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Response of Neem Plants to Applied Salinity and Sprayed Riboflavin Derivatives

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Abstract

This study was carried out at greenhouse on National Research Centre during two successive seasons of 2021-2022 in Giza, Egypt. The aim of this work was to investigate the effect of spraying different riboflavin derivatives (Riboflavin, Ribo. $NH_2.NH_2$ [derivative (1) riboflavin], and Ribo. $NH_2.OH$ [derivative (2) riboflavin]) concentrations (0, 1000 ppm) on vegetative growth and chemical constituents of *Azadirachta indica* L. seedlings grown under three salinity concentrations (0, 6000 and 8000 ppm). Our results showed that, spraying riboflavin of derivative (1), lead to increasing allof (plant height, leaves number/plant, stem diameter, branches number/plant, fresh and dry weight of leaves, stems and roots, chlorophyll a, b, a+b and carotenoids, water leaf content and total carbohydrates percentage), while a significant reduction in all the same parameters, were occurred by using high salinity level (8000 ppm). The combined proline and phenols contents decreased by using derivative (1) or derivative (2) riboflavin, compares of treated as the control. The results suggested that *Azadirachta indica* L. seedlings benefited the application of riboflavin, especially under salinity condition

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إستجابة نباتات النيم للملوحة التطبيقية ورش مشتقات الريبوفلافين

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الملخص العربى:

أجريت هذه الدراسة في صوبة المركز القومي للبحوث خلال موسمين متتاليين 2021-2022 في الجيزة، مصر. الهدف من هذا العمل هو دراسة تأثير الرش بمشتقات الريبوفلافين المختلفة (Riboflavin, Ribo. $NH_2.NH_2$ [مشتق (1) للريبوفلافين] ، و Ribo. $NH_2.OH$ [مشتق (2) للريبوفلافين]) بتراكيز (0، 1000 ppm) في النمو الخضري والمكونات الكيميائية لشتلات *Azadirachta indica* L. المزروعة تحت ثلاثة تراكيز من الملوحة (0، 6000، 8000 جزء في المليون). أظهرت النتائج أن رش مشتق الريبوفلافين (1) يؤدي إلى زيادة كل من (إرتفاع النبات، عدد الأوراق/نبات، قطر الساق، عدد الأفرع/نبات، الوزن الطازج والجاف للأوراق والسيقان والجذور، الكلوروفيل أ، ب). و a+b والكاروتينات ومحتوى الأوراق من الماء ونسبة الكربوهيدرات الكلية)، في حين حدث انخفاض معنوي في جميع المؤشرات نفسها عند استخدام مستوى ملوحة عالي (8000 جزء في المليون). انخفض محتوى البرولين والفينولات الكلية بإستخدام مشتق (1) أو مشتق (2) من الريبوفلافين، مقارنة بمعاملة الكونترول. أشارت النتائج إلى أن شتلات *Azadirachta indica* L. إستفادت من إضافة الريبوفلافين، خاصة تحت ظروف الملوحة.

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الكلمات المفتاحية: منشط النمو، منتجات مشتقات الريبوفلافين (فيتامين ب$_2$)، الملوحة؛ نباتات الزينة، *L. Azadirachta indica* ، النيم.

INTRODUCTION:

Azadirachta indica, neem tree or Indian lilac, is a medium size in the mahogany family: Meliaceae and is specie in the genus *Azadirachta* and in appearance is Chinaberry, **(Deepika and Paul, 2013)**. It thrives grown fast in tracts of land, semi-tropical regions, humid conditions, with an annual rainfall or ground water and is anti-desertification properties and a carbon dioxide sink in Iran islands, **(The Food and Agriculture Organization of the United Nations and Earthscan, 2011)**.

Neem has a mycorrhizal deep taproot and seeds cake is an organic manure added to N nitrogenous fertilizers (urea) as it delays the nitrification inhibitor of soil and into powder in water and sprayed onto the crop and protected from damage, repellent and die within a few days, **(Abdel-Salam, *et al.*, 2018)**. Neem trees thrive in full sunlight and provide shade when lining streets in their early years. They are adept at extracting nutrients from leached soils with extreme pH levels ranging from 3 to 9, particularly in shallow, stony, poor, and drained soils, **(Zulfiqar, *et al.*, (2022)**. They can also grow on marginal slopes, rocky crevices, and dunes for land reclamation purposes, exhibiting some tolerance to soil salinity. Neem trees are beneficial in sugarcane cultivation, and their bark extracts can protect foliage from being eaten by pests without harming pollinators such as honeybees. They can be grown from seeds, cuttings, or root suckers and benefit from limonoids such as *Azadiracht indica*, **(Ahmad, *et al.*, (2023)**, *salannin*, *nimbin* which have anticancer properties and can cause low blood sugar. However, neem oil is toxic to children and can be fatal. Additionally, the upper leaves of neem trees have been studied for their potential benefits in combating COVID-19 due to the presence of approximately 300 safe compounds, **(Osman, *et al.*, 2018)**.

