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Effect of Mycorrhizal Fungi Inoculation and Humic Acid on Vegetative Growth and Chemical Composition of *Acacia saligna* Labill. Seedlings under Different Irrigation Intervals

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Abstract: A pot experiment was conducted to investigate the effect of mycorrhizal fungi inoculation and humic acid on growth, chlorophylls and total carbohydrates content of *Acacia saligna* Labill. seedlings under different irrigation intervals. The seedlings were subjected to four irrigation intervals (I1, I2, I3 and I4) where the seedlings were irrigated every 3or 5days (I1), every 5or8 days(I2), every 7or11days (I3) and every 9or 14 days(I4) according to the climatic conditions. Results showed that 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. Mycorrhizal fungi inoculation had significantly positive effects than humic acid on plant height, stem diameter, leaf area, fresh and dry weights of stems and roots, chlorophyll content and total carbohydrates content in all seedling parts, while humic acid treatment gave the highest fresh and dry weights of leaves under all irrigation treatments. The irrigation treatment I1 combined with humic acid gave the highest values of plant height, fresh and dry weights of leaves. While mycorrhizal inoculation combined with I1 or I2 gave the highest values of leaf area, stem and roots fresh and dry weights, chlorophyll a, b, carotenoids content and total carbohydrates content in leaves and stems. The highest total carbohydrates in the roots was obtained with humic acid treatment combined with irrigation interval I3.

Key words: *Acacia saligna* · Irrigation intervals · Mycorrhizal inoculation · Humic acid · Plant growth · Chemical composition

INTRODUCTION

Acacia saligna Labill. belongs to family *Mimosaceae***.** It is a dense, evergreen shrub or small tree, usually 2–5 m tall. It is native to the Southwestern corner of Western Australia and planted in many countries in Asia and Africa. It is tolerant to drought, alkalinity and salinity. It has been used as an ornamental species, for shade, fodder and firewood, for erosion control, sand dune stabilization, reduction of nutrient runoff and as a windbreak, It improves the soil through nitrogen fixation. In many arid and semiarid regions of the world, drought is considered probably the most important factor limiting crop productivity [1]. It reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ions uptake, carbohydrates, nutrient metabolism and growth promoters [2]. Plants under drought condition change their metabolism by decreasing water loss or by increasing water absorption and the morphological and physiological adaptations. Prior researches [3-8] conducted reductions in total biomass, biomass of leaves, stems and roots, leaf area, seedling height and stem diameter under water stress conditions. Water stress reduces the total dry weight of plant [9, 10]. Generally, the dry weight of leaves stems and roots of seedlings in most ornamental plants species decreases with increasing the interval between irrigations. Seedling height, dry biomass, leaf number, leaf area decreases significantly with increasing water stress [11-14]. Soha and Atef [15] on *Hibiscus subdariffa* subjected seedlings to different soil moisture stress levels (70%, 50% and 30% depletion of the available soil water). They found that the lowest significant means of all growth parameters observed under the lowest soil moisture level, except for number of branches. chlorophylls (a and b) content increases with reducing availability of water [16-18]. Mazhar *et al*. [13] on *Jatropha curcas,* found that the chlorophyll a, b and carotenoids were increased as soil moisture content increased. Total soluble carbohydrates decreased as the

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water stress increased [9, 19]. Khalid [10] on *Ocimum americanum* and *O. basilicum* found that the total carbohydrates increased under water stress.

