DISTRIBUTION, CHARACTERIZATION AND PROPAGATION METHODS FOR CONSERVATION AND UTILIZATION OF OYSTER NUTS *Telfairia pedata* IN TANZANIA

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ABSTRACT

Oyster nut (*Telfairia pedata*) is a native East African climber, growing naturally or planted in association with tall trees Tanzania (including Zanzibar island), Kenya and Uganda. Plot-less sampling method within different land use (farmlands, homesteads, and along riverbanks) was used to understand the spatial distribution of oyster nuts across elevation and rainfall gradients and a cross-sectional survey using semi-structured questionnaire was conducted on 346 respondents to assess the utilization and conservation status of oyster nuts in northern Tanzania. In addition, phylogenetic analysis were used to assess the genetic variability of oyster nuts collected from the study location and lastly developing local propagation methods for the production of oyster nut seedlings for farmers in northern Tanzania. The findings on the spatial distribution of oyster nuts indicated that more oyster nut plants grow in higher elevations and areas with higher rainfall. The findings on utilization and conservation status of the oyster nuts reveal that about 21% of respondents used oyster nuts for cooking with other staple foods while (18%) claimed that the nuts are used by pregnant and lactating mothers for medicinal and breast milk stimulation and nine percent indicated that the nuts are used for ritual and cultural devotions. Also, respondents within the 36-50 age groups reported the greatest diversity of uses of oyster nuts were (51%) compared with those aged below 36 years old (21%). The findings on genetic analysis suggest that the oyster nut (*Telfairia pedata*) is the only specie that exists in Tanzania with similarity of >99% to known accessions as deposited in the National Center for Biotechnology Information (NCBI). Although the accessions were obtained from different districts in Northern Tanzania, their close genetic similarity indicates that they might have originated from the same parents and were introduced to other sites through cultivation by human interventions. The findings on propagation methods for the production of oyster nut seedlings indicate that mother plant topsoil and Nitrogen, Phosphorus and Potassium fertilizer levels and mother plant topsoil and cow manure are the best media for the propagation of oyster nuts using stem cuttings. The study concludes that oyster nuts offers multiple benefits to farmers and is the only species that exist in Tanzania. The study recommends: (i) Promote the multipurpose and only oyster nut plant that exist in Tanzania through workshops and seminars to contribute to food security and biodiversity conservation; (ii) mother plant topsoil and NPK or cow manure are the most suitable soil mixture for raising oyster nuts from stem cuttings; and (iii) Research institutions can take an interest in both in-situ and ex-situ long-term monitoring trends of oyster nuts (*Telfairia pedata*) distribution as it is the only specie that exists in the country.

DECLARATION

I, Philipina Fenest Shayo, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this Thesis is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Philipina Fenest Shayo — Date: 14.04.2023

The above declaration is confirmed by

Prof. Anna C. Treydte — Date: 14.04.2023

Prof. Ernest R. Mbega — Date: 14.04.2023

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CERTIFICATION

The undersigned certify that they have read and hereby recommended for acceptance by the Nelson Mandela African Institution of Science and Technology a Thesis titled: *"Distribution, Characterization and Propagation Methods for Conservation and Utilization of Oyster Nuts in Tanzania"* in Fulfillment of the Award of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology (NM-AIST).

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Prof. Ernest R. Mbega Date

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This work is dedicated to my father, Mr. Fenest Mtuweta Shayo, my son Junior Roy Moshi and my brother Mr. Ludwig Fenest Shayo for their endless prayers, encouragement and support throughout the study period.

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LIST OF SYMBOLS AND ABBREVIATIONS

^{o}C	Degrees Centigrade
°F	Degrees Fahrenheit
%	Percentage
ANOVA	Analysis of Variance
AAS	Atomic Absorption Spectroscopy
BS	Base Saturation
Ca	Calcium
CEC	Cation Exchange Capacity
CIP	International Potato Center
CLC	Community Learning Center
Cm	Centimeter
CM	Cow Manure
C/N	Carbon to Nitrogen ratio
COVID-19	Coronavirus Disease
CTAB	Cetrimonium Bromide
DBH	Diameter at Breast Height
df	Degrees of Freedom
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetraacetic Acid
ESP	Exchangeable Sodium Percentage
FAO	Food and Agriculture Organization
FS	Forest Top Soil
FASTA	Format used to Specify the Reference Sequence for an Imported Genome
FTI	Forestry Training Institute-Olmotonyi
GLM	General Linear Model
GPS	Global Positioning System
H_2O	Chemical Formula for Water
IBM	International Business Machines Corporation
IDT	Integrated DNA Technologies
IPCC	Intergovernmental Panel on Climate Change
ISSR	Inter Simple Sequence Repeat
Kg	Kilogram

M.a.s.l	Height above Mean Sea Level
MEGA	Molecular Evolutionary Genetics Analysis
Mg	Magnesium
mL	Milliliter
mm	Millimeter
M	Mother Plant Topsoil
Mt	Mountain
MUSCLE	Multiple Sequence Comparison by Log-Expectation
N	Represents the population size or total number of observations
Na	Sodium
NCBI	National Center for Biotechnology Information
NJ	Neighbor-Joining
OC	Organic Carbon
NPK	Nitrogen, Phosphorus and Potassium
P	Probability
Pers. obs.	Personal Observation
pH	Potential Hydrogen
Pty	Proprietary Limited
rRNA	Ribosomal Ribonucleic Acid (rRNA)
R	The Correlation between the Predicted Values or Observed Values of the Response Variable Made by the Model
r	Correlation Coefficient
RAPD	Relative Afferent Pupillary Defect
RS	River Sand
SAS	Statistical Analysis Software
SD	Decomposed Saw Dust
Sp	Species
SPSS	Statistical Package for Social Science
TBE	Tris-Borate-EDTA
TE	Tris-EDTA Buffer
v/v	Measure of the Concentration of a Substance in a Solution
WAP	Week after Planting
w/w	Proportion of a Particular Substance within a Mixture
X^2	Chi-square

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The oyster nuts, *Telfairia pedata*, consist of the husk (11%), shell (38%) and kernel (51%) and are consumed either raw, boiled, fried, salted, roasted or grounded to thick paste to staple meals including soups (Odiaka *et al.*, 2008). The nuts are much respected by the East African natives and owing to their value from lactogenic properties that are in great demand by pregnant and lactating women. It is also usual for pregnant women to store huge amount of nuts before delivery as it is part of the traditional food breastfeeding mothers (Ajayi *et al.*, 2004). The nuts also contain carbohydrates, protein, fat, and essential amino acids (Mwakasege *et al.*, 2021). It is suggested that the nut cake, which is left after pressing the edible oil (approx. 60 % of the kernel), is also suitable as livestock feed (Ajayi *et al.*, 2004). Succinctly, oyster nuts are known to be good carbon dioxide sink with low maintenance and are for ornamental activities due to their beautiful foliage (Garrity, 2004).

Moreover, Van der Vossen and Mkamilo (2007), clarified that commonly, oyster nuts are directly sown along the drip line of large trees (Plate 1). Bamidele and Dania (2011), further asserts that domestication has been an important target for agro-ecosystem conservation since oyster nuts provide a wide range of ecological benefits as well as socio-economic benefits derived from a valuable set of products and services (Ajayi *et al.*, 2004). Even, Obute *et al.* (2001) described that *T. occidentalis* plants, which are in the same family as oyster nuts, can be used for bio-remediating heavily polluted soils.

Furthermore, oyster nuts are also valued for biodiversity conservation, livelihood improvement, and household income (particularly among women) by East African communities (Ashagidigbi *et al.*, 2018). Because of their economic and ecological value, they also safeguard and protect saplings, trees and forests in areas where the nuts are grown through host trees or wooden structures (Ajayi *et al.*, 2004). Hence, this specie has become part of the rich agroforestry systems of Mt Kilimanjaro and Arumeru in Tanzania, where it is grown in combination with banana (*Musa sp*) and coffee (*Coffea sp*) (Garrity, 2004).

Oyster nuts are usually cultivated by small holder farmers at the subsistence level to improve nutrition, household income and sustainable biodiversity conservation. Further, Fubara-Manuel *et al.* (2012) clarified that oyster nuts have been neglected as they do not fall into the large export group. Hammer *et al.* (2001), additionally describes neglected crops as crops that are

believed to be used in places where they are competitive and well-adapted, whereas underutilized crops are those that were once widely farmed and consumed but have since gone out of favor. The repercussions of the neglect of usable plant species on human nutrition may be particularly severe for the least fortunate members of society, such as children, the elderly and women, given that they are often endowed with valuable micronutrients that assist in dietary health (Tabuti *et al.*, 2004).

Regardless of these benefits, the spatial distribution, utilization and conservation status, genetic variability and propagation techniques of oyster nuts in Tanzania remain unknown.

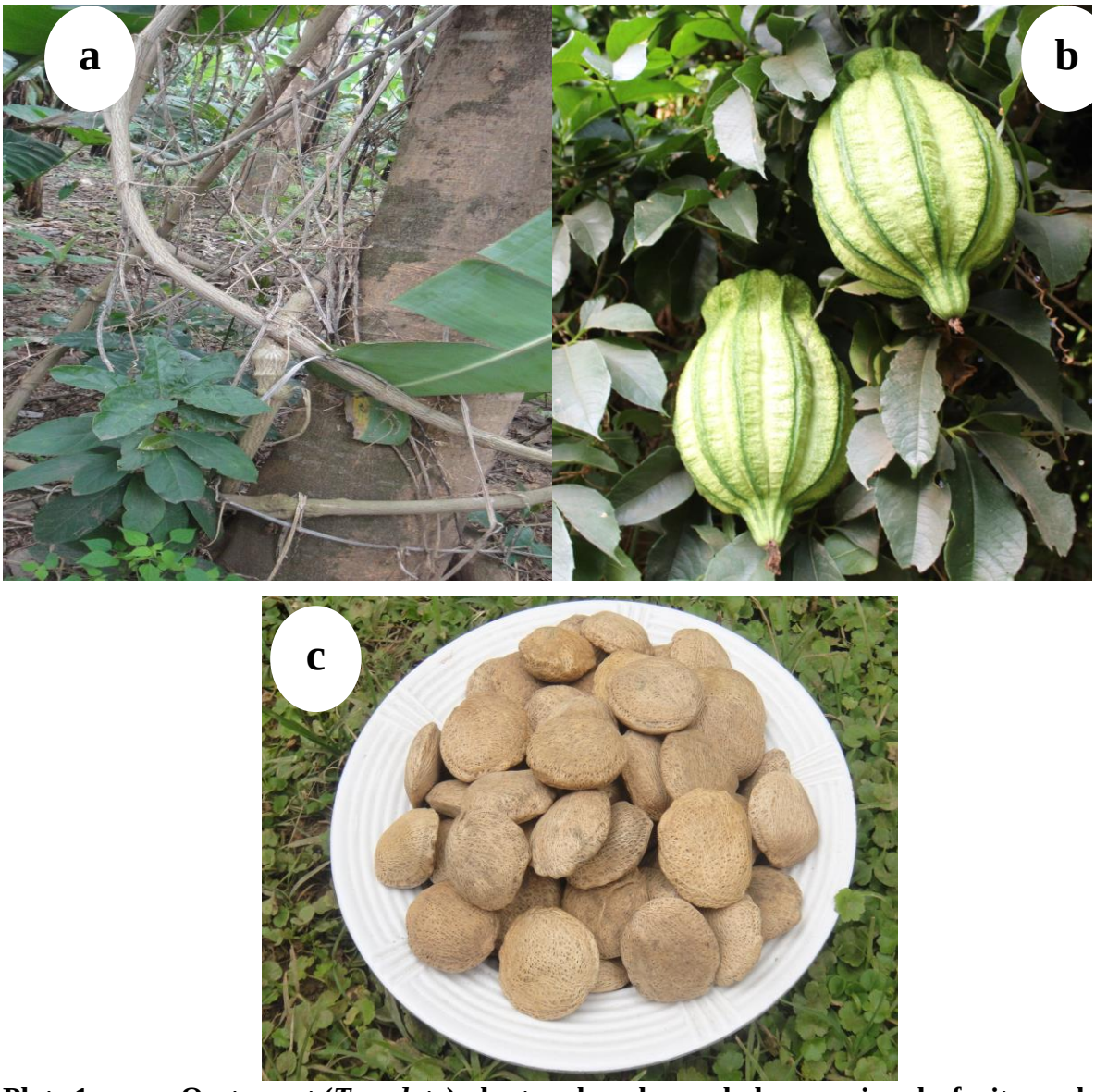

Plate 1: **Oyster nut (*T. pedata*) plant and seed morphology a: vines b: fruits, each can contain up to 200 nuts c: nuts, Pictures were captured in Northern Tanzania (March, 2020)**

1.2 Statement of the Problem

Despite the fact that Oyster nut (*Telfairia pedata*) is a multipurpose plant native to East Africa, there is scanty information on the distribution, utilization and propagation techniques of *Telfairia pedata* in Tanzania. No studies analyzed spatial distribution on *T. pedata* on elevation and rainfall gradients, assessed ethnobotanical uses and genetic variability of *T. pedata* and developed propagation methods for raising *T. pedata* from stem cuttings to the best of my knowledge that is backed by systematic review. To bridge this knowledge gap, this study generate information on oyster nut distribution and status on utilization and conservation, and developed propagation techniques to enhance its cultivation by farmers in northern Tanzania.

1.3 Rationale of the Study

The East African natives use oyster nuts for their nourishment, environment, and health which enhance the nutritional status of rural farmers as well as conserve tall trees they climb on (Ajayi *et al.,* 2007). Since, oyster nut is used as a source to achieve both food and livelihood security to farmers, appropriate propagative methods are needed for effective and efficient domestication and commercialization of the oyster nuts. Understanding the distribution and abundance of different genetic variations, conservation efforts for *T. pedata* plant can be targeted to the areas where unique or endangered genetic variations are found. Mapping the distribution and abundance of *T. pedata* across different landscape gradients provide insight into the ecological and adaptive traits that are important for the species' survival and growth in different environmental conditions. In some ecological and ethnobotanical findings from this study can enhance national food security and economic growth, which is currently reliant on only a few key plant species (Ajayi *et al.,* 2004).

1.4 Research Objectives

1.4.1 General Objective

To generate information on oyster nut distribution and status on utilization and conservation, and to develop propagation techniques to enhance its cultivation by farmers in northern Tanzania.

1.4.2 Specific Objectives

(i) To map the distribution of oyster nut populations across environmental factors (elevation, rainfall).

(ii) To determine ethnobotanical values of oyster nuts in northern Tanzania to livelihood (food, health).

(iii) To assess the genetic variability of oyster nuts collected from different areas of northern Tanzania.

(iv) To assess conservation and management strategies of oyster nuts by different ethnic communities of northern Tanzania.

(v) To develop local propagation methods for the production of oyster nut seedlings to farmers in northern Tanzania.

1.5 Research Questions

(i) What is the status and distribution of the oyster nuts population across different environmental factors (elevation and rainfall)?

(ii) Which are the ethno-botanical values of oyster nuts in northern Tanzania in relation to livelihood (food, health)?

(iii) Are there genetic differences in oyster nuts collected from different locations of study sites?

(iv) What are the management and conservation strategies of oyster nuts practiced by different ethnic communities?

(v) How do you develop improved seedlings/cuttings for farmers?

1.6 Significance of the Study

The study contributes to the understanding of oyster nut populations' status, distribution and genetic differences among the study sites. Due to the crop's cultural and socio-economic importance, different ethnic communities tend to utilize it while practicing suitable propagation methods, management and conservation strategies to ensure the species' future regeneration potential. Therefore, before the oyster nut can attain its full potential and be harvested sustainably, a deeper understanding of its local uses and population dynamics is required. Furthermore, it is important to understand how local communities choose the host hardwood tree species for oyster nut population growth, as this will have an impact on management strategies for the species' conservation and sustainable utilization.

1.7 Delineation of the Study

It has been recognized since the inception of the study that there is a socio-economic and conservation need to learn more about oyster nuts, which has been neglected and underutilized due to inadequate literature. As a result, it was important to undertake a thorough literature review inorder to solidify the study's design. Propagation methods, management and conservation measures were also unknown due to inadequate literature on oyster nuts. The genetic diversity of the collected samples was also a focus of the research. While it was clear that the interviewed farmers were eager to understand more about oyster nuts, there was a need to ensure that the information provided was accurate. As a result, the study focused on areas where oyster nuts had been identified. In obtaining the information from farmers, the study was purposively and cross-sectional conducted among oyster nut growers (both males and females) in regions of Arusha, Kilimanjaro and Tanga in Northern Tanzania where data was collected only once. Farmers were questioned using structured and semi-structured questionnaires on socio-economic and conservation status, distribution, cultural practices and utilization of oyster nuts, inorder to represent long-term observation and keep the interview focused (Fig. 1).

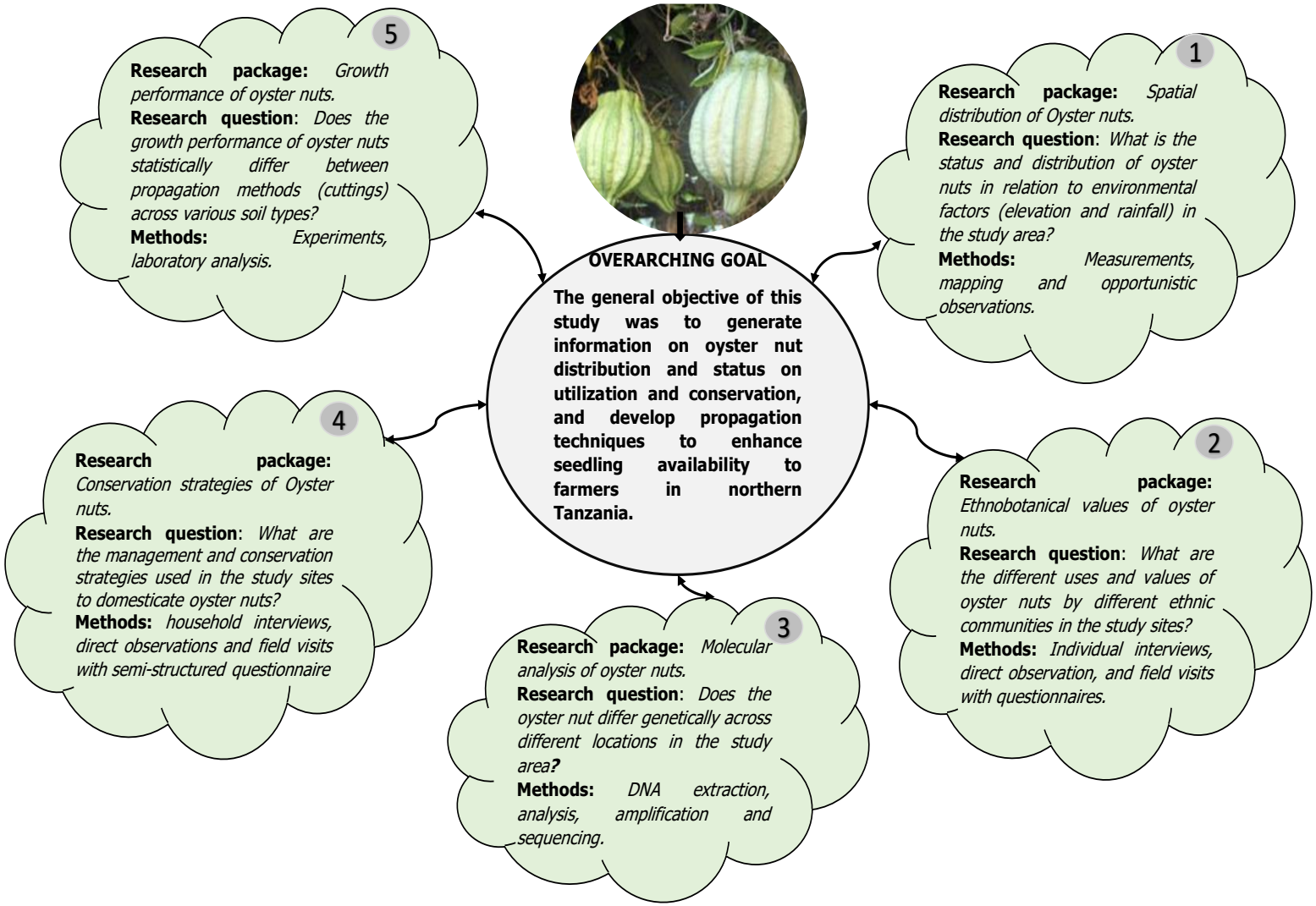

Figure 1: Graphical presentation of research on Oyster nuts (*Telfairia pedata*) in Tanzania overarching research questions and goals. The research packages represents the specific objectives and their respective questions and number

CHAPTER TWO

LITERATURE REVIEW

2.1 The Origin and Diversity of Oyster Nuts

The genus name *Telfairia* was named after Charles Telfair, an Irish surgeon, naturalist, botanist, and plant collector who lived from 1778 to 1833 (Aregheore, 2012; Eseyin *et al.,* 2014). The genus includes two other major species, *T. occidentalis* Hook. F and *T. batesii*, in addition to *T. pedata*. The former is a leafy vegetable plant that is also known as fluted pumpkin or ugu in the West African vernacular (Aregheore, 2012). Only Cameroon and Equatorial Guinea have *T. batesii* specie, which is on the verge of extinction (Schippers, 2000). *Telfairia pedata* is farmed in East Africa for its protein-rich oily nuts, while *T. occidentalis* is grown in most areas of West Africa for both its oily nuts and nutritious vegetable leaves (Okoli & Nyananyo, 1988). In Tanzania, oyster nuts are found in Arusha, Kilimanjaro, Tanga, Njombe, Mbeya, Ruvuma, Morogoro, and Zanzibar Island (Unguja and Pemba). It is also known as “mkweme”, “ngoimee” or “ikwemee”, “kirambaa”, “makunguu”, “nhahani”, “matando” and so forth. The plant is a woody vine that produces large, non-edible squash-like fruits with hefty nutritious oily nuts that take 120-180 days to mature and harvest (Kayode & Kayode, 2011). *Telfairia pedata* belongs to Cucurbitaceae family and a small genus of flowering plants which are native to Tanzania (including Zanzibar Island), northern Mozambique, and Uganda, have a fairly limited geographical distribution compared to other common oily nuts (Ajayi *et al.,* 2004; Aregheore, 2012).

2.2 Taxonomy, General Biology and Propagation Methods of Oyster Nuts

The oyster nut belongs to Kingdom: Plantae; Class: Magnoliopsida; Order: Cucurbitales; Family: Cucurbitaceae; Tribe: Joliffieae; Genus: *Telfairia* Hook; Species: *pedata*; Botanical name: *Telfairia pedata* (Smiths ex Sim) Hook; English name: Oyster nut (Onovo *et al.,* 2010; Odiyi *et al.,* 2014). The oyster nut is a dioecious (2n = 24) climber with female and male flowers borne in different plants (Akoroda *et al.,* 1990; Fayeun *et al.,* 2016). The Cucurbitaceae family has been among the first cultivated plants in the world, especially around the tropics (Odiyi *et al.,* 2014; Akwukwaegbu *et al.,* 2016). It has been an incredible plant family to humans due to its economic, medicinal, aesthetic, botanical and cultural significance as they have been related to human culture and nourishment for over 1200 years (Kocyan *et al.,* 2007; Lebeda *et al.,* 2007). Within East Africa, the plant is native to Tanzania (including Zanzibar Island) and Uganda, where it is grown for its seed oil (Agoyi *et al.,* 2019). On the other hand, *Telfairia* species were

originally wild plants, that were harvested to extinction and are now being replaced by cultivated varieties (Okoli & Mgbeogu, 1983). Whereas in Nigeria, *T. occidentalis* is cultivated in most parts of the country (Igbozulike, 2015; Sakpere *et al.*, 2019).