Leaves oil has toxic and a botanical broad and repellent for insects, **(Saad, *et al.*, 2017)**. The biopesticide contains triterpenes from cell suspension and hairy root cultures in bioreactors of a two process of growth and the opportunities by the multi-biological and pharmacological to solve of problems in the agriculture, health, and economic sectors, **(Eibl, *et al.*, 2018)**.

Salinity conditions with salt-sensitive microalgae organisms trigger metabolic and structural changes that affect cell division and cell size, leading to low growth rates, **(Choix, *et al.*, 2012)**. Saline stress induces osmotic and ion stress and triggers the antioxidant system, **(Okon, (2019))**. The adjustment of the osmotic gradient to maintain cell turgor and extension mainly involves changes in membrane permeability to favor adequate intracellular ionic ratios (K/Na, Mg/Ca) and the production of compatible solutes, among them free amino acids, proline, and soluble sugars, **(Ji, *et al.*, 2018; Heo, *et al.*, 2019 and Zhang, *et al.*, 2018)**. In addition to providing intracellular ion balance, these compounds also serve as osmoprotectants of essential molecules, such as DNA, protecting them from reactive oxygen species (ROS). ROS trigger the antioxidant defense system through the biosynthesis of enzymatic antioxidants, such as superoxide dismutase (SOD), catalase and ascorbate peroxidase, or of non-enzymatic antioxidants, such as carotenoids, anthocyanins, polyphenols, and proline, **(Karan and Subudhi (2012) and Peng, *et al.*, 2020)**.

Riboflavin (vitamin B2) is recognized as an essential bio-sensitized product in plants and

many microorganisms. The high concentration of riboflavin has no negative side effect, **Batool, *et al.*, (2014)**. It contributes many ethnobotanicals uses and pharmacological activities, **(Deng, *et al.*, 2013)** and modulates several different physiological processes such as growth of plants and defense responses, **(Taheri and Tarighi, 2011)**. Several studies have illustrated the effect of riboflavin in improving disease resistance against a broad-spectrum pathogen, **(Conrath, 2011)**. Riboflavin is participated in the anti-oxidation, **(Perumal, *et al.*, 2005)** and peroxidation processes, **(Nazarul, *et al.*, 2006)** which both enhance the reactive oxygen reactions and other plant defense mechanisms, **(Nie and Xu, 2016)**. Moreover, treatment with riboflavin induces defensive responses in plants instead of directly inhibition of pathogens growth, **(Rodrigues, *et al.*, (2017) and Taheri and Höfte, 2006)**. Recently, riboflavin activated PR-genes in Arabidopsis and induces SAR to pathogens, **(Boubakri, *et al.*, 2013)**.

The relation among vitamin B and abiotic stresses was reported, **(Hanson, *et al.*, 2016)**. Riboflavin supplementation mitigated the negative effect of salinity abiotic stress in rice seedlings by ameliorating oxidative stress and inducing riboflavin biosynthesis. B2 treatment significantly improved the salt tolerance of winter wheat seedlings by elevating the biomass. The physiological analysis found that B2 reduced the generation rate of O_2^-, electrolyte leakage, the content of proline, and the accumulation of malonaldehyde (MDA) and H_2O_2 and also significantly increased the contents of endogenous hormones zeatin riboside (ZA) and gibberellic acid (GA), as well as biochemical analysis revealed that enhanced the activities of various antioxidant enzymes system, including superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX), also antioxidase isozymes such as SOD3, POD1/2, and APX1/2, so response-related gene *TaSOS1* and *TaTIP2*, **Yu, *et al.*, (2022)**. The role of riboflavin in increasing drought tolerance in tobacco plants accumulated higher levels of ROS (O2− and H2O2), lipid peroxide and the activities of catalase (CAT), glutathione reductase (GR), chlorophyll degraded, survival times and ROS scavenger, **(Deng, *et al.*, 2013)**.

