UNIVERSITI PUTRA MALAYSIA

GENETIC DIVERSITY, GROWTH EFFECTING FACTORSAND NUTRITIONAL VALUE OF MORINGA

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GENETIC DIVERSITY, GROWTH EFFECTING FACTORS AND NUTRITIONAL VALUE OF MORINGA

By

SHAMSUDDEEN RUFA'I

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

September 2016

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DEDICATION

This research work is dedicated to my dear and caring parents, my 3 wives, and my 9 children; it is also dedicated to two distinguished personalities: Prof Madya Dr Syed Omar Syed Rastan (Diversatech Sdn. Bhd.) and Prof Shamsher Mohamad (International Centre for Education in Islamic Finance - INCEIF)

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

GENETIC DIVERSITY, GROWTH EFFECTING FACTORS AND NUTRITIONAL VALUE OF MORINGA

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SHAMSUDDEEN RUFA'I

September 2016

Chairman : Professor Mohamed Hanafi Musa, PhD
Institute : Tropical Agriculture

Taking into account the various cases of malnutrition affecting many impoverished nations of the developing world, the current research effort was conceived with the objective of appraising the nutritive potential of 'Moringa' (*Moringa oleifera* L.) plant through the evaluation of proper agronomic practice that will enhance its nutritive potential. Various accessions were first tested for genetic diversity using RAPD markers; afterwards, growth and development pattern were determined through the assessment of the effect of applied phosphorus (P) and potassium (K) nutrient levels on the 'Moringa' performance. Applications of 5 levels of P and K revealed significant variations for plant height, stem diameter, crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR), leaf area index (LAI), SPAD chlorophyll content, root length, root volume, root diameter, stem dry weight, leaf dry weight, root dry weight, and total dry matter (TDM). Average values of 0.279 g cm^{-2} day^{-1}, 0.029 mg g^{-1} day^{-1}, and 0.149 g cm^{-2} day^{-1} were obtained for CGR, RGR, and NAR, respectively. The LAI values ranged from 1.43 to 2.13, while TDM values varied from 17.36 to 22.17 g $plant^{-1}$. Evaluating measured quantity of dried roots, stem, and leaves portions for essential and non-essential amino acids content through high-performance liquid chromatography (HPLC) analysis revealed the presence of 9 essential and 7 non-essential amino acids, with a range of 4.24 to 10.04 g kg^{-1} recorded for leucine, threonine, histidine, glutamic acid, aspartic acid, and glycine. The *2,2′- diphenyl-1- picrylhydrazyl* (DPPH) and the ferric reducing antioxidant power (FRAP) assay revealed strong antioxidants presence (up to 68% DPPH scavenging activity) and a high level FRAP potential of 2500 mg ascorbic acid equivalent (AAE) / 100 g dry weight (DW). Folin-Ciocalteu's and aluminium chloride calorimetric assay revealed high levels of secondary metabolites (ranging from 1000 – 1350 mg gallic acid equivalent (GAE)/100 g DW total phenolics and up to 2400 mg quercetin equivalent (QE)/1 mg DW total flavonoids), and the wet digestion procedure revealed the presence of 9 mineral nutrient elements (with significant values of up to 19.13 g kg^{-1} maximum) content acquired from calcium (Ca) and up to 9.90 g kg^{-1} recorded for iron (Fe). These nutritive components are noted to be highest (312.5% higher in terms of

antioxidants) in the leaves portion, followed by the roots, and then the stem parts. Influence of growth media, stem diameter, and growth regulators were evaluated using two growth media (with and without biochar) and three levels of indole 3-butyric acid (IBA). Plant height, percentage of primary branch produced, leaf area, and dry matter (DM) were found to be significantly ($P<0.05$) influenced by variation in stem diameter magnitude. The use of 2 growth media (munchong series soil [M] and munchong series soil mixed with Biochar [MB]) revealed a significant influence of the MB growth media on plant height, percentage root number, and root length as compared to the M growth media, but the trial record no significant variation in growth parameters in terms of IBA applications on the stem cuttings.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KEPELBAGAIAN GENETIK, FAKTOR-FAKTOR YANG MEMPENGARUHI PERTUMBUHAN DAN NILAI NUTRISI 'MORINGA'

