PRODUCTION, UTILIZATION, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF TAMARIND (*Tamarindus indica*) ACCESSIONS FROM SEMI-ARID EASTERN KENYA

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JOMO KENYATTA UNIVERSITY OF

AGRICULTURE AND TECHNOLOGY

2022

Production, Utilization, Characterization and Antimicrobial Activity of Tamarind (*Tamarindus Indica*) Accessions from Semi-Arid Eastern Kenya

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A Thesis Submitted in Partial Fulfilment of the Requirements for The Degree of Doctor of Philosophy in Horticulture of the Jomo Kenyatta University of Agriculture and Technology

2022

DECLARATION

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

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DEDICATION

This thesis is dedicated to my dad, Albert Kidaha Govoga for the encouragement to work hard and be different and to my mother Sarah Munga, for pushing me to accomplish what she did not. May God give them fruitful long lives!!

ACKNOWLEDGEMENT

I wish to express my profound gratitude to my supervisors Prof. Fredah K. Rimberia Wanzala, Dr Remmy Kasili Wekesa and Prof. Wariara Kariuki for their great support, guidance and encouragement during the entire study period. I wish to thank the Institute for Biotechnology Research staff and the Department of Horticulture and Food Security, JKUAT, particularly Prof. Turoop Losenge and Prof. Aggrey Nyende. I wish to appreciate DAAD for sponsoring my PhD program and JKUAT for giving me ample time to study. I thank Dr. Grace Wacheke, Dr. Dadjo Columbe, Mr. Samuel Mwaura, Mr. Richard Rotich whose advice was helpful in molecular work. I wish to appreciate my beloved family and friends for their love and constant encouragement, as well as advice throughout this work. Above all, I give thanks to God Almighty for His grace, uncountable blessings, strength, love and energy which made this research possible.

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ACRONYMS AND ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribose Nucleic Acid
EDTA	Ethylene diamine tetraacetic acid
GPS	Global Positioning System
ISSR	Inter Simple Sequence Repeats
MAS	Metres Above the Sea level
PC	Principal Component
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
SSR	Simple Sequence Repeats
TBE	Tris borate EDTA

ABSTRACT

Tamarind (*Tamarindus indica L*) is a fruit tree native to tropical parts of Africa and Asia. It is used as food and a source of raw material in the food industry, pharmaceuticals as well as providing ecosystem services. Despite the great value of tamarind, there is limited information related to its utilization, production, morphological diversity, genetic diversity and antimicrobial potential in Kenya. The objectives of this study were to evaluate production and utilization, morphological diversity, genetic diversity and antimicrobial activity of tamarind extracts from semi-arid Eastern Kenya. A total of 89 trees were sampled and studied. Data on production and utilization were collected through personal interviews with the farmers and the use of questionnaires and the data was analyzed using SPSS software at a significance of $P<0.05$. Standard descriptors for tamarind were used in morphological diversity. The data were subjected to Exlstat software for Principal Component Analysis (PCA), correlation and cluster analysis and Genstat for descriptive statistics and analysis of variance and at the significance of $P<0.05$. DNA was extracted using the CTAB method and amplified using ISSR markers. The molecular data were analyzed using GeneAlex and R software. Antimicrobial compounds were extracted sequentially from fruits and leaves using methanol and water. The pathogenic bacteria (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli*) were cultured in nutrient agar while the pathogenic *Penicillium digitatum, Colletotrichum gloeosporioides and Alternaria solani*) were cultured on Potato Dextrose Agar, Malt Extract Agar and Sabouroud Glucose Agar respectively. Data on inhibition zones were collected and analyzed using SPSS and the significance of $P<0.05$. This study revealed that all farmers used seeds from tamarind trees as their source of planting material. Tamarind was grown for market and subsistence use, the fruit was utilized as a dessert, an ingredient in porridge and as a source of herbal medicine. The cropping system used

by most farmers was intercropping with crops like cereals and legumes. Weed control and harvesting practices were carried out when necessary. Time taken for the trees to mature was approximately 5-6 years. Maturity indicators included changes in fruit color and pod brittleness. Farmers harvested less than 180kgs of tamarind fruits per tree in a season. The challenges of tamarind production included tamarind weevil infestation, harvesting from tall trees, marketing and transportation. Morphological results revealed that there were significant variations in trunk diameter at the ground, pod length, color and shape, seed shape across the counties. There were no significant differences in terminal shoot length, trunk diameter at the neck and height to the first branch, pod weight, pulp length, seed weight and the number of seeds per pod, primary and secondary branches, growth habit, seed color, roughness and brilliance, pod shape and seed shape across the counties. Quantitative PCA revealed 5 PCs. Agglomerative Hierarchical Clustering revealed 3 major clusters. Morphological variation within clusters was 66.12% and between the cluster was 33.1%. The ISSR markers revealed polymorphism of 68.7-84.7%, PIC ranges of 0.72-0.89 and genetic diversity of 0.74-0.9. Cluster analysis showed 7 distinct clusters that indicated that tamarind accessions were diverse. There were no significant differences in the inhibition zones between leaf and fruit extracts against *B. subtilis*. There were significant differences in the inhibition of tamarind extracts from the study regions and the extraction solvents against *B. subtilis*. There were significant inhibition differences in the extracts from study regions, leaf and fruit extracts and the extraction solvents against *P. aeruginosa*. Tamarind extracts were not effective against *S. aureus, E. coli, P. digitatum, C. gloeosporioides and A. solani*. Tamarind fruit and leaf extracts of accessions KB004, KB005, KB011, KB012, KB014 and KB015 had higher inhibition than ampicillin streptomycin, kanamycin and cotrimoxazole against *B. subtilis* and *P. aeruginosa*. In conclusion, tamarind was produced as an intercrop and utilized as an ingredient. The accessions showed morphological and genetic diversity and antimicrobial activity in *B. subtilis and P. aeruginosa*. There is a need to establish management practices in tamarind production,

sensitize the public on tamarind fruit value addition. Morphological and genetic diversity data can be used in breeding for improved tamarind varieties. Antimicrobial activity information will be utilized in herbal medicine.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background of the study

Tamarind (*Tamarindus indica* L.) belongs to the family Fabaceae and subfamily Caesalpinioideae (Khanzada *et al.*, 2008). Tamarindus has been reported by Mbora and Bernekov, (2006) to be a monotypic genus containing only T. indica. It is considered indigenous to tropical, subtropical and semi-arid areas of Africa (Bibitha *et al*., 2002). It was also cultivated in South East Asia, Australia and America (Rao and Mary, 2012). The plant has 24 chromosomes with 2n=24 (Purseglove *et al*., 1987). It is cross-pollinated resulting in variation within populations (Nandini et al., 2011).

Cross-pollination is affected by pollinators as selective agents, floral characteristics, geographical selection on floral characters and the role of pollinators in the evolution of reproductive isolation. Pollinator-driven speciation is among the most widely spread form of ecological speciation (Forest *et al.,* 2014). The pollinators can initiate diversification at several different levels (Schiestl & Johnson 2013) and have revealed a potential of pollinator-driven micro-evolution. Evidence from phylogenetic splitting in many flowering plants is associated with changes in the pollination system and correlated changes in flower characteristics (Schiestl & Johnson 2013). Predictions in ecological speciation are mediated when 1) different pollinators act as agents of divergent selection on floral traits 2) geographic mosaic of divergent selection which involves adaptation to the most efficient pollinator may occur and is aided by variable

pollinator distributors leading to the formation of pollinator ecotypes (Goodwillie *et al*., 2010). Differences in floral characters may result from new pollinators as a by-product which may lead to reduced or increased pollination by the original pollinator. When plants depend on pollinators for reproduction the pollinators shift will contribute to reproductive isolation hence speciation. The absence of both original and alternative pollinators may lead to the evolution of self-pollination which will lead to reproductive isolation (Goodwillie *et al.,* 2010).

Cross-pollination bears phenotypic variability that enables the plant to adapt to different environments and increases the likelihood of survival and evolutionary changes (Simpson, 2019). It's enhanced by floral changes in structure especially the separation between the anthers and the stigma (Simpson, 2019)

For breeding purposes, both quantitative and qualitative traits are considered. Qualitative traits are easily selected for breeding to the next generation because they have a profound effect on plant value and utilization and are governed by one or a few major genes. Quantitative traits of economic importance are governed by many genes each having a small effect and are hard for a breeder to control because:1) the number of genes involved makes heredity change slow and difficult to detect, 2) differences in the traits involved are detectable through measurements and statistical analysis and 3) 95% of the variation is due environmental changes while 5% is brought about by genes (Allard, 2019).

Selection involves choosing traits of importance and evaluating them in successive generations. Plants with desirable traits are selected and the unwanted ones discarded. Commercial growers ensure uniformity in the plants from season to season and from one plant to another. Uniformity and stability are enhanced by intense selection over many

seasons. Common methods of selection are mass selection, family selection, backcrossing, pure line selection and line-breeding (Loria, 2019)

1.1.1 Morphological description

The tamarind tree is large, evergreen and grows up to 30 m tall. The leaves are pinnately arranged with opposite leaflets. The flowers are yellow and are produced in racemes. Tamarind fruit is a brown pod and the fruit shape varies from straight to curved to a sausage shape. The pod contains many hard-coated seeds (Hemshekhar *et al*., 2011). *Tamarindus indica* plants are morphologically different in terms of fruit color and shape, crown diameter, foliage density, trunk size, seed color and seed shape as well as flower characteristics (Nandini *et al*., 2011). Schabel (2004) reported that fruit taste ranged from sweet to acidic. Parrotta (1990) reported red fruits in India. In Kenya, tamarind has been reported to be sour and is harvested when the pod is brittle and brown (Plate 1.1). The pulp color is red-brown and sticky (Betser, 2009; Wanjala, 2019). Morphological descriptors have enhanced the selection of superior cultivars for the market in terms of fruit taste and pulp thickness (Elsiddig *et al.,* 2006). In Africa, many studies have been done on biochemical compounds of tamarind (Soloview *et al*., 2004).

Morphological markers utilize visible traits and represent genetic polymorphism which is easily identified and manipulated. They have been utilized in the construction of linkage maps by classical 2-3-point test. Some are linked with agronomic characters and can be used in indirect selection criteria in practical breeding. Morphological markers are limited and many are not associated with economic traits such as yield and have undesirable effects on the growth and development of plants and are highly influenced by environmental conditions (Bekele and Bekele 2014; Chesnokov *et al.,* 2020)

Cytological markers have been used in characterization and utilize structural features of chromosomes shown by chromosome karyotype and bands. The banding patterns are displayed in color, width order and position. The patterns depict differences in the distribution of euchromatin and heterochromatin. Chromosome landmarks are useful in the characterization of normal chromosome detection of mutation, physical mapping and linkage group identification. Physical maps derived from morphological and cytological markers lay a basis for genetic linkage mapping with the help of molecular techniques. Their use is limited in genetic mapping and plant breeding (Kwiatek *et al.,* 2019)

Plate 1.1: Tamarind tree in semi-arid Eastern Kenya: (A) flowering and (B) fruiting.

1.1.2 Importance of tamarind

Tamarind fruits are either eaten fresh or processed into juices, jams or chewing gums (NRC, 2008; Nazir *et al.*, 2017). Fruits are rich in carbohydrates, Vitamin C, Calcium, tartaric acid and Potassium (El-Siddig *et al.*, 2006; Azad, 2018). Leaves are used as

vegetables and fodder for animals (Maundu *et al*., 2005). The plant is considered a source of food in the marginalized areas, especially during the dry spells when other sources of food were scarce (Maundu *et al*., *2005*). The stem is used as a source of hardwood timber while the leaves and bark are used in traditional medicine for the treatment of ailments such as jaundice and dysentery (Srinivasan *et al.,* 2001). Industrially, tamarind is used in the production of tamarind pulp powder, tamarind kernel powder and alcohol (Azad, 2018). The seed is important in the production of preservatives, jute, paper, adhesives for textile sizing and printing (Leakey, 2017). In India, tamarind is used in the production of fortified wine where seed, wood, leaves, bark, roots in dry form were used to flavor it (Panesar *et al*., 2017).

1.1.3 World production and utilization

Commercial cultivation of tamarind is carried out in Asia. In India, it is considered an important cash crop and ranked 6th in the export market (Pal and Mukherjee, 2020). In 2017-2018, tamarind was produced on an acreage of 74.26 hectares with a production of 309.44 metric tonnes (Israel *et al.,* 2019). India was closely followed by Thailand and Mexico. In America, Costa Rica showed a potential of producing up to 200 tonnes annually which would be exported to North America and Europe (Rao and Mary, 2012).

Tamarind is native and widespread in Africa from Sudan, Ethiopia, Kenya, Tanzania, Sub-Sahelian Africa to Senegal (Yahia and Salih, 2011). In West Africa it is produced in Benin, Nigeria, Mali and Senegal where it is utilized as fodder, food, medicine as well as spiritual and in ethnoveterinary purposes (Van der Stege *et al*., 2011). In East Africa, tamarind trees grow in the semi-arid areas and upon maturity, the fruits are collected and utilized in homes or sold through informal channels (Omari, 2016). In Kenya, tamarind is mainly found in wild woodlands in dry areas of Kitui, Tharaka Nithi, Baringo, West Pokot, Turkana, Homa Bay, Taita Taveta, Kwale, Lamu, Makueni and Embu (Wanjala,

2019). The fruits are collected by middlemen and sold in Mombasa where they are used locally in coastal dishes and some are exported to Asian countries (Wanjala, 2019). Tamarind is among the top 10 prioritized fruits for future crop diversification programs and development in Sub-Saharan Africa (NRC. 2008).

1.1.4 Molecular markers

Molecular markers are used in many tropical fruits to determine their genotypes, biodiversity evaluation, germplasm conservation and understanding genetic backgrounds (Ouédraogo *et al*., 2019). Molecular markers reveal polymorphism between different genotypes or alleles of a gene of a particular sequence of DNA in a population of a gene pool. Polymorphism is detected by PCR and Southern blotting markers play a key role in molecular breeding. For them to be effective in marker-assisted breeding they should show high levels of polymorphism, be evenly distributed in the entire genome, be codominant in expression, clear distinct allelic features, have no detrimental effect on the phenotype and be genome-specific (Jiang, 2013; Nybom, 2017). The molecular markers include; Inter Simple Sequence repeats (ISSRs), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphism DNA (RAPDs) and Simple Sequence Repeats (SSRs). Inter Simple Sequence repeats (ISSRs) are highly polymorphic, reproducible, efficient and offer a quick method of determining diversity by combining SSRs, AFLPs and RAPDs (Ng and Tan, 2015; Sarmieto *et al.*, 2017). ISSRs are used in phylogenic analysis, gene tagging, genome mapping, evolutionary biology, cluster analysis and plant breeding (Pradeep *et al*., 2002; Alansi *et al*., 2015). Sarmieto *et al.,* (2017) used ISSR markers and determined the diversity of tamarind in Ecuador and identified important tamarind trees for breeding, but no markers have been used to determine the diversity of Kenyan tamarind.

Molecular markers assist in the selection of desired traits simultaneously using 2nd generations back cross populations near-isogenic links, double haploids and recombinant inbred lines (Nadeem *et al*., 2018). Molecular markers should be near the target genes. The selection of the marker ensures success in the selection of the genes. Linkage maps provide a framework that detects marker-trait association and for the selection of markers to be used in assisted breeding (Platten *et al*., 2019). High-density maps derived from genetic linkage maps are important in molecular-assisted breeding. Only markers that are closely related to the related genes provide success in practical breeding (Jiang, 2013).

1.1.5 Antimicrobial activity

Antimicrobial compounds in plants can control diseases caused by pathogenic micro-organisms (Hayashi *et al*., 2013; Gonelimali *et al*., 2018). In tamarind, extracts from different plant parts have shown the ability to control different pathogenic bacteria (Escalona-Arranz *et al*., 2010; Abdallah & Muhammad, 2018). Studies on antimicrobial compounds in plants have increased the use of herbal medicine since most bacteria have become resistant to commercial antibiotics (CDC, 2018; WHO, 2018). Studies on antibacterial compounds of tamarind have been carried out in Sudan and India (Sanaa and Yagoub, 2008; Pauldas and Banerjee, 2014).

Plant fungal pathogens cause plant diseases and have increased yield losses in recent years as well as limiting storage and post-marketing period (Yang *et al.,* 2017). Control of fungal pathogens has largely depended on synthetic chemicals that are a threat to the environment and humans. In addition to this, pathogens have developed resistance to synthetic chemicals (Droby *et al*., 2009; Hua *et al*., 2018). The challenges in the chemical control of fungal pathogens have increased research towards control using plant extracts that may be used as alternative fungicides. Tamarind extracts have been

reported to be effective against *Alternaria solani, Fusarium solani and C. gloeosporioides* (John *et al*., 2004; Garcia, 2011; Alcasid *et al*., 2016). There is no available information on the antimicrobial activity of Kenyan tamarind and this has limited the exploitation of its extracts in the pharmaceutical industry.

1.2 Statement of the problem

Tamarind is abundant in the semi-arid areas of Nyanza, Eastern, Coastal and North-Eastern parts of Kenya (Infonet Biovision, 2019). The study focused on Eastern Kenya due to the availability of the trees and improvement would solve food security in the region and promote ecosystem service. There is limited information on tamarind production and utilization. Tamarind trees grow in the wild as forest trees, in the homesteads along with other farm crops and along the roads. In these semi-arid areas, production practices of the crop are limited even though it is in abundance. Improved varieties are present in India, Mexico and Thailand in terms of flavor and yield (Reddy 2017) but in Kenya, there are no improved varieties and even information on what is available is still scanty which has limited production, utilization and commercialization of tamarind in Kenya (Wanjala, 2019).

Tamarind has been distinguished based on morphological descriptors of seed, fruit, pod and floral morphology in India, Mexico and Venezuela (Osorio *et al*., 2018). In Kenya, tamarind has been known to be sour and there is limited information available to distinguish tamarind in different regions based on morphological descriptors.

Molecular markers have been used to identify and conserve tamarind genotypes (Algabal *et al*., 2011) in Bangalore. Research has been done to distinguish tamarind using AFLPs, RAPDs and ISSRs (Algabal *et al*., 2011; Kumar *et al*., 2015; Sarmiento *et al.,* 2017) in Bangalore, India and Ecuador. The diversity of Kenyan tamarind has not been studied at a molecular level (Wanjala, 2019). There is limited information on the

available tamarind genotypes and this has limited the possibility of improving the accessions using conventional methods of breeding.

Antimicrobial compounds in plants have been exploited in the production of herbal medicine against bacteria in Brazil (Hayashi *et al*., 2013; Bhardwaj *et al*., 2016; Anand *et al*., 2019). Tamarind extracts have been reported to be effective against gram-positive (*B. subtilis, S. aureus*) and negative (*E. coli, P. aeruginosa*) bacteria (Doughari, 2006). In Nigeria, Nwodo *et al*. (2011) reported that extracts of tamarind bark, fruit pulp, stems and leaves were effective against both gram-negative and positive bacteria. In India, Gupta *et al*. (2015) reported that extracts of tamarind were active against gram-positive and negative bacteria. Tamarind extracts were reported to be effective against *C. gloeosporiodes, A. Solani, F. solani* (Bautista-Baños *et al*., 2003; Gatan and Jonnalaxer, 2013; Alcasid *et al*., 2016). There is no available information on antimicrobial compounds of Kenyan tamarind and this has limited its utilization for antibiotic and fungicidal purposes.

1.3 Justification

Tamarind is an economically important plant with many uses ranging from food, ecosystem uses, ethnomedicine, ethnoveterinary, timber and fungicides. An increase in production would provide income to farmers in the arid and semi-arid areas of Kenya. Production of tamarind in India, Thailand and Mexico has been commercialized because of the improved varieties and the farmers have benefited by exporting tamarind products (Rao & Mary, 2012) while in Kenya there are no improved varieties. An increase in utilization would promote industrialization due to an increase in value-added products. Tamarind has a great impact on the ecosystem such as windbreaks, control of soil erosion, provision of shade and increase in its production would increase the ecosystem uses in the environment.

Morphological descriptors have been used by Fadohan *et al.* (2010) to distinguish tamarind cultivars in Benin. The authors reported that both quantitative and qualitative traits were important in distinguishing cultivars. Floral morphology, fruit size, pulp color, pulp taste, and the number of seeds per pod were used in Venezuela, Columbia, Mexico and India to distinguish between varieties (Osorio *et al.*, 2018). Studying morphological differences of tamarind from Kitui, Kibwezi, Mwingi, Embu and Masinga could permit initial identification of what is available in the different regions. The information will enhance crop improvement through breeding programs and conservation strategies.

Molecular markers have been used in the determination of genetic diversity, cluster analysis, identification of plants, conservation and breeding programs in China and America (Grover & Sharma, 2016; Jo *et al.*, 2017). Molecular markers are highly polymorphic, not influenced by the environment, are not limited and can detect variation at the DNA level (Samantaray, 2017). ISSRs have revealed higher polymorphism due to high annealing temperatures and longer sequences (Qian *et al.*, 2001). Inter Simple Sequence Repeat (ISSR) markers have been used by Sarmiento *et al*.(2017) for diversity determination and the results showed diversity among the Ecuadorian tamarind genotypes this was useful in the selection of trees for clonal propagation and identification of diverse trees for hybridization programs. The molecular characterization will help establish the genotypes present in Eastern Kenya and enhance tamarind improvement through marker-assisted breeding.

Studies on antimicrobial activity of tamarind have been carried out in West Africa, Sudan, India and America and the results revealed that they are more effective compared to commercial antibiotics and fungicides (John *et al*., 2004, 2004; Nwodo *et al*., 2011). Antimicrobial activity determined will improve the consumption of tamarind for health benefits, have tamarind used as raw materials in pharmaceutical industries and improve

the economic standards of farmers. Tamarind herbal medicine will be readily available at a cheaper cost and the side effects induced by the synthetic antibiotics and fungicides will be reduced.

1.4 Objectives

1.4.1 General objective

To determine production, utilization, morphological and genetic diversity and antimicrobial activity of tamarind (*Tamarindus indica*) accessions found in the semi-arid Eastern Kenya.

1.4.2 Specific Objectives

1. To evaluate tamarind (*Tamarindus indica*) production and utilization in semi-arid Eastern Kenya
2. To assess the morphological diversity of tamarind accessions in semi-arid Eastern Kenya
3. To evaluate the genetic diversity of tamarind accessions in semi-arid Eastern Kenya
4. To evaluate the antibacterial and antifungal activity of extracts from tamarind accessions collected from semi-arid Eastern Kenya against bacterial and fungal pathogens.

1.5 Null hypotheses (H_0)

1. There is no production and utilization of tamarind in semi-arid Eastern Kenya.
2. There are no morphological differences among *T. indica* accessions in semi-arid Eastern Kenya.

3. There is no genetic diversity among *T. indica* accessions in semi-arid Eastern Kenya.
4. Extracts of *T. indica* accessions in semi-arid Eastern Kenya have no anti-bacterial and antifungal activity against pathogens.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and taxonomy of tamarind

Tamarind (*Tamarindus indica L*) is native to Madagascar. It belongs to the family *Fabaceae*, subfamily *Caesalpinioideae*, genus *Tamarindus* and species *indica*. The genus Tamarindus is monotypic (Khanzada *et al.*, 2008). It was introduced to India from Africa and India is believed to be its secondary origin (Du Preez, 2003). It was also introduced to America and Mexico by Spanish and Portuguese colonialists and cultivated in the tropics (Azad, 2018).

The tree is multipurpose, wind-resistant, evergreen and best known for its fruits (Yahia and Salih, 2011; Infonet Biovision, 2019). It grows up to a height of 24 - 30m with pinnate leaves. The leaves have oblong leaflets that are 2.5 - 25 mm long and 5 – 6 mm wide (Du Preez, 2003). Flowers are born on small racemes with 5 petals where two of them are reduced to bristles and are yellow with orange or red streaks. Fruits have brown pods like legumes with soft acidic pulp with shapes varying from curved to semi-curved to straight (Hemshekhar *et al*., 2011; Infonet Biovision, 2019).

The pod length is 50 - 150mm long and 20-30mm wide with seeds that are hard coated with blackish to brown pulp (Hemshekhar *et al*., 2011; Infonet Biovision, 2019). Tamarind grows in an acid climate and tolerates humid to dry hot regions. Optimum rainfall should be 750-1900mm but still does well at low amounts of 500-750mm. Altitude requirements are 0-1600 meters asl, soils should be deep loamy or alluvial but can still tolerate slightly saline and alkaline soils. The plants are pollinated by honey

bees (Samake *et al.,* 2014). Flowering starts after 5-8 years for plants raised from seeds during the dry season. Flowering lasts for 2-3 months' fruits set in at the inception of rains and gets to the 6-8 months later (Samake *et al.,* 2014; Fandohan *et al*., 2015).

2.2 Utilization of tamarind

In homes, tamarind is used as an ingredient in sauces, porridge and juices and as vegetables during lean periods. Additionally, it is utilized in the treatment of ailments such as jaundice and dysentery (Srinivasan *et al.,* 2001). The leaves are also fed to animals as fodder, the wood is used in charcoal production which served as a major source of energy used in urban areas of Senegal (Fandohan *et al*., 2010a). The tamarind tree is tolerant to adverse environmental conditions that make them possible to be used in shade provision and the shaded areas were utilized as meeting places in villages (Ranaivoson *et al*., 2015).

Industrially, tamarind has been used in the production of tartaric acid, tartrates, wine, vermouth and tamarind pulp powder (Singh *et al*., 2007; Panesar *et al*., 2017; Nazir *et al*., 2017). The pulp has been used in vinegar production and herbal therapies (Taha *et al.,* 2016; Abdallah & Muhammad, 2018). Dried tamarind pulp has been used as layer chicken feed as a supplement to reduce egg yolk cholesterol by 12-14% without affecting egg production qualities (Biradar and Kanduri, 2016).

Tamarind seeds are separated from the pulp manually or mechanically and used to produce tamarind kernel powder that is important in sizing material in textile, paper and jute industries (Kumar & Bhattacharya, 2008). The tamarind seed gum is used as a polysaccharide to improve the texture and viscosity of processed food (Nazir *et al*., 2017). The polysaccharide is also used in the production of gels with a wide range of pH that could be used as an alternative to pectin (Azad, 2018). The seed is used as an

adhesive filler in the plywood industry and as a stabilizer for the brick industry (Nazir *et al*., 2017).

In pharmaceutical treatment, tamarind leaves, pulp, seeds and bark extracts have been effective in the control of gram-positive and gram-negative bacteria pathogens (Sanaa and Yagoub, 2008; Escalona-Arranz *et al*., 2010). Extracts of tamarind leaves, barks, stems and seeds have been used in the control of plant fungal pathogens such as *Colletotrichum spp, Alternaria spp, Fusarium* spp *and Penicillium* spp (Bautista-Baños *et al*., 2003; John *et al*., 2004).

