.40 ± 0.51^{ab}
P value	**< 0.05**

The number of *M. volkensii* trees observed varied among transects (Figure 4.1). *Melia volkensii* trees found in the bushland had the highest relative abundance (24%, 12 % and

11 %) in transects 2, 3 and 4 respectively. It was least represented in transects 5 and 6 at 3 % and 4 % respectively. The trees that were found on the farmland had a moderate representation of relative abundance (6 % and 7 %) in transects 1 and 7 respectively.

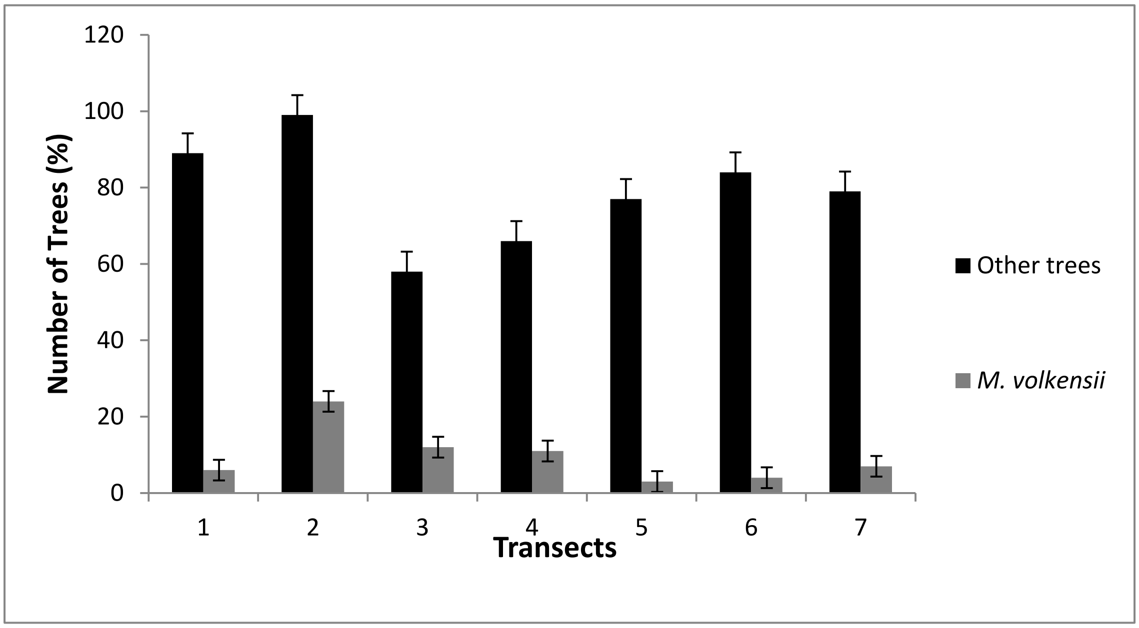


Figure 4.1: Relative abundance of M. volkensii and other tree species in Kasaala Location

4.1.3: Height of *Melia volkensii* in Kasaala Location

The mean height of *M. volkensii* trees were highest in transects 1, 3, 4 and 6 and lowest in transect 2. The differences were significant at ($P < 0.05$) as shown in Figure 4.2.

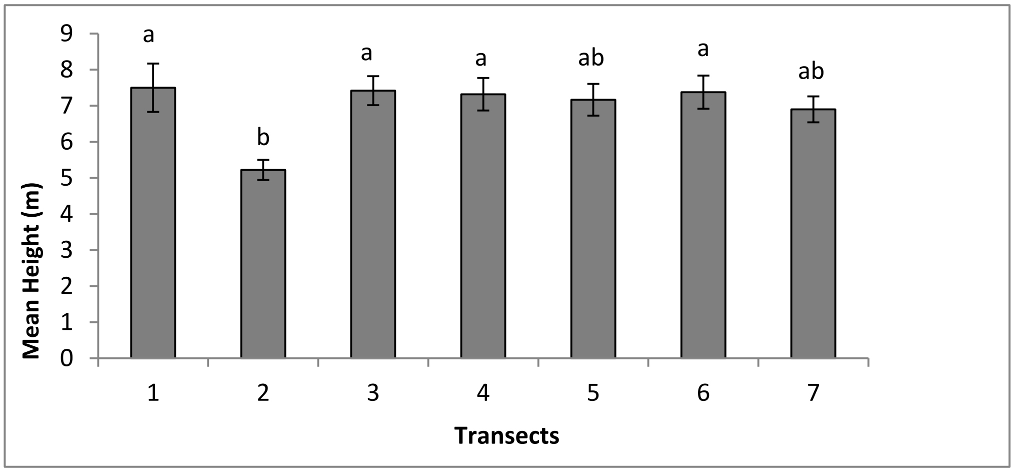


Figure 4.2: Mean height of M. volkensii in Kasaala Location

4.1.4 Mean DBH (diameter at breast height) of *M. volkensii* in Kasaala Location

At $P<0.05$, the DBH of trees within transects showed significant differences. The mean DBH of *M. volkensii* trees in transect 1 (40.8 cm) was significantly highest compared to the other transects at $p \leq 0.05$. Transects 3, 4, 5, 6 and 7 had mean DBH of between 24.0 to 31.4 cm, while transect 2 had the lowest mean DBH of 19.2 cm (figure 4.3).