Oleh

SHAMSUDDEEN RUFA'I

September 2016

Pengerusi : Profesor Mohamed Hanafi Musa, PhD
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Mengambil kira pelbagai kes kekurangan nutrien yang menjejaskan banyak negara membangun miskin di dunia, usaha dan kajian ini telah dilakukan dengan tujuan untuk menilai potensi tumbuhan 'Moringa' (*Moringa oleifera* L.) yang melibatkan penilaian amalan agronomi untuk meningkatkan potensi nutrisi yang betul. Pelbagai aksesi terlebih dahulu diuji kepelbagaian genetik yang mengguna penanda RAPD, yang mana selepas itu, keputusan dan perkembangan corak aksesi ditentukan melibatkan penilaian kesan penggunaan nutrien fosforus (P) dan kalium (K) pelbagai tahap terhadap prestasi 'Moringa'. Penggunaan P dan K pada 5 tahap mendedahkan satu keputusan ketara dari segi ketinggian tumbuhan, diameter batang, kadar pertumbuhan (CGR), kadar pertumbuhan relatif (RGR), kadar asimilasi bersih (NAR), index kawasan daun (LAI), kandungan klorofil SPAD, kepanjangan akar panjang, isipadu akar, diameter akar, berat kering batang, berat kering daun, berat kering akar dan berat kering keseluruhan tumbuhan (TDM). Purata nilai 0.279, 0.029 dan 0.149 g cm^{-2} $hari^{-1}$ diperolehi bagi CGR, RGR dan NAR, masing-masing. Nilai LAI adalah daripada 1.43 2.13, manakala nilai-nilai TDM berbeza-beza dari 17.36 ke 22.17 g^{-1} $tumbuhan^{-1}$. Menilai diukur kuantiti kering akar, batang dan daun bahagian-bahagian penting dan amino acids tak-penting kandungan melalui analisis kromatografi cecair berprestasi tinggi (HPLC) mendedahkan kewujudan 9 yang penting dan asid amino tak-penting 7, dengan pelbagai 4.24 untuk 10.04 g kg^{-1} dicatatkan leucine, threonine, histidine, asid glutamik, asid aspartic dan glycine. 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) dan ferik mengurangkan antioksidan kuasa (FRAP) cerakinan mendedahkan kehadiran antioksidan yang kuat (sehingga 68% DPPH) dan potensi FRAP tinggi 2500 mg asid askorbik setaraf (AAE) / 100g berat kering (DW). Folin-Ciocalteu dan crakinan kalorimeter aluminium klorida menurunkan paras metabolit sekunder (terdiri dari 1000 – 1350 mg asid gallik setaraf (GAE) / 100 g DW jumlah fenolik dan sehingga 2400 mg quercetin setara (QE) / 1 mg DW jumlah flavonoids dan kaedah pengabuan basah mendapati kehadiran 9 jenis unsur-unsur nutrien mineral (dengan nilai-nilai penting sehingga 19.13 g kg^{-1} maksimum) kandungan diperolehi dari kalsium (Ca) dan sehingga 9.90 g kg^{-1} dicatatkan bagi ferum (Fe). Komponen-komponen nutrien tersebut dicatatkan

sebagai paling tinggi di bahagian daun, di ikuti oleh akar, dan kemudian bahagian batang. Pengaruh media pertumbuhan, diameter batang dan pengawal selia pertumbuhan telah dinilai menggunakan dua jenis media pertumbuhan dan tiga peringkat "indole 3-butyric asid" (IBA). Ketinggian tumbuhan, peratusan cawangan utama dihasilkan, daun kawasan, dan perkara kering (DM) didapati signifikan ($P < 0.05$) dipengaruhi oleh perubahan dalam magnitud diameter batang. Penggunaan 2 media pertumbuhan (siri munchong tanah [M] dan siri munchong tanah bercampur dengan Biochar [MB]) mendedahkan pengaruh media pertumbuhan MB pada ketinggian tumbuhan, peratusan nombor akar dan akar panjang berbanding media pertumbuhan M, tetapi perbicaraan itu merekodkan Tiada variasi ketara dalam parameter pertumbuhan dari segi aplikasi IBA.

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I certify that a Thesis Examination Committee has met on 19 May 2016 to conduct the final examination of Hayati binti Mohamad Mukhair on her thesis entitled "Modification of Coconut Coir as Adsorbent in Oil Spill Removal" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AA	Auto Analyser
AAE	Ascorbic Acid Equivalent
AAS	Atomic Absorption Spectrophotometer
ADP	Adenosine Diphosphate
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ATP	Adenosine Triphosphate
AVRDC	Asian Vegetable Research and Development Centre
B	Boron
BRIS	Beach Ridges Interspersed with Swales
C	Carbon
Ca	Calcium
CEC	Cation Exchange Capacity
CGR	Crop Growth Rate
Cl	Chlorine
CO_2	Carbon dioxide
CRD	Completely Randomised Design
CTAB	Cetyl Trimethyl Ammonium Bromide
Cu	Copper
DNA	Deoxyribonucleic Acid
DPPH	*2,2′- diphenyl-1- picrylhydraz*
DW	Dry Weight
EC	Electrical Conductivity
EDTA	Ethylene Diamine Tetra Acetic Acid
F	Fluorine
FAO	Food and Agriculture Organisation
Fe	Iron
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalent
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulphuric Acid
IBA	Indole 3-butyric acid
ISSR	Inter Simple Sequence Repeat
ITA	Institute of Tropical Agriculture
K	Potassium
LAI	Leaf Area Index
LSD	Least Significant Difference
Mg	Magnesium
MOCP	Moringa Oleifera Cationic Protein
MOP	Muriate of Potash
Mn	Manganese
N	Nitrogen
Na	Sodium
NaCl	Sodium Chloride

