REPRODUCTIVE PHENOLOGY AND MOLECULAR CHARACTERISATION OF *Moringa oleifera* LAM. LANDRACES IN RAIN FOREST ZONE OF SOUTHWESTERN NIGERIA

BY

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CERTIFICATION

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DEDICATION

To God the creator of heaven and earth, who raised me like a unicorn to stand tall on the mountain, much more than I could ever imagine I can be and to all who love and believe in the supremacy of God in nature

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ABSTRACT

Local adaptation of multipurpose tree species such as *Moringa oleifera* has resulted in the emergence of landraces, which could influence selection for mass propagation. It has been established that knowledge on landraces affects decisions on germplasm collection for propagation of *Moringa oleifera*. However, critical information on the reproduction and genetic characteristics of different landraces are limited. Therefore, the flowering and fruiting patterns, pod morphology, seedling growth and genetic characteristics of *Moringa oleifera* in Southwestern Nigeria were investigated.

Two Moringa plantations were purposively selected from each of eight locations: Abeokuta, Akure, Erinjiyan, Ijare, Ijari, Ijaye, Omu, and Oyo, based on availability. Five trees were randomly selected from four corners and the centre of a 20m by 20m plot, demarcated at the centre of each plantation. These trees were used to assess phenology: onset and duration of flowering and fruiting (days), pod morphology and maturity index [duration before pod colour change (days)], for 24 months. Pods (300) were collected from each location and measured for length (cm), diameter (mm), seed weight (g) and number of seed/pod (NS). Seeds extracted at each stage of maturity were subjected to germination test using standard procedures. Sixty uniformly growing seedlings were selected per location and monitored for height (cm), collar diameter (mm) and number of leaves for six months. Genetic characteristics of five accessions/plantation (n=80) were determined using five microsatellite markers (MO8, MO15, MO48, MO61, MO64). Number of Alleles (NA), allele frequency, genetic diversity and Polymorphic Information Content (PIC) were determined. Data were analysed using descriptive statistics, Analysis of Molecular Variance (AMOVA), Cluster Analysis and ANOVA at $\alpha_{0.05}$.

Flowering (April-June; August-October) and fruiting (June-September; October-February) occurred twice a year; while duration of flowering (43.5-44.3) and fruiting (154-160) in days were similar across locations. Three stages of pod colour change: green (26.5-28.8), yellow (82.6-92.3) and brown (25.8-31.8) were observed. Abeokuta (40.0±1.7) had the highest pod length, while Erinjiyan (27.6±0.6) had the least. Pods from Akure (16.4±3.2) had the least diameter while Abeokuta (19.5±2.9) had highest. Seed weight was significantly highest (31.4±1.7) at Omu and least (17.2±1.0) at Ijari, while NS ranged from 12.9±3.3 (Erinjiyan) to 17.9±3.3 (Akure). Seed germination was highest (90.7±0.3%) for yellow pods and least (30.7±0.9%) for green. Height was significantly highest (112.7±1.4) for seedlings from Ijari and least (76.9±0.8) for those from Omu. Seedling collar diameter ranged from 21.7±0.6 (Akure) to 37.4±0.9 (Erinjiyan). Ninety-six alleles with an average of 9.6±0.6 alleles/locus in each accession were amplified. Allele frequency and gene diversity ranged from 0.2% to 0.5% and 0.7% to 0.9%, respectively. The highest PIC (0.9) occurred in MO64, while MO48 had the least (0.6). There were significant differences in intra-specific (48%) and inter-specific (52%) genetic diversity of the sampled populations. Five clusters were identified with similarity coefficients that ranged from 0.1 to 0.4.

Moringa oleifera population in Southwestern Nigeria exhibited extensive and simultaneous flowering and fruiting patterns. Pod and seed morphology were location dependent and influenced seedling growth. Five landraces of *Moringa oleifera* were identified and this has implications in germplasm selection for propagation.

Keywords: *Moringa oleifera*, Microsatellite markers, Pod maturity index, Germplasm variation, Genetic diversity

Word count: 498

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LIST OF ABBREVIATIONS

ADP	Agricultural Development Program
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
BFA	Biosciences for Farming in Africa
CA	Cluster Analysis
CRD	Complete Randomise Design
CTAB	Cetyltrimethyl Ammonium Bromide
DMRT	Duncan Multiple Range Test
DNA	Deoxyribonucleic Acid
EDTA	Ethylene DeminTetraacetic Acid
FAO	Food Agricultural Organisation
FRIN	Forestry Research Institute of Nigeria
GAE/g	Gallic Acid Equivalent per grain
GFU	Global Facilitation Unit
IITA	International Institute of Tropical Agriculture
MDAN	Moringa Development Association of Nigeria
NRC	National Research Council
PAGE	Polyacrylamide Gel Electrophoresis
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction

PIC	Polymorphic Information Content
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
SC	Similarity Coefficient
SSR	Simple Sequence Repeat
Taq	Enzyme that withstands high temperature (95^{o}) in PCR and retains its functions
TBE	Tris Borate
UV	Ultra Violent Transilluminator

CHAPTER ONE
INTRODUCTION

1.1 Background

Landrace variation is a noticeable disparity between and within individual species whose origin is not in the environment where they exist (BFA, 2015). These species have formed some attributes that make them adjust to these new surroundings. Landraces of tree species existing in different ecological parts of Nigeria were introduced based on their economic importance and health benefits. Landrace variation can be quantified both in phenotypic and genetic measurements. This promotes the management of plant genetic resources, by aiding the identification of seeds which is capable of producing forest that will comprise variants of high survival rate; yield, as well as, resistance to pest and adverse environmental conditions.

Moringa oleifera Lam. is an example of a landrace introduced to the Nigerian environment. The tree species is an out-crossing diploid (2n=28) native to Northwest India and the most commonly grown species in the monogeneric family Moringaceae of all 13 species (Ozumba, 2008). This evergreen and the drought-resistant tree grows to heights of 5m to 10m and is always in seasons all year-round (Fuglie, 2001 Bhuptawat *et al*., 2007). The fast-growing species tolerates a wide range of soil and rainfall conditions, attaining sexual maturity in 36 weeks and starts to flower sometimes before a year of development (Ozumba, 2008). *Moringa oleifera* is a tree species introduced to the northern part of Nigeria for agroforestry systems, particularly as a home garden, for hedges and livestock forage because of its multiple-use (Amaglo, 2006).

However, it is now distributed throughout Nigeria and in many parts of the tropics. The multiple uses of the tree species have engendered research interests with various research carried out to separate bioactive compounds from their different parts (Guevara *et al*., 1999). It has also been widely accepted as one of the alternative measures for health therapies in the medical field, because of its reasonable cost (Abalaka *et al.,* 2009). It is believed to be among the world's most useful plants owing to its outstanding nutritional values (Zaku *et al.*, 2015). The increase in awareness of

Moringa oleifera and its usefulness have led to a continuous distribution of its landraces across ecological zones in Nigeria (Popoola *et al*., 2014). *Moringa oleifera*, a cross-pollinated plant, is expected to have a wide gene pool and genetic base. However, research findings on its conservation, management and genetic composition have revealed that its genetic pool among different landraces in Nigeria is narrow.

For example, the exchange of planting materials from one location to another (Popoola *et al*., 2017). The continuous utilization and consumption of *Moringa oleifera* necessitate the need for elaborate research on the degree of genetic diversity within and among populations of the plant. Furthermore, baseline information that would assist germplasm utilization, tree improvement, hybridization and increase value of *Moringa oleifera* are required. Hence, this study examined the reproductive phenology and conducted molecular characterisation of different populations from southwestern Nigeria.

1.2 Statement of the problem

Moringa oleifera is regarded to be an under-exploited, under-utilised and under-researchable plant species in Africa (NRC, 2006; GFU, 2012). This is partly because data collection and records on its existence and population distribution pattern, particularly in monocultures (where high yield and uniform products can be obtained) is limited. Many farmers cultivating *Moringa oleifera* in southwestern Nigeria have substituted it with other plant species because of the poor markets for their produce. Despite the availability and easy access to Moringa plant materials, knowledge about their uses among different populations is unequal (Omonhinmin, 2012). Besides, limited information on the population distribution of monoplantations of *Moringa oleifera* hinders the effort aimed at germplasm improvement.

Seeds of *Moringa oleifera* are recalcitrant, losing their viability within a short length of time (Mubvuma *et al*., 2013; Csurches and Navie, 2016). Hence, seeds lose viability quickly even when enclosed in pods under storage and their maturity indices are not known. To this end, the germination percentage obtained does not justify the efforts put into seed collection and the quantity of the seeds collected. Also, the floral and fruiting duration which could provide insights into conditions for the harvesting of seeds is not clearly understood.

Research efforts aimed at breeding improved varieties of *Moringa oleifera* landraces in Nigeria are scarce. For instance, the morphological variability of local

accession that controls its survival and productivity have not been adequately investigated. Much of the information recorded about the species morphology relates to the landraces of other regions (Daoebou and Kabore, 2015). This limits the multiple benefits of the species and the effective harvesting of the plant for product development in Nigeria.

The xenogamous nature of *Moringa oleifera* increases its ability to produce different varieties. However, information on the characteristics of the variants concerning survival rate, yield and disease resistance have not been properly documented (Popoola *et al*., 2017). In particular, the impact of the source of seed on germination and early seedling growth of accessions is not well known.

The demands for utilizing the leaves of *Moringa oleifera* as the source of a nutrient supplement is generally high according to Plant Resources of Tropical Africa (Prota, 2017). The species has a rapid coppice potential that allows for the regrowth of lopped branches and shoots. However, the cost of harvesting on the growth of the plant and its nutrient content has not been fully investigated. To this effect, the rate of foliage production is essential for quantifying *Moringa* leaf production. Likewise, a clear understanding of the nutritional and health benefits accrual from lopped trees vis-a-vis the knowledge of proximate and phytochemical properties of *Moringa oleifera* leaves have not been properly investigated.

Moringa oleifera is known to exhibit a narrow genetic pool in Nigeria accessions (Popoola *et al*., 2017). The classification of its genetic diversity in populations has not been adequately addressed in Nigeria. The situation is further compounded by the lack of information on the use of molecular marker which generates random and unstable genetic estimates and those that could reveal genetic variation in a population without the influence of the environment. Therefore, it has been difficult to fully capture different possible forms of a gene that could enhance stable genetic investigations (Ojuederie *et al*., 2013). with only southwestern varieties found to exhibit unique genetic information (Popoola *et al*., 2017). Therefore, this study aims to undertake an accurate molecular characterisation of the landraces of *Moringa oleifera*, in Southwestern Nigeria.

1.3 Main objective

This study investigated the genetic variation among populations of *Moringa oleifera* from various locations in Nigeria to find suitable sources of seeds for breeding and improvement.

1.3.1 Specific Objectives:

This study was carried out to:

(i) determine the population distribution and tree density of *Moringa oleifera* in monoculture;

(ii) assess the flowering and fruiting patterns and duration of *Moringa oleifera* trees;

(iii) determine fruit and seed morphology of *Moringa oleifera*;

(iv) evaluate the effect of seed source on germination and early growth of seedlings;

(v) assess the effect of seed source, seedling age and lopping height on proximate, phytochemical and biomass production; and

(vi) characterise the genetic variation of *Moringa oleifera* from the selected sources.

1.4 Justification for the study

Moringa oleifera is a species that has naturalized itself in Nigeria. It is widely distributed due to deliberate introduction and planting across the landscape (Popoola *et al*, 2014; Csurches and Navie, 2016). A properly managed forest requires adequate information from a reliable database to show the level, condition and potentials of its resources. Unfortunately, Leone *et al.* (2015) asserted that no records of *M. oleifera* active germplasm banks around the world. Consequently, information on the geographical distribution and population density of this species, particularly in southwestern Nigeria, will support the selection of seed source and tree improvement efforts.

The success of breeding and improvement programmes for plant species depend on an adequate understanding of their floral biology (Jyothi *et al*., 1990). Hence a detailed knowledge of the reproductive biology of the candidate species is essential for producing unique variants of high economic value. The developmental stages, flowering and fruiting patterns as well as the optimum point at maturity where harvesting would give maximum seed germination of *Moringa oleifera* landraces in

Nigeria have received little attention. Therefore, an assessment of the species' developmental stages is imperative to determine the appropriate timing for seed collection to prevent forfeiting pods that could produce variety suitable to local needs.

The morphological variation that is observed in the pods and seeds (reproductive organs) of *Moringa oleifera* is not likely to be environmentally influenced but genetically both within and across populations from different landraces. This makes the selection of reliable seed sources a major prerequisite for understanding the level of variation among populations during breeding or propagation. Hence, morphological characteristics assessment among *Moringa* populations is essential for the determination of the level of genetic diversity.

Seed sources significantly affect seedling morphology and development (Aderounmu and Adegeye, 2011). This makes seedling vigour and subsequent development important tools in genetic classification, particularly in plantation establishment. An assessment of the growth performance of *M. oleifera* from different sources will provide in-depth knowledge of the variations that occur based on seed source.

The foliage from *Moringa oleifera* has been considered as a potentially inexpensive source of protein along with high amounts of vitamins (ß-carotene, ascorbic acid, B1, B6 and niacin). Coppicing is a traditional management technique, which enhances forest persistence under unfavourable climatic conditions and has been shown to potentially increase biomass accumulation. Coppicing involves cutting and regrowth which enhances new leaf flush in growing trees (Price, 2007). These leaves are vigorously produced by the tree and can be harvested several times within an annual cycle (Sarwatt *et al*., 2004). The production of succulent tissues (new leaf flush) in coppices of *Moringa oleifera* would greatly affect the nutritional status due to its age thus, these could enhance an increase in its economic value as well as its nutritional and medicinal qualities. Besides, the influence of age on leaf biomass production from coppiced stems would help in management and planning efforts.

The use of molecular markers in forest genetics has revolutionized studies on mating systems of plants, pollen movement, seed dispersal and genetic processes (FAO, 1994). Previous efforts made to characterised *Moringa oleifera* had a major weakness in which gene profiling was dependent on reaction condition, which may vary from laboratory to laboratory (Popoola *et al*., 2014). However, the study of the

distribution of *Moringa oleifera* population in the six geopolitical zones in Nigeria by Popoola *et al*. (2017) revealed five zones out of six to have exhibited similarities in the genetic information; only the southwest accession displayed and detected unique genetic information. Therefore, characterizing additional accessions from the southwestern population with the use of a very competent molecular marker tool will generate an improved estimate of genetic diversity.

1.5 Scope of the study

The study assessed the genetic variation of *Moringa oleifera* tree populations with the use of competent molecular marker tools from sixteen farms within eight sources in the rainforest zone of Southwestern Nigeria. The assessment was based on the population estimate of the species, floral and fruiting duration, seed and pod morphology, germination and growth studies and leaf quality and quantity production ability through coppicing potential.

CHAPTER TWO
LITERATURE REVIEW

2.1 Botanic description of *Moringa oleifera*

Moringa oleifera is a miracle tree, known as ewe igbale (in Yoruba) and zogali in the Hausa language. It is fast in growing, reaches a height of 10-12 metres with a girth of 45 cm but drops its leaves in the dry season (Parrotta, 1993). It is a cross-pollinated plant (2n = 28 chromosome) from the *Moringaceae* family that is made up of only one genus (Moringa) with 13 species; *Moringa oleifera*, *M. arborea, M. borziana, M. concanensis, M. drouhardii, M. hildebrandtii, M. longituba, M. ovalifolia, M. peregrine, M. pygmaea, M. rivae, M. ruspoliana, and M. stenopetala* that are native to Africa, Madagascar, Western Asia and India (Mahmood *et al*., 2010) which has been grouped into three based on habit and wood anatomy. The first group consists of four species and referred to as the bottle tree; *Moringa stenopetala, M. drouhardii, M ovalifolia* and *M. hildebrandtii*, having the characteristics of trunks that are swollen and radially symmetric flowers. The second group (tuberous clade) is six in number; *Moringa arborea, M. borziana, M. longituba, M. pygmaea, M. rivae* and *M. ruspoliana.* They are shrubs with thick and fleshy tuberous root. The third group is referred to as the slender trees which possess bilaterally symmetrical flowers and tough root, members are *Moringa oleifera*, *M. concanensis* and *M. peregrine* (Olson and Rosell, 2006).

Moringa oleifera can be cultivated from seed or stem cuttings. The percentage of germination is high and germinates throughout the year. It tolerates different types of soil conditions but prefers sandy or loamy soil that is well-drained with a pH level of 6.3 to 7.0 which is either neutral or slightly acidic. It thrives well where there are light and heat, it can cope with drought and this has made it cultivation suitable in dry regions without expensive irrigation techniques but with rainwater (Jyothi *et al*., 1990). However, it cannot withstand an ice-cold or waterlogged environment to avoid roots rot (Radovich, 2009).

Nevertheless, well-distributed annual rainfall (1000-2000mm) is needed for optimum leaf and pod production. Growth tends to slow down significantly when the temperature is less than 20°C. *Moringa oleifera* undergo leaves fall once a year during the dry season and new leaves start growing at the onset of the rainy season. As for fruits, the first harvest may occur 6 to 8 months after planting. Most times, there is no fruit production in the first year, and fruits are commonly grown in the early years. Around 300 pods are produced in the second year while between 400 and 500 pods are produced in the third year. A healthy tree of *Moringa oleifera* should be able to produce 1000 pods or more within a year (FAO, 2014).

2.2 Plant population distribution and density of *Moringa oleifera*

The knowledge about the forces driving species distributions is a prerequisite to understanding species abundance and distribution (Chesson, 2000). Methods of pollination and environmental factors are the main primary causes of species distribution (Smith and Lundholm, 2010). Moreover, these environmental factors have been reported and supported by many authors as the primary factor influencing species distribution. (Gilbert and Lechowicz, 2004; Tuomisto *et al*, 2003)

Moringa oleifera is known to be well adapted and distributed in all ecological zones of Nigeria because it can tolerate varied climatic conditions. Among Nigerian, the species has been reported to be widely accepted, recognized and useful. The distribution pattern across the geopolitical zone in Nigeria has been influenced by the source of introduction, domestication and ethnic differentiation (Popoola and Obembe, 2013). The population density of a plant can be described as the amount of space that is left between plants when establishing a plantation. The more closely spaced standing trees are, the higher the density and this can be measured between trees. Planting density can impact the overall health of the plant and its yields. Planting density that is too sparse may be susceptible to weed, while planting density that is too dense might force plants to compete over nutrients (Gregory, 2018).

The existence of many plant species and organisms is a function of its rate of survival and the existence of climate that is favourable for the growth and development of seedlings. (Olajide *et al*., 2008). An important silvicultural variable is planting density which manipulates the microenvironment of the field and influence tree crop growth, development and yield (Rahman and Hosain, 2011). However, the density of plants or species of timber tree standing volume often estimates and reveal the multiple

values of a forest (Udo *et al*., 2009). Moreover, the intensity and pattern of exploitation a forest is subjected to is determined by the surplus and scarcity of the species within the forest as well as its values or economic importance (Udo *et al*., 2009).

Plant genotype and geographical location influence optimum density distribution and variation in plantations (Mabapa *et al*., 2017; Patricio *et al*., 2017). Plant population density is also being affected by spacing which could result in high yield when maximum interception of all the available photosynthetically active radiation (PAR) is allowed (Rahman and Hosain, 2011). Adegun and Ayodele (2015) reported that the spacing adopted during the cultivation of *Moringa oleifera* greatly affected its yield and population density while with low spacing resulting in small stem girth, and the reverse was the case for high spacing.

Planting densities of 167,000 trees ha^{-1} resulted in 27 tonnes of biomass in a *Moringa oleifera* plantation while planting a density of 100,000 ha^{-1} produced 11 tonnes ha^{-1} (Medieta-Araica, 2012). Also, the frequency of cutting biomass favourably affected the nutritional quality and yield of *Moringa oleifera* under high plant density (Mabapa *et al*., 2017). Although, an increase in density had no positive effect on leaf biomass production (Patricio *et al*., 2017). In southwestern Nigeria; the main practice of cultivating Moringa is through agroforestry: where smallholder farmers integrate the plant on their farms alongside their crop (Adegun and Ayodele, 2015).

2.2.1 Implication of spacing and tree density estimation in forest management

In recent time, forest plant production strategy has shifted from natural forests to plantation establishment and its role in meeting future needs will continually increase (Brown, 2001 and Alfred, 2007). Effective stand management involves controlling the spacing of the growing stock by varying planting density to regulate tree growth and wood quality (Kenk, 1990). Enspacement is one of the silvicultural techniques adopted to ensure rigorous forest management that is practised to improve the productivity of forest plantations (Jiang *et al.,* 2007).

Spacing is simply defined as the distance between rows and between the plant in forest plantations. Various factors are responsible for different spacing adopted. For instance, characteristics of species, species tolerance to the environmental factors, growth rate, the condition of the land area and the objectives of plantation establishment such as production of timber, fuelwood, fruits production e.t.c (Naeem,2018).

In a natural forest, it is observed that trees establish themselves widely apart and increased infiltration of rainwater and decrease of evaporation from soil is perceived. However, the amount of water and nutrient available to plant in an established plantation is proportional to the stand density which varies with species, site and the objective of the established plantation (FAO,1987). Serious attention needs to be paid to spacing before embarking on any plantation establishment because spacing has controlling effects on tree growth and development. Besides, planting spacing regimes plays an important role in tree growth because it influences the quantity and quality of wood produced (Iddis *et al*., 1996).

The size and height of trees at maturity are proportional to the spacing adopted. For instance, when a tree at maturity is expected to be 5meters in girth (GBH) then, it would be planted at 5meters from the next plant of the same species (Nuga *et a*l., 2010). Therefore, to maintain a good balance between an established plantation and the trees growing on it for desired economic and silvicultural benefits, the growth, quality and health of trees can be manipulated by regulating the stand density (Etigale *et al*, 2013)

There is a limit to the number of trees that can be planted in a given area and this can be determined by prior knowledge of the tree morphology (Klaus and Heyns, 2014). At lower spacing, there is high plant density therefore, trees tend to grow faster, taller with straight bole, competing for light, soil moisture and nutrients (Woods *et al*., 1992; Smith *et al*., 2014). Therefore, populations of trees growing at high densities are vulnerable to self-thinning and as such, the survival and mortality rate is determined by an increase or decrease in the number of planted trees per unit area.

Moreover, when the initial plant density of an established plantation is reducing, then the surviving rate which is a subject of the spacing methods adopted is revealed (Nwoboshi, 1982). Although, various factors could be responsible for the reduction of trees in a plantation such as nutrient deficiencies. There could also be high competition among trees for growth resources and this could result in the rigorous natural selection which could favour the most vigorous trees that survive the intense competition (Smith, 1962).

On the other hand, trees with wider spacing resulted in lower planting density during plantation establishment but these trees effectively absorb sunlight, sufficient moisture and nutrients, thereby producing trees with larger bole and crown size (Jiang

et al. 2007). Higher spacing is recommended for timber production and fruit trees, especially when thinning cannot be done earlier. This influence a steady increase in total Dbh and enhances branching for massive fruit production. However, such growing trees at an early stage are exposed to weed encroachment which tends to compete with trees for space and nutrient (Westfall *et al*.,2004)

Tree density estimation is an important operation that provides insights into suitable procedures required for activities such as planting, beating-up, thinning and pruning. Invariably, tree density will determine the amount of space available for each tree to grow in a location and the level of competition for light, soil moisture and nutrients (Etigale *et al*., 2014). Tree density can be by counting trees in the sample plot, and use the estimated number of trees per hectare, to extrapolate the total number of tree enumerated in respective plots (Etigale *et al*., 2014).

2.3 Reproductive phenology, cross mating and progeny variation in forest trees

Plants express their maturity by developing floral structures specially configured for sexual reproduction (pollination and fertilization). Flowers which are the reproductive organs consist of four main important parts: sepals, petals, stamen and carpel. The stamen, which is the male part, is structurally divided into two parts anther and stigma. Pistil, a female part, is sub-divided into three parts: stigma, style and ovary. A flower-bud develops from a bud and results in a tiny complete flower. As time goes on, the tiny flower develops into a mature flower. When pollens are trapped at the centre of the matured flower, available eggs are fertilized to become ovule. Every ovule increases in length after fertilisation and ripens to become fruit that encloses the seeds. Every seed has a tiny rudiment that is called an embryo. Fruit formation commences with the formation of flower but not all flower formed develop into a fruit.

The time it takes for the embryo to develop varies among different species and can be from several days to many months, and even years (Dumas and Rogowsky, 2008). Seeds of different species vary enormously in their structural and anatomical complexity and size. The weight of seed varies from 0.003mg for orchids to over 20kg for the double coconut palm (*Lodoicea maldvica*). Seed development is divided into three stages: Phase I – formation of the different tissues within the embryo and surrounding structures (histo-differentiation, which is characterized by extensive cell divisions); Phase II – cell enlargement and expansion (little cell division, dry weight

increase due to reserve deposition, water content decline); and Phase III – dry matter accumulation slows and ceases at physiological maturity (Black *et al.*, 2006). All plants that produce flower go through a similar life cycle while there is variation in the length of time it takes. For some plants, the life cycle will be completed within a few weeks while it will take several years for other plants.

Fertilized flowers develop into a fruit set (fruit initiation) and progressively grow into mature fruit (Mathew and Rajamony, 2004). The flower bud and fruit set in *Moringa oleifera* are influenced by irrigation (Muhl *et al*., 2013). *Moringa oleifera* is made up of male and female flower parts (monoecious) and flowers within the first six months after planting. In the temperate, it flowers only once a year between April and June while in the tropics flowering can happen twice or even all year-round with constant rainfall (Parrotta, 1993). There are two peaks of flowering: October – November and April – May with two corresponding fruiting peaks that are during October and May. Moreover, continuous flowering and fruiting have been reported by Pushpangathan *et al.* (1996) and Sindhu (2002).

The process of fruit formation from a flower is affected by climatic conditions which may favour or limit fruits developmental process. *Moringa oleifera* commences bud and flower formation processes with the on-set of the calyx (outmost whorls of flower parts). At this stage, the buds are green and not prominent. As times goes on, the buds bulge out with a light green colour to express their full formation. At this stage, changes (both in colour and size) at every other part become visible. This development proceeds steadily until the bud becomes slightly opened into flower bud and at last, fully opened into flowers.

2.3.1 Cross mating in forest plants

Pollination is an important process in the reproduction of flowering plants. It involves the transfer of pollen grains to the stigma (the receptive surface of the pistil). This is followed by the growth of a pollen tube through the style to the ovule. Pollination is referred to as autogamy or self-pollination if occurs within the same plant or the pollen can be delivered from a different plant (cross-pollination, allogamy). Pollination can be affected by wind, water, insects, and animals, such as bats and birds. Most angiosperms (over 70%) depend on insects for cross-pollination (Faheem *et al.*, 2004). The effectiveness of pollinators depends on flower structure and characteristics, such as colour, scent, shape, size, nectar and pollen production. Wind

pollination is likely due to insect pollination in response to limitations of pollinators and changes in the abiotic environment, particularly in families with small single flowers and dry pollen (Culley *et al.*, 2002).

Deliberate manipulation of plants to create desired genotype and phenotypes for a specific purpose is referred to as cross mating. It is an application of genetic principles to produce a plant with desirable characteristics for specific objectives to humans (Allard, 2019). This manipulation involves controlled pollination, genetic engineering and artificial progeny selection. The mating system in plant populations is influenced by genetic and environmental factors. These factors mostly control the mating system in the plant population (Clegg,1980). Knowing the rate of outcrossing is important in breeding and tree improvement programmes (Loveless and Hamrick, 1984). Bisexual flowering plants like *Moringa oleifera* modify floral parts that determine the mating system in their population (Muluvi *et al.,* 2004).

The mating system may also be sensitive to plant density and size of population (Goodell *et al*., 1997), types of pollination vector and abundance, flower colour, size of floral display and anther-stigma separation. Variation in the timing of flowering can lead to seasonal changes in the mating patterns and composition of the outcross pollen pool (Mitchell and Marshall, 1998). Autogamy is a mating system that leads to the production of true-to-type offspring; this is disadvantageous when autogamous offspring harbour recessive traits, but it may be advantageous for reproduction under unfavourable environmental conditions. Conversely, allogamy can introduce traits that increase resistance to diseases and predation, as well as seed and fruit yield (Sliwinka and Bewley, 2014).

In *Moringa oleifera* a, pollination happens between two flowers of the same plant and results in offspring that are genetically identical (geitonogamous**).** Similarly, flowers from different plants resulting in genetically different offspring (xenogamous). The mode of pollination, propagation methods (sexual and vegetative), easy fruit crack and explosive method of seed dispersal have collectively and efficiently supported gene flow. These attributes help to increase diversity within the population and reduce heritable attributes among populations (Popoola *et al*., 2017).

2.4 Morphology of forest tree species

Morphology is the study of the shape, form, structure and arrangement of parts of an organism to determine their function, development and how they have been

shaped by evolution (Mariam, 2006). Morphological variation enhances the adaptation of species to their environment and helps to improve genetic potentials (Safia, *et al*., 2017). For identifying species in the forest and classifying plants into ecological succession classes, prior knowledge of the morphology of fruits, seeds, and seedlings in their early stages of development is a valuable tool (Feguson *et al*., 1991).

The fruit and seed morphology of plants has provided useful information on the characteristics that have solved taxonomical challenges (Gontcharova *et al*., 2009). Therefore, the difficulties encountered in providing solutions to minute taxonomical variations have been successfully resolved through morphological evaluations (Taia, 2004). The use of scanning electron microscopy (SEM) has made the examination of seed morphology less complicated (Ozkan *et al*., 2015).

Plant morphology provides information for the estimate of the size, maturity as well as density (Brasil, 2009). Seed morphology is strongly influenced by the genetic diversity of the species and it relates to the maturation process with the seed development and the number and size of the cells in the embryo, endosperm and tegument (Ohto *et al*., 2009). According to the recommendations from the Rules for Seed Testing Brasil, (2009) seed morphology of tree species is dependent on temperature and moisture content which may vary according to the conditions of the collection site, the age, and the maturity of seeds (Marcos-Filho, 2015).

2.4.1 Morphology of fruit and seeds of *Moringa oleifera*.

In a natural population of *M. oleifera*, the morphology of fruit and seed help to identify the variation occurring from the inherited trait. The interrelationship of traits, however, is typically expressed by phenotypic, genotypic and environmental associations. Fruit morphology is one of the major qualitative and quantitative characters used to define and identify structures of *Moringa oleifera* in a population; the most important analytical features are the fruit/ pod shape and the number of seed (s) per fruit /pod (Daniel *et al*., 2015).

