MORPHO-MOLECULAR CHARACTERISTICS AND PHYSICO-CHEMICAL PROPERTIES OF *BALANITES AEGYPTIACA* (L.) DELILE IN THE SAHELIAN ZONE OF NIGERIA

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CERTIFICATION

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DEDICATION

This research work is dedicated to the Omnipotent, Omnipresent, and Omniscient God who made everything beautiful in His time.

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ABSTRACT

Balanites aegyptiaca (BURKAN) is a wild fruit tree of high ethnomedicinal importance in the Sahelian zone of Nigeria for treating ailments like typhoid and malaria. The wild populations of the species are threatened by overexploitation and habitat loss. Variation in plant leaf, fruit morphology, genetic diversity, and physico-chemical properties from different locations which are essential for superior trait selection and vital to developing strategies for its domestication and conservation, is limited in Nigeria. This study was therefore conducted to determine the fruit and leaf morphology, physico-chemical and molecular characteristics of *Balanites aegyptiaca* in the Sahelian zone of Nigeria.

Eight locations in five states: Baure and Mashi (Katsina), Buratai (Borno), Dumsai and Gashua (Yobe), Gamawa (Bauchi), Guri and Kirikasama (Jigawa) were purposively selected, based on the availability of *Balanites aegyptiaca* trees. Ten mature trees were randomly selected from each location. Thirty ripe fruits and leaves were randomly collected from each tree. Fruit Length (FL, cm), Fruit Weight (FW, g), Fruit Thickness (FT, cm), and Pulp Weight (PW, g) were determined. Leaf morphology: Leaf Length (LL, cm), Leaf Width (LW, cm), and Leaf Thickness (LT, cm) were measured. Oil was extracted from the fruit kernels obtained from each location using soxhlet extraction method. Physico-chemical properties of extracted oil: refractive index, viscosity (cP), acid, and iodine values (mgKOH/g) were analysed. Genetic characteristics of selected trees were determined using chloroplast gene sequences of matK region. Nucleotide diversity (Pi), Parsimony informatics sites (Ps), Polymorphic sites (S), and average number of nucleotide difference (k) were determined following standard procedures. Data were analysed using descriptive statistics, cluster analysis, and ANOVA at $\alpha_{0.05}$.

The FL varied significantly from 2.45±0.31 (Dumsai) to 3.08±0.26 (Kirikasama), while FW ranged from 1.77±0.19 (Gashua) to 2.13±0.16 (Baure). Baure had the highest FT (1.84±0.39), while Guri had the least, (1.41±0.15). The PW significantly decreased from 4.44±1.87 (Gamawa) to 2.18±1.33 (Buratai). The LL, LW, and LT significantly increased from 0.41±0.05 (Dumsai) to 0.62±0.35 (Baure); 0.21±0.13 (Buratai) to 0.44±0.19 (Baure); and 0.41±0.05 (Dumsai) to 0.62±0.35 (Baure), respectively. This supported the feasibility of location as a criterion for selection in trait improvement. Refractive index and viscosity increased from 1.36±0.15 (Mashi) to 1.48±0.06 (Gamawa) and 41.33±2.08 (Gashua) to 48.67±2.52 (Buratai), respectively. Acid and iodine values varied significantly from 1.36±0.07 (Dumsai) to 2.11±0.07 (Baure) and 67.07±1.53 (Gamawa) to 85.33±2.52 (Baure), respectively. The Pi and Ps varied from 0.002 (Dumsai) to 0.264 (Mashi) and 0.00 (Dumsai) to 2.00 (Mashi), respectively. The S and k ranged from 3.0 (Dumsai) to 302.00 (Mashi) and 2.00 (Dumsai) to 151.70 (Mashi), respectively. The high genetic diversity in Mashi signified germplasm potential for species improvement. Species population in Baure and Mashi formed a distinct cluster with the highest bootstrap value (100), while the other populations formed a single cluster with bootstrap value of 4.

The genetically diverse population in Mashi is a potential source for superior germplasm required for the domestication and improvement of *Balanites aegyptiaca* and could play vital roles in germplasm collection.

Keywords: Desert date, Plant genetic diversity, *MatK* gene, Underutilised tree species

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CHAPTER 1
INTRODUCTION

1.1 Background to the study

Unpredictable rainfall pattern occasioned by climate change has worsened food scarcity, particularly in Africa (Ojelel *et al*., 2019), pushing many households to depend on edible wild fruit trees for survival. Unfortunately, these important wild food trees are in decline because of overexploitation and lack of deliberate and adequate strategies for their conservation and/or domestication. Wild Fruit Trees (WFTs) support household finances through the sale of fruit and provide the needed nutritional requirements of the people, particularly in rural communities (Lockett *et al*., 2000; Ramadhani, 2002). Rural dwellers usually fall back on common WFTs for their survival during a famine (Egeru *et al*., 2014). Leaf, root, stem, bark, etc of WFTs are used in the treatments and management of common diseases (Ojelel *et al*., 2019) and also contribute to supplementing household income (Bharucha and Pretty 2010). For example, in some parts of Southwest Nigeria, trading in WFTs such as *Chrysophyllum albidum, Irvingia gabonensis*, and *Garcinia kola* was reported to contribute about 20–60% annually to household income (Onyekwelu *et al*., 2015). In Southern Africa, WFTs were also reported to contribute to household income (Ramadhani, 2002) thereby enhancing their living standard.

The Sahelian Zone of Nigeria, which comprises states in the Northeast and Northwest, is reported to house several important wild fruit tree species of high nutritional, medicinal, and economic value (Lockett *et al*., 2000). Notable among these species is *Balanites aegyptiaca* (L) Delile a tree belonging to the family Zygophyllales/Balanitaceae and it is commonly referred to as desert date, soapberry, and thorn tree. In Nigeria, it is known as Aduwa in Hausa. *Balanites aegyptiaca* is one of the common WFTs of dryland environments serving multiple purposes. The species has a wide distribution range, covering Africa, Asia and the Middle East (Varshney and Anis, 2014). *Balanites aegyptiaca* is a drought tolerant tree and thrives in the Sahara where other plants hardly

survive providing several uses of immense importance to the community (NRC, 2008). It is valued for its nutritional (Okia *et al*., 2013) and medicinal benefits (Chapagain and Wiesman, 2005). Apart from the nutritional and medicinal value highlighted and the economic benefits that could be derived from it, *Balanites aegyptiaca* tree can be used in fighting some of the environmental challenges of dryland areas such as desertification and soil erosion (NRC, 2008). However, this important tree is yet to be domesticated, neither is there any documented conservation effort made so far.

Unlike crops that have a long history of domestication (Haines, 1994), the domestication of forest tree species started much later around the 1950s (Mullin *et al*., 2011). A lot of efforts have been made particularly in developed countries on tree domestication and breeding (Mansfield, 2009). However, there is inadequate information on some fruit trees native to the tropics. The few studies carried out tend to focus on some selected trees regarded as economic species. This is not surprising because farmers who are the direct beneficiaries are more interested in fruit trees which provide food and income for them (Tchoundjeu *et al*., 2004).

Domestication is the process of taking a wild plant species and bringing it under management and cultivation. This process involves identification, characterisation, selection, and multiplication of desired tree species (Garrity 2008). Variability existing within and among species populations is exploited to improve desired traits (Elfeel *et al*., 2009) during the process of domestication and improvement programmes. The process of domestication and/or conservation starts with sourcing high-quality seeds within the natural population and then designing suitable silvicultural techniques for it and propagation.

1.2 Statement of problem

In Africa, many rural dwellers rely on WFTs to augment their meager income and provide nutritional and medicinal needs. However, these WFTs have not received adequate attention from researchers and development partners (NRC, 2008). So far, domestication effort has been focused on forest fruit trees like *Irvingia gabonensis* and *Dacryodes edulis* which are considered to be placed high on the priority list (Leakey and

Tchoundjeu 2001) neglecting other important WFTs such as *Balanites aegyptiaca* despite its enormous benefits. *Balanites aegyptiaca* is yet to be domesticated; it is only found in the wild, and in some cases retained on farmland.

Variation in fruit/seed size, growth rate, and composition of nutrients was reported in some parts of Africa such as Niger, Sudan, and Ethiopia (Abasse *et al*., 2011; Elfeel *et al*., 2009). Similarly, variation in the amount and quantity of oil extracted from *Balanites aegyptiaca* in Israel, Burkina Faso, Senegal, Mali, Niger, and India was reported (Chapagain and Wiesman, 2005). The density and calorific value of *Balanites aegyptiaca* wood in Niger were also observed to vary based on their locations (Montes *et al.*, 2010). Concerning the genetic characteristics of *Balanites aegyptiaca*, very little is known though some investigations were conducted on the species from Sudan, Cairo, Ethiopia, Saudi Arabia, Yemen, and Ghana origin (Khamis *et al.*, 2017). However, in Nigeria, apart from Aviara *et al*. (2005) who assessed the physical properties of two different shape nuts sourced from a market in North-East Nigeria, very little is known on the level of variation at both morphological and molecular levels among and within *Balanites aegyptiaca* population. This is one of reasons impeding domestication, breeding and/or improvement effort of this important tree species.

The population of *Balanites aegyptiaca* trees has reduced significantly over time due to habitat destruction and overexploitation for food, medicinal and energy purposes (Retallick and Sinclair 1992; FAO 2001). In Nigeria, the population of *Balanites aegyptiaca* is fast declining due to over-exploitation by traditional healers, clearing on farmland by farmers for growing arable crops, felling by herders, and the wood merchant for feeding animals and fuelwood, respectively. If nothing is done to checkmate these activities, this species might go into extinction in the not-too-distant future. One of the reasons militating against the conservation, domestication, and possible improvement of this species is that we are yet to appreciate the extent of variations within and among its populations which is important for designing domestication and improvement strategies. Assessment of morphological variation required assemblage or collection of samples (fruit, leaf, seed, etc.) from the species for quantitative/qualitative measurement and provenance study or field trial, this is necessary to assess their performance, while

assessing genetic diversity may require the use of molecular markers to determine the level of variability.

1.3 Objectives

Main objective: The study assessed the morphology and growth characteristics, genetic diversity, physical and chemical properties of *Balanites aegyptiaca* with a view to identifying suitable seed sources and improvement strategies for domestication and conservation purposes.

The specific objectives were to:

i. determine morphological characteristics of *Balanites aegyptiaca* from different locations
ii. investigate germination and early growth characteristics of *Balanites aegyptiaca* from different locations
iii. examine molecular characteristics of *Balanites aegyptiaca* from different locations
iv. assess chemical and physical properties of soil under *Balanites aegyptiaca* trees from different locations
v. assess the oil yield, physical and chemical properties of *Balanites aegyptiaca* kernel oil from different locations

1.4 Justification of the study

Wild fruit trees are good sources of fruits and other non-wood products (Onyekwelu *et al*., 2015). They play important roles as they are major sources of medicinal, nutritional, and cultural treasures. They provide sources of income and alternative sources of food in terms of fruits and vegetables (Ndah *et al.*, 2013). This role is increasingly becoming vital due to the variability in climatic factors as a result of a change in climate leading to erratic rainfall resulting in crop failure.

Balanites aegyptiaca tree was characterised as one of the overexploited species in Africa that required conservation measures (FAO, 2001). Many people in dryland regions of

Africa and the Middle East rely on the leaf, fruit, nut, and kernel of *Balanites aegyptiaca* for nutritional and medicinal purposes. The leaf and fruit pulp provide cheap and rich sources of nutrients and mineral supplies (Lockett *et al*., 2000; NRC 2008; Okia *et al.,* 2013). Parts of the tree are also used traditionally in the control/management of several ailments such as anxiety, malaria, oedema, stomach pains, chest pains, and deworming (Lockett *et al*., 2000; Orwa *et al*., 2009). It also provides an alternative means of preventing the menace of desertification and soil erosion (NRC, 2008). Resources derived from *Balanites aegyptiaca* when fully harnessed have the potential to provide a cheap and all-year-round supply of essential nutrients and minerals as well as provide income to household.

Variation is the basis for any tree domestication and improvement programme (Elfeel *et al*., 2009). However, *Balanites aegyptiaca* species remains undomesticated. One of the factors militating against the domestication of the species is the dearth of information concerning the extent of morphological and molecular variations in Nigeria. *Balanites aegyptiaca* was ranked second only to *Adansonia digitata* as a priority species in Niger (ICRAF, 1996). In the dry region of Eastern Africa, *Balanites aegyptiaca* was also ranked among the top three species (Chikamai *et al.,* 2005). In Nigeria, it is one of the favourite indigenous fruit tree species, particularly in Northern Nigeria where it is common. It was ranked among the top 15 species in the Sahel agroecological zone of Nigeria (ICRAF, 1996). Therefore, it becomes imperative to develop an appropriate conservation strategy that will ensure a continuous supply of goods and services from this important species.

For successful conservation, domestication, breeding, and/or improvement strategies, it is imperative to appreciate the level of genetic diversity among and within the species (Leakey and Tchoundjeu, 2001). Collection and management of genetic resources is an important process of conservation. When this information is lacking, domestication and improvement become impossible, while strategies to be used for conservation are dependent on genetic diversity. Therefore, this study assessed the extent of variation in *Balanites aegyptiaca* species in Nigerian using both morphological and molecular

techniques. This will serve as the basis for developing appropriate improvement and domestication strategies for the conservation of *Balanites aegyptiaca* in Nigeria.

1.5 Scope of the study

Fruits and leaves samples of *Balanites aegyptiaca* tree were collected from eight locations based on abundance in five states: Baure and Mashi (Katsina), Buratai (Borno), Dumsai and Gashua (Yobe), Gamawa (Bauchi), Guri and Kirikasama (Jigawa).

Fruit, leaf, kernel, and nut were used for the morphological characterisation of *Balanites aegyptiaca*. The molecular marker technique using matK gene loci was used to isolate DNA from leaves for molecular characterisation and assessment of genetic diversity. The germination percentage, mean germination time and germination speed of *Balanites aegyptiaca* seeds from different locations were investigated for 4 weeks. Growth characteristics such as stem height, number of leaves, root length, root: shoot ratio, collar diameter, absolute growth rate by plant height, and biomass assessment of *Balanites aegyptiaca* seedlings from different locations were assessed for 12 months. Variation in oil yield and physicochemical properties of *Balanites aegyptiaca* among sources were determined using standard methods.

CHAPTER 2
LITERATURE REVIEW

2.1 Morphological description of *Balanites aegyptiaca*

Balanites aegyptiaca tree can grow up to 10 m and attain a diameter at a breast height (Dbh) of 40 cm under favorable conditions. It is characterised by multiple branches and spines. The crown is a dense, round, or spherical shape. The bole is usually short and bends from the base though it can grow straight without necessary branching (Orwa *et al*., 2009). The bark of the stem has a dark brown or grey appearance (Plate 2.1). Leaves are bi-foliolate and spirally arranged on the shoots, green–dark green, leaflets are bright green colour dark, fleshy succulent with 2 firm coriaceous leaflets (Plate 2.2). Fruit is yellow at maturity, it is long, and narrow (Plate 2.3). The pulp is edible and has a bitter-sweet taste. The nut comes in different sizes and shapes with light brown, fibrous, and very hard outer layer, the average length ranged from 1.5 to 3 cm (Plate 2.4). There are about 500 to 600 seeds per kg equivalent to 1.6-2 g per seed.

2.2 Species of *Balanites aegyptiaca*

The following species: *aegyptiaca*, *ferox, pallida, quarrei*, and *tomentosa* have been reported in Africa (Sands, 2001) but *aegyptiaca* is the most commonly known variety and is distributed throughout most parts of Africa including Nigeria (Keay, 1989).

2.3 Flowering and fruiting phenology of *Balanites aegyptiaca*

Fruiting and flowering begin when the tree is 5 – 7 years (Varshney and Anis, 2014). A period of one year is required for the fruit to mature and ripen (Orwa *et al.*, 2009). Flowering has no definite time; it varies across the different environments in the Sahel. In Nigeria, flowering was reported to commence around November and April but the ripe fruits will become available by December and January, though fruits sometimes can be available from March to July (Orwa *et al.*, 2009).

Plate 2.1: Mature tree of *Balanites aegyptiaca*

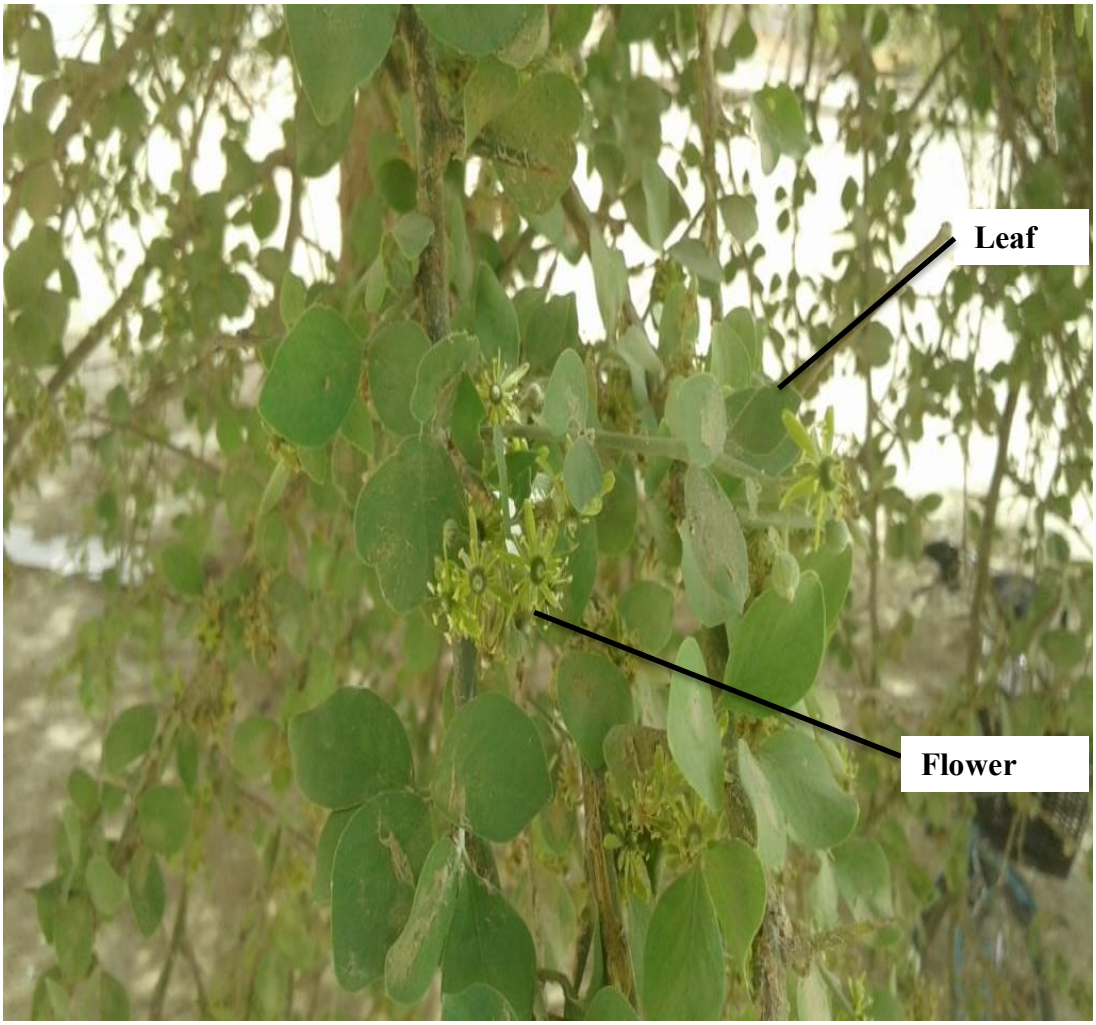

Plate 2.2: Leaves and flowers of *Balanites aegyptiaca*

Plate 2.3: Fruits of *Balanites aegyptiaca*

Plate 2.4: Seeds (nuts) of *Balanites aegyptiaca*

2.4 Ecology of *Balanites aegyptiaca* tree

Balanites aegyptiaca has wide ecological distribution; however, it achieves optimum growth when grown on clay (NRC, 2008). *Balanites aegyptiaca* does not tolerate shade at the seedling stage. It thrives very well in open woodland or savannah (Orwa *et al.*, 2009). It can survive in the waterlogged area (Sands, 2001) but prolonged exposure to flood can cause the plant to wither (Hines and Eckman 1993). *Balanites aegyptiaca* grows in locations with the minimum annual rainfall requirement of between 250 – 1200 mm and a mean annual temperature of 20 -30^{0}C (Orwa *et al*., 2009), and maximum temperatures of 40-46 ℃ (Hall, 1992).

2.5 Distribution of *Balanites aegyptiaca*

Balanites aegyptiaca is widely distributed across Africa; it is also native to some countries in the Middle East such as Israel, Saudi Arabia, and Yemen. In Asia, it is native to Myanmar and India (Orwa *et al.*, 2009). In Nigeria, *Balanites aegyptiaca* is predominantly found in the Sahel agroecological zone of the country, though few populations were reported in other parts of the country.

2.6 Propagation of *Balanites aegyptiaca*

There is a dearth of information on the optimum cultivation methods of *Balanites aegyptiaca* (NRC, 2008). The information available showed that propagation can be done by seedlings, cuttings, potted stock, and root suckers (Hines and Eckman, 1993). Average rooting success from stem cutting was reported to be about 60% without the inducement of growth by any growth hormones (Ladipo, 1989). Regeneration through direct sowing of seeds is also practiced. However, the seeds' dormancy has to be compromised to induce fast and synchronised germination. Seeds can germinate even without pretreatment but it can take as long as 6 weeks or more before it germinates (Mahgoub and Daffaalla, 1996).

Various pretreatment methods have been used in experiments to determine the effective method for breaking dormancy in *Balanites aegyptiaca* seeds. These methods include; soaking in water at ambient temperature, boiling water, and sulphuric acid (Elfeel, 2012; Mahgoub and Daffaalla, 1996). Seeds can be scarified manually followed by 24 hours of soaking in 30°C water for 12 hours (NRC, 2008). Soaking in water for 18 hours after

extraction was reported to result in 88% germination after three weeks of sowing. The direction or sowing position was also found to influence germination. Seeds sown vertically and horizontally with stalk upward resulted in better germination (Elfeel, 2012). Dormancy can also be broken biologically when seeds passed through the intestinal tract of ruminants (Hines and Eckman, 1993).

2.7 Management of *Balanites aegyptiaca* seedlings

Balanites aegyptiaca grows slowly but it is very resilient to extreme climatic conditions (Hines and Eckman, 1993). Weeding and protecting young seedlings from animals is important during the developmental stage after which it becomes tolerant of browsing and weeds. Weeding is important due to the slow growth rate (FAO 1988). Before transplanting young seedlings to the field, they should be between 18 to 28 weeks in the nursery. This is because they do not withstand transplanting well because of the presence of a deep taproot. Matured *Balanites aegyptiaca* trees can withstand fire but young trees can easily be damaged when exposed to fire (Hines and Eckman 1993). Pruning is usually adopted as a silvicultural/management strategy to help the trees survive drought associated with arid environment (NRC, 2008).

2.8 Uses of *Balanites aegyptiaca* tree

The roots, leaves, fruits, nuts, and bark of *Balanites aegyptiaca* are traditionally used in the management of several diseases such as anxiety, malaria, oedema, stomach pains, chest pains, and deworming (Orwa *et al*., 2009). Scientific findings have demonstrated the efficacy of *Balanites aegyptiaca* against microbial, viral organisms (Chothani and Vaghasiya, 2011). It also possesses psychoactive, anti-cancerous, and anti-proliferative and antidiabetic (Morsy *et al*., 2010) properties. Diosgenin compound was reported to be present in oil extracted from *Balanites aegyptiaca* kernel (Chapagain and Wiesman, 2005). This compound is not common in tree species and has attracted significant interest in the scientific community because of its medicinal properties (Liu *et al*., 2005).

Leaves and fruits of *Balanites aegyptiaca* are edible and are consumed as food. The leaves and fruit pulp contains Fe, K, Mn, Zn, and Cu (NRC 2008, Okia *et al*., 2013).

Nutrients from the leaves and fruits can help in supplementing other food sources, especially during the dry season and famine. *Balanites aegyptiaca* was reported to be one of the important species relied on by some communities when vegetables and fruits harvested during the farming season are not available (Lockett *et al*., 2005)

The timber from the *Balanites aegyptiaca* tree is small in diameter, hard, and durable. It is usually used in constructing minor products such as yokes for animals and other household tools (Orwa *et al*., 2009).

2.9 Conservation status of *Balanites aegyptiaca*

Conservation status simply refers to the present state of a living organism which can be a plant or animal and the risk of depletion it will face in the future (Kjaer and Graudal, 2000). FAO (2001) categorised *Balanites aegyptiaca* as overexploited species in Africa that required conservation measures. In Sudan, the species according to Warrang *et al*., (2002) is considered an endangered priority species while Elfeel and Warrag (2011) based on the study they carried out categorised it as threatened. In Nigeria, there seems to be no information about its conservation status. However, from field observation, the species population has been observed to be declining at an alarming rate. The cause of this decline as observed on the field includes; felling by herdsmen who use the tree as forage for their animals, clearing of lands for arable farming, and wood fetching by fuelwood merchants, and woodcarvers. This contributes immensely to the decline of the *Balanites aegyptiaca* population in Nigeria. Unless a deliberate conservation strategy for *Balanites aegyptiaca* is developed in Nigeria, the species may soon go into extinction in no distance time.

2.10 Assessment of plant genetic diversity

Genetic diversity is the amount of variability existing in a population of plants or animals. This variability can be at both morphological and molecular levels. The presence of significant variation in genetic traits presents an opportunity to improve these traits/characters through the selection of a seed source of high/desired quality. Conservation or improvement programmes will be unsuccessful without variability in

traits or characters (Elfeel *et al*., 2009). Genetic diversity does not only play a part in breeding or improvement programs, but it is also essential to the survival of the plant population. Information on genetic variation in plant species will help in developing appropriate conservation and improvement strategy for plant species (Iloh *et al.,* 2016) Genetic variations are assessed using genetic markers. Two major groups of genetic markers have been identified; they are classical and molecular markers (Xu, 2010).

2.11 Plant characterisation for conservation purposes

In a broad sense, characterisation is the process by which plant or animal specimens are identified or differentiated from one another. Molecular characterisation refers to the identification of variation in genetic makeup or DNA sequences or specific gene. Molecular characterisation has the power of identifying variation at the DNA level devoid of environmental influence (de Vicente *et al*., 2005). Morphological characterisation can be defined as the differentiation or identification of characters that can be seen and appreciated physically. These characters are highly heritable and expressed in all environments (IPGRI/CIP 2003)

The process of plant conservation includes the collection and management of genetic resources. This is made possible through gene identification and value addition (de Vicente *et al*., 2005). Prioritising sites for conservation begins with the identification of sites harboring desirable traits for the collection of plant genetic material (Lidder and Sonnino, 2012). Our understanding of taxonomy, domestication, and evolution process which are crucial in enhancing conservation efforts in plants or animals cannot be complete without molecular and morphological characterisation. Molecular characterisation alone or together with morphological characterisation gives reliable information for assessing genetic diversity among populations (Figliuolo and Perrino, 2004). Identification of real or potential variation in germplasm is one of the most challenging aspects guiding conservation decisions (de Vicente *et al*., 2005).

2.12 Morphological markers

Morphological markers are the oldest marker used in characterising or identifying the organism. They are biological features that can be easily appreciated physically such as growth habits, fruit color, length, weight, size, etc and are used in identifying plants with desirable traits. They are traditionally used in assessing genetic variation in forest tree species (NRC, 1991). They do not require the use of sophisticated technology, however, this method is slow, very expensive to conduct, and difficult to isolate the influence of environmental factors (Runo *et al.*, 2004).

2.13 Morphological variations in plants

Morphological variation can be quantitative or qualitative. Quantitative variations are those differences that can be easily seen and assigned values such as diameter, height, weight, etc, while qualitative variation cannot be easily measured or assigned value for example fruit color and taste. Variations in forest tree species are generally high (Tsobeng *et al.*, 2015).