NAR	Net Assimilation Rate
P	Phosphorus
PAR	Photosynthetically Active Radiation
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PPB	Percentage Polymorphism of Bands
ppm	parts per million
PVP	Polyvinyl Pyrrolidone
QE	Quercetin Equivalent
RAPD	Random Amplified Polymorphic DNA
RGR	Relative Growth Rate
SAS	Statistical Analysis Software
TDM	Total Dry Matter
TPTZ	Tripyridyl-s-triazine
TSP	Triple Superphosphate
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UNICEF	United Nations International Children Education Fund
USDA	United States Department of Agriculture
UV	Ultraviolet
Zn	Zinc

CHAPTER 1

INTRODUCTION

'Moringa' is a small, fast growing, deciduous (von Maydell, 1990) tropical tree belonging to the family Moringaceae. The Moringaceae is a monotypic family of sole genus 'Moringa' containing a range of 10 to 12 species (Dhakar et al., 2011). The plant has many common names such as miracle tree, horseradish tree, ben oil tree, drumstick tree, and cabbage tree. It is one of the most beneficial multipurpose tree for semiarid areas (von Maydell, 1990) that has been found to fulfill many basic human needs, with an inherent potential of being an economically supportive tree of the tropics. 'Moringa' possess the very important attribute of having all of its parts (leaves, flowers, bark, pods, nuts, seeds, tubers, and roots) as edible. 'Moringa' tropical tree is shown in Figure 1.1 and Figure 1.2.

Figure 1.1 : A young 'Moringa' tree in UPM farm, Puchong, Malaysia

The leaves can be used fresh or can be dried then ground into powder. The seed pods can be picked while in their green shape and eaten cooked or fresh (Figure 1.3 and Figure 1.4), while the seed oil is discovered to be sweet, non-drying, non-sticking oil that resists rancidity, and found to be enriched with about 70% oleic acid and almost all the other fatty acids that are obtained from olive oil. The cake from the seed is used in purifying drinking water (FAO, 2014).

The water clarifying ability of the 'Moringa' seed powder, as per studies in recent years, was linked to a positively-charged protein called the *Moringa oleifera* Cationic Protein (MOCP).When the seeds are crushed and added to water, the protein kills some of the microbial organisms, then bundle them together, making them to settle at the bottom of the container. Though, the research further stressed

that the water purified this way should not be stored for a long period, because the organic matter residue from the seed will serve as a food source for any remaining bacteria that were not killed (Jerri et al., 2012).

Figure 1.2 : 'Moringa' tree bearing developing fruits

'Moringa' is a particularly nutritional tree readily found in the regions of Asia, Africa, and Latin America, with many notable benefits to human health. Analysis of its leaf samples revealed the presence of 9 essential and 7 non-essential amino acids (Shamsuddeen et al., 2015) that serves as proteins building blocks within the human body. The leaves are equally good sources of vitamins (A and E) and antioxidants that have been linked to improvement of vision and cancer prevention respectively.

Pharmacologically, the leaf extract of 'Moringa' is rubbed over skin as treatment to paralysis and skin rashes in folk medicine (Mooza et al., 2014). The juice extracted from the bark of 'Moringa' tree is employed as a disinfectant for swift healing of wounds (Marwah et al., 2007). In Egypt, it is locally used for the treatment of headache, fever, constipation, slimness, back and muscle pains (Abdel-Rahman et al., 2010). Its seeds were considered of economic value and medicinal significance due to its unique composition of macro and micronutrients (Hanif et al., 2011). The pharmacological actions were attributed to the existence of phenolic compounds in its leaves extract. Similarly, it is associated with the existence of antioxidants and mineral nutrients that assist in curbing the damage free radicals usually cause to skin.

The lack of potable drinking water is an enormous problem in lots of developing countries. Based on UNICEF estimates, there exists 783 million people worldwide without improved drinking water, and the report from World Health Organization

estimates that scarcity of suitable drinking water lead to 1.6 million deaths every year as a result of diarrheal and parasitic infections (FAO, 2015).

Figure 1.3 : Sauce curry from 'Moringa' green immature fruits

The noted malnourishment related high mortality rate prevalent among the children of African countries as well as other communities around the world, can be significantly checked through the spread and nourishment of the 'Moringa' plant to those communities in an competent approach.

Figure 1.4 : Sauce curry from 'Moringa' green immature fruits

It was reported that introducing the tree leaves into the diets of malnourished breastfeeding mothers, and other family members in Senegal, led to a significant reduction in the malnourishment with a corresponding increment in the health and energy of the affected communities (Fuglie, 2001). The recognised ability associated with the 'Moringa' to combat malnutrition and provide food, water and

income is very ideal for communities in developing countries where these basic necessities are a major issue.

