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Response of *Khaya senegalensis* Seedlings to Irrigation Intervals and Foliar Application of Humic acid

M.M. Farahat, Azza A.M. Mazhar and Mona H. Mahgoub

Department of Ornamental Plants and Woody Trees, National Research Centre, Dokki, Giza, Egypt

Abstract: This study was carried out during two successive seasons of 2011 and 2012 at National Research Centre (Research and Production Station, Nubaria), Egypt to evaluate the effects of irrigation intervals and foliar application of humic acid (HA) on growth and chemical composition of *Khaya senegalensis* seedlings. Experiment treatments included three irrigation intervals (3, 6 and 9 days) and four concentrations of humic acid (0, 2, 3 and 4%). Water deficit reduced the growth of plant characters. Plant height, stem diameter, leaves fresh and dry weights under water-stressed plants were less than the equivalent growth in the well-watered plants. The plants responded to water stress by the development of longer roots and roots fresh and dry weights. Water stress (9 days) resulted in decreases in chlorophyll (a and b), carotenoids content and soluble sugars % in leaves and roots. Water stress increased macro elements concentration (N, P and K) and proline content in the leaves. The well-watered plants showed less nutrient concentrations than the water-stressed plants. Foliar application of humic acid at 4% gave the highest values of plant height, stem diameter, leaves fresh and dry weights and root length. Also, it increased pigments content, soluble sugars, N, P and K % in leaves and roots. While, humic acid treatments decreased proline content in leaves and Na% in leaves and roots compared with control. The combination of irrigation at 3 days interval and 4% humic acid resulted in highest values of plant height; stem diameter; fresh and dry weights of leaves; chlorophyll a and b and carotenoids in leaves and soluble sugars in leaves and roots contents. The longest irrigation interval (9 days) with high humic acid level (4%) treatment resulted in the highest N, P and K % in leaves and roots. The highest value of proline content in leaves resulted from the longest irrigation interval (9 days) without application of humic acid.

Key words: Khaya senegalensis · Irrigation · Humic acid · Proline

INTRODUCTION

Khaya senegalensis (Desr.) A. Juss., also known as African mahogany, is a member of the Meliaceae family, which contains many of world's most highly valued timber species [1]. *Khaya senegalensis* is often planted by the roadsides for shade in countries of its natural range (in Africa) and also increasingly in tropical zones of other continents [2]. Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world [3]. Trees and shrubs have much deeper and more extensive root systems than turf grass so they should be watered less frequently but for longer periods of time. Symptoms of drought can appear on a wide range of deciduous and evergreen trees and shrubs. The effects of drought are particularly severe on all types of seedlings and new transplants. El-Khateeb *et al.* [4] on *Acacia saligna,* reported that prolonging the irrigation intervals had negative effects on plant height, fresh and dry weights of leaves, stems and roots and total carbohydrates content in leaves and stems and the opposite trend was recorded in the root.

Humic substances (HS) represent the organic material mainly widespread in nature. HS have positive effects on plant physiology by improving soil structure and fertility and by influencing nutrient uptake and root growth. The biochemical and molecular mechanisms underlying these events are only partially known. HS have been shown to contain auxin and an "auxin-like" activity of humic substances has been proposed [5]. Numerous studies have shown that HS enhance root, leaf and shoot growth but also stimulate the germination of various crop species [6]. The addition of HS stimulate nutrient uptake

Corresponding Author: M.M. Farahat, Department of Ornamental Plants and Woody Trees, National Research Centre, Dokki, Giza, Egypt.

[6, 7] and seems to regulate mechanisms involved in plant growth stimulation [8]. Most experiments designed to elucidate potential ameliorative effects of humic substances on drought stressed crops is limited to their foliar application in pot studies. So, the purpose of this research is to study the effects of humic acid growth and chemical constituents of *Khaya senegalensis* seedlings under irrigation intervals.

