

Effect of nanoparticle on growth, biochemical and anatomical characteristics of moringa plant (*Moringa oleifera* L.) under salinity stress condition

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Abstract

Two pot experiments were carried out at the Experimental Farm of the Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University (Assiut branch), Assiut, Egypt, during two successive seasons of 2018 and 2019. The main aim of this study was to find out the effect of both of foliar application with some growth promoters (using nanotechnology) *i.e.*, zinc, iron, copper oxide nanoparticles at 50, 100 and 150 mg l⁻¹ of each and silica nanoparticles at 20, 40 and 60 mg l⁻¹ as well as salinity stress level of irrigation water at 9000 mg l⁻¹ individually and their interactions on growth, biochemical and anatomical characteristics of *Moringa oleifera* L. plant. The results showed that individually salinity stress level of irrigation water at 9000 mg l⁻¹ decreased all studied vegetative growth parameters of moringa plant *i.e.*, plant height (cm), stem diameter (cm), leaves number plant⁻¹, leaf area plant⁻¹ as well as root, stem and leaves dry weights plant⁻¹ compared with the control. The same trend was obtained in photosynthetic pigments content (total chlorophyll) as well as the studied anatomical characteristics of root section (*i.e.*, root diameter of V.C and length of xylem arch), stem section (*i.e.*, stem diameter, Ø of V.C, length of V.B in the stem) and Leaf blade section (*i.e.*, upper epidermis thickness, lower epidermis thickness and mesophyll tissue for leaflet) at 50 days of *Moringa oleifera* leaves compared with unstressed plant. On other hand, salinity stress level at 9000 mg l⁻¹ resulted in increasing antioxidant activity as well as total phenolic contents at 60 days of *Moringa oleifera* leaves compared with unstressed plant. Data also indicated that the individually foliar application treatments with Zinc, Iron, Copper oxide nanoparticles at 50, 100, 150 of each and Silica at 20, 40, 60 mg l⁻¹ nanoparticles increased studied vegetative growth as well as anatomical characteristics, chemical compositions, photosynthetic pigments (total chlorophyll), antioxidants activity and total phenolic contents of moringa plant compared with the control treatment. In this respect, Silica nanoparticles at 60 mg l⁻¹ followed by zinc and iron oxide at 150 mg l⁻¹ nanoparticles of each were the most effective treatments, respectively. Finally, Based on these results, it could be concluded that the foliar application with nanoparticles gave the highest Valois of morphological, chemical compositions and anatomical characteristics its that reduce the harmful effects of salinity stress of *Moringa oleifera* L. plant specially at silica 60 mg l⁻¹,. Data recorded that this results using it under the same field conditions (Assiut governorate, Egypt).

Keywords: *Moringa oleifera*, nanoparticles, salinity, growth, bioconstituents, anatomy.

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1. Introduction

Moringa (Moringa oleifera L.) is the most widely cultivated species of Moringace family due to its easy propagation, fast growth and its numerous economic uses. *Moringa oleifera L.* in the botanical scientific taxonomy belong to Kingdom: Plantae, Division: Magnoliophyta, Class: agnoliopsida, Order: Brassicales, Family: Moringaceae, Genus: *Moringa*, Species: *M.oleifera* (Fahey, 2005). *Moringa oleifera L.* is one of the most useful tropical trees, its leaves are extremely valuable source of nutrition for people of all ages. Nutritional analysis indicates that moringa leaves contain affluence of essential disease preventing nutrients. They even contain all of the essential amino acids which are unusual for a plant source. The young leaves are edible and are commonly cooked and eaten like spinach or used for making soups and salads. It is an exceptionally good source of antioxidant compounds such as flavonoids, ascorbic acid, carotenoids phenolics and some mineral nutrients (in particularly iron) and the sulphur-containing amino acids methionine as well as cystine. The composition of amino acids in the leaf protein is well balanced; they contain high amounts of many of these nutrients and total phenols also a very low source of fat (Abdull Razis *et al.*, 2014; Mishra *et al.*, 2012; Osman and Abohassan, 2012). Soil salinity threshold levels depend on a crop species, variety, developmental stage and environmental factors. One of the most important a biotic stress factors is soil salinity. It causes great effects on growth development, yield of crops (Chaparzadeh

et al., 2004) and causes great losses in crops yield (Smirnoff, 1998). Seed germination also affected by the excessive content of salt in soil solution, particularly in case of sensitive plants. Regarding, the interaction between plants and salinity stress, there are several mechanisms: (1) selective ions accumulation/exclusion (2) control of ion uptake by roots and their transport into leaves (3) prevention of Na⁺ and Cl⁻ accumulation in the cytoplasm (4) synthesis and accumulation of nontoxic (compatible) osmolytes in the cytosol (5) change in photosynthetic pathway (6) induction of antioxidative system (7) stimulation of phytohormones production, such as abscisic and jasmonic acids. All these mechanisms are realized at the levels of whole plant and plant tissues as well as the cell molecular level (Dajic, 2006). The effect of salinity on root (An *et al.*, 2003) and leaf anatomy (Hu and Schmidhalter, 2001; Kiliç *et al.*, 2007) of plants had already been reported in previous works. Many researchers reported that with an increasing salinity there was a decreasing in xylem tissue development. Pimmongkol *et al.* (2002) stated that width and diameter of the vascular bundles of rice stem decreased in NaCl medium. Junghans *et al.* (2006) showed that high salt concentrations reduced the cambial activity in *Populus euphratica*. Salinity causes total leaf area reducing (Awang *et al.*, 1993) as well as leaf thickness increasing (Raafat *et al.*, 1991). It is also reducing the vascular tissues development (Belda and Ho, 1993). It increases trichome density and decreases or has no effect on stomatal density (Ludders and Kaminski, 1991). Salinity affects ion accumulation in

leaves, thereby membrane permeability and chlorophyll synthesis. Micronutrients are playing important roles in crop yield increasing. Micronutrients have prominent effects on dry matter accumulation and yield of plants (Asad and Rafique, 2000). As for Iron, it plays roles in biological redox system, enzyme activation and oxygen carrier in nitrogen fixation (Romheld and Marschner, 1991). Zinc is important to membrane integrity and phytochrome activities (Shkoinik, 1984). Also Zinc plays an important role in the plant production, and chlorophyll synthesis (Kaya and Higgs, 2002). It can reduce the negative effects of ROS in environmental stresses and its deficiency decreases plant resistance (Cakmak and Marschner, 1988; McCauley *et al.*, 2009). Cu is one of the most essential elements in chlorophyll synthesis, enhance plant photosynthesis and carbohydrate metabolism, help to regulate water movement through plant and play vital role in seed production, in addition to it is vital for physiological redox processes, pollen viability and lignification as well as increasing yield components (Marschner, 1995). Therefore, much attention is needed for adequate and balanced use of micronutrients along with macronutrients to enhance the response to organic fertilizers (Baddaruddin *et al.*, 1999). Silicon (Si) is an environmental friendly and ecologically compatible for stimulating plant growth. It was reported that silicon plays a role in reducing the hazard effects of several a biotic and biotic stresses such as drought stress (Etesamy and Jeong, 2018; Laing and Adandonon, 2005). It has emerged as an important mineral for many horticultural

crops (Ma, 2004). It is contributing elasticity of the cell wall during extension growth. It is interacting with cell constituents as polyphenols and pectins and this increasing elasticity of the cell wall. Also, increasing of silicon absorption led for maintaining erect leaves and important for leaf angle to photosynthesis (Emadian and Newton, 1989). Foliar spray with silicon significantly increased yield and its components of pea plant (Gharib and Hanafy, 2005). Foliar application with potassium silicate (KSiO_3) increased growth of sunflower plant (Kamenidou and Cavins, 2008). Sayed *et al.* (2018) found that globe artichoke plant sprayed with silicon at 2000 mg l^{-1} recorded the highest increasing in all studied characteristics *i.e.*, growth aspects, chlorophylls content, nitrogen, phosphorus, potassium, total sugars and total amino acids concentrations as well as the yield parameters compared with untreated plant. Remero Aranda *et al.* (2006) reported that, Si is improving the storage of water within plant tissues, which allows a higher rate of growth. Abd El-Aal *et al.* (2019) confirmed that spraying taro plant grown under water stress levels with potassium silicate at 2500 mg l^{-1} improved plant tolerability to the harmful effects of water stress. Hence, the present study was conducted to evaluate the effects of different salinity level of irrigation water and foliar spray with some stimulant nutrients nanoparticle *i.e.*, zinc, iron, copper and silica treatments individually and the combination between the foliar application treatments within the salinity level on vegetative growth parameters,

chemical compositions and anatomical characteristics of moringa plant for studying the possibility for improving plant tolerability to the harmful effects of salinity stress conditions.

