Balanites aegyptiaca (L.) Del. *var. aegyptiaca* seed composition and variability among three different intra-specific sources

Elfeel AA and Sherif Z. Hindi

Dept. of Arid land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia
aidris@kau.edu.sa

Abstract: The aim of this study was to investigate intra-specific variability among three geographical sources in fruit morphology and seed chemical composition. Morphological traits of various fruit layers (epicarp, mesocarp, endocarp and kernel) were measured. Seed kernel was analyzed for oil, saponin, protein, macro (Ca, Mg, Na, K, P) and micro (Fe, Mn, Zn, Co, Cu) nutrients. Endocarp wood was characterized for (specific gravity, gross heat of combustion, ash content and biocarbon yield). Biocarbon volatile matter, fixed carbon and ash contents were determined. The seed kernel contained high oil, saponin and minerals that were significantly differed among sources. The protein was not significantly different among sources Endocarp revealed good properties and relatively high biocarbon yield. There was observed relationship between sources with morphological and chemical properties. Damazin source (dark cracking clay) showed higher saponin, woody portion and biocarbon yield, while Hada Alsham source (lighter soil) had higher pulp content. The high contents in most of seed kernel constituent's highlights the suitability of *Balanites* for very wide range of products and the remaining woody part can be used for potential biocarbon products. Whereas the high variability observed among sources, indicates the importance of considering suitable sources for domestication.

[Elfeel AA and Hindi SZ. ***Balanites aegyptiaca* (L.) Del. *var. aegyptiaca* seed composition and variability among three different intra-specific sources.** *Life Sci J* 2014;11(7):160-166]. (ISSN:1097-8135). http://www.lifesciencesite.com. 20

Keywords: intra-specific source; fruit morphology; chemical contents; Variability; domestication; biocarbon.

1. Introduction

The genus *Balanites* is composed of nine species and 11 intra-specific taxa (Sands, 2001). *Balanites aegyptiaca* is one of most drought resistance tree species widely distributed in Africa, Arabian peninsula and other Asian countries (Arbonnier, 2004). Adopt many adaptive mechanisms to grow in arid saline soil (Elfeel et al., 2013). The wide range under which this species is found was reflected in a very wide variability in its morphology and products (Abasse et al., 2011). For any successful domestication, conservation or transfer of seeds between sites, it is important to know the extend and magnitude of variation among different geographical sources (Zobel and Talbert, 2003).

Balanites was known to be an all purpose tree with various uses and values. However, the most important valued part is its fruits (National Research Council, 2008). Fruit from *Balanites*, known as desert date (common) and lalobe (Arabic) is widely used for food, fodder and traditional medicine (Elfeel and Warrag, 2011). The fruit is a drupe, pubescent when green, becoming yellow and glabrous, after ripening (Arbonnier, 2004). It contains four layers (the outer skin called epicarp, the fleshy pulp called mesocarp, the woody shell called endocarp and the inner seed called kernel). The fleshy pulp of the fruit contains a large amount of carbohydrates. The kernel produces a high quality oil that can be used in human food (Obidah et al., 2009), medicine (Hanan et al., 2010) or biodiesel production (Gutti et al., 2012, Chapagain et al, 2009). The kernel is also rich in protein and minerals contents (Elfeel, 2010). The hardy wood shell is used for fuel. However, one of the most valued products of the pulp and kernel is the saponin, a source of many pharmaceutical products (Farid et al., 2002). Recently saponin proved to have wide range of uses, including protection of crops from mealy bugs (Patil et al., 2010), antitumor effects (Gnoula et al., 2008), effective antischistosomal remedy (Koko et al., 2005) and mosquito control (Wiesman and Chapagain, 2003). Despite all these products *Balanites* is considered as one of the underutilized, neglected arid zone tree species need to be domesticated (Wiesman, 2007). Schmidt and Joker (2000) reviewed high variability in fruits and seeds of *Balanites* between and within geographical sources. Determination of such variation is very important for successful utilization or conservation plans (Zobel and Talbert, 2003).