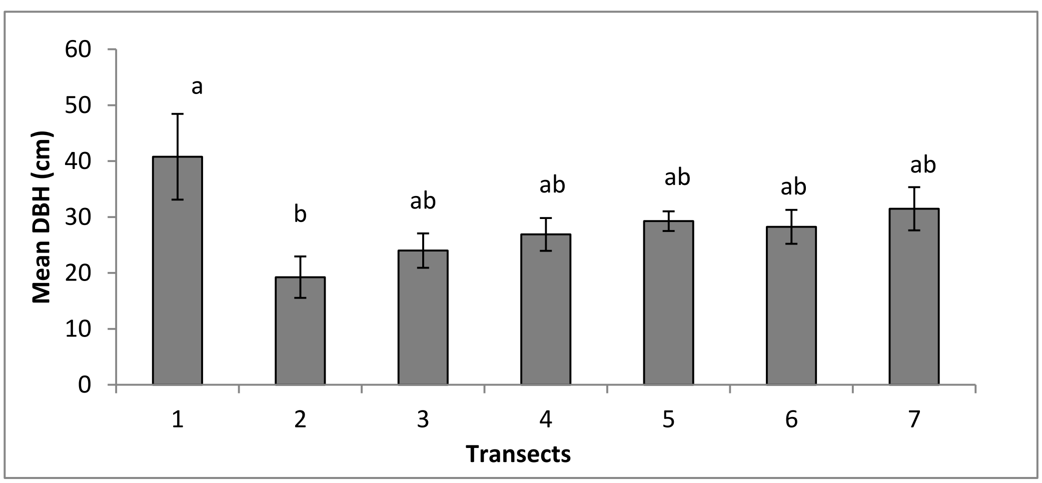


Figure 4.3: Mean DBH (cm) of *M. volkensii* in Kasaala Location

4.1.5 DBH size-class distribution in Kasaala Location

Distribution of diameter at breast height of *M. volkensii* trees was computed for the all transects combined. The highest DBH size class was in the cluster ranging between 20.5 and 30 cm and the lowest was in the cluster that ranging between 70.5 and 80 cm with the latter having only 1 tree. The size class distribution ranging between 40.5-50 cm and between 50.5- 60.0 cm had the second lowest representation of 3 trees each (Figure 4.4).

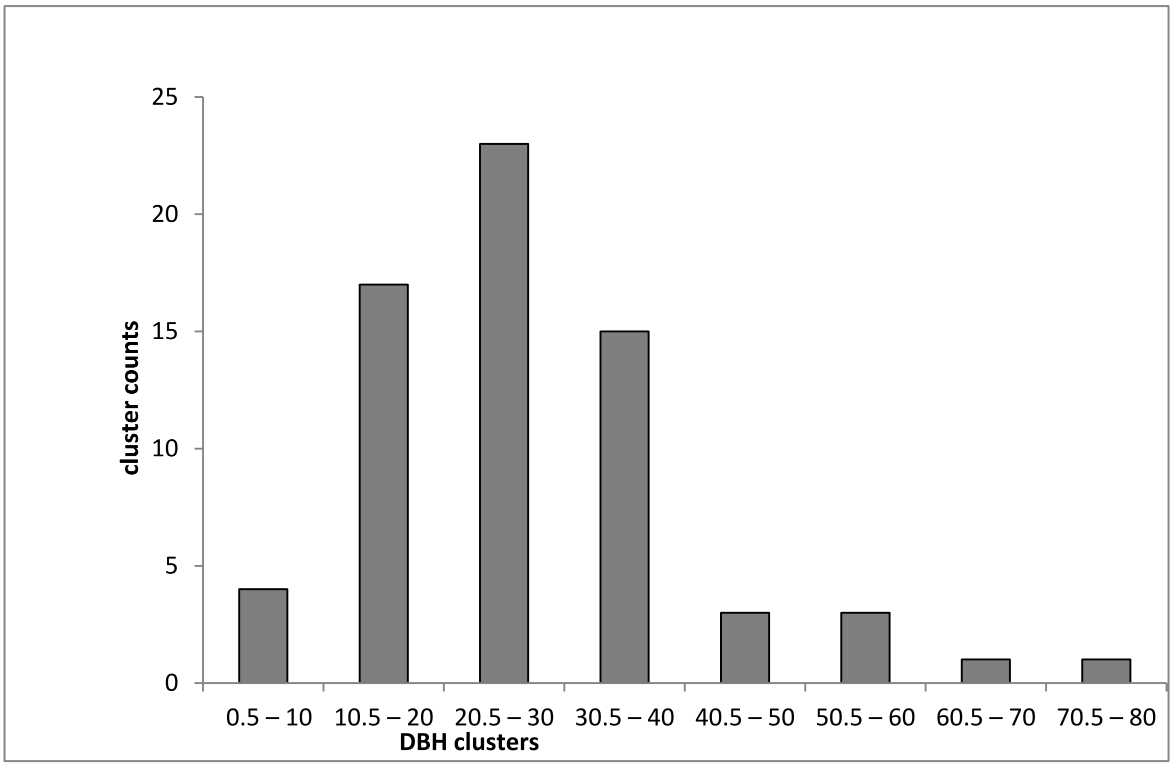


Figure 4.4: DBH size-class distributions of *Melia volkensii* in Kasaala Location

4.1.6 Natural regeneration of *M. volkensii in* Kasaala Location

Coppicing and natural seed germination of *M. volkensii* were recorded in the study area. The mean number of coppices found in the study area were significantly different between transect 1 and 2 at $P<0.05$. At $P >0.05$ there were no significant differences in the mean number of seedlings that were found in Kasaala Location (Table 4.3).

