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Effect of irrigation water quantity and salinity level on growth and internal chemical contents of Moringa plants

Hesham S. Abdelaty[1]*, Abdelaziz M. Hosny[1], Ahmed N. Abdelhamid[1], Aboelfetoh M. Abdalla[2]

[1] *Horticulture Dept., Faculty of Agriculture, Ain shams Univ., Cairo, Egypt.*

[2] *Horticultural Crops Technology Dept., National Research Centre, Giza, Egypt.*

Abstract

Moringa 'the miracle tree' is a relatively new promising plant because of its rich nutritional value, viz. mineral nutrients, protein containing all amino acids, vitamins and antioxidants. The objective of this study was to determine the optimal water amount for Moringa productivity even under salinity conditions and to ensure getting better quality and more active substances from it for human nutrition. During the growing seasons of both 2020 and 2021, Moringa seedlings were irrigated with 12 different combinations using three water quantities, i.e. 100, 75 and 50% (of Class A pan evaporation equation) and four salinity levels, i.e. non-saline control, 2000, 3000 and 4000 ppm (NaCl from Rashid salt). The obtained results indicated that decreasing irrigation water quantity from 100 to 50% of pan evaporation negatively affected all vegetative growth characters of Moringa and decreased the contents of essential nutrients, crude protein, vitamin C and total chlorophyll content, albeit the variations between 100 and 75% of pan evaporation sometimes were not significant. On the other hand, increasing salinity levels gradually reduced all vegetative growth characters of Moringa and decreased the essential nutrients, crude protein, vitamin C and total chlorophyll. Notably, Na and proline contents were increased with both decreasing irrigation water quantity and increasing of salinity level.

Keywords: *Moringa oleifera*, Irrigation water, Salinity levels, Yield, Quality, Proline, Protein, Vitamin C, Chlorophyll content
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1. Introduction

Moringa known as the miracle tree, earns its title from being a dietary supplement for both human and animals, not to mention its importance in folk medicine [1, 2, 3]. All plant parts of the Moringa tree are edible and have long been consumed by humans [1]. Several studies have been conducted for the sake of expanding Moringa cultivation in Egypt [4, 5, 6, 7, 8, 9).

The world's population is expected to grow to nearly 10 billion by 2050 [10], boosting food demand by 50 percent compared to 2013 [11]. Most of the predicted population growth will take place in the developing countries. In addition, developing countries, in particular, will be strongly confronted with severe food-security challenges.

The agricultural sector has been impacted by climate change and climate variability events [11, 12]. In turn, food security is and will be challenged by climate change impacts. The environmental stress like for example increasing salinity and rising temperatures and reduced irrigation water availability are major limiting factors in sustaining and increasing plant productivity in many arid and semi-arid areas including Egypt, where rainfall is normally lower than evapotranspiration [13, 14]. Environmental stress is the primary cause of crop losses worldwide, reducing average yields for most major crops by more than 50% [15, 16].

In Egypt, new reclaimed lands are suitable and promising areas to expand Moringa cultivation [8]. According to [17], the notable problems facing Moringa cultivation in reclaimed lands are water deficit and salinity stress, which adversely affect the growth and productivity. Declining water availability is one of the challenges facing Egypt for cultivation expansion to meet the needs of high population density [18]. Water share reached 860 m^3 $capita^{-1}$ and is expected to decrease to 582 m^3 by the year 2025 [18, 19], taking into account the less water coming from Ethiopia after the Ethiopian renaissance dam when completed and our high population rate [18]. The limited water resources require and dictate to increase water use efficiency [20, 21] and use marginal land and waters [19, 21] to satisfy the high rates of population growth. This may help to minimize water consumption, reduce the losses of irrigation water and increase the cultivated area. Therefore, the main goal

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of the current research was to determine the optimum irrigation water requirement per season under three other saline conditions for high Moringa productivity taking into account water use efficiency.