Taking into account all the afore mentioned advantages of this all important tree crop, still there is a general **problem** of lack of research work on the various planting materials (accessions) present with respect to genetics and breeding and agronomic performance, eventhough a significant segment of population here are utilising its leaves and young fruits for consumption, this research was, therefore, undertaken with the aim of achieving the following **objectives:**

i) To evaluate and establish the genetic diversity and to identify potential genotypes for future breeding work,
ii) To assess the influence of P and K fertilisation on total dry matter yield and nutritional quality,
iii) To measure the potential of various plant growing parts of 'Moringa' (leaves, stem, and roots) for use as natural plant sources of amino acids, secondary metabolites, antioxidants, and mineral nutrients and subsequent recommendation for utilization as nutraceuticals among impoverished communities,
iv) To determine the effect of growth media, stem diameter and indole-3-butyric acid (IBA) on growth and development of 'Moringa' stem cuttings,

REFERENCES

Abdel-Rahman T.M.A, Hegazy A.K, Sayed A.M, Kabiel H.F, Alfy E.T, and Komy E.S.M (2010). Study on combined antimicrobial activity of some biologically active constituents from wild *Moringa peregrina* Forssk. *Journal of Yeast and Fungal Research* 1(1):15-24.

Ahmed A.K., Amanullah, S.K., Basharat H.S., Munir A.K (2010). Effect of indole butyric acid (IBA) on rooting of olive stem cuttings. *Pakistan Journal of Agricultural Research* 23:3-4.

Amoo S.O, Aremu A.O, Moyo M, and Staden J.V (2012). Antioxidant and acetylcholinesterase-inhibitory properties of long-term stored medicinal plants. *BMC Complementary and Alternative Medicine* 12:87 doi:10.1186/1472-6882-12-87.

Amtmann A, Troufflard S, Armengaud P (2008). The effect of potassium nutrition on pest and disease resistance in plants. *Physiologia Plantarum* 133:682–691

Ana VCS, Allivia R.F.S, Ana S.L, Rosano B.F, Camila S.A, Gilvania M.S, and Maria S.A.R (2012). Moringa genetic diversity from germplasm bank using RAPD markers. *Tropical and Subtropical Agroecosystems* 15:31-39.

Anwar F, Hussain A.I, Ashraf M. Jamail A, and Iqbal S (2006). Effect of salinity on yield and quality of Moringa oleifera seed oil. *Grasas Y Aceites* 57:394-401.

Anwar F and Bhanger M.I (2003) Analytical characterisation of M. oleifera seed oil grown in temperate regions of Pakistan. *Journal of Agriculture and Food Chemistry* 51(22):6558-63

AOAC (1990). AOAC official methods of analysis. *Association of Official Analytical Chemists* Arlington, Virginia. 15:84–85.

Ao J, Fu J, Tian J, Yan X, Liao H (2010). Genetic variability for root morph-architecture traits and root growth dynamics as related to phosphorus efficiency in soybean. *Functional Plant Biology* 37:304–312

Arash K.E, RosnaMat T, Sadegh M, and Behrooz B (2015). Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from in vivo and In vitro grown *Trifolium pratense* L. (Red Clover). *BioMed Research International* 2015:643285.

Beadle C.L (1993): Growth analysis. Photosynthesis and production in a changing environment: *a field and laboratory manual* (ed. by D.O. Hall), pp. 36–46. Chapman & Hall, London.

Benzie I.F.F, and Strain J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of ‘‘antioxidant power’’: the FRAP assay. *Analytical Biochemistry* 239:70–76.

Berger M. R, Habs M, Jahn S.A.A, and Schmahl, D. (1984). "Toxicological assessment of seeds from *Moringa oleifera* and M. stenopetala, two efficient primary coagulants for domestic water treatment of tropical raw waters", *East African. Medical Journal* 9:712-716.

Booth F. E. M; and Wickens G. E (1988): Non-timber uses of selected arid zone trees and shrubs in Africa. FAO Conservation Guide 19, *Food and Agriculture Organisation* United Nations, Rome.

Brady NC, Weil RR (2007). The nature and properties of soils. 14th ed. Englewood Cliffs (NJ):Prentice-Hall.

Chorage P, Kadam D.A, Kadam A.S, Ghule Y.A and Aparadh V.T. (2013). Free Radical Scavenging (DPPH) and Ferric Reducing Ability (FRAP) of Some Gymnosperm species. *International Journal of Research in Botany* 3:34-36.

Daniel B. A., Naalamle A., Julius Y., and Essie B (2014). Effect of indole 3-butyric acid and media type on adventitious root formation in Sheanut tree (*Vitellaria paradoxa* C. F. Gaertn.) stem cuttings. *American Journal of Plant Sciences*, 2014, 5, 313-318.

DelRio D, Rodriguez-Mateos A, Spencer J.P.E, Tognolini M, Borges G, and Crozier A. (2013). Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. *Antioxidants and Redox Signaling* 18:1818-1892.

Dhakar R.C, Maurya S.D, Pooniya B.K, Bairwa N, and Gupta M. S. (2011). Moringa: the herbal gold to combat malnutrition. *Chronicles of Young Scientists* **2**(3):119-125.

Doruska P. F. and Burkhard H. E. (1994). Modeling the diameter and locational distribution of branches within the crowns of loblolly pine trees in unthinned plantations. *Canadian Journal of Forest Research* 24: 2362-2376.