Table 4.3 Mean number of coppices and seedlings in Kasaala Location

Transect Number	Mean number of coppices	Mean number of seedlings
1	0.60 ± 0.4^{b}	0.40±0.4
2	9.20 ± 1.3^{a}	2.40±0.9
3	1.20 ± 0.5^{b}	1.80±0.2
4	8.20 ± 1.0^{a}	1.00±0.6
5	2.20 ± 0.9^{b}	1.60±0.5
6	1.00 ± 0.5^{b}	1.40±0.7
7	1.40 ± 0.7^{b}	0.60±0.6
P value	**<0.05**	**> 0.05**

4.1.7 Diversity indices of tree species in Kasaala Location

The highest Shannon diversity index of 2.23 was recorded in transect 7. The lowest Shannon diversity index was recorded in transect 3 (1.76). Shannon Wiener values as a measure of species evenness indicates that there was greater diversity in transect 7 compared to transect 3. Other (transects 1,2,4,5 and 6) had Shannon diversity indices ranging from 1.98 to 2.2. The highest Simpson's index was recorded in transect 7 (0.74), while the lowest Simpson's index was recorded in transect 3 (0.69), other transects (transects 1,2,4,5 and 6) had Simpson's diversity indices ranging from 0.69 to 0.73 (Table 4.4). Simpson's index calculates the species evenness and richness as a measure of diversity. In this study thus transect 7 recorded the highest diversity and transect 3 the lowest. Diversity was therefore not determined by disturbance of the study site since transects 7 and 1 were established in farmland while transect 3 was in bushland.

Table 4.4: Diversity indices of tree species in Kasaala Location

Transects	**Shannon-Wiener Index (H')**	**Simpson's Index (λ)**
1	2.03	0.70
2	2.02	0.72
3	1.76	0.69
4	1.98	0.71
5	2.13	0.71
6	2.20	0.73
7	2.23	0.74

4.2: Effect of storage conditions on germination of *M. volkensii* seeds

4.2.1 Initial germination percentage of *M. volkensii* nuts and seeds

Initial germination test was done before storage of nuts and seeds. Nuts and seeds were dried to a moisture content of 6 %. At $p \leq 0.05$, there was no significant difference in initial germination percentage between dried extracted seeds and dried nuts (Table 4.5).

Table 4.5: Initial germination percentage of *M. volkensii* nuts and seeds

Seed type	Germination %
Nuts	57.7
Seeds	30.5
P value	**>0.05**

4.2.2 Germination percentage of *M. volkensii* nuts and seeds after storage

Nuts and extracted seeds were stored in different conditions (30^{o}C and -20^{o}C) and in closed and open containers for 6 months and germination tests done at 2, 4 and 6 months interval. There was a gradual increase in germination percentage over the 6 months experimental period (figure 4.5 and 4.6).

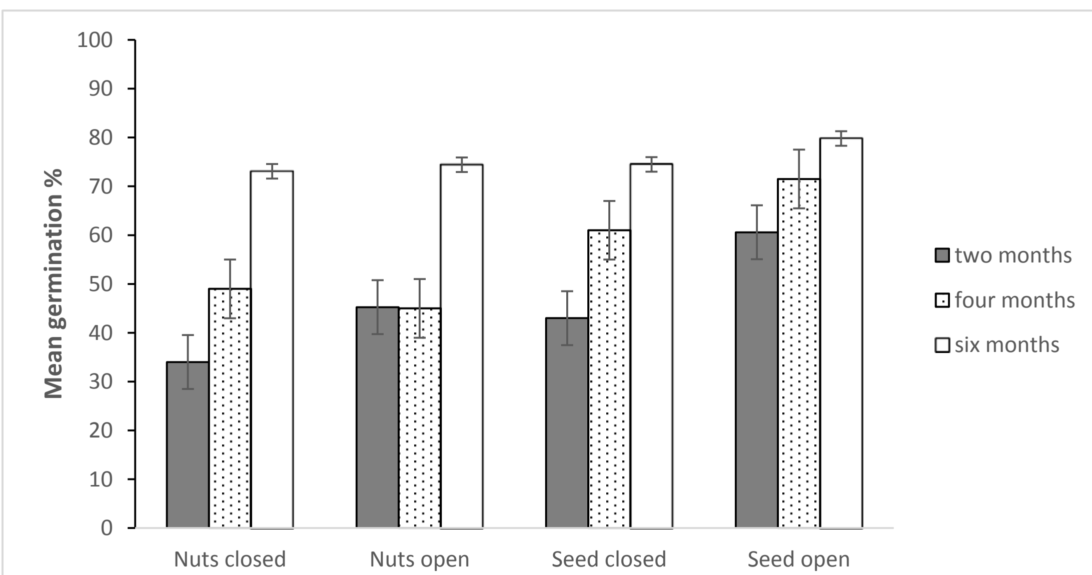


Figure 4.5: Mean germination percentage of M. volkensii under cold temperature (-20oC) over a period of 2-6 months.