2. Materials and methods

Two pot experiments were carried out at the experimental farm of the agricultural botany department, faculty of agriculture, Al-Azhar university, Assiut branch, Egypt during the two successive seasons of 2018 and 2019 to investigate the effect of foliar spray with some nanoparticles and salinity level of irrigation water individually as well as the combination between the foliar application treatments within salinity level 9000 mg^l⁻¹ on vegetative growth characteristics, chemical constituents at 60 days and anatomical characteristics of *Moringa oleifera* plant at 50 days. Seeds of moringa (*Moringa oleifera* L.) were obtained from National Research Center, Dokki, Giza, Egypt. While, zinc, iron, copper oxide and Silica nanoparticles were obtained from Nano technology Company (3 Ibn-Eyas. St., Roxy, Heliopolis, Cairo). Moringa seeds were sowed at 1th March for 2018 and 2019 seasons in plastic pots of 30 cm in diameter which filled with 6 kg with a mixture of 1 clay: 1 sand :1 peat moss (v:v:v), then seeds were sowed (2-3 seeds / pot). After emergences of seedlings, were thinned to one plant per pot, this experiment included 26 treatments, each treatment was about 3

replications (4 pots for each replicate). The pots were arranged in split plot design. Cultivation and all cultural practices except irrigation *i.e.*, weeding, fertilization and pest control and so on were performed according to the recommendations as usual. Foliar application with nanoparticle treatments were carried out four times, first at 30 days and repeated every 15 days as foliar spray to cover completely the plant foliage.

2.1 The experiment treatments

This experiment included 26 treatments, which were the foliar application with some nanoparticle and salinity level individually treatments as well as their interactions.

2.1.1 Salinity level of irrigation water

NaCl was used to apply the level of salinity stress, salinity level of irrigation water were used in addition to the control (tap water) *i.e.*, 9000 mg^l⁻¹ as well as the control of water requirements of moringa plants in both seasons. All the experimental units were received equal amounts of water until complete germination at 30 days, then applying of water irrigation salinity treatment were started in both seasons.

2.1.2 Application of nanoparticle treatments

Some nutrients nanoparticle individually

treatments i.e., zinc, iron, copper oxide nanoparticles at 50, 100 and 150 mg^l⁻¹ of each and silica nanoparticles at 20, 40 and 60 mg^l⁻¹ in addition to the control (tap water) were used as foliar applications. Plants were sprayed with the foliar application treatments four times after 30, 45, 60 and 75 days.

2.2 Sampling and collecting data

2.2.1 Growth characteristics

Different morphological characteristics of moringa plant were measured and calculated at 60 days. Three plants from each treatment were randomly taken and then separated into their organs and the following characteristics were recorded: plant height (cm), stem diameter (cm), leaves number plant⁻¹, leaf area plant⁻¹, root as well as stem and leaves dry weight plant⁻¹. The samples were dried in the oven-dried for 48 h in 75°C to a constant weight and then the dry weight per plant was calculated. These dry samples were kept in for chemical analysis.

2.2.2 Physiological characteristics

Chemical analyses were carried out in the leaves sample at 60 days during both seasons of 2018 and 2019. Total chlorophyll was calorimetrically determined in the fresh leaves according to the method described by Sadasivam and Manickam (1997). The antioxidants activity was determined in dry leaves

according to the method by Saint-Cricq De Gaulejae *et al.* (1999) modified by Lu *et al.* (2007). Total phenolic compounds content was determined according to Folin-Ciocalteu spectrophotometric method (Lu *et al.*, 2007).

2.2.3 Anatomical study

Specimens of moringa (*Moringa oleifera* L.) root, stem and terminal leaflet were collected in the second season only. The root samples were taken 0.5 cm from the root tip and the 5th apical internode of the main stem and its corresponding leaf of treated plants with salinity level i.e., 9000 mg^l⁻¹ and applied nanoparticle i.e., zinc, iron, copper at 150 of each mg^l⁻¹ as well as silica at 60 mg^l⁻¹ and their interaction in addition to those of the control at 50 days. The specimens were taken then killed and fixed in FAA (5 ml. formalin, 15 ml. glacial acetic acid and 90ml. ethyl alcohol 70%, distilled water 35 ml), washed in 50% ethyl alcohol, dehydrated in series of ethyl alcohols 70, 80,90,95 and 100%, infiltrated in xylene, embedded in paraffin wax with a melting point of 40-45°C , sectioned on a rotary microtome at a thickness of 5-7 µm., stained with the double method (light green and safranin), cleared in xylene and mounted in Canada balsam (Johanson, 1940) .Sections were read to detect histological manifestation of noticeable responses resulted from treatments. The prepared sections were microscopically examined; counts and measurements (µ) were taken by

computerized morphometrical analysis, the morphometrical analysis was done by research microscope type Axiostar plus made by Zeiss transmitted light bright field examinations upgrade able to professional digital image analysis system (Carl Zeiss Axiovision Product Suite DVD 30).

2.2.4 Statistical analysis

Statistical analysis conducted using Costat computer statistical software package, Data were statistically analyzed according to the analysis of variance (ANOVA) of the completely randomized design, applied in both laboratory and greenhouse experiments according to Gomez and Gomez (1984). Least Significant Difference (LSD) test at 5% was used to determine genotypic differences among all means of pathological, morphological and anatomical traits under each treatment.

3. Results and Discussion

3.1 Vegetative growth characteristics

Data presented in Table (1a, b) show the effect of the individually treatment of irrigation water salinity level *i.e.*, 9000 mg l^{-1} and applied nutrients nanoparticle *i.e.*, zinc, iron, copper and silica as well as the effect of the applied nanoparticles on the vegetative growth characteristics *i.e.*, plant height (cm), stem diameter (cm), leaves number plant $^{-1}$, leaf area plant $^{-1}$, root, stem and leaves dry weight

plant $^{-1}$ of moringa plant grown under salinity stress level 9000 mg l^{-1} at 60 days during the two growing seasons of 2018 and 2019.

3.1.1 Effect of salinity stress

All pot experiments under this study indicated that salinity level of irrigation water at 9000 mg l^{-1} have decreased all studied vegetative growth characteristics of moringa plant. The same data also cleared that the level of salinity *i.e.*, 9000 mg l^{-1} was the most effective treatment in this respect when compared with unstressed plant (the control) at 60 days during the two experimental seasons. Plants growing under saline conditions are stressed basically in three ways; (1) reduced water potential in the root zone causing water deficit, (2) phytotoxicity of ions such as Na $^{+}$ and Cl $^{-}$, and (3) nutrients imbalance by uptake depression and shoot transport (Marschner, 1995). Salinity became a basic problem when sufficient salts accumulate in the root zone to negatively affect plant growth. Excess salts in the root zone prevent plant roots from withdrawing water from the surrounding soil. This lowers the amount of water available to the plant, regardless of the amount of water actually in the root zone (Abdelhamid *et al.*, 2010). The effect of salinity on plant growth is related to the stage of plant development at which salinity is imposed (Ayres and Westcot, 1985). The reduction in most vegetative growth parameters may be caused by the

reduction in the cell size which might be attributed to changes in osmotic cell enlargement dependent on solute accumulation (Asin *et al.*, 2007) or due to drastic changes in ion relationship (Grossmann *et al.*, 1994). High salinity causes both hyper-ionic and hyper-osmotic stress and can lead to plant demise (Wilson *et al.*, 2006). Moreover, salt treatment affects differently early growth stages of plants and has both osmotic and specific ion effects on plant growth (Dionisio-Sese and Tobita, 2000). Moreover, the reduction in growth is generally the consequences of several physiological responses including modification of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency and carbon allocation and utilization (Greenway and Munns, 1980). In salt-sensitive plant,

shoot and root growth is permanently reduced within hours of salt stress and this does not appear to depend on Na⁺ concentrations in the growing tissues, but rather is a response to the osmolality of the external solution (Munns, 2003). The decrease in growth due to salinity may be attributed to an increase in respiration rate resulting from higher energy requirements (Sakr *et al.*, 2013). These results may attributed to the effect of salinity stress on the water content of the leaves, as suggested by Hu *et al.* (2007). High level of salinity negatively affected shoot dry weight. Salinity can damage the plant through its osmotic effect, which is equivalent to a decrease in water activity through specific toxic effects of ions and by disturbing the uptake of essential nutrients (Dorais *et al.*, 2001; Gomaa *et al.*, 2008).

