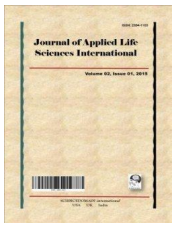

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Chemical Analysis of *Moringa oleifera* and *M. peregerina* and their Growth Responses to Water Stress under Semi-desert Condition of Sudan

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NBH and SOY designed the study, and wrote the first draft of the manuscript. Author HAMA managed the literature searches, managed the experimental process and performed the analyses with authors NBH and SOY. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The objective of this study was to evaluate the growth and chemical analysis of two of *Moringa oleifera* and *Moringa peregerina* under three watering intervals.
Place and Duration of Study: Field experiments were conducted for two consecutive seasons (2011\2012- 2012\2013) in the at the Demonstration Farm at Shambat, Sudan University of Science & Technology College of Agricultural Studies, Sudan.
Methodology: Complete randomized block design with four replications was used to execute the experiment for three irrigation intervals (control irrigated every 10 days, and the other two watering intervals extended for every 20, and 30 days). *M. peregrina* in the field delayed in emergence in both seasons. The field parameters were taken only for *M. oleifera.* Chemical and proximate

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analyses were carried out for the two species of *Moringa* to determine, protein, ash, fiber, fat. The minerals, calcium, magnesium, sodium, potassium, and ion.

Results: Results of this experiment demonstrated no significant differences within or between parameters of plant height, number of leaves per plant, fresh weight, plant dry weight, and root dry weight among the three watering intervals for the two seasons for *Moringa oleifera*. However, stem diameter in the first season showed significant variations only in results for the first four weeks from starting watering interval treatment. The watering interval every 30 days showed the highest plant height in both seasons, whereas, number of leaves was the highest for the 30 days watering interval only in the first season, were not significantly different between watering treatments.

Conclusion: The findings of this study proved that growth of *Moringa oleifera* grown in Sudan are not affected by water interval up to 30 days. The nutritive value of *M. oleifera* was significantly higher than *M. peregrina* especially in protein, calcium, potassium and iron.

Keywords: Chemical analysis; M. oleifera; M .peregrina, watering interval; growth; semi desert conditions.

1. INTRODUCTION

The family Moringaceae*,* consists of only one genus called *Moringa*, the genus holds fourteen species [1]. The family Moringaceae includes species exhibiting a wide range of forms: bottle trees to slender trees, sarcorhizal trees or tuberous shrubs [2]. All these species are native to the Indian subcontinent, the Red Sea area and parts of Africa, including Madagascar. Although *Moringa* is native to India and Pakistan [3], it is widely cultivated especially in dry tropical areas of the Middle East and Africa [4] 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 [4]. Moringa can also be found as far away as the Caribbean, purported to be in Jamaica since 1817. With the folk use of promotes digestion and appetite, pain and headache [5]. Besides being consumed by humans [6], it is also used as animal fodder [7,4] a natural coagulant of turbid water [8] and source of phytomedical compounds [9].

Most of the previous studies on Moringa concentrated on *M. oleifera* because of its prevalence in the poor areas in Africa and Asia, where 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. *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 [10]. Its leaves are incomparable as a source of the sulfur-containing amino acids methionine and cystine. 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 alleviation of malnutrition, [11]. Those studies revealed beyond any doubt that *M. oleifera* is the miracle or the life tree [12].

Moringa has been reported to exist in the 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, and Blue Nile [13]. The objective of this work was to study the growth responses of *Moringa oleifera* & *M. peregrina* to water stress under semi-desert condition of Shambat, Sudan and to compare the chemical constituents for both *Moringa oleifera and M. peregrina*, as the latter is poorly studied.

2. MATERIALS AND METHODS

Field experiments were conducted for two consecutive seasons (2011\2012- 2012\2013) in the Demonstration Farm at Shambat (latitude 15º40´ N, longitude 32º32´ E and altitude 386m above sea level) at the Sudan University of Science & Technology, College of Agricultural Studies, to study two *Moringa* species. The *Moringa* seeds that were used in this study were obtained from the Seed Research Centre (Soba),

Khartoum, Sudan. Complete randomized block design with four replications were used to execute this experiment using three irrigation intervals (control irrigated every 10 days [irrigation 1], the other watering intervals extended every 20 days [irrigation 2] and 30 days [irrigation 3]). The spacing between ridges was 70 cm. The plot size was 3 x 3 m ridge. Seed rate was two seeds sown per hole 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, and after the seed germinated, irrigation intervals of the treatments was every 10 days, 20 and 30 days. The fertilizer was applied after 2 months. The Harvesting was carried out after four to five months.