2. Materials and Methods:

2.1 Location and duration:

Two pot experiments in the open field were carried out on Moringa plants (two-month old seedlings) during the two successive cropping seasons of 2020 and 2021. The experiments took place at a farm in the Delta region, El-Menoufia Governorate.

2.2 Plant materials:

Moringa *(Moringa oleifera*) seeds were obtained from the Egyptian Scientific Association for Moringa in National Research Centre, Dokki, Cairo, Egypt. For sowing, wet seeds were placed into black plastic bags 9 cm in diameter (two seeds per bag) filled in advance with silty-clay soil on the first of April during the two cropping seasons. Two months later, on the first of June, uniform Moringa seedlings (35 cm tall) with uniform and healthy growth were transferred from the bags into plastic pots (30 cm in diameter), one seedling per pot. A soil sample was collected before plant cultivation and analyzed. The soil texture was silty-clay containing 55.2% clay, 24.1% silt and 20.7% sand. Soil moisture at field capacity was 30% and at wilting point was 18% of soil dry weight. The pH was 7.3 and EC was 1.4 EC. After transplanting, all seedlings were first irrigated by the same regime with fresh water for good plant establishment for the duration of two weeks.

2.3 Irrigation water calculation:

The irrigation water for Moringa was calculated based on Class A pan evaporation equation for 100% treatment according to [22] as follows:

$$ET_0 = E_{pan} \times K_p$$
$$CU = ET_0 \times K_c$$
$$WR = CU \times L\%$$

Where:

ET_o: Reference evapotranspiration.
E_{pan}: Pan evaporation in mm.
K_p: Pan coefficient (constant, 0.85).
CU: Water consumption.
L%: Leaching factor (1.25%).
WR: Water requirement (L m^{-2}).
K_c: Crop coefficient (variable 0.7 :1.2 during growing season according to [23].

The water volume of 100% of Class A pan evaporation was reduced to 75 and 50%, and Moringa plants were subjected to three irrigation treatments as moderate water regime (75%) and low water regime (50%), compared to high water regime (100% of Class A pan evaporation), which served as control.

2.4 Salinity levels specifics:

At the same time, NaCl (coarse salt from Rashid marshes) as a source of salinity was added to the fresh water, to make up for the three saline brackish water concentrations, i.e. 2000, 3000 and 4000 ppm compared to non-saline treatment (control).

2.5 Treatments:

Two-month old Moringa seedlings were irrigated by 12 different combinations created from three water quantities (100, 75 and 50% of Class A pan evaporation) and four saline treatments (0, 2000, 3000 and 4000 ppm). The irrigation water treatments were weekly supplied at 7 days intervals. The NPK fertilizers were added at the proper time as recommended for all pots.

2.6 Data recorded:

Representative samples were collected 90 days from transplanting in the two growing seasons, by harvesting three plants from each plot. The whole plant canopy was cut 10 cm above soil surface to evaluate vegetative growth, and chemical contents as follows:

2.6.1 Vegetative growth characters:

Vegetative growth parameters, recorded plant height (cm), number of leaves per plant, in addition to fresh and dry weights characters of plant (g) were recorded.

2.6.2 Chemical analysis:

Samples were collected from leaves and shoots for chemical analysis to determine the essential nutrients, i.e. K (%), Ca (%), Fe (ppm), Zn (ppm) and Na (%). Also, Crude Protein (%), Proline (mg/100g), Vitamin C (mg/100g) and total Chlorophyll content (SPAD) were estimated. For chemical analyses, plant samples were dried for 3 days in an oven at 70°C, then fine grinded and used to find out ion contents on a dry weight basis. K, Ca, Mg, Fe, Zn and Na were determined by an atomic absorption spectroscope (VARINA Spectra AA 100) after 6 h ashing at 550°C and digestion with concentrated HCl as described by [24]. Proline content (g/100g F.W.) was conducted according to [25]. The rate of crude protein was calculated according to the method of [26]. Vitamin C content was determined in fresh leaves (g/100g F.W.) using dye 2, 4, 6-di-chlorophenolindophenol method as described in [26]. Total Chlorophyll content (SPAD) was performed using a Minolta Chlorophyll Meter (SPAD-501).