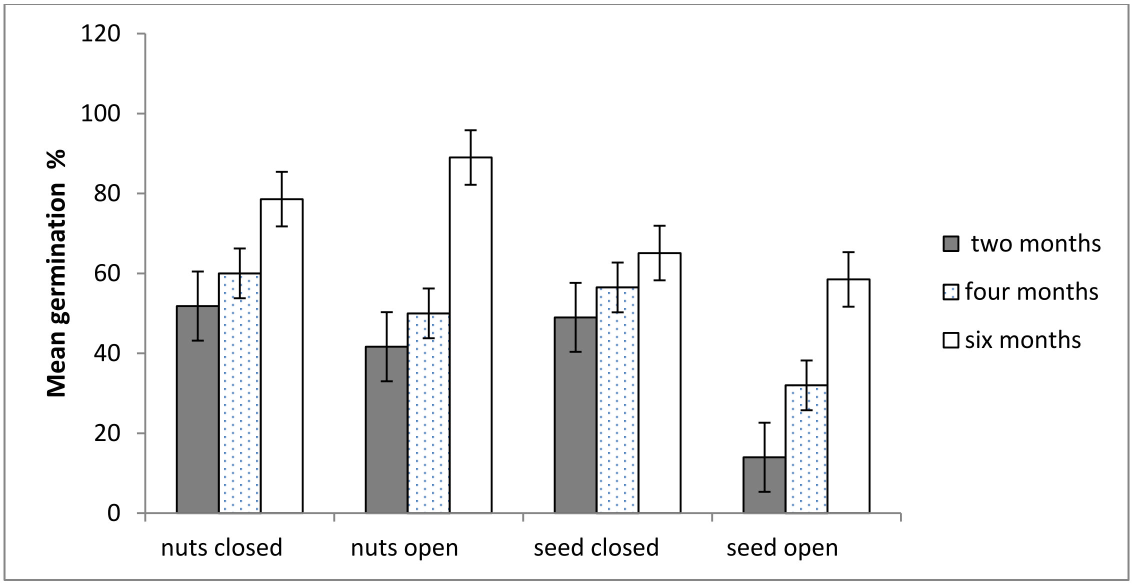


Figure 4.6: Mean germination percentage of *M. volkensii* in room temperature (30°C) over a period of 2-6 months

At two months storage, seeds stored in open containers in cold room (-20°C) had the highest mean germination percentage of 60.58 whilst seeds in open room showed the least mean germination percentage of 14.00. The mean germination percentage under the different storage conditions after two months were in the following decreasing order: seed open cold room > nuts closed room > nuts open room > seeds closed cold room > seed closed room > nuts open cold room > nuts closed cold room > seed open room.

For the two-month period, significant differences at ($P< 0.05$) were generally observed between seeds stored in open containers under cold room (-20°C) and nuts stored in closed containers under room temperature (30°C). This was also observed between nuts in closed containers under room temperature and nuts stored in open containers under room temperature (30°C). There was also significant difference between nuts stored in closed containers under cold room and seed open room. There was no significant difference (P

>0.05) between nuts stored in open containers in ambient temperature and seeds kept in closed containers under cold room. Seeds stored in closed containers in room temperature and nuts stored in open containers under cold room had the intermediate germination means (table 4.6).

The highest mean germination percentage after a four-month period of storage was for seed that were stored in open containers in cold room (71.5 %) and the least mean germination percentage was for seed that was stored in open containers in room temperature (32 %). The mean germination percentage after four months were in the following decreasing order: seed open cold room > nuts closed room > seeds closed cold room > nuts closed room > seed closed room> nuts open room> nuts closed cold room> nuts open cold room > seed open room. At $P < 0.05$, there were significant differences between seed kept in unsealed containers at ambient temperature and nuts stored in room temperature in closed containers. The significant differences were also noted in nuts stored in room temperature in closed containers and those stored in open containers in room temperature (table 4.6).

The highest mean germination percentage after a six-month period of storage was recorded in nuts stored at room temperature (30^{o}C) in open containers (89 %), while the lowest was in seeds stored in closed containers at room temperatures (58.5 %). Nuts stored in cold room (-20^{o}C) in closed containers and those stored in open containers at room temperatures (30^{o}C) had the intermediate mean germination percentages. At $P > 0.05$, there was no significant difference between seeds that were stored in closed containers in room temperature (30^{o}c) and nuts that were kept in unsealed containers in room temperature (table 4.6).

Table 4.6: Germination means of *M. volkensii* during storage

Storage conditions			Months in storage		
Extraction	**Temperature**	**Container**	**2**	**4**	**6**
Seeds	Room	Open	14.0±1.37^{f}	32.0±2.7^{e}	58.5±4.1^{bc}
	(**30**^{o}C)	Closed	49.0±0.58^{bc}	56.5±0.36^{bc}	65.1±1.8^{c}
	Cold ((**20**^{o}C)	Open	60.58±2.4^{a}	71.5±1.9^{a}	79.8±3.7^{ab}
		Closed	43±9.6^{d}	61.0±2.3^{b}	74.5±3.1^{ab}
Nuts	Room (**30**^{o}C)	Open	41.7±2.6^{d}	50.0±3.1^{cd}	89.0±3.2^{a}
		Closed	51.8±3.2^{b}	60.0±2.7^{b}	78.6±3.5^{ab}
	Cold	Open	45.1±3.3^{bcd}	45±1.0^{d}	74.4±5.1^{ab}
	(**20**^{o}C)	Closed	34.0±1.8^{e}	49.0±2.3^{cd}	73.1±5.2^{ab}
P value			**<0.05**	**<0.05**	**<0.05**

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Status and Population structure of *Melia volkensii* in the Study Area

In this study, *M. volkensii* was the most abundant species in the study area, followed by *Acacia tortilis.* These results are related to findings of Anjarwalla *et al.* (2016) who observed that *M. volkensii* is found growing alongside the Acacia-Commiphora vegetation, along uncultivated land which is covered with natural vegetation. The study also agrees with Kamondo *et al.* (2016), who reported that the tree species are drought tolerant and adaptable to the semi-arid weather conditions in Kitui County. Additionally, there were significant differences in the mean number of *M. volkensii* trees found in the study area, though the differences were not however consistent with where transects were established. It was expected that transects laid in the farmland would register lower mean number of *M. volkensii* trees than those in the bushland which wasn't the case. This can be attributed to the fact that the ecological patterns of a plant population are characterized by its population dynamics, as well as the correlation between each individual plant and the external environments (Odum, 1971).