The oyster nut is a herbaceous woody vine and dioecious plant (female plants bear fruits) which endures squash-like fruits with huge and nutritious oily nuts (Odiyi *et al.,* 2014). Of the edible nuts, the oyster nuts or Zanzibar oil climber which are locally called *Mkweme* (in kiswahili), can be stored up to eight years and the plants have a life cycle of up to 20 years (Fayeun *et al.*, 2016). The fruits are fleshy and large, green in color, with a diameter of 20-30 cm, a length of 40 - 60 cm, usually have 10 deep ridges, hard outer cover and they are among the largest known fruits (Eseyin *et al.,* 2010; Ajuru & Nmom, 2017). They have a white-yellowish pulp, fibrous flesh with brownish seeds which are fairly flat, and their numbers are proportional to the size of the fruit although the pods and pulp are thrown away after seed removal (Essien & John, 2010). The nuts are rich in oil, fats (68%), carbohydrates (5%), protein (25%), ash (2%), polyunsaturated fatty acids mainly linoleic acid (47%), monounsaturated fatty acids, minerals including magnesium, phosphorous (Akoroda, 1990; Mwakasege *et al.,* 2021). Ibeawuchl *et al.* (2012) explained that the fruits of *Telfairia* species are set by around 20% of female flowers and male to female flower ratio is 800:1. As a result, two to three fruits are produced per plant, indicating that the pollination mechanism is ineffective. According to Akoroda (1990), this indicates that over than 800 male flowers open to a single female flower, and that male flowers open in the evening and female flowers open in the morning and late evening (they tend to alternate in flower opening), so male flowers continue to flower after female flowers have stopped and vice versa is true. Every year, a solitary plant produces 10 to 30 fruits, each containing around 70 to 180 nuts (Akoroda, 1990). Because it is a climbing vine that ascends by leaning on, entangling with, or looping around trees to give animals access from one canopy to the next, it contributes significantly to species diversity (Schnitzer & Bongers, 2002; Muoghalu & Okeesan, 2005).

The oyster nuts are also noted for being aesthetic due to their beautiful foliage, as well as being a good carbon dioxide sink that requires minimum management (Garrity, 2004). The climbers are aggressive, fast-growing plants that prefer growing in shades rather than in full sunshine. They can be supported by artificial stakes and trees by hanging trunks up to 30 meters long, as their fruits can weigh up to 15 kg. The seeds are viviparous, meaning they can germinate in the fruit, and they are naturally recalcitrant at 20% (Ajayi *et al.*, 2007). Van der Vossen and Mkamilo (2007) also described that the most effective method of propagation for oyster nuts is by seeds, which are easily collected once the fruits ripens and gradually split while pruning activities erode

the plants. However, there is scarce information about oyster nut cuttings. Further, Qadri *et al.* (2018) explains that cuttings allow for the propagation of true-to-type plants in just one growing season.

2.3 Nutritional Composition and Socio-economic Importance of Oyster Nuts

Oyster nuts are utilized for medicinal purposes, as well as for income generation and commercialization through seed sales (Msuya *et al.,* 2010; Minzangi *et al.,* 2015), and Mwakasege *et al.* (2021) stated that, oilseeds contain energy, minerals, vitamins, and antioxidants and that they can be used for medicinal and industrial purposes. It is also revealed that a third of the global population, especially women and children, are deficient in at least one critical nutrient that can be obtained from oilseeds.

Telfairia species are known to have abundant essential fatty acids and iron, making them suitable for use as cooking oil. The essential amino acid content is also high when compared to other crops including legumes (Fasuyi & Nonyerem, 2007). However, some studies have revealed that oyster nuts on the other hand, include oleic acid (11.5%), which can be used to substitute commercial sources of fatty acids, as well as omega-3, omega-6 (Mwakasege *et al.,* 2021); palmitic acid (24.5%); linoleic acid (32.5); Alpha-linoleic acid (five percent) and stearic acid (18%) (Okoli & Mgbeogu, 1983; Mwakasege *et al.,* 2021).

Furthermore, oyster nuts oyster nuts feature fibrous, leathery, and tough shells that are used as a source of fuel, and finely crushed oyster nut powder is used as a filler or extender in the production of plastics, paints, varnishes, and adhesives (Eseyin *et al.,* 2010). Additionally, the growing significance of *Telfairia* nuts and oils importance as raw materials is creating International Trade prospects for nations that grow them (Nwonuala & Obiefuna, 2015). Likewise, Christian (2006) proposed that, given the rising trend in demand for cooking oil, which has resulted in a high level of cooking oil importation, there is a need to search for more indigenous oil-yielding nuts for both industrial and consumer use. Further, Mgeni *et al.* (2019) explained that Tanzania's significant national need for edible oil necessitates importation to meet around 60% of demand and supports the use of edible oilseed crops. Whereas, the demand for imported edible oils has grown resulting in an annual outflow of nearly US$ 294 billion in foreign currency reserves. Also, *Telfairia* plant has a considerable agronomic superiority over many other plant protein sources, in addition to nutritional and economic benefits (Fasuyi & Nonyerem, 2007). Oyster nut farming is a significant economic activity and a source of livelihood for many rural populations in East Africa, in addition to its nutritional benefits and contribution to food security

2.4 Ethnobotanical and Ecological Importance of Oyster Nuts

Local communities are known to have ethnobotanical knowledge of medical, cultural, ecological, and economic benefits which is passed down through word of mouth from generation to generation (Young, 2007; Tamalene *et al.,* 2016). Ethnobotany assists in the explanation of plant preservation and utilization, hence maintaining local ecological systems and culture (Reid *et al.,* 2009). For more than 12 000 years, *Telfairia* species have been linked to human diet and culture (Okoli & Nyananyo, 1988). For health, economic, ecological, and cultural reasons, they grow close to individuals' residences, including for use in staple foods, as snacks, and by expectant and lactating women to promote milk production and recoup strength after childbirth and during lactation (Ajayi *et al.,* 2004; Mwakasege *et al.,* 2021). Also, the nuts are used in various traditional dishes including cooked banana plantain, thickening traditional soup, cooked beans, green vegetables, mixed maize and beans (*makande*), and specific meals for lactating mothers such as *kibibi* for the *Sambaa* tribe. For example women from the Tanga, Zanzibar, and Pare communities, use the thick crushed paste of pounded oyster nuts for skin protection, skin and hair cosmetics, aromatherapy, to massage girls and women (Akpabio *et al.,* 2008). Therefore, it makes a substantial contribution to human health and livelihood diversification (Fasuyi & Nonyerem, 2007).

Oyster nuts are high in protein (>25%) and oil that can be extracted (55% – 60%) (Mwakasege *et al.,* 2021). They are eaten fresh or roasted for delicacy (Musalima *et al.,* 2019) and contain a versatile, slightly sweet oil with content in the kernel (51%) that is used for cooking, cosmetics (it encourages soft and elastic skin), soap and candle making, and medication for rheumatism and stomach ailments (Aregheore, 2012). Oyster nuts also function as a novel oil that provides skin conditioning and protection against the loss of essential skin hydration (Athar & Nasir, 2005). The oyster nut fruit pulp which accounts for 64% of the total weight of the fruit, is used to feed livestock (Nwonuala & Obiefuna, 2015). Fatty acids such as palmitic acid, oleic acid, stearic acid, linoleic acid, and linolenic acid are also present (Odiaka *et al.,* 2008). They have a secondary function as a panacea (panacea of witchcraft, couth, and friendship oaths), in addition to being grown as a spice for local foods and used by lactating and pregnant mothers to increase milk flow and fast healing after giving birth and as a substitute for cooking oil (Furusawa *et al.,* 2014: Minzangi *et al.,* 2015). Also suggested that habits like respecting forest reserves and other species aid in the preservation of local biodiversity, particularly among endemic species. Even for *T. occidentalis* from the same family, can be used to treat a variety of diseases, including arthritis, high blood pressure, anemia, cardiovascular disease, inflammatory conditions, liver difficulties, and convulsions (Oluwole *et al.,* 2003).

In Sub-Saharan Africa, oyster nuts are underutilized oilseed crop (Mwakasege *et al.,* 2021) with a high yield per acre, drought resistance, and low input and maintenance requirements (Christian, 2006). In addition, the climber can fight invading undergrowth weeds by competing for resources such as nutrients, water, and light (Paul & Yavitt, 2011). The crop's capacity to fight disease is attributed to its high vitamin (A and C) content, protein content, and antibacterial and antioxidant capabilities (Kayode & Kayode, 2011; Chukwudi & Agbo, 2014). The plant is mainly propagated by seeds, which are frequently planted directly along the drip line of large host trees (Okoli & Nyananyo, 1988) on wooden structures, trellis, and as live hedges (Aregheore, 2007). The plants have robust and deep root systems, allowing them to be both a carbon dioxide sink and an aesthetic plant due to their attractive foliage. It is cultivated in combination with beans (*Phaseolus vulgaris*), banana (*Musa* sp), maize (*Zea mays*), and coffee (*Coffea* sp) plantations on Mt Kilimanjaro and Arumeru in Tanzania's rich agroforestry systems (Garrity, 2004). Kanyua (2016), explains that, in its third year, it produces up to 30 fruits, each of which contains approximately 80 – 180 nuts and has an oil content of 60 –66 (w/w). Furthermore, smallholder farmers in East Africa also cultivate oyster nut saplings to earn additional income from the selling of the seeds while growing them on forest verges to protect the trees and forests, thereby safeguarding their contribution to community livelihood and ecological benefits (Ajayi *et al.,* 2004).

2.5 Genotypic Traits for Characterization of Oyster Nuts

Morphological features like growth habit, floral morphology and leaf type have traditionally been employed to define taxa. The majority of plant taxa were classified based on their morphology which is still a poor taxonomic description in most cases. For a plant breeder, data on genetic similarities among crop cultivars is critical. Researchers can use molecular DNA techniques to detect diverse accessions for breeding purposes, identify genotypes at the taxonomic level, evaluate relative diversity within and among species, and identify genotypes at the taxonomic level. Furthermore, the commercial benefit of identifying favorable features has a direct impact on gene banks, guaranteeing the long-term preservation of the collection. Plant breeders are being pressed to increase production while maintaining biodiversity. As a result, genetic diversity is required to generate composite cultivars that can meet a variety of environmental and market demands (Chukwudi & Agbo, 2016). Plant breeding resources are the foundation of diversified, dynamic, and adaptable agriculture, and they are critical to national food security, therefore their use and conservation are critical components of any national development strategy aimed at achieving self-sufficiency (FAO, 1996). As Lewis (1985) stated that an acceptable gene resource conservation program is to genetic engineering what a library is

to information. Some of the measures used to maintain plant genetic resources both ex-situ and in vitro include botanical gardens, field gene banks, and seed storage (Ajayi *et al.,* 2004). However, over years the exploitation of genetic resources has aided in the development of agriculture, particularly in the variety of food crops including cow pea (*Vigna unguiculata*), cassava (*Manihot esculenta*), maize (*Zea mays*) and rice (*Oryza sativa*) unfortunately little effort has been made to characterize and conserve biodiversity in the *Telfairia* genus (Esuoso *et al.*, 2000).

Germplasm characterization is an important link amongst plant genetic resource utilization and conservation. The utility of PCR-based random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) variations at the genomic DNA level as phylogenetic markers for studying plant evolutionary relationships has been well established (Srivastava *et al.,* 2004). The conservation and characterization of indigenous genetic resources are critical to meet the needs of breeders for current and future generations (Thul *et al.,* 2017). When a high degree of genetic diversity is kept and made available, the chances of fulfilling future demand for genetic resources improves (Given, 1994). Hence, this is an important challenge that should not be missed, especially for the neglected and underutilized crops such as *T. pedata*. Also, their diverse agronomic and economic potential make them materials for which increased production and utilization can result in food security and income generation for farmers (Adjatin *et al.*, 2012).

However, the limiting constraint for long-term conservation and utilization of *Telfairia* species, particularly for nursery establishment, plant export, and industrialization, is the differentiation of sexes, both male and female, at an early stage of plant growth (Ajayi *et al.,* 2006). The diverse use of different parts of *Telfairia* species suggests that there is a great potential for isolating chemical compounds with significant value including pharmaceutical substances. Though such a process necessitates the characterization and isolation of plant chemical components hence it confers a high medicinal value (Akwaowo *et al.,* 2000). Characterization, on the other hand, can be defined and achieved by recording highly heritable descriptors that are easily visible to the naked eyes and can be represented in all environments (Goda *et al.,* 2007). As a result, efforts must be made to meet the need for oyster nut genetic improvement to enhance growth and seed yields, as this plant family requires special attention due to its botanical, medical, cultural, and aesthetic characteristics. Further, Adebooye and Opabode (2004) emphasized that for Africa to solve its problems, it must develop and adapt novel applications of technologies and scientific procedures, such as the use of molecular marker technologies in the assessment of marker-assisted selection, core collections germplasm development, functional genomics, gene mining, characterization and evaluation, and intra-specific diversity assessment.

Furthermore, to increase output, desirable genotypes from the existing gene pool must be selected, and superior materials must be used in vine improvement programs. However, due to lack of information about the genetic diversity of the existing germplasm, the genetic improvement of oyster nuts has been limited. As a result, oyster nut breeding will be focused on the selection of useful morphological, genotypic, and agronomic features. However, Schut *et al.* (1997) stressed that in most cases, genetic diversity in crop species should be evaluated based on differences in morphological and qualitative features.

However, there are several limitations to the use and conservation of *Telfairia* species including a limited and confined genetic base and natural diversity. A good example is the seeds of *T. occidentalis* which are generally recalcitrant and their germination capability is lost when seed moisture falls below 30% (Schippers, 2000). The best technique for preserving such challenged species is through cryopreservation, which requires a significant investment in human skills and resources to be successful (Adebooye & Opabode, 2004). Conversely, identifying and conserving the available germplasm is still critical for Tanzania's agricultural viability.

2.6 Associated Challenges Faced by Oyster Nuts Growers

There is a growing interest in neglected and underused crop species (NUS) around the world, which represents a growing trend in agriculture crops to explore and produce novel crops for domestic and export markets (Padulosi *et al.,* 2011). However, factors such as nutritional properties, taste, processing qualities, physical appearance, environmental adaptability, cultivating techniques, storability, and range of possible uses have driven the promotion of the "major crops," ensuring their acceptance by so many different cultures and success across continents. Unfortunately, oyster nuts have fallen out of favor due to their low or non-existent financial trade value, putting food and nutrition security at risk (Tabuti *et al.,* 2004). Despite their minor importance and consumption in comparison to other important oil and food crops, oyster nuts can provide a well-balanced diet, add variety to the diet, likely improve nutrition by contributing essential fats, nutrients, and minerals (Giday *et al.,* 2003) and contribute to biodiversity conservation (Ajayi *et al.,* 2007; Powell *et al.*, 2015).

Farmers in Tanzania have faced similar challenges, including low rainfall due to changing weather patterns and variability, which may be due to change of climate (Chukwudi, 2009; Padulosi *et al.,* 2011), the absence of large host trees capable of carrying the large fruit weight of oyster nuts, which can reach up to 20 kg (Akoroda, 1990) and seasonal fungal and pest attacks including *Telfairia* mosaic virus on vines, immature fruits, and flowers (Ajayi *et al.,* 2007). Subsequently, farmers tend to sell their nuts for human consumption resulting into serious seed

scarcity in subsequent planting seasons (Ajayi *et al.,* 2004). Oyster nuts must be promoted by developing initiatives and programs on food security for a diversified balanced diet, better use of marginal land and changing environments, better safeguarding of our agro-biodiversity, associated cultural heritage, contribution to agricultural diversification, an additional source of income for farmers, employment opportunities, and agricultural self-reliance systems (Padulosi *et al.,* 1999).

2.7 Emerging Challenges Related to the Environment

Climate change, water scarcity, and land degradation have sparked a surge in interest in plant species and crops that have adapted to harsh conditions (Padulosi *et al.,* 1999). Most researchers believe that the earth's average surface temperature has risen by around 0.74 degrees Celsius over the last 100 years, and that temperature changes for the next 30 to 50 years will range between 2 and 3 degrees Celsius (Ifeanyi-Obi *et al.,* 2013). Furthermore, when global temperatures rise, more alterations are anticipated to be so life-threatening that plants may struggle to adapt (Ozor *et al.,* 2010). Similarly, countries in Sub-Saharan Africa, including Tanzania are projected to suffer the most as a result of their geographic location, greater reliance on climate-sensitive renewable natural resource sectors like agriculture, low institutional capacity, and low incomes, to mention a few (Ifeanyi-Obi *et al.,* 2013). Farmers in Tanzania, for example, rely on climatic signals as the main predictor of their farming activities, making climate a crucial factor in crop production. Farmers are exposed to the effects of climate change since climatic impacts are no longer predictable as they were in the past (Apata, 2011). Droughts connected to climate change have had an impact on people and ecosystems (Msalilwa *et al.,* 2013).

In contrast to *T. occidentalis* which has been affected by climate change at all phases of production, the oyster nut is known to be drought-tolerant to an extent (Ifeanyi-Obi *et al.,* 2013). Due to the importance of oyster nuts to the local community, it is necessary to develop adaptation strategies for farmers to cope with climate change’s consequences. As a result, studies on the effect of climate change are required to create adequate recommendations for the conservation of important oily crops.

2.8 Farmers’ Practices and Conservation Strategies

Certain crops are grown all year round in many parts of Africa, although main food crops like cereal grains and tubers, especially potatoes are frequently seasonal. However, food harvested during a short period of time, such as a few weeks, must be preserved for slow consumption until

the next harvest, while seeds must be kept for the following season's crop. Furthermore, if a surplus commodity is marketable in an uncontrolled market, its value tends to rise during the off-season (Midega *et al.*, 2016). As a result, the primary goal of any storage system should be to keep the crop including oyster nuts in the best possible condition for as long as possible (Abitew *et al.*, 2020). Because most nuts such as oyster nuts are maintained in local and traditional storage facilities such as plastic containers, metal cans, and perforated bags without pesticides, *Telfairia* species conservation is based on indigenous knowledge (Odiaka *et al.*, 2008). The development of appropriate techniques and tools for effective storage facilities and methods is required to maximize the crop's potential, ensuring food security and economic gain for small-scale farmers and entrepreneurs. According to Adebooye and Opabode (2004), if current traditional postharvest methods and operations are not effectively addressed, it could result in a significant loss of *T. occidentalis* and similar related crops including oyster nuts throughout the crop production chain and storage. As a result, there is a need to develop pest management methods and techniques that are easy for small holder-farmers in Africa, keeping in mind the simultaneous imperative of ensuring food safety and security. Giving value to oyster nuts and promoting their conservation and management could aid in preserving the agroecological zones in which they thrive.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the Study Area

The socio-economic and ecological survey was conducted between September 2019 and July 2021 in the regions of Arusha, Kilimanjaro and Tanga of northern Tanzania. A total of 384 oyster nut growers (female and male) were purposely selected and surveyed in the districts of Arumeru, Same, Muheza, and Lushoto (Bumbuli inclusive) in northern Tanzania (Table 1 and Fig. 2). Table 1 Summarizes different study sites where the growth and properties of oyster nuts were assessed, in addition to their climatic data and elevations in Northern Tanzania. Population = human population numbers according to estimates of the year 2012, Rainfall = average annual rainfall range, Elevation = range of locations of oyster nut growth.

Table 1: Summary of the different study sites where the growth and properties of oyster nuts were assessed

Region	Population	Rainfall (mm)	Elevation (m. a. s.l)
Arusha (Arumeru and Meru districts)	1 694 310	800 to 1200	900 to 1600
Kilimanjaro (Same district)	1 640 087	200 to 2300	100 to 2400
Tanga (Lushoto, Bumbuli and Muheza districts)	2 045 000	1100 to 1400	700 to 1200

The research area's altitude spans from 800 to 1800 meters above mean sea level, with annual rainfall ranging from 800 to 1200 mm and mean monthly minimum and maximum temperatures are 16 and 26 degrees Celsius, respectively (URT, 2006). The main anthropogenic activities practiced in the farmlands of surveyed areas are mainly the subsistence and rain-fed farming practices growing seasonal crops such as maize (*Zea mays*), potatoes (*Solanum tuberosum*), banana (*Musa sp*), and coffee (*Coffea sp*) which are sometimes grown together with oyster nuts (Garrity, 2004).

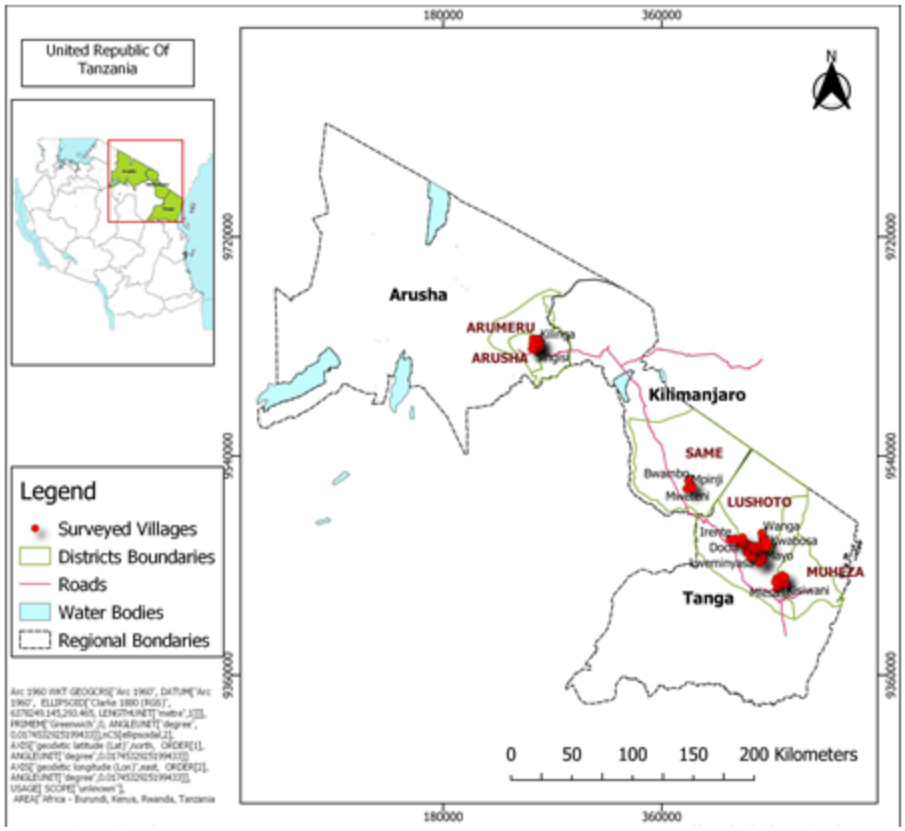

Figure 2: Map of study area indicating the geographical position of the surveyed villages, N=346, during 2019-2020 ecological survey in northern Tanzania

3.2 Research Design and Sampling Strategy

Plot-less sampling method within different land use (farmlands, homesteads, and along riverbanks) was used to understand the spatial distribution of oyster nuts across elevation and rainfall gradients. Purposive sampling was employed to obtain the districts where oyster nuts were grown from various land use categories (farmlands, homesteads and along river banks). The snowballing sampling technique was used to sample the respondents whereby the sample size of the respondents, for unknown populations (Smith, 2013), was calculated from the formula:

$$n = \frac{Z^2 Pq}{e^2}$$

Whereby: n = sample size, Z= the value on the Z table at 95% confidence level =1.96, Confidence level P = maximum variability of the population at 50% (0.5), e = sampling error at 5% and q = 1-P = 0.5.

The reason for choosing the unknown population formula is that the district consist of unknown number of farmers who grow oyster nuts. Therefore, the number of interviewees summed up to $(1.96)^2$ x 0.5 (1-0.5) $(0.05)^2$ = 384. The written informed consent was derived to eligible farmers

who signed their consent to participate or not in the study (Ryan & Silvanto, 2009). In addition, phylogenetic analysis was used to assess the genetic variability of oyster nuts collected from the study location and lastly developed local propagation methods for the production of oyster nut seedlings for farmers in northern Tanzania.

3.3 Overall Data Collection Procedure and Analysis

To acquire the data needed to respond to each specific objective, a variety of approaches were used in this research. The relevant information was collected through ecological and questionnaire surveys, laboratory analysis and open field experiment. After completing a one month reconnaissance survey in the oyster nut-growing areas of northern Tanzania, the major data was collected for two years. The main goal was to acquire fundamental data on plant parts, topographical characteristics, land-use systems, and the distribution of oyster nuts. Data collection was done based on each objective as follows:

3.3.1 Training of the Research Assistants and Pretesting of Research Tools

Research assistants who were foresters with sufficient knowledge of forests and environmental issues were instructed on the study design and how to use the research tools prior to the actual data gathering process. The training took five days and was conducted in Kiswahili. The questionnaire was then translated into Kiswahili as it was developed in English. Following the training, all trainees participated in pre-testing the tools with 20 randomly selected farmers from the Olmotonyi ward who were not part of the study, and the results were not used in the actual study. The results of the pre-test were discussed, and appropriate changes were made to improve the research instruments as needed (Appendix 1).