Vesicular arbuscular mycorrhizal (VAM) fungi are important in sustainable agriculture because they improve plant water relations and thus increase the drought resistance of host plants, they improve disease resistance and they increase mineral uptake by increased acquisition of phosphorus and other low mobile mineral nutrients, which reduce the use of fertilizers. It is a well documented fact that Arbuscular Mycorrhizal Fungi can improve the drought resistance of host plants under ample water and drought stress conditions [20]. Humic acid (HA) is one of the major components of humus. Humates have long been used as a soil conditioner, fertilizer and soil supplement, humic acid can be used as growth regulate-hormone improve plant growth and enhance stress tolerance [21]. Morte *et al*. [22] on *Pinus halepensis,* reported that inoculation seedling with mycorrhizal fungi increased height, biomass, number of shoots, total dry mass and chlorophyll content more than nonmycorrhizal plant. The inoculated seedlings had higher stem growth, chlorophyll content and leaf number than those without inoculation under two weeks irrigation. Oyun *et al*. [23] on *Acacia Senegal* and Morte *et al*. [24] on *Helianthemum almeriense* stated that the chlorophyll content, fresh weight and leaf area were higher in mycorrhizal plants than in non-mycorrhizal plants, but differences were significant only under draught stress conditions. Kung'u *et al*. [25] on *Senna spectabilis* grown under drought conditions, stated that inoculating plants increased total shoot length, root collar diameter, shoot dry weight, root dry weight and plant leaves number and improved its drought resistance. Wu and Zou [26] on *Poncirus trifoliate,*found that the Mycorrhizal seedlings had greater growth characteristics in either non-drought stress or under drought stress conditions. Manoharan *et al*. [27] on *Cassia siamea, Delonix regia, Erythrina variegata, Samanea saman* and *Sterculia foetida*, showed that the contents of chlorophyll a, chlorophyll b, total chlorophylls and carotenoid increased in mycorrhizal seedlings compared with non-mycorrhizal tree seedlings. Nikbakht *et al*. [28] on *Gerbera jamesonii* found that the application of humic acid increased fresh and dry weights of roots, but had no significant effect on leaf fresh and dry weights. Application of HA increased plant height, leaves number, stem thickness, fresh and dry weights of leaves, stems and roots in comparison to control [29-31]. Ferrara *et al*. [32] on grape, found that treated with HA increased grapevine shoot growth and increased chlorophyll contents in the leaves.

The aim of the search is to investigate the effects of humic acid and inoculation with mycorrhizal fungi on growth and chemical composition of *Acacia saligna* Labill. under drought conditions.

MATERIALS AND METHODS

This study was carried out at the Experimental Nursery of Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, during the period from June 2010 to October 2011(16 months). The main objective of this study was to investigate the effects of both humic acid and inoculation with mycorrhizal fungi on growth under drought condition on *A. saligna* Labill. One year-old seedlings of *A. saligna* were obtained from the local market. The seedlings were planted on 27th June 2010 in plastic pots 30 cm in diameter (one plant/pot), the average height of seedlings were 79 cm), each pot filed with a mixture of sand: clay (1: 1 v/v). A plastic sheet were put under the pots. After planting the seedlings were left for one month and irrigated every three days then the irrigation intervals applied from August as following: the first interval irrigation (I1) the treatment irrigated every 3 or 5days, the three days interval started from August to 6th November 2010, the five days interval started from7th November 2010 to 30th March 2011 and three days from 30th March to September 2011, the second interval irrigation (I2) irrigated every 5or 8days, the third interval irrigation (I3) irrigated every 7 or 11days and the fourth interval irrigation(I4) irrigated every 9 or 14days as the same time as of the first interval.

Mycorrhizal spores suspension (10^{5}spores/ml) of *Glomus sp.* was obtained from the Unit of Biofertilizers, Faculty of Agriculture, Ain Sham University. It was used twice at the same rate of 2.5 ml/pot in mycorrhizal treatments. Humic acid (powder) were obtained from local market, it used at 4gm/pot at 21 days intervals in humic acid treatments. The following data were recorded: Plant height (cm), stem diameter (mm), leaf area (cm^{2}), fresh and dry weights of leaves, stems and roots (g). The following chemical analysis was determined: Chlorophyll a, b and carotenoids contents were determined according to Saric *et al*. [33]. Total carbohydrates in dried leaves, stems and roots were determined according to Herbert *et al*. [34]. The layout of the experiment was split plot design, where the irrigation intervals were chosen as main plots and the treatments of mycorrhiza and humic were the subplots and were arranged in a complete randomized design where the treatment were replicated three times (5 pots in each replicate). The differences between the means of the different treatments were compared by using L.S.D test at 5% probability, according to Snedecor and Cochran [35].