The tree has a vast effect on the improvement of soil organic matter, soil biological properties and is considered an ornamental tree due to its evergreen nature and windbreak in farms (Faust *et al*., 2015).

2.3 Morphological characterization of tamarind

Characterization describes the entire plant germplasm. Morphological, physiological, agronomical and molecular markers have been used in characterization (Engels & Visser, 2003; El-Esawi, 2019). Characterization has enhanced accession identification, utilization and conservation (Khan *et al*., 2015).

Plant morphological characterization utilizes plant vegetative and reproductive characters. The characters determine plant phenotype and reflect plant genetics and ecology (Engels & Visser , 2003; Cervantes & Diego,2010; Wyatt 2016). Morphological markers should be easy to observe and score by naked eyes and expressed in all environmental conditions (Khan *et al*., 2015; Martinez *et al*., 2017). In Ecuador, quantitative and qualitative traits were used to study the diversity of tamarind and the results revealed six dissimilar clusters with stem height, fruit, flower and leaf descriptors being significant (Alvarez *et al*., 2019). In Columbia, Osorio *et al*. (2018) used morpho-

agronomic descriptors to study diversity among the sweet and sour tamarind and they observed significant diversity in the two groups. In Uganda, Nyadoi, *et al*. (2010) and Okello *et al*. (2018) evaluated twelve quantitative morphological descriptors of tamarind fruit and seed and their results depicted morphological variations and correlation relationships that could be used for the selection of trees for breeding. In West Benin, Fandohan *et al*. (2011) studied the impact of habitat type on conservation of tamarind and the results revealed that the variations were significantly correlated with the ecological factors which indicated that fruit and seed size as well as mass increased with an increase in humidity (Fandohan *et al*., 2011).

2.4 Molecular characterization of tamarind

A molecular marker is a DNA sequence that has a known chromosome location and can control a particular trait (Nadeem *et al.,* 2018). Molecular markers have been used in cultivar identification, biodiversity evaluation, germplasm conservation and understanding genetic backgrounds of living things (Grover & Sharma, 2016; Jo *et al.*, 2017; Sharma, 2019). Genetic diversity can alter species populations through hybridization, recombination, gene flow and introgression (Coates *et al*., 2018). Maintenance of genetic diversity is important in breeding programs (Ma *et al*., 2008, Nara *et al*., 2009). Geographical differences may cause changes in populations of the same species (Biron *et al*., 2002).

Amplified Fragment Length Polymorphism (AFLPs) were used by Algabal *et al*. (2011) and revealed diversity among the 36 tamarind genotypes. The authors found out that AFLPs were unsuitable for diversity studies for they were to be scored dominantly, required the development of locus-specific markers from individual fragments and used different kits adapted to the size of the genome that was analyzed.

Random Amplified Polymorphic DNA (RAPDs) are PCR-based techniques that are adapted for the rapid detection of polymorphism. The markers use single or multi short oligonucleotides primers of random or arbitrary sequence (Krawczyk & Kur 2018; Chatterjee *et al.,* 2019). RAPDs have been useful in determining the genetic diversity of *C, brasiliense, C. colocynthis*, pome, papaya and tamarind in Brazil and India (Mendonca *et al* 2014; Kumar *et al*., 2015; Verma *et al*., 2017; Zarei *et al*., 2017; Kumar *et al.,* 2019). The results revealed the diversity and could be utilized in hybridization programs.

Simple sequence repeats (SSRs) are regions in the genome where a group of bases of 1-8 is repeated in tandem. SSRs are codominant, multi-allelic, reproducible and have high resolutions (Cai *et al*., 2019). SSR markers have been used in trait and marker association of plants, cultivar identification, genetic diversity studies and marker-assisted breeding for variety development (Lou *et al.,* 2015: Biswas *et al*., 2019). Thirty-five SSR markers were used by Biswas *et al*. (2019) in the USA to determine diversity among the cultivated and wild strawberry varieties and the results revealed genetic diversity. Twenty SSR markers were used by Israr *et al*. (2019) in India to determine the diversity of traditional mango cultivars and the results revealed high intraspecies diversity between monoembryonic and polyembryonic mango cultivars. In Taiwan, Ma *et al*., (2019) used SSR markers to determine diversity among 45 guava cultivars and the authors reported that they were able to distinguish seedling strains with unclear parental origin and accurately identify the cultivars.

Inter simple sequence repeats (ISSRs) markers use primers containing only the repetition of a particular SSR di, tri, tetra or pentanucleotide of 16-25bp. The markers are polymorphic, reproducible and efficient. The markers combine the variability of SSRs, AFLPs and RAPDs and do not require prior information about the species genome (Sarmieto *et al.,* 2017; Pena *et al.,* 2020). ISSR markers are used in phylogenic analysis,

gene tagging, genome mapping, taxonomy studies, population genetics and plant breeding (Alansi *et al*., 2015; Pena *et al.*, 2020).

Ten ISSR primers were used to study the genetic diversity of mango cultivars in Vietnam by Ho and Tu (2019) and the results depicted dissimilarities within the cultivars. In Ethiopia 17 ISSR primers were used by Indracanti *et al*. (2019) to determine the diversity among 5 landraces and introduced cultivars of date palm and the results indicated high diversity. ISSR markers were able to differentiate the five cultivars of *Ziziphus jujubi* species from Spain (Reche *et al*., 2019). In Brazil, Jamile da Silva Oliveira *et al*. (2019) studied the genetic diversity of *Passiflora spp using* ISSR markers and the results revealed high genetic variability and adequate accession differentiation.

2.5 Antimicrobial compounds of tamarind

Most bacterial infections are caused by micro-organisms that have developed resistance to antibiotics and this has increased the cost of treatment (Angiolella *et al*., 2018). The emergence of natural products has significantly increased the production of antimicrobial compounds that have been effective against micro-organisms (Boakye, 2019). Antimicrobial compounds are easily obtained from herbal plants as well as terrestrial and marine organisms (Angiolella *et al*., 2018). Herbal plants are readily available, less expensive and contain active compounds that have antimicrobial activity (Cheesman *et al*., 2017). Most rural communities still depend on herbal medicine in primary health care (Rahayu *et al*., 2020).

Antimicrobial compounds are secondary metabolites such as alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins and quinones. Antimicrobials are used to control microbial growth by altering membrane permeability and reduction of pH (Cheesman *et al.*, 2017; Elisha *et al.*, 2017).

Alkaloids are naturally occurring compounds in flowering plants. They have been reported to be drug stimulants that have shown antimicrobial, anti-cancer and analgesic activity. They have been useful in diet ingredients, supplements and pharmaceuticals (Cushnie *et al*., 2014; Hussain *et al*., 2018). Flavonoids are polyphenols, widely found in vegetables, fruits and some beverages. They are found as flavones, flavanones, flavanes, isoflavones, bio flavones and chalcones (Panche *et al.*, 2016). They are found in most plant parts such as the flowers leaves, roots, seeds and barks (Ruiz-Cruz, 2017). They have been reported to have antimicrobial, anti-cancer, anti-viral, anti-mutagenic and anti-inflammatory activities (Djouossi *et al*., 2015).

Tannins are water-soluble natural polyphenols in plants. They are often applied as medicinal agents such as anti-oxidants, anti-inflammatory, anti-carcinogenic and antiseptics. They have properties that make them suitable candidates for pharmaceutical the industry (Singh, 2020). The compounds are accumulated in flowering plant parts such as bark, wood, fruits and leaves (Krzyzowska *et al*., 2017; Ribeiro *et al*., 2018).

Saponins are detergent-like substances with the capability to kill and destroy bacteria and cancer cells, protozoa and mollusca (Desai *et al*., 2009; Kregiel *et al*., 2017). They are found in plant products as secondary metabolites (Hussain *et al*., 2019). They have exhibited antioxidant, antimicrobial, anti-inflammatory and analgesic activity (Kregiel *et al*., 2017). Saponins antibacterial activity has often been limited but it has an excellent antifungal activity (Guil-Guerrero *et al*., 2016).

Quinones are secondary metabolites isolated from aromatic plants. They are products of hydroquinone oxidation (Eyong *et al.*, 2013). They are grouped into benzoquinone and hydroquinone. They are found in nature as a pigment in animals, plants and microbes and they are the main ingredients in many herbs (Eyong *et al*., 2013).

In Nigeria, Abdalla and Muhammad, (2018) carried out phytochemical screening of tamarind leaves and fruits and the results revealed that the two parts contained alkaloids, glycosides, saponins, tannins, flavonoids, terpenoids, phenols and anthraquinone. In India, the active compounds of tamarind shells were screened by Gomathi *et al.* (2017) and the results revealed the presence of alkaloids, cardiac glycosides, saponins, tannins, flavonoids, terpenoids, phenols and anthraquinone, steroids and xanthoproteins.

In Nigeria, tamarind seed coats, pulp and leaves showed the presence of flavonoids, saponins and terpenoids (Adeniyi *et al*., 2017). In Nigeria, tamarind pulp extracted using hot water contained saponins at 2.2%, alkaloids at 4.32% and glycosides at 1.59% (Abukakar *et al*., 2008).

2.5.1 Bacteria pathogens

A pathogen is an organism that is capable of causing a disease in a host (Piglowski, 2019). *Pseudomonas aeruginosa* is a gram-negative bacterium that is rod-shaped, mono flagellated and obligate. It can live in water, rhizosphere and humans (Paškevičius *et al*., 2017). It causes infections in plants and animals such as sepsis, pneumonia and burn wound infections (Paškevičius *et al*., 2017). The pathogens have developed resistance to synthetic antibiotics (Bassetti *et al*., 2013). Reports by WHO declared that *P. aeruginosa* was a problem of clinical importance (Tacconelli & Magrini, 2017).

Escherichia coli belongs to the family *Enterobacteriaceae* that is composed of gram-negative, facultative anaerobic rod-shaped bacteria. The bacterium does not produce enzyme oxidase and is either motile or non-motile (Desmarcheller & Fegan, 2016). It causes diarrhea, hospital-acquired pneumonia, urinary tract infections, bacteremia and neonatal meningitis. It has caused up to 70% mortality in delayed treatment (Poolman.2017; Farver, 2018; Madappa, 2019). Infections are prevalent in young

children with weakened immune systems and older adults after consumption of contaminated food and beverages (Lim *et al*., 2010; Richter *et al.*, 2018). Prevention mechanism has been used against it *E.coli* (Christian, 2017).

Bacillus subtilis is a gram-positive bacterium that is positive for catalase, forms endospores in unfavorable conditions, is aerobic to facultative (Du *et al.,* 2019). The bacterium is concentrated in soil, water and food products with a plant origin (Schultz *et al*., 2017). It affects humans through food poisoning, infection is evident by diarrhea and vomiting (Elshaghabee *et al*., 2017).

Staphylococcus aureus is a facultative gram-positive anaerobe that occurs singly, in pairs or irregular clusters. It is a non-motile, spore-forming bacterium found in the environment and humans (Taylor and Unakal, 2018). *S. aureus* multiplies rapidly at room temperature and produces toxins that cause infections (Taylor & Unakal, 2018). Treatment of *S. aureus* infections depends on the type of infections as well as the presence or absence of drug-resistant strains. Resistance has increased morbidity, mortality and treatment cost (Gnanamani & Hariharan, 2017).

2.5.2 Control of bacterial pathogens

Anti-biotics have been used in the control of bacterial pathogens, but bacteria have developed resistance to antibiotics (Das & Patra, 2017; Aslam *et al*., 2018). Resistance may be due to excessive use of antibiotics, increased international travel, poor sanitation and release of non-metabolized antibiotics and their residues to the environment (Aslam *et al*., 2018). Antibiotic resistance has threatened the effectiveness of antibiotics and has limited the treatment of common infections (Liu *et al*., 2017). The resistance has shifted attention to natural products which could be used as effective drugs to treat human

diseases with high efficiency against pathogens and negligible side effects (Liu *et al*., 2017).

Tamarind fruits extracted using water and ethanol have been reported by Warda *et al*. (2007) to be effective against gram-positive and negative bacteria. Tamarind aqueous leaf extracts have been reported to be effective against gram-positive (Escalona-Arranz *et al*., 2010). Tamarind bark and leaf extracts have been reported by Abdallah & Muhammad (2018) to be effective against gram-positive and gram-negative bacteria. Tamarind ethanol extracts from Sudan have been reported to be more effective than common antibiotics (Sanaa & Yagoub, 2008). Tamarind fruit pulp from India, extracted using methanol showed higher activity against *B. subtilis* compared to the commercial antibiotics (Pauldas and Banerjee, 2014).

2.5.3 Control of plant fungal pathogens

Fungal plant pathogens are fungi that cause plant diseases and they have increased yield losses in recent years (Yang *et al.*, 2017). Fungal pathogens cause both field and post-harvest losses limiting storage and post-marketing period (Zhang *et al*., 2019). The fungi use various mechanisms to infect the plant such as killing the host and feeding on the dead materials, others colonize the living tissue (Doehlemann *et al.*, 2017). Fungi infect unripe fruits but the symptoms become visible in ripening due to the favorable conditions of the fruit (Buchholz *et al.*, 2018). The pathogens are spread by wind, rainwater and vectors (Jain *et al*., 2019).

Control of fungal pathogens has been done using chemicals, biological agents, cultural practices and integrated disease management (IDM). Integrated disease management aims at the optimization of the pathogen in an ecologically and economically viable manner. IDM is accomplished by coordinated use of multiple tactics that ensure stable

crop production and maintenance of pathogen damage below economic injury levels while reducing harm to animals and the environment (Bittman *et al*., 2019).

Biological control is emphasized to reduce the effects of chemical fungicides. Pathogenic organisms are suppressed and their effects on the host are reduced and beneficial organisms are favored. Biological control agents used include fungi, bacteria and viruses. Common bacteria used include; *Bacillus* spp, *Pseudomonas* spp, *Rhizobium and Streptomyces.* Common fungi used are *Trichoderma spp, Gliocladium spp. Arbuscular mycorrhizal fungi* and *Chaetomium spp (*Rashad and Moussa, 2020*).* Cultural control methods include crop rotation, proper spacing, proper watering and planting disease-resistant cultivars (Hosack & Miller, 2017).

Control of fungal pathogens has mainly depended on synthetic chemicals that are a threat to the environment and humans (Droby *et al*., 2009). Chemical control is not satisfactory due to the development of resistance hence the need to search for an alternative fungicide (Mahlo *et al*., 2016; Hua *et al*., 2018). Pathogenic fungi of economic importance include; *Colletotrichum* spp, *Alternaria* spp, *Fusarium* spp, *Penicillium* spp.

Anthracnose *(Colletotrichum gloeosporioides*) is the most economically important destructive disease in mango, papaya and avocado (Gatan and Jonnalexer, 2013). It affected both pre and post-harvest quality of fruits and vegetables (John *et al*., 2004). Conventional control has been done using synthetic chemicals which are not only expensive but have proved to be hazardous to the user and plants (Alcasid *et al*., 2016).

Aqueous tamarind leaf and stem extracts have been reported by Bautista-Baños *et al*., (2003) to reduce conidial germination of *C. gloeosporioides*. Aqueous and ethanolic tamarind extracts were evaluated for their activity against *C. gloeosporioides* and both

revealed significant inhibition with leaf extracts having higher inhibition zones compared to the commercial fungicide mancozeb (Gatan & Jonnalaxer, 2013). In the Philippines, Alcasid *et al.* (2016) studied the activity of aqueous and ethanolic extracts against anthracnose and the results revealed that aqueous extracts were not active whereas ethanolic extracts showed 42% activity indicating that they could be used as fungicide if the mode of application could be enhanced. In a study by John *et al*., (2004) antifungal activity of tamarind extracts was evaluated and the results showed that they were effective against a wide range of fungal pathogens including *C. gloeosporioides and A. solani*. The researchers concluded that tamarind extracts offered a great opportunity to be used as an antifungal to control soil, seed and airborne phytopathogenic fungi. Garlic and tamarind extracts were evaluated against *C. gloeosporioides* in bananas and garlic was shown to be more effective than the tamarind (Garcia, 2011).

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Description of the study area

The study was carried out in semi-arid areas of Kitui, Embu, Machakos and Makueni counties in Kenya. The counties lie within an altitude of 833-1377 meters above sea level. Machakos county is majorly arid and semi-arid (ASAL) and it receives an annual rainfall of 500-1300mm. The rains are bimodal with long rains from March to May and short rains from October to December. The temperatures range from 18-29˚C and the coldest month is July. Agro ecological zones (AEZ) in this county include upper midland (UM), UM2-3, UM5-6, lower medium (LM), LM3, LM4 and LM5 (Machakos county, 2015). Makueni county is fairly hot and dry, it receives rainfall of 500 to 750 mm, temperatures range from 21-25˚C. The wet season is from January to June (GoK, 2013). The long rains are from March to April and short rains are experienced in November and December whereas June to December are dry periods. Makueni county has eight AEZ of lower highland (LH), LH2, UM3, UM4, LM3, LM4, LM5 and inner lowland 6 (IL6). LM4, LM5, LM6 and IL6 are ASAL and form 80% of the county (Maluki *et al*., 2016). Embu county receives an annual rainfall of 600-1800 mm which is bimodal. Temperatures range from 12 to 26˚C, in the cold season temperatures average up to 11˚C and in the hot season they average up to 25˚C. AEZ in Embu county include LH1, UM1, UM2, UM3, UM4, LM3, LM4 and LM5 (Mburu *et al.*,2016). Kitui county is drought tolerant with bimodal rainfall. Annual rainfalls range from 500-1050 mm. Annual mean minimum temperatures range from 22 to 28˚C and the annual maximum temperatures range from 28 to 32˚C. AEZ in Kitui county includes UM3, UM4, LM3, LM4, LM5 and LM6 (Mugo, 2014). Dominant soils are alfisols, ultisols, oxisols, lithic

soils. The soils are highly erodible with low levels of fertility (Obiero and Onyando, 2013).

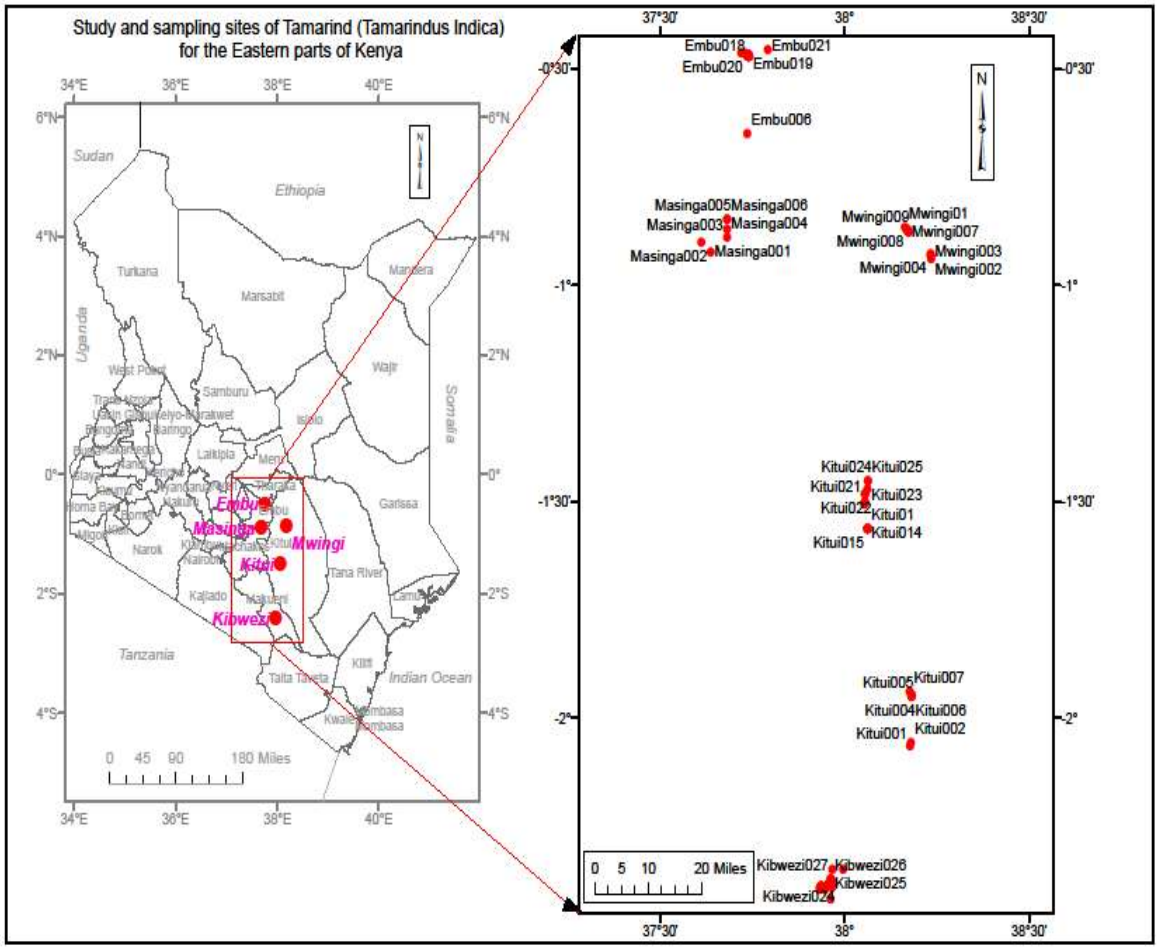


Figure 3.1: Tamarind sampling and study sites in semi-arid Eastern Kenya

The Global Positioning System (GPS) and location coordinates were obtained for each tree (Table 3.1).

Table 3.1: Global positioning system coordinates of the study sites in semi-arid Eastern Kenya.

County	Sub-county	Latitude	Longitude	Elevation (MAS)	Temp	Soils
Kitui	Kitui central	S01.451-S02.056	E038.054-E038.181	1335-1375	27°C	oxisols
	Mwingi	S00.865-S00939	E037. 637–E38.234	1365-1377	25°C	vertisols
Embu	Ishiara	S00.455-S00.470	E037.733-E037.791	1328-1377	21°C	**V**ertisols
Machakos	Masinga	S00.846-S00.924	E037.610-E37.681	1130-1376	23°C	**A**ndosols
Makueni	Kibwezi	S02.372-S02.397	E037.932-E037.996	833-1121	23°C	sandy clays

3.2 Sampling

A field survey was carried out from December 2015 to August 2016 (Figure 3.1). Multi-stage sampling method was used starting with purposive with the help of key informants such as administrative chiefs, village elders and Kenya Wildlife Service (KWS) officers who identified tamarind farms. This was then followed by random sampling in the farms. In Masinga and Embu most of the sampling was on-farm, while in Kitui, Mwingi and Kibwezi it was either on-farm or in the forest reserves. In Kitui county, 35 trees were sampled and studied; 25 trees from Kitui central and 10 trees from Mwingi. In Embu county a total of 21 trees were sampled and studied from the Ishiara location. In Machakos county, a total of 6 trees were sampled in Masinga. Most of the plants in Masinga were sparse as they had been cleared to pave way for other crops. In Makueni county, a total of 27 trees were sampled and studied in the Kibwezi location.

3.3 Labelling of samples

Samples from Kitui sub-county were labeled as KT001-KT025. Samples from Mwingi sub-county were labeled as MW001-MW010. Samples from Embu were labeled as

E001-E021. Samples from Machakos were labeled as MS001- MS006. Samples from Makueni county were collected in Kibwezi and labeled as KB001- KB027.

3.4 Evaluation of tamarind production and utilization

Data were collected on the production system, cultural practices, source of planting material, time taken to maturity, maturity indices, fruit yield per tree per season, number of harvests per year, the portion of fruit sold, uses of the tamarind fruit, use of tamarind tree, challenges in tamarind production. Data were collected by observation and through personal interviews (I asked the farmers the questions as they responded) with the farmers using structured and semi-structured questionnaires.

3.5 Morphological characterization of tamarind

Characterization was done according to the International Union of Plant protection of new Vegetal Variants (Tripp,*et al.*,2007)., the International Committee of Plant Genetic Resources of plant for the description of tropical plants (IPGR, 1991) and according to the procedure by (Fandohan *et al*., 2010). Qualitative and quantitative descriptors of the stem, fruit and seeds were used. Data on the stem were measured as an average of three readings. Pod length, width, weight, pulp weight and seed number were determined as an average of five pods. Seed weight was determined as an average of seeds in an entire pod. A standard color chart was used to determine color variations. Data were analyzed to get Analysis of Variance (ANOVA), Principal Component Analysis PCA and Agglomerative Hierarchal Clustering (AHC) using Genstat version 12.1 and Xlstat software respectively.

3.6 Molecular characterization of tamarind

Young apical leaves were used and DNA extracted using modified CTAB as described by Doyle & Doyle 1990 and visualized in 0.8% agarose after 45 minutes. DNA was

amplified using ISSR primers as described by Sarmiento *et al*. (2017) and visualized on 2% agarose. Band sizes were estimated by comparing with 100bp ladder. Data collected were either (1) for the presence of bands and (0) for the absence of bands. Genalex 6.5 software (Peakall & Smouse, 2012) was used to estimate pairwise individual relatedness, genetic diversity, Principal Coordinate Analysis (PCoA) and Analysis of Molecular Variance (AMOVA). Phylogenetic clusters were obtained using R software.

3.7 Antimicrobial evaluation of tamarind

Leaf and fruit samples were dried under shade and antimicrobial compounds were extracted sequentially using methanol and water as described by Uthayarasa *et al*. (2010). The extract was dried and reconstituted as described by Predrag *et al*. (2005) and stored at 4°C. *Bacillus subtilis, S. aureus, E. coli* and *P. aeruginosa* were preserved in nutrients broth and stored at 4˚C and cultured on 28g/l of nutrient agar. Pathogenic fungi *Penicillium digitatum, Alternaria solani* and *Colletotrichum gloeosporioides* were isolated from an orange, tomato and avocado and cultured PDA, Malt extract agar and Sabouraud glucose agar respectively.

Disc diffusion method was used to determine the antimicrobial activity of tamarind extract against pathogens (Sandle, 2016). The bacteria were incubated at 24°C for 48 hrs and fungi at 37℃ for 96 hrs. The experiment was done in 3 replicates in a split block. Data on inhibition zones were collected in millimeters and analyzed using Two –way ANOVA at a significant level of $P< 0.05$ using SPSS version 12.