The Objective of this study was to investigate variation between three sources of *B. aegyptiaca* in seed morphological and chemical properties and to highlight their suitability for industrial utilization.

2. Materials and Methods

Seed Sources

Fruits of *Balanites aegyptiaca* were collected in 2011 season from three sources. The first sources

(Code SA) is Hada Al-sham Experimental Research Station, King Abdulaziz University, Saudi Arabia. Sources two (Code SD1) was Damazin area, Blue Nile state, Sudan. The fruits were collected from natural forest on dark cracking clay soils in one of the largest mechanized rainfed farming areas in low rainfall savannah of the Sudan. Sources three (Code SD2) is Umbeir area, South Kordofan State, Sudan. In this area the trees were found in gardud soil (red hard compact soil) on Nuba Mountains vegetation zone. The tree is found associated with *Acacia seyal, Acacia Senegal* and some *Combretum* species. Locations and site description of the sources is shown in Table 1.

Weights of different fruit layers

For determination of different fruit layers weight, a sample of 30 fruits were drawn per each source and weighed on individual fruit basis with electronic balance (0.00 g). After that the epicarp (outer shell) were removed by hand and weighed. Then the remaining fruits were soaked in water for 24 hours and thoroughly cleaned by washing with water and sun dried for 72 hours, after which the whole seed weight was obtained. The weight of the mesocarp (fleshy pulp) was determined by subtraction (weight of whole from – weight of epicarp – weight of the seed). The hard woody shell (endocarp) was then broken using small electrical power saw and the kernel was removed out. The endocarp and kernels were then weighed separately. The corresponding percentages of epicarp, mesocarp, endocarp and kernel were determined (weight of layer/weight of fruit * 100). Also, fruit and seed number per kilogram was calculated by the general equation: number of (fruits) or (seeds) in sample * 1000/weight of sample in gram.

Fruit length, diameter and size index

Fruit length and diameter were measured with digital verneir caliper (Digmatic caliper, Model CD-6"-CX,2011, Mitutoya Corporation). Fruit size index was calculated as fruit length divided by fruit diameter.

Seed kernel mineral contents

Seed kernel was analyzed for macro (P, Ca, Na, K, Mg), and micro elements (Co, Cu, Mn, Zn, Fe). Ca, Na, K, Mg, Co, Cu, Mn, Zn and Fe were analyzed using atomic absorption spectrophotometery according to the method described by (Hanlon, 1998). While P was determined according to (Bhargava and Raghupalhi, 1993).

Protein content

Protein content was determined as N content multiplied by 6.25. N was analyzed by micro-Kjeldahl method (Horneck and Miller, 1998),

Oil content

Kernel oil content was extracted by soxhlet apparatus for 6 hours using Petroleum ether. The percentage of ether extract (E.E.) was calculated as: weight of E.E. * 100/ weight of sample.

Saponin content

Crude saponin content was determined according to the methods described by Wiesman (2007). In brief the kernel left after the oil extraction (defatted kernel) was used for saponin determination. Four samples of defatted kernel per each source was weighed in tube and methanol was added (at ratio of 1 : 10) to each tube and kept in the high speed electric shaker overnight followed by centrifugation at 3500 rpm for 20 minutes. The supernatant of the methanol extract was collected and evaporated using a rotary evaporator to obtain crude saponin.

Endocarp (wood shell) properties

Samples Preparation for Wood Properties Determinations

To prepare the samples for the different tests 30 g of wood from each source was converted into meal by frequent crosscut using a disc saw machine. The produced wood meal was screened using different sieves depending on the standard methods for each test.