2.7 Experimental design and statistical analysis:

Two-factorial experiments, which included 12 combinations as treatments were carried out in a randomized complete block design (RCBD) with three replications. The three water amounts: 100, 75 and 50% of Class A pan evaporation were assigned to the main- blocks (Factor A). While, the four salinity levels: non-saline treatment (0), 2000, 3000 and 4000 ppm occupied the sub-plots (Factor B). Each experimental plot consisted of 18 pots, with plant density of 9 plants/m^2.

The obtained data were statistically analyzed using analysis of variance (ANOVA). The mean values were compared using Duncan's multiple range test at P<5% as reported by [27].

3. Results

3.1 Vegetative growth characters:

Results data presented in Table (1) show gradual reductions in all measured vegetative growth characters, i.e. plant height, number of leaves per plant, and also plant fresh and dry weights with the gradual decreasing of irrigation water from 100 to 50% of pan evaporation equation. This was valid and true for both seasons 2020 and 2021. Whereas, reduction values in plant height and plant fresh weight during second season and number of leaves per plant and plant dry weight in both seasons were not significant between the higher water regime (100%) and the moderate water regime (75%).

On the other hand, increasing salinity levels caused significant reductions in vegetative growth characters of Moringa plants. Plant height was gradually reduced by increasing salinity levels from 2000 to 4000 ppm when compared to each other and the non-saline control in both seasons studied (Table, 1). Whereas, it was realized that there were no significant variations in number of leaves per plant, plant fresh weight and plant dry weight in the two cultivated seasons between the lowest salinity level (2000 pm) and the non-saline treatment (control), which in the case of both number of leaves and plant dry weight they were still higher in values when compared to each of the higher level of salinity (3000 and 4000) in both season investigated. For plant fresh weight, this was valid only in the first season only.

Concerning the interaction effects (Table 1), application of the highest water regime (100%) with no-salt control resulted in the tallest plant height in 2020 season and heaviest plant fresh weight in both 2020 and 2021 seasons when compared with lowest (50%) water regime and highest (3000 and 4000 ppm) salinity levels combinations and a little few of the remaining treatments.

3.2 Chemical analysis characters

Results data illustrated in Table (2) and (3) show clearly that decreasing water amounts from 100 to 50% based on pan evaporation equation negatively affected chemical contents of all nutrient elements: macronutrients, i.e., K and Ca, and micronutrients, i.e., Fe and Zn (Table, 2) as well as decreased chemical compounds of crude protein, vitamin C and total chlorophyll content (Table, 3). Meanwhile, both Na and proline increased by decreasing irrigation water gradually from 100% to 50% of pan evaporation (Table 2 and 3). The differences in the contents of K in the second season, and Ca and Zn in both seasons between higher 100% and moderate 75% water regimes were insignificant. Notably, in both seasons the differences between the moderate (75%) regime and low (50%) regime in Na % were insignificant.

As for as salinity levels are concerned, the obtained results indicate that increasing salinity levels from 2000 to 4000 ppm in both seasons reduced all nutrient elements: macronutrients, i.e. K and Ca and micronutrients i.e., Fe and Zn (Table, 2) as well as crude protein, vitamin C and total chlorophyll content (Table, 3). Whereas, it increased both Na and proline (Table 2 and 3). The differences between the lowest salinity levels (2000 ppm) and non-saline control were not statistically significant concerning their effect on the contents of Ca in the first season, vitamin C in the second season and Zn, protein and chlorophyll content in both the two cultivated seasons.

With regards to the interaction effects, application of the highest water regime (100%) with no salt control increased the contents of two essential nutrients, i.e. Ca and Fe, and total chlorophyll content in the first season (Table, 2) as well as it increased crude protein in both seasons (Table, 3) when compared to low (50%) water regime and salinity levels (2000, 3000, 4000 ppm) combinations. Whilst, Na in the first season and proline in both seasons were increased at the low (50%) water regime when combined with highest (4000 ppm) salinity level when compared with all combinations of the higher (75 and 100%) water regimes and all remaining salinity levels.