CHAPTER FOUR

EVALUATION OF TAMARIND (*Tamarindus indica*) PRODUCTION AND UTILIZATION IN SEMI-ARID EASTERN KENYA

Abstract

Tamarind is a multipurpose, evergreen tree, in the *Fabaceae* family. It is mainly used as a fruit, with the other parts having limited exploitation. This study aimed at evaluating tamarind production and utilization in semi-arid Eastern Kenya. Data were collected through personal interviews with the selected farmers using questionnaires. Data collected were on social factors of farmers, production systems, planting material, cultural practices, the proportion of tamarind sold, time taken to maturity, maturity indices as well as uses and challenges in tamarind production. Results indicated that 33.78 % of the respondents were female and 66.22% were male. Tamarind was mainly produced for home use and local market (48.3%). Tamarind was produced by 72.2% of the respondent as an intercrop. Many respondents (44.9%) had farm sizes of 2 to 4 acres but tamarind occupied the least percentage of the farm with 76.4% of the respondents having tamarind on 2.1 to 4% of the farm. Land preparation was mainly done using handheld implements (61.8%) such as hoes and machetes. All farmers (100%) reported that they did not propagate, but tamarind grew naturally from seeds. Weed control was practiced by 68.5% of the respondents. Most of the farmers (57.3%) reported that pod brittleness and change in pod color were the maturity indicators. The majority of the respondents (60.7%) were not sure of the actual time tamarind trees took from germination to maturity, but 20.7% approximated it to be 5 to 6 years. A larger number of the respondents (34.8%) harvested less than 180kg of tamarind fruits per tree per season. All the respondents (100%) reported that they harvested tamarind once a year.

Approximately 46.1% of the respondents did not sell tamarind fruits harvested. A greater number of the farmers (76.4%) reported that they utilized tamarind as a dessert and an ingredient in porridge preparation while 49.4% used the fruit for medicinal purposes. Most of the farmers (71.9%) had a negative attitude towards tamarind farming. More than half of the farmers (67.6%) reported that they had transport difficulties and almost all (98.9%) had challenges with tamarind weevil (*Sitophilus lineans*) attacks during post-harvest handling. The majority of the farmers (60.7%) reported challenges in harvesting from tall trees and (79.8 %) reported a lack of links to urban markets.

4.1 Introduction

Production of tamarind is characterized by wild growth with no or minimal supervision. Traditionally the tree is considered a forest tree (Ranaivoson *et al*., 2015). Cultivation of the tree requires 40 to 50 trees per acre. The plant is considered hardy which requires less irrigation (Ledesma, 2013). Irrigation is important if the tree is raised from seeds in a seedbed and during transplantation for four years. True to type tamarind plants are raised by vegetative means (Ledesma, 2013). Red loamy and alluvial soils are suitable for its cultivation with pH 4.5-9 (Reddy, 2015). Dry weather is critical for the ripening of the fruits during the harvesting period. Tamarind trees remain productive for up to a century (Reddy, 2015).

Tamarind is cultivated by Asian countries with India as a leading producer whereby production in India has increased with an increase in awareness in cultivation (Panesar *et al.*, 2017). The more tamarind was produced the more it was utilized both locally and internationally and this increased the market price of the crop (Du Preez, 2003). In India, tamarind is produced commercially in several districts with a total production of 98.16 tonnes in 2017-2018 (National Horticulture Board, 2017). Du Preez, (2003) reported that tamarind from India was imported by Australia, Canada, Germany, America, Pakistan and Qatar. Two varieties have been reported in India; PKM1, which matured

early and produced up to 25 tonnes of pods/ha and Urigam, which was the local, sweet, long variety (Reddy, 2015). In India, the leaves and the flowers are utilized as food and a source of herbal medicine. Kumar and Bhattacharya (2008) reported that the seeds had various uses but had not been fully utilized in oil, textile, food and tanning industries.

In Africa tamarind is grown in the tropics; in the woodlands and semi-arid areas where the leaves, bark, flowers and seeds are used as food and in traditional medicine for the treatment of jaundice and dysentery as was reported by El-Siddig *et al.*, (2006). Tamarind was found planted for landscaping due to its ability to tolerate extreme environmental conditions and its evergreen nature and provide shade on country roads, highways and homesteads. For example in Kenya tamarind tree provided shade for meeting places in the villages and was used as a source of food in the semi-arid regions during the lean periods (Maundu *et al*., 2005; NRC, 2008; Orwa *et al.*, 2009). It was used for the control of soil erosion in areas prone to mudslides and was a source of firewood and timber for construction (Orwa *et al.,* 2009). Fandohan *et al*., (2010a) reported that farmers benefited from sales in charcoal and the leaves were fed to goats and sheep.

In Kenya, research findings showed that tamarind was grown in the Coastal, Eastern, Nyanza and North Eastern regions. In the Coastal region, tamarind had equal importance with the mangoes and cashew nuts as it was utilized as a spice in most of the dishes (El-Siddig *et al.*, 2006). Tamarind fruits collected from Tharaka in Kenya were sold in the Coastal market (Betser, 2009).

4.2 Materials and methods

4.2.1 Survey data collection

Data were collected by observation, personal interviews with the farmers using structured and semi-structured questionnaires **(**Appendix I**)**. Farmers provided information on the type of production system, cultural practices, source of planting material, time taken to maturity, maturity indices, fruit yield per tree per season, number of harvests per year, the portion of fruit sold, uses of the tamarind fruit, use of tamarind tree, challenges in tamarind production; market, transport, pests and diseases.

4.2.2 Data analysis

Data were summarized using cross-tabulations and processed descriptively using means, frequencies and percentages, Chi-square X^2, F test and one-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) Version 18 (SPSS Inc. Chicago USA). Analysis of variance was conducted to assess the differences between counties, gender, age of farmers, crops grown, type of production, farm size in acres, nature of production, the proportion of tamarind sales, the proportion of farm under tamarind and land preparation methods. Source of planting materials, crop management practices, maturity indices, use of the tamarind fruit, medicinal uses, maturity length, source of planting seed, opinion on tamarind farming and challenges in tamarind production were also analyzed using ANOVA.

4.3 Results

4.3.1 Social characteristics of tamarind respondents

Most of the respondents (61.90 %) were male and middle-aged while females were only 38.10% (Table 4.1).

Table 4.1: Social characteristics of tamarind farmers in semi-arid Eastern Kenya

Variable	County				
	Embu N=21	**Kitui N=35**	**Machakos N=6**	**Makueni N=27**	**Mean N=89**
Gender %					
Female	38.10	23.81	33.33	38.46	33.78
Male	61.90	76.19	66.67	61.54	66.22
Age of the farmers in years	42.14	41.62	47.83	39.54	41.54

N is the number of respondents

4.3.2 Crops intercropped with tamarind in semi-arid Eastern Kenya

Crops that were intercropped with tamarind included maize, beans, mangos, pawpaw, khat, pigeon peas and other trees. Maize was grown in all the regions, mangoes, avocadoes and pawpaw were highly produced in upper Embu. Beans were planted in all regions; pigeon peas were mostly grown in the lower eastern regions with Makueni recording the highest production than the upper region of Embu that had the least production.

Table 4.2: Crops intercropped with tamarind in semi-arid Eastern Kenya

Variable (%)	County				
	Embu N=21	**Kitui N=35**	**Machakos N=6**	**Makueni N=27**	**Mean N=89**
Maize	95.2	51.4	66.7	18.5	52.8
None/trees	0	48.6	0	25.9	27.0
Beans	28.6	17.1	50.0	7.4	19.1
Pigeon peas	9.5	22.9	16.7	85.2	38.2
Mangoes	57.1	5.7	16.7	0	16.9
Khat	0	0	0	16.7	1.1
Avocados	23.8	0	0	0	5.6
Pawpaw	33.3	0	0	0	7.9

N is the number of respondents

4.3.3 Production of tamarind in semi-arid Eastern Kenya

There was a significant difference in the types of tamarind production. A great proportion of tamarind produced in Embu was for home use. A great proportion of tamarind fruits produced in Makueni and Kitui and Machakos were for both market and home use. There was a significant difference in the nature of production. Tamarind farms were intercropped with legumes and cereals across the region with Machakos and Makueni recording 100%. Abandoned production was observed in Embu and Kitui especially Mwingi areas with forest reserves. There was a significant difference in the farm sizes across the counties. Very few farmers in Machakos and Makueni reported farm sizes of greater than 8 acres. In Machakos the respondents had between 4-8 acres. An acreage of 2-4 acres was reported in all the regions except in Machakos and a high number was reported in Makueni. Out of 2-4 acres' farmers had only a small 2.1-4% percentage of it that was on tamarind production which was high in Embu, Makueni and

Machakos (Table 4.3 and Plate 4.1). All farmers reported that tamarind grew from seeds upon falling and obtained favorable growth conditions.

Table 4.3: Type of production, nature of production, farm size in acres and proportion of farm occupied by tamarind in semi-arid Eastern Kenya

Variable (%)	County					Significance	
	Embu N=21	Kitui N=35	Machakos N=6	Makueni N=27	Mean N=89	chi-square tests	*P*-value
Type of production (A)							
None	38.1	0	0	3.7	10.1	42.761	0.00 **
Subsistence	61.9	45.7	0	29.6	41.6		
Subsistence +Market	0	54.3	100	66.7	48.3		
Nature of production							
Intercrop	33.3	71.4	100	100	72.2	119.363	0.000 **
Abandoned	66.7	28.6	0	0	26.7		
Farm size in acres							
0-2	38.1	25.7	16.7	33.3	30.3	25.669	0.002 **
2-4	33.3	45.7	0	63.0	44.9		
4-8	28.6	28.6	66.7	0	22.5		
>8	0	0	16.7	3.7	2.2		
The proportion of land under tamarind							
0-2%	0	45.7	0	0	18	39.480	0.000 **
2.1-4%	100	42.9	83.3	100	76.4		
4.1-8%	0	11.4	16.7	0	5.6		

**significant difference, Chi-square analysis and F-test at $P<0.05$, N is the number of respondents

(A) Abandoned **(**B) Intercropped

Plate 4.1: Nature of tamarind production in semi-arid Eastern Kenya:

4.3.4 Land preparation methods and crop management practices of tamarind in semi-arid Eastern Kenya

There was a significant difference in the methods used in land preparation. Embu (100%) and Kitui (20%) were abandoned hence the land was not prepared. The simple implements included hoes, machetes which were used across the region with high percentages in Makueni. Animal-drawn implements were used across the region except upper for region Embu and highly used in Machakos. Machine usage was only reported in Machakos (16.7%). There was a significant difference in crop management practices across the region. Weed control was mainly done in tamarind farms with intercrops which was high in Machakos and Makueni. Cultural practices such as pruning, fertilizer application and pesticide application were not carried out in tamarind production. All farmers reported that tamarind grew from seeds of the same tree naturally (Table 4.4 and Plate 4.2).

Table 4.4: Land preparation method and crop management in tamarind production in semi-arid Eastern Kenya

Variable (%)	County					Significance chi-square tests	*P* value
	Embu N=21	Kitui N=35	Machakos N=6	Makueni N=27	Mean N=89		
Land preparation method							
None	61.9	25.7	0	0	24.7	49.285	**0.000****
Simple implements	38.1	60.0	33.3	88.9	61.8		
Animals	0	14.3	50.0	11.1	12.4		
Machine	0	0	16.7	0	1.1		
Crop Management							
None	100	20	0	0	31.5	68.03	**0.000****
Weed control	0	80	100	100	68.5		

**significant difference, Chi-square analysis and F-test at $P<0.05$, N is the number of respondents

A Weeds not controlled

B Weeds controlled

Plate 4.2: Tamarind management practices in semi-arid Eastern Kenya:

4.3.5 Maturity and harvesting of tamarind in semi-arid Eastern Kenya

There was a significant difference in maturity indicators across the region. Both indices of pod brittleness and pod color change from green to brown were observed across the counties. Most farmers were not sure how long tamarind took to maturity with a high number in Embu (100%). Farmers in Makueni reported that it took 5-6 years to get to maturity. There was a significant difference in fruits harvested across the counties. Farmers across the region were not keen on the quantity of tamarind fruits they harvested with high numbers in Embu (71.4%). Those who kept records reported they harvested less than 180 kg per season per tree with a greater percentage from Kitui (42.9%). Farmers in Makueni reported higher yields of more than 271kg per plant per season. The fruits were harvested by shaking, climbing and handpicking (Table 4.5 and Plate 4.3).

Table 4.5: Maturity indices, maturity length, yield per plant per season of tamarind in semi-arid Eastern Kenya

Variable	County					Significance	
	Embu N=21	Kitui N=35	Machakos N=6	Makueni N=27	Mean N=89	chi-square tests	P value
Maturity indices %							
Pod brittleness	**19.0**	**20.0**	**0**	**33.3**	**22.5**	**24.407**	**0.00****
Pod color change	52.4	11.4	50	0	20.2		
Both	28.6	68.6	50	66.7	57.3		
Maturity length of tamarind (years)							
Not sure	100	74.3	50	14.8	60.7	50.916	0.00**
5-6	0	17.1	0	66.7	27.0		
6-8	0	8.6	50	18.5	12.4		
Yield per plant per season (kgs)							
Not sure	71.4	5.7	16.7	25.9	28.1	43.247	0.00**
<180	28.6	42.9	33.3	29.6	34.8		
181-270	0	22.9	33.3	0	11.2		
>271	0	28.6	16.7	44.4	25.8		

**significant difference, Chi-square analysis and F-test at $P<0.05$, N is the number of respondents

(A) Harvesting of tamarind fruits by hand picking

(B) Semi-arid Eastern Kenya

Plate 4.3: Maturity indicator of brown color pods

Harvesting of tamarind fruits was done once a year, the fruits were either for home use or sold in Nairobi or Mombasa by middlemen who determined the market price. There was a significant difference in the proportion of tamarind sold across the region. Farmers across the region had limited tamarind fruit sales with a high number from upper Embu (90.5%). Most sales were less than 25% of the fruits harvested with high a percentage from Makueni (74.1%). Few farmers sold above 75% of the harvest and this was highly reported in Machakos (33%) (Table 4. 6).

Table 4.6: Proportion of tamarind fruit sales in semi-arid Eastern Kenya.

Variable	County					Significance	
	Embu N=21	Kitui N=35	Machakos N=6	Makueni N=27	Mean N=89	chi-square tests	*P* value
The proportion of sales in%							
No sales	90.5	40	16.7	25.9	46.1	66.089	0.000**
0-25	9.5	31.4	0	74.1	37.1		
26-50	0	20	50	0	11.2		
51-75	0	5.7	0	0	2.2		
>75	0	2.9	33	0	3.4		

**significant difference, Chi-square analysis and F-test at $P<0.05$, N is the number of respondents

Fruits were harvested and stored in gunny bags. For preservation purposes the fruits were dehusked and stored in polythene packing bags and stored at room temperatures.

4.3.7 Utilization of tamarind fruit and tree in semi-arid Eastern Kenya

Tamarind was predominately used as a dessert and in porridge preparation. It was only in Embu where it was preferred as a dessert. The leaves were used for medicinal purposes across the region except for Embu. The opinion on tamarind farming was negative (71.8%). A positive opinion was only reported in Kitui (48.6%) and Makueni (29.6%) (Table 4.7).

Table 4.7: Utilization of tamarind fruit, medicinal use and opinion on tamarind farming in semi- arid Eastern Kenya

Variable	County					Significance	
	Embu N=21	Kitui N=35	Machakos N=6	Makueni N=27	Mean N=89	chi-square tests	P value
Use of tamarind fruit							
Dessert	66.7	8.6	0	0	19.1	41.333	0.00**
Porridge	0	11.4	0	0	4.5		
Both	33.3	80.0	100.00	100	76.4		
Medicinal use							
Not used	100	14.3	33.3	63	50.6	41.33	0.00**
Used	0	85.7	66.7	37.0	49.4		
Opinion on tamarind farming							
Negative	100	51.4	100	70.4	71.9	17.847	0.00**
Positive	0	48.6	0	29.6	28.1		

**significant difference, Chi-square analysis and F-test at $P<0.05$, N is the number of respondents

Tamarind trees were reported to have various uses in the area; used as landscaping trees, as a source of fuel in form of charcoal, timber, shade, in control of soil erosion during flash floods and as a storage area for animal feeds (Plate 4.4).

(A) Shade for animal feeds (B)source of firewood (C) source of human shade

Plate 4.4: Utilization of tamarind tree in semi-arid Eastern Kenya

4.3.8 Constraints in tamarind production in semi-arid Eastern Kenya.

Transporting tamarind fruits was a challenge in Makueni and Kitui. Tamarind weevil attacked the fruits throughout the region at maturity before harvesting and in storage. Harvesting the fruits from the trees was a challenge experienced in all the counties with greater effects in Kitui (100%). All four counties had a challenge with marketing tamarind fruits. (Table 4.8).

Table 4.8: Constraints in tamarind production in semi-arid Eastern Kenya

Variable	County					Significance	
	Embu N=21	Kitui N=35	Machakos N=6	Makueni N=27	Mean N=89	chi-square tests	P value
Challenges in tamarind production							
Transport							
Not a challenge	100	0	100	34.3	67.4	53.103	0.00**
Challenge	0	100	0	65.7	32.6		
Pests							
Not a challenge	0	2.9	0	0	1.1	1.560^{*}	0.668^{ns}
Challenge	100	97.1	100	100	98.9		
Harvesting							
Not a challenge	38.1	2.9	83.3	77.8	39.3	41.123	0.00**
Challenge	61.9	97.1	16.7	22.2	60.7		
Market							
Not a challenge	0	42.9	50	0	20.2	72.484	0.00**
Challenge	100	57.1	50	100	79.8		

^{ns} no significant difference, **significant difference, Chi-square analysis and F-test at $P<0.05$, N is the number of respondents

4.4 Discussion

Tamarind in semi-arid Eastern Kenya has vast uses ranging from food to ecosystem services. Most uses were skewed towards the ecosystem which includes: control of soil erosion as the area is prone to mudslides, source of firewood, use as shelter and shade.

This observation was also reported by Ranaivoson *et al*. (2015) where the authors reported that tamarind in West Africa provided more ecosystem services.

Tamarind fruit was either eaten as a dessert or used as an ingredient in porridge preparation which was also reported by Singh *et al*. (2007). The authors reported that in India, the fruit pulp could be eaten raw when dipped in a sweetener or salt as a snack while in the Bahamas it was dipped in wood ash and also used in the preparation of other foods. Havinga *et al*. (2010) also reported that in Africa, tamarind fruit is a useful source of food during lean periods. The authors stated that tamarind was also used in cultural activities such as worship which was not evident in semi-arid Eastern Kenya. In this study tamarind was used as a cure to mouth rash which was similar to (Pinar, 2014) who reported that tamarind fruits and leaves in Turkey were effective against fungal pathogens.

In Kitui, Machakos, Embu and Makueni land was prepared manually using simple implements like hoes and machetes which were readily available. Farmers in Machakos (50%), Kitui (14.4%) and Makueni (11.1%) used animal-drawn plow donkeys that were readily available. Farmers reported they were not able to afford and maintain farm machinery in the production of tamarind.

Due to the low acreage of productive land, farmers were forced to intercrop tamarind with other crops. Short season intercrops (pigeon pea, beans and maize) provided food and income to the farmers unlike tamarind which is perennial. The pigeon peas and other intercrops benefited as they did not get scorching from the sun. Weed control was mainly practiced due to the presence of intercrops.

Farmers (100%) reported that they did not have a particular season when they planted tamarind as it grew on its own upon seeds falling on the ground and having obtained

favorable germination conditions. This finding was also reported by Singh *et al*. (2007), that commercial use of tamarind in Africa was still underdeveloped. Infonet Biovision (2019), reported that in the Philippines tamarind was grown on a large scale, propagation was done by vegetative means that included; cleft grafting, shield and patch budding.

Farmers (60.7%) were not sure of the period tamarind took to mature but most (27%) of them reported 5-6 years which was contrary to reports by Infonet Biovision, (2019) which indicated 3-4 years since the trees were raised by vegetative means. The fruit was harvested for market and home use and this was similarly reported by Rao and Kumar, (2015). The authors reported that tamarind produced in Africa was mainly for domestic consumption. Fruits from Kitui and Makueni were sold to traders from coastal Kenya, who used the fruits for making jams, juices and sauces for making dishes. This was also reported by Ranaivoson *et al*. (2015), who reported that tamarind fruit could be used to make sauces and juice. High (42.9%) yields and positive opinions on tamarind production in Kitui were associated with the ready market to the coastal Kenya traders. The ready market in the Kenyan coast was also reported by Betser, (2009) who stated that tamarind could be collected around Tharaka in Kenya and sold to the coast and used in making dishes. The yields in this study were slightly lower (not more than 270kgs) than yields reported by Rao and Kumar, (2015). They reported that each tamarind tree in India yielded 150-500kg per season. Alveraz *et al*. (2019) reported fruit yields of up to 200 kg/tree per season in Asia where the fruit varied based on the age of the tree, genetic potential and climatic condition. These authors reported that after 50 years' production decreased. Low yield also could be influenced by poor management practices such as pruning, fertilization and phytosanitary control.

Challenge in transport experienced was associated with the poor infrastructure of the region. The most dominant mode of transport was human and animal transport. There were no reported mechanical means of transporting the produce to the market.

Marketing tamarind fruits was a challenge and this was similar to the findings by Betser, (2009) who reported that the ready market for tamarind in the Tharaka region was only available in the coast region of Kenya. This forced the traders to travel to the Kenyan coast, which is far away from the region of production.

Harvesting tamarind fruits was a challenge mainly in Kitui as this region had huge, tall trees (more than 1.5M) where climbing, picking by hand and shaking of the branches was difficult. The trees are huge and tall because they are raised from seeds and pruning was not done which is in agreement with reports by Infonet Biovision, (2019). In this report trees that were raised from seeds grew taller than their counterparts raised by vegetative means. The taller they are the more difficult it is to harvest using the common methods of climbing, picking and shaking. In Embu tamarind was in abundance due to the favorable climate. The fruit trees were abandoned as the farmers had a negative opinion on tamarind production. The negative opinion was associated with the fact that Embu is a high agricultural potential area that has a diversity of products to choose from such as mangoes, maize, beans, avocado and pawpaw. The tamarind trees were majorly used as a source of firewood which was similar to the findings by Ranaivoson *et al*. (2015), who reported that tamarind trees in West Africa were heavily used as a source of fuel.

The farmers (100%) did not report any incidence of diseases and similar findings were made by *(Singh et al.,* 2007), in India where the authors reported that tamarind trees were free from serious diseases. Reports by Infonet Biovision, (2019) in Kenya indicated that tamarind was attacked by powdery mildews and leaf spots, a sooty mold, stem disease, stem, root and wood rot, stem canker, a bark parasite and a bacterial leaf-spot. A serious pest in tamarind production was reported to be the weevil that attacked the fruits when they mature on the trees and after harvesting which was similar in Nigeria and India (Ojo & Omoloye 2015; Mercedo-Mesa *et al*., 2018). This was

contrary to the reports by Soemardji, (2007) in Japan who reported that the major pests that attacked tamarind were mealy bugs, seed beetles and bruchid pod beetles.

4.5 Conclusion

Tamarind was not produced as the main crop in semi-arid Eastern Kenya. It was intercropped with the other crops (legumes and cereals). Tamarind occupied less acreage of the total farms, management practices such as fertilizer application, pruning and propagation were not carried out hence the less yields. Tamarind was not considered as the main food it was utilized as an ingredient in juices, sources and only considered as an important vegetable during the lean periods. Tamarind production was constrained by weevils and poor marketing channels

4.6 Recommendation

Production information collected can be used in the improvement of tamarind yields by developing crop management practices such as vegetative propagation, pruning, pest control (tamarind weevil), fertilization e.t.c. Utilization information could be used to sensitize the public on the importance of tamarind and develop commercialization and industrialization. There is a need to develop proper marketing strategies so that the farmers can realize the full potential of tamarind.

CHAPTER FIVE

EVALUATION OF MORPHOLOGICAL DIVERSITY OF TAMARIND (*Tamarindus indica*) ACCESSIONS IN SEMI-ARID EASTERN KENYA

Abstract

The morphological diversity of tamarind in Kenya has not been evaluated. The aim of this study was to evaluate the morphological diversity of tamarind accessions in semi-arid Eastern Kenya. Standard descriptors of tamarind stems, seeds and fruits were used. Data were collected on the number of primary and secondary branches, height to the first branch, growth habit, number of seeds per pod, seed color, seed shape, seed weight, seed brightness, seed roughness, pod color, pod shape, pod length, pod width and pulp color. Data were analyzed using unbalanced one-way analysis of variance (ANOVA) to compare differences across the counties and F test and PCA analysis, correlations and cluster analysis using Genestat and Exlstat at a significant level of ($P \leq 0.05$). There was significant variation in terminal shoot length, trunk diameter at ground, trunk diameter at the neck, pod length, pod width, irregular and ovate seed shape, dark brown, brown and black seed colors, curved and semi-curved pod shape, pod color and pulp color. There were no significant differences in number of primary and secondary branches, height to the first branch, pod weight, seed weight, number of seeds per pod, growth habits, seed color of dark brown at center and brown at the edges and light brown, quadrant and D-shape, seed brightness and straight pod shape. Quantitative PCA revealed 5 PCs. Descriptors that contributed positively to PC1, PC2, PC3, PC4 and PC5 were trunk diameter at the ground, pod weight, number of seeds per pod, height to the first branch

and pod width respectively. Agglomerative Hierarchical clustering revealed 3 major clusters. The variation within the clusters was 66.12% and 33.88% between clusters. A high correlation was observed between terminal shoot length and trunk diameter at the ground, between pod length and the seed number per pod. Morphological diversity was observed among tamarind accessions in semi-arid Eastern Kenya. The differences in tamarind morphology across the counties can be used in selecting accessions with desirable traits for molecular-assisted breeding.

5.1 Introduction

Morphological descriptors are used as basic characters in the identification of plants, breeding, commercialization, cluster analysis, genetic diversity and conservation of plant resources *(*Khan *et al*., 2015; Martinez *et al.,* 2017*)*. Components of fruits such as fruit size, shape, color and general appearance of the plants are important in description (Nasution & Yapwattanaphun, 2017). Morphological descriptors have been used to differentiate between many plant species such as capsicum which have been classified based on fruit shape (Nasution & Yapwattanaphun, 2017). Morphological descriptors have limitations in distinguishing subfamilies and tribes as the traits are similar (Swenson & Anderberg, 2005). *Tamarindus indica* is morphologically different in terms of fruit; color, shape, taste, crown size and density, foliage color, trunk diameter, flower characteristics as well as seed color, weight and shape (Nandini *et al*., 2011). Schabel, (2004) evaluated fruit taste, which ranged from sweet to acidic and Parrotta, (1990) reported red fruits in India. Obulesi, (2011) also documented light brown-reddish fruits in India while Vanden, (2014) recorded sour, small and large fruits in Mali.

Morphological and physiochemical traits were used to study Asian tamarind populations and the results revealed the existence of morphological and genetic differences (Fandohan *et al*., 2010). These descriptors enabled the authors to choose superior cultivars for the market in terms of taste, pulp thickness and taste (Elsiddig *et al.*, 2006).

In West Africa, most studies have been carried out on biochemical compounds of tamarind (Adeola & Aworh, 2012). In Columbia, India, Mexico and Venezuela, tamarind varieties have been differentiated using fruit taste, pulp color, seed number, pod color and floral morphology (Du Preez, 2003; Osori*o et al.*, 2018). Fandohan *et al.* (2011) suggested that quantitative and qualitative descriptors should be combined in distinguishing varieties. In Kenya, there is no information on research aspects available that can be used to compare morphological differences among tamarind populations.

