CHAPTER ONE

INTRODUCATION

The family Moringaceae consists of only one genus called *Moringa* the genus holds fourteen species, that is native to the sub Himalaya tracts of India, Pakistan, Bangladesh and Afghanistan(Verdcourt, 2000). This rapidly growing tree, was utilized by the ancient Romans, Greeks and Egyptians; Although the species indigenes to northwest India, it is now cultivated through the Middle East and almost the whole atropical belt (Ramachandran *et al.,* 1980; Jahn *et al*., 1986; Khantharajah *et al*., 1991), it is now widely cultivated and has become naturalized in many locations in the tropics. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses. All parts of the *Moringa* tree are edible and have long been consumed by humans' Multi-purpose crop indigenous to Northwest India. Tender pods, leaves and flowers are consumed as vegetables (Green pods, leaves, flowers and roasted seeds), the twigs and leaves as fodders. *Moringa* has excellent nutritive characteristics so it can easily be used as a fresh forage material for cattle, both economically and productively. Leaves and branches are very useful to feeding all farm animals and poultry especially if it mixed with other fodder (*Moringa* 70%, Alfa Alfa 10% and any other fodder 20 %.).

The leaves are rich in protein, carotene, iron and ascorbic acid and the pod is rich in the amino acid lysine (CSIR, 1992; Chawla *et al*., 1998; Dogra *et al*., 1975). Another important advantageous characteristic of *Moringa* is high productivity of fresh height per unit area compared with other forage crops.

The seed contains 40 percent of oil, where are used in cooking, soap manufacture, cosmetic base and in lamps and oil extracted from the seeds is used as spice, in illumination and cosmetics (Concha, 1980).

The plant is used as tonic to enhance lactation, as poultice to reduce glandular swelling, and as purgative. The roots when chewed and applied to snake bite, can prevent the poison from spreading (De Paduals *et al*., 1983).

Nutritional analysis shows that, the leaves are very high in protein and contain all of the essential amino acids, including two amino acids that are especially important for children's diets. This is most uncommon in a plant food. *Moringa* leaves are also packed with essential vitamins and minerals especially vitamins A and C. delivering such powerful nutrition, these leaves could prevent the scourge of malnutrition and related diseases. *M. peregrina leaves* and pods have high nutritional value for humans and livestock. It is a good source of vitamins A, C, and B, minerals, and Calcium its leaves are an excellent source of protein and are very low in fat and carbohydrates (Al Kahtani , *et al*., 1993). Its leaves are incomparable as a source of the sulfur-containing amino acids methionine and cystine.

M. peregrina grows naturally in north Sudan in Karima city (Casengar village), in Wady Halfa it is called "khoar Alban". It also exists in Alkro (Khoar Alshkul) and in western desert Altmam area, in the desert area of the red sea, Kassala, North kordofan, Darfur, Blue Nile (Hussein Y, 2009).

The tree exists in a reserved forest in Casengar area; farmers use it as a natural fodder for animals, use the wood for industrial purposes and extraction of oil from seeds. Oil produced by traditional methods, produced oil is called (Smn Alawd). It is used for food making, treatment of the flu infection. However, a common use of *M. oleifera* is in water purification, known as "Alrawag". There are many local names of *Moringa* in Sudan *M. oleifera* (shagar alrawag- akseer alhiaa) And *M. peregrina* (Al bân tree) , Al Yassar, Habb al bân, Jasaar, Yassar).(Hussein Y ,2009).

Moringa has been reported to exist in Sudan since 1887 (Jahn's, 1986). *Moringa* became most popular plant in Sudan consume by humans it's multipurpose tree every family wants to plant, recently been most important, all part of tree has many

commercial using, seed oil, water purification, cosmetic, fodder and traditional medicine.

Sudan owns a huge head of animal wealth estimated to be around 116 million head. It ranks first in the Arab World and second in Africa in livestock population. The natural pasture sector and crop residues are responsible for sustaining more than 90% of this wealth (N.C.S., 1999). The area under forage crops has recently witnessed a rapid increase both in the small holders and large specialized systems as well. Reliable records of acreage and tonnage at the national level are not available in the literature. However, taking Khartoum State as an example, the statistics of the Ministry of Agriculture and Animal Health for 2003 showed that, the total area devoted to forage crops amounted to 120,000 feddan representing around 52% of the total area cultivated. A forage gap of about 800 thousand metric ton still exists despite this expansion (Ibrahim, 2004). There are several problems facing fodder, like typical, non-diversity, low productivity and quality. Therefore, it is very important to search for other fodder alternatives.

Sudan is considered the most suitable environments for growing *Moringa,* because it can grow in desert climate and hard climate conditions, adaptable to a wide range of environmental conditions from hot and dry to hot, humid and wet conditions. It's originally considered a tree of hot semi-arid regions (annual rainfall 250-1500 mm), it can germinate without irrigation if it's sown during the rainy season. Whereas, the tree it very sensitive to freezing condition.

The medicinal value of different parts of the plant has long been recognized in folk medicine and is extensively used in the treatment of ascites, rheumatism, venomous bites and as a cardiac and circulatory stimulant. The seed oil is a strong anti-inflammatory agent and used for skin diseases, as they are often the only source of extra protein, vitamins and minerals.

In recent years, DNA–based techniques e.g: (RAPD, AFLPs, SSRs, ISSR and CAPS) have been employed to study genetic variation in tree species. Advantages of these methods over isozyme include their increased saturation of the genome therefore, better representation of the variation present within species.The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one most significant development in the field of the molecular genetics. RAPD and ISSR are very useful DNA-based methods for assessment of genetic diversity in plant species, owing to their technical ease and speed and the wide availability of universal primers. Despite these practical advantages, however, their value in diversity studies has been questioned, owing to the dominant nature of the bands, which can lead to underestimation of recessive allele frequencies. However, this potential skewing can be reduced by examining a large number of RAPD and ISSR loci and using data analysis techniques.

The main Objectives of this study were to:

(I) Evaluate growth of two commonly known *Moringa* species in Sudan *Moringa oleifera* & *Moringa peregrina* under watering intervals.

(ii)Study chemical analysis and compare between the two *Moringa* species

(iii) Assessment of genetic diversity between two *Moringa* species, using molecular marker (ISSR)

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution and Habitat

Moringa oleifera La is native from India, occurring wild in the sub-Himalayan regions of Northern India, and now grown world-wide in the tropics and sub-tropics. It is already an important crop in India, Ethiopia, the Philippines and the Sudan and is being grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands. Commonly known as the 'horse-radish' tree (arising from the taste of a condiment prepared from the roots) or 'drumstick' tree (arising from the shape of the pods), While in the Nile village, the name of the tree is ‘Shagara al Rauwaq’, which means ‘tree for purifying’ (Von Maydell, 1986). Where as in others it is known as the kelor tree (Anwar *et al.*, 2003).In Pakistan, *M. oleifera* is locally known as ‘Sohanjna’ and is grown and cultivated all over the country (Qaiser 1973; Anwar *et al.*, 2005). *M.oleifera,* has a host of other names, an indication of the significance of the tree around the world.

M. oleifera belongs to the mono-generic family Moringaceae, consists of only one genus called *Moringa*, the genus holds fourteen species (Verdcourt,2000). The family Moringaceae includes species exhibiting a wide range of forms, bottle trees to slender trees, sarcorhizal trees or tuberous shrubs (Olson *et al.*, 2001). All these species are native to the Indian subcontinent, the Red Sea area and parts of Africa, including Madagascar. Although *Moringa* is native from India and Pakistan (Verdcourt 1985; Morton 1991; Duke, 2001) but it is widely cultivated especially in dry tropical areas of Middle East and Africa (Fahey 2005; Palada *et al.*, 2007;Nouman *et al.*, 2012) and more recently in many countries located within the Tropics, like Nicaragua because its pods, seeds, leaves and roots are useful as fodder, vegetable and plant growth enhancer (Kantharajah *et al.,*1991; Veeraragava 1998; Mughal *et al.*, 1999; Anhwange *et al.*,

2004, Sanchez *et al.,* 2006; Nouman *et al*., 2012).Besides being consumed by humans, (Bennett *et al.*, 2003; Gidamis *et al.,* 2013). It is also used as animal fodder (Sanchez *et al.*, 2006;Nouman *et al.,* 2012b). Suarez *et al.*, 2003, used as natural coagulant of turbid water and source of phytomedical compounds (Anwar *et al.*, 2006).

All parts of the *Moringa* tree are edible and have long been consumed by humans. Tender pods, leaves and flowers are consumed as vegetables, the twigs and leaves as fodders, and oil extracted from the seeds is used as spice, in illumination and cosmetics (Concha, 1980). The plant is used as tonic to lactation, as poultice to reduce glandular swelling, and as purgative. The roots when chewed and applied to snake bite, can prevent the poison from spreading (De Padua *et al*., 1983). The medicinal value of different parts of the plant has long been recognized in folk medicine and is extensively used in the treatment of ascites, rheumatism, venomous bites and as a cardiac and circulatory stimulant. The seed oil is a strong anti-inflammatory agent and used for skin diseases, as they are often the only source of extra protein, vitamins and minerals.

Moringa is a drought tolerant plant which can be grown in diverse soils, except that water logged. Slightly alkaline clay and sandy loam soils are considered the best media for this species due to their good drainage (Ramchandran , 1980; Abdul 2007).

M. oleifera does not grow properly under water logged conditions, its roots get rotten. This species can tolerate water with an electrical conductivity (EC) of 3 dS m^{-1} during its germination phase, while at later stages its resistance to saline water increase (Oliveira *et al.*,2009). Once it has established, its strong antioxidant system helps it to cope with moderate saline conditions (EC 8 dS m^{-1}), experiencing only a mild reduction in its mineral quality (Nouman *et al.*, 2012a). So, *Moringa* can be grown on different conditions including hot, humid, dry tropical and subtropical regions except water logged conditions. It can perform better under marginal conditions with ample nutritional quality.

2.2 *Moringa peregrina*

Most of the previous studies on *Moringa* concentrated on *M. oleifera* because of its prevalence in the poor areas in Africa and Asia, whereas , most of the people in the rural areas search for edible natural food resources to support their living and fill the stomach in respective of the nutritive value of these resources. The reliance of some African tribes on the leaves of *Moringa* as a local food and an income source motivated some of the scientific bodies to assess the nutritive value of the tree and its possible role in the compact of malnutrition, (Scrimshaw *et al*., 1983). That studies revealed beyond no doubt that *M. oleifera* is the miracle or the life tree (Booth *et al*., 1988).

In the Arabian desert, the home of (*M. peregrina* or *M. Arabica)*, it is observed that residents in Medinah, Wajah and Tehama areas, recognize the *Moringa* tree under various names such as Alyser, Alban and Baan tree and extract an valuable oil from its mature pods the traditional methods that involves crushing and boiling of the seeds and collection of the floating oil in the next day. It is noticed that the baan oil has a special place in the areas and it is sold high prices and used in cooking, medicine and cosmetics and skin softening (Le Poole, 1996). Other uses of pods or leaves were not reported in the areas and no research activities were carried out to assess the Arabian tree. However, a recent visit carried out to the area extending between Al-Medinah Almonawarah and al Wadjah indicated that, the *Moringa* trees are heavily grazed by sheep, goat, camel and cattle stocks and that grazing *Moringa* trees increased both milk production, fat deposition, strength and fertility in grazing livestock's. Drinking milk of animal stocks fed on *Moringa* increased body strength and overall health conditions. These preliminary observations are in accordance with previous studies that indicated the nutritive value of the vegetative parts of *Moringa* and its use in both man and animal nutrition. Thus, the present work is conducted to assess forage yield, growth rates and nutritive components of two *Moringa* species namely, *M. peregrina* and *M. oleifera.* (Elata *et al*., 2009), reported it is hoped that the results of study will lead to

the utilization of the tree in human nutrition and in feeds and forage production in the Kingdom of Saudi Arabia

2.3 *Moringa* in Sudan

Moringa has been reported to exist in Sudan since 1887. However, there is no enough knowledge or research done on *Moringa* in Sudan. *M. oleifera* spread all over the country and used mainly as fences and for traditional water purification" Rawag tree". Another species *M. peregrina* grow*s* naturally in north Sudan in Karima city (Casengar), in" khoar Alban" area it is called "Ban Alkhla". It also exists in Alcaro area (Khoar Eshkol) and in western desert Altmam area, in the desert area of the red sea, Kassala, North kordofan, Darfur, Blue Nile (Hussein , 2009). There are many local names of *Moringa* in Sudan *M. oleifera* (Shajar Alrwag- Acsear Alhiaa) and *M. peregrina* called (Al ban tree), Al Yassar, Habb al Baan, Jasaar, and Yassa (Hussein , 2009).

Moringa can grow in the desert climate and in harsh climatic conditions, like the (big desert North east of Kordofan - Northern State - Red Sea Mountains (Hussein , 2009).The seed oil of *M. peregrina* contains a high level of oleic (70.5%), followed by gadoleic (1.5%) and while the dominant saturated acids was plasmatic (8.9%) and stearic (3.82%),and d-tocopherols were also detected.B-sitosterol was found as the most predominant component of the sterolic fraction of the oil campesterol, stigmasterol, brassicasterol and cholesterol were also found another important advantageous characteristic of *Moringa* its high productivity of fresh material per unit area compared with other forage crops. *Moringa* is most well known for having more vitamins and minerals per weight than many other nutritious foods that are well known to provide an abundance of specific nutrients. These include: more calcium than is found in milk, more Vitamin A than is found in carrots, more Vitamin C than is found in oranges, more potassium than is found in bananas, more iron than is found in spinach (Palada *et al*.,2003).