Table 1: Effect of mycorrhizal inoculation and humic acid on plant height, stem diameter and leaf area of *A. saligna* under different irrigation intervals

	Plant height (cm)					Stem diameter (mm)					Leaf area (cm^2)				
	Irrigation intervals (I)					Irrigation intervals (I)					Irrigation intervals (I)				
Treatments (T)	I1	I2	I3	I4	Mean	I1	I2	I3	I4	Mean	I1	I2	I3	I4	Mean
Control	125.82	120.33	110.44	112.76	117.34	16.52	16.13	15.76	14.69	15.78	20.35	21.77	20.87	20.97	20.99
Mycorrhiza	134.22	141.53	125.92	128.20	132.47	18.40	19.91	16.89	16.06	17.81	25.77	25.05	25.23	22.00	24.51
Humic acid	146.25	125.85	120.22	127.83	130.04	17.89	18.22	16.65	16.60	17.34	24.32	24.16	23.59	22.42	23.62
Mean	135.43	129.24	118.86	122.93	126.62	17.60	18.09	16.44	15.78	16.98	23.48	23.66	23.23	21.80	23.04
LSD at 5% for (I)	4.24					0.84					1.72				
For (T)	4.53					0.67					1.60				
For (I)*(T)	9.06					1.33					3.20				

Table 2: Effect of mycorrhizal inoculation and humic acid treatments on leaves, stems and roots fresh weight of *A. saligna* under different irrigation intervals

	Leaves fresh weight (g)					Stems fresh weight (g)					Roots fresh weight (g)				
	Irrigation intervals (I)					Irrigation intervals (I)					Irrigation intervals (I)				
Treatments (T)	I1	I2	I3	I4	Mean	I1	I2	I3	I4	Mean	I1	I2	I3	I4	Mean
Control	45.38	40.44	34.19	25.72	36.43	63.54	43.65	59.19	39.70	51.52	36.72	32.67	30.83	28.30	32.13
Mycorrhiza	50.10	43.62	47.05	30.10	42.72	72.54	76.47	46.93	40.02	58.99	48.48	53.01	38.32	29.44	42.31
Humic acid	59.50	46.31	43.29	36.40	46.37	65.62	65.53	50.71	42.60	56.12	50.63	49.03	30.70	31.40	40.44
Mean	51.66	43.46	41.51	30.74	41.84	67.23	61.88	52.27	40.77	55.54	45.28	44.90	33.28	29.71	38.29
LSD at 5% for (I)	6.75					5.41					7.88				
For (T)	4.99					3.55					3.21				
For (I)*(T)	9.99					7.09					6.41				

Table 3: Effect of mycorrhizal inoculation and humic acid on leaves, stems and roots dry weight of *A. saligna* under different irrigation intervls

	Leaves dry weight (g)					Stems dry weight (g)					Roots dry weight (g)				
	Irrigation intervals(I)					Irrigation intervals(I)					Irrigation intervals(I)				
Treatments (T)	I1	I2	I3	I4	Mean	I1	I2	I3	I4	Mean	I1	I2	I3	I4	Mean
Control	13.05	15.26	12.62	7.91	12.21	25.22	16.88	24.40	15.92	20.60	16.91	11.19	13.28	11.94	13.33
Mycorrhiza	17.58	11.01	15.00	8.89	13.12	31.65	34.00	19.92	15.29	25.21	22.98	22.30	17.49	11.57	18.59
Humic acid	21.26	18.98	14.10	12.20	16.63	27.03	27.30	23.39	17.00	23.68	21.12	21.10	11.51	13.71	16.86
Mean	17.29	15.08	13.91	9.67	13.99	27.96	26.06	22.57	16.07	23.17	20.34	18.20	14.09	12.41	16.26
LSD at 5% for (I)	1.90					1.96					2.20				
For (T)	1.25					1.73					1.50				
For (I)*(T)	2.49					3.46					3.00				

RESULTS AND DISCUSSION

Plant Growth: The results obtained in Tables 1- 3 showed that there were significant differences between irrigation intervals, the highest values for plant height were obtained with treatment I1, while the highest values of stem diameter and leaf area were obtained with I2 irrigation treatment. Furthermore, fresh and dry weights of leaves, stems and roots were significantly decreased with increasing irrigation intervals. Prolonging irrigation intervals reduced the abovementioned growth parameters. These results are in accordance with previous studies [3-6].