The aim of the present work is evaluating the influence of different levels of riboflavin derivatives (1 and 2) on vegetative growth and chemical constituents of *Azadirachta indica* L. seedlings irrigated with various concentrations of saline water condition.

MATERIALS AND METHODS:

The experimental design employed a randomized complete block design (RCBD) with three replicates. The experiment was conducted at the greenhouse of National Research Centre, Egypt, during 2020 and 2021 seasons. The experimental design employed a randomized complete block design (RCBD) with three replicates. The experiment was conducted at the greenhouse of National Research Centre, Egypt, during 2020 and 2021 seasons. The factors included "salinity levels" (main plot) i.e., 0, 4000, 6000 and 8000 ppm, and "riboflavin derivatives" (subplot) i.e., Control, Riboflavin, Ribo. NH2.NH2, and Ribo. NH2.OH. Saline water was prepared by using a mixture of sodium chloride (NaCl) and calcium chloride ($CaCl_2$) 1:1 w/w. Irrigation with saline water treatments were started after one month from planting.

The Physical and Chemical Analysis of the Soil: the soil samples were collected from different locations in the plantation at many pots and analysed for physical and chemical characters according to the standard procedures that mentioned by **(Wilde, *et al.*, 1985)**.

Analysis of soil (Average of two seasons)

Appreciation Sample	
pH (1:2.5)	7.66
EC (dSm-1) (1:5)	0.72
OM	0%
Soluble cations (ml, milliliter (mm) equivalent / liter) mEq/L-1	
($CaCO_2$) Ca^{++}	1.0
Mg^{++}	0.8
Na^{+}	5.6
K^{+}	0.05
Dissolved soluble anions (mEq/L-1)	
$CO_3^{=}$	-
HCO_3^{-}	1.5
Cl^{-}	2.5
$SO_4^{=}$	3.45
Pb	-
Soil types	
Sand	56 %
Silt	0%
Clay	44 %

Vegetative growth:

One year-old seedlings of *Azadirachta* seedlings were obtained from Nursery of Forestry Department, Horticulture Research Institute, Agriculture Research Centre, Giza, Egypt. The plants were transplanted on 15th March in free draining plastic pots (25 and 18 cm top and bottom) which filled with 10 kg soil (one plant/pot, the average height of seedlings was 12-15 cm).

The experiment was factorial design in two factors: A) three riboflavin derivatives (0, 1 and 2) (riboflavin, derivatives1, derivatives 2) and B): four salinity levels (0, 4000, 6000 and 8000 ppm) with control (tap water).

The following data were recorded at the second week of October of 2020 and 2021: plant height (cm), stem diameter (mm), number of branches / plants (No.), number of leaves / plants (No.), fresh and dry weight of leaves (g), fresh and dry weight of stems (g) and fresh and dry weight of roots (g).