3.3.2 Oyster Nut Plant Collection and Measurements

During the survey, oyster nuts were measured and collected in the districts of Lushoto, Muheza, Same and Arumeru. The morphological characteristics involved the measurement of the vine to determine: (a) number of leaves; (b) vine thickness; (c) furrow distance; (d) fruit weight; (e) seed diameter (girth); (f) seed weight and (g) seed length. Seed shape and color, days to fruit maturity, flesh texture and color, and disease resistance were among the other features identified as by Levi *et al.* (2001).

Plate 2: **Measurement of tree height using Blume-leiss hypsometer (a) and taking coordinates using GPS -Garmin 64S (b)**

3.4 Mapping the Distribution of Oyster Nut Populations

Plot-less sampling method was used to quantify the abundance and assess the distribution of oyster nuts and measure all tree species associated with oyster nuts. The recorded data included GPS coordinates, the major land-use categories (farmlands, homesteads, and along the river, the vine stem diameter at breast height (1.3 m above the ground surface: hereafter DBH) was measured using calipers, fruit diameter using diameter taper and the number of fruits and seeds as well as the number of vines per tree (Plate 1). The GPS coordinates were used to extract rainfall data (https://www.worldclim.org) and elevation data (http://srtm.csi.cgiar.org) at a spatial resolution of ~1 km^2. All tree species associated with oyster nuts were identified with the help of a field guidebook (Yineger *et al.*, 2007) and farmers were interviewed to understand which tree species preferably hosted the vines.

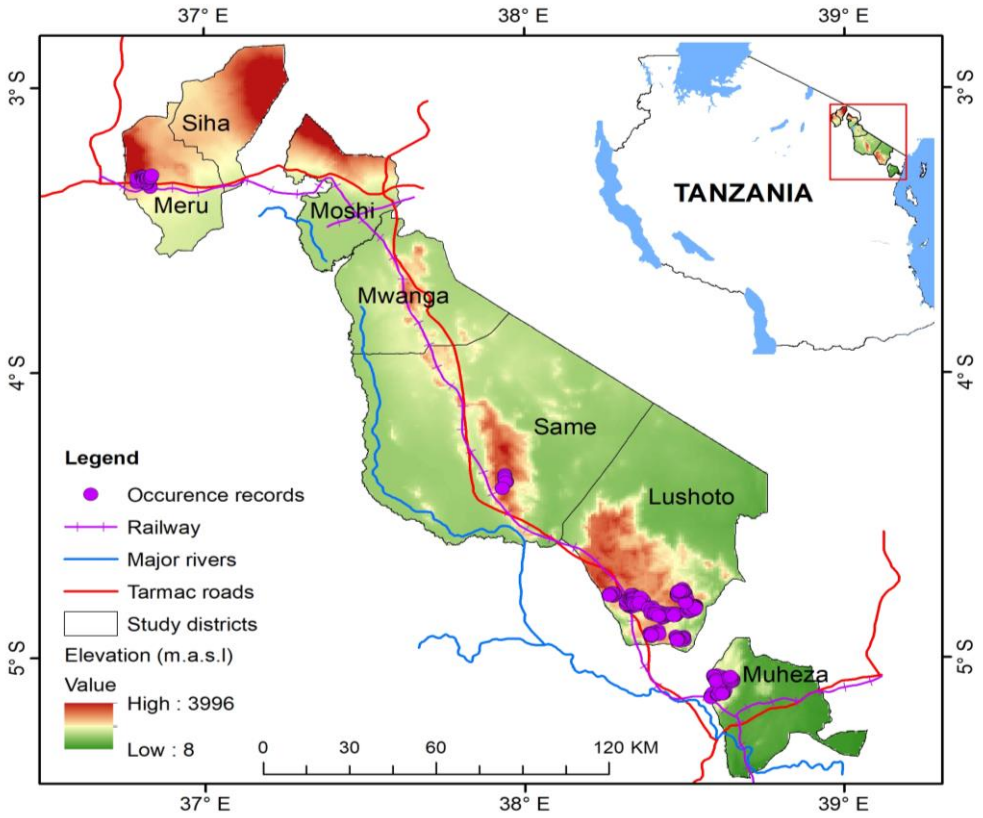

Figure 3: Location of study sites across districts in Northern Tanzania, where we assessed the use, management, climate and rainfall parameters of oyster nuts. Sites covered four districts (Arumeru, Muheza, Lushoto and Same) and were visited from September, 2019 to February 2020 (N =346)

3.4.1 Data Analysis

Pearson's correlation analysis was used to explore the relationship between environmental variables (elevation, rainfall) and vine parameters (fruit diameters, number of seeds, number of fruits and diameter of vines). Also, linear regression in R, Core Team 2020 was used to define the effects of environmental variables on fruit diameter, number of seeds, number of fruits, and

diameter of vines. Frequencies of host tree species associated with oyster nuts were descriptively analyzed and expressed as percentages. The GPS locations for each vine were overlaid with an elevation and rainfall map using ArcMap 10.6.

3.5 Determining the Ethnobotanical Values of Oyster nuts in Northern Tanzania in Relation to Livelihood (Food and Health)

Purposive sampling was used in the selection of villages where oyster nuts were found based on proximity, availability, cultural relevance and accessibility of the plants based on literature, unpublished reports and recorded conversations. A total of 346 oyster nut farmers (female and male) across 57 villages were randomly chosen for interviews. Semi-structured questionnaires and direct observations were used to collect data related to farming systems, socio-economic characteristics, seed source, and cultural practices reflecting diverse age and gender groups, consumption, postharvest handling and storage, and associated constraints in oyster nut production.

Furthermore, local names (Kiswahili and ethnic) of oyster nut plant, uses and parts of the plant, their abundance and whether they were perceived to be steady or diminishing, the reasons for such changes if known, and the types of host tree species in the area were noted. The demographic information of the respondents was also gathered through key informants and focus group discussions (Plate 3).

The informants were divided into groups based on their age, length of residence in the area (more than 20 years), gender, educational attainment, and marital status. The respondents were split into three age groups: 18–35 years old, 36–50 years old, and older than 50 years old. The respondents were selected through the snowball sampling technique based on procedures described by N'Danikou *et al.* (2015), and Adigoun-Akotegnon *et al.* (2019). The questionnaire that was used during the interview session was first run as a pilot before the final questionnaire was administered. During the surveys, the questionnaires were conducted with the assistance of three trained enumerators who were familiar with the research area, the local language could comprehend cultural norms and provide comments during the interviewing process. The majority of the farmers were chosen with the assistance of the village leaders, Agricultural Extension Officers and by *snowball* sampling approach (Loko *et al.,* 2019). When the selected farmer was unavailable, the enumerators rescheduled the interviews at their convenient time. The ward and village Administrative leaders and Agricultural Extension Officials assisted in attaining the cooperation of the respondents by assuring them that there was no harm would be done to the

community and biodiversity. All data were collected with each respondent's prior consent and trust was maintained throughout the field measurements and interview procedure.

3.5.1 Data Analysis

Statistical Package for Social Sciences (IBM SPSS ver. 20.0) was used to code, organize and analyze data from the field survey, both qualitatively and quantitatively. The analysis consisted of descriptive statistics (response frequencies, means, and percentages) and inferential statistics (Chi-square test) and the results were presented in form of graphs and tables constructed with Excel software (Microsoft office 2016).

Plate 3 (a&b): Focus group discussion (a) and key informants interviews (b)

3.6 Assessing Conservation Strategies of Oyster Nuts by Different Ethnic Communities

The field survey was conducted using a semi-structured questionnaire methodology which included household interviews, direct observations and field visits. A total of 346 farmers were interviewed by three trained enumerators who were familiar with the research area, the local language could comprehend cultural norms and provide comments during the interviewing process. The questionnaire was translated to Kiswahili and pre-tested before the actual survey.

The majority of the farmers were chosen with the assistance of the village leaders, Agricultural Extension Officers and by *snowball* sampling approach (Loko *et al.*, 2019). When the selected farmer was unavailable, the enumerators rescheduled the interviews at their convenient time. The ward and village Administrative leaders and Agricultural Extension Officials assisted in attaining the cooperation of the respondents by assuring them that there was no harm would be done to the community and biodiversity. All data were collected with each respondent's prior consent and trust was maintained throughout the field measurements and interview procedure.

3.6.1 Data Analysis

All collected data, were coded, organized, and analyzed both qualitatively and quantitatively by using Statistical Package for Social Sciences (IBM SPSS ver. 20.0). Excel software (Microsoft office 2016) was used to ascertain the relationship between the socio-demographic characteristics of the households and the differences among districts, education levels, age, and gender. Socio-demographic characteristics (age, gender, education level, size of household, land size, farming practice) of surveyed farmers, storage containers and methods (perforated plastic sacks, buckets, earthen jars, sisal sacks, tray driers, ventilated cribs, on the floor surface, under the roof) Jamovi (V. 1.2).

3.7 Molecular Identification and Diversity of Oyster Nuts in the Study Area

3.7.1 Leaf Sample Collection, DNA Preparation and Polymerase Chain Reaction (PCR) Amplification

Mature leaf samples were collected from Arusha, Tanga and Kilimanjaro regions in Northern Tanzania (Fig. 1). Silica gel was used to dry and preserve the leaf samples from rotting. The samples were transported to the NM-AIST laboratory for further processing. Before the extraction of DNA, dried leaf materials were grounded using a motor and pestle to obtain the homogenous powder. For the DNA extraction, 100 mg for each of the grounded leaf samples and 700 µL of extracted buffer (CTAB) were transfered in 2.0 mL Eppendorf tube and then mixed thouroughly by vortexing. The mixture was incubated for 30 mins in water bath at 60ºC and vigorously vortexed for every 10 mins. A homogenous mixture was cntrifuged for 5 mins at 14 000 x g. Then, 500 µL of supernatant was transfered to a new tube. To each of the transfered supernatant, 5 µL of Rnase A soultion was added and the mixture incubated at 37ºC for 20 mins.

The DNA purification was carried out by adding 500 µL of Chloroform: Isoamyl (24:1, v/v) to each 500 µL sample. The mixture was vigourously vortexed until the color turned homogenous light-green. The mixture was then centrifuged for 20 mins at 12 500 rpm. Then, 500 µL of the

supernatant was transferred from each sample to a new 1.5 mL Eppendorf tube, and 500 µL of cold isopropanol was added and mixed gently by inversion, then the mixture was incubated for 20 mins at -20 ºC and centrifuged for 15 mins at 12 500 rpm. The supernatant was poured and the pellet was washed by adding 500 µL of 70% ethanol followed by centrifugation for 10 mins at 12 500 rpm. The supernatant was poured and, the pellet was left long enough to dry the alcohol while monitoring to avoid complete drying of pellet. The dry pellet was suspended in 100 µL of TE buffer (pH 8.0) and then incubated at 37ºC in a water bath for one hour, in order to dissolve. The extracted DNA was stored at -20ºC.

The concentration and purity of DNA were checked using the Nano-Drop Lite Spectrophotometer. The A260/A280 ratio less than 1.8 suggested phenol or protein contamination while A260/A280 ratio to 1.8 – 2.0 corresponded to pure double-stranded DNA (Plate 4). The quality and quantity of genomic DNA bands was determined by running an electrophoresis on 0.8% agarose gel at 50V for one hour in 0.5X Tris-borate – Ethylenediaminetetraacetic acid - EDTA (TBE). The bands were viewed using a UV trans-illuminator (Cole-Parmer).

Plate 4: **Pipetting extracted DNA into Eppendorf tube (a) and setting PCR gel analysis (b) Oyster nut DNA labelled samples (c) and oyster nut colored samples ready for PCR analysis (d)**

3.7.2 Polymerase Chain Reaction (PCR) and DNA Sequencing

The targeted gene for PCR amplification and DNA sequencing was the 16S rRNA. The PCRS amplifications of 16S rRNA was accomplished using the two primer set, 5'-ATT TAT GCG TTG GAG AGA CCG- 3' 600F and 5'-CAA TAA CRG CAG GCA TYG CAC GRT-3' 800R; 5'-TAT ACA CCG GAG CTC YTT C-3', 384F and 5'-GTT TCT ATT GGT GYA AAT CC-3 717R. The PCR mixture were done by mixing readymade master mix (One Taq Quick-load 2X master mix with standard buffer), primers (forward and reverse). The DNA template and PCR grade water to make up to volume. The amplifications were done using C1000 touch^{TM} Thermal Cycler, BIORAD to the 30 samples. The amplification reaction was carried out using the protocol of initial denaturation step at 94°C for 3 min, 30 cycles comprising denaturation at

94°C for 30s, optimum annealing temperature for particular ISSR primer for 45s, and extension at 68°C for 1 min and a final extension step at 68°C for 5 min. The RAPD and ISSR amplified products were electrophoresed in 1.0% agarose in 0.5×TBE buffer (Plate 5). The amplified 27 samples were sent for sequencing at the Inqaba biotechnological industries (Pty) Ltd, Pretoria, Republic of South Africa (Plate 5). The Purification and sequencing of PCR amplicons were done by Inqaba biotechnological industries (Pty) Ltd, Pretoria. Aliquots of successful purified PCR products as revealed by agarose gel electrophoresis were pipetted into 10 µL Epperndoff tubes and submitted to the Sequencing Unit, Inqaba biotechnological industries (Pty) Ltd, Pretoria, South Africa, capillary sequencing on a 3 730 xL DNA Analyzer (Thermo Fisher Scientific Inc. USA).

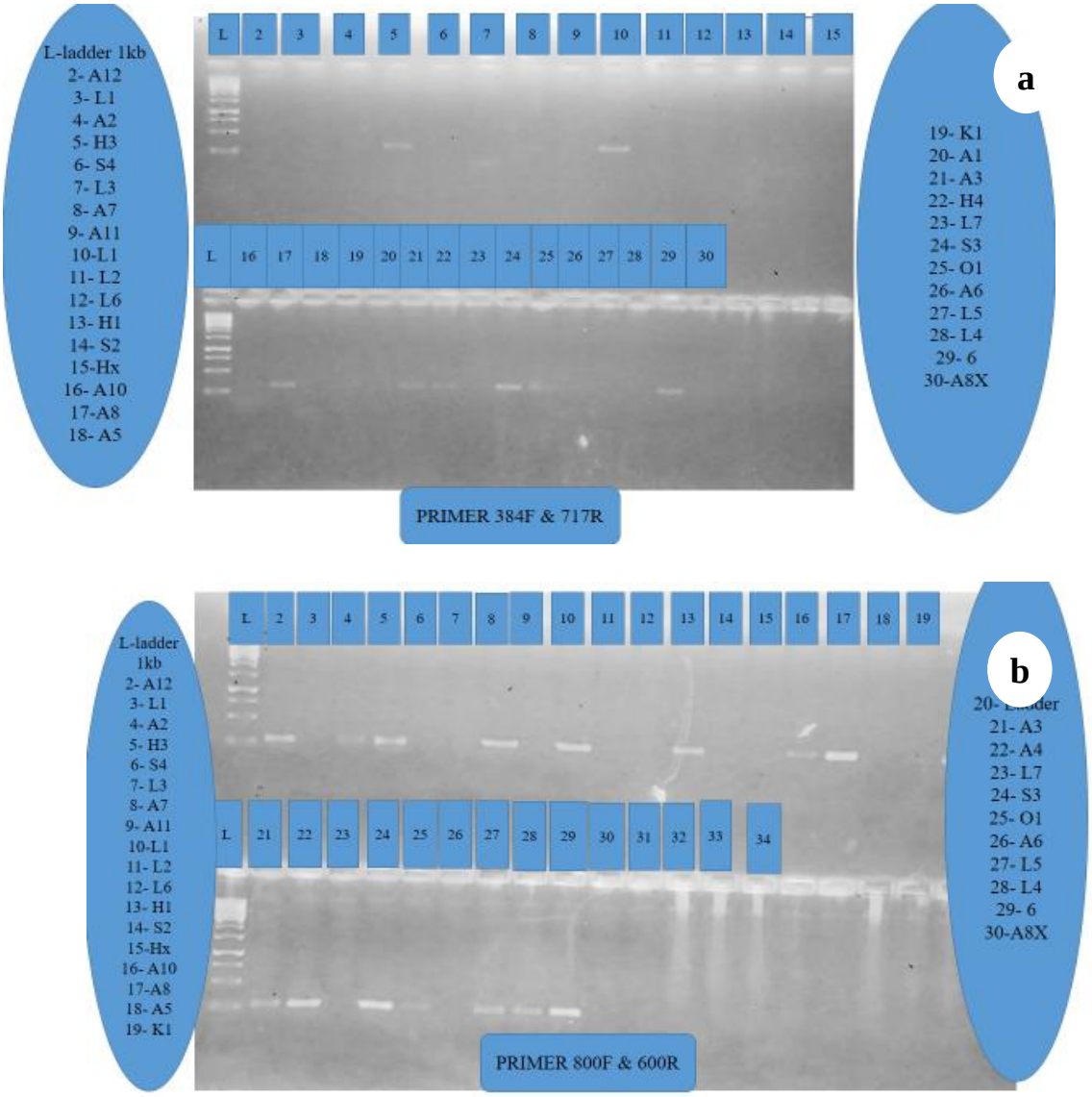

Plate 5: Rep-PCR fingerprints patterns collected in 2020

All isolates fell into 34 patterns. Primer 384F & 717 (a) and primer 800F & 600R (b) whereas for villages (Districts) are A12=Ngyani (Arumeru) ; L1=Yoghoi (Lushoto); A2=Songoro (Arumeru); H3=Masama Boma (Hai); S4=Ivulu (Same); L3=Kilangwi (Lushoto); A7=Njoro (Arumeru); A11=Seela (Arumeru); L1*=Yamba (Lushoto); L2=Kongei; L6=Kwembago (Lushoto); H1=Modio (Hai); S2=Bwambo (Same); HX=Kisawero (Hai); A10=Singisi (Arumeru); A8=Urisho (Arumeru); A5=Poli (Arumeru); K1=Kibosho singa kati (Moshi rural); A3=Kimundo (Arumeru); A4=Nkoarua (Arumeru); L7=Ngulu (Lushoto); S3=Vugwama (Same); O1=Olmotonyi chuo (Arumeru); A6=Nkoarisambu (Arumeru); L5=Irente juu (Lushoto); L4=Boholoi (Lushoto) and A8*=water

3.7.3 Sequencing Analysis and Species Identity

Forward and reverse sequences were assembled and trimmed on the CLC Main Workbench (CLC Bio, V to obtain 6.8.3) the consensus sequences as queries. The taxonomic nomenclature for each specie was designated based on the homology of their 16S rRNA to its known genera and species were done by performing the Basic Search Tool (BLAST®) (V. 9. 2.0) (Zhang *et al.,* 2000) at the National Centre for Bioinformatics (NCBI) GenBank: www.ncbi.blast.lnm.nih.gov (Altschul *et al.,* 1990). Sequences with greater than 97% similarity were retrieved for phylogenetic analysis. The assembled sequences were transferred into the Molecular Evolutionary Genetics Analysis (MEGA) (V.11.0) software for evolutionary analysis. The sequences were subjected to multiple and pairwise alignments using the MUSCLE algorithm in MEGA (V.11) (Tamura *et al.,* 2021). The evolutionary distances were computed using the Maximum Composite Likelihood and the evolutionary histories were inferred using the Neighbor-Joining (NJ) method (Tamura *et al.,* 2011).

3.7.4 Phylogenetic Analysis

Phylogenetic analysis was performed through the Molecular Evolutionary Genetics Analysis (MEGA) (V. 11.0) software (Tamura *et al.,* 2021). The process of multiple alignments of the gene sequences of the 20 *T. pedata* isolates is presented in Fig. 4 (a) and Fig. 4 (b) before and after the alignment in (MEGA) (V.11), respectively. To evaluate the relationship between *T. pedata* species and related genera and specie in the NCBI gene bank, the blastin suite was performed on the NCBI gene bank using the sequence of the *T. pedata*, and the search was optimized for high sequence similarity. The search was filtered to include only sequences with a similarity of more than 91% from the results, in this manner a total of 16 gene sequences were obtained. The sequences were downloaded in FASTA files and aligned against the gene sequence of *T. pedata* on MEGA 11 software. The gene sequences of *T. pedata* and the MEGA 11 alignment explorer shows related gene sequences as displayed in Fig. 4, whereas the blank regions show areas where DNA bases are similar.

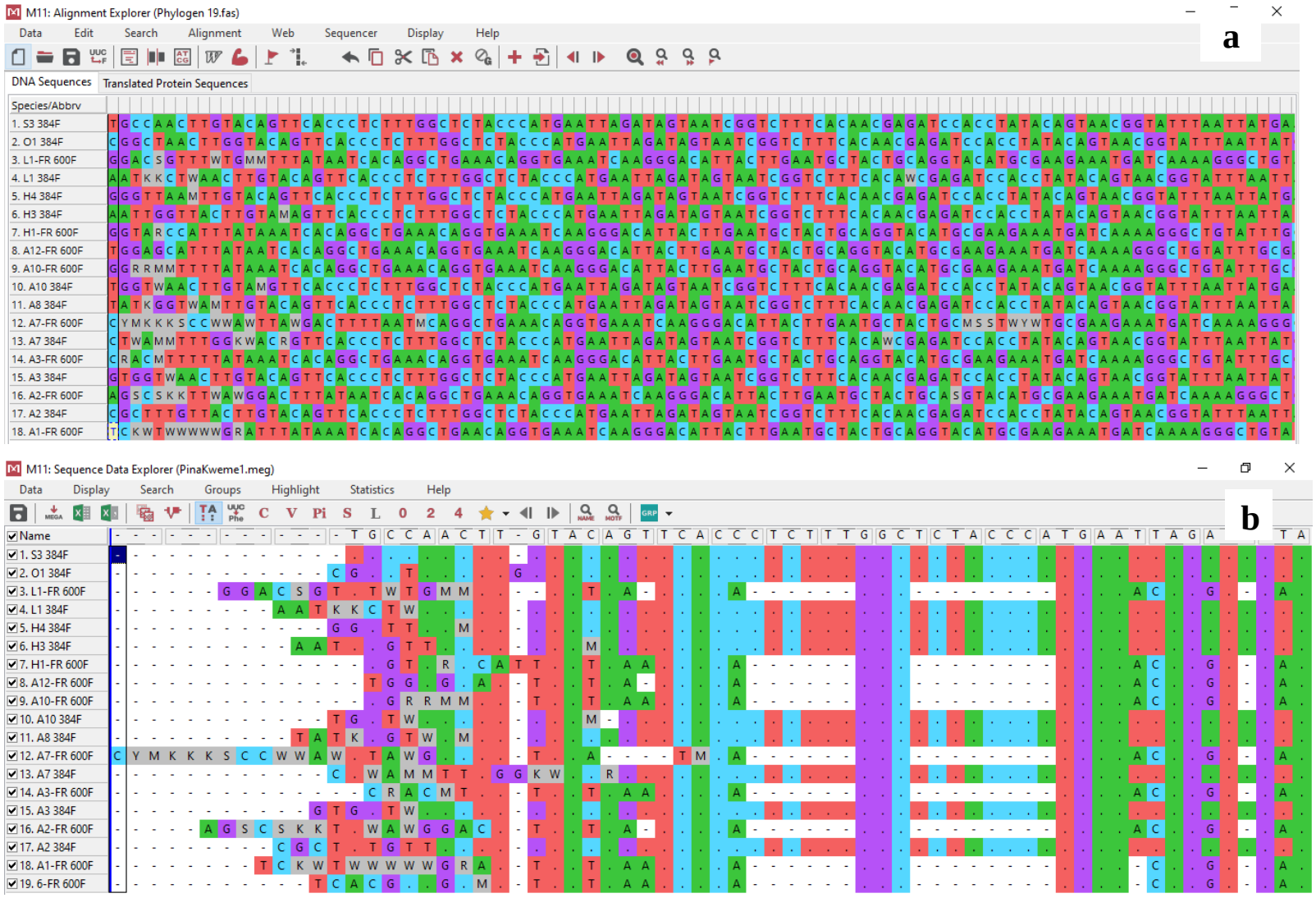

Figure 4: **Phylogenetic analysis of the 20 *T. pedata* gene sequences using MEGA 11 software during the 16S rRNA sequences**

3.8 Developing Oyster Nut Seedlings for Farmers

The experiment was conducted at the nursery of Forestry Training Institute-Olmotonyi (FTI), from September to December 2020. The institute is situated 17 kilometers north of Arusha Municipality and 3.2 kilometers north of Ngaramtoni on the Arusha – Nairobi highway. The Institute dwells in an area of about 325 hectares and located at 1670 m.a.s.l on the foothills of Mt. Meru (4979 m), Tanzania's second-highest mountain and is located along latitude 3°17′S and longitude 36°40′ E. The weather varies throughout the year, with a maximum temperature of around 28°C and a minimum temperature of 14°C and the mean annual rainfall is 800-1100 mm (Kayombo *et al.,* 2022). Simple farm equipment were used to manually clean the nursery site and packing polythene tubes. The media used were sand collected from the river bank and forest top soil which were sieved to improve porosity and aeration, and remove stones and other exogenous materials.