Specific Gravity (SG)

Air-dried wood samples were saturated by water under vacuum and saturated volume was determined following Pycnometric displacement of water (ASTM, D 2395-84, 1989). The samples were then oven-dried to a constant weight at 103°±2°C, and weighed. The specific gravity of wood was calculated based on oven-dry weight and saturated volume, which considered as a green volume in this study.

Determination of wood biocarbon yield

Wood samples for biocarbon yield were placed in the heated chamber of the muffle e which was connected to the carbon dioxide source in a pre-determined sequence. Digital thermometer was positioned in the muffle sample chamber through the ceiling hole together with the copper pipe connected to the CO_2-source. The inert gas flow used was 354 ml/minute until a steady gas flow was obtained. Then increased to 825 ml/minutes for 10 minutes. The temperature program was set to maximum final temperature (MFT) OF 500ºC and heating rate (HR) of 25ºC/min. After the desired MFT was reached, the samples were pyrolyzed during the duration of 45 min residence time (RT), before the heating system was shut off. The samples were allowed to cool to 100°C with maintenance of CO_2 flow at 354 ml/minute. The charcoal yield (CY) was calculated according to the following equation: CY, % = (Oven-dry weight of charcoal sample, g / Oven-dry weight of wood sample, g) * 100.

Ash Content

Ash content of wood was determined according to the ASTM, D 1102-84 (2007) standard method. In brief, 2 g of prepared wood meal sample was put in a porcelain crucible. The sample in an uncovered crucible was heated gradually, and then ignited at

600°C using tube furnace until all carbon was eliminated. Alternate heating for 30 minutes and weighing was done frequently until constant weight was obtained. Ash content was calculated as a percentage of residues based on the oven-dry wood meal weight. For biocarbon the sample was heated at 500°C for 6 hours and burning repeated until succeeding heating results in a loss of less than 0.0005 g.

Gross Heat of Combustion

The gross heat of combustion was determined by adiabatic oxygen bomb calorimeter, following the procedure described in ASTM, D2015-85, (1987) standard test. About one gram sample was used without grinding and subsequent pressing into a pellet, oven-dried and weighed. The sample was then placed in a capsule and combusted in the oxygen bomb, Parr 1108 under controlled conditions. The temperature was recorded before, during, and after combustion. The heat produced due to the combustion was estimated and converted into calories per g on a moisture free basis. Correction factors for the combustion of fusing wire and thermometer were included in the gross heat of combustion calculations. On the other hand, the correction factors for nitric and sulfuric acids formation were not considered as they are too small (Neenan and Steinback 1979).

Determination of Moisture Content

For moisture content determination about 0.8g meal sample was placed in a small crucible and oven-dried at 103°±2°C for 2 hours. The moisture content (MC %) was calculated [(W_1 - W_2) / W_1] X 100. Where: W_1 = The air-dry weight of the sample. and W_2 = The oven-dry weight of the meal sample.

Determination of Volatile Matter

For biocarbon volatile matter (VM), the same crucibles containing moisture content samples were used. The sample was preheated in the tube furnace for 2 minutes at 300°C, followed by 3 minutes at 500°C and 6 minutes at 950°C. Volatile matter content (VM) was calculated as follow: VM = [(W_2 - W_3) / W_2] X 100. Where: W_2 = The oven-dry weight of the meal sample and W_3 = Weight of the sample after the three steps of heating, g.

Determination of Fixed Carbon Content

Biocarbon fixed carbon content (FC %) was calculated as [100 - (moisture content + volatile matter + ash content)].

Statistical Analysis:

All the data were subjected to analysis of variance (ANOVA) to test the significance of the main treatments and the means were separated by Duncan's multiple range test. The data were analyzed by SAS Statistical Analysis Software (SAS System, Version 9.1, 2008).