4. Discussion

Water is the most vital factor affecting plant growth and its productivity. The general trend of results in the current study concluded that all vegetative growth characters, essential nutrient elements, protein and vitamin C contents in addition to chlorophyll were decreased by lowering the amount of supplied water to irrigate Moringa plants. Whilst vice versa, Na and proline contents were increased. A similar trend to decrease growth with decreasing irrigation water was reported by several previous authors [19, 28, 29, 30]. So, the enhancing effect of increasing irrigation water amounts on Moringa plant growth can be explained by the fact that water is a major constituent of growing plant tissues and involved in many biochemical processes. Water has a crucial role in the process of photosynthesis and acts as a translocation agent of organic and mineral constituents. Hence, the number, size and turgor of the cells increased, resulting finally in increases in most vegetative growth parameters. The beneficial effect of highest water regime (100% of evaporation) on plant vegetative growth resulted in more accumulation of dry matter which is possibly the main reason for firstly increasing plant productivity and secondly improving the nutrient status of Moringa plants leading to more quality.

Table (1): Effect of irrigation water quantity and salinity level on vegetative growth of Moringa plants during the two cultivated seasons of 2020 and 2021.

Treatments		Plant height (cm)		No. of leaves per plant		Plant fresh weight (g)		Plant dry weight (g)	
		2020	2021	2020	2021	2020	2021	2020	2021
Water amounts:									
100% of *P. Eva.		111.0a	117.3a	12.8a	12.5a	158.1a	151.3a	39.5a	36.3a
75% of *P. Eva.		108.3b	116.7a	11.9ab	12.1a	149.8b	150.8a	38.0a	37.1a
50% of *P. Eva.		104.2c	111.0b	11.2b	11.3b	133.4c	138.3b	34.7b	33.7b
Salinity levels:									
Control		114.2a	121.3a	12.9a	13.0a	159.9a	162.8a	40.4a	39.4a
2000 ppm		111.0b	118.2b	12.4a	12.9a	155.6a	156.7ab	39.6a	38.6a
3000 ppm		106.4c	113.9c	11.7b	11.8b	144.5b	147.4b	36.8b	35.7b
4000 ppm		99.7d	106.6d	10.9b	10.1c	128.3c	120.3c	32.7c	29.1c
Interactions:									
100% of *P. Eva.	Control	116.3a	122.3a	13.7a	13.0a	167.8a	163.9ab	42.0a	39.0a
	2000 ppm	113.3a	120.3a	13.3a	13.3a	165.4ab	155.6b	41.3a	39.3a
	3000 ppm	110.0b	115.7a	12.7a	12.3a	156.8b	152.7b	39.1a	36.3a
	4000 ppm	104.3d	111.0a	11.7a	11.3a	142.3cd	132.9c	35.6a	30.7a
75% of *P. Eva.	Control	115.0a	122.7a	13.0a	13.3a	165.1ab	170.0a	41.2a	40.8a
	2000 ppm	111.3ab	119.7a	12.3a	13.0a	160.9b	163.7ab	40.9a	39.7a
	3000 ppm	106.7c	115.0a	11.7a	12.0a	145.5cd	149.2bc	37.1a	37.2a
	4000 ppm	100.3e	109.3a	10.7a	10.0a	127.6e	120.3d	32.6a	30.5a
50% of *P. Eva.	Control	111.3ab	119.0a	12.0a	12.7a	146.7c	154.3b	38.1a	38.4a
	2000 ppm	108.3bc	114.7a	11.7a	12.3a	140.7d	150.7b	36.6a	36.6a
	3000 ppm	102.7de	111.0a	10.7a	11.0a	131.2e	140.3c	34.1a	33.7a
	4000 ppm	94.2f	99.3a	10.3a	9.0a	115.0f	107.6e	29.9a	26.2a

Means within each column and factor followed by the same letter are not significantly different at $P<5\%$ [27].
*P. Eva. : Pan Evaporation

Table (2): Effect of irrigation water quantity and salinity level on nutrient elements of Moringa plants during the two cultivated seasons of 2020 and 2021.