3.8.1 Preparation of Cuttings

Two hundred and ten cuttings, in 20 centimeter long were taken from vigorous parent stock (to avoid the effects of genetic variation, a single parent stock was used (Ofodile *et al.,* 2013). Lower leaves were removed, and to make a hole for the cuttings, a dibber was used into each polythene tube. The growing media were then filled in perforated polythene bags (2.5 by 12 cm) in size, firmly leaving 2.5 cm space at the top. A dibber was used to make a hole to insert the cuttings which had a length of about 20 cm acquired from a healthy parent stock, was done very early in the morning to ensure the plant is fully turgid. A sharp knife was used to make a smooth slanting cut at a distal lower end just below the node. Also, a veneer caliper was used for shoot diameter measurement, wheel barrow for ingredient collection, a spade or shovel was used for collecting and mixing ingredients, sieve wire for sieving the ingredients, stationary (pencil, pen and notebook) for data recording, scale rule for height measurement and watering can for watering.

3.8.2 Growth Media

Seven different growth media were prepared such as washed river sand (RS); mother plant topsoil (M), cow manure (CM), decomposed saw dust (SD), forest top soil (FS), mother plant topsoil (M) + cow manure (CM) at 1:1, river sand (RS) + cow manure (CM) at 1:1 and mother plant topsoil (M) + Nitrogen, Phosphorus and Potassium (NPK) fertilizer. For the experiment, they were separated to eliminate debris, rotten plant roots and leaves, and then sieved using 2 mm sieve were used for experiment. All types of media were sterilized by heating for 1 hour at 80°C, cooled for 24 hours to 15°C and then moistened to 20 to 35% moisture content (Magesa *et*

al., 2018). The chemical and physical characteristics of the growth media were determined at the beginning of the experiment (Table 2).

Soil samples were analyzed as follows: (a) pH 1:2.5 soil/water ratio suspension; (b) percentage of organic carbon was by using methods as described by Walkley-Black (2019), where by soil is treated with potassium dichromate and concentrated sulphuric acid, then titrated with ferrous sulphate using diphenylamine indicator to obtain pale green color (c) Available soil P using the Bray I protocol, where by soil is extracted with Bray 1 solution (1.0 M ammonium Fluoride and 0.5 M Hydrochloric acid), and blue color is developed using mixed indicator with Ammonium molybdenum solution and then measured in color spectrometer at 882 nm (d) percentage N measured by Kjeldahl method where soil sample is digested with concentrated sulphuric acid, distilled and then vapors trapped with boric acid and titrated with dilute sulphuric acid (e) soil exchangeable soil bases (Ca, Mg, K, Na) using 1 M ammonium acetate extraction and then measured by using atomic absorption spectrometer (AAS) (f) Soil texture by the Bouyoucos hydrometer method. Exchangeable cations and the cation exchange capacity (CEC) were also determined and element analysis of the decomposed sawdust was done using the digestion method.

Table 2: Physical and chemical properties of the study's growth media

Block	pH (H2O)1:2.5	EC1:2.5(mS/cm)	Exchangeable cation in me/100g Sample				Percentage						Percentage Texture			
			Ca^{2+}	Mg^{2+}	K^{+}	Na	BS	CEC	ESP	OC	Total N	C/N Ratio	Sand	Silt	Clay	Texture Class
1	7.16	0.105	13.24	4.39	8.22	6.23	50	64	9.7	2.97	0.202	15	63	29	7	sandy loam
2	7.22	0.044	4.69	1.46	6.04	3.36	41	38	8.8	3.50	0.050	69	59	27	13	sandy loam
3	7.11	0.003	9.81	3.30	4.39	3.65	88	24	15.2	4.08	0.098	42	67	27	5	sandy loam
4	4.89	0.120	2.48	1.62	0.00	2.58	33	20	12.9	1.03	0.014	74	41	31	27	Loam
5	6.47	0.081	5.27	2.66	0.00	2.52	37	28	9.0	1.07	0.022	48	65	25	9	sandy loam
6	5.50	0.236	6.20	4.05	0.00	2.66	26	50	5.3	4.90	0.165	30	41	31	27	Loam
7	6.86	0.156	12.00	6.06	0.00	2.72	40	52	5.2	3.38	0.008	402	39	27	33	Clay loam
8	6.37	0.120	3.83	2.67	0.00	3.07	40	24	12.8	1.73	0.006	309	83	13	3	sandy loam
9	6.68	0.313	14.07	5.00	0.47	3.10	33	68	4.6	3.29	0.255	13	61	21	17	sandy loam
10	6.42	0.885	24.55	4.88	0.08	3.16	31	104	3.0	6.47	0.008	770	69	25	5	sandy loam
11	6.31	0.086	5.60	2.63	0.00	3.14	14	82	3.8	1.48	0.006	265	63	29	7	sandy loam

3.8.3 Treatments and Data Collection

The experimental site for the nursery was manually cleared and packed using simple farm tools. All media except composite sawdust were either on mixtures (1:1 and v/v) or their own, were cleaned and sieved before use to improve porosity and aeration. Seven different propagation media namely river sand (RS); mother plant topsoil (M), forest top soil (FS), saw dust (SD), a 50:50 mother plant topsoil and cow manure (M+CM); a 1:1 mixture of washed river sand and decomposed saw dust (RS+SD) and a ratio of 2:1 of top forest soil and Nitrogen, Phosphorus and Potassium-NPK (TFS+NPK) were prepared. All types of media were sterilized by heating at 80°C for 1 hour, cooled at 15°C for 24 hours, and then moistened to 20-35% moisture content (Yeboah *et al.,* 2011; Magesa *et. al.,* 2018). The river sand was obtained from a nearby *Engare Olmotoni* river bank while the composted sawdust from the Forestry Training Institute-Olmotonyi training sawmill.

Two hundred and ten single node terminal stem cuttings were harvested from the stock plants and randomly allocated to the media (RS, M, FS, SD, M+CM, RS+SD and FS+NPK) as described above. The cuttings were inserted in the media under the nursery weaning shed and arranged in a randomized complete design with three replicates. Cuttings were assessed bi-weekly for the presence of shoot formation. The cuttings were planted out into polythene tubes, one cutting per tube which were previously filled with soil mixtures and the river sand was collected from the *Engare Olmotoni* river bank (Plate 6). The treatments were stem cuttings, control (no soil mixture) and soil media where 10 cuttings were planted in each media, making a total of 70 polythene pots The collected data were on: The number of leaves per cutting by counting; germination rate (%) and leaf length (cm) using a ruler; the number of weeks to sprout by counting. Data were obtained at 2, 4, 6 and 8 weeks after planting (WAP) for the presence of leaves on cuttings.

3.8.4 Data Analysis

After the data collection, survival percentage, leaf length, and the number of emerging leaves were calculated and analyzed. The analysis on leaf emergence was done by using ANOVA in Excel software (Microsoft 2016), comparisons of different means of different treatments were done by Duncan's Multiple Range Test (DMRT). Differences were considered to be significant at $P < 0.05$ and the differences between the treatment means were separated based on Tukey's test at $P \leq 0.05$.

Plate 6: **Oyster nut cuttings layout in different soils mixtures (a) Four weeks of oyster nut buds (b) and (c) and germinated oyster nut cuttings in different soil mixtures (d) and (e)**

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Mapping the Distribution and Abundance of Oyster Nut Population in Northern

(i) Oyster Nut Distribution and Abundance in Northern Tanzania

The survey findings indicated that the distribution of oyster nuts along the elevation and rainfall gradient ranged from 900 – 1800 m. a. s. l and 1000-1400 mm, respectively (Table 3). The oyster nuts abundance was about 79% higher at >1500 m. a. s. l than in lower altitudes (< 900 m. a. s. l) (Table 3). Also, oyster nuts were more abundant in the southern part of Lushoto district (44%), in the eastern part of Arumeru district (28%), and in the western part of Muheza district (23%) (Fig. 5 a-d). A high abundance of oyster nuts was recorded in Lushoto (46%) and Arumeru (28%) (Table 3). The majority of farmers (88%) grew more than one vine per host tree but a maximum of three vines per tree were recorded across all districts (Fig. 5 a-d). In addition, oyster nuts and the host trees were mainly planted, particularly in agroforestry farmlands, where other crops were grown such as maize (*Zea mays L.*), banana (*Mussa* species) and coffee (*Coffea*), intercropped with cauliflower (*Brassica oleracea* var. *botrytis*), carrots (*Daucus carota* subsp. *sativus*), common beans (*Phaseolus vulgaris*), potatoes (*Solanum tuberosum*) and onions (*Allium cepa L.*). Most of the vines (81%) were planted near the homesteads, in the middle of the farm, and on-farm boundaries while few vines (20%) were planted more than 200 m away from the farm lands.

The findings indicated that more oyster nuts plants grow in higher elevations up to 2000 m. a. s. l and in areas with higher rainfall. This was similar to *T. occidentalis* which is grown in the Niger Delta region where it is abundant with rainfall throughout the year (Fubara-Manuel *et al.,* 2012). Even for lianas, it has been documented that their abundance strongly increases with mean annual precipitation (Birhane *et al.,* 2020). Moreover, in Lushoto, the average number of vines per tree was three which was slightly higher than in the other districts due to presence of higher rainfall. This was also shown by species richness for climbers tend to increase with elevation up to 2760 m a. s. l (Cirimwami *et al.,* 2019). Even, Hemp (2006) revealed the same trend in similar species in Mt. Kilimanjaro. In contrast, Schnitzer (2005) reported that the diversity and abundance of climbers tend to decrease with decreasing temperature and rainfall in tropical forests.

Table 3: **Oyster nut abundance across different districts, elevation and rainfall gradients in northern Tanzania during the assessment from September 2019 to February 2020 (N = 346)**

Districts	Abundance	Elevation range (m. a. s. l)	Rainfall range (mm)
Arumeru	107	1 160-1 740	1 000-1 400
Lushoto	170	1 100-1 730	1 000-1 400
Muheza	87	900-1 720	1 100-1 500
Same	22	1 300-1 600	1 150-1 350

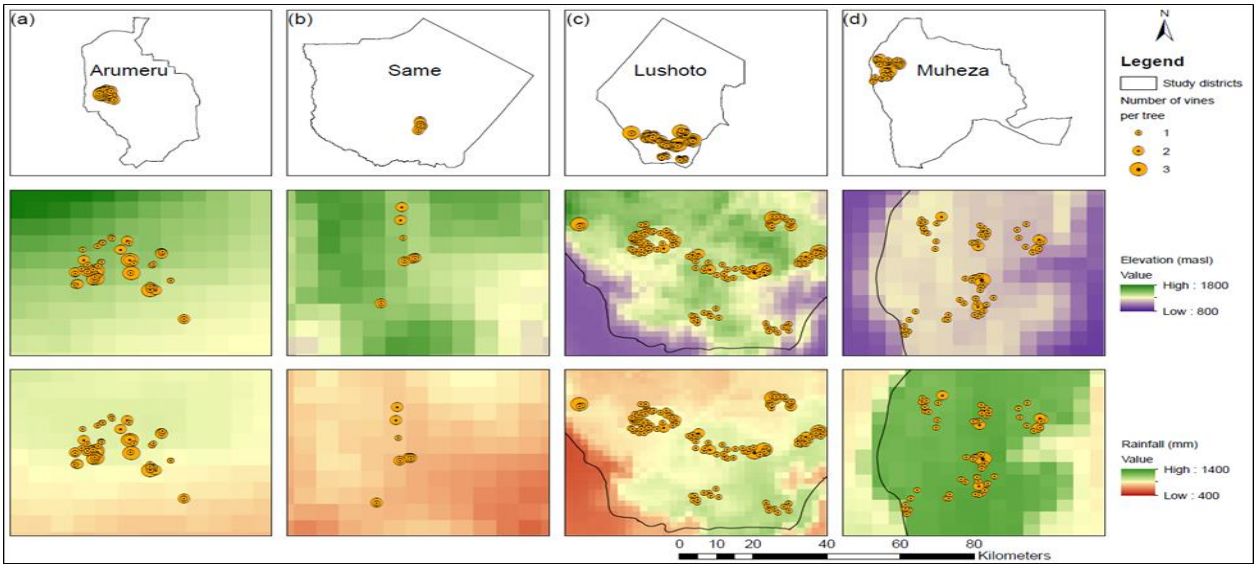

Figure 5 (a-d): **Maps showing the distribution of oyster nut population across the four districts surveyed from September, 2019, to February, 2020, in Northern Tanzania**

(ii) Relationship between Fruit Diameter, Number of Seeds and Environmental Factors

The study observed a significant positive relationship between fruit diameter and elevation for Arumeru district (r = 0.90 p <0.001), Muheza district (r = 0.84, p <0.001), Same district (r = 0.89, p = 0.001), and Lushoto district (r = 0.67, p <0.001) (Fig. 6a). Similarly, a significant positive relationship between fruit diameter and rainfall were found in Arumeru district (r = 0.83 p <0.001), Muheza district (r = 0.84, p <0.001), Same district (r = 0.88, p = 0.001), and Lushoto district (r = 0.71, p <0.001) (Fig. 6b). Further, a significant positive relationship was observed between the number of seeds and elevation for Arumeru district (r = 0.90 p <0.001), Muheza district (r = 0.88, p <0.001), Same district (r = 0.92, p < 0.001), and Lushoto district (r = 0.5, p <0.001) (Fig. 6c) as well as for number of seeds and rainfall for Arumeru district (r = 0.87 p <0.001), Muheza district (r = 0.87, p <0.001), Same district (r = 0.80, p = 0.002), and Lushoto district (r = 0.57, p <0.001) (Fig. 6d). The rainfall also has an effect on number of seeds in a fruit, as the number of seeds within a fruit increased with increasing rainfall across the study

sites. Even the size of seeds has commonly been associated with increased water availability and vice versa is true (Parciak, 2002).

It was further found that the fruit diameter increased with increasing elevation (>900 m. a. s. l) as did the number of seeds and the number of fruits. Parciak (2002), also described that the seeds of *P. virginiana* size and numbers varied with environmental conditions and annual resource availability such as rainfall availability, soils levels to mention a few. While there was similar trends for the three districts Lushoto, Muheza and Lushoto though Same showed a slightly opposite trend due to low rainfall availability of >1300 mm per annum. Guo *et al.* (2010) elucidated that the mean number and size of seeds per fruit of *Pedicularis* species increase with elevation and they also exhibit larger seeds hence seeds that are large exhibit superior survivor mechanisms. Even, Sivirihauma *et al.* (2016) explained the growth and yield of banana (*Mussa* spp) plantain cultivars performed well at a higher elevation of 1815 m. a. s. l.

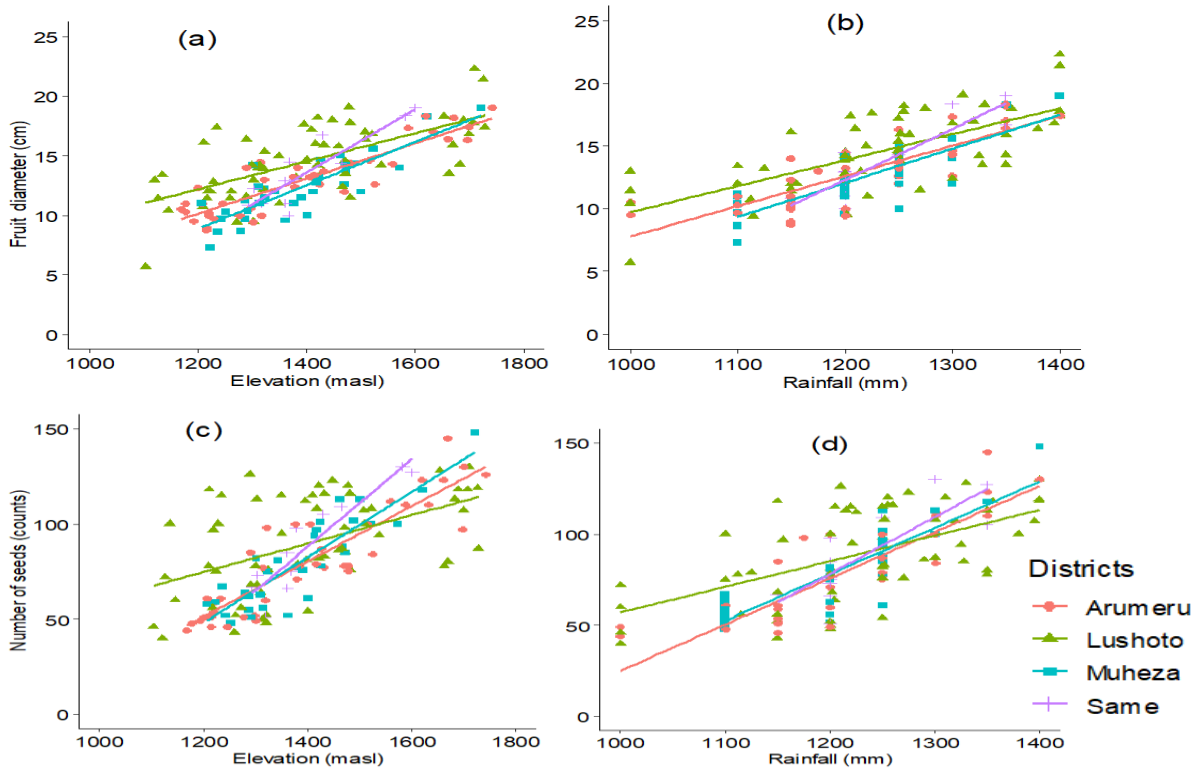

Figure 6 (a-d): Relationship between oyster nuts fruit diameter with elevation (a) and rainfall (b) as well as between the number of seeds with elevation (c) and rainfall (d) during the survey in Arumeru, Lushoto, Muheza, and Same districts, from September 2019 until February 2020 (N = 346)

(iii) Relationship between, Number of Fruits, Diameter of Vines and Environmental Factors

A significant positive relationship between number of fruits and elevation in Arumeru district (r = 0.47 p = 0.002) and Lushoto district (r = 0.35, p <0.007), Muheza district (r = 0.32, p = 0.03)

and Same district (r = 0.23, p = 0.04) was observed (Fig. 7a). Likewise, a significant positive relationship between number of fruits and rainfall for Arumeru district (r = 0.40 p = 0.011), Lushoto district (r = 0.28, p = 0.03) and Muheza district (r = 0.35, p <0.001) was recorded, but not for Same district (r = -0.06 p = 0.85) (Fig. 7b). Further, a significant positive relationship was observed between diameter of vines and elevation for Arumeru district (r = 0.37 p = 0.019), Lushoto district (r = 0.52, p <0.001), and Muheza district (r = 0.46, p = 0.007), but not for Same district (r = -0.27, p = 0.40) (Fig. 7c) as well as for diameter of vines and rainfall for Arumeru district (r = 0.38 p = 0.016), Lushoto district (r = 0.42, p = 0.001) and Muheza district (r = 0.51, p = 0.002), but not for Same district (r = -0.38 p = 0.23) (Fig. 7d). This study concurrently agrees with Nwangburuka *et al.* (2014) that by understanding vegetative traits including vine length and width, number of fruits, fruit width and length, seed size per *Telfairia* plant in different environmental factors will be effective in predicting its yield.

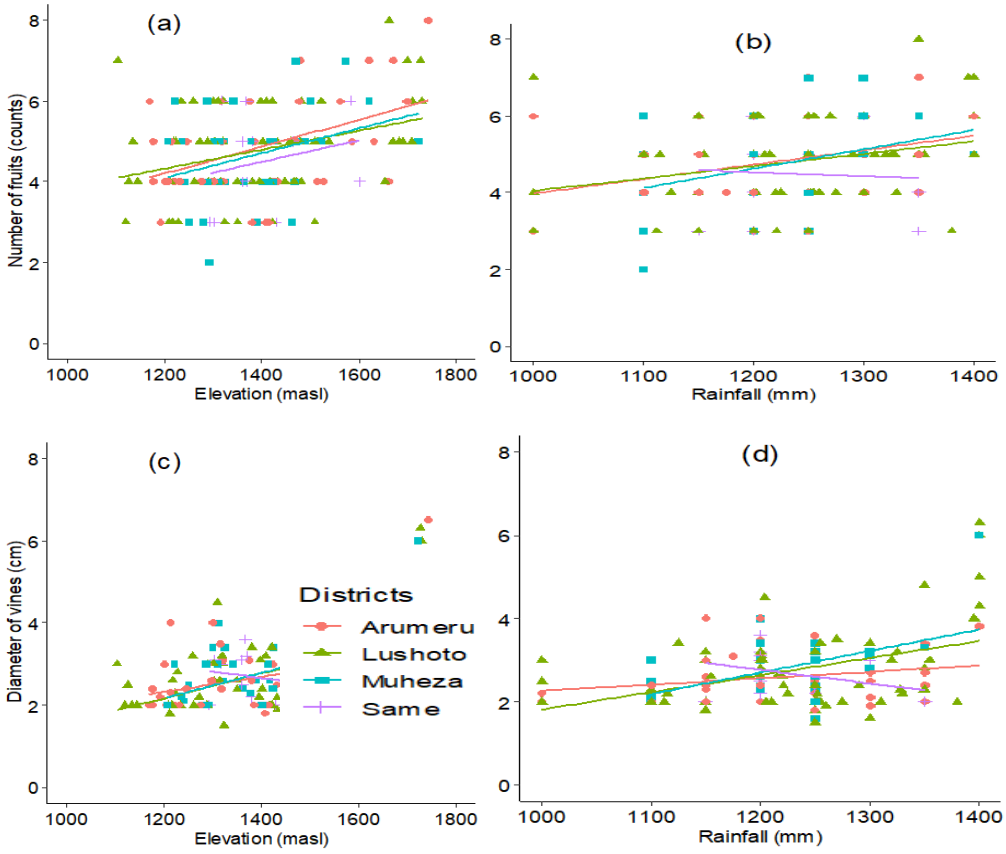

Figure 7 (a-d): Relationship between number of fruits with elevation (a) and rainfall (b) as well as between diameter of vines with elevation (c) and rainfall (d) during the survey in Arumeru, Lushoto, Muheza, and Same districts, from September 2019 until February 2020 (N = 346)

(iv) Variations of Fruit Diameter, Number of Seeds with Environmental Factors

From the study, a significant main effect of elevation on fruit diameter across the study districts ($F_{1, 136}$ = 217.9, p < 0.001) with larger fruit diameter at higher elevations across the study districts

was observed. Similarly, there was a significant main effect of rainfall on fruit diameter across the study districts ($F_{1, 136}$ = 228.2, p < 0.001) with larger fruit diameter increasing with rainfall across the study districts. In addition, significant districts × elevation interaction was observed on fruit diameter ($F_{3, 136}$ = 3.57, p = 0.015) with Lushoto and Same showing higher positive slopes than the other two districts, highlighting that elevation played a stronger role in these two districts. It was not found to have a significant rainfall × districts interaction on fruit diameter ($F_{3, 136}$ = 2.21, p = 0.08).