Table (1a): Effect of salinity stress level, applied nanoparticle and their interaction treatments on moringa growth parameters at 60 days during 2018 and 2019 seasons.

Treatments	Con. mg l ⁻¹	2018							2019						
		Plant height (cm)	Stem diameter (cm)	Leaves number plant ⁻¹	Leaf area Cm ² plant ⁻¹	Root dry weight g plant ⁻¹	Stem dry weight g plant ⁻¹	Leaves dry weight g plant ⁻¹	Plant height (cm)	Stem diameter (cm)	Leaves number plant ⁻¹	Leaf area cm ² plant ⁻¹	Root dry weight g plant ⁻¹	Stem dry weight g plant ⁻¹	Leaves dry weight g plant ⁻¹
Effect of salinity															
Tap water	-	47.89	2.00	30.33	168.12	2.06	2.35	3.52	50.97	2.20	32.28	173.31	2.48	2.77	4.03
Salinity	9000	30.51	1.18	16.82	103.58	1.07	1.35	2.09	32.36	1.38	18.82	108.82	1.49	1.77	2.49
L.S.D	0.05	1.33	0.26	1.02	3.22	0.24	0.27	1.00	2.44	0.28	2.33	1.99	0.33	0.29	0.23
Effect of applied nanoparticles															
Control	-	26.67	1.00	14.50	91.69	1.06	1.23	1.80	30.17	1.20	16.50	97.49	1.38	1.63	2.20
Zn	50	37.83	1.45	22.83	125.43	1.41	1.69	2.57	40.33	1.65	24.83	130.66	1.85	2.17	3.04
	100	41.83	1.62	26.33	144.77	1.63	1.93	2.93	44.33	1.87	28.33	149.96	2.06	2.40	3.39
	150	46.33	1.82	29.83	162.17	1.89	2.17	3.30	48.83	2.02	31.83	167.28	2.27	2.64	3.75
Fe	50	36.33	1.52	21.33	120.97	1.36	1.63	2.49	38.33	1.72	23.33	126.23	1.81	2.09	2.93
	100	40.33	1.73	24.33	140.16	1.57	1.87	2.84	42.33	1.93	26.33	145.34	2.00	2.34	3.30
	150	44.83	1.93	27.83	157.72	1.80	2.11	3.20	46.83	2.13	29.83	163.06	2.22	2.58	3.64
Cu	50	34.83	1.37	19.83	116.64	1.31	1.57	2.37	36.83	1.57	21.83	121.81	1.75	2.03	2.82
	100	38.83	1.55	22.83	135.65	1.52	1.81	2.77	40.83	1.75	24.50	141.01	1.95	2.28	3.23
	150	43.33	1.77	26.33	153.19	1.73	2.05	3.11	45.33	1.97	28.33	158.34	2.16	2.53	3.56
Si	20	35.33	1.43	20.33	119.98	1.46	1.75	2.67	38.33	1.63	22.33	124.98	1.90	2.24	3.13
	40	39.33	1.65	23.33	141.13	1.69	1.99	3.02	42.33	1.85	25.33	146.13	2.12	2.48	3.48
	60	43.83	1.87	26.83	156.57	2.00	2.23	3.42	46.83	2.07	28.83	161.57	2.33	2.72	3.90
L.S.D 0.05		0.88	0.08	0.57	2.02	0.05	0.06	0.07	0.99	0.14	0.27	1.34	0.19	0.08	0.12

Table (1b): Effect of salinity stress level, applied nanoparticle and their interaction treatments on moringa growth parameters at 60 days during 2018 and 2019 seasons.

Treatments		Con. mg l ⁻¹	2018							2019						
			Plant height (cm)	Stem diameter (cm)	Leaves number plant ⁻¹	Leaf area Cm ² plant ⁻¹	Root dry weight g plant ⁻¹	Stem dry weight g plant ⁻¹	Leaves dry weight g plant ⁻¹	Plant height (cm)	Stem diameter (cm)	Leaves number plant ⁻¹	Leaf area cm ² plant ⁻¹	Root dry weight g plant ⁻¹	Stem dry weight g plant ⁻¹	Leaves dry weight g plant ⁻¹
Effect of applied nanoparticles																
Tap water	Control	-	38.67	1.60	24.33	137.20	1.52	1.82	2.78	42.67	1.80	26.33	142.30	1.90	2.29	3.23
		50	47.33	1.90	31.33	159.26	1.90	2.18	3.22	50.33	2.10	33.33	164.66	2.34	2.66	3.75
		100	51.33	2.07	34.33	177.33	2.12	2.42	3.62	54.33	2.27	36.33	182.59	2.56	2.89	4.14
		150	56.33	2.27	37.33	196.35	2.42	2.66	4.04	59.33	2.47	39.33	201.31	2.76	3.13	4.56
	Zn	50	45.33	1.93	29.33	154.30	1.84	2.12	3.12	48.33	2.13	31.33	159.77	2.29	2.58	3.63
		100	49.33	2.13	31.33	173.13	2.06	2.36	3.52	52.33	2.33	33.33	178.67	2.50	2.83	4.03
		150	54.33	2.33	34.33	191.44	2.30	2.60	3.93	57.33	2.53	36.33	196.69	2.71	3.07	4.44
	Fe	50	43.33	1.77	27.33	149.61	1.78	2.06	3.00	46.33	1.97	29.33	154.76	2.22	2.51	3.51
		100	47.33	1.97	29.33	169.45	2.02	2.30	3.44	50.33	2.17	30.67	174.60	2.45	2.77	3.95
		150	52.33	2.20	32.33	186.51	2.22	2.54	3.82	55.33	2.40	34.33	191.66	2.65	3.02	4.33
	Cu	20	41.33	1.73	25.33	144.33	1.96	2.24	3.33	44.33	1.93	27.33	149.33	2.40	2.51	3.84
		40	45.33	1.93	27.33	165.33	2.18	2.48	3.73	48.33	2.13	29.33	170.33	2.62	2.77	4.24
		60	50.33	2.17	30.33	181.36	2.57	2.72	4.16	53.33	2.37	32.33	186.36	2.82	3.02	4.70
	Si	-	14.67	0.40	4.67	46.19	0.602	0.642	0.822	17.67	0.60	6.67	52.69	0.863	0.973	1.17
		50	28.33	1.00	14.33	91.59	0.922	1.20	1.92	30.33	1.20	16.33	96.66	1.36	1.67	2.32
100		32.33	1.17	18.33	112.22	1.14	1.44	2.24	34.33	1.37	20.33	117.33	1.55	1.91	2.63	
Salinity 9000 mg l ⁻¹	Zn	150	36.33	1.37	22.33	127.98	1.36	1.68	2.55	38.33	1.57	24.33	133.24	1.78	2.15	2.93
		50	27.33	1.10	13.33	87.64	0.88	1.14	1.85	28.33	1.30	15.33	92.68	1.32	1.60	2.22
		100	31.33	1.33	17.33	107.19	1.08	1.38	2.16	32.33	1.53	19.33	112.01	1.49	1.86	2.57
	Fe	150	35.33	1.53	21.33	124.00	1.30	1.62	2.46	36.33	1.73	23.33	129.42	1.72	2.09	2.84
		50	26.33	0.967	12.33	83.67	0.84	1.08	1.74	27.33	1.17	14.33	88.86	1.28	1.55	2.12
		100	30.33	1.13	16.33	101.86	1.02	1.32	2.09	31.33	1.33	18.33	107.43	1.44	1.79	2.50
	Cu	150	34.33	1.33	20.33	119.86	1.24	1.56	2.39	35.33	1.53	22.33	125.01	1.66	2.04	2.79
		20	29.33	1.13	15.33	95.64	0.962	1.26	2.00	32.33	1.33	17.33	100.64	1.40	1.55	2.42
		40	33.33	1.37	19.33	116.92	1.20	1.50	2.31	36.33	1.57	21.33	121.92	1.62	1.79	2.71
	Si	60	37.33	1.57	23.33	131.78	1.42	1.74	2.67	40.33	1.77	25.33	136.78	1.84	2.04	3.09
		L.S.D 0.05		0.98	0.10	0.49	1.33	0.018	0.06	0.011	0.81	0.07	0.42	2.22	0.09	0.010