M. peregrina leaves and pods have high nutritional value for humans and livestock. It is a good source of vitamins A, C, and B, minerals, and calcium (Price, 2000). Its leaves are excellent source of protein and are very low in fat and carbohydrates (Al Kahtani *et al.,* 1993). Its leaves are incomparable as a source of the sulfur-containing amino acids methionine and cystine.

Particular interest in *M. oleifera* concerned its role as 'purification tree' (*shagarat al rauwaq*) in the northern Sudan. After scientific confirmation of the flocculating properties of the seeds, which the village women had so far mainly used to treat the highly turbid water of the Nile (Jahn , 1979; Jahn, 1981). Systematic search for natural coagulants was also extended to seeds from other *Moringa* species. So far, success has been obtained with *M.peregrina* (Forssk) Fiori (Egypt), *M. stenopetala* (Bak. f) cut. (Kenya), *M. longituba* Engl. (Somalia), *M. drouhardii* Jumelle (Madagascar) and *M. ovalifolia* Dinter & Berger (Namibia).

As with *M.oleifera* seeds, all investigated seed types contained primary coagulants comparable to the conventional coagulant alum. Applied in doses of 30 to 200 mg/litre in accordance with raw water quality, the *Moringa* seed powder suspensions could clarify different types of tropical surface waters with low, medium and high turbidities to tap-water quality within one to two hours (Jahn, 1984). As efficient elimination of turbidity is accompanied by a 98-99 percent elimination of indicator bacteria, domestic water treatment with *Moringa* seeds became a low-cost technology to be utilized in improving water and health in rural communities of tropical developing countries (Jahn, 1981; Sattaur, 1983; Jan, 1986). The main objective for cultivation trials with *M. oleifera* and its relatives was therefore, to find out which M*oringa* species could provide the essential raw material for water purification in the shortest time and with maximum yield. The trials were aimed, in addition, at finding out whether certain *Moringa* species were more suitable for semi-arid zones and marginal soils and more resistant to plant diseases than the traditional *M. oleifera.* In the course of their research, and travels by one of their number, the authors discovered that other

Moringaceae species have several similar traditional uses. This means that, the chemotaxonomic relationship detected in connection with the presence of flocculants in the seeds also exists for other chemical constituents in this family, such as the seed-oils and anti-microbial substance in one or several plant organs. Thus most or perhaps all Moringaceae deserve to be considered multipurpose trees or shrubs. For water purification purpose and traditionally performed, woman and men participated in planting one or more *Moringa* tree in their compounds (Jahn, 1981), in forestry Research station at Soba few Sudanese were involved in the basic cultivation studies. Supplementary comparative experiments with different *Moringa* species were later also carried out between 1982 and 1984 in the framework of the (GTZ) plant protection project (Jahn, 1986).

The traditional cultivation of *M.oleifera* is only from seeds in Sudan, whereas, vegetative propagation was very common in North Sudan, the lack of parent trees mean that only a few trials could be made with vegetative propagation. Sowing was tested during the cool dry season the hot dry season and the rainy season. Apart from the *M.peregrina* seeds, which were supplied from the herbarium in Cairo, the collection dates for the other types of *Moringa* seeds were known and fresh seeds were used unless otherwise stated (Jahn, 1986).

2.4 Agronomy practices:-

2.4.1 Climate

Moringa is widely adapted to the tropics and subtropics. Optimum leaf and pod production requires high average daily temperatures of 25–30°C (77–86°F), well distributed annual rainfall of 1000–2000 mm (40–80 in), high solar radiation and well-drained soils. Growth slows significantly under temperatures below 20°C (68°F). Minimum annual rainfall requirements are estimated at 250 mm with maximum at over 3,000 mm, but in waterlogged soil the roots have a tendency to rot. It can be found from 30º North to 20º South and it grows best at altitudes up to 600 m but it will grow

at altitudes of 2000 m. It is usually found in areas with a temperature range of 25°C to 40° C but will tolerate 48° C and light frosts. *Moringa* is relatively tolerant of drought and poor soils and responds well to irrigation and fertilization. *Moringa* agronomy and pH(5-9)tolerance no much literature exists regarding agronomic practices for production, the little literature available is based on Indian conditions. Some agronomic trials with *Moringa* showed that the plant can grow well in hilly areas and in weathered soils of low fertility (Tinh Bien districts, India) (Manh *et al.*,2003). Not much literature exists regarding agronomic practices for *Moringa* production. However, information about growth of *Moringa* in the acid regions of India is almost absent. The information available on cultivation procedures for *Moringa* is limited, except in certain regions of India where it's cultivated on a large scale (Bezerra *et al.,* 2004). Since conclusive information on responses of *Moringa* to pH is absent, responses of other Multi-Purpose Tree Species (MPTS) to pH may be instructive. Nutrition however, might also have an influence on the growth aspects of *Moringa,* (Fuglie, 1999).

2.4.2 Soil and land preparation:-

The ease with which the roots spread is a necessary condition in plant growth and development. *Moringa* therefore requires a well-drained loamy or sandy soil for optimal growth. The land should be slide where necessary and all unwanted materials removed from the field. If planting density is high, the land must be ploughed and harrowed to a maximum depth of 30 cm. If planting density is low (1 m x 1 m), it is better to dig pits and refill them with the soil. This ensures good root system penetration without causing too much land erosion (plugging can be risky in some tropical environments, in the cases of heavy rains, wind or sloping). In this case, the pits must be 30 to 50 cm deep, and 20 to 40 cm wide. When refilling the pit, mix the soil with manure. For the farmer producing *Moringa* on a large scale it is recommended that soil and seed testing are done to ensure good returns on investment, (Amaglo *et al.*, 2007).

2.4.3 Soil

Moringa tolerates a wide range of soil types and pH (4.5–9), but prefers well-drained soils in the neutral pH range. It can grow well in heavy (clay) soils provided that they do not become saturated for prolonged periods of time. Light (sandy) soils are preferred for rooting branch cuttings directly in the ground. It can be established in slightly alkaline soils up to pH .9. In areas with heavy rainfall, trees can be planted on small hills to encourage water run-off). Presence of a long taproot makes it resistant to periods of drought, (Bezerra *et al.,* 2004).

2.4.4 Irrigation

Moringa can germinate and grow without irrigation if it is sown during the rainy season. Its tuberous root develops in twenty days and allows young plants to endure drought. However, for optimal growth, it is advisable to irrigate regularly during the first 3 months after seeding. Irrigation is also necessary to produce leaves all year long, including during dry seasons. Another option is to stop producing during these periods: the trees will shed their leaves but will not die. At the onset of the rains, a good pruning and adding some organic manure will ensure that the trees start producing many new branches and leaves. Any suitable irrigation system can be used e.g. rubber hose, watering can, sprinkler or drippers. Ideally, irrigation should be done in the early morning, night or evening, to reduce evaporation. If water is scarce, mulching or a very superficial weeding will also decrease evaporation (Craker *et al.*, 2007).

2.4.5 Irrigation needs according to climatic zone

In the Coastal zone (south Ghana) the leaf production is possible all year long without irrigation, with a lower production during the dry season. Whereas, in the Savannah zone (north Ghana), Production is possible without irrigation but harvests will stop during the dry season. In the Sahelian zone (Niger, Burkina Faso). plantations must be irrigated almost all year long (every day during dry seasons, two or three times a week

during wet seasons).It is also possible to irrigate only when water is available and to stop production during the dry season.

2.4.6 Fertilizing

Moringa can produce large quantities of leaves, but only if it receives enough organic supplements. Its leaves are rich in proteins and minerals, which means that the soil needs to provide enough nitrogen and minerals to the plants. Instead of chemical fertilizer, farmyard manure (animal dung mixed with plant residue) or compost (plant residue left to decompose on a heap) can provide the necessary nutrients as well as improve the soil structure. The best fertilization is ensured by mixing fast decomposing residue (animal dung, green and soft plant residue) with slow decomposing residue (straw, dry plant residue and thin branches). Fertilization must be done during land preparation, before seeding. After it is important to apply manure or compost at least once a year, for instance before the rainy season, when the trees are about to start an intense growth period (pruning can also be done at this time). If there are two rainy seasons, two applications are advised.(Sontakke *et al.*, 2006).

2.4.7 Pest and disease control

The most common pests are grasshoppers, crickets and caterpillars. These insects bite and chew parts of the plant, causing the destruction of leaves, buds, flowers, shoots, fruits or seeds as well as the interruption of sap flow. These outbreaks are frequent in dry zones where *Moringa* leaves strongly attract insects. It seems that these outbreaks occur at the beginning of the dry season when insects cannot find other tender, green material to feed. The best solution, in this case, is to cut back the trees, leaving no green part apparent. The following growth is very vigorous if conditions permit (sufficient water supply). Concerning the Lepidoptera caterpillar, it is imperative to detect the outbreak at the beginning at the shoot centre in order to act before it is too late. Spraying must be aimed at the centre and the extremity of the shoots to reach the young caterpillars (Kokou *et al.*, 2001).

2.5 *Moringa* as fodder

Various research reports and reviews highlighted the importance of trees and shrubs being used as livestock fodder or supplementing the low value fodders or rations in dry season (Atta-Krah 1990; Lefroy *et al.*, 1992; Otsyina and Dzo'pwela 1995). As mentioned previously that *Moringa* leaves, fresh pods, seeds and roots are being widely and increasingly used by humans and animals, because of their higher contents of essential nutrients (Hammouda CSIR *et al.,* 1962; Hartwell 1971). However, scientists devoted to livestock research are not only interested on finding good quality fodders which can increase milk and meat production, but they are also looking for species which can be grown and exploited as friendly to environment and cultivated in a cheap way, such features are also met in *Moringa.*

Moringa trees are used for diverse purposes because they are easy to maintain once their roots have developed and established. *Moringa* trees have deep tap root system when they are grown from seeds and adventitious root system when those are grown from stem cuttings, its roots penetrate deep into soil to search for water and nutrients, which enable *Moringa* trees to tolerate severe conditions. Besides the features of its root system, this species has a fast growing habit, low requirement of maintenance on late stages, reduced necessities of fertilizers and irrigation and a high capacity to re-sprout after harvesting. Relatively low requirement of irrigation makes *Moringa* superior to some other livestock meals like soybean, cotton seed cake and range grasses, which require relatively high irrigation to avoid reduced livestock production (Benavides, 1994). For instance, soybean requires intensive irrigation which makes it too difficult to cultivate for small livestock farmers. Forage in Sudan was severing many problems like typical, non-diversity, low productivity and quality. There is a pattern there is a productive one in Khartoum state it's green chopping or cut and carry either other systems such as, the production of fodder for storage or cultivation of fodder for grazing a few that are not non-existent there of two or three varieties of fodder and not take advantage of the benefits of other fodder alternatives (Ibrahim ,

2004). Although *Moringa* has a nutritional characteristics of the tree are excellent so it can easily be used as a fresh forage material for cattle. The leaves are rich in protein, carotene, iron and ascorbic acid and the pod is rich in the amino acid lysine(CSIR, 1992; Chawla *et al.*, 1998; Dogra *et al.*, 1975). Another important advantageous characteristic of *Moringa* is its high productivity of fresh material per unit area compared with other forage crops. *Moringa* is especially useful as forage for cattle both economically and productively given the problems facing typical cattle breeders (70 % of the national herd in Nicaragua is in the hands of these small cattle producers). Major among these problems several places in the world are now developing intensive cultivation of *Moringa*, branches serve as food for each small farm animals and poultry, especially if mixture with *Moringa* by 70% , Brsum 10% and 20% of any other fodder.

2.6 Traditional uses of *Moringa peregrina* in Sudan

Some of the traditional uses of *M. peregrina in* Sudan are as follows, in Casengar area, farmers use it as a natural fodder for animals, and use wood for industrial purposes. Whereas oil produced by traditional methods, produced oil is called "Smn Alawd" It is used for food making, treatment of the flu infection, protection skin and hair oil (Jan *et al.* ,1980). As well as, the common use of *M.peregrina is* in water treatment, known as "Alrawag".

Also they used to remove freckles. The wood is seen as a good source for firewood and charcoal. The traditional medicinal uses such as, abdominal pain, Burns, Constipation, Febrifuge, Laxative, and Headache (Batanouny, 1999).

2.7 Uses of *Moringa oleifera*

M.oleifera was originally an ornamental tree in the Sudan, planted during British rule. That was where, Jahn's (a german Scientist) laboratory tests confirmed the presence of a very efficient coagulant. In the Sudan, dry *M.oleifera* seeds are used in place of alum

by rural women to treat highly turbid Nile water (Jahn, 1986).It is a multipurpose tree for semi-arid and drought-prone areas. Even though it is Non nitrogen fixing tree, its different parts can be useful for other purposes. Pods, leaves and seeds can be eaten as vegetable and are highly nutritious. The extracted oil from the seeds is used for cooking, soap making, cosmetics, fuels and lamps. Wood pulp may be used for paper making. (Schneider *et al*., 1995), the wood is light and can´t be used for heavy constructions but it provides a fairly good fuel for cooking. *Moringa* wood makes acceptable firewood but poor charcoal because it is very soft. It also makes excellent pulp for paper manufacture. The bark is sometimes used for tanning and to make mats and rope. The leaves can be also used as fertilizer. Powered seeds are used to heal bacterial skin infection (all parts of the plant are used in a variety of traditional medicines (Folkard *et al.,* 1999).