2.13.1 Quantitative morphological variation

Fruit

Variations in fruit traits exist among and within fruits from different sources or provenances. For example, significant variation in fruit length of *Sclerocarya birrea* among different provenances was reported by Mkwezalamba *et al*. (2015). A similar observation was made for *Balanites aegyptiaca* fruits sourced from different sites in Sudan, the fruits' lengths range between 1.82 cm and 5.79 cm with a mean length of 3.36 cm (Abdoun 2005). In Niger and Uganda, mean *Balanites aegyptiaca* fruit lengths of 2.78 cm and 3.2 cm were recorded respectively (Abasse *et al*., 2011; Okia 2010). Fruits of *Sclerocarya birrea* collected among different sites in Malawi were reported to vary significantly in their diameter or thickness (Mkwezalamba *et al*., 2015). In Costa Rica, Wheelwright (1993) also reported that fruits of *Ocotea tenera* varied significantly in their diameter. The average fruit diameter of *Balanites aegyptiaca* collected from different sources in Sudan was found to be 2.08 cm (Abdoun, 2005).

Fruit weight is a very important trait when it comes to fruit selection for consumption. Fruits with higher weight would be expected to have large mesocarp or pulp which will be preferable when it comes to selection for consumption by people. Oboh *et al*. (2008) observed a significant difference in the fruit weight of *Terminalia catappa* from southwestern Nigeria. Mkwezalamba *et al.* (2015) also observed a significant variation in the fruit weight of *Sclerocarya birrea* among different provenances in Malawi. In *Balanites aegyptiaca*, fruits collected from different sources in Sudan were reported to vary significantly, with a weight range between 2.43 and 10.91 g with a mean weight of 6.64 g (Abdoun, 2005). This is similar to what was reported by Elfeel (2004) who reported a mean fruit weight of between 5.5 g and 9 g in Sudan. Fruits of *Balanites aegyptiaca* in Uganda and Niger were reported to have a mean weight of 7.2 g and 6.28 g respectively (Okia 2009; Abasse *et al*., 2010).

Just like fruit weight, fruit pulp also varies within and between species. A significant variation in fruit pulp of *Sclerocarya birrea* among different provenances was reported (Mkwezalamba *et al*., 2015). Chapagain and Wiesman, (2005) reported *Balanites aegyptiaca* mesocarp or pulp to represent 28.33% of total fruit weight. In Uganda *Balanites aegyptiaca* pulp accounts for 22.7% of the total fruits (Okia 2010). The pulp is the fleshy part (mesocarp) of the fruits which is consumed by man and animals. Human's and animals' choice of fruits is usually a result of the taste of this part of the fruit. Therefore it is an important part of fruit composition.

Nut (seed)

Nut (seed) or stone as is called by some people is the hard remaining part of the fruit after removing the mesocarp or pulp. After fruit consumption, the nut is usually discarded. The nut contains the kernel when oil is extracted which is used in oil extraction. The nut of *Balanites aegyptiaca* has been reported to vary in size. In Niger, a mean nut length of 2.51 cm was recorded among *Balanites aegyptiaca* population in different land management systems (Abasse *et al*., 2011) while a mean nut length of 2.89 cm was reported by Aviara *et al*. (2005) from *Balanites aegyptiaca* population in Nigeria. The difference in nut weight has been observed in *Balanites aegyptiaca* in Uganda, the nuts were reported to account for 47.2% of the weight of the total fruit

(Okia, 2010). In Nigeria, 3.13g was reported as the mean weight of *Balanites aegyptiaca* (Aviara *et al*., 2005).

Kernel

Kernels obtained from *Balanites aegyptiaca* are edible and contain oil that is used as vegetable oil. Variation in the kernel weight of *Balanites aegyptiaca* has been reported. Elfeel (2004) reported that the kernel represents 15% of the total fruit weight in Sudan. Chapagain and Wiesman, (2005) reported that *Balanites aegyptiaca* kernel sourced from different countries represents 8.12% of total fruits weight while In Uganda, it accounts for 12.4% of the total fruits (Okia, 2010). A mean kernel weight of 0.71g was reported by Aviara et al. (2005) from fruits collected from Nigeria while in Niger, a mean kernel weight of 0.58 g was reported by Abasse et al. (2011).

Leaf

Photosynthetic activities in plants are largely determined by the leaf which is responsible for producing organic matter (Bojović and Stojanović 2006). Photosynthesis in plants is controlled by the pattern of light intercepted which is also determined by leaf architecture (Nyarko *et al*., 2012). This implies that plants having smaller size leaves could results in less transpiration and photosynthetic activities compared to those having larger leaf sizes (England and Attiwill 2006) while those having large areas will be expected to have more area to transpire and photosynthesize.

Leaf morphological traits within and among plant populations have been shown to vary. Kolawole et al. (2016) reported variations in vegetative parts of Jatropha species. Significant variation in leaf traits of *Vitellaria paradoxa* was observed between three (3) locations by Nyarko et al. (2012) and Oboh et al. (2008) also observed remarkable differences in leaf morphology in *Terminalian catappan* from southwestern Nigeria. Significant variations were also reported in leaf traits of *Hippophae rhamnoides* between the village and wild stands and among populations (Nawaz *et al*., 2018). Leaf size was observed to also vary in *Milicia excelsa* population across different biogeographical

zones in Benin (Ouinsavi and Sokpon 2010). The leaves of different seedlings of *Balanites aegyptiaca* in Sudan were found to vary in shape and size (El-Amin 1990).

2.13.2 Qualitative morphological variations

The fruit of different plants has different shapes, even among the same species fruits shape can vary. Fruits' shape was found to vary significantly within different locations in Uganda (Okia 2010). Two shapes, tapered oblong, and spheroidal were reported for *Balanites aegyptiaca* fruits by Aviara *et al*. (2005) in Nigeria while four fruits shape; elongate, oblong, oval, and spherical reported in Sudan by Abdoun (2005).

Fruits taste is a very good criterion for fruit selection; the general assumption is that a sweet taste will be preferred in comparison with a bitter taste. Not much has been done in the assessment of fruit taste. Taste is a qualitative trait and most researchers tend to focus on quantitative traits because it is easy to assess. *Balanites aegyptiaca* fruits were reported to have an astringent and bittersweet taste (Von Maydell 1986). Variations in fruit taste provide a good selection trait for improvement purposes thereby boosting the marketing opportunity of the fruits since people generally preferred sweet fruits.

Fruit colour is a qualitative trait that could offer a competitive advantage when it comes to marketing fruits. Variations in fruit colour have been reported, for example, the fruit of *Arbutus andrachne* in the southern part of Macedonia was reported to vary significantly (Markovski 2017). In Southwestern Nigeria, the fruit of *Terminalia catappa* was also reported to vary in colour (Oboh *et al*., 2008). They observed a shade of green, yellow, and yellowish-green fruits colour. Kala and Dubey (2014) also observed significant within-species variation in the fruit colour of *Balanites aegyptiaca* in India. *Balanites aegyptiaca* fruits according to Thirakul (1984), are yellow when ripe (epicape) while the edible flesh (mesocarp) is yellow-brown.

Environmental factors such as latitudinal and altitudinal ranges or contrasting climatic conditions could be responsible for both quantitative and qualitative variations discussed above (Beaulieu *et al*., 2004). According to Ouinsavi and Sokpon (2010), environmental factors instead of geographical location are responsible for variation in morphological traits of *Milicia excelsa* in the Benin republic. Their finding was corroborated by Carter

et al. (1987) who show that moisture stress and nutritional deficiencies are responsible for the change in morphological features of the tree population.

Soil physical and chemical properties vary within and between locations; this could influence tree morphological characteristics. The soil is the medium through which the plant drives its nutritional requirements. Deficiency and an adequate supply of important nutrients in the soil could affect morphological features positively or negatively. According to Meril and Hendry (2014), phenotypic variation is a response to climatic condition which affects their adaptive evolution or phenotypic plasticity, or both. Plant genetic makeup can also be responsible for these variations. Variation in morphological traits could also be a function of resource allocation at the time of fruiting (Shu *et al*., 2012) since different provenances or locations may have different climatic variables such as precipitation, temperature, etc during the time of fruiting which could play a significant role in resource allocation.

2.14 Correlation between plants morphological traits

Correlation analysis is very useful in tree improvement programs since the improvement of one character can cause simultaneous changes in the other characters (Shu *et al*., 2012). Correlations existing between plant traits are important in planning any breeding or improvement program Knowledge of the relationship existing between these traits provides an avenue to predict the behaviour of one trait using another (Khan *et al*., 2009). Even though this relationship might not always be accurate, it at least gives us a clue.

A significant positive correlation was reported in the seed traits of fifteen Chinese provenances of *Magnolia officinalis.* Seed width positively correlates with seed length and seed length/width ratio (Shu *et al*., 2012). Intra-specific genetic variation in Arbutus *andrachne* (Greek strawberry tree) species from Macedonia was observed and a significant correlation between fruit length and pulp ration, fruit width, and pulp ration, and fruit mass and pup ration were also observed (Markovski 2017). Fruits diameter was found to correlate positively with fruit length, fruit mass, seed diameter, seed length, pulp mass, and seed mass (Wheelwright, 1993). In *Terminalia catappa*, leaf length correlates

positively with leaf width while mesocarp weight correlates positively with plant height. Fruit length correlates positively with fruit breath, fruit width, and fruit weight (Oboh *et al*., 2008). The seed weight of *Argania spinosa* was found to correlate positively with the percentage of oil content (Ait Aabd *et al*., 2010). A significant correlation between fruit length and fruit mass, pulp mass, and fruit breadth in *Tamarindus indica* population from different Agroecological zones in Uganda was reported by Okello *et al*. (2018).

In *Balanites aegyptiaca*, the following correlation relationship in fruits, nuts, and kernel from within and among parkland agroforests in Niger was reported by Abasse *et al*. (2011); Fruits weight correlates positively with fruit length, fruits width, nut length, nut width. Kernel weight correlates positively with fruit length, and nut length. Fruit length correlates positively with fruit width, nut length, and nut width. Fruit width correlates positively with nut length and nut width. Nut length correlates significantly positively with nut width. However, a non-significant correlation between fruit length and kernel weight was observed, kernel weight and fruit width, kernel weight, and nut width (Abasse *et al*., 2011). Okia (2010) in Uganda also reported a negative relationship between *Balanites aegyptiaca* fruit length and fruit weight. He observed that as fruit length increases the fruit's weight decrease while the fruit's weight was found to be positively related meaning as the fruit's width increase so does the weight of the fruit.

2.15 Seed source and its effect on germination behaviour

Germination is the beginning of life in a plant, the quality of seeds is therefore very important for the success of any forest establishment programme. The source of seeds has been shown to affect seed germination behavior by several authors. For example, Dangasuk *et al.* (1997) observed a significant variation in seed germination of *Faidherbia albida* from different provenances in Kenya. Seeds of *Faidherbia albida* collected from different sources were also reported by Fredrick *et al*. (2015) to exhibit different germination patterns. A significant variation in germination capacity and germination energy was observed on day 14. On the 9^{th} day, both germination capacity and germination energy were insignificant, showing that the number of days possibly affects seed germination response. The mean germination time of seeds among provenance did not show any significant variation too. The seeds of *Balanites aegyptiaca*

sourced from different locations in Sudan were observed to show significant variation in germination behaviour as germination was observed to commence at day 10 and end on day 31 in mast of the provenances. The highest percentage of mean germination was 60.6 while the least was 17.1 % (Elfeel *et al*., 2009). However, in seeds of *Tamarindus indica*, the variation recorded in germination characteristics among seed sources was not significant (Azad *et al*., 2013)

Differences in seed germination might be attributed to different environmental factors as suggested by Shu *et al*. (2012), while the lack of significant difference could be attributed to the similarity in microclimate among the seed sources (Azad *et al*., 2013). The genetic makeup of seeds from different sources could also be responsible for these variations. This is likely since the seeds though collected from different locations are sown in the same environment thereby possibly eliminating the influence of the environment. However, plant genetic makeup itself could be a product of the environment. Variations in seeds' responses from different sources or provenance present an opportunity for identifying sources or locations with high-quality seeds that possess superior germination indices that could be used to establish a plantation.

2.16 Effects of seed sources on the growth characteristics of plants

Source or provenances where seeds are collected does affect seedlings response. This has been documented by several researchers, for example, Shu et al. (2012) reported that shoot height, collar diameter, number of leaves, root length, and number of lateral roots of *Magnolia officinalis* from different provenance vary significantly. Akinyele and Adegeye (2012) also observed significant variation among different sources in many growth characteristics (leaf area, root dry weight, total biomass, stem dry weight, leaf dry weight, collar diameter, leaf production, and seedlings height) of *Buchholzia coriacea*. A similar trend was reported by Fredrick et al. (2015) as they observed a significant variation in leaf number and seedlings collar diameter of *Faidherbia albida* within and among provenances. Elfeel *et al*. (2009) also observed a significant difference in *Balanites aegyptiaca* seedlings' height and the number of branches from Sudan. However, Shu *et al.* (2012) observed a lack of significant difference in relative growth rates and net assimilation rates. A similar result was also reported by Fredrick *et al*.

(2015) who reported that seedlings' height of *Faidherbia albida* did not vary significantly within and among provenances after 5 months of growth. Both inter and intra-specific variations in seedlings' growth from different sources or provenances signify a potential for selecting seeds from sources or provenances with early or faster growth attributes for plantation establishment. This information is also important for any improvement program.

2.17 Cytological markers

Variations in chromosome attributes such as numbers, banding patterns, size, shape, order, position, arm ratio, and total genomic chromosome strength are some of the common cytological markers (Bhanu, 2017; Nadeem *et al.*, 2018). The differences in these attributes (cytological markers) is been utilized in assessing genetic variation in organisms. It is used in identifying normal and mutated chromosomes, identification of linkage groups, and physical mapping (Nadeem *et al.,* 2018). The major drawback of the cytological marker is its low resolution which limits its application in diversity studies (Bhanu, 2017).

2.18 Biochemical markers

Isozymes otherwise known as biochemical markers are made up of multiple molecular enzymes with similar functions (Nadeem *et al*., 2018). Genes in populations, frequencies of genotypes, genetic differentiation, heterozygosity, and genetic structure of the population can be determined with isozymes (Mondini *et al*., 2009). One of the advantages of biochemical markers is, their co-dominant nature, it is also cheap and easy to use. However, its application in genetic diversity studies is limited due to the availability of a few enzyme markers and the complexity of their structure (Govindaraj *et al*., 2015; Nadeem *et al*., 2018

2.19 Molecular markers

A molecular or DNA marker is an advanced method or technique for assessing variation in an organism. Unlike morphological markers which focused on the physical (phenotypic) appearance to assess variation, molecular markers make use of DNA

extracted from organisms to identify, characterize or understand the extent of variation. Several DNA markers exist but priority should be given to co-dominant markers, which can detect a high level of polymorphism and reproducibility and be evenly distributed throughout the genome when selecting markers (Mondini *et al.,* 2009; Nadeem *et al*., 2018).

2.19.1 Restricted fragment length polymorphism (RFLP)

The restricted fragment length polymorphism markers had been in use as far back as 1975 for the genetic marking of serotypes, an adenovirus (Semagn *et al*., 2006). It was subsequently deployed in human and plant genetic studies (Weber and Helentjaris, 1989). Two organisms are differentiated from each other by the difference in DNA fragment sizes (Semagn *et al*., 2006). RFLP markers are now the most widely used hybridization-based molecular marker (Semagn *et al*., 2006). Its popularity is based on its flexibility and reliability, its co-dominant nature, its ability to detect a large number of loci, and high reproducibility. However, several challenges are associated with the use of RFLP markers such as high cost, labor and time consumption, limited polymorphism, and requirement for a huge quantity of DNA.

2.19.2 Random amplified polymorphic DNA (RAPD)

The random amplified polymorphic DNA has been successfully used to assess the genetic diversity of several plants such as *Jatropha curcas* in India (Gopale and Zunjarrao 2013), RAPD was used in assessing the genetic variation and genetic relationships among 54 accessions of *Azadirachta indica* in Brazil (da Silva *et al*., 2013), *Syncepalum dulcificum* in Nigeria (Iloh *et al.,* 2016), *Melia volkensii* in Kenya (Runo *et al.,* 2004). RAPD was used by Fathy and Abd El-Kader (2012) to assess the level of genetic diversity of *Balanites aegyptiaca* plantlet after being subjected to varying conservation procedures. The RAPD was observed to successfully determine the level of genetic stability among the different treatments. However, it should be noted that *Balanites aegyptiaca* plant samples used for their research were basically from one location and thus their work only shows evidence of intra-genetic diversity which was affected by preservation protocols used.

El-Domyati *et al.*, (2011) also used RAPD to assess the level of Genetic diversity in some selected plant germplasm along the western Red Sea coast of Sinai of which *Balanites aegyptiaca* was among the selected plant germplasm used. They were able to determine the level of intra-genetic diversity. Their results indicated less than 10% intra-plants polymorphism (within). RAPD has found wide acceptance because it is simple to conduct and yields a high level of polymorphism but its major drawback is its low reproducibility and detection of allelic differences is not possible.

2.20 DNA Sequencing

The DNA of an organism is made up of several nucleotide bases such as (adenine), G (guanine), C (cytosine), and T (thymine) embedded in a molecule. Arrangements of these nucleotide bases from one species to another vary. DNA Sequencing is the technique used in determining the order of the arrangement of nucleotide bases to differentiate one species from another or identify variation within members of the same species. There are two major categories or types of DNA Sequencing: next-generation sequencing technique (NGS) and conventional sequencing technique.

2.20.1 Next-Generation Sequencing Technique (NGS)

The next-generation sequencing techniques are playing a significant role in understanding variation among individuals in a population (Govindaraj *et al.*, 2015). The NGS offered the possibility of sequencing a complete genome instead of short sequences of a single gene; it possesses the potential to determine how genotypic variation translates into phenotypic characteristics as well as understand the evolutionary process of a plant (Arif *et al.*, 2010). Unlike first-generation sequencing, NGS is independent of Sanger chemistry (Sanger *et al.*, 1977; Arif *et al.*, 2010). The use of NGS techniques reduces error, has broader exploration, is cost-effective and saved time compared to first-generation sequencing (Arif *et al.*, 2010).

2.20.2 Conventional sequencing technique

Dye-terminator sequencing technique is the commonest method of conventional sequencing technique used presently for phylogenetic analysis. The combination of a dye

terminator with automated high-throughput DNA sequence analyzers is generally used for most sequence work. In dye-terminator sequencing techniques, the chain terminator ddNTPs are labeled and sequenced in a single reaction, unlike the formerly used labeled-primer method which required four reactions (Arif *et al*., 2010). To perform dye-terminator sequencing, labeling of four dideoxynucleotide chain terminators with fluorescent dyes is done with each having a different wavelength of fluorescent emission. This technique is automatic, robust, and has a high accuracy level (>98%), however, it cannot handle long sequences and is prone to dye effects caused by differences in the incorporation of the dye-labeled chain terminators into the DNA fragment (Arif *et al*., 2010).

2.21 DNA barcoding

The introduction of DNA barcoding has generated a lot of interest in the area of plant identification and characterisation. DNA barcoding can be referred to as the technique of identifying or characterizing plant or animal samples of unknown origin using a short DNA sequence and comparing it with a known sample from a library of DNA barcodes (Wilson *et al*., 2018). Knowledge of molecular biology is essential in DNA barcoding, for the extraction and amplification of DNA barcode sequence fragments from the unknown specimen. Amplified samples are usually taken to companies that specialised in Sanger sequencing or next-generation sequencing (Wilson *et al*., 2018). Results from sequencing are subjected to tools such as the basic local alignment search tool (BLAST) for species-level assignment (Kress *et al*., 2005; Arif *et al*., 2010). Common gene region or primer used for DNA barcoding includes *matK*, psbA, and rbcL (Kress *et al*., 2009)

2.22 Use of matK in DNA barcoding

The selection of the appropriate gene region (primer) for sequencing is a challenging task because none of the genes (loci) available is perfect. This is because each region has its strength and weakness and the result obtained is determined by the targeted regions in the gene, (Tallei and Kolondam 2015). According to Plant Working Group (PWG-CBOL), three criteria must be satisfied for a DNA barcode to be considered perfect for use in successful sequencing (Janzen 2009). These criteria are; minimalism,

standardization, and scalability. As mentioned earlier, none of the available genes can meet all these requirements even though a single primer was reported to successfully amplify 90% of angiosperm species (Janzen 2009). This necessitates the recommendation of combining two loci of rbcL (ribulose-1, 5-bisphosphate carboxylase oxygenase large subunit) _ matK (maturase K) as the plant barcode as suggested by PWG-CBOL. For example, the combination of rbcL and matK was reported to give better results because they have a high degree of segregation among species (Bafeel *et al*. 2011).

Each one of trnH–psbA, rbcL, and matK can successfully be used in a DNA barcoding system even though none meet all the requirements (Janzen 2009) but matK unlike rbcL and atpB (ATPase) can analyse evolution below family level (Barthet 2006). MatK is becoming one of the sought-after plastid coding regions because of the high level of discrimination it exhibits among angiosperm species (Lahaye *et al*., 2008). It has been successfully used in differentiating *Vachellia* species from other *Acacia* species. This makes it suitable for taxa separation at the genus level (Newsmaster and Ragupathy, 2009). 66% discrimination in plant species after sequencing was reported for matK while rpoC1, psbK–psbI, the–psbA, and rbcL recorded 43%, 68%, 69%, and 61% discrimination respectively (Janzen, 2009). Among single barcode loci of ITS, trnL, and matK used, matK had the highest success rate (82.4%) of discrimination among *Daniellia ogea* and *Daniellia oliveri* species (Onefeli 2021). MatK compared to rbcL is opined to provide more information in plant systematic due to its high phylogenetic signal; it is a suitable gene for the phylogenetic analysis below the family level (Johnson and Soltis (1994). MatK has been successfully used to amplify several species belonging to the dicot family and a monocot family (Johnson and Soltis, 1994). 88% and 69% success rate was reported by rbcL and matK gene, respectively in amplifying 26 different plant species (covering 14 families) including *Zygophyllum propinquum* species a plant belonging to the family of Zygophyllaceae from Saudi Arabia (Bafeel *et al*., 2011). 93.1% amplification and 92.6% sequencing success in 58 species from 47 families of angiosperm plants were recorded from the use of matK (Jing *et al*., 2011).

However, some researchers have some reservations about the use of matK as a universal primer owing to conflicting reports from different studies (Kress and Erickson 2007; Lahaye et al. 2008). For example, in *Myristica fragrans,* the pairs of MatK-1RKIM-f and MatK-3FKIM-r succeeded in only amplifying partial *mat*K gene and are unable to distinguish intra and interspecific variation (Tallei and Kolondam 2015). Bafeel *et al*. (2011) reported that matK failed to amplify *Anthemis desert, Pulicaria undulate,* and *Sonchus oleraceus* species which belong to the family Asteraceae. They opined that a mismatch of primer at the annealing site could be responsible (Bafeel *et al*., 2011).

2.23 Soil properties

The growth of plants is influenced by several factors in which soil plays indispensable roles and low productivity in trees has been attributed to low soil quality. Therefore, for optimal plant production, it is necessary to understand the soil in which plant grows (Fasina *et al.,* 2015). The primary source of soil is from weathering of rocks while some fractions of soil on the earth's surface are contributed from dead organic materials.

Soil quality for plant growth is defined by the physical and chemical properties of the soil. The soil's physical properties include; the soil bulk density, soil texture, soil porosity. They define the compaction of the soil, movement of water, and soluble nutrients into the soil (Chaudari *et al.,* 2013) while the soil chemical properties split into macro and micronutrients depending on their levels of requirements by plants. The macronutrients are nitrogen, potassium and phosphorus, calcium, magnesium, and sulfur. These nutrient elements are required in large amounts for plant growth while the micronutrients are copper, iron manganese, boron, and zinc. These nutrient elements are required in a small quantity by plants for reproductive and physiological growth.

2.23.1 Soil texture

It is either individual or combinations of clay, silt, and sand in the soil. It dictates soil water infiltration and air holding potentials; hence, the easiness with which soil can be worked. This consistently affects how water and nutrients are taken up by plants for physiology and morphological growth. There are four major soil textural classes and include; sandy, silt, clay, and loamy are the four broad classes of soil textural classes.

However, there are variants of soil textural classes based on the proportion of sand, silt, and clay in the soil. Sandy soil is soil with over 80% sand particles and less than 15% clay while soil containing over 80% silt with less than 12% is silty soil. In the same vein soils with a minimum of 35% clay is clayey soil. Loamy soils are soils with an equal proportion of sand, silt, and clay particles. The sandy soils are lower in major nutrients elements required for plant growth whereas compared to the loamy. Trees generally can be distributed over a wide range of soil textural classes. For instance, *Adansonia digitata* had been found on silty, clayey, sandy, and loamy soils (Bouda, 2014; Sidibe and Williams, 2002).

2.23.2 Soil organic carbon

There are two types of carbon in the soil pool based on their sources. They are organic carbon and inorganic carbon. The soil organic carbon is vital in soil health and productivity (Corsi *et al.*, 2012). Its adequacy in the soil enhances soil structure, improves soil chemical and biological activities. Soil organic carbon is derivable from the decay of plants and animal residues (Wang *et al.*, 2011). The quantity of organic carbon in the soil is usually determined by factors such as temperature, rainfall, sunlight, land use types. Hence, there is a potential occurrence of the large variation in soil organic carbon within a short location. The range of organic carbon required for trees is 7g/kg to 20g/kg of soil (Abam and Orji, 2018). It had been asserted that trees are capable of generating this quantity for optimal growth (Pardon *et al.*, 2017). However, an instance of the inability of soil under tree crop to meet the range had been documented (Ogeh and Ipinmoroti, 2015). This had been attributed to soil textural class as sandy soils are inherently poor in organic carbon retention (Blanchart *et al.*, 2005). Soil in Northern Nigeria are characterised by high sand contents (Salako et al., 2001). Soil dominated by sand is usually associated with low organic carbon which makes it prone to degradation because of its fragility

2.23.3 Soil chemical requirements and trees growth

The tree requires soil chemicals in adequate amounts for growth and productivity. Some of these chemicals are mostly inadequate whereas others are in excess in the soil. Either

of the scenarios affects tree growth and productivity. Nutrients have been classified into essential and non-essential. In commercialised plants the focus of supplements is the essential nutrients and whereas the non-essential nutrients are ignored. However, shreds of evidence of improved productivity from supplementing plants with non-essential have been reported (Dimkpa and Bindraban, 2016).

Although, trees can generate and absorb nutrients from litter decomposition in adequate amounts in plantation through nutrient cycling (Carrnaca *et al.*, 2018). However some nutrients, such as potassium and phosphorus may not meet the plant nutrients demand through nutrient cycling (Ma *et al.*, 2002; Wolde, 2016). Conversely, elements such as manganese and copper are at toxicant levels in most soils (Millaleo *et al.*, 2010; Chiou *et al.*, 2019). Therefore, there is the need to ascertain the concentration of the nutrient elements in *Balanites aegyptiaca* growing soil to ascertain the nutrient elements that the species might be able to absorb from the soil nutrients pool and those that might need to be supplemented or control its toxicity for the species.

2.24 Edible oil yield of some plants

Animals and vegetables are the major sources of oil. Oil seeds crops are defined as those seeds which contain oil in appreciable quantity or sufficient enough to be exploited for commercialisation (Aremu *et al*., 2015). Commonly used oil seeds include groundnut, soybean, palm kernel, sesame seed, olive, etc. Oil yield varies from one seed to another; this variation is even possible in the seed of the same species grown in different sites. For example, the oil yield of the Argan tree (*Argania spinosa*) was reported by Ait Aabd *et al*. (2010) to vary within the population. The percentage of oil yield recorded was in the range of 39.2% to 58.0%.

Different percentage of oil yield was reported when oil was extracted from *Balanites aegyptiaca* in several countries. For example, in Sudan, 19.8 – 40% oil yield was reported by Elfeel (2004) while Chapagain and Wiesman (2005) reported a significant variation in the percentage oil yield of *Balanites aegyptiaca* sourced from Israel, Africa and India. The percentage oil yield they reported was in the range of 39.20 and 50.22. Okia (2010) reported a mean oil yield of 44.5% from different locations in Uganda while

a 49.9 % oil yield was reported in Nigeria from *Balanites aegyptiaca* seeds sourced in one location (Manji *et al*., 2013). The standard range of percentage oil yield according to the association of analytical chemists (AOAC (1990) is ≥32%. However, according to Kyari (2008), an oil yield of 26 to 42% can be considered to be a reasonable yield level.