In the study area, there existed vast farmlands with tall well-groomed cultivated *Melia volkensii* trees which were found growing on both the farmlands and the natural bushland vegetation. The bushland possessed a high H^1 index of tree species and supported a range of vegetation cover unlike the farmland that exhibited a lower H^1 index of tree species and a limited range of vegetation cover. This is probably because of anthropogenic activities that were carried out in the farmland for instance cultivation, logging, overgrazing, and other habitat degradation activities thus causing impact on the trees and undercover biological species. These results are related to the observation of Caviedes and Ibarra (2017) who stated that environmental change caused by human beings have the ability to degenerate and simplify forest stands by accelerating the loss of trees, coppices and seedlings. These results also agree with the study carried out by Tsechoe (2014) that species richness increases at a lower elevation but decreases at a higher elevation. He also stated that the slope and aspect affect plant diversity in many ways, consequently affecting the formation of soils from parent rock. This is due to the soil physicochemical properties and microbial activities that are usually abundant at the topsoil leading to an abundance of tree species on flat bushland topography.

According to Borda-Nino *et al.* (2021) fluctuations in the sloppiness influence the vegetation composition and structure, leading to a lower diversity. *Melia volkensii* trees had high DBH and height indicator of the high rate of trees' growth, which were probably controlled by combined effects of biotic and abiotic factors, particularly soil microorganisms, soil nutrients, water, weather patterns, sunlight, and competition among trees (Stephenson, *et al*., 2014).These results are also in agreement with Pang *et al.* (2017) who observed that the total tree height averages for assessment that was carried out on indicator species of a natural forest was between 0.2 -0.5 meters.

From this study, it was evident that, the steep slope farmlands carried a few species adapted to a narrow range of prevailing conditions. This can be attributed to the fact that soil erosion was prevalent on the farmland, leading to soil structure loss, nutrient degradation, and soil compaction. Similar results were confirmed by Yang Liu *et al.* (2019), who observed that the topographical features were essential for soil formation. As well, they affect the soil condition and bacterial communities. Congruently, the steep soils may be eroded and thus lose their topsoil during the formation process. Therefore, they become thinner than the more nearly level grounds that receive deposits from areas upslope Flores *et al.* (2020).

A considerable portion of the bushland had a big number of mature trees, coppices, and seedlings of *Melia volkensii*. Thus, the positive association between mature individuals and juveniles of this tree species in the bushland suggests that *M.volkensii* juveniles are successfully establishing under the canopy of other associating trees that were found growing in the study area. This shows the role of trees in generating vegetation cover that plays a vital role in the maintenance of plant diversity in the study area. These results are congruent with Xu *et al.* (2020) who found evidence of positive association between tree over story-layer composition and the understory layer diversity in different forest types.

5.1.2 Effect of seed extraction and storage conditions on viability of *M. Volkensii*

The extracted seeds and those enclosed in nuts and stored for a period of two, four and six months significantly improved under all storage conditions from the initial germination of 30.5% to 89%. *Melia volkensii* seeds stored in open containers under cold storage recorded a higher mean germination percentage as compared to seeds stored in closed containers under cold storage. These results are akin to findings according to a *Melia volkensii* growing guide in Kenya's dryland regions (2018). From the study, it is evident that storing extracted seeds under air-tight conditions damages the seeds, leading to a lower germinability. These results are related to Kamondo *et al*. (2016) who observed that extracted seeds are vulnerable to external conditions and lose viability in one month's period. As observed, *M. volkensii* seeds that were held in the form of nuts (un-extracted) were highly viable compared to those that were stored in form of seeds. This trend was ascribed to the stony layer covering *M. volkensii* seeds, enabling them to preserve the right moisture content, light and temperature for a longer time.

The nuts stored for a period of 2, 4 and 6 months in open containers under room temperature had a higher mean germination percentage as compared to seeds stored under similar storage conditions. These results are in agreement with the findings of Jaoko *et al.* (2020) who reported that *M. volkensii* extracted seeds possess a high oil content, which comes from health delicate unsaturated fats. Upon thermal shock, sunlight, and air, the seeds degrade, turning rancid. In reaction, the seeds lose germination and consequently have a limited shelf life that results to a reduced germination rate. In my study, *M. volkensii* nuts that are protected by the outer hardcover exhibited prolonged viability. This is probably because the stony layer that covers the *M. volkensii* seeds protects the seeds from adverse conditions for instance very high temperatures and humidity. These results are akin to findings of Chen *et al.* (2013) that seeds which are enclosed by shell are shielded from exposure to oxygen and light. The findings confirmed that nuts are free from damage caused by pests, mold and excess external moisture; hard shell provides greater protection against microbial contamination. Considering that *M. volkensii* thrives in arid and semi-arid habitats, the pulp and the hard seed coat enables this tree species to remain viable until the conditions are right for germination.