The ability of the plant response to saline stress can be hardly explained by the fact that salinity imposes both an ionic and osmotic stress, which causes reduction in roots growth and weights (Pasternak, 1987). More in these respect, salinity was found to reduce shoot and root weights as well as photosynthates reduction (Brugnoli and Lauteri, 1991). Moreover, the reduction in shoot and root dry weight accumulation might be attributed to a decrease in either leaf number and leaf area, decreasing in CO₂ uptake in leaves mainly because Na Cl treatment, decrease stomatal conductance and consequently less CO₂ is available for carboxylation reaction in the

photosynthesis apparatus (Sakr *et al.*, 2013).

3.1.2 Effect of applied nanoparticles treatments

In both seasons of study, all foliar application with nanoparticle treatments increased root, stem and leaves dry weight as well as total leaf area when compared to the untreated plant (the control) at 60 days. In this respect, the solely treatments of zinc, iron, copper oxide at 150 mg l⁻¹ of each as well as silica nanoparticles at 60 mg l⁻¹ recorded the highest values of vegetative growth parameters of moringa plant. Moreover,

obtained data cleared that foliar spraying treatments have important roles in alleviating and mitigating the harmful effects of salinity stress when compared with the control. Our results go on line with those reported that application of zinc and iron resulted in useful changes in growth attributes. The possible cause for such positive role is enhancing in the activity of bio-substances or activity of photosynthetic system (Mekkdad, 2017; Quary *et al.*, 2006) or might be due to the active role of these trace-elements in metabolic processes of plants and photosynthesis and thus, reflected to increase the growth attributes. Abou El-Nasr *et al.* (2015) found that foliar sprays with iron nanoparticles increased stem diameter, leaf area and dry weights. Prasad *et al.* (2012) found that zinc oxide nanoparticles increased plant growth parameters. Sedghi *et al.* (2013) found that zinc oxide nanoparticles increased plant growth and development. Therefore, it could be concluded that increasing leaf area as well as increment of dry matter accumulation Table (1a, b) as well as photosynthetic pigments Table (2) in leaves of moringa plants reverse the stimulating effect of these treatments on the photosynthetic efficiency process, thereby more photosynthates being created as well as enhancing minerals translocation from root to leaves.

3.1.3 Effect of the interactions (salinity x nanoparticles)

With regarding to the interaction

treatments *i.e.*, the effect of foliar application treatments with nanoparticles on vegetative growth characteristics of moringa plant grown under the level of used salinity stress *i.e.*, 9000 mg l^{-1} , the data indicated that the individually foliar spray treatments of zinc, iron, copper at 150 mg l^{-1} of each and silica nanoparticles at 60 mg l^{-1} significantly enhanced growth attributes of moringa plant under salinity stress level at 9000 mg l^{-1} which were the most effective treatments when compared with other treatments and the control during the two growing seasons. In this regard, the foliar application treatments with nanoparticles basically mitigate the harmful effect of salinity stress on growth attributes. Silica at 60 mg l^{-1} followed by zinc, iron and copper oxide nanoparticles at 150 mg l^{-1} of each respectively, were the most effective in this respect.

3.2 Bioconstituents content

3.2.1 Photosynthetic pigments content

Data presented in Table (2) show the effect of irrigation water salinity level at 9000 mg l^{-1} and applied nutrients nanoparticle *i.e.*, zinc, iron, copper and silica in addition to the effect of the applied nanoparticle treatments on photosynthetic pigments content (total chlorophyll content mg g $^{-1}$ f.wt.) in leaves of moringa plant grown under salinity stress level at 9000 mg l^{-1} at 60 days during the two growing seasons of 2018 and 2019.

Table (2): Effect of salinity stress level, applied nanoparticle and interaction treatments on some bioconstituents of moringa leaves at 60 days during 2018 and 2019 seasons.

Treatments	Con. mg ^l ⁻¹	2018			2019			
		Total chl. Mg g ⁻¹ f.wt	Antioxidants activity Mg g ⁻¹ d.wt.	Total phenolic mg g ⁻¹ d.wt.	Total chl. Mg g ⁻¹ f.wt	Antioxidants activity Mg g ⁻¹ d.wt.	Total phenolic mg g ⁻¹ d.wt.	
Effect of salinity								
Tap water	-	2.145	67.93	19.63	1.986	69.04	20.75	
Salinity	9000	1.337	60.43	17.01	1.360	61.49	18.11	
L.S.D 0.05		0.304	1.11	1.05	0.313	1.22	1.04	
Effect of applied nanoparticles								
Control	-	1.284	60.26	13.45	1.309	61.11	14.57	
Zn	50	1.512	62.26	17.70	1.535	63.36	18.80	
	100	1.773	64.26	18.63	1.796	65.36	19.73	
	150	2.048	66.26	19.51	2.074	67.36	20.61	
Fe	50	1.577	63.01	18.06	1.602	64.11	19.16	
	100	1.840	65.01	18.98	1.862	66.11	20.08	
	150	2.108	67.01	19.83	2.132	68.11	20.93	
Cu	50	1.467	62.51	17.83	1.490	63.61	18.93	
	100	1.679	64.51	18.70	1.731	65.61	19.80	
	150	1.977	66.51	19.66	1.997	67.61	20.76	
Si	20	1.550	62.26	17.68	1.574	63.36	18.78	
	40	1.781	64.27	18.69	1.804	65.37	19.86	
	60	2.034	66.26	19.51	2.057	67.36	20.61	
L.S.D 0.05		0.007	0.88	0.62	0.022	0.45	0.33	
Effect of interactions								
Tap water	Control	-	1.831	64.01	11.88	1.857	65.21	13.02
	Zn	50	1.960	66.00	19.25	1.982	67.10	20.35
		100	2.203	68.02	20.20	2.226	69.12	21.30
		150	2.455	70.00	21.00	2.479	71.10	22.10
	Fe	50	2.010	66.50	19.51	2.038	67.60	20.61
		100	2.257	68.52	20.40	2.279	69.62	21.50
		150	2.514	70.50	21.25	2.537	71.60	22.35
	Cu	50	1.928	67.00	19.75	1.950	68.10	20.85
		100	2.068	69.02	20.60	2.149	70.12	21.70
		150	2.400	71.00	21.50	2.419	72.10	22.60
	Si	20	1.876	65.51	19.00	1.899	66.61	20.10
		40	2.068	67.52	20.08	2.091	68.62	21.31
60		2.313	69.50	20.80	2.336	70.60	21.90	
Salinity 9000 mg ^l ⁻¹	Control	-	0.737	56.51	15.01	0.762	57.02	16.11
	Zn	50	1.065	58.51	16.15	1.088	59.61	17.25
		100	1.343	60.50	17.05	1.367	61.60	18.15
		150	1.640	62.52	18.01	1.668	63.62	19.11
	Fe	50	1.144	59.51	16.60	1.166	60.61	17.70
		100	1.423	61.50	17.55	1.446	62.60	18.65
		150	1.702	63.52	18.40	1.726	64.62	19.50
	Cu	50	1.007	58.01	15.91	1.030	59.11	17.01
		100	1.290	60.01	16.80	1.314	61.11	17.90
		150	1.553	62.02	17.81	1.575	63.12	18.91
	Si	20	1.225	59.01	16.35	1.248	60.11	17.45
		40	1.495	61.02	17.30	1.517	62.12	18.40
60		1.755	63.01	18.21	1.778	64.11	19.31	
L.S.D 0.05		0.022	0.53	0.15	0.010	0.48	0.09	