Duan H.Y, Shi L, Ye X.S, Wang Y.H, Xu F.S (2009). Identification of phosphorous efficient germplasm in oilseed rape. *Journal of Plant Nutrition* 32:1148–1163

Dumroese, R.K., Heiskanen, J., Englund, K., and Tervahauta, A (2011). Pelleted biochar: Chemical and physical properties show potential use as a substitute in container nurseries. *Biomass Bioenergy* 35:2018–2027.

Ehsan K, Ahmad A, Hamid R.S, and Arash A (2012). The potential of glauconitic sandstone as a potassium fertilizer for olive plants. *Archives of Agronomy and Soil Science* **58**(9):983–993.

Eugenio J.G, Tatiane L.C.O, Severino M.A, Alessandra R, Alessandro D.L, and Rosa H.M.G. (2012). Antioxidant Activity by DPPH Assay of Potential Solutions to be Applied on Bleached Teeth. *Brazilian Dental Journal* 23:1.

FAO. (2014). Moringa – FAO Crop of the Month. Cited from: *http://Moringafacts.net/Moringa-fao-crop-month/*

Farooq A, Sajid L, Muhammad A, Anwarul H.G. (2007). *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytotheraphy Research* 21:17-25.

Freddo A, Cai C, Reid B.J (2012). Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar. *Environmental Pollution* 171: 18-24

Fries C, Johansson O, Pettersson B, Simonsson P (1997). Silvicultural models to maintain and restore natural stand structures in Swedish boreal forests. *Forest Ecology and Management* 94: 89-103.

Fuglie L.J and Sreeja K.V. (2001). Cultivation of Moringa. In: The miracle tree – The multiple attributes of Moringa. L.J. Fuglie (ed.) CTA, U.S.A. pp 153-158.

Fuglie, Lowell J. (2001). "The miracle tree." *Moringa oleifera.* ACS Publications: **28**(4):2262-8. doi: 10.1021/la2038262.

Foidl N, Makkar H.P.S, Becker K. (2001). The potential of Moringa oleifera for agricultural and industrial uses. In: The miracle tree – The multiple attributes of Moringa. L.J. Fuglie (ed.) CTA, U.S.A.

Gardner F.B, Pearce R.B, Mitchell R.L. (1985). Physiology of crop plants. Iowa State University Press, Ames, Ia. P 293.

Gostner J.M, Becker K, Ueberall F, and Dietmar F. (2015). The good and bad of antioxidant foods: An immunological perspective. *Food and Chemical Toxicology* 80:72-79.

Hammond J.P, Broadley M.R, White P.J, King G.J, Bowen H.C, Hayden R, Meacham M.C, Mead A, Overs T, Spracklen W.P, Greenwood D.J (2009). Shoot yield drives phosphorus use efficiency in *Brassica oleracea* and correlates with root architecture traits. *Journal of Experimental Botany* 60:1953–1968.

Hanif M.A, Al-Maskri A.Y, Al-Mahruqi Z.M, Al-Sabahi J.N, Al-Azkawi A, Al-Maskari M.Y (2011). Analytical evaluation of three wild growing Omani medicinal plants. *Natural Product Communications* **6**(10):1451-1454.

Hou W.C, Lin R.D, Cheng K.T, Hung Y.T, Cho C.H, Chen C.H, Hwang S.Y, and Lee M.H. (2003). Free radical scavenging activity of Taiwanese native plants. *Phytomedicine* 10:170-175.

Humayun M.A, Elango R, Ball R.O, and Pencharz P.B (2007). Reevaluation of the protein requirement in young men with the indicator amino acid oxidation technique 1,2,3. *Journal of American Society For Clinical Nutrition* 137:1874-1879.

Hunt R. (2003).: Growth Analysis, Individual Plants. From: Thomas, B., Murphy, D.J., Murray, D. (eds.). *Encylopaedia of Applied Plant Sciences*. Academic Press, London p 588-596.

Jahn, S. A.; Musnad, H. A.; Burgstaller, H (1986): The tree that purifies water: cultivating multipurpose Moringaceae in the Sudan. *Unasylva* **38(**2):23–28.

Janick J, and Paull R.E (2008). The encyclopedia of fruit and nuts. 1st Edition, Cabi Publishing. Oxfordshire, UK, pp. 800.

Jaykumar J.C, Nikhil B.G, Parthraj R.K, and Ghansham B.D. (2013). Total phenolics, flavonoids and antioxidant properties of three Ceropegia species from Western Ghats of India. *South African Journal of Botany* 88:273-277.

Jerri H.A, Adolfsen K.J, McCullough L.R, Velegol D, Velegol S.B (2012). Antimicrobial sand via adsorption of cationic *Moringa oleifera* protein.nLanqmuir **28**(4):2262-8. doi: 10.1021/la2038262.

Jones B.J. (1998). Plant nutrition manual. CRC Press New York p 149.

Jones B.J. (2005). Hydroponics: A practical guide for the soilless grower. 2nd Edition, CRC Press. New York, pp. 71-77.