Five tagged plants from each plot were chosen randomly for each plot treatment to monitor the growth characteristics every week.Plant height was measured from the soil surface to the upper plant recorded as the average plant height expressed in centimeters.

Also all the leaves in the five tagged plants and the average number of leaves per plant was determined. The thickness of the stalk at the middle of internodes from the plant base was measured using digital vernier calliper. The fresh weight of the total number of plants (five plants/plots), was taken per square meter, by cutting plants from each plot and weighting using a balance. The fresh forage yield was left to dry in the air and then weighed again to give the dry weight by using sensitive balance. The same plants which were harvested to take fresh weight were taken to estimate root dry weight, the roots were separated from fresh plant stems and dried by air, dry weight was measured by using sensitive balance.

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

The samples taken (dried plant and root) to estimate the dry matter yield were used for the analysis. The percentages of the following quality traits were determined. The protein content was determined for both *M. oleifera* & *M. peregrina* by macro – Kjeldahl method using a copper sulphate catalyst according to the official method [14]. The sample was accurately weighed and transferred together with 2g of Kjeldahl catalysts (No. 33064, BDH, England) and 20 ml of concentrated sulphuricacid (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 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.

Fat content was determined according to the official method of [14]. 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.

Ash content was determined according to the method described by [15]. The inorganic materials are customary determined as a residue after being ignited at specified heat degree. (No. 20. 301870, carbolated, England). The total carbohydrates content of the *Moringa* samples were calculated by subtracting the total sum of (Moisture, Fat, Protein and Ash as percentages) from 100% according to [15].

Proximate analysis for minerals in *Moringa* were determined, the dry aching method that was described by [16] was used for determination of minerals; *Moringa* samples were weighed in clean dry porcelain crucible and placed into muffle furnace (No.20. 301870, 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 and the concentrations of Sodium, Potassium, Magnesium, Calcium and Iron determined by using atomic Absorption Spectrophotometer (3110-Perkin Elmer).

The data collected for the different 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).

3. RESULTS AND DISCUSSION

Moringa peregrina germination was very slow and showed delayed growth in two months. Whereas, *Moringa oleifera* showed fast germination and good establishment in 4 weeks (Figs. 1 and 2). This might be due to the genetic characteristics of growth of *Moringa peregrina.* [17] concluded that the phonological development of *Moringa peregrina* was delayed with increasing altitude. Apparently *Moringa peregrina* is more drought tolerant than *Moringa oleifera* which is commercially planted in a large scale in tropical and sub tropical areas [18]. Due to the delay of *Moringa peregrina* growth in the field, the parameters were taken only for *Moringa oleifera.*

Results demonstrated that there were no significant differences in plant height for the *Moringa oleifera* for the two seasons under the three watering intervals. As presented in Table 1, the irrigation treatment 30 days gave the highest plant height of the three treatment for all weeks except weeks 5^{th} and 8^{th} in which irrigation every 20 days gave the highest plant height on the first season although these differences were not significant. In the second season, irrigation every 10 days (control) showed the highest plant height for all weeks except in the first week for all watering treatments. In this study, the average plant heights ranged from 45.7 - 62.7 cm in watering interval one (10 days), to 47.8- 76.9 cm in water interval two (20 days) 54.1-62.7 cm, in watering interval three (30 days). The plant height was higher than the previous 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 [19]. This phenomenon, known as hardening, suggests that plants have greater tolerance to stress from biochemical changes and/or epigenetic alterations that occur after the first exposure to environmental disturbance [20].

The effect of different irrigation intervals (10, 20, and 30 days) on the number of leaves of *Moringa oleifera* is presented in Table 2. There were no significant differences 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) for all weeks 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.