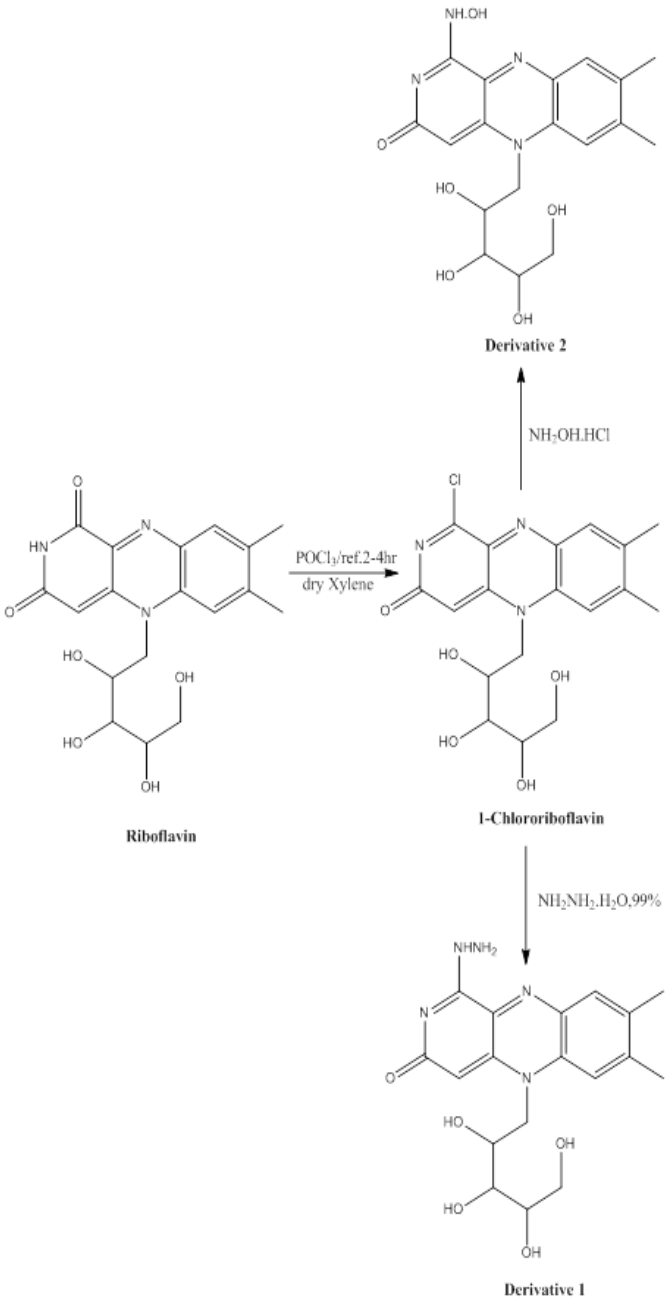
The following chemical analyses were determined: pigments (chlorophyll a, b) content (mg / g F.W.), total carbohydrates (% D.W.), carotenoids (% F.W.), proline content (ug / g) and phenols content (mg / g F.W.).

Chemical constituents:

Determination of photosynthetic pigments content (mg / g F.W.) of chlorophyll (a, b) and carotenoids was carried out according to the method described by **Saric, *et al.*, (1967)** and **Zhang and Shi, (2013)** by using Acetone solvent. Total carbohydrates percentage (% D.W.) was determined according to **Dubios, *et al.*, (1956)** by using Phenol solution. The proline content (micromole / g F.W.) was determined using fresh material according to **Bates, *et al.*, (1973)** by using Ninhydrin solution. Total soluble phenols content (mg / g F.W.) were determined calorimetrically by using Follin Ciocalta reagent, **A.O.A.C. (1985)**. Water content (mg / g F.W.) were determined according to **A.O.A.C. (1999)**. The protein content (mg / g D.W.) was determined in dry material by using Ethanol solvent according to **Gupta, *et al.*, (2011)**.

As we mentioned before, riboflavin possesses many biological activities, meanwhile we decided to prepare some new derivatives and use them in comparison with riboflavin (scheme 1), **Desk and Te (1969)**; **Batool, *et al.*, (2014).** 1-hydrazinylriboflavin (derivative 1) was prepared by reacting riboflavin (3.76 g) and phosphorous oxychloride ($POCl_3$) in 30 ml dry xylene under

reflux for 2-4 hr, **Lambooy and Rivlin (1975)**. Let the reaction to cool to room temperature and pour over crushed ice/water, **Li, *et al.*, (2022)**.



Scheme 1

The solid so formed was collected upon filtration, dried, and recrystallized in benzene to obtain 1-chlororiboflavin in good yield, mp, 276-77 ^{o}C, **Gul, *et al.*, (2022)**. Its IR spectra showed 3380-3388 cm^{-1} broad signal corresponding to OHs group, 2980 cm^{-1} corresponding to aliphatic (–CH_3, -CH_2), 1680 cm^{-1} for -C=O group, **Muchate, *et al.*, (2016)**. When the latter compound was reacted with hydrazine hydrate (99 %) in dry dioxane / absolute ethanol mixture (3:1) (30 ml; 10 ml) under reflux for 4-6 hr, the target compound 1-hydrazinylriboflavin was produced in moderate yield, mp; 269-71 ^{o}C. Its IR reveals bands at; 3380-3388 (OHs), 3340 (NH), 3326, 3324 (NH_2), 2898 (-CH_3, -CH_2), 2687 (-C=O), **Arzani and Ashraf (2016)**. On other hands, when 1-chlororiboflavin was heated under reflux with hydroxylamine hydrochloride in absolute ethanol for 6-8 hr, the desired derivative 1-hydroxylamineylriboflavin (derivative 2) was produced in low yield, mp; 291-93 ^{o}C, **Oyebamiji, *et al.*, (2024).**. Its IR gives bands at; 3377-3380 (OHs), 3338 (NH), 2898 (-CH_3, -CH_2), 2700 (-C=O), **Shams and Khadivi, (2023); Chourasia, *et al.*, (2022) and Chourasia, *et al.*, (2022)**.