Nutritional analysis shows that the leaves are very high in protein and contain all of the essential amino acids, including two amino acids that are especially important for children’s diets. *Moringa* leaves are also packed with essential vitamins and minerals especially vitamins A and C, delivering such powerful nutrition, these leaves could prevent the scourge of malnutrition and related diseases (Misra *et al.,* 1976).

2.7.1 Medicinal Applications

Moringa flowers, leaves and roots are used in folk remedies for tumors, whilst the seed is used specifically for abdominal tumors. *Moring*a root is used in Nicaragua for dropsy and the root juice can be applied externally to counter irritations of the skin, while the leaves are applied as poultices to sores, rubbed on the temples for headaches and are said to have purgative properties. *Moringa* bark leaves and roots can be taken to promote digestion, while the *Moringa* oil can be applied externally for skin diseases (Ramachandran *et al.*, 1980). The bark can also be used to treat diarrhea cases. *Moringa* flowers and roots contain a compound, pterygospermin, which has powerful antibiotic and fungicidal effects which have been put to various usages in the health

sector (Hartwell,1967-1971). The roots from young plants can also be dried and ground for use as a hot seasoning base with a flavor similar to that of horseradish. This is why the *Moringa* tree has been given the name "Horseradish Tree" (Delaveau *et al.,* 1980).

2.7 .2 Cosmetic and Industrial Uses

Moringa seed contains bean oil used in making perfumes and soap, and can also be used as a preservative and as a machinery and watch lubricant because it won't spoil (Fuglie, 1999).

2.7. 3 Sanitation and Agricultural Uses

One crushed (*Moringa*) seed can clear 90 percent of the total coliform bacteria in a liter of river water within 20 minutes (Wilson, 1992). The seedcake left after oil extraction can be used as a soil fertilizer or in the treatment of turbid water where it is applied at a dose not exceeding 250 mg/liter of water (Price, 1985). It coagulates the solid matter and suspended bacteria and these impurities then sink to the bottom. The juice from fresh *Moringa* leaves can be used to produce an effective plant growth regulator which increases yields by 25 – 30 percent for many crops: onions, bell pepper, soya, maize, sorghum, coffee, tea, chili, melon, since it contains Zeatin, a plant hormone belonging to the cytokinines group, which can be fairly applied (Price, 1985).

2.8 Food and Nutritional Uses

Moringa leaves are used for food, with almost every part of the plant having a food value Palada *et al.*, (2003) Ramachandran *et al.,* 1980). For instance, seed is said to be eaten like a peanut in Malaya while the leaves are widely used as, a highly nutritious vegetable, a supplement as greens in salads, in vegetable curries, as pickles and also for seasoning. *Moringa* leaf has significant quantities of vitamins A, B and C, calcium, iron and protein Misra *et al*., (1976), qualities which assist in combating malnutrition in humans. *Moringa* flowers are cooked and consumed either mixed with other foods or fried in a flour batter and have shown to be rich in potassium and calcium which have

proven to be vital sources of these minerals especially for developing countries. *Moringa* seeds are utilized either as a green 'pea' in their immature state or fried in their mature state and they are added to curries, canned and sold in stores in India, a vital side dish or vegetable. The dried *Moringa* leaves are used to make herbal tea or *Moringa* powder which is used to increase energy or as a spice. The mature *Moringa* seed contains about 40 percent oil which is of excellent quality (73 percent oleric acid similar to olive oil) for cooking and has been sold for many years as ''ben oil'' used in cooking. It takes approximately 11kg of *Moringa* seed to produce 2.6 liters of oil (Price, 1985).

2.9 Mineral and chemical compounds

2.9 .1 Mineral

M. oleifera tree has probably been one of the most underutilized tropical crops. Leaves of *M. oleifera* could serve as a valuable source of nutrient for all age groups. In some parts of the world for example Senegal and Haiti, health workers have been treating malnutrition in small children, pregnant and nursing women with *Moringa* leaf powder (Price, 1985). The leaves of *M. oleifera* can be eaten fresh, cooked, or stored as a dried powder for many months reportedly without any major loss of its nutritional value (Arabshahi *et al.*, 2007 ; Fahey, 2005). The leaves are known as great source of vitamins and minerals being served raw, cooked or dried (Fuglie ,2005), reported that 8 g serving of dried leaf powder will satisfy a child within ages 1-3 with 14% of the protein, 40% of the calcium, 23% of the iron, and nearly all the vitamin A that the child needs in a day. One 100 g portion of leaves could provide a woman with over a third of her daily need of calcium and give her important quantities of iron, protein, copper, sulfur, and B-vitamins. Introduction of *Moringa* leaves as part of the diet in Senegal has been successful despite the fact that new foods are often very difficult to introduce in West Africa (Price, 1985).

The higher crude protein content of the meal as compared to kernels together with the higher solubility of proteins from meal suggested that the coagulants used for the purification of water which are proteins in nature can be recovered efficiently from meal. Higher recovery of the active proteinous coagulants from meal would benefit the overall economy of the system. The oil recovered can be used for human consumption and other purposes such as, illumination and lubrication. The residues left after extraction of coagulants from the meal can form a good source of protein supplement because of high crude protein content (approximately 70 %), all of which is in the form of true protein, high availability of protein postruminal (69 % of the total protein) and high pepsin digestibility, virtual absence or presence of negligible levels of antinutrional factors such as tannins, saponins, alkaloids, inhibitors of trypsin and amylase, lectin, cyanogenic glucosides and glucosinolates, and higher concentration of sulphur-containing amino acids than that of the recommended amino acid pattern of FAO/WHO/UNO reference protein for 2 to 5 years old child. Presence of phytate at about 6.7 % might decrease bio availability of minerals. The residue obtained after extraction of coagulants from the defatted *Moringa* kernels (meal) could replace some of these conventional seed meals. This may be a good source of sulphur amino acids for fiber-producing animals (i.e. Angora rabbits, sheep and goats) in a mixed diet containing sufficient levels of other essential amino acids. However, before recommendations are made to farmers, in vivo experiments are required to study various performance parameters and possible toxicity arising due to factors not studied in the present investigation. It may be noted that the presence of high levels of sulphur-containing amino acids would offer the animal some protection against toxic factors since these acids are known to enhance the detoxification process of the animal by acting as methyl donors in various organs.

The kernels of the *M. oleifera* variety used as bitter but the bitter taste was almost absent in the residue left after extraction of coagulants from the defatted kernels. The bitter taste is generally attributed to alkaloids, saponins, cyanogenic glucosides,

glucosinolates which were removed by the treatment, suggesting that the bitter taste would not limit the use of this material in animal diets. Considerable genetic diversity exists within and between *M. oleifera* and *M. stenopetala* (Odee, 1998; Muluvi *et al.,* 1999). Perusal of the literature reveals that many different varieties exist whose kernels taste from sweet to very bitter (CSIR 1962; Dogra *et al.*, 1975). Seeds of some varieties are consumed by humans after roasting and taste like peanuts(Ramachandran *et al.*, 1980). The study has shown that the kernel's antinutrional components or their degraded products, for example of glucosinolates which are known to cause various adverse effects (Mawson *et al.*, 1994, 1995), would be consumed by humans through drinking water, which might produce clinical or sub-clinical changes in internal organs. Workers in this area are aware of this problem and studies are available where kernel has been fed to rats and mice without any apparent toxic symptoms (Barth *et al.,* 1982; Berger *et al.*, 1984). However, in depth studies are required in this direction especially in light of the fact that various *M. oleifera* varieties are presently in use.

Ogbe *et al.,* (2011) ,showed that *Moringa* leaves contained appreciable amounts of crude protein (17.01% ±0.1) and carbohydrate (63.11% ±0.09). The leaves also contained appreciable amounts of crude fiber (7.09% ±0.11), ash (7.93% ±0.12), crude fat (2.11% ±0.11) and fatty acid (1.69% ±0.09). The dry matter (96.79±0.10). The contained essential of minerals, Ca (1.91% ±0.08), K (0.97% ±0.01), Na (192.95±4.4), Fe (107.48±8.2), Mn (81.65±2.31), Zn (60.06±0.3) and P (30.15±0.5) parts per million (ppm). Magnesium (0.38% ±0.01) and copper (6.10±0.19) were the least. The presence of these essential nutrients and minerals implies *M. oleifera* leaves could be utilized as a nutritionally valuable and healthy ingredient for poultry. These nutrients may not be strictly medicinal but could be valuable in preventing diseases that are related to malnutrition. The dry matter (DM) value of the *Moringa* leaves in (Olugbemi *et al.,* 2010) and Mutayoba *et al.,*(2011), study was 93.7% and 87.20%, respectively. However, the crude protein value reported by (Olugbemi *et al.,* 2010) was (27.44%), and (Ogbe*, et al.*, *2011*), (17.01%).

Mutayoba *et al.*, (2011) also reported (30.65%) crude protein in *M. oleifera* leaves. The previous studies on *Moringa* minerals obtained the minerals values for, Mn (81.65 ±2.3), Zn (60.06 ±0.3) and Cu (6.1±0.2) (Ogbe *et al.,* 2011). Mutayoba *et al.*,(2011) reported values for Mn 57.34, Zn 21.70 and Cu 5.73 parts per million. Fe (318.81 ppm), Ca (2.47%), K (1.63%) and Mg (1.03%). Minerals are required for normal growth, activities of muscles and skeletal development (such as calcium), cellular activity and oxygen transport (copper and iron), chemical reaction in the body and intestinal absorption (magnesium), fluid balance and nerve transmission (sodium and potassium), as well as the regulation of acid-base balance (phosphorus). Iron is useful in prevention of anemia and other related diseases (Oluyemi *et al.*, 2006). Manganese plays a role in energy production and in supporting the immune system (Muhammad *et al.,* 2011). It also works with vitamin K to support blood clotting, and with B complex vitamins to control the effects of stress (Muhammad *et al.*, 2011). Zinc is useful for protein synthesis, normal body development and recovery from illness Muhammad *et al., (*2011). Deficiency of these nutrients and minerals are known to affect the performance and health of poultry (Merck, 2005).

Melesse *et al .,* (2011) report that, evaluating the nutritional value of indigenous shrubs, trees and browse plants is important in Ethiopian situation where availability and quality of forages severely limited during long and dry seasons. A comparative assessment was thus conducted to investigate the nutrient compositions and feeding values of *M. stenopetala* and *M. oleifera* leaves. Feed samples were analyzed for proximate nutrients, minerals and amino acid profiles using official methods. In addition, the metabolizable energy (ME), organic matter digestibility (OMD) and short chain fattyacids (SCFA) were predicated using the Hohenheim in vitrogas test method. *M. stenopetalahad* 26.6% crude protein (CP), 3.36% fat, 17.9 KJ/kg dry matter gross energy, 45% nitrogen free extract (NFE), and 38.4% non fiber carbohydrate (NFC). Melesse *et al.*, 2011, report that *M. oleifera* , contents of CP, fat, NFE, NFC and gross energy were 28.9%, 6.73%, 45%, 38.4% and 17.9 MJ/kg DM, respectively*, M.*

stenopetala leaves contained significantly higher crude fiber, acid detergent fiber (ADF) and cellulose than those of *M. oleifera* .However, the acid detergent lignin (ADL) and hemicelluloses contents of *M. oleifera* were significantly higher than those of *M. stenopetala*. The contents of calcium, phosphorous, magnesium, potassium and sodium in *M. stenopetala* were 2.47%, 0.57%, 0.76%, 2.45% and 0.11%, respectively. The values of the corresponding minerals in *M. oleifera* were 2.62%, 0.43%, 0.56%, 2.0% and 0.03%. The concentrations of essential amino acids were significantly higher in *M. oleifera* than those of *M. stenopetala* and were generally comparable with the contents of soybean meal. The highest in virago's production of 47.9 ml was recorded for *M. stenopetala*, being significantly higher than for *M. oleifera* (40.6 ml). Similarly, values of ME (9.83 MJ/kg DM), OMD (76.4%) and SCFA (101 mmol) in *M. stenopetala* were significantly higher than those of *M. oleifera.* Although not significant, organic matter, CP, fat, ADF and cellulose contents were positively correlated with in viragos production while DM, NFE, neutral detergent fiber, ADL and hemicelluloses contents were negatively correlated with gas production. The chemical compositions showed the potential of leaves of both *Moringa* species to be used as a protein supplement in ruminant and non-ruminant feeding during the dry season. Moreover, the enhanced values of ME, OMD and SCFA in leaf suggest its ability to meet the energy requirements of tropical livestock.