The treatments of mycorrhizal inoculation and humic acid significantly increased plant growth parameters including plant height, stem diameter, leaf area and fresh and dry weights of leaves, stems and roots. But the mycorrhizal inoculation had better effects than humic acids in all growth parameters studied, except for fresh and dry weights of leaves. The positive effects of mycorrhizal inoculation and humic acids on plant growth were also confirmed by prior studies [22, 28, 29, 32].

Interaction between irrigation intervals and mycorrhizal inoculation and humic acid had a significant effect on growth parameters. The combined treatment of I1with humic acid gave the highest values of plant height, fresh and dry weight of leaves. While the treatment mycorrhizal inoculation combined with I1 gave the highest values of leaf area, stem fresh and dry weights and roots dry weight compared with irrigation at the longest interval and control which gave the lowest values.

Table 4: Effect of mycorrhizal inoculation and humic acid photosynthetic pigments in fresh leaves (mg/g fresh weight) of *A. saligna* under different irrigation intervals

	Chlorophyll A (mg/g fresh weight)					Chlorophyll b (mg/g fresh weight)					Carotine (mg/g fresh weight)				
	Irrigation intervals(I)					Irrigation intervals(I)					Irrigation intervals(I)				
Treatments (T)	I1	I2	I3	I4	Mean (I)	I1	I2	I3	I4	Mean (I)	I1	I2	I3	I4	Mean (I)
Control	0.944	1.112	1.145	1.120	1.080	0.155	0.258	0.253	0.164	0.208	0.332	0.401	0.451	0.631	0.454
Mycorrhiza	1.445	1.814	1.242	1.162	1.416	0.215	0.303	0.219	0.128	0.216	1.325	1.242	0.741	0.684	0.998
Humic acid	1.230	1.528	1.169	1.133	1.265	0.245	0.261	0.210	0.156	0.218	0.456	0.339	0.316	0.423	0.384
Mean (T)	1.206	1.484	1.185	1.138	1.254	0.205	0.274	0.228	0.149	0.214	0.705	0.661	0.503	0.579	0.612
LSD at 5% for (I)	0.036					0.115						0.365			
for (T)	0.027					0.009						0.009			
for (I)*(T)	0.055					0.017						0.017			

Table 5: Effect of mycorrhizal inoculation and humic acid on total carbohydrates contents (% DM) in leaves, stems androots of *A. saligna* under different irrigation intervals

		Total carbohydrates (% DM)				
		Irrigation intervals(I)				
Seedling parts	Treatments (T)	I1	I2	I3	I4	Mean
Leaves	Control	26.89	21.69	19.45	22.82	22.71
	Mycorrhiza	28.28	38.37	21.83	27.04	28.88
	Humic acid	30.67	22.93	28.32	29.26	27.79
	Mean	28.61	27.66	23.20	26.37	26.46
Stems	Control	28.45	23.32	22.95	20.52	23.81
	Mycorrhiza	33.84	34.02	27.87	22.69	29.60
	Humic acid	30.45	29.35	23.84	22.09	26.43
	Mean	30.91	28.90	24.88	21.77	26.61
Roots	Control	21.85	23.35	25.78	20.65	22.91
	Mycorrhiza	33.06	36.52	27.04	24.59	30.30
	Humic acid	31.76	35.08	40.04	34.39	35.32
	Mean	28.89	31.65	30.95	26.55	29.51

Pigments Content: Data in Table 4 indicate that there were significant differences among the photosynthetic pigment contents in all treatments. Also, there were significant differences among the irrigation intervals on pigments content. The highest chlorophyll a and b contents were obtained when plants were treated with I2, while I1 gave the highest carotenoids content. Prolonging irrigation intervals significantly decreased pigments content. I4 irrigation treatment gave the lowest values of pigments. Similar results were obtained by Mazhar *et al.* [13] on *Jatropha curcas*.