The shape of the *M. oleifera* fruit /pods is straight and pointed at both ends. Pod length could vary across the collection area but mostly commensurate with the numbers of seed. *Moringa oleifera* produces two types of seed shapes; ovate and isodiametric which are identified with wings that are conspicuous or unnoticeable and has tan or cream colour (Daniel *et al*., 2015). Its most important features are shape,

size, seed coat surface, placement of the hilum, and presence or absence of structures such as aril, caruncle or elaiosome.

2.5 Concept of provenance and landrace and in forest trees

Provenance is the original native source of a population, where trees or any stands exist, either indigenous or non-indigenous, (Ahmad, 2013). It is the region or geographical source, where the plant was found originally while the genetic makeup has developed as a result of natural selection over some time (Dunster and Dunster, 1996). When a population is removed from its source and has grown or it has been introduced and cultivated elsewhere for many generations, it is referred to as a landrace (Danida, 1997). *Moringa oleifera* is an exotic species originally native to India, but was introduced to Nigeria and has developed landraces all over Nigeria.

Generally, variation can be categorised into three types; permanent component (inherited and genetic), component stimulated by the environment (non-heritable) and a developmental component. The existing variation among the population of forest trees in Nigeria has been documented: Oni and Gbadamosi (1998), observed significant differences among provenances as well as the growth parameters of both *Dacryodes edulis* and *Terminalia ivorensis* seedlings in Southwestern Nigeria. Muluvi *et al*., 1999) also assessed the level of variation among the *Moringa oleifera* population both in India and Kenya and reported that *M. oleifera* was an outcrossing tree species, which exhibited high variation among populations. Comparably, the morphological analysis of *Moringa oleifera* trees from Southern Benin populations revealed significant differences in the leaves, leaflets and fruits of samples evaluated (Agoyi *et al*., 2015).

2.5.1 Effect of seed source on germination and early growth of tree species

The seeds of widely distributed species exhibit high levels of variation among populations because of the difference in geographical locations. Therefore, the selection of the right seed source is a major factor affecting the germination, growth, development and productivity of tree species (Ahmad *et al*., 2013).

Evidence on the significant effect of seed source variation on the physiological and phenological characteristics of tree species exist in several studies. For instance, *Pinus sylvestrics* (Ratio and Sargala, 2000) collected from different sources revealed a wide variation in its nutrient quantity and; there were significant variations among seed

sources of *Tamarindus indica* seedling emergence and early growth (Ugese and Dennis 2006). Also, seed morphology affected seedling growth and development of *Vitellaria paradoxa* collected from different sources (Aderounmu and Adegeye, 2011).

Skivanna *et al.* (2002) reported significant differences that occurred in the germination of seed and seedling of *Acacia nilotica* from different provenance. Baiyeri *et al*. (2015) collected three accessions of *Moringa oleifera* from northern Nigeria and revealed that significant differences occurred in their growth performance and nutrient quality. Particularly, one of the sources had the highest cumulative percentage (97%) of seedlings.

2.6 Decapitation effect on coppicing potential and bioactive components in plants

Coppicing is the renewal or rebuilding process of replacing a severely or deliberately damaged plant that still has its stem and root in contact with the soil (Forrester *et al*., 2003). It is a constant old custom way of managing the woody plants in such a way that when these plants are cut down, another new plant grows from the stump or roots of the damaged one (Coles, 1978). This practice can help to improve the amount of browse during the dry season or under extreme weather conditions as browse trees regenerate after lopping. However, Primefacts (2009) opined that not more than 60% of tree/shrub foliage should be removed. Trees with coppicing potential regenerate well, but, when cut close to the ground (<5cm), they produce less coppice growth than those cut at heights of 1.3 m (Jimu, 2010**).** Self restrain carbons of trees are heavily exhausted when decapitated and the growth ability of such tree is highly reduced. On the other hand, the ability of such a tree to restock the stored carbohydrate reserve depends mainly on the soil moisture and nutrient content as well as its ability to photosynthesize competently and develop adequately (Cruz and Moreno, 2001).

For example, shoot growth and coppice potential of *Plukenetia conophora* seedlings were influenced significantly by the decapitation heights (Amadi, 2014). In that study, decapitated height at 20 cm gave the highest value for seedlings shoot development, collar diameter, number of coppices and number of leaves. Also, in *Buchholzia coriacea* juvenile seedlings, the number of coppices and the number of leaves varied and were significantly affected by the height of decapitation. It was reported that the height and number of leaves were found in eight-month-old seedings while 6-month-old seedlings were recorded for the highest coppice number (Akinyele,

2007). The point of cutting should not be more than two-thirds of the crown length for coppice stands to achieve better yield (Kumar and Tewari 2000). Trees cut at lower heights, may experience stress in optimum shoot development and makes them less competitive for nutrients. This is because auxins which are naturally produced at the tip of shoot and plant root to promote cell division in stem and root growth may have been disrupted. *Moringa oleifera* has exhibited high coppicing potentials and stimulations of new leafy growth when decapitated. The spacing of 0.75m by 1m in *Moringa oleifera* trees to positively respond to increased leaf production after decapitation was recommended by Radovich (2009).

2.6.1 Proximates and bioactive composition in leaves of forest plants

The proximate and bioactive composition in leaves of forest plants is the main nutrient and health-benefiting elements available in leaves. They are measured through the evaluation of proximate and phytochemical analysis (Gokmen, 2016). A set of methods used to obtain the mass percentage of information about the nutritional value of the samples through the estimation of chemical composition is known as proximate analysis.

Bioactive compounds are additional nutrients available in foods in little quantity especially in fruits, vegetables, and whole grains (Bamishaiye *et al*.,2011). They are derived through phytochemical analysis and provide health benefits outside the basic nutritional values they offer (Gokmen, 2016). They include beneficial and detrimental compounds and their identification at a particular time in plant parts depends on the type of extracting solvent used (Tijjani *et al*., 2009). They contain antioxidant, anticarcinogenic, anti-inflammatory, and antimicrobial properties. *Moringa oleifera* is noted to be highly nourishing and rich in proximates and phytochemicals (Tetteh, 2008; Bamishaiye *et al*., 2011). Romero *et al*. (2019) among others affirmed that high consumption of food rich in bioactive compound prevents the risk of numerous diseases.

Generally, proximate analysis involves the process of determining crude protein, ash content, ether, crude fibre, total carbohydrates and moisture content while phytochemicals which are classified various groups based on the chemical makeup and attributes detects various compounds such as; Saponin, tannin, phenolics, terpenoids, cardiac glycosides, carotenoids, flavonoids, steroids, alkaloids, and other nitrogen-containing compounds (Campos-Vega and Oomah, 2013).

Proximates and bioactive compounds vary widely in chemical structure and functions. Protein is large complex organic compounds; the basic component of living cells that boost the immune system, act as tissue binder and helps in growth (Okeke and Elekta, 2006). Fibre is undigested or unabsorbed materials gotten from food that functions to slow down the rate of glucose absorption into the bloodstreams and preventing diseases (Buttwell,1998). Carbohydrates are the major and cheapest source of energy in foods and feeds (Okeke *et al*., 2008).

Moisture content or water is a universal solvent that aid digestion, dissolves other substances, carries nutrients and other materials throughout the body, making it possible for every organ to perform its function effectively (McDonald et al., 1998). Ash content destroys useful organic materials in the plant. Its presence in a plant inhibits the quantity and determines the amount of minerals present (Archa, 2010). Ether is a pleasant smelling great solvent for fats, waxes, oils, perfumes, alkaloids, and gums. Some of its vapour is used as insecticides, miticides and fumigants for soil (Wade, 2019).

The Phytochemical screening of *Schoenoplectus lacustris* and *Oroxylum indicum* discovered the presence of saponin, tannin, phenolics, terpenoids, cardiac glycosides, carotenoids, flavonoids, steroids and alkaloids (Samatha *et al.,* 2012 and Amir *et al*., 2020). Schneider and Wolfling (2004) reported that saponin inhibits the growth of cancer cells, boost the immune system and lower cholesterol. Tannins that function to give protection against microbiological degradation of dietary proteins in the semen and hasten the healing of wounds was detected in *Oroxylum indicum* at low concentration. The phenolic compound is known to play an active role in the quenching of free radicals and prevent the body from oxidative stress. Terpenoids are involved in the weakening of the cell wall and tissue of the microorganisms. It is anti-viral, anti-fungal and anti-inflammatory. Glycosides contain anti-HIV, anti-leukaemia, antidiabetic, antibacterial, analgesic, antipyretic, aphrodisiac, laxative, anti-cardiac and anti-stress characters which help to prevent heart failure and irregular heartbeat. Flavonoids play a role in antioxidant potential and help in quenching free radicals. Maturation of sperm cells and inflammatory potency was found with steroids while carotenoids boost the immune system and vitamins essential for growth and eye health. Alkaloids have been reported by Edeoga and Enata (2001) to have stimulating effects and powerful pain relief.

2.7 Genetic make-up, gene flow and diversity in tree species

Genetic diversity is the appearance of different kinds of a gene in a population and the rate at which they occur (BFA, 2017). Every species have different individuals that have their unique genetic configuration. Genes are made up of a molecule (DNA) that is composed of genetic directives needed by all living organisms to grow, develop, functions and reproduce. Several aggregates of nucleotides form this molecule called Deoxyribonucleic acid (DNA). These directives remain on the inside of every cell and are transferred from every parent to their offspring (Adam, 2018). A group of phosphate, sugar and a nitrogen base is in every single nucleotide content. There are four types of nitrogen bases; adenine (A), thymine (T), guanine (G) and cytosine (C). Several combinations of these nitrogen bases and their order of arrangement is what decides DNA's instruction, or genetic code. The arrangement is what instruct cells on how or what type of protein to produce (Racheal, 2017).

In a population, the genetic makeup of a tree is determined by some genetic processes such as the mating system, gene flow, selection, and migration (Degen *et al*., 2014). In time past, genetic variation of forest plants have been investigated through various quantitative methods: morphological and physiological techniques such as; germination and growth studies, species adaptation to environmental factors, pod and seed characterization among others (Hamrick *et al*.,1990). As a result of the lack of understanding of these assessments, the findings obtained are insufficient for genetic studies. However, in recent time, the modern method of assessing genetic variation has become extensive and have provided a lot of advantages over the previous methods, thereby making it easier to identify the varying amount of genetic variation within and among the population (Lemes *et al*., 2003)

2.7.1 Genetic flow in forest tree species

The movement of genetic material from one population to another is known as gene flow or gene migration. This can happen between two populations of the same species through reproduction and vertical gene transfer from parent to offspring, or between two species through horizontal gene transfer (Supratim, 2014). Gene flow can increase (within a population) or decrease (between genetically distant populations) the genetic variation of a population, it can also be hindered by incompatible reproductive activities among individuals in populations. In the process of accumulating change in a population, gene flow is a critical determinant of genetic structure.

Among plant population, there is substantial variation in the frequency of gene flow and this can be estimated through species, population, seasons, and even individual plants. However, physical proximity or barriers between populations can either facilitate or suppress gene flow. This is when plants are separated at a range of distance between hundreds to thousands of meters, the level and the rate at which gene flow are sufficient to alter genetic inferences as well as the levels and direction of selection (Norman,1992).

Gene flow is a fundamental evolutionary agent that occurs when genes are distributed between populations of a species. In this process, individual plants and their reproductive organelles are moved actively or passively. Besides, gene flow does not only involves distribution but also active in the establishment of immigrant genotypes in the new population (David, 2001). Understanding the historical pattern and gene flow frequency is important in preserving a population. This is because gene flow is often limited to certain phases of the life cycle and may be accelerated under certain climatic conditions that occur at frequencies or irregular intervals of many years.

2.7.2 Genetic diversity

The percentage of significant differences in genetic variation among plant species can be traced to their life history in relations to their environment. Features such as nutrition, gestation, reproduction, e.t.c are imperative and control major genetic variation in plants. Plants that live for a long time and have a high reproductive ability usually have large and stable populations which are resistant to accidental fluctuations in gene or genotype frequencies, such plant species would maintain more genetic variation among the population.

Longevity also ensures resilient relationships among several cohorts within a community, enabling them to withstand several hardships and individuals that survive to a maturity level within this phase, will maintain a high record of genetic variation in a continuously established population. For example, Hamrick *et., al* (1979) observed varying amount of genetic variation in different plant species and indicated more genetic variation of about 60 per cent in trees than in herbaceous plants because woody plants are exposed to environmental fluctuations throughout the year and possess numerous combine features that are associated with high levels of genetic variation.

Previous research carried out in tree improvement programmes were mainly based on phenotypic selection and morphological characteristics. These attempts were

repeated several times and many cycles of breeding and backcrossing were done before new varieties were obtained. In contrastingly, scientific knowledge has helped solve the problems and provide a better way of understanding the process of hereditary which involves the structure, function and behaviour of an organism. Moreover, professional breeders now have chances of altering the existence of previous varieties.

Therefore, it has been established that the approach in today's plant genetics, consist of important tools such as molecular markers and genetic maps which enhances choosing and combining very useful naturally occurring genes in different trees (Rafii *et al*., 2012). Given this, plant genetics has become more easily achievable with the use of important tools derivable from the relationship between molecular markers and genetic maps. This approach easily combines favourable genes that exist naturally within a tree species.

2.8 Molecular marker and its application in tree improvement programmes

A molecular marker is known as a genetic marker; a DNA or gene sequence, a piece or fragment of DNA that is found on a chromosome within a sample taken at a particular position from the entire hereditary information fixed in the DNA (genome) of an organism (Gaurab, 2020). It helps to reveal some specific information about such an organism or identify the DNA sequence in an unknown population (Barcaccia *et al*., 2000). The molecular marker helps to mark out differences between individuals through the nucleic sequence, discover genes that are involved in genetic disorders and identify any alteration in a DNA sequence.

A molecular marker must be polymorphic and equally spread all over the genome. It should not require prior information about the genome of an organism. It should be able to utilise small portions of DNA samples and tissues to link noticeably different phenotypes. However, it may or may not agree or reflect the outward appearance or the phenotypic expression of a genomic trait but generally, it must be simple, fast and cheap, separations of constituent parts to display the genetic differences must be visible and must create more other reliable and selfstanding markers (Mondini *et al*., 2009).

Several molecular markers have been developed for ecological, evolutionary, taxonomical, phylogenetic and genetic studies in plant science. Some of these identified as PCR and hybridization-based and available for genetic studies are; Random Amplified Amplified Fragment Length Polymorphism (AFLP), Inter Simple

Sequence Repeat (ISSR) markers, Microsatellites or simple sequence length polymorphisms (SSLPs)- short tandem repeats (STRs), or simple sequence repeats (SSRs), Cleaved Amplified Polymorphic Sequence (CAPS), Expressed Sequence Tags (ESTs), Sequence Characterized Amplified Region (SCAR) and Single nucleotide polymorphism (SNP). These genetic markers, however, have their various advantages and disadvantages and level of preferences in diversity studies (Gaurab, 2020)

2.8.1 Application of molecular techniques in tree improvement programmes

Tree improvement is an advancement in the genetic quality of a forest which involves the application of guidelines in forest genetics. This aims at caring, developing, obtaining products or providing benefits from the forest while maintaining its diversity. Moreover, it aims at making provision for improved varieties of suitable germplasm for afforestation programmes. The use of molecular markers in breeding programmes started in the 1980s. It accelerated the plant breeding process by generating high-density linkage maps. These maps contain traits and markers, used in breeding programmes (Mahajan and Guptag, 2012). The use of molecular marker involves the ability to understand the genetic basis of the quantitatively inherited trait (Mahajan and Guptag, 2012). This facilitates the selection of favourable combinations of genes that occur naturally within a tree species (Wu *et al*., 2000).

The use of molecular technique involves extrapolating information about what an individual gene is and how it is likely to behave in an environment. This technique allows adjusting or altering some traits in living organisms (Doty *et al*., 2007). Analyses of molecule comprise a variety of large markers that can be adopted for analysis of differences. The two molecular genetic markers (those obtained from analysing polymorphism directly in DNA sequences) and biochemical markers (those obtained from chemical product study of gene expression). However, it has been established that DNA markers are preferable in the estimation of differences in heritable traits within and among species because, over time, they have been proven to be a better tool (Song *et al*., 2003). Also, it directly measures genetic variation and provides an adequate resolution of genetic difference that is found in a population while preventing interruptions from the environmental factors (Karp *et al*., 1996).

Molecular markers are greatly important for forest tree improvement (Manoj *et al*., 2014). They offer hope of circumventing some restrictions, complex and varied challenges faced by tree breeders (Sniezko and Koch, 2017). DNA markers are

potentially unlimited and are rapidly developed to monitor forest improvement activities, such as estimating genetic diversity in breeding populations, identifying genetic material, verifying controlled crosses and estimating the effectiveness of seed orchards. Among populations of trees, genetic diversity is been estimated using molecular markers. For instance, the genetic pattern of *Khaya grandifoliola* populations was revealed by (Okere, 2014). Simple Sequence Repeat markers were used for determining the genetic diversity and population structure of *Moringa oleifera* accessions in India (Santhosh *et al*., 2014). Muluvi *et al*. (1999*)* reported diversity evaluation among and within populations of *M. oleifera* in Kenya using AFLPs. In Nigeria, RAPD and SSR markers have been used to evaluate the genetic diversity of *Moringa oleifera* (Amao *et al*., 2017; Popoola *et al*., 2017).

SSR marker was recommended as a useful marker by Wu *et al*. (2010), who first developed the ones specific to *M. oleifera* and noted it for its detailed genetic population studies as well as the movement of pollen-mediated genes in a population. Moreover, results of SSR's analysis distinctively pronounce it as the most suitable due to its ability to discover reproducible and highly polymorphic sequence using little amounts of DNA samples (Ellwood *et al*., 2006; Wu *et al*. 2010). The evaluation of genetic characterization of *Moringa oleifera* populations could promote *in situ* and *ex situ* conservation efficiently using SSR marker. Information on population differentiation based on genetic relationship, possible breeding and genetic improvement plans as well as management and conservation of the species will be provided.

2.9 Extraction and quality assessments of DNA

Deoxyribonucleic acid (DNA) extraction refers to the process of separating DNA from proteins, membranes and other cellular materials that are in a cell. DNA extraction is an important component of modern molecular biology. Careful handling of biological properties is required to avoid contamination of the sample. Generally, DNA extraction is done using three basic steps: lyse (break open) the cells; separate the DNA from the other components of the cell, and isolation of the DNA. The ability to extract and purify DNA most time is the crucial point of starting for different experimental procedures, such as polymerase chain reaction (PCR) (Cseke and Joseph, 2012). The reaction of the polymerase chain is the most common analysis that is observed after DNA extraction. The method creates multiple copies of a particular or

target DNA region and aims to undergo further analysis to make it available for sequencing, visualization using gel electrophoresis, and plasmid cloning for further experiments (Khan, 2018). DNA primers designed are required specifically for the targeted DNA region of interest and are based on DNA polymerase (enzymes) that is not physically or chemically resistant to high temperatures (thermostable). DNA polymerase is quite thermally stable and the most active at a temperature of about 70 ° C. *Taq* polymerase, primers, template DNA and nucleotides (DNA building blocks) are important components of the PCR reaction. The basic PCR step is denaturation (96 ° C), during which the reaction is strongly heated to separate or denature the DNA strand and provide a single-stranded template for the next step. Annealing is the next basic step (55-65°C); at this stage, the reaction is allowed to cool so that the primers can bind to their complementary sequences on the single-stranded template DNA while the third basic step is the extension (72°C); the temperature of the reaction is increased which will result to the *Taq* polymerase extending the primers with new strands of DNA produced.

This cycle will be repeated 25 to 35 times in typical PCR primers and it usually takes 2 to 4 hours, based on the length of the copy DNA region. If the move is successful (works well), the area at the end of the trip can go from one or more copies to billions. Indeed, not the original DNA was used as a template at the same time. Instead, the newly synthesized DNA can serve as a model for the next stage of DNA replication. Many copies of the primer and several Taq polymerase moves around in the process so that number of DNA molecules can be almost doubled in each cycle. Polymerase Chain Reaction requires a post-analysis that detects amplicons (PCR products) size. This is achieved through electrophoresis: an insensitive method for the determination of sample integrity which is done by using gels, agarose (for longer DNA) and polyacrylamide (for shorter DNA) (Burak and Fazilet, 2018).

2.9.1 Statistical tools used for Evaluation of genetic diversity

The diversity indicator is a mathematical measure of the diversity of species in a community. It provides more information on the composition of communities and takes into account the relative abundance of various species and provides critical information on the rarity and universality of species in the community. The Shannon Diversity Index (H) is a type of diversity indicator commonly used to characterize the diversity of species in a community. It takes into account the abundance and equality

of the species present. The proportion of species (*i*) to the total number of species (*pi*) is calculated and multiplied by the natural logarithm of this proportion (*lnpi*). The resulting product is added between the species and multiplied by -1 (Beals *et al*., 2000).

The number of observed or actual alleles is also important in determining the specific number of alleles in any given population. It is the number of common alleles needed to achieve a certain level of genetic diversity (heterozygosity) and permits comparison within populations where there was a significant difference in the number and distributions of alleles (Weir, 1990). Analysis of Molecular Variance (AMOVA) is a tool used to calculate the level of genetic differentiation among different populations. It uses molecular markers to reveal the degree of differentiation between populations, between samples, within populations and/or within samples. The method directly estimates population differentiation from molecular data while the hypotheses are tested about such differentiation. The value of polymorphic information content (PIC) indicates the usefulness of a genetic marker for binding analysis (Shete *et al*., 2000; Elston, 2005). Generally, this is a measure of how instructive the marker is, irrespective of the inheritance mode of the combined feature. The marker genotype of a given offspring is likely to suggest which of the two marker alleles of the affected parent received. The high PIC mean-value (79.3%) in the genetic structure of scot pine population by Justyna (2016) is an indication that molecular markers were highly informative for the genotypes of different genetic backgrounds.

Principal Coordinate Analysis is a multidimensional metric and classical scaling technique used to study and visualize data similarities or discrepancies. It begins with matrix similarity or dissimilarity (= distance matrix) and assigns each element a location in a dimensional space that is low. It helps to visualize individual and/or group differences by calculating the distance matrix and showing how dissimilar the families are with a graphical representation for better interpretation. Then the Jaccard index is used to measure the association among the families (Zuur *et al*., 2007). Cluster analysis is an exploratory analysis that tries to identify structures within data and uses it in conjunction with other analyses (James, 2018). It is a statistical tool that group objects into categories so that objects which belong to a particular group are similar to one another but different from objects that belongs to a different group.

A diagram that shows the hierarchical relationships between objects, the correlation of data and how clusters are merged in a visual representation is called a dendrogram. It helps to work out the best way to allocate objects to clusters by focusing on the height at which two objects are joined together and summarises distance matrices. It is the most important result of cluster analysis that lists all samples and has a line on the scale which indicates the distance and level of similarity of any two clusters joined. However, the shape of dendrogram does not give a clue as to many clusters that exist but suggests a correct number of clusters when there is no real evidence to support the conclusion (Bock, 2017).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site selection

The distribution of *Moringa oleifera* in Southwestern Nigeria was investigated through a Reconnaissance survey. The sampling sites were selected based on information received from the Moringa Development Association of Nigeria (MDAN), State Ministries of Agriculture and Agricultural Development Programme (ADP) in four states of Southwestern Nigeria. In the rain forest region of southwestern Nigeria, clusters of Moringa farms were chosen, with 60 per cent of the farms being managed for seed production. A multistage random sampling technique was used to select eight locations from four states (Ekiti, Ogun, Ondo and Oyo) (Fig. 3.1). Two Moringa farms that have been managed for seed production from each sampling location and not less than 0.4 hectares in size were selected. Mature pods of *M. oleifera* were collected from the eight locations (Table 3.1).

3.2 Description of the Study Area

Southwestern Nigeria is one of the geopolitical zones of Nigeria comprising six states; Ekiti, Lagos, Ogun, Ondo. Osun and Oyo. The weather conditions ranged between two distinct seasons; rainy (March-November) and dry season (November-February). The dry season ranged between 2-5 months; a period of harmattan dust and cold dry winds. The Rain Forest of the whole of south-western Nigeria is situated near its climatic limits. The annual rainfall varies between 2600 mm. to 1600 mm .while the dry season has less than 50 mm. Where the annual rainfall falls below 1600 mm, the rain forest supports mixed deciduous forest,

3.3 Selection of mother trees and seed collection

At every farm, five trees were randomly selected from four cardinal points and the centre of a 20m by 20m plot, purposively laid at the centre of each plantation. This was done by measuring 5 m at each cardinal point from the edge of the farm (North, East, West and South.) as the buffer (Fig. 3.2). At these points, one tree each was selected within an established 5 m circumference and the fifth one at the centre of the farm. Harvest of mature pods from these selected mother trees was done separately and labelled. The genetic information for each farm was fully documented.

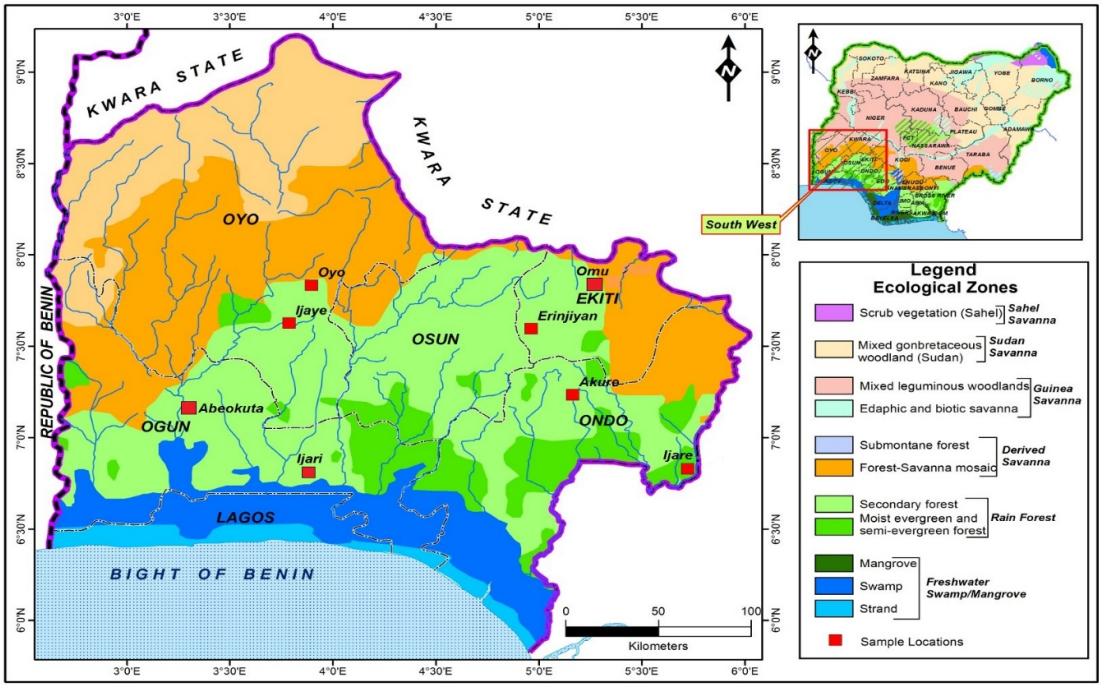


Figure 3.1: **Selected *Moringa oleifera* farms in Southwestern, Nigeria (inset map of Nigeria)**

Source: **(Field survey (2015)**

Table 3.1: Selected *Moringa oleifera* plantations in states of Southwestern Nigeria

Locations	Farm Sites	Latitude	Longitude	Local Government Area	State	Land Area (ha)
Oyo	Owode odo-Eran	8°29.817′N	3°24.254′E	Oyo West	Oyo	0.69
	Oko-oba Akinmorin	8°36.817′N	3°36.254′E	Oyo East	Oyo	6.00
Ijaye	Oloode Village	7°49.714′N	3°42.254′E	Akinyele	Oyo	0.54
	Igboole village	4°36.817′N	3°36.254′E	Akinyele	Oyo	0.80
Akure	FUTA area	7°15.817′N	5°42.254′E	Akure South	Ondo	1.40
	Orita Obele	7°36.817′N	5°36.254′E	Akure South	Ondo	5.00
Ijare	Ijare	7°22.817′N	5°10.254′E	Ifedore	Ondo	0.60
	Ijare	7°36.817′N	5°36.254′E	Ose	Ondo	1.00
Erinjiyan	Iwaro	4°36.817′N	7°36.254′E	Ekiti West	Ekiti	6.00
	Ojuurin	4°59.799′N	7°36.243′E	Ekiti West	Ekiti	3.20
Omu	Oye	7°53.817′N	5°36.254′E	Oye	Ekiti	2.58
	Irepodun	7°36.817′N	5°24.254′E	Irepodun	Ekiti	0.40
Abeokuta	Osiele	4°36.817′N	7°36.254′E	Odeda	Ogun	0.49
	Alabata	4°36.817′N	7°36.254′E	Odeda	Ogun	0.40
Ijari	Ogbogbo	3°94.850′N	6°84.681′E	Ijebu North East	Ogun	0.40
	Bisorod Enterprises	3°95.056′N	7°36.254′E	Ijebu North East	Ogun	0.40

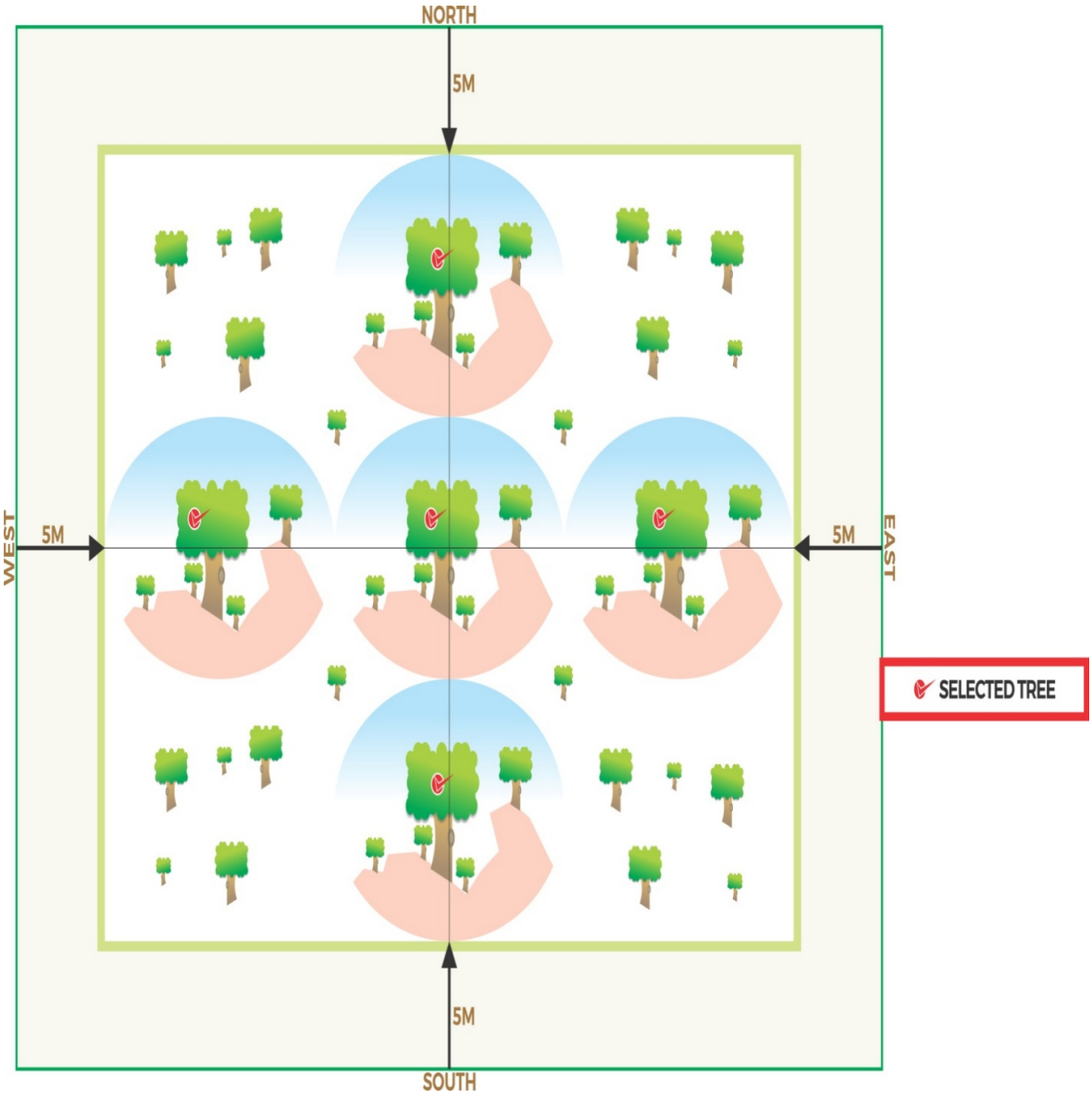


Figure 3.2: Tree selection from *Moringa oleifera* plantation in Southwestern Nigeria

3.4 Sites for experimental studies

3.4.1 Nursery and field experiments

Growth experiments and field trials were carried out at the central nursery and arboretum of the Department of Sustainable Forest Management, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. It is situated in the Southwestern part of Ibadan, at 7 ° 24 'north and 33 ° 55' east. The climate of this region is tropical and has an average annual rainfall of around 1,309 mm and a maximum average temperature of 33.95 ° C and a minimum average of 22.35 ° (NIMET).

