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Increasing Water Deficiency Tolerance of *Melia azedarach* Seedlings Through Application of Iron

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Abstract: A pot experiment was conducted during the summer growing seasons of 2004 and 2005 in the greenhouse of National Research Centre, Dokki, Giza, Egypt, to study the effect of foliar application with ferrous sulfate at a rate of (0, 50 and 100 ppm) on the growth and chemical constituents of Neem-tree grown under four levels of water regimes (100, 80, 60 and 40% of W.H.C). Results observed that, decreasing water supply gradually decreased significantly stem length, stem diameter, leaves number/plant, fresh and dry weight of leaves and stem. The same tendency was observed regarding N, P, K, Fe, Zn and Mn uptake in leaves. While, root length, fresh and dry weight of roots as well as N, P and K percentage and Fe, Zn and Mn content in leaves increased as water levels were decreased. Data also revealed that growth parameters, chlorophyll (a), (b), carotenoids, Fe, Zn and Mn content as well as total sugar, N, P and K percentage tended to increase by increasing the concentration of iron up to 100 ppm as compared with the untreated one. Iron application can be used to overcome the reduction effect of water deficiency up to 40% W.H.C. These treatments may be recommended for inducing the growth of *Melia azedarach* seedlings in sandy soil under water deficiency.

Key words: *Melia azedarach* · iron · water deficiency

INTRODUCTION

Melia azedarach is among the main timber trees tended to be cultivated as a source of woods, shade, forage and insecticides in sandy soil. The Neem tree (*Azadirachta indica* juss. var. Simensis vaieton) is of ornamental, revegetation, medicinal and biomass value in India, Burma (Myanmar) and south-east Asia The compound azadirachtin which is arrived from Neem seeds, is commercially used for insecticidal properties is, the botanical insecticide Margosan-O for control of trips, whiteflies and other insects in nurseries and greenhouse.

Stress has been defined as any environmental factor capable of inducing a potentially injurious strain in plants. Water is a major constituent of tissue, a reagent in chemical reaction, a solvent for and mode of translocation for metabolites and minerals within plant and is essential for cell enlargement through increasing turgor pressure. With the occurrence of water deficits many of the physiological processes associated with growth are affected and under severe deficits, death of plants may result. Metwally *et al.* [1] found that plant height of roselle and number of leaves decreased with prolonging the water intervals. Shehata [2] on *Cupressus simpervirens* and *Eucalyptus camaldulensis*, Sayed [3] on *Khaya senegalensis*, Uday *et al.* [4] on *Simmondsia chinesis* link supplied seedlings with different irrigation levels and found that highest values for plant height and diameter and fresh and dry weights of stem and leaves were obtained due to the use of high irrigation levels. On the contrary, root length and fresh and dry weights were reduced by the increasing in water supply up to the high level. Shehata [2] on *Cupressus sempervirens* and *Eucalyptus camaldulensis*, El-Tantawy *et al.* [5] on *Eucalyptus camaldulensis*, Sayed [3] on *khaya senegalensis* and Soad [6] on *Simmondsia chinensis*, irrigated seedlings with different soil moisture contents. They observed that chlorophyll a, b and carotenoids contents were increased as soil moisture content decreased in both seasons. They added that total sugars and N, P and K% in the leaves were also stimulated gradually by decreasing water supply. While, leaf contents of nitrogen, phosphorus and potassium were increased by increasing water supply.

Iron is among the essential micronutrients needed for better plant growth and high quality. Hussein *et al.* [7] on *Hibiscus sabdariffa* reported that Fe at the concentration of 25 ppm stimulated growth characters. Misra and Srivastava [8] and Misra [9] on *Mentha arvensis* L. indicated that plants grown under iron

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deficiency had smaller leaves with smaller mesophyll cells compared with plants given sufficient Fe. Also the maximum biomass was produced at 5.6 mg L^{-1}. Leithy [10] on *Nigella sativa* found that application of Fe at 25 ppm was affecting positively on plant height, number of leaves and fresh and dry weight/plant through its effect on enhancement of cell division and / or cell enlargement. Abd El-Salam and Inas [11] Azza and El-Mesiri [12]on *Foeniculum vulgare* found that Fe at 50 or 100 ppm in most cases led to increase the plant height, fresh and dry weight of all plant organs. Misra and Srivastava [8] on *Mentha arvensis* lu, Srivastava and luthra [13] on *Mentha peperita* cv. MSLP and Leithy [10] on *Nigella sativa* L. stated that application of Fe increased the chlorophyll contents, carotenoids and total carbohydrates.