5.2 Materials and methods

5.2.1 Sampling

Sampling was done as shown in Chapter 3 (Section 3.2).

5.2.2 Morphological characterization of tamarind

Characterization was done according to the International Union of Plant Protection of New Vegetal Variants (Tripp *et al*., (2007). International Plant Genetic Resources Institute (IPGRI, 1991) and according to the procedure by (Fandohan *et al*., 2010).

Table 5.1: Morphological descriptors used to study tamarind accessions in semi-arid Eastern Kenya

Plant part	Quantitative	Qualitative
Stem	Terminal shoot length (cm)	Growth habit
	Trunk diameter at ground (cm)	
	Trunk diameter at the neck(cm)	
	Height to first branch (cm)	
	Number of primary branches	
	Number of secondary branches	
Seed	Number/pod	Shape, color, brightness, roughness
	Weight (g)	
Fruit	Length(cm), width(cm) weight(g)	Shape and color
Pulp	Weight(g)	Color

Qualitative descriptors used included: growth habit, pulp color, seed color, pod color, seed shape, fiber color, seed brightness and seed roughness. Quantitative descriptors used included: trunk diameter at the ground and neck, height to the first branch, pod length, width, weight, seed weight, seed number per pod and pulp weight (Table 5.1). A standard color chart was used to determine the color shades.

5.2.3 Data collection and analysis

Data were collected on mature fruiting tamarind trees. Trunk diameter at the ground was measured as an average of three readings at the ground. Trunk diameter at the neck was an average of three readings of the diameter where the tree started branching. Height to the first branch was measured as an average distance from the ground to the first branch from three angles of the tree.

Pod length was determined as an average of five pods from pole to pole. Pod width was determined as a mean of five pods from the equator of the cross-section of the fruit and pod weight was determined as a mean of five pods of the same tree. Seed weight was determined as an average of seeds in an entire pod. Pulp weight was determined as the average of pulp in 5 pods per accession. Seed number was determined as an average of seeds in 5 pods from the same accession. Growth habit was determined as either orthotropic if the branching started above the ground or plagiotropic if the branching started at the ground level.

Data were summarized using cross-tabulation and processed using unbalanced one-way analysis of variance ANOVA to compare differences across the counties and F test using Genstat software version 12.1. Quantitative data were submitted to principal component analysis (PCA), correlation of traits was carried out and cluster analysis using Agglomerative Hierarchical Clustering (AHC). This was done using Xlstat 2021.1.1 software.

5.3 Results

5.3.1 Quantitative morphological results for tamarind in semi-arid Eastern Kenya

There were significant variations in tamarind tree terminal shoot length, trunk diameter at the ground, trunk diameter at the neck, pod length and pod width across the counties. There was no significant variation in height to the first branch, number of primary and secondary branches, number of seeds/pod, seed weight, pulp weight and seed weight (Table 5.3, Plate 5.1 and Appendix II-XII).

Table 5.2: Quantitative Morphological variation of tamarind in semi-arid Eastern Kenya

Variable	Minimum	Maximum	Mean	Standard deviation	F. Value
Terminal shoot length	340	2400	842.7	339.76	0.002**
Trunk diameter at the ground	54	590	203.2	114.39	0.013**
Trunk diameter at the neck	43	590	196.4	119.45	0.001**
Height to the first branch	28	420	148.7	61.22	0.664 ^{ns}
Number of primary branches	1	3	1.11	0.38	0.246 ^{ns}
Number of secondary branches	1	12	3.39	1.94	0.319 ^{ns}
Number of seeds per pod	1	12	6.87	1.76	0.308 ^{ns}
Pod length	3.3	20.83	11.49	2.78	0.030**
Pod weight	3	41.59	15.3	6.73	0.671 ^{ns}
Pod width	2.6	10.7	5.97	1.98	<0.001**
Seed weight	0.27	1.16	0.65	0.19	0.350 ^{ns}
Pulp weight	0.28	2.5	0.76	0.33	0.284 ^{ns}

^{ns} not significant at $p< 0.05$ and ** significant difference

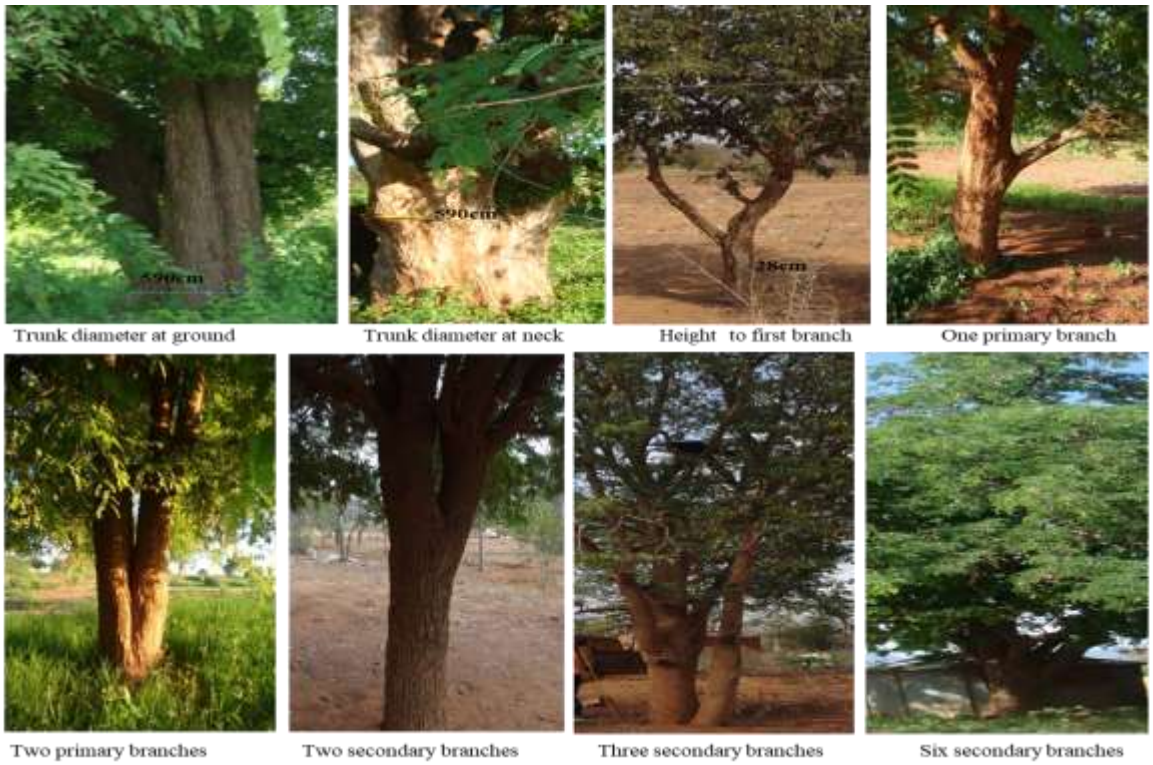


Plate 5.1: Tamarind quantitative morphological variation in semi-arid Eastern Kenya

5.3.2 Qualitative morphological results for tamarind in semi-arid Eastern Kenya.

Significant variations were observed in pulp color, pod color, irregular and ovate seed shape, dark brown, brown and black seed color and in curved and semi-curved pod shape (Table 5.4 and Plate 5.2). There was no significant difference in the growth habit across the counties, orthotropic habit was predominantly observed. Plagiotrophic was not observed in Embu and Machakos. Irregular and ovate seed shapes recorded a significant variation across the counties while there was no significant variation in quadrant and D shape. Dark brown, brown and black seed colors significantly varied across the counties while there was no significant variation in seed colors of brown and dark brown at the center and brown to the edges. Pod shapes of curved and semi-curved

showed variations across the regions while straight shapes did not show significant variation. Pod colors of cinnamon-brown and greyish brown significantly varied in the counties. Greyish brown was not observed in Machakos while cinnamon-brown was not observed in Embu. Pulp color significantly varied across the counties. Dark brown pulp color was predominant across the counties while brown was not observed in Embu and Makueni.

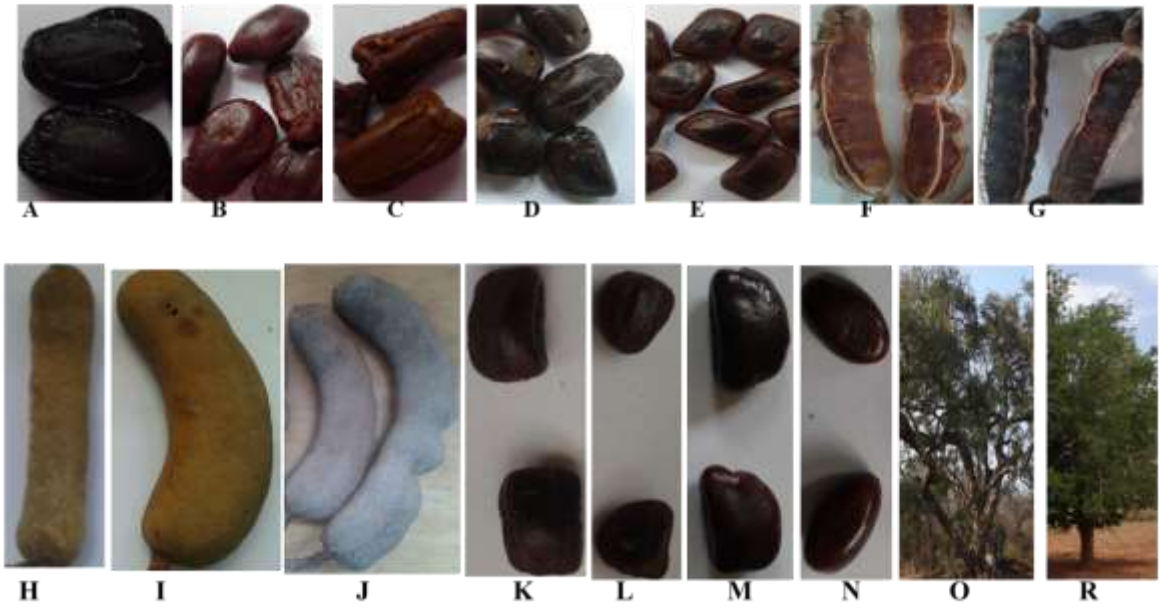


Plate 5.2: Tamarind qualitative morphological variations in semi-arid Eastern Kenya: Color of seeds: Black (A), Brown (B), light brown (C), dark brown (D) and dark brown at the center and brown outside (E), Color of tamarind pulp: brown (F) and Dark brown (A). Tamarind pod shape: straight (H), semi-curved (I) and curved (J). Tamarind pod color: cinnamon brown (H and I) and greyish brown (J). Tamarind seed shape: Quadrant (K), irregular (L), D shape (M) and ovate (N). Tamarind growth habits: Orthotropic (O) and plagiotropic (P).

Table 5. 3: Qualitative Morphological Variations in Tamarind from Semi-arid Eastern Kenya

Variable	Counties								Significance
	Kitui N=35	**Embu N=21**	**Makueni N=27**	**Machakos N=6**	**Means N=89**	**Minimum**	**Maximum**	**STD**	***p* value**
Growth habit (%)						1	2	0.3	
Orthotropic	85.74	100	88.89	100	93.65				0.267^{ns}
Plagiotropic	14.26	0.00	11.11	0.00	6.35				0.267 ^{ns}
Seed shape (%)						1	4	1	
Quadrant	54.29	19.05	33.33	50.00	39.16				0.054 ^{ns}
Irregular	14.29	76.2	11.11	50.00	37.90				<0.001**
D shape	8.57	4.75	25.93	33.30	18.19				0.112 ^{ns}
Ovate	0.00	0.00	25.93	0.00	6.48				<0.001**
Seed color (%)						1	5	1	
Dark brown	71.43	38.10	29.63	50.00	47.29				0.006**
Dark brown/brown	17.14	42.86	33.33	0.00	23.33				0.066 ^{ns}
Brown	11.43	19.05	11.11	50.00	22.89				0.0094 **
Light brown	0.00	0.00	11.11	0.00	2.78				0.068 ^{ns}
Black	0.00	0.00	14.81	0.00	4.94				0.006**
Pod shape (%)						1	3	0.54	
Curved	82.86	100	66.67	0.00	62.38				0.012 **
Semi curved	0.00	0.00	18.52	0.00	11.77				0.031**
Straight	17.14	0.00	14.81	100	32.99				0.177 ^{ns}
Pod color(%)						1	2	0.3	
Grayish brown	40.00	100.00	15.9	0.00	30.28				**<0.001****
Cinnamon brown	60	0	74.1	100	60.83				**<0.001****
Pulp color (%)						1	2	0.37	
Brown	28.57	0.00	85.19	0.00	30.97				**<0.001****
Dark-brown	71.43	100	14.81	100	71.56				**<0.001****

^{ns} not significant at $p< 0.05$ and ** significant difference, STD- standard deviation

5.3.3 Principal component analysis of tamarind using quantitative traits

Components with SS loadings of greater than one and cumulative variations of greater than 75% are considered. Eleven traits contributed 84.35% variation in the first 5 PCs. PC1 contributed 25.77% of the total variation. A positive correlation was contributed by trunk diameter at the ground, trunk diameter at the neck and terminal shoot length. PC2 contributed 20.56% of the total variation. Pod weight, number of seeds per pod and trunk diameter at the ground contributed positively to the PC. PC3 contributed 19.56% of the variation and the positive contributors in the component were the number of seeds per pod, pod length and number of secondary branches. PC4 traits contributed 10.78% of the variation with height to the first branch, the number of seeds per pod and pod length as positive contributors. PC5 contributed 7.70% of the total variation with positive contributors as the number of secondary branches, trunk diameter at the neck and ground.

Table 5.4: Principal components loadings of 12 quantitative traits in 89 tamarind accessions in semi-arid Eastern Kenya

Variable	PC1	PC2	PC3	PC4	PC5
					-0.19
					-0.11
Terminal shoot length	0.78	0.07	0.18	-0.20	-0.38
Trunk diameter at ground	0.73	0.49	-0.12	0.21	0.39
Trunk diameter at neck	0.71	0.45	-0.13	0.29	0.40
Height to the first branch	0.59	-0.28	-0.08	0.47	-0.28
No of the primary branches	0.16	-0.74	0.37	0.16	0.25
No. of secondary branches	-0.34	-0.45	-0.19	-0.30	0.54
No of seeds/pod	-0.55	0.50	0.12	0.49	0.03
Sd. weight	0.26	-0.31	0.76	0.05	0.08
Pulp weight	0.39	-0.08	0.81	-0.27	0.07
Pod length	-0.59	0.11	0.48	0.44	-0.08
Pod width	-0.02	0.77	0.10	-0.53	-0.03
Pod weight	-0.28	0.48	0.78	-0.03	0.10
SS Loadings	3.09	2.47	2.34	1.29	0.92
Variability %	25.77	20.56	19.53	10.78	7.70
Cumulative variability %	25.77	46.33	65.86	76.65	84.35

Values in bold represent the most relevant trait that contributed to a particular principal component

The first two principal components contributed to 46.33% of the variation. A high correlation was observed between terminal shoot length (TSL), trunk diameter at the neck (CDN) and trunk diameter at the ground (CDG), between pod length (PDL) and the number of seeds per pod (SD/pod). Terminal shoot length (TSL) and the seed number per pod, Terminal shoot length (TSL) and pod length (PDL) are not connected. Pod width (PDW), secondary branches (SB), height to the first branch (HB) and the number of seeds per pod (SD/pod) are well represented for they are in the 5th, 3rd and 4th principal components. The variables to the right (CDG, TSL, CDN, No. of SB, HB, No. of PB, Pd Width and pp weight) of the plot are correlated and the ones on the left (SD/pod, PDL, PDWT and sd weight) are not connected to the ones on the right. (Fig 5.1).

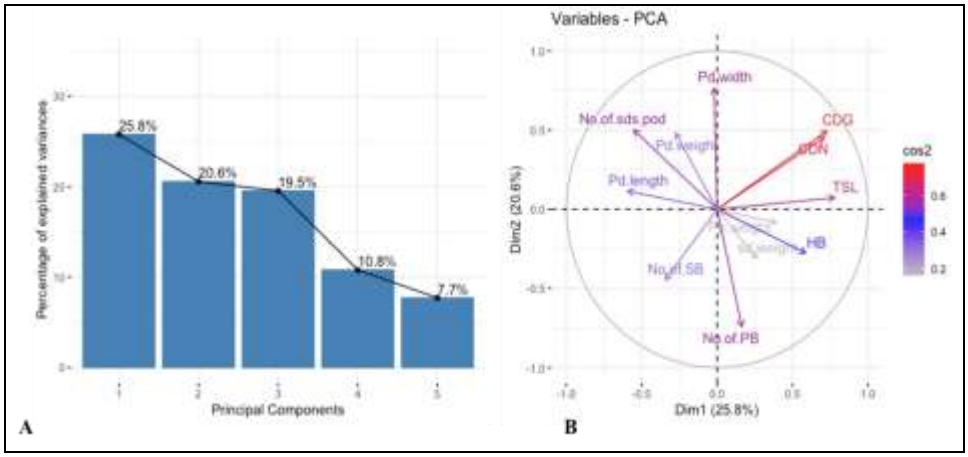


Figure 5.1: Scree plot of 12 quantitative variables (A) and correlation variable plot of among 12 quantitative characters of tamarind associated with the first and second principal component.

NB Pd- pod, Pp-pulp, PB- primary branches, CDG- crown diameter at the ground, CDN- trunk diameter at the neck, Sd -seed, SB –secondary branches, HB – height to the first branch, No-number

5.3.4 Cluster analysis of tamarind using Agglomerative Hierarchal clustering (AHC)

Agglomerative Hierarchal Clustering (AHC) distinguished three major clusters. Variation within the classes was 66.12% and between classes was 33.88% and the distance between the cluster 1 and 3 was 0-5.712 (Appendix XIV-XV). Cluster 1 had 45 accessions from all counties with curved pods. The accessions included: E001, E002, E003, E004, E006, E007, E013, E016, E017, E018, E019, E021, KT001, KT003, KT004, KT005, KT012, KT014, KT017, KT018, KT020, KT021, KT023, KT027, KT028, KT029, KT030, KT031, KT032, KT033, KT034, KT035, MS04, MK002, MK004, MK009, MK010, MK012, MK015, MK021, MK022, MK023, MK024, MK025 and MK027. Cluster 2 consisted of 36 accessions from all four counties: E005, E008, E009, E010, E011, E012, E014, E015, E020, KT002, KT006, KT008, KT011, KT013, KT015, KT016, KT019, KT024, KT025, MS001, MS002, MS003, MS005, MS006, MK005, MK007, MK008, MK011, MK013, MK014, MK016, MK017, MK018, MK019, MK020 and MK025. Predominant in cluster 2 was dark-brown pulp and long pod. Cluster 3 had 8 accessions from two counties Kitui and Makueni; KT007, KT009, KT010, KT022, KT026, MK001, MK003, MK006. The accession in the third cluster had curved pods (Fig 5.2).

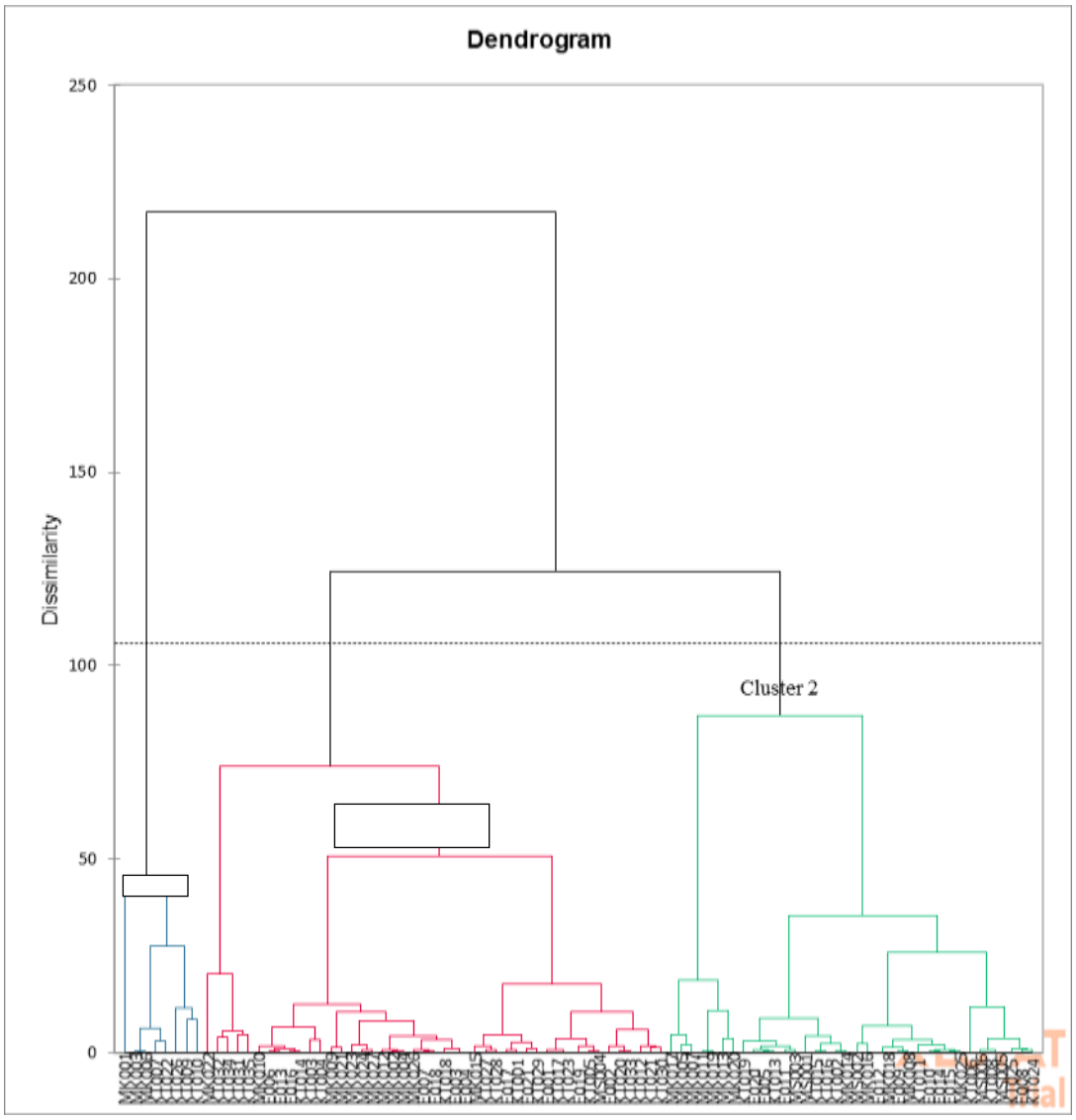


Figure 5.2: Dendrogram constructed based on morphological characters of 89 tamarind accessions from Eastern Kenya and the Euclidian average distances. Green – cluster 2, red –cluster 1 and blue –cluster 3.

5.4 Discussion

Morphological descriptors have been used in the initial identification of organisms (Piyasundura *et al*., 2008). In this study, morphological diversity was reported among accessions collected from semi-arid Eastern Kenya, similar variations were reported by Nyadoi *et al*. (2014). The authors reported that there was a great diversity among the tamarind populations collected in Uganda. Fandohan *et al*. (2011) in Benin reported 3 to 8 primary branches and 30 to 60 secondary branches, but my study revealed that the number of primary branches ranged from one to two and secondary branches from 2 to 12. Trunk diameters varied greatly where tamarind trees in intercrop farms were shorter with smaller diameters than those in forest reserves. These findings were similar to reports by Nyadoi *et al*. (2014) where the authors reported that trunk diameter varied greatly with the type of vegetation in the habitat. The authors studied farmlands, savanna and forests in Uganda and observed that tamarind trees in the forests were bigger compared to those in the farmlands. The growth habit of orthotropic and plagiotropic branches in my study were in line with the findings by Ali *et al*. (2010) which were reported in Southern India. The authors indicated that the growth habit of tamarind was not influenced by changes in the environment and cultural practices. Three pod shapes were observed in my study: curved, semi-curved and straight and these were similar to the findings by Algabal *et al*. (2011) in Bangalore. Fandohan *et al*. (2010) in Benin, reported two pod shapes: curved and straight. Pod shape differences were affected by the seed number and seed shapes which are components of genetics (Fawzi, 2011). Pod colors in my study were either greyish brown or cinnamon brown, which was similar to the findings by Ayala-Silva *et al*. (2016) and Toungos (2019) in Florida and Nigeria. Variations in pod color are highly influenced by the age of the pod and environmental changes (Bhatangar *et al*. 2013). Pulp color varied from brown to dark brown in my study which slightly varied from the findings by Ayala- Silva *et al*. (2016) in Florida,

where the authors reported pulp colors of reddish-brown and brown and also indicated that pulp color was influenced by the genetic make-up of the plant (Bilcke *et al*., 2014).

The diversity observed in seed color was more compared to the findings by Fandohan *et al*. (2011) in Benin. In their study they reported three seed colors of black, brown and dark brown but this study revealed additional colors of dark brown at the center and brown outside and light brown. Seed colors reported in this study were similar to the findings by Fawzi, (2011) in England. Seed color was an inherited trait and affected by the environment, and in different environments different colors could be observed (Fawzi, 2011). Fandohan *et al*. (2011) also observed seed shapes of quadrant, bowl shape and irregular while Fawzi (2011) reported seed shapes that were oblong, asymmetrical, ovate and rhomboid but from this study, seed shapes of ovate, irregular, quadrant and D-shape were reported. Seed shape was an inherited trait that was affected by the environment (Fandohan *et al*., 2011). In this study, pod weight was 3 to 31.4 g, while Bhatangar *et al*. (2013) recorded pod weight of 5.49 to 24.55 g in India. Pod weight was directly correlated to pulp weight and seed number. Pulp weight ranged from 0.28 to 1.92 g while Bilcke *et al*. (2014) reported pulp weight of 1.96-4.65 g in Mali. Pulp weight was a factor of management practices given to the tree. Osorio *et al*. (2018) reported 6 seeds per pod in Columbia while this study depicted seed range of one to twelve per pod. The number of seeds per pod coincides with the reports by Tounges 2019 who also found 1-12 seeds per pod in Nigeria. This was highly influenced by the nutrition available for the plant and the management practices that also influence the length of the pod directly (Bilcke *et al*., 2014).

Diversity was not observed in fiber color, seed roughness, seed brilliance and pulp taste. Fiber color observed was yellow-brown, all seeds were rough, non-brilliant and pulp was sour. These factors were not altered in Makueni, Machakos, Kitui and Embu. Fandohan *et al*. (2011) reported both brilliant and non-brilliant seeds, rough and

polished seeds and this could be affected by different environmental factors. AHC clustering grouped the accessions into three major clusters. The samples were from all four counties indicating that the diversity was not based on the specific county. These findings were similar to the reports by Idi Garba *et al*. (2015) where the authors reported that accessions did not cluster based on their collection sites.