3.4.1 Laboratory Experiments and Molecular studies

Leaf quality screening; proximate and phytochemical analysis were carried out in the Kappa Laboratory. The molecular analysis of the different landraces was performed in the Bioscience Laboratory of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. It is situated north of Ibadan, along Ibadan - Oyo express road, about 7 ° 28'N latitude and 3 ° 52'E longitude, at an altitude of 277 m above sea level. The climate is typically a heavy rain season with a dry season. The dry season usually lasts between November and March and is characterized by the dry, cold Harmattan wind. From April to October, the rainy season normally starts, often with heavy winds and storms. The annual precipitation is approximately 1300 mm, with an average annual temperature of at least 22 °C and a high of 34 °C. (NIMET).

3.5 Data collection

3.5.1 Population distribution and tree density of *Moringa oleifera* in the selected plantations in Southwestern Nigeria

Two *Moringa* farms were selected within each location and 20m x 20m sample plots (at 30% proportion to the size of each farm) were laid (Plate 3.1). All trees within the sample plots were enumerated and the values were recorded. The population density was calculated from the data obtained using the average number of trees per hectare (Equation 3.1).

$N = \frac{h}{a} \times c$ ---------- Equation (3.1),

where: h =Total land area a = area of all sampled plot

C = mean number of trees counted in selected sample plots.

N =average number of trees per hectare.

The results were computed and presented in a table.

A

B

Plate 3.1: Laying of plots and tagging of selected trees for population distribution, tree density and phenological characteristics on *Moringa oleifera* farms in Southwestern Nigeria.

A Plots laying

B Tree tagging

3.5.2 Assessment of floral and fruiting duration and fruit maturity index evaluation of *Moringa oleifera* in the selected plantations

On each farm, branches from the selected five mother trees were randomly selected and tagged. The timing and period of flowering, as well as fruit development stages, were observed for two seasons (dry and rainy) in 2015/2016 and 2016/2017. The phenological characteristics (Plate 3.2), including Bud initiation (BI), Bud formation (BF), flower initiation (FI), flower formation (FF), fruit initiation (FTI), fruit formation (FTF), fruit maturation (FRM), green pod (GP), yellow/light brown pod (YP) and brown pod (BP) were noted. Between each developing stage, the time interval was observed and recorded. Pod maturity evaluation assessment was carried out by extracting seeds from the three different colour maturity index (green, yellow/light brown and brown pod) and subjected them to a germination test using washed and sterilised river sand. This was done to ascertain the best time for harvesting pods of *M. oleifera*. The collected data were subjected to analysis of variance (ANOVA) using Statistical package for social sciences (SPSS) while significant means were subjected to a control test using the Duncan Multiple Range Test (DMRT).

3.5.3 Determination of seeds and pods morphology of *Moringa oleifera* in the selected plantations

At each farm site, pods were collected from each of the five selected mother trees; and kept separately. Thereafter, thirty pods were randomly selected from the harvested pods for each tree. Thus, having an aggregate of 150 pods per farm. The length of the pods (cm), the diameter of the pods (cm), the number of seeds per pod and the weight of seeds (g) (per 100 seeds) were measured (Plate 3.2). The dimensions of the pods were determined using a meter rule and vernier callipers, and the weight of the seeds was measured using the weighing balance. The experiment was a Completely Random Design (CRD). The data collected were subjected to Analysis of Variance (ANOVA) using SPSS and a control test was performed using the Duncan Multi-Range Test (DMRT) to separate the means that were found to be significantly different.

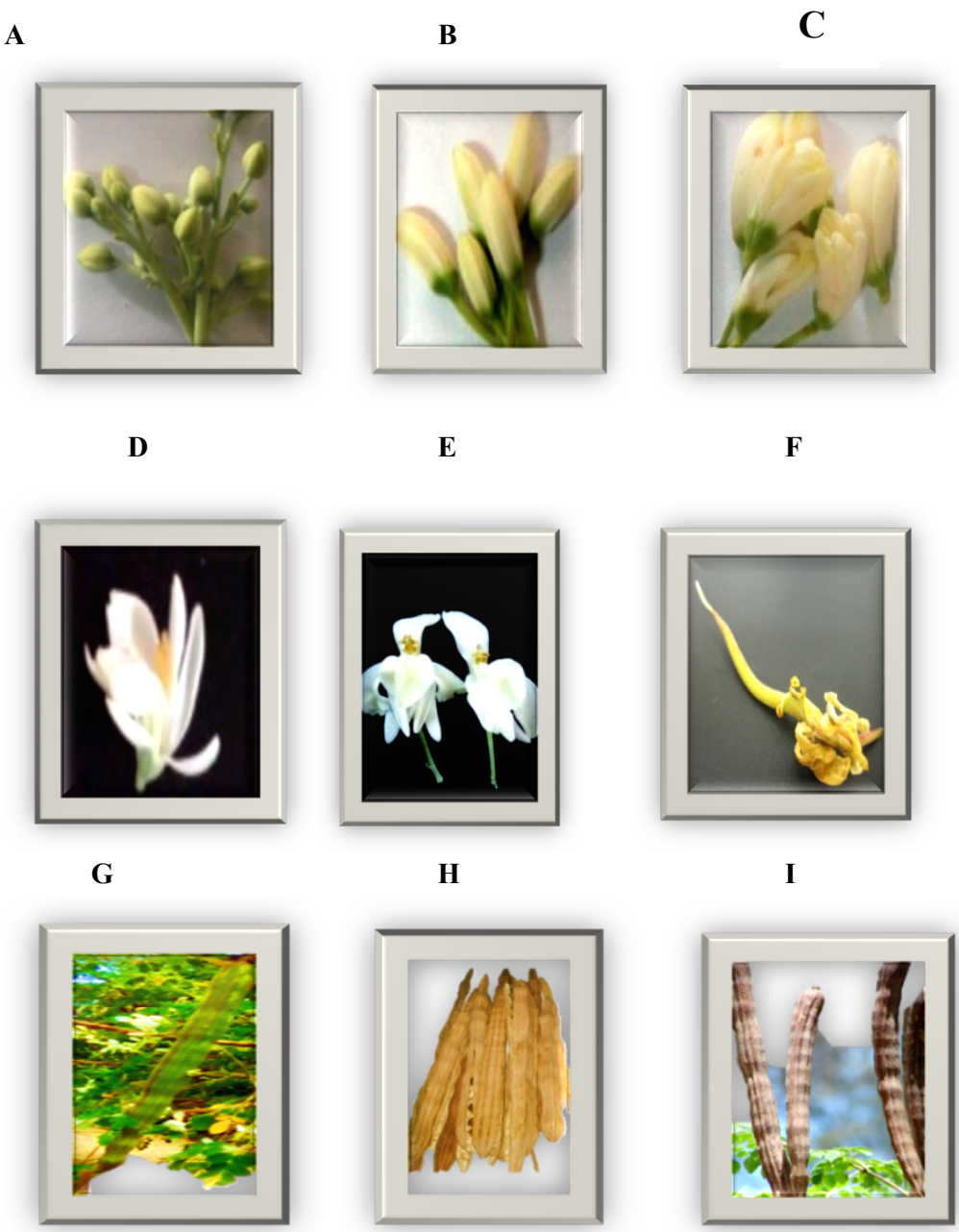


Plate 3.2: Stages of flower and fruit development in M*oringa oleifera* from the selected farms in Southwestern Nigeria

A: Bud initiation (BI) **B:** Bud formation (BF) **C:** Flower initiation (FI)

D: Flower formation (FF) **E:** Fruit initiation (FI) **F:** Fruit formation (FTF)

G: Green pod (GP) **H:** Yellow pod (YP) **I:** Brown Pod (BP)

A 

B 

C 

D 

Plate 3.3: Characterization of pods from selected *Moringa oleifera* plantation

A: pod length

B: pod diametre

C: number of seed per pod

D: seed weight

$Y_{ij} = \mu + T_i + e_{ij}$ ---------- Equation (3.2).

Where:

Y_{ij} = individual observation

μ = Mean

T_i = Treatment effect

e_i = experimental error

3.5.4 Influence of seed source on germination and early seedling performance

For each farm within each location, one hundred seeds extracted from pods within each farm were randomly selected. These were prepared and sown in an appropriately labelled germination trays containing sterilized river sand. Germination was observed daily and recorded until no germination was noticed. Germination was taken to have occurred when the plumule emerged from the river sand. Germination was taken to have ceased when there was no plumule emergence for two weeks. Thereafter, at two leaf-stage, sixty uniformly growing seedlings were transplanted into polythene bags (24cm x18cm) filled with topsoil.

In total, 960 seedlings were prepared for the study. The growth experiment was laid out in a Completely Random Design (CRD) under a winning shed in the nursery and watered once a day. Thereafter, the uniformly growing seedlings were allowed to stabilize for two weeks before the evaluation of growth variables commenced. For six months at an interval of two weeks, the total height, collar diameter and the number of leaves were assessed. Height measurements were made from the root collar to the apical apex of the bud using a meter rule. The collar diameter was measured with a digital veneer calliper and the number of leaves on each seedling was counted and recorded.

The collected data were subjected to an Analysis of Variance (ANOVA) using SPSS and significant means were subjected to a control test using the Duncan Multiple Range Test (DMRT).

3.5.5 Effect of seed source, age and lopping height on proximate, phytochemical and biomass production

A plot size of 20m by 18m was established and replicated three times at the field site. Twenty (20) uniformly growing seedlings each were selected at three different ages (4, 6 and 8 months old) from the selected eight sources (making a total

of sixty (60) seedlings each per source). A total number of 480 seedlings were transferred to each plot at a spacing of 1m x 0.75m and left for two weeks to stabilise to field condition. Five seedlings each from the different ages were decapitated at heights of 20cm, 40cm and 60cm from plant base at soil level with a pair of secateurs and the control plants were not decapitated. The decapitated seedlings were left to grow and laid out in a Completely Randomised Design (CRD) in the arboretum. Biomass estimation began four weeks after and the experiment was carried out for 24 weeks. The Physico-chemical properties of the soil used for the field experiment were analysed. Samples were taken with an auger from six points at a depth of 0-20cm, these were dried and subjected to chemical analysis.

At four weeks interval, leaves were collected per treatment and subjected to biomass estimation with the fresh weight of the harvested leaves determined before oven-drying at 103^{o}C to constant weight. Proximate and phytochemical analysis (leaf quality assessment) was carried out in the laboratory using standard procedure. The data collected were subjected to Analysis of Variance (ANOVA) using SPSS and the means found to be significantly different were subjected to follow-up test, using Duncan Multiple Range Test (DMRT)

$Y_{ij} = \mu + T_i + e_{ij}$ ---------- Equation (3.5).

Where:

Y_{ij} = individual observation

μ = Mean

T_i = Treatment effect

e_i = experimental error

3.5.6 Determination of genetic variability of *Moringa oleifera* using Simple Sequence Repeat (SSR) molecular markers in Southwestern Nigeria

Fresh juvenile leaves from seedlings of *Moringa oleifera* were randomly collected from 80 accessions across 8 populations, kept separately in labelled laboratory 'tea' bags, lyophilized for three days and stored at -20°C.

The protocol of Cetyltrimethyl Ammonium Bromide (CTAB) plant extraction buffer facilitated the capturing of the DNA. Genogrinder-2000 was used to grind 100mg of each of dried sample tissue in a test tube (from the 80 accessions) separately into a fine powder. Pre-heated 450µl plant extraction buffer was added to each test tube, mixed occasionally by inverting the tubes to homogenize the sample and were

incubated at 65°C for 20 minutes. Two minutes after cooling down, 200µl of ice-cold 5M potassium acetate was then added and incubated on ice for 20 minutes to precipitate the protein.

Tubes containing this mixture were centrifuged for 10 minutes at 3500rpm and the supernatant transferred into freshly labelled tubes. 450µl chloroform Isoamyl alcohol at ratio 24:1 was added, mixed gently (for the further precipitation of protein and lipids) and was centrifuged again for 15 minutes at 3500rpm. Three volumes of cold Isopropanol was added, mixed gently and incubated at -80^{o}C for 15 minutes to precipitate the DNA. The supernatant was centrifuged at 3500rpm for 15minutes and decanted until the last drop. 400µl of 70% ethanol was used to wash the DNA pellet. The supernatant was centrifuged at 3500rpm for 15 minutes again and decanted completely. The pellet was air-dried until all had evaporated ethanol. The DNA was re-suspended by adding 60µl of ultra-pure water or low salt TE and 2µl of RNase and was incubated at 37^{o}C for 30-40 minutes. 0.8% agarose gel was used for checking the DNA quality while 0.8 gram of agarose was boiled in 100ml of 1X TBE to removed RNA (Ribonucleic acid), cooled to about 60^{o}C. Ethidium bromide (5 µL) was added, allowed to flow gently to mix and poured onto the gel shelf before polymerization.

Afterwards, air bubbles were ensured not to remain inside the gel and a mixture of 3 ml DNA and 3 ml dye was briefly applied to settle to the bottom of the plate. 6 ml of this product was placed on a 0.8% agarose gel and allowed to run at 80 volts for 60 minutes. To verify the quality of the extracted DNA and whether the RNA was completely removed before switching to Nanodrop, the gel photo was captured, saved and visualized under UV light. A Nanodrop spectrophotometer with the DNA-50 option was used to quantify the DNA concentration**.** After the quality purity of approximately 1.8 sample absorbance at A260 / 280 was verified, DNA was selected to proceed with polymer chain reaction (PCR). Twelve (12) microsatellite polymorphic SSR markers unique to *M. oleifera*, based on Popoola *et al*. (2017) were adopted because of their effectiveness, efficiency and large coverage ability (Table 3.2). After PCR optimization, five SSR polymorphic microsatellite markers that produced clear and bright fragments were selected. Six per cent Polyacrylamide Gel Electrophoresis (PAGE) was filled with the PCR product and the gel images were captured using 50 base pairs of Thermo Scientific's gene ruler (ladder).

Table 3.2: Twelve SSR primer sequences and repeat motif proposed for this study

Locus	Primer sequence forward (5' – 3') Reverse (5' – 3')	Repeat motif
MO1	**F** TTGTCTGCCTCCTTTTGTCA **R** AACTGTCACCCTCCTATCCA	$(AG)_T(AG)_6$
MO6	**F**GCATAGCCACCTTTACTCCT **R**GACTTTTGAACTCCACCACC	$(AG)_T(AG)_6$
MO8	**F** GTAGATGGTGCAGCTACTCA **R** TGGGGTTCTTGTTCTTTATT	$(CT)_{13}$
MO12	**F** ACCGAAGATGATAAGGTGGG **R** CAAAAGGAAGAACGCAAGAG	$(CT)_{11}$
MO13	**F** TTTCGGGTTTTCTTTCACGG **R** AGCTCACTTTCCATCTCCAT	$(CT)_{15}$
MO15	**F** CCCCTCTATTTCCATTTTCC **R** GCTCCATAAACCCTCTTGCT	$(TC)_{10}$ CCT $(TC)_6$
MO18	**F** TTTTCCTCCCTTATTGTGCC **R** CCGTTGCCCTTTGTGGTTCA	$(GA)_6$ A $(AG)_{16}$
MO46	**F** ACCAAGGGTTTCAACTGCTG **R** CATTTTGCGACGGTCTCACG	$(AG)_5(GA)_6$
MO48	**F** AGAAGAACCCAACAGAGGAT **R** CTTTTCACTAACCACCACCC	$(TC)_8$ C $(CT)_{15}$A $(AC)_7$
MO58	**F** TGGATTTCTTCTCCTGCTAT **R** CACAGTTCTTATTGTATTGG	$(CT)_6$ T $(TC)_9$
MO61	**F** TGTGGGTCCTGCCTTTTCTC **R** CTTCTGTCTTTCTTCCTGCT	$(TC)_{11}$
MO64	**F** TCGGCACCTTCTTCCTCTTT **R** AATCCCTTGACGGACACCAG	$(TC)_{14}$ G $(CT)_9$

The images captured showed distinct, well-resolved and unambiguous bands which were counted; but faded bands were discarded. Simple Sequence Repeat fragments were scored for their presence (1) /absence (0), size and percentage polymorphism. The total number of alleles, to determine the mean allelic pattern was evaluated at three frequencies; ≥5%, ≥5% but ≤25% and ≥5% but ≤50%. Effective alleles, unique alleles, Shannon information index, genetic similarity/identity and genetic distance/dissimilarity and Allelic Polymorphic Information Content (PIC) were all estimated. To determine the genetic variation in the accessions, Molecular Variance Analysis (AMOVA), Principal Coordinates Analysis (PCoA) and cluster analysis (CA) were used.

CHAPTER FOUR

RESULTS

4.1 Population distribution and tree density of *Moringa oleifera* from selected plantations in Southwestern Nigeria

The largest plantation of *Moringa oleifera* was found at Erinjiyan, followed by Oyo, Akure, Omu, Ijare, Ijaye and Abeokuta with the total land area of 9.20ha, 6.69 ha, 6.40ha, 2.98ha, 1.60ha and 1.34ha and 0.89ha per hectare, respectively (Table 4.1). However, it was observed that the tree density varied significantly across locations due to the lack of uniformity in the enspacement adopted by different farmers (Table 4.1). There were four types of spacing adopted by farmers across the selected locations; 2X2, 4X4, 4X5 and 8X5 meters. Oyo, Ijaye, Abeokuta and Ijari farmers adopted 2X2 enspacement. 4X5 meters enspacement was adopted in Ijare and Omu plantation while Akure and Erinjiyan adopted 4X4 and 8X5 meters enspacement respectively.

Considering the number of trees per plot as a common denominator across the locations (Table 4.1), 100 trees are expected to be in a 2X2 enspacement. Given this, Abeokuta and Ijari had the highest number of trees per plot (83) followed by Ijaye (76) and the lowest was found in Oyo with 75 trees per plot. Akure with the enspacement of 4X4 meters had 20 trees per plot. For 4X5 meters enspacement, Ijare had the highest number of trees per plot (13 trees) while the lowest (8trees) was found in Omu. Erinjiyan with the largest enspacement (8X5) had 8 trees per plot. Among the plantation with 2X2 enspacement, Oyo had the highest density/ha of 12,525 and also had the highest population distribution of *M. oleifera* in Southwestern Nigeria. The next to this was Ijaye with a value of 2,584 while the lowest value of 1,600 was obtained in Ijari. The density/ha of trees in Omu plantation (600) was higher than that of Ijaye (520) in the 4X5 enspacement. *M. oleifera* plantation in Akure with 4X4 meter spacing had a density of 3,200 and Erinjiyan plantation with a spacing of 8X5 meters had the density of 1,840 trees.

Table 4.1: Tree density of *Moringa oleifera* in the selected plantations in Southwestern Nigeria

Location	Total Land Area /ha	Number of plot in total land area /ha	Spacing Adopted	N0 of trees /plot	Total No of trees/ Density /ha
Oyo	6.69	167	2x2	75	12,525
Ijaye	1.34	34	2x2	76	2,584
Akure	6.40	160	4x4	20	3,200
Ijare	1.60	40	4x5	13	520
Erinjiyan	9.20	230	8x5	8	1,840
Omu	2.98	75	4x5	8	600
Abeokuta	0.89	22	2x2	83	1,826
Ijari	0.80	20	2x2	83	1,660

Sample plot size 0.04ha

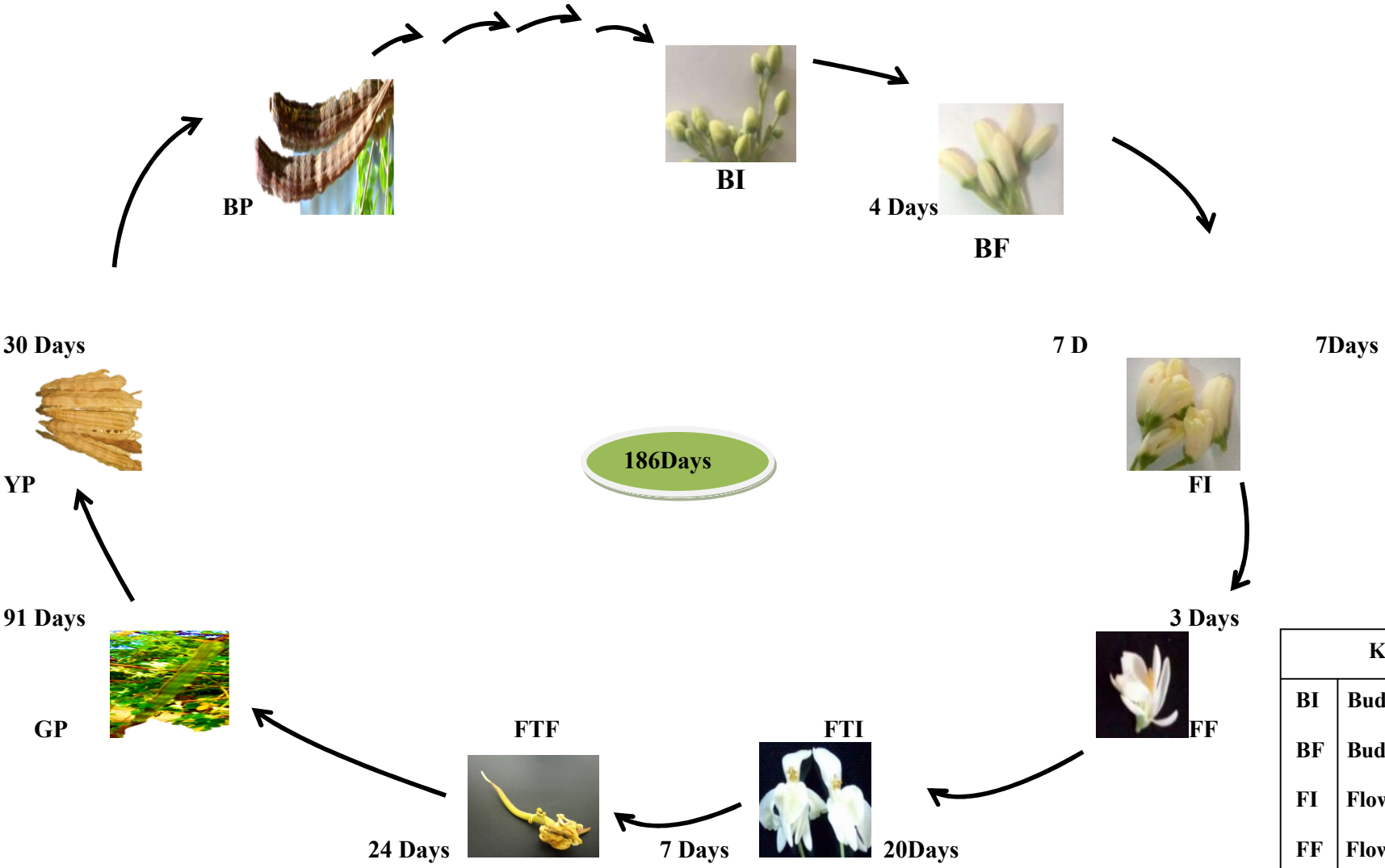
4.2 Flower and fruiting duration and fruit maturity index evaluation of *Moringa oleifera* in selected plantations in Southwestern Nigeria

There were 9 phenological stages in the process of flower and fruit formation of *Moringa oleifera*. Approximately 186 days were required for the completion of all phenological processes before the full maturity of *Moringa oleifera* pods was achieved (Plate 4.1). The pheno-phases commenced with a growth of a long leaf-like structure from the anterior nodal points of *Moringa oleifera* branches. A globule-like structure was noticed at the tip of the leaf-like structure, this appeared like a modified leaf (Bud emergence). After the Bud emergence(Bud initiation (BI), it took an average of about 4 days to develop into a fully formed bud (Bud formation (BF). Flower initiation (FI) commenced with a gradual opening of the bud and was completed with a wholly opened bud within an average of 7 days. Three days after, a copiously formed flower (flower formation (FF) emerged. From the centre of the flower, pollen grains were noticeable and with continuous observation and imperceptible fertilization process, the fruit initiation (FTI) stage became visible within an average of 20 days; shortly after which (about 7 days after), fruits were fully formed (Fruit formation (FTF). The maturity index stage assessment commenced 24 days after, from the fully matured green pod to the yellow pod (90 days) and finally to the brown pod 30 days after. Stages of reproductive phenology across all the selected locations were alike but varied in timing; as in some locations, these developing stages were found to occur differently or at the same time.

4.2.1 Seasonal variation in the floral and fruiting duration of *Moringa oleifera* in the rainy season

There were significant differences in the development stages of *Moringa oleifera* across the locations during the first rainy season (2015/2016). These significant differences were observed only in four stages; Flower initiation (FI), Flower formation (FF), Fruit initiation (FTI) and Fruit formation (FTF). Duration in the formation of other development stages during the first rainy season (2015/2016) was similar and no significant variation was recorded across all the locations.

The period (in days) for flower initiation (FI) was earliest initiated in Oyo (5.00 ± 0.07 days) while flower was initiated in Omu and Ijaye at 8.50 ± 0.70 days (Table 4.2). Flower formation (FF) started earliest and occurred concurrently in Oyo and Erinjiyan within 3.00±0.19 days after flower initiation (FI) but did not start until 5.50±0.35 days in Omu. Moringa fruits/pods were initiated earliest (FTI) and occurred concurrently in Oyo



KEY	
BI	Bud initiation
BF	Bud formatio
FI	Flower initiation
FF	Flower formation
FTI	Fruit initiation
FTF	Fruit formation
GP	Green pod
YP	Yellow pod
BP	Brown pod

Plate 4.1: The flower and fruiting cycle of *Moringa oleifera* in selected plantations of Southwestern Nigeria

Table 4.2: Variation in floral and fruiting duration of *Moringa oleifera* across eight locations during the rainy season in Southwestern Nigeria

Year	2015/2016				2016/2017		
Developing Stages	**FI**	**FF**	**FTI**	**FTF**	**FI**	**FF**	**FTF**
LOCATION							
Omu	8.50 ± 0.70 [a]	5.50 ± 0.35 [a]	30.00 ± 0.55 [a]	8.50 ± 0.26 [a]	8.00 ± 0.65[a]	4.50 ± 0.19[ab]	8.50 ± 0.70[a]
Ijaye	8.50 ± 0.70[a]	5.00 ± 0.31 [ab]	28.50 ± 0.52[ab]	7.00 ± 0.16 [b]	8.50 ± 0.72[a]	3.00 ± O.07[C]	7.00 ± O.46[b]
Akure	8.00 ± 0.50 [a]	5.00 ± 0.31[ab]	28.50 ± 0.52[ab]	8.50 ± 0.26 [a]	7.50 ±0.61[ab]	4.00 ± 0.17[b]	8.50 ± 0.70[a]
Ijare	7.00 ± 0.35[b]	4.50 ± 0.25[ab]	28.50 ± 0.52[ab]	7.00 ± 0.16 [b]	6.50 ±0.61[b]	5.00 ± 0.21[a]	7.00 ± 0.46[b]
Abeokuta	6.50 ± 0.31[b]	4.00 ± 0.23[ab]	28.00 ± 0.43[b]	8.50 ± 0.26 [a]	6.50 ± 0.61[b]	5.50 ± 0.25[a]	8.00 ± 0.62[ab]
Ijari	6.00 ± 0.19[bc]	3.50 ± 0.21[ab]	28.00 ± 0.43[b]	8.50 ± 0.26 [a]	6.00 ±0.57[bc]	5.00 ± 0.21[a]	8.00 ± 0.62[ab]
Oyo	5.00 ± 0.07[c]	3.00 ± 0.19[ab]	27.00 ± 0.40[bc]	8.50 ± 0.26 [a]	4.50 ± 0.49[c]	3.50 ± 0.13[bc]	8.50 ± 0.70[a]
Erinjiyan	5.00 ± 0.60[c]	3.00 ± 0.19[ab]	27.00 ± 0.40[bc]	8.50 ± 0.26 [a]	5.00 ± O.51[c]	3.00 ± 0.07[c]	8.00 ± 0.62[ab]

Means with the same letter in a column are not significantly different across locations

FI-Flower initiation

FF-Flower formation

FTI-Fruit initiation

FTF-Fruit formation

and Erinjiyan at 27.00±0.40 days after the previous development stage (Flower formation), but did not start until 30.00±0.55 days in Omu. The earliest number of days for fruit formation (FTF) was recorded for Ijaye and Ijare (7.00±0.16 days) while fruits were formed concurrently at all other locations at 8.50±0.26 days.