2.25 Physical and chemical properties of some edible oil

The qualities of edible oil are determined by its physico-chemical properties (Zahir *et al*., 2017) and these properties are not evenly distributed; they vary based on location and species

2.25.1 Refractive index

The Refractive index (RI) of oil can be defined as the ratio of the speed of light at a defined wavelength to its speed in the oil/fat itself (Aremu *et al*., 2015). The RI indicates the purity of oil (Hoffman, 1986). In Nigeria, groundnut (*Arachis hypogeal*) seed oil was reported to have a RI of 0.147-1.472 (Andrew *et al.*, 2012) while Castor (*Ricinus communis*) seed oil had 1.467-1.792 RI according to Akpan et al. *(*2006) and Nangbes et al. *(*2013). Sesame (*Sesamum indica*) and soybean (*Glycine max*) seeds oil was reported to record 1.464 and 1.430-1.466 RI respectively (Njoku *et al.*, 2010; Akanni *et al.*, 2005). A similar refractive index of 1.46 from different sites in Uganda was reported in oil extracted from *Balanites aegyptiaca* (Okia 2010) while Manji *et al*., (2013) reported a value of 1.49 in Nigeria. The acceptable refractive index value according to the American Society for Testing Material (ASTM 2002) ranged from 1.476 to 1.479.

2.25.2 Viscosity

Viscosity is a measure of the resistance of a fluid to deform under stress (Aremo *et al.*, 2015) and it is used in determining the quality of oil (Okia 2010). Chapagain *et al*., (2009) reported a viscosity of 49 cp from *Balanites aegyptiaca* oil obtained from different countries while Okia (2010) reported a value of between 18.94 – 23.04 cSt in *Balanites aegyptiaca* oil extracted from different sources in Uganda. In Nigeria, a viscosity of 46.8 cSt in *Balanites aegyptiaca* oil was reported (Eromosele and Paschal 2003). The viscosity of castor (*Ricinus communis*) seed oil was reported to be 0.43-9.42

cSt (Akpan *et al.,* 2006; Nangbes *et al.,* 2013) while soybean (*Glycine max*) seed oil had 7.99 cSt (Akanni *et al.,* 2005). The viscosity of oil is used in determining its suitability as a lubricant, the higher the viscosity of oil the better (Belewu *et al.,* 2010). The acceptable viscosity value according to Eze (2012) is 6.3 to 8.8 St.

2.25.3 Acid value

The acid value is a good indication of the quality of fatty acids in the oil and determines the stability of oil when stored for a while (Aremu *et al*., 2015). The acid value of oil can also be used as an indication of its edibility (Tesfaye and Abebaw 2016). For an oil to be acceptable for consumption, it must have a value below 0.6 mgKOH/g (AOCS 2003). The acid value of *Hildegardia barteri* was found to range between a mean value of 0.032 – 0.034 mgKOH/g (Aremo and Oluwadare, 2016), 11.60 mgKOH/g was reported for palm kernel (Atasie and Akinhanmi, 2009). The acid value reported for *Balanites aegyptiaca* oil from Uganda was 1.33 – 1.954 mgKOH g^{-1} (Okia 2010).

2.25.4 Iodine value

The iodine value can be used to categorise oil as drying or none drying. The iodine value in *Hildegardia barteri* was found to range between a mean value of 57.95 – 61.85 (Aremo and Oluwadare, 2016). In *Balanites aegyptiaca* oil, iodine value ranged from 98.20 – 103.32 I_2g/100 g in Uganda (Okia 2010) and 78.7 gI_2/100 g in Nigeria (Manji *et al*., 2013). According to AOAC (1990), 80 – 100 gI_2/100 g is the standard range of iodine value. The iodine values of some common Nigeria vegetable oil are as follow: Groundnut (*Arachis hypogeal*) seed oil 38.71-110.70 I_2g/100 g (Musa *et al.,* 2012; Atasie *et al.,* 2009), Castor (*Ricinus communis*) seed oil 58.64-87.72 I_2g/100 g (Akpan *et al.,* 2006; Nangbes *et al.,* 2013), sesame (*sesamum Indica* L.) seed oil 103.00-121.40 I_2g/100 g (Mohammed and Hamza, 2008; Njoku *et al.,* 2010; Ogbonna and Ukaan, 2013), Soybean (*Glycine max*) seed oil 73.02-136.81 I_2g/100 g (Akanni *et al.,* 2005), sunflower (*Helianthus annuus L.*) seed oil 78.45-119.92 I_2g/100 g (Aboki *et al.,* 2012; Ologunde *et al.,* 2008).

2.25.5 Saponification value

The suitability of oil to be used for the industrial manufacture of soap is often assessed by its saponification value. The saponification value of *Balanites aegyptiaca* oil from different countries was reported to vary, for example, a range of 180.50 – 192.80 mgKOH g^{-1} was reported by Okia (2010). A saponification value of 168.6 mgKOH g^{-1} was reported by Manji *et al*. (2013) and 200.02 mgKOH/g was reported by Zang *et al* (2017) both in Nigeria. A saponification value of 170-227.49 mgKOH/g was reported for groundnut (*Arachis hypogeal*) seed oil in Nigeria (Musa *et al.,* 2012; Atasie *et al.,* 2009) while sesame (*sesamum Indica* L.) seed oil had 189.00-191.00 mgKOH g^{-1} (Njoku *et al.,* 2010). The standard saponification value is ≥ 180mgKOH/g according to AOAC, (1990).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in the Sahel zone of Nigeria. The Sahel zone of Nigeria is found in the Northern part of the country. States in the Northwest and Northeast geopolitical zones fall within this zone (Abdulkadir *et al*., 2015). The Sahelian zone is characterised by definite wet and a marked dry season. The rainfall pattern varies and is unpredictable. The amount of rainfall ranges from 500 mm to 800 mm while the temperature ranges between 29^{o}C to 30^{o}C (Atedhor, 2014).

3.2 Site selection

According to Keay (1989), *Balanites aegyptiaca* tree species are found in the Sahelian zones of Northern Nigeria. A reconnaissance survey of the geographical distribution of *Balanites aegyptiaca* was therefore carried out in the Sahelian zone of Nigeria before flowering and fruiting to ascertain stands with desirable traits and appropriate periods for fruit collections. Consequently, eight locations were purposively sampled from these states based on the abundance of *Balanites aegyptiaca* trees (Figure 3.1). The locations selected were: Bauchi (Gamawa), Borno (Buratai), Jigawa (Guri and Kirikasama), Katsina state (Baure and Mashi), and Yobe state (Dumsai and Gashua). The coordinates (Latitude and Longitude), elevation, and name of each location were recorded (Table 3.1).

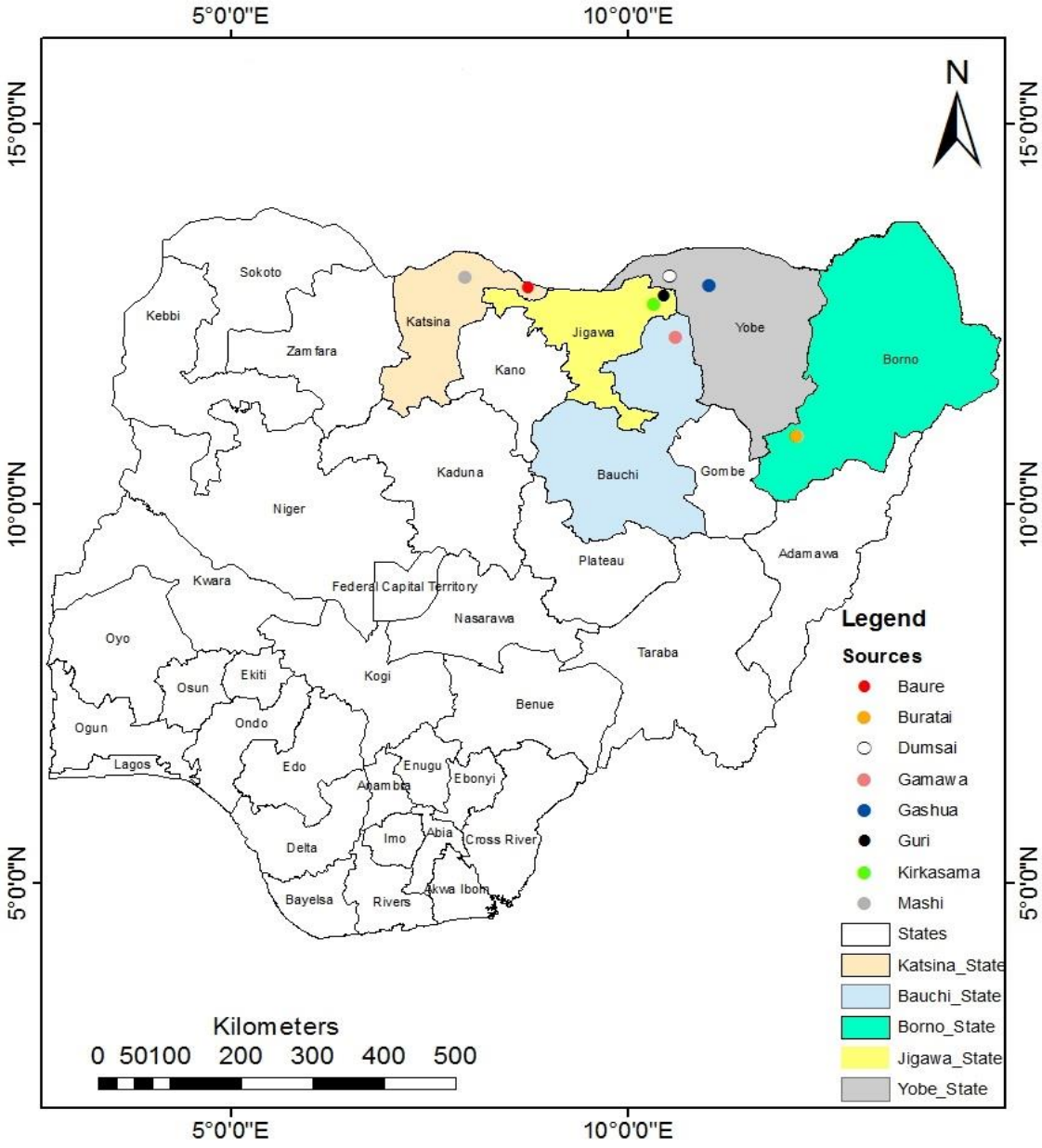

Fig. 3.1: Sources of *Balanites aegyptiaca* seeds

Table 3.1: Coordinates and weather characteristics of locations of *B. aegyptiaca* seed collection

Source (s)	Longitudes	Latitudes	Elevation	Pre. (mm)	Tem. (^{0}C)
Baure	008°43.496	12°50.407	403.7	747	28
Buratai	012°02.535	11°01.488	537.8	850	28
Dumsai	010°33.325	12°51.615	344	540	28
Gamawa	010°35.661	12°11.397	355.5	479	29
Gashua	011°00.770	12°52.496	334.1	500	29
Guri	010°24.755	12°45.313	347.9	479	29
Kirikasama	010°20.296	12°40.213	387.2	479	29
Mashi	007°56.316	12°57.681	507.2	844	28

Source: Field survey, 2018. Pre. = Precipitation, Tem. = Temperature

3.3 Experiment 1: Morphological characterisation of *Balanites aegyptiaca*

3.3.1 Sample collection and preparation

Ten matured trees with sufficient fruits from each of the eight locations were purposively sampled based on their abundance. Voucher specimens of collected samples were preserved, authenticated, and accessioned in the herbarium unit, Bayero University Kano, Nigeria with the voucher identity number: BUKHAN 0359. A minimum distance of 100 m apart was adopted when selecting the trees from each of the locations. This was done to avoid inbreeding (Abasse *et al*., 2011). From each tree, 5 kg of matured and ripe fruits were collected. Fresh matured leaves were also collected from the selected trees. The coat (epicarp) of the fruits collected was removed after which it was soaked overnight in water and de-pulped the next day by hand washing to obtain the nuts (seeds). The kernel was obtained by breaking the nut manually with a hammer. The fruits, leaves, nuts and kernels were assessed immediately after collection to avoid deterioration of the samples.

3.3.2 Characterisation of the tree, fruit, leaf, nut, and kernel of *Balanites aegyptiaca*

Tree: Ten trees from each source were used for the characterisation. The trees were characterised according to the descriptor used by Kehlenbeck (2015). The descriptors include:

- **Total height (m)**
- **Crown diameter (m)**: The crown diameter of each tree was assessed determined using spiegel relaskop.
- **Diameter at breast height (cm):** Stem diameter at breast height (Dbh) of sampled trees was measured using tree caliper.

Fruit: Thirty fruits were randomly sampled from each tree in each location. The following descriptors were assessed:

- **Fruit length and width (cm):** These were measured with the aid of a vernier caliper.

- **Fruit thickness (cm):** The spike attached to the vernier caliper was used to measure fruit thickness (Onyekwelu *et al*., 2015).
- **Fruit weight (g):** Electronic sensitive weighing balance was used to determine fruit weight.
- **Fruit shape:** Fruit shape was determined by assigning an appropriate geometric shape through physical observation.
- **Fruit colour:** The color of fruit/pulp was assessed by using the Methuen code of color (Kornerup and Wanscher, 1978; Atangana *et al.*, 2001)
- **Fruit taste:** Palatability taste (Scored 1 [very bitter] – 5 [very sweet]) was used in assessing fruit taste (Atangana *et al.*, 2001)
- **Fruit pulp weight (g):** This was obtained by subtracting the weight of the nut (seed) from the fruit

Nut: Thirty seeds from each source were used to characterised *B. aegyptiaca* seeds based on the following descriptors:

- **Nut length and width (cm):** Electronic vernier caliper was used to measure these traits
- **Nut weight (g):** Sensitive electronic weighing balance was used in determining nut weight

Leaf: Characterisation of *Balanites aegyptiaca* leaf was done using thirty randomly selected leaves samples from each source using the descriptors below:

- **Leaf length (cm):** The leaf length without petiole was measured from the base to the apex using centimeter rule.
- **Leaf width (cm):** Centimeter rule was placed horizontally along the midrib of the leaf to determine the leaf width
- **Leaf thickness (cm):** This was assessed using a vernier caliper

Kernel weight (g): Thirty kernels extracted from each source were used to determine its weight using a sensitive weighing balance.

3.3.3 Data analysis

One way analysis of variance at $\alpha_{0.05}$ was used to analyse quantitative data collected. Means separation where applicable was done using Duncan Multiple Range Tests (DMRT). Descriptive statistics, mainly frequency bar chat was used to represent qualitative data from different locations. Correlation analysis among morphological traits and between soil physical and chemical properties and morphological characteristics was investigated to determine the relationship existing among and between them. Hierarchical cluster analysis was conducted using squared Euclidian distances to group quantitative characters according to their morphological similarities.

The coefficient of variation (CV %): CV was calculated according to Munilla and Guitián (2014) formula:

$$CV = \frac{SD}{X} \times 100 \qquad \text{eq. (3.1)}$$

X = the phenotypic mean of the trait, *SD*= the standard deviation of the trait

Heritability (h^2): h^2 among sources for each trait was based on Xu (2006) formula:

$$h^2 = 1 - \frac{1}{F} \qquad \text{eq. (3.2)}$$

F= is the F-test value in the ANOVA

Genetic gain (ΔG): ΔG was computed according to Silva *et al*. (2008) formula:

$$\Delta G = \frac{h^2 \Delta S}{X} \times 100 \qquad \text{eq. (3.3)}$$

X = the phenotypic mean of the trait, *h2* = heritability of the trait, Δ*S* = the selection differential.

3.4 Experiment 2: Germination characteristics of *Balanites aegyptiaca* seeds from different locations

3.4.1 Experimental site

This research was conducted at Federal University, Gashua, Yobe State which is located on approximately Latitude 12°51'.723"- 12°54'.723" N and longitude 11°00'.024" - 11°03'.475" E. The climate is characterised by the wet and dry seasons with a minimum temperature that ranges from 23-28°C and a maximum temperature of between 38-40°C. Average annual rainfall falls between 500 and 1000 mm.

3.4.2 Samples collection and preparation

Balanites aegyptiaca seeds obtained from each source were de-pulped by soaking in water overnight and scrubbing by hand. Five hundred visibly healthy seeds from each location were selected and subjected to a floatation test to ascertain their viability (Wakawa and Akinyele 2016). Fifty viable seeds were randomly selected from each source and soaked for 24 hours to reduce the effects of dormancy (Elfeel, 2012). The seeds were then sown on germination trays of 24 cm × 24 cm size in diameter and 10 cm in depth, filled with sterilized river sands. This was replicated five times which translated to 250 seeds for each source and 2000 seeds for all the sources. The germination trays were watered in the morning and evening for 31 days while the study lasted. The seeds were considered to have germinated when the plumule emerges. The experiment was arranged in a completely randomised design.

3.4.3 Germination indices assessed

1. ***Germination Percentage (GP)***: The formula given below was used to determine the germination percentage

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100 \qquad \text{eq. (3.8)}$$

2. ***Germination Speed (GS):*** The equation derived by Maguire (1962) as shown below was used to calculate GS

$$\text{GS} = \frac{\text{No.of seeds germinated}}{\text{Days of first count}} + \cdots + \frac{\text{No.of seeds germinated}}{\text{days of final count}} \qquad \text{eq. (3.9)}$$

3. ***Mean Germination Time*** **(MGT):** This was assessed according to the formula given by Al-mudaris (1998) as shown below

$$MGT = \Sigma f.x \div \Sigma f \qquad \text{eq. (3.10)}$$

Where f = seeds germinated on day x

3.4.4 Data analysis

Data were analysed using Analysis of variance at $\alpha_{0.05}$ and Duncan multiple range test was used for mean separation. The results were then represented in form of bars and line graphs using MS Excel.

3.5 Experiment 3: Early growth performance of *Balanites aegyptiaca* seedlings from different seed locations

3.5.1 Experimental setup

Polythene bags of 15 × 8.5 cm size were filled with topsoil and cattle cow dung, mixed in a ratio of 5:1 respectively. Ten seedlings of similar height growth from each source were collected from the germination beds and transplanted into polythene bags. This was replicated ten times, making a total of 100 seedlings for each source (treatment) and 800 seedlings for all the sources. After twenty weeks, the seedlings were transferred to another polythene bag measuring 28 cm × 12.4 cm in size. This was done to avoid growth restriction because the seedlings were found to have outgrown the size of the polythene bags they were earlier transplanted into. Seedlings were watered once daily during the 12 months the study lasted. This experiment was arranged completely randomised design.

3.5.2 Growth characteristics of *Balanites aegyptiaca* seedlings assessed

Ten seedlings from each treatment were randomly sampled for growth assessment. The following variables were assessed:

1. **Stem diameter (mm):** Digital vernier caliper was used to measure the diameter at the collar
2. **Stem height (cm):** The centimeter rule was used to measure the stem height. Measurement was taken from the soil level to the apex.

3. **The number of leaves:** The number of leaves was counted by physical observation.
4. **Root length:** The root length was measured using the centimeter rule at the termination of the experiment.
5. **Root: shoot ratio:** The shoot and root of each seedling sampled for analysis were collected and washed with water to remove soil particles. The shoots and roots were then packaged in a separate envelope and dried to constant weight at 70^0C in the oven for 72 hours. Root: shoot ratio was then calculated as shown below

$$\frac{\text{Dry weight of the root}}{\text{Dry weight of the shoot}} \qquad \text{eq. (3.11)}$$

6. **Absolute growth rate by plant height (AGR_{PH}) (cm day^{-1})**

   AGR_{PH} was calculated according to the formula used by Redford (1969) given below:

$$AGR_{PH} = \frac{H2-H1}{t2-t1} \qquad \text{eq. (3.12)}$$

   H_1 = Initial plant height

   H_2 = final plant height

   t_1= initial time

   t_2 = final time

7. **Biomass assessment**

   A biomass assessment was conducted at the termination of the experiment (12 months). Seedlings used for growth assessment were subjected to destructive sampling. The leaves stems, and roots of selected seedlings were cut with a sharp knife. The roots were washed with tap water to remove debris after which their fresh weight was determined. The fresh weight of leaves and stems was also determined separately. The samples were then oven-dried to constant weight for twenty-four hours at 70^0C to ascertain their dry weight. Plant total fresh weight was the summation of the fresh weight of roots, stems, and leaves.

   i. Relative growth rate by dry weight (RGR_{DW}) (mg g^{-1} day^{-1})

   RGR_{DW} was determined using the formula below (Gbadamosi, 2014)

$$RGR = \frac{\text{In (final dry weight)} - \text{In (initial dry weight)}}{\text{Duration of the experiment (Days)}} \qquad \text{eq. (3.9)}$$

ii. Absolute growth rate by dry matter* (AGR_{DM}) *(g day^{-1})

AGR_{DM} was calculated based on the formula of Redford (1969) as shown below

$$AGR_{DM} = \frac{W2 - W1}{t2 - t1} \qquad \text{eq. (3.10)}$$

W_1 = Plant dry matter weight at the start of the experiment

W_2 = Plant dry matter weight at end of the experiment

t_1= Time at beginning of the experiment

t_2 = Time at end of the experiment

3.5.3 Data analysis

Analysis of variance was used to analyse data, while Duncan multiple range test was employed for mean separation where applicable. Simple correlation analysis was used to determine the strength of the association between selected growth variables (seedlings' height, diameter, and the number of leaves) and the weather characteristics of the seed sources.

3.6 Experiment 4: Molecular characteristics of *Balanites aegyptiaca* tree populations

3.6.1 Samples collection and preparation

Leaf samples of *Balanites aegyptiaca* were purposively selected from the seedlings used for the growth study based on their availability. Twenty-nine (29) seedlings were selected; Baure (4), Buratai (3), Dumsai (3), Gamawa (3), Gashua (4), Guri (4), Kirikasama (4), and Mashi (4). 29 Snap-top containers measuring 25 mL each were filled with 15 mL of silica gel and covered with 5 mL of cotton wool. One gram of a fresh young leaf was collected from each sample and shredded into small sizes. The leaf samples were then placed on top of the cotton wool inside the snap-top plastic containers and closed tightly. The snap-top containers were labeled properly and put inside a big zip lock bag and zipped. Another batch of fresh leaf samples was collected and put inside small zip-lock bags, zipped, and labeled properly. The samples were stored in a vaccine

storage cooler filled with ice blocks and labeled accordingly before being transported to the Laboratory for DNA analysis.

Samples stored in the vaccine storage cooler were still fresh when they get to the laboratory. The samples were transferred into a fridge in the laboratory while the samples in snap-top plastic containers which have already dried because of the silica gel were left inside the big zip-lock bag in the laboratory before DNA extraction.

3.6.2 Laboratory analysis

Laboratory analyses to determine the molecular characteristics of *B. aegyptiaca* leaf samples from different locations were carried out at the Biotechnology Laboratory, National Horticultural Research Institute, Ibadan, Nigeria

3.6.3 Buffer preparation

The buffer solution was prepared by diluting 2.5% cetyltrimethyl ammonium bromide (CTAB), 2 mL of 0.5M ethylenediamine tetraacetic acid (EDTA), 1% polyvinylpyrrolidone (PVP), 4 mL of 1 M trismabase, 0.2% mercaptoethanol and 12 mL of 5 M sodium chloride (NaCl) in 17 mL of sterile distilled water.

3.6.4 DNA extraction

A slightly modified CTAB protocol of DNA extraction used by Varshney and Anis (2014) was adopted in this study. The procedure is explained below:

To each leaf sample, 2 mL of buffer was added and ground using mortar and pestle. 1 mL of the ground sample was emptied into 2 mL extraction tubes (labeled). The samples were incubated in a water bath set at 65^0C for 1 hour. Samples were shaken at 20 minutes intervals to ensure an adequate mixture of chemical constituents. Chloroform isoamyl alcohol (CIA) measuring 900 µL was added to each sample and shaken thoroughly until it mixed very well. This was done to avoid possible protein contamination, debris, and interphase material, Samples were then centrifuged at 15,000 rpm at 4^0C for 10 min and the supernatant was transferred to new tubes. 170 µL of 5 M of NaCl and 340 µL of chilled isopropanol was added to each sample, respectively and kept at -4^0C overnight in

a freezer. Samples were removed from the freezer the next day and centrifuged at 15,000 rpm for 10 min after which the supernatant was decanted to recover the white pellet. 70 µL TE buffer was added to the samples and incubated at -4^{0}C for 2 hours. After incubation, 25 µL and 600 µL of potassium acetate and ethanol were added to each sample respectively and incubated again for 2 hours at -4^{0}C. The dissolved pellets were spun at 15,000 rpm for 10 min at 4^{0}C. Pellets were air-dried after discarding the supernatants. Lastly, 50 µL of sterile distilled water was used to dissolve the pellets.

3.6.5 Preparation of gel (1.2%) for electrophoresis

Agaros powder measuring 0.6 g was dissolved in 60 mL of TBE buffer to give 1% gel for a 60 mL tray. To get 1.2 % gel, 0.6 g Agaros powder was multiplied by 12 g. After cooling, 5 µL ethidium bromides were added and then poured into the tray for use.

3.6.6 Electrophoresis

Each DNA sample of 2 µL was mixed with 2 µL of gel-loading dye on a parafilm paper and loaded into each well of the electrophoresis machine and allowed to run at a constant voltage of 80 V for 15 minutes twice (i.e. 30 minutes).

3.6.7 Integrity test

Samples on a gel tray were loaded in a gel documenting system (E-BOX CXG.TS Made in France) and photographed to assess DNA quality. Illuminating bright light reflection of the sample indicates quality DNA.

3.6.8 Polymerase chain reaction (PCR), optimisation, and DNA amplification

A polymerase chain reaction (PCR) cocktail of 12.5 µL volume was used. The constituents include the following 1.25 µL Buffer, 0.8 µL $MgCl_2$, 0.25 µL dNTPs, 0.25 µL *MatK* F, 0.25 µL *MatK* R, 6.64 µL sterile distilled water, 0.06 µL Taq, 3 µL DNA template. PCR was done using the Eppendorf master cycler (Eppendorf AG 22331 Hamburg). Initial denaturation took 1 min at 94^{0}C, followed by 35 cycles of DNA denaturation at 94^{0}C for 4 secs. Annealing of primers took 40 secs at 51^{0}C; the initial extension took 40 secs at 72^{0}C while the final extension lasted for 5 mins at 72^{0}C.

A pair of Mat*K* primers was used for amplification. The sequence of the primers is shown below:

MATK-KIM3F (5'-CGTACAGTACTTTTGTGTTTACGAG-3')

MATK-KIM1R (5'-ACCCAGTCCATCTGGAAATCTTGGTTC-3')

3.6.9 Data analysis

Base calling using the BioEdit sequence alignment editor was carried out on the sequence data manually (Hall, 1999). Matching (BLAST) of sequences with similar sequences was performed on National Centre for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences belonging to the same Genus (Balanites) of 625 lengths (bp) and above that used matK gene loci only were retrieved from the NCBI gene bank on 15th September 2023 and used for the authentication (identification) of sample species. A phylogenetic tree of the gene sequence was generated using Molecular Evolutionary Genetics Analysis software (MEGA 7). The pairwise distance was computed using maximum composite likelihood (Kumar *et al.*, 2004). DnaSP 6.12.03 was used to determine the molecular characteristics and population size change (Rozas *et al.*, 2017).

3.7 Experiment 5: Physical and chemical properties of soil under *B. aegyptiaca* tree from different locations

3.7.1 Samples collection and preparation

Soil samples were collected under selected mother trees at 0-15 cm and 16-30 cm depths at four different points in each location using a soil auger (0.5 cm in diameter and 0.5 cm in height). Samples under each tree in each location were bulked and packaged in a Ziploc sample bag and properly labeled. Samples were air-dried in a ventilated room and crushed for the separation of non-soil particles. Composite soil samples were sieved with 5 mm and 2 mm sieves.