Subrahamanyam *et al.* [14] on Japanese mint reported that Fe application increased both the concentration and total uptake of Fe by the crop. Azza [15] on *Ammi visanga* and Azza and El-Misiri [12] on *Foeniculum vulgare* showed that iron application gave the highest values of N, P and K percentage. Also, Zn, Mn and Fe ppm increased by different levels of Fe.

Therefore, the present investigation aims to study the effect of water deficiency tolerance on *Melia azedarach* seedlings through application of iron.

MATERIALS AND METHODS

The present investigation was carried out in the greenhouse of National Research Centre, Dokki, Cairo, Egypt, during the two successive seasons of 2004 and 2005, to study the response of *Melia azedarach* grown under four contrasting soil moisture regimes to iron application through measurements of growth parameters and some chemical constituents. The soil of the experimental was sandy in texture of the following characteristics 84.6% coarse sand 6.3% fine sand 3.8% silt and 5.3% clay, pH 7.6, EC 1.3 dSm^{-1}, $CaCO_3$ 2.35%, K^+ 0.2, Na^+ 2.1, Ca^{++} 1.0, Mg^{++} 0.8, $HCO3^-$ 2.5, Cl^- 1.5, SO^{--} 0.1 meq L^{-1}.

Plant materials and procedures: *Melia azedarach* fam Meliaceae, the seeds were sown on 15th March of the 2004 and 2005 seasons. Five seeds were planted in each pot and after one month thinned to one plant/pot. Plants were subjected to four levels of soil moisture till the end of experiment (15th November). The four soil moisture contents are (100, 80, 60 and 40% of the water holding capacity W.H.C). Water amounts were supplied to the time of the seedlings of each treatment to reach the previously mentioned percentages of the water holding capacity.

The source used for Fe was ferrous sulfate as foliar spray at rates of 0, 50 and 100 ppm. The plants were treated with Fe three times of 30 days intervals starting on the 15th of May in both seasons.

The experiments were sit in a Completely Randomized Design with four irrigation levels and sprayed with three concentrations of Fe to give 4x3 factorial with 6 replicates.

The available commercially fertilizer used through this experimental work was kristalon (N: P: K, 19: 19: 19) were used at a rate of 5.0 g per pot in four doses. The plants were fertilized after 4, 8, 16 and 20 weeks from sowing. Stem length (cm), stem diameter (mm), root length (cm), leaves number/pot, fresh and dry weights of plant organs (root, stem and leaves "g"). The data were statistically analyzed according to Snedecor and Cochran [16] using the least significant different (L.S.D) at 5%.

Chlorophyll (a), (b) and carotenoids content were determined in fresh leaves according to the method described by Saric *et al.* [17]. The following data were determined in dry leaves. Total sugars percentage were determined according to the method described by Dubios *et al.* [18].

N, P and K were determined according to the method described by Cottenine *et al.* [19]. Fe, Zn and Mn were determined by Atomic Absorption described by Chapman and Pratt [20].

RESULTS AND DISCUSSION

Effect of the soil moisture content on the growth characteristics: Data in Table 1-3 indicated that, low level of irrigation caused an obvious reduction in all growth characters including stem length and thickness, leaves number/plant and fresh and dry weights of leaves and stem. These characters were decreased by decreasing water supply as compared with those grown under normal condition. The highest values for all these traits were obtained due to the use of the high irrigation level (100% W.H.C). This may be due to the vital roles of water supply at adequate amounts for different physiological processes such as photosynthesis, respiration, transpiration, translocation enzyme reactions, cells turgidity occur simultaneously.

On the contrary, root length, fresh and dry weights of roots were gradually decreased by increasing water supply up to the high level. This may be attributed to the reduction in water supply could be explained by the fact that the lower water supply causes the root system to penetrate deeper and extending wider in the soil with higher root system researching for moisture in lower. These results are in agreement with those obtained by Shehata [2] on *Cupressus sempervirens* and *Eucalyptus*

Table 1: Stem length (cm), stem diameter (mm) leaves number/plant and root length (cm) of *Melia azedarach* as affected by irrigation treatments and iron (combined data for two seasons 2004 and 2005