According to Chatfield and Collins (1980) components with eigenvalues less than one should be eliminated; so those with eigenvalues of one and above were considered to be more significant. The eigenvalues decreased from PC1 to PC5 showing a decrease in variation. The first five components explained 76% of total variations among the accessions (Table 5.6). Variables that contributed to variations in the PCAs and clusters: trunk diameter at the ground, trunk diameter to the neck, terminal shoot length pod weight, pod length, seed weight, pulp weight, number of seeds per pod, height to the first branch and pod shape could be explored further to realize the full potential of tamarind in molecular-assisted breeding programs.

5.5 Conclusion

There exists morphological diversity among tamarind accessions in semi-arid Eastern Kenya. Cluster analysis revealed three clusters with 66.12% variation within the clusters and 33.88% among the clusters and the clusters had accessions across the counties. The 5 PCs contributed a variation of 84.35% which were contributed by trunk diameter at the ground, trunk diameter to the neck, terminal shoot length pod weight, pod length, seed weight, pulp weight, number of seeds per pod, height to the first branch and pod shape. Variation across the counties was contributed by seed dispersal by animals from one locality to another and the agents of pollination. The number of seeds and pod length traits, terminal shoot length, trunk diameter at ground and neck were highly correlated whereas vegetative and fruit characters were not correlated.

5.6 Recommendation

Diversity information will be used in the improvement of the crop through conventional breeding methods.

CHAPTER SIX

EVALUATION OF GENETIC DIVERSITY OF TAMARIND (*Tamarindus indica*) ACCESSIONS IN SEMI-ARID EASTERN KENYA USING ISSR MARKERS

Abstract

There is limited information on the genetic diversity of tamarind in Kenya. The objective of this study was to evaluate the genetic diversity of 89 tamarind accessions from Eastern Kenya using 12 Inter Simple Sequence Repeats (ISSRs). DNA was extracted from apical leaves using the CTAB method and amplified using ISSR markers. Data collected were scored as presence (1) or absence (0) of bands and analyzed using GeneAlex and R software. Twelve ISSR primers were used and only seven of them amplified the DNA of 64 accessions. A total of 46 alleles were produced for the 7 loci with an average of 6.5 per loci. Polymorphic information content varied from 0.72 to 0.89 and genetic diversity of 0.74 to 0.9. The ISSR markers revealed effective polymorphism of 40.87-101.46% and the band sizes varied from 100-1000 bp. Analysis of molecular variance revealed high variation within the population at 90% least variation among the population at 10%. Principal coordinate analysis revealed that the first three components contributed 40.83% of the variation. Cluster analysis showed that tamarind accessions were diverse and were grouped into seven major distinct groups. Tamarinds were different within counties but the variations were minimal among counties. There exists genetic diversity among the tamarind accessions in semi- Eastern Kenya.

6.1 Introduction

Tamarind pulp is used in food, pharmaceuticals, textile, cosmetic, oil, paper and printing industries (Chawananorasest *et al.,* 2016; Altrafine, 2018). Tamarind leaves are used as vegetables and are reported to contain vitamins and minerals such as calcium, iron and ascorbic acid (Narina *et al.,* 2019). Tamarind is grown in home gardens, farmlands, roadsides and on common lands (Algabal *et al*., 2011). Tamarind is mostly sown from seeds of unknown parentage and this has resulted in wide variation among the progenies. Wide genetic variation is also aided by the wide geographical distribution and adaptation and cross-pollination nature of the tree. (Algabal *et al*., 2011; Kumar *et al*., 2015). Trees with wide variation within a population offer opportunities in selecting the best trees in relation to crop improvement (Kumar *et al.,* 2015). Very little is known about its genetic improvement and farmers choose cultivars based on observable desirable traits especially of pulp (Algabal *et al*., 2011). These observable traits are highly altered by environmental factors and have many limitations in perennial crops (Algabal *et al*., 2011). Very little has been studied on tamarind conservation, genetic characters and population biology (Sarmiento *et al*., 2017).

Characterization based on DNA markers is more reliable and not hindered by environmental factors (Nadeem *et al*., 2018). A clear and detailed study of the molecular diversity of Kenyan tamarind has not been done. Molecular characterization has been carried out in Bangalore using AFLPs by Algabal *et al*. (2011). RAPDs have been used in Burkina Faso and India by Diallo *et al.* (2007) and Kumar *et al*. (2015) respectively. ISSRs have been used in Ecuador by Sermiento *et al*. (2017) due to their superiority over SSRs, RAPDs and AFLPs. The ISSRs are highly polymorphic, simple, reproducible and use a primer length of 16-25 mers (Pena *et al.,* 2020). Twelve ISSR primers were used to determine the diversity of 89 tamarind accessions from Eastern Kenya.

6.2 Materials and methods

6.2.1 Sample preparation

Five apical leaves from the tip of the leaf were collected and placed in falcon tubes containing silica gel and transported to the laboratory. The leaves were crushed in liquid nitrogen and stored for further extraction as described by Doyle & Doyle 1990.

6.2.2 DNA extraction

DNA extraction was done using 0.4 g of leaves that were ground in 3ml of extraction buffer (CTAB) as described by Doyle & Doyle 1990. The buffer contained (1M Tris HCL (pH 8), 0.5M Ethylene diamine tetraacetic acid (EDTA) (pH 8.0), 5M Sodium Chloride ($NaCl_2$), Sodium Sulphate ($NaSO_4$), Polyvinyl pyrrolidone (PVP10) and 2% CTAB and then incubated at 65°C for 30 minutes. The samples were then centrifuged at 13000 revolutions per minute (rpm) for 12 minutes and the supernatant was mixed with equal volumes of chloroform: Isoamyl (24:1). The mixture was centrifuged at 13000 rpm for 10 minutes and the chloroform: Isoamyl step was repeated. The supernatant was mixed with equal volumes of cold isopropanol and incubated at room temperature. The nucleic acid was pelleted at 13000 rpm for 5 minutes and then washed with 70% ethanol twice. The pellet was air-dried and re-suspended in 50 µl of sterile distilled water. Visualization gel was prepared by weighing 0.8g of agarose in 100ml of Tris Borate EDTA (TBE) buffer and heated for 2 minutes using microwave and Ethidium bromide (EtBr) added. Loading dye of 3 µl was mixed with 7 µl of re-suspended pellet in distilled water and loaded. Observations were made and the presence and absence of bands was scored after 45 minutes.

6.2.3 PCR reaction

DNA amplification was done using ISSR primers as described by Sarmiento *et al*. (2017) in (Table 6.1). Each 20 µl of PCR mix comprised of 10 µl of 2X Bioneer ready mix with 2 µl of primer, 2 µl of DNA and 6 µl of PCR water. Twelve primers were used to screen for more polymorphic primers. The PCR reaction was: initial denaturation at 94˚C for 5 minutes, denaturation at 94˚C for 30 seconds annealing at 54 to 44˚C for 1 minute, extension at 72˚C for 2 minutes and final extension at 72˚C for 7 minutes as described by Sarmiento *et al*. (2017). Amplified DNA was visualized on 2% agarose. Band sizes were estimated by comparing with 100 bp DNA ladder.

Table 6.1: ISSR primers used in characterization of 89 tamarind accessions from semi-arid Eastern Kenya.

S/NO	Name	Sequence
1	ISSR807	AGA GAG AGA GAG AGA CT
2	ISSR814	CTC TCT CTC TCT CTC TA
3	ISSR836	AGA GAG AGA GAG AGA GCTA
4	ISSR860	TGT GTG TGT GTG TGT GAGA
5	ISSRHB11	GT GT GT GT GT GT CC
6	ISSR808	AGA GAG AGA GAG AGA GC
7	ISSR844	CT CT CT CT CT CT CT CT AC
8	ISSR835	AGA GAG AGA GAG AGA GCTC
9	ISSR17899A	CA CA CA CA CA CA AG
10	ISSR17899B	CA CA CA CA CA CA GG
11	ISSR848	CAC ACA CAC ACA CAC AGC
12	ISSR 842	GAG AGA GAG AGA GAG ACTG

6.2.4 Data analysis

Data from ISSR primers were generated by scoring (1) for presence and (0) for absence of bands. The binary data was used to obtain polymorphic information content (PIC) according to Liu *et al.*,2011. PIC=1- $\sum_{j-1}^{n} P2ij$ where $\mathbf{P_{ij}}$ is the frequency of the **jth** allele for ith locus and summation extends **n** alleles scored for ISSR locus. Genetic diversity was obtained using. Genotypic richness (number of multilocus genotypes observed per population MLG). Genotypic diversity was estimated as the percentage of polymorphism observed detected by each population %Pj, ShanWeiner index of MLG diversity per population. Simposons index per population Lambda, Evenness index per population –E. Expected heterozygosity or unbiased gene diversity for each population –He. Observed heterozygosity per population –Ho were analyzed using R3.6.3 software

Genalex 6.5 software (Peakall & Smouse, 2012) was used to estimate pairwise individual relatedness, Principal Coordinate Analysis (PCoA), Analysis of Molecular Variance (AMOVA) was also carried out to give the difference between populations and between the accessions. The obtained data were subjected to R software to obtain phylogenetic clusters using Hierarchical cluster analysis. Accessions from Mwingi were denoted as population 1, Masinga denoted as population 2, Kibwezi as population 3 and Embu as population 4.

6. 3 Results

6.3.1 Selection of polymorphic primers from candidate ISSR primers

Optimization was done using touch-down PCR at annealing temperatures of 54 to 44 ˚C for 35 cycles. Primers ISSR 807, ISSR 836, ISSR 842, ISSR 844, ISSR HB11, ISSR 17899A and ISSR 17899B produced reproducible bands while primers ISSR 808, ISSR 814, ISSR 835, ISSR848 and ISSR 860 did not amplify DNA products (Plate 6. 1). Amplification was only done to 64 accessions from Mwingi, Masinga, Kibwezi and Embu. Accessions from Kitui were not amplified with the ISSR primers.

For all the plates in this chapter, M denotes 100bp molecular weight ladder, Mw denotes accessions from Mwingi, Ms denotes accessions from Masinga, E denotes accessions from Embu and KB denotes accessions from Kibwezi.

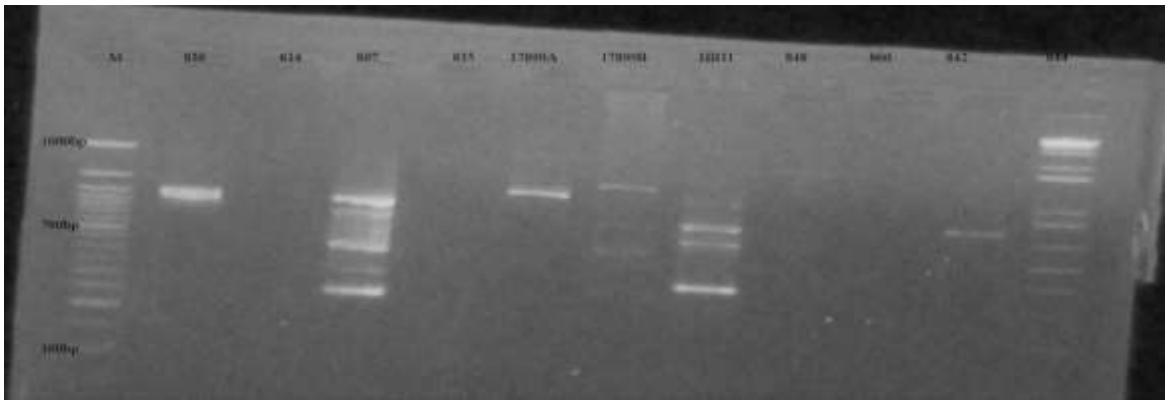


Plate 6.1: A representation of ISSR Primers ISSR (836, 814, 807, 835, 17899A, 17899B, HB11, 848, 860 842 and 844) pattern used for screening polymorphic primers used to analyze 64 tamarind accessions in semi-arid Eastern Kenya

6.3.2 Level of polymorphism in tamarind using ISSR markers

The seven ISSR primers produced 545 scorable bands which were used for the estimation of Analysis of Molecular Variance, estimation of genetic diversity, principal coordinate analysis, analysis of relatedness within populations and cluster analysis. The banding pattern is represented in Plates 6.2-6.8. In all the plates M denotes 100 bp molecular weight ladder, Mw are accessions from Mwingi, Ms are accessions from Masinga, E are accessions from Embu and KB are accessions from Kibwezi.

ISSR807 produced 43 loci with 32 polymorphic loci with band sizes were 200, 300, 500, 600 and 700 bp. Accession of MW004, E009, E010, E017 and E021. 300 bp allele was present in MS006, E005 and E009. 500 bp was present in MW004, E001, E009, E010 and E021. Allele of 600 bp was observed in MW002, MW007, KB001, KB002, KB004, KB005, KB006, KB010, KB011, KB012, KB018, KB026, E001, E009, E010 and E0021. Band size of 700 bp was observed in KB001, KB004, KB005, KB006, KB010, KB011, KB012, KB022, E001, E002, E003 and E021.

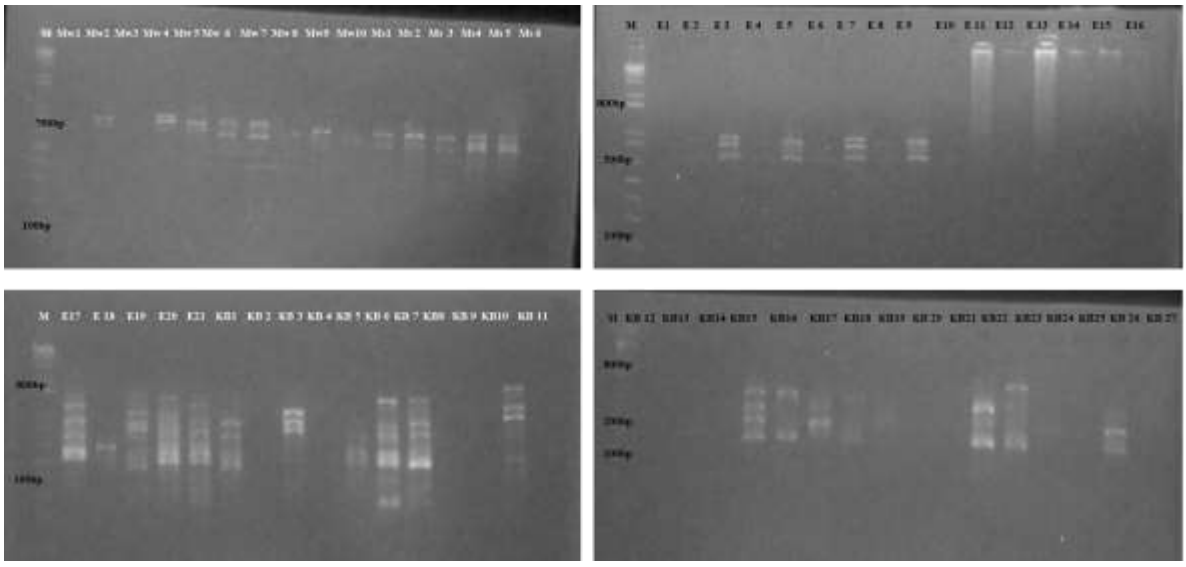


Plate 6.2: ISSR primer pattern for polymorphic primer ISSR 807 used to analyze 64 tamarind accessions in semi-arid Eastern Kenya.

ISSR 836 produced bands of 400, 500, 600, 700 and 800 bp. Accessions with the 400 bp allele included MW005, MS001, E001, E009 and E010. 500 bp allele was observed in accessions from MS001, E001, E007, E009 and E010. Accessions with 600 bp bands included MW10, MS002, MS006, KB004, KB005, KB006, KB007, KB009, KB011, KB012, KB013, KB014, KB011, KB015, KB019, KB021, KB023, KB025, KB027, E001, E007, E009, E010 and E017. Accessions with the allele size of 700 bp included MW04, MW009, KB002, KB004, KB005, KB006, KB007, KB009, KB011, KB012, KB013, KB014, KB015, KB016, KB019, KB021, KB023, KB025, KB027, E001, E007, E009 and E010. 800 bp allele was observed in KB010, KB012, KB013, KB014, KB015, KB016, KB019, KB021, KB023, KB025, KB027, E001, E003, E007, E009, E010 and E021

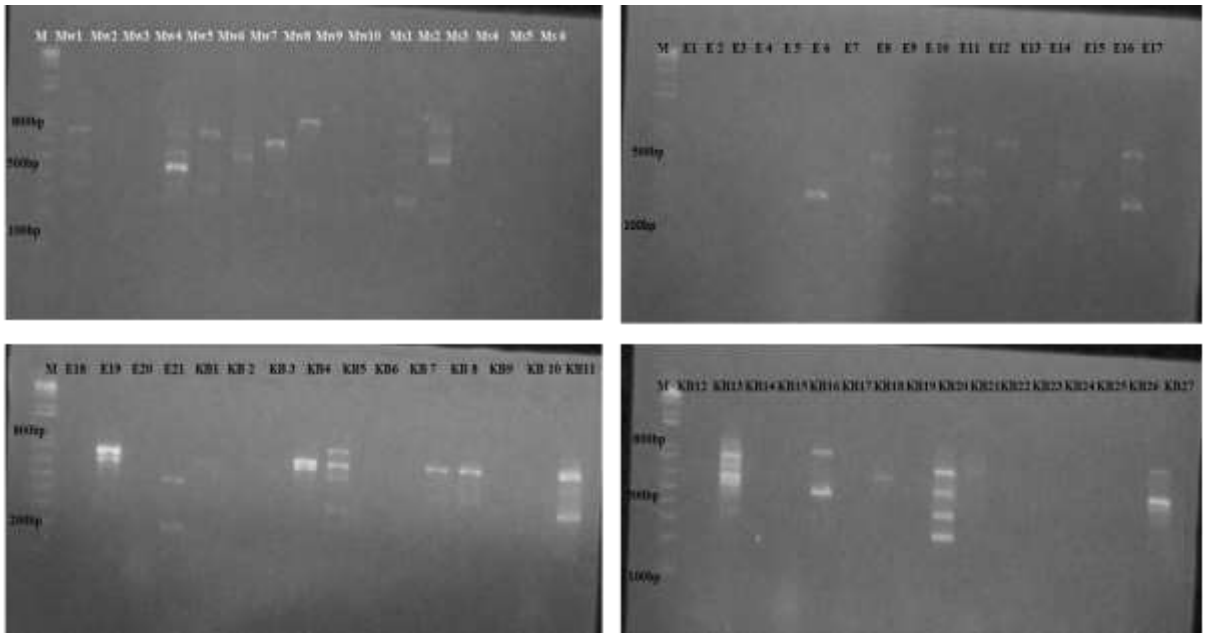


Plate 6.3: ISSR primer pattern for polymorphic primer ISSR 836 used to analyze 64 tamarind accessions in semi-arid Eastern Kenya.

ISSR 17899B produced bands that ranged from 100-1000 bp. The primer was highly polymorphic with 79 polymorphic bands. 100 bp band was observed in MW001, MW006, MW007, MW008, MW010, E001, E004, E008, E009, E012, E013 and E017. 300 bp was observed in MW006, MW007, MW008, MS003, KB022, KB023, KB024, KB025, KB026, KB027, E001, E008, E009, E012, E013 and E014. The band size of 400 was observed in MS003, KB004, KB005, KB006, KB012, KB013, KB016, E001, E008, E009, E011, E012, E013 and E014. Band size of 500 bp was present in the following accessions; MS001, KB004, KB006, KB007, KB016, E003, E008, E009 and E016. Band size of 600 bp was present in the following accessions; MS003, KB004, KB005, E001, E007, E008, E009, E013, E014 and E021. Band size of 700 was observed in MS003, KB004, KB005, KB016, E001, E002, E003, E008, E009, E014, E015, E021.800 bp band was observed in MS003, KB005, KB006, KB017, E001, E008, E009, E013, E014, E015 and E021. 900bp was present in E001, E008, E009, E014, E015,

E019, E020, E021, MS005 and MS006. 1000 bp was reported in E001, E008, E009, E021, MS001, MS002 and MS003.

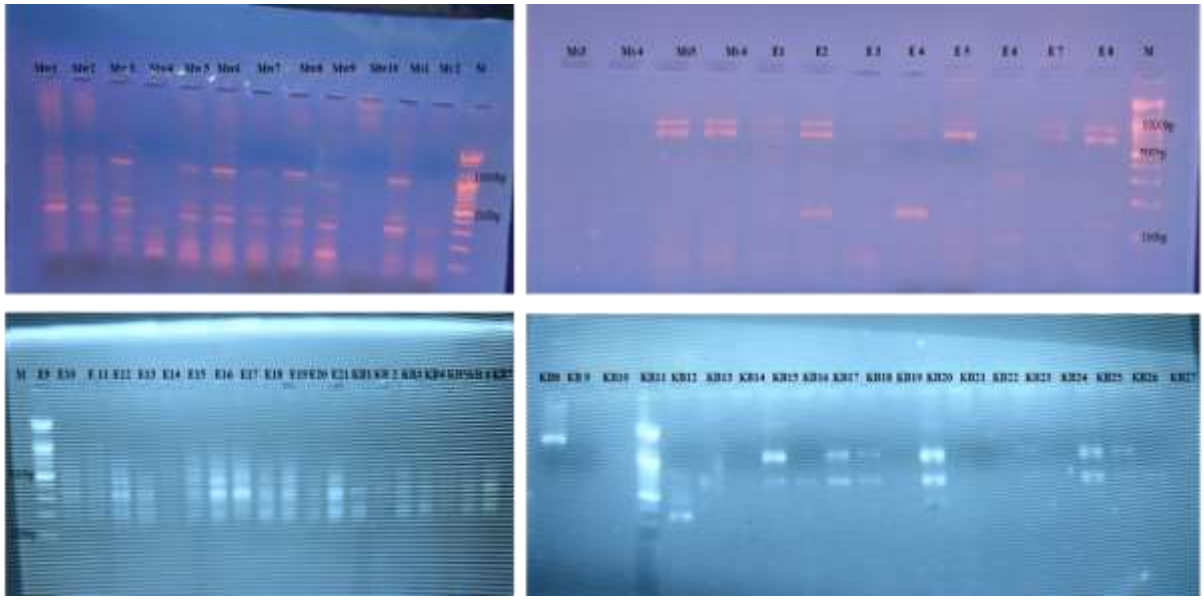


Plate 6.4: ISSR primer pattern for polymorphic primer ISSR 17899B used to analyze 64 tamarind accessions in semi-arid Eastern Kenya.

ISSR17899A produced 63 polymorphic loci. The band sizes were 100, 300, 400, 500, 600, 700 and 800 bp. Accessions that had 100 bp included MW002, MW003, MW004, MW008, MW009, KB007, KB016, E001, E008, E009, E015, E021. Accessions with 300 band size included MW004, MW005, MW006, MW007, MW010, KB011, KB012, KB015, E001, E008, E009, E011, E012, E013, E021. 400 band size was observed in KB011, E001, E009, E011, E012, E013 and E021. 500 bp was observed in MW005, MW006, MW008, KB011, E001, E003, E008, E009, E010, E011, E012 and E013. Band size of 600 was observed in KB011, KB020 E001, E003, E008, E009, E010, E011, E012, E013, E014, E015 and E016. 700 bp was observed in KB021, E001, E005, E008, E009, E011, E012, E013, E014 and E015. Band size of 800 was recorded in KB022, E001, E005, E008, E009, E012 and E013.

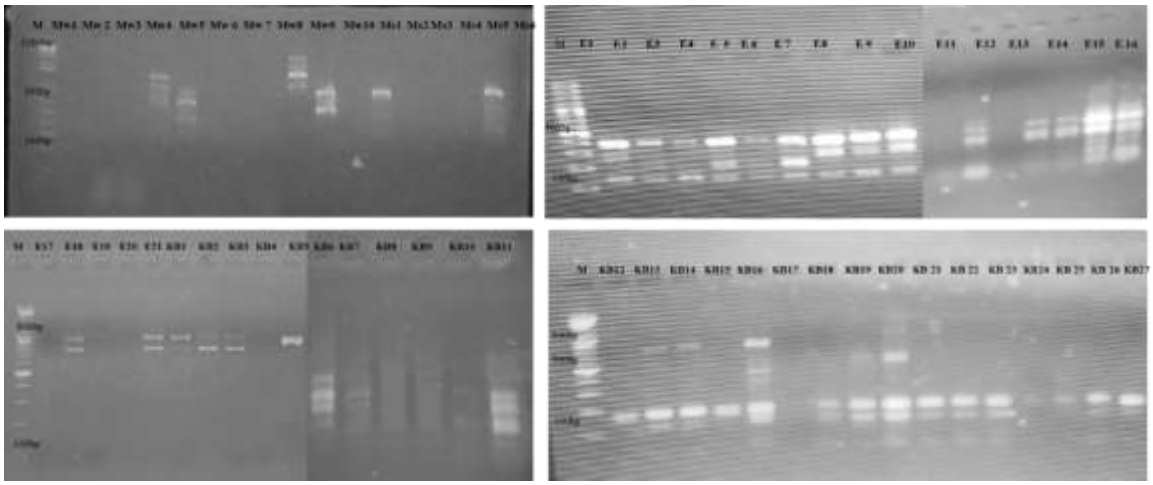


Plate 6.5: ISSR primer pattern for polymorphic primer 17899A used to analyze 64 tamarind accessions in semi-arid Eastern Kenya.

ISSR 844 produced band sizes of 200, 500, 600,700, 800 and 900.The primer had 66 polymorphic loci. Band size of 200 bp was observed in MW002, MW003, KB027, E001, E009 and E010 accessions. 500 bp band was observed in in MW002, MW003, MS005, KB007, KB016, KB020, KB022, E001, E009, E010, E012 and E015 accessions. Band size of 600 was recorded in MW002, MW003, MS003, MS004, MS005, KB008, KB016, KB020, KB023, E001, E003, E009, E010, E014 and E016 accessions. Band size of 700 bp was observed in MW001, MW002, MW003, MW004, MW008, MW009, MW010, MS001, MS003, MS004, MS005, MS006, KB001, KB002, KB004, KB005, KB006, KB010, KB011, KB012, KB013, KB014, KB015, KB016, KB017, KB020, KB021, KB024, E001, E003, E009, E010, E012 and E013.