3.7.2 Analysis of soil samples

The hydrometer method was used for bulk density determination following the method of (Brian, 1997). Soil pH was determined using the soil water-extracting methods. Soil organic carbon was determined with the chromic acidic wet oxidation method (Walkley and Black, 1935). Nitrogen was determined using the micro Kjeldahl method (AOAC, 1997). Available phosphorus was determined using the Bray-2 extractant method. Soil potassium quantity was determined by a flame photometer (Toth and Princem, 1949). Manganese was determined with the diethylene triamine penta-acetic acid (DTPA) extraction method. Calcium and magnesium were determined with NH4OAc base saturation methods. Manganese, calcium, magnesium were determined in solution using Atomic Absorption Spectrophotometer (AAS) method.

3.7.3 Data analysis

The data obtained were analysed using one way analysis of variance at $\alpha_{0.05}$. Duncan Multiple Range Tests (DMRT) was used to separates means.

3.8 Experiment 6: Oil yield, physical and chemical properties of *Balanites aegyptiaca* kernel oil from different sources

3.8.1 Sample preparation

Balanites aegyptiaca kernels from different locations were extracted manually using a hammer. The kernels were sundried for 3 days after which it was blended into powder. Dried samples were stored in airtight containers and labeled accordingly for oil extraction.

3.8.2 Laboratory analysis

- **Oil extraction**

  The extraction thimble was loaded with 500 g of *Balanites aegyptiaca* kernel powder (M1) from each of the eight locations which were then loaded into a soxhlet apparatus for extraction. Hexane was used as a solvent for the extraction. Oil was successfully extracted after 6-8 hours of the distillation process. After

the extraction, the solvent was separated from the oil with the aid of a rotary vapourator. The extracted oil was weighed and labeled as M2.

- **Determination of oil yield (%)**

  Oil yield was determined gravimetrically and expressed in percentage using the formulae below:

$$\text{Yield}(\%) = \frac{\text{M2}}{\text{M1}} \times 100 \qquad \text{eq. (3.4)}$$

M1 = weight of kernel power, M2 = weight of oil exacted

- **Physical and chemical properties assessment**

  Refractive index, viscosity, acid value, saponification, and iodine value were analysed using standard methods described by AOAC (1984)

  1. **Refractive index:** *Balanites aegyptiaca* oil sample of 0.5 g from each location was melted at 25°C in a water bath after which the refractive index was analysed using a refractometer.
  2. **Viscosity:** Three hundred milliliter of *B. aegyptiaca* oil from each location was melted at 29°C in a water bath and then empty into a beaker for viscosity assessment using viscometer.
  3. **Acid value:** Five grams of *B. aegyptiaca* oil from each location was dissolved in a solvent ethanol and diethyl ether (95 %) in the ration of 1:1. The solution was then titrated with 0.1 M potassium hydroxide (KOH) using 1cm^3 of 1 % (w/v) of phenolphthalein as an indicator until a pink colouration was obvious. Acid value was then calculated using the equation given below:

$$\text{Acid value} = (\text{mgKOH/g oil}) = \frac{56.1 \text{ x V x C}}{\text{W}} \qquad \text{eq. (3.5)}$$

V = Volume of standard potassium hydroxide used

C = Concentration of potassium hydroxide used

W = Weight of oil (g)

4. **Saponification:** Two grams each of *B. aegyptiaca* oil from eight locations were weighed in a conical flasks. Each oil samples were dissolved in 25 mL of 0.5 N alcoholic potassium-hydroxide. The mixture in each flask was refluxed for about an hour in a water bath and shaken regularly. The end products were then allowed to cool before titrating against 0.5 M hydrochloric acid (HCl). Two drops of phenolphthalein was used as an indicator for the titration. A blank determination was carried out and the saponification value determined from the relationship given below:

$$\text{Saponification value (mgKOH/g oil)} = \frac{56.1 \text{ x C x (Vo – V)}}{\text{W}} \qquad \text{eq. (3.6)}$$

C = Concentration of HCl used

Vo = Volume of HCl used for blank

V = Volume of HCl used for oil sample

W = Weight of oil (g)

5. **Iodine value:** Two gram of *B. aegyptiaca* oil from each location was weighed into a 500 ml conical flask after which 10 ml of chloroform (CCl_4) was added. This was followed by the addition of 15 % potassium iodine, the solution was shaken very well and titrated with 0.1 M sodium thiosulphate ($Na_2S_2O_3$) until a yellow solution turned almost colourless. Starch of three drops which served as an indicator was added and while the titration continues until the colour turns colourless. A blank determination was done and recorded. The iodine value was calculated as shown below:

$$\text{Iodine value (mgKOH/g oil)} = \frac{(\text{B}-\text{S})\times\text{M}\times 12.69}{\text{W}} \qquad \text{eq. (3.7)}$$

B= volume of $Na_2S_2O_3$ used for blank titration

S= volume of $Na_2S_2O_3$ used for oil sample

M= molarity of $Na_2S_2O_3$ (0.1)

12.69 = constant (meq weight of iodine)

W = Weight of oil (g)

3.8.3 Data analysis

The data were analysed using one way analysis of variance. Duncan multiple range test was used to separate means of treatments found to differ significantly. The association between oil yield, physical and chemical properties with some weather variables of the kernel locations was determined using correlation analysis at $\alpha_{0.05}$.

CHAPTER 4

RESULTS

4.1 Morphological characteristics among *Balanites aegyptiaca* populations

4.2 Quantitative morphological characteristics

4.2.1 Tree characteristics

The morphological characteristics (height, diameter at breast height (DBH), and crown diameter) of *Balanites aegyptiaca* trees among locations were observed to vary significantly (Table 4.1). Tree height (m) ranged from 5.57±0.79 (Gashua) to 12.67±2.85 (Gamawa), the DBH (cm) ranged from 27.96±9.62 in Gashua to 44.16±6.77 in Gamawa, while the crown diameter (m) was discovered to increase from 7.68±1.28 (Kirikasama) to 10.49±2.92 (Baure). The coefficient of variation (CV) was highest in DBH (33.02) compared with height and crown diameter which had 16.36 and 22.53 CV, respectively (Table 4.1).

4.2.2 Fruits characteristics

The fruit of *Balanites aegyptiaca* comes in different sizes, this variation was discovered to be significant among locations (Table 4.2). Fruit of *Balanites aegyptiaca* had a mean fruit pulp thickness that ranged from 1.41±0.15 cm at Guri to 1.84±0.39 cm at Baure. Similarly, the fruit was also found to have different width sizes across the various sources of seeds. Fruits from Baure recorded the highest mean fruit width of 2.13±0.16 cm, while that of Gashua recorded the lowest mean fruit width of 1.77±0.19 cm. *Balanites aegyptiaca* mean weight of fruit in the study area increased from 4.29±1.77 g to 7.20±2.26 g. Baure fruits recorded the highest mean weight of fruit (7.20±2.26 g) but were not significantly different from the weight from fruits in Dumsai (5.89±1.28 g), Gamawa (6.73±2.66 g), Gashua (5.64±1.42 g), Kirikasama (6.12±1.47 g), and Mashi source (6.15±1.99 g). Fruit from Buratai which recorded 4.29±1.77 g had the lowest weight of fruit. Fruit length was found to range from 2.45±0.31 cm in Dumsai to

3.08±0.26 cm in Kirikasama (Table 4.2). The level of variability (CV) among fruit traits was highest in fruit weight (29.68) and lowest in fruit width (10.79) (Table 4.2).

4.2.3 Pulp and leaf characteristics

The fruit of *Balanites aegyptiaca* has an edible mesocarp (pulp), and the pulp weight was found to vary significantly among locations (Table 4.3). Fruits from Gamawa recorded the highest pulp weight of 4.44±1.87 g and varied significantly compared with fruits from Buratai which had the lowest pulp weight of 2.18±1.33 g. The leaves of *Balanites aegyptiaca* are generally small in size. Leaf length and leaf width of *Balanites aegyptiaca* varied significantly among locations (Table 4.3). Leaf length ranged from 0.14±0.02 cm (Guri) to 0.18±0.04 cm (Baure), while the leaf width ranged from 0.22±0.13 cm (Buratai) and 0.44±0.19 cm (Baure and Gamawa). There was no significant difference in leaf thickness of *Balanites aegyptiaca* among the locations. Leaf thickness decreased from 0.62±0.35 cm in Baure to 0.41±0.05 cm in Dumsai. The coefficient of variation (CV) in fruit pulp weight was 37.07, while the leaf length, leaf width and leaf thickness had a CV of 19.50, 40.20 and 18.51, respectively (Table 4.3).

Table 4.1: Diameter at breast height, height and crown diameter of *Balanites aegyptiaca* trees across different locations

Source	Diameter at breast height (cm)	Height (m)	Crown diameter (m)
Guri	32.7±7.65^{abc}	6.76±0.91^{bc}	10.91±1.95^{a}
Kirikasama	40.32±8.28^{ab}	7.25±1.39^{b}	7.68±1.28^{c}
Gamawa	44.16±6.77^{a}	12.67±2.85^{a}	9.68±1.63^{abc}
Buratai	28.47±11.20^{bc}	6.12±1.28^{bc}	8.98±2.28^{abc}
Mashi	30.45±12.05^{bc}	6.11±0.84^{bc}	9.13±2.88^{abc}
Baure	31.71±10.27^{bc}	6.53±1.0^{bc}	10.49±2.92^{ab}
Gashua	27.96±9.96^{c}	5.57±0.79^{c}	8.19±1.94^{bc}
Dumsai	33.51±19.47^{abc}	6.53±0.78^{bc}	8.83±1.80^{abc}
CV (%)	33.02	16.36	22.53
F-Sta	2.29	26.65	2.16
Sig	0.04*	0.00*	0.05*

Source: Field survey (2019). CV = coefficient of variation, F= test value in the ANOVA table. Mean values are followed by the standard deviation. Values with the same letter across column are not significantly different (P≤0.05).

Table 4.2: Quantitative characteristics of the fruit of *Balanites aegyptiaca* from different locations

Source	Fruit pulp thickness (cm)	Fruits width (cm)	Fruit weight (cm)	Fruit length (cm)
Baure	1.84±0.39^{a}	2.13±0.16^{a}	7.20±2.26^{a}	2.83±0.34^{ab}
Buratai	1.47±0.45^{b}	1.89±0.31^{bc}	4.29±1.77^{c}	2.51±0.52^{bc}
Dumsai	1.51±0.21^{b}	1.87±0.15^{bc}	5.89±1.28^{abc}	2.45±0.31^{c}
Gamawa	1.45±0.36^{b}	1.97±0.34^{abc}	6.73±2.66^{ab}	2.78±0.43^{abc}
Gashua	1.62±0.18^{ab}	1.77±0.19^{c}	5.64±1.42^{abc}	2.53±0.31^{bc}
Guri	1.41±0.15^{b}	1.98±0.16^{abc}	5.14±1.13^{bc}	2.65±0.17^{bc}
Kirikasama	1.69±0.24^{ab}	2.10±0.18^{ab}	6.12±1.47^{abc}	3.08±0.26^{a}
Mashi	1.45±0.31^{b}	2.05±0.20^{ab}	6.15±1.99^{abc}	2.71±0.26^{bc}
CV	18.49	10.79	29.68	12.91
F-stat	2.25	2.74	8.15	2.88
Sig.	0.04*	0.01*	0.03*	0.01*

Source: Field survey (2019). CV = coefficient of variation, F= test value in the ANOVA table. Mean values are followed by the standard deviation. Values with the same letter across column are not significantly different (P≤0.05).

Table 4.3: Quantitative characteristics of *Balanites aegyptiaca* pulp and leaf across different locations

Sources	Pulp weight (g)	Leaf length (cm)	Leaf width (cm)	Leaf thickness (cm)
Baure	4.38±1.92[a]	0.18±0.04[a]	0.44±0.19[a]	0.62±0.35
Buratai	2.18±1.33[b]	0.15±0.05[b]	0.22±0.13[b]	0.48±0.03
Dumsai	3.83±1.09[a]	0.15±0.02[b]	0.38±0.11[a]	0.41±0.05
Gamawa	4.44±1.87[a]	0.14±0.04[b]	0.44±0.19[a]	0.45±0.09
Gashua	3.48±1.04[ab]	0.16±0.02[ab]	0.35±0.10[ab]	0.45±0.07
Guri	2.98±1.07[ab]	0.14±0.02[b]	0.30±0.11[ab]	0.46±0.06
Kirikasama	3.61±1.12[ab]	0.17±0.02[ab]	0.36±0.11[ab]	0.47±0.05
Mashi	3.46±1.78[ab]	0.15±0.03[b]	0.35±0.18[ab]	0.43±0.06
CV	37.07	19.50	40.20	18.51
F-stat	2.52	2.25	2.52	2.08
Sig.	0.02*	0.04*	0.02*	0.06**ns**

Source: Field survey (2019). CV = coefficient of variation, F= test value in the ANOVA table. Mean values are followed by the standard deviation. Values with the same letter across column are not significantly different (P≤0.05).

4.2.4 Nut and kernel characteristics

The nut of *Balanites aegyptiaca* also referred to as the seed comes in different sizes (length, width, thickness) and weights based on the observation from this study. Nut length and thickness were observed to vary significantly among the locations, while nut width did not vary significantly among locations (Table 4.4). *Balanites aegyptiaca* nut in the study area had a mean length that ranged from 2.31±0.14 cm (Guri) to 2.90±0.26 cm (Kirikasama), the nut thickness increased from 0.81±0.12 cm (Guri) to 1.24±0.09 cm (Baure), while the width ranged from 1.33±0.22 at Gamawa to 1.49±0.13cm at Mashi. *Balanites aegyptiaca* nut and kernel weight did not vary significantly among locations. The mean nut weight ranged from 2.06±0.62 g (Kirikasama) to 2.82±0.61 g (Baure), while the kernel weight ranged from 0.45±0.06 g (Gashua) to 0.70±0.18 g (Baure). The coefficient of variation (CV) in nut traits was highest (27.07) in nut weight, compared with nut length (12.52), nut width (12.87) and nut thickness (15.73). Kernel weight had a CV of 27.76 (Table 4.4).

4.3 Hereditary and genetic gain in traits

The level of heredity among traits was highest in nut thickness (0.90) and lowest in nut width (0.06). Kernel weight recorded the highest genetic gain (14.47) among the traits, while nut width had the lowest (2.46) (Table 4.5).

Table 4.4: Morphological characteristics of nut and kernel of *Balanites aegyptiaca* from different sources

Source	Nut length (cm)	Nut width (cm)	Nut weight (g)	Nut thickness (cm)	Kernel weight (g)
Baure	2.71±0.33^{ab}	1.48±0.09	2.82±0.61	1.24±0.09^{a}	0.70±0.18^{a}
Buratai	2.53±0.38^{bc}	1.39±0.19	2.21±0.75	1.19±0.15^{a}	0.49±0.15^{a}
Dumsai	2.37±0.36^{c}	1.40±0.14	2.06±0.62	1.16±0.20^{a}	0.53±0.16^{bc}
Gamawa	2.61±0.38^{abc}	1.33±0.22	2.28±0.87	0.84±0.33^{b}	0.49±0.19^{bc}
Gashua	2.45±0.31^{bc}	1.40±0.15	2.17±0.55	1.20±0.12^{a}	0.45±0.06^{c}
Guri	2.31±0.14^{c}	1.43±0.43	2.16±0.52	0.81±0.12^{b}	0.56±0.11^{abc}
Kirikasama	2.90±0.26^{a}	1.39±0.10	2.50±0.46	0.85±0.12^{b}	0.63±0.24^{ab}
Mashi	2.55±0.26^{bc}	1.49±0.13	2.69±0.67	1.14±0.12^{a}	0.70±0.18^{a}
CV (%)	12.52	12.87	27.07	15.73	27.76
F-Stat	2.075	0.602	1.744	10.263	3.62
Sig.	0.09**ns**	0.75**ns**	0.11**ns**	0.00*	0.00*

Source: Field survey (2019). CV = coefficient of variation, F= test value in the ANOVA table. Mean values are followed by the standard deviation. Values with the same letter across column are not significantly different (P≤0.05).

Table 4.5: Hereditary and genetic gain of traits in *B. aegyptiaca* in the source population

Trait	Hereditary (h^2)	Genetic gain (ΔG)
Fruit thickness	0.56	6.73
Fruit width	0.63	4.35
Pulp weight	0.60	11.76
Fruit weight	0.59	11.76
Fruit length	0.65	5.74
Leaf length	0.56	7.9
Leaf width	0.60	12.34
Leaf thickness	0.52	-
Nut length	0.52	3.06
Nut width	0.06	2.46
Nut weight	0.43	6.96
Nut thickness	0.90	13.83
Kernel weight	0.72	14.47

4.4 Qualitative morphological characteristics of *Balanites aegyptiaca* fruit from different locations

Balanites aegyptiaca fruit exhibited different qualitative traits among sources. The fruits had different tastes; colours and shapes. Forty eight percentages of fruits from Gamawa had a very sweet taste, while 62% of fruits from Mashi had sweet taste. The highest percentage (26.67%) of fruits with a bitter-sweet taste was recorded in Kirikasama, and 3.33% of fruits in Gashua had very bitter taste (Fig. 4.1). Four fruit shapes were identified in the study areas: elongates, oblong, oval, and spherical (Plate 4.1). The 4 shapes (elongates, oblong, oval, and spherical) were found in Mashi, Kirikasama, and Gamawa, while only 3 shapes (oblong, oval and spherical) were found in Dumsai, Gashua, Guri, and Buratai. *Balanites aegyptiaca* fruits in Baure all had oblong shape (Fig. 4.2). The fruit colour of *Balanites aegyptiaca* fruit (pulp) was observed to vary among sources but the predominant colour was yellow (Plate 4.2). Yellow constituted 53 %, 67.5 %, 89 %, 65 %, 65 %, 91 %, 86.67 %, and 87 % of fruit colour in Baure, Buratai, Dumsai, Gamawa, Gashua, Guri, Kirikasama and Mashi, respectively (Fig. 4.3).

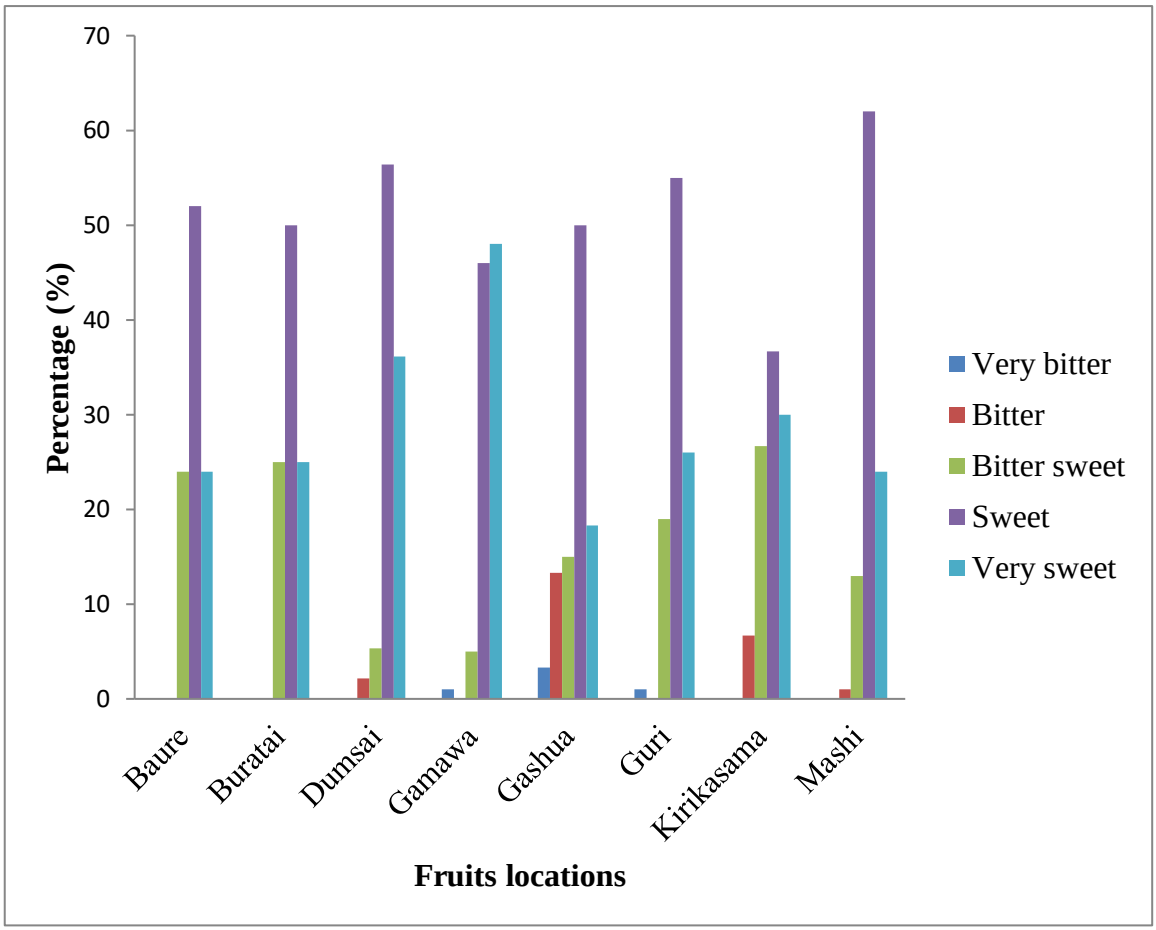

Fig. 4.1: Taste of *B. aegyptiaca* fruit from different locations

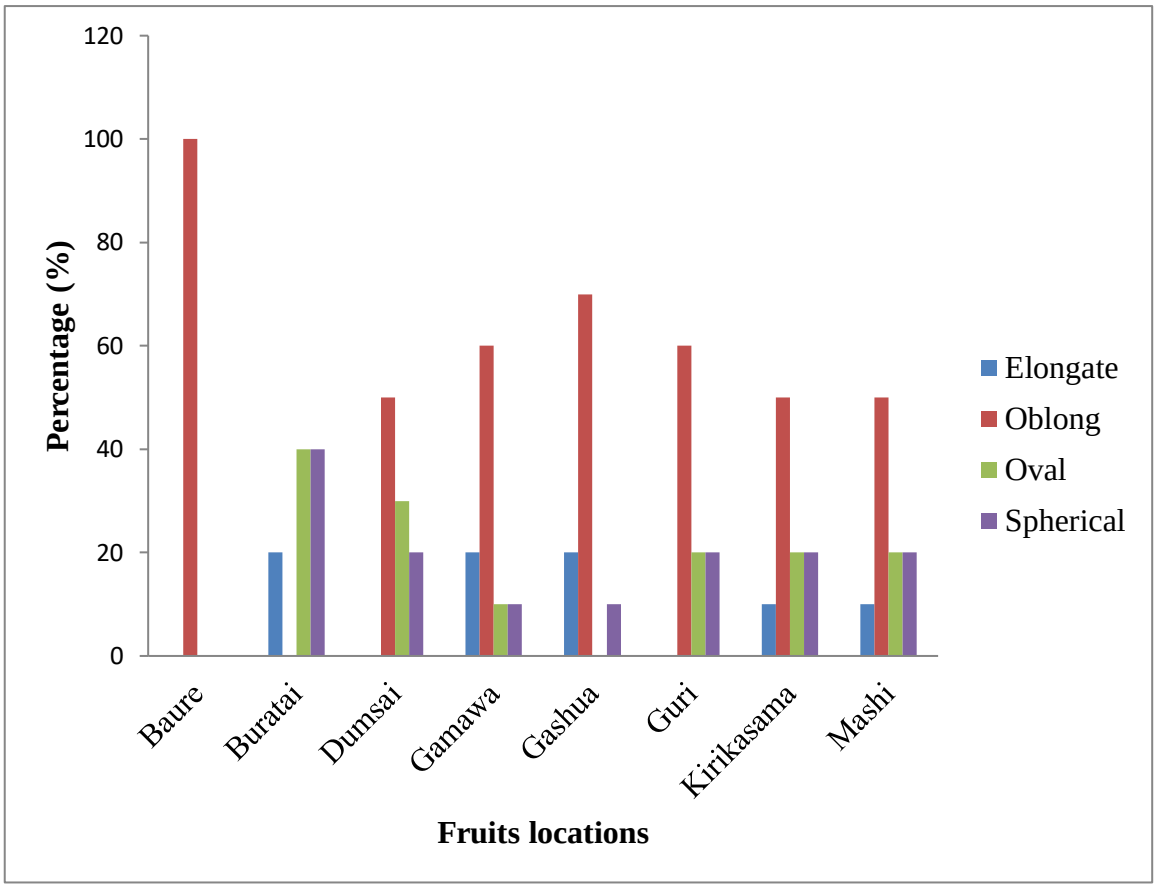

Fig. 4.2: Shape of *B. aegyptiaca* fruit from different locations

Plate 4.1: Shape of *B. aegyptiaca* fruits in the study area (a) Spherical (b) Elongate (c) Oval (d) Oblong

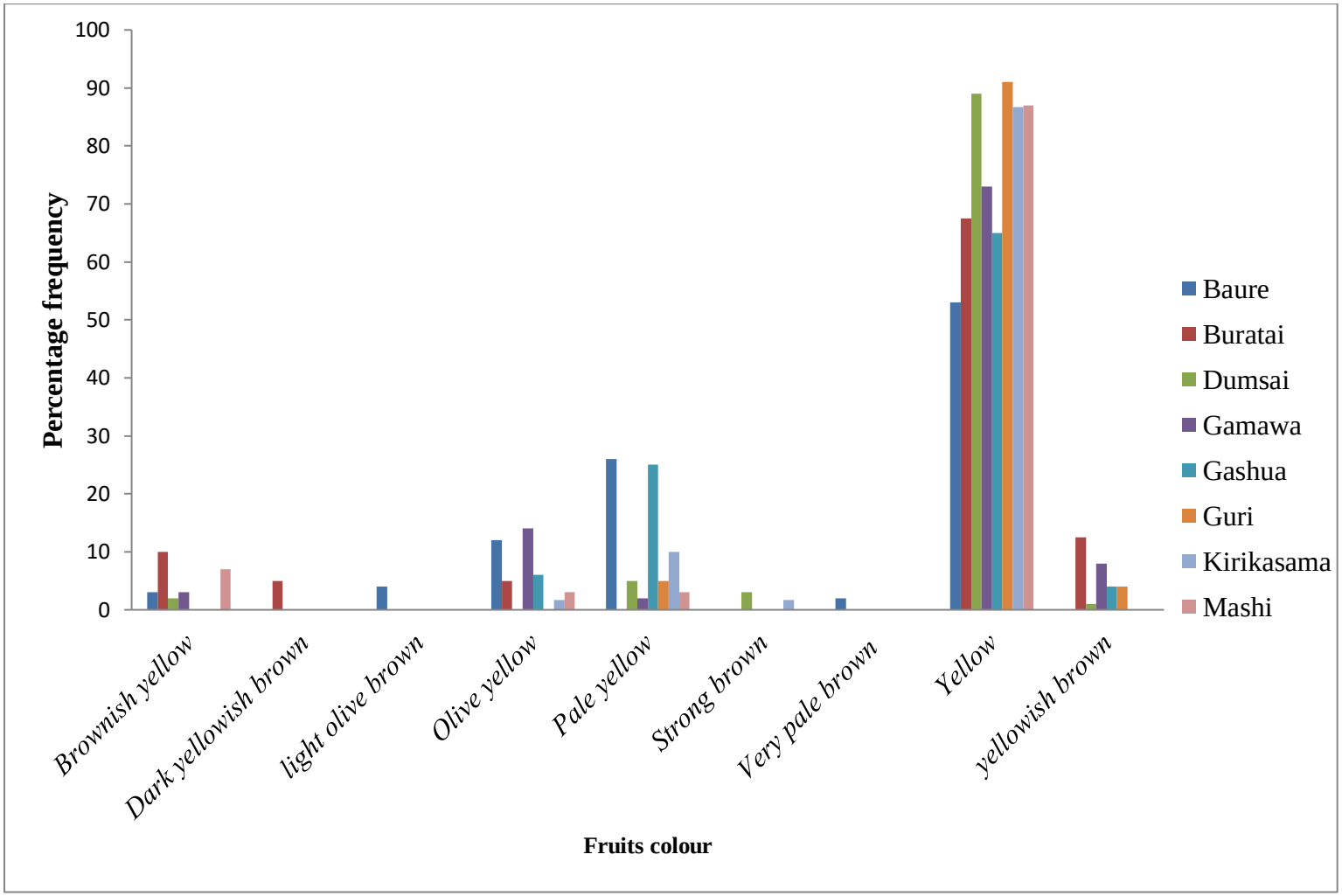

Fig. 4.3: Colour of *B. aegyptiaca* fruit from different locations

Plate 4.2: Colours of *B. aegyptiaca* fruit from different locations. (a) Light olive brown (b) Yellow (c) yellowish brown (d) Strong brown

4.5 Correlations between *Balanites aegyptiaca* morphological traits

The correlation between *Balanites aegyptiaca* morphological traits varied. The length of *Balanites aegyptiaca* fruit showed a significant positive correction with pulp weight, fruit weight, nut length, nut weight, leaf length, and leaf width. Pulp weight was also found to have a significant positive correlation with fruit weight, nut length, nut weight, leaf length, and leaf width, while fruit weight has a positive significant correlation with nut length, nut weight, leaf length, and leaf width. Nut length significantly correlated positively with nut weight, leaf length, and leaf width, a significant positive correlation was also observed between nut weight and leaf length, and leaf width (Table 4.6).