There were significant differences in the photosynthetic pigments content among the treatments of mycorrhizal inoculation, humic acid and control. Chlorophyll a and b contents increased significantly in the mycorrhizal inoculation and humic acid treatments, but carotene content increased significantly in the mycorrhizal inoculation treatment and decreased significantly in the humic acid treatment. The highest values of chlorophyll a and carotenoids content were obtained due to mycorrhizal inoculation treatment. While the highest chlorophyll- b content was obtained due to humic acid treatment. Accordingly it can be stated that the mycorrhizal inoculation treatment was the most effective one for promoting the synthesis and accumulation of the three photosynthetic pigments. These results are in accordance with those obtained by Morte *et al.* [22] on *Pinus halepensis,* Oyun *et al.* [23] on *Acacia senegal* and Morte *et al.* [24] on *Helianthemum almeriense*. Also, the highest total chlorophylls content of asparagus was found in plants fertilized with humic acid substance. Ferrara *et al.* [32] on grape as well as Tejada and Gonzalez [36] obtained the same result.

The interaction between irrigation intervals and mycorrhizal inoculation as well as humic acid had a significant effect on pigments content. The irrigation treatment of I2 combined with mycorrhizal inoculation gave the highest chlorophylls a and b contents. While I1 treatment combined with mycorrhizal inoculation gave the highest carotenoids content.

Carbohydrates Content: The obtained data in Table 5 indicate that total carbohydrates content in the leaves and stems decreased as the irrigation intervals increased, but it increased in roots at I2 and I3, whereas it decreased at I4 in comparison to I1 treatment. Similar findings were obtained by Khalid [10] on *Ocimum americanum* and *O. basilicum*. Total soluble carbohydrates decreased as the water stress increased [9, 19].

The treatments of mycorrhizal inoculation and humic acids increased the total carbohydrates content in the leaves, stems and roots compared to the control. Mycorrhizal inoculation treatment gave the highest total carbohydrates content in leaves and stems, while humic acid treatment gave the highest value in roots. These positive increments in total carbohydrates content in leaves and roots due to mycorrhizal inoculation treatment is in harmony with El-Khateeb *et al*. [37] on *Chamaedorea elegans*, who found that was total carbohydrates content increased significantly with inoculation with mycorrhizal fungi. Also El-Khateeb *et al*. [38] on *Calia secundiflora,* found that total carbohydrates content increased with humic acid and mycorrhizal inucolation.

The combined effect of mycorrhizal inoculation and humic acids increased the total carbohydrates content in the leaves, stems and roots compared to the control at all irrigation intervals. The highest values of total carbohydrates content in the leaves and stems were obtained due to the mycorrhizal treatment combined with the irrigation treatment of I2. While the highest value of total carbohydrates in the roots was obtained with humic acid treatment combined with irrigation interval (I3).

CONCLUSION

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. Mycorrhizal fungi inoculation had significantly positive effects than humic acid on plant height, stem diameter, leaf area, fresh and dry weights of stems and roots, chlorophyll content and total carbohydrates content in all seedling parts, while humic acid treatment gave the highest fresh and dry weights of leaves under all irrigation treatments. The irrigation every 3or 5days combined with humic acid gave the highest values of plant height, fresh and dry weights of leaves. While mycorrhizal inoculation combined with irrigation every 3or 5days or every 5or 8 days gave the highest values of leaf area, stem and roots fresh and dry weights, chlorophyll a, b and carotenoids content and total carbohydrates content in leaves and stems. The highest total carbohydrate in the roots was obtained with humic acid treatment combined with irrigation interval every 7or11days.

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