3. Results

Fruit morphology:

ANOVA results showed a very high significant differences between sources in fruit morphology (p= < 0.01) (Table 2). It seems that soil has a great effect in seed morphology, as fruits collected from dark cracking clay soil (SD1), had higher values in most of the traits studied (Table 2). However, most of this additional weight came as the results of epicarp and endocarp weights (the woody parts). For mesocarp (the pulp), SA has a remarkably higher weight followed by SD2. The seed kernel showed no significant differences between sources. Figures 1, 2 and 3, showed the content of the different fruit layers as percent of total fruit weight. Where in SA the mesocarp represents the higher percent, in SD1 and SD2 the endocarp represents the highest percent. SD1 and SD2 are from natural stands where SA is from irrigated planted trees. Fruits obtained from SD1 are longer, but with shorter diameter. While those obtained from SA has longer diameter, but shorter in length, indicating that SD1 are more elongate while SA fruits are ovate (Table 2).

Seed kernel mineral contents

Analysis of variance revealed a very high affect of sources on seed kernel mineral contents (p = < 0.01)(Table 3), except N whereas the sources were not differed significantly.

Oil and protein content

The oil content was significantly differed between the three sources studied (Table 3). The oil content ranged from 49% of SD1 to 47% SD2.The protein content showed no significant differences among sources. The range of protein was relatively higher in the three sources (Table 3).

Saponin

The seed kernel crude saponin content was significantly differed among the three sources (Table 3). The highest content of saponin was recorded in SD1 sources, whereas the lowest content in SA.

Endocarp wood properties

Table 4 revealed significant differences between sources in ash content whereas the other parameters showed no significant differences between sources. However, comparison of means using Duncan multiple range test showed that specific gravity was significantly affected by the geographical sources (Table 4). Also, there was strong relationship between specific gravity and biocarbon production of the three sources. Sources with higher specific gravity had higher biocarbon production (Table 4). The highest biocarbon yield of 27.9% was recorded by SD1, while the lowest was SD2 (26.3%). Similar to biocarbon SD1 wood had the highest ash content. The cross heat of combustion showed no significant differences among the three sources.

4. Discussion

Balanites fruit is considered as the most important part of the tree (Elfeel and Warrag, 2011). All three of the four layers of the fruit, except the epicarp has a diversified products. Between source variations in fruit characterization is very important for successful domestication and conservation of the species. Without this variation, any attempt for domestication or conservation programs is unsuccessful (Zobel and Talbert, 2003).The present study showed a considerable variation in seed morphology between different sources. This variation, may suggests genetic variation among sources. SD1 and SA sources had higher fruits and seed weights, hence less number of fruits and seeds per kilogram. However, when the fruits were partitioned into four layers (epicarp, mesocarp, endocarp and kernel), there was a difference between SD1 and SA in some layers (Figure 1, 2, 3). Where in SA the mesocarp represents the higher percent, in SD2 the endocarp represents the highest percent. This may indicates that soil has a great effect in seed morphology. SD2 fruits were collected from natural forests with dark cracking clay soil, while SA was collected from light soils with supplementary irrigation SD2 from hard compact soil had least values in most of the traits. The seed kernel contains very high contents of oil, protein and minerals. The almost, similar amount of seed kernel as percentage of total fruit weight between the three sources, reflects that same quantities can be obtained from different sources. However, the composition was differed among sources.

The seed kernel had moderately higher contents of oil in the three sources. Many studies revealed the high value of *Balanites* oil. The oil can be used in food with no any serious safety concern (Obidah et al, 2009). Also, the oil can be used for medicinal purposes (Hanan et al., 2010), or as source of biodiesel production (Chapagain et al., 2009). The remaining cake had very high protein and minerals contents, which make the kernel cake useful nutrient source. Therefore the kernel cake can used for animal feed supplement as it contains high saponin or can be used as fertilizer. The variability observed among the three sources in oil and minerals, indicate that the tree can be improved by selection of best seed sources or provenances

The seed kernel crude saponin content was significantly differed among the three sources. The importance of the saponins in plants came as the results of their biological activity, which led to saponins commercially significant compounds with many applications in food, cosmetics, and pharmaceutical (Sparg et. al., 2004; Vincken et. al., 2007). This will highlight the importance of *Balanites* as a source of saponin. Recently many studies revealed the application of *Balanite*s saponin in many fields such as botanical insecticides for the protection of crops from mealy bugs (Patil et al, 2010), anti-tumor activity in vitro and in vivo (Gnoula et al., 2008), effective antischistosomal remedy (Koko et al, 2005) and are efficient in mosquito control (Wiesman and Chapagain, 2003).

