Middle East Journal of Agriculture Research
ISSN 2077-4605
Volume : 06 | Issue : 03 | July-Sept. | 2017
Pages:757-765

Riboflavin minimizes the deleterious effects of salinity stress on growth, chloroplast pigments, free proline, activity of antioxidant enzyme catalase and leaf anatomy of *Tecoma capensis* (Thumb.) Lindl.

Mona A. Darwish[1], Rania M. A. Nassar[2], Nahed G. Abdel-Aziz[3] and Ahmed S. Abdel-Aal[3]

[1]*Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Giza, Egypt.*
[2]*Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt.*
[3]*Department of Ornamental Plants and Woody Trees, National Research Center, Giza, Egypt*.

Received: 29 June 2017 / Accepted: 31 July 2017 / Publication date: 28 August 2017

ABSTRACT

The present search was carried out through the two successive seasons of 2014 and 2015 in order to enhancement the growth of tecoma plants grown under different concentrations of salinity (2000, 4000, 8000 and 12000 ppm of salt mixture, NaCl:$CaCl_2$,1:1w/w) by foliar application of 2000 ppm riboflavin. Results revealed that increasing salt concentration in irrigation water induced significant reduction in tecoma growth and in concentrations of photosynthetic pigments in tecoma leaves. By contrast, concentration of free proline and activity of antioxidant enzyme catalase in tecoma leaves were increased significantly with increasing salt concentration in irrigation water up to 12000 ppm. Results also indicated that the thinner leaflets induced by salinity stress could be attributed mainly to the decrement induced in all included tissues (palisade and spongy tissues of the mesophyll, dimensions of midrib bundle, xylem rows/midrib bundle and vessel diameter). It is clear that spraying riboflavin at concentration of 2000ppm on salinized tecoma plants overcomes the harmful effects of salinity stress on growth, physiological characteristics and leaflets anatomy of tecoma plants exposed to salinity stress.

Key words: *Tecoma capensis*, Salinity, Growth, Photosynthetic pigments, Free proline, Catalse activity, Anatomy

Introduction

Tecoma capensis (Thumb.) Lindl., often called tecoma, is a member of the family Bignoniaceae. Tecoma is an erect fast growing, scrambling shrub which may grown up to 2-3 m in height and spread more than 2 m and normally evergreen. Leaves are pinnately compound comprised of 5-9 oblong leaflets. Flowers are grouped in terminal clusters, vary in colour from red, deep orange, yellow to salmon. It is an ornamental garden plant commonly used for screening and decorative purposes, used for hedging. The powdered bark of this attractive garden plant is used as a traditional medicine to relieve pain and sleeplessness (Thompson, 2001).

Such attractive shrub is also used as a ground cover on steep slopes or rocky banks. It is tolerant to salt stress and recommended to cultivated in areas assigned for landscape especially in newly reclaimed soils which considered salt-affected (Steve, 2003).

It is well established that salinity retarded growth and productivity in many plant species. In Egypt, one of the new strategies for facing salinity problem is the use of salt-tolerant species, especially woody plants, for cultivation in newly reclaimed soils. *Tecoma capensis* is one of the most promising shrubs in this concern.

Recently, the use of vitamins for counteracting the harmful effects of salinity stress on growth and productivity of many plant species is highly recommended. Vitamin contents of plants are known to show altered metabolism under the influence of salinity stress (Ratnakar and Rai, 2013). Riboflavin (vitamin B_2) has antioxidant nature and is essential for several oxidative processes occurring inside the cell. In this respect, Azooz (2009) stated that foliar application with 100 ppm riboflavin enhancing the resistant of *Hibiscus sabdariffa* to salinity stress and promoting growth of salinized plants.

Therefore, the present search is designed to disclose the impact of foliar spray with riboflavin on growth, chloroplast pigments, free proline, activity of antioxidant enzyme catalase and leaf

Corresponding Author: Mona A. Darwish, Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Giza, Egypt. E-mail: tantawe2006@hotmail.com

anatomy of *Tecoma capensis* plants grown under salinity stress hoping to counteracting the harmful effects of salinity stress on tecoma growth by foliar application with riboflavin.