Chemical and mineral compositions the proximate nutrients and fiber fractions of *Moringa* leaves the contents of crude protein (CP) and fat in *M. oleifera* was significantly ($p<0.05$) higher than those of *M. stenopetala*. The average CP content in *M. oleifera* was comparable with that of (Oduro *et al.,* 2008) for the same *Moringa* specie. (Sanchez *et al.*, 2006) reported CP contents of 22.8% and 23.3 % for found 30.6% CP for *M. stenopetala* leaves, which are higher than those of both *Moringa* species in the current study. The variations in CP contents of the reported values may be due to differences in agro-climatic conditions or to different ages of trees, and possibly due to different stages of maturity.

2.10 Molecular characterization of *Moringa*

2.10.1 Molecular Makers

Molecular markers are segments of chromosomes which don't necessarily encode any traits and are not affected by the environment but which are inherited in a Mendelian In the last decade, the use of DNA markers for the study of crop genetic diversity has become routine, and has revolutionized biology. Increasing, techniques are being developed to more precisely, quickly and cheaply assess genetic variation. These techniques have changed the stand equipment of many labs, and most germplasm scientists are accepted to be trained in DNA data generation and interpretation (Spooner, 2005). In recent years, different marker system have been developed and applied to arange of crop species. Some segments of the chromosome change faster than others (i.e. coding vs. noncoding DNA). As a result it is recommended to use fast changing markers for closely related individuals and slow changing markers for less related individuals (different species). Different marker types therefore, have different usefulness in fingerprinting. The following techniques are those most used in genetic diversity studies.

2.10.2 Assessment of Genetic diversity using molecular markers

The genetic diversity of tree crop is very important for breeding and improvement yield and quality of its produce. Conservation strategies, it's very important for breeding and improvement program for the purpose of improving the yield , quality and varieties ,Its most elementary level is represented by differences in the sequences of nucleotides that form DNA within the cells of the organism . Nucleotides variation is measured of discrete sections of the chromosome called genes. Thus, each gene compromises a heredity sections of DNA that occupies a specific place of the chromosome and control a particular characteristic of an organism .

Useful genetic diversity traditionally produced by crossing parents to create a segregating population choice of parents is key to achieving targeted priding objectives.

One or more parents must provide a source of favorable alleles for each of the traits of interest and mean performance must also be major consideration for improvement of quantitive traits (Eathington *et al.*, 2007; Johnson and Mumm , 2006).

2.10.1.1 Inter-Simple Sequence Repeat (ISSR)

ISSR-PCR is a genotyping technique based on variation found in the regions between microsatellites. It has been used in genetic fingerprinting, gene tagging, detection of clonal variation, cultivar identification, phylogenetic analysis; detection of genomic instability, lgenotyping technique makes ISSR useful for researchers interested in diverse fields such as, conservation biology and cancer research.

ISSR-PCR uses a single fluorescently labeled primer to target the region between identical microsatellites. An ISSR-PCR primer, there are over 100 primers developed for use in ISSR techniques, (Brenner, 2011). Since ISSRs are dominant markers, the amplified regions in an ISSR-PCR are scored as diallelic. Between individuals within a population, changes in the amplified products can arise through structural changes to the region (insertions or deletions) or the loss of primer binding sites.The ISSR workflow is faster and requires a lower startup investment than other genotyping methodologies such as, amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP). In addition, since there is no need to characterize microsatellites, ISSR is also considerably less expensive and less time-consuming than microsatellite-based genotyping. Several studies have compared AFLP and ISSR results and have found ISSRs preferable because of the reduced number of protocol steps required and the smaller amounts of DNA consumed. Additionally, using capillary electrophoresis (CE) to detect amplified DNA fragments helps deliver significantly higher resolution than traditional agarose gel electrophoresis, thus

increasing the amount of information obtained from each experiment. Despite all of the potential advantages of ISSR-PCR, there are some drawbacks to the technique that highlight the need for improvements in the workflow. The primers that are designed to anneal to the di- or trinucleotide repeats can lack specificity in PCR and are a major contributor to the lack of reproducibility. Also, the lack of complexity of the ISSR primers can lead to nonspecific amplification, particularly if coupled to poor-quality DNA extraction methods and suboptimal PCR amplification conditions.

2.10.1.2 Randomly Amplified Polymorphic DNA (RAPD)

The random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence. RAPD markers are decamer (10 nucleotide length) DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and which are able to differentiate between genetically distinct individuals, Amplification products are generally separated on agarose gels and stained with ethidium bromide. Decamer primers are commercially available from various sources, (Williams, 1990).

Welsh and McClelland ,(1991) ,independently developed a similar methodology using ,primers about 15 nucleotides long and different amplification and electrophoresis conditions from RAPD and called it the arbitrarily primed polymerase chain reaction (AP-PCR) technique. PCR amplification with primers shorter than 10 nucleotides DNA amplification fingerprinting (DAF) has also been used producing more complex DNA fingerprinting profiles .Although these approaches are different with respect to the length of the random primers, amplification conditions and visualization methods, they all differ from the standard PCR condition in that only a single oligonucleotide of random sequence is employed and no prior knowledge of the genome subjected to

analysis is required. One of the most widely used applications of the RAPD technique is the identification of markers linked to traits of interest without the necessity for mapping the entire genome.(Martin *et al.*,1991).

2.10.1.3 Amplified Fragments Length Polymorphism (AFLP)

Is a recent DNA fingerprinting technique developed by (Zabeau , 1993); Kuiper, 1997). This method is based on PCR amplification of selected restriction fragments of a total digested genomic DNA.Once labelled, amplified products are separated by electrophoresis. DNA fragments obtained range from 60 to 500 base pairs. To be visualised, DNA polymorphism, which is usually made of small DNA fragments of few base pairs (up to 500), must be amplified. This amplification is commonly done by Polymerase Chain Reaction (Mullis *et al.*, 1986; Mullis and Faloona, 1987). The PCR method can amplify specific DNA fragments through a precise priming of the polymerisation reaction occurring at each end of the target DNA.

This precise priming is done by short oligonucleotidic sequences (Primers) able to anneal to the template DNA in the target zone. Primers are 18-24 base pairs long, synthesised in laboratory and correspond to a complementary DNA sequence designed in the flanking regions of the heavy strand of the target DNA. The Polymerase Chain Reaction starts first with a high temperature phase (denaturation) that produces single-stranded DNA. Then, once temperature has reached, primers will bind to the template DNA .The Taq polymerase recognises each double-stranded DNA as a start of synthesis and will continue the polymerisation reaction in the direction 5' -3' as soon as the temperature has reached 72°C (optimal elongation temperature). Therefore, in order to design specific primers,the sequences of the flanking regions of the target DNA must be known. This supposes detailed knowledge about the genome or further elaborated investigations to get it. These steps usually require high laboratory equipments and are most of the time, time consuming. The originality of the AFLP method was to design and synthesise arbitrary primers first, and then to ligate them to

target DNA fragments .The AFLP arbitrary primers are called “adapters” and consist of a known sequence of 20 nucleotides. The target DNA sequences are DNA fragments generated by restriction enzymes. Fragments are produced from total genomic DNA by the combined action of two restriction enzymes. Then, adapters are ligated at each end of a restriction fragment by a protein ligase . Finally, adapters are used in a PCR as priming sites to amplify the restriction fragments. AFLP markers reveal a “restriction site” polymorphism and must be treated as dominant markers, since homozygotes and heterozygotes cannot be established unless breeding/pedigree studies are carried out to determine inheritance patterns of each fragment. However, the large number of fragments gives an estimate of variation across the entire genome, which thus, gives a good general picture of the level of genetic variation of the studied organism.

2.10.1.4 Simple Sequence Repeats (SSR)

The development of the polymerase chain reaction (PCR) (Saiki *et al.*, 1988) has led to the development of many techniques to detect polymorphism at the DNA level (Powell *et al.*, 1995; Rafalski *et al.*, 1996). Of these techniques, some require the use of sequence-specific primers, such as simple sequence repeats (SSRs) or micro satellites(Powell *et al.*, 1996), sequence-tagged sites (STSs) (Williams, 1995), and cleaved amplified polymorphisms (CAPs) (Konieczny *et al.,* 1993). Other PCR-based techniques utilise random PCR priming and thus the same set(s) of primers can be used across a wide range of species. For most among these techniques are randomly amplified polymorphic DNA (RAPDs),(Williams *et al.*,1990; Welsh and McClelland 1990), arbitrarily-primed PCR (AP-PCR), (Welsh *et al.*, 1993), and amplified fragment length polymorphisms (AFLPs) Vos *et al.*,(1995). The major attractions of PCR-based marker techniques are that they require relatively small amounts of DNA and are for the most part, technically straight forward. Those techniques which utilize anonymous primers, such as RAPDs and AFLPs, have high. Multiplex ratios, i.e., multiple polymorphic loci can be amplified and subsequently analyzed by gel electrophoresis using a single primer or pair of primers (Rafalski *et al.*, 1996).

However, both techniques have their drawbacks: RAPDs are notoriously difficult in terms of reproducibility and transfer ability and the AFLP technique requires the laborious preparation of template DNA for each individual, rather than being applicable to total genomic DNA. Recently, a modification of the AFLP technique known as, sequence-specific amplification polymorphism (SSAP) has been reported (Waugh *et al.*, 1997). This involves the substitution of one of the standard AFLP primers with a primer complementary to a specific sequence, in this case, the long terminal repeat (LTR) region of the Bare Ty1Copiaretro transposon in barley. These transposable elements are found dispersed throughout the euchromatic regions of the genome (Kumar, 1996). as a result S-SAPs exhibit wide spread genome coverage. We have developed a PCR-based assay which utilizes a Copia specific primer with anchored simple-sequence repeat primers Zietkewicz *et al.,* (1994), to generate multiple polymorphic products. Unlike the SSAP technique, this procedure utilises total genomic DNA with no need for the generation of template DNA fragments associated with such AFLP-based assays.

2.10.1.5 Restriction fragment length polymorphism (RFLP)

originally developed for mapping human genes, has much greater power and was than anything previously available (Botstein *et al.*, 1980). This technique quickly proved their utility in virtually all species.(O'Brien, 1991) groups genetic markers into two types: Type I markers are associated with a gene of known function and Type II markers are associated with anonymous gene segments of one sort or another. For now, RFLP remain the most common Type I marker presently used in many eukaryotic organisms, variations in the characteristic pattern of a RFLP digest can be caused by base pair deletions, mutations, inversions, translocations and transpositions which result in the loss or gain of a recognition site resulting in a fragment of different length and polymorphism, only a single base pair difference in the recognition site will cause the restriction enzyme not to cut. If the base pair mutation is present in one chromosome but not the other, both fragment bands will be present on the gel and the

sample is said to heterozygous for the marker. Only codominant markers exhibit this behavior which is highly desirable, dominant markers exhibit a present/absent behavior which can limit data available for analysis.

RFLP has some limitations since it is time consuming. Moreover, in some organisms such as wheat, RFLP is of Low frequency which is attributed to the polyploidy nature of wheat, and large genome size. However; in the past RFLP was used for several purposes including genome mapping, varietal identification, identification of wheat rye recombinants, and identification of homologous chromosome arm (Tankesley *at al.*, 1989).RFLP was also used for varietal identification, for example, it has been used for mapping different storage protein loci polymorphic probes was used to identify 54 common wheat cultivar, mostly Italian type (Vaccino *et al.*, 1993).

2.11 Techniques used for studying *Moringa*

In recent years DNA based methods such as RAPDs (Gjuric and Smith, 1996) and AFLPs Gaiotto *et al.*, (1997) have been used, to estimate out crossing rates. However, due to their dominance behavior, RAPD and AFLP markers provide less information per locus than codominant markers (Gaiotto *et al.*, 1997). This is particularly relevant for applications that require genotype discrimination, as in the case of out crossing-rate estimation (Gaiotto *et al.*, 1997). However, (Ritland *et al.*, 1981) demonstrated that this limitation could be readily overcome by multi locus estimation of out crossing with dominant markers having intermediate gene frequencies.

Shamsuddeen *et al.,* (2012) studied the genetic diversity and analysis of genetic relationship among 20 *M. oleifera* were carried out with the aid of twelve primers from random amplified polymorphic DNA marker. The seeds of twenty *M. oleifera* genotypes from various origins were collected and germinated and raised in nursery before transplanting to the field at University Agricultural Park (TPU). Genetic diversity parameter, such as Shannon's information index and expected heterozygosity, revealed the presence of high genetic divergence with value of 1.80 and 0.13 for

Malaysian population and 0.30 and 0.19 for the international population, respectively. Mean of Nei's gene diversity index for the two populations was estimated to be 0.20. In addition, a dendrogram constructed, using UPGMA cluster analysis based on Nei's genetic distance, grouped the twenty *M. oleifera* into five distinct clusters. The study revealed a great extent of variation which is essential for successful breeding and improvement program. From their study *M. oleifera* genotypes of wide genetic origin, were recommended to be used as parent in future breeding program.

A total of 12 RAPD primers were used to investigate the genetic diversity between and within cultivated and non-cultivated provenances of *M.oleifera* Lam (Nyomora *et al.*, 2010).

Muluvi *et al.*, (1999) study the utility of dominant AFLP markers in estimating outcrossing rates in *M. oleifera* and then use them (AFLP markers) to obtain estimates of outcrossing rates in an *M. oleifera* seed orchard from Mbololo, Kenya.