Statistical Analysis: the data were statistical analysed for each season and them combined analysis of the two seasons was carried out according to the procedure outlined by **Steel and Torrie (1980)**.

RESULTS AND DISCUSSION:

Vegetative growth:

Data on morphological parameters of vegetative growth of *Azadirachta indica* plants as affected by salinity stress and sprayed with riboflavin in two successive seasons are given in Tables (1 & 2). The studied parameters of vegetative growth included plant height, leaves and branches number, stem diameter, fresh and dry weight of leaves, stems, and roots/plant in two successive seasons. It is realized from Tables (1 & 2) that the concentrations of salinity in irrigation water induced decrements in all investigated parameters of vegetative growth in both seasons. Worthy to mention that increasing salt level significantly

regarded all investigated parameters. The maximum salinity level of 8000 ppm in both seasons. The decrements were (17.22 % for plant height), (31.15 % and 9.52 % for leaves and branches number), (22.14 % for stem diameter), (22.94 % and 23.98 % for fresh and dry weight of leaves), (21.91 % and 26.57 % for F.W. and D.W. of stems) and (28.66 % and 33.07 % for F.W. and D.W. of roots), respectively, in both seasons. Similar results were also reported on other ornamental plants by **Al-Shaharani and Shetta (2011), Hardikar and Pandey (2011), Langroudi and Sedaghathoor (2012), Ratnakar and Rai (2013), Alam, *et al.*, (2014), Amirjani (2015), Nassar, *et al.*, (2016) and Nermeen, *et al.*, (2023)**.

Table (1): Effect of salinity concentrations and riboflavin forms on growth parameters of *Azadirachta indica* plants (Means of two seasons)

Salinity ppm / Ribo. form	Plant height				Stem diameter			
	0	6000	8000	**Mean**	0	6000	8000	**Mean**
Control	66.37	59.61	55.85	**60.61**	2.31	2.02	1.63	**1.99**
Riboflavin	98.31	90.31	81.71	**90.11**	2.37	2.29	1.86	**2.17**
Ribo. $NH_2.NH_2$	111.11	107.21	92.12	**103.48**	2.45	2.36	1.97	**2.26**
Ribo. $NH_2.OH$	126.71	116.67	103.51	**115.63**	2.63	2.51	2.12	**2.42**
Mean	**100.63**	**93.45**	**83.30**	**92.46**	**2.44**	**2.30**	**1.90**	**2.21**
L.S.D. 0.05:	SA: **06.12**	Ribo: **07.07**	SA X Ribo.: **12. 30**		SA: **0.11**	Ribo: **0.16**	SA X Ribo: **0.28**	
Salinity ppm / Ribo. form	**Number of leaves**				**Number of branches**			
	0	6000	8000	**Mean**	0	6000	8000	**Mean**
Control	35.27	30.61	24.21	**30.03**	2.47	2.36	2.30	**2.38**
Riboflavin	42.61	37.67	30.73	**37.00**	2.75	2.69	2.63	**2.69**
Ribo. $NH_2.NH_2$	49.75	42.17	34.25	**42.06**	3.06	3.02	2.68	**2.92**
Ribo. $NH_2.OH$	61.35	55.21	40.93	**52.50**	3.47	3.45	3.01	**3.31**
Mean	**47.25**	**41.42**	**32.53**	**40.40**	**2.94**	**2.88**	**2.66**	**2.82**
L.S.D. 0.05:	SA: **02.75**	Ribo.: **03.18**	SA X Ribo.: **05.50**		SA: **0.02**	Ribo.: **0.03**	SA X Ribo.: **0.05**	

Values shown in table are the means of three replicates. Where SA= salinity levels; Ribo. = riboflavin forms.

The significant decrease in growth parameters at higher salinity levels may be attributed to the osmotic effects **(Abbasi, *et al.*, (2016)**. Reduced rate of new cell production may make additional contributions to inhibition of growth **(Liang, *et al.*, (2018)**.