Kaur S and Mondal P (2014). Study of total phenolic and flavonoid content, antioxidant activity and antimicrobial properties of medicinal plants. *Journal of Experimental Microbiology* 1(1): 00005. DOI:10.15406/jmen.2014.01.00005.

Kobayashi K. D, McConnel J, and Griffis J (2007). Bougainvillea. Cooperative Extension Service. College of tropical Agriculture and Human Resources. University of Hawai'i at Monoa, OF-38.

Kshirsagar, C. R.; and D'souza, T. F (1989): A new disease of drumstick. *Journal of Maharashtra Agricultural Universities* **14(**2):241–242.

Kuzyakov Y, Subbotina I, Chen H, Bogomolova I, and Xu X (2008): Black carbon decomposition and incorportaion into soil microbial biomass estimated by 14C labeling, *Soil Biology and Biochemistry* 41:210–219, doi:10.1016/j.

Lalas, S and Tsaknis J (2002). Characterization of *Moringa Oleifera* Seed Oil Variety "Periyakulam 1"."*Journal of Food Composition and Analysis* **15**(1):65-77.

Leigh, R,A (2001). Potassium homeostasis and membrane transport. Journal of Plant Nutrition and Soil Science 164:193–198.

Leigh R.A, and Wyn J.R.G (1984): A hypothesis relating critical potassium concentration for the growth and distribution of this ion in the plant cell. *New Phytology* 97:1–13.

Lynch J.P (2007). Roots of the second green revolution. *Australian Journal of Botany* 55:493–512.

Makkar H.P.S and Becker K. (1996). Nutritional value and antinutritional components of whole and ethanol extracted Moringa oleifera leaves. *Animal Feed Science Technology*: 63, 211-228.

Marschner H. (1995). Mineral nutrition of higher plants. Academic Press Intl., San Diego, CA, USA, pp. 261-274.

Marwah R.G, Fatope M.O, Al Mahrooqi R, Varma G.B, Al Abadi H, and Al-Burtamani S.K.S. (2007). Antioxidant capacity of some edible and wound healing plants of Oman. *Food Chemistry* **101**(2): 465-470.

Marzieh B., Somayeh S., and Vida V. (2012). Effects of indole-3-butyric acid on the rooting ability of semi-hardwood Bougainvillea sp. cuttings. *www.ccsenet.org/mas*. ISSN 1913-1844. Vol. 6, No. 5.

Mehta L.K, Balaraman R, Amin A.H, Baffa P.A, and Gulati O.D (2003). Effects of fruits of M. oleifera on the lipid profile of normal and hypercholesterolaemic rabbits. *Journal of Ethnopharmacology* 86, 191 – 195.

Meister A, and Tate S.S (1976). Glutathione and related gamma-glutamyl compounds: biosynthesis and utilization. *Annual Review of Biochemistry* 45:559-604.

Mengel K and Kirkby E.A (2001). Principles of plant nutrition, 5th edn. Kluwer Academic Publishers, Dordrecht, p 849

Mengel K (2007). Potassium. In: Barker AV, Pilbeam DJ, editors. Handbook of plant nutrition. Boca Raton (FL): CRC Press. p. 91–121.

Mgendi M.G, Manoko M.K, Nyomora A.M (2010). Genetic diversity between cultivated and non-cultivated *Moringa oleifera* Lam. provenances assessed by RAPD markers. *Journal of Cell and Molecular Biology* 8:95-102.

Mooza A.O, Nora A.H, and Shah A.K (2014). GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of Moringa peregrina (Forssk.) Fiori leaves. *Asian Pacific Journal of Tropical Biomedicine* 4:964-970.

Morton J. F. (1991). The horseradish tree, Moringa pterygosperma (Moringaceae) – a boon to arid lands? *Economic Botany* **45(**3):318–333.

Muluvi G.M, Sprent J.L, Soranzo N, Provan J, Odee D, Folkard G, McNicol J.W, Powell W (1999). Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. *Molecular Ecology* 8:463-470.

Munyanziza E, and Sarwatt S. V (2003). The evaluation of *Moringa oleifera* for food security and environmental rehabilitation in Tanzanian rural areas. *Journal of Tropical Forest Science* **15(**3):450–456.

Musa K.H, Abdullah A, Jusoh K, and Subramaniam V. (2010). Antioxidant activity of pink-flesh guava (*Psidium guajava*L.): effect of extraction techniques and solvents. *Food Analytical Methods*. DOI 10.1007/s12161-010-9139-3.

Nass L.L (2007). Recursos genéticos vegetais. *Embrapa Recursos Genéticose Biotecnologia*, Brasília.

National Research Council. (2006). *Lost Crops of Africa*, Vol. 1, National Academy Press, Washington DC.

Nikkon F, Saud Z.A, Haque M.E, Karagianis G, and Mosaddik M.A. (2003). Isolation of aglycone of deoxy-Niazimicin from *Moringa oleifera* Lam. and its cytotoxicity. *Revista Latinoamericana de Química* 31:5-9.