A significant main effect of elevation on the number of seeds across the study districts ($F_{1, 136}$ = 166.9, p < 0.001) with the number of seeds increased at higher elevations across the study districts was observed. Likewise, a significant main effect of rainfall on the number of seeds across the study districts ($F_{1, 136}$ = 171.9, p < 0.001) with the number of seeds increased with increasing rainfall was observed across the study districts. In addition, a significant districts × elevation interaction on fruit diameter ($F_{3, 136}$ = 7.9, p = <0.001) with Muheza and Same showing higher positive slopes than the other two districts, highlighting that elevation played a stronger role in these two districts was observed. In addition, there was a significant rainfall × districts interaction on the number of seeds ($F_{3, 136}$ = 4.9, p = 0.003) Arumeru, Muheza, and Same showed higher positive slopes than Lushoto district. But, Sakpere *et al.* (2019) also reported that it is not always the case that large fruits are associated with the total number of seeds as small fruits but can also have a higher number of seeds than large fruits. Contrary to Chukwudi and Agbo (2014), they found out that large-sized fruit gave bigger seed size in *Benincasea hispida* (ash guard) similar to oyster nuts both members of Cucurbitaceae family. Even with *T. occidentalis* large-sized fruits gave higher marketable leaves and fruit yield than small-sized fruits.

(v) Variations of Number of Fruits, Diameter of Vines with Environmental Factors

There was a significant effect of elevation on number of fruits across the study districts ($F_{1, 136}$ = 21.7, p < 0.001) with more than average of five fruits at higher elevations across the study districts. Similarly, rainfall significantly increased the number of fruits across the study districts ($F_{1, 136}$ = 14.3, p < 0.001). In addition, a non-significant districts × elevation interaction on number of fruits ($F_{3, 136}$ = 0.18, p = 0.90) and districts × rainfall interaction on number of fruits ($F_{3, 136}$ = 0.5, p = 0.63) across study districts was observed.

Moreover, there was a significant effect of elevation on the diameter of vines across the study districts ($F_{1, 136}$ = 33.25, p < 0.001) with larger vine diameters at higher elevations across the study districts. Similarly, a significant main effect of rainfall on the diameter of vines across the study districts ($F_{1, 136}$ = 26.44, p < 0.001) with larger vine diameter at increasing rainfall across

the study districts was observed. In addition, a non-significant districts × elevation interaction on vine diameter ($F_{3, 136}$ = 0.18, p = 0.90) and districts × rainfall interaction on vine diameter ($F_{3, 136}$ = 0.5, p = 0.63) across study districts was observed. Further, there was a significant positive relationship (r = 0.55, p <0.001) between vine size and fruit numbers. However, other studies revealed that, vine size, fruit size, and numbers are traits that are vital information used to distinguish *T. occidentalis* and used for the conservation of genetic materials (Chukwudi & Agbo, 2016).

(vi) Availability of Host Tree Species in Different Land use Categories/Systems

The findings observed a total of 280 trees representing seven families as hosts for oyster nut vines across the surveyed districts (Table 4). Most of the time, the oyster nut was associated with *Albizia schimperiana* (39%) of the cases, followed by *Persea americana* (14%), and the least common was *Mangifera indica*, three percent (Fig. 8). It was also expected that tall native tree species were had dominant host plants across all elevation levels due to the nature of the crop self-support from their climbing ways.

Table 4: Names, frequency and average diameter at breast height (DBH) of host tree species preferred by farmers for growing oyster nut vines as found during the study in Northern Tanzania from 2019 to 2020

Tree species	Family	Frequency	Relative frequency (%)	Mean DBH (cm)
Albizia schimperiana	Fabaceae	110	39	40
Persea americana	Lauraceae	40	14	36
Croton macrostaychs	Euphorbiaceae	24	9	38
Artocarpus heterophyllus	Moraceae	23	8	38
Cordia africana	Boraginaceae	23	8	37
Terminalia superba	Combretaceae	16	6	19
Ficus sur	Moraceae	14	5	62
Rauvolfia caffra	Apocynaceae	12	4	35
Ficus thonningii	Moraceae	11	4	60
Mangifera indica	Anacardiaceae	7	3	52
Total		**280**	**100**	

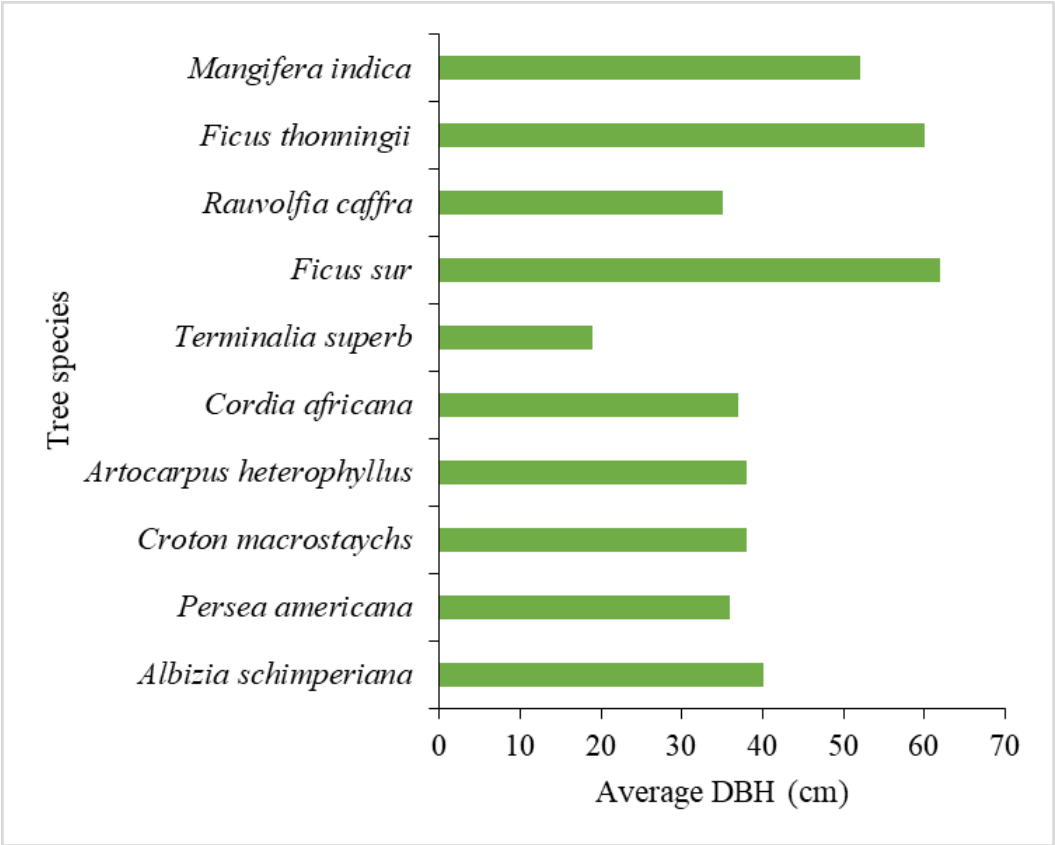

Figure 8: Average diameter at breast height (DBH) of hardwood tree species that were highly preferred by farmers as host species for the oyster nut vines

4.2 Ethno-Botanical Values of Oyster Nuts in Northern Tanzania in Relation to Livelihood (Food And Health)

4.2.1 Socio-Demographic Characteristics of Study Respondents

Out of the 346 interviewed respondents, 87% and 13% were female and male respectively. The majority of respondents 68% of those surveyed were middle age category (30-50), while 20% were above 50 years and 12% were youths between the age of 18 -36 years. A majority of respondents, more than 80% had completed primary school. Furthermore, more than 40% of the household members had 6-10 people, 20% had less than 6 members and 10% had more than 10 family members (Fig. 9).

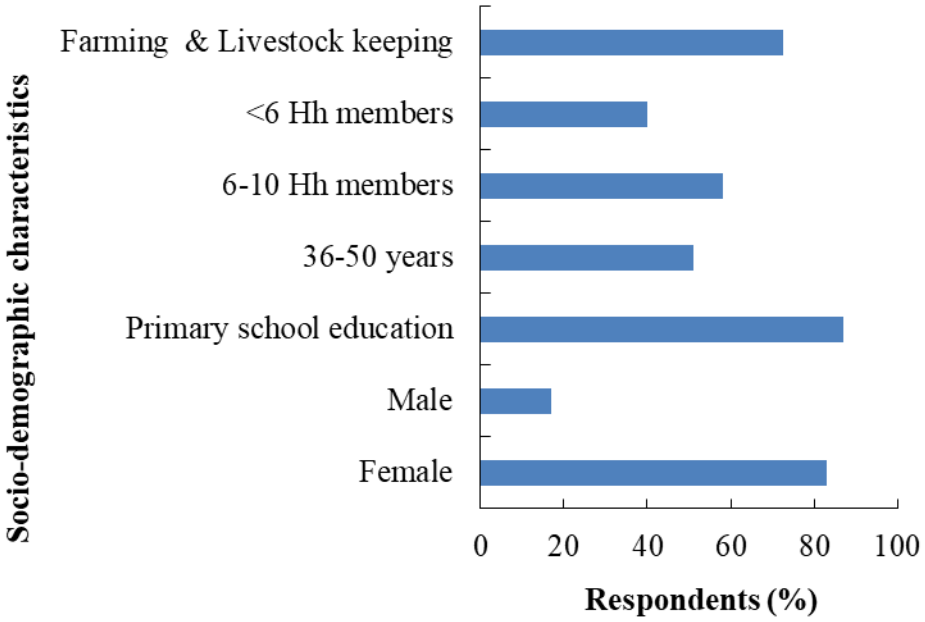

Figure 9: **Socio-demographic characteristics of respondents (N = 346) across the regions that were interviewed from September 2019 to March 2020. Whereas >10 Hh (Household) members means having more than 10 family members; <6 Hh members means having less than 6 family members**

In this study there was no statistical significance between prior knowledge and gender influence on oyster nuts, meaning that whether being a man or a woman does not influence on likelihood of knowing about oyster nuts. Although from the findings, women were more involved in oyster nut production than men. On the other hand, the participation of women in agricultural activities, particularly on the African continent is highly valued (Harouna *et al.,* 2019). Women were also found out to be the primary growers of oyster nuts, confirming Vodouhè *et al.* (2011; 2007) and Adjatin *et al.* (2012) results that, *T. occidentalis* is primarily grown by women in West Africa. Even in the production of other agricultural products, such as Bambara nuts, women are known to cultivate them in the County of Kakamega, Kenya, the similar crop is completely a female crop, and their male counterparts have forgotten to farm it (Ashagidigbi *et al.,* 2018; Ibrahim *et al.,* 2018). Therefore, this implies that the majority of the population were female farmers and hence continue to sustainably cultivate oyster nuts for household income if offered the same advantages as males.

4.2.2 Oyster Nuts Awareness

The results showed that oyster nut farming was also observed to be more engaged among middle-aged and older participants. This shows that, younger generations (18–36 years old) had little interest in oyster nut farming or other crops. Participants in the middle and older age groups, on the other hand, had a substantial impact on passing on knowledge about oyster nut

cultivation and the benefits derived from it to the younger generation, as they know the most about oyster nuts. Respondents' previous knowledge of oyster nuts was unaffected by their farming experience or education level, implying that significant expertise and education in oyster nut production did not influence on their understanding of oyster nuts. This clearly showed that illiterate and educated responders, as well as new and experienced farmers, may have similar prior knowledge and experience about oyster nuts. This indicates that, education and farming experience has a slight role in oyster nut policy formation, implementation, or adoption as other major agricultural oilseed crop such as peanuts. In contrast to *Telfairia occidentalis*, a closely related crop, is a valuable cash crop in West Africa due to the sale of its leaves which is regarded a potential oilseed crop (Ajayi *et al.,* 2007; Odiaka & Odiaka, 2011).

4.2.3 Ethnobotanical uses of Oyster Nut Crop as Practiced by Respondents

Oyster nuts grow close to the respondents' homes, and each person listed an average of ten uses for economic, environmental, health, and cultural reasons. Table 5 shows that, the most common uses were for cooking with staple foods (21%) (of use records), followed by expectant and nursing mothers (18%) for stimulating milk production and regaining strength after child-birth and lactation. Oyster nuts were also found to be commonly used as snacks (16%) and for various purposes such as cultural and ornamental purposes (17%) (Table 5 and Plate 7). Cooking was significantly higher than other uses (X^2=431, df = 9, p< 0.001).

Table 5: Ethnobotanical uses of oyster nuts as named by respondents in the study area (N=346)

Uses	Frequency	Percent of responses
Cooking	324	21
Lactations	281	18
Snacks	249	16
Conservation	234	15
Medicine/herbs	200	13
Culture	131	9
Ornamental	116	8
Total	**1535**	**100**

Field survey (2020)

Although the nuts are currently consumed primarily by children, youths, adults, and the elderly, they have the potential to enhance local people's income through the selling of seeds and the manufacturing of oil for food, cosmetics, and industries (Okoli & Nyananyo, 1988; Mwakasege *et al.,* 2021). They are generally grown for use as a spice in meals, used by pregnant and lactating women, and as a cooking oil substitute. They also act as panacea (panacea of

witchcraft, couth, and friendship oaths). Furusawa *et al.* (2014) argued that behaviors, such as respecting forest reserves and the semi-domestication of some species can aid in the preservation of confined biodiversity, particularly endemic species. If rural farmers are mentored and encouraged, their deep knowledge of the use of oyster nuts may drive them to cultivate this crop. Thus, there is a chance to increase production, post-harvesting and marketing potential.

The nuts are used in a variety of traditional dishes such as soups, cooked banana plantain, cooked beans, green vegetables, mixed maize, and beans (*makande*), and special meals for lactating mothers, such as *kibibi* from the *Sambaa* tribe which is pounded like peanuts (*Arachis hypogaea* L.). However, the nuts can be consumed fresh or roasted for confectionery and snacks (Ajayi *et al.,* 2004; Plate 8 a-e). Several studies have found that eating nuts reduces the risks of degenerative diseases like diabetes, stomach problems, and rheumatism (Okoli & Nyananyo, 1988). It is also used for a variety of other purposes including traditional rituals, skin care (aroma-therapy) and hair care (Akpabio *et al.*, 2008; Aregheore, 2012). As a result, it makes a substantial contribution to human health and livelihood diversification (Fasuyi & Nonyerem, 2007). Nonetheless, it is important to understand that, tribes have extremely strong traditions attached to both rural and urban geographical areas. One example that stands out as common practice for pregnant women in parts of East Africa, is having a large stockpile of oyster nuts before birth since it is part of the traditional nourishment for lactating mothers and weaned infants (Ajayi *et al.,* 2007).

Plate 7 (a-e): Photos of various uses of oyster nuts a) Shelled and unshelled raw nuts b) nuts coated with chocolate (c) pounded nuts known as *kibibi* (d) oyster nut cake (e) pastries with nuts (f) oyster nut flour

4.2.4 Gender-Based Cultivation Areas in the Study Area

Of the 346 interviewees, a majority of farmers (89%) cultivated the plant on their farms or family inherited farm lands while 11% did so elsewhere such as on neighbors' or relatives' land. The common planting procedure was direct seeding (100%), in the farm fields next to tall trees

94% or close to wired fences or live fences was six percent. Moreover, the results demonstrate that females (83%) engaged more in oyster nut cultivation than male (17%) counterparts. There was a significant difference between female and male respondents in terms of frequency of cultivating oyster nuts (χ^2=431, df= 9, p<0.001), as most of those who often used and engaged in farming were female. All growers that were studied planted oyster nuts by sowing the seeds in their fields, and Gil-Loaiza *et al.* (2016) stated that direct seeding is a simple, cost-efficient alternative to germination in a nursery.

4.2.5 Perceptions of the Abundance of Oyster Nuts within the Study Area

Overall, more than half of respondents (54%) said that oyster nuts' availability had decreased over the past ten years, 32% indicated it had not changed (remained the same), and (14%) said they were unsure whether oyster nut plant abundance was changing or not (Fig. 10). The absence of large trees as a result of agricultural activities, variations in weather patterns, the existence of pests, and small animals (rodents and squirrels) that eat the seeds and bury them underground for their future consumption, according to the respondents who perceived a decrease in oyster nut abundance. Even though, the peoples' interest was to manage the trees that the oyster nuts grew on for timber purposes (Chaturvedi *et al.,* 2016).

The oyster nut is a multipurpose agroforestry plant that may be grown alongside other crops to reduce carbon emissions, and the kernel can be utilized as a source of fuel to improve ignition for kitchen cooking. Fortunately, the *Telfairia* specie production is throughout the year (Odiaka *et al.,* 2008). In addition, oyster nut species serve as soil stabilization and bioremediation (Wegwu *et al.,* 2002). Local ecological knowledge about oyster nuts in the study area, along with the trees they grow on, for biodiversity conservation could benefit local biodiversity.

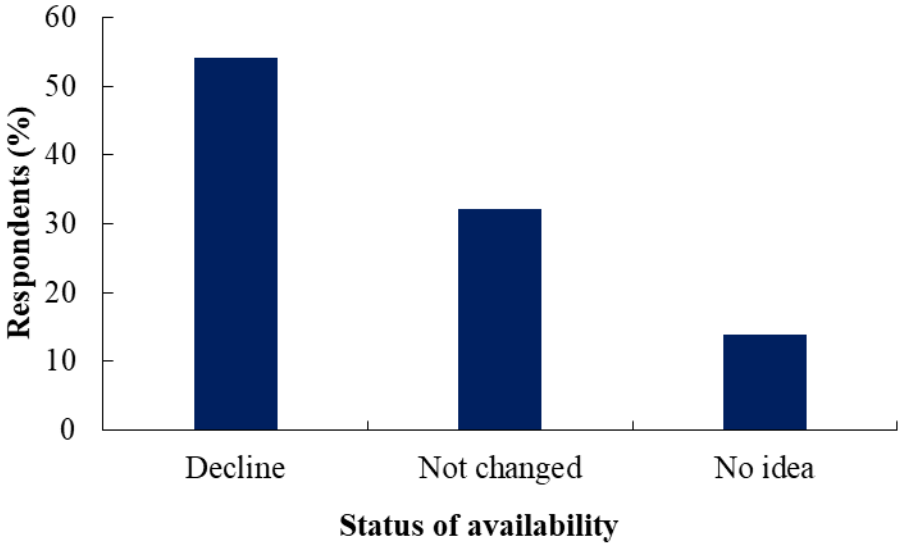

Figure 10: Status of availability of oyster nuts in the study area based on interviews conducted in districts of Lushoto, Same, Arumeru, Muheza in 2020-2021(N=346)

4.2.6 Oyster nut Storage Methods as Performed by Respondents in the Study Area

The findings revealed that the majority of respondents (36%) used perforated plastic sacks to store the nuts while 26% put them in plastic buckets or metal tins (usually 20 kg capacity), 22% used traditional handmade pans and 16% placed them under the roof to dry. This could have been the case because they were more readily available, simple to use, and inexpensive to purchase than alternative facilities such as jute bags (Fig. 11). The indicated storage methods and facilities, according to respondents, might keep the seeds viable for up to a year.

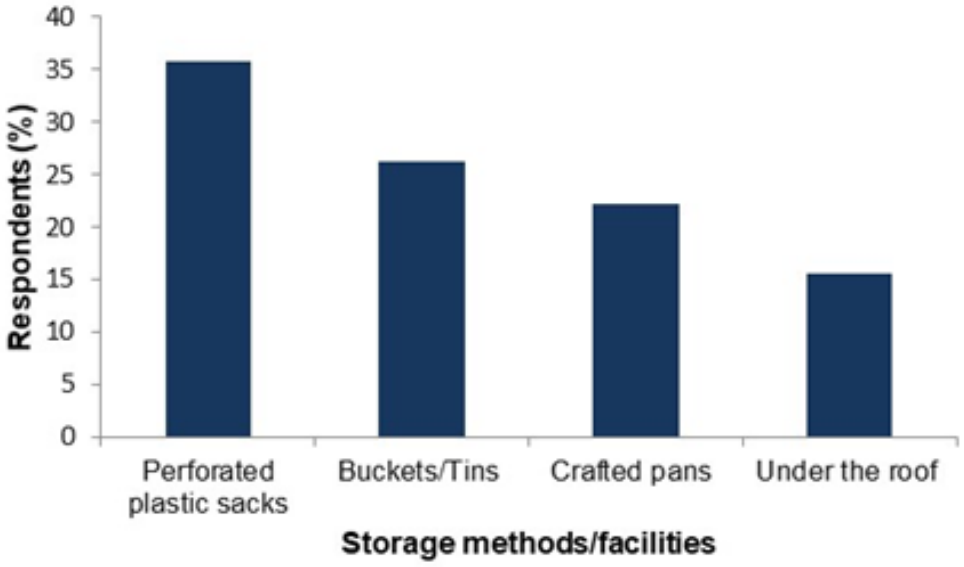

Figure 11: Storage methods and facilities of oyster nuts in the study area based on interviews conducted in districts of Lushoto, Same, Arumeru, Muheza in 2020-2021 (N=346)

The findings also comprehended that, in many parts of Africa, certain crops are grown year round, while the main food crops such as tubers, grains, and cereals, especially potatoes, are often seasonal (Adjatin *et al.,* 2012; Midega *et al.,* 2016). As a result, harvests collected during a short period of time such as few weeks, must be kept for gradual consumption until the next harvest, while seeds must be saved for the following season's crop. Furthermore, in an un-regulated market, the value of surplus products, if marketable, rises during the off-season period. As a result, the fundamental purpose of any storage system must be to keep the crop in peak condition for as long as feasible (Abtew *et al.,* 2016). The methods of storage and handling should reduce losses and be appropriate in terms of other factors including labor, machinery, construction expenses, and economies of scale. Despite the nutritional value of *Telfairia* species, their seeds are difficult to store due to their nature of being recalcitrant (Akoroda, 1990; Nwonuala & Obiefuna, 2016).

Furthermore, because the collected seeds are preserved in local and traditional storage facilities such as plastic containers, metal cans, perforated plastic bags, earthen-ware containers, sacks, buckets and perforated bags (they were not in combination) with no pesticides added to the nuts, the conservation of oyster nut variety is reliant on traditional knowledge. Since, only a small amount of oyster nuts are stored and the majority of those collected are sold one to three months after harvesting to meet family requirements or avoid storage losses. As a result, developing appropriate techniques and equipment for efficient storage facilities and methods will boost the crop's potential, ensuring food security and economic gain for small-scale farmers and entrepreneurs. *Telfairia occidentalis* and similar crops, could suffer large losses in the crop production chain and storage if present traditional postharvest methods and procedures are not addressed (Adebooye & Opabode, 2004; Igbozulike, 2015).

4.3 Conservation Strategies of Oyster Nuts by Different Ethnic Communities

4.3.1 Farmers' Oyster Nut Growing Practices in the Study Area

According to the findings, the majority of farmers (89%) cultivated the crop on family lands which were mostly inherited, while only seven percent of farmers used neighbors' lands. In addition, only four percent of the farmers planted the nuts on lands that belonged to other areas which included relatives lands (Fig. 12). Intercropping with key crops such as maize (*Zea mays*) banana (*Musa sp*) and coffee (*Coffea sp*) was well adopted by all farmers. Since women were the principal growers of oyster nuts, they planted the crop around the farms or on marginal lands because they did not have access to lands as well as hardwood tree species that provide support to their heavy and large hanging fruits.

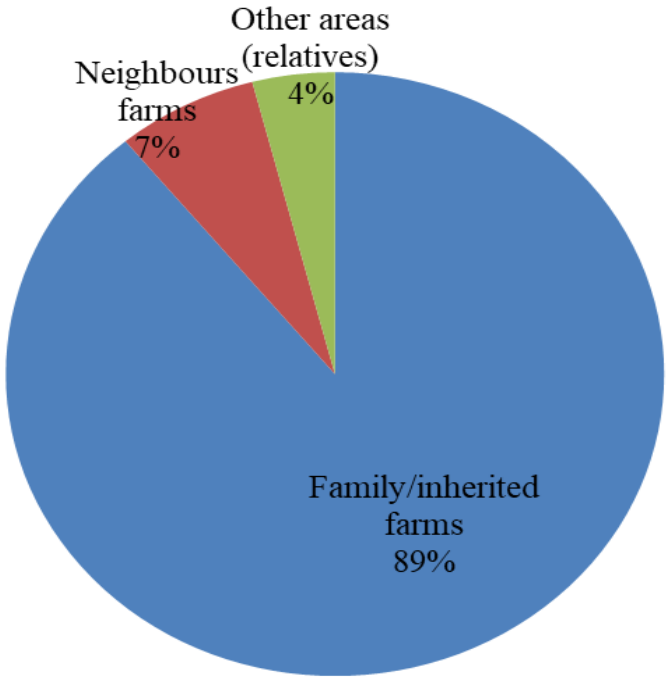

Figure 12: Areas of cultivation

4.3.2 Utilization by Gender and Conservation of Oyster Nuts

According to the study, the majority of female respondents (313) utilized the nuts for cooking with staple meals, 251 lactating and weaning mothers used the nuts and whereas 131 men consumed the nuts as snacks and 109 used them for conservation purposes (Fig. 13). In terms of frequency of the use of oyster nuts, the study found a significant difference between male and female respondents (χ^2=431, df= 9, p<0.001), with females accounting for the majority of those who utilized and engaged in the production of oyster nuts (Fig. 13).