3.2.1.1 Effect of salinity stress

Regarding to the effect of irrigation water salinity level, obtained data clearly

indicate that stressed plants grown under salinity stress level recorded lowest values of total chlorophyll content when compared with the control. In other

words, salinity level of irrigation water *i.e.*, 9000 mgL⁻¹ decreased total chlorophyll content of moringa leaves compared with the control. In this respect, salinity level at 9000 mgL⁻¹ treatment recorded the highest reduction in the content of total chlorophyll. Increasing of sodium concentration in plant tissues could be resulted in increasing the oxidative stress, which causes deterioration in chloroplast structure and an associate losing in chlorophyll, this leads to a decrease in chlorophyll content, while carotenoids increased (Khosravinejad and Faboondia, 2008). Increasing salinity under stress conditions significantly reducing photosynthesis by closure of the stomata, in which decreasing carbon dioxide supply and then growth is reducing (Jonas *et al.*, 1992). During water stress produced by salt stress, production of reactive oxygen species (ROS) and reduction of chloroplast stromal volume are also thought to play an essential role in inhibiting and limiting photosynthesis (Price and Hendry, 1991). ROS causes chlorophyll deterioration and membrane lipid peroxidation. So, accumulation of lipid peroxidation and chlorophyll retention are two oxidative stress indicators (Yildirim *et al.*, 2008).

3.2.1.2 Effect of applied nanoparticle treatments

Concerning the effect of stimulants foliar spray, the same data in Table (2) also, clear that all applied nanoparticle

treatments recorded significant increases of photosynthetic pigments compared with untreated plant. In this regard, iron followed by zinc, copper oxide at 150 mgL⁻¹ of each and silica nanoparticles at 60 mgL⁻¹ recorded the highest values compared with the other treatments and control.

3.2.1.3 Effect of the interaction

As for the effect of interaction, data clearly showed that the foliar application nanoparticle treatments led for enhancing the photosynthetic pigments content of moringa leaves grown under salinity stress level at 9000 mgL⁻¹ compared with untreated plant. Applied nanoparticle treatments basically mitigate the harmful effect of salinity stress on photosynthetic pigments. Silica at 60 mgL⁻¹ followed by Iron, Zinc and Copper oxide nanoparticles at 150 mgL⁻¹ of each respectively, were the most effective treatments in this respect.

3.2.2 Antioxidants activity and total phenolic content

Data presented in Tables (2) indicate the effect of irrigation water salinity level at 9000 mgL⁻¹ and applied nutrients nanoparticle *i.e.*, zinc, iron, copper and silica furthermore the effect of applied nanoparticle treatments on antioxidants activity as well as total phenolic content (mg g⁻¹ D.wt.) in leaves of moringa plant grown under salinity stress level 9000 mgL⁻¹ at 60 days during the two growing

seasons of 2018 and 2019.

3.2.2.1 Effect of salinity stress

Regarding the effect of irrigation water salinity level, obtained results clearly show that stressed plants grown under salinity stress level at 9000 mgL⁻¹ recorded highest values of antioxidants activity and total phenolic content when compared with the control. Moreover, salinity level of irrigation water increased antioxidants activity and total phenolic content of moringa leaves compared with the control. Highest salt concentration normally impair the cellular electron transport within the different sub-cellular compartments and lead to the generation of reactive oxygen species (ROS) such as singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals. ROS led for enhancing antioxidants system defense by increasing the accumulation of non-enzymatic antioxidants such as phenolic compounds, vitamin C and tocopherol (Bellaire *et al.*, 2000; Mckersie *et al.*, 1996; Tanaka *et al.*, 1994).

3.2.2.2 Effect of applied nanoparticle treatments

Concerning the effect of stimulants foliar spray, the same data in Table (2) also, clear that all applied nanoparticle treatments recorded significant increases of antioxidants activity and total phenolic content compared with untreated plant. In this regard, copper followed by iron, zinc

oxide at 150 mgL⁻¹ of each and silica nanoparticles at 60 mgL⁻¹ recorded the highest values compared with the other treatments and control.

3.2.2.3 Effect of the interaction

With regarding to the effect of interaction, results clearly show that the foliar application nanoparticle treatments led for enhancing the antioxidants activity and total phenolic content of moringa leaves grown under salinity stress level at 9000 mgL⁻¹ compared with untreated plant. Applied nanoparticle treatments basically mitigate the harmful effect of salinity stress on antioxidants activity and total phenolic content. iron at 150 mgL⁻¹ followed by silica at 60 mgL⁻¹, zinc and copper oxide nanoparticles at 150 mgL⁻¹ of each respectively, were the most effective treatments in this respect. In general, increasing antioxidants activity and total phenolic content with different applied treatments considered as a direct result of increasing both photosynthesis rate and efficiency. Also, that was preceded with large photosynthetic area Table (1a, b) and high concentration of photosynthetic pigments (Table 2) under the application of foliar spray with some stimulant nutrients nanoparticle in moringa plant grown under salinity stress level 9000 mgL⁻¹.

3.2.3 Anatomical study

The aim of this study was to follow up

the internal structure characteristics of moringa (*Moringa oleifera* L.) root, stem and leaflet which exhibited the most noticeable response to salinity stress level at 9000 mg^l⁻¹ and nutrients nanoparticle foliar application (*i.e.*, zinc, iron and copper oxide nanoparticles at 150 mg^l⁻¹ of each as well as silica nanoparticles at 60 mg^l⁻¹) treatments compared with the control. Individually or in combinations on the mean counts and measurements in micron (μ) at 50 days as a step towards understanding the effect of salinity stress, foliar spray with nutrients nanoparticle and their interactions on moringa internal morphology characteristics based on transverse sections.

3.2.3.1 Effect of applied treatments on anatomical characteristics of moringa root

3.2.3.1.1 Response to Salinity stress

Obtained results in Table (3) as well as Figure (1) showed that salinity stress level treatment at 9000 mg^l⁻¹ decreased the most of moringa root anatomical features compared with the control. Obvious reductions were recorded in the thickness of many anatomical features with salinity stress at 9000 mg^l⁻¹. Among these anatomical features were the most important ones *i.e.*, root diameter, diameter of vascular cylinder and xylem arch length which were greatly decreased with salinity treatment compared with the

control. In other words, control of moringa plant manifested the best results as it exceeded that of salinity level at 9000 mg^l⁻¹ in terms of most studied anatomical features. In this respect, Neumann (1995) reported that salinity reduces root length and diameter anatomically, it affects cell division and expansion processes. Also, Reinhardt and Rost (1995) as some morphological parameters were negatively affected by salinity; some anatomical variables were diminished as well. Cortical parenchyma and vascular cylinder were reduced. Younis *et al.* (2014) found that salinity showed a subtractive effect on root anatomy. The statistical data indicate that with the increase in salinity level, there was a significant decrease in root xylem, phloem, cortex and epidermis Akram *et al.* (2002). Increasing salt levels in roots caused reduction in epidermal area due to cell injuries. Halophytic or salt tolerant species generally possess thick epidermis and this serves as an effective mechanism against water loss during limited moisture availability, but at high salinity levels thickness of epidermis decreased Botti *et al.* (1998) however, in the salt tolerant species, epidermis thickness greatly increased, which showed its better adaptability because thick epidermis is a specific character of the salt tolerant species (Awasthi and Pathak, 1999; Curtis and Lauchli, 1987; and Kanwal *et al.*, 2012; Nawaz *et al.*, 2006; 2012a,b).

Table (3): Effect of salinity stress level, applied nanoparticle and interaction treatments on mean counts and measurements of certain histological features of moringa root, stem and leaflet at 50 days during 2019 seasons.