Northup J (2013). Biochar as a replacement for perlite in greenhouse soilless substrates, Graduate Theses and Dissertations, Paper 13399, Iowa State University,

Nuruzzaman M, Lambers H, Bolland M.D.A, Veneklaas E.J (2006) Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. *Plant and Soil* 281:109–120.

Palada M.C., and Chang L.C (2003). Suggested cultural practices for Moringa. Asia Vegetable Research Development Centre, International guide. *AVRDC* publication 03-545.

Park E.J., Pezzuto JM (2002). Botanicals in cancer chemoprevention. *Cancer and Metastasis Reviews* 31(3-4):231-255.

Parrotta, J. A. (1993): *Moringa oleifera* Lam. Reseda, horseradish tree. Research Note SO-ITF-SM-61, South. For. Research Station, Forestry Services, U.S. Dep. Agric., New Orleans, LA, USA.

Passwater R.A. (1998). The Antioxidants: The amazing nutrients that fight dangerous free radicals. McGraw-Hill edition p 48.

Pathan S.M, Aylmore L.A.G, and Colmer T.D. (2003). Soil properties and turf growth on a sandy soil amended with fly ash. *Journal of Plant and Soil* 256:103–114.

Percival G.C, Keary I.P, and Noviss K (2008). The potential of a chlorophyll content SPAD meter to quantify nutrient stress in foliar tissue of sycamore, *Acer pseudoplatanus* Arboric. *Urban Forestry* 34(2):89-100.

Prendergast-Miller, M.T., Duvall M., and Sohi S.P. (2014). Biochar-root interactions are mediated by biochar nutrient content and impacts on soil nutrient availability. *European Journal of Soil Science* 65.

Radford R.J. (1967). Growth analysis formulae – their use and abuse. *Crop Science* 7:171–175.

Rafii M. Y, Arolu I. W, Omar M. H. A, and Latif M. A. (2012). Genetic variation and heritability estimation in Jatropha curcas L. population for seed yield and vegetative traits. *Journal of Medicinal Plants Research*, 6(11), 2178-2183.

Raja S, Bagle B.G, and More T.A (2013). Drumstick (*Moringa oleifera* Lamk.) improvement for semiarid and arid ecosystem: Analysis of environmental stability for yield. *Journal of Plant Breeding and Crop Science* **5**(8):164-170.

Ramachandran C, Peter K.V, and Gopalakrishnan P (1980). *Moringa oleifera*. *Economic Botany* 34:276–283.

Razali I., Norhaya H., and Norasimah A.S (1997). Determination of antioxidants in palm oil products by high performance liquid chromatography, *Elaeis*, 9, 25 – 33.

Roloff A, Weisgerber H, Lang U, and Stimm B (2009). *Enzyklopädie der Holzgewächse, Handbuch und Atlas der Dendrologie.* WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. ISBN: 978-3-527-32141-4.

Saffari M., and Saffari V.R (2012). Effects of media and indole butyric acid (IBA) concentrations on hopbush (Dodoneae viscosa L.) cuttings in green house. *Annals of. Forest. Research* 55(1): 61-68, 2012.

Sahreen S, Khan M.R, and Khan R.A (2010). Evaluation of antioxidant activities of various solvent extracts of Carissa opaca fruits. *Food Chemistry* 122:1205–1211.

Saran S, John G and John A (2013). Biochar in growing media: A sustainability and feasibility assessment. Defra project ref. SP1213, UK Biochar Research Centre, www.biochar.org.uk

Scherer R, and Godoy H.T. (2009). Antioxidant activity index (AAI) by the 2, 2-diphenyl-1- picrylhydrazyl method. *Food Chemistry* 112:654–658.

Seggewiss B, and Jungk A (1988). Influence of potassium dynamics at the soil-root interface on magnesium uptake of plants. *Z Pflanzenernaehr Bodenkd.* 151:91–96.

Shahzad U, Khan M A, Jaskani M J, Khan I A and Korban S S (2013). Genetic diversity and population structure of *Moringa oleifera.* Conserv Genet **14**(6):1161-1172.

Shamsuddeen R, Hanafi, M.M, Rafii M.Y, et al., (2013). Genetic Dissection of New Genotypes of Drumstick Tree (*Moringa oleifera* Lam.) Using Random Amplified Polymorphic DNA Marker. *BioMed Research International* (Hindawi publishing corporation). Volume 2013, Article ID 604598, 6 pages http://dx.doi.org/10.1155/2013/604598

Sharma G. K; and Raina V (1982). Propagation techniques of *Moringa oleifera* Lam. In: High value vegetables in Southeast Asia: Production, supply, and demand. Copyright, world vegetable centre (AVRDC).

Siddhuraju P, and Becker K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of Agriculture and Food Chemistry* **51**(8):2144-55.

Silveira D.G, Amorim E.P, Jesus O.N, Souza F.V.D, Pestana K.N, Santos V.J, Santana J.R.F (2009). Variabilidade genética de populações naturais de caroá por meio de marcadores RAPD. *Pesquisa Agropecuária Brasileira*. 44:283-290.