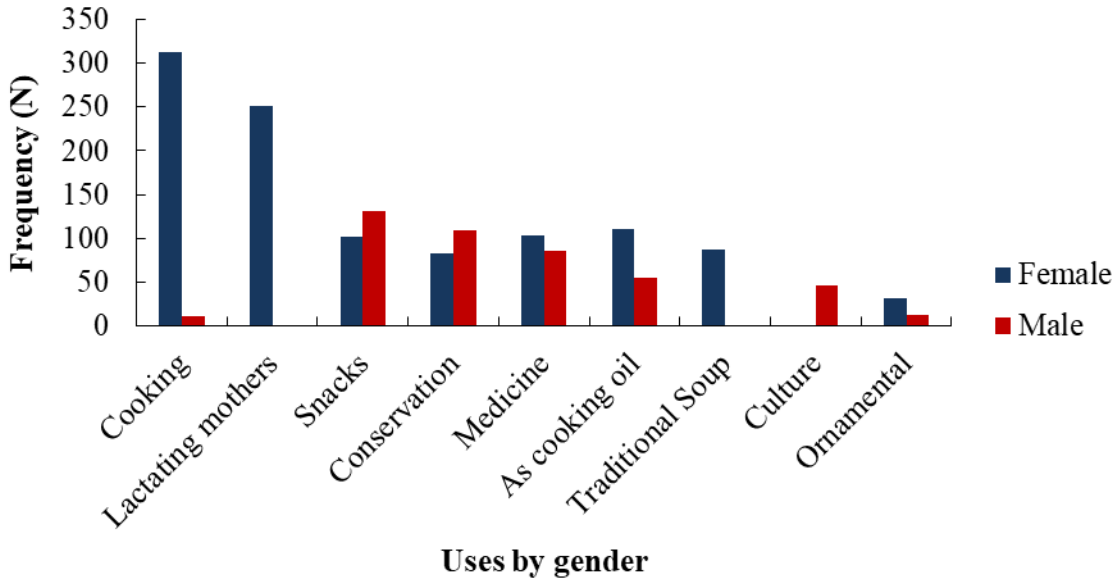

Figure 13: Ethnobotanical uses of oyster nuts by gender (N=346)

The findings noted that, farmers have their traditional methods for opening the seed, which usually involves heating it on low heat or smashing the dry pods. The guard-shaped fruit of oyster nuts likes to detach itself and tumble or drop on the ground surface, breaking open, once it has reached maturity. Once the fruit ruptures, the full seeds are collected (unfilled seeds are discarded), the interior section is removed, the pulp is cleansed, and the nuts are sun-dried before being stored, consumed, or sold. Furthermore, oyster nuts are mostly sold as dry nuts with little formal marketing, whereas fluted pumpkin leaves (*T. occidentalis*) are highly advertised and marketed with formal marketing (Alegbejo, 2012). However, according to the study, any income earned from the sale of oyster nuts was spent on household consumption. It was also found that oyster nut growers used only seeds as a technique of propagation, and that no pesticides, fungicides, industrial fertilizer, or organic manure were used in oyster nut planting. As the crop is grown without the use of chemicals, this suggests that oyster nuts are grown utilizing indigenous knowledge and organic methods. According to the study, oyster nuts can be cultivated without the use of agrochemicals or fertilizers (Ajayi *et al.*, 2007; Mwakasege *et al.*, 2021). Similar findings were revealed by Garrity (2004) in northern Tanzania, where the agroforestry system is practiced in combination with oyster nuts, coffee (*coffea sp*), and bananas (*Musa sp*).

Plate 8: **Ripe oyster nut fruit harvested wooden structure (a) and methods of storing oyster nuts for future use (b-e), fiber pan, plastic bag, earthed pot and on floor respectively**

4.4 Molecular Difference and Phylogenetic of Oyster Nuts Samples

4.4.1 Specie Identification Analysis using 16S rRNA Gene Sequences

According to Felsenstein (1985), the BLASTn algorithm at the NCBI GenBank database was used to conduct an initial sequence similarity search to ascertain *T. pedata* identities, resulting in matches for the DNA component and their respective source of material. These results ascertain that the collected samples matched *T. pedata* with >90% similarly, Table 6. The predicted open reading frames (ORFs) were identified using the Open Reading Frame Finder (ORF Finder) tool, also available at the NCBI website (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The DNA sequences were aligned using the MUSCLE algorithm in MEGA (V. 11) (Tamura *et al.,* 2021) and the phylogenetic analysis was carried out using maximum likelihood (ML) parsimony to reconstruct the relationship of the DNA sequences with their closest relatives using the NJ algorithm.

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model (Tamura, 1992). The tree with the highest log likelihood (-483.95) is shown in Fig. 14. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model, and then selected the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 27.75% sites). This analysis involved 20 nucleotide sequences. All positions with less than 95% site coverage were eliminated, i.e., fewer than five percent alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There were a total of 191 positions in the final dataset.

The identification by using 16S rRNA gene sequences appeared to coincide with the BioEdit sequence alignment editor results that T. pedata was the main plant species. All of the accessions collected from Northern Tanzania had the highest similarity (>95%). The major clades of equal numbers of sequences were obtained in the analysis with one of the major clades consisting of 2 sub-clades of 5 sequences each. These results show that the obtained T. pedata sequences only had minor genetic variations. Although the accessions were obtained from different districts in Northern Tanzania, their close genetic similarity indicates that they might have originated from the same parents and made to migrate to other sites for cultivation by human intervention.

Table 6: *Telfairia pedata* specie identified by 16S rRNA sequence analysis

SN	Organism	GenBank accession	16S rRNA gene analysis		
			Query coverage (%)	E-value	SIM ID (%)
1	*Telfairia pedata*	DQ535853.1	94	1e-61	98.60
2	*Telfairia pedata*	DQ535853.1	95	1e-108	98.28
3	*Telfairia pedata*	DQ374439.1	98	6e-133	99.27
4	*Telfairia pedata*	DQ535853.1	93	6e-107	97.84
5	*Telfairia pedata*	DQ374439.1	98	2e-133	99.27
6	*Telfairia pedata*	DQ374439.1	98	1e-67	99.33
7	*Telfairia pedata*	DQ535853.1	97	3e-110	98.71
8	*Telfairia pedata*	DQ374439.1	95	6e-127	98.86
9	*Telfairia pedata*	DQ535853.1	88	2e-99	95.93
10	*Telfairia pedata*	DQ374439.1	99	6e-133	97.86
11	*Telfairia pedata*	DQ374439.1	98	3e-130	98.53
12	*Telfairia pedata*	DQ535853.1	97	3e-110	98.71
13	*Telfairia pedata*	DQ535853.1	97	4e-109	98.29
14	*Telfairia pedata*	DQ535853.1	100	2e-112	97.92
15	*Telfairia pedata*	DQ374439.1	99	2e-132	98.55
16	*Telfairia pedata*	DQ374439.1	98	2e-133	99.27
17	*Telfairia pedata*	DQ374439.1	97	7e-132	98.90
18	*Telfairia pedata*	DQ535853.1	94	5e-108	98.28
19	*Telfairia pedata*	DQ374439.1	97	2e-65	99.33
20	*Telfairia pedata*	DQ374439.1	98	2e-132	99.63

SIM = similarity index

4.4.2 Phylogenetic Analysis

(i) Genetic Diversity of Oyster Nuts

In this study, the phylogenetic analyses of the detected oyster nut isolates were based on estimates of genetic divergence between the respective sequences. The number of base changes per site was calculated using the p-distance method between the sequences. The analysis used 20 nucleotide sequences that corresponded to the 20 oyster nut isolates which were studied. The final dataset comprised a total of 619 positions after all ambiguous positions were removed from each sequence pair (pair-wise deletion option). The Neighbor-Joining (NJ) method was used to

infer the evolutionary history (Saitou & Nei, 1987). The evolutionary history of the taxonomic analysis was represented by a bootstrap consensus tree generated from 1000 replicates (Felsenstein, 1985). Branches that corresponded to partitions that were replicated in less than 50% bootstrap replicates were collapsed. Next to the branches is the proportion of replicate trees in which the related taxa clustered together in the bootstrap test (1000 replicates) (Felsenstein, 1985). The evolutionary distances were quantified in base differences per area and were measured using the p-distance approach (Kumar & Gadagkar, 2000). A total of 20 nucleotide sequences were used in this study. For each sequence pair, all ambiguous positions were deleted (pair-wise deletion option). The final data set contained 348 positions, and evolutionary analysis was performed in MEGA 11 (Kumar *et al.*, 2018).

(ii) Evolutionary Relationships between Oyster Nut Gene Sequence and Related Gene Sequence in the NCBI Gene Bank

The numbers of nucleotide variations per site between Oyster nuts sequences and similar gene sequences in the NCBI gene bank were at (similarity >91%). Evolutionary relationships between the 20 oyster nut isolates and the corresponding gene sequences in the NCBI gene bank were visualized using a phylogenetic diagram (Fig. 14). Phylogenetic analysis revealed that the identified oyster nut strains were all strongly related to other gene bank strains. The MEGA (V. 11) was used to conduct evolutionary analyses, and the NJ method was used to refer to the evolutionary history. The evolutionary history was inferred using the Neighbor-Joining method (Kumar & Gadagkar, 2000). The evolutionary history of the taxa studied is represented by bootstrap consensus tree inferred from 1000 replicates (Felsenstein, 1985). Branches that correspond to partitions that have been replicated in less than 50% of bootstrap replicates have been collapsed. Next to the branches are the percentage of replicate trees in which the related taxa clustered together in the bootstrap test (1000 replicates) (Felsenstein, 1985). The evolutionary distances were calculated using the Maximum Composite Likelihood method (Nei & Kumar, 2000) and are in the base substitutions per site units. There were 20 nucleotide sequences in this study. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). The total number of positions in the final dataset were 314. The MEGA 11 was used to conduct evolutionary analyses (Kumar *et al.*, 2018).

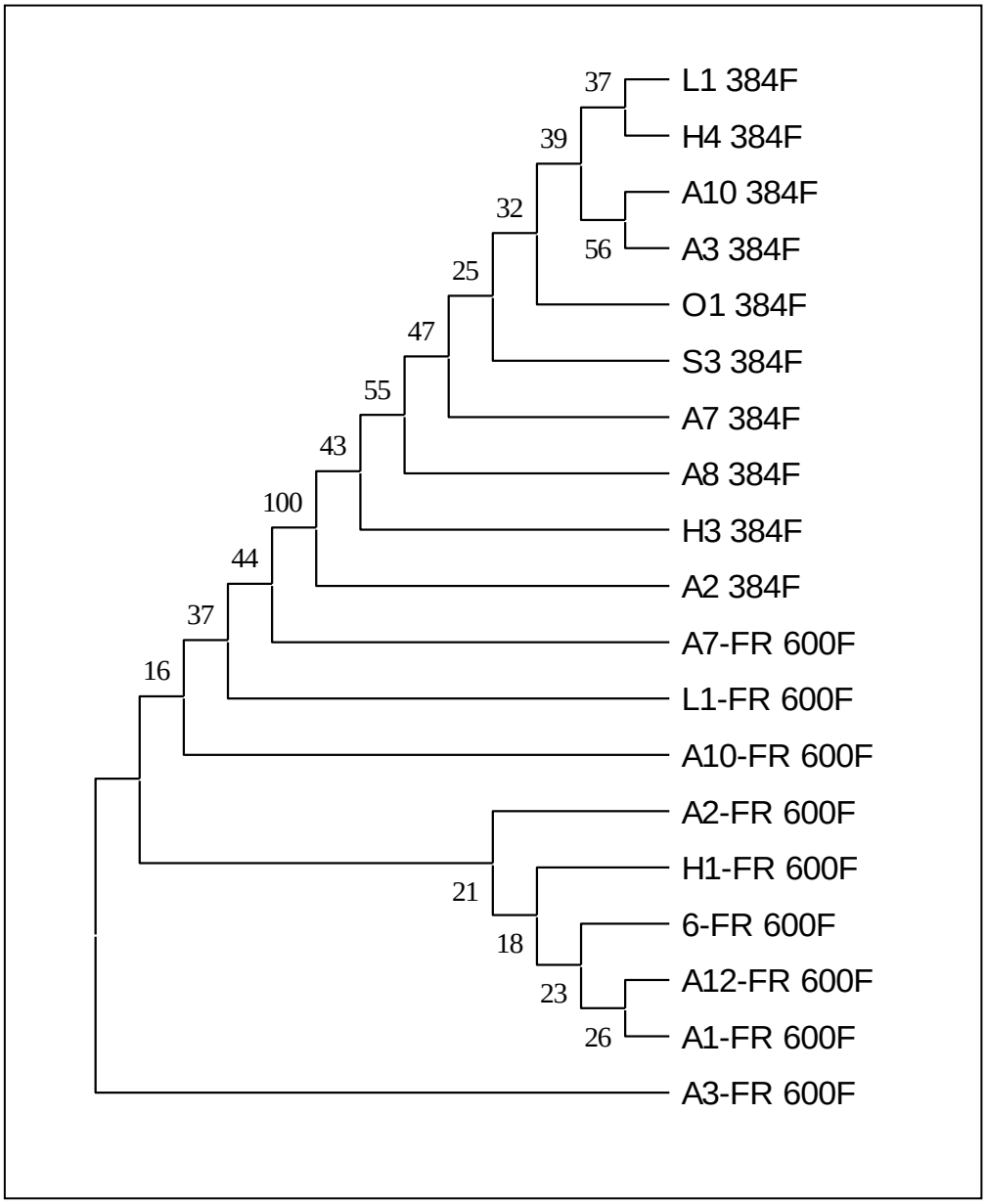

Figure 14: Genetic relationship of the 20 collected of *T. pedata* taxa collected from different areas in Northern Tanzania

Key: A8= Urisho village, Arumeru; A3= Kimundo village, Arumeru District; A10= Singisi village, Arumeru District; H3= Masama Boma village, Hai District; S4= Ivulu village, Same District; L1=Yoghoi village, Lushoto District; S3= Vugwama village, Same District; O1= Olmotonyi village, Arumeru District; A2= Songoro village, Arumeru District; A7= Njoro village, Arumeru District; H1= Modio village, Hai District; L1; A12= Ngyani village, Arumeru District; A7/L7= Ngulu village, Lushoto District; A10/A11= Seela village, Arumeru District; A3/L3= Kilangwi village, Lushoto District; A1/L1*= Yamba village, Lushoto District; A6= Nkoarisambu village, Arumeru District.

For clarity, the sequences of T. pedata were obtained by Sanger sequencing. The horizontal lines are proportional to the number of nucleotide substitutions per site/study area. The values at each major node represent values of 95 % or greater in 100 replicates. The NCBI GenBank accession number for each T. pedata is indicated, with the name of each isolate included in the tree. The evolutionary analysis was conducted in MEGA 11 (Tamura *et al.,* 2021).

4.5 Improved Propagation Methods to Develop Oyster Nut Seedlings to Farmers

4.5.1 Number of Leaves per Cutting

The results of this experiment on the number of leaves produced per cutting in different growth media including mother plant topsoil and Nitrogen, Phosphorus and Potassium, mother plant topsoil and cow manure (M+NPK, M+CM), mother plant topsoil (M) and forest top soil (FS) were measured at 2, 4, 6 and 8 weeks after planting (WAP) as shown in Fig. 15. All cuttings in the growth media did not produce leaves during the first week. From the second week, all the cuttings showed promise in supporting the number of leaves except for saw dust (SD) and river sand (RS) growth media which generated a low number of leaves. It was also observed that the cuttings in mother plant topsoil and Nitrogen, Phosphorus and Potassium (M+NPK) had an average of 2.0 leaves, mother plant topsoil and cow manure (M+CM) had an average of 1.2, mother plant topsoil (M) had an average of 1.1 leaves and forest top soil (FS) had an average of 1.0 leaf (Fig. 15). In saw dust (SD) and river sand (RS) media the cuttings had average leaves of 0.4 and 0.3 respectively. The result showed a significant difference at 2, 4, 6 and 8 weeks after planting (WAP), whereas, the leaf production was significantly higher in mother plant topsoil and Nitrogen, Phosphorus and Potassium (M+NPK), mother plant topsoil and cow manure (M+CM), mother plant topsoil (M) and forest top soil (FS) growth media than in forest decomposed saw dust (SD) and river sand (RS) (F =72.04, df = 6, $p \leq 0.05$).

When compared to the other growing media, decomposed saw dust (SD) had a significantly lower percentage in the number of leaves, which might be due to the physical quality of the growth media on which the plants' supply of water and air is dependent (Baiyeri, 2005). Qadri *et al.* (2018), reported that good porosity of rooting media promotes rapid absorption of nutrients leading to growth, which is mainly due to having adequate aeration, drainage and low bulk density of media. Although, the porosity of the various growth media was not tested in this study, the lowest growth attributes were in the decomposed saw dust (SD) and river sand (RS) maybe it was most likely attributed to low porosity and aeration. As Amri and Shahsavar (2009) pointed out, soils that lack enough required aeration and porosity for proper gas exchange can cause the cuttings to rot, which was also observed in this study.

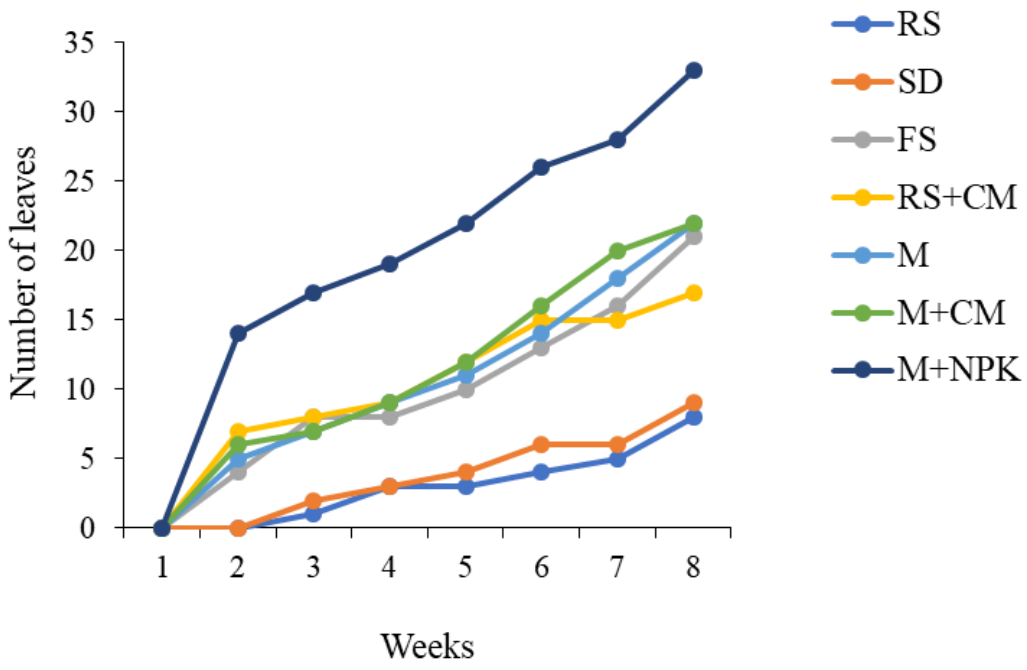

Figure 15: The average number of leaves produced in oyster nut cuttings under different growth media

Whereas, M+NPK stands for Mother Plant Tree Soil with NPK, M+CM stands for Mother Plant Tree Soil with Cow Manure, M stands for Mother Plant Tree Soil, FS stands for Forest Top Soil, SD stands for decomposed Saw Dust and RS stands for River Sand

4.5.2 Percentage of Leaf Emergence Increased in M+NPK and M+CM in Polythene Tubes

The results of leaf emergence percentage from polythene tubes containing cuttings (Fig. 16), wherein, the leaf emerging percentage for each treatment was recorded on the second week after planting (2WAP) and had attained their maximum percentage on the eighth week after planting (8WAP). For example, mother plant topsoil and Nitrogen, Phosphorus and Potassium (M+NPK) had the maximum leaf emergence of 29%, mother plant topsoil and cow manure (M+CM) recorded 17%, and the least leaf emergence was river sand (RS) and decomposed saw dust (SD), which were had four and five percent, respectively. However, all the treatments did not record any leaf emergence for the first week after planting (1WAP). In general, oyster nut cuttings grown in mother plant topsoil (M) treatments treated with NPK and cow dung manure sprouted at a much higher rate than those grown in mother plant topsoil (M) (control) or forest top soil (FS) alone. The result showed a significant difference at 2, 4, 6 and 8 weeks after planting (WAP). Whereas, the leaf sprouting was significantly higher in mother plant topsoil and Nitrogen, Phosphorus and Potassium (M+NPK), mother plant topsoil and cow manure (M+CM), mother plant topsoil (M) and forest top soil (FS) growth media than in SD and RS (F =33.52, df = 6, $p \leq 0.05$).

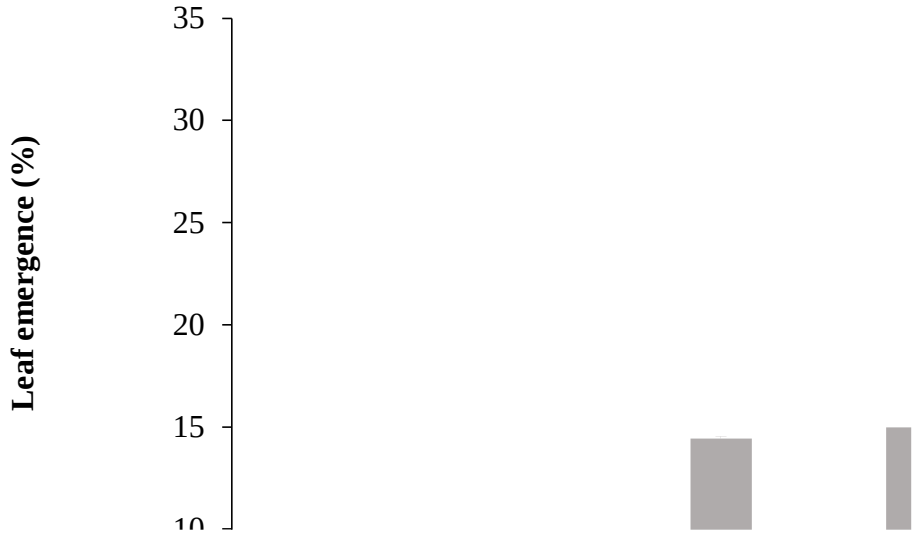

Figure 16: Sprouting rate of oyster nut cuttings in different growth media

Whereas, M+NPK stands for Mother Plant Tree Soil with NPK, M+CM stands for stands for Mother Plant Tree Soil with Cow Manure, M stands for Mother Plant Tree Soil, FS stands for Forest Top Soil, SD stands for decomposed Saw Dust and RS stands for River Sand (N=210)

From the experiment, found that the commencement of foliage production was fastest in mother plant topsoil and Nitrogen, Phosphorus and Potassium fertilizer (M+NPK); mother plant topsoil and cow manure (M+CM) and mother plant topsoil (M), which may be attributed to better aeration, water drainage and higher total porosity as reported by Adams and Pandey (2003). Even the observed highest number of leaves produced in mother plant topsoil and nitrogen, phosphorous, and potassium fertilizer (M+NPK) compared to other growth media may be attributed to the length of both lateral/tap roots, which favours the absorption of nutrients (Qadri *et al.,* 2018). The most essential macronutrients in inorganic fertilizers are nitrogen, phosphorous, and potassium fertilizer (NPK), which boost soil fertility, plant growth, and soil structural strength (Khan *et al.,* 2019). Nitrogen (N) is the most important nutrient for runner production, plant development, fruit and bud formation (Idem *et al.,* 2012). This is in consistent with Ofodile *et al.* (2013), who claimed that limiting N application may harm plant vegetative growth development in plants. Phosphorus (P) is also an important ingredient for strawberry growth and development (Simons & Leakey, 2004), and showed that such elements also helps with photosynthesis, energy transmission, sugar conversion to starches, and nutrient translocation. Among other things, potassium (K) is vital for cell elongation, carbohydrate, and sugar synthesis. According to Yoshida *et al.* (2016), NPK nutrient is important for optimal oyster nut development as well as boosting the quality of the fruit and seed firmness. However, growth analysis, which evaluates the yield and physiological phenomena of the plant, defines the primary productivity of plants.