Concerning the effect of riboflavin forms on vegetative growth of *Azadirachta indica* plant from data in Tables (1 & 2) showed that foliar spraying riboflavin NH_2OH led to significantly increased in all vegetative growth. The increments were (90.08 %, 21.61 %, 74.83 %, 39.08 %, 78.39 %, 109.56 %, 57.56 % 81.30 %, 72.48 % and 92.25 %) for plant height, stem diameter, leaves and branches number, fresh and dry weight of leaves, stems, and roots, respectively, compared with control plants. These increments with spraying of B_2 vitamin, might be attributed to that the vitamin is one of carbonation process components, participates in the transfer of electrons (oxidation and reduction), and acts as

catalysis which are used in the biological oxidation within the plant and have a role information of auxins within the plant elongation of cells and cells preservation of side effects, **Abood and Abdulhameed (2017)**. These results are in good agreement with those obtained by **El-Lethy, *et al.*, (2011) and Anjali and Aruna (2013)**.

Table (2): Effect of salinity concentrations and riboflavin forms on growth parameters of *Azadirachta indica* plants (Means of two seasons)

Salinity ppm	**Fresh weight of leaves**				**Fresh weight of stems**				**Fresh weight of roots**			
Ribo. form	0	6000	8000	**Mean**	0	6000	8000	**Mean**	**0**	6000	8000	**Mean**
Control	37.61	33.61	30.11	**33.78**	42.17	35.17	29.96	**35.77**	29.01	24.31	20.35	**24.56**
Riboflavin	53.31	46.71	40.11	**46.71**	49.74	43.91	39.93	**44.53**	33.76	30.61	25.11	**29.83**
Ribo. $NH_2.NH_2$	56.75	51.31	43.35	**50.47**	55.71	49.12	43.35	**49.39**	40.71	36.67	30.61	**36.00**
Ribo. $NH_2.OH$	67.31	61.12	52.36	**60.26**	62.17	56.31	50.61	**56.36**	50.31	43.13	33.63	**42.36**
Mean	**53.75**	**48.19**	**41.48**	**47.81**	**52.45**	**46.13**	**40.96**	**46.51**	**38.45**	**33.68**	**27.43**	**33.18**
L.S.D. 0.05:	SA: **3.11**	Ribo: **4.35**	SA X Ribo: **7.11**		SA: **03.52**	Ribo: **04.61**	SA X Ribo.: **07.34**		SA: **1.67**	Ribo: **1.93**	SA X Ribo: **3.33**	
Salinity ppm	**Dry weight of leaves**				**Dry weight of stem**				**Dry weight of roots**			
Ribo. form	0	6000	8000	**Mean**	0	6000	8000	**Mean**	0	6000	8000	**Mean**
Control	9.44	8.11	6.93	**8.16**	12.69	10.20	8.39	**10.43**	10.56	8.46	6.92	**8.65**
Riboflavin	14.74	12.52	10.43	**12.56**	16.02	13.66	11.86	**13.85**	12.90	11.39	8.94	**11.08**
Ribo. $NH_2.NH_2$	13.95	13.85	11.36	**13.05**	18.51	15.62	13.35	**15.83**	15.92	14.19	11.30	**13.80**
Ribo. $NH_2.OH$	19.52	17.48	14.29	**17.10**	21.14	18.98	16.60	**18.91**	20.12	17.08	12.68	**16.63**
Mean	**14.41**	**12.99**	**10.75**	**12.72**	**17.09**	**14.62**	**12.55**	**14.75**	**14.88**	**12.78**	**9.96**	**12.54**
L.S.D. 0.05:	SA: **0.03**	Ribo.: **0.04**	SA X Ribo.: **0.07**		SA: **0.03**	Ribo.: **0.04**	SA X Ribo.: **0.06**		SA: **0.13**	Ribo: **0.14**	SA X Ribo: **0.25**	

Values shown in table are the means of three replicates. Where SA= salinity levels; Ribo. = riboflavin forms.

Regard to the effect of interaction between salinity irrigation and foliar spraying of riboflavin forms, data presented in Tables (1 & 2) indicated that vegetative growth characteristics including plant height, stem diameter, number of leaves and branches, fresh and dry weight of leaves, stems and roots, foliar spray of riboflavin (NH_2OH) combined with un-irrigation salinity followed by foliar spraying of riboflavin (NH_2OH) combined with salinity at 6000 ppm significantly increased all the vegetative parameters compared with other treatments. **Kumari, *et al.*, (2012)** reported that foliar application with 100 ppm riboflavin enhances the resistance of *Hibiscus sabdariffa* L. to salinity stress by promoting growth of salinized plants, agreeing with our findings.