In the 2016/2017 rainy season, significant differences were observed in the duration of only three developing stages of *Moringa oleifera*; Flower initiation (FI), Flower formation (FF) and Fruit formation (FTF). Duration for all other developing stages was not significantly different across the locations. The earliest flower initiation occurred in Oyo, with a mean of 4.50± 0.49 number of days while in Ijaye, flower initiation occurred late with a mean number of days of 8.50 ± 0.72 days (Table 4.2). The period of flower formation ranged from 3.00 ± 0.07 to 5.50±0.25 days, with the earliest flower formation recorded for both Ijaye and Erinjiyan. Longer periods of formation occurred at Oyo, Omu and Akure. Fruit formation occurred earliest at Ijaye and Ijare and later at Ijari, Erin and Abeokuta, with a mean number of days of 7.00 ± 0.46 and 8.00± 0.62 respectively.

In the dry season of 2015/2016, significant differences were observed in flower initiation (FI), fruit initiation (FTI) and yellow pod formation (Table 4.3) other developing stages were not significantly different. The shortest duration for flower initiation was obtained in Erinjiyan (5.00 ± 0.60 days), while the longest duration occurred concurrently in Omu, Ijaye, Akure and Oyo with 7.00 ± 0.32 mean days.

The shortest time for fruit initiation (FTI) occurred in Abeokuta (27.00± 0.41), while in Omu and Oyo, the longest period for fruit initiation occurred concurrently at 31.00±0.70 mean days. The period for yellow pod colouration started earliest in Abeokuta (88.00±1.41 days) while the longest mean days of 98.00±2.82 days were recorded in Omu.

In 2016/2017, only flower initiation and flower formation varied significantly among all the selected locations, other phenology indices were not significant across all the selected locations. Moringa flowers were earliest initiated at Ijare (5.00±0.31) while it took a longer period at Akure (7.50±0.52 days).

However, the mean values for flower formation (FF) ranged from 3.00 ± 0.09 to 5.50 ± 0.33 days; the earliest flower was found in Ijare and the longest mean days in Abeokuta. For fruit maturity index evaluation, the highest germination percentage occurred for yellow pod seeds (90.66 ±0.33%) followed by brown (80.00±0.57%), with the green pods having the lowest (30.66±0.88%) (Table 4.4).

Table 4.3: Variation in floral and fruiting duration of *Moringa oleifera* across eight locations during the dry season in Southwestern Nigeria

Year	2015/2016			2016/2017	
Developing stages	**FI**	**FTI**	**YP**	**FI**	**FF**
LOCATION					
Omu	7.00 ± 0.32 [a]	31.00 ± 0.70 [a]	98.00 ± 2.82 [a]	7.00 ± 0.49[ab]	5.00 ± 0.31[a]
Ijaye	7.00 ± 0.32 [a]	29.00 ± 0.61[ab]	91.00 ± 2.63 [b]	7.00 ± 0.49[ab]	3.50 ± 0.11[bc]
Akure	7.00 ± 0.32 [a]	29.00 ± 0.61[ab]	91.00 ± 2.63 [b]	7.50 ±0.52[a]	4.00 ± 0.13[b]
Ijare	6.00 ± 0.15[c]	28.50 ± 0.52[b]	91.00 ± 2.63 [b]	5.00 ±0.31[b]	3.00 ± 0.09[c]
Abeokuta	6.50 ± 0.31[b]	27.00 ± 0.41[bc]	88.00 ± 1.41 [c]	6.50 ± 0.43[b]	5.50 ± 0.33[a]
Ijari	6.00 ± 0.19[b]	28.00 ± 0.43[b]	90.50 ± 2.18 [b]	6.50 ±0.43[b]	5.00 ± 0.31[a]
Oyo	7.00 ± 0.32 [a]	31.00 ± 0.70 [a]	93.00 ± 2.75 [ab]	7.00 ± 0.49[ab]	4.00 ± 0.13[b]
Erinjiyan	5.00 ± 0.60[c]	29.50 ± 0.66[ab]	90.00 ± 2.03 [c]	5.50 ± 0.43[b]	5.00 ± 0.15[a]

Mean with the same letter in a column are not significantly different across locations

FI-Flower initiation

FF-Flower formation

FTI-Fruit initiation

YP-Yellow pod

Table 4.4 Effect of pod colouration on germination of *Moringa oleifera* seeds

Pod colour	germination (%)
Brown	80.00±0.57 [a]
Yellow	90.66±0.33 [a]
Green	30.66±0.88 [b]

Mean with the same letter in a column are not significantly different

4.3 Seed and pod morphology of *Moringa oleifera* from selected plantations of Southwestern Nigeria

There were significant differences in the pod length and weight of *Moringa oleifera* seeds but other morphological characteristics were not significant across the locations. Abeokuta had the longest pods (40.04 ± 1.66cm) while Erinjiyan (27.63±0.64cm) had the shortest (Table 4.5). The weight (100 seeds) varied among locations with the highest mean value was obtained found in Omu (31.37 ± 1.69g) while the lowest value was recorded in Ijari (17.19 ± 1.0g).

4.4 Influence of seed source on germination and early seedling performance.

There were significant differences in the effect of seed source on germination and growth variables. The highest germination percentage was recorded for seeds collected from Ijare (92.50±2.71%) while the lowest was found in Omu (5.50±5.50%) (Table 4.6). The highest mean seedling height was found in Omu (112 ± 1.39cm), followed by Ijaye (106.51 ± 1.26cm) while the lowest for Ijari (76.92±0.79cm). Seedlings highest mean collar diameter occurred for seedlings from Ijari (15.85±0.92 mm) and Ijare (15.38 ±0.91mm) while the lowest was for Abeokuta seedlings (9.29±0.57 mm). For leaf production, Erinjiyan had the highest mean value (37.25±0.87), followed by Ijaye (30.57±0.73), while the lowest was for seedlings from Abeokuta (20.57±0.51). Most of the locations experienced a decrease in the number of leaves particularly between 6 and 10 weeks after planting. However, for Ijaye and Ijare locations, a drastic decrease in the number of leaves was observed. Commonly, seedling height, collar diameter and the number of leaves produced increased with time across the studied locations (Figures 4.1, 4.2 and 4.3).

4.5 Effect of seed source, age and lopping height on leaf quality and biomass production

4.5.1 Soil physicochemical and Proximate analysis

The soil analysis conducted to show the fertility status of the experimental plot revealed its physicochemical properties (Table 4.7).

The soil pH, organic carbon, organic matter, total nitrogen, potassium and sodium had the values of; 5.72 mg/kg, 1.62 g/kg, 2.79g/kg, 0.14g/kg 1.05mg/kg and 0.46 cmol/kg respectively. Tha calcium (2.74cmol/kg), Magnesium (1.40cmol/kg), Manganese (26.50cmol/kg), Copper (4.20 mg/kg), Zinc (12.30mg/kg), and Iron (64.00mg/kg) were also recorded. In addition, the soil varied in texture from sand, silt and clay with the values 80.50g/kg, 5.00g/kg and 14.50g/kg respectively.

Table 4.5: Effect of location on pod length and seed weight of M*oringa oleifera*

LOCATION	Pod length (cm)	Seed weight (g)	Pod diameter (mm)	Number of seed per pod
Omu	39.28±1.27[a]	31.37±1.69[a]	18.00±5.63	15.39±3.57
Ijaye	38.25±1.19[ab]	23.30±1.09[d]	17.33±3.43	15.57±4.23
Akure	35.64±1.06[b]	29.63+1.36[a]	16.30±3.19	17.96±3.34
Ijare	36.03±1.09[b]	27.27±1.20[b]	18.59±10.27	15.90±3.44
Abeokuta	40.04±1.66[a]	31.37±1.69[a]	19.45±2.89	16.74±4.26
Ijari	30.57±0.79[d]	17.19±1.03[e]	18.47±2.44	14.86±6.86
Oyo	33.71±0.98[c]	25.40±1.16[c]	18.31±4.81	15.11±3.85
Erinjiyan	27.63±0.64[e]	27.63±0.64[e]	16.99±3.23	12.91±3.28

Mean with same letter in a column are not significantly different across locations

Table 4.6: Effect of seed source on germination and growth variables of *Moringa oleifera* in Southwestern Nigeria

LOCATION	Germination (%)	Seedlings height (cm)	Collar diameter (mm)	Leaf count
Omu	55.50±5.50^{d}	112.72±1.39^{a}	13.71±0.73^{c}	28.32±0.65^{c}
Ijaye	63.00±5.87^{cd}	106.51±1.26^{b}	10.71±0.66^{d}	30.57±0.73^{b}
Akure	80.00±4.65^{b}	98.61±0.98^{c}	13.76±0.71^{c}	21.69±0.57^{f}
Ijare	92.50±2.71^{a}	103.02±1.19^{b}	15.38±0.91^{ab}	25.90±0.60^{d}
Abeokuta	74.00±5.3^{bc}	90.54±0.86^{d}	9.29±0.57^{e}	20.57±0.51^{g}
Ijari	78.50±5.37^{b}	76.92±0.79^{e}	15.85±0.92^{a}	27.91±0.63^{c}
Oyo	68.00±4.84^{c}	102.64±1.34^{b}	10.19±0.62^{d}	24.25±0.53^{e}
Erinjiyan	73.50±5.47^{bc}	98.71±1.98^{c}	14.54 ±0.83^{b}	37.25±0.87^{a}

Means with the same letter in a column are not significantly different across locations

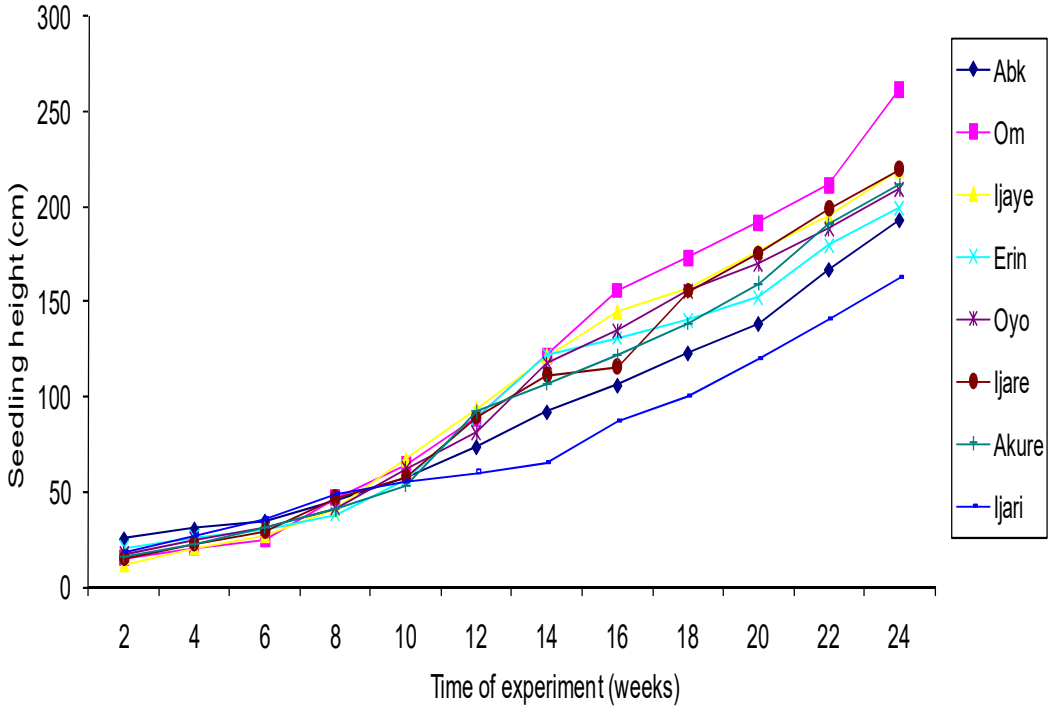


Figure 4.1: Seedling height of *Moringa oleifera* from eight plantations in South West Nigeria

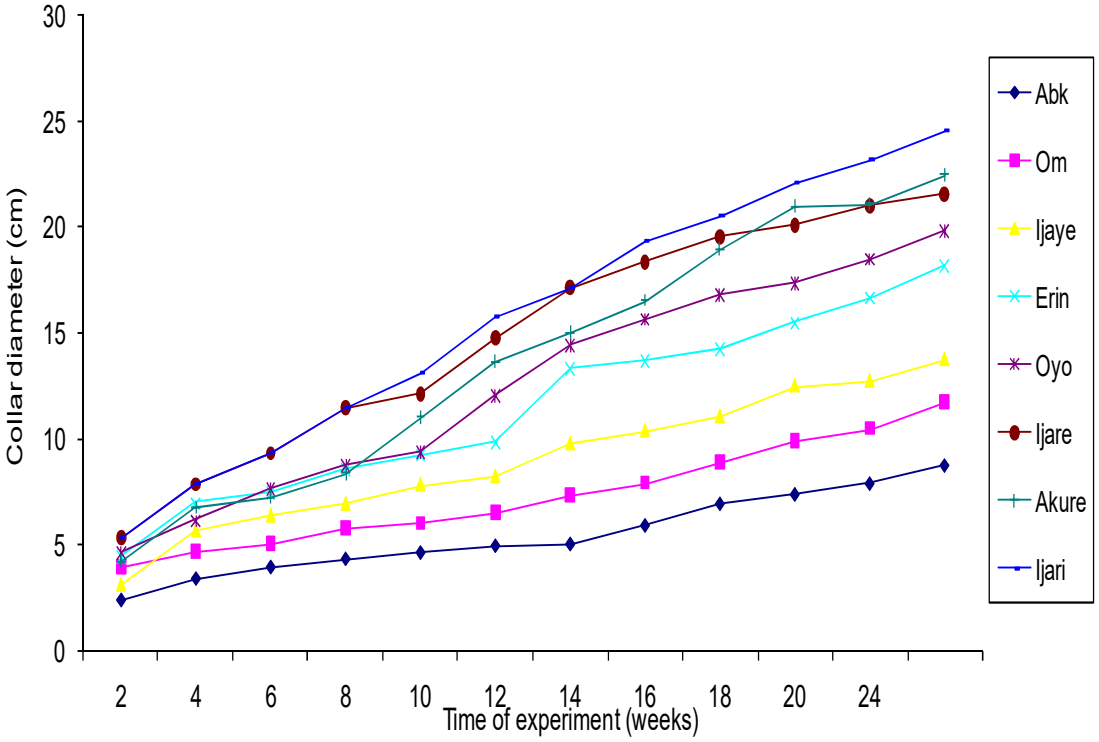


Figure 4.2: Collar diameter of *Moringa oleifera* seedlings from eight plantations in Southwestern Nigeria

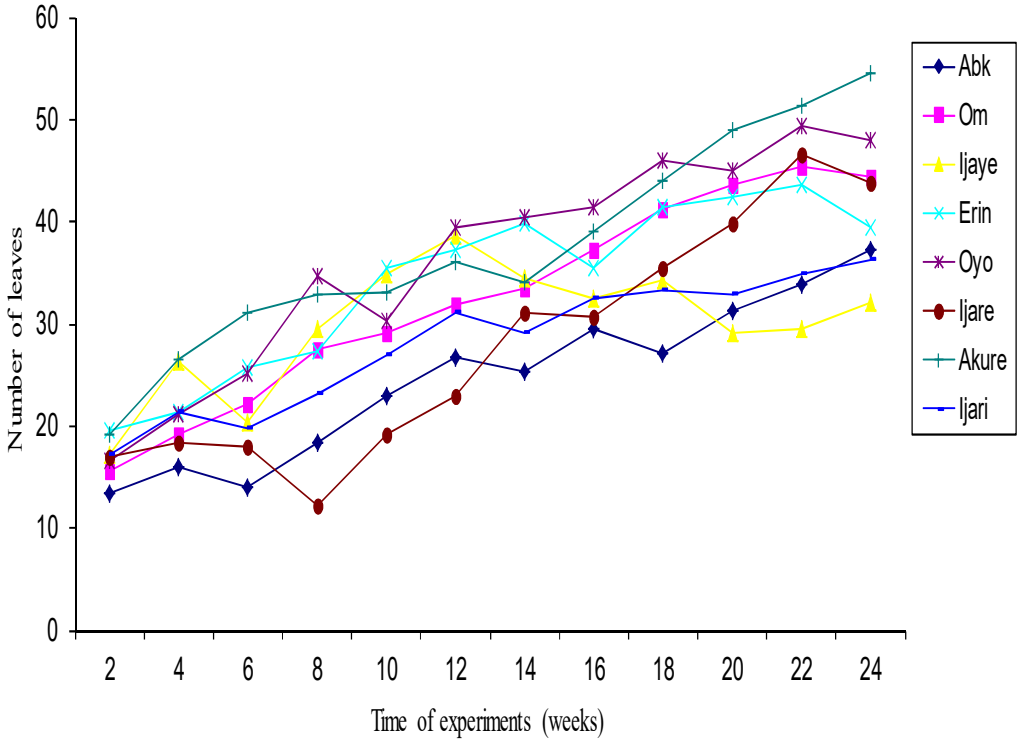


Figure 4.3: Number of leaves of *Moringa oleifera* seedlings from plantations in Southwestern Nigeria

Table 4.7: Physico-chemical soil properties of the site used for field trials to determine leaf quality and biomass production of decapitated *Moringa oleifera* seedlings

Soil parameters	Nutrient Values
pH (mg/kg)	5.72
Organic Carbon (g/kg)	1.62
Organic Matter (g/kg)	2.79
Total Nitrogen (g/kg)	0.14
Sodium (cmol/kg)	0.46
Calcium (cmol/kg)	2.74
Magnesiumg (cmol/kg)	1.40
Manganese (cmol/kg)	26.50
Copper (mg/kg)	4.20
Zinc (mg/kg)	12.30
Iron (mg/kg)	64.00
Potassiun (Mg/kg)	1.05
Sand (g/kg)	80.50
Silt (g/kg)	5.00
Clay (g/kg)	14.50

Source: (Field survey, 2017)

The analysis of variance on the effect of seed source, age and lopping height on all proximate parameters showed significant differences across all the locations.

4.5.1.1 Protein

The protein content of *Moringa oleifera* leaves for four-month-old seedlings ranged from 8.45 to 9.05%, six-month-old seedlings ranged from 8.45 to 9.10% while for eight-month-old seedlings, it ranged from 8.20 to 9.10% (Table 4.8).

The protein content also varied across locations. For Akure, it ranged from 8.30 to 9.10%; Ijare: 8.45 to 9.10%; Ijaye: 8.55 to 8.95%, Oyo: 8.45 to 9.05%; Omu: 8.45 to 8.70) %; Erinjiyan: 8.70 to 9.05%; Abeokuta: 8.20 to 8.90 % and Ijari: 8.55 to 8.85%. Protein content of sprouts varied across the lopping height. It ranged from 8.40 and 9.00% in sprouts from 20cm lopping height, 8.20 and 9.10% for sprouts from 40cm and 8.45 to 9.10% for sprouts from 60cm lopping height.

4.5.1.2 Ash

The ash content of *Moringa oleifera* in a four months old seedling ranged from 2.20 to 3.12% that of six-month-old seedlings ranged from 2.35 to 3.10 % while eight-month-old seedlings ranged from 2.20 to 3.10% (Table 4.9). However, variation was observed across the locations: Akure (2.70 to 3.10%), Ijare (2.60 to 3.10%), Ijaye (2.60 to 2.90%), Oyo (2.70 to 3.10%), Omu (2.20 to 2.60%), Erinjiyan (2.40 to 2.80%), Abeokuta (2.70 to 3.10%) and Ijari (2.09 to 3.12%). Ash content in sprouts of *Moringa oleifera* across the lopping height ranged between 2.09 and 3.10% (seedling at 20 cm), sprouts from 40cm lopping height (2.30 and 3.12 %) and 60 cm lopping height produced 2.20 and 3.10% sprouts of Ash content.

4.5.1.3 Ether

Ether content in the four-month-old seedlings ranged from 0.50 to 0.90%. For six-month-old seedling, it was 0.50 to 2.55 % and 0.55 to 0.95% for eight-month-old seedlings (Table 4.10). Similarly, variations were observed across the locations; Akure (0.55 to 0.95%), Ijare (0.75 to 0.90%), Ijaye (0.65 to 0.85%), Oyo (0.60 to 0.90%), Omu (0.50 to 0.75%), Erinjiyan (0.50 to 0.95%), Abeokuta (0.50 to 0.80%) and Ijari (0.50 to 0.95%). Ether content in sprouts of *Moringa oleifera* across the lopping height ranged from 0.50 to 0.85% for seedling lopped at 20cm, at 40cm, sprouts of Moringa contained 0.50 and 0.95 % while 0.50 and 0.85% Ether content was found in sprout of seedling lopped at at 60cm.

Table 4.8: Protein content (%) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling age (months)	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	8.85 ± 0.07	8.45 ± 0.07	8.55 ± 0.07	8.75 ± 0.07	8.45 ± 0.07	8.85 ± 0.07	8.85 ± 0.07	8.85 ± 0.15
	20	8.75 ± 0.07	8.65 ± 0.03	8.65 ± 0.07	8.75 ± 0.07	8.65 ± 0.07	8.70 ± 0.07	8.75 ± 0.07	8.76 ± 0.15
	40	9.00 ± 0.07	8.55 ± 0.04	8.55 ± 0.07	8.95 ± 0.07	8.50 ± 0.07	8.85 ± 0.07	8.95 ± 0.07	8.80 ± 0.05
	60	9.00 ± 0.07	8.65 ± 0.07	8.75 ± 0.07	9.05 ± 0.07	8.45 ± 0.07	8.75 ± 0.07	8.90 ± 0.00	8.75 ± 0.70
6	Control	8.65 ± 0.07	8.80 ± 0.00	8.75 ± 0.07	8.60 ± 0.00	8.60 ± 0.00	8.95 ± 0.07	8.85 ± 0.07	8.50 ± 0.13
	20	8.65 ± 0.07	8.50 ± 0.00	8.55 ± 0.07	8.85 ± 0.07	8.70 ± 0.00	9.00 ± 0.14	8.65 ± 0.07	8.65 ± 0.19
	40	8.75 ± 0.07	8.65 ± 0.07	8.70 ± 0.00	8.70 ± 0.00	8.55 ± 0.07	8.70 ± 0.00	8.50 ± 0.00	8.60 ± 0.21
	60	9.10 ± 0.00	8.45 ± 0.07	8.60 ± 0.00	8.65 ± 0.07	8.50 ± 0.00	9.05 ± 0.07	8.50 ± 0.00	8.65 ± 0.17
8	Control	8.30 ± 0.00	9.00 ± 0.00	8.85 ± 0.07	8.45 ± 0.07	8.45 ± 0.07	8.85 ± 0.07	8.25 ± 0.07	8.55 ± 0.00
	20	8.60 ± 0.00	8.95 ± 0.07	8.75 ± 0.07	8.55 ± 0.07	8.55 ± 0.07	8.75 ± 0.07	8.40 ± 0.00	8.75 ± 0.00
	40	8.60 ± 0.00	9.10 ± 0.00	8.95 ± 0.07	8.75 ± 0.07	8.40 ± 0.00	8.95 ± 0.00	8.20 ± 0.00	8.80 ± 0.00
	60	8.50 ± 0.00	8.90 ± 0.00	8.85 ± 0.07	8.50 ± 0.00	8.50 ± 0.00	8.95 ± 0.07	8.50 ± 0.00	8.75 ± 0.00

Table 4.9: Ash content (%) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling age (months)	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	2.80 ± 0.00	2.85 ± 0.07	2.70 ± 0.00	2.90 ± 0.00	2.20 ± 0.00	2.40 ± 0.00	3.05 ± 0.07	2.90 ± 0.02
	20	2.70 ± 0.00	2.85 ± 0.07	2.80 ± 0.00	3.05 ± 0.07	2.40 ± 0.00	2.50 ± 0.00	3.10 ± 0.00	3.10 ± 0.00
	40	2.80 ± 0.07	2.70 ± 0.00	2.60 ± 0.00	2.90 ± 0.08	2.30 ± 0.00	2.40 ± 0.00	3.00 ± 0.00	3.12 ± 0.02
	60	2.90 ± 0.00	2.90 ± 0.00	2.75 ± 0.01	2.75 ± 0.07	2.20 ± 0.00	2.40 ± 0.00	2.90 ± 0.00	2.95 ± 0.00
6	Control	2.75 ± 0.07	3.00 ± 0.00	2.85 ± 0.07	2.80 ± 0.07	2.35 ± 0.07	2.65 ± 0.07	2.95 ± 0.07	2.65 ± 0.04
	20	2.80 ± 0.00	3.10 ± 0.00	2.70 ± 0.00	2.70 ± 0.00	2.35 ± 0.07	2.55 ± 0.07	2.95 ± 0.07	2.70 ± 0.07
	40	2.90 ± 0.00	3.00 ± 0.00	2.90 ± 0.00	3.05 ± 0.07	2.50 ± 0.00	2.50 ± 0.00	2.85 ± 0.07	2.85 ± 0.09
	60	2.85 ± 0.07	2.95 ± 0.07	2.90 ± 0.07	2.95 ± 0.07	2.60 ± 0.00	2.45 ± 0.07	3.10 ± 0.00	2.80 ± 0.07
8	Control	3.00 ± 0.00	2.70 ± 0.00	2.60 ± 0.00	3.05 ± 0.07	2.25 ± 0.07	2.80 ± 0.00	2.80 ± 0.00	2.80 ± 0.10
	20	3.10 ± 0.00	2.60 ± 0.00	2.70 ± 0.00	3.10 ± 0.00	2.30 ± 0.00	2.70 ± 0.00	2.90 ± 0.00	2.09 ± 0.03
	40	3.00 ± 0.14	2.80 ± 0.00	2.60 ± 0.00	3.10 ± 0.00	2.30 ± 0.00	2.60 ± 0.00	2.70 ± 0.00	2.95 ± 0.09
	60	2.90 ± 0.00	2.70 ± 0.00	2.50 ± 0.00	2.90 ± 0.00	2.20 ± 0.00	2.50 ± 0.00	2.80 ± 0.00	2.95 ± 0.06

Table 4.10: Ether content (%) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling age (months)	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	0.75 ± 0.00	0.75 ± 0.00	0.65 ± 0.00	0.90 ± 0.00	0.50 ± 0.00	0.75 ± 0.00	0.65 ± 0.00	0.90 ± 0.00
	20	0.55 ± 0.00	0.75 ± 0.00	0.65 ± 0.00	0.80 ± 0.00	0.55 ± 0.00	0.65 ± 0.00	0.60 ± 0.00	0.85 ± 0.00
	40	0.75 ± 0.00	0.75 ± 0.00	0.80 ± 0.00	0.85 ± 0.00	0.60 ± 0.00	0.50 ± 0.00	0.65 ± 0.00	0.90 ± 0.00
	60	0.70 ± 0.00	0.70 ± 0.00	0.70 ± 0.00	0.70 ± 0.00	0.50 ± 0.00	0.75 ± 0.00	0.50 ± 0.00	0.95 ± 0.00
6	Control	0.75 ± 0.01	0.87 ± 0.07	0.65 ± 0.07	0.75 ± 0.07	0.60 ± 0.00	0.55 ± 0.07	0.55 ± 0.07	0.50 ± 0.01
	20	0.85 ± 0.01	0.70 ± 0.00	0.75 ± 0.07	0.85 ± 0.07	0.50 ± 0.00	0.65 ± 0.07	0.55 ± 0.07	0.65 ± 0.01
	40	0.95 ± 0.02	0.90 ± 0.00	0.85 ± 0.07	0.60 ± 0.07	0.70 ± 0.00	0.50 ± 0.00	0.55 ± 0.07	0.60 ± 0.02
	60	0.85 ± 0.02	0.80 ± 0.00	0.65 ± 0.07	0.75 ± 0.07	0.50 ± 0.00	0.55 ± 0.07	0.50 ± 0.00	0.65 ± 0.02
8	Control	0.60 ± 0.00	0.65 ± 0.07	0.85 ± 0.07	0.65 ± 0.07	0.55 ± 0.07	0.85 ± 0.07	0.75 ± 0.07	0.75 ± 0.02
	20	0.55 ± 0.07	0.75 ± 0.07	0.75 ± 0.07	0.65 ± 0.07	0.55 ± 0.07	0.85 ± 0.07	0.75 ± 0.07	0.85 ± 0.03
	40	0.55 ± 0.07	0.85 ± 0.07	0.70 ± 0.14	0.70 ± 0.14	0.75 ± 0.07	0.70 ± 0.00	0.80 ± 0.00	0.85 ± 0.05
	60	0.65 ± 0.07	0.60 ± 0.00	0.65 ± 0.07	0.75 ± 0.07	0.75 ± 0.07	0.95 ± 0.07	0.70 ± 0.00	0.80 ± 0.03

4.5.1.4 Crude fibre

In four-month-old seedlings, Crude fibre ranged from 1.95 to 3.90%. For six-month-old seedlings, it ranged from 2.25 to 4.35 %, and 2.15 to 4.35% for eight-month-old seedlings (Table 4.11). Crude fibre content varied across all the locations: Akure (2.30 to 2.85%), Ijare (2.30 to 2.50%), Ijaye (1.95 to 2.65%), Oyo (2.05 to 2.55%), Omu (2.45 to 3.30%), Erinjiyan (2.45 to 2.75%), Abeokuta (3.45 to 4.35%) and Ijari (2.45 to 3.95) %. The crude fibre content in sprouts of *Moringa oleifera* across the lopping height ranged from 2.15 and 4.20% (seedlings lopped at 20cm); 2.10 and 3.85% for sprouts of seedlings lopped at 40cm and 2.05 and 4.15% for sprouts of seedling lopped at 60 cm.