Materials and methods

The present investigation was carried out at the Nursery of Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the two successive seasons of 2014 and 2015 in order to improve the growth of tecoma plants (*Tecomacapensis* (Thumb.) Lindl.) grown under different levels of salinity stress (2000, 4000, 8000 and 12000 ppm) by foliar application with riboflavin at concentration of 2000 ppm.

Source of plant material and riboflavin:

Uniform seedlings of tecoma, six months old and 20 cm height, were obtained from private farm at El-Qanater El-Khayreya, Qalyubia Governorate, Egypt and used as a plant material. Riboflavin, vitamin B_2, was obtained from Electro Sciente Company, Egypt. It is a powder contain 99.9% active ingredient.

Procedure of the experiment:

The obtained uniform seedlings of tecoma were transplanted to plastic pots (30 cm diameter), one seedling per pot, on fourth March of both studied seasons (2014 and 2015). Each pot was filled with about 9 kg of clean sand and compost at the ratio of 2:1 by weight and fertilized by NPK in form of kristalon which contained NPK (19:19:19). Kristalonwas obtained from Phayzon Company, Hollaand and used at the rate of 5g / pot added in three times through the whole growing season.

The experiment was made in a randomized complete block design with three replicates. The replicate contained 45 pots, each 5 pots were assigned for one treatment. The treatments were nine as follows:

1. Control, plants were irrigated with tap water.
2. Four levels of salinity in irrigation water; namely, 2000, 4000, 8000 and 12000 ppm of salt mixture (NaCl : $CaCl_2$, 1:1 w/w).
3. One level of riboflavin (2000 ppm) was applied on each of the tested four levels of salinity.

The pots were located under open field conditions. Each level of salinity in irrigation water was added regularly (750ml/pot/week) during the whole period of the experiment (eight months from transplanting). Irrigation treatments were applied four times with salinized water followed by one irrigation with tap water (for leaching the accumulated salts) and then repeated in the same manner till the end of the experiment.

In order to improve the growth of tecoma plants grown under stress of salinity in irrigation water, riboflavin at concentration of 2000 ppm was sprayed twice. The first spray was done after one month from transplanting and the second one was applied two months from the first spray. Tween-20 was added as a spreading agent for tested treatments.

Recording of data:

I- Vegetative growth characters:

At the end of the experiment in each of the two growing seasons (eight months from transplanting). Nine plants from each treatment, three from each replicate, were lifted from pots for recording the data of vegetative growth. The recorded data includes:

1. Plant height (cm).
2. Total number of compound leaves developed per plant.
3. Fresh weight of branches (g)/plant.
4. Fresh weight of leaves (g)/plant.
5. Fresh weight of shoots (g)/plant.

II- Physiological studies:

Photosynthetic pigments, free proline and activity of the antioxidant enzyme catalase were determined in leaves of treated and untreated plants at the age of five months from transplanting in the second growing season of 2015.

1- Photosynthetic pigments:

Chlorophyll a, chlorophyll b and carotenoids were extracted from fresh leaves by using dimethyl formamide and determined according to Nornai, (1982) as mg/g fresh weight (F.W.) of tecoma leaves.

2- Free proline:

Free proline was determined in fresh leaves according to the method described by Bates *et al*. (1973). Bush and Lomb spectrophotometer (model spectranic 2000) was used. The absorbance was measured at 520 nm. Free proline was estimated as mg/g fresh weight of tecoma leaves.

3- Activity of antioxidant enzyme catalase:

Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the consumption of H_2O_2 (NaKano and Asada, 1981). The reaction mixture consisted of 2.5mMTris-acetate buffer, pH 7.5, 0.8 mM Na-EDTA and 20mMH_2O_2 .The enzyme assay was performed at 25^o C. Catalase activity was expressed as A_{290}/min/g protein. Protein was estimated in crude enzyme extract by dye binding assay (Bradford, 1976).

III- Anatomical studies:

Specimens were taken from the terminal leaflet of the compound leaf developed on the median portion of the main stem of treated and untreated plants at the age of five months from transplanting of the second growing season.

Specimens were killed and fixed for one week in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The tested materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56^{o}C, sectioned to a thickness of 20 micrometers (μm), double stained with crystal violet-erythrosin, cleared in xylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998). Sections were read to detect histological manifestations of the noticeable responses resulting from tested treatments and photomicrographed.