MATERIALS AND METHODS

Two pot experiments were conducted at National Research Centre (Research and Production Station, Nubaria), Egypt, during two successive seasons of 2011 and 2012 to study the individual and combined effects of foliar application of humic acid (HA) and irrigation intervals on growth and chemical composition of *Khaya senegalensis* seedlings. The soil of the experimental site was sandy. The investigated soil characterized by 80.5% coarse sand, 9.4% fine sand,4.5% silt and 5.6% clay, pH 7.8, EC 1.4 dS/m, $CaCO_3$ 46%, K^+ 0.3, Na+ 2.3, Ca^{++} 1.0, Mg^{++} 0.6, HCO_3 2.3,Cl^- 1.8, SO_4^{--} 0.1 meq/L. The physical and chemical analyses of the experimental soil were determined according to the methods described by Chapman and Pratt [9]. One year old seedlings of *Khaya senegalensis* were obtained from Nursery of Forestry Department, Agriculture Research Centre, Giza, Egypt. The seedlings were transplanted on the first week of March during the two successive seasons in plastic pots 30 cm. in diameter filled with 10 kg of soil, one seedling/pot(replicate), the average heights of seedlings were 15- 20 cm. The available commercially fertilizer used through this experimental work was Kristalon (NPK 19:19:19) produced by Phayzon Company, Holland. The fertilizers rate (5.0 g /pot) was applied in four equal doses after 4,8,16 and 20 weeks from transplanting. The seedlings were watered with three treatments of irrigation intervals (3, 6 and 9 days).Plants were sprayed with four concentrations of humic acid (HA) as potassium humate HA_0:0, HA_1: 2%,HA_2: 3% and HA_3:4%). Plants were sprayed twice with humic acid until run-off occurred; the first spraying was in the second week of April. One month later the second spray was performed. The experiment was set in a completely randomized design with twelve treatments and six replicates of each treatment. The agricultural processes were performed according to normal practice.

The following data were recorded at the first week of October of 2011 and 2012: plant height (cm), stem diameter (mm), root length (cm), fresh and dry weights of leaves and roots (g). The following chemical analyses were determined: total soluble sugar percentages were determined according to the method of Dubois *et al.* [10], photosynthetic pigments as well as carotenoids content were determined in fresh leaves as mg/g fresh weight, according to the procedure achieved by Saric *et al.* [11]. Nitrogen, Phosphorus, Potassium and Sodium were determined in leaves and roots according to the method described by Cottenie *et al.* [12]. Proline concentration was determined according to Bates *et al.* [13].

Statistical Analysis: The data were statistically analyzed for each season and then a combined analysis of the two seasons was carried out according to the procedure outlined by Steel and Torrie [14].

RESULTS AND DISCUSSION

Vegetative Growth: Data on vegetative growth characters in response to irrigation intervals and foliar application of humic acid are presented in Table 1. Irrigation the plants every 9 days caused a significant decrease in plant height, stem diameter, fresh and dry weights of leaves compared with the other irrigation intervals. These results may be due to the fact that cell growth is considered one of the most drought sensitive physiological processes due to the reduction in turgor pressure. Growth is the result of daughter- cell production by meristematic cell divisions and subsequent massive expansion of the young cells. Under severe water deficiency, cell elongation of higher plants can be inhibited interruption of water flow from the xylem to the surrounding elongating cells [15]. On the contrary, the same treatment (9 days) gave the highest values of root length, fresh and dry weights of roots. Seedlings under limited watering regime developed longer roots to uptake limited water available in the soil. The rapid development of a deep root system that can access water stored lower in the soil profile may be essential for successful seedling establishment [16, 17]. All vegetative growth characters were increased significantly by foliar application of humic acid at 2, 3 and 4% compared with control and the highest values were obtained at 4%humic acid. Humic substances may play an important role in regulating the plant root metabolism by inducing or repressing the mechanism of protein synthesis, enzyme activation or inhibition resulting in morpho-functional changes in plant root tissues [18].

The interaction between irrigation and foliar application of humic acid treatments indicated that the combination of irrigation interval at 3 days and 4% humic

Table 1: Growth parameters of *Khaya senegalensis* seedlings as affected by irrigation intervals (days) and humic acid (Average two seasons 2011 and 2012)