4.5.1.5 Carbohydrates

Carbohydrates in a four-month-old seedling of *Moringa oleifera* ranged from 10.30 and 12.35%; 10.45 to 12.90% for -six-month-old seedlings and 10.70 to 12.30%for eight-month-old seedlings (Table 4.12). It was observed that across locations, the carbohydrates varied; Akure (12.15 to 12.50%) Ijare (10.65 to 12.20%), Ijaye (10.45 to 11.45%), Oyo (10.30 to 12.90%), Omu (10.70 to 11.90%), Erinjiyan (11.45 to 11.90%), Abeokuta (11.65 to 12.35%) and Ijari (10.70 to 12.42%). Carbohydrates content in sprouts of *Moringa oleifera* among the three different lopping heights ranged from 10.35 and 12.35% for sprouts from 20cm. For sprouts from 40cm, it was 10.85 and 12.65% while 10.30 to 12.90 % of sprouts from seedlings lopped at 60cm.

4.5.1.6 Moisture content

The moisture content of *Moringa oleifera* in a four-month-old seedling ranged from 71.60 to 76.50%; that of six-month-old seedlings was 71.20 to 76.85 %; and 72.30 to 76.70% for eight-month-old seedling (Table 4.13). However, variation was observed across the locations: Akure (72.30 to 76.70%), Ijare (71.60 to 76.90%), Ijaye (71.20 to 75.80%), Oyo (71.93 to 76.70%), Omu (73.36 to 75.96%), Erinjiyan (72.80 to 76.40%), Abeokuta (73.73 to 76.50%) and Ijari (72.10 to 76.70%). Moisture content in sprouts of *Moringa oleifera* across the lopping heights ranged between 73.16 and 76.70% (sprouts from 20cm), 74.33 and 76.90 %, sprouts from 40cm and 73.83 to 76.85 % for sprouts from 60cm lopping height.

Table 4.11: Crude Fibre content (%) of *Moringa oleifera* seedlings and sprouts at different ages, sources and looping heights

Seedling age (months)	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	2.35 ± 0.07	2.35 ± 0.07	1.95 ± 0.07	2.05 ± 0.07	3.05 ± 0.07	2.45 ± 0.07	3.45 ± 0.07	3.40 ± 0.05
	20	2.80 ± 0.14	2.45 ± 0.07	2.15 ± 0.07	2.25 ± 0.07	3.15 ± 0.07	2.50 ± 0.00	3.85 ± 0.07	3.45 ± 0.04
	40	0.80 ± 0.14	0.35 ± 0.07	2.10 ± 0.00	2.30 ± 0.00	3.15 ± 0.07	2.75 ± 0.07	3.95 ± 0.07	3.40 ± 0.03
	60	0.85 ± 0.07	0.45 ± 0.07	2.05 ± 0.07	2.35 ± 0.07	3.15 ± 0.07	2.65 ± 0.07	3.90 ± 0.00	3.45 ± 0.03
6	Control	2.40 ± 0.14	2.45 ± 0.07	2.45 ± 0.07	2.55 ± 0.07	2.95 ± 0.07	2.55 ± 0.07	4.35 ± 0.07	2.45 ± 0.13
	20	2.30 ± 0.00	2.35 ± 0.07	2.45 ± 0.07	2.25 ± 0.07	3.05 ± 0.07	2.55 ± 0.07	4.20 ± 0.07	3.20 ± 0.11
	40	2.85 ± 0.07	2.35 ± 0.07	2.60 ± 0.00	2.35 ± 0.07	3.20 ± 0.00	2.75 ± 0.07	3.85 ± 0.07	3.55 ± 0.14
	60	2.75 ± 0.07	2.45 ± 0.07	2.65 ± 0.07	2.45 ± 0.07	3.30 ± 0.07	2.75 ± 0.07	4.15 ± 0.07	3.55 ± 0.13
8	Control	2.30 ± 0.00	2.45 ± 0.07	2.55 ± 0.07	2.15 ± 0.07	2.45 ± 0.07	2.45 ± 0.07	3.95 ± 0.07	2.90 ± 0.00
	20	2.65 ± 0.07	2.30 ± 0.00	2.45 ± 0.07	2.25 ± 0.07	3.15 ± 0.07	2.50 ± 0.14	4.10 ± 0.28	3.95 ± 0.10
	40	2.55 ± 0.07	2.50 ± 0.00	2.40 ± 0.00	2.40 ± 0.00	3.15 ± 0.07	2.50 ± 0.14	4.35 ± 0.07	3.95 ± 0.20
	60	2.45 ± 0.07	2.45 ± 0.07	2.55 ± 0.07	2.40 ± 0.00	3.05 ± 0.07	2.65 ± 0.07	4.25 ± 0.07	3.40 ± 0.20

Table 4.12: Carbohydrate content (%) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling age (months)	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	12.00 ± 0.00	10.85 ± 0.07	10.85 ± 0.07	10.35 ± 0.07	11.75 ± 0.07	11.75 ± 0.07	12.15 ± 0.07	12.17 ± 0.08
	20	12.35 ± 0.07	11.40 ± 0.00	11.40 ± 0.00	10.35 ± 0.07	11.75 ± 0.07	11.90 ± 0.00	12.23 ± 0.07	12.40 ± 0.06
	40	12.15 ± 0.07	10.85 ± 0.07	10.85 ± 0.07	10.35 ± 0.07	11.75 ± 0.07	11.65 ± 0.07	12.35 ± 0.07	12.42 ± 0.40
	60	12.20 ± 0.07	11.85 ± 0.07	11.85 ± 0.07	10.30 ± 0.00	11.75 ± 0.00	11.90 ± 0.00	12.35 ± 0.07	12.35 ± 0.03
6	Control	12.20 ± 0.14	10.65 ± 0.92	10.45 ± 0.07	12.35 ± 0.07	11.45 ± 0.07	11.45 ± 0.07	11.65 ± 0.07	11.10 ± 0.10
	20	12.35 ± 0.07	11.45 ± 0.14	11.15 ± 0.07	12.55 ± 0.07	11.75 ± 0.07	11.55 ± 0.07	11.90 ± 0.00	11.90 ± 0.10
	40	12.50 ± 0.00	11.60 ± 0.14	11.25 ± 0.07	12.65 ± 0.07	11.80 ± 0.14	11.85 ± 0.07	11.85 ± 0.07	11.85 ± 0.10
	60	12.35 ± 0.07	11.50 ± 0.14	11.25 ± 0.07	12.90 ± 0.00	11.90 ± 0.00	11.88 ± 0.07	11.95 ± 0.07	12.25 ± 0.10
8	Control	12.15 ± 0.21	11.55 ± 1.06	11.20 ± 1.20	10.75 ± 0.07	10.70 ± 0.28	11.45 ± 0.07	11.65 ± 0.07	10.70 ± 0.00
	20	12.30 ± 0.14	12.20 ± 0.28	10.90 ± 0.57	11.05 ± 0.07	10.95 ± 0.07	10.85 ± 0.07	10.85 ± 0.07	10.95 ± 0.00
	40	12.25 ± 0.07	12.05 ± 0.07	11.40 ± 0.00	11.75 ± 0.07	11.75 ± 0.07	11.80 ± 0.14	11.75 ± 0.07	11.25 ± 0.00
	60	12.30 ± 0.14	12.15 ± 0.07	11.45 ± 0.07	11.85 ± 0.07	11.90 ± 0.00	11.64 ± 0.21	11.75 ± 0.07	11.45 ± 0.00

Table 4.13: Moisture content (%) of *Moringa oleifera* seedlings and sprouts ages, sources and lopping height

Seedling age (months)	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	73.20 ± 1.95	71.60 ± 1.35	73.20 ± 1.10	71.93 ± 1.35	73.36 ± 0.71	72.90 ± 0.71	73.73 ± 0.71	73.20 ± 1.35
	20	74.80 ± 1.15	73.16 ± 1.45	74.40 ± 1.05	73.96 ± 1.45	74.46 ± 1.41	74.70 ± 1.41	74.33 ± 1.41	74.50 ± 1.45
	40	75.36 ± 1.10	74.70 ± 1.35	74.60 ± 1.35	74.56 ± 1.95	74.90 ± 1.35	74.90 ± 1.35	75.10 ± 0.71	75.90 ± 1.55
	60	75.20 ± 1.05	73.83 ± 1.45	74.50 ± 1.45	75.73 ± 1.15	75.60 ± 1.45	75.60 ± 1.45	76.50 ± 0.71	76.70 ± 1.75
6	Control	74.20 ± 1.10	74.20 ± 1.35	71.20 ± 1.35	72.30 ± 0.71	73.43 ± 0.07	73.20 ± 1.10	74.60 ± 0.10	72.10 ± 0.95
	20	74.60 ± 1.05	76.30 ± 1.45	75.30 ± 1.45	73.56 ± 1.41	74.16 ± 1.45	74.70 ± 1.05	75.20 ± 1.05	73.60 ± 1.15
	40	76.70 ± 1.35	76.90 ± 1.35	75.80 ± 1.55	74.33 ± 0.71	74.33 ± 1.95	74.60 ± 1.35	75.30 ± 1.35	73.90 ± 1.10
	60	75.50 ± 1.14	76.85 ± 1.45	74.90 ± 1.75	75.10 ± 0.71	75.96 ± 1.15	72.50 ± 1.45	75.60 ± 1.45	74.50 ± 1.05
8	Control	72.30 ± 1.10	73.30 ± 0.55	73.60 ± 1.80	73.50 ± 1.70	73.50 ± 0.55	72.80 ± 1.10	73.53 ± 0.55	73.66 ± 0.55
	20	73.40 ± 1.05	74.50 ± 0.55	74.90 ± 1.70	76.70 ± 1.60	74.20 ± 0.55	73.95 ± 1.05	75.26 ± 0.75	75.33 ± 0.55
	40	74.50 ± 1.35	74.90 ± 0.75	75.00 ± 1.80	75.60 ± 1.80	75.60 ± 0.75	75.30 ± 1.35	75.40 ± 0.70	75.16 ± 1.05
	60	74.90 ± 1.45	75.00 ± 0.75	74.50 ± 1.70	76.50 ± 1.70	75.70 ± 0.75	74.80 ± 1.45	75.56 ± 1.60	75.16 ± 1.35

4.5.2 Phytochemical analysis

The phytochemical analysis conducted for *Moringa oleifera* leaves showed significant differences across all the locations. Each parameter was expressed in milligram per 100grams of the leaf sample collected.

4.5.2.1 Saponins

The saponin content in four-months-old seedlings ranged from 226.00 to 335.50mg/100g; 291.00 to 235.50 mg/100g, for six-month-old seedlings; and 256.00 to 285.50mg/100g for eight-month-old seedlings (Table 4.14). Variation was observed across the locations. The saponin content in sprouts of *Moringa oleifera* at 20cm from ground level at four months old ranged between 265.00 mg/100g and 297.50 mg/100g, those at 40cm was between 264.00 mg/100g and 305.50 mg/100g while sprout at 60cm had saponin content that ranged from 271.00 mg/100g to 305.50 mg/100g at four months old. For six months old seedlings lopped at 20cm, 40cm and 60cm, saponin content in sprouts of *Moringa oleifera* ranged between 260.00 mg/100g and 301.00 mg/100g, 255.34 mg/100g and 306.00 and 261.40 mg/100g and 300.30 mg/100g respectively. At 20cm, 40cm and 60cm lopping height of eight-month old seedlings, saponin content in the sprout of *Moringa oleifera* ranged from 270.00mg/100g to 295.00 mg/100g, 266.00 mg/100g to 306.00 mg/100g and 280.50 mg/100g to 306.00 mg/100g respectively.

4.5.2.2 Tannins

The tannins content in four-months-old seedlings ranged from 78.42mg/100g to 97.00mg/100g; 85.35 to 95.50 mg/100g, for six-month-old seedlings; and 85.10 to 99.50mg/100g for eight-month-old seedlings (Table 4.15). Variation was observed across the locations. The tannin content in sprouts of *Moringa oleifera* at 20cm from ground level at four months old ranged between 84.61mg/100g and 111.00mg/100g, those at 40cm was between 91.00 mg/100g and 305.50 mg/100g while sprout at 60cm had tannin content that ranged from 91.24mg/100g to 105.50 mg/100g at four months old. For six months old seedlings lopped at 20cm, 40cm and 60cm, tannins content in sprouts of *Moringa oleifera* ranged between 89.50 mg/100g and 111.00 mg/100g, 92.40 mg/100g and 115.50 and 95.60 mg/100g and 115.50 mg/100g respectively. At 20cm, 40cm and 60cm lopping height of eight-month old seedlings, tannins content in the sprout of

Table 4.14: Saponin content (mg/100) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling age (months)	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	275.50 ± 0.71	261.50 ± 2.12	281.00 ± 1.41	335.50 ± 2.42	292.50 ± 0.71	281.00 ± 1.41	226.00 ± 4.41	256.00 ± 0.71
	20	291.00 ± 1.41	266.00 ± 1.41	290.50 ± 0.71	290.50 ± 0.70	297.50 ± 0.71	288.50 ± 1.41	270.50 ± 0.71	265.00 ± 1.29
	40	289.00 ± 2.67	296.00 ± 1.41	305.50 ± 0.71	285.00 ± 2.15	301.00 ± 1.41	281.00 ± 1.41	266.00 ± 1.41	264.00 ± 1.41
	60	288.00 ± 2.82	275.00 ± 0.71	300.50 ± 0.71	300.50 ± 0.70	305.50 ± 0.71	295.50 ± 0.71	273.00 ± 1.41	271.00 ± 1.41
6	Control	261.50 ± 2.95	260.50 ± 0.71	275.50 ± 0.71	281.00 ± 1.41	291.00 ± 1.41	280.50 ± 0.71	235.50 ± 0.71	235.60 ± 2.15
	20	260.00 ± 1.41	266.00 ± 1.41	300.00 ± 1.41	291.00 ± 1.41	301.00 ± 1.41	281.00 ± 1.41	266.00 ± 1.41	264.50 ± 2.60
	40	276.00 ± 1.41	275.50 ± 0.71	306.00 ± 1.41	285.50 ± 0.71	305.50 ± 0.71	295.50 ± 0.71	272.50 ± 0.71	255.34 ± 2.72
	60	291.00 ± 1.41	280.50 ± 0.71	291.00 ± 1.41	300.30 ± 0.71	295.50 ± 0.71	295.50 ± 0.71	291.00 ± 1.41	261.40 ± 2.43
8	Control	265.50 ± 0.70	256.00 ± 1.41	276.00 ± 1.41	275.50 ± 0.71	285.50 ± 0.71	271.00 ± 1.41	263.00 ± 2.83	265.00 ± 0.12
	20	271.00 ± 1.41	271.00 ± 1.41	281.00 ± 1.41	295.00 ± 1.29	291.00 ± 1.41	278.00 ± 1.41	286.00 ± 1.41	270.00 ± 0.12
	40	275.50 ± 0.70	266.00 ± 1.41	290.50 ± 0.71	306.00 ± 1.41	295.00 ± 0.71	289.00 ± 1.41	271.00 ± 1.41	286.00 ± 0.12
	60	285.50 ± 0.70	286.00 ± 1.41	280.50 ± 0.71	306.00 ± 1.41	296.00 ± 1.41	280.50 ± 0.71	291.00 ± 1.41	286.00 ± 0.12

Table 4.15: Tannin content (mg/100g) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling Age Months	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	97.00 ± 1.41	95.50 ± 0.71	85.50 ± 0.71	91.00 ± 1.41	85.60 ± 0.71	95.50 ± 0.71	80.50 ± 0.71	78.42 ± 0.71
	20	95.50 ± 0.71	105.50 ± 0.71	111.00 ± 1.41	95.50 ± 0.71	101.00 ± 1.41	105.50 ± 0.71	85.50 ± 0.71	84.61 ± 0.71
	40	100.50 ± 0.71	115.50 ± 0.71	111.00 ± 1.41	101.00 ± 1.41	111.00 ± 1.41	111.00 ± 1.41	91.00 ± 1.41	92.48 ± 0.10
	60	95.50 ± 0.71	101.00 ± 1.41	105.50 ± 0.71	105.50 ± 0.71	105.50 ± 0.71	105.50 ± 0.71	95.50 ± 0.71	91.24 ± 0.71
6	Control	95.50 ± 0.71	90.50 ± 0.71	91.00 ± 1.41	90.50 ± 0.71	91.00 ± 1.41	93.50 ± 0.71	85.50 ± 0.71	85.30 ± 1.42
	20	101.00 ± 1.41	105.50 ± 0.71	105.50 ± 0.71	111.00 ± 1.41	106.00 ± 1.41	105.50 ± 0.71	105.50 ± 0.71	89.50 ± 1.52
	40	115.50 ± 0.71	111.00 ± 1.41	111.00 ± 1.41	105.50 ± 0.71	111.00 ± 1.41	101.00 ± 1.11	98.50 ± 0.71	92.40 ± 1.60
	60	115.50 ± 0.71	101.00 ± 1.41	101.50 ± 0.71	100.50 ± 0.71	100.50 ± 0.71	103.50 ± 2.12	100.50 ± 0.71	95.60 ± 1.72
8	Control	85.50 ± 0.71	99.50 ± 0.71	85.50 ± 0.71	90.50 ± 0.71	85.50 ± 0.71	85.50 ± 0.71	85.50 ± 0.71	85.30 ± 1.15
	20	91.00 ± 1.41	100.50 ± 0.71	96.00 ± 1.23	95.00 ± 0.71	101.00 ± 1.41	101.00 ± 1.41	106.00 ± 1.41	105.30 ± 1.34
	40	91.00 ± 1.41	105.50 ± 0.71	95.50 ± 0.71	85.00 ± 0.10	105.50 ± 0.71	105.50 ± 0.71	105.50 ± 0.71	107.42 ± 1.41
	60	95.00 ± 0.71	105.50 ± 0.71	105.50 ± 0.71	16.50 ± 0.71	115.50 ± 1.41	115.50 ± 0.71	99.00 ± 1.41	110.35 ± 1.38

Moringa oleifera ranged from 91.00 mg/100g to 106.00 mg/100g, 85.00 mg/100g to 107.42 mg/100g and 95.00 mg/100g to116.50.00 mg/100g respectively.

4.5.2.3 Phenolics

Phenolic content (expressed in Gallic Acid Equivalent per gram (GAE/g)) in seedlings of *Moringa oleifera* varied at different seedling ages. In four-month-old seedlings, phenolics content ranged from 75.50 to 79.50 GAE/g, for six-month-old seedlings it was 76.00 to 80.50 GAE/g; and 75.75 to 78.50 GAE/g for eight-month-old seedling (Table 4.16). Variation was observed across the locations. The Phenolic content in sprouts of *Moringa oleifera* at 20cm from ground level at four months old ranged between 77.50 GAE/g and 85.50 GAE/g, those at 40cm was between 78.50 GAE/g and 82.00 GAE/g while sprout at 60cm had phenolic content that ranged from 79.00GAE/g to 90.50 GAE/g at four months old. For six months old seedlings lopped at 20cm, 40cm and 60cm, phenolic content in sprouts of *Moringa oleifera* ranged between 78.50 GAE/g and 85.20 GAE/g, 76.50 GAE/g and 84.50 and 79.00 GAE/g and 85.60 GAE/g respectively. At 20cm, 40cm and 60cm lopping height of eight month old seedlings, phenolic content in the sprout of *Moringa oleifera* ranged from 77.75 GAE/g to 80.50 GAE/g, 78.35 GAE/g to 82.50 GAE/g and 78.80 GAE/g to 84.25 GAE/g respectively

4.5.2.4 Terpenoids

The terpenoids content in four-months-old seedlings ranged from 125.50 to 147.50mg/100g; 126.60 to 186.50 mg/100g, for six-month-old seedlings; and 115.50 to 151.00mg/100g for eight-month-old seedlings (Table 4.17). Variation was observed across the locations. The terpenoids content in sprouts of *Moringa oleifera* at 20cm from ground level at four months old ranged between 140.50 mg/100g and 160.50 mg/100g, those at 40cm was between 145.50 mg/100g and 163.50 mg/100g while sprout at 60cm had terpenoids content that ranged from 155.50 mg/100g to 186.50 mg/100g at four months old. For six months old seedlings lopped at 20cm, 40cm and 60cm, terpenoids content in sprouts of *Moringa oleifera* ranged between 131.00 mg/100g and 206.00 mg/100g, 150.50 mg/100g and 171.00 and 155.50 mg/100g and 185.50 mg/100g respectively. At 20cm, 40cm and 60cm lopping height of eight-month old seedlings, terpenoids content in the sprout of *Moringa oleifera* ranged from 126.00 mg/100g to 159.00 mg/100g, 136.00 mg/100g to 161.00 mg/100g and 141.50 mg/100g to 186.00 mg/100g respectively.

Table 4.16: Phenol content (%) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling Age Months	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	75.50 ± 0.71	78.50 ± 0.71	75.50 ± 0.71	76.50 ± 1.41	79.50 ± 0.71	75.50 ± 0.71	78.50 ± 0.71	79.20 ± 0.71
	20	80.50 ± 0.71	77.50 ± 0.71	81.50 ± 0.71	78.50 ± 0.71	80.50 ± 0.71	80.50 ± 0.71	80.50 ± 0.71	85.50 ± 0.71
	40	81.50 ± 0.71	78.50 ± 0.71	82.00 ± 1.41	79.00 ± 1.41	80.50 ± 0.71	78.50 ± 0.71	78.50 ± 0.71	80.25 ± 0.71
	60	80.50 ± 0.71	80.00 ± 0.71	81.50 ± 0.71	79.50 ± 0.71	90.50 ± 0.71	79.00 ± 1.41	81.50 ± 0.71	81.50 ± 0.71
6	Control	76.00 ± 0.00	78.50 ± 0.71	79.50 ± 0.71	77.00 ± 1.41	79.50 ± 0.71	71.50 ± 0.71	80.50 ± 0.71	80.50 ± 1.61
	20	79.50 ± 0.71	78.50 ± 0.71	80.50 ± 0.71	79.40 ± 0.57	82.50 ± 0.71	79.00 ± 0.00	79.00 ± 0.00	85.20 ± 1.72
	40	79.50 ± 0.71	80.50 ± 0.71	82.50 ± 0.71	79.50 ± 0.71	80.50 ± 0.71	76.50 ± 0.71	82.00 ± 1.41	84.50 ± 1.43
	60	82.50 ± 0.71	83.50 ± 0.71	83.50 ± 2.21	79.35 ± 0.92	82.50 ± 0.71	79.00 ± 1.41	84.00 ± 1.41	85.60 ± 1.62
8	Control	75.75 ± 0.35	77.20 ± 0.28	76.10 ± 0.71	77.50 ± 0.71	78.50 ± 0.71	77.50 ± 0.71	77.50 ± 0.71	76.50 ± 1.52
	20	77.75 ± 0.71	78.20 ± 0.85	79.50 ± 0.71	79.50 ± 0.71	80.50 ± 0.71	79.50 ± 0.71	79.50 ± 0.71	79.25 ± 1.30
	40	79.40 ± 0.57	78.35 ± 0.49	79.65 ± 0.21	80.50 ± 0.71	82.50 ± 0.71	81.50 ± 0.71	81.50 ± 0.71	79.50 ± 1.40
	60	79.50 ± 0.71	78.80 ± 0.14	80.55 ± 0.71	79.35 ± 0.71	82.00 ± 0.21	83.00 ± 0.71	83.00 ± 1.40	84.25 ± 1.34

Table 4.17: Terpenoid content (mg/100g) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling Age Months	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	135.50 ± 0.71	125.50 ± 1.71	126.50 ± 1.41	135.50 ± 0.71	135.50 ± 0.71	140.50 ± 0.71	147.50 ± 0.71	140.40 ± 0.71
	20	151.00 ± 1.41	140.50 ± 0.71	140.50 ± 0.71	156.50 ± 1.41	141.00 ± 1.41	160.50 ± 0.71	155.50 ± 0.71	145.30 ± 1.41
	40	161.00 ± 1.41	145.50 ± 0.71	145.50 ± 0.71	145.50 ± 0.71	151.00 ± 1.41	163.50 ± 0.71	161.00 ± 1.41	155.70 ± 1.41
	60	161.00 ± 1.41	156.00 ± 1.41	156.00 ± 1.41	160.50 ± 0.71	155.50 ± 0.71	186.50 ± 0.71	160.50 ± 0.71	156.60 ± 0.71
6	Control	136.00 ± -	155.20 ± 0.71	126.00 ± 1.41	140.50 ± 0.71	130.50 ± 0.71	145.50 ± 0.71	186.50 ± 0.71	145.62 ± 2.40
	20	156.00 ± 1.41	206.00 ± 1.41	131.00 ± 1.41	151.00 ± 1.41	155.50 ± 0.71	151.00 ± 1.41	145.50 ± 0.71	149.71 ± 2.60
	40	166.00 ± 1.41	171.00 ± 1.41	155.50 ± 0.71	150.50 ± 0.71	156.00 ± 1.41	155.50 ± 0.71	150.60 ± 0.71	156.53 ± 2.32
	60	155.50 ± 0.71	185.50 ± 0.71	156.00 ± 1.41	160.50 ± 0.71	160.50 ± 0.71	165.50 ± 0.71	185.50 ± 0.71	159.46 ± 2.50
8	Control	132.50 ± 2.54	135.00 ± 1.32	115.50 ± 0.71	135.50 ± 0.71	151.00 ± 0.71	151.00 ± 2.49	140.50 ± 0.71	145.42 ± 2.83
	20	141.60 ± 1.41	139.00 ± 1.41	126.00 ± 1.41	146.00 ± 1.41	156.00 ± 1.41	159.00 ± 1.41	149.00 ± 1.41	149.51 ± 1.41
	40	141.00 ± 1.41	141.00 ± 1.41	136.00 ± 1.41	151.00 ± 1.41	161.00 ± 1.41	156.00 ± 1.41	156.00 ± 1.41	152.43 ± 1.41
	60	141.50 ± 1.41	155.50 ± 0.71	146.00 ± 0.71	151.00 ± 1.41	156.00 ± 1.41	151.50 ± 0.71	186.00 ± 1.41	164.51 ± 1.41

4.5.2.5 Cardiac glycosides

The cardiac glycosides content in four-months-old seedlings ranged from 0.20 to 0.40mg/100g; 0.20 to 0.40 mg/100g, for six-month-old seedlings; and 0.07 to 1.20mg/100g for eight-month-old seedlings (Table 4.18). Variation was observed across the locations. The cardiac glycosides content in sprouts of *Moringa oleifera* at 20cm from ground level at four months old ranged between 0.35 mg/100g and 1.20 mg/100g, those at 40cm was between 0.25 mg/100g and 0.35 mg/100g while sprout at 60cm had cardiac glycosides content that ranged from 0.20 mg/100g to 0.35 mg/100g at four months old. For six months old seedlings lopped at 20cm, 40cm and 60cm, cardiac glycosides content in sprouts of *Moringa oleifera* ranged between 0.20 mg/100g and 1.15 mg/100g, 0.15 mg/100g and 0.50 and 0.25 mg/100g and 1.25 mg/100g respectively. At 20cm, 40cm and 60cm lopping height of eight-month old seedlings, cardiac glycosides in sprout of *Moringa oleifera* ranged from 0.25mg/100g to 0.45 mg/100g, 0.20 mg/100g to 1.45 mg/100g and 0.20 mg/100g to 1.30 mg/100g respectively.

4.5.2.6 Carotenoids

The carotenoid content in four-months-old seedlings ranged from 163.00 to 169.00mg/100g; 162.50 to 170.00mg/100g, for six-month-old seedlings; and 161.00 to 168.60mg/100g for eight-month-old seedlings (Table 4.19). Variation was observed across the locations. The carotenoid content in sprouts of *Moringa oleifera* at 20cm from ground level at four months old ranged between 161.50 mg/100g and 170.50 mg/100g, those at 40cm was between 162.00 mg/100g and 170.00 mg/100g while sprout at 60cm had carotenoid content that ranged from 163.00 mg/100g to 171.50 mg/100g at four months old. For six months old seedlings lopped at 20cm, 40cm and 60cm, carotenoid content in sprouts of *Moringa oleifera* ranged between 163.00 mg/100g and 172.00 mg/100g, 163.50 mg/100g and 170.50 and 164.00 mg/100g and 171.50 mg/100g respectively. At 20cm, 40cm and 60cm lopping height of eight-month old seedlings, carotenoid content in the sprout of *Moringa oleifera* ranged from 161.50 mg/100g to 169.50 mg/100g, 162.00 mg/100g to 169.50 mg/100g and 161.50 mg/100g to 169.50 mg/100g respectively.