Statistical analysis:

Data on vegetative growth characters, photosynthetic pigments, free proline and catalase activity were subjected to conventional methods of analysis of variance according to Snedecor and Cochran (1982). The least significant difference (LSD) at 0.05 level was calculated for each investigated character under different tested treatments.

Results and discussion

I- Vegetative growth characters:

Data on morphological characters of vegetative growth of tecoma plants as affected by salinity stress and sprayed with riboflavin in two successive seasons are given in Table (1). The studied characters of vegetative growth included height of tecoma plant, total number of compound leaves developed / plant, branches fresh weight /plant, leaves fresh weight/ plant and shoots fresh weight/plant at the age of eight months from transplanting in the two investigated seasons.

It is realized from Table (1) that all tested concentrations of salinity in irrigation water induced decrements in all investigated characters of vegetative growth in both studied seasons. The significant decrease was recorded at salinity level of 2000 ppm for tecoma height and at 4000 ppm for the reset characters in both studied seasons. Worthy to mention that increasing salt level retarded significantly all investigated characters and reached its maximum at salinity level of 12000 ppm in both growing seasons, being 35.7 and 36.5% for plant height , 40.4 and 38.3% for number of leaves /plant, 44.8 and 42.8% for fresh weight of branches, 42.9 and 39.0% for fresh weight of leaves/plant and 43.8 and 41.0% for fresh weight of shoots / plant less than those of the normal untreated plants in the first and second season; respectively.

Table 1: Mean values of growth characters of *Tecoma capensis* plants, aged eight months from transplanting, as affected by salinity stress and sprayed with riboflavin in two studied seasons of 2014 and 2015

Treatments	Vegetative growth characters				
	Plant height (cm)	Total number of leaves / plant	Branches fresh weight (g)/ plant	Leaves Fresh weight (g)/plant	Shoots fresh weight (g) / plant
First season (2014):					
Control	131.3	340.3	119.9	112.9	232.8
2000 ppm salinity	116.3	317.9	108.6	103.6	212.2
4000 ppm salinity	107.7	281.2	92.4	89.9	182.3
8000 ppm salinity	97.7	239.5	78.7	77.4	156.1
12000 ppm salinity	84.4	202.8	66.2	64.5	130.7
2000 ppm salinity +2000 ppm riboflavin	133.0	351.5	146.9	131.5	278.4
4000 ppm salinity +2000 ppm riboflavin	123.3	314.9	131.2	117.7	248.9
8000 ppm salinity +2000 ppm riboflavin	109.5	277.7	118.6	103.9	222.5
12000 ppm salinity +2000 ppm riboflavin	96.3	239.2	97.5	89.5	187.0
L.S.D. (0.05)	11.25	33.81	12.18	11.37	22.97
Second season (2015):					
Control	141.7	364.7	131.8	123.5	255.3
2000 ppm salinity	129.0	336.5	124.2	112.4	236.6
4000 ppm salinity	111.6	302.7	106.5	102.5	209.0
8000 ppm salinity	103.3	261.4	88.9	88.9	177.8
12000 ppm salinity	90.0	225.2	75.4	75.3	150.7
2000 ppm salinity +2000 ppm riboflavin	146.5	403.9	158.9	178.2	337.1
4000 ppm salinity +2000 ppm riboflavin	135.9	363.4	141.3	163.1	304.4
8000 ppm salinity +2000 ppm riboflavin	122.9	311.7	129.2	134.6	263.8
12000 ppm salinity +2000 ppm riboflavin	103.7	276.3	112.8	117.5	230.3
L.S.D. (0.05)	9.84	35.77	13.26	12.59	24.12

Similar results were also reported on other timber, ornamental and medicinal plants by Maximous and Abd-El-Dayem (1998), Reda (2007), Azooz (2009), Al-Shaharani and Shetta (2011), Hardikar and Pandey (2011), Langroudi and Sedaghathoor (2012), Ratnakar and Rai (2013), Farahat *et al*. (2013), Ivanova *et al*. (2013), Khafagy *et al*. (2013), Alam *et al*. (2014), Amirjani (2015), Shanan (2015) and Nassar *et al*. (2016).

Results also indicated that tecoma plants subjected to different levels of salinized water and sprayed with 2000 ppm riboflavin showed better growth behavior than those unsprayed with riboflavin. Riboflavin overcomes the deleterious effects of salinity stress on tecoma growth. In this connection, Azooz (2009) stated that foliar application with 100 ppm riboflavin enhancing the resistant of *Hibiscus sabdariffa* L. to salinity stress by promoting growth of salinized plants, being in agreement with our findings.