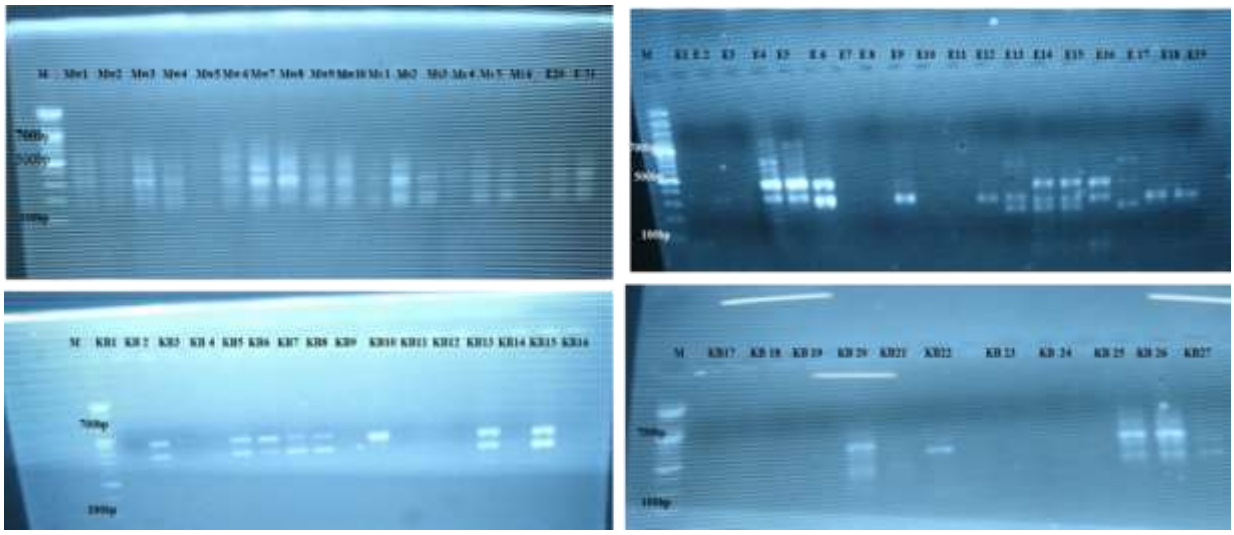


Plate 6.6: ISSR primer pattern for polymorphic primer ISSR 844 used to analyze 64 tamarind accessions in semi-arid Eastern Kenya.

ISSR 842 produced band sizes of 300, 400, 500, 600 and 700 bp with 66 polymorphic loci. 300 band size was observed in MW009, E002, E005, E007, E008, E015 and E016. 500 bp was observed in MW002, MW005, MW009 MS006,KB001, KB002, KB004, KB005, KB006, KB011, KB012, KB015, KB024, E002, E003, E007, E008, E015 and E016. Band size of 600bp was observed in MW007, MW009, MS001, KB001, KB003, KB004, KB005, KB008, KB011, KB012, KB015, KB024, E002, E007, E013 and E018. Band size of 700 bp was present in MW002, MW005, KB011, KB012, KB015, E002, E007, E013 and E014.

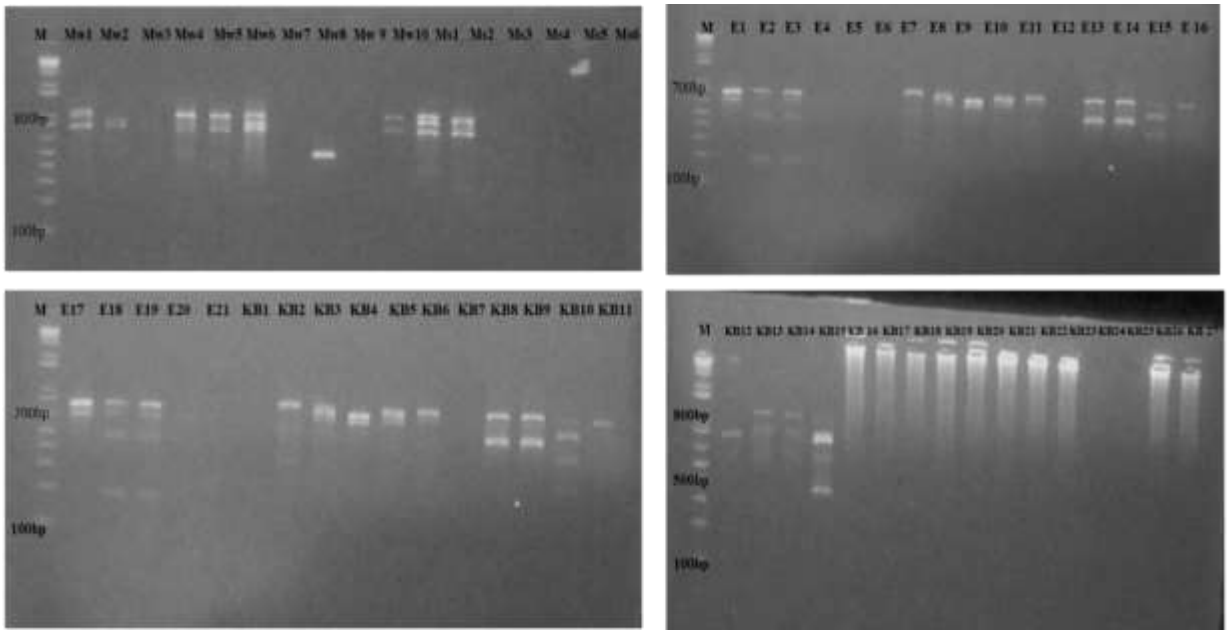


Plate 6.7: ISSR primer pattern for polymorphic primer ISSR 842 used to analyze 64 tamarind accessions in semi-arid Eastern Kenya.

ISSR HB11 produced band sizes of 100, 200, 300, 400, 500, 600,700, 800, 900 and 1000 bp with 68 polymorphic loci. Band size of 100 was present in accessions of KB020, KB021, KB026,E001, E008, E009,E011, E019 and E020. Band of 200 bp was observed in KB005, KB006, KB011, KB020, KB021, E001, E008, E009, E012 and E013. 300 bp band size was present in accessions of MW002, MW009, KB020, E001, E008, E009. 400 bp band was present in accessions of KB001, KB004, KB013, KB018, KB020, KB026, E001, E008 and E009. Band size of 500 bp was observed in MW002, MW004, MS001, MS005, KB004, KB007, KB022, E001, E005, E008 and E009. Band size of 600 b was observed in MW003, MW009, KB001, KB004, KB005, KB006, KB008, E001, E003, E008, E009, KB011, KB012 and KB013. The band size of 700 bp was present in MW002, MW008, MW009, KB009, E001, E008, E009 and E011. Band size of 800 bp was observed in MW010, MS001, MS005, E001, E003, E008, E009 and

E011. Band size of 900 bp was observed in MW009, E001,E008, E009 and E011. Band size of 1000 bp was observed in MW009, E001, E003, E008 and E009.

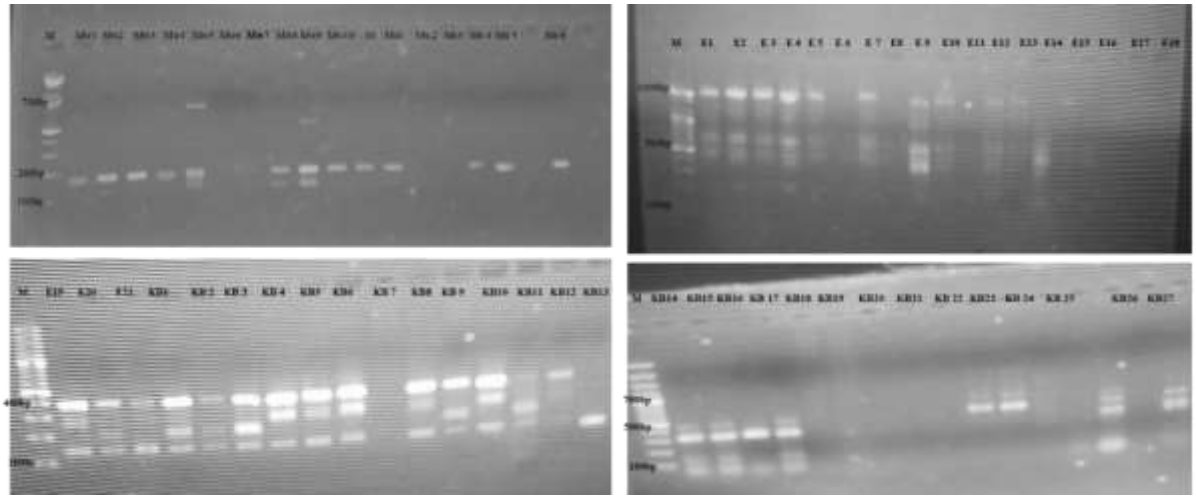


Plate 6.8: ISSR primer pattern for polymorphic primer ISSR HB11 used to analyze 64 tamarind accessions from semi-arid Eastern Kenya.

The size of bands amplified ranged from 100 bp to 1000 bps. Primer ISSR17899B and ISSRHB11 had the highest effective polymorphism of 101.43% and 87.33% respectively while ISSR807 had the least effective polymorphism (Table 6.2).

Table 6.2: Analysis of polymorphism obtained using 7 ISSR primers in 64 tamarind accessions from semi-arid Eastern Kenya

ISSR Primers	Number of amplified loci(a)	Number of polymorphic loci (b)	Effective Polymorphism %	Min band	Max band
ISSR807	43	32	40.87	200	800
ISSR836	72	61	78.33	400	800
ISSR842	78	66	84.76	300	700
ISSR844	83	57	73.24	200	900
ISSR17899A	80	63	80.92	100	800
ISSR17899B	103	79	101.46	100	1000
ISSRHB11	86	68	87.33	100	1000

The seven scorable primers resulted in 7 loci with a total of 46 alleles. The average number of alleles was 6.5 alleles per locus. The alleles ranged from 5 for 807 to 10 alleles for ISSRHB11. ISSRHB11 had the highest polymorphism of 0.89 and the highest gene diversity of 0.90. ISSR807 showed the least polymorphism of 0.73 with the least gene diversity of 0.74 (Table 6.3).

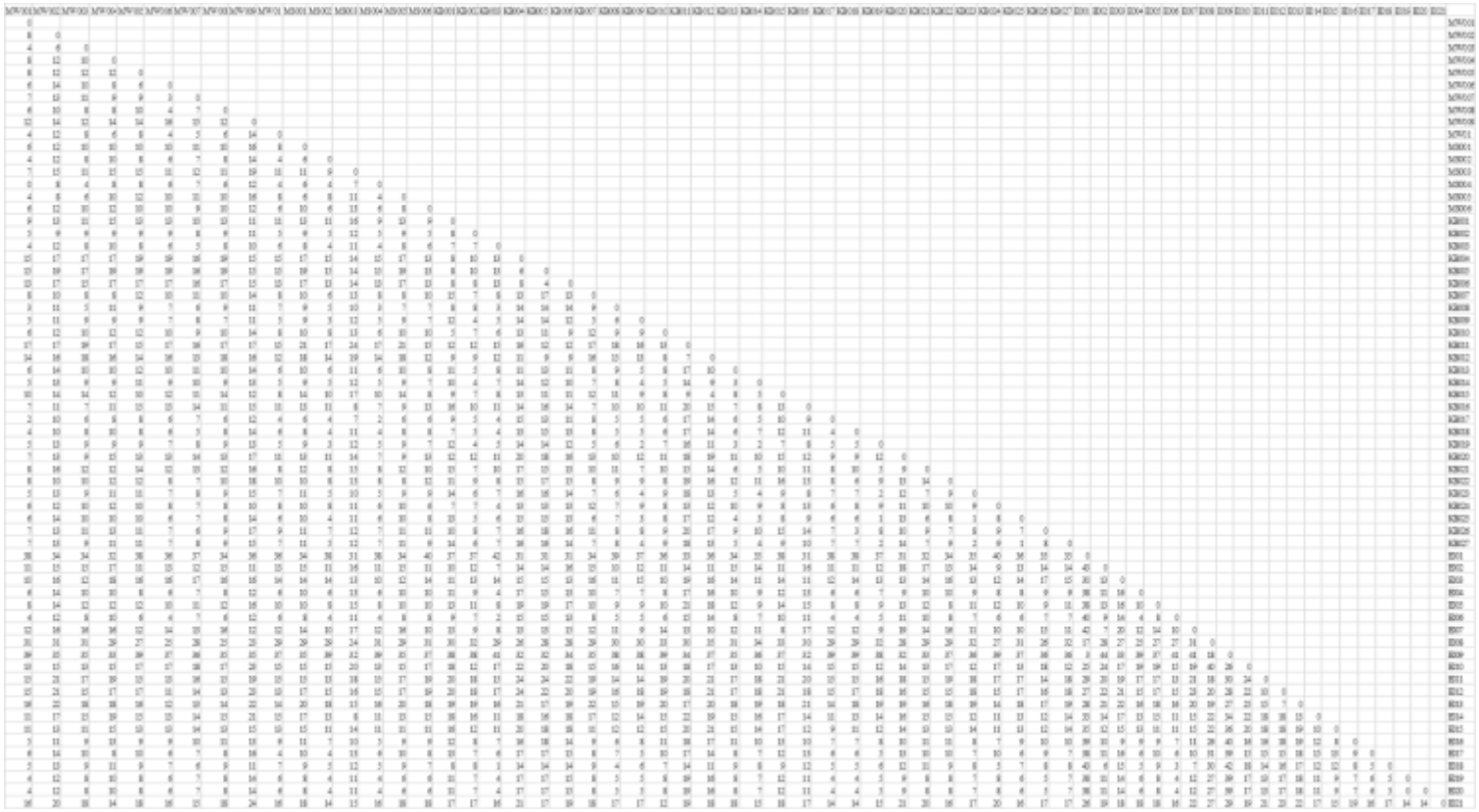
Table 6.3: Analysis of loci and the total number of allele frequencies using 7 ISSR primers from semi-arid Eastern Kenya

Loci	allele	1-D (PIC)	Hexp	Evenness
ISSR807	5	0.727	0.7452	0.8376
ISSR836	5	0.735	0.7454	0.8740
ISSR844	6	0.773	0.7814	0.8282
ISSR842	5	0.775	0.7859	0.9372
ISSR17899A	6	0.825	0.8348	0.9720
ISSR17899B	9	0.885	0.8937	0.9812
ISSRHB11	10	0.891	0.9016	0.9552
Mean	6.571	0.802	0.8126	0.9122
Total	46			

KEY: allele = Number of observed alleles, 1-D = Simpson index (Simpson, 1949), Hexp = Nei's 1978 gene diversity, Evenness of allele distribution

Genetic distance between the 64 tamarind accession ranged from 2-44. The highest genetic distance was between E009 and E002 of 44, E18 and E001 of 43, E002 and E001 of 43, E001 and KB003 of 42. The lowest genetic distance was between KB017 and MW001 of 2, KB008 and MW001 of 3, MW007 and MW006 of 3 (Table 6.4).

Table 6.4: Genetic distance among the 64 tamarind accessions from semi-arid Eastern Kenya



6.3.3 Analysis of molecular variance (AMOVA) in tamarind accessions from semi-arid Eastern Kenya

Analysis of molecular variance revealed more variation within a population than among populations. Variation within a population was 90% while among the population was 10% (Table 6.5). Principal coordinate analysis revealed that the first three components of two-dimensional PCoA contributed to 40.83% variation (Table 6.6). Accessions in populations 1, 2, 3 (Mwingi, Masinga and Kibwezi) were closely related while accessions from Embu were further apart (Fig 6.1).

Table 6.5: Analysis of molecular variance in 64 tamarind accessions from semi-arid Eastern Kenya

Source	df	SS	MS	Est. Var.	%
Among Pops	3	49.764	16.588	0.712	10%
Within Pops	60	374.846	6.247	6.247	90%
Total	63	424.609		6.959	100%

Table 6.6: Principal coordinate analysis of 64 tamarind accessions from semi-arid Eastern Kenya

Axis	1	2	3
%	21.20	11.05	8.58
Cum %	21.20	32.25	40.83

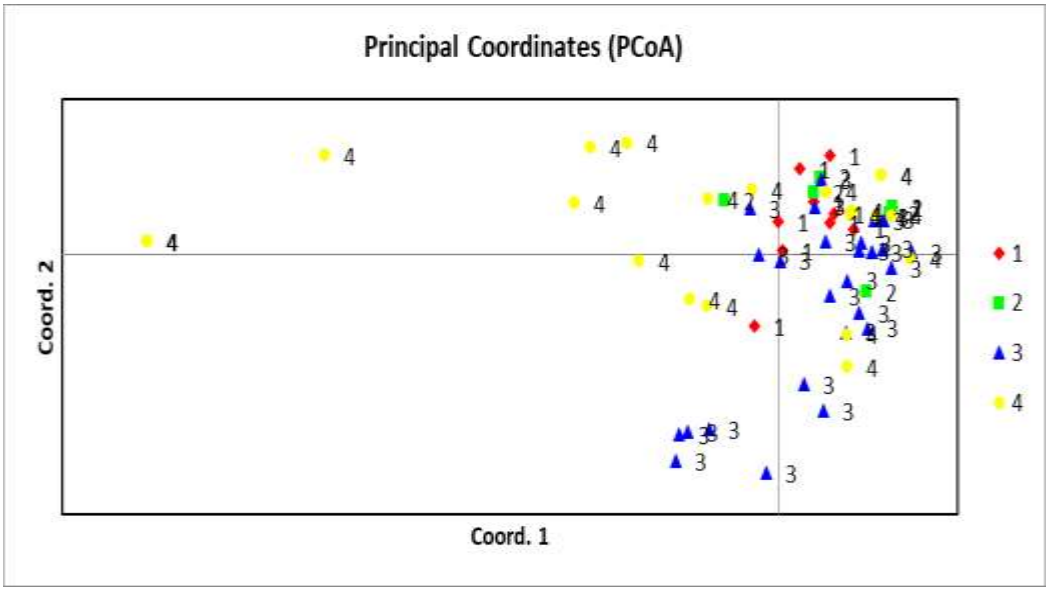


Figure 6.1: Principal coordinate analysis (PCoA) of tamarind populations from semi-arid Eastern Kenya (1-Mwingi, 2-Masinga, 3-Kibwezi, 4- Embu)

6.3.5 Cluster analysis of tamarind accessions from semi-arid Eastern Kenya

HAC clustered the 64 accessions into 7 major clusters. Cluster one comprised of accessions from Embu which included; E008, E001 and E009. Cluster two comprised of accessions from Mwingi only and one from Masinga which include; MW009, MW008, MW010, MW006, MW007, MW005, MW002, MW003 and MS004. Cluster 3 comprised of accessions from Embu and Masinga which included; E011, E012, E013, E021, E003, MS003 and E014. Cluster four comprised of accessions from Kibwezi which included; KB004, KB005, KB006, KB012, KB015, KB001, KB010, KB011, KB002 and KB007. Cluster five comprised only one accession from E010. Cluster six comprised of accessions from Kibwezi and Masinga which included; KB020, MS01, KB008, KB017, MS002, KB009, MS006, KB024, KB021, KB024, KB027, KB023, KB019, KB025, KB002, KB013 and KB014. Cluster seven comprised of accessions

from Embu and Kibwezi which included E015, E016, KB022, KB018, KB026, E005, E017, KB019, KB020, KB004, KB006, KB003 and E018.

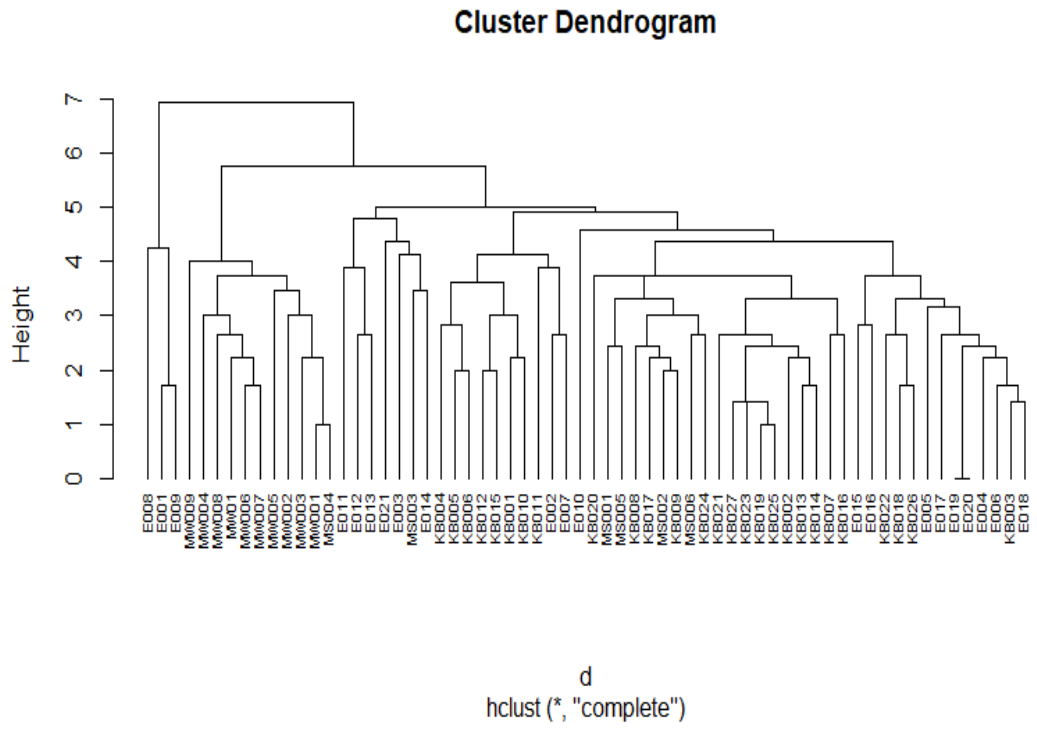


Figure 6.2: HAC dendrogram of 64 tamarind accessions from semi-arid Eastern Kenya amplified using 7 ISSR markers.

6.4 Discussion

ISSR markers were used in characterization studies and revealed genetic diversity in Opuntia (Valadez-Moctezuma *et al.*, 2014), in cucumber (Kumari *et al.*, 2016), hassawi rice (Al-Turki & Basahi, 2015) and in tamarind (Sarmiento *et al.*, 2017). Genetic variation analysis for the four populations revealed high genetic differences within populations and this was similar to the findings by (Chen *et al.*, 2014; Nilkanta *et al.*,

2017) where the authors found high differences within populations of *M. baccifera* using ISSR markers.

Self-incompatible plants had genetic differences at species level and lower differences among populations (Zawko *et al*., 2001). Genetic differences in tamarind were expected since it is a cross-pollinated plant and propagated using seeds thereby displaying significant variation within the population (Borba *et al*., 2001). The presence of pollinators promotes diversity and decreases inbreeding. Sufficient pollinators promote gene flow which in turn promotes diversity (Ksiazek-Mikenas *et al*., 2019). Plants with high geographical ranges tend to maintain high genetic diversity than geographically localized species (Wang *et al.,* 2018). Genetic diversity within a population is also influenced by population size, genetic drifts, gene flow and extended periods with a low number of individuals (Sheeja *et al*., 2009; Nilkanta *et al*., 2017)). When the population size is large the genetic variation is also high and the plants can adapt to climatic changes unlike small populations which are threatened by genetic drifts that led to inbreeding depression and loss of diversity (Soo-Rang *et al*., 2018). Extended long periods with a low number of individuals in an area can also minimize diversity.

Genetic clusters revealed more diversity compared to the morphological cluster which is in agreement with reports by Masumbuko and Bryngelsson, (2006); Gajera *et al*., (2014) whereby genetic relationship and revealed high genetic diversity among cowpeas and coffee. Very few accessions clustered based on the counties of collection but most of them clustered across the counties which was supported by the fact that the tamarind tree is self-incompatible (Govindaraj *et al*., 2015) and propagated using seeds (Hogbin & Peakall, 1999). The presence of pollinators that promote gene flow within populations, tamarind populations are still large. This clustering was contrary to reports by Wu *et al.*, (2018) who reported that plum varieties evaluated clustered based on the regions of study. Perennials are also able to maintain high levels of variation compared to annuals

and short-lived perennials (Ledig, 1986). High levels of variation were also associated with the fact that the tree was able to adapt to different environmental conditions (Algabal *et al*., 2011). Tamarind populations were genetically isolated by mutation and genetic drift that lead to differences in the allele frequencies at selectively natural loci. The least diversity was Masinga and this is attributed to habitat loss, small population, degradation, exploitation and introduction of crop plants in the region.

6.5 Conclusion

Genetic diversity was demonstrated among the tamarind accessions in semi-arid Eastern Kenya. Populations from Embu were more diverse as they clustered in 10 groups and PCoA they clustered differently from the rest while least diversity was in Masinga and Mwingi population. AMOVA revealed variation within a population as 90% while among populations to be 10%. PIC values for the 7 loci varied from 0.72-0.89 and diversity from 0.74-0.9. High polymorphism is attributed to pollinators that promote gene flow within populations and also climatic conditions of different regions. Cluster analysis 7 major and 19 subclasses with accessions from different regions grouping in different clusters. Genetic distance between the 64 tamarind accessions ranged from 2-44. The highest genetic distance was between E009 and E002 of 44, E18 and E001 of 43, E002 and E001 of 43, E001 and KB003 of 42. The lowest genetic distance was between KB017 and MW001 of 2, KB008 and MW001 of 3, MW007 and MW006 of 3.

6.6 Recommendation

High diversity in Embu can be exploited in marker-assisted breeding. High PIC produced by primer ISSR17899A and ISSRHB11 can be used to study the genes that code for important traits in tamarind.

CHAPTER SEVEN

EVALUATION OF ANTI-MICROBIAL ACTIVITY OF EXTRACTS FROM TAMARIND *(Tamarindus indica)* IN SEMI-ARID EASTERN KENYA AGAINST BACTERIA AND PLANT FUNGAL PATHOGENS.