Table 4.18: Cardiac glycoside content (mg/10g) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling Age Months	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	0.40 ± 0.00	0.35 ± 0.00	0.40 ± 0.00	0.30 ± 0.00	0.30 ± 0.00	0.20 ± 0.00	0.30 ± 0.00	0.30 ± 0.00
	20	0.35 ± 0.00	0.70 ± 0.00	1.20 ± 0.00	1.15 ± 0.00	1.15 ± 0.00	0.35 ± 0.00	1.15 ± 0.00	0.35 ± 0.00
	40	0.30 ± 0.00	0.25 ± 0.00	0.35 ± 0.00	0.35 ± 0.00	0.30 ± 0.00	0.30 ± 0.00	0.35 ± 0.00	0.33 ± 0.00
	60	0.20 ± 0.00	0.25 ± 0.00	0.30 ± 0.00	0.30 ± 0.00	0.25 ± 0.00	0.30 ± 0.00	0.30 ± 0.00	0.35 ± 0.00
6	Control	0.30 ± 0.00	0.25 ± 0.00	0.40 ± 0.00	0.30 ± 0.00	0.35 ± 0.00	0.35 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
	20	1.15 ± 0.00	0.20 ± 0.00	0.40 ± 0.00	0.25 ± 0.00	0.20 ± 0.00	0.40 ± 0.00	0.35 ± 0.00	0.25 ± 0.00
	40	0.30 ± 0.00	0.35 ± 0.00	0.50 ± 0.00	0.25 ± 0.00	0.35 ± 0.00	0.15 ± 0.00	0.30 ± 0.00	0.31 ± 0.00
	60	0.25 ± 0.00	0.25 ± 0.00	0.35 ± 0.00	0.70 ± 0.00	1.20 ± 0.00	1.25 ± 0.00	0.45 ± 0.00	0.34 ± 0.00
8	Control	0.30 ± 0.00	0.30 ± 0.00	0.35 ± 0.00	0.07 ± 0.00	1.20 ± 0.00	1.15 ± 0.00	0.35 ± 0.00	0.32 ± 0.00
	20	0.25 ± 0.00	0.45 ± 0.00	0.30 ± 0.00	0.25 ± 0.00	0.35 ± 0.00	0.30 ± 0.00	0.30 ± 0.00	0.42 ± 0.00
	40	0.20 ± 0.00	0.40 ± 0.00	0.20 ± 0.00	0.25 ± 0.00	0.30 ± 0.00	0.25 ± 0.00	0.45 ± 0.00	1.45 ± 0.00
	60	0.35 ± 0.00	0.40 ± 0.00	0.35 ± 0.00	0.40 ± 0.00	1.30 ± 0.00	0.20 ± 0.00	0.40 ± 0.00	0.40 ± 0.00

Table 4.19: Carotenoids content (mg/100g) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling age (months)	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	167.50 ± 2.36	165.50 ± 2.18	169.00 ± 2.36	163.00 ± 1.87	168.00 ± 1.97	167.00 ± 2.05	160.50 ± 2.55	166.00 ± 2.18
	20	169.00 ± 1.87	167.50 ± 1.97	170.50 ± 1.87	163.50 ± 2.05	169.50 ± 2.55	167.50 ± 2.33	161.50 ± 2.18	167.50 ± 2.36
	40	168.00 ± 2.05	168.50 ± 2.14	170.00 ± 2.05	164.00 ± 2.55	169.50 ± 2.18	168.50 ± 2.55	162.00 ± 2.36	168.00 ± 1.97
	60	169.00 ± 2.33	168.00 ± 2.14	171.50 ± 2.33	164.50 ± 2.18	170.00 ± 1.97	168.00 ± 2.14	163.00 ± 1.87	169.00 ± 2.55
6	Control	167.50 ± 2.36	168.00 ± 2.18	170.00 ± 1.87	163.35 ± 1.87	167.50 ± 2.18	167.00 ± 1.97	162.50 ± 1.97	163.00 ± 1.87
	20	169.50 ± 1.87	168.50 ± 2.36	172.00 ± 2.05	164.50 ± 2.05	167.50 ± 2.18	168.00 ± 2.55	163.00 ± 2.55	164.00 ± 2.05
	40	169.00 ± 2.05	169.00 ± 1.97	170.50 ± 2.05	165.00 ± 2.55	169.00 ± 1.97	167.50 ± 2.05	163.50 ± 2.18	164.50 ± 1.97
	60	170.00 ± 2.33	169.00 ± 2.55	171.50 ± 2.33	164.50 ± 2.18	169.50 ± 2.55	170.50 ± 2.55	164.00 ± 2.36	165.00 ± 2.55
8	Control	12.15 ± 0.21	11.55 ± 1.06	11.20 ± 1.20	10.75 ± 0.07	10.70 ± 0.28	11.45 ± 0.07	161.00 ± 1.87	162.00 ± 2.36
	20	12.30 ± 0.14	12.20 ± 0.28	10.90 ± 0.57	11.05 ± 0.07	10.95 ± 0.07	10.85 ± 0.07	161.50 ± 2.05	164.50 ± 1.87
	40	12.25 ± 0.07	12.05 ± 0.07	11.40 ± 0.00	11.75 ± 0.07	11.75 ± 0.07	11.80 ± 0.14	162.00 ± 2.55	165.00 ± 2.05
	60	12.30 ± 0.14	12.15 ± 0.07	11.45 ± 0.07	11.85 ± 0.07	11.90 ± 0.00	11.64 ± 0.21	161.50 ± 2.18	165.50 ± 2.33

4.5.2.7 Flavonoids

The flavonoid content in four-months-old seedlings ranged from 675.00 to 690.00mg/100g; 680.00 to 705.00 mg/100g, for six-month-old seedlings; and 657.00 to 695.00mg/100g for eight-month-old seedlings (Table 4.20). Variation was observed across the locations. The flavonoid content in sprouts of *Moringa oleifera* at 20cm from ground level at four months old ranged between 680.00 mg/100g and 700.00 mg/100g, those at 40cm was between 685.00 mg/100g and 705.00 mg/100g while sprout at 60cm had flavonoid content that ranged from 695.00 mg/100g to 705.00 mg/100g at four months old. For six months old seedlings lopped at 20cm, 40cm and 60cm, flavonoid content in sprouts of *Moringa oleifera* ranged between 685.00 mg/100g and 705.00 mg/100g, 695.00 mg/100g and 710.00 and 685.00 mg/100g and 715.00 mg/100g respectively. At 20cm, 40cm and 60cm lopping height of eight-month old seedlings, flavonoid content in the sprout of *Moringa oleifera* ranged from 670.00 mg/100g to 695.00 mg/100g, 680.00mg/100g to 705.00 mg/100g and 685.00 mg/100g to 700.00 mg/100g respectively.

4.5.2.8 Steroids

The steroids content in four-months-old seedlings ranged from 60.00 to 90.00 mg/100g; 65.00 to 85.00 mg/100g, for six-month-old seedlings; and 65.00 to 85.00mg/100g for eight-month-old seedlings (Table 4.21). Variation was observed across the locations. The steroids content in sprouts of *Moringa oleifera* at 20cm from ground level at four months old ranged between 65.00 mg/100g and 95.00 mg/100g, those at 40cm was between 65.00 mg/100g and 100.00 mg/100g while sprout at 60cm had steroids content that ranged from 70.00 mg/100g to 95.00 mg/100g at four months old. For six months old seedlings lopped at 20cm, 40cm and 60cm, steroids content ranged between 70.00 mg/100g and 90.00 mg/100g, 70.00 mg/100g and 95.00 and 75.00 mg/100g and 95.00 mg/100g respectively. At 20cm, 40cm and 60cm lopping height of eight-month old seedlings, steroids content in the sprout of *Moringa oleifera* ranged from 70.00mg/100g to 95.00 mg/100g, 70.00 mg/100g to 90.00 mg/100g and 75.00 mg/100g to 95.00 mg/100g respectively.

Table 4.20: Flavonoid content (mg/100g) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling age (months)	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK
4	Control	690.00 ± 2.36	690.00 ± 2.67	685.00 ± 2.78	680.00 ± 2.19	680.00 ± 2.98	680.00 ± 2.59	675.00 ± 2.33
	20	695.00 ± 1.87	675.00 ± 2.78	700.00 ± 2.69	690.00 ± 2.05	690.00 ± 2.33	685.00 ± 2.64	680.00 ± 2.16
	40	700.00 ± 2.05	700.00 ± 2.69	705.00 ± 2.09	695.00 ± 2.45	695.00 ± 2.35	690.00 ± 2.55	685.00 ± 2.70
	60	695.00 ± 2.33	705.00 ± 2.79	695.00 ± 2.98	705.00 ± 2.98	695.00 ± 2.44	705.00 ± 2.43	705.00 ± 2.11
6	Control	680.00 ± 2.83	695.00 ± 2.67	695.00 ± 2.67	705.00 ± 2.19	690.00 ± 2.78	680.00 ± 2.33	680.00 ± 2.67
	20	690.00 ± 1.41	705.00 ± 2.78	700.00 ± 2.78	705.00 ± 2.05	695.00 ± 2.69	690.00 ± 2.16	685.00 ± 2.78
	40	695.00 ± 1.41	710.00 ± 2.69	705.00 ± 2.69	710.00 ± 2.45	695.00 ± 2.09	695.00 ± 2.7	695.00 ± 2.69
	60	685.00 ± 1.41	715.00 ± 2.79	715.00 ± 2.79	710.00 ± 2.22	705.00 ± 2.98	690.00 ± 2.11	695.00 ± 2.79
8	Control	685.00 ± 2.19	685.00 ± 2.59	685.00 ± 2.33	685.00 ± 2.13	695.00 ± 2.09	685.00 ± 2.67	675.00 ± 2.98
	20	685,00 ± 2.05	690.00 ± 2.16	690.00 ± 2.16	695.00 ± 2.78	690.00 ± 2.98	670.00 ± 2.78	685.00 ± 2.33
	40	690.00 ± 2.45	705.00 ± 2.55	695.00 ± 2.70	690.00 ± 2.35	700.00 ± 2.33	680.00 ± 2.69	705.00 ± 2.35
	60	695.00 ± 2.22	700.00 ± 2.43	695.00 ± 2.11	695.00 ± 2.44	685.00 ± 2.56	695.00 ± 2.79	700.00 ± 2.44

Table 4.21: Steriod content (mg/100g) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling age (months)	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJRI
4	Control	85.00 ± 1.02	90.00 ± 1.41	75.00 ± 1.67	60.00 ± 1.32	70.00 ± 1.13	75.00 ± 1.15	75.00 ± 1.56	80.00 ± 1.00
	20	90.00 ± 1.04	95.00 ± 1.41	80.00 ± 2.09	65.00 ± 1.34	65.00 ± 1.34	85.00 ± 1.34	85.00 ± 1.47	85.00 ± 1.00
	40	95.00 ± 1.34	100.00 ± 0.71	85.00 ± 1.99	65.00 ± 1.34	75.00 ± 1.57	85.00 ± 1.27	90.00 ± 1.76	90.00 ± 1.00
	60	95.00 ± 1.43	95.00 ± 1.35	85.00 ± 2.56	70.00 ± 0.71	70.00 ± 1.66	90.00 ± 1.89	95.00 ± 1.89	90.00 ± 1.00
6	Control	65.00 ± 1.67	80.00 ± 1.15	75.00 ± 1.47	65.00 ± 1.02	65.00 ± 1.56	80.00 ± 1.41	80.00 ± 1.67	85.00 ± 1.41
	20	70.00 ± 2.09	85.00 ± 1.34	80.00 ± 1.76	70.00 ± 1.04	75.00 ± 1.47	85.00 ± 1.41	85.00 ± 2.09	90.00 ± 1.41
	40	75.00 ± 1.99	85.00 ± 1.27	85.00 ± 1.34	75.00 ± 1.34	70.00 ± 1.76	90.00 ± 0.71	90.00 ± 1.99	95.00 ± 1.41
	60	80.00 ± 2.56	90.00 ± 1.89	75.00 ± 1.27	75.00 ± 1.43	75.00 ± 1.89	95.00 ± 1.34	90.00 ± 2.56	85.00 ± 1.41
8	Control	85.00 ± 0.71	80.00 ± 1.15	80.00 ± 1.15	65.00 ± 1.02	65.00 ± 1.47	75.00 ± 2.09	80.00 ± 1.15	85.00 ± 1.67
	20	95 ± 1.41	85.00 ± 1.34	85.00 ± 1.34	70.00 ± 1.04	70.00 ± 1.76	80.00 ± 2.56	85.00 ± 1.34	95.00 ± 2.09
	40	90.00 ± 0.71	90.00 ± 1.27	90.00 ± 1.27	70.00 ± 1.34	75.00 ± 1.34	90.00 ± 1.99	90.00 ± 1.27	90.00 ± 1.99
	60	90.00 ± 1.41	90.00 ± 1.89	95.00 ± 1.89	75.00 ± 1.43	75.00 ± 1.27	95.00 ± 1.33	95.00 ± 1.89	95.00 ± 2.56

4.5.2.9 Alkaloids

The alkaloid content in four-months-old seedlings ranged from 170.00 to 192.50mg/100g; 175.00 to 185.83 mg/100g, for six-month-old seedlings; and 170.00 to 195.00 mg/100g for eight-month-old seedlings (Table 4.22). Variation was observed across the locations. The alkaloid content in sprouts of *Moringa oleifera* at 20cm from ground level at four months old ranged between 184.17 mg/100g and 195.00 mg/100g, those at 40cm was between 182.50 mg/100g and 205.00 mg/100g while sprout at 60cm had alkaloid content that ranged from 180.00 mg/100g to 197.50 mg/100g at four months old. For six months old seedlings lopped at 20cm, 40cm and 60cm, alkaloid content ranged between 175.00 mg/100g and 195.00 mg/100g, 175.00 mg/100g and 193.33 and 178.00 mg/100g and 195.00 mg/100g respectively. At 20cm, 40cm and 60cm lopping height of eight-month old seedlings, alkaloid content in the sprout of *Moringa oleifera* ranged from 185.00 mg/100g to 195.00 mg/100g, 180.00 mg/100g to 205.00 mg/100g and 180.00 mg/100g to 208.00 mg/100g respectively.

4.5.3 Leaf biomass production

Analysis of variance conducted to show the effect of seed source, age and lopping height on leaf biomass production of *Moringa oleifera* showed significant difference across all the locations (Table 4.23). Leaf production was poor at four-month old seedlings, only two locations; Akure and Ijare produce leaves hence, leaf biomass was 8.21g and 7.10g respectively. For six-month old seedlings, leaf production was fair; 50% of the location produced leaves and leaf biomass ranged from 0.90g to 2.34g. For eight-month old seedling, leaf production was fairly-good and leaf biomass ranged between 2.42g and 20.91g. The sprouts of *Moringa oleifera* at 20cm,40cm and 60cm from ground level at four months were poor across the location. It ranged between 004g and 5.76g, 0.51g and 6.41g and 0.22g and 6.55g respectively among three locations. Sprout from six-months old seedlings lopped at 20cm, 40cm and 60cm, produced leaf biomass that ranged from 0.90g to 4.83g, 0.13g to 1.23g and 0.37g to 3.32g respectively within five locations. At 20cm, 40cm and 60cm lopping height of eight-month old seedlings, leaf biomass in the sprout of *Moringa oleifera* ranged among six locations from 2.23g to 11.31g, 2.42g to 20.91g and 0.07g to 3.32g respectively.

Table 4.22: Alkanoid content (mg/100g) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling Age Months	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJR
4	Control	180.83 ± 1.67	191.67 ± 2.37	192.50 ± 2.13	170.00 ± 2.98	170.00 ± 2.45	185.15 ± 2.45	185.05 ± 2.33	190.50 ± 1.00
	20	185.00 ± 2.09	184.17 ± 1.87	186.25 ± 2.45	195.00 ± 2.33	180.00 ± 2.17	190.00 ± 2.17	190.00 ± 2.35	195.00 ± 2.00
	40	182.50 ± 1.99	185.83 ± 2.05	188.75 ± 2.17	190.00 ± 2.35	185.00 ± 1.87	195.00 ± 2.35	195.00 ± 2.13	205.00 ± 2.00
	60	180.83 ± 2.56	189.17 ± 2.33	192.50 ± 2.33	190.00 ± 2.44	180.00 ± 2.05	197.00 ± 2.44	195.00 ± 2.45	197.50 ± 2.00
6	Control	175.00 ± 2.36	175.00 ± 2.45	182.50 ± 1.87	177.50 ± 2.36	185.83 ± 2.33	182.50 ± 2.36	175.00 ± 1.67	180.00 ± 2.36
	20	190.00 ± 1.87	175.00 ± 2.17	190.00 ± 2.05	183.33 ± 1.87	189.17 ± 2.35	185.00 ± 1.87	195.00 ± 2.09	195.00 ± 1.86
	40	190.00 ± 2.05	175.00 ± 2.35	190.00 ± 2.33	184.17 ± 2.05	193.33 ± 2.13	187.50 ± 2.05	190.00 ± 1.99	192.00 ± 2.05
	60	190.50 ± 2.33	180.00 ± 2.44	185.62 ± 2.11	179.17 ± 2.33	190.33 ± 2.45	183.50 ± 2.33	178.00 ± 2.56	195.00 ± 2.33
8	Control	185.00 ± 2.45	175.00 ± 2.17	170.00 ± 2.45	190.00 ± 1.67	195.00 ± 1.87	186.25 ± 2.13	183.33 ± 2.45	189.17 ± 2.13
	20	190.00 ± 2.17	195.00 ± 2.35	185.00 ± 2.17	195.00 ± 2.09	195.00 ± 2.05	193.00 ± 2.45	188.33 ± 2.17	195.00 ± 2.45
	40	200.00 ± 2.35	180.00 ± 2.67	180.00 ± 2.35	205.00 ± 1.99	190.00 ± 2.33	187.50 ± 2.17	188.83 ± 2.35	193.33 ± 2.17
	60	208.00 ± 2.44	185.00 ± 2.44	180.00 ± 2.44	205.00 ± 2.56	185.00 ± 2.45	183.25 ± 2.33	184.64 ± 2.44	187.00 ± 2.33

Table 4.23: Leave biomass content (mg/100g) of *Moringa oleifera* seedlings and sprouts at different ages, sources and lopping heights

Seedling age (months)	lopping height (cm)	AK	IJR	IJY	OY	OM	ERI	ABK	IJRI
4	Control	8.22 ± 0.23	7.10 ± 0.35	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	20	5.76 ± 0.19	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.00	0.26 ± 0.00	0.00 ± 0.00
	40	6.41 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.51 ± 0.01	1.65 ± 0.02
	60	4.71 ± 0.15	6.55 ± 0.26	0.00 ± 0.00	3.14 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.00
6	Control	0.90 ± 0.03	0.00 ± 0.00	2.29 ± 0.03	2.34 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	2.07 ± 0.04	0.00 ± 0.00
	20	0.90 ± 0.03	0.00 ± 0.00	1.80 ± 0.01	2.38 ± 0.02	0.00 ± 0.00	0.97 ± 0.01	4.83 ± 0.06	0.00 ± 0.00
	40	0.90 ± 0.03	0.00 ± 0.00	0.13 ± 0.00	1.23 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	60	0.90 ± 0.03	3.32 ± 0.04	0.07 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.99 ± 0.03	0.37 ± 0.01	0.00 ± 0.00
8	Control	5.57 ± 0.17	20.91 ± 0.56	2.42 ± 0.01	5.00 ± 0.90	0.00 ± 0.00	4.81 ± 0.25	9.90 ± 0.47	0.00 ± 0.00
	20	11.31 ± 0.31	9.14 ± 0.34	0.00 ± 0.00	2.23 ± 0.03	0.00 ± 0.00	6.44 ± 0.31	10.09 ± 0.51	0.00 ± 0.00
	40	5.57 ± 0.17	20.91 ± 0.56	2.42 ± 0.01	5.00 ± 0.90	0.00 ± 0.00	4.81 ± 0.25	9.90 ± 0.47	0.00 ± 0.00
	60	0.69 ± 0.01	3.32 ± 0.05	0.07 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.99 ± 0.04	0.37 ± 0.01	0.00 ± 0.00

4.6 Genetic characterisation of *Moringa oleifera* using Simple Sequence Repeat (SSR) molecular markers

Distinct, well-resolved and unambiguous scorable polymorphic bands were observed from the genetic characterization of eighty accessions of *Moringa oleifera* collected from different locations in southwestern Nigeria (Plate 4.2). Five SSR markers formed these polymorphic bands and ranged in size from 80 base pairs (bp) to 350bp. (Table 4.24).

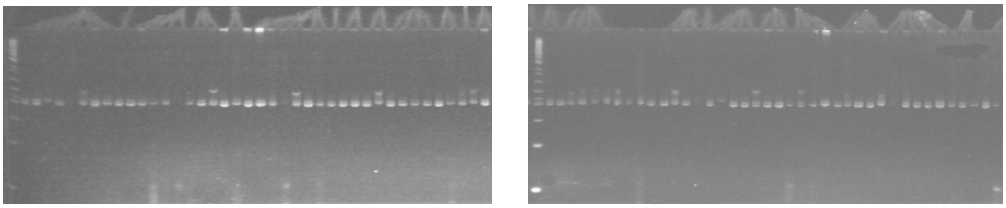
The polymorphic loci percentage variance ranged from 34.78 per cent in ABK to 91.30 per cent in IJY, with a mean value of 61.41 per cent. (Table 4.25). In the 80 accessions, specific alleles (SA) with a range of values from 8 to 11 per locus were detected through the marker MO15 to MO64, with a total number of 96 alleles (AN) (Table 4.26). On average, 9.6 alleles were amplified by loci. The major allele (MA) frequency ranged from 0.24 for the MO64 marker to 0.54 for the MO48 marker, with a mean of 0.407. Genetic diversity (DG) was high and ranged from 0.66 for marker MO48 to 0.85 for marker MO64 with an average of 0.75. Shannon Information Index (I) ranged from 0.24 in marker MO61 to 0.41 in marker MO8, with a mean value of 0.37.

4.6.1: Allelic patterns of *Moringa oleifera* populations in Southwestern Nigeria

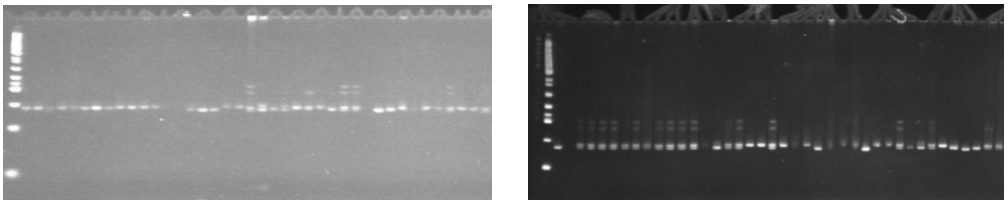
The number of different alleles (NB) found across the three categories of frequency; (≥5%, ≥5% but ≤25% and ≥5% but ≤50%) in the population of *M. oleifera* in Southwestern Nigeria is shown in (Table 4.27). In the first category (≥5 %,), the number of alleles was the same (15) for Akure, Erinjiyan, and Ijare and similar for Omu and Oyo while the lowest was found in Abeokuta (10).

The highest number of alleles was recorded for Ijaye (22). The number of effective alleles (Ne) was the same (0.50) for Abeokuta, Akure and Ijare and also the same (1.00) for Erinjiyan, Ijari, Ijaye and Oyo. However, it was absent in Omu. In this result, a higher number of different alleles were discovered in both Ijari and Ijaye (19 and 22) with one unique allele each which was absent in other populations (Table 4.27). In the second category (≥5% but ≤ 25%), only AK and IJY had one allele each within the eight populations. In the third category (≥5% but≤50%), alleles were present in all, except Abeokuta (Table 4.27). The mean heterozygosity (more) ranged from 0.107 (Abeokuta) to 0.314 in the Ijaye population.

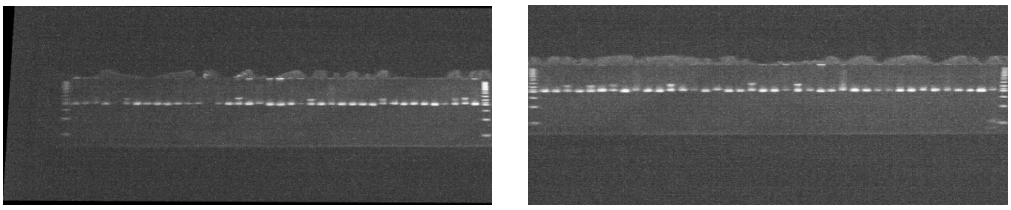
MO8 samples 1-40 41-80



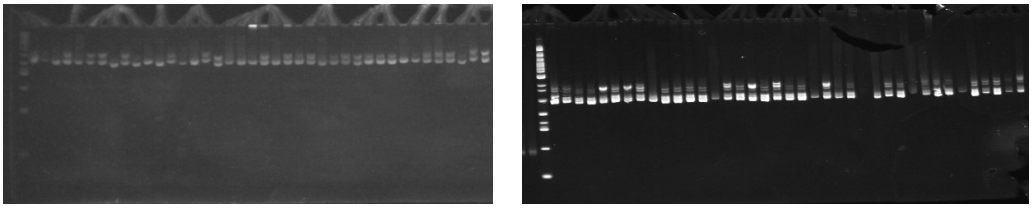
MO15 Samples 1-40 41-80



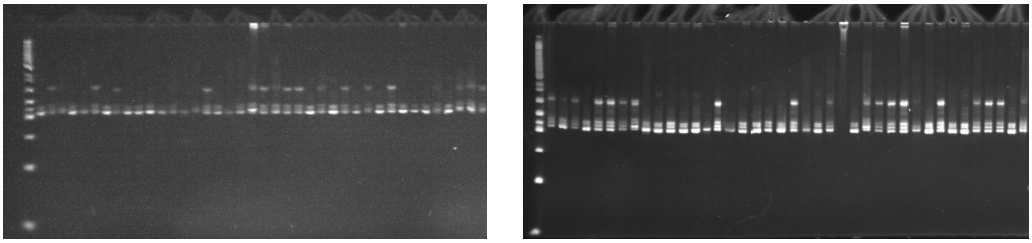
MO48 samples1-40 41-80



MO61 samples 1-40 41-80



MO64 samples 1-40 41-80



Plates 4.2: Five Simple Sequence Repeat (SSR) reproducible scorable bands produced from 80 accessions of *Moringa oleifera* collected from eight locations in Southwestern Nigeria.

Table 4.24: The sequences and repeat motif of the five selected markers used for this study

Locus	Marker sequence forward (5' – 3') Reverse (5' – 3')	Repeat motif
MO8	F GTAGATGGTGCAGCTACTCA R TGGGGTTCTTGTTCTTTATT	$(CT)_{13}$
MO15	F CCCCTCTATTTCCATTTTCC R GCTCCATAAACCCTCTTGCT	$(TC)_{10}$ CCT $(TC)_{6}$
MO48	F AGAAGAACCCAACAGAGGAT R CTTTTCACTAACCACCACCC	$(TC)_{8}$ C $(CT)_{15A}$ $(AC)_{7}$
MO61	F TGTGGGTCCTGCCTTTTCTC R CTTCTGTCTTTCTTCCTGCT	$(TC)_{11}$
MO64	F TCGGCACCTTCTTCCTCTTT R AATCCCTTGACGGACACCAG	$(TC)_{14}$ G $(CT)_{9}$

Table 4.25: Polymorphic loci of *Moringa oleifera* across the selected locations in Southwestern Nigeria

LOCATIONS	PERCENTAGE (%)
Abeokuta	34.78
Akure	60.87
Erinjiyan	60.87
Ijare	56.52
Ijari	69.57
Ijaye	91.30
Omu	69.57
Oyo	47.83
Mean	61.41
SE	5.89

Table 4.26: Genetic parameters estimates of the five SSR markers used for this study

M	MA	SA	AN	GD	PIC	I
MO15	0.50	8	16	0.69	0.66	0.34
MO61	0.46	10	22	0.72	0.71	0.24
M08	0.30	9	17	0.78	0.75	0.41
MO64	0.24	11	26	0.85	0.83	0.28
MO48	0.54	10	15	0.66	0.62	0.27
Mean	0.4075	9.6	9.6	0.75	0.71	0.31

M= Markers, MA= Major Allele Frequency, SA=Specific allele, AN = Number of alleles per locus, GD = Gene Diversity, PIC = Polymorphic Information Content, I = Shannon's Information Index.

Table 4.27: Categories of band patterns for populations of *Moringa oleifera* in Southwestern Nigeria

Population (P)	ABK	AK	ER	IJR	IJRI	IJY	OM	OY
Alleles at Freq.≥5%	10	15	15	15	19	22	16	14
Effective Alleles (Ne)	0.50	0.50	1.00	0.50	1.00	1.00	0.00	1.00
Private Alleles (PA)	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00
Alleles (≥5%; ≤ 25%)	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00
Alleles (≥5%; ≤ 50%)	0.00	2.00	1.00	1.00	3.00	5.00	3.00	2.00
(mHe)	0.107	0.145	0.192	0.133	0.257	0.314	0.259	0.192

Foot note:

P=population

Alleles at Freq.≥5% = No. of Different alleles with a Frequency >= 5%

Ne = No. of Effective bands
PA (Private bands) =No. of alleles unique to a single population
Alleles (≥5% but ≤ 25%) = Number of alleles found in 25% or fewer populations
Alleles (≥5% but ≤ 50%) = Number of alleles found in 50% or fewer populations
(mHe) = Mean Expected Heterozygosity=Level of intraspecific diversity among populations
ABK=Abeokuta
AK=Akure
ER=Erinjiyan
IJR=Ijare
IJRI=Ijari
IJY=Ijaye
OM=Omu
OY=Oyo

4.6.2 Nei Genetic Identity (I) and Distance (D) among and within the population of *Moringa oleifera*

The genetic similarity or identity (I) ranged from 0.826 (lowest) to 0.992 (highest) (Table 4.28) among the locations. The closer genetic frequencies occurred between Ijare and Abeokuta with a value of 0.992 followed by Ijare and Akure (0.986), Akure and Abeokuta (0.980) while the least was found between Ijari and Akure (0.826). However, where I= 1.00 or closer to 1.00, shows equal gene frequencies or how genetically closely related they are while where I= 0.00 or closer to 0.00 indicates that there are no common alleles or how genetically the locations are far from each other.

4.6.3 Percentages of Molecular variance among and within the populations of *Moringa oleifera*

The result of the analysis of molecular variance (AMOVA) among and within the populations of *M. oleifera* is shown in table 4.29. Approximately, genetic diversity was 48 % among the individual populations while variation (significant at p = 0.001) across the populations was 52 %.

4.6.4 Principal Coordinate Analysis (PCoA) of the 80 accessions of *Moringa oleifera*

The scattered plot of the PCoA presented the 80 accessions based on their genetic similarity and distances (Figure 4.4). On both axes, similar accessions and those that are far apart genetically are revealed by the scattered plot. The numerical arrangement of the serial numbers is gathered in such a way that some accessions that belong to the same population (Table 4.30), are genetically similar to one another but far from accession that belongs to another population.

On-axis 1&2, the scattered plot illustrates the level of genetic similarities (Figure 4.4) by gathering together some accessions; 57&45, 74&52, 46&59, 26&62 and 28&71 that belongs to; Ijaye and Ijari, Oyo and Ijaye, Erinjiyan and Omu and Erinjiyan and Oyo respectively even when they do not belong to the same population (Table 4.30). However, some accessions which belong the same population; 2,3&5 (Abeokuta), 14&16 (Akure), 31&32 (Ijare), 51&54 (Ijaye) and 71&73Oyo) are shown to be genetically far from each other.