4.5.3 Average Leaf Length in Centimeter

The length of leaves was measured at 2, 4, 6 and 8 weeks after planting (WAP) (Fig. 17). The mean leaf length for the cuttings grown in mother plant topsoil and Nitrogen, Phosphorus and Potassium (M+NPK) (9.0 cm ±0.26), M+CM (7.3 cm ± 0.26) were the longest compared to the cuttings grown in river sand (RS) (2.7 cm ± 0.78) and SD (3.1 cm ± 0.57) at 8WAP (Table 7). The average leaf length was significantly higher in cuttings grown in mother plant topsoil and Nitrogen, Phosphorus and Potassium (M+NPK) and mother plant topsoil and cow manure (M+ CM) growth media compared to the cuttings grown in other media during the experiment ($F = 21.2$, $df = 6$, $p < 0.001$, Table 8).

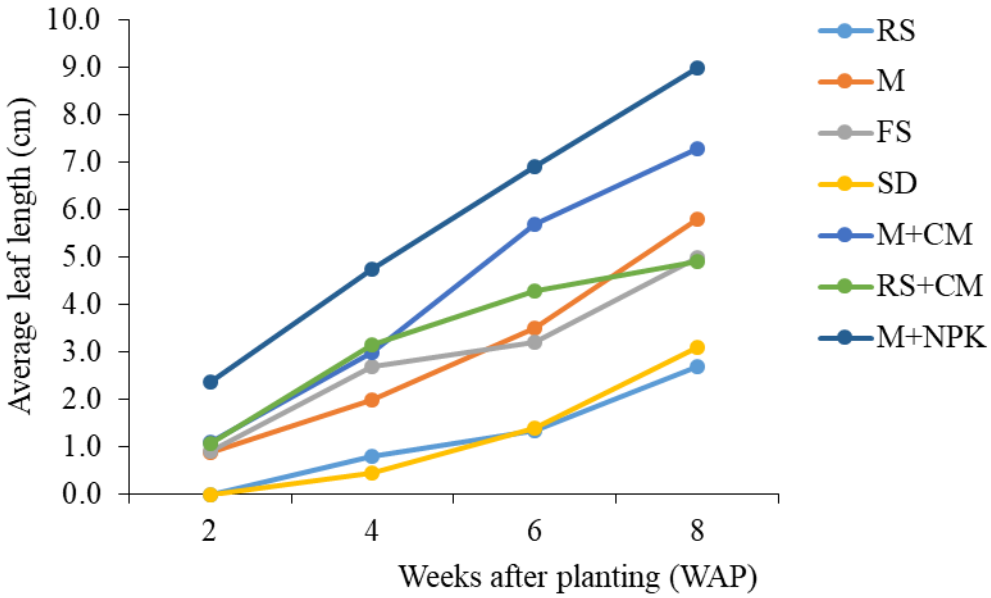

Figure 17: The average leaf length in oyster nut cuttings under different growth media

Whereas, M+NPK stands for Mother Plant Tree Soil with NPK fertilizer, M+CM stands for Mother Plant Tree Soil with Cow Manure, M stands for Mother Plant Tree Soil, FS stands for Forest Top Soil, SD stands for decomposed Saw Dust and RS stands for River Sand (N=210)

The mother plant topsoil and Nitrogen, Phosphorus and Potassium fertilizer (M+NPK) and mother plant topsoil and cow manure (M+CM) produced longer leaves compared to all other media. This is in consistent with Adams and Pandey (2003) findings that good soil media is a necessary resource for the production of healthy and successful plants.

Table 7: The average leaf length ±SE of the cuttings grown in different growth media (N=210)

	Duration	RS	M	FS	SD	M+CM	RS+CM	M+NPK
Mean	Week 2	0.00	0.880	0.900	0.00	1.10	1.08	2.36
	Week 4	0.800	2.00	2.70	0.450	3.00	3.15	4.75
	Week 6	1.35	3.50	3.20	1.40	5.70	4.30	6.90
	Week 8	2.70	5.80	5.00	3.10	7.30	4.90	9.00
Std. error mean	Week 2	0.00	0.403	0.267	0.00	0.288	0.270	0.148
	Week 4	0.389	0.650	0.597	0.241	0.447	0.597	0.359
	Week 6	0.495	0.477	0.611	0.452	0.300	0.367	0.379
	Week 8	0.775	0.327	0.365	0.567	0.260	0.348	0.258

Table 8: Games-Howell Post-Hoc Test for comparison of mean leaf length (cm) of Cuttings grown in different growth media (N=210)

GROWTH MEDIA		M	FS	SD	M + CM	RS + CM	M + NPK
RS	Mean difference	-1.83**	-1.74**	-0.025	-3.06***	-2.15***	-4.54***
	p-value	0.004	0.003	1	< .001	< .001	< .001
MPTS	Mean difference	—	0.095	1.8075**	-1.23	-0.313	-2.71***
	p-value	—	1	0.003	0.307	0.995	< .001
TFS	Mean difference		—	1.7125**	-1.33	-0.41	-2.8***
	p-value		—	0.002	0.174	0.971	< .001
SD	Mean difference			—	-3.04***	-2.12***	-4.52***
	p-value			—	< .001	< .001	< .001
MPTS + CM	Mean difference				—	0.918	-1.48
	p-value				—	0.567	0.174
RS + CM	Mean difference					—	-2.4***
	p-value					—	< .001

Note: * p < .05, ** p < .01, *** p < .001

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Based on the findings the study concludes the following:

(i) There is a substantial variation in abundance and occurrence of oyster nut climber and associated trees among different gradient and environmental factors with the highest oyster nut density being observed in higher elevations.

(ii) The oyster nut plant as a lesser known and underutilized crop provides evidence of its potential as a highly nutritious food source as well as providing medicinal, oil and manufacturing products.

(iii) Oyster nut plants have an environmental conservation value due to their dependence on compatible large trees across land use and environmental gradients of Northern Tanzania. The magnitude and patterns of genetic variation and DNA profile of the oyster nut plant have significant implications for breeding programs and genetic resource conservation.

(iv) The mother plant topsoil and NPK or cow manure are the best growth media for oyster nut cuttings in the nursery to ensure the successful establishment homestead specie, and

(v) Higher elevations and areas with higher rainfall are suitable for the growth of oyster nut plant in northern Tanzania.

5.2 Recommendations

Based on the findings, the study recommends the following:

(i) Promote the multipurpose and only oyster nut plant exist in Tanzania through workshops, and seminars to contribute to food security, environmental sustainability, and income generation for small-scale farmers in rural areas.

(ii) Mother plant topsoil and NPK or cow manure are recommended as the most suitable media for raising oyster nuts from stem cuttings.

(iii) Given that *Telfairia pedata* is the only specie that exist in the country, research institutions can take an interest in both in-situ (focusing on the “recruitment and

regeneration" of the specie) and ex-situ (promote tree retention on farms, or advocate further planting) long-term monitoring trends of *T. pedata* distribution in the country.

(iv) Exploration of the medicinal, religious, industrial, and nutritional values of oyster nuts, cuisine recipes, is necessary to expand their production areas, raise the number of consumers, and urge researchers and policymakers to consider it as a priority crop.

(v) Grafting and other micro-propagation procedures can be developed alongside genetic characterization and barcoding across and within populations in order to establish the best lines that are both climate change adaptable and quickly establish in the regions where they are planted, and

(vi) Replication to other oyster nut growing regions of Tanzania to develop sustainable production systems.

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APPENDICES

Appendix 1: Research tool (Questionnaire)

Section A: Background information

Name of Interviewer…… Date……. Household's ID number……

Village…… Hamlet……..…….. Ward…… District ……...Division ……

Name of household head ……Name of respondent…….……

Sex of respondent: 01 Male 02 Female

1. Age of a household head: 01. 18 - 30 years 02. 31 – 60 years

   03. Above 61 years

2. Education level of the respondent? 01 Primary school level 02 Secondary school level

   03 College/University level 04 Adult education 05 Non formal education

   06 Others (***specify***)……..

3. Occupation: 01 Employed 02 Farmer 03 Fisherman 04 Business/Entreprenuer

   05 Jobless (***specify***)……

4. Marital status: 01. Single 02 Married 03 Widowed 04 Divorced

   05. Other (*specify*)……..

5. Household size (number of people in household)

Age	Male	Female
< 18 years		
19 – 45 years		
>46 years		

6. Major sources of income: 01 Farming 02 Livestock keeper 03 Employed

   04 Business 05 Others **(*specify*)**……

Section B: Differences in use value and use patterns of oyster nuts in the village

1. Do you know oyster nut plant (have you seen an oyster nut plant)? 01. Yes 02. No

2. Have you ever seen oyster nut products? 01. Yes 02. No

   If **YES**, where? 01. In my farm 02. In the forest 03. Relative/Neighbors

   04. Market 05. Others (*specify*)

3. Do you use oyster nut products? 01. Yes 02. No

If **YES**, fill in the table below.

Plant part (*circle*)	General Use (*tick*)						Specific Use (*tick*)				
	Cooking	Medicine	Culture	Conservation	Ornamental	Others (*specify*)	Staple food	Soup	Salad	Barbecue	Others (*specify*)
Leaves											
Flower											
Seedilings											
Fruits											
Seeds											
Branches											
Roots											
Barks											

4. Which part of oyster nut plant do you harvest? Fill in the table below.

Part collected (*circle*)	**Season harvested	Source of collection (*Use tick*)					Where to be sold (*Use tick*)			
		From market	Collectors	Forest	Farms	Others (*specify*)	Individuals	Market	Collection points	Others (specify)
Leaves										
Flowers										
Seedlings										
Fruits										
Seeds										
Branches										
Roots										
Barks										
Others *(specify)*										

**** Mention the season of collection (*months*):……**

5. Do you sell oyster nut products? 01. Yes 02. No

If **YES,** fill in the table below.

Plant part / Activity	Leaves	Flower	Seedlings	Fruits	Branches	Roots	Bark	Others *(specify)*
What is the unit of selling *?								
What is the price per unit sold? Tsh								
How much Tsh do you get per month?								

*Unit of selling could be 01. Gm/Kg 02. Pieces 03. Bunches 04. Others *(specify)*

6. How frequent do you sell the oyster nut products? Fill in the table below by ***ticking***.

Frequency

Plant part

	Daily	Weekly	Bi-weekly	Monthly	Others (*specify*)
Leaves					
Flower					
Seedlings					
Fruits					
Seeds					
Branches					
Roots					
Bark					
Others *(specify)*					

7. Out of the amount accrued from selling oyster nuts, what is the percentage used for purchasing items? Fill in the table below.

Activity Plant part	Percentage used for home consumption	Percentage used for other items/uses	Others (*specify*)
Leaves			
Flower			
Seedlings			
Fruits			
Seeds			
Branches			
Roots			
Bark			
Others *(specify)*			

8. What are the constraints to expand the uses of the oyster nut products in this village? Fill in the table below.

Reasons for constraints							Reasons & Source for Irrigation	Reasons for using fertilizer	Type of fertilizer used	Reasons for using manure	Type of manure used	Others (specify)
Pests	Diseases	Insects	Weather pattern	Tree species &size	Land issues (*specify*)	Others (specify)						

9. How is the market demand of oyster nut products in your village/area? Fill the table below.

Frequency

Activity	Decreased	Low	Increased	High	very high	Not changed (the same)	Others (specify)
Market demand of Oyster nut products in the village							
Distance for collecting oyster nut products							

10. Who collects and sell the oyster nut products in your household? Fill in the table below.

Product (*circle*)	**Anae kusanya/okota**					**Anae Uza**				
	Husband	**Wife/ mothers**	**Children**	**Neighbors**	**Others (*specify*)**	**Husband**	**Wife/ mothers**	**Children**	**Neighbors**	**Others (*specify*)**
Leaves										
Bark										
Fruits										
Seeds										
Roots										
Flowers										
Branches										
Seedlings										
Others (specify)										

11. Do you store/preserve the oyster nut products? 01. Yes 02. No

If Yes, why do you store/preserve them? 01. To use during shortage period 02. For selling

03. Eat/cook 04. Others (*specify*)……..

12. Storage/preservation methods for oyster nut products? Fill in the table below.

Activity / Frequency	1 Week	2 weeks	1 month	3 months	6 months	1 year	> 1 year	Others (specify)
How long do you store the oyster nut products								
Methods of storage								

Section C: Domestication and conservation strategies by different ethnic communities and predict the future management of oyster nuts in the villages

1. For how long have you been residing in this village/area? …….

2. What is the current availability of oyster nut plants compared to the past ten years?

01. Decrease 02. Increase 03. Same/Constant 04. I don't know

05. Others ***(specify)***

If it is **decreasing,** what are the reasons? ……..…

3. If it is increasing, what are the reasons (plant/product)? ……

4. What are the major threats of oyster nut plants in this village/area?

Major threats causing oyster nuts to	Decrease	Low	Increase	High	very high	Not changed (the same)	Others (specify)
Disease (specify)							
Pests (specify)							
Climate change							
Wildlife (specify)							
Soil nutrients (specify)							
Land issues (specify)							
Others (specify)							

5. If you collect oyster nut products from the forest. How far is the forest? ……(km)

6. Has the distance changed? 01. Yes 02. No 03. I don't know 04. Reasons *(if any)*

If Yes, fill in the table below.

Reasons of change of distance to the forest	Decreased	Increased	Not changed (the same)	Others (specify)
Relocated to another hamlet/village				
The plants died				
Trees harvested				
Drought				
Diseases/pests				
Others (specify)				

6. If you have planted the oyster nut plants ? Fill the table below.

Propagation methods (*Tick*) and Mention its source				Interval of planting (*Tick*)			
Seeds	Wildings	Cuttings	Others (Specify)	Yearly	Within 2 years	> 2 years	Others (specify)

8. How many fruits do you collect per season. Fill in the table below.

Collection/Duration	Daily	Weekly	Bi-weekly	Monthly	Others (*specify*)
Number of fruits collected					
Number of seeds per fruits					
Others *(specify)*					

9. Details of tree species.

Tree species (provide names)		
Grows well (Preferable ones)	Doesn't grow well (Not preferable ones)	Reasons (if any)

10. Are there any traditions believes based on oyster nut plants ? 01. Yes 02. No

03. I don’t know

If **YES**, which are those believes? …….

11. Are there any sacred activities which are take place on trees with oyster nut plant?

01. Yes 02. No 03. I don't know

If **YES**, what are the reasons? …….

12. Do you agreed that harvesting of the oyster nut plant parts can threaten the plant? 01. Yes 02. No 03. I don't know

If **YES**, mention any conservation strategies used for the oyster nut plants in this village.

……..

…….

……..

SECTION D: Local people's perception on measured ethno-botanical importance of different parts.

1. Do you agree that oyster nut seeds has a lot of nutrients compared to other nuts?

01. Yes 02. No 03. I don't know

If **YES**, What types of nutrients does the oyster nut/seeds has?

i) …….…….

ii) …….

iii) …….

iv) …….

2. Which types of nuts/seeds you think has low nutrients content compared to the oyster nuts/seeds?

i) …….…….

ii) …….

iii) …….

iv) …….

3. Do you use oyster nut leaves? 01. Yes 02. No

If **YES**, Mention the uses.

i) …….

ii) …….

iii) …….

iv) …….

4. Do you agree that oyster nuts have medicinal values? 01. Yes 02. No

03. I don't know

If **YES**, fill in the table below.

Plant part	Processed form			Curing purposes (medicinal)						
	Powder form	Small blocks (pieces)	Others (specify)	Stomach aches	Head aches	Back pains	Chest pains	Pregnant women	Arthritis	Others (specify)
Leaves										
Bark										
Fruits										
Seeds										
Roots										
Flowers										
Branches										
Seedlings										
Others (specify)										

5. Do you have any opinions/suggestions on increasing the number of trees and oyster nuts in the village?

i) ……..

ii) ……..

iii) ……..

iv) ……..

6. Are ther any other observations/comments you want to add which are not in the questionnaire?

i) ……..

ii) ……..

iii) ……..

iv) ……..

Appendix 2: Focus group discussion checklist

1. What are the livelihoods resources present in the area?
2. What types of *Oyster nuts* products do you collect in your village?
3. Where do you collect the *Oyster nuts* products from?
4. Which are the parts and uses of *Oyster nuts* plant collected?
5. Which plant parts of the *Oyster nuts* tree is collected and how is processed?
6. What are the seasons of collection of the *Oyster nuts* products?
7. What are nutrients contents obtained from the *Oyster nuts* leaves and fruit pulp?
8. What are the medicinal values obtained from the *Oyster nuts* products?

Appendix 3: Consent form for the cross-sectional study

I..... voluntarily agree to participate in the oyster nut research. I understand that the participation involves:

(a) I agree that my interview be audio-recorded and photos taken;

(b) I agree my real or nick names and family names be used in the research;

(c) I agree to pick some plant parts, seeds or soil samples for the research and

(d) I agree my photos, names videos/clips be used in seminars, workshops, media and social media for training purposes.

Participants' statement

I …….have read and understood the explained information provided by the investigator, and I agree to participate in this study. I have the option to withdraw at any time without giving reasons.

Participant's Name:……Signature…… Date……..

Investigator's Name :……Signature ……....Date……..

Appendix 4: The consensus sequences of the studied 20 *T. pedata* strains

A1-FR_600F_H01_3730XL

TCKWTWWWWWGRATTTATAAATCACAGGCTGAACAGGTGAAATCAAGGGACATT
ACTTGAATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCG
AGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGTGGATTCACTGCA
AATACTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCAYCGTG
CRATGCATGCYGTTATTGA

A2_384F_A02_3730XL

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CGGTCTTTCACAACGAGATCCACCTATACAGTAACGGTATTTAATTATGAAGATTAG
TTGGGTAGCTGACCCTCTTAGTCCGTTCTTGGAAGAATAAGGCCATAATCTTTCTGTT
AAATAGGATTTCCTCTGCTTAATGGATAAGCATTTGTTACCAATGGGGAATTCTTTCT
CATCTAAAATTGAAAATTGAGGTGATTGGATTTRCACCAATAGAAACA

A2-FR_600F_G01_3730XL

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AGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGTGGATTCACTGCA
AATACTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCAYCGTG
CRATGCATGCYGTTATTGA

A2-FR_600F_G01_3730XL

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GGTCTTTCACAACGAGATCCACCTATACAGTAACGGTATTTAATTATGAAGATTAGT
TGGGTAGCTGACCCTCTTAGTCCGTTCTTGGAAGAATAAGGCCATAATCTTTCTGTTA
AATAGGATTTCCTCTGCTTAATGGATAAGCATTTGTTACCAATGGGGAATTCTTTCTC
ATCTAAAATTGAAAATTGAGGTGATTGGATTTRCACCAATAGAAACA

A3_384F_C02_3730XL

GTGGTWAACTTGTACAGTTCACCCTCTTTGGCTCTACCCATGAATTAGATAGTAATC
GGTCTTTCACAACGAGATCCACCTATACAGTAACGGTATTTAATTATGAAGATTAGT
TGGGTAGCTGACCCTCTTAGTCCGTTCTTGGAAGAATAAGGCCATAATCTTTCTGTTA
AATAGGATTTCCTCTGCTTAATGGATAAGCATTTGTTACCAATGGGGAATTCTTTCTC
ATCTAAAATTGAAAATTGAGGTGATTGGATTTRCACCAATAGAAACA

A3-FR_600F_D01_3730XL

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TGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCGAGAGAAT
TGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGTGGATTCACTGCAAATACTA
GCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCAYCGTGCRATGCA
TGCYGTTATTGA

A7_384F_C05_08

CTWAMMTTTGGKWACRGTTCACCCTCTTTGGCTCTACCCATGAATTAGATAGTAAT
CGGTCTTTCACAWCGAGATCCACCTATACAGTAACGGTATTTAATTATGAAGATTAG
TTGGGTAGCTGACCCTCTTAGTCCGTTCTTGGAAGAATAAGGCCATAATCTTTCTGTT
AAATAGGATTTCCTCTGCTTAATGGATAAGCATTTGTTACCAATGGGGAATTCTTTCT
CATCTAAAATTGAAAATTGAGGTGATTGGATTTRCACCAATAGAAAC

A7-FR_600F_H12_3730XL

CYMKKKSCCWWAWTTAWGACTTTTAATMCAGGCTGAAACAGGTGAAATCAAGGG
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TTGCGAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGTGGATTCA
CTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCA
YCGTGCRATGCATGCYGTTATTGA

A8_384F_E05_14

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CGGTCTTTCACAACGAGATCCACCTATACAGTAACGGTATTTAATTATGAAGATTAG
TTGGGTAGCTGACCCTCTTAGTCCGTTCTTGGAAGAATAAGGCCATAATCTTTCTGTT
AAATAGGATTTCCTCTGCTTAATGGATAAGCATTTGTTACCAATGGGGAATTCTTTCT
CATCTAAAATTGAAAATTGAGGTGATTGGATTTRCACCAATAGAAACAATG

A10_384F_B02_3730XL

TGGTWAACTTGTAMGTTCACCCTCTTTGGCTCTACCCATGAATTAGATAGTAATCGG
TCTTTCACAACGAGATCCACCTATACAGTAACGGTATTTAATTATGAAGATTAGTTG
GGTAGCTGACCCTCTTAGTCCGTTCTTGGAAGAATAAGGCCATAATCTTTCTGTTAA
ATAGGATTTCCTCTGCTTAATGGATAAGCATTTGTTACCAATGGGGAATTCTTTCTCA
TCTAAAATTGAAAATTGAGGTGATTGGATTTRCACCAATAGAAACA

A10_FR_600F_C01_3730XL

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TGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCGAGAGAAT
TGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGTGGATTCACTGCAAATACTA
GCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCAYCGTGCRATGCA
TGCYGTTATTGA

A12_FR_600F_F05_17

TGGAGCATTTATAATCACAGGCTGAAACAGGTGAAATCAAGGGACATTACTTGAAT
GCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCGAGAGAATT
GGGAGTTCCTATCGTAATGCATGACTACTTAACAGGTGGATTCACTGCAAATACTAG
CTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCAYCGTGCRATGCAT
GCYGTTATTGA

H1_FR_600F_B01_3730XL

GGTARCCATTTATAAATCACAGGCTGAAACAGGTGAAATCAAGGGACATTACTTGA
ATGCTACTGCAGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTGCGAGAGAA
TTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGTGGATTCACTGCAAATACT
AGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCAYCGTGCRATGC
ATGCYGTTATTGA

H3_384F_B05_05

AATTGGTTACTTGTAMAGTTCACCCTCTTTGGCTCTACCCATGAATTAGATAGTAAT
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TTGGGTAGCTGACCCTCTTAGTCCGTTCTTGGAAGAATAAGGCCATAATCTTTCTGTT
AAATAGGATTTCCTCTGCTTAATGGATAAGCATTTGTTACCAATGGGGAATTCTTTCT
CATCTAAAATTGAAAATTGAGGTGATTGGATTTRCACCAATAGAAACA

H4_384F_D02_3730XL

GGGTTAAMTTGTACAGTTCACCCTCTTTGGCTCTACCCATGAATTAGATAGTAATCG
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GGGTAGCTGACCCTCTTAGTCCGTTCTTGGAAGAATAAGGCCATAATCTTTCTGTTA
AATAGGATTTCCTCTGCTTAATGGATAAGCATTTGTTACCAATGGGGAATTCTTTCTC
ATCTAAAATTGAAAATTGAGGTGATTGGATTTRCACCAATAGAAACA

L1_384F_D05_11

AATKKCTWAACTTGTACAGTTCACCCTCTTTGGCTCTACCCATGAATTAGATAGTAA
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GTTGGGTAGCTGACCCTCTTAGTCCGTTCTTGGAAGAATAAGGCCATAATCTTTCTGT
TAAATAGGATTTCCTCTGCTTAATGGATAAGCATTTGTTACCAATGGGGAATTCTTTC
TCATCTAAAATTGAAAATTGAGGTGATTGGATTTRCACCAATAGAAACA

L1_FR_600F_A01_3730XL

GGACSGTTTWTGMMTTTATAATCACAGGCTGAAACAGGTGAAATCAAGGGACATTA
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GAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGTGGATTCACTGCAA
ATACTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCAYCGTGC
RATGCATGCYGTTATTGA

O1_384F_F02_3730XL

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GGTCTTTCACAACGAGATCCACCTATACAGTAACGGTATTTAATTATGAAGATTAGT
TGGGTAGCTGACCCTCTTAGTCCGTTCTTGGAAGAATAAGGCCATAATCTTTCTGTTA
AATAGGATTTCCTCTGCTTAATGGATAAGCATTTGTTACCAATGGGGAATTCTTTCTC
ATCTAAAATTGAAAATTGAGGTGATTGGATTTRCACCAATAGAAAC

S3_384F_E02_3730XL

TGCCAACTTGTACAGTTCACCCTCTTTGGCTCTACCCATGAATTAGATAGTAATCGGT
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GTAGCTGACCCTCTTAGTCCGTTCTTGGAAGAATAAGGCCATAATCTTTCTGTTAAAT
AGGATTTCCTCTGCTTAATGGATAAGCATTTGTTACCAATGGGGAATTCTTTCTCATC
TAAAATTGAAAATTGAGGTGATTGGATTTRCACCAATAGAAACA

RESEARCH OUTPUTS

(i) Journal Papers

Shayo, P. F., Mbega, E. R., & Treydte, A. C (2021). The Potential of Oyster Nuts (*Telfairia pedata*) for Environmental Conservation and Food Security in Tanzania: A Review. *Human Ecology*, 49, 495-504. https://doi.org/10.1007/s10745-021-00249-6

Shayo, P. F., Treydte, A. C., & Mbega, E. R. (2022). The Ethnobotany of oyster nuts (*Telfairia pedata*) in Northern Tanzania. *Biodiversity and Environmental Sciences*, 20 (1), 43-52.