4.6 Morphological similarities of *Balanites aegyptiaca* trees from different locations

Three distinct clusters based on morphological characters were established. Trees in Guri, Dumsai, Gashua, Mashi, and Kirikasama formed a cluster. *Balanites aegyptiaca* trees in Gamawa and Baure were clustered together, while Buratai stand on its own without forming a cluster with any source (Fig. 4.4).

4.7 Germination characteristics of *Balanites aegyptiaca* seeds from different locations

4.7.1 Seed germination percentage (GP)

The source of seed collection was found to significantly influence the germination of *Balanites* aegyptiaca seeds. Seeds from Guri recorded the highest mean germination percentage (91.96) and differed significantly from all the other sources except for Gashua which recorded 91.14 %. Seeds from Baure source recorded the lowest (52.94) germination percentage in this study (Fig. 4.5).

4.7.2 Mean germination time (MGT days)

The average time (days) required for the seeds of *Balanites aegyptiaca* to complete germination (MGT) after sowing varied significantly among seed sources**.** Gashua seed took a longer period (9.06 days) to complete germination, while seed from Baure completed germination in 5.07 days (Fig. 4.6)

Table 4.6: Correlation between morphological traits of *Balanites aegyptiaca* trees

	FL	PWG	FWG	NL	NWG	KWG	LL	LWD	LTH
FL	1.000								
PWG	0.446*	1.000							
FWG	0.529*	0.951*	1.000						
NL	0.836*	0.321*	0.433*	1.000					
NWG	0.446*	0.392*	0.651*	0.511*	1.000				
KWG	0.126	0.082	0.109	-0.010	0.118	1.000			
LL	0.308*	0.651*	0.694*	0.232*	0.470*	0.090	1.000		
LWD	0.446*	0.631*	0.951*	0.321*	0.392*	0.082	0.651*	1.000	
LTH	0.139	0.031	0.074	0.145	0.150	-0.053	0.189	0.031	1.000

Source: Field survey (2019). FL = Fruit length (cm), PWG = Pulp weight (g), FWG = Fruit weight (g), NL = Nut length (cm), NWD = Nut width (cm), KWG = Kernel weight (g), LL = Leaf length (cm), LWD = Leaf width (cm), LTH = Leaf thickness (cm).

* Correlation is significant at the 0.05 level

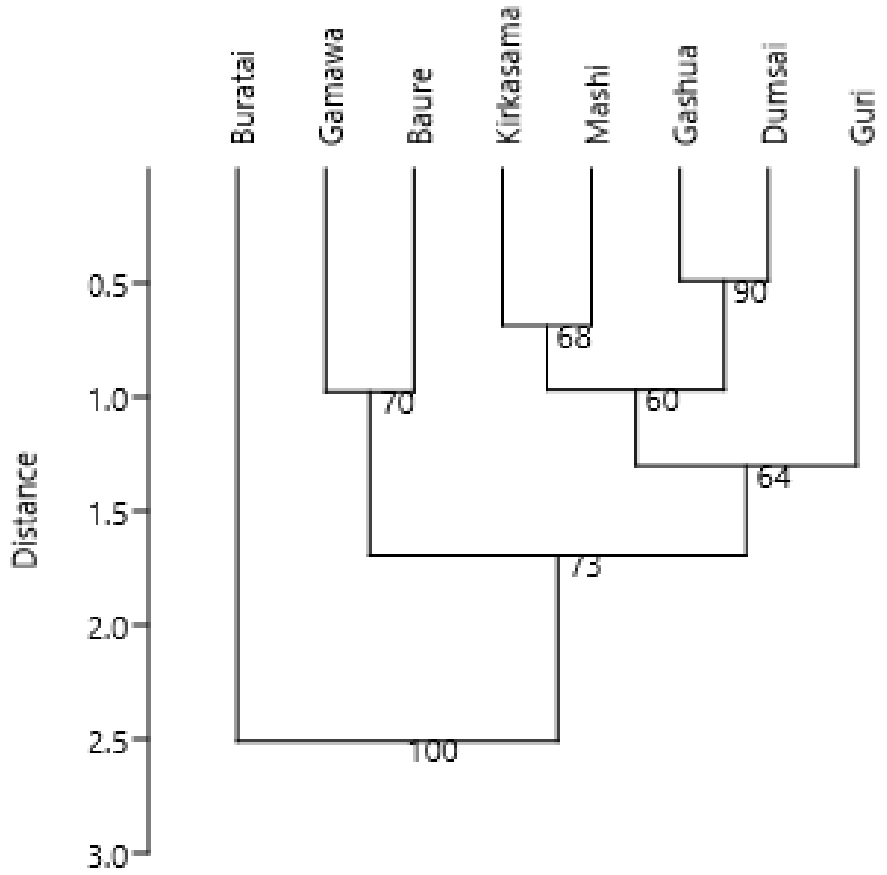

Fig. 4.4: Hierarchal clustering showing variation in morphological traits of *Balanites aegyptiaca* from different locations

4.7.3 Germination speed (GS)

Seed sources significantly affected the germination speed of *Balanites aegyptiaca*. Seed from Guri recorded the highest mean germination speed (0.87) and differed significantly with all the other sources. This was followed by Gashua (0.82), Mashi (0.78), Buratai (0.69), Kirikasama (0.68), and Dumsai (0.65). Seeds from Baure and Gamawa with 0.47 GS each had the lowest value (Fig 4.7).

4.8 Growth performance of *Balanites aegyptiaca* seedlings among seed sources

4.8.1 Stem height growth

Significant variation in seedlings height growth was observed among the sources of *Balanites aegyptiaca* seedlings. Seedlings from Dumsai had the highest mean stem height of 60.82 cm and differed significantly from the other sources except for Baure and Guri sources which had 54.30 cm and 57.24 cm respectively. Seedlings from Mashi had the lowest mean stem height of 43.57 cm (Fig. 4.8).

4.8.2 Collar diameter growth

Stem height growth of *Balanites aegyptiaca* seedlings varied significantly among seed sources. Seedlings from Guri had the highest mean collar diameter of 4.91 mm but were not significantly different with seedlings from Baure, Buratai, Dumsai, Kirikasama, and Mashi which had 4.37, 4.38, 4.83, 4.32, and 4.38 mm, respectively (Fig. 4.9).

4.8.3 Number of leaves

Number of leaves in *Balanites aegyptiaca* seedlings varied significantly among seed sources. Seedlings from Mashi recorded the highest mean number of leaves with 166.60 even though it did not show any significant variation with seedlings from Buratai and Dumsai which had 144.10 and 150.40 mean number of leaves respectively. Seedlings from Gamawa were observed to have the lowest mean number of leaves with 104.70 (Fig. 4.10).

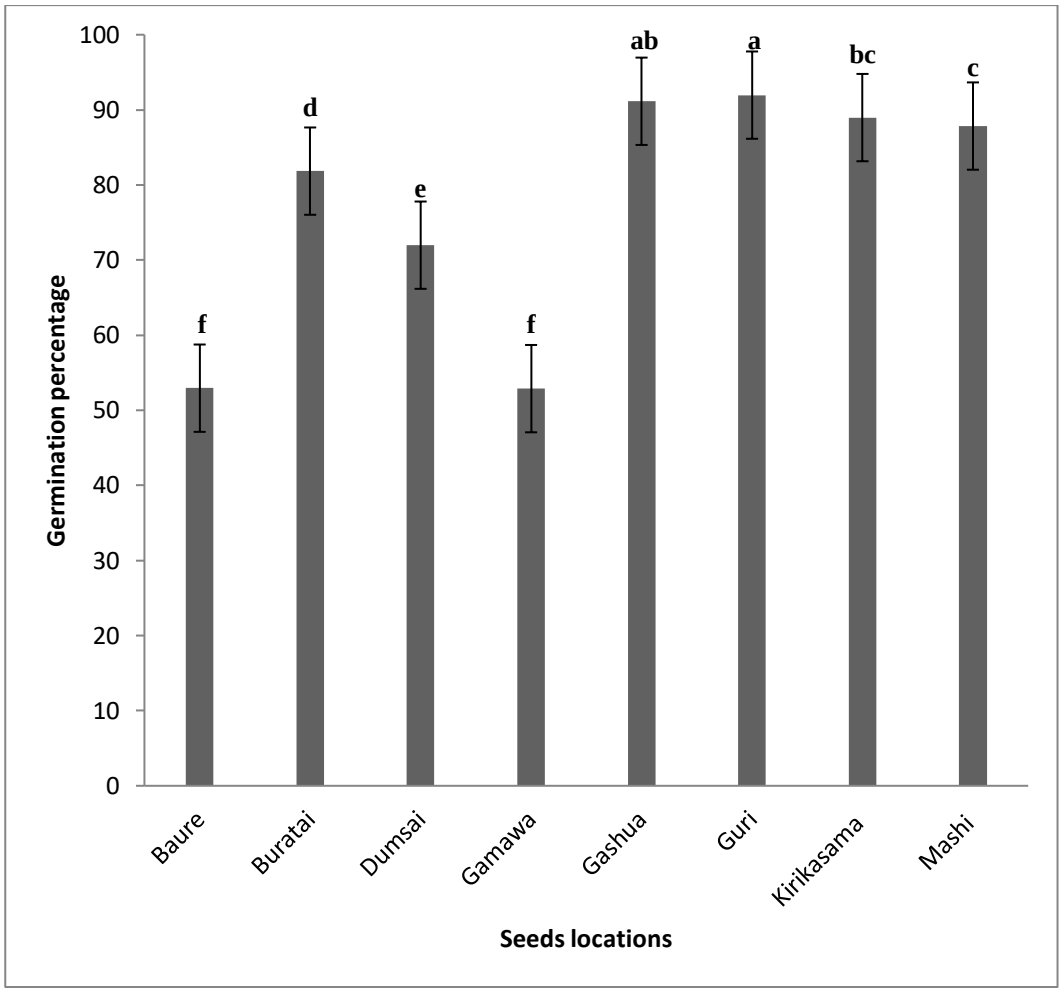

Fig. 4.5: Germination percentages of *B. aegyptiaca* from different seed locations

Means sharing a similar alphabet did not differ significantly from each other ($P \leq 0.05$). The line through a point on the graph represents standard deviation error bars

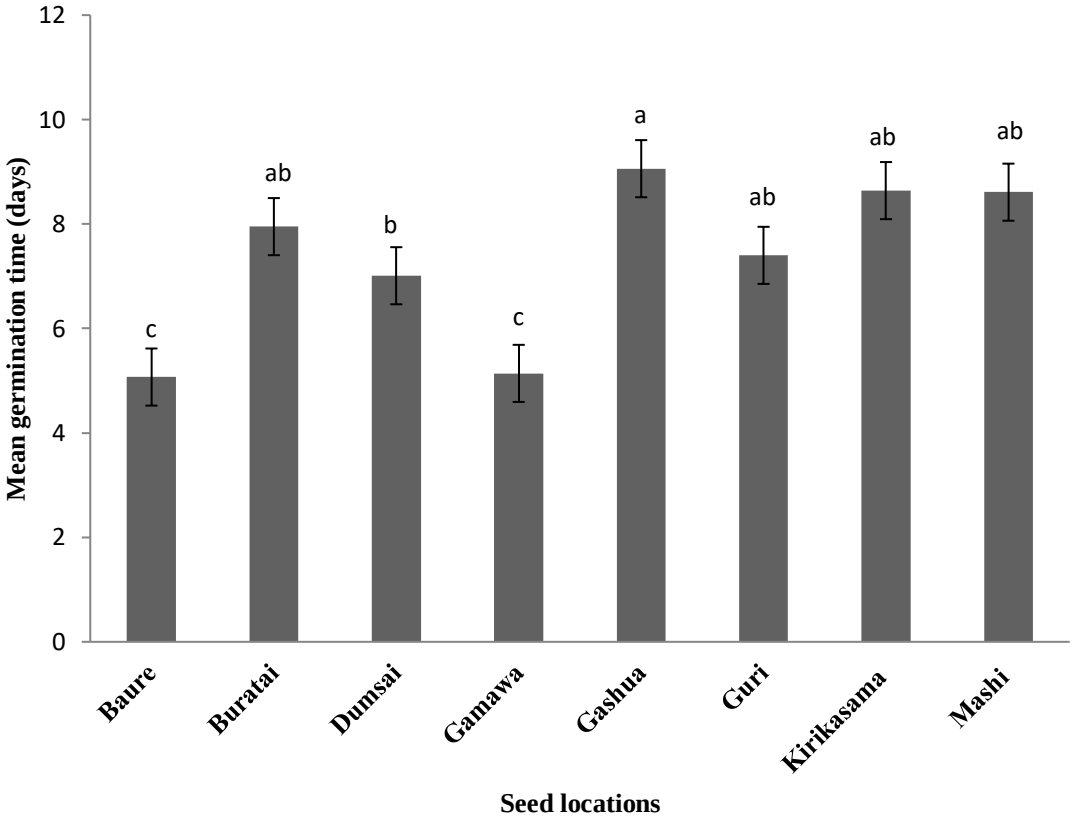

Fig. 4.6: Mean germination time of *B. aegyptiaca* from different seed locations

Means sharing a similar alphabet did not differ significantly from each other ($P \leq 0.05$). The line through a point on the graph represents standard deviation error bars

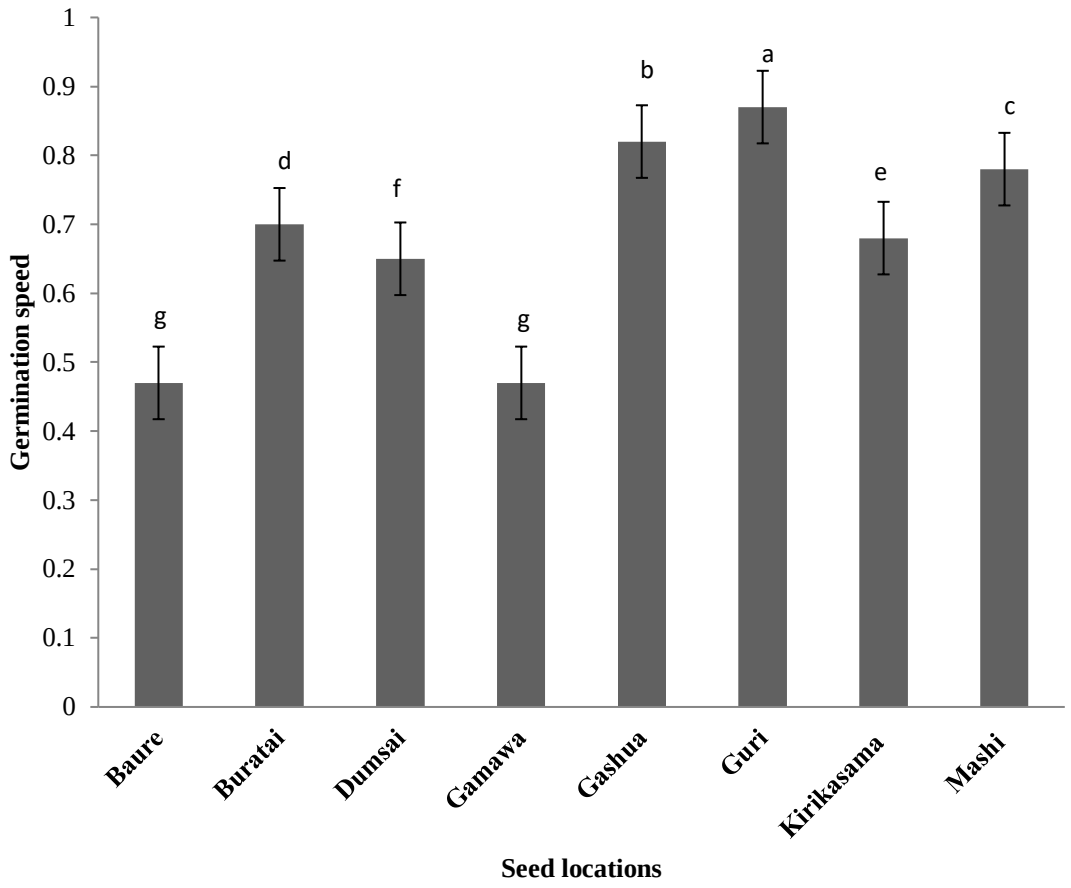

Fig. 4.7: Germination speed of *B. aegyptiaca* seeds from different seed locations

Means sharing a similar alphabet did not differ significantly from each other ($P \leq 0.05$). The line through a point on the graph represents standard deviation error bars

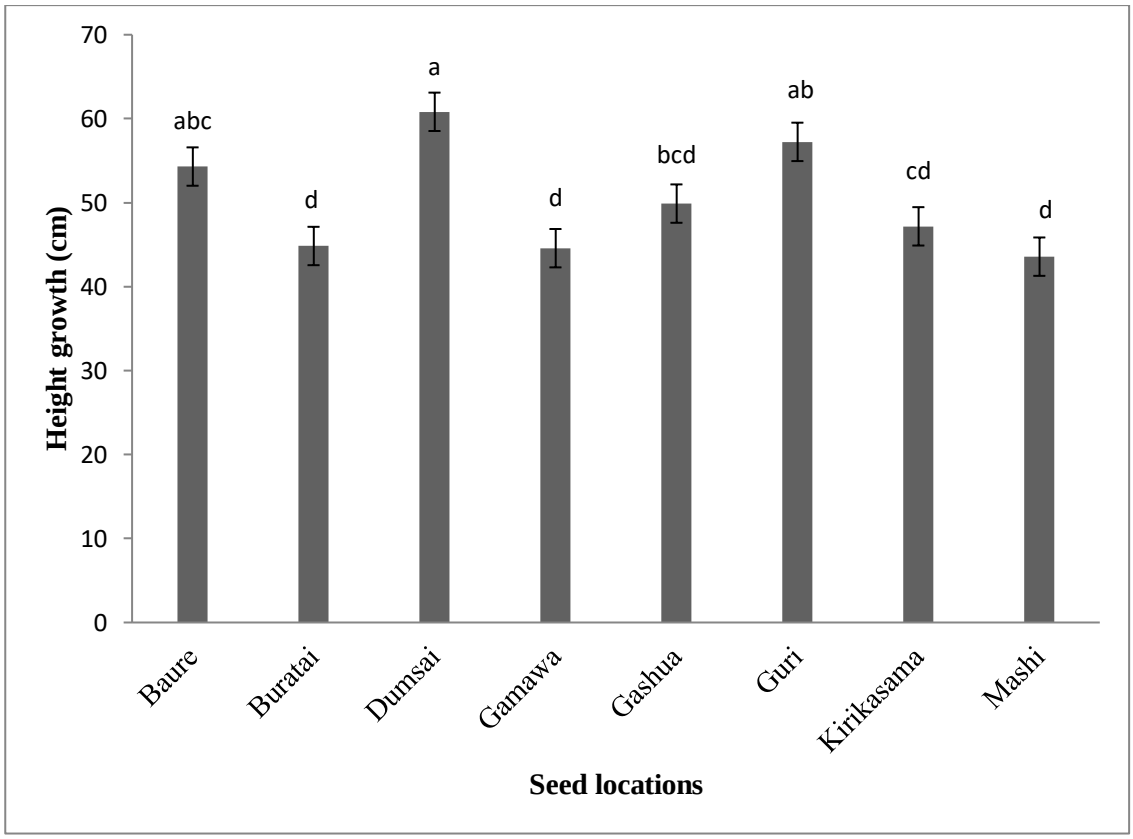

Fig. 4.8: Height growth of *B. aegyptiaca* seedlings from different seed locations

Means sharing a similar alphabet did not differ significantly from each other (P≤0.05). The line through a point on the graph represents standard deviation error bars

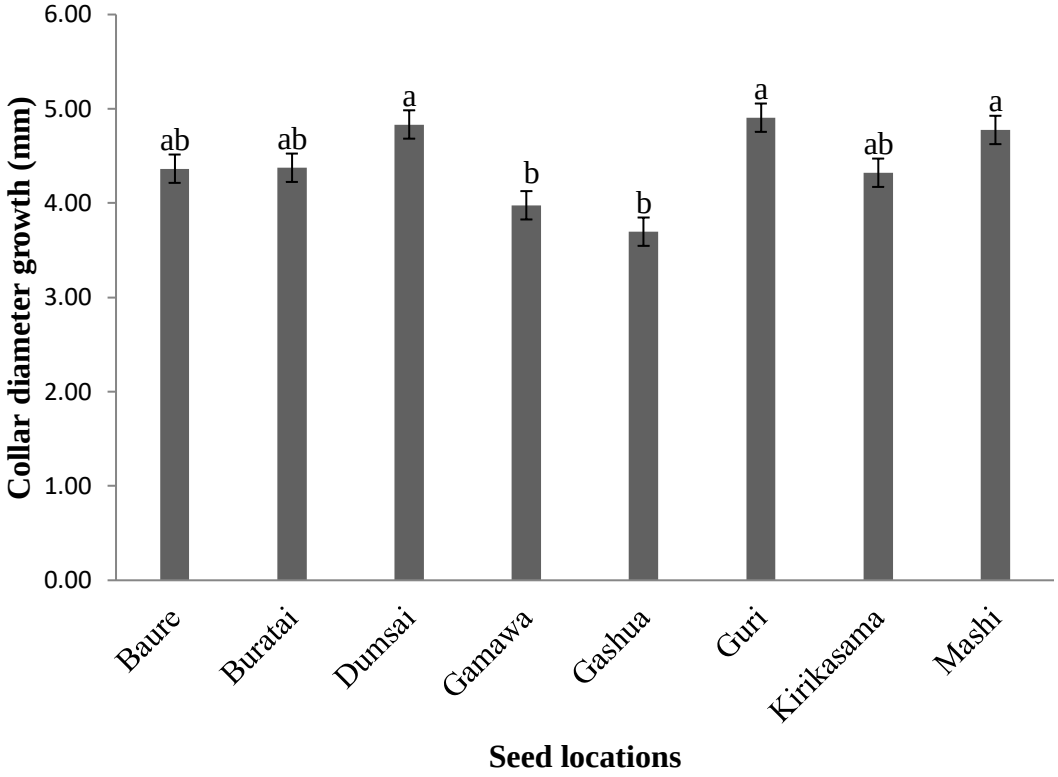

Fig. 4.9: Collar diameter growth of *B. aegyptiaca* seedlings from different seed locations

Means sharing a similar alphabet did not differ significantly from each other (P≤0.05). The line through a point on the graph represents standard deviation error bars

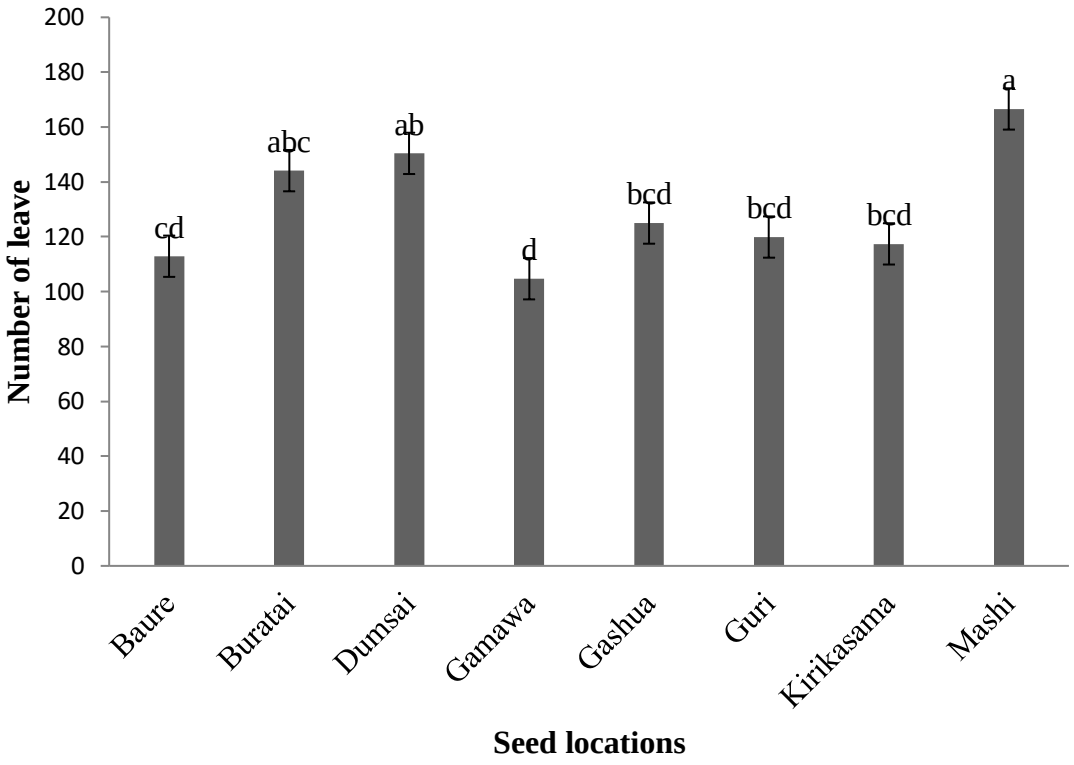

Fig. 4.10: Leaf production in *B. aegyptiaca* seedlings from different seed locations

Means sharing a similar alphabet did not differ significantly from each other ($P \leq 0.05$). The line through a point on the graph represents standard deviation error bars

4.8.4 Root shoot ratio by dry weight (RSR_{DW})

There was no significant difference in RSR_{DW} of *Balanites aegyptiaca* seedlings from different sources. However, seedlings from Mashi had the highest mean RSR_{DW} of 3.77±2.03 g, while seedlings from Dumsai had the lowest mean RSR_{DW} having recorded 2.26±0.79 g (Table 4.7).

4.8.5 Dry weight of leaf

Significant difference in the dry weight of *Balanites aegyptiaca* leaves from different locations was observed. Seedlings from Guri had the highest mean leave dry weight of 2.65±0.78 g and differed significantly with leaf dry weight of *B. aegyptiaca* from Baure, Buratai, Gamawa, Gashua, and Kirikasama which had 1.62±0.70 g, 1.67±0.70 g, 1.85±0.82 g, 1.54±0.73 g, and 1.69±0.47 g respectively. However, it did not vary significantly with leaf dry weight of Dumsai and Mashi which recoded mean leaf dry weight of 2.25±0.92 g and 2.39±0.80 g respectively (Table 4.7)

4.8.6 Dry weight of stem

The dry weight of *Balanites aegyptiaca* stem differed significantly from different locations. Seedlings from the Dumsai recorded the highest mean stem dry weight of 3.52±1.08 g but did not differ significantly from that of Guri which had 2.91±0.88 g. However, it was found to differ significantly from all the other sources. Seedlings from Gamawa which had 1.66±0.43 g recorded the lowest mean stem dry weight (Table 4.7).