Characters	Stem length (cm)				Stem diameter (mm)				Leaves number/plant				Root length (cm)			
	Fe ppm				Fe ppm				Fe ppm				Fe ppm			
Irrigation treatments	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
100	56.31	63.76	69.11	63.06	4.3	4.7	5.0	4.7	17.63	19.15	21.74	19.51	12.61	12.82	13.11	12.85
80	49.54	54.11	58.31	53.99	4.1	4.5	4.8	4.5	15.41	15.71	17.68	16.27	14.12	14.76	15.03	14.64
60	43.45	46.91	51.32	47.23	3.2	3.6	4.0	3.6	12.11	13.75	14.19	13.35	15.11	15.71	16.16	15.66
40	36.12	42.00	45.54	41.22	2.6	2.9	3.1	2.9	9.71	11.12	11.76	10.86	17.31	17.96	18.45	17.91
Mean	46.36	51.70	56.07		3.6	3.9	4.2		13.72	14.93	16.34		14.79	15.31	15.69	
L.S.D 0.05																
Irrigation treatments (A)	9.04				0.97				3.09				2.43			
Fe (B)	11.07				1.18				3.78				2.97			
(A) X (B)	15.65				1.67				5.35				4.20			

Table 2: Fresh weight of Leaves, stem and root (g) of *Melia azedarach* as affected by irrigation treatments and iron (combined data for two seasons 2004 and 2005)

Characters	Leaves				Stem				Root			
	Fe ppm				Fe ppm				Fe ppm			
Irrigation treatments	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
100	6.31	6.91	7.39	6.87	13.31	13.91	14.39	13.87	4.35	4.67	5.11	4.71
80	5.61	5.93	6.43	5.99	11.61	11.93	12.43	11.99	4.76	4.93	5.45	5.05
60	5.00	5.33	5.76	5.36	9.00	9.33	10.76	9.70	5.14	5.65	6.05	5.61
40	4.12	4.43	4.79	4.45	7.12	8.43	8.79	8.11	5.91	6.77	6.91	6.53
Mean	5.26	5.65	6.09		10.26	10.90	11.59		5.04	5.51	5.88	
L.S.D 0.05												
Irrigation treatments (A)	1.52				2.93				0.88			
Fe (B)	1.87				3.59				1.08			
(A) X (B)	2.64				5.07				1.53			

Table 3: Dry weight of leaves, stem and root (g) of *Melia azedarach* as affected by irrigation treatments and iron (combined data for two seasons 2004 and 2005)

Characters	Leaves				Stem				Root			
	Fe ppm				Fe ppm				Fe ppm			
Irrigation treatments	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
100	3.14	3.56	4.15	3.62	7.17	7.68	8.11	7.65	2.61	2.69	2.93	2.74
80	2.45	2.81	3.38	2.88	6.41	6.53	7.15	6.70	2.96	3.43	3.67	3.35
60	2.03	2.45	2.74	2.41	5.01	5.43	5.74	5.39	3.11	3.31	3.68	3.37
40	1.65	1.93	2.07	1.88	3.53	3.84	4.15	3.84	3.65	3.81	4.03	3.83
Mean	2.32	2.69	3.09		5.53	5.87	6.29		3..08	3.31	3.58	
L.S.D 0.05												
Irrigation treatments (A)	0.69				0.74				0.46			
Fe (B)	0.84				0.90				0.56			
(A) X (B)	1.19				1.28				0.79			

camaldulensis and El-Tantawy *et al.* [6] on *Eucalyptus camaldulensis*, supplied seedlings with three soil moisture content (40, 60 and 80% of field capacity). They observed that plant height, stem diameter, fresh and dry weight of leaves, stem and roots were increased by increasing soil moisture but root length was decreased. Also, Uday *et al.* [4] studied the effect of irrigation at different levels of (field capacity 10.4% w/w), 2 F.C., 5 F.C. and 10 F.C. levels) on growth of *Simmondsia chinensis* seedlings and found that growth was increased with the increase of irrigation levels.

Effect of the foliar application of Fe on growth: The results in Table 1 showed that, all growth characters increased by increasing Fe concentration compared with the untreated plants. This may be due to the positive affect of Fe on enhancement of cell division and/or cell enlargement. Deficiency of iron inhibits cell division. Iron spraying could be explained in the light of its role in the oxidation or reduction reactions in respiration and photosynthesis and hence, it causes a marked effect on photosynthetic efficiency. The role of Fe enhancing the synthesis of chlorophyll and protein for optimum growth as well as increasing the enzyme systems activity. Fe enhancing the metabolism of carbohydrates and protein as well as the enzyme systems, consequently the vegetative growth, therefore, the fresh and dry weights of leaves [21]. These results were on line with those reported by Abd El-Salam and Inas [11] and Azza and El-Mesiri [12].