Shayo, P. F., Mbega, E. R., & Treydte, A. C. (2022). Growth performance of the neglected crop *Telfairia pedata* across elevation and climate in Northern Tanzania. *Trees, Forests and People*, 7, (2022), 1-8. https://doi.org/10.1016/j.tfp.2022.100216

(ii) Poster Presentation

Poster and Conference Presentations

Presentation 1

Presented poster on the Potential of Underutilized *Telfairia pedata* crop for Nutrition, Food Security and Livelihood in Northern Tanzania, during the XV World Forestry Congress on 5th May 2022 at Coex, Seoul-Korea

XV WORLD FORESTRY CONGRESS
Building a Green, Healthy and Resilient Future with Forests
2–6 May 2022 | Coex, Seoul, Republic of Korea

Potential of Underutilized *Telfairia pedata* crop for Nutrition, Food security and Livelihoods in Northern Tanzania

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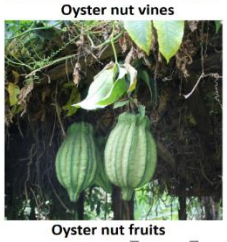
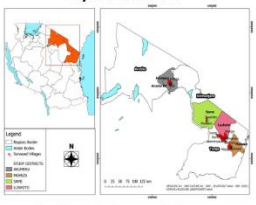
Introduction

- Neglected or underused plants are those ignored by science and development although are well adapted and competitive in nature.
- Telfairia belongs to Curcurbitaceae family and small genus of flowering plants in the squash family, which are native to Africa.
- *Telfairia pedata* (Oyster nut or Mkweme) is native to Tanzania and has important nutritional and healthy oily seeds.
- It is woody and herbaceous dioecious vine, its gourd encloses 80-150 edible nuts and can weigh 7 to 20 kg.
- The seeds can last up to 8 years and have a life cycle up to 20 years.

Oyster nut vines



Oyster nut fruits



Map of Study area Northern Tanzania (Arumeru, Same, Lushoto , Bumbuli and Muheza Districts)

Seeds and Kernel

It represents a very hard seed with perforated skin that needs to be removed. Cracking the nut with something hard is the most effective way of removing the kernel from the nut.

Split Oyster nut fruit

Oyster nut seeds

Benefits

Medicinal benefits:
- Gives a tonic skin to women who have just given birth;
- The nuts are valuable to pregnant and lactating mothers to facilitate milk production (lactogenic properties).
- Have medicinal value e.g. anti-rheumatism, gastric, healing after giving birth

Agroforestry, climate change & ornamental benefits:
- Known to be good sink with low maintenance;
- Is used as an ornamental due to its beautiful foliage;
- It is planted alongside host tree species in combination with banana and coffee in parts of Mt Kilimanjaro and Mt Meru's rich agroforestry systems.

Human and Livestock benefits:
- The nuts are edible and can be eaten either raw, pickled or roasted for confectionery purposes;
- Have versatile, mildly sweet oil from the oyster seeds, used in cooking, cosmetics, soap and candle-making;
- Food for livestock fodder (after pressing the edible oil from the kernels);

Justification

- Unknown distribution (naturally and cultivated)
- Little is known on how growth, yield etc..
- Depends on environmental and agricultural factors
- Promote strategies for cultivating this native and highly nutritious food species
- Characterize morphological and genetic diversity of oysternuts which are important for long-term biological conservation
- Unknown propagation methods and post-harvest technologies

Therefore, this study will generate information that can be integrated into sustainable conservation and utilization of oyster nuts.

Objectives

General Objective:
The main objective of this study is to investigate the natural and farmed distribution, plant characteristics, assess the local knowledge and develop propagation methods for conservation of oyster nuts by small holder farmers in northern Tanzania.

Specific Objectives:
- To map the distribution of oysternut populations across different land uses and environmental factors (soil, elevation, temperature, rainfall, relative humidity);
- To assess the growth pattern and yield of oysternut under different cultural and environmental (soil, elevation, temperature, rainfall) conditions;
- To examine the perceptions of local communities on the values of oysternut for their livelihood (food, health, income);
- To characterize oysternut (morphological/phenotypic and genotypic) populations from the study sites;
- To develop improved propagation methods for improved seedlings availability to farmers and
- To examine the extent of domestication and conservation strategies by different ethnic communities and suggest management of oysternut.

Methodology

- Data were collected between September 2019 and March 2020.
- Socio-economic survey was conducted through questionnaires, Key informant interviews and focus group discussions.
- A total of 346 oyster nut farmers were purposively interviewed throughout the study area.
- Respondents were selected through snow ball method
- Lab samples were collected purposively from three sites (Kilimanjaro, Tanga and Arusha regions) during harvest period.
- 6 plants were selected from each site, to give 18 total plants and from each plant, 1 kg of nuts were weighed to give a total of 18 kg of samples
- Collected samples were cleaned, sun-dried
- Proximate analysis (protein, fats, carbohydrates, ash) was done by AOAC, 2000 (Official method of analysis)
- Fatty acid analysis was done by Gas chromatography
- Mineral analysis was done by Inductively coupled plasma - optical emission spectrometry (ICP-OES)
- Free fatty acids (FFA) was done through titration methods.

Data analysis

- Data collected through questionnaires were subjected to both qualitative and quantitative analysis in SPSS vs. 16 computer software.
- Frequencies of responses were descriptively analyzed and expressed as percentages.
- Data were tested for normality using QQ plot test
- Inferential statistics were performed using Chi-square (X^2) to ascertain the statistical differences between ethnobotanical uses
- Cross-tabulation was used to summarize the descriptive information from the respondents in form of frequency, percentage form.
- Descriptive analysis was used to obtain mean nutritional values.

Results, conclusion and recommendation

Out of 100g kernel, oyster nuts are highly nutritious oilseeds with:
- ✓ Water 3.6%
- ✓ Protein 25%
- ✓ Fat 68%
- ✓ Minerals,: Phosphorus, Magnesium, Potassium, Calcium, Zinc, Selenium, Iron, Sodium

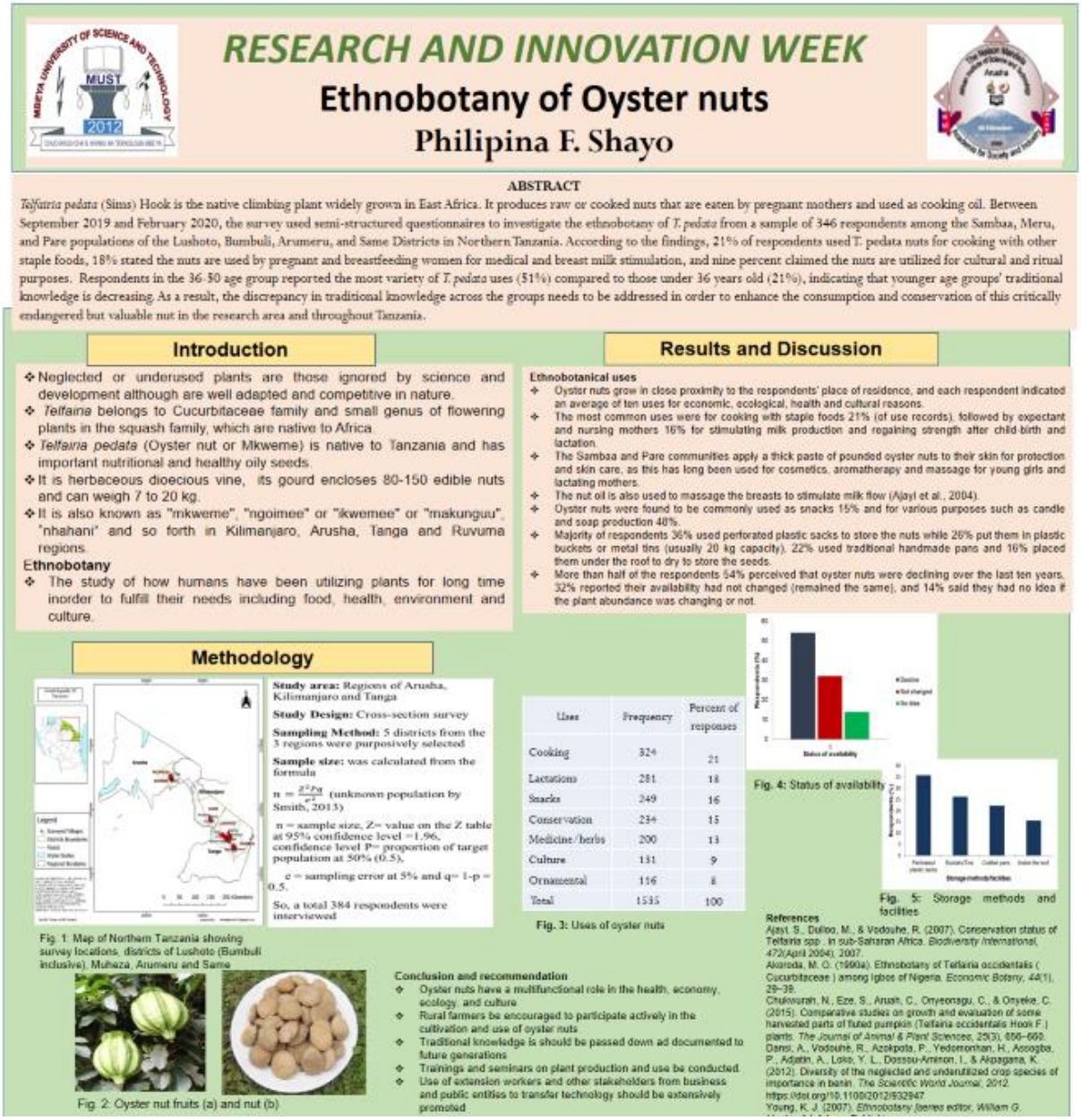
Fatty acid:
-Oleic acid 11.5%; Linoleic acid 48%; Linolenic acid 5%, Palmitic acid 28%, Omega 3 and Omega 6 and others 8.5%

- Oysternuts can be an additional source of income generation as well as for agricultural bio-diversification if the importance of optimizing growing conditions is considered.
- Research and development be promoted e.g. improved propagation methods (seedlings, cuttings, grafting) for varieties be available to low income farmers
- To promote oyster nuts to be primarily grown to be used as spice to foods, by pregnant and lactating mothers and as a substitute for cooking oil;

Acknowledgment: The Nelson Mandela African Institution of Science and Technology (NM-AIST); Centre for Research, Agricultural Advancement, Teaching Excellence and Sustainability in Food and Nutrition Security (CREATES); IDEAL WILD Inc. and The Mbeya University of Science and Technology MUST) are acknowledged for the their cordial support.

Presentation 2

Poster on the Ethnobotany of Oyster nuts during MUST Research and Innovation Week, 3rd to 7th December, 2021 at MUST, Mbeya (on my absence).



RESEARCH AND INNOVATION WEEK

Ethnobotany of Oyster nuts

Philipina F. Shayo

ABSTRACT

Telfairia pedata (Sims) Hook is the native climbing plant widely grown in East Africa. It produces raw or cooked nuts that are eaten by pregnant mothers and used as cooking oil. Between September 2019 and February 2020, the survey used semi-structured questionnaires to investigate the ethnobotany of *T. pedata* from a sample of 346 respondents among the Sambaa, Meru, and Pare populations of the Lushoto, Bumbuli, Arumeru, and Same Districts in Northern Tanzania. According to the findings, 21% of respondents used T. pedata nuts for cooking with other staple foods, 18% stated the nuts are used by pregnant and breastfeeding women for medical and breast milk stimulation, and nine percent claimed the nuts are utilized for cultural and ritual purposes. Respondents in the 36-50 age group reported the most variety of *T. pedata* uses (51%) compared to those under 36 years old (21%), indicating that younger age groups' traditional knowledge is decreasing. As a result, the discrepancy in traditional knowledge across the groups needs to be addressed in order to enhance the consumption and conservation of this critically endangered but valuable nut in the research area and throughout Tanzania.

Introduction

- Neglected or underused plants are those ignored by science and development although are well adapted and competitive in nature.
- *Telfairia* belongs to Cucurbitaceae family and small genus of flowering plants in the squash family, which are native to Africa.
- *Telfairia pedata* (Oyster nut or Mkweme) is native to Tanzania and has important nutritional and healthy oily seeds.
- It is herbaceous dioecious vine, its gourd encloses 80-150 edible nuts and can weigh 7 to 20 kg.
- It is also known as "mkweme", "ngoimee" or "ikwemee" or "makunguu", "nhahani" and so forth in Kilimanjaro, Arusha, Tanga and Ruvuma regions.

Ethnobotany

- The study of how humans have been utilizing plants for long time inorder to fulfill their needs including food, health, environment and culture.

Methodology

Study area: Regions of Arusha, Kilimanjaro and Tanga

Study Design: Cross-section survey

Sampling Method: 5 districts from the 3 regions were purposively selected

Sample size: was calculated from the formula

$n = \frac{Z^2 Pq}{e^2}$ (unknown population by Smith, 2013)

n = sample size, Z= value on the Z table at 95% confidence level =1.96, confidence level P= proportion of target population at 50% (0.5),

e = sampling error at 5% and q= 1-p = 0.5.

So, a total 384 respondents were interviewed

Fig. 1: Map of Northern Tanzania showing survey locations, districts of Lushoto (Bumbuli inclusive), Muheza, Arumeru and Same

Fig. 2: Oyster nut fruits (a) and nut (b)

Results and Discussion

Ethnobotanical uses

- Oyster nuts grow in close proximity to the respondents' place of residence, and each respondent indicated an average of ten uses for economic, ecological, health and cultural reasons.
- The most common uses were for cooking with staple foods 21% (of use records), followed by expectant and nursing mothers 16% for stimulating milk production and regaining strength after child-birth and lactation.
- The Sambaa and Pare communities apply a thick paste of pounded oyster nuts to their skin for protection and skin care, as this has long been used for cosmetics, aromatherapy and massage for young girls and lactating mothers.
- The nut oil is also used to massage the breasts to stimulate milk flow (Ajayi et al., 2004).
- Oyster nuts were found to be commonly used as snacks 15% and for various purposes such as candle and soap production 48%.
- Majority of respondents 36% used perforated plastic sacks to store the nuts while 26% put them in plastic buckets or metal tins (usually 20 kg capacity), 22% used traditional handmade pans and 16% placed them under the roof to dry to store the seeds.
- More than half of the respondents 54% perceived that oyster nuts were declining over the last ten years, 32% reported their availability had not changed (remained the same), and 14% said they had no idea if the plant abundance was changing or not.

Uses	Frequency	Percent of responses
Cooking	324	21
Lactations	281	18
Snacks	249	16
Conservation	234	15
Medicine/herbs	200	13
Culture	131	9
Ornamental	116	8
Total	1535	100

Fig. 3: Uses of oyster nuts

Fig. 4: Status of availability

Fig. 5: Storage methods and facilities

Conclusion and recommendation

- Oyster nuts have a multifunctional role in the health, economy, ecology, and culture.
- Rural farmers be encouraged to participate actively in the cultivation and use of oyster nuts.
- Traditional knowledge is should be passed down ad documented to future generations.
- Trainings and seminars on plant production and use be conducted.
- Use of extension workers and other stakeholders from business and public entities to transfer technology should be extensively promoted.

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Presentation 3

Poster on Nut and Oil seed: The Oysternuts, presented at the Terra Madre Slow Food Conference, Turin-Italy on 25th September 2019 (On my absence)

Flyer (English version)

Potential of OYSTER NUTS
To improve Community livelihoods and Biodiversity Conservation

What is an Oysternut (Telfairia pedata)?

- ✓ *It is a perennial and herbaceous dioecious vines, its gourd encloses 80-150 edible nuts and can weigh 7 to 20kg.*
- ✓ *Belongs to Cucurbitaceae family and small genus of flowering plants in the squash family, which are native to Tanzania (Tanga, Arusha, Mbeya, Morogoro, Ruvuma, Njombe and Kilimanjaro regions), Uganda and Mozambique.*
- ✓ *The seeds can last up to 8 years and have life span up to 20 years*
- ✓ *It has a very important nutritional and healthy oily seeds.*

It's Uses:

- ✓ *The nuts are edible and can be eaten either fresh, raw, pickled or roasted for confectionery*
- ✓ *purposes;*
- ✓ *The nuts are valuable to pregnant and lactating mothers to facilitate milk production*
- ✓ *(due to lactogenic properties);*
- ✓ *It is children's favorite snack;*
- ✓ *Versatile, mildly sweet oil from the oyster seeds is used for cooking, cosmetics, soap and*
- ✓ *candle-making;*
- ✓ *Anti-rheumatism medicine, gastric problems;*
- ✓ *Gives a tonic oil from the seeds and given to women who have just given birth;*
- ✓ *Food for livestock fodder (after pressing the edible oil from the kernels);*
- ✓ *Known to be good Carbon dioxide sink with low maintenance;*
- ✓ *Is ornamental due to its beautiful foliage;*
- ✓ *In areas of Mt Kilimanjaro and Mt Meru its part of the rich agroforestry systems where it is grown in combination with banana and coffee.*

Did you know for an Oyster nut with 100g kernel, you can get!

- *Ash 2%*
  Protein
  25% Fat
  68% Carbohydrates
  5%
- ***Fatty acid:*** *Linoleic acid 47%*
- ***Minerals:*** *magnesium (Mg) 150mg/100g*

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Magazine (Swahili version)

The Mkulima Mbunifu magazine is a monthly publication that provides East African smallholder farmers with educational information on Ecological Sustainable Agriculture (ESA).

https://mkulimambunifu.org/

biovision

Mkulima Mbunifu

Jarida la Kilimo Endelevu Afrika Mashariki | Toleo la 103 | Aprili, 2021

Mkweme, mmea wa asili wenye faida lukuki

Erica Rugabandana

Kweme ni nini?

- Kweme (Telfairia pedata, Kiingereza – oysternut) ni mmea wa asili utambaao, uliopo kwenye familia ya Curcurbitaccae ambao unapatikana duniani, Tanzania (Tanga, Arusha na Kilimanjaro), pamoja na nchi za Msumbiji na Uganda.
- Kawaida tunda lake linakuwa na uzito wa kilo 7 hadi 20 na lina mbegu au karanga yapata 80-150
- Mbegu zikihifadhiwa vizuri kwenye mazingira yasiyokuwa na unyevunyevu zinaweza kaa hadi miaka 8 bila kuharibika wala kupoteza ubora wake
- Mmea huo unaweza ishi hadi miaka 20 pasipo kushambuliwa na wadudu wala magonjwa

Matumizi na faida za mkweme

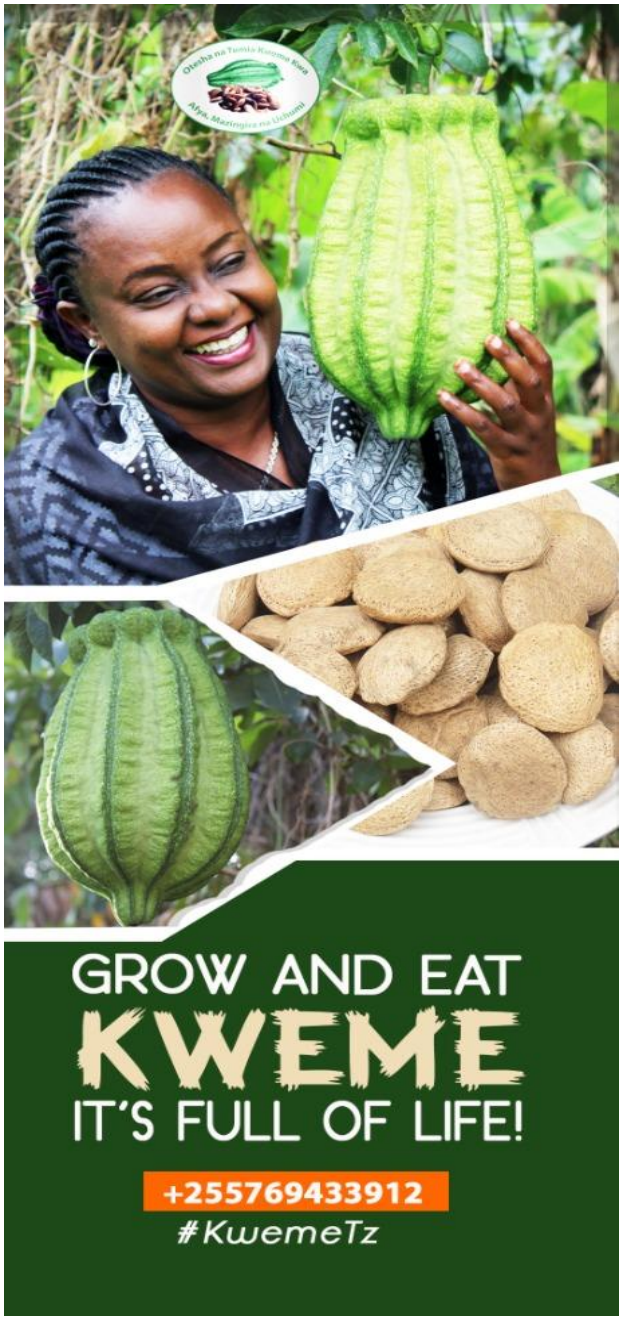
- Mbegu zake zinaweza kuliwa zikiwa mbichi, kukaangwa, kuchomwa kwenye majivu, kuchanganywa na chakula kama ndizi, mboga za majani nk. Pia, hutumika kwenye vyakula vya kuokwa jamii ya biskuti, mikate na keki;
- Mbegu au karanga zake zinatumika sana kwa wakina mama wanyonyeshao watoto ili kuongeza wingi wa maziwa kwa sababu zitokanazo na lactogenic properties,
- Mbegu au karanga zake zinatumika kwenye kupikia, kutengeneza sabuni, mishumaa na urembo malimbali;
- Mbegu au karanga za kweme zinasaidia kutibu magonjwa mbalimbali mwilini ikiwa ni Pamoja na ugonjwa wa jongo (rheumatism), matatizo ya tumbo;
- Baada ya kukamua mbegu au karanga za mkweme, mabaki yake hutengeneza chakula cha mifugo;

... inaendelea uk. 8.

Banners

Were used during exhibitions and side events inorder to promote Oyster nut to the audience.

(a) English version



(b) Swahili version