4.8.7 Dry weight of root

Variation in root dry weight of *Balanites aegyptiaca* seedlings among sources was found to be significant. Mashi had the highest mean root dry weight of 7.86±2.64 g but did not differ significantly with Baure (6.73±2.00 g), Dumsai (7.66±2.52 g), Gamawa (5.94±1.80 g), and Guri (7.43±1.70 g), however, it differed significantly with Buratai (4.92±1.74 g), Gashua (5.43±2.27 g) and Kirikasama (4.55±1.54 g). Kirikasama source with 4.55±1.54 g, had the lowest mean dry weight (Table 4.7)

4.8.8 Total dry weight (TDW)

A significant difference in the total dry weight of *Balanites aegyptiaca* seedlings from different sources was observed. The highest mean total dry weight was recorded by the Dumsai source which had 13.44±3.84 g, though it did not show any significant difference

with those of Guri (12.99±2.13) and Mashi (12.75±3.82) sources. Kirikasama source which recorded 8.21±2.20 g had the lowest TDW value (Table 4.7).

4.8.9 Relative growth rate by dry weight

The relative growth rate by dry weight (RGR_{DW}) of *Balanites aegyptiaca* seedlings among sources did not differ significantly. However, seedlings from Mashi had a higher RGR_{DW} of 0.04±0.00 mg g^{-1} day^{-1} in comparison with seedlings from Baure, Baure, Buratai, Dumsai, Gashua, Guri and Kirikasama which recorded 0.03 mg g^{-1} day^{-} RGR_{DW} each (Table 4.8)

4.8.10 Absolute growth rate by plant height

Seed sources did not significantly affect the absolute growth rate by plant height of *B. aegyptiaca* seedling's (Table 4.8). However, seedlings from Dumsai recorded the highest mean absolute growth rate by plant height of 0.12±0.04 cm day^{-1}. Seedlings from Kirikasama had the lowest (0.07±0.02 cm day^{-1}) absolute growth rate by plant height (Table 4.15)

4.8.11 Root length

Significant difference in root length of *Balanites aegyptiaca* seedlings was observed among sources of seed collections. Seedlings from Mashi source which had 46.71±20.18 cm were observed to record the highest mean root length after 12 months of assessment but they did not differ significantly with seedlings from Kirikasama, Guri, Gashua, and Buratai which recorded 43.24±12.14 cm, 45.54±8.87 cm, 43.56±9.45 cm, and 34.01±6.46 cm respectively. Seedlings from Baure had the lowest mean root length having recorded the value of 34.01±6.46 cm (Table 4.8)

Table 4.7: Root: shoot ratio and dry biomass of *B. aegyptiaca* seedlings from different locations

Source	RSR_{DW}	LDW	SDW	RDW	TDW
Baure	3.58±1.87^{a}	1.62±0.70^{c}	2.18±0.77^{bcd}	6.73±2.00^{ab}	10.52±2.06^{bc}
Buratai	3.47±1.60^{a}	1.67±0.70^{bc}	1.70±0.97^{cd}	4.92±1.74^{bc}	8.28±2.88^{c}
Dumsai	2.26±0.79^{a}	2.25±0.92^{abc}	3.52±1.08^{a}	7.66±2.52^{a}	13.44±3.84^{a}
Gamawa	3.67±1.04^{a}	1.85±0.82^{bc}	1.66±0.43^{d}	5.94±1.80^{abc}	9.44±2.49^{c}
Gashua	3.03±1.41^{a}	1.54±0.73^{c}	1.88±0.54^{cd}	5.43±2.27^{bc}	8.85±2.13^{c}
Guri	2.81±1.23^{a}	2.65±0.78^{a}	2.91±0.88^{ab}	7.43±1.70^{a}	12.99±2.13^{ab}
Kirikasama	2.36±0.77^{a}	1.69±0.47^{bc}	1.98±0.59^{cd}	4.55±1.54^{c}	8.21±2.20^{c}
Mashi	3.77±2.03^{a}	2.39±0.80^{ab}	2.50±1.09^{bc}	7.86±2.64^{a}	12.75±3.82^{ab}
CV (%)	42.30	38.72	35.17	33.55	25.82
F-stat.	1.780	3.090	6.264	3.912	5.949
Sig	0.11**ns**	0.01*	0.00*	0.00*	0.00*

Source: Field survey (2020). RSR_{DW} = Root: Shoot ratio by dry weight, LDW (cm) = Leave dry weight (g), SDW = Stem dry weight (g), TDW = Total dry weight (g) RDW = Root dry weight (g). Mean values are followed by the standard deviation. Values with the same letter across column are not significantly different ($P \leq 0.05$)

Table 4.8: Relative growth rate, absolute growth rate by dry weight and length of root *B. aegyptiaca* from different seed sources

Source	RGR_{DW}	AGR_{PH}	RL
Baure	0.03±0.00	0.09±0.03	34.01±6.46^{c}
Buratai	0.03±0.01	0.07±0.03	45.69±7.83^{ab}
Dumsai	0.03±0.01	0.12±0.04	34.63±9.67^{bc}
Gamawa	0.03±0.01	0.07±0.05	35.22±7.83^{bc}
Gashua	0.03±0.00	0.07±0.04	43.56±9.45^{abc}
Guri	0.03±0.00	0.10±0.03	45.54±8.87^{ab}
Kirikasama	0.03±0.01	0.07±0.02	43.24±12.14^{abc}
Mashi	0.04±0.00	0.10±0.03	46.71±20.18^{a}
CV (%)	33.33	39.74	24.84
F-stat.	2.40	2.02	2.44
Sig	0.22**ns**	0.06**ns**	0.03*

Source: Field survey (2020). RGR_{DW} (mg g^{-1} day^{-1}) = Relative growth rate by dry weight, AGR_{PH} (cm day^{-1}) = Absolute growth weight by plant height, AGR_{DW} (g day^{-1}) = Absolute growth rate by plant dry weight (g day^{-}), RL = Root length. Mean values are followed by the standard deviation. Values with the same letter across column are not significantly different (P≤0.05).

4.9 Molecular characteristics of *Balanites aegyptiaca* populations among sources

4.9.1 DNA quality

The quality of the DNA samples extracted from the leaves of *Balanites aegyptiaca* was good as can be seen in the result of the integrity test in Plate 4.3. This makes the sample suitable for amplification. All the samples were successfully amplified except for JK1 (Plate 4.4).

4.9.2 Phylogenetic analysis of *Balanites aegyptiaca* trees population

Balanites aegyptiaca trees populations in the study area were grouped into two clades. Buratai, Guri, Gamawa, and Kirikasama formed a clade (cluster) with a weak bootstrap value of 4, while Mashi, Baure, Gashua, and Dumsai formed another clade with a 0 bootstrap value attached. Guri and Kirikasama formed a monophyletic clade with a weak bootstrap value of 26, and Buratai, Guri, and Kirikasama formed another monophyletic clade with a very weak bootstrap value of 13. *Balanites aegyptiaca* tree population in Baure and Mashi formed a monophyletic clade having a very high support (bootstrap) value of 100. *Balanites aegyptiaca* tree population in Gashua, Baure, and Mashi also formed a monophyletic clade supported by a bootstrap value of 73 (Fig. 4.11)

4.9.3 Identification of *Balanites aegyptiaca* species from different sources

The DNA samples of *Balanites aegyptiaca* species from the different locations in the study area matched with the DNA samples of *Balanites aegyptiaca* species deposited in the NCBI gene bank. The percentage match was 99.04% and 98.06%, while the average percentage match was 98.55%. All the samples in the gene bank had an accession length of 726 bp and were originally from Kenya (Table 4.9).

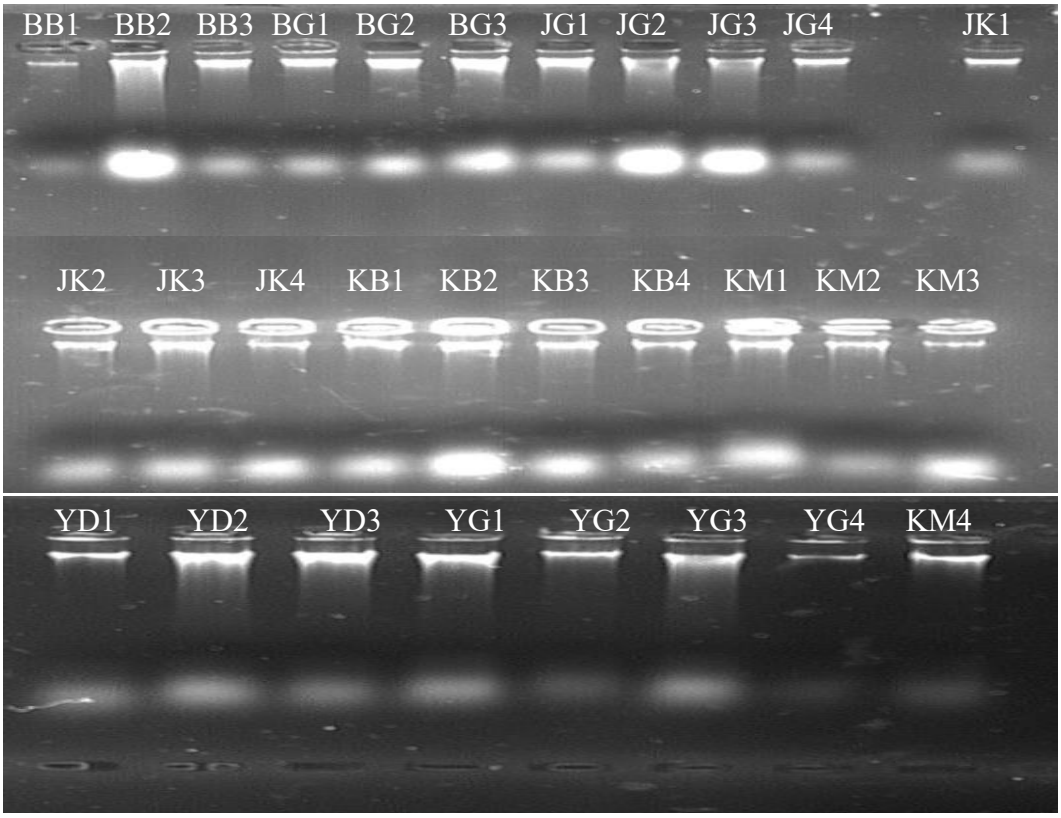

Plate 4.3: Integrity test of *Balanites aegyptiaca* DNA samples

BB = Buratai, BG = Gamawa, JG =Guri, JK = Kirikasama, YD = Dumsai, YG = Gashua, KM = Mashi

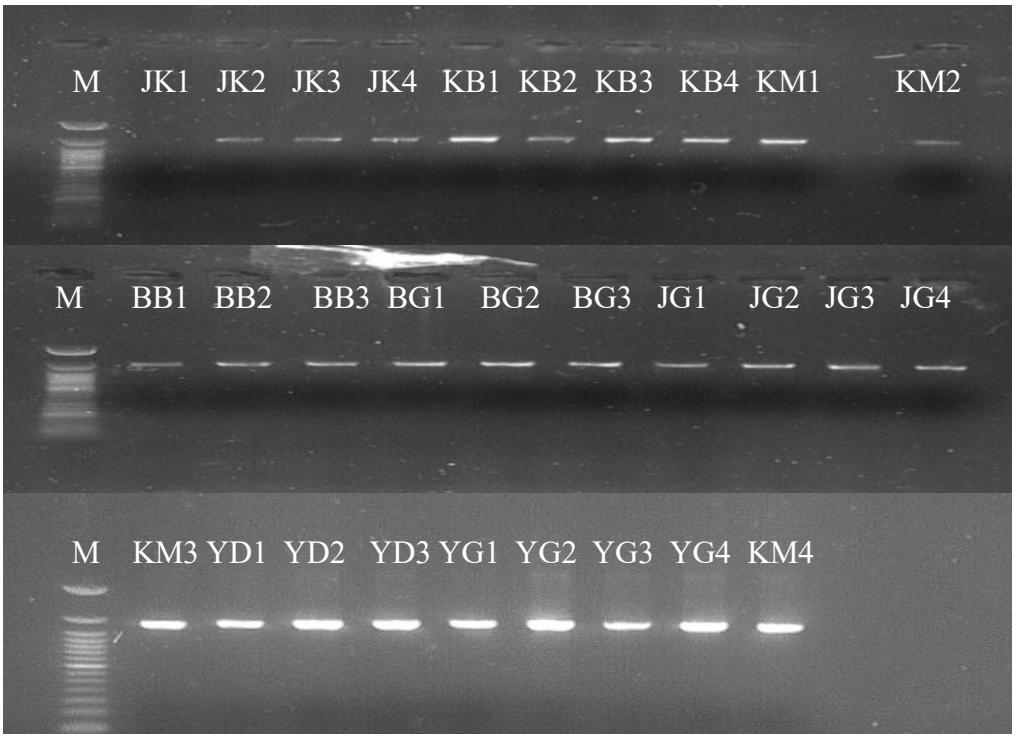

Plate 4.4: PCR amplification of *Balanites aegyptiaca* DNA samples

M= ladder, BB = Buratai, BG = Gamawa, JG =Guri, JK = Kirikasama, YD = Dumsai, YG = Gashua, KM = Mashi

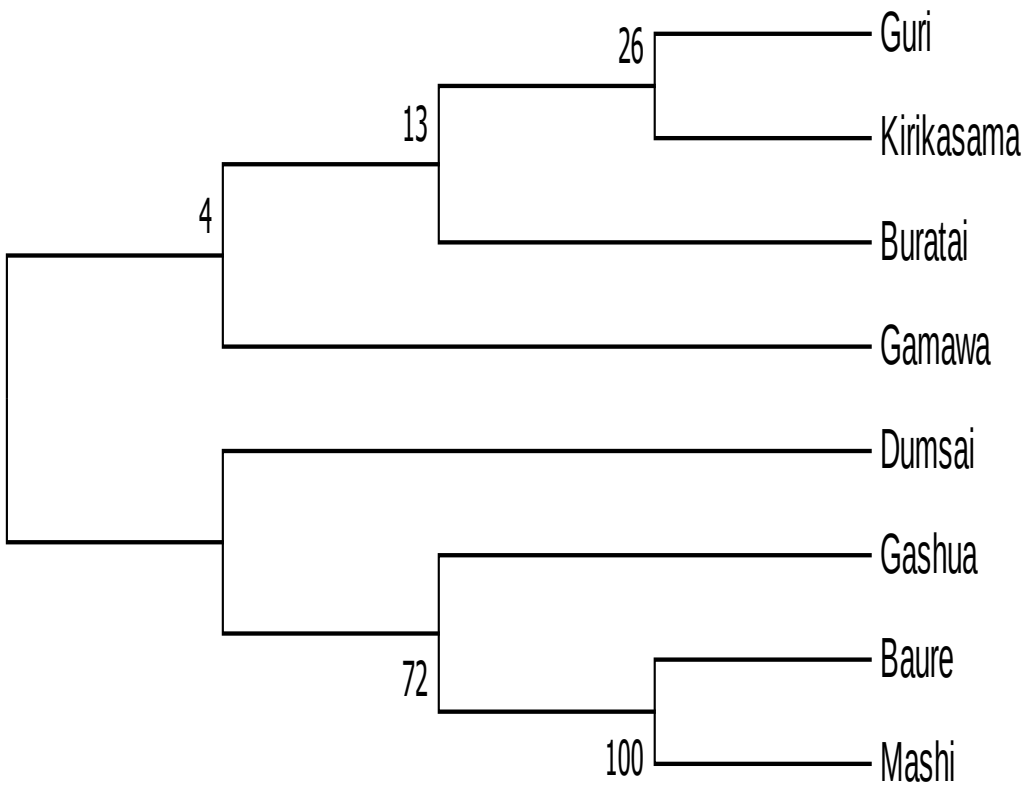

Fig. 4.11: Phylogenetic trees among sources of *Balanites aegyptiaca* trees

Table 4.9: Percentage match of *Balanites aegyptiaca* gene sequences from Nigeria with DNA sequences on National Centre for Biotechnology Information (NCBI) gene bank

S/N	Matched species	% M	A. No.	A.L (bp)	Country
1	*B. aegyptiaca*	99.04	KR735118.1	726	Kenya
2	*B. aegyptiaca*	98.06	KR735118.1	726	Kenya
Average (%)		**98.55**			

Source: NCBI (2021). Note: M = Match, A. No. = Accession Number, A.L = Accession length (bp)

4.9.4 Distance matrix of *Balanites aegyptiaca* nucleotide from different locations

The highest nucleotide distance (0.120) was observed between the sample from Baure and Guri. This was followed by the nucleotide distance between Baure and Buratai, Baure and Gamawa, Baure and Kirikasama, and Baure and Dumsai, each had a nucleotide distance of 0.117 between them. Nucleotide distance of 0.0 was observed between Buratai and Gamawa, Buratai and Kirikasama, Buratai and Dumsai, Kirikasama and Gamawa, Dumsai and Gamawa, and Dumsai and Kirikasama (Table 4.10).

4.9.5 Genetic characteristics among *Balanites aegyptiaca* tree populations

The number of haplotypes (Nh) in *Balanites aegyptiaca* gene in the study area ranged from 2 to 4. The gene from Baure, Gashua, Guri, Kirikasama and Mashi had 4 Nh each, Buratai and Gamawa recorded 3 Nh each, while Dumsai had 2 Nh (Table 4.11). Variable or polymorphic sites (S) in the study area increased from 3 (Dumsai) to 302 (Mashi). All the DNA samples in the study area lacked parsimony-informative sites except for Guri and Mashi which had 2 parsimony-informative sites. *Balanites aegyptiaca* tree populations in the study area had diverse nucleotide diversity (Pi). Populations from Mashi had the highest Pi (0.264), followed by Baure (0.095), Dumsai had 0.002 Pi. Unlike the results of Pi which was diverse among sources, only 2 groups of haplotype diversity (Hd) were observed, i.e. 0.667 and 1.000. Baure, Buratai, Gamawa, Gashua, Guri, Kirikasama, and Mashi each recorded an Hd of 1.0, while Dumsai had 0.667 Hd. The average number of nucleotide differences (k) among *Balanites aegyptiaca* tree populations varied, the populations in Mashi had the highest (151.667) k, while the lowest (2.0) k was observed in Dumsai populations. A significant negative Tajima test value was observed in Gashua (-1.202**)** and Kirikasama (-1.217). Baure, Guri, and Mashi sources recorded a non-significant negative Tajima test value of -0.868, -0.403, and -0.864 respectively (Table 4.11).

Table 4.10: Pairwise distance matrix of *B. aegyptiaca* nucleotide from different locations

Source	Buratai	Gamawa	Guri	Kirikasama	Baure	Mashi	Dumsai	Gashua
Buratai	-							
Gamawa	0.000	-						
Guri	0.002	0.002	-					
Kirikasama	0.000	0.000	0.002	-				
Baure	0.117	0.117	0.120	0.117	-			
Mashi	0.111	0.111	0.114	0.111	0.004	-		
Dumsai	0.000	0.000	0.002	0.000	0.117	0.111	-	
Gashua	0.002	0.002	0.004	0.002	0.114	0.108	0.002	-

Source: Field survey (2021)

Table 4.11: Genetic diversity among *Balanites aegyptiaca* tree populations

Source	Nh	S	Ps	Pi	Hd	k	Tajima's D Test
Baure	4	55	0	0.095	1.000	27.5	-0.868**ns**
Buratai	3	10	0	0.008	1.000	6.667	-
Dumsai	2	3	0	0.002	0.667	2.0	-
Gamawa	3	7	0	0.006	1.000	5.333	-
Gashua	4	7	0	0.005	1.000	3.833	-1.202*****
Guri	4	14	2	0.009	1.000	7.333	-0.403**ns**
Kirikasama	4	13	0	0.009	1.000	8.167	-1.217*****
Mashi	4	302	2	0.264	1.000	151.667	-0.864**ns**

Source: Field survey (2021). Significant **"*"** represents significance, and **ns** indicates not significant, at $\alpha_{0.05}$. Nh = number of haplotype, S = variable or polymorphic sites, Ps = parsimony-informative sites, k = average number of nucleotide difference, Pi = nucleotide diversity, and Hd = haplotype diversity

4.9.6 Population size changes of *Balanites aegyptiaca* from different locations

Observed and expected mismatch distribution analysis to measure changes in the population size of *Balanites aegyptiaca* among sources showed a varied distribution pattern. A bimodal pattern of mismatch distribution curve was observed in Baure and Dumsai, Gashua displayed a multimodal distribution pattern, while a unimodal pattern was observed in Buratai populations. *Balanites aegyptiaca* populations in Gamawa, Kirikasama, and Mashi displayed bimodal patterns of distributions, while the population in Guri had a multimodal distribution pattern (Fig. 4.12).

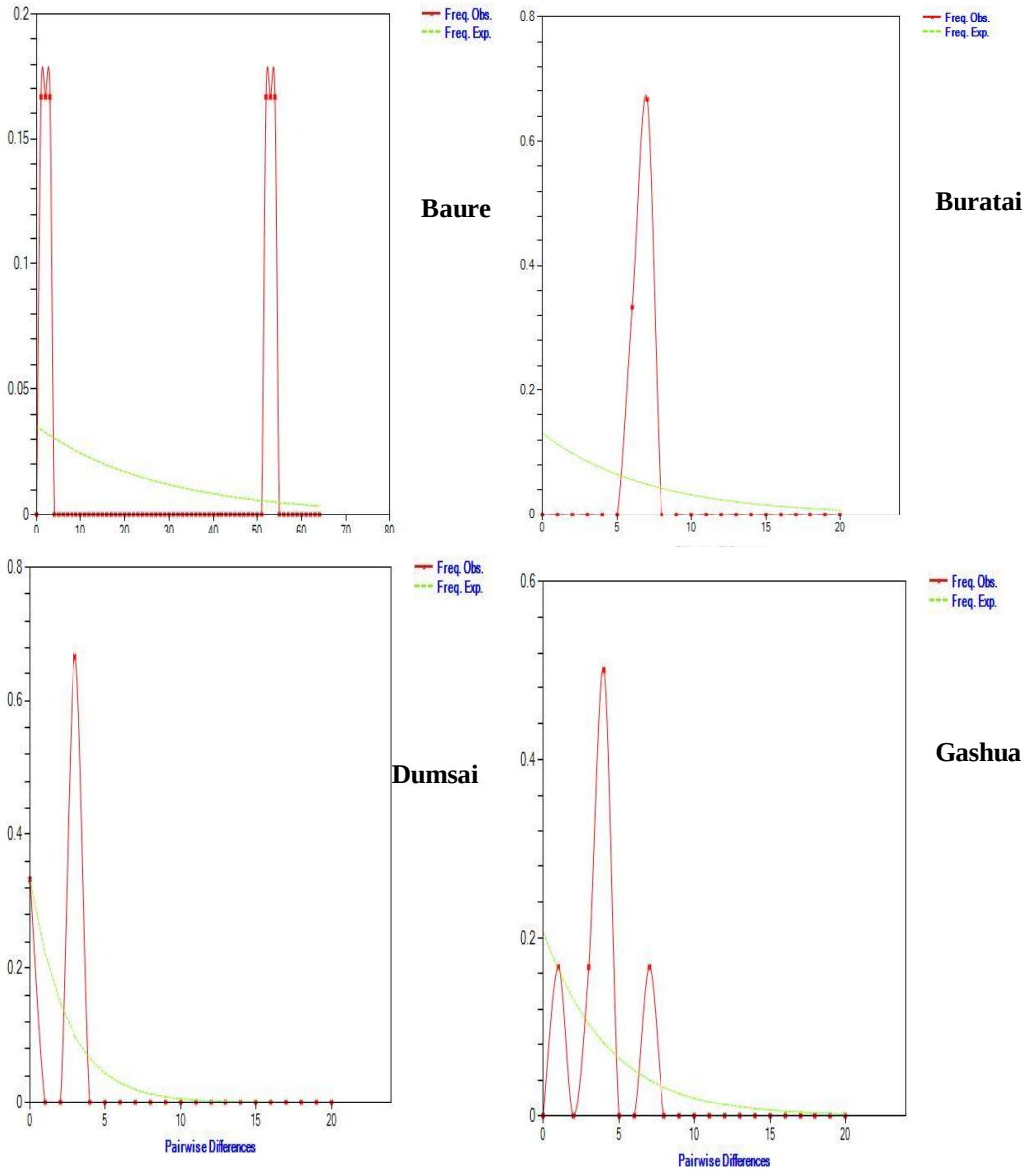

Fig. 4.12a: The mismatch distribution curve of *Balanites aegyptiaca* tree populations

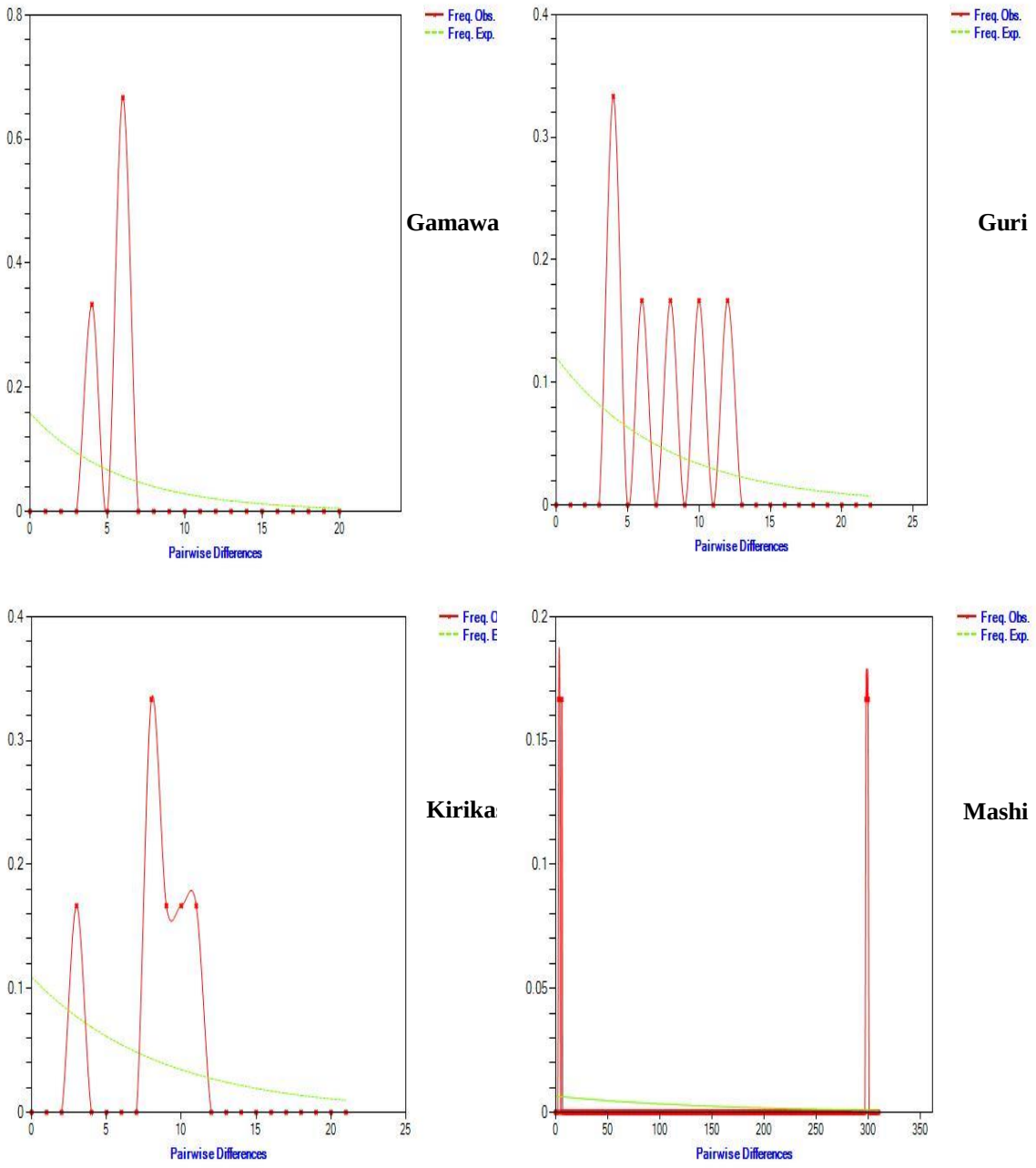

Fig. 4.12b: The mismatch distribution curve of *Balanites aegyptiaca* tree populations

4.10 Physical and chemical properties of soil under *Balanites aegyptiaca* trees from different locations

4.10.1 Soil physical properties

The soil under *Balanites aegyptiaca* trees across the Sahelian zone in Nigeria is characterised by sand, silt, and clay. The percentage of sand was high in all the locations, followed by silt, then clay. The percentage of sand and silt vary significantly among locations, but the percentage of variation in clay among locations was not significant. The highest percentage of sand was recorded in Gamawa (87.8±2.45), while Buratai and Gashua recorded the highest percentage of silt (45.8±5.55) and clay (16.02±9.11) respectively (Table 4.12)

4.10.2 Soil chemical properties of soil under *B. aegyptiaca* trees from different locations

Soil chemical properties varied significantly among *Balanites aegyptiaca* sources in most of the properties assessed (Table 4.13). The soil pH was found to range from 6.13±0.50 (Gamawa) to 7.67±0.55 (Kirikasama), while organic carbon increased from 0.40±0.22 % (Gamawa) to 1.64±1.09 % (Guri). Exchangeable acidity in the soil ranged from 0.24±0.06 cmol/kg (Dumsai) to 0.46±0.10 cmol/kg (Kirikasama) (Table 4.13). Calcium (Ca) content in the soil increased from 5.01±2.87 mg/kg (Gashua) to 23.90±11.99 mg/kg (Buratai), soil Magnesium (Mg) content ranged from 3.08±1.22 mg/kg (Gashua) to 21.77±8.23 mg/kg (Gamawa), while Potassium (K) and Sodium (Na) content varied from 0.52±0.12 mg/kg (Gamawa) to 8.18±4.26 mg/kg (Buratai) and 0.35±0.09 (Dumsai) to 2.88±1.27 mg/kg (Buratai), respectively (Table 4.14). Manganese (Mn) content in the soil ranged from 78.52±17.99 mg/kg (Buratai) to 18.5±5.38 mg/kg (Dumsai), available phosphorus (Av.P) was highest (44.48±24.98 mg/kg) in Kirikasama and lowest (3.23±0.64 mg/kg) in Buratai, while soil total Nitrogen was ranged from 0.38±0.11 (Guri) to 0.04±0.02 (Gamawa) (Table 4.14).