Abstract

Natural products are alternatively used in the control of pests and diseases because they are highly available, cheap and environmentally friendly. This study aimed at evaluating the antimicrobial activity of leaf and fruit extracts from tamarind trees growing in semi-arid Eastern Kenya. The extracts were tested for their activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli, Penicillium digitatum, Colletotrichum gloeosporioides and Alternaria solani*. Fruits and leaves were sequentially extracted using methanol and water and evaporated using a rotary evaporator at 40°C. The extracts were then reconstituted using the solvent and stored at 4°C. The pathogenic bacteria were cultured on 28g/l of nutrient agar and the extract-impregnated discs were inoculated on the plates and cultured at 37°C. *Penicillium dumigitatum* was isolated from an orange fruit and cultured on 39g/l of PDA, *A. solani* was isolated from tomato fruits and cultured on 50g/l of Malt extract agar. *Colletotrichum gloeosporioides* was isolated fran avocado fruit and cultured on 65g/l of Sabouraud glucose agar. The media were supplemented with chloramphenicol 500mg/l. Pathogens were incubated at 37℃ for 96 hrs. Sub-culturing was done to obtain pure isolates of the pathogens. Data on bacteria inhibition zones were recorded after 24 hrs while data on fungal inhibition were collected after 96 hrs and analyzed using SPSS Version 12. The results of the study revealed that there was no significant difference in

inhibition between the leaf and fruit extracts. However, there was a significant inhibition difference between the five study regions and a significant difference in water and methanol extracts against *B. subtilis*. There was significant inhibition of *P. aeruginosa* in the five study regions, fruits and leaves and water and methanol. Tamarind extracts were not effective against *S. aureus* and *E. coli*. When compared to common antibiotics Ampicillin, Methanol leaf extracts from accessions KT007, E017 and E020 had a higher inhibition to *B. subtilis* and water fruit extracts from accessions E008 and E014 had a higher inhibition to *B. subtilis*. Additionally, methanol leaf extracts from accessions KT012, E001, KB008 and KB011 had higher inhibition against *P. aeruginosa* compared to Streptomycin, Kanamycin and Co-trimoxale. Water fruit extracts from the accession of KT012 had a higher inhibition of *P. aeruginosa* compared to Streptomycin, Kanamycin and Co-trimoxale. Additionally, waterleaf extracts from accessions of KT001, KB004, KB005, KB011, KB012, KB014 and KB016 had a higher inhibition of *P. aeruginosa* compared to Kanamycin, Gentamycin, Streptomycin, Ampicillin, and Co-trimoxale. There was no significant inhibition of *S. aureus* and *E. coli* by tamarind extracts. There was no significant inhibition of fungal pathogens by tamarind extracts. The results of this study revealed that Kenyan tamarind had limited potential to be used as a biological control agent against *B. subtilis* and *P. aeruginosa*. However, using tamarind leaf and fruit extracts as fungicides against *P. digitatum, A. solani* and *C. gloeosporioides* proved to be ineffective.

7.1 Introduction

Tamarind has been used for many years to control fungal and bacterial pathogens as different parts of tamarind contain different medicinal properties (Escalona Arranz *et al*., 2010). The increased antimicrobial properties of tamarind increased its ethnobotanical use in Latin America, Asia and Africa (Meléndez & Capriles, 2006). Tamarind fruit extracts are used as refrigerants in fever and as laxatives and carminatives alone or as a combination. In southeast Asia the pulp has been used to cure sore throats (Du Preez,

2003). Tamarind pulp is composed of tartaric acid, malic acid, citric acid, pectin gum, potassium bitartrate and parenchymatous fiber (Nazir *et al*., 2017).

In West Africa tamarind has been used as food and in herbal therapies (Nwodo *et al*., 2011). In Nigeria the pulp is used in the production of the local drink, preservation of food and general traditional medicine as a drug conveyor. A combination of tamarind with other herbs was reported to be effective against constipation, fever and sore throats (Abukakar *et al*., 2008).

Most rural communities worldwide depended on traditional medicines for health solutions (Nwodo *et al*., 2011), which were more effective compared to the predominant synthetic drugs popularly found in urban areas where resistance to conventional medicine was a challenge. The resistance increased research in herbal medicine (Paul Das & Banerjee, 2014). Extracts of biologically active compounds were reported to offer a new source of antibacterial and antifungals (Abukakar *et al*., 2008).

Pathogenic fungi of economic importance in horticulture include; *Anthracnose* spp, *Alternaria* spp, *Fusarium* spp, *Penicillium* spp that cause crop disease and post-harvest losses (Yang *et al*., 2017). Fungi infect the plants by killing the host, feeding on the dead materials and others colonizing the living tissue (Doehlemann *et al*., 2017). Most pathogens infect unripe fruits and the symptoms become visible as the fruit ripens due to the favorable conditions of the fruit (Buchholz *et al*., 2018). The pathogens are spread by wind, water and vectors (Narayanasamy, 2011). Control of fungal pathogens has depended on synthetic chemicals that are a threat to the environment and humans (Droby *et al*., 2009). Most pathogens have developed resistance to chemicals used in control (Hua *et al*., 2018) and this has led to resistance to chemical control has increased research towards using plant extracts that offer alternative bio-fungicide that are relatively nontoxic (Mahlo *et al*., 2016).

In the Philippines, young tamarind leaf extracts were evaluated in different solvents and they produced a strong inhibitory effect against *C. gloeosporioides*, the inhibition was higher compared to the commercial fungicide Mancozeb (Gatan & Jonnalaxer, 2013). John *et al*. (2004), evaluated the antifungal activity of tamarind extracts and the results showed that the extracts were effective against a wide range of fungal pathogen; *C. gloeosporioides, A. solani, F. solani*. The author concluded that the extracts offered a great opportunity to be used as a bio-fungicide against soil, seed and airborne phytopathogenic fungi.

Plant extracts have been used in the control of *P. digitatum* and proved to be more effective than the commercial fungicides or equal to their activity. In Nigeria, Adeola & Aworh. (2012) evaluated the activity of tamarind fruits extracted using methanol and hexane and the results showed that hexane fruit pulp extracts were effective against *Penicillium* spp. Gupta *et al*. (2014) evaluated the activity of tamarind ethanol extracts against food fungal and bacterial pathogens reported that *Penicillium spp* and *Aspergillus* were partially inhibited. Leaves, seeds and fruits of forty-five plant samples in Morocco were evaluated for antifungal activity against *P. digitatum*. Their fruits, leaves, stems and seeds were evaluated and 23 plants reported a significant antifungal activity of more than 50%. The other 22 aqueous extracts showed reduced inhibitory effect or enhanced mycelia growth (Askarne *et al.,* 2011). In Kenya tamarind is present in the arid and semi-arid areas and there is limited information on its antimicrobial activity.

7.2 Materials and methods

7.2.1 Sample preparation

Leaf and fruit samples were collected from tamarind trees as described in Chapter 3 (Section 3.2). The pods and the leaves were collected and dried under shade, the pods were dehusked and the pulp separated and the leaves pulverized and used for evaluation.

7.2.2 Extraction of anti-microbial compounds

Twenty grams of the leaves and fruit from each accession were weighed and each dissolved in 120 ml of solvent. This was extracted sequentially using methanol and water as described by Uthayarasa *et al*. (2010). The extract was dried using a rotary evaporator at 30-40°C and 0.2 gms of the extract was dissolved in 1ml of the solvent as described by Predrag *et al*., (2005) and stored at 4°C.

7.2.3 Pathogen for the study

Two gram-positive bacteria (*B. subtilis* and *S. aureus)* and two gram-negative bacteria (*E. coli* and *P. aeruginosa*) were used. The micro-organisms were collected from National Public Health laboratories then preserved in nutrients broth and stored at 4˚C and cultured on 28g/l of nutrient agar. Pathogenic fungi were isolated from several horticultural crops; oranges, tomatoes and avocados. *Penicillium digitatum* was isolated from an orange fruit. The infected orange was surface sterilized with distilled water and cultured on 39g/l of Potato dextrose agar (PDA). *Alternaria solani* was isolated from a tomato fruit and cultured on 50g/l of Malt Extract Agar (MEA). *Colletotrichum gloeosporioides* was isolated from avocado fruit and cultured on 65g/l of Sabouraud Glucose Agar (SGA). The three media: PDA, MEA and SGA were supplemented with

500mg/l of chloramphenicol. The pathogens were subsubcultured to obtain pure cultures which were identified based on morphology as described by Samson and Verga, (2007).

7.2.4 Pathogen inoculation

Disc diffusion method was used to test the antimicrobial potential of tamarind extract against the selected bacteria species as described by (Sandle, 2016). The pathogens were inoculated on nutrient agar media onto which extract impregnated discs were placed and incubated at 24°C for 48 hrs. Plant pathogens were inoculated into PDA, MAE and SGA media and incubated at 37°C for 96 hrs.

The antimicrobial potential of the tested extract was validated by measuring the magnitude of a clear zone of inhibition around the point of application of the disc with the extract. The solvents were used as negative control while streptomycin, kanamycin and co-trimoxale, tetracycline, ampicillin, gentamycin, sulfamethoxazole and benomyl were used as control antibiotics and antifungal.

7.2.5 Data collection and analysis

The experiment was done in 3 replicates in a split-block design (two main blocks of leaves and fruits, each block divided into methanol and water as solvents, then solvent tested against the seven pathogens. Data on inhibition zones were collected as the diameter of the zone in millimeters (mm) and analyzed using two –way ANOVA followed by Post Hoc Test using Chi-squares are to compare mean inhibition zones of tamarind plant part and solvent extracts. The significance level was set at $p< 0.05$. This was done by SPSS Version 12.

7.3 Results

Inhibition was observed *against B. subtilis and P. aeruginosa* while there was no activity against *E. coli, S. aureus, Penicillium digitatum, Alternaria solani and Colletotrichum gloeosporioides.*

There was a significant difference in inhibition of tamarind extracts from study regions; Kitui, Mwingi, Embu, Masinga and Kibwezi (Table 7.1). Tamarind leaf and fruit extracts were not significantly different but there was significant inhibition in water and methanol extracts against *B. subtilis* at $p<0.05$.

Table 7. 1: Inhibition of *B.subtilis* by tamarind extracts from semi-arid Eastern Kenya

Source	Wald Chi-Square	Sig.
Study regions	65.484	.000
Plant parts (leaves and fruits)	.001	.973
Extraction solvent (water and methanol)	22.456	.000

There was a significant inhibition of *P. aeruginosa* by tamarind extracts from study regions; Kitui, Mwingi, Masinga, Embu and Kibwezi extracts. Tamarind leaf and fruit extracts showed significant inhibition. The extraction solvents; water and methanol revealed significant inhibition against *P. aeruginosa* (Table 7.2).

Table 7. 2: Inhibition of *P.aeruginosa* by tamarind extracts from semi-arid Eastern Kenya.

Source	Wald Chi-Square	Sig.
Study sites	16.460	.002
Plant parts (leaves and fruits)	242.176	.000
Extraction solvents (methanol and water)	207.033	.000

7.3.1 Inhibition of *Bacillus subtilis* by tamarind extracts from semi-arid Eastern Kenya

Methanol Leaf extracts that were active against *B. subtilis* included accessions of KT001, KT002, KT004, KT007, KT011, KT012, KT015, KT018, KT020, E001, E003, E004, E005, E008, E009, E010, E012, E014, E015, E016, E017, E018, E020, E021, MW002, MW005, MW006, MW010, MS004, KB002, KB004, KB006, KB009, KB010 and KB022 (Fig 7.1 and Plate 7.1 A).

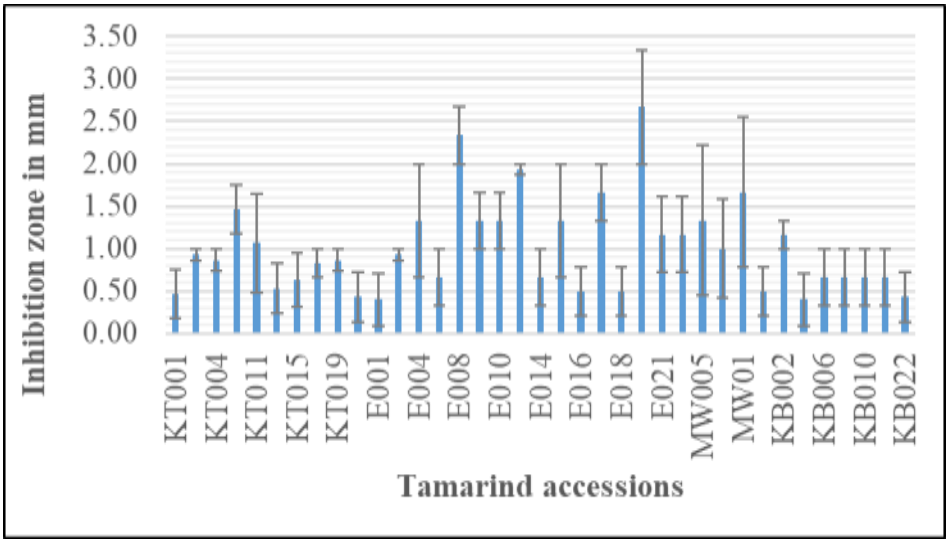


Figure 7.1: Inhibition of B. subtilis by tamarind leaves extracted using methanol

Tamarind methanol leaf extracts of accessions of KT007, E017 and E020 inhibited *B. subtilis* better than ampicillin (Table 7.3). All the other common antibiotics showed a higher inhibition activity compared to the tamarind extracts.

Table 7.3: Inhibition of *B.subtilis* with methanol leaf extracts from semi-arid Eastern Kenya compared to common antibiotics

Leaf samples	Mean (mm)	Leaf samples	Mean (mm)	Common antibiotics	Mean (mm)
KT007	1.47±0.29	E004	1.33±0.67	Gentamycin	22.67±0.67
KT011	1.07±0.58	E008	2.33±0.33	Tetracycline	22.33±0.33
MW002	1.17±0.44	E009	1.33±0.33	Ampicillin	1.33±0.33
MW005	1.33±0.88	E010	1.33±0.33	Co-trimoxale	23.67±0.88
MW006	1.00±0.58	E012	1.93±0.07	Chloramphenicol	19.33±1.33
MW01	1.67±0.88	E015	1.33±0.67	Sulfamethoxazole	2.67±0.44
KB002	1.17±0.17	E017	1.67±0.33	Streptomycin	21.67±1.20
E020	2.67±0.67	E021	1.17±0.44	Kanamycin	20.67±0.67

Tamarind methanol fruit extracts that were active against *B. subtilis* were from Kibwezi (KB001, KB002, KB003, KB004, KB005, KB006, KB007, KB008, KB009, KB011, KB012, KB013, KB014, KB015, KB016 and KB017) and Embu (E003 and E005) (Fig 7.2 and Plate 7.2 B**)**.

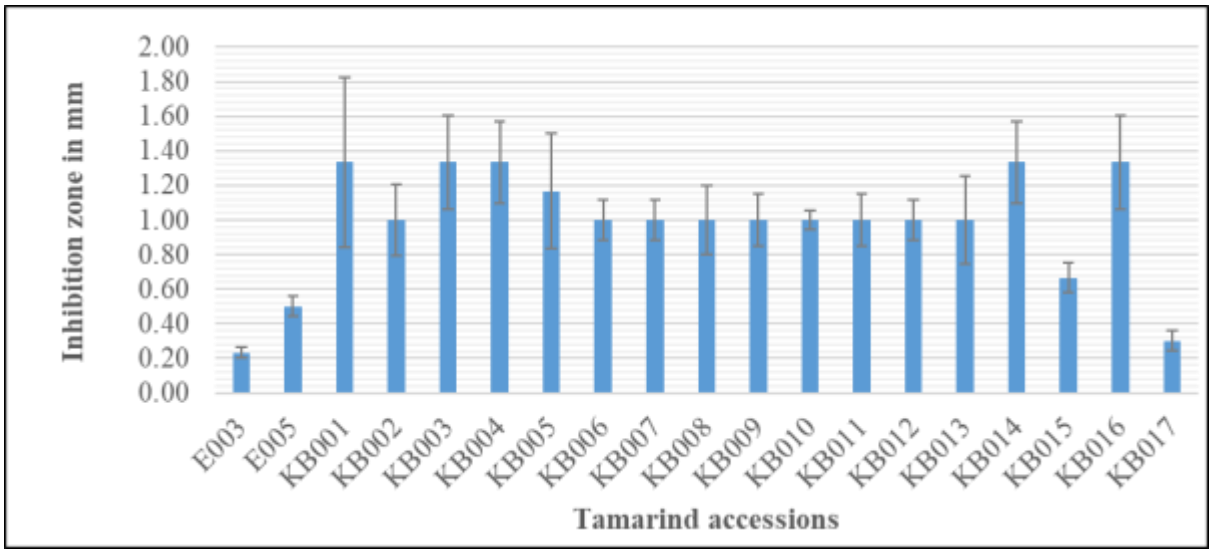


Figure 7.2: Inhibition of *B. subtilis* by tamarind fruits extracted using methanol

All fruit extracts from methanol as a solvent had inhibition zones less than the common antibiotics. Leaf extracts from methanol showed some inhibition against *B. subtilis* (Table 7.2).

Table 7. 4: Inhibition of *B. subtilis* with methanol fruit extracts from semi-arid Eastern Kenya compared to standard antibiotics.

Fruit samples	Mean (mm)	Fruit samples	Mean (mm)	Common antibiotics	Mean (mm)
E003	0.23±0.03	KB010	1.00±0.06	Gentamycin	22.67±0.67
E005	0.50±0.06	KB011	1.00±0.15	Tetracyclin	22.33±0.33
KB001	1.33±0.49	KB012	1.00±0.12	Ampicillin	1.33±0.33
KB002	1.00±0.21	KB013	1.00±0.25	Co-trimoxale	23.67±0.88
KB003	1.33±0.27	KB014	1.33±0.24	Chloromphenical	19.33±1.33
KB004	1.33±0.24	KB015	0.67±0.09	Sulfamethoxazole	2.67±0.44
KB005	1.17±0.33	KB016	1.33±0.27	Streptomycin	21.67±1.20
KB006	1.00±0.12	KB017	0.30±0.06	Kanamycin	20.67±0.67
KB007	1.00±0.12	KB009	1.00±0.15		
KB008	1.00±0.20				

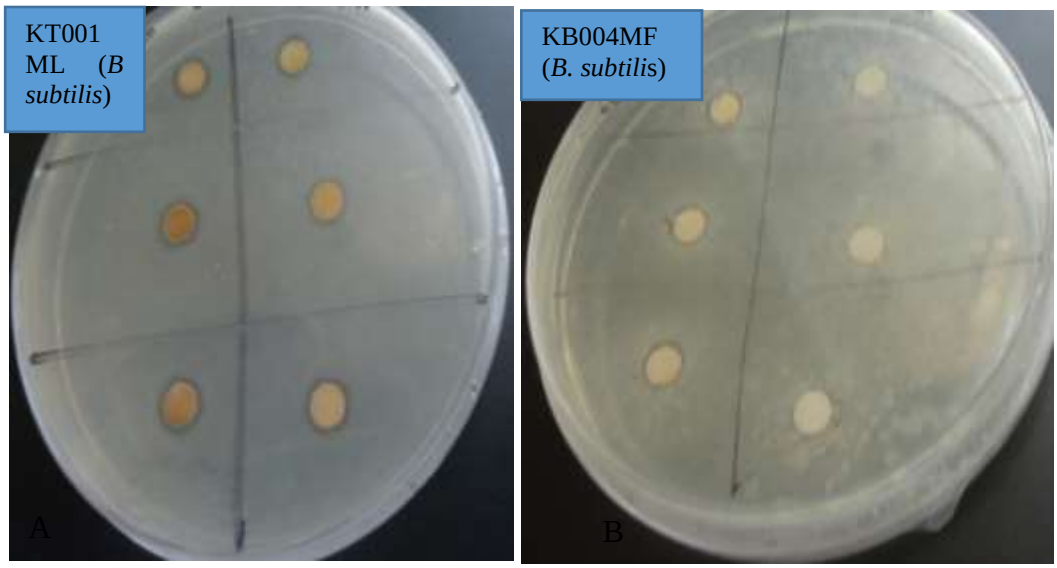


Plate 7. 1: Inhibition of *B.subtilis* by tamarind leaves (A) and fruits (B) extracted using methanol

Tamarind water leaf extracts that were active against *B. subtilis* were from Embu (E003, E005) and Kibwezi (KB001, KB002, KB003, KB004, KB005, KB006, KB007, KB008, KB009, KB011, KB012, KB013, KB014, KB015, KB016 and KB017) (Fig 7.3).

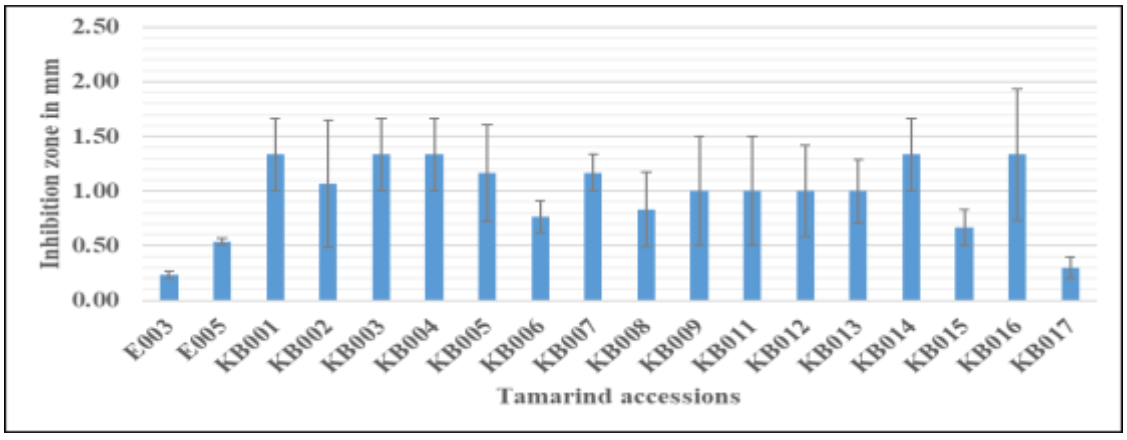


Figure 7. 3: Inhibition of *B. subtilis* by tamarind leaves extracted using water

Almost all tamarind leaves extracted using water had less inhibition zones compared to common antibiotics (Table 7.3).

Table 7. 5: Inhibition of *B.subtilis* with tamarind water leaf extracts from semi-arid Eastern Kenya compared with common antibiotics.

Inhibition zones					
Leaf samples	**Mean(mm)**	**leaf samples**	**Mean (mm)**	**Common antibiotics**	**Mean (mm)**
E003	0.23 ± 0.03	KB008	0.83±0.34	Kanamycin	20.67±0.67
E005	0.53 ± 0.03	KB009	1.00±0.5	Gentamycin	22.67±0.67
KB001	1.33±0.33	KB011	1.00±0.5	Tetracycline	22.33±0.33
KB002	1.07±.58	KB012	1.00±0.42	Ampicillin	1.33±0.33
KB003	1.33±0.33	KB013	1.00±0.29	Co-trimoxale	23.67±0.88
KB004	1.33±0.33	KB014	1.33±0.33	Chloramphenicol	19.33±1.33
KB005	1.17±0.44	KB015	0.67±0.17	Sulfamethoxazole	2.67±0.44
KB006	0.77±0.15	KB016	1.33±0.60	Streptomycin	21.67±1.20
KB007	1.17±0.17	KB017	0.30±0.10		

Tamarind fruit extracted using water that were active against *B. subtilis* were from Kitui (KT004, KT009, KT011, KT015, KT025), Embu (E003, E005, E006, E007, E008, E010, E013, E014, E017, E018, E019, E021) and Kibwezi (KB007 and KB022). Fruits extracted using water had less inhibition compared to common antibiotics except for extracts from accessions E008, E014 that performed better than ampicillin (Fig 7.4, Table 7.6 and plate 7.2)

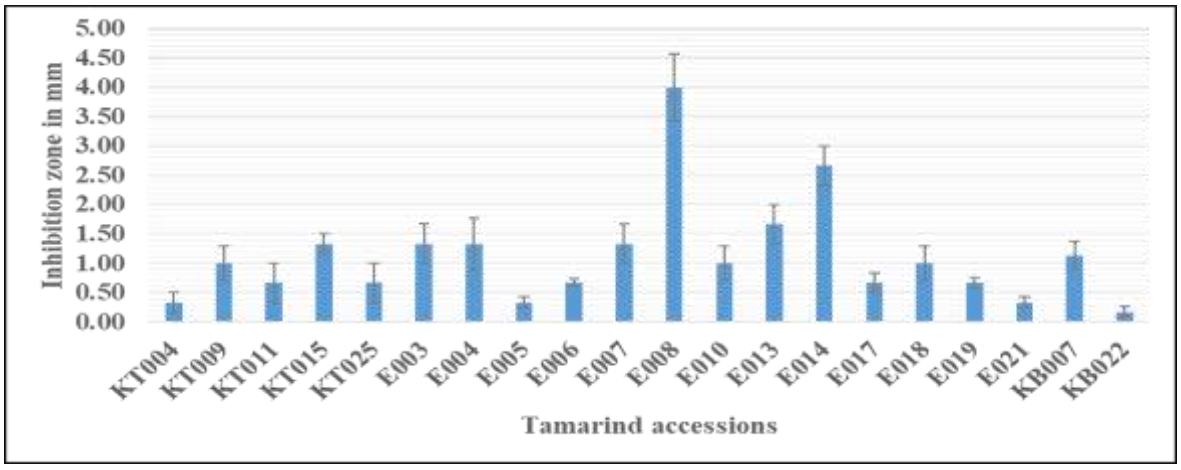


Figure 7.4: Inhibition of *B. subtilis* by tamarind fruits from semi-arid Eastern Kenya extracted using water

Table 7.6: Inhibition of *B.subtilis* with tamarind water extracts from semi-arid Eastern Kenya compared to common antibiotics

Fruit samples	Mean(mm)	Common antibiotics	Mean (mm)
KT015	1.33±0.17	Kanamycin	20.67±0.67
E003	1.33±0.33	Gentamycin	22.67±0.67
E004	1.33±0.44	Tetracycline	22.33±0.33
E007	1.33±0.33	Ampicillin	1.33±0.33
E008	4.00±0.58	Co-trimoxale	23.67±0.88
E013	1.67±0.33	Chloramphenicol	19.33±1.33
E014	2.67±0.33	Sulfamethoxazole	2.67±0.44
KB007	1.13±0.24	Streptomycin	21.67±1.20

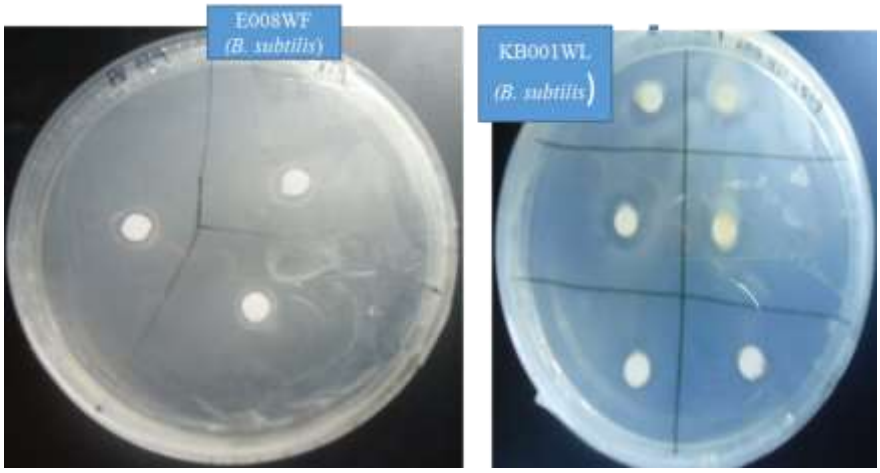


Plate 7. 2: Inhibition of *B.subtilis* by tamarind fruits (A) and leaves (B) extracted using water