Effect of the interactions between moisture levels and Fe application: The results obtained in Tables 1, 2 and 3 indicated that, the interaction between different involved factors (irrigation and Fe) were almost significant for vegetative growth characters. The highest values due to the irrigation x Fe were obtained due (100% W.H.C X 100 ppm Fe) for stem length, stem diameter, number of leaves and fresh and dry weights of stem and leaves and (40% W.H.C X 100 ppm Fe) for root length and fresh and dry weights of roots.

Chemical composition:

Pigments content: Data in Table 4 showed that, the leaves contents of three photosynthetic pigments (chlorophyll (a), (b) and carotenoids) were gradually increased as the irrigation was sloping downward. These results were in agreement with those obtained by Sayed [3] and Soad [6]. Also, the results were also revealed that, spraying plants with Fe caused an increase in Chlorophyll (a), (b) and carotenoids content as compared with control one. This phenomenon may be due to a good correlation between the levels of Fe supply and chlorophyll content. Plant well supplied with Fe being high in chlorophyll content [21]. The important role of iron on the photosynthesis of chlorophyll [22]. The effective role of Fe for increasing the synthesis of chlorophylls in the plant tissues [21]. Similar results were reported by Leithy [10]. Considering the interaction, the maximum values of the three photosynthetic pigments content was obtained at irrigation (40% W.H.C) and 100 ppm Fe.

Total sugar content: Effect of water regime and foliar application with different levels of Fe were presented in Table 4 indicated that, total sugar as affected by different irrigation levels treatments, followed the same trend obtained previously on photosynthetic pigments, were gradually decreased by increasing the level of irrigation. The obtained results were in harmony with the findings obtained by Sayed [3] and Soad [6].

Iron at both used concentration caused an increase in total sugar percentage as compared with untreated seedlings. Increase in total sugar percentage being gradual and parallel to increase in the applied concentration up to 100 ppm.

The positive effect of Fe on enhancing the total sugar percentage may be due to the importance of Fe has important functions in plant metabolism [23]. Fe enhancing the metabolism of carbohydrates [21]. These results were in accordance with those recorded by Leithy [10].

As for the interaction between irrigation and iron application, the higher values were provided when adding 100 ppm Fe and irrigation (40% W.H.C).

Minerals percentage: Effect of irrigation treatments and foliar application of different levels of Fe were presented in Tables 5 and 6 showed that a gradual reduction in the leaves percentage of the applied nutrients, nitrogen, phosphorus, potassium, iron, manganese and zinc were parallel to the increase in the irrigation level. Increasing water regime (100% W.H.C) decreased N, P and K

Table 4: Chlorophyll (a), (b) and carotenoids content (mg g^{-1} F.W.) and total sugar percentage of *Melia azedarach* as affected by irrigation treatments and iron (combined data for two seasons 2004 and 2005)

Characters	Chlorophyll (a)				Chlorophyll (b)				Carotenoid				Total sugar			
	Fe ppm				Fe ppm				Fe ppm				Fe ppm			
Irrigation treatments	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
100	1.893	2.236	2.307	2. 145	0.512	0.591	0.637	0.580	0.813	0.923	0.976	0.904	10.01	10.96	11. 87	10.95
80	2.118	2.385	2.443	2.315	0.673	0.725	0.767	0.722	0.935	1.053	1.121	1.036	12.46	13.06	14.12	13.21
60	2.315	2.379	2.456	2.383	0.696	0.754	0.811	0.754	1.113	1.128	1.209	1.150	14.19	15.45	16.31	15.32
40	2.367	2.412	2.516	2.432	0.735	0.821	0.906	0.82	1.265	1.306	1.417	1.329	16.60	17.03	18.18	17.27
Mean	2.173	2.353	2.431		0.654	0.723	0.780		1.032	1.103	1.18		13.32	14.13	15.12	

Table 5: Nitrogen, phosphorus and potassium percentage of *Melia azedarach* as affected by irrigation treatments and iron (combined data for two seasons 2004 and 2005)

Characters	N %				P %				K %			
	Fe ppm				Fe ppm				Fe ppm			
Irrigation treatments	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
100	1.231	1.346	1.369	1.531	0.383	0.457	0.497	0.446	1.131	1.256	1.367	1.251
80	1.311	1.367	1.406	1.361	0.437	0.513	0.568	0.506	1.411	1.521	1.678	1.537
60	1.369	1.421	1.446	1.412	0.523	0.573	0.617	0.572	1.671	1.731	1.763	1.722
40	1.383	1.452	1.531	1.455	0.578	0.618	0.667	0.621	1.813	1.867	1.915	1.865
Mean	1.324	1.397	1.479		0.481	0. 540	0.587		1.507	1.594	1.681	