The good properties observed in seed endocarp reflect that no waste product can be lost from the seeds. The seed which have high proportion of woody endocarp (ranged from (29% to 37% in this study) can be a good source of biocarbon that can be used for heating or further be converted to activated charcoal that has wide industrial applications. Specific gravity differed according to the different sources studied. The high specific gravity value indicates that more cell wall materials can be used industrially. This was apparently reflected in high biocarbon yield. Damazin source has high specific gravity (0.75) and high charcoal yield (27.9). In addition to the morphological data which revealed that Damazin has a high percent of endocarp as percentage of total fruit weight.

This study revealed that different layers of *Balanites* fruit have important products with potential industrial applications. Thus value addition by processing of the different fruit products or by conversion of the fruits to different products will help in domestication of this underutilized and neglected arid zone tree species.

5. Conclusion

The study showed very high variation among the three sources that were associated with the original soil of the sources. The high oil content and the high protein and minerals in the remaining cake, may suggests that the kernel can be used for oil production and the cake as nutrient. The saponin can be applied for medicinal uses. The woody endocarp can be used for biocarbon production. The variation among sources suggests that for domestication and conservation of this species among source variation must be taken into account.

Table 1. Locations and site description of the three sources of *Balanites aegyptiaca* from which fruits were collected.

Location	Lat.°N	Long.°E	Soil	Rainfall mm	Elevation Masl
Umbeir	11.7330	30.8000	Gardud	800	615
Ad Damazin	11.7670	34.3500	Clay	700	493
Hada Alsham	21.4830	39.4325	Salty silt	< 100	240

Table 2. Differences in *Balanites aegyptica* fruits and seed morphology among three sources (Hada Alsham (SA), Damazin (SD1) and Umbeir (SD2).

Source	Fruit wt (g)	Fruit No./kg	Seed wt (g)	Seed No./kg	Fruit length cm	Fruit Diameter cm	Fruit size index
SA	8.87a	113b	3.45b	290a	3.61b	2.23a	1.63b
SD1	9.67a	104b	4.55a	220b	4.11a	2.06b	2.02a
SD2	6.57b	153a	3.02b	339a	3.39b	1.90c	1.81b
Significance	**	**	**	**	**	**	**

* ≤ 0.05; ** ≤ 0.01; **ns** = not significant
Means with different letters are significantly different at 0.05 using Duncan's Multiple Range Test

Table 3. Differences in seed kernel minerals, protein, oil and saponin contents among three sources of *Balanites aegyptiaca* (Hada Alsham (SA), Damazin (SD1) and Umbeir (SD2)).

Source	Co (Mg/L)	Cu (Mg/L)	Mn (Mg/L)	Zn (Mg/L)	Fe (Mg/L)	Ca (Mg/L)	Mg (Mg/L)	Na (Mg/L)	K (Mg/L)	P (%)	Protein %	Oil %	Saponin %	N %
SA	0.038a	0.425a	0.056b	0.478a	0.352b	2.55a	112.43b	178b	484b	0.91a	25.20a	48.57b	12.05c	4.03a
SD1	0.033ab	0.234c	0.079a	0.431b	0.490a	0.901b	119.05a	196a	454c	0.81b	25.38a	49.32a	14.45a	4.06a
SD2	0.028b	0.319b	0.050b	0.410c	0.522a	2.07a	108.50b	178b	493a	0.85b	24.50a	46.93c	13.34b	3.92a
P	*	**	**	**	**	**	**	**	**	**	ns	**	**	ns

* ≤ 0.05; ** ≤ 0.01; **ns** = not significant
Means with different letters are significantly different at 0.05 using Duncan's Multiple Range Test

Table 4. Endocarp wood properties (specific gravity, wood biocarbon yield, gross heat of combustion, ash content) and biocarbon volatile matter, fixed carbon, moisture content and as content among three sources of *Balanites aegyptiaca* (Hada Alsham (SA), Damazin (SD1) and Umbeir (SD2)).