7.3.2 Inhibition of *P. aeruginosa* by tamarind extracts from semi-arid Eastern Kenya

Tamarind methanol leaf extracts from Kitui (KT005, KT007, KT0012, KT013, KT016, KT022, KT023), Embu (E001, E002, E005, E006, E009, E010, E015, E016 and E020) and Kibwezi (KB008, KB011, KB016 and KB017) were active against *P. aeruginosa*. accessions (Fig 7.6**)**. Tamarind fruits extracted using methanol were not active against *P. aeruginosa*

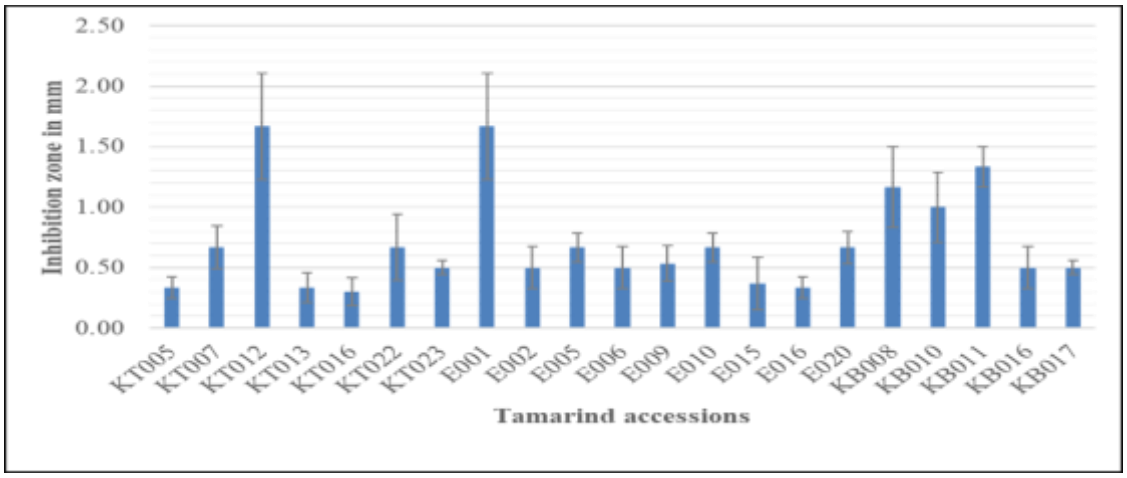


Figure 7.5: Inhibition of *P. aeruginosa* by tamarind leaves extracted using methanol from semi-arid Eastern Kenya

Methanol leaf extracts of accessions KT012, E001, KB008 and KB011 had inhibitions greater than streptomycin, kanamycin and co-trimoxale (Table 7.7)

Table 7.7: Inhibition of *P.aeruginosa* by methanol leaf extracts from semi-Eastern Kenya compared to common antibiotics

Leaf samples	Mean(mm)	Leaf samples	Mean (mm)	Common antibiotics	Mean(mm)
KT007	0.67±0.18	E009	0.53±0.15	Co-trimoxale	1.00±0.10
KT012	1.67±0.44	E010	0.67±0.12	Chloramphenicol	6.00±2.00
KT022	0.67±0.27	E020	0.67±0.13	Sulfamethoxazole	4.67±1.33
KT023	0.50±0.06	KB008	1.17±0.33	Streptomycin	1.00±0.12
E001	1.67±0.44	KB010	1.00±0.29	Kanamycin	1.00±0.23
E002	0.50±0.17	KB011	1.33±0.17	Ampicillin	1.87±0.23
E005	0.67±0.12	KB016	0.50±0.17	Gentamycin	1.67±0.33

Leaves extracted using water that were active against *P. aeruginosa* included extracts of accessions; KT001, KT002, KT003, KT004,KT005, KT008, KT009, KT011, KT012, KT013, KT014, KT015, KT016, KT017, KT018, KT019,KT020, KT022, KT023,

KT024, KT025, E001, E002, E003, E004, E005, E006, E007, E008,E009, E010, E011, E012, E013, E015, E016, E019, E020, E021, MW001, MW003, MW004, MW006, MW007, MW008, MS001, MS002, MS003, MS004, MS005, MS006, KB001, KB002, KB003, KB004, KB005, KB006, KB008, KB009 KB010, KB011,KB012, KB013, KB015, KB014, KB016, KB017, KB018, KB019, KB020, KB021, KB022, KB023,KB024, KB025, KB026 and KB0027 (Fig7.6).

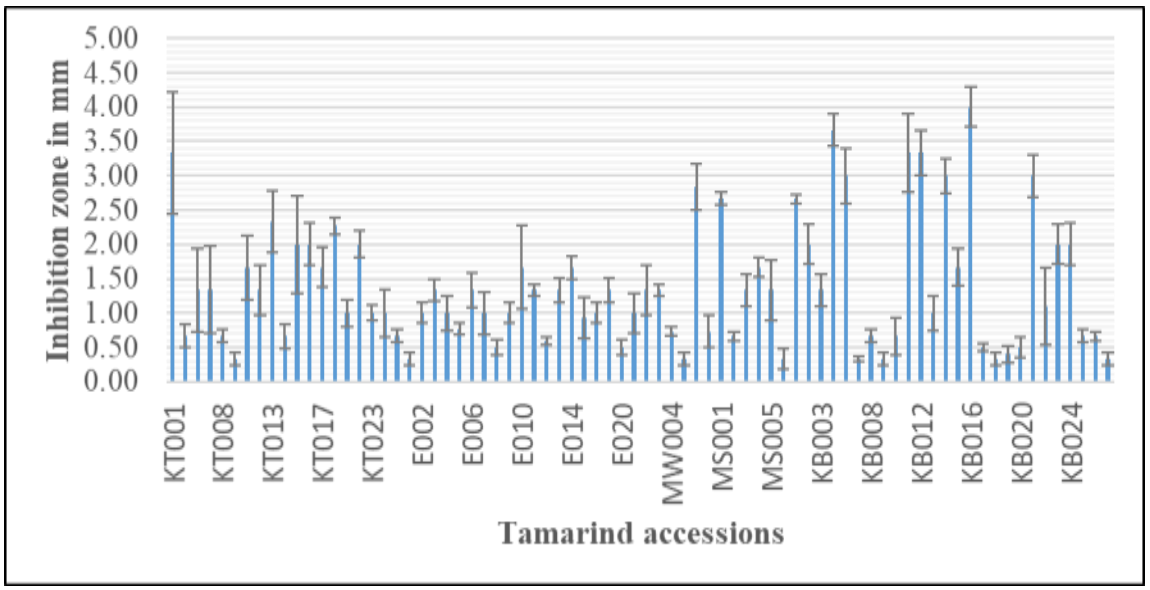


Figure 7.6: Inhibition of *P. aeruginosa* by tamarind leaves extracted using water from semi-arid Eastern Kenya

Leaf Extracts of accessions KT001, KB004, KB005, KB011, KB012, KB014 and KB016 had inhibition zones greater than kanamycin, gentamycin, streptomycin, ampicillin, and co-trimoxale (Table 7.8).

Table 7.8: Inhibition of P. aeruginosa by tamarind leaves extracted using water from semi-arid Eastern Kenya compared to common antibiotics.

Samples	Mean(mm)	Samples	Mean (mm)
KT001	3.33±0.88	Chloramphenicol	6.00±2.00
KB004	3.67±0.23	Kanamycin	1.00±0.23
KB005	3.00±0.40	Sulfamethoxazole	4.67±1.33
KB011	3.33±0.57	Gentamycin	1.67±0.33
KB012	3.33±0.33	Streptomycin	1.00±0.12
KB014	3.00±0.25	Tetracycline	20.67±0.67
KB016	4.00±0.29	Ampicillin	1.87±0.23
		Co-trimoxale	1.00±0.10

Tamarind fruits extracted using water that were active against *P. aeruginosa* were from Kitui, Embu and Kibwezi which included KT011, KT012, KT024, E004, E005, E006, E007, E011, E012, E013, E014, E015, KB010, KB017 and KB019 (Fig7.7**)**.

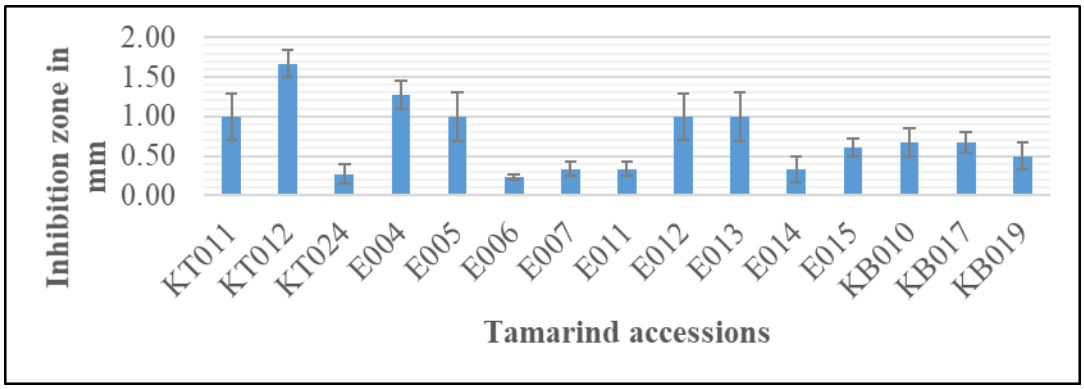


Figure 7.7: Inhibition of *P. aeruginosa* by tamarind fruits extracted using water from semi-arid Eastern Kenya.

Fruits extracted using water had less inhibition zones compared to the common antibiotics except for extract of accession KT012 that performed better than streptomycin, kanamycin and co-trimoxale (Table 7.9).

Table 7.9: Inhibition of *P.aeruginosa* by tamarind fruits extracted using water compared to common antibiotics

Fruit samples	Mean(mm)	Fruit samples	Mean(mm)	Common antibiotics	Mean(mm)
KT011	1.00±0.29	E015	0.60±0.12	Co-trimoxale	1.00±0.10
KT012	1.67±0.17	KB010	0.67±0.18	Chloramphenicol	6.00±2.00
KT024	0.27±0.12	KB017	0.67±0.13	Sulfamethoxazole	4.67±1.33
E004	1.27±0.18	KB019	0.50±0.17	Streptomycin	1.00±0.12
E005	1.00±0.31	E012	1.00±0.29	kanamycin	1.00±0.23
E006	0.23±0.03	E013	1.00±0.31	Ampicillin	1.87±0.23
E007	0.33±0.09	E014	0.33±0.17		
E011	0.33±0.09				

7.3.3 Inhibition activity of tamarind extracts from Kitui, Mwingi, Embu, Kibwezi and Masinga), solvents (Water and methanol) and plant parts (leaves and fruits)

Tamarind extracts from Embu had high inhibition while the least inhibition was from Mwingi (Fig 7.8A). Water extracts had higher inhibition than methanol (Fig 7.8B). The leaves as plant parts had higher inhibition than fruits (Fig 7.8 C). Tamarind extracts inhibited *P. aeruginosa* highly followed by *B. subtilis* and there was no inhibition of *E. coli* and *S. aureus* (Fig 7.8D).

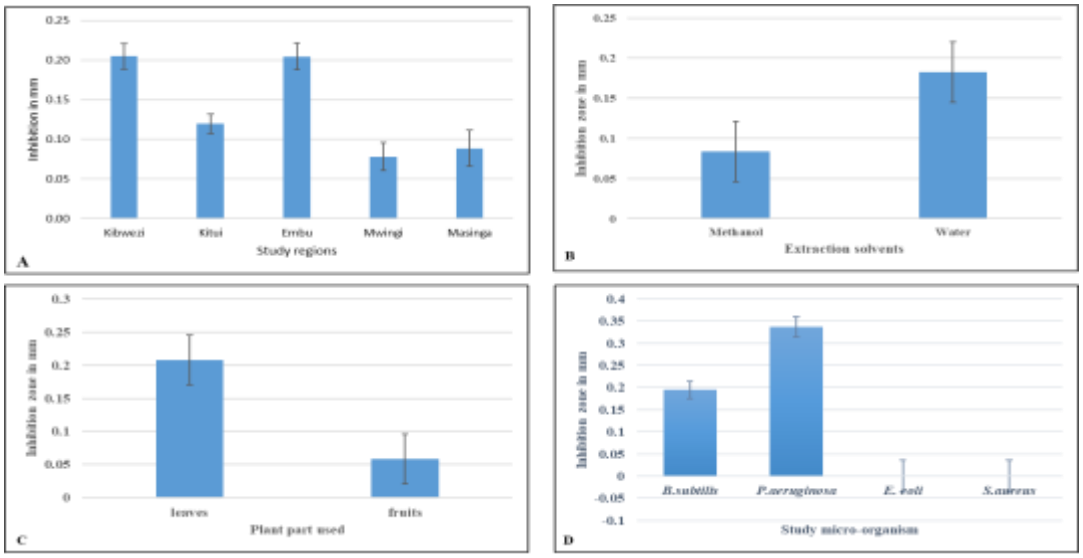


Figure 7.8: Inhibition zones of tamarind extracts: Study regions (A), extraction solvent (B), plant parts (C) and study micro-organism (D)

7.4 Discussion

7.4.1 Inhibition of bacteria (*B. subtilis, S. aureus, E. coli, P. aeruginosa*) by tamarind extracts

Plant extracts are considered active against micro-organisms when they have inhibition zones of more than 6mm (Saadabi and Ayoub, 2009). In this study, both gram-positive and negative were inhibited by the extracts but all the inhibitions were less than 6mm.

The five regions of the study had significant inhibition against *B. subtilis* and *P. aeruginosa* with Embu having the highest inhibition, followed by Kibwezi, then Kitui, Masinga and least inhibition in Mwingi which was attributed to the differences in soil types, rainfall availability, temperatures and humidity as this factors contribute greatly to the availability and different antimicrobial compounds in different plants (Yahia *et al*., 2020).

Leaf extracts had higher inhibition compared to fruit extracts which was contrary to the reports by Abdallah & Muhammad, (2018) who reported that fruits had a higher inhibition than the leaves. Similarly, reports by Nwodo *et al*. (2011) showed that fruit and bark which are storage organs had higher inhibition zones.

It was observed that fruits extracted using methanol had no inhibition while reports by Ali *et al*. (2015) indicated high inhibition in fruits extracted using methanol against *S. aureus, B. subtilis, E.coli* and *P aeruginosa*. Abdallah & Muhammad, (2018) also indicated that tamarind fruits extracted using methanol were effective against *E. coli*. Paul das and Banerjee, (2014) reported that tamarind fruits extracted using methanol were effective against *B. subtilis* with an inhibition zone of 15.6 mm which was higher compared to 1.66 mm from my study.

Aqueous fruit extracts exhibited insignificant zones of inhibition but a report by Ali *et al*. (2015) revealed there was no inhibition. Aliyu *et al*. (2017) and Compean and Ynalves, (2014) reported that aqueous fruit extracts of tamarind were active against *S .aureus* and *E.coli* which was contrary to my findings where *S. aureus* and *E. coli* were not inhibited at all. Aqueous leaf extracts were active against *B. subtilis* and *P. aeruginosa* but the findings of Ali *et al*. (2015) revealed that the extracts were inactive against all micro-organisms. Different inhibition abilities could be associated with

different compositions of antimicrobial compounds in different regions (Yahia *et al*., 2020).

Water extracts had a significant inhibition compared to methanol. This is supported by findings of the study by Hijazi *et al.* (2013) who reported that polar solvents had a higher ability to extract more compounds though this would have a combination of high numbers of impurities. This was in agreement with the findings by Esimone *et al*., (2012). Saadabi & Ayoub, (2009) also showed that water extracts inhibited seven strains of *S. aureus.*

Water and methanol solvents were able to extract compounds that were active against the microbes. In this study, water had significant inhibition compared to methanol. This finding was in agreement with Obeidat *et al*. (2012) who reported that water extracts of *A. discondis* had a high inhibition against *P. aeruginosa*. Conversely reports by Mudzengi *et al*. (2017) showed that aqueous extracts of *D. cinera, S. persica* and *C. mpone* inhibited *E. coli* higher than *S. aureus*. This could be associated with the polarity of water to extract and dissolve more antimicrobial compounds than methanol (Thouri *et al.*, 2017).

Methanol extracts had the least inhibition against the pathogens. This finding was contrary to the reports by Bacon *et al*. (2016) who revealed that most antimicrobial compounds of Japapeno were extracted using methanol had a high inhibition against the pathogens. Additionally, Alo *et al*. (2012) reported that *Ocinum gratissimum* and *Vernonia amygdalin* extracted using methanol highly inhibited *E. coli.* Experiments by Mariita *et al*. (2011) showed that methanol extracts of *T. africanum, B. angustifolia, S. multiflorus, A. nilotica* and *G. simi* had high inhibition against *S. aureus, E. coli* and *P. aeruginosa.*

Commercial antibiotics had higher inhibition than most of the extracts. These results were similar to Abdallah & Muhammad, (2018) report. Tamarind extracts hardly inhibited *E. coli* and *S. aureus* which indicates that these extracts could not be used in treating diseases caused by the two micro-organisms. Extracts of KT001, KB004, KB005, KB011, KB014, KB016, E008 and E014 could be exploited more as they were effective against *P. aeruginosa* than K, Gen, S, Amp and COT.

7.4.2 Inhibition of plant fungal pathogens (*P. digitatum, C. gloeosporioides, A. solani*) by tamarind extracts

Neither tamarind fruit nor leaf extracts showed inhibition against *P. digitatum*, similar results were observed by Raju and Naik, (2006) who evaluated the effects of pre-harvest sprays of fungicides and botanicals on storage diseases of onion. The authors observed that all concentrations of 1%, 2.5% and 5% of tamarind extracts did not inhibit the growth of mycelia. The results were closely similar to the findings by Necha *et al*. (2002), where the authors evaluated the influence of leaf, fruit and seed powders and extracts of *P. dulce* on the invitro vegetative growth of seven post-harvest fungi. The results revealed that seed powders had higher inhibition compared to fruits and leaves. Seed powders of *P. dulce* at 10mg/ml stimulated mycelial growth of *P. digitatum* when extracted sequentially using hexane, dichloromethane, acetone, methanol and water. *P. digitatum* was found to be the least sensitive fungi since it had only two fractions that were fungistic. These results contradicted the findings Tequida-Meneses *et al.* (2002). They reported that some wild plants extracted using methanol and ethanol had antifungal activity against *Penicillium spp*.

Alternaria solani was not inhibited by any of the tamarind extracts. Similar results were obtained by Necha *et al.* (2002). Their study showed that *P. dulces* seeds powder at 10mg/ml extracted using methanol and water significantly stimulated the growth of

Alternaria spp. This was contrary to Mishra *et al.* (2009) findings who reported that; bark and leaf extracts of *C. zeylanium* inhibited 100% of the spore germination of *Alternaria spp*. Methanol extracts of paper mint, ravandula and eucalyptus inhibited mycelial growth as well as spore germination of *Alternaria* spp. Cabrera *et al*. (2009) also reported contrary results from this study wherein their findings leaves of *S. officinalis* and *R. officinalis* and the seed extracts of *Salivia scolymus* had higher inhibition even compared to conventional fungicide captan and it could be used instead.

Colletotrichum gloeosporioides was not inhibited by any tamarind extracts. This was contrary to the reports by Thangavelu *et al.* (2004). The authors reported that extracts of *S. torvum* inhibited significantly the incidence of *Colletotrichtricum spp* in bananas and extended the shelf life from 15-20 days which was much better than benomyl which is the commercial fungicide. Chen & Dai, (2012) also reported that *C. camphora* extracts exhibited great inhibition against *Colletotrichum spp* in cucumber which was better compared to the commercial fungicide.

7.5 Conclusion

Tamarind extracts of KB004, KB005, KB011, KB012, KB014, KB015 E008 and E014 have antimicrobial activity against *B. subtilis* and *P. aeruginosa*. Tamarind extracts from semi-arid Eastern Kenya are not effective against *E. coli* and *S. aureus* and horticultural fungal pathogens of *A. solani, P. digitatum* and *C. gloeosporioides*

7.6 Recommendation

The activity of tamarind extracts against *B. Subtilis* and *P. aeruginosa* is important in ethnobotany. However further study is necessary to identify antimicrobial compounds in tamarind parts such as roots and bark using other extraction solvents. Additionally, testing tamarind extracts against plant bacterial pathogens is recommended.

CHAPTER EIGHT

GENERAL CONCLUSION AND RECOMMENDATIONS

8.1 Conclusions

Tamarind was not produced as a main crop in the semi-arid Eastern Kenya. It was intercropped with the other crops and majorly legumes and cereals which were considered important. Tamarind occupied less acreage of the total farms because it was not considered as the main crop. Management practices such as fertilizer application, pruning and propagation were not carried out. Tamarind was not considered as the main food, it was utilized as an ingredient in juices, sources and only considered as an important vegetable during the lean periods. Tamarind production was constrained by weevils and poor marketing channels.

There exists morphological and genetic diversity among tamarind accessions in semi-arid Eastern Kenya. Significant variation was recorded in trunk diameter at the ground, pod length, pulp color, pod color and seed shape of irregular and ovate across the counties. Tamarind accessions clustered in 3 major groups and the clusters had a variation of 66.12 within clusters and 33.18 among the clusters.

Genetic diversity was revealed among accessions from semi-arid Eastern Kenya. There was high variation within populations and least among the population. Seven major clusters revealed high genetic diversity. Furthermore, accessions from Embu were more diverse they clustered in 10 sub-clusters. Loci ISSR17899B and ISSRHB11 revealed high PIC and genetic diversity among the tamarind accessions.

Tamarind extracts of KB004, KB005, KB011, KB012, KB014, KB015, E008 and E014 showed antimicrobial activity against *B. subtilis* and *P. aeruginosa*. Tamarind extracts were not effective against *E. coli*, *S. aureus, A. solani, P. digitatum* and *C. gloeosporioides.*

8.2 Recommendations

Production data collected could be used in the improvement of tamarind yields by developing crop management practices such as vegetative propagation, pruning, pest control (tamarind weevil), and fertilization e.t.c. Utilization information could be usedsensitize the public on the importance of tamarind and develop commercialization. There is a need to develop proper marketing strategies so that the farmers can realize the full potential of tamarind.

Morphological and genetic diversity observed among the Embu accessions will be utilized in breeding programs for the improvement of tamarind using marker-assisted programs. PIC information obtained will be used in building tamarind genetic banks and breeding. Locus HB11 can be exploited further to determine the genes present in tamarind and their functions. Extracts from accessions of Kibwezi (KB004, KB005, KB011, KB012, KB014 KB015) and Embu (E008 and E014) can be explored in ethnobotany against *B. Subtilis* and *P. aeruginosa* infections. There is need to evaluate tamarind bark and bark extracts using other solvents and also evalauate the antimicrobial activity of the tamarind extracts against plant bacterial pathogens. Additionally, further work can be carried out on the evaluation of composition of tamarind seed powder.

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APPENDICES

Appendix I: Questionnaire on production and utilization of tamarind in semi-arid Eastern Kenya

AREA

County.....

Subcounty.....

Date.....

HOUSEHOLD

Farmers' name.....

Age of farmer.....Gender.....

TAMARIND PRODUCTION

Main crop grown.....

Farm production: subsistence () subsistence + market () only market ()

Nature of farm production: Intercrop () Main crop () Abandoned ()

What proportion of tamarind do you sell 0-25% () 26-50% () 51- 75% () > 76%?

FARM DESCRIPTION

Size (Acreage) of farm 0-2 () 2.1-4 () 4.1 – 8 () > 8 ()

Proportion of farm under tamarind 0-2% () 2.1-4% () 4.1-8% () >8% ()

How is land prepared: By hand () animal () power machine ()

What is the source of planting material? Seeds () others specify () .....

Source of seeds used.....

Field management e.g. Weeding (), fertilizer application () Pruning ()

How long does tamarind take to mature? .....

What are the maturity indices? ……

Number of harvests per year: ones () 1-2 () continuous ()

Yields per plant. Not sure (), <180 Kg (), 181-270Kg (), >271kg () …….....

Uses of tamarind plant.....

Uses of tamarind fruit.....

Medicinal uses of tamarind.....

Opinion on tamarind farming……

Other uses of tamarind

.....

Major constraints of tamarind production

Pests ()

Major pests that affect tamarind production.....

Major pests that affect tamarind fruits.....

Control measures of the pests……....

Disease ()

Major disease that affects tamarind production.....

Major disease that affects tamarind fruits.....

Market ()

Transport ()

Other challenges

i)

ii)

Appendix II: ANOVA table for trunk diameter at the ground variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	137256.	45752.	3.83	0.013
Residual	85	1014230.	11932.		
Total	88	1151487.			

Appendix III: ANOVA tables for trunk diameter at the neck variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	216197.	72066.	5.89	0.001
Residual	85	1039459.	12229.		
Total	88	1255656.	14269.		

Appendix IV: ANOVA table for height to the first branch variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	6046.	2015.	0.53	0.664
Residual	85	323821.	3810.		
Total	88	329867.	3748.		

Appendix V: ANOVA table for number of primary branches variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	0.6097	0.2032	1.41	0.246
Residual	85	12.2667	0.1443		
Total	88	12.8764	0.1463		

Appendix VI: ANOVA table for number of secondary branches variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	13.333	4.444	1.19	0.319
Residual	85	317.903	3.740		
Total	88	331.236	3.764		

Appendix i: ANOVA table for the number of seeds per pod variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	11.198	3.733	1.22	0.308
Residual	85	260.442	3.064		
Total	88	271.640	3.087		

AppendixVIII: ANOVA table for number of seeds per pod variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	67.233	22.411	3.12	0.030
Residual	85	611.006	7.188		
Total	88	678.239	7.707		

Appendix IX: ANOVA table for pod weight variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	71.57	23.86	0.52	0.671
Residual	85	3919.45	46.11		
Total	88	3991.02	45.35		

Appendix X: ANOVA table for pod width variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	282.5134	94.1711	127.82	<.001
Residual	85	62.6225	0.7367		
Total	88	345.1359	3.9220		

Appendix ii: ANOVA table for terminal shoot length variate

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Sample	3	1581639.	527213.	5.22	0.002
Residual	85	8577113.	100907.		
Total	88	10158753.	115440.		

Appendix XII: ANOVA table for seed weight variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	0.11545	0.03848	1.11	0.350
Residual	85	2.94816	0.03468		
Total	88	3.06360	0.03481		

Appendix XIII: ANOVA table for pulp weight variate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	3	0.4275	0.1425	1.29	0.284
Residual	85	9.3989	0.1106		
Total	88	9.8264	0.1117		

Appendix iii: Variance between morphological clusters

	Absolute	Percent
Within-class	7.258	66.12%
Between-classes	3.719	33.88%
Total	10.977	100.00%

Appendix XV: Distance between morphological clusters

	1	**2**	**3**
1	0	2.494	5.513
2	2.494	0	5.712
3	5.513	5.712	0