

Effect of Foliar Spraying with Ascorbic Acid on Growth and Chemical Constituents of *Khaya senegalensis* Grown under Salt Condition

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Abstract: A pot experiment was conducted during 2004 and 2005 seasons in the National Research Centre, Dokki, Cairo, Egypt (Research and production station, Noharia). The aim of this work is to study the effect of foliar spraying with Ascorbic acid (0, 200 and 400 ppm) on growth and chemical constituents under three levels of salinity (1000, 2000 and 3000 ppm) and tap water served as control. Salinity treatments have a depressing effect on various growth parameters (i.e. stem length, stem diameter, root length, leaves number/plant, leaf area and fresh and dry weight of all plant organs). The same tendency was observed regarding total sugar, chlorophyll a, b, carotenoids content as well as the percentage and uptake of N, P and K. Such depressive effect was increasingly prominent with increasing salinity level. While, proline content and the percentage and uptake of Na increased by increasing salinity levels. On the contrary, all previous growth parameters and chemical constituents, except the percentage and uptake of Na, tended to increase by increasing the concentration of Ascorbic acid up to 400 ppm as compared to the untreated ones. It could be recommended to spray plants, grown in regions irrigated with saline water, with Ascorbic acid to overcome destructive effect of salinity.

Key words: Ascorbic acid · *Khaya senegalensis* · salt condition · growth · chemical constituents

INTRODUCTION

Khaya senegalensis is a loft West African American tree, related to the mahogany, which it resembles in the quality of the wood, widely grown as a street and timber tree and has the potential to cadonize disturbed tropical rainforests.

Salinity is considered a significant factor affecting plant production and agricultural sustainability in many regions of the world as it reduces the value and productivity of the affected land. Salinity mainly occurs in arid and semiarid conditions where the precipitation is not enough to leach the excess soluble salts from the root zone. Salinity problems can also occur in irrigated agriculture, particularly when poor quality water is used for irrigation. Shehata *et al.* [1] on *Ficus nitida*, Bondok *et al.* [2] on peach, EL-Mahrouk *et al.* [3] on *Thevitia nereifolia*, Roussos and Pontikis [4] on *Simmondsia chinensis* and Azza *et al.* [5] on *Sesbainia aegyptiaca* reported that salinity caused reduction in seedlings height, stem diameter, number of leaves/plant, fresh and dry weights of stem, leaves and roots. This

reduction increased by increasing salinity levels. Asmaael [6] on *Casuarina equisetifolia*, Abd El-Fattah [7] on *Adhatoda*, *Hibiscus* and *Phyllanthus shrus* and Azza *et al.* [5] *Sesbania aegyptiaca* declared that saline water decreased the various chemical constituents such as chlorophyll a and b, soluble and non sugar and N, P and K percentage but increased Na percentage. Talaat [8] on sweet pepper and Azza *et al.* [5] on *Sesbania aegyptiaca* found that proline gradually decreased by increasing salinity levels.

Ascorbic acid is currently considered to be regulators on plant growth and development owing to their effects on cell division and differentiation. Moreover the changes in the level of ascorbic acid in response to ionic stress might be important in the regulation of the ionic environment within the cell. Hanafy Ahmed [9] on lettuce, Tarraf *et al.* [10] on lemongrass and found that foliar application of Asc. had positive effects on growth parameters. Golan-Goldhirsh *et al.* [11] on soybean indicated that, plants treated with Asc. showed increases in photosynthesis processes. Biacs *et al.* [12] on tomato stated that sugar content was increased by

foliar spray of Asc. Singh *et al.* [13] on *Cassia augustifolia* and Talaat [8] on sweet pepper detected that Asc. foliar application increased the content of macronutrients (N, P and K).