Table 4.12: Percentage of sand, silt, and clay of soil under *Balanites aegyptiaca* trees in different locations

Source	% Sand	% Silt	% Clay	Textural class
Baure	82.08±11.53^{a}	14.2±10.15^{de}	3.76±2.83	Loamy sand
Buratai	44.6±3.34^{c}	45.8±5.55^{a}	9.6±7.56	Loam
Dumsai	50.2±15.58^{bc}	38.2±13.76^{ab}	11.6±2.28	Loam
Gamawa	87.8±2.45^{a}	4.6±1.79^{e}	7.6±1.09	Sand
Gashua	61.4±11.61^{b}	22.6±6.72^{cd}	16.02±9.11	Sandy loam
Guri	55.14±2.68^{bc}	34.32±6.72cd	10.66±3.06	Sandy loam
Kirikasama	61.48±8.29^{b}	31.8±5.90^{bc}	6.84±4.6	Sandy loam
Mashi	77.2±11.00^{a}	12.6±5.02^{e}	10.2±6.84	Sandy loam

Source: Field survey (2019). Mean values are followed by the standard deviation. Values with the same letter across column are not significantly different (P≤0.05).

Table 4.13: Chemical properties of soil under *Balanites aegyptiaca* trees in different locations

Source	pH	%OC	Exch. Acidity(cmol/kg)	Exch. H+ (cmol/kg)
Baure	6.86±0.63^{abc}	0.74±0.79^{b}	0.42±0.09^{ab}	1.14±1.60
Buratai	7.05±0.53^{ab}	1.21±0.09^{ab}	0.30±0.07^{cd}	0.30±0.30
Dumsai	6.84±0.47^{abc}	0.55±0.11^{b}	0.24±0.06^{d}	0.24±0.06
Gamawa	6.13±0.50^{d}	0.40±0.22^{b}	0.26±0.04^{d}	0.26±0.04
Gashua	7.08±0.49^{ab}	0.90±0.71^{ab}	0.35±0.04^{bc}	1.07±1.64
Guri	7.39±0.40^{ab}	1.64±1.09^{a}	0.38±0.04^{abc}	0.38±0.04
Kirikasama	7.67±0.55^{a}	1.21±0.45^{ab}	0.46±0.10^{a}	0.46±0.10
Mashi	6.77±0.97^{cd}	0.78±0.30^{b}	0.37±0.07^{bc}	0.37±0.07

Source: Field survey (2019). Mean values are followed by the standard deviation. Values with the same letter across column are not significantly different (P≤0.05).

Table 4.14: Soil mineral elements under *Balanites aegyptiaca* trees in different locations

Source	Ca	Mg	K	Na	Mn	%TN	Av P
Baure	18.30±6.72^{a}	11.45.47^{b}	6.89±4.22^{a}	1.62±1.46^{b}	71±21.73^{a}	0.15±0.16^{b}	5.71±2.29^{b}
Buratai	23.90±11.99^{a}	10.30±5.04b^{c}	8.18±4.26^{a}	2.88±1.27^{a}	78.52±17.99^{a}	0.13±0.01^{b}	3.23±0.64^{b}
Dumsai	7.18±10.80^{b}	5.06±4.99^{cd}	1.46±1.86^{b}	0.35±0.09^{c}	18.5±5.38^{c}	0.06±0.01^{b}	8.41±8.41^{b}
Gamawa	23.46±5.56^{a}	21.77±8.23^{a}	0.52±0.12^{b}	0.43±0.11^{c}	39.58±7.71b^{c}	0.04±0.02^{b}	6.44±2.47^{b}
Gashua	5.01±2.87^{b}	3.08±1.22^{d}	1.03±0.68^{b}	0.43±0.24^{c}	37.64±13.27^{bc}	0.09±0.08^{b}	6.71±2.63^{b}
Guri	5.95±4.50^{b}	3.63±2.19^{d}	1.88±2.98^{b}	0.37±0.21^{c}	34.18±31.60^{bc}	0.38±0.11^{a}	25.41±18.12^{ab}
Kirikasama	14.17±14.17^{ab}	3.59±1.84^{d}	1.18±1.18^{b}	0.55±0.22^{c}	44.42±14.07^{b}	0.13±0.05^{b}	44.48±24.98^{a}
Mashi	15.66±15.67^{ab}	6.32±1.28^{bcd}	0.59±0.15^{b}	0.36±0.36^{c}	32.96±10.54^{bc}	0.09±0.03^{b}	5.83±2.09^{b}

Source: Field survey (2019). Ca (mg/kg) = calcium, Mg (mg/kg) = magnesium, Na (mg/kg) = sodium, Mn (mg/kg) = manganese TN (mg/kg) = total nitrogen, Av.P = available phosphorus. Mean values are followed by the standard deviation. Values with the same letter across column are not significantly different (P≤0.05).

4.11 Oil yield and physical and chemical properties of *Balanites aegyptiaca* kernel from different locations

4.11.1 Oil yield (%)

Locations significantly influenced the oil yield of *B. aegyptiaca* in this study. Guri recorded the highest oil yield (43.95 %) and differed significantly from Baure, Buratai, Dumsai, Gamawa, Gashua, Kirikasama and Mashi which recorded 20.84 %, 21.09 %, 31.21 %, 22.45 %, 23.11 %, 30.15 % and 23.47 %, respectively (Fig 4.13)

4.11.2 Refractive index

The refractive index of *Balanites aegyptiaca* oil among sources was not significantly different from each other. However, oil from Gamawa source had the highest mean refractive index of 1.48±0.06, while oil from Mashi had the lowest with 1.36±0.15 (Table 4.15).

4.12.3 Viscosity

There was no significant difference in the viscosity of *Balanites aegyptiaca* oil extracted from different sources of the kernel. Mean oil viscosity ranged from 41.33±2.08 cP in Gashua to 48.67±2.52 cP in Buratai (Table 4.15).

4.12.4 Acid value

A significant variation in the acid value of *Balanites aegyptiaca* oil was observed among kernel oil sources. Baure source recorded the highest mean acid value of 2.11±0.07 mg KOH/g but did not differ significantly from Buratai which had 1.96±0.07 mg KOH/g. Dumsai had the lowest acid value (1.36±0.07 mg KOH/g) and differed significantly from all the other sources (Table 4.15)

4.12.5 Saponification value (mg KOH/g)

The saponification value was significantly higher (179.85±0.90) in Kirikasama than in the other locations. The lowest mean saponification value (76.35±0.22) was recorded by Guri source (Table 4.15)

4.12.6 Iodine value (mg KOH/g)

Balanites aegyptiaca oil was observed to differ significantly in their iodine content among locations. The iodine value in *Balanites aegyptiaca* oil varied significantly from 67.07±1.53 mg KOH/g in Gamawa to 85.33±2.52 mg KOH/g in Baure (Table 4.15).

4.13 Association between weather factors and properties of *B. aegyptiaca* oil

The association between elevation and oil yield and viscosity was significantly negative (-0.465) and significantly positive (0.491), respectively. Rainfall had a significant positive correlation relationship with viscosity (0.476) and iodine value (0.439). However, the correlations between rainfall and oil yield were significantly negative (-0.530). Temperature had a positive correlation with oil yield (0.386), refractive index (0.391), and saponification value (0.159). However, temperature had a significant negative correlation with viscosity (-0.449) (Table 4.16)

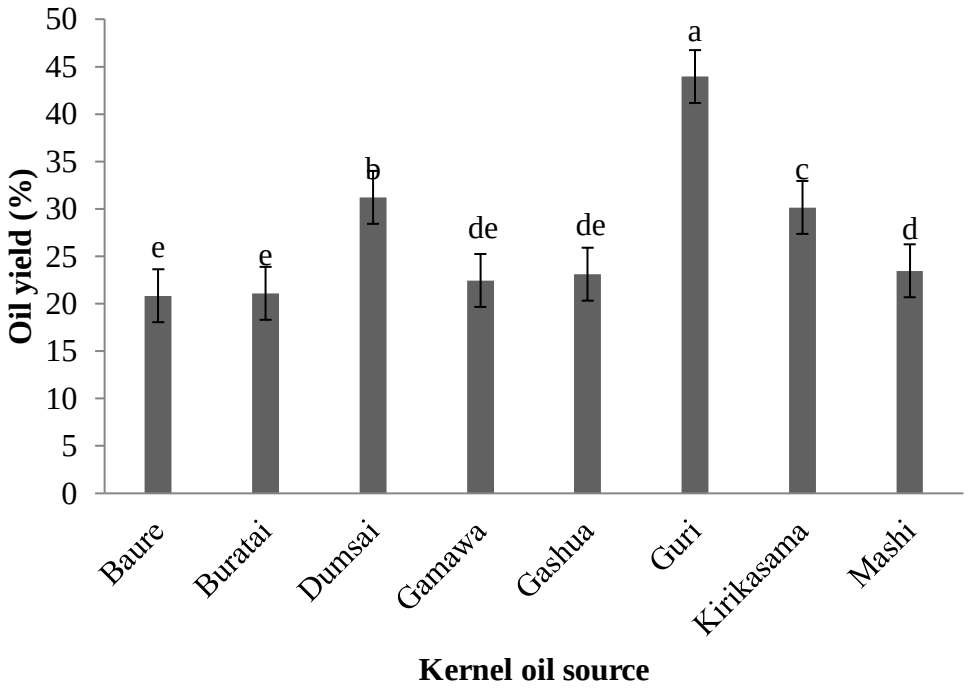

Fig. 4.13: Oil yield among sources of *Balanites aegyptiaca* kernel

Different letters indicate significant variation among sources (P≤0.05). The error bars indicate the standard error.

Table 4.15: Physical and chemical properties of *B. aegyptiaca* oil from different locations

Source	RI	Vis	AV	SV	IV
Baure	1.39±0.06	42.33±4.93	2.11±0.07^{a}	129.85±0.65^{c}	85.33±2.52^{a}
Buratai	1.43±0.0	48.67±2.52	1.96±0.07^{ab}	107.74±1.42^{e}	71.43±1.59^{bc}
Dumsai	1.45±0.26	44.0±2.65	1.36±0.07^{e}	114.31±0.37^{d}	67.93±1.10^{d}
Gamawa	1.48±0.06	36.67±2.52	1.92±0.15^{bc}	154.48±0.77^{b}	67.07±1.53^{d}
Gashua	1.45±0.03	41.33±2.08	1.62±0.06^{d}	89.55±0.45^{f}	70.80±1.37^{bc}
Guri	1.44±0.05	45.0±5.0	1.64±0.11^{d}	76.35±0.22^{g}	72.11±1.39^{b}
Kirikasama	1.46±0.04	41.67±2.52	1.84±0.09^{bc}	179.85±0.90^{a}	69.06±0.65^{cd}
Mashi	1.36±0.15	44.67±1.53	1.76±0.13^{cd}	108.06±0.54^{e}	71.65±0.54^{bc}
CV (%)	9.12	6.91	5.28	0.55	1.83
F-Stat	1.07	3.55	16.92	6112.7	46.27
Sig	0.43^{ns}	0.02*	0.00*	0.00*	0.00*

Source: Field survey (2019). RI = Refractive index, Vis = Viscosity (cP), AV = Acid value (mg KOH/g), Iodine value (mg KOH/g), SV = Saponification value (mg KOH/g). Mean values are followed by the standard deviation. Values with the same letter across column are not significantly different (P≤0.05).

Table 4.16: Correlation between weather factors and oil yield and properties of *B. aegyptiaca* oil

Variables	OY	RI	Vis	AV	SV	IV
Elevation	-0.465*	-0.354	0.491*	0.407	-0.157	0.187
Precipitation	-0.530*	-0.458*	0.476*	0.419	-0.181	0.439*
Temperature	0.386	0.391	-0.449*	-0.093	0.159	-0.394

Source: Field survey (2019). RI = Refractive index, Vis = Viscosity (cP), AV = Acid value (mg KOH/g), Iodine value (mg KOH/g), SV = Saponification value (mg KOH/g).

* Correlation is significant at the 0.05 level

CHAPTER 5

DISCUSSION

5.1 Morphological characteristics of *Balanites aegyptiaca* tree populations from different locations

The thickness, length, width, and weight of *Balanites aegyptiaca* fruits varied with the location. The presence of variation in fruit traits among and within plant sources has been well documented (Mkwezalamba *et al*., 2015; Hounkpèvi *et al*., 2020) and is usually attributed to many factors such as individual plant genetic makeup and environment factor (Shu *et al*., 2012). The mean fruit length, thickness, and width recorded in this study were similar to what was reported for *Balanites aegyptiaca* fruits by Abdoun (2005), Okia (2010), and Abasse *et al*. (2011) in Sudan, Uganda, and Niger, respectively.

The high coefficient of variation (CV) recorded by fruit weight in this study was consistent with the findings of Li *et al*. (2022) who reported high CV in fruit weight of *Juglans mandshurica* compared to other traits. Fruit weight is likely to have an effect on pulp (mesocarp) weight which is one of the attributes of choice when it comes to fruit selection for consumption. Therefore, it is a very important trait when it comes to selection for domestication and improvement programmes. The variation in fruit traits observed in this study is an indication that improvement of these characters is possible through selection. Variability in traits among and within plant populations is regarded as the building block of plant improvement programme (Elfeel *et al*., 2009). Fruits from Baure were longer, bigger, and have more weight compared to other sources. These made it a suitable source for seed selection.

Balanites aegyptiaca fruits in Baure had a significantly higher pulp weight compared with the other sources. Pulp or mesocarp weight constitutes one of the vital parts of *Balanites aegyptiaca* fruit because it is edible and is consumed by both humans and animals. It accounts for about 22.7% - 31% of the total fruit weight (Chapagain and Wiesman, 2005;

Okia 2010) and it is a good source of food for the people in the arid zone region where *Balanites aegyptiaca* is common. This implies that Baure could provide a good source of fruit for consumption and pulp improvement. This result was in agreement with Mkwezalamba *et al*. (2015) who reported a significant difference in fruit pulp weight of *Sclerocarya birrea* sourced from different provenances. Variation in fruit pulp of *Balanites aegyptiaca* was also reported in Israel, India, Niger, Mali, Senegal, Burkina Faso, and Uganda (Chapagain and Wiesman 2005; Okia, 2010). This signifies a wide range of variability in fruit morphology which needs collaboration at both national and regional levels when it comes to selection for improvement or breeding programmes.

Variations were observed in leaf traits among sources of *Balanites aegyptiaca* in this study. This conforms to the findings of Nyarko *et al.* (2012), Nawaz et al. (2018), and Ouinsavi and Sokpon (2010) who reported that leaf traits vary significantly among sources. Leaves from Baure were longer and thicker in comparison with other sources. This implies leaf from Baure may have a better leaf area in comparison with other sources. The leaf is a vital organ in a plant; organic carbon is produced by the leaf during photosynthesis (Bojović and Stojanović 2006). Since the leaf is the organ responsible for light interception during photosynthesis, plants with higher leaf areas would be expected to intercept more sunlight which may result in an increased growth rate. The rate of transpiration and photosynthesis in plants with smaller leaf sizes is low (England and Attiwill 2006) therefore, those with large leaf sizes will have higher rates of transpiration and photosynthesis.

The highest mean nut length of 2.90 cm recorded by Kirikasama was similar to the 2.89 cm reported by Aviara *et al.* (2005) but higher than the 2.51 cm reported in the Niger Republic (Abasse *et al.,* 2011). This implies that the Nigerian source of *Balanites aegyptiaca* possesses better nut attributes in comparison with those in Niger. The variations among locations could be attributed to the difference in geographical and climatic factors or the inherent genetic makeup of trees in the different locations. Many researchers have also opined that seed origin has an impact on the seed and seedlings characteristics of many plant species (Loha *et al*., 2006). Nut weight is very important

because it can affect the weight of the kernel which is used for oil extraction. The weight of seeds has been shown to influence seeds' germination behaviour (Owoh *et al*., 2011).

Balanites aegyptiaca kernel is one of the most important parts of the fruits because it is used for oil extraction. The oil extracted from the kernels has various applications ranging from home use and industrial application, especially in pharmaceutical industries. The kernels are also consumed raw in Borno and Yobe states of Nigeria just like groundnuts after it has undergone soaking in water to reduce the bitterness. Kernels from Guri had better weight in comparison with other sources. Therefore kernels in Baure may give a higher percentage oil yields in comparison with other sources. Variations in the kernel weight of *Balanites aegyptiaca* from different sources have also been reported in other countries (Elfeel 2004; Chapagain and Wiesman, 2005; Abasse *et al*., 2011).

High heritability in traits of *B. aegyptiaca* was observed in this study, this is an indication that these characters can be easily pass to the offspring. Therefore it is advisable to select traits with high heritability during selection for improvement. Zhang *et al*. (2022) reported similar result in seed and seed length of *Juglans mandshurica*.

Variations in qualitative morphological traits of *Balanites aegyptiaca* fruit were observed in this study. The taste of the fruit is one of the factors used during selection because people generally prefer fruits with sweet taste though, in some fruit bearing trees, the fruits have a sour or bitter taste. For instance, *Tamarindus indica* fruits are usually sour. Von Maydell (1986) reported the taste of *Balanites aegyptiaca* fruit is stringent and bittersweet. However, a different taste was observed in this study. This difference might be due to variations in the source of fruit. Gamawa source had a higher percentage of fruits with a sweet taste while Gashua source had a higher percentage of fruits with bitter taste.

Balanites aegyptiaca fruits have a wide variety of shapes, four different shapes were identified in this study namely; elongate, oblong, oval, and spherical shape. Similar shapes were reported by Abdoun (2005) in Sudan. Yellow was found to be the predominant colour of *Balanites aegyptiaca* fruits in Nigeria. Variation in the colour of the fruit of *Arbutus andrachne, Terminalia catappa* has been reported by Markovski (2017) and Oboh

et al. (2008) respectively. Thirakul (1984) reported yellow-brown as the colour of *Balanites aegyptiaca* fruit when ripe. This is a clear indication of the wide variation in colour from different sources and could be attributed to environmental and genetic factors.

5.2 Association between morphological traits of *Balanites aegyptiaca*

The significant positive correlation between fruit weight of *Balanites aegyptiaca* and pulp weight and nut weight implies that, fruit weight of *Balanites aegyptiaca* may be an indicator of the pulp weight and nut weight of *Balanites aegyptiaca*. A similar association was reported by Abasse *et al*. (2011) and Okia (2010) in *Balanites aegyptiaca* fruits from Niger and Uganda respectively. Reports on other species by several authors validate this finding. For example, Markovski (2017) reported a significant positive correlation between fruit length and pulp ratio, fruit width and pulp ratio, and fruit mass and pup ratio. Okello et al. (2018) also observed a significant correlation between fruit length and fruit mass, pulp mass, and fruit breadth in *Tamarindus indica*. A significant positive correlation between fruit weight and fruit width, fruit weight and fruit length, and fruit weight, and leaf length was also observed in fig trees (Bostan, 2002).

The result from this study implies that the fruit length may positively influence the pulp weight, fruit weight, nuts length, nuts weight, leave length, and leave width. Correlation analysis is usually employed during tree improvement activities to understand the relationship between traits or how the behavior of a trait affects another (Khan *et al*., 2009). Therefore, fruit length could be used as a good indicator for the selection of fruit with better weight and pulp.

5.3 Germination characteristics of *Balanites aegyptiaca* among seed sources

Variation in seed germination among sources of *Balanites aegyptiaca* seeds was observed in this study. Seeds sourced from Guri had a higher germination percentage than all the other sources though its performance did not significantly differ from that of Gashua source. Location of seed collection has been documented to influence the germination of seed many tree species. Dangasuk et al. (1997) and Fredrick et al. (2015) both reported a variation in the germination response of *Faidherbia albida* seeds from different sources in Kenya. Elfeel *et al*. (2009) also reported that seed sources significantly affect germination

of *Balanites aegyptiaca* seeds collected from different locations in Sudan. The highest mean germination percentage they recorded was 60.6 which is less than what was reported in this study (91.96 %). This implies that seeds from Nigerians demonstrated a better germination capacity as can be seen in this study in comparison with those from Sudan.

Seeds of *Balanites aegyptiaca* from different sources differed significantly in the number of days it took to complete germination (mean germination time). This implies that seeds from different sources have different germination energy. This result was in accordance with Loha *et al*. (2006) who observed MGT of *Cordia africana* seed to be significantly influenced by provenance. However, a contradictory result was reported by Fredrick *et al.* (2015) who observed no significant difference in the MGT of *Faidherbia albida* seeds from different sources. Bognounou *et al*. (2010) also reported a lack of significant difference in MGT of some selected species from different provenances in Burkina Faso.. The germination pattern of species is expected to vary from one another. The environmental effects to which the seed is subjected during maturity in the different sites of seed collection may be responsible of the variation.

Seeds from Baure source recorded the lowest (5.07) mean germination time which implies that the average time it took for the seeds to commence and complete germination is shorter. Therefore, in terms of the average number of days required to complete germination, seeds from Baure performed better.

Seeds from Guri recorded the highest germination speed (0.87) which implies that they germinate faster than the other sources. Germination speed can be used as an indication of seedlings' vigour (Wakawa and Akinyele, 2016) and under field conditions, seedlings of seeds that germinate faster are likely to establish easily on the field (Schmidt 2000). Seeds from Guri performed better both in germination percentage and germination speed which implies that seeds with better germination capacity are also likely to have faster germination speed. This observation is in agreement with that of Fredrick et al. (2015) who observed a significant difference in germination capacity and germination energy of *Faidherbia albida* from different sources in Kenya.

5.4 Growth performance of *Balanites aegyptiaca* among seed sources

5.4.1 Stem height

Seedlings of *Balanites aegyptiaca* from different sources vary in their height growth. The effect of source or environment on seedling height performance has been documented by several authors on different species. For example, Akinyele and Adegeye (2012) observed significant variation in stem height growth of *Buchholzia coriacea* from different sources. Shu et al. (2012) and Saklani et al. (2012) observed significant variations in stem height growth behaviour of *Magnolia officinalis* and *Quercus leucotrichophora* seedlings respectively from different sources. Since all the species reported by these researchers are different, it is logical to attribute the contradictions to species differences.

The initial seedling height of *Balanites aegyptiaca* seems to determine future height growth as can be seen from the result of this study. Seedlings with superior height growth at the initial stage of growth maintained their superiority up to the end of the experiment. This finding is consistent with several findings which demonstrated that seedlings with better initial height growth are likely to maintain their performance over time (Jayasankar *et al*., 1999; Ivetić *et al*., 2016). This implies that seedlings from Dumsai that have superior height throughout the study would likely outperform seedlings from other sources in terms of height growth when transplanted on the field and would therefore be recommended for selection during planting programme.

5.4.2 Collar diameter increment

Seedlings with superior performance during the first or early month (Baure) failed to maintain their superiority and were outperformed at the end of the experiment by Guri source. However, it should be noted that even though seedlings from the Guri source outperformed that of Baure source at the end of the study, the variation was not significant. This implies that initial collar diameter increment could still be regarded as a good indicator in predicting diameter increment in *Balanites aegyptiaca*. Significant variations in plant collar diameter have been reported in several plants such as *Magnolia officinalis* (Shu *et al.,* 2012), *Buchholzia coriacea (*Akinyele and Adegeye 2012), *Quercus leucotrichophora* (Saklani *et. al*., 2012) and *Faidherbia albida* (Fredrick *et al*., 2015).

This variation could be attributed to differences in environmental factors and plant genetic makeup. According to Loha *et al.* (2006), collar diameter increment is easily affected by environmental conditions.

Diameter increment did not follow a similar pattern as height growth in *Balanites aegyptiaca* seedlings where seedlings with superior growth at the initial month maintained their height advantage throughout the study (1-12 months). The inconsistency in collar diameter increment in *Balanites aegyptiaca* seedlings was contradictory to the findings of Fredrick *et al*. (2015) who reported consistency in the performance of collar diameter growth in seedlings of *Faidherbia albida*. This difference could be due to differences in genetic makeup. Furthermore, the genes controlling height and diameter growth in a plant are different thereby resulting in different behavioral patterns.

5.4.3 Leaf production

Sources of seed collections affected the number of leaves produced in *Balanites aegyptiaca* seedlings. This conforms to the results of Fredrick et al. (2015) who observed provenance to affect leaf production significantly in *Faidherbia albida*. Shu et al. (2012) also reported source to significantly affect the number of leaves produced in *Magnolia officinalis.* The number of leaves in *Quercus leucotrichophora* seedlings from different sources was found to vary significantly after one year of assessment in the nursery according to Saklani et al. (2012). Akinyele and Adegeye (2012) also reported source of seed collection to influences the number of leaves produced in the *Buchholzia coriacea* plant.

However, there was a lack of consistency concerning sources having better performance. Seedlings from Dumsai produced more leaves during the first to fifth months but from the 6 – 12 months seedlings from the Mashi source had more leaves. Seedlings from Mashi which have more leaves would be expected to have a high photosynthetic ability since the leaf is the organ that facilitates carbon fixation in plants, however as can be seen from the results, it did not have better performance for collar diameter, and height growth.

5.4.4 Root length

A longer root system is expected to favour water and nutrients uptake by the plant. Seedlings from Mashi have longer roots and would be expected to have a better uptake of water and nutrients in comparison with others. However, since all the plants were subjected to similar conditions, water, and nutrient supply may not be an issue at the seedling stage. Seedlings from Mashi may have a better chance of survival on the field, in the long run, especially when water and nutrient supply become scarce. Plants with developed root systems have a better chance of survival and resistance to fire which is very important in the savannah region. Significant variation in root length of plants from different sources has been reported by Saklani *et al*. (2012) in *Quercus leucotrichophora* and Jayasankar *et al*. (1999) in teak.