Fig. 1. *Moringa oleifera* growth after two months

Fig. 2. *Moringa peregrina* growth after two months

Table 1. Effect of three irrigation intervals on plant height of *Moringa oleifera* grown in two seasons (2011\2012- 2012\2013)

Season 2011/12								
Treatments	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week7**	**Week 8**
Irrigation1	45.7a	51.7a	55.2a	47.0a	42.9a	61.3a	62.0a	62.7a
Irrigation 2	47.8a	53.5a	59.9a	64.4a	72.7a	64.6a	63.9a	76.9a
Irrigation 3	62.7a	69.9a	70.4a	68.6a	69.3a	73.4a	68.1a	54.1a
S.E.±	7.4 ns	10.0ns	12.5ns	8.7ns	11.8ns	11.7ns	11.3ns	10.5ns
LSD	43.96	59.22	74.07	51.50	69.79	69.31	66.69	62.26
Season 2012/13								
Irrigation 1	60.4a	82.2a	89.4a	93.7a	86.9a	95.7a	95.4a	95.9a
Irrigation 2	69.9a	80.4a	88.1a	80.7a	85.8a	90.3a	92.6a	88.8a
Irrigation 3	57.0a	69.2a	74.5a	72.5a	67.8a	70.7a	76.1a	80.1a
S.E.±	9.3ns	11.7ns	10.2ns	9.6ns	10.6ns	10.8ns	11.7ns	12.1ns
LSD	55.26	69.65	60.81	57.04	63.01	64.54	69.53	72.23

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 2. Effects of three irrigation intervals on the number of leaves of *Moringa oleifera* grown in two seasons (2011\2012- 2012\2013)

Season 2011/12								
Treatments	**Week 1**	**Week 2**	**Week 3**	**Week4**	**Week 5**	**Week6**	**Week7**	**Week 8**
Irrigation 1	10.0a	08.1a	07.4a	06.3a	04.8a	06.2a	05 .5a	04.7a
Irrigation 2	09.7a	07.7a	05.7a	04.9a	06.7a	06.2 a	04.9a	04.5a
Irrigation 3	11.1a	09.9a	07.2a	05.2a	06.0a	0 5.0a	04.9a	03.9a
S.E.±	1.2 ns	1.2ns	0.8ns	0.9ns	1.1ns	0.7ns	0.6ns	0.6ns
LSD	7.264	6.751	4.407	5.086	6.169	4.121	3.623	3.752
Season 2012/13								
Irrigation 1	08.1a	08.5a	08.5a	07.9a	07.5a	07.5a	06.6a	06.4a
Irrigation 2	07.1a	06.5a	07.0a	06.1a	05.6a	05.2a	05.6a	06.1a
Irrigation 3	07.2a	06.8a	06.7a	07.0a	05.4a	06.1a	05.9a	05.7a
S.E.±	1.1ns	1.1ns	0.9ns	0.8ns	0.5ns	0.7ns	0.5ns	0.8ns
LSD	6.830	6.867	5.666	4.789	2.762	4.111	3.277	4.816

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

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

Effect of the three irrigation intervals on plant fresh weight, dry weight and root dry weight of *Moringa oleifera* grown in two seasons are presented in Table 4. The results obtained revealed no significant differences among all parameters for the two seasons.

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 [21]. 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 [19]. [22] 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 their study compared with this study.

[23] suggested that seeds of *M. oleifera* subjected to mild water deficit have had

increased the ability for drought tolerance when young plant.

The results of the chemical composition of *M. peregrina* and *M. oleifera* leaves and roots are presented in Tables 5 and 6. The analyses showed the variations in some minerals between the two species. *M. oleifera* leaves had 23.5% protein, 9.7% Ash, 17.6%, 14.6% Fiber CHO and 7.7% Fat. Whereas, *M. peregrina* had15.9%, 9.6%, 11.7%, 11.2% and 7.5% respectively.

[7] reported protein contents of 22.8% and 23.3% for found 30.6% protein for *M. stenopetala* leaves. They attributed 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.

[24] showed that *Moringa* leaves are rich in protein and amino acid composition which is suitable for human and animal nutrition. Results illustrated that *Moringa* leaves can be considered as good fodder as it contain essential nutrients that can improve growth performance of animals. These results indicated that the *M. oleifera* was slightly higher than the *M. peregrina* in contents. *Moringa* being extremely rich in nutrients is an excellent source of mineral for feeding human and animals. *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%). However roots of the two species were not significantly different for any parameters measured.