Regarding the interaction, Shaddad *et al.* [14] on both lupine and broad bean and Talaat [8] on sweet pepper, mentioned that Asc. counteracted the suppressive effect of the higher salinity levels on seedlings growth. Also, the interaction showed a markedly decreases in Na concentration, while, total carbohydrates, N, P and K percentage increased. Therefore, the aim of this investigation is to study the influence of Ascorbic acid application on growth and chemical constituents of *Khaya senegalensis* seedlings irrigated with saline water.

MATERIALS AND METHODS

The experimental trials were carried out 2004 and 2005 seasons at National Research Centre (Research and production station, Nobarria). It was intended to find out the individual and combined effect of different levels of salinity and ascorbic acid doses as foliar application on growth and chemical constituents of *Khaya senegalensis* seedlings.

Plant material and procedure: One year old seedlings (15-20 cm height) were planted on the 15th of March each season, as one seedling / pot 30 cm diameter filled with ten kg sandy soil + compost. Three salinity levels were prepared (1000, 2000 and 3000 ppm) by adding sodium chloride for irrigating seedlings with previously prepared salinized. The untreated plants (control) were irrigated with tap water. 250 ml of water was added to each pot twice a week throughout the course of the study (6 months). On the 10th of April the seedlings were sprayed monthly intervals by three concentrations of Ascorbic acid namely (0, 200 and 400 ppm). The statistical layout of the experiment was a Completely Randomized in factorial experiments Design of 2 factors (3 Ascorbic acid x 4 salinity concentrations) each treatment included 6 replicates. Starting from March until one month before ending experiment, the seedlings received NPK (4.0 g ammonium nitrate 33.5% N, 4.0 g calcium super phosphate 15% P₂O₅ and 2.0 g potassium sulphate 48.5 K₂O pot in four doses. The following data were recorded, stem length (cm), stem diameter (mm), root length (cm), number of leaves/plant, leaf area and fresh and dry weight (g) of plant organs. All previous data were subjected to statistical analysis according to procedure outlined by Snedecor and Cochran [15]. Treatments means were

compared by L.S.D. test and the combined analysis of the two seasons was calculated according to the method of Steel and Torrie [16]. Chlorophyll (a, b) and carotenoids contents were determined in fresh leaves according to Saric *et al.* [17]. Total sugar percentage was determined in dry leaves according to Dubois *et al.* [18]. The proline concentration was determined by using dry material (leaves) according to Snell and Snell [19]. Nitrogen, phosphorus, potassium and sodium were determined according to the method described by Cottenie *et al.* [20].

RESULTS AND DISCUSSION

Growth characters: The growth parameters as affected by saline water irrigation treatments are showed in Tables 1-4. However, all growth parameters, stem length, stem diameter, root length, number of leaves/plant, leaf area and fresh and dry weight of all plant organs were reduced by irrigation with different levels of saline water as compared with the control plants. The reduction in stem length might be due to that salinity which decreased each of cell division, cell elongation and meristemic activity [21, 22]. Also, under salinity conditions, reduction of leaves number/plant might cause a disturbance in natural hormones leading to unbalanced growth of the plant. Bernstien *et al.* [23] found that, the decrease in root length due to salinity treatments might be attributed to the inhibition in cell division and/or cell enlargement caused by salinity. Also, the effect of high salt concentration in the rooting media on growth might be due to an osmotic inhibition of water absorption, specific ions concentration in the saline media, or a combination of both. Moreover, the decrease in fresh and dry weight of all plant organs due to the Cl or Na accumulation in leaves might cause injury by interfering with normal stomatal closure causing excessive water loss and leaf injury symptoms like those of drought and CO₂ fixation might be reduced under high level of salinity which led to lower metabolism. In this respect, such decrease in fresh and dry weight of stem might be due to the inhibition of water absorption and/or distribution of mineral balance and/or absorption and utilization under salinity conditions. In the same time, the decrease in fresh and dry weights of root might be due to the reduction in water and minerals absorption and/or the reduction in upper ground growth under salinity conditions. Similar results were obtained by Roussos and Pontikis [4] and Azza *et al.* [5]. Concerning the effect of Ascorbic acid in growth parameters, data in Tables 1-4 reveal that using Ascorbic acid as a foliar application at 200 or 400 ppm had