Histological characteristics (μ) Treatments	Root			stem			leaflet				
	Ø of root	Ø of V.C.	Length of xylem arch	Ø of stem	Ø of V.C.	Length of V.B.	Upper epidermis thickness	Lower epidermis thickness	Spongy tissue thickness	Palisade tissue thickness	
Effect of salinity											
Tap water	1760.72	1313.08	534.74	1194.08	815.66	310.4	12.17	13.23	88.96	110.17	
Salinity 9000 mg ^l ⁻¹	1397.88	1001.92	373.72	1045.46	688.16	227.1	7.84	10.74	62.97	72.36	
L.S.D. at 5 %	44.629	14.621	97.405	75.953	37.486	55.32	3.9040	NS	18.77	3.5563	
Effect of applied nanoparticles											
Control	1397.2	975.5	378.15	1060.0	687.75	224.45	7.98	10.20	61.16	77.58	
Zn 150 mg ^l ⁻¹	1755.3	1351.1	490.2	1155.0	836.8	292.7	11.96	13.04	84.79	106.5	
Fe 150 mg ^l ⁻¹	1610.3	1213.4	481.7	1188.8	756.6	289.5	10.77	12.51	78.66	96.3	
Cu 150 mg ^l ⁻¹	1497.9	1079.3	431.5	1080.7	735.3	271.7	9.61	11.40	76.45	85.5	
Si 60 mg ^l ⁻¹	1635.9	1168.2	489.7	1114.4	743.2	265.5	9.71	12.77	78.79	90.4	
L.S.D. at 5 %	115.2	70.8	N.S.	53.3	38.5	N.S.	N.S.	N.S.	N.S.	6.4	
Effect of interactions											
Tap water	Control	1620.3	1183.5	467.3	1125.0	739.0	265.6	9.38	12.62	72.66	91.06
	Zn 150 mg ^l ⁻¹	2041.3	1591.7	582.7	1258.0	968.8	347.0	15.41	14.00	98.53	132.1
	Fe 150 mg ^l ⁻¹	1754.7	1332.7	566.0	1298.3	813.7	345.7	13.62	13.65	94.90	126.1
	Cu 150 mg ^l ⁻¹	1695.8	1250.8	532.7	1156.3	795.8	314.0	12.21	12.94	93.05	105.5
	Si 60 mg ^l ⁻¹	1691.5	1206.7	525.0	1132.8	761.0	279.7	10.25	12.94	85.67	96.1
Salinity 9000 mg ^l ⁻¹	Control	1174.0	767.5	289.0	995.00	636.5	183.3	6.58	7.78	49.66	64.11
	Zn 150 mg ^l ⁻¹	1469.3	1110.5	397.7	1052.0	704.8	238.3	8.51	12.09	71.04	80.9
	Fe 150 mg ^l ⁻¹	1465.8	1094.2	397.3	1079.3	699.5	233.3	7.93	11.37	62.41	66.4
	Cu 150 mg ^l ⁻¹	1300.0	907.7	330.3	1005.0	674.7	229.3	7.02	9.86	59.84	65.6
	Si 60 mg ^l ⁻¹	1580.3	1129.7	454.3	1096.0	725.3	251.3	9.16	12.60	71.91	84.8
L.S.D. 0.05%	142.06	106.63	128.24	64.540	75.758	43.729	4.3058	3.4602	22.81	8.7502	

Ø = .0.5 Diameter of root and stem, (X=12.5 = 1mm and Leaf= X=100 =200mm), V.C.= vascular cylinder and V.B.= vascular bundle.

3.2.3.1.2 Response to foliar application nanoparticle treatments

Same data in Table (3) and Figure (1) obviously indicate the effect of applied nutrients nanoparticle i.e., zinc, iron and copper oxide nanoparticles at 150 mg^l⁻¹ of each as well as silica nanoparticles at 60 mg^l⁻¹ individually treatments on different anatomical features of moringa root. In this respect, most of these applied treatments have positively impacts on the studied histological characteristics of *Moringa oleifera* L. root i.e., root diameter, diameter of vascular cylinder and xylem arch length

when compared with the control. Data also clearly indicate that foliar applications with nutrient nanoparticle of zinc, iron, copper oxide nanoparticles at 150 mg^l⁻¹ of each and silica nanoparticles at 60 mg^l⁻¹ recorded highly values of the root studied anatomical characteristics compared with the control treatment. In general, the stimulatory effects of applied treatments upon the anatomy features of treated plants could be attributed to the effect upon cambium activity. Increment of cambium activity could mainly attributed to the increase of endogenous hormones level especially cytokinins and auxins (Abd El-Aal and Eid, 2017;

Sotiropoulos et al., 2002).

3.2.3.1.3 Response to salinity stress and applied nanoparticle interaction

As shown in Table (3) as well as Figure

(2) The obtained data indicate the effect of different applied nutrient nanoparticle treatments upon alleviating the adverse effects of salinity stress on different studied anatomical features of *Moringa oleifera* L. root.

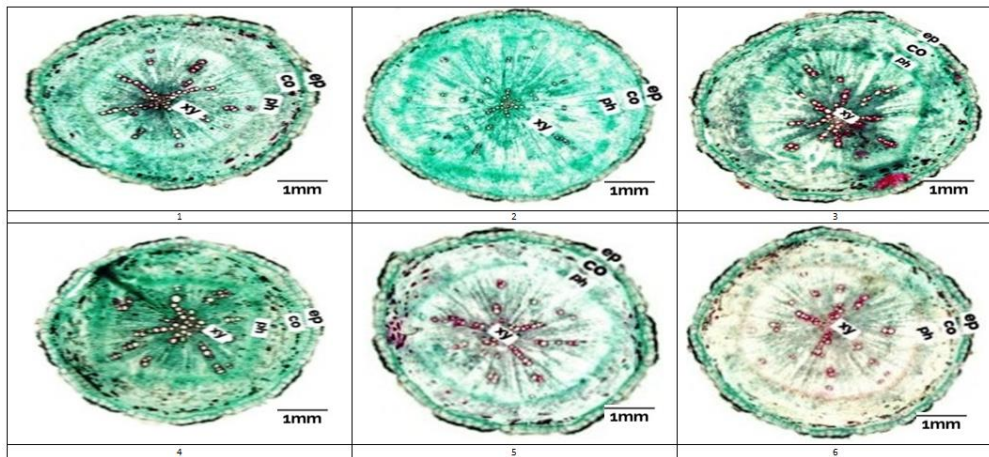


Figure (1): Transverse sections (X 12.5) of moringa root at 50 days as affected by different applied treatments. Where: (1): Control, (2): Salinity, 9000 mg^l⁻¹, (3): Zinc 150 mg^l⁻¹, (4): Iron 150 mg^l⁻¹, (5): Copper 150 mg^l⁻¹, (6): Silica 60 mg^l⁻¹, ep= epidermis, co= cortex tissue, ph= phloem tissue, xy= xylem tissue.

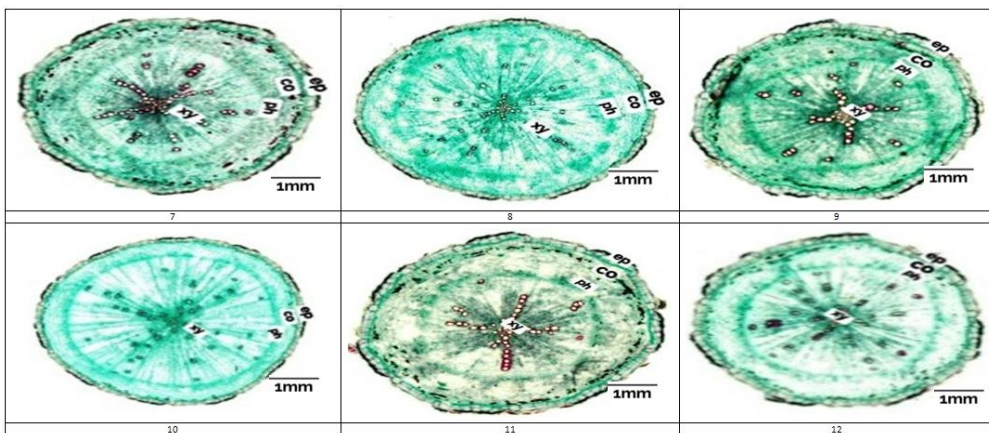


Figure (2): Transverse sections (X 12.5) of moringa root at 50 days as affected by different applied treatments. Where: (7): Control, (8): Salinity 9000 mg^l⁻¹, (9): Salinity with zinc 150 mg^l⁻¹, (10): Salinity with iron 150 mg^l⁻¹, (11): Salinity with copper 150 mg^l⁻¹, (12): Salinity with silica 60 mg^l⁻¹, ep= epidermis, co= cortex tissue, ph= phloem tissue, xy= xylem tissue.