II- Physiological investigations:

Chloroplast pigments, free proline and activity of antioxidant enzyme catalase were determined in leaves of salinized and unsalinized tecoma plants aged five months from transplanting in the second investigated season 2015. Results on these physiological aspects are shown in Table (2).

1- Photosynthetic pigments:

It is noted from Table (2) that the first used concentration of 2000 ppm salinity did not show statistical effect on photosynthetic pigments of tecoma leaves. Whereas, other used concentrations of salinity (4000, 8000 and 12000 ppm) induced significant decrease in this respect. It is clear that the rate of reduction increased proportionally as the rate of salinity increased. The maximum decrement in concentration of chloroplast pigments was detected at salinity level of 12000 ppm, being 41.7, 38.1 and 34.3% less than the control for chlorophyll a , chlorophyll b and carotenoids; respectively. Our results are in accordance with those recorded on other timber plants by Darwish and Reda (2000) as well as by Reda (2007) and by Nassar *et al*. (2016).

Table 2. The effect of foliar application with riboflavin on concentrations of chloroplast pigments and free proline as well as on activity of antioxidant enzyme catalase in leaves of *Tecoma capensis*, aged five months from transplanting, grown under stress of different levels of salinity in the second growing season of 2015

Treatments	Chloroplast pigments (mg/g F.W.)			Free proline (mg/g F.W.)	Catalase activity (A_{290}/min/g protein)
	Chl a	Chl b	Carotenoids		
Control	0.947	0.438	0.513	0.672	86.9
2000 ppm salinity	0.943	0.431	0.508	0.683	88.2
4000 ppm salinity	0.806	0.382	0.459	0.771	98.3
8000 ppm salinity	0.692	0.329	0.401	0.869	113.8
12000 ppm salinity	0.552	0.271	0.337	0.991	129.7
2000 ppm salinity +2000 ppm riboflavin	1.054	0.507	0.575	0.656	87.5
4000 ppm salinity +2000 ppm riboflavin	0.938	0.429	0.499	0.714	96.8
8000 ppm salinity +2000 ppm riboflavin	0.812	0.387	0.452	0.796	97.1
12000 ppm salinity +2000 ppm riboflavin	0.679	0.334	0.408	0.847	102.4
L.S.D. (0.05)	0.092	0.041	0.048	0.072	8.95

Data also revealed that foliar spray of riboflavin at concentration of 2000 ppm minimizes the deleterious effect of salinity stress and raise the concentration of chloroplast pigments in leaves of salinized tecoma plants. Information about the effect of riboflavin on the concentration of chloroplast pigments in leaves of tecoma plants grown under salinity stress are not available.

2- Free proline:

It is realized from table (2) that increasing salinity level in irrigation water induced significant increase in concentration of free proline in leaves of tecoma plants aged five months from transplanting. Worthy to note that the rate of proline accumulation in tecoma leaves increased steadily

as salinity level increased and reached its high level at salinity concentration of 12000 ppm, being 47.5% more than that of untreated plants. The obtained results are in conformity with those recorded by Reda (2007) on *Senna occidentalis* as well as by Azooz (2009) on *Hibiscus sabdariffa* and by Nassar *et al*. (2016) on *Leucaena leucocephala*.Likewise, Pessarakli (1994) stated that abiotic stresses like salinity , drought, light or temperature increased concentration of soluble proline in leaves of higher plants. In this respect, Flowers *et al*. (1977) as well as Ridge *et al*. (1993) postulated that proline play an important role in many physiological processes for recovering plant after stress.

Results also indicated that spraying riboflavin at concentration of 2000 ppm resulted in reduction of free proline concentration in leaves of tecoma plants grown under salinity stress. In this connection, Azooz (2009) suggested that riboflavin (vitamin B_2) may have a potential role as an effective antioxidant by regulating osmotic and ion balance and enhancing the resistance of *Hibiscus sabdariffa* seedlings to salinity stress.