Table 1: Stem length (cm), stem diameter (mm) and root length (cm) of *Khaya senegalensis* as affected by salinity and Ascorbic acid (combined data of two seasons 2004 and 2005)

Characters	Ascorbic (ppm) (B)											
	Stem length (cm)				Stem diameter (mm)				Root length (cm)			
	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean
Salinity (ppm) (A)												
Control	42.90	50.67	54.25	49.27	12.10	14.70	15.50	14.10	24.77	35.00	37.00	32.26
1000	42.30	45.00	49.97	45.76	11.70	14.00	14.30	13.30	23.40	33.50	34.67	30.52
2000	37.90	39.83	48.33	42.02	11.00	13.00	13.30	12.40	22.73	30.83	31.73	28.43
3000	33.27	35.60	44.57	37.81	10.00	12.00	12.70	11.60	17.47	24.90	28.10	23.49
Mean	39.09	42.78	49.28		11.20	13.40	14.00		22.09	31.06	32.28	
L.S.D. 5%												
A				3.05				0.26				2.64
B				2.49				0.21				2.15
(A) x (B)				4.32				0.37				3.73

Table 2: Leaves number/plant and leaf area of *khaya senegalensis* as affected by salinity and Ascorbic acid (combined data of two seasons 2004 and 2005)

Charcters	Ascorbic acid (ppm) [B]							
	Leaves number/plant				Leaf area (cm ²)			
	0	200	400	Mean	0	200	400	Mean
Salinity (ppm)								
Control	13.30	21.00	21.67	18.67	10.23	14.53	15.53	13.43
1000	12.70	18.67	20.00	17.11	9.60	9.60	13.49	112.11
2000	11.00	16.00	17.67	14.89	9.34	11.93	13.23	11.50
3000	8.67	13.67	14.67	12.34	7.00	9.86	11.67	9.51
Mean	11.40	17.34	18.50		9.04	12.39	13.48	
L.S.D. 5%								
(A)				1.60				0.90
(B)				1.31				0.74
(A) x (B)				2.26				1.28

Table 3: Fresh weight of stem, leaves and root (g) of *Khaya senegalensis* as affected by salinity and Ascorbic acid (combined data of two seasons 2004 and 2005)

Characters	Ascorbic (ppm)											
	Stem				Leaves				Roots			
	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean
Salinity (ppm)												
Control	33.30	38.77	50.44	40.83	31.7	48.38	54.64	44.91	31.65	46.53	48.45	42.20
1000	25.50	33.67	43.85	34.34	20.4	26.40	39.80	28.87	30.43	38.67	44.10	37.70
2000	25.10	27.00	38.07	30.06	17.35	23.47	36.62	25.81	27.40	38.60	43.67	36.60
3000	23.10	26.30	29.56	26.31	14.67	21.67	29.73	22.02	25.13	34.90	37.60	32.50
Mean	26.70	31.44	40.48		21.03	29.98	40.20		28.65	39.68	43.60	
L.S.D. 5%												
(A)				2.53				2.17				1.68
(B)				2.07				1.77				1.37
(A) x (B)				3.58				3.07				2.37

Table 4: Dry weight of stem, leaves and root (g) of *Khaya senegalensis* as affected by salinity and Ascorbic acid (combined data of two seasons 2004 and 2005)

Characters	Ascorbic (ppm)											
	Stem				Leaves				Roots			
	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean
Salinity (ppm)	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean
Control	15.80	19.16	24.96	19.97	12.04	19.09	21.73	17.62	16.80	25.30	26.50	22.90
1000	11.90	16.58	21.18	16.55	7.43	10.15	15.46	11.01	17.90	20.70	23.80	20.80
2000	11.80	12.77	18.04	14.20	6.34	8.78	13.89	9.67	14.40	20.60	23.50	19.50
3000	10.70	12.43	13.63	12.27	5.39	8.10	11.20	8.23	13.10	18.70	20.10	17.30
Mean	12.60	15.24	19.45		7.80	11.53	15.57		15.55	16.18	23.48	
L.S.D. 5%												
(A)				1.31				0.70				0.82
(B)				1.07				0.57				0.67
(A) x (B)				2.62				0.99				1.16