Source	Endocarp wood properties				Biocarbon properties			
	Specific gravity g cm^{-3}	Biocarbon yield %	Gross heat of combustion cal g^{-1}	Ash content %	Volatile matter	Fixed carbon	Moisture content %	Ash content %
SA	0.625b	26.42b	4700.1a	2.051b	40.02b	53.23a	3.55a	3.311b
SD1	0.754a	27.89a	4649.8a	3.625a	43.01a	48.70b	3.40a	4.881a
SD2	0.689b	26.33b	4633.3a	1.888b	39.31b	53.49a	3.67a	3.538b
Significance	ns	*	ns	**	**	**	ns	**

* ≤ 0.05; ** ≤ 0.01; **ns** = not significant
Means with different letters are significantly different at 0.05 using Duncan's Multiple Range Test

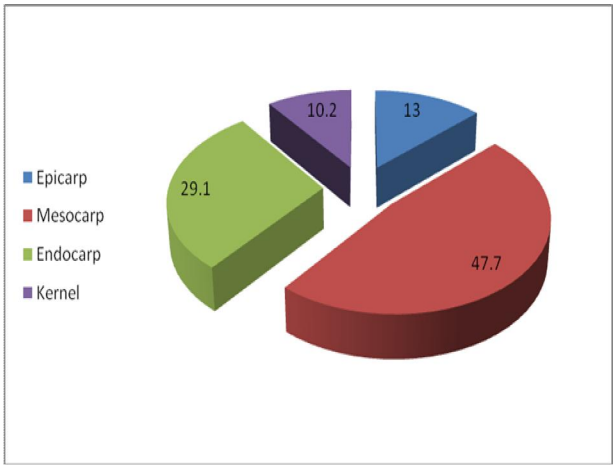

Fig. 1. Composition of different fruit layers (epicarp, mesocarp, endocarp and kernel) as percent of fruit weight of *Balanites aegyptiaca* from Hada Alsham source (SA)

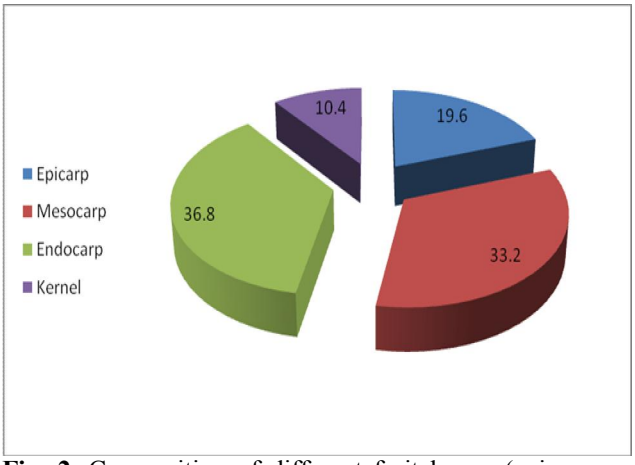

Fig. 2. Composition of different fruit layers (epicarp, mesocarp, endocarp and kernel) as percent of fruit weight of *Balanites aegyptiaca* from Damazin source (SD1)