The present work demonstrates that AFLP markers, though dominant with a lower information content than co-dominant markers are adequate for the study of the mating system in *M. oleifera.*

CHAPTER THREE

MATERIALS AND METHODS

3.1. Experimental site

The field experiments were conducted for two consecutive seasons **(2011\12-2012\13)** in the Demonstration Farm at Shambat (Latitude 15º 40´ N, Longitude 32º 32´ E and A latitude 386m above sea level) of the, Sudan University of Science & Technology, College of Agricultural Studies, to study **Agromorplological , Molecular and Quality investigation on *Moringa oleifera* and *Moringa peregrina* as affected by water intervals**.The soil is montmorillonitic clay soil with a pH in the range of 7.8-8.5 (Abdelhafiz, 2001). The climate of the locality has been described by (Adam, 2002) as semi-desert and tropical with low relative humidity. The mean annual rainfall is about 160 mm and the mean maximum temperature is more than 40º C in summer and around 20 º C in cool season. Solar radiation is about 400-500 cal cm^2 days. The growing season of the year 2011/2012 compared to that of 2012/2013 was characterized by increased maximum temperature, reduced total rain fall relative humidity. In the year 2011/2012, sowing date was on the 29 November and the year 2012/2013 was on 29 November at Shambat respectively.

3.2. Cultural practices and treatments of the experiments

The land was prepared by disc plough; disc harrowed and leveled ridging up north – south arranged in a complete randomized block design with four replicates. The spacing between ridges was 70cm. The plot size was 3 x 3 m ridge, seed rate was two seeds from each variety (*M.oleifera & M, peregrina*) were sown per holes spaced at 15 cm along the ridge. Nitrogen (urea) was applied four weeks after sowing at a rate of 112g N/ plot. The experiment was irrigated firstly every seven days after the germinations

established, irrigation interval treatment was control irrigated every 10 days, and the watering intervals every 20 and 30 days respectively. The fertilizer Nitrogen was applied after 2 months.

3.3. Plant materials

The *Moringa* seeds that used in the study were obtained from the seed Research Centre (Soba).The experimental design applied was complete randomized block design with four replications.

3.4. Layout of experiments

The two Moringa species were subjected to three irrigation intervals (control irrigated every 10 days, and the watering intervals every 20 and 30 days) in four replications. Two seeds per hole were sown from both species in a soil depth of 2 cm.

3.5. Characters studied

3.5.1 Growth attributes

After the establishment stage five plants were randomly chosen from each plot and tagged in each plot treatment to monitor the growth characters samples were taken every week. However, the two species were grown under the same cultivation practises.

3.5.1.1 Plant height (cm)

Plant height was measured from the soil surface to the upper plant. Five plants was selected at random in each plot expressed in centimeter were taken weekly.

3.5.1.2 Number of leaves plant

Were taken weekly by counting all leaves in the five tagged plants and the average number of leaves plant was determined.

3.5.1.3 Stem diameter (mm)

Taken as the thickness of the stalk at the middle of internodes from the plant base using digital Vernier, five were selected from tagged plants which was taken weekly.

3.5.1.4. Plant fresh weight (kg)

These character was taken as the fresh weight of the total number of plants were taken in square meter by cutting plants from each plot and weight using a balance and estimate the fresh weight.

3.5.1.5. Plant dry weight (kg)

The fresh forage yield was left to dry in the air and then weighed again to give the dry weight by using sensitive balance.

3.4.1.6. Root dry Weight (g)

The same plants were harvested to take fresh weight were taken to estimate root dry weight. The roots were separated from fresh plant stems and been dried by air dry weight was measured by using sensitive balance.

3.6 Proximate Chemical Analysis

The chemical analyses were carried out in the laboratory of the Sudan University of Science and Technology, University of Khartoum, and Food and Technology Research Centre at Shambat.

3.6.1 Proximate analysis for *Moringa oleifera and Moringa peregrina*

The samples taken (dry weight and root) to estimate the dry matter yield were used for the analysis. The percentages of the following quality traits were determined:

3.6.1.1 Crude fiber (CF)

The crude fiber content was determined according to the official method of the AOAC (1984). It was determined gravimetrically after the sample is chemically digested in chemical solutions. The weight of the residue after ignition is then corrected for ash content and is considered as crude fiber.

3.6.1.2 Crude protein (CP)

The protein content was determined for both *M. oleifera &M pergreina* by macro – Kjeldahl method using a copper sulphate catalyst according to the official method of the AOAC (1990). The sample was accurately weighed and transferred together with 2 g of Kjeldahl catalysts (No. 33064, BDH , England) and 20 ml of concentrated sulphuric acid (No. 66700, BH 15, England) into a Kjeldahl digestion flask . After the flask was placed into Kjeldahl (No. 01186069, Tape KI 16, Gerhart Bonn) for about 2 h, until a colorless digest was obtained and the flask was left at cool room temperature. The distillation of ammonia was carried out boric acid by using distilled water and sodium hydroxide solution, finally the distillate was titrated with standard solution in the presence of drops of mixed (Bromocreasol green and methyl red) until a pink reddish color was observed .

3.6. 1.3 Fat content (FC)

Determined according to the official method of AOAC (1990) .The method determined the substances which are soluble in petroleum ether (No. 28111, Doo, England) (B.P , 40-60C) and extractable under the specific conditions of Soxhlet extraction method , then dried ether extract (fat content by Electro thermal, England) is weighed and reported as percentage of initial dry matter.

3.6.1.4 Ash content

Determined according to the method described by Pearson (1981), the inorganic materials which are concentration and composition, are determined as a residue after being ignited at specified heat degree. No. 20. 30(1870, carbolated, England).

3.6.1.5 Carbohydrate

The total carbohydrates content of *Moringa* samples were calculated by subtracting the total sum of (Moisture, Fat, Protein and Ash as percentages) from 100% according to (promeran and Meloan, 1992).

3.6.2 Proximate analysis for Minerals in *Moringa*

The dry aching method that was described by (Chapman *et al.*, 1961) was used for determination of minerals. *Moringa* samples were weighed in clean dry porcelain crucible and placed into muffle furnace No.20. 30 (1870, carbolated, England), then the ash content was cooled and placed in a hot sand bath. After that the ash solution of each sample was filtrated with ash less filter paper the concentrations of Sodium, Potassium, Magnesium, Calcium and Iron were determined by using Atomic Absorption Spectrophotometer (3110-Perkin Elmer).

3.7 DNA extraction

Genomic DNA was extracted from fresh leaves tissue 22 samples of both *M.oleifera* & *M peregrina* individuals using modified CTAB method (Porebski *et al.,* 1997). The modification was made in intention to improve the quantity and the quality of DNA. In this method the fine powdered plant materials were transferred into 13 ml Falcon tubes containing 6 ml of pre-warmed buffer solution. Tubes containing the samples were then incubated in a water bath at 65°C with gentle shaking for 30 min and left to cool at room temperature for 5 min. Isoamyl alcohol chloroform mixture (1:24) was added to each tube and the phases were mixed gently for 5 min at room temperature to make a

homogenous mixture. The cell debris was removed by centrifugation at 5000 rpm for 15 min and the resulted clear aqueous phases (containing DNA) were transferred to new sterile tubes. The step of the chloroform: isoamyl alcohol extraction was repeated twice. The nucleic acids in the aqueous phase were precipitated by adding equal volume of deep cooled isopropanol. The contents were mixed gently and collected by centrifugation at 4000 rpm for 10 min. The formed DNA pellet was washed twice with 70% ethanol and the ethanol was discarded after spinning with flash centrifugation. The remained ethanol was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in TE buffer (10 mM Tries, 1 mM EDTA, pH 8) and stored at -20°C for further use. The extracted DNA samples were observed under UV illumination after staining with ethidium bromide and agarose gel electrophoresis. The purity and the concentrations of the DNA were then spectrophotometrically assessed following (Sambrook *et al.*, 1989) method.

3.8. Agarose Gel for Electrophoresis

First, the ends of the tray were sealed with a masking tape. 1% agarose gel solution was prepared in a flask (by weighing 1g of agarose in 100 ml 1XTBE buffer). The powder was placed in a conical flask and 100 ml TBE were added.The solution was heated using ,the microwave oven for 2 min, to dissolve completely the agarose particles and clear solution was made.

The flask was left at room temperature until the solution become warm enough. Then 2 µl of Ethidium Bromide (diluted) was added and shacked gently. The solution was poured into gel tray and the comb was placed in the tray to make wells after the gel cools.

The tank was filled with 1x TBE buffer. The samples (DNA) were loaded using micropipette. The power supply was connected and operated at 75 volts for 3 hours.

3.9 DNA by the Gel Documentation Apparatus

After electrophoresis was finished, gloves were used to take the gel and place it in the gel documentation chamber. The camera was operated and image was adjusted on it. The chamber was closed and the U.V. light was turned on. The picture was viewed on the camera screen and captured. The camera USB was connected with USB Flash for saving image and the image was viewed after connecting the USB Flash to the computer.

This is very important step where, it is useful to know the amount of present DNA before performing a PCR or RAPDs techniques. In this study, DNA quantification was done by the comparison of the extracted DNA and comparing it with several aliquots of lambda DNA, at known concentrations using gel electrophoresis. 1.5% agarose gel was prepared (with added 2 µl of Et Br). 3µl of loading dye were mixed with 5µl of DNA and loaded onto the gel. 2µl of Hind III digested DNA, (2 µg) were loaded into one of the wells, the gel was run at 75 V until the dye is 5cm away from the wells, then the gel was illuminated with U.V. The intensity of the DNA bands of the samples was compared with the intensity of the bands. As the amount of DNA present in each band is known, the amount of DNA sample can be calculated by comparing the fluorescent yield of the sample with those of the bands.

3.10 Inter-Simple Sequence Repeat Techniques (ISSR)

20 ISSR primers were used for PCR amplification**.** The PCR reactions were carried out in 25 µl volume containing 15µl sterile distilled water, 2.5 µl 10X taq buffer, 2.5 µl (2 mM/µl) dNTPs, 1.5 µl (50 mM) $MgCl_2$, 2 µl (10 pmol/µl) primer, 0.5 µl(5u/ µl) Taq DNA polymerase and 1 µl (10 to 30 ng/µl) template DNA, for each sample.

The PCR amplification protocol was programmed for 5 min at 94°C for initial denaturation, followed by 40 cycles of 1min at 94°C, 1 min at (42°C for ISSR) and 1

min at 72°C, final extension was programmed for 7min at 72°C followed by hold time at 4°C until samples were collected.

3. 11. Statistical analysis

The data were collected for the different growth characters were subjected to analysis of variance (ANOVA) using the standard procedure of the complete randomize plot design and means separation was done by Duncan Multiple Range Test (DMRT) for the main and interaction effects.(Gomez and Gomez, 1984). Statistic analysis of molecular markers (FSSR) Data was scored as (0) for absence of a fragment and (1) for presence of a fragment. The software ***STATISTICA* ver. 6** was used to reflect the genetic relationships among the 22 studied date Moringa genotypes based on twenty ISSR primers data.

CHAPTER FOUR

RESULTS

4.1 Agro- morphological Chartres:

The results showed that, there were highly significant differences between two species of Moringa. However *M. peregrina* germination was very slow and showed delay in growth which took two months. On the other hand, *M. oleifera* showed fast germination and good establishment in 4 weeks. Due to weak performance of *M. peregrina* in the field, the parameters were taken only from *M. oleifera.* However, chemical and molecular analyses were done for the two *Moringa* species. (Fig .1 & Fig.2)

Figure (1) *Moringa oleifera* under field Experiment

Figure (2) *Moringa peregrina* under field Experiment

4.2 Plant height (cm)

Results showed that, there were no significant differences within plant height for the *M.oleifera* during the two seasons under three watering intervals. Whereas presented in Table (1), the treatment of irrigation 30 days gave the highest plant height (73.4 cm) for all weeks except weeks 5th and 8th. Which irrigation every 20 days gave the highest plants height on the first season. In the second season irrigation every 10 days (control) showed the highest plant highest (95.9cm) for all weeks except week 1th in each irrigation due to interval irrigations.

4.3 Number of leaves/ plant

The effect of different irrigation intervals (10, 20, and 30 days) on *M. oleifera* on number of leaves was presented in Table (2). There was no significant difference for all readings due to the effect of watering intervals. The first week presented, the highest average of number of leaves than the last weeks. Irrigation every 30 days showed the highest number of leaves (11.1) in first week in the first season. However, watering every 10 days in the second season showed the highest number of leaves (8.5) during the whole experiment.

4.4 Stem diameter (mm)

Table (3) The analysis of variance of *M. oleifera* in stem diameter, showed that, no significant differences between water treatments during two seasons, except in **first, second, third, and sixth** weeks of the first season. Watering interval of 30 days showed significant difference between other treatments, with highest (6.4 mm) in irrigation every 30 days during season (2011\12).

Table 1. Effect of different irrigation intervals in plant height (cm) of *Moringa oleifera* during two seasons (2011/12 and 2012/13).