Table 3. Effects of three irrigation intervals on stem diameter of *Moringa oleifera* grown in two Seasons (2011\2012- 2012\2013)

Season 2011/12								
Treatments	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week7**	**Week 8**
Irrigation 1	4.2ab	3.7b	4.2ab	4.0a	3.2a	4.4ab	4.1a	5.4a
Irrigation 2	3.2b	3.6b	3.6b	3.3a	5.1a	3.7b	4.5a	4.8a
Irrigation 3	6.4a	6.4a	6.2a	6.2a	5.6a	6.4a	4.2a	5.9a
S.E.±	0.6*	0.4*	0.4*	0.6ns	1.0ns	0.4*	0.7ns	0.9ns
LSD	3.07	2.25	2.15	2.98	5.11	2.25	3.63	4.36
Season 2012/13								
Irrigation	5.9a	6.1a	6.3a	7.5a	5.3a	7.0a	7.0a	7.0a
Irrigation	6.1a	6.1a	6.1a	6.7a	7.2a	7.0a	7.0a	7.1a
Irrigation	5.0a	4.8a	5.5a	6.0a	6.2a	6.2a	5. 9a	5.4a
S.E.±	0.9ns	0.5ns	0.6ns	0.9ns	1.4ns	1.1ns	1ns	1ns
LSD	5.14	2.97	3.31	5.24	7.75	6.23	5.85	5.60

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 4. Effect of three irrigation intervals on plant fresh weight, dry weight and root dry weight of *Moringa oleifera* grown in two seasons (2011\2012- 2012\2013)

	Season 2011/12			**Season 2011/13**		
Treatments	**Plant Fresh weight**	**Plant Dry weight**	**Root dry weight**	**Plant Fresh weight**	**Plant Dry weight**	**Root dry weight**
Irrigation 1	591.0a	73.7a	105.7a	1274.0a	291.6a	264.6a
Irrigation 2	436.6a	38.5a	82.8a	1870.0a	306.4a	500.6a
Irrigation 3	437.9a	50.4a	87.00a	1352.0a	277.9a	402.0a
S.E±	97ns	15.6ns	23.3ns	210ns	70.5ns	73.1ns
LSD	572.6	92.2	137.6	120.9	406.1	420.5

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 20 days, Irrigation3 = every 30 days

Table 5. Chemical composition of leaves and roots of *Moringa oleifera* & *Moringa peregrina* in seasons (2011\2012- 2012\2013)

Element	Protein (%)	Ash(%)	Fiber(%)	CHO(%)	Fat(%)
Moringa oleifera- leaves	23.5	9.7	17.6	14.6	7.7
Moringa oleifera - roots	-	11.2	18.6	-	-
Moringa peregrina- leaves	15.9	9.6	11.7	11.2	7.5
Moringa peregrina- roots	-	13.1	13.6	-	-

Table 6. Mineral composition of leaves and roots of *Moringa oleifera* & *Moringa peregrina* grown in seasons (2011\2012- 2012\2013)

Element	Ca(%)	Mg(%)	Na(%)	K(%)	Fe(%)
Moringa oleifera - leaves	3.5	0.10	1.06	3.80	2.38
Moringa oleifera - roots	0.15	0.20	0.75	0.75	0.13
Moringa peregrina- leaves	0.74	0.34	3.44	0.59	-
Moringa peregrina- roots	0.11	0.21	1.13	0.28	0.19

4. SUMMARY AND CONCLUSION

Based on the result of this study, we recommend to grow *M. Oleifera* under the condition of Khartoum state, due to easiness in germination and fast growth rate. Also, *Moringa oleifera* can be used as a fodder crop, because it continued to grow up within 30 days, whereas, *M. peregrina* had drastic drop in growth. *Moringa oleifera* is not affected by watering intervals up to 30 days. *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 in Protein, Cacium, Potassium and Iron.

Moringa leaves can be considered as good fodders as it contain essential nutrients that can improve growth performance of animals the percentage of protein is higher in *Moringa oleifera*. Whereas, *Moringa oleifera* roots showed higher fiber content.

Mineral composition showed that, there was high variation between leaves of the two *Moringa* species. *Moringa oleifera* gave higher percentage of Ca, K, Fe, and Mn. Whereas, *Moringa peregrina* contained higher percentage in Na and Mg. On the other hand, root analysis indicated that *Moringa oleifera* contents of K was slightly higher than in the *Moringa peregrina*. *Moringa peregrina* showed higher percentage in Na.

The different species of *Moringa* need more research concerning agronomy and cultural practices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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