In this respect, most of the applied treatments have a positively impact on most histological characteristics of moringa root *i.e.*, diameter of both of root and vascular cylinder as well as length of xylem arch under used salinity stress level at 9000 mg^l⁻¹ compared with the untreated plant. Also, obtained results clearly show that *Moringa oleifera* L. plant irrigated with salinity at 9000 mg^l⁻¹ in combination with Silica nanoparticles at 60 mg^l⁻¹ followed by zinc and iron oxide nanoparticles at 150 mg^l⁻¹ of each treatments appeared to be the most effective treatments upon alleviating the adverse effects of the highest salinity stress. Generally, the obtained results clearly indicate that different applied nutrient nanoparticle treatments play a defensive protective role against adverse effects of highest salinity stress level via its regulatory functions.

3.2.3.2 Effect of applied treatments on anatomical characteristics of moringa stem

3.2.3.2.1 Response to Salinity stress

As shown in Table (3) and Figure (3) Data indicate the effect of salinity stress at 9000 mg^l⁻¹ treatment compared with the control upon different anatomical features of moringa stem. In this respect, most of the applied treatments have a negatively impact on most studied histological characteristics of *Moringa oleifera* L. stem *i.e.*, stem diameter and Ø of V.C. as well as length of the V.B. at

50 days. The obtained results show that, the treatment of salinity stress decreased most of moringa stem anatomical features compared with control. Obvious reduction was recorded in the thickness of many anatomical features. Among these anatomical features were the most important ones, *i.e.*, stem diameter, Ø of V.C. and length of V.B. were greatly decreased with salinity stress level 9000 mg^l⁻¹ compared with control. In other words, moringa plant irrigated with tap water (control) manifested the best results as it exceeded that of salinity 9000 mg^l⁻¹ in terms of most studied anatomical features. Generally, stem anatomical features decreased with salinity at 9000 mg^l⁻¹ which recorded lower values in the studied anatomical characteristics when compared with control.

3.2.3.2.2 Response to foliar application nanoparticle treatments

Data in same Table (3) and Figure (3) obviously indicate the effect of applied nutrients nanoparticle individually treatments *i.e.*, zinc, iron and copper oxide nanoparticles at 150 mg^l⁻¹ of each as well as Silica nanoparticles at 60 mg^l⁻¹ on different anatomical features of moringa stem . In this respect, most of these applied treatments have a positively impact on studied histological characteristics of stem *i.e.*, stem diameter, Ø of V.C. and length of V.B. when compared with control. Also, the obtained results clearly show that

Moringa oleifera L. plants treated with zinc foliar spray followed by iron and copper oxide nanoparticles at 150 mg l⁻¹ of each appeared to be the most effective treatments compared with silica nanoparticles at 60 mg l⁻¹ and control.

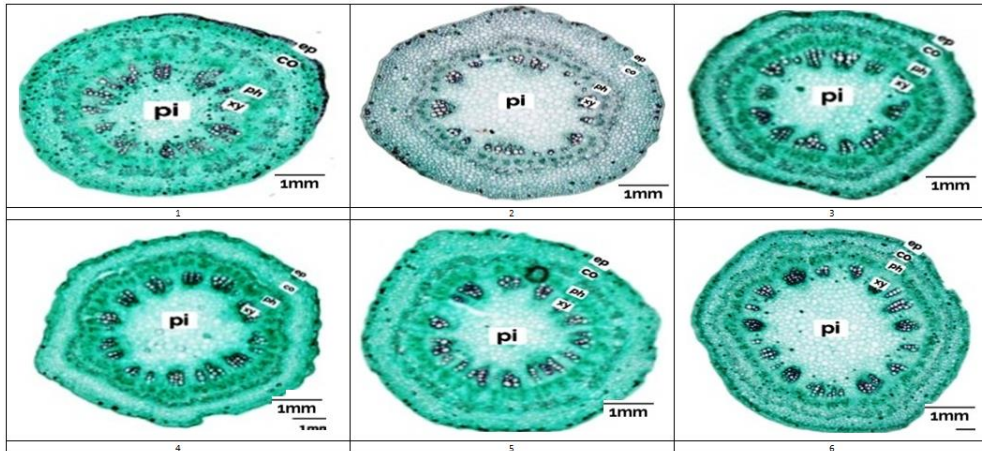


Figure (3): Transverse sections (X 12.5) of moringa stem at 50 days as affected by different applied treatments. Where: (1): Control, (2): Salinity 9000 mg l⁻¹, (3): Zinc 150 mg l⁻¹, (4): Iron 150 mg l⁻¹, (5): Copper 150 mg l⁻¹, (6): Silica 60 mg l⁻¹, ep= epidermis, co= cortex tissue, ph= phloem tissue, xy= xylem tissue, pi= pith tissue.

Foliar application treatments with Zinc, Iron, Copper oxide nanoparticles and Silica nanoparticles recorded highly values of the stem studied anatomical characteristics, respectively. Our results are in harmony with those reported by El Feky *et al.* (2013) found that epidermis cells of the control were similar in shape and size, while the epidermal cells of the NP-treated leaves became larger in size and reached a maximum size when 3 mg l⁻¹ Fe₃O₄ Nano Particles NPS foliar spray was used. In addition, the thickness of mesophyll tissue, which is specialized photosynthetic tissue that contains chloroplasts in palisade and spongy parenchyma tissue, was greater in Fe₃O₄

treated leaves compared to control leaves. Abou-Shlell (2017) indicated that foliar application of moringa plant with Lithovit (Fe-nano particles) at 500 mg l⁻¹ increased moringa stem and leaf anatomical measurements *i.e.*, stem diameter, phloem and xylem tissues thickness as well as vessel diameter.

3.2.3.2.3 Response to salinity stress and nanoparticle interaction

As shown in same Table (3) and Figure (4) Data indicate the effect of different applied nutrient nanoparticle treatments upon alleviating the adverse effects of salinity stress on different studied

anatomical features of *Moringa oleifera* L. stem. In this respect, most of the applied foliar spray treatments have a positively impact on most histological characteristics of moringa stem *i.e.*, stem diameter and Ø of V.C. as well as length of V.B. under used salinity stress level at 9000 mg^l⁻¹ compared with the control plant. Also, obtained results clearly show that *Moringa oleifera* L. plant irrigated with water salinity at 9000 mg^l⁻¹ in combination with Silica nanoparticles at 60 mg^l⁻¹ foliar sprays followed by Zinc and Iron oxide nanoparticles at 150 mg^l⁻¹ of each treatment appeared to be the most effective treatments upon alleviating the

adverse effects of the highest salinity stress. In general, the stimulatory effects of applied treatments upon the anatomy features of treated plants could be attributed to the effect upon cambium activity. Increment of cambium activity could mainly attributed to the increase of endogenous hormones level especially cytokinins and auxins (Abd El-Aal and Eid, 2017; Sotiropoulos *et al.*, 2002). Generally, the obtained results clearly indicate that different applied nutrient nanoparticle treatments play a defensive protective role against adverse effects of highest salinity stress level via its regulatory functions.