Seeds stored in open containers under cold room temperature had a significance difference and a higher mean germination percentage as compared to seeds stored in open containers under room temperature. This can be attributed to the ability of a cold room to store seeds at the correct temperature while controlling the moisture level unlike the case of ambient temperature. The results agree with the findings by Hirano, *et al*. (2009) who reported that seeds should be dried and stored at cold temperatures for long-term preservation. Cryopreservation, or freezing in liquid nitrogen at -180°C can also be used for extremely long-term storage. The results are entirely in line with what Vasques *et al.* (2014) observed in seeds of three Mediterranean shrub species stored at a low temperature. The stored seeds in their study maintained a high viability and vigor for a couple of years but ambient temperature storage led to a marked decline after one year ending in almost complete mortality after 4 years of shelf storage. By contrast, Croft *et al.* (2012) stated that very cold storage for instance refrigerators consists of quite high relative humidity which can damage seeds due to high moisture content, therefore according to their study, storing seeds in cold storage is not fit for seed viability.

Nonetheless, seeds stored at room temperature in sealed containers for 2, 4, and 6 months showed a higher germination percentage than those kept in open containers but the same temperature conditions. This is probably due to lack of a barrier upon the open containers (between the seed and the outside atmosphere), thus permitting movement of oxygen and water leading to irreversible seed deterioration. The low germination agrees with findings of Jour *et al.* (2018) that open containers are unable to create a barrier between the seed and the outside atmosphere, which allows movement of oxygen and water between the outside aerosphere and the stored seed. Consequently, this causes a rise of metabolic activity, high respiration and fungal attack causing the seeds to die and as a result low germination is observed. The results are related to the observation of Alemayehu *et al.* (2020), who observed that agriculture seeds placed in atmospheric temperatures in open bins sometimes result in limited storage life and seed degeneration in hot, humid locations. These findings are more or less similar to the study of Steven *et al*. (2014) who reported that air is made up of 21 % by volume of oxygen, which reacts with essential materials found in the seeds. The reaction of oxygen with seeds decreases the grade of seeds and their germination rate.

Similar findings were also observed by Jour *et al.* (2018) that when seeds are stored at 7-8% MC in closed containers, they maintain over 50 % germination for a considerable number of months. There is some indication that a further reduction of MC will conserve viability even better. Sealed or hermetic storage systems are a very effective means of controlling seed moisture and insect activity, especially in tropical conditions (Pan et al., 2019). According to Mattana, *et al*. (2019), reduction of moisture content of seeds up to 10 percent after seeds are dry, make the seeds to achieve moderate germination of seeds under diverse conditions of storage. The study as well agrees with the findings of Fenolossa *et al*. (2020), who stated that viability and germination tested in two different populations of *Carissa edulis* revealed germination of 70–90% for seeds which have led to reduction of moisture content. In comparison, percentage of germination condensed to 20 percent in population two, which had higher moisture content. This study is related to findings of (Himstedt, 2002) who stated that Macadamia kernels rapidly develop rancidity when stored at room temperature at higher moisture content.

5.2 Conclusions

i. The seed storage study showed that the storage conditions of seeds and nuts significantly affected the seed germination capacity of *M. volkensii*, therefore seed germination depends on storage conditions. The study concludes that *M. volkensii* seeds and nuts stored for six months had the highest germination levels as compared to storage at 2 and 4 months in regardless of the storage conditions.

ii. From the study of assessing the population status of *Melia volkensii*, it is evident that the risk of tree depletion has no significant effects on the population status of *Melia volkensii* and associated tree species in Kasaala Location of Kitui County. Findings from this study will help improve the production of *M. volkensii* and associated tree species thus promote its propagation and conservation.

5.3 Recommendations

5.3.1. Recommendations from the study

i. *Melia volkensii* seeds should be stored and distributed as extracted seeds as opposed to current practice of storing and distributing these seeds when enclosed in nuts. This will reduce cost of storage and transportation due to bulkiness of *M. volkensii* nuts.

ii. There is a great need to conduct more extensive research on the population status of *M. volkensii* and associated tree species in Kitui County. Tree species with poor regeneration should be prioritized during rehabilitation and conservation of the tree species in the study area.

5.3.2 Areas for further research

i. Detailed research should be done to evaluate the biotic and abiotic factors that affect seed germination and growth of *Melia volkensii.*

ii. Further research should be done to assess the dependence between germination and seed reserves as well as their mobilization during germination of different species of Melia tree.

iii. Further research is required to quantitatively assess the impacts of storing *M. volkensii* seeds for more than 6 months in order to determine whether germination of this tree species will rise above the 89% that was recorded at 6 months storage.

iv. Detailed regeneration studies should be done in Kitui County so as to fully understand the mechanisms that promote natural regeneration.

v. Conservation stakeholders should device alternative source of livelihood for local community so as to discourage anthropogenic activities that lead to overconsumption, pollution and overexploitation of forest products. This will help to reduce the pressure on the forest and hence conserving the environment.