Season 2011/12

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week7	Week 8	Average
Irrigation1	45.7a	51.7a	55.2a	47a	42.9a	61.3a	62a	62.7a	53.6
Irrigation 2	47.8a	53.5a	59.9a	64.4a	72.7a	64.6a	63.9a	76.9a	62.9
Irrigation 3	62.7a	69.9a	70.4a	68.6a	69.3a	73.4a	68.1a	54.1a	67.0
S.E.±	**7.4 ns**	**10 ns**	**12.5 ns**	**8.7 ns**	**11.8 ns**	**11.7 ns**	**11.3 ns**	**10.5 ns**	**-**
C.V %	27.8	33.4	39.4	28.2	37.2	34.3	33.9	31.7	-

Season 2012/13

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week7	Week 8	Average
Irrigation 1	60.4a	82.2a	89.4a	93.7a	86.9a	95.7a	95.4a	95.9a	87.4
Irrigation 2	69.9a	80.4a	88.1a	80.7a	85.8a	90.3a	92.6a	88.8a	84.6
Irrigation 3	57a	69.2a	74.5a	72.5a	67.8a	70.7a	76.1a	80.1a	70.9
S.E.±	**9.3 ns**	**11.7 ns**	**10.2 ns**	**9.6 ns**	**10.6 ns**	**10.8 ns**	**11.7 ns**	**12.1 ns**	
C.V %	29.1	29.6	23.8	22.8	25.8	24.8	26	26.9	

Means with the same letter(s) within each column are not significantly different according to Duncan Multiple Range Test (DMRT) at the 0.05 level.

Where: -Irrigation 1 =every 10days, Irrigation2 = every 20days and Irrigation3 = every 30 days

Table 2. Effects of different irrigation intervals in number of leaves of *Moringa oleifera* during two seasons (2011/12 and 2012/13).

Season 2011/12

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week7	Week 8	Average
Irrigation 1	10a	8.1a	7.4a	6.3a	4.8a	6.2a	5.5a	4.7a	6.6
Irrigation 2	9.7a	7.7a	5.7a	4.9a	6.7a	6.2a	4.9a	4.5a	6.3
Irrigation 3	11.1a	9.9a	7.2a	5.2a	6a	5a	4.9a	3.9a	6.7
S.E.±	**1.2 ns**	**1.2 ns**	**0.8 ns**	**0.9 ns**	**1.1 ns**	**0.7 ns**	**0.6 ns**	**0.6 ns**	**-**
C.V %	23.3	25.9	21.4	30.7	34.7	23.2	23.3	27.8	-

Season 2012/13

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week7	Week 8	Average
Irrigation 1	8.1a	8.5a	8.5 a	7.9a	7.5a	7.5a	6.6a	6.4a	7.6
Irrigation 2	7.1a	6.5a	7a	6.1a	5.6a	5.2a	5.6a	6.1a	6.1
Irrigation 3	7.2a	6.8a	6.7a	7a	5.4a	6.1a	5.9a	5.7a	6.3
S.E.±	**1.1 ns**	**1.1 ns**	**0.9 ns**	**0.8 ns**	**0.5 ns**	**0.7 ns**	**0.5 ns**	**0.8ns**	**-**
C.V %	30.1	31	25.2	22.4	14.8	21.5	17.8	26.2	-

Means with the same letter(s) within each column are not significantly different according to Duncan Multiple Range Test (DMRT) at the 0.05 level

Where: -Irrigation 1= every 10 days, Irrigation2 =every 20days and Irrigation3 =every 30 days

Table 3. Effects of different irrigation intervals in stem diameter of *Moringa oleifera* during two seasons (2011\12 and 2012\13).

Season 2011/12

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week7	Week 8	Average
Irrigation 1	4.2ab	3.7b	4.2ab	4a	3.2a	4.4ab	4.1a	5.4a	4.2
Irrigation 2	3.2b	3.6b	3.6b	3.3a	5.1a	3.7b	4.5a	4.8a	3.9
Irrigation 3	6.4a	6.4a	6.2a	6.2a	5.6 a	6.4a	4.2a	5.9a	5.9
S.E.±	**0.6***	**0.4***	**0.4***	**0.6 ns**	**1 ns**	**0.4***	**0.7 ns**	**0.9 ns**	**-**
C.V %	21.7	16.1	15.2	21.8	36.4	15.3	24.2	26.7	-

Season 2012/13

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week7	Week 8	Average
Irrigation 1	5.9a	6.1a	6.3a	7.5a	5.3a	7a	7a	7a	6.5
Irrigation 2	6.1a	6.1a	6.1a	6.7a	7.2a	7a	7a	7.1a	6.6
Irrigation 3	5a	4.8a	5.5a	6a	6.2a	6.2a	5.9a	5.4a	5.6
S.E.±	**0.9 ns**	**0.5 ns**	**0.6 ns**	**0.9 ns**	**1.4 ns**	**1.1 ns**	**1 ns**	**1 ns**	**-**
C.V %	29.9	17.3	18.2	25.6	40.8	30.4	29	28.5	-

Means with the same letter(s) within each column are not significantly different according to Duncan Multiple Range Test (DMRT) at the 0.05 level

Where:-Irrigation 1= every 10 days, Irrigation2 = every 20days and Irrigation3 =every 30 days

4.5 Fresh weight (kg)

Fresh weight parameter for both seasons (Table 4), the analysis variance showed that, there was no significant effect of watering intervals between all treatments (10, 20, and 30 days) on *M. oleifera* fresh weight cultivated during two seasons.

4.6 Dry weight (kg)

The results revealed that , there was non significant difference for dry weight of *M.oleifera* which was cultivated under the three irrigation interval (10, 20, and 30 days) during two seasons (2011/12), Table (5). However, the lower dry weights were observed in treatments 20 and 30 days irrigation intervals in compared with control which showed the highest weight. (Table 5)

4.7 Root dry weight (kg)

Data in table (6) illustrate the results of root dry weight cultivated under the three watering intervals. The analysis of variance showed no significant difference during two seasons. Watering interval every 10 days showed that, the highest root dry weight value, whereas, watering every 20 days showed the least weight in the first season. In the second season, watering interval every 20 days showed the highest weight and every 10 days showed the lowest weight.

Table 4. Effects of different irrigation intervals in fresh weight of *Moringa oleifera* during two seasons (2011\12 and 2012\13).

Treatment s	**Season 2011/12**	**Season 2011/13**
Irrigation 1	591.0 a	1274.0 a
Irrigation 2	436.6 a	1870.0 a
Irrigation 3	437.9 a	1352.0 a
S.E±	**97 ns**	**210 ns**
C.V%	**38.4**	**26.5**

Means with the same letter(s) within each column are not significantly

different according to Duncan Multiple Range Test (DMRT) at the 0.05 level .

Where: - Irrigation 1 = every 10 days

Irrigation2 = every 20days

Irrigation3 = every 30 days

Table 5. Effects of different irrigation intervals in dry weight of *Moringa oleifera* during two seasons (2011\12 and 2012\13).

Treatments	**Season 2011/12**	**Season 2011/12**
Irrigation 1	73.7 a	291.6 a
Irrigation 2	38.5 a	306.4 a
Irrigation 3	50.4 a	277.9 a
S.E±	**15.6 ns**	**70.5 ns**
C.V%	**55.9**	**45.7**

Means with the same letter(s) within each column are not significantly different according to Duncan Multiple Range Test (DMRT) at the 0.05 level

Where: - Irrigation 1 = every 10 days

Irrigation2 = every 20days

Irrigation3 = every 30 days

Table 6. Effects of different irrigation intervals in root dry weight of *Moringa oleifera* during two seasons (2011\12 and 2012\13).

Treatments	**Season 2011/12**	**Season 2011/12**
Irrigation 1	105.7 a	264.6 a
Irrigation 2	82.8 a	500.6 a
Irrigation 3	87.00 a	402.0 a
S.E±	**23.3 ns**	**73.1 ns**
C.V	**49.2**	**35.5**

Means with the same letter(s) within each column are not significantly different according to Duncan Multiple Range Test (DMRT) at the 0.05 level

Where: - Irrigation 1 = every 10 days

Irrigation2 = every 20days

Irrigation3 = every 30 days

4.8 Chemical composition analysis

4.8.1 Leaves

Analysis was done to know the suitability of the *Moringa* leaves of both species as animal fodder. Chemical analysis Results showed that *M.peregrina* and *M. oleifera* Protein, Fiber, Carbohydrate and Fat, was presented in table (7). Results showed that *Moringa* leaves can be considered as good fodder as it contain essential nutrients that can improve growth performance of animals. The percentage of protein is higher in *M. oleifera* . (Table 7).

4.8.2 Roots

Moringa oleifera roots showed higher fiber content than *Moringa peregrina* as presented in table (8).

4.9 Mineral composition analysis of *Moringa oleifera* and *Moringa peregrina*

4.9.1 Leaves mineral composition of *Moringa oleifera* and *Moringa peregrina*

Mineral composition show high variation between the two Moringa species (Table 9). *Moringa oleifera* gave higher percentage of Ca, K and Fe whereas, *Moringa peregrina* contained higher percentage in Na and Mg.

4.9.2 Root mineral composition of *Moringa oleifera* and *Moringa peregrina*

The analysis of the roots of the two *Moringa* species were presented in table (10). The analysis showed that, there were highly variations between the two species in some mineral contents . These results indicated that the *M. oleifera* was slightly higher than the *M. peregrina* in contents of K. Where, *M. peregrina* showed higher percentage in Na

Table 7. Chemical composition of *Moringa oleifera and Moringa peregrine* which was cultivated during two seasons *(2012\13)*.

Moringa species	Protein(%)	Ash (%)	Fiber (%)	CHO(%)	Fat (%)
Moringa oleifera	23.5	9.7	17.6	14.6	7.7
Moringa peregrina	15.9	9.6	11.7	11.2	7.5

Table 8 .chemical composition of *Moringa oleifera* and *M. peregrina*s which was cultivated during season 2012\ 2013

Element	*Moringa oleifera*	*Moringa peregrina*
Ash (%)	11.2	13.1
Fiber (%)	18.6	13.6

Table 9. Mineral composition of *Moringa oleifera* and *Moringa peregrina* which were cultivated during during season 2011/12.

Moringa species	Ca(%)	Mg(%)	Na(%)	K(%)	Fe(%)
Moringa oleifera	3.5	0.10	1.06	3.80	2.38
Moringa peregrina	0.74	0.34	3.44	0.59	-

Table 10. Mineral composition of *Moringa oleifera* & *Moringa peregrina* Roots grown in season 2011/12.

Element	Ca	Mg	Na	K	Fe
Moringa oleifera (%)	0.15	0.20	0.75	0.75	0.13
Moringa peregrina(%)	0.11	0.21	1.13	0.28	0.19

4.10 Molecular analysis

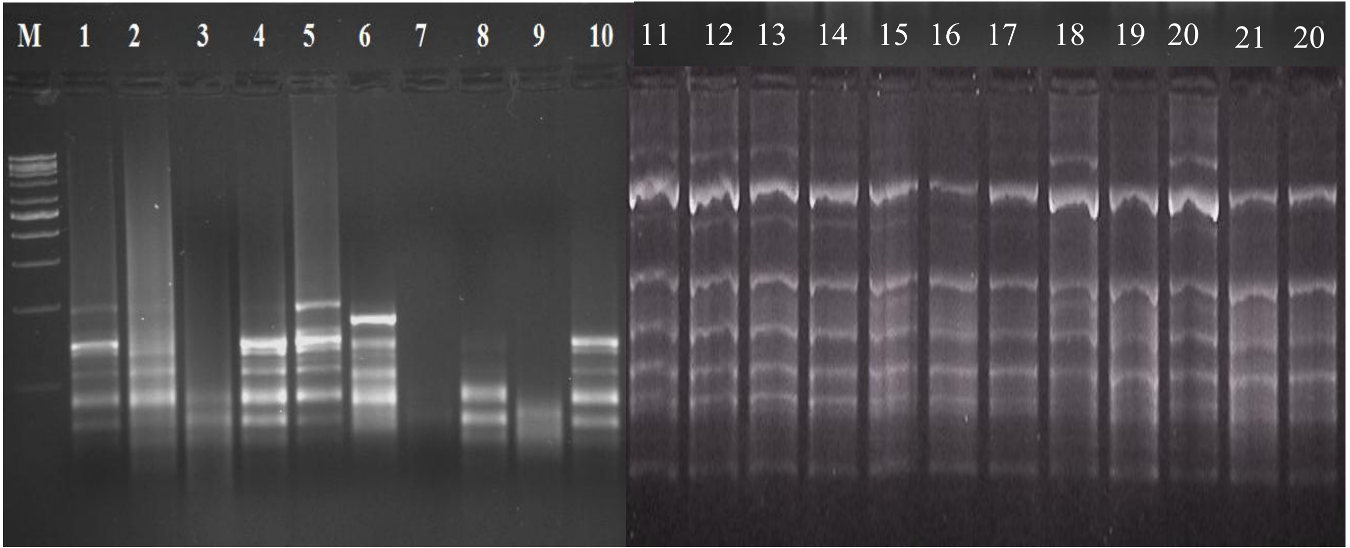
20 ISSR primers were tested to investigate the genetic diversity between *M. oleifera &* *M. peregrina*. Four primers showed polymorphism within and among the two species. The four ISSR primers showed number of bands (45). Out of the 43 polymorphic bands, and only one band was identified as a fingerprint as shown in figure (3) and in table (12).