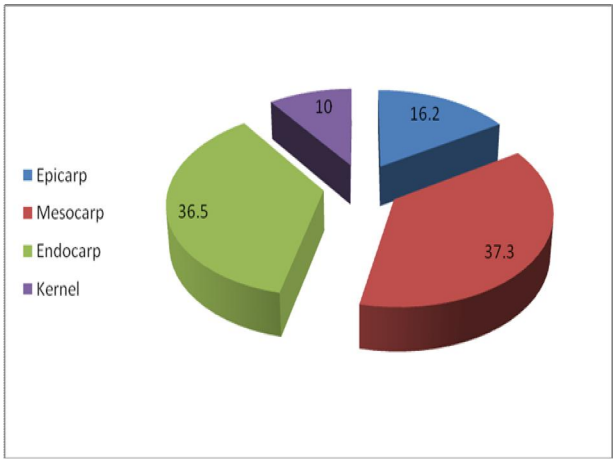

Fig. 3. Composition of different fruit layers (epicarp, mesocarp, endocarp and kernel) as percent of fruit weight of *Balanites aegyptiaca* from Umbeir sources (SD2)

Acknowledgements

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah under grand **no**. **261/155/1431**. The authors therefore, acknowledge with thanks DSR technical and financial support.

Corresponding author:
Elfeel, A. Ahmed
Department of Arid land Agriculture, King Abdulaziz University
P.O. Box 80208, Jeddah 21589
Saudi Arabia
Cell Phone: +966-567420763
email: (aidris@kau.edu.sa)

References

1. Sands MJ. The desert date and its relatives: A revision of the genus *Balanites*. Kew Bulletin 2001; 56: 1-128.
2. Arbonnier M. Trees, Shrubs and Lianas of West African Dry Zones. PP 573, CIRAD, MARGRAF PUBLISHERS GMBH 2004.
3. Elfeel AA, Hindi SZ and Abohassan RA (2013). Stomatal conductance, mineral concentration and condensed tannins in three *Balanites aegyptiaca* intra-specific sources affected by salinity stress. Journal of Food, Agriculture and Environment-JFAE, 11 (1): 46 - 471.
4. Abasse T, Weber JC, Katkore B, Boureima M, Larwanou M and Kalinganire A Morphological variation in *Balanites aegyptiaca* fruits and seeds within and among parkland agroforests in eastern Niger. Agroforestry Systems 2011; 81(1): 57 – 66.
5. Zobel B, Talbert J. Applied Forest Tree Improvement. The Blackburn Press, pp 505 2003.
6. Natinal Research Council. Lost Crops of Africa: Volume III, Fruits, Development, Security and Cooperation. The national Academies Press, Washington, D.C. 2008
7. Elfeel AA, Warrag EI. Uses and conservation status of *Balanites aegyptiaca* (L.)Del. (Hegleig Tree) in Sudan: Local people perspective. Asian Journal of Agricultural Sciences 2011; 3(4): 386 – 390.
8. Obidah W, Nadro MS, Tiyafo GO, Wurochekke AU. Toxicity of Crude *Balanites aegyptiaca* Seed Oil in Rats. Journal of American Science 2009; 5(6):13-165.
9. Hanan AA, Ayman A, Farghaly MM, Abd El Aziz MA. Phytochemical investigation and medicinal evaluation of fixed oil of *Balanites aegyptiaca* fruits (Balantiaceae). Journal of Ethnopharmacology 2010; 127 (2): 495 – 501.
10. Gutti B, Bamidele SS, Bagaje IM. Characterization and composition of *Balanites aegyptiaca* seed oil and its potential as biodiesel feedstock in Nigeria. Journal of Applied Phytotechnology in Environmental Sanitation 2012; 1(1): 29-35.
11. Chapagain P Bishnu, Yehoshua Hariv, Wiesman Zeev. Desert date (*Balanites aegyptiaca*) as an arid lands sustainable bioresource for biodiesel. Bioresource Technology 2009; 100 : 1221 – 1226.
12. Elfeel AA..Variability in *Balanites aegyptiaca var. aegyptiaca* seed kernel oil, protein and minerals contents between and within locations. Agriculture and Biology Journal of North America (ABJNA) 2010; 1(2), 170 – 174.
13. Farid H, Haslinger E, Kunert O, Wegner C, Hamburger M New steroidal glycosides from *Balanites aegyptiaca*. Helvetica Chimica Acta 2002;.88(4): 1019-1026.
14. Patil SV, Salunke BK, Patil CD, Salunkhe RB, Gavit P, Maheshwari VL. Potential of extracts of the tropical plant *Balanites aegyptiaca* (L) Del. (Balanitaceae) to control the mealy bug, Maconellicoccus hirsutus (Homoptera: Pseudococcidae). Crop Protection 2010; 29: 1293 – 1296.
15. Gnoula GM, Megalizz V, De Neve N, Sauvage S, Ribaucoure F, Guissou P, Duez P, Dubois J, IngrassiaL, Lefrance F, Kiss R, Mijatovic T. Balanitin-6 and -7: Diosgenyl saponins isolated from *Balanites aegyptiaca* Del. display significant anti-tumor activity in vitro and in vivo International Journal Of Oncology 2008; 32: 5-15.
16. Koko WS, Abdallab HS., Galala M, Khalida HS. Evaluation of oral therapy on Mansonial Schistosomiasis using single dose of Balanites