Table 4.28 : Pairwise population matrix of genetic distance and identity along and across accessions of *Moringa oleifera in* Southwestern Nigeria

(A) Genetic distance

	ABK	AK	ER	IJR	IJRI	IJY	OM	OY	
ABK	0								ABK
AK	0.02	0							AK
ER	0.059	0.047	0						ER
IJR	0.008	0.014	0.053	0					IJR
IJRI	0.191	0.178	0.155	0.176	0				IJRI
IJY	0.16	0.126	0.124	0.154	0.073	0			IJY
OM	0.113	0.09	0.091	0.124	0.182	0.075	0		OM
OY	0.118	0.119	0.097	0.132	0.112	0.047	0.081	0	OY

(B) Genetic Identity

	ABK	AK	ER	IJR	IJRI	IJY	OM	OY	
ABK	1								ABK
AK	0.98	1							AK
ER	0.942	0.954	1						ER
IJR	0.992	0.986	0.949	1					IJR
IJRI	0.826	0.837	0.856	0.838	1				IJRI
IJY	0.852	0.882	0.883	0.857	0.929	1			IJY
OM	0.894	0.914	0.913	0.883	0.834	0.927	1		OM
OY	0.889	0.887	0.908	0.877	0.894	0.954	0.922	1	OY

ABK=Abeokuta Ak= Akure ER= Erinjiyan IJR= Ijare IJRI= Ijari IJY= Ijaye OM= Omu OY= Oyo

Table 4.29: Analysis of Molecular Variance (AMOVA) among and within populations of *Moringa oleifera* from southwestern Nigeria

Source	Df	SS	MS	Est.Var.	Percentage of Molecular Variance (%)
Among population	7	15.28	2.18	0.19	48
Within population	72	15.40	0.21	0.21	52
Total	79	30.68		0.411	100

(Significant at P= 0.001)

Table 4.30: Numerical arrangment of 80 acessions of *Moringa oleifera* from Southwestern Nigeria

s/n	Acc.no	s/n	Acc.no	s/n	Acc.no	s/n	Acc.no	s/n	Acc.no	s/n	Acc.no	s/n	Acc.no	s/n	Acc.no
1	ABK 1	11	AK 1	21	ERI 1	31	IJR 1	41	IJRI 1	51	IJY 1	61	OM 1	71	OY 1
2	ABK 2	12	AK 2	22	ERI 2	32	IJR 2	42	IJRI 2	52	IJY 2	62	OM 2	72	OY 2
3	ABK 3	13	AK 3	23	ERI 3	33	IJR 3	43	IJRI 3	53	IJY 3	63	OM 3	73	OY 3
4	ABK 4	14	AK 4	24	ERI 4	34	IJR 4	44	IJRI 4	54	IJY 4	64	OM 4	74	OY 4
5	ABK 5	15	AK 5	25	ERI 5	35	IJR 5	45	IJRI 5	55	IJY 5	65	OM 5	75	OY 5
6	ABK 6	16	AK 6	26	ERI 6	36	IJR 6	46	IJRI 6	56	IJY 6	66	OM 6	76	OY 6
7	ABK 7	17	AK 7	27	ERI 7	37	IJR 7	47	IJRI 7	57	IJY 7	67	OM 7	77	OY 7
8	ABK 8	18	AK 8	28	ERI 8	38	IJR 8	48	IJRI 8	58	IJY 8	68	OM 8	78	OY 8
9	ABK 9	19	AK 9	29	ERI 9	39	IJR 9	49	IJRI 9	59	IJY 9	69	OM 9	79	OY 9
10	ABK 10	20	AK 10	30	ERI 10	40	IJR 10	50	IJRI 10	60	IJY 10	70	OM 10	80	OY 10

S/N=Serial number

Acc.No=Accession number

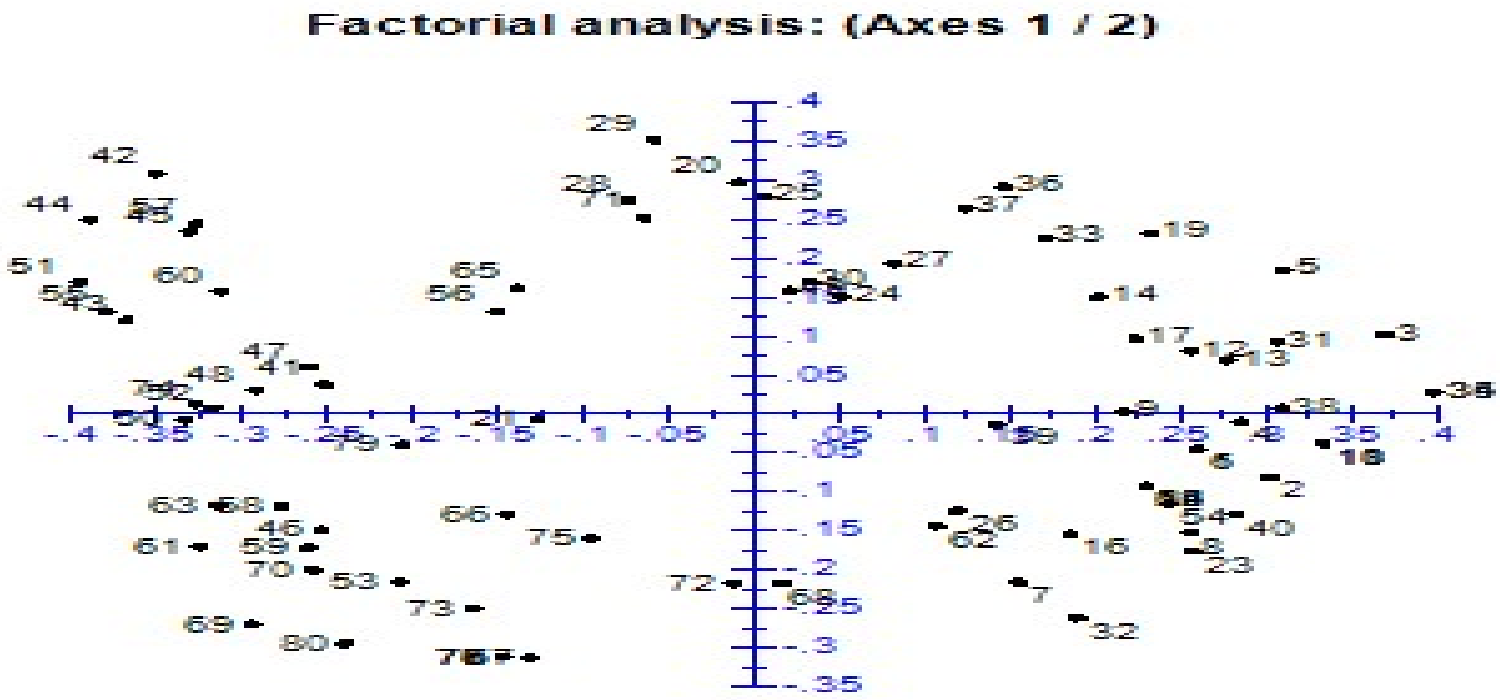


Figure: 4.4: Scatter plot of 80 accessions of *M. oleifera* in Southwestern Nigeria based on 1st and 2nd axes of Principal coordinate analysis using Simple Sequence Repeat marker

4.6.5 Cluster analysis of *Moringa oleifera* accessions from selected sources in Southwestern Nigeria

The cluster analysis partitioned the population of *Moringa oleifera* into five major clusters; A, B, C, D and E (Figure 4.5) with a similarity coefficient of 0.10. Cluster A consist of 16 accessions (ER1, IJRI1, OM5, IJY5……IJY1), cluster B had 36 accessions, ranging from IJY9 to OY4; cluster C consisted of one accession only (IJR9); while cluster D had the largest number of accessions(36), with accession numbers ranging from ER6, ABK2, IJR2, OM6……to AK2 and cluster E having 12 accessions, ranging from OM2 to ER9.

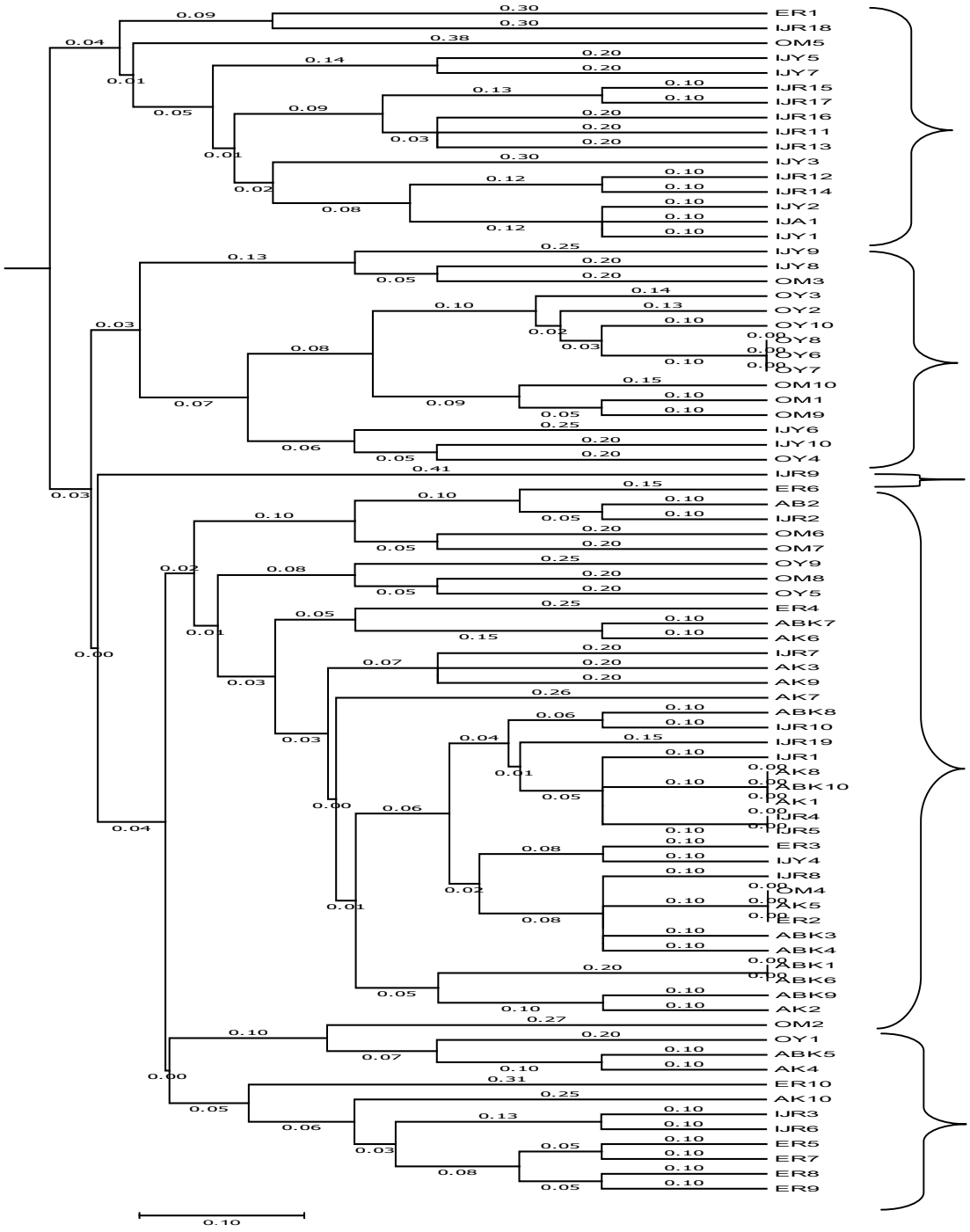


Figure 4.5: Dendrogram generated from simple sequence repeat markers used for 80 accessions of *Moringa oleifera* from Southwestern Nigeria.

CHAPTER FIVE
DISCUSSION

5.1 Population distribution and tree density of *Moringa oleifera* in monoculture plantation

The population of *Moringa oleifera* trees in established plantations in Southwestern Nigeria was not evenly distributed. It was established with different spacing methods. The distribution and density were greatly affected by location with a larger land area having low population density due to spacing. The source of planting materials and the initial advice given for continuous supply of leaves and fruit of *Moringa oleifera* as raw materials to some specific organization may be responsible for different spacing methods adopted by the farmers. Popoola and Obembe (2013) affirmed that the distribution pattern of *Moringa oleifera* across locations in Nigeria is influenced by the source of introduction, domestication and ethnic differentiation. The largest plantation (9.20 ha) found in Erinjiyan had a tree density of 1,848/ha owing to large spacing (8x5m). Wider spacing resulted in lower planting density during plantation establishment (Jiang *et al.* 2007).

However, it was observed from the established plantations that the survival rate of *Moringa oleifera* with reduced spacing was higher than that with high spacing. This agrees with Gregory (2018) who stated that the more closely spaced a plant is, the higher the density. It also supported the assertions that: At lower spacing, there is high plant density because trees tend to grow faster and taller as they are competing for light, soil moisture and nutrients (Woods *et al*., 1992; Amaglo, 2006; Smith *et al*., 2014). The planting densities of 167,000 trees ha^{-1} resulted in 27 tonnes of biomass in a *Moringa oleifera* plantation while planting density of 100,000 ha^{-1} produced 11 tonnes ha^{-1} (Medieta-Araica, 2012).

Besides, considering the four different types of spacing methods adopted across the location; 8m X 5m, 4mX5m, 4m X 4m and 2m X 2m, the percentage of survival rate in each spacing types, commensurate with the rate of reduction from the initial planting density across the location. This result is in line with Nwoboshi, (1982) who

reported that: when the initial plant density of an established plantation is reducing, then the surviving rate which is a subject of the spacing methods adopted is revealed.

On the contrary, species tolerance to the environmental factors at different locations may also account for the tree density in the monoplantation of *Moringa oleifera* across southwestern Nigeria. This is confirmed by Naeem, (2018) that: various factors are responsible for tree density such as; characteristics of species, species tolerance to the environmental factors, growth rate, the condition of the land area and the objectives of plantation establishment such as production of timber, fuel wood, fruits production e.t.c.

5.2 Floral and fruiting patterns of *Moringa oleifera* from Southwestern Nigeria

Flowering is a central element in the life cycle of all angiosperms because it ensures the preservation of species through the formation of seeds (Bareke, 2018). Plants have evolved mechanisms to ensure timely flowering and ensure reproductive success, and the development of fruit or seeds is a direct function of flower induction. Also, environmental stimuli, such as photoperiod, temperature and water availability, are the main factors answerable for flower induction (Muhl *et al*., 2013). Hence, plant's age size or vigour, hormones and nutrient flows, which are endogenous physiological signals are rather derivative factors that further ensure flowery commencement at the appropriate time (Dean and Levy, 1998; Ainsworth, 2006).

Seasonal variation of floral and fruiting patterns of *Moringa oleifera* in Southwestern Nigeria revealed two cycles occurring both during rainy and dry seasons. Parrotta (1993) opined that flowering could occur two times in a year or continuously throughout the year with two peaks of flowering and fruiting, during the rainy and dry season. In Southwestern Nigeria, the rainy season influenced flower initiation (4-9 days), flower formation (3-6 days), fruit initiation (27-30 days) and fruit formation (7-9 days) across the locations. Conversely, in the dry season, flower initiation, flower formation, fruit initiation and yellow pod formation required an average of 5-8 days, 3-6 days, 27-31 days and 88-98 days respectively for completion of the cycle at each developing stage. Time for embryo development varies among different species; it could take several days to many months, and even years for completion (Dumas and Rogowsky, 2008).

The floral and fruiting duration of *Moringa oleifera* cumulative occurred over an average of 186 days. This is required for the completion of all developing stages of *Moringa oleifera* from Bud initiation to matured pod formation. This is in agreement with Resmi *et al*., (2005) who studied the flowering pattern of *Moringa oleifera* for one year in central and southern Kerela and observed variability in its flowering attributes. The duration of the developing stages from flower initiation to fruit formation was comparable within the selected locations, although the timing for every development varied significantly. The development stages were found to occur at slightly different intervals or occurred at the same time at different locations. Location, environmental and edaphic factors tend to induce flowering and fruiting initiation and formation of *Moringa oleifera* in Southwestern Nigeria.

The highest germination percentage was obtained for yellow pods (90.66 ±0.33) followed by brown pods (80.00±0.57) while green pods (30.66±0.88) had the least. This result confirmed the assertion that most fruits announce their maturity through colour changes. Some plant species produce mature green fruits while some immature fruits produce bright colours (Schaefer *et al*., 2008). Maximum germination of harvested seeds at or after the yellow pod stage, under a wide range of field conditions, have been reported for many leguminous plants (Samarah and Mullen, 2004).

5.3 Seed and pods morphology of *Moringa oleifera* from eight locations in Southwestern Nigeria

The pod length values fall within the range (20-50cm) recorded by Foidl *et al*. (2001), Rollof *et al*. (2009) and Dolapo *et al.* (2015). However, the results are contrasts with those (30 – 120cm) reported by Ashfaq *et al*., (2012). This difference may be because trees in the plantations where the pods were collected had a spacing of 2m x 2m and the leaves were not regularly harvested. Also, marked differences were revealed in the pod and seed character analyzed from 40 accessions of *M oleifera* in Nigeria, where the number of pods was significantly correlated with pod length while seed weight was significantly correlated with pod length, pod weight and the number of seed per pod (Popoola *et al*.,2016).

In this study, pod length and seed weight varied significantly however, pod length commensurate with the number of seed per pod even though there was no significant difference in the number of seeds per pod and pod diameter across the

locations. Pod length could vary across the collection area but mostly commensurate with the numbers of seed (Daniel *et al*., 2015). The pod length from Daniel *et al* (2015) study ranged from 19.87cm to 49.50cm with the mean number of seeds of 23.40 falls in line with the value(40.04cm) recorded in this study. Variation in seed weight across the study locations agrees with Nkongmeneck *et al*. (1996), which recorded that the weight of the seed of *M. oleifera* was different from one location to another.

Spacing adopted in a plantation influences tree growth and morphological variation (Hebert *et al*., 2016). The rate of branching among all was the effects of spacing reported by Shaltout *et al* (2017) as the main factor that affected pod morphology of *Moringa oleifera*. He further reported significant variations in pod length, seed weight and the number of seed per pod. The mean pod length of 50.4 cm was higher than the 40.04cm reported in this study while the seed weight (27.00g) was lower to 31.37g reported in this study. Popoola *et al*., (2014) recorded the highest number of seed per pod for southwestern accessions with the highest pod length of 50.00 cm and 35.9g seed weight.

5.4 Seed source effect on germination and early growth of seedlings of *Moringa oleifera* from Southwestern Nigeria

Variation was observed on the effect of seed source on seedling height, collar diameter and the number of leaves across the selected location. These seedling morphological traits increased with time across the selected locations. Edward *et al*. (2014) reported variations in the morphological characteristics of *Moringa oleifera* seedlings based on seed source.

Seasonal variation effects on the mother plants and the variation in the time interval of the developmental stages across the location where seeds were collected may result in different germination rate and growth performances. A previous report by Palada *et al* (2007) showed that Moringa production is dependent on the season and climatic conditions. This observation confirms the assertion that the rate of seedling emergence in *M. oleifera* is influenced by accession and plant growth rate which is determined by climatic and edaphic factors (Nwoboshi 1982; Nkongmeneck *et al*., 1996).

Variation observed in the morphological traits of seedlings across location could also be a result of seed size. Emrah and Fahrettin (2007) revealed the effect of seed size on germination, survival and seedling growth of *Castanea sativa*. The

germination percentage (98.9%) was higher in large seed size than small seed (91.3%). In forest tree species, large seed is regarded to germinate better and produce better seedlings, survival rate and grow stronger. (Karrfalt, 2004). Seed size significantly affect the germination and early growth rate of *Gmelina arborea*. The earliest and highest germination percentage was reported for large seed size. The seedling height, stem collar diameter and number of leaves of large seed size recorded the highest values (Owoh *et al*.,2011).

5.5 Leaf quality and biomass production based on seed source, age and lopping height

Significant variations occurred in the proximate and phytochemical analyses of *Moringa oleifera* across different sources, ages and decapitating heights. Sprouts from 60cm and 40cm lopping heights contained the highest nutrient values across all locations and seedling ages.

The analysis of the soil Physico-chemical properties of the experimental plot revealed the fertility status of the soil. Soil provides the most essential elements needed for plants growth and the fertility of a soil is determined by both its physical properties and its nutrients (Atiku and Noma, 2011). Slightly acidic soil with a pH of 5.72mg/kg was recorded in this study. This was lower than 6.94mg/kg but higher than 3.77mg/kg and 2.41mg/kg of Jamijimi, Yartagimba and Wassaniya respectively (Atiku and Noma, 2011). The plant growth rate has been reported to be faster at soil pH of 5 and below than soil pH of 5 and above (Gentili *et al*., 2018). This value of soil pH makes plants accessible to essential nutrient however, a very high or low pH can distrust plant nutrient uptake.

The soil of the experimental plot in this study varied in texture and ranged from sand, silt to clay with organic matter. Essential elements such as; carbon, nitrogen, sodium, calcium, magnesium, potassium, copper, manganese, zinc, iron and phosphorus were also discovered. Essential elements form part of plant tissues, they act as a catalyst in the various metabolic process and are very important for plants growth and developments (Atiku and Noma, 2011). Nitrogen, phosphorous and potassium are important nutrients needed by plants in large quantity than other elements (Tisdale *et al*., 1993). However, the values recorded for the experimental plot of this study was quite low. This may be due to the previous cultivation methods which

might have exposed the soil to constant leaching. This result agreed with the findings of Noma, (1998) where soil degradation was prominent.

Moringa leaves are highly rich in nutrient and provide an outstanding concentrated protein, vitamins and minerals (Armelle and Melanie, 2010). The high-quality protein is easily digested (Foidl *et al*., 2001) and ranged from 8.45 to 9.10% in this study. This value is higher than 7.2 and 8.1% reported by Kathryn (2013). It, however, conforms to 9.4% recorded in the USDA National Nutrient Database and 8.3 +/- 0.7% of the FAO West African Food Composition Table. Variations exist in the protein content of fresh and dry leaf powder of *Moringa oleifera* (Kumar *et al*., 2016). The protein content in sprouts of *Moringa oleifera* was influenced by decapitating height. However, Bamishaye *et al*. (2011) reported higher protein content of 28.2% in the leaves of *Moringa stenopetala* and also a similar value (28.08%) for *Moringa oleifera.* Although, a condition of differences in species, delay in the harvest (20th week after pruning) and analysis on dry leaf powder of *Moringa oleifera* was stated.

The ash content obtained in this study ranged from 2.95 to 3.12%. It was observed that ash content in fresh leaves of *M. oleifera* was lower than that with dry leaf powder. Onu and Aniebo (2011) as well as Busani *et al*.(2011), reported high ash content values of 7.13% and 10.60 per cent respectively. The ether and crude fibre ranged from 0.70 to 0.95 and 3.45 to 4.35%, respectively. The crude fibre values gotten were comparable to 5.51% reported by Debebe and Eyobel (2017). Related values were obtained by Ijarotimi *et al*. (2013) who gave a detailed report about crude fibre values of powdered leaves of *Moringa stenopetala* which ranged from 3.65 and 4.29%. Epidemiological studies have shown that high dietary fibre intake helps to prevent or treat cardiovascular disease, hypertension, obesity, certain cancers, gastrointestinal disorders and diabetes (Ijarotimi *et al*., 2013).

The carbohydrate content ranged from 12.15 to 12.90% and was higher than 7.6% reported by Kathryn (2013) and 9.1% reported by Abass *et al*. (2018). Although there were significant differences in Moringa leaves from different locations and sprouts from different levels of decapitation even though they grew under the same climatic conditions, it may be due to different seedling age and stages of maturity (Yang *et al*., 2006).

The moisture contents obtained (73.2% to 76.9%) in this study were higher than that of Anthonia (2012), who reported 3.21% of moisture content in dry leaf

powder of *M. oleifera*. Related research findings have also reported different moisture content, which ranged from 9.53 to 11.76%. This was quite lower because it was conducted for dry leaf powder of *M. oleifera* (Ogbe and John, 2011; Busani *et al*., 2011). This is consistent with Debebe and Eyobel's results who was unable to significantly differentiate the moisture content (6.88 and 6.60%) in the dry leaf powder of *M. oleifera* gotten from two agro-ecological zones.

Phytochemicals are the chemical constituents in plants with distinct physiological action on the human body (Vimala *et al*., 2013). The geographic location of the plant and the solvent system used in the extraction process may act as a determining factor for the distribution of these phytochemicals (Deshpande and Kadam, 2013).

Metabolites, such as proteins, vitamins and phenolic compounds, contributed immensely to the derivable benefits of *M. oleifera* (Goyal *et al.,* 2007; Adedapo *et al*., 2009). Measurement of phytochemicals is a widely accepted procedure for identifying the anti-nutritional benefits of plants (Yemis *et al.,* 2008). *Moringa oleifera* leaves could be considered as an antioxidant source with high antioxidant activity reported in earlier studies (Chumark *et al*, 2008; Sreelatha and Padma, 2009).

In the course of quantitative screening of various available phytochemicals in sprouts of *Moringa oleifera* at different ages (4, 6, 8 months), a high presence of different levels of phytochemicals was revealed. These varied significantly across locations and at different decapitating levels. The phytochemical analysis revealed the presence of cardiac, carotenoids, flavonoids, terpenoids, steroids, saponins, tannins, alkaloids and polyphenolic compounds.

The fresh leaves of *Moringa oleifera* collected from 4-months-old seedlings gave the highest flavonoids (675 to 745) and phenolics (75.50 to 90.50mg/100g). *Moringa oleifera* leaves from 6-months-old seedlings gave the highest content of alkaloids (170 to 208), Tannins (91 to 115.50), carotenoids (164 to 172) and terpenoids (126 to 206 mg/100g). Eight-month-old seedlings had the highest content of saponins (256 to 306), cardiac glycosides (0.20 to 1.30) and steroids (70-95 mg/100g)

Moreover, the decapitation level of *Moringa oleifera* seedlings influenced the level of phytochemical content in fresh leaves. The lowest level of phytochemical content was observed in sprouts at 20cm decapitation level across all the accessions, while sprouts at 40cm had highest alkaloids and tannins sprout at 60 cm had optimum

flavonoids, saponins, cardiac glycosides, phenolics, carotenoids, terpenoids and steroids.

Phenolic compounds are usually related to several biological activities and the method by which they put forth their activities differs (Terres-castillo *et al*., 2013). Reportedly, herbs containing tannin as their major components were used for intestinal disorders such as diarrhoea and dysentery (Oluduro, 2012). Alkaloids have analgesic effects (Edeoga *et al*., 2005) and known for their antimicrobial activities, against gram-negative bacteria (Sutradhar *et al*., 2007). Flavonoids are common in plants due to their antifungal activity (Galeotti *et al*., 2008) and induce mechanisms that kill cancer cells and inhibit tumour invasion (Williams *et al*., 2004). Saponins illustrated their beneficial effects on blood cholesterol, cancer, bone health and immune system stimulation; their ability to form froth has made them relevant in producing bathing soap locally from them (Bamishaiye *et al*., 2011).

Steroids improve sex hormones and increase protein synthesis, thereby promoting the growth of muscles and bones. Furthermore, this study reported that *Moringa oleifera* leaves consist of some large phytoconstituents that can produce functional foods and nutraceuticals. The presence of these essential amino acids and carotenoids in the leaves, support the suggestion that they could be used as nutritional supplements or constituents in the preparation of food (Adedapo *et al*., 2015).

Some authors (Adedapo *et al*, 2009, Goyal *et al*, 2007) observed that phytochemicals in leaves of *M. oleifera* cured snake bites, rheumatism pains, asthma, cardiac and circulatory problems. The presence of flavonoids, alkaloids, steroids and carotenoids in *Moringa oleifera* which functions as a powerful antioxidant, anti-inflammatory, aphrodisiac and boost immune, suggest that moringa leaves that they could be recommended for ethnomedicinal use.

5.6 Genetic variability of *Moringa oleifera*

The existence and extent of genetic diversity of *M. oleifera* in Southwestern Nigeria were revealed through the use of five SSR primers. These primers (MO8, MO15, MO48, MO61 and MO64) discovered 96 alleles with a mean of 9.6 per locus. This was greater than 1.84 alleles and 4.75 alleles per locus in 300 accessions and 31 accessions recorded by Ganensa *et al.* (2014) and Amao *et al.* (2017), respectively. It was comparable to the 7.4 and 7 alleles found by Popoola *et al*. (2017) and Rajakakshim *et al*. (2017), which used SSR markers to examine genetic intraspecific

relationships and genetic diversity of the *Moringa oleifera* population structure. The level of the multiple forms of genes (polymorphism) that exist among the eight collection sources was relatively high. The polymorphism of the Ijaye accession was the highest; the next to it was that of Ijari; others displayed similarity, and the least was found in Abeokuta. This is in an agreement with Popoola *et al.* (2017), who observed and that in the populations from the six geopolitical zones (North Central, Northeast, Northwest, Southsouth, Southeast and Southwestern) he worked on, only the Southwestern subgroup displayed the utmost genetic variability.

The level of genetic diversity of *Moringa oleifera* in this study was 52% higher within-population (within farms from one location to another) than 48% among the population (between farms within each location). This similarity among sources revealed that Abeokuta, Akure, Erinjiyan and Ijare were genetically similar at the value range of 0.094 to 0.992 similarity coefficients; while Ijari, Ijaye, Omu and Oyo accessions were genetically far from other accessions, with a value range of 0.889 to 0.193. Principal Coordinate Analysis (PCoA) and SSR markers indicated that Southwestern accessions of *Moringa oleifera* were rich in alleles and implies a large genetic pool of *Moringa oleifera* population in Southwestern Nigeria across the two axes. This result confirms the assertion that consistency in genetic diversity within the population of *Moringa oleifera* is quite higher than that among the population (Muluvi *et al.,* 1999).

Five major groups (obtained from the Unweighted Pair Group Method with Arithmetic (UPGMA) was created from the cluster analysis of 80 accessions in 8 populations from South-West Nigeria. The constructed dendrogram exposed the degree of similarity among these five clusters: although they fell into cluster groups that are made up of accessions from different populations. The findings conform with Rajakakshim *et al.* (2017), who noted that grouping individuals from the same population in different clusters indicate a large genetic diversity in the population, which can be attributed to the use of seed sources, a system of mutations or breeding. This supports the results of Yang *et al*. (2006), which reported a link between the collection and geographical distribution of germplasm.

From these findings, IJR accession (IJR9) appeared the most pronounced of all and existed as a separate cluster. This means that numerous intraspecific variations leading to the formation of other cluster groups may have originally been created from

this accession. This accession from its main location (Ijare) performed excellently well in germination percentage, growth parameters, and morphological characterization. It also had high values from proximate and phytochemical analysis which is an indication of the degree of leaf quality in *Moringa oleifera*. This is closely linked to the biological behaviour and the cultivation mechanism of *Moringa oleifera* (Jyoth *et al*., 1990). *Moringa oleifera* allows two flowers of the same plant (purple and purple flower on the same tree) to be pollinated, which produces genetically identical flowers (geitonogamy) and flowers from a different plant (tree A with purple flower and tree B with white flower) of the same species to be cross-pollinated, resulting in genetically different flowers (xenogamy). These methods of pollination and mechanisms of seed dispersion as well as other methods of propagation in *Moringa oleifera* have been effective in regulating gene flow and increasing gene pool in the population.

Cluster Group C (IJR9) may thus be exceptional and could be adopted for breeding and genetic enhancement. Therefore, by combining this group with other groups, such as Group B (with a small number of populations), variants with high economic value can be formed.

CHAPTER SIX

SUMMARY AND CONCLUSION

6.1 Summary of results

Reproductive phenology and molecular characterisation of *Moringa oleifera* LAM. landraces from eight sources in the rain forest zone of Southwestern Nigeria were carried out.