Characters Treatments	Plant height (cm)	Stem diameter (mm)	Root length (cm)	Leaves fresh weight (g)	Leaves dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Irrigation intervals (days)							
3	87.9	11.80	45.7	78.1	30.2	20.8	7.80
6	80.4	9.70	51.8	70.5	25.3	25.3	9.50
9	74.0	8.10	57.6	63.3	21.2	32.2	11.60
LSD 5%	2.8	0.80	2.7	1.24	0.93	0.67	0.55
Humic acid %							
0	70.4	7.90	40.9	56.8	16.3	21.8	7.90
2	75.5	8.90	46.9	68.3	22.8	24.0	8.80
3	82.9	10.50	56.3	71.0	29.83	26.9	10.40
4	94.1	12.00	62.7	78.9	33.4	31.6	12.40
LSD 5%	2.4	0.60	2.3	1.13	0.56	0.45	0.43
Interaction							
3 days+ HA 0%	76.3	9.30	35.2	65.3	21.1	16.7	5.70
3 days+ HA 2%	81.1	10.70	41.6	73.6	26.3	18.5	6.90
3 days+ HA 3%	88.7	12.40	49.3	81.7	35.1	21.6	8.50
3 days+ HA 4%	105.3	14.60	56.7	83.6	38.3	26.5	10.10
6 days+ HA 0%	71.5	8.00	39.9	59.3	15.3	21.3	7.30
6 days+ HA 2%	75.1	9.10	46.6	68.8	22.6	23.6	8.10
6 days+ HA 3%	83.3	10.10	57.3	75.1	30.1	25.5	9.90
6 days+ HA 4%	91.7	11.40	63.4	78.6	33.3	30.6	12.60
9 days+ HA 0%	63.4	6.30	47.6	45.8	12.6	27.5	10.70
9 days+ HA 2%	70.3	7.00	52.4	62.6	19.5	29.9	11.50
9 days+ HA 3%	76.7	9.10	62.3	70.0	24.3	33.7	12.70
9 days+ HA 4%	85.4	9.90	67.9	74.6	28.5	37.6	14.40
LSD 5%	3.5	1.12	3.8	1.84	1.66	1.35	0.63

Table 2: Chlorophyll (a), (b), carotenoids (mg/g F.W) and soluble sugars% of *Khaya senegalensis* seedlings as affected by irrigation intervals (days) and humic acid (Average two seasons 2011 and 2012)

Characters Treatments	Chlorophyll (a)	Chlorophyll (b)	Carotenoids	Soluble sugars % Leaves	 Root
Irrigation intervals (days)					
3	1.66	0.58	0.37	14.32	14.75
6	1.39	0.48	0.34	11.95	12.17
9	1.21	0.46	0.31	10.02	10.93
Humic acid %					
0	1.29	0.45	0.29	10.77	10.25
2	1.38	0.48	0.33	11.46	11.82
3	1.45	0.52	0.36	12.52	13.31
4	1.56	0.57	0.38	13.63	15.08
Interaction					
3 days+ HA 0%	1.58	0.51	0.33	13.51	12.12
3 days+ HA 2%	1.62	0.56	0.36	13.93	13.55
3 days+ HA 3%	1.69	0.62	0.39	14.51	15.67
3 days+ HA 4%	1.76	0.63	0.41	15.33	17.64
6 days+ HA 0%	1.27	0.45	0.28	10.73	10.31
6 days+ HA 2%	1.35	0.46	0.33	11.34	11.71
6 days+ HA 3%	1.44	0.49	0.37	12.41	12.56
6 days+ HA 4%	1.51	0.51	0.39	13.31	14.11
9 days+ HA 0%	1.03	0.39	0.25	8.07	8.33
9 days+ HA 2%	1.17	0.43	0.37	9.11	10.20
9 days+ HA 3%	1.23	0.46	0.32	10.63	11.70
9 days+ HA 4%	1.40	0.56	0.35	12.25	13.50

acid resulted in highest values of plant height; stem diameter, fresh and dry weights of leaves. A significant increase in root length, fresh and dry weights of roots was occurred by increasing both the interval between irrigation (9 days) and humic acid (4%) compared with the other treatments. Researchers speculate that the hormone-like properties of humic substances may play a causal role in drought stress amelioration [19-22].