Table 5: Chlorophyll a, b and carotenoids (mg g⁻¹ F.W.) of *Khaya senegalensis* as affected by salinity and Ascorbic acid (combined data of two seasons 2004 and 2005)

Characters	Ascorbic acid (ppm)											
	Chrophyll a				Chrophyll b				Carotenoids			
	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean
Salinity (ppm)	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean
Control	0.340	0.356	0.399	0.365	0.165	0.176	0.184	0.175	0.590	0.890	0.910	0.800
1000	0.280	0.339	0.200	0.318	0.118	0.149	0.160	0.142	0.380	0.560	0.580	0.510
2000	0.270	0.283	0.316	0.288	0.117	0.144	0.145	0.135	0.350	0.470	0.490	0.440
3000	0.220	0.780	0.283	0.261	0.115	0.120	0.121	0.119	0.330	0.420	0.460	0.400
Mean	0.280	0.314	0.335		0.129	0.147	0.153		0.413	0.585	0.610	

a significant favourable effect on all growth parameters of *Khaya senegalensis* seedlings during the two growing seasons. The same trend was also found by Tarraf *et al.* [10] and Talaat [8]. In this respect, Smirnoff [24] mentioned that ascorbate has been implicated in regulation of cell division. In this connection, the author pointed out that cell wall ascorbate and cell wall localized ascorbate oxidase has been implicated in control of growth, high ascorbate oxidase activity is associated with rapidly expanding cells. Accordingly, these increments in growth parameters by Asc. treatments might be attributed to the postulation of Shaddad *et al.* [14] who assumed that the effect of Asc. on plant growth may be due to the substantial role of Asc. in many metabolic and physiological processes. Under irrigation with saline water conditions, spraying 200 and 400 ppm Asc. increased all growth parameters. In this respect, it can be assumed that the depressive effects of salinity on plant growth and other relevant physiological activities can be alleviated and/or modified to some extent, by spraying

plants by the appropriate concentrations of Asc. These results are in line with those obtained by Shaddad *et al.* [14] who mentioned that Asc counteracted the adverse effect of salinity on plant growth as well as on some metabolic mechanisms in the plants.

Chemical composition:

Photosynthetic pigments: It is clear from Table 5 that, increase of salt concentration in the irrigation water gradually decreased the content of photosynthetic pigments (chlorophyll a, b and carotenoids). These results are in agreement with those obtained by Patil and Potil [25] and Batanouny *et al.* [26] mentioned that the lowered photosynthetic ability under salt stress condition was due to stomata closure, inhibition of chlorophyll synthesis, a decrease of carboxylase and due to high chlorophyllase activity.

As for the effect of Asc on all photosynthetic pigments, Asc. acid at both used rates caused an increase in chlorophyll a, b and carotenoides content as compared

Table 6: Total sugar percentage and proline content of *khaya senegalensis* as affected by Ascorbic acid and salinity (combined data of two seasons 2004 and 2005)

Charcters	Ascorbic acid (ppm)							
	Total sugar %				Proline (μmg^{-1})			
	0	200	400	Mean	0	200	400	Mean
Salinity (ppm)	0	200	400	Mean	0	200	400	Mean
Control	18.10	18.44	18.63	18.38	0.22	0.29	0.34	0.28
1000	15.10	17.26	17.39	16.58	0.37	0.43	0.47	0.42
2000	14.70	16.56	16.62	15.95	0.39	0.55	0.58	0.51
3000	13.10	15.20	16.21	14.83	0.57	0.63	0.86	0.69
Mean	15.20	16.87	17.21		0.39	0.48	0.56	