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APPENDICES

Appendix 1: Composition of tree species in transect 1

Species	Number	Percentage
Grewia tephrodermis	12	13.48
Acacia tortilis	10	11.24
Adansonia digitata	7	7.87
Melia volkensii	6	6.74
Tennantia sennii	6	6.74
Albizia anthelmintica	5	5.62
Cassia abbreviate	5	5.62
Acacia nilotica	4	4.49
Combretum aculeatum	4	4.49
Acacia Senegal	3	3.37
Balanites aegyptiaca	3	3.37
Commiphora Africana	3	3.37
Commiphora baluensis	3	3.37
Delonix alata	3	3.37
Tamarindus indica	3	3.37
Delosperma cooperi	2	2.25
Grewia bicolor	2	2.25
Grewia villosa	2	2.25
Acacia mellifera	1	1.12
Bauhinia coriacea	1	1.12
Bauhinia taitensis	1	1.12
Boscia angustifolia	1	1.12
Commiphora edulis	1	1.12
Tephrosia villosa	1	1.12
Total	**89**	**100**

Appendix 2: Composition of tree species in transect 2

Species	**Number**	**Percentage**
Melia volkensii	24	24.24
Acacia tortilis	11	11.11
Sterculia rhynchorcarpa	10	10.10
Adansonia digitata	7	7.07
Acacia nilotica	4	4.04
Cassia abbreviate	4	4.04
Combretum aculeatum	4	4.04
Grewia villosa	4	4.04
Tamarindus indica	4	4.04
Bauhinia nataliensis	3	3.03
Grewia tephrodermis	3	3.03
Berchemia discolor	2	2.02
Commiphora Africana	2	2.02
Commiphora baluensis	2	2.02
Commiphora edulis	2	2.02
Erythrina melanacantha	2	2.02
Grewia flava	2	2.02
Terminalia prunioides	2	2.02
Acacia Senegal	1	1.01
Calyptrotheca taitensis	1	1.01
Commiphora eminii	1	1.01
Euphorbia ingens	1	1.01
Euphorbia tirucalli	1	1.01
Lannea schimperi	1	1.01
Thylachium thomasii	1	1.01
Total	**99**	**100**

In transect 2, *Melia volkensii* was most represented tree species 24.24% (n=24), followed by *Acacia tortilis* 11.11% (n=11). There was a total of 99 tree species.

Appendix 3: Composition of tree species in transect 3

Species	**Number**	**Percentage**
Melia volkensii	12	20.69
Acacia tortilis	10	17.24
Adansonia digitata	8	13.79
Delonix alata	4	6.90
Grewia tephrodermis	4	6.90
Acacia busciae	3	5.17
Grewiavillosa	3	5.17
Acacia nilotica	2	3.45
Berchemia discolor	2	3.45
Lannea schimperi	2	3.45
Terminalia brownie	2	3.45
Boscia angustifolia	1	1.72
Commiphora africana	1	1.72
Commiphora baluensis	1	1.72
Grewia calymmatosepala	1	1.72
Grewia flava	1	1.72
Maerua crassifolia	1	1.72
Total	**58**	**100**

In transect 3, *Melia volkensii*was most represented tree species 20.69% (n=12), followed by *Acacia tortilis* 17.24% (n=10) and *Adansoniadigitata* 13.79% (n=8). There was a total of 58 tree species.

Appendix 4: Composition of tree species in transect 4

Species	Number	Percentage
Acacia tortilis	11	17.46
Melia volkensii	11	17.46
Adansonia digitata	10	15.87
Acacia nilotica	3	4.76
Balanites aegyptiaca	3	4.76
Lannea schimperi	3	4.76
Bauhinia nataliensis	2	3.17
Berchemia discolor	2	3.17
Boscia angustifolia	2	3.17
Commiphora africana	2	3.17
Grewia villosa	2	3.17
Cassia abbreviate	1	1.59
Combretum aculeatum	1	1.59
Commiphora baluensis	1	1.59
Duosperma cooperi	1	1.59
Erythrina melanacantha	1	1.59
Grewia calymmatosepala	1	1.59
Grewia tephrodermis	4	6.06
Maerua decumbens	1	1.59
Solanum campylacanthum	1	1.59
Sterculia rhynchorcarpa	1	1.59
Tamarindus indica	1	1.59
Tephrosia villosa	1	1.59
Total	**66**	**100**

In transect 4, both *Acacia tortilis* and *Melia volkensii* were equally represented amongst tree species 17.46% (n=11) being the highest in the population, followed by *Adansoniadigitata* 15.87% (n=10). There was a total of 63 tree species.

Appendix 5: Composition of tree species in transect 5

Species	Number	Percentage
Grewia tephrodermis	8	10.39
Commiphora edulis	7	9.09
Grewia calymmatosepala	7	9.09
Acacia tortilis	6	7.79
Adansonia digitata	6	7.79
Combretum aculeatum	6	7.79
Bauhinia nataliensis	3	3.90
Commiphora africana	3	3.90
Melia volkensii	3	3.90
Acacia Senegal	2	2.60
Albizia anthelmintica	2	2.60
Boscia angustifolia	2	2.60
Calyptrotheca taitensis	2	2.60
Commiphora eminii	2	2.60
Delonix alata	2	2.60
Duosperma cooperi	2	2.60
Lannea schimperi	2	2.60
Maerua decumbens	2	2.60
Cassia abbreviate	1	1.30
Commiphora baluensis	1	1.30
Commiphora campensis	1	1.30
Grewia africana	1	1.30
Grewia flava	1	1.30
Grewia villosa	1	1.30
Maerua holstii	1	1.30
Solanum taitensis	1	1.30
Sterculia rhynchorcarpa	1	1.30
Tennantia sennii	1	1.30
Total	**77**	**100**

In transect 5, both *Grewiatephrodermis* 10.39% (n=8), *Commiphoraedulis* 9.09% (n=7) and *Grewia calymmatosepala*9.09% (n=7) were most represented amongst the population. *Melia volkensii* was 3.90% (n=3) of the population. There was a total of 77 tree species.