5.4.5 Root shoot ratio by dry weight

The root shoot ratio by dry weight (RSR_{DW}) relationship is a good measure of the ability of a plant to cope with limited resources required for growth and development in the environment (Maskova and Herben 2018). The variation existing in RSR_{DW} among sources of *Balanites aegyptiaca* seedlings was not significant. This is contrary to the report by Jayasankar et al. (1999) and Saklani et al. (2012). The higher RSR_{DW} recorded by *B. aegyptiaca* seedlings in Mashi could enhanced the ability to the species to withstand excess wind, erosion or flood when transplanted on the field. Root growth is essential for stability (Fredrick *et al*., 2015)

Variation in the stem, leaf, root, and total dry mass among sources was significant which is in agreement with the report of Akinyele and Adegeye (2012) and Saklani et al. (2012) who found significant variation in leaf, stem, root, and total dry weight of *Buchholzia coriacea* and *Quercus leucotrichophora* respectively from different sources. Fornah et al. (2017) also observed significant variation in shoot dry weight and total dry weight of *Gmelina arborea* seedlings from different provenances. However, they could not establish any significant variation in root dry weight among provenances. Biomass allocation in seedlings is thought to be controlled by environmental conditions and changes over time according to Parker *et al.* (2006). However, it is not clear how these changes happened among species (Mašková and Herben 2018). It will not be out of place to suggest genetic

influence as the major factor controlling biomass partitioning in *Balanites aegyptiaca* seedlings among sources since they were grown under similar conditions.

Guri source which had more allocation of biomass to its leaves in comparison to others would be expected to exhibit greater photosynthetic ability while seedlings from Dumsai which had a higher allocation of biomass to its stem in comparison with others may maintain such superiority in the future. However, Mashi source which had higher biomass allocation to its root may have a competitive advantage to survive over others on the field, especially where water and nutrients are limited. This is because of its ability to explore water and nutrients deep down because of its extensive root network. The root is one of the major organs through which a plant allocates resources (water and nutrients) to itself and determined its success (Maskova and Herben 2018)

5.4.7 Relative growth rate by dry weight (RGR_{DW} mg g^{-1} day^{-1})

Seedlings from Mashi had better RGR_{DW} even though the variation was not significant. This finding is in agreement with that of Jayasankar et al. (1999) in a study done to evaluate the role of provenance in the growth characteristics of *Tectona grandis*. However, a contrary result was obtained by Bognounou et al. (2010) for *Anogeissus leiocarpa* and *Combretum aculeatum* species from different provenance. The number of resources acquired by the plant can be determined indirectly by RGR (Didon 2002) and determine the competitive ability of the plant (Lowry and Smith 2018). This means seedlings from Mashi may have a competitive edge over others on the field since it has superior RGR.

5.5 Molecular characterisation of *Balanites aegyptiaca* tree among sources

5.5.1 Genetic characteristics among *Balanites aegyptiaca* tree populations

Genetic characteristics such as nucleotide diversity, and haplotype diversity have been successfully used to assess genetic diversity in several organisms (Ndoye *et al*., 2013; Tamboli *et al*., 2016; Zhang *et al*., 2022) while gene frequencies are used to established genetic relationship among populations. The level of genetic diversity among *Balanites aegyptiaca* populations in this study was high. Nucleotide diversity increased from 0.002 to 0.264 while haplotype diversity ranged from 0.667 to 1.000. This was higher than what

was recorded in *Adansonia digitata* (Baobab), another important indigenous food tree species of the Sahel. The authors reported a nucleotide diversity ranging from 0.00060 - 0.00527 and a haplotype diversity of 0.363 - 0.648 (Ndoye *et al*., 2013). Similarly, the average nucleotide diversity recorded in the population of Scottish pine according to Wachowiak et al. (2011) was 0.0078. However, a genetic similarity index that ranged from 0.246 to 0.591 was reported among species of *Balanites aegyptiaca* sourced from Sudan, Egypt, Ethiopia, Saudi Arabia, and Yemen (Khamis *et al.*, 2017). However, it is difficult to compare their results with that of this study since the authors used different indices for assessing genetic diversity, as well as a different molecular marker (AFLP) which is less discriminative than the sequencing used in this study. MatK gene loci used in this study has been reported to be successful in the amplification of several species such as *Zygophyllum propinquum* of the family Zygophyllaceae which share the same family as *Balanites aegyptiaca* (Bafeel *et al*., 2011).

Balanites aegyptiaca populations in Mashi have high genetic diversity compared with the other sources. This is an indication that Mashi could serve as a potential source of germplasm required for domestication and improvement purposes. Baure and Mashi populations formed a clade different from the other populations. Genetic distance between Baure and Mashi, from the pairwise genetic distance matrix, likewise revealed closeness among them an indication of genetic similarities. *Balanites aegyptiaca* populations in Mashi and Baure have a better chance of survival from unfavourable environmental conditions and offer greater opportunity for selection during improvement or breeding programme. This is because the level of genetic diversity in a species is regarded as an important factor that determined its response to unfavourable environmental factors (Ndoye *et al*., 2013). The more diverse a population is, the more likely it is to evolve and survive when faced with undesirable conditions.

5.5.2 Phylogenetic characteristics of *Balanites aegyptiaca* trees populations

Balanites aegyptiaca trees in Buratai, Guri, Gamawa, and Kirikasama source have similar genetic makeup, likewise trees in Mashi, Baure, Gashua, and Dumsai based on the phylogenetic tree. However, the bootstrap values on both clades were very week. This means the level of confidence supporting the similarities of samples in each clade was

very low, as such it cannot be said with certainty that the samples are siblings even though they share similar genetics attributes. According to Hillis and Bull (1993), a bootstrap value lower than 73 signifies week bootstrap support or is considered statistically not significant as such the level of confidence in the phylogenies was low.

Balanites aegyptiaca tree population in Baure and Mashi which share similar genetic attributes seem to form different ecotype. This could be attributed to the closeness between the two populations which can encourage cross-pollination and seed dispersal between the two populations. The phylogenetic tree of DNA sequences has been reported to help identify ecotypes in the population of *Adansonia digitata* sourced from a different location in Senegal (Ndoye *et. al.,* 2013).

5.5.3 Species identification

The high percentage match (98.55%) of *Balanites aegyptiaca* samples collected from different locations in the Sahelian zone of Nigeria with samples of *Balanites aegyptiaca* species on the NCBI gene bank confirmed the identity of the species used in this study to be *Balanites aegyptiaca*. This gives credence to the opinions held by most scientists that molecular markers are more reliable means of authenticating the identity of species (Tamboli *et al*., 2016). The matK gene loci used in this study is regarded as one of the best because of its high precision rate when it comes to identification, more especially in angiosperm species (Lahaye *et al*., 2008). It has been reported to have a high success rate in the discrimination of several species such as *Daniellia ogea* and *Daniellia oliveri* (Onefeli 2021) and *Vachellia* species (Newsmaster and Ragupathy, 2009). Furthermore, about 26 species including *Zygophyllum propinquum* which belongs to the same family as *Balanites aegyptiaca* have been successfully amplified using *MatK* (Bafeel *et al*., 2011).

5.5.4 Population size change among *Balanites aegyptiaca* tree populations

The distribution pattern of mismatch analysis gives us insight into possible changes in populations over time, which could be attributed to several factors. A unimodal pattern signifies population expansion while a bimodal or multimodal pattern is an indication that the population is in a state of equilibrium (Xue *et al*., 2014). *Balanites aegyptiaca*

population in Buratai had a unimodal distribution pattern of mismatch. This implies that *Balanites aegyptiaca* populations in Buratai may have experienced some changes in their genetic makeup over time as a result of population expansion. According to Harpending et al. (1998), changes in genetic diversity are commonly associated with changes in population size. A bimodal pattern of mismatch distribution curve was observed in all the other sources. This implies that the populations of *Balanites aegyptiaca* in these locations have not encountered significant changes over time. Equilibrium in population size is a characteristic associated with populations that are free from human disturbance or other factors. This is usually found in organisms that are under protection. *Balanites aegyptiaca* species in most of the location are not under any form of protection, however, in Baure and Dumsai the species are protected by the local because of the benefits they derived from the trees.

To confirm the mismatch distribution curve results that indicate stability or lack of change in the *Balanites aegyptiaca* population in Baure, Dumsai, Gashua, Gamawa, Guri, Kirikasama, and Mashi, a Tajima D test was carried out on the populations that have at least four (4) haplotypes which are necessary for the neutrality test. A non-significant Tajima D test signifies a lack of expansion in population, while a significant negative Tajima D test result indicates a population expansion or population change (Tamboli *et al*., 2016). Non-significant results were obtained in Baure, Dumsai, Gamawa, Guri, and Mashi which is a confirmation of the mismatch results. Therefore it can be said with certainty that these populations of *Balanites aegyptiaca* have not experienced a change in their population. However, significant negative Tajima D test results were obtained for the *Balanites aegyptiaca* population in Gashua and Kirikasama which indicates a sign of significant expansion or change. This is in contrast to the results of the mismatch test which shows stability in these populations. In Baobab, a similar contradiction between the Tajima D test and mismatch analysis was also reported (Ndoye *et al*., 2013). This contradiction could be a result of recent population growth or expansion in Gashua and Kirikasama that was not dictated by mismatch analysis. The sensitivity of the Tajima D test to recent population expansion pushes results to more negative values (Tajima, 1989).

5.6 Physical and chemical properties of soil under *Balanites aegyptiaca* trees

Soil physical and chemical properties vary among locations, and this could influence tree morphological characteristics. *Balanites aegyptiaca* trees seem to prefer soil with a large percentage of sand in comparison with silt and clay. The majority of the trees were found growing in sandy loam soil. NRC (2008) observed the ability of *Balanites aegyptiaca* trees to survive in different types of soil. Soils in Northern Nigeria are characterised by high sand content according to Salako et al. (2001). This is in agreement with the results of this study. Soil texture is regarded as the most important property of soil because it affects physical and chemical properties and biological activities taking place within the soil, thereby playing a significant part in soil quality. Soil organic carbon across the locations was low (6.13±0.50 - 7.67±0.55). This was not surprising since the soil is dominated by sand content. Soil dominated by sand is usually associated with low organic carbon which makes it prone to degradation because of its fragility (Salako *et al*., 2001).

The soil under *Balanites aegyptiaca* trees among the locations varies from moderately acidic to alkaline. Soil pH affects the availability of mineral elements such as Ca, Mg, K, Na, Mn, and P in plants. Low pH encourages Ca, Mg, and K deficiency in the soil, and the availability of P also decreases due to a decline in solubility (Delgado and Gomez, 2016). Ca, Mg, K, Na, Mn, and P content in the soil samples varied among locations. Ca content was high in Buratai (23.90±11.99 mg/kg) and low in Gashua (5.01±2.87 mg/kg). Additions of Ca and Mg in soil have been reported to improve fruit production in *Mangifera indica* (Njana, 2017). The variability in soil texture in the locations could be responsible for the differences in mineral elements recorded. Soil texture is one of the most important determinants of soil chemical properties.

5.7 Oil yield among sources of *Balanites aegyptiaca* kernel

The significant variation in oil yield among locations observed in this study was in agreement with that of Ajayi (2010) who reported a significant variation in the seed oil yield of some underutilized indigenous crops of Nigeria. The yield of 23.11 - 43.95 % recorded in this study was higher than 19.8 to 40 % reported in desert date kernel oil from Sudan by (Elfeel 2010) but lower than 39.20 - 50.22 % recorded by Chapagain and

Wiesman (2005) in desert date kernel oil sourced from different countries. According to AOAC (1990), an oil yield of ≥32 % is the standard range of yield accepted for commercial production. This implies that Guri could serve as a good source of the kernel for the commercial production of desert date oil.

5.8 Physical and chemical properties of *Balanites aegyptiaca* oil from different locations

The values of the refractive index of *Balanites aegyptiaca* oil in this study (1.36 - 1.48) was within the acceptable range of 1.45 to 1.48 according to ASTM International (2002). The values reported are similar to those of some popular seed oil in Nigeria. For example, in groundnut oil, an RI of 0.15-1.47 was reported (Andrew *et al.,* 2012), in castor oil, an RI of 1.45-1.79 was reported (Akpan *et al.,* 2006; Nangbes *et al.,* 2013) and in Sesame oil, the value of 1.46 was reported (Njoku *et al.,* 2010).

The viscosity of *Balanites aegyptiaca* oil from different location in the study was within the range of 41.33 to 48.67 cP. The variation observed among sources was not significant. A similar value of 49 cP was reported by Chapagain et al. (2009) in *Balanites aegyptiaca* oil collected from Egypt. However, the value recorded in this study was higher than what was reported by Okia (2010) who recorded 18.94 – 23.04 cSt from *Balanites aegyptiaca* oil in Uganda. The difference observed between this study and that of Okia (2010) could be a result of variation in environmental factors since the source of collection varied. The temperature used during oil extraction could also be responsible for the variation observed since different temperature was used. Temperature affects fluid products, the higher the temperature the lower the viscosity. The viscosity of the oil is one of the indicators used in assessing the suitability of oil as a lubricant (Belewu *et al.,* 2010).

The acid value of *Balanites aegyptiaca* oil from different sources ranged from 1.36 to 2.11 mg KOH/g. This result was collaborated by the work of Okia (2010) who reported an acid value of 1.33 to 1.95 mgKOH g^{-1} from *Balanites aegyptiaca* oil extracted from different sources in Uganda. However, it is higher than what was reported by Aremo and Oluwadare (2016) in *Hildegardia barteri* seed oil in Nigeria, they recorded a value of 0.032 – 0.034 mgKOH g^{-1}. The acid value of sesame oil in Nigeria was reported to range from 0.45-2.63 mgKOH g^{-1} (Njoku *et al.,* 2010). The edibility of oil can be determined by

its acid value (Tesfaye and Abebaw 2016) and the acceptable acid value recommended for consumption is 0.6 mgKOH/g (AOCS 2003). This implies that the oil from *Balanites aegyptiaca* kernel in all the locations need refining because of the high acid content recorded before it can be suitable for consumption.

The potential use of oil for soap production is ascertained by its saponification value. The saponification value recorded in this study was lower than the recommended value of ≥ 180 mgKOH/g for use as raw material for soap production (AOAC, 1990). However, it was higher than the saponification value of corn oil (153.8 mg KOH/g) and mustard oil (125.6 mg KOH/g) according to Zahir et al. (2017). Oil with a high saponification value usually contains a high proportion of lower fatty acids which makes it suitable for consumption. Oil from Kirikasama source which recorded the highest saponification value 179.85 mgKOH/g could be good for soap production.

The kernel oil in this study could be considered non-drying based on Ouattara *et al*. (2015) classification. This implies that *Balanites aegyptiaca* kernel oil in all locations could be suitable for the production of vegetable oil-based ice cream. The iodine value recorded in this study was less than what was reported by Okia (2010) in Uganda where a value of 98.20 – 103.32 I_2 g/100 g was recorded.

5.9 Effects of geo-climatic factors on yield and properties of *Balanites aegyptiaca* kernel oil

The influence of elevation on the viscosity of *Balanites aegyptiaca* kernel oil was positive, which implies that as elevation increases viscosity may also likely increase. The temperature of a location may positively influence oil yield, refractive index, and saponification value in *Balanites aegyptiaca* kernel oil based on the result of the correlation analysis conducted. The lipid content of pine nuts grown in different locations in Chile was found to correlate significantly with temperature by Lutz et al. (2016) validating the result of this study. However, this result contradicts that of Zohara et al. (1995) who observed a decrease in oil yield with an increase in temperature. *Balanites aegyptiaca* is an arid tree species; therefore it might have developed a mechanism that favours better productivity in high temperatures and little amount of precipitation that

characterised a dry environment. However, high temperature was shown to affect viscosity negatively. This finding was in agreement with Qin *et al*. (2018) who stated that the viscosity of oil decrease with increasing temperature.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study was conducted to assess morphological and molecular variations, germination and early growth characteristics as well as physical and chemical properties of *Balanites aegyptiaca* soil and oil from different locations in the Sahelian zone of Nigeria. The fruit, nut, kernel, and leaf traits of *Balanites aegyptiaca* tree populations in the study area were found to vary among sources. The level of variation in fruit weight, pulp weight, leaf width, and nut weight was very high in comparison with the other traits based on the result of the coefficient of variation. *Balanites aegyptiaca* populations in Baure had superior quantitative morphological characteristics compared with the other sources. Fruit length of *Balanites aegyptiaca* was discovered to have a significant positive correlation with pulp weight, fruit weight, nut length, nut weight, leaf length, and leaf width. This implies that *Balanites aegyptiaca* fruit length can be used as criteria for selecting fruit with better pulp weight, fruit weight, nut length, nut weight, leaf length, and leaf width characteristics for improvement programme.

High genetic variation was observed among *Balanites aegyptiaca* tree populations in the study area. This confirmed the complementary role of morphological and molecular markers in diversity study. Barcoding using matK gene loci has proved useful in assessing the level of genetic diversity among *Balanites aegyptiaca* populations. It was also able to confirm (authenticate) with certainty, the identity of the species used in this study. Mashi population recorded the highest genetic characteristics, therefore it should be prioritized during conservation, breeding, and/or improvement programmes. *Balanites aegyptiaca* population size seems to be stable in most of the locations assessed. This

implies that they are less disturbed by human activities but further analysis revealed the presence of recent population expansion in some locations like Kirikasama and Gashua that was not captured by mismatch distribution curve analysis indicating the possibility of recent expansion (change) in the population. However, there was a significant change in the population size of Buratai population as indicated by the unimodal pattern in the mismatch distribution curve.

Seeds sourced from Guri germinated faster and resulted in better germination percentage but in the long run, seeds from Dumsai performed better in comparison with the others in many of the seedling early growth variables such as height growth, stem dry weight, total dry weight, and absolute growth rate by plant dry weight assessed. Seedlings from Mashi had a better root shoot ratio by dry weight and relative growth rate by dry weight. This is an indication of the ability of seedlings from Mashi to tolerate or withstand harsh conditions on the field over seedlings from other sources. The relationship between geographical and climatic characteristics of the seed sources with growth variables varied. Seedling height increased with an increase in longitude and latitude, while the number of leaves was found to increase with an increase in elevation and precipitation.

Balanites aegyptiaca kernel oil yield of was discovered to vary among locations with a percentage yield suitable for commercial production. The physical and chemical properties of the oil vary widely among kernel sources, indicating the feasibility of improvement in kernel oil properties through selection. The refractive index, acid value, and iodine value of the oil were within the acceptable recommended range. This supported the feasibility of the utilisation of kernel oil for domestic and industrial purposes. Climatic and geographical factors of kernel source demonstrated both positive and negative correlations with oil yield and physical and chemical properties of the kernel oil. An increase in temperature at the source affected oil yield positively, however, rainfall had a negative effect on oil yield.

The results of this study portray a broad morphological and molecular diversity as well as high variation in oil yield and physico-chemical properties among *Balanites aegyptiaca* seed sources. The presence of wide morphological, molecular, and physical and chemical variations observed among *Balanites aegyptiaca* populations in this study presents a great

opportunity for selection of seeds with superior characteristics for domestication and breeding and/or improvement programmes. Variations are the basis for designing any successful breeding and conservation programme. It gives us an idea about the population that should be targeted based on the level of diversity existing among and within them. Selection of populations with desired attributes and the application of silvicultural techniques for improvement is a common practice in silviculture for improving trees, likewise crossing of characters among parents to obtain superior offsprings. Crossing more diverse parent ensure the level of variation is maximised in the segregating population. Therefore germplasm collection from Baure, Dumsai, and Mashi should be prioritized during conservation, breeding, and/or improvement programmes.

6.2 Recommendations

- Selection of seed from Baure is recommended for fruit production.
- Breeding programme to improved morphological traits of *Balanites aegyptiaca* species should be through the crossing of Baure, Mashi, and Dumsai genotypes.
- Seeds from Baure source which was identified as a plus tree should be mass propagated to establish a seed orchard for seed collection activities of the species
- Intra-specific molecular diversity of *Balanites aegyptiaca* tree species should be investigated.
- Seed selection from Guri is recommended for oil production

6.3 Contributions to knowledge

1. The morphological characters for description and delimitation of *Balanites aegyptiaca* species in the Sahelian zone of Nigeria were documented
2. The pattern of genetic characteristics in *Balanites aegyptiaca* populations in the Sahelian zone of Nigeria was documented
3. The effect of seed sources on the yield and physico-chemical properties of oil extracted from *Balanites aegyptiaca* kernels were determined
4. The effectiveness of matK gene loci in the discrimination of *Balanites aegyptiaca* at the species level was established

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Appendix I

Tagging of *Balanites aegyptiaca* tree

Appendix II

Samples of fruits collected

Appendix III

Kernels of *Balanites aegyptiaca*

Appendix IV

Measuring fruit of *Balanites aegyptiaca*

Appendix V

Leaf length measurement of *Balanites aegyptiaca*

Appendix VI

Taking weight of *Balanites aegyptiaca* fruits

Appendix VII

Seedlings of *Balanites aegyptiaca* uprooted for transplanting

Appendix VIII

Transplanted seedlings of *Balanites aegyptiaca*

Appendix IX

Blended toast samples of *Balanites aegyptiaca* kernels

Appendix X

Balanites aegyptiaca oil extraction using soxhlet apparatus

Appendix XI

Extracted oil samples of *Balanites aegyptiaca*

Appendix XII

Fresh leaf samples of *Balanites aegyptiaca* in zip lock bags before grinding in mortar and pestle

Appendix XIII

Loading of DNA samples for electrophoresis

Appendix XIV

Analysis of Variance (ANOVA) table for morphological characters of *Balanites aegyptiaca* fruits and leave from different sources

	DF	SS	MS	F	P
Fruits thickness					
Source	7	1.485	0.212	2.254	0.040*
Error	68	6.398	0.094		
Total	75	7.883			
Fruit width					
Source	7	0.963	0.138	2.739	0.014*
Error	68	3.417	0.050		
Total	75	4.380			
Pulp weight					
Source	7	37.885	5.412	2.520	0.023*
Error	68	146.036	2.148		
Total	75	183.921			
Fruits length					
Source	7	2.412	0.345	2.876	0.011*
Error	68	8.146	0.120		
Total	75	10.558			
Leave length					
Source	7	0.015	0.002	2.254	0.040*
Error	68	0.064	0.001		
Total	75	0.079			
Leave width					
Source	7	0.379	0.054	2.520	0.023*
Error	68	1.460	0.021		
Total	75	1.839			
Leave thickness					
Source	7	0.280	0.040	2.075	0.058**ns**
Error	68	1.313	0.019		
Total	75	1.594			
Fruit weight					
Source	7	57.024	8.146	2.424	0.028*
Error	68	228.521	3.361		
Total	75	285.545			

Source: Field Survey 2019. * and ns = significant and not significant respectively $\alpha = _{0.05}$

Appendix XV

ANOVA table for morphological characteristics of *Balanites aegyptiaca* nuts from different sources

	DF	SS	MS	F	P
Nut length					
Source	7	0.280	0.040	2.075	0.058**ns**
Error	68	1.313	0.019		
Total	75	1.594			
Nut width					
Source	7	0.194	0.028	0.602	0.752**ns**
Error	68	3.123	0.046		
Total	75	3.317			
Nut weight					
Source	7	5.205	0.744	1.744	0.113**ns**
Error	68	28.989	0.426		
Total	75	34.194			
Nut thickness					
Source	7	2.217	0.317	10.263	0.000*
Error	68	2.099	0.031		
Total	75	4.316			
Kernel weight					
Source	7	0.657	0.093	3.616	0.002**ns**
Error	68	1.765	0.026		
Total	75	2.421			

Source: Field Survey 2019* and ns = significant and not significant respectively $\alpha = _{0.05}$

Appendix XVI

ANOVA table for germination behaviour of *Balanites aegyptiaca* seed from different sources

	DF	SS	MS	F	P
Germination percentage					
Source	7	9460.9	1351.6	476.30	0.00*
Error	32	90.8	2.8		
Total	39	9551.7			
Mean germination time					
Source	7	83.870	11.981	7.246	0.00*
Error	32	52.913	1.654		
Total	39	136.783			
Germination speed					
Source	7	0.775	0.111	564.14	0.00*
Error	32	0.006	0.000		
Total	39	0.781			

Source: Field Survey 2018. * and ns = significant and not significant respectively $\alpha = {}_{0.05}$

Appendix XVII

ANOVA table for growth characteristics of *Balanites aegyptiaca* seedlings from different sources

	DF	SS	MS	F	P
Stem height					
Source	7	2924.8	417.8	5.747	0.000*
Error	72	5234.5	72.7		
Total	79	8159.3			
Collar diameter					
Source	7	12.665	1.809	3.769	0.002*
Error	72	34.565	0.480		
Total	79	47.230			
Number of leave					
Source	7	31727	4532	3.299	0.004*
Error	72	98922	1374		
Total	79	130649			

Source: Field Survey 2020. * and ns = significant and not significant respectively $\alpha = {}_{0.05}$

Appendix XVIII

ANOVA table for some growth parameters and biomass production of *Balanites aegyptiaca* seedlings from different sources

	DF	SS	MS	F	P
Root length					
Source	7	2095.8	299.4	2.443	0.026*
Error	72	8824.4	122.6		
Total	79	10920.2			
Root: shoot ratio					
Source	7	24.849	3.550	1.780	0.105**ns**
Error	72	142.599	1.994		
Total	79	168.448			
Absolute growth rate by plant height					
Source	7	0.016	0.002	2.017	0.064**ns**
Error	72	0.080	0.001		
Total	79	0.095			
Relative growth rate					
Source	7	0.003	0.000	2.400	0.219**ns**
Error	72	0.0020	0.000		
Total	79	0.0023			
Absolute growth rate by dry weight					
Source	7	0.002	0.002	5.604	0.000*
Error	72	0.004	0.001		
Total	79	0.005			
Leave dry weight					
Source	7	12.178	1.740	3.090	0.007*
Error	72	40.533	0.563		
Total	79	52.711			
Stem dry weight					
Source	7	30.005	4.286	6.264	0.000*
Error	72	49.266	0.684		
Total	79	79.271			
Root dry weight					
Source	7	116.327	16.618	3.912	0.001*
Error	72	305.837	4.248		
Total	79	422.164			
Total dry weight (Total Biomass)					
Source	7	339.370	48.481	5.949	0.000*
Error	72	586.785	8.150		
Total	79	926.155			

Source: Field Survey 2020. * and ns = significant and not significant respectively $\alpha = {}_{0.05}$

Appendix XIX

ANOVA table for oil yield and physicochemical properties of *Balanites aegyptiaca* oil from different sources

	DF	SS	MS	F	P
Oil yield					
Source	7	1308.086	186.869	116.913	0.000*
Error	16	25.574	1.598		
Total	23	1333.660			
Refractive index					
Source	7	0.164	0.164	16.917	0.000*
Error	16	0.155	0.010		
Total	23	1.301			
Viscosity					
Source	7	254.958	36.423	3.553	0.017*
Error	16	164.000	10.250		
Total	23	418.958			
Acid value					
Source	7	1.146	0.164	16.917	0.00*
Error	16	0.155	0.010		
Total	23	1.301			
Saponification value					
Source	7	24077.9	3439.7	6112.7	0.00*
Error	16	9.0	0.6		
Total	23	24086.9			
Iodine value					
Source	7	687.5	98.2	46.27	0.000*
Error	16	34.0	2.1		
Total	23	721.5			

Source: Field Survey 2019. * and ns = significant and not significant respectively $\alpha = {}_{0.05}$

Appendix XX

ANOVA table for proximate and mineral composition of *Balanites aegyptiaca* oil from different sources

	DF	SS	MS	F	P
Moisture content					
Source	7	80.220	11.460	57300.274	0.000*
Error	16	0.003	0.000		
Total	23	80.224			
Ash content					
Source	7	0.066	0.009	28.098	0.000*
Error	16	0.005	0.000		
Total	23	0.071			
Protein					
Source	7	8.172	1.167	221.1	0.000*
Error	16	0.084	0.005		
Total	23	8.256			
Calcium					
Source	7	46.851	6.693	660.944	0.000*
Error	16	0.162	0.010		
Total	23	47.013			
Sodium					
Source	7	7.479	1.068	86.76	0.000*
Error	16	0.197	0.012		
Total	23	7.676			
Potassium					
Source	7	155.386	22.198	137.59	0.000*
Error	16	2.581	0.161		
Total	23	157.967			
Magnesium					
Source	7	0.576	0.082	172.353	0.00*
Error	16	0.008	0.000		
Total	23	0.583			
Copper					
Source	7	0.020	0.003	118.94	0.000*
Error	16	0.000	0.6		
Total	23	0.020			
Zinc					
Source	7	0.029	0.004	5233.403	0.000*
Error	16	0.000	0.000		
Total	23	0.029			

Source: Field Survey 2019. * and ns = significant and not significant respectively $\alpha = _{0.05}$