The distance matrix values ranged between 1.41 to 5.48, which reflected high range of variation Table (12). The tree diagram unweight pair gr (UPGMA) for the 22 individuals of the two *Moringa* species showed clear clustering, as each species clustered separately. Cluster one contained all *M.oleifera* individuals (from 1-10) except individual 11. Cluster two contained all *M. peregrina* individuals (from 12- 22). Only one individual of the *M. oleifera* (11) was different from the two *Moringa* individuals and appeared as out-group.

Some of the *M.oleifera* individuals within the same cluster were genetically close to each other and appeared as sisters, e.g. (1 &4), (2& 5), (3&8). Some individuals of *M.peregrina* were genetically close e.g. (17& 18& 19) as appeared as sisters, also individuals (14 & 20& 21) were closely related than others within the same cluster.

Table12. Total number of bands, number of Polymoriphic and Monomorphic bands and number of fingerprint for ISSR primers

Primer	Sequence (5'-3')	Total Number of bands	Number of polymorphic bands	Number of monomorphic Bands	Number of fingerprint	Percentage of polymorphic bands
807	**(AG)8 T**	**12**	**11**	**1**	**1(sample7)**	**92%**
814	**(CT)8 A**	**13**	**13**	**0**	**0**	**100%**
841	**(GA)8YC**	**11**	**11**	**0**	**0**	**100%**
848	**(CA)8 RG**	**9**	**8**	**1**	**0**	**89%**
Total		**45**	**43**	**2**	**1**	**381%**



Figure(3) Amplification of *M. oleifera* individuals ISSR primer 807

figure(4) Amplification of *M. Peregrina* individuals ISSR primer 807

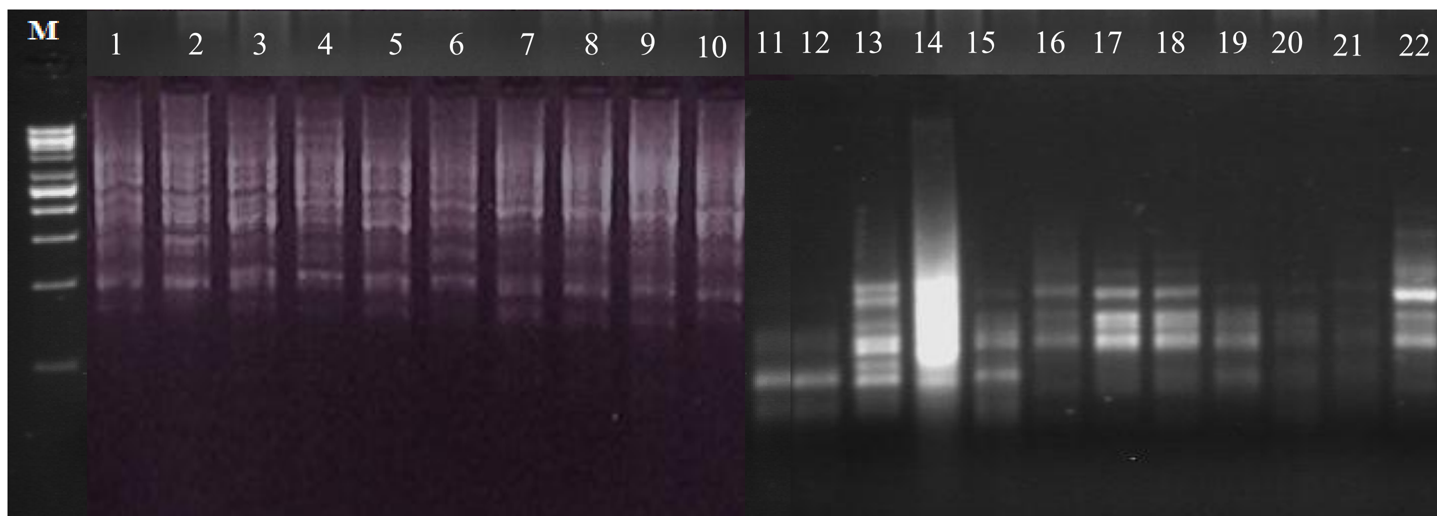
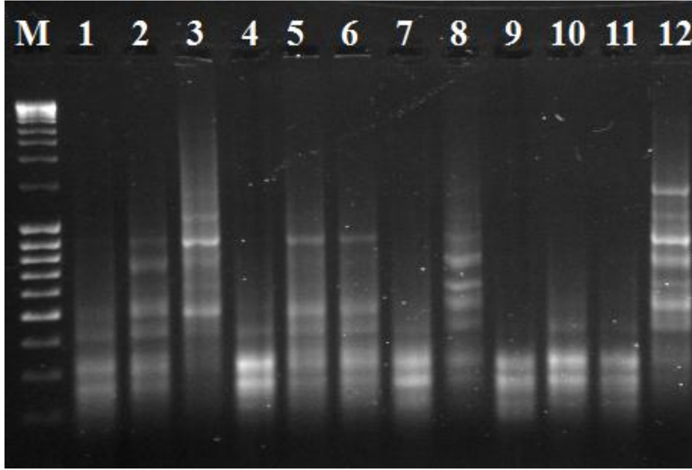
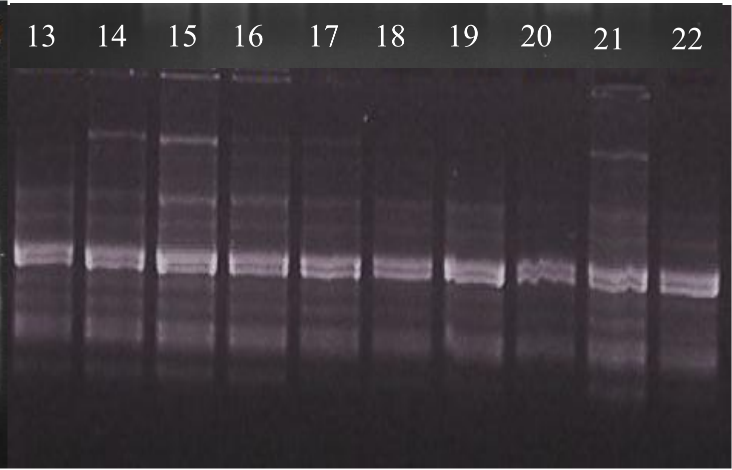


Figure (5) Amplification of *M.oleifera* individuals ISSR primer 814

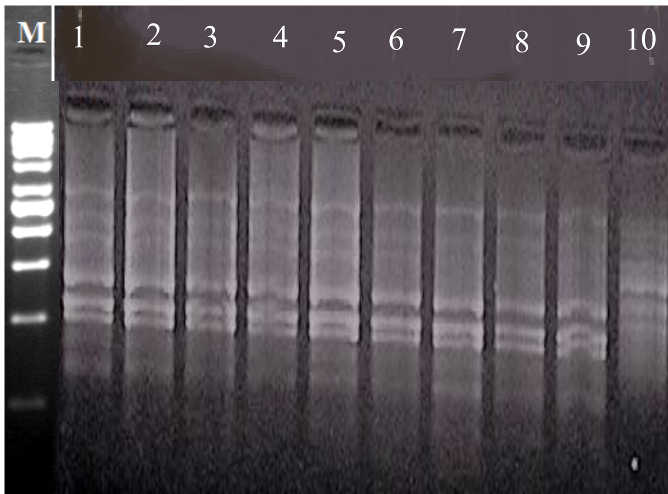
Figure (6) Amplification of *M.Peregrina* individuals ISSR primer 814

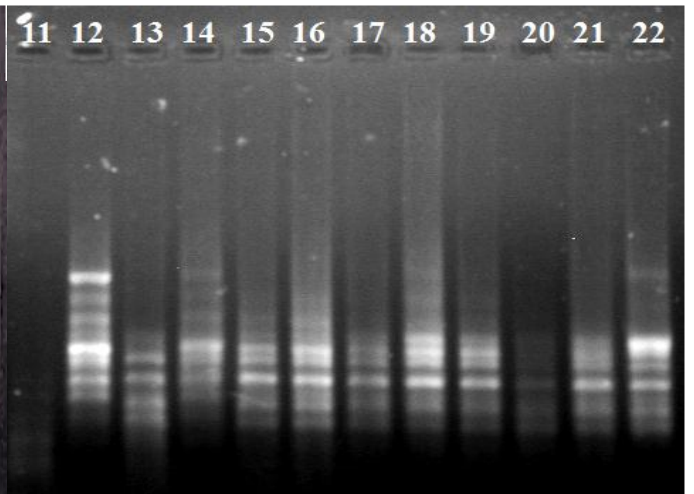




Figure(7) Amplification of *M. oleifera* individuals ISSR primer 841

Figure(8) Amplification of *M. Peregrina* individuals ISSR primer 841





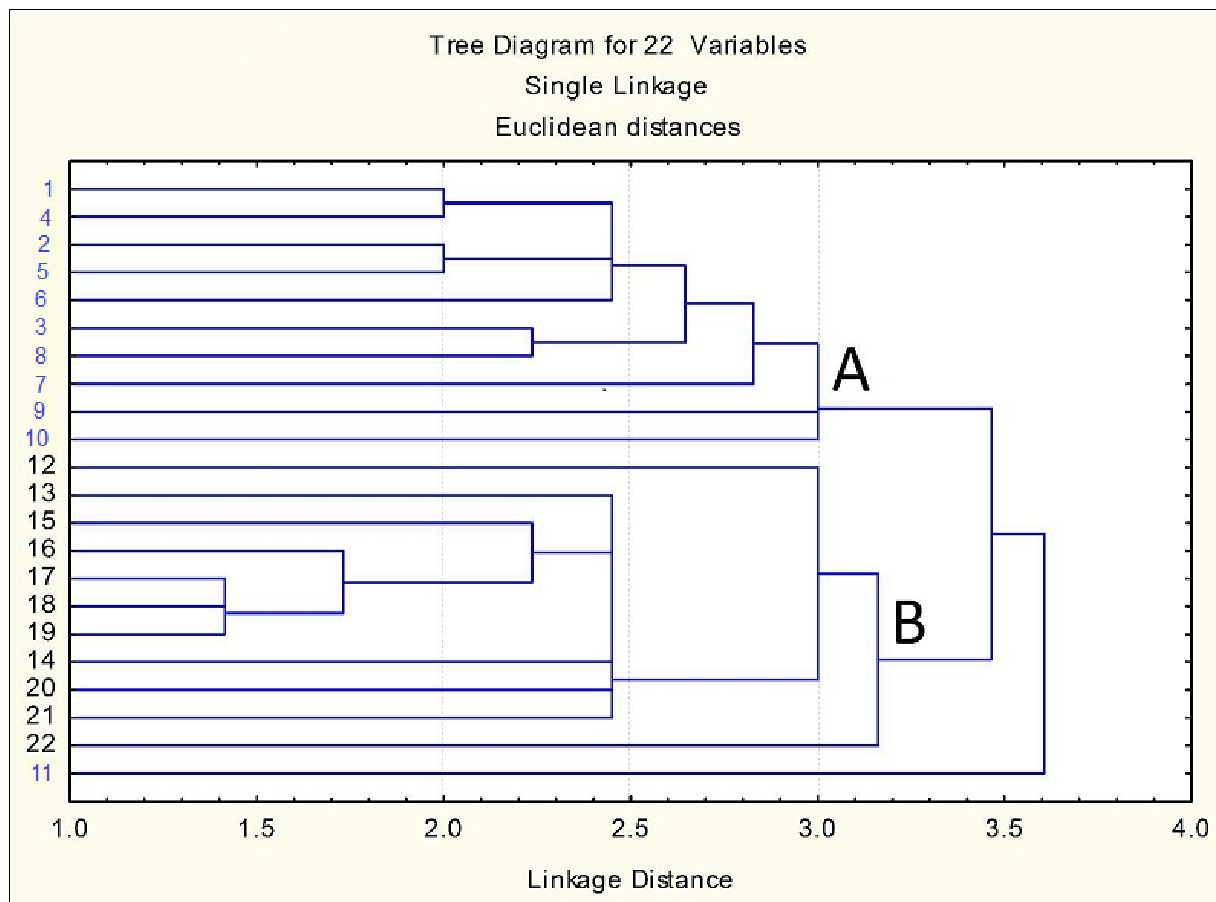
Figure(9)Amplification of *M. oleifera* individuals ISSR primer 848