Plant Constituents

Pigments and Soluble Sugars Content: The results on the contents of chlorophyll a and b and carotenoids in leaves and soluble sugars in leaves and roots of *Khaya senegalensis* seedlings as affected by irrigation and foliar application of humic acid are presented in Table 2. Chlorophyll a, b and carotenoids in leaves and soluble sugars in leaves and roots contents were decreased by extending the intervals between irrigations. Drought stress may decrease stomatal conductance [23] and concentration of chlorophyll *a* and *b* and carotenoids [24], leading to a decrease (3-7 folds) in photosynthetic activity in *Acacia auriculiformi* [25]. The plants which were irrigated at short intervals (3 days) contained higher concentrations of chlorophyll a and b and carotenoids in leaves and soluble sugars in leaves and roots than those irrigated at relatively long intervals. The highest values of chlorophyll a and b and carotenoids in leaves and soluble sugars in leaves and roots were recorded in plants sprayed with humic acid at 4%. This result is in agreement with that found by Zaghloul *et al.* [26], who stated that foliar application of potassium humate increased soluble sugars of *Thuja orientalis* shoots. The increments of chlorophyll a and b and carotenoids in leaves might be lead to positive effects on growth parameters and increased total soluble sugars content. As for the effect of interaction, the results indicated that raising the concentration of humic acid under the same irrigation interval increased gradually chlorophyll a and b and carotenoids in leaves and soluble sugars in leaves and roots contents. The highest values of these contents resulted from foliar application of humic acid at 4% with short irrigation interval (3 days). Humic substances (HS) have positive effects on plant physiology [27, 28], so that, concentration of 4% humic acid was effective on increasing of chlorophyll content.

Minerals Concentration: The results presented in Table 3 shows N, P, K and Na % in leaves and roots of *Khaya senegalensis* in response to irrigation and foliar application of humic acid treatments. Prolonging irrigation interval increased N, P, K and Na % in leaves and roots. The greatest reduction was associated with the shortest interval (3 days). This may be due the leaching of the minerals from soil. These results were on line with those reported by Mazher and Zaghloul [29]. High humic acid level (4%) recorded the highest N, P and K % in leaves and roots, while the lowest Na % in leaves and roots was recorded with the same high humic acid (4%). N, P and K % in leaves and roots were increased gradually with increasing humic acid level up to (4%). The opposite trend was observed with Na%. The stimulatory effect of humic substances have been directly correlated with enhancing uptake of macronutrients such as nitrogen, phosphorus, potassium and sulfur [18, 22]. The effect of interaction results between irrigation intervals and humic acid concentrations showed that the longest irrigation interval (9 days) with high humic acid level (4%) treatment resulted in the highest N, P and K % in leaves and roots. Whereas, the longest irrigation interval (9 days) without humic acid (HA_0) treatment recorded the highest Na % in leaves and roots. De Souza *et al.* [30] showed that the magnetic treatments led to a remarkable increase in plant root and stem length as well as fresh and dry weight during the nursery period. These initial effects are very positive since they appear to induce an improved capacity for nutrient and water uptake, providing greater physical support to the developing shoot.

Proline Content: The effects of irrigation intervals and foliar application of humic acid treatments on proline content in leaves and roots are shown in Fig. 1. Proline content in leaves was increased by in prolonging the interval between irrigations. The maximum increase occurred when the interval was prolonged from 6 to 9 days. The proline content was increased with increasing water stress [31]. When plants are subjected to water stress, proline is synthesized from glutamic acid to act as osmoprotectant for keeping the water balance in cells and the outer environment [32]. Farahat [33] reported that the extension of irrigation intervals increased proline content of *Myoporum acuminatum* leaves. Increasing foliar application of humic acid level gradually decreased proline content in leaves relative to control. So, the highest values resulted from untreated plants (HA_0). The highest values of proline content in leaves resulted from the longest irrigation interval (9 days) without humic acid (HA_0) treatment. The combination between the shortest irrigation interval (3 days) and highest level of humic acid (4%) produced the minimum values of proline content in leaves.

Table 3: Nitrogen (N), phosphorus (P), potassium (K) and sodium (Na) % of *Khaya senegalensis* seedlings as affected by irrigation intervals (days) and humic acid.) Average two seasons 2011 and 2012)