3- Activity of antioxidant enzyme catalase:

It is noted from Table (2) that increasing salinity level up than 2000 ppm increased significantly the activity of antioxidant enzyme catalase and the rate of activation increased linearly as salinity level increased and reached its maximum at salinity level of 12000 ppm which induced significant increase in catalase activity in tecoma leaves by 49.3% over the control.

The previous report of Azooz (2009) found that salinity increased activity of antioxidant enzymes in leaves of *Hibiscus sabdariffa*, being in harmony with the present findings. It is obvious that spraying riboflavin at concentration of 2000 ppm on salinized tecoma plants induced slight increases in the activity of catalase compared to control.

III- Leaflet anatomy:

Results on the influence of spraying riboflavin at concentration of 2000 ppm on anatomical structure of leaflet blade of tecoma plant grown under salinity stress of 8000 ppm are given in Table (3) and Figure (1).

Table 3: The effect of riboflavin on anatomical structure of leaflet blade of tecoma plants grown under salinity stress

Investigated characters	Treatments					
	Untreated (Control)	8000 ppm salinity	± % to control	8000 ppm salinity + 2000 ppm riboflavin	± % to control	± % to 8000 ppm salinity
Midvein thickness	954.7	678.4	-28.9	932.8	-2.3	+37.5
Lamina thickness	318.5	233.2	-26.8	391.6	+23.0	+67.9
Palisade tissue thickness	116.6	84.8	-27.3	127.2	+9.1	+49.8
Spongy tissue thickness	137.8	127.2	-7.7	159.6	+15.8	+25.5
Dimensions of midvein bundle						
Length	212.3	169.6	-20.1	254.4	+19.8	+50.0
Width	530.5	381.4	-28.1	472.7	-10.9	+23.9
No. of xylem rows/midvein bundle	15.6	9.4	-39.7	11.5	-26.3	+22.3
Vessel diameter	21.4	17.7	-17.3	32.3	+50.9	+82.5

It is noted from data presented in Table (3) and from microphotographs illustrated in Figure(1) that salinity stress at 8000 ppm reduced thickness of medvein and lamina of tecoma leaflet by 28.9 and 26.8% less than recorded in the control ; respectively. It is clear that the thinner leaflets produced by salinity stress could be attributed mainly to the decrease observed in thickness of both palisade and spongy tissues of the mesophyll as well as to the decrements induced in size of the main vascular bundle and its included tissues.