aegyptiaca fruits and praziquantel Fitoterapia 2005; 76: 30–34
17. Wiesman Zeev, Chapagain BP Laboratory Evaluation of Natural Saponin as a Bioactive Agent against Aedes aegypti and Culex pipiens. Dengue Bulletin 2003; 27.
18. Wiesman Z Metabolomic analysis of Balanites aegyptiaca plant tissue by LC-ESI/MS and MALDI-TOF/MS. Phytochemical Society of Europe conference. Cambridge. UK 2007; 11-14.
19. Schmidt LH, joker D *Balanites aegyptiaca* (L) Dell. Danida Forest Seed Centre seed leaflet No. 21. DFSC, Denmark 2000.
20. Hanlon EA Elemental Determination by Atomic Absorption Spectrophotometry In: Kalra, Y.P. (ed.), handbook of reference methods for plant analysis. CRC Press, Taylor and Francis Group, PP 157 – 164 1998.
21. Bhargava BS, Raghupalhi HB. Analysis of plant materials for macro and micro nutrients. In: Tandon, H.L.S. (ed.), Methods of analysis of soils, plants, waters and fertilizers. Development consultation organization, New Delhi, India, PP 285 1993.
22. Horneck DA, Miller RO Determination of total nitrogen in plant tissue In: Kalra, Y.P. (ed.), handbook of reference methods for plant analysis. CRC Press, Taylor and Francis Group, PP 75 – 83 1998.
23. ASTM International. ASTM D2395 - 84 Standard Test Methods for Specific Gravity of Wood and Wood Based Materials. ASTM.org, USA 1989.
24. ASTM International. ASTM D1102 – 84 Standard Test Method for Ash in Wood. ASTM.org, USA 2007.
25. ASTM International. ASTM D2015 - 85 Standard Test Method for Gross Calorific Value of Coal and Coke by the Adiabatic Bomb Calorimeter. ASTM.org, USA 1987.
26. Neenan, M, Steinbeck K Caloric values for young sprouts of nine hardwood species. Forest-Science1979; 25: 455-461
27. SAS Institute (2008). SAS Statistical analysis, Version 9.1, SAS institute Inc., Cary, NC 27513, USA.
28. Sparg SG, Light ME, Stadem J. Biological activities and distribution of plant saponins. Journal of Ethnopharmacology 2004; 94: 219 – 243.
29. Vincken JP, Heng L, Groot A, Gruppen H. Saponins, classification and occurrence in the plant kingdom. Phytochemistry 2007; 68: 275 – 297.

4/11/2014