Sinclair, T.R. and Muchow, R.C. 1999. Radiation use efficiency. *Advances in Agronomy* **65**:215265.

Somali M.A, Bajneid M.A, and Ai-Fhaimani S.S (1984). "Chemical composition and characteristics of *Moringa peregrina* seeds and seeds oil". *Journal of the American Oil Chemists Society* 1:85-86.

Soong Y.Y, and Barlow P.J (2004). Antioxidant activity and phenolic content of selected fruit seeds. *Food Chemistry* **88**(3):411-7.

Stipanuk M.H (2007). Leucine and protein synthesis: mTOR and beyond. *Nutrition Reviews* 65:122–129.

Sun HX, Qin F & Pan YJ (2005). *In vitro* and *in vivo* immunosuppressive activity of Spica prunellae ethanol extract on the immune responses in mice. *Journal of Ethnopharmacology* 101:31-36.

Suzuki D, and Grady W (2004). . Tree: a biography. *Greystone* Books, Vancouver, BC, V5T 4S7, Canada.

Taiz L, and Zeiger E (2006). Plant physiology. 4th Edn. Sinauer Associates, Inc., Sunderland,MA, 764 p.

Templer P.H. and Dawson T.E (2004). Nitrogen uptake by four tree species of the Catskill Mountians, New York: implications for forest N dynamics. Plant Soil 262: 251-261.

Tan E.S, Aminah A, Khalid H.M, Mohammad Y.M, and Maaruf A.G. (2012). Antioxidant Properties of Three Banana Cultivars (*Musa acuminata* 'Berangan', 'Mas' and 'Raja') Extracts. *Sains Malaysiana* 41:319–324.

Teoh C.C, Hassan D.A, Radzali M.M, and Jafni J.J. (2012). Prediction of SPAD chlorophyll readings using remote sensing technique. *Journal of Tropical Agriculture and Food Science* **40**(1):127-136.

Thaipong K, Boonprakob U, Crosby K, Cisneros L.Z, and Hawkins D.B. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis* 19:669–675.

Thoo Y.Y, Ho S.K, Liang J.Y, Ho C.W and Tan C.P. (2010). Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (*Morinda citrifolia*). *Food Chemistry* 120:290-295.

Tian Y, Sun X, Li S,Wang H,Wang L, Cao J, and Zhang L (2012). Biochar made from green waste as peat substitute in growth media for *Calathea rotundifola cv. Fasciata, Scientia Horticulturae* 143: 15–18, doi:10.1016/j.

Thomas P.A. (2014). Trees: Their Natural History. Cambridge University Press p 409.

Twarakavi N.K.C, Imunek J.S, and Schaap M.G. (2010). Can texture-based classification optimally classify soils with respect to soil hydraulics?, *Water Resource Research* 46, W01501.

Ullasa B. A, and Rawal R. D (1984). Papaver rheoeas and Moringa oleifera, two new hosts of papaya powdery mildew. *Current Science* **53(**14):754–755.

USDA (2005). Proximate fruit composition of Moringa pods and leaves. In: Lost crops of Africa, National Academies Press, p. 247-269. http://www.nap.edu/catalog/11763.htm

von Maydell, H.J. (1990). Trees and Shrubs of the Sahel: their characteristics and uses. Verlag Josef Margraf. ISBN-10: 3823611984.

Wiesman Z, and Lavee S. (1994). Vegetative growth-retardation, improved rooting and viability of olive cuttings in response to application of growth-retardants. *Plant Growth Regulation* 14:83–90.

Wiesman Z and Lavee S (1995). Enhancement of IBA stimulatory effect on rooting of olive cultivar stem cuttings. *Scientia Horticulturae* 62:189–198.

Wilson, T (1999). Whole foods, antioxidants, and health. *Antioxidants for Human Health and Diseases*, 1, 141 – 150.

Wu H, Kongvui Y, Kong Z, Li C.Z, Liu D, Yu Y, Gao X (2011). Removal and recycling of inherent inorganic nutrient species in mallee biomass and derived biochars by water leaching. *Industrial and Engineering Chemistry Research* 50:12143-12151

Wu J, Yang J, Gu Z and Zhang Y (2010). Isolation and characterization of twenty polymorphic microsatellite loci for *Moringa oleifera* (Moringaceae). *Horticultural Science* 45:690–692.

Yi Z, Zhichun Z and Qing Y (2013). Genetic variations in root morphology and phosphorus efficiencyof *Pinus massoniana* under heterogeneous and homogeneous low phosphorus conditions. *Plant and Soil* 364:93–104, doi 10.1007/s11104-012-1352-y

Zarkadas C.G, Gaganon C, Gleddie S, Khanizadeh S, Cober E.R, and Guillemette R.J.D (2007). Assessment of the protein quality of fourteen soyabean (Glycine max (L.) Merr.) cultivars using amino acid analysis and two dimensional electrophoresis. *Food Research International* 40:129-146.