Chemical constituents:

Photosynthetic pigments:

According to the data in Table (3), it is evident that all salinity concentrations caused a decrement of chlorophyll (a), (b), (a+b) and carotenoids content compared with untreated plants. The lowest Photosynthetic pigment's ability under salinity was due to stomata closure, inhibition of chlorophyll synthesis, a decrease of carboxylase and due to high chlorophyllase activity as reported by **Tiwari, *et al.*, (2020)** and **Hanin, *et al*, (2016)**. Our results agree with the finding of **Farahat, *et al*, (2013), Ibrahem, *et al*, (2013), Sayed, *et al*, (2014), Nisha (2015), Nahed, *et al.*, (2020) and Acosta-Motos, *et al.*, (2017),** they found that pigment content was decreased with increasing salinity levels.

Table (3): Effect of salinity concentrations and riboflavin forms on chemical constituents of *Azadirachta indica* plants (Means of two seasons)

Salinity ppm	**Chlorophyll (a)**				**Chlorophyll (b)**			
Ribo. form	0	6000	8000	**Mean**	0	6000	8000	**Mean**
Control	1.91	1.76	1.55	**1.74**	0.56	0.52	0.41	**0.50**
Riboflavin	2.34	2.13	1.81	**2.09**	0.67	0.61	0.54	**0.61**
Ribo. $NH_2.NH_2$	2.41	2.21	1.93	**2.18**	0.73	0.68	0.63	**0.68**
Ribo. $NH_2.OH$	2.53	2.37	2.08	**2.33**	0.79	0.73	0.66	**0.73**
Mean	**2.30**	**2.12**	**1.84**	**2.09**	**0.69**	**0.64**	**0.56**	**0.63**
L.S.D. 0.05:	**SA: 3.30**	**Ribo.: 3.81**	**SA X Ribo.: 3.10**		**SA: 3.99**	**Ribo.: 4.60**	**SA X Ribo.: 7.98**	
Salinity ppm	**Total chlorophyll (a + b)**				**Total carotenoids**			
Ribo. form	0	6000	8000	**Mean**	0	6000	8000	**Mean**
Control	2.47	2.28	1.96	**2.24**	0.92	0.85	0.79	**0.85**
Riboflavin	3.01	2.74	2.35	**2.70**	1.31	1.21	0.96	**1.16**
Ribo. $NH_2.NH_2$	3.14	2.89	2.56	**2.86**	1.50	1.42	1.13	**1.35**
Ribo. $NH_2.OH$	3.32	3.1	2.74	**3.05**	1.55	1.47	1.28	**1.43**
Mean	**2.99**	**2.75**	**2.40**	**2.71**	**1.32**	**1.24**	**1.04**	**1.20**
L.S.D. 0.05:	**SA: 0.02**	**Ribo.: 0.03**	**SA X Ribo.: 0.04**		**SA: 0.02**	**Ribo.: 0.03**	**SA X Ribo.: 0.04**	

Chlorophyll content in leaves is the biochemical indicator of the plant's tolerance to salinity, **Rahneshan, *et al.*, (2018)**. The degradation of photosynthetic pigments lowers the photoreception effeciency of photosystems, which reduces the overall level of photosynthesis, **Deinlein, *et al.*, (2014)**. **and Zhang, *et al.*, (2012)**.

Regarding the effect of Vit. B_2 (Riboflavin) forms on photosynthetic pigments, the recorded data in Table (3) retreated the positive and active effect of the riboflavin (NH_2OH) on chls (a), (b), (a+b), and carotenoids content in leaves of *Azadirachta indica* plants, as compared with other treatments and untreated plants. Our results agree with **Jahan, *et al.*, (2018) and El-Shazly and Abd El-Wahab (2017)**.

They reported that application of riboflavin led to the highest increase in biosynthesis of pigments.

Concerning the effect of interaction between salinity and riboflavin forms the results in Table (3) showed that, the highest chls (a), (b), (a+b) and carotenoids in both seasons were obtained from unsalinized plants sprayed with riboflavin (NH_2OH) compared with other treatments. The lowest pigments in both seasons were obtained from plants which salinized with 8000 ppm and without spraying with riboflavin. These results agreed with **Kumari, *et al.*, (2012)** on *Hibiscus sabdariffa* L. **and Darwish, *et al.*, (2017)** on *Tecoma capensis*.