Appendix 6: Composition of tree species in transect 6

Species	Number	Percentage
Acacia nilotica	10	11.90
Acacia mellifera	6	7.14
Acacia Senegal	6	7.14
Acacia tortilis	6	7.14
Adansonia digitata	6	7.14
Lannea schimperi	5	5.95
Albizia anthelmintica	4	4.76
Boscia angustifolia	4	4.76
Melia volkensii	4	4.76
Balanites aegyptiaca	3	3.57
Berchemia discolor	3	3.57
Cassia abbreviate	3	3.57
Grewia villosa	3	3.57
Solanum campylacanthum	3	3.57
Commiphora edulis	2	2.38
Delonix alata	2	2.38
Erythrina melanacantha	2	2.38
Grewia calymmatosepala	2	2.38
Maerua decumbens	2	2.38
Terminalia prunioides	2	2.38
Calotropis strophela	1	1.19
Calyptrotheca taitensis	1	1.19
Dalbergia melanoxylon	1	1.19
Duosperma cooperi	1	1.19
Grewia tephrodermis	1	1.19
Thylachium thomasii	1	1.19
Total	**84**	**100**

In transect 6, *Acacia nilotica* 11.90% (n=10) had the highest representation in the population, followed by *Acacia mellifera* 7.14% (n=6), *Acacia senegal* 7.14% (n=6), *Acacia tortilis* 7.14% (n=6) and *Adansoniadigitata* 7.14% (n=6). *Melia volkensii* was 4.76% (n=4) of the population. There was a total of 84 tree species.

Appendix 7: Composition of tree species in transect 7

Species	Number	Percentage
Adansonia digitata	8	10.53
Melia volkensii	7	9.21
Acacia nilotica	6	7.89
Bauhinia nataliensis	6	7.89
Commiphora edulis	5	6.58
Commiphora eminii	4	5.26
Delonix alata	4	5.26
Acacia senegal	3	3.95
Balanite aegyptiaca	3	3.95
Cassia abbreviate	3	3.95
Erythrina melanacantha	3	3.95
Grewia villosa	3	3.95
Terminalia prunioides	3	3.95
Albizia anthelmintica	2	2.63
Boscia angustifolia	2	2.63
Combretum aculeatum	2	2.63
Lannea schimperi	2	2.63
Maerua decumbens	2	2.63
Maerua holstii	2	2.63
Sterculia rhynchorcarpa	2	2.63
Acacia tortilis	1	1.32
Grewia africana	1	1.32
Grewia calymmatosepala	1	1.32
Solanum taitensis	1	1.32
Eurphobia schefferi	1	1.32
Bridelia taitensis	2	2.63
Total	**79**	**100**

In transect 7, *Adansonia digitata* (n=8) had the highest numbers in the population (10.53%) and *Melia volkensii* 9.21% (n=7) had the second highest numbers in the population. There was a total of 76 tree species.

Appendix 8: DBH size-class distribution of *Melia volkensii* in the study area (Kasaala Location)

	Transects							
DBH clusters	1	2	3	4	5	6	7	**Total**
0.5 – 10	0	3	1	0	0	0	0	4
10.5 – 20	1	10	3	2	0	0	1	17
20.5 – 30	1	3	4	7	2	2	4	23
30.5 – 40	1	6	3	1	1	2	1	15
40.5 – 50	0	0	1	1	0	0	1	3
50.5 – 60	2	1	0	0	0	0	0	3
60.5 – 70	1	0	0	0	0	0	0	1
70.5 – 80	0	1	0	0	0	0	0	1
Total	**6**	**24**	**12**	**11**	**3**	**4**	**7**	**67**

Appendix 9: Diversity of *M. volkensii* and associated tree species in Kasaaala Location

Tree species	Species richness (mean values)	Groups
Adansonia digitata	0.51	a
Acacia busciae	0.48	ab
Acacia tortilis	0.44	ab
Albizia anthelmintica	0.38	ab
Acacia nilotica	0.37	ab
Acacia senegal	0.37	ab
Terminalia prunioides	0.36	ab
Tamarindus indica	0.36	ab
Balanite aegyptiaca	0.35	ab
Delosperma cooperi	0.30	ab
Grewia bicolor	0.30	ab
Terminalia brownii	0.30	ab
Acacia mellifera	0.30	ab
Tennantia sennii	0.30	ab
Erythrina melanacantha	0.27	ab
Delonix alata	0.26	ab
Grewia tephrodermis	0.26	b
Bauhinia taitensis	0.20	b
Maerua holstii	0.20	b
Sterculia rhynchorcarpa	0.18	b
Commiphora edulis	0.16	b
Boscia angustifolia	0.15	b
Grewia calymmatosepala	0.15	b
Lannea schimperi	0.15	b
Commiphora eminii	0.15	b
Combretum aculeatum	0.14	b
Cassia abbreviata	0.13	b
Commiphora africana	0.11	b
Grewia villosa	0.11	b
Commiphora baluensis	0.10	b
Duosperma cooperi	0.10	b
Grewia flava	0.10	b
Berchemia discolor	0.09	b
Maerua decumbens	0.05	b
Bauhinia coriacea	0.01	b
Calotropis strophela	0.01	b
Calyptrotheca taitensis	0.01	b
Commiphora campensis	0.01	b
Dalbergia melanoxylon	0.01	b
Euphorbia ingens	0.01	b
Euphorbia tirucalli	0.01	b
Eurphobia scheffleri	0.01	b
Grewia africana	0.01	b
Maerua crassifolia	0.01	b
Melia volkensii	0.01	b
Solanum campylacanthum	0.01	b
Solanum taitensis	0.01	b
Tephrosia villosa	0.01	b
Thylachium thomasii	0.01	b

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