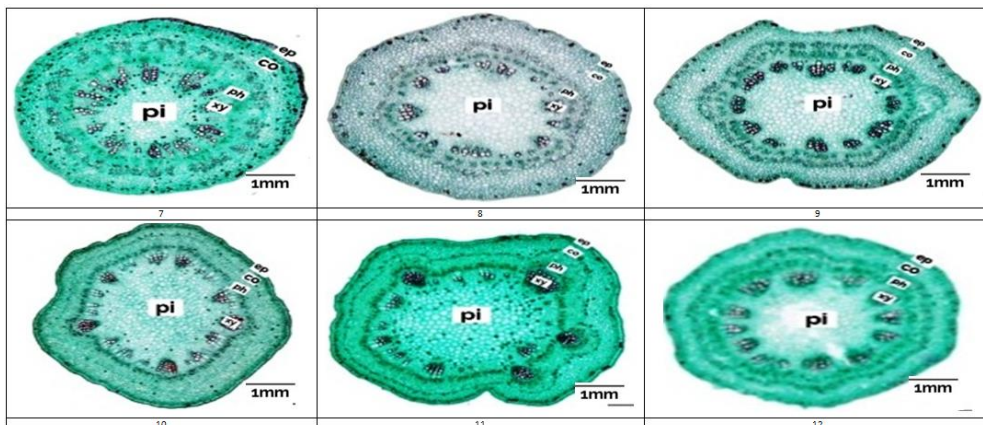


Figure (4): Transverse sections (X 12.5) of moringa stem at 50 days as affected by different applied treatments. Where: (7): Control, (8): Salinity 9000 mg^l⁻¹, (9): Salinity with zinc 150 mg^l⁻¹, (10): Salinity with iron 150 mg^l⁻¹, (11): Salinity with copper 150 mg^l⁻¹, (12): Salinity with silica 60 mg^l⁻¹, ep= epidermis, co= cortex tissue, ph= phloem tissue, xy= xylem tissue, pi= pith tissue.

3.2.3.3 Effect of applied treatments on anatomical characteristics of moringa leaflet

3.2.3.3.1 Response to salinity stress

Data presented in Table (3) and Figure (5) illustrate that studied histological features of moringa leaflet also behaved as the same as root and stem anatomical features. Since, the treatment of salinity

stress level at 9000 mg l^{-1} decreased most of *Moringa oleifera* L. leaflet histological features compared with control. High reductions were recorded in many anatomical features thickness with salinity stress level which negatively affected on studied anatomical characteristics. Among these anatomical features were the most important ones *i.e.*, upper and lower epidermis thickness as well as mesophyll tissues (palisade and spongy tissues) were decreased when compared with the control. In other

words, *Moringa oleifera* L. plant of control recorded best results as it exceeded that of salinity stress level at 9000 mg l^{-1} in terms of most studied anatomical features. In general, the above mentioned results clearly indicate that salinity stress level of irrigation water had opposite effect on those leaflet anatomical characteristics of *Moringa oleifera* L. plant. The obtained results are in harmony with those reported by Child *et al.* (2003), Abdel and Al-Rawi (2011) and El-Afry *et al.* (2012).

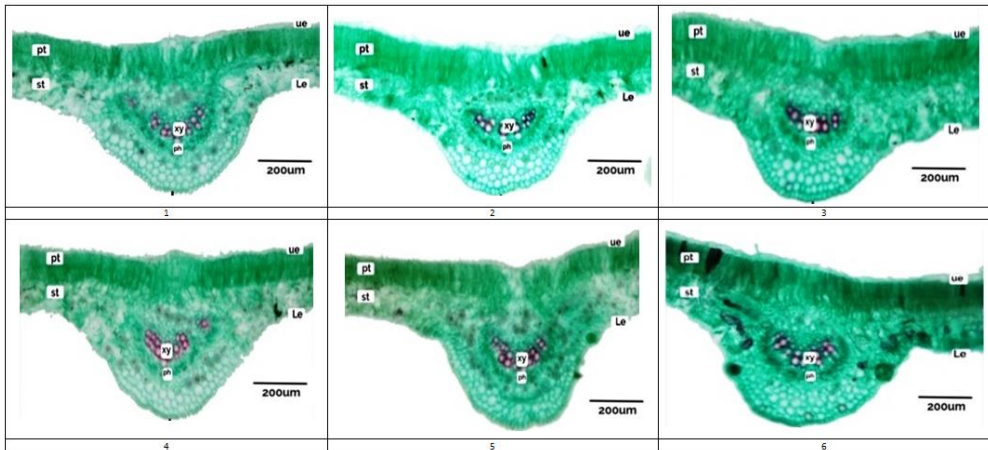


Figure (5): Transverse sections (X 12.5) of moringa leaflet at 50 days as affected by different applied treatments. Where: (1): Control, (2): Salinity 9000 mg l^{-1} , (3): Zinc 150 mg l^{-1} , (4): Iron 150 mg l^{-1} , (5): Copper 150 mg l^{-1} , (6): Silica 60 mg l^{-1} , ue= upper epidermis, pt= palisade tissue, st= spongy tissue, le= lower epidermis, ph= phloem tissue, xy= xylem tissue.

3.2.3.3.2 Response to foliar application nanoparticle treatments

Data presented in Table (3) and Figure (5) clearly show the simulative effect of different applied nanoparticle individually treatments on some anatomical features of *Moringa oleifera*

L. leaflet compared with the control plant. Same data clear the simulative effect of foliar spray with nanoparticle treatments which led to maintain the highest values of studied histological features of moringa leaflet *i.e.*, upper and lower epidermis thickness as well as mesophyll tissues (palisade and spongy

tissues) compared with control. In general, foliar application with zinc followed by iron oxide nanoparticles at 150 mg^l⁻¹ of each treatment recorded the

highest values as exceeded that of other foliar applications with copper and silica nanoparticle treatments in terms of the most studied anatomical features.

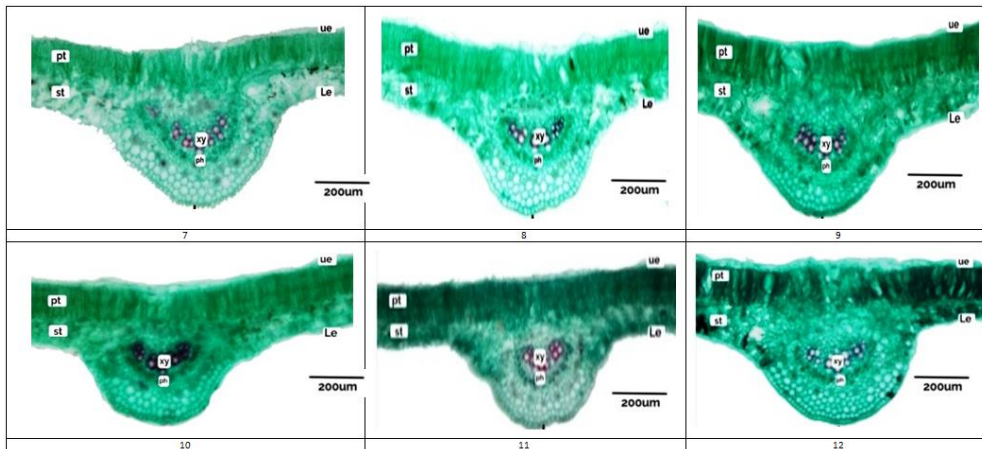


Figure (6): Transverse sections (X 12.5) of moringa leaflet at 50 days as affected by different applied treatments. Where: (7): Control, (8): Salinity 9000 mg^l⁻¹, (9): Salinity with zinc 150 mg^l⁻¹, (10): Salinity with iron 150 mg^l⁻¹, (11): Salinity with copper 150 mg^l⁻¹, (12): Salinity with silica 60 mg^l⁻¹, ue= upper epidermis, pt= palisade tissue, st= spongy tissue, le= Lower epidermis, ph= phloem tissue, xy= xylem tissue.

3.2.3.3.3 Response to salinity stress and nanoparticle interactions

Data presented in Table (3) and Figure (6) clearly indicate the simulative effect of different applied nanoparticle treatments upon alleviating the adverse effects of salinity stress on some anatomical features of *Moringa oleifera* L. leaflet. Once again, same data clear the positive effect of foliar applications with zinc, iron, copper and silica nanoparticle treatments which recorded the highest values of moringa leaflet histological features *i.e.*, upper and lower epidermis thickness as well as mesophyll tissues (palisade and spongy tissues)

under salinity stress compared with the control. Also, the obtained results clearly show that moringa plant irrigated with water salinity at 9000 mg^l⁻¹ in combination with Silica nanoparticles at 60 mg^l⁻¹ foliar sprays followed by Zinc oxide nanoparticles at 150 mg^l⁻¹ appeared to be the most effective treatments upon alleviating the adverse effects of the salinity stress compared with other treatments and control. Finally we can conclude with, improving moringa plant tolerance to such salinity stress level can be achieved by application of some simulative substances to enhance its growth and maximizing plant yield.

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