Table 7: Nitrogen, phosphorous, potassium and sodium percentage of *Khaya senegalensis* as affected by salinity and Ascorbic acid (combined data of two seasons 2004 and 2005)

Caharcters	Ascorbic acid (ppm)															
	N %				P %				K %				Na%			
	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean
Salinity (ppm)	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean
Control	0.84	0.91	0.97	0.91	0.413	0.665	1.117	0.732	3.95	4.17	4.48	4.23	0.10	0.06	0.03	0.06
1000	0.73	0.85	0.91	0.83	0.387	0.556	0.882	0.603	3.35	3.51	3.75	3.54	0.18	0.12	0.05	0.12
2000	0.66	0.78	0.84	0.76	0.382	0.502	0.828	0.571	2.92	3.19	3.33	3.15	0.22	0.15	0.07	0.15
3000	0.62	0.66	0.80	0.69	0.283	0.382	0.615	0.427	2.29	2.71	2.89	2.63	0.28	0.18	0.11	0.09
Mean	0.71	0.80	0.88		0.366	0.526	0.861		3.13	3.40	3.64		0.20	0.13	0.07	

Table 8: Nitrogen, phosphorous, potassium and sodium uptake of *Khaya senegalensis* as affected by salinity and Ascorbic acid (combined data of two seasons 2004 and 2005)

Caharcters	Ascorbic acid (ppm)															
	N				P				K				Na			
	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean
Salinity (ppm)	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean	0	200	400	Mean
Control	101.00	173.73	210.78	161.90	49.73	127.00	242.70	139.80	475.58	796.10	995.23	756.00	12.00	11.45	6.52	10.00
1000	54.20	86.28	140.69	93.74	28.75	56.43	136.40	73.85	248.91	340.00	579.75	390.00	13.40	12.18	7.73	11.09
2000	41.80	68.48	116.68	75.67	24.22	44.08	115.00	61.10	185.13	280.10	462.54	309.00	14.00	13.17	9.72	12.28
3000	33.40	53.46	89.60	58.83	15.25	30.94	42.78	29.65	123.43	219.50	323.68	222.00	15.10	14.58	12.32	14.00
Mean	57.70	95.49	139.44		29.49	64.60	134.20		258.26	408.90	590.30		13.60	12.85	9.07	

with the untreated seedlings. The interaction between two factors (Salinity x Asc) was increased all photosynthetic pigments content compared with using each alone.

Total sugar percentage: The results of total sugar percentage presented in Table 6 show that growing seedlings under salinity conditions led to a reduction in leaves total sugar percentage compared to control at the two seasons. This results agreed with those obtained by Bernstein *et al.* [23] suggested that, the obtained reduction total sugar percentage as salinity levels increasing, might have relation to respiration processes since the free sugar were the main sugar pattern involved in the mechanism of respiration.

As regarding the effect of Asc. foliar application on total sugar concentrations, it is clear from data that total sugar percentage in the two growing seasons, were

increased by using Asc. foliar application, especially by using the higher rate of Asc. (400ppm). In this manner, Smironoff [24] stated that ascorbate has a central role in photosynthesis, as the high concentration in chloroplasts would imply.

Regarding the effect of interaction, Asc applications were also effective on total sugar percentage of salinized water irrigated plant, total sugar percentage were greatly induced. These trends point out by Shaddad *et al.* [14] Asc. treatments under non-saline or saline conditions resulted in an increase in seedlings growth. This stimulatory effect was accompanied by the observed increases in carbohydrates.