Treatments		K (%)		Ca (%)		Fe (ppm)		Zn (ppm)		Na (%)	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Water amounts:											
100% of *P. Eva.		2.04a	2.25a	2.09a	1.98a	73a	83a	51a	47a	0.60b	0.65b
75% of *P. Eva.		1.96b	2.20a	2.00a	1.95a	67b	79b	47ab	46a	0.68a	0.69ab
50% of *P. Eva.		1.79c	1.98b	1.76b	1.74b	61c	72c	44b	44b	0.72a	0.75a
Salinity levels:											
Control		2.17a	2.35a	2.25a	2.15a	78a	89a	55a	50a	0.50d	0.48d
2000 ppm		2.05b	2.20b	2.14ab	2.02b	73b	83b	52a	49a	0.61c	0.63c
3000 ppm		1.86c	2.09c	1.81b	1.79c	64c	75c	45b	43b	0.72b	0.78b
4000 ppm		1.65d	1.93d	1.58c	1.61d	53d	64d	39c	40b	0.83a	0.90a
Interactions:											
100% of *P. Eva.	Control	2.25a	2.45a	2.39a	2.24a	82a	91a	59a	53a	0.43d	0.49a
	2000 ppm	2.18a	2.34a	2.26a	2.12a	78a	88a	55a	50a	0.55cd	0.60a
	3000 ppm	1.97a	2.18a	2.05b	1.93a	72ab	83a	49a	46a	0.67bc	0.71a
	4000 ppm	1.75a	2.02a	1.65ce	1.64a	59bc	69a	42a	39a	0.74b	0.78a
75% of *P. Eva.	Control	2.19a	2.36a	2.32a	2.21a	78a	90a	54a	48a	0.50d	0.46a
	2000 ppm	2.07a	2.28a	2.26a	2.11a	74ab	83a	53a	51a	0.62c	0.59a
	3000 ppm	1.92a	2.13a	1.79c	1.81a	62bc	75a	44a	42a	0.74b	0.80a

	4000 ppm	1.67a	2.03a	1.63ce	1.67a	55c	66a	38a	43a	0.85a	0.92a
50% of *P. Eva.	Control	2.06a	2.25a	2.05b	2.02a	73ab	85a	51a	50a	0.56cd	0.49a
	2000 ppm	1.89a	1.97a	1.92bc	1.83a	67b	79a	47a	46a	0.67bc	0.68a
	3000 ppm	1.68a	1.96a	1.59ce	1.62a	56c	68a	43a	42a	0.76b	0.84a
	4000 ppm	1.53a	1.75a	1.46e	1.51a	46d	55a	36a	38a	0.89a	0.99a

Means within each column and factor followed by the same letter are not significantly different at *P*<5% [27].
*P. Eva. : Pan Evaporation

Table (3): Effect of irrigation water quantity and salinity level on chemical constitutes of Moringa plants during the two cultivated seasons of 2020 and 2021.