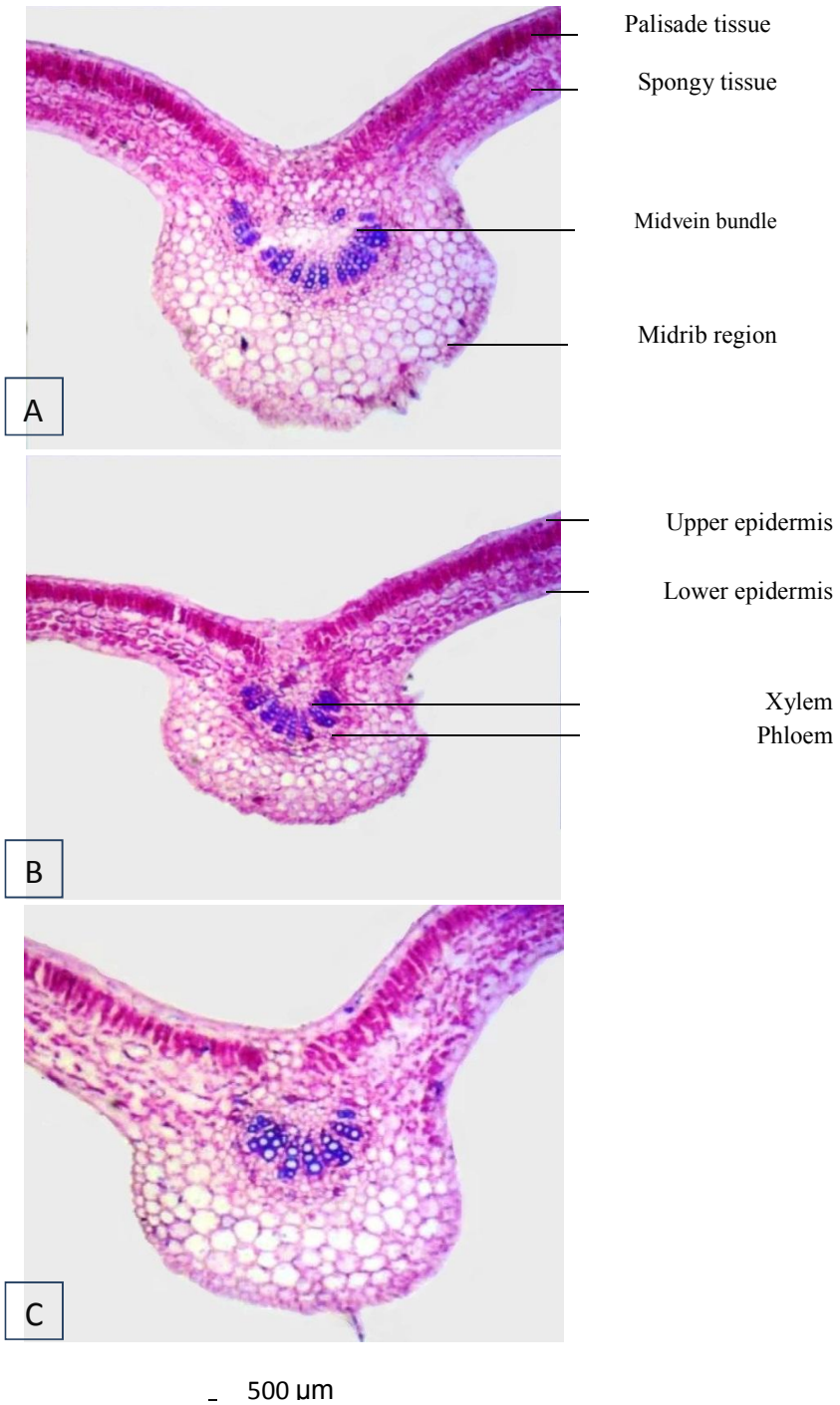

Fig. 1: The effect of foliar spray with riboflavin on anatomical structure of leaflet blade of tecoma plant, aged five months from transplanting, grown under salinity stress.
A- Cross section of leaflet blade from untreated plant (control).
B- Cross section of leaflet blade from plant grown under salinity stress of 8000 ppm.
C- Cross section of leaflet blade from plant grown under salinity stress of 8000 ppm and sprayed with 2000 ppm riboflavin.

The decrements below the control were 27.3 and 7.7% for the thickness of palisade and spongy tissue; respectively. Also, the dimensions of midvein bundle were decreased below the control by

20.1% in length and by 28.1% in width. Moreover, the number of xylem rows/midvein bundle and the mean diameter of vessel were decreased by 39.7 and 17.3 % less than those of the control; respectively. Similar results were also reported by Reda *et al*. (2000) on leucaena as well as by Boghdady (2009) on mung bean and by Dawood *et al*. (2014) on faba bean.

Worthy to note that the foliar application of 2000 ppm riboflavin on tecoma plants grown under salinity stress of 8000 ppm showed favourable changes in anatomical structure of tecoma leaves. Riboflavin have the ability to counteract the deleterious effects of salinity stress on anatomical structure of tecoma leaves. Riboflavin caused slight decrease in midvein thickness of salinized plants by 2.3% less than that of the control. Whereas, such treatment induced prominent increase in lamina thickness of salinized plants by 23% over the control and increased length of midvein bundle and vessel diameter by 19.8 and 50.9% over the control ; respectively . However, the width of midvein bundle and number of xylem rows/ midvein bundle were decreased below the control by 10.9 and 26.3% ; respectively. At the same time, the mean values of all included tissues in leaflet blades of salinized plants which were treated with riboflavin were decidedly higher over those of salinized plants. Thickness of midvein, lamina, palisade and spongy tissue were increased by 37.5, 67.9, 49.8 and 25.5% over those of salinized plants; respectively. Likewise, length and width of midvein bundle as well as number of xylem rows/midvein bundle and mean vessel diameter were increased over those of salinized plants by 50.0, 23.9, 22.3 and 82.5 % ; respectively. Previous information about the influence of spraying riboflavin on the anatomical structure of lealet blades of tecoma plants grown under salinity stress are not available.

Conclusion

Foliar application of riboflavin overcomes the deleterious effects of salinity stress on growth, chloroplast pigments, free proline, antioxidant enzymes activity and leaf anatomy of *Tecoma capensis* plants.

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