Characters	Leaves				Roots			
Treatments	N %	P%	K%	Na%	N%	P%	K%	Na%
Irrigation intervals (days)								
3	1.65	0.67	0.65	0.17	1.52	0.58	0.65	0.26
6	1.85	0.73	0.71	0.22	1.74	0.66	0.71	0.33
9	2.04	0.83	0.78	0.25	2.00	0.81	0.85	0.39
Humic acid %								
0	1.55	0.68	0.60	0.28	1.39	0.58	0.63	0.40
2	1.73	0.72	0.66	0.23	1.63	0.64	0.70	0.34
3	1.83	0.76	0.73	0.18	1.79	0.73	0.76	0.30
4	2.27	0.81	0.85	0.16	2.19	0.77	0.85	0.26
Interaction								
3 days+ HA 0%	1.35	0.59	0.55	0.21	1.12	0.43	0.53	0.31
3 days+ HA 2%	1.63	0.66	0.61	0.18	1.41	0.53	0.59	0.28
3 days+ HA 3%	1.66	0.69	0.67	0.15	1.63	0.65	0.69	0.23
3 days+ HA 4%	1.96	0.73	0.75	0.13	1.91	0.69	0.77	0.20
6 days+ HA 0%	1.53	0.67	0.59	0.29	1.37	0.57	0.59	0.41
6 days+ HA 2%	1.69	0.69	0.66	0.23	1.65	0.61	0.67	0.36
6 days+ HA 3%	1.85	0.75	0.73	0.18	1.80	0.71	0.73	0.30
6 days+ HA 4%	2.31	0.79	0.86	0.16	2.12	0.74	0.85	0.25
9 days+ HA 0%	1.76	0.77	0.67	0.33	1.69	0.75	0.78	0.47
9 days+ HA 2%	1.88	0.80	0.71	0.27	1.83	0.78	0.83	0.39
9 days+ HA 3%	1.99	0.85	0.79	0.22	1.95	0.83	0.86	0.37
9 days+ HA 4%	2.53	0.91	0.95	0.16	2.53	0.87	0.93	0.32

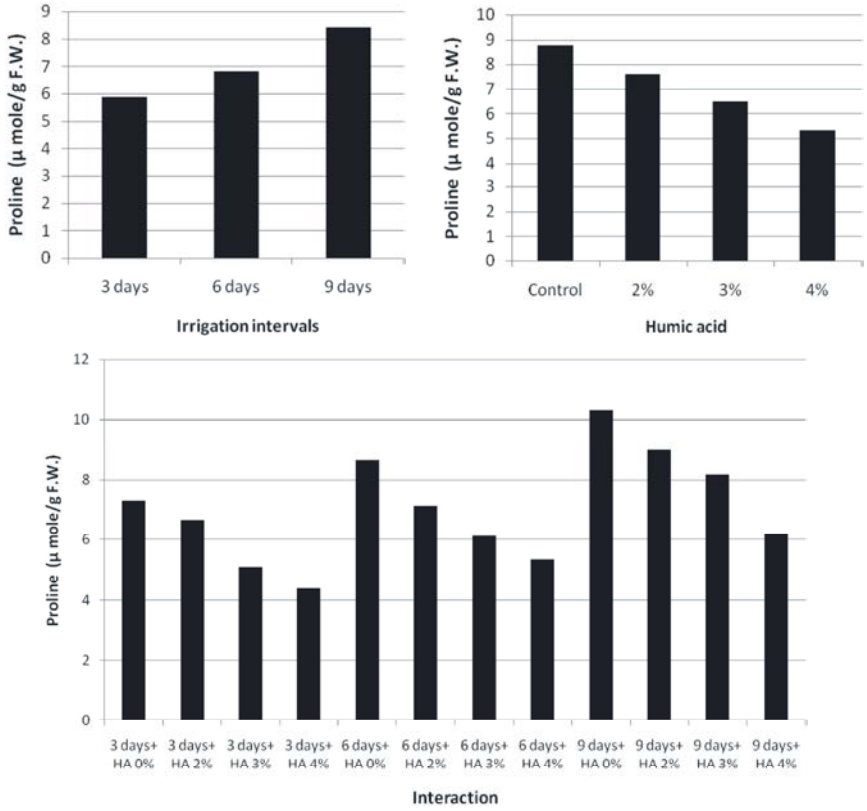

Fig. 1: Proline (µmole/g F.W.) in leaves of *Khaya senegalensis* seedlings as affected by irrigation intervals (days) and humic acid. (Average two seasons 2011 and 2012)

CONCLUSION

The results indicated that humic acid treatments can ameliorate the harmful effects of water stress by increasing vegetative growth and minerals content, thus inducing drought tolerance in *Khaya senegalensis* seedlings. The beneficial effects of humic acid on plant growth may be related to their indirect (increase of fertilizer efficiency), or direct (improvement of the overall biomass) effects.

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