The distribution of the *Moringa oleifera* population and its tree density from selected plantations in Southwestern Nigeria were determined. Population distribution assessment showed that Erinjiyan had the largest plantation of *Moringa oleifera*, followed by Oyo, Akure, Omu, Ijare, Ijaye and Abeokuta with the total land area of 9.20ha, 6.69 ha, 6.40ha, 2.98ha, 1.60ha and 1.34ha and 0.89ha per hectare respectively while the smallest plantation (0.8ha) was found at Ijari. The tree density varied significantly across locations. This was as a result of lack of uniformity in the enspacement adopted across the locations by the farmers. Erinjiyan had the highest enspacement of 8m by 5m and the density of 1,848 trees/ha. Oyo, Ijaye, Akure, Abeokuta, Omu, and Ijari had density/ha of 12,525, 2,584, 3,200, 1,826, 600 and 1,660, respectively and the least density was found in Ijare (520/ha).

Stages of reproductive phenology across all the selected locations were approximately 186 days for the completion of all phenological processes before fully matured pods of *Moringa oleifera* was achieved. Floral and fruiting duration and fruit maturity index evaluation were alike but varied in timing in some locations because they were found to either occur differently or at the same time.

Seasonal variation influenced the developing stages of *Moringa oleifera* across the selected locations. Significant differences were observed across the two seasons. Developing stages across selected locations were initiated within 3.00$\pm$0.19 and 30.00$\pm$0.55 days during the rainy season while during the dry season, it ranges between 5.00 ± 0.60 and 98.00$\pm$2.82 days. For fruit maturity index evaluation, the highest germination percentage occurred for yellow pod seeds (90.66 ±0.33%) followed by brown (80.00±0.57%), with the green pods having the least (30.66±0.88%) respectively for both year 2015/2016 and 2016/2017.

Morphological variation was observed only in pod length and seed weight across the eight locations. Abeokuta had the longest pods (40.04 ± 1.66cm) while Erinjiyan (27.63±0.64cm) had the shortest. The seed weight (100 seeds) varied

among locations with the highest found in Omu (31.37 ± 1.69g) while the least was found in Ijari (17.19 ± 1.0g).

The influence of seed source on germination and early seedling performance were significantly different across the locations. The result showed that seedling height, collar diameter and the number of leaves produced increased with time across the studied locations. The highest germination percentage, seedling height, seedlings collar diameter and leaf productions were found in Ijare, Omu, Ijari and Erinjiyan with values of 92.50±2.71%, 112 ± 1.39cm, 15.85±0.92 mm and 37.25±0.87 respectively while the least values 5.50±5.50%, 76.92±0.79cm, 9.29±0.57 mm and 20.57±0.51 were found in Omu, Ijari, Abeokuta and Abeokuta respectively.

Leaf quality and biomass production of *Moringa oleifera* was greatly influenced by seed source, seedling age and seedling lopping height. The soil Physico-chemical properties of the experimental plot in this study showed the soil fertility status and revealed the available quantity of the essential elements needed by plants for growth and developments. Analysis of variance conducted on all proximate and phytochemical parameters showed a significant difference across the locations. For proximate analysis, the highest value for protein, ash, ether, crude fibre, carbohydrate and moistures content in *Moringa oleifera* were found in Ijare (8.45 to 9.10%;), (Akure, Oyo and Abeokuta)(2.70 to 3.10%), Ijare (0.75 to 0.90%), Abeokuta (3.45 to 4.35%), Akure (12.15 to 12.50%) and Abeokuta (73.73 to 76.50%). For seedling age, six-month seedlings produced the highest protein (8.45 to 9.10%), ether (0.50 to 2.55 %), and crude fibre (2.25 to 4.35 %) while the highest ash content (2.20 to 3.12%) was gotten in four-month-old seedlings. Carbohydrate (10.70 to 12.30%) and moisture content (72.30 to 76.70%) highest value was produced in eight-month-old seedlings. Seedlings lopped at 60cm produced the highest protein (8.45 to 9.10%), at 40cm, ash, ether, carbohydrate and moisture content had the values of 2.30 to 3.12 %, 0.50 to 0.95 %, 10.85 to 12.65 and 74.33 to 76.90 % respectively while the highest value of crude was produced by seedlings lopped at 20cm across the locations.

The phytochemicals screening of cardiac, carotenoids, flavonoids, terpenoids, steroids, saponins, tannins, alkaloids and polyphenolic compounds in *M. oleifera* leaves at different ages 4, 6 and 8 months varied significantly across locations and at different decapitating levels. Fresh leaves of *Moringa oleifera* collected from 4-months-old seedlings gave the highest flavonoids (675 to 745) and phenolics (75.50 to

90.50mg/100g). Six-months-old gave the highest content of alkaloids (170 to 208), Tannins (91 to 115.50), carotenoids (164 to 172) and terpenoids (126 to 206 mg/100g) while eight-month-old seedlings had the highest content of saponins (256 to 306), cardiac glycosides (0.20 to 1.30) and steroids (70-95 mg/100g).

For lopping height, seedlings lopped at 60 cm produced the highest values of flavonoids, saponins, cardiac glycosides, phenolics, carotenoids, terpenoids and steroids. 40cm produced the highest values of alkaloids and tannins, while the lowest level of phytochemical content was observed in 20cm decapitation level across all the locations**.** Generally, the biomass production of *Moringa oleifera* showed significant difference across all the location. Biomass accumulation was higher in eight-month-old seedlings, followed by four-month and the least was found in six with values 20.9g, 8.22g and 4.83g respectively. For lopping height, seedlings with 40cm had the highest (12.73g), followed by 20cm (11.31g) while the least was found in 60cm(6.55g). Across the locations, Ijare had the highest biomass accumulation with a value of 20.91g while the least(1.65g) was found in the Ijari location.

The presence and level of genetic diversity of *M. oleifera* in Southwestern Nigeria were discovered through the use of five SSR primers (MO8, MO15, MO48, MO61 and MO64). These primers discovered 96 alleles with a mean of 9.6 per locus. Genetic diversity of *Moringa oleifera* was 52% higher within the population (within farms from one location to another) and 48% among the population (between farms within each location). There was a significant difference among the multiple forms of genes (polymorphism) across 80 accessions in 8 populations from South-West Nigeria. Ijaye accession displayed the highest form of polymorphism. The next to it was Ijari and while others exhibited similarity, the least was found in Abeokuta accession. The level of similarity among sources revealed that Abeokuta, Akure, Erinjiyan and Ijare were genetically similar at the value range of 0.094 to 0.992 similarity coefficients; while Ijari, Ijaye, Omu and Oyo accessions were genetically far from other accessions, with a value range of 0.889 to 0.193. Principle Coordinate Analysis (PCoA) with SSR markers indicated that Southwestern accessions of *Moringa oleifera* were rich in alleles which indicates a large genetic pool of *Moringa oleifera* population in Southwestern Nigeria.

The Arithmetic of Unweighted Pair Group Method (UPGMA) through cluster analysis that formed the 80 accessions into five main groups revealed that there is a

degree of similarities among the populations, however, IJR accession (IJR9) was discovered as a separate cluster that exists as a single member of the five clusters. This suggests that various intraspecific variations leading to the creation of other cluster groups may have been initially calved after this accession (IJR9). This accession from its main location (Ijare) performed excellently well in germination percentage, growth parameters, and morphological characterization and had high values from proximate and phytochemical analysis which indicate the leaf quality of *Moringa oleifera*. Therefore, cluster Group C (IJR9) was identified as a unique cluster, which may be used for breeding and genetic enhancement.

6.2 Conclusion

The assessment on variation and molecular characterisation among landraces of *Moringa oleifera* from Southwestern Nigeria has provided essential information towards its values and usage.

The population of *Moringa oleifera* in a monoculture was distributed across eight sources: Oyo, Ijaye, Abeokuta, Ijari, Ijare, Akure, Omu and Erinjiyan, in Southwestern Nigeria. The largest plantation was found in Erinjiyan, while the highest tree density of *Moringa oleifera* was found in Oyo. This variation was a result of different spacing adopted by farmers. The tree density found in each location was commensurate with the spacing adopted for each land area.

Fully mature pods of *Moringa oleifera* required an average of 186 days for the completion of all the developmental stages. Stages of flowering and fruit forming were identical across the sources, but the timing of each reproductive stage across sources differed considerably. The developing stages occurred at a different time interval or occurred at the same time at different locations.

Pod and seed morphology varied for *Moringa oleifera* in Southwestern Nigeria. Pod length and seed weight (100 seeds) varied from one location to another. Pod maturity evaluation was found in the yellow pods, which confirmed that *Moringa oleifera* announces its maturity through a colour change; and its maximum seed germination was got at the yellow pod maturity stage.

Germination and growth of *Moringa oleifera* were influenced by seed source. The highest germination percentage was recorded for seeds collected from Ijare and the least occurred in Omu. The mean height, collar diameter and number of leaves varied significantly across the locations with the highest value found in Omu (height),

Ijari (collar diameter) and Erinjiyan (number of leaves). Across the selected locations per time, the height of seedlings, diameter and leaf number produced increased steadily.

Fresh leaves of *Moringa oleifera* belonging to Southwestern Nigeria at different seedling ages (4, 6, 8 months) are nutritionally rich and an excellent source of concentrated proximate and phytochemicals, with variations across different sources in Southwestern Nigeria. Quantitative screening of both proximates and phytochemicals revealed that leaves harvested from 4-month-old seedlings gave the highest content of flavonoids and phenolics; 6-month-old seedlings gave the highest content of protein, crude fibre, carbohydrate, alkaloids, tannins, carotenoids, and terpenoids; while 8-month-old seedlings influenced the highest content of ash, ether, moisture content, saponins, cardiac glycosides, and steroids.

Simple Sequence Repeat (SSR) markers in their broad exposure capacity were remarkably useful and competent particularly MO64, which discovered the extent and reality of genetic diversity within populations of *M. oleifera* in Southwestern Nigeria. However, Ijare accession was discovered as the most distinct of all because it existed as a separate group in the cluster. It is therefore recommended for adoption as it may possess a unique trait which may be exploited to suggest different genotypes for breeding and genetic improvement program.

6.3 Recommendation

The results obtained from this study showed that significant variation exists in the landraces and genetic composition of *Moringa oleifera* from Southwestern Nigeria. It is therefore recommended that monoculture plantations of *Moringa oleifera*, which has detailed nutritional, medicinal, and genetic information are available in Southwestern Nigeria for germplasm collection and improvement programs. At the maturity level, *Moleifera's* lightly dried or yellowish pods are recommended for harvest and planting because seeds extracted from them gave the best germination percentage. Long pods of Moringa with an average diameter range (27-40) mm is thereby recommended for planting as it has been identified with a large number of seeds. *Moringa oleifera* seedlings decapitated at 60cm or 40cm are thereby recommended for adoption because they produce reasonable quantity and leave quality in terms of proximate and phytochemical potentials. For valuable and proficient genetic diversity studies at the molecular level in *Moringa oleifera*, the MO64 SSR marker is

recommended for use. Across all the selected populations, it was discovered that Ijare accession produced the most distinct genetic information with a unique trait among southwestern Nigerian accessions, therefore, further assessment and adoption inbreeding and genetic improvement program is thereby recommended.

6.4 Contribution to the knowledge

The following are contributions to knowledge:

Current information on the available tree density of *Moringa oleifera* in a monoculture with detailed nutritional, medicinal, and genetic information has been provided. Empirical information on the flora and fruiting duration of *Moringa oleifera* trees has been documented. Pod size-range values that enhance multiple numbers of seeds in Southwestern Nigeria were provided. Improved knowledge of a reliable seed source selection and its effect on germination and early growth of *Moringa oleifera* seedlings is available. Leaf quality and biomass production of *Moringa oleifera* as it is being affected by seed source, seedling age, and lopping height has been provided. Baseline information has been made available for further genetic characterization of *Moringa oleifera* from southwestern Nigeria.

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APPENDIX

Appendix 1: Analysis of variation for the effect of location on bud formation, flower initiation, flower formation and fruit initiation during the rainy season in the year 2015/2016

SV	SS	Df	MS	F	P-Value
(a)Bud formation					
Intercept	315.063	1	315.063	750.787	.000
Location	2.438	7	.348	.830	.594ns
Error	2.938	7	.420		
Total	321.000	16			
(b)Flower initiation					
Intercept	742.563	1	742.563	3615.957	.000
Location	28.938	7	4.134	20.130	.000*
Error	1.438	7	.205		
Total	773.000	16			
(c)Flower formation					
Intercept	280.563	1	280.563	1366.217	.000
Location	12.938	7	1.848	9.000	.005*
Error	1.438	7	.205		
Total	295.000	16			
(d)Fruit initiation					
Intercept	12712.563	1	12712.563	30293.766	.000
Location	12.938	7	1.848	4.404	.035*
Error	2.938	7	.420		
Total	12729.000	16			

*= significant at P≤0.05
ns= not significant at p≤0.05

Appendix 2: Analysis of variation for the effect of location on fruit formation, green pod, yellow pod and brown pod during the rainy season in the year 2015/2016

(a)Fruit formation

SV	SS	Df	MS	F	P-value
Intercept	1008.063	1	1008.063	4908.826	.040
Location	5.438	7	.777	3.783	.040*
Error	1.438	7	.205		
Total	1015.000	16			
(b) Green pod					
Intercept	13225.000	1	13225.000	24686.667	.000
Location	7.000	7	1.000	1.867	.215ns
Error	3.750	7	.536		
Total	13236.000	16			
(c)Yellow pod					
Intercept	127270.563	1	127270.563	14863.715	.000
Location	153.938	7	21.991	2.568	.118ns
Error	59.938	7	8.563		
Total	127485.000	16			
(d) Brown Pod					
Intercept	12656.250	1	12656.250	4991.197	.000
Location	35.750	7	5.107	2.014	.188ns
Error	17.750	7	2.536		
Total	12716.000	16			

Appendix 3: Analysis of variation for the effect of location on bud formation, flower initiation, flower formation and fruit initiation during the rainy season in the year 2016/2017

(a)Bud formation

SV	SS	Df	MS	F	P-value
Intercept	306.250	1	306.250	779.545	.000
Location	4.750	7	.679	1.727	.244ns
Error	2.750	7	.393		
Total	314.000	16			
(b)Flower initiation					
Intercept	689.063	1	689.063	1086.972	.000
Location	27.438	7	3.920	6.183	.014*
Error	4.438	7	.634		
Total	721.000	16			
(c)Flower formation					
Intercept	280.563	1	280.563	1366.217	.000
Location	12.938	7	1.848	9.000	.005*
Error	1.438	7	.205		
Total	295.000	16			
(d)Fruit initiation					
Intercept	12544.000	1	12544.000	17561.600	.000
Location	15.000	7	2.143	3.000	.085*
Error	5.000	7	.714		
Total	12564.000	16			

Appendix 4: Analysis of variation for the effect of location on fruit formation, green pod, yellow pod and brown pod during the rainy season in the year 2016/2017

SV	SS	Df	MS	F	P-value
(a)Fruit formation					
Intercept	1008.063	1	1008.063	4908.826	.040
Location	5.438	7	.777	3.783	.040*
Error	1.438	7	.205		
Total	1015.000	16			
(b) Green pod					
Intercept	13282.563	1	13282.563	15659.442	.000
Location	7.938	7	1.134	1.337	.356ns
Error	5.938	7	.848		
Total	13297.000	16			
(c)Yellow pod					
Intercept	126025.000	1	126025.000	12468.905	.000
Location	152.000	7	21.714	2.148	.167ns
Error	70.750	7	10.107		
Total	126250.000	16			
(d) Brown Pod					
Intercept	12544.000	1	12544.000	3991.273	.000
Location	34.000	7	4.857	1.545	.290ns
Error	22.000	7	3.143		
Total	12604.000	16			

Appendix 5: Analysis of variation for the effect of location on bud formation, flower initiation, flower formation and fruit initiation during the dry season in the year 2015/2016

(a)Bud formation

SV	SS	Df	MS	F	P-value
Intercept	272.250	1	272.250	2541.000	.000
Location	.750	7	.107	1.000	.500ns
Error	.750	7	.107		
Total	274.000	16			
(b)Flower initiation					
Intercept	637.563	1	637.563	10201.000	.000
Location	10.938	7	1.563	25.000	.000*
Error	.438	7	.063		
Total	649.000	16			
(c)Flower formation					
Intercept	315.063	1	315.063	641.582	.000
Location	8.438	7	1.205	2.455	.130ns
Error	3.438	7	.491		
Total	327.000	16			
(d)Fruit initiation					
Intercept	13572.250	1	13572.250	14074.926	.000
Location	26.750	7	3.821	3.963	.045*
Error	6.750	7	.964		
Total	13606.000	16			

Appendix 6: Analysis of variation for the effect of location on fruit formation, green pod, yellow pod and brown pod during the dry season in the year 2015/2016

(a)Fruit formation

SV	SS	Df	MS	F	P-value
Intercept	1008.063	1	1008.063	3642.032	.000
Location	3.438	7	.491	1.774	.234ns
Error	1.938	7	.277		
Total	1015.000	16			
(b) Green pod					
Intercept	13053.063	1	13053.063	31105.170	.000
Location	8.438	7	1.205	2.872	.094ns
Error	2.938	7	.420		
Total	13065.000	16			
(c)Yellow pod					
Intercep	133773.063	1	133773.063	46967.345	.000
Location	125.437	7	17.920	6.292	.013*
Error	19.938	7	2.848		
Total	133929.000	16			
(d) Brown Pod					
Intercept	15500.250	1	15500.250	4931.898	.000
Location	64.750	7	9.250	2.943	.089ns
Error	22.000	7	3.143		
Total	15588.000	16			

Appendix 7: Analysis of variation for the effect of location on bud formation, flower initiation, flower formation and fruit initiation during the dry season in the year 2016/2017

(a)Bud formation

SV	SS	Df	MS	F	P-value
Intercept	289.000	1	289.000	674.333	.000
Location	2.000	7	.286	.667	.697ns
Error	3.000	7	.429		
Total	294.000	16			
(b)Flower initiation					
Intercept	650.250	1	650.250	2275.875	.000
Location	11.750	7	1.679	5.875	.016*
Error	2.000	7	.286		
Total	664.000	16			
(c)Flower formation					
Intercept	306.250	1	306.250	779.545	.000
Location	10.750	7	1.536	3.909	.046*
Error	2.750	7	.393		
Total	320.000	16			
(d)Fruit initiation					
Intercept	13110.250	1	13110.250	7810.362	.000
Location	29.750	7	4.250	2.532	.122
Error	11.750	7	1.679		
Total	13154.000	16			

Appendix 8: Analysis of variation for the effect of location on fruit formation green pod, yellow pod and brown pod during dry season in the year 2016/2017

(a)Fruit formation

SV	SS	Df	MS	F	P-value
Intercept	992.250	1	992.250	2315.250	.000
Location	3.750	7	.536	1.250	.388ns
Error	3.000	7	.429		
Total	1000.000	16			
(b) Green pod					
Intercept	12996.000	1	12996.000	24259.200	.000
Location	10.000	7	1.429	2.667	.109ns
Error	3.750	7	.536		
Total	13010.000	16			
(c)Yellow pod					
Intercept	131406.250	1	131406.250	18125.000	.000
Location	72.750	7	10.393	1.433	.323ns
Error	50.750	7	7.250		
Total	131530.000	16			
(d) Brown Pod					
Intercept	15190.563	1	15190.563	3493.517	.000
Location	96.938	7	13.848	3.185	.075ns
Error	0.438	7	4.348		
Total	15323.000	16			

Appendix 9: Analysis of variation for the effect of pod length, pod diameter, number of seed per pod and seed weight of *Moringa oleifera* in Southwestern Nigeria

(a)Pod length

SV	SS	Df	MS	F	P-value
Location	39334.463	7	5619.209	162.697	.000*
Farm	4016.343	1	4016.343	116.288	.000*
Location * Farm	22888.918	7	3269.845	94.674	.000*
Error	82338.317	2384	34.538		
Total	148578.042	2399			
(b) Pod Diameter					
Location	9107.083	7	1301.012	.819	.571ns
Farm	3779.832	1	3779.832	2.379	.123ns
location * Farm	10826.087	7	1546.584	.973	.449ns
Error	3787677.512	2384	1588.791		
Total	3811390.514	2399			
(c) Number of Seeds per pod					
Location	4089.536	7	584.219	.902	.504ns
Farm	3699.158	1	3699.158	5.709	.017*
Location * Farm	11200.743	7	1600.106	2.470	.016*
Error	1527105.317	2357	647.902		
Total	1545245.642	2372			
(d)100 seed s weight					
location	4055.037	7	579.291	18.919	.000*
Farm	81.162	1	81.162	2.651	.105ns
Location * Farm	3497.466	7	499.638	16.318	.000*
Error	6889.338	225	30.619		
Total	14533.780	240			

Appendix 10: Analysis of variation for the effect of location and farm on growth variables of *Moringa oleifera* in southwestern Nigeria

(a) Seedling height

Source	Sum of Squares	df	Mean Square	F	P-value
Intercept	9355970.817	1	9355970.817	4.657E4	.000
Location	99877.948	7	14268.278	71.025	.000*
Farms	1513.028	1	1513.028	7.532	.006*
location * farms	5412.149	7	773.164	3.849	.000*
Error	154284.604	768	200.891		
Total	1.354E7	960			
(b) Seedling collar diameter					
Intercept	160623.467	1	160623.467	1.942E4	.000*
Location	5293.744	7	756.249	91.414	.000*
Farms	74.209	1	74.209	8.970	.003*
location * farms	39.581	7	5.654		
Error	6353.523	768	8.273		
Total	215329.934	960			
(c) Leaf count					
Intercept	703029.626	1	703029.626	7.863E4	.000*
Location	23831.349	7	3404.478	380.788	.000*
Farms	46.376	1	46.376	5.187	.023*
location * farms	149.499	7	21.357	2.389	.020*
Error	6866.400	768	8.941		
Total	770113.000	960			

Appendix 11: Analysis of variation for the effect of location on biomass assessment of *Moringa oleifera* in southwestern Nigeria

Leaf Biomass

Sources of variation		Sum of Squares	df	Mean Square	F	P-value
Month 1	Between farms	.391	7	.056	2.914	.036*
	Within farms	.307	16	.019		
	Total	.698	23			
Month 2	Between farms	.780	7	.111	3.811	.013*
	Within farms	.468	16	.029		
	Total	1.247	23			
Month 3	Between farms	2.688	7	.384	1.881	.140ns
	Within farms	3.267	16	.204		
	Total	5.955	23			
Month 4	Between farms	8.783	7	1.255	7.272	.001*
	Within farms	2.761	16	.173		
	Total	11.544	23			
Month 5	Between farms	7.069	7	1.010	3.416	.020*
	Within farms	4.730	16	.296		
	Total	11.799	23			
Month 6	Between farms	7.069	7	1.010	3.416	.020*
	Within farms	4.730	16	.296		
	Total	11.799	23			

* = significant at P≤0.05

ns = not significant at P≥0.05

Appendix 12: Analysis of variation for the effect of location overtime on biomass assessment of *Moringa oleifera* in southwestern Nigeria

Stem Biomass

Source of Variation		Sum of squares	Df	Mean square	F	P-value
Month 1	Between farms	.391	7	.056	2.914	.036*
	Within farms	.307	16	.019		
	Total	.698	23			
Month 2	Between farms	.642	7	.092	4.476	.006*
	Within farms	.328	16	.020		
	Total	.970	23			
Month 3	Between farms	.642	7	.092	4.476	.006*
	Within farms	.328	16	.020		
	Total	.970	23			
Month 4	Between farms	.645	7	.092	4.498	.006*
	Within farms	.328	16	.021		
	Total	.973	23			
Month 5	Between farms	.629	7	.090	4.805	.004*
	Within farms	.299	16	.019		
	Total	.929	23			
Month 6	Between farms	.989	7	.141	8.094	.000*
	Within farms	.279	16	.017		
	Total	1.269	23			

Appendix 13: Analysis of variation for the effect of location overtime on biomass assessment of *Moringa oleifera* in southwestern Nigeria

Root biomass

Source of Variation		Sum of Squares	Df	Mean Square	F	P-value
Month 1	Between farms	.222	7	.032	1.771	.163ns
	Within farms	.286	16	.018		
	Total	.508	23			
Month 2	Between farms	.694	7	.099	.829	.578ns
	Within farms	1.913	16	.120		
	Total	2.608	23			
Month 3	Between farms	1.054	7	.151	1.349	.292ns
	Within farms	1.786	16	.112		
	Total	2.840	23			
Month 4	Between farms	.952	7	.136	1.206	.354ns
	Within farms	1.803	16	.113		
	Total	2.754	23			
Month 5	Between farms	.897	7	.128	1.095	.411ns
	Within farms	1.871	16	.117		
	Total	2.768	23			
Month 6	Between farms	1.331	7	.190	1.557	.219ns
	Within farms	1.954	16	.122		
	Total	3.286	23			

Apendix 14: Analysis of variation for the effect of location overtime on proximate assessement of *Moringa oleifera* in southwestern Nigeria

(a)Protein

Source	SS	df	Mean Square	F	P-value
	12689.833				
Month	.200	2	.100	27.492	.000*
Location	1.723	6	.287	79.082	.000*
Cutlevel	.088	3	.029	8.060	.000*
month * location	3.200	12	.267	73.451	.000*
month * cutlevel	.156	6	.026	7.142	.000*
location * cutlevel	.528	18	.029	8.073	.000*
month * location * cutlevel	.998	36	.028	7.633	.000*
Error	.305	84	.004		
Total	12697.030	168			
(b)Ether					
Intercept	80.233	1	80.233	2.140E4	.000
Month	.028	2	.014	3.762	.027*
Location	.552	6	.092	24.534	.000*
Cutlevel	.036	3	.012	3.190	.028*
month * location	.915	12	.076	20.336	.000*
month * cutlevel	.042	6	.007	1.857	.098ns
location * cutlevel	.337	18	.019	4.993	.000*
month * location * cutlevel	.432	36	.012	3.197	.000*
Error	.315	84	.004		
Total	82.890	168			
(c) Ash					
Intercept	1258.976	1	1258.976	4.919E5	.000*
Month	.187	2	.093	36.488	.000*
Location	7.589	6	1.265	494.178	.000*
Cutlevel	.033	3	.011	4.302	.007*
month * location	1.352	12	.113	44.004	.000*
month * cutlevel	.277	6	.046	18.070	.000*
location * cutlevel	.220	18	.012	4.773	.000*
month * location * cutlevel	.561	36	.016	6.087	.000*
Error	.215	84	.003		
Total	1269.410	168			

(d) Carbohydrate					
Intercept	22873.667	1	22873.667	4.342E5	.000
Month	1.451	2	.725	13.771	.000*
Location	17.957	6	2.993	56.814	.000*
Cutlevel	4.718	3	1.573	29.853	.000*
month * location	23.093	12	1.924	36.532	.000*
month * cutlevel	.608	6	.101	1.923	.086ns
location * cutlevel	1.816	18	.101	1.915	.025*
month * location * cutlevel	3.455	36	.096	1.822	.013*
Error	4.425	84	.053		
Total	22931.190	168			
(e) Crude fibre					
Intercept		1283.734	1	1283.734	2.201E5
Month	.502	2	.251	43.071	.000*
Location	53.978	6	8.996	1.542E3	.000*
Cutlevel	.930	3	.310	53.143	.000*
month * location	1.812	12	.151	25.893	.000*
month * cutlevel	.378	6	.063	10.786	.000*
location * cutlevel	.548	18	.030	5.214	.000*
month * location * cutlevel	1.388	36	.039	6.607	.000*
Error	.490	84	.006		
Total	1343.760	168			

Appendix 15: Analysis of variation for the effect of location overtime on phytochemical assessment of Moringa oleifera in southwestern Nigeria

(a)Saponin

Source	SS	df	Mean Square	F	P-value
Month	590.250	2	295.125	4.697	.012*
Location	18925.655	6	3154.276	50.196	.000*
Cutlevel	7647.256	3	2549.085	40.565	.000*
month * location	3763.167	12	313.597	4.990	.000*
month * cutlevel	482.226	6	80.371	1.279	.276ns
location * cutlevel	4820.536	18	267.808	4.262	.000*
month * location * cutlevel	7166.357	36	199.065	3.168	.000*
Error	5278.500	84	62.839		
Total	1.355E7	168			
(b) Tannin					
Intercept	1663839.054	1	1663839.054	1.635E6	.000
Month	602.179	2	301.089	295.807	.000*
Location	1031.988	6	171.998	168.981	.000*
Cutlevel	5755.208	3	1918.403	1.885E3	.000*
month * location	1687.155	12	140.596	138.130	.000*
month * cutlevel	339.202	6	56.534	55.542	.000*
location * cutlevel	692.917	18	38.495	37.820	.000*
month * location * cutlevel	1371.798	36	38.105	37.437	.000*
Error	85.500	84	1.018		

Total	1675405.000	168			

(c) Phenolics

Intercept	1065016.229	1	1065016.229	1.831E6	.000
Month	21.281	2	10.640	18.293	.000*
Location	153.392	6	25.565	43.952	.000*
Cutlevel	276.385	3	92.128	158.387	.000*
month * location	48.073	12	4.006	6.887	.000*
month * cutlevel	28.119	6	4.687	8.057	.000*
location * cutlevel	66.477	18	3.693	6.349	.000*
month * location * cutlevel	73.803	36	2.050	3.525	.000*
Error	48.860	84	.582		
Total	1065732.620	168			

(d) Terpenoid

Intercept	3854463.149	1	3854463.149	1.764E6	.000
Month	2912.226	2	1456.113	666.559	.000*
Location	7746.810	6	1291.135	591.037	.000*
Cutlevel	11354.589	3	3784.863	1.733E3	.000*
month * location	6895.940	12	574.662	263.060	.000*
month * cutlevel	576.107	6	96.018	43.954	.000*
location * cutlevel	3184.619	18	176.923	80.989	.000*
month * location * cutlevel	7052.060	36	195.891	89.672	.000*
Error	183.500	84	2.185		
Total	3894369.000	168			

(e) Cardiac

Intercept	29.417	1	29.417	180.698	.000
Month	.070	2	.035	.215	.807ns
Location	.467	6	.078	.478	.823ns
Cutlevel	1.075	3	.358	2.201	.094ns
month * location	1.077	12	.090	.551	.874ns
month * cutlevel	4.240	6	.707	4.341	.001*
location * cutlevel	1.195	18	.066	.408	.983ns
month * location * cutlevel	5.113	36	.142	.872	.670ns
Error	13.675	84	.163		
Total	56.330	168			