Treatments		Proline (mg/100g)		Crude Protein (%)		Vitamin C (mg/100g)		Chlorophyll (SPAD)	
		2020	2021	2020	2021	2020	2021	2020	2021
Water amounts:									
100% of *P. Eva.		53.4c	62.0c	25.5a	23.6a	51.4a	57.6a	42.6a	41.8a
75% of *P. Eva.		59.3b	66.8b	24.7b	23.0a	49.5b	56.9a	41.8a	41.1ab
50% of *P. Eva.		70.6a	76.5a	23.1c	21.9b	46.3c	53.3b	39.5b	39.5b
Salinity levels:									
Control		45.1d	50.1d	25.7a	23.9a	54.5a	60.9a	43.5a	42.3a
2000 ppm		54.9c	59.9c	25.4a	23.5a	52.4b	59.3a	42.5a	41.5a
3000 ppm		66.0b	76.6b	24.2b	22.5b	48.1c	55.2b	41.0b	40.4b
4000 ppm		78.3a	87.2a	22.4c	21.4c	41.3d	48.2c	38.1c	38.9c
Interactions:									
100% of *P. Eva.	Control	39.7f	44.3d	26.6a	24.3ab	57.3a	62.7a	45.1a	43.6a
	2000 ppm	48.3e	53.3cd	27.0a	24.6a	54.4a	60.0a	44.5ab	42.7a
	3000 ppm	57.0d	71.3b	25.3b	23.5b	50.2a	57.3a	42.6b	41.4a
	4000 ppm	68.7c	79.0b	23.1c	21.9cd	43.9a	50.3a	37.9d	39.4a
75% of *P. Eva.	Control	42.7f	46.3d	26.1ab	24.0ab	55.3a	61.0a	44.1ab	42.6a
	2000 ppm	50.3e	54.7cd	25.6b	23.6ab	53.1a	61.7a	42.8cd	41.7a
	3000 ppm	67.0c	78.3b	24.4bc	22.8bc	49.1a	55.7a	41.5bc	40.8a
	4000 ppm	76.7b	88.0ab	22.7c	21.6cd	40.7a	49.3a	38.7cd	39.2a
50% of *P. Eva.	Control	53.0de	59.7c	24.3bc	23.3bc	51.1a	59.0a	41.3bc	40.7a
	2000 ppm	65.7c	71.7b	23.6c	22.3c	49.7a	56.3a	40.1c	40.2a
	3000 ppm	74.0b	80.0b	22.9c	21.2d	44.9a	52.7a	39.0cd	39.1a
	4000 ppm	89.3a	94.7a	21.4d	20.9d	39.4a	45.0a	37.6d	38.2a

Means within each column and factor followed by the same letter are not significantly different at *P*<5% [27].
*P. Eva. : Pan Evaporation

Also, the current study concluded that increasing salinity levels generally reduced vegetative growth and decreased essential nutrient elements, protein and vitamin C compound contents in addition to chlorophyll content. Whilst in the opposite direction, Na and proline contents were increased. These obtained results are in good accordance with those reported by [17, 19, 31]. High saline levels in irrigation water have adverse impacts, which affect water and nutrient uptake leading to decreasing assimilation rate and dry matter accumulation, and accordingly reducing plant growth and essential nutrients. The adverse effect of increasing salinity on plant growth was possibly mainly due to the low osmotic potential of the nutrient solution in the root zone [19]. Also, an osmotic shock in the root may have occurred directly after exposure to the highest salinity level (4000 ppm). The root hair zone are most important for nutrient and water uptake. High salt concentrations resulting in low water potentials may reduce root elongation [33]. The decrease in water uptake is strongly correlated to the concentration of salinity [33]. At high salinity level, toxicity reactions may contribute to growth depression [34]. This assumption is supported by more pronounced reduction in plant growth obtained in this study under the highest salinity level (4000 ppm). The decrease of essential nutrients, i.e. K, Ca, Fe and Zn in Moringa plants were most likely due to the antagonism between Na and K or Ca at the sites of uptake in the roots and the effect of Na on the K and Ca transport in the xylem [35]. In addition, the competitive uptake of Na against essential nutrients K and Ca, may also have contributed to limited vegetative growth, causing drops in Moringa plant productivity due to osmotic, toxic and nutritional damage to the crop. This common effect deriving from uptake competition between Na element and essential nutrients at the roots was also observed by previous researchers [17, 19, 31]. Irrigation has proven to be one of the most important factors in crop management when using saline water for irrigation [36]. Saline water requires more frequent irrigation than for fresh water because salts in the water and the soil increase the osmotic potential of the soil water, which makes water uptake by the plant roots more difficult [37]. Therefore, when saline waters are used for irrigation, much attention should be given to minimize root-zone salinity as has been shown and verified in this research study and was proven earlier too [28, 29, 38].

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5. Conflicts of interest

There are no conflicts to declare.

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