Table 6: Iron, zinc and manganes (ppm) of *Melia azedarach* as affected by irrigation treatments and iron (combined data for two seasons 2004 and 2005)

Characters	Fe ppm				Zn ppm				Mn ppm			
	Fe ppm				Fe ppm				Fe ppm			
Irrigation treatments	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
100	110.0	144.0	167.0	140.33	45.0	53.0	61.0	53.00	17.0	19.0	20.0	18.67
80	135.0	151.0	169.0	151.67	57.0	64.0	68.0	64.00	18.0	20.0	22.0	20.00
60	158.0	172.0	196.0	175.33	61.0	73.0	78.0	70.67	20.0	22.0	26.0	22.67
40	176.0	195.0	220.0	197.00	69.0	79.0	85.0	77.67	23.0	26.0	31.0	26.67
Mean	144.75	165.50	188.0		58.00	68.00	73.00		19.50	21.75	24.75	

Table 7: Nitrogen, phosphorus and Potassium uptake of *Melia azedarach* as affected by irrigation treatments and iron (combined data for two seasons 2004 and 2005)

Characters	N				P				K			
	Fe ppm				Fe ppm				Fe ppm			
Irrigation treatments	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
100	38.7	47.9	63.5	50.0	12.03	16.27	20.63	16.31	35.51	44.71	56.73	45.65
80	32.1	38.4	47.5	39.3	10.71	14.42	19.20	14.78	34.57	42.74	56.72	44.68
60	27.8	34.8	39.6	34.1	10.67	14.04	16.91	13.87	33.92	42.41	48.31	41.55
40	22.8	28.0	31.7	27.5	9.54	11.93	13.81	11.76	29.91	36.03	39.64	3.519
Mean	30.4	37.3	45.6		10.74	14.17	17.64		33.48	41.47	50.35	

Table 8: Iron, zinc and manganes uptake of *Melia azedarach* as affected by irrigation treatments and iron (combined data for two seasons 2004 and 2005)

Characters	Fe				Zn				Mn			
	Fe ppm				Fe ppm				Fe ppm			
Irrigation treatments	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
100	345.40	512.64	693.05	517.03	141.30	188.68	253.15	194.38	53.38	67.64	83.00	68.01
80	330.75	424.31	571.22	442.09	139.65	179. 84	229.48	183.11	44.10	56.20	74.36	58.22
60	320.74	421.40	537.04	426.39	123.83	178.85	213.72	172.13	40.60	53.90	71.24	55.25
40	290.40	376.35	455.40	374.05	113.85	152.47	175.95	147.42	37.95	50.18	64.17	50.77
Mean	321.82	433.68	564.18		129.66	174.96	218.17		44.01	56.98	73.19	

percentage as compared with decreasing water regime (40% and 60% W.H.C). This may be due to the leaching of the minerals from soil. Regarding Fe, Zn and Mn concentration in plant, pronounced increase in Fe, Zn and Mn was observed with increasing water regime.

In relation to the effect of iron concentrations, the percentage of the previous minerals in the leaves were gradually increased by increasing Fe level. Foliar application with Fe were increased the previous minerals due to the enhancing effects of Fe on the absorption and/or translocation of these minerals. Such phenomenon may be due to its effect on enhancing the plant metabolism [23].

As for the interaction, between the 100 ppm Fe application and irrigation at (40% W.H.C) showed that maximum percentage for each elements were noticed.

Minerals uptake: Data in Tables 7 and 8 mentioned that, N, P, K, Fe, Zn and Mn uptake gave the opposite results to the previous treatment. Increasing water regime increased N, P, K, Fe, Zn and Mn uptake. These results may be due to the reduction of the dry matter of leaves.

On the other hand, increasing iron level up to 100 ppm increased N, P and K and Fe, Zn and Mn uptake. The interaction between the 100 ppm application and irrigation at (100% W.H.C) showed the maximum concentration for each.

The results in this study indicated that iron application can be used to overcome the reduction effect of water deficiency up to 40% W.H.C. This treatment may be recommended for inducing the growth of *Melia Azedarach* seedlings in sandy soil under water deficiency.

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