Proline content: From the data given in Table 6 it can be concluded that, irrigation with saline water at all levels increased proline content in the leaves as compared with

the unstressed plants. Following this, Ackerson [27] argued that, cellular osmotic adjustment occurs in response to stress via an active or passive accumulation of solutes. It has been assumed that salt stress enhanced the production of proline, which causes osmotic adjustment [28]. With respect to the effect of Asc applications on proline contents, it is evident that detected an increase in the concentration of endogenous proline when treated with the two different levels of Asc. (200 or 400 ppm). In this connection, this enhancement in the proline concentration by the application of Asc. emphasizes the assumption of Anderson *et al.* [29] suggested that organic acids may be the principal C source for proline accumulation in stressed plants. The data of interaction effect among the two studied factors (level Asc rates) on proline content show that, high proline values were found when using Asc at a rate of 400 ppm combined with 300 ppm salinity.

Nutrients: Data of nutrients (N, P, K and Na) in the leaves as affected by different salinity levels and various Asc foliar application treatments in both seasons throughout experimental periods are presented in Tables 7 & 8. It is clear from data that increasing salinity levels reduced the percentage of N, P and K and the uptake in the two growing seasons. In close agreement with these results were the findings reported on *Sesbania aegyptiaca* [5]. In this connection, Hanafy Ahmed *et al.* [9] pointed out that salinization impaired N accumulation and incorporation into protein and raised total free amino acid accumulation in salinized plants. Also, it can be suggested that amino acids can act as components of salt tolerance mechanism and build up a favourable osmotic potential inside the cell in order to combat the effects of which replaced nitrate in the vacuoles. Furthermore, the reduction in P uptake under saline condition could be explained on the fact that Na salt raised the pH of the soil, which in turn reduced the availability of P to the plants [30]. In this context, Gomea *et al.* [31] found an increase in Na concentration and a decrease in K concentration in the leaves with salinity, this result may be due to a possible antagonism between K and Na. This antagonism could be due to the direct competition between K and Na at the site of ion uptake at plasmalemma. Data in Table 7 indicated that there was a gradual increase in the Na percentage and uptake in the leaves with increasing salt concentration in irrigation water. Accordingly, the increase in Na concentration in plant with salinity may be a result of the ability of plants to use Na to maintain an adequate osmotic potential gradient between the plant tissues and the external solution [32]. In this respect, Flowers *et al.* [33] noted that

osmotic adjustments are essential for a plant to survive in saline environments. As regarding the effect of Asc. foliar application on N, P, K concentration, it is to be noticed from the results that, the percentage and the uptake of N, P and K gradually increased by increasing Asc. levels compared with the untreated plant. This increment in N concentration by Asc. treatments could be explained by the findings of Talaat [8] who showed that the accumulation of nitrate by Asc. foliar application may be due to the positive effect of Asc. on root growth which consequently increased nitrate absorption. In this context, the increase in P concentration by Asc. treatments may be attribute to the postulation of Hanafy Ahmed *et al.* [9] who mentioned that foliar spray with Asc might increase the organic acids excreted from the roots into the soil and consequently increase the solubility of most nutrients which release slowly into the rhizosphere zone where it may be utilized by the plants. In addition, Hiatt and Lowe [34] mentioned that electrostatic binding of inorganic ions by organic ions such as organic acid is undoubtedly involved in the process of K-ions accumulation. On the other hand, *Khaya* seedlings exhibited a decrease in the percentage and uptake of Na ions when sprayed with the two different levels of Asc. (200 or 200 ppm) which declares the assumption of Hallberg *et al.* [35] who noted that the significant counteracting effect of Asc. has wide nutritional implications. Furthermore, the combination between salinity levels and Asc. levels were almost positive for the percentage and uptake of N, P and K. Therefore, it can be postulated that Asc. treatments might increase the rate of incorporation of free amino acid into protein [8]. Also, the protection of plant against salt stress by an exogenous supply of Asc. is believed to be caused indirectly as a result of its effect on K uptake which plays an essential role in many metabolic processes such as photosynthesis process and hence the formation of starch. On the other hand, the interaction between salinity levels and Asc. foliar application rates decreased Na percentage and uptake in leaves compared with untreated plants. Hence, it could be recommended to spray plants, grown in regions irrigated with saline water, with Ascorbic acid to overcome destructive effect of salinity.

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