Figure (10) Amplification of *M. Peregrina* individuals ISSR primer 848

Table 13. Genetic distance matrix between 22 individuals of *M. oleifera* and *M. peregrina* based on data of four ISSR primers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	0.00																					
2	3.00	0.00																				
3	4.36	3.16	0.00																			
4	3.16	3.00	3.87	0.00																		
5	3.32	2.45	3.74	3.00	0.00																	
6	3.74	3.00	3.61	2.83	2.65	0.00																
7	4.24	3.61	3.87	3.16	3.61	3.16	0.00															
8	4.12	3.16	2.83	3.61	3.74	3.32	3.61	0.00														
9	4.24	4.36	4.80	3.74	4.36	4.24	4.24	4.36	0.00													
10	3.61	3.46	4.47	4.12	3.74	4.36	4.80	4.69	4.58	0.00												
11	5.10	5.20	5.20	4.90	5.00	5.10	4.69	5.20	4.69	4.80	0.00											
12	5.48	4.80	4.36	5.10	4.80	5.10	5.48	4.80	5.48	5.74	5.66	0.00										
13	5.10	4.80	5.20	4.47	4.80	4.90	5.10	4.80	4.90	5.57	5.48	3.74	0.00									
14	4.69	4.58	5.00	4.47	4.36	4.69	5.29	4.80	5.10	5.20	5.48	3.74	2.83	0.00								
15	5.00	4.90	5.10	4.36	4.69	4.80	5.39	4.90	5.00	5.29	5.20	3.87	2.65	2.65	0.00							
16	4.69	4.58	4.80	4.00	4.58	4.90	4.90	4.58	4.90	5.20	5.10	4.24	2.83	2.45	2.24	0.00						
17	4.90	4.80	4.58	4.24	4.80	5.10	4.90	4.58	4.90	5.20	5.29	4.24	3.16	3.16	3.00	2.00	0.00					
18	4.69	4.80	4.58	4.24	5.00	5.10	4.90	4.58	4.90	5.39	5.10	4.24	3.16	3.74	3.61	2.83	2.00	0.00				
19	5.20	5.10	5.10	5.00	5.29	5.74	5.39	5.29	5.20	5.10	5.20	4.36	3.32	4.12	4.00	3.61	3.32	2.65	0.00			
20	5.20	5.10	5.29	5.20	5.29	5.57	5.39	5.10	4.80	4.90	4.36	5.00	4.58	5.00	4.90	4.58	4.12	3.87	4.00	0.00		
21	5.20	5.29	5.10	5.00	5.29	5.57	5.00	4.90	5.00	5.29	4.80	5.00	3.61	4.12	3.74	3.32	3.32	3.32	3.74	4.24	0.00	
22	5.10	5.20	5.20	5.10	5.20	5.29	5.48	5.20	4.90	5.57	5.48	4.47	3.74	4.00	4.36	4.24	4.00	3.46	3.61	4.12	4.58	0.00

figure (11): UPGMA- Dendogram results from analysis of four ISSR primers showing the relationship among 22 *Moringa oleifera & Moringa prergrina* individual



Whereas:

M. oleifera individuals (1-11)

M. peregrina individuals (12-22)

CHAPTER FIVE

DISCUSSION

Two species of *Moringa* were cultivated in the field, *M. oleifera* and *M. peregrina*. As the results obtained , *M. peregrina* showed slow growth , with creeping stand and there were no clear observation in growth, this may be due to genetic characters of growth of *M. peregrina.* (Brisibe *et al.,*2009) concluded that, the phenological development of *M. peregrina* was delayed with increasing altitude. This seems to be a consequence of the lower water supply and lower temperature at higher latitude. Moisture availability and temperature have been recognized to control the phenological behavior of plant in arid regions (Hagazy *et al.*,2008). The results showed that *M. oleifera* was early and fast growing compared with *M. peregrina,* it took four weeks for germination. Whereas, *M. peregrina* took more than Two months. In this study due to the poor performance of *M. peregrina* in the field, however, were taken parameters form *M. oleifera* only. Apparently *M. peregrina* is more drought tolerant than *M. oleifera* which is commercially planted in a large scale in tropical and sub tropical areas (Abohassan *et al.,* 2012).

Quintin,.2009, reported that, the average height and stem diameter of *Moringa* tree were 1.10m and 16.11 mm respectively. On the other hand , (Goss, 2007) detected that *Moringa* tree ranges in height from 5-12m with an open umbrella shaped crown, straight trunk (10-30 cm thick) with corky whitish park. Also Plada *et al*., 2003 found that, the fast growing, drought-tolerant *M. oleifera* can tolerate poor soil, it has a wide rainfall range (25 to 300 cm per year), and soil pH range from 5.0 to 9.0 .

In this study, the average plant heights ranged from 45.7 - 62.7cm in watering interval one (10 days), to 47.8- 76.9cm in water interval two (20 days), whereas in watering interval three (30 days) the average of plant heights range

between 54.1 -73.4 cm, the plant height was higher than the two intervals. Recent studies have shown that when a plant undergoes prior exposure to stress, it has the ability to respond faster and more vigorously to a recurring stress event (Walter *et al.,* 2011). This phenomenon, known as hardening, that plants have greater tolerance to stress from biochemical changes or epigenetic alterations that occur after the first exposure to environmental disturbance(Bruce *et al.,* 2007), (Elata *et al*., 2009) found that plant height of *M. oleifera* under semi desert were 237.5 cm and *M. peregrina* were 264.0 cm. *M. oleifera* was in line with the plant height of study, however *M. peregrina* showed different manner of growth and very tall in his study compared with this study. Results of *M. oleifera* showed, no significant difference among parameters of plant height , number of leaves per plant , stem diameter, fresh weight , dry weight and root dry weight due to effects of watering intervals in two seasons. There were exception on stem diameter in the first season (First , second , third and six weeks), which were showed significance.

Rivas *et al.,*2013 suggested that, seeds of *M. oleifera* subjected to mild water deficit have had increased the ability for drought tolerance young plant.

M. oleifera kept high germination percentage only until the potential of 0.2 Mpa which may be one indicative of low osmotic capacity due to high oil content Rivas *et al*.,(2013). Fantinatti *et al.*2005) suggested that oil seed species absorb less water because of they are hydrophobic. Young *M. oleifera* plant submitted to drought showed stomata closures as a first line of defiance, which is common in woody species . Thus it is vital for plants to reduce the transpiration rate under low water availability conditions. However, *M.oleifera* showed strong leaf senescence , which greatly reduced the total leaf area. Young plant of *M. oleifera* exhibited a height plasticity to carry out metabolic adjustment to recover during the hydration period. (Rvias *et al*.,

2013) suggested an increased tolerance of *M.oleifera* after repeated cycle of drought due to more than one mechanism.

Moringa being extremely rich in nutrients is an excellent source of mineral for feeding human and animals. In this study, mineral composition showed high variation between two *Moringa* species in leaves and root. *M. oleifera* gave higher percentage of Ca(3.5%) , K (3.8%) and Fe (2.38%), and, whereas, *M. peregrina* contained higher percentages in Na (3.44%), and Mg (0.34 %). On the other hand, Soliva *et al.*,(2005) showed that, of *Moringa* leaves are rich in protein and amino acid composition which is suitable for human and animal nutrition.

The results of the chemical composition of *M.oleifrea* leaves was obtained higher value of protein 23.5%, Ash 9.7%, Fiber 17.6%,CHO 14.6% and Fat 7.7%. Compared with *M.peregrina* which obtained 15.9%, 9.6%, 11.7%, 11.2% and 7.5% respectively. However, roots of two species were not different, *M.oleifera* obtained Ash 11.2% and fiber 18.6 % and *M.peregrina* Ash 13.1% and 13.6%. This results were similar to(Elata *et al.,* 2009) who reported that, the percentage of protein of *M. oleifera* leaves was 25 ,56 % whereas, fiber , Ash and Fat were 8.47% ,11.73% and 12.77%, respectively, and for *M. peregrina* percentage of protein in leaves were 23, 31 %, however, Fiber 6, 39%, Ash 11, 72%, and Fat 5, 81 %. Also Aja *et al.*,(2013) who reported that, both leaves and seeds of *M. oleifera* contain an appreciable amount of Fat and Oil, Crude, fibre, Carbohydrate, Moisture, Ash, serving as a good source of these nutrients with low amount of protein in the seeds and the least in the leaves. On the other hand,(Elata *et al.*, 2009) confirmed that, *M.oleifera* had greater values in chemical composition than *M. perigerna.* Most reports indicate that, *Moringa* leaves are rich in protein and amino acid composition, which is suitable for human and animal nutrition. Similar results was obtained by (Ogbe *et al.*, 2011) who reported that, *Moringa* leaves contained appreciable amounts of crude protein (17.01% ±0.1) and carbohydrate

(63.11% ±0.09, crude fiber (7.09% ±0.11), ash (7.93% ±0.12), crude fat (2.11% ±0.11) and fatty acid (1.69% ±0.09). Furthermore the Leaves of *Moringa* represent an important source of nutrients for rural populations (Gupta *et al*., 1989 and Lockett *et al*., 2000).

Mubark, *et al.*, (2010) who showed the different between leaves and other morphological part of *M. oleifera* the Ash 139.8g/kg Protien 267.9g/kg Fiber 210.0g/kg however, while Plant (forage)Ash 123.7g/kg protein 200.0 g/kgFiber 760.9g/kg .Whereas, Mutayoba *et al.*, (2011) how reported that, the crude protein was 30.65% in *M. oleifera* leaves which is more than we found in this study. However Ogbe *et al*., (2011), how reported that most of the Moringa *previous* studies on considered to leaves minerals values such as , Mn (81.65 ±2.3), Zn (60.06 ±0.3) and Cu (6.1±0.2).

Mutayoba *et al.,* (2011) who reported values for Mn 57.34, Zn 21.70 and Cu 5.73 parts per million. Fe (318.81 ppm), Ca (2.47%), K (1.63%) and Mg (1.03%).

In the present study, 20 ISSR primers were tested to investigate the genetic diversity between *M. oleifera & M. peregrina*. The four ISSR primers showed a total of 45 bands. Out of the 43 polymorphic bands, one band was identified as a fingerprint, in the agreement with (Abubakar, *et al*.,2011), who studied genetic diversity of *Moringa oleifera* on 75 accessions from Sudan and Guinea savanna zones of Nigeria, where six polymorphic primers of RAPD origin gave a total of 42 polymorphic bands. On the other hand, (Rufai *et al.*, 2013), studied the genetic diversity of twenty genotypes of *M. oleifera,* reported that,24 RAPD primers identified total of 108 polymorphic bands. Whereas, the percentage of polymorphism ranged between 32.70 to 75.73. Moreover, (Mgendi *et al.*, 2010) founded the total of 98 fragments were scored and 86 of them (89.6%) were polymorphic by using 12 RAPD primers.

The distance matrix values ranged between 1.41 to 5.48. also the tree diagram (UPGMA) for the 22 individuals of the two *Moringa* species showed clear clustering, as each species clustered separately. Cluster one contained all *M.oleifera* individuals and cluster two contained *M. peregrina* individuals. This reflects the clear genetic variation between the two species.

This finding was agreed the UPGMA clustering for *Moringa oleifera* obtained different level of clusters regarding the primers techniques, (Silva *et al.,* 2011) who reported that the clustering for *M. oleifera* were identified three distinct groups The first group formed by ten accessions, second by four and third that only two accessions. Among the groups three stood alone, indicating that these accessions are the most genetically distant. On the other hand, (Rufai *et al.,* 2013), who studied twenty genotypes of *M.oleifera* by using RAPD technique, the genotypes were grouped into five clusters.Whereas, Al Khateeb *et al.*, (2012) used 10 ISSR primers to study the genetic stability of *M.peregrina* , showed monomorphism in all in vitro grown plants and two fingerprints in vitro micropropagated .However the ISSRs used in the recent study, only one fingerprint was found for *M .oleifera* .Although Ravishankar *et al.*,(2013) used the three DNA markers (ISSR, RAPD and cytochrome P_{450} gene-based) were used for genetic variability in eight *M.oleifera* cultivars showed a total of 17 RAPD, 6 ISSR and 7 pairs of cytochrome P-based markers generated 48.68, 48.57 and 40.00 % polymorphisms, respectively. There are many studies on the investigation of genetic variation in *Moringa* species by using DNA techniques, such the study of (Smith, 1996), who used RAPDs, (Gaiotto *et al.*, 1997), who used AFLPs. (Gaiotto *et al.*, 1997), who used both RAPD and AFLP concluded that both markers provide less information per locus than codominant markers

Shamsuddeen *et al.*, (2012) studied the genetic diversity and analysis of genetic relationship among 20 *M. oleifera* were carried out with the aid of twelve primers from, random amplified polymorphic DNA marker.

The present study finding that, PCR based on ISSR technique was found very useful in revealing genetic variation between the two species. These significant differences of polymorphism detected by using ISSR can be used for studying both *Moringa* species; this was in agreement with (Al Khateeb *et al.*, 2012) who reported that the ISSR was successful and quick protocol for large scale in vitro propagation method *M.peregrina*. Ravishankar *et al.*, (2013) suggested that ISSR markers are the most effective for assessment of genetic diversity for eight cultivars of *M. oleifera,* where they were grouped into four sub-clusters in a dendrogram, but without any distinct geographical pattern.

CHAPTER SIX

CONCLOUSIN AND RECOMMENDATIONS

- Moringa *oleifera* can be used as a fodder crop , because it continued to grow with drowned up to 30 days , whereas , *M. peregrina* had drastic drop in growth is not affected by water intervals up to 30 days watering interval. *Moringa* leaves can be considered as good fodder as it contains essential nutrients that can improve growth performance of animals.
- The nutritive value of *M. oleifera* was significantly higher than *M.peregrina* especially protein , Ca, K and Fe.
- Based on the results of this study, we recommended to grow *M. oleifera* due to easiness in germination and fast growth rate , in addition to its higher nutrition content than *M. peregrina* the later , did not with steed the field condition.
- The ISSR technique was very useful in revealing genetic variation between the two *Moringa* species, the significant differences found in this study and the polymorphic ISSR can be used in future studies of the *Moringa* species.

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