Total carbohydrates %:

According to the Data in Table (4), it is evident that all salinity treatments caused a decrement on total carbohydrate contents compared with untreated plants with clear effect when high levels were used. The decrement was (20.21 %) compared with control plants. These results agreed with **Hamay, Lopez, *et al.*, (2008), Azza, *et al.*, (2011 a), Nahed, *et al.*, (2011), Azza, *et al.*, (2011 b), Azza, *et al.*, (2012) and Hashish, *et al.*, (2015)**. The reduction in total carbohydrate as salinity levels increased may be related to respiration processes science free sugar was the main sugar pattern involved in the mechanism of respiration, **Gupta and Huang (2014)**.

From the data given in Table (4) it can be concluded that foliar spraying of riboflavin in all forms increased carbohydrates % compared with control plants. The highest values of carbohydrates were obtained when plants were sprayed with riboflavin (NH_2OH) compared with other forms and control plants. The increment was (75.95 %) compared with control. Higher accumulation of soluble carbohydrates and protein might play an important role in osmotic adjustment **Shaddad, *et al.*, (1990)**. The stimulation effect of riboflavin on the biosynthesis of carbohydrates and protein may be taken as further evidence of the role played by the vitamin in plant adaptation mechanisms, the accumulation of carbohydrate due to vitamin treatment might be attributed to the increase in green area, which consequently leads to increase in photosynthetic activity and plant productivity, **Afzal, *et al.*, (2022)**.

These results are agreement with **Ahmed (2017)**.

Concerning the effect of interaction between salinity irrigation and foliar spray of riboflavin forms, data presented in Table (4) show that the plants spraying with riboflavin (NH_2OH) combined with unsalinized water gave the highest value of carbohydrates followed by irrigation salinity at 6000 ppm combined with riboflavin (NH_2OH). Vit. B_2 treatment ameliorated the inhibitory effects of salinity on the contents of insoluble protein at most of the salinity levels used **Ahmed (2017)**.

The Proline and Phenol content:

Data presented in Table (4) showed that irrigation with different salinity levels increased proline and phenol content in the leaves of *Azadirachta indica* plants as compared to control plants. The highest values of proline and phenol were obtained when plants were irrigated with 8000 ppm. The increments were (46.11 % & 10.41 %), respectively, compared with control plants. Salt stress inhibits growth and protein synthesis preventing the utilization of proline and thus leading to its accumulation, **Hussain, *et al.*, (2021) Hamay, *et al.*, (2020) ,** found that cellular osmotic adjustment occurs in response to stress via an active or passive accumulation of saltues**.** It has been assumed that salt stress euhanced the production of proline, which causes osmotic

adjustment, **Mesquita, *et al.*, (2019)**. The response of different plants to salt stress depends on the degree of their tolerance and on type, level and duration of osmotic substrate as reported by **Haider, *et al.*, (2023)** and **Chen, *et al.*, (2018)**. Similar results were found by **Watanabe, *et al.*, (2000), El-Quessni, *et al.*, (2015) and Magdalene and Barabara (2022)**. Regarding the effect of riboflavin on proline and phenol content, the results presented in Table (4) showed the foliar spraying of riboflavin (NH_2OH) decreased in the amount of proline and phenol contents, the decrement was (50.76 % and 25.32 %), respectively, compared with control plants.

Table (4): Effect of salinity concentrations and riboflavin forms on chemical constituents of *Azadirachta indica* plants (Means of two seasons)

Salinity ppm / Ribo. form	Total carbohydrates %				Water content of leaf			
	0	6000	8000	**Mean**	0	6000	8000	**Mean**
Control	23.61	21.97	16.67	**20.75**	54.61	51.01	44.37	**50.00**
Riboflavin	33.41	31.00	26.63	**30.35**	68.11	65.12	53.35	**62.19**
Ribo. $NH_2.NH_2$	35.12	33.61	29.71	**32.81**	70.12	66.21	58.17	**64.83**
Ribo. $NH_2.OH$	40.25	36.63	32.64	**36.51**	76.63	70.03	63.73	**70.13**
Mean	**33.10**	**30.80**	**26.41**	**30.10**	**67.37**	**63.09**	**54.91**	**61.79**
L.S.D. 0.05:	**SA: 00.24**	**Ribo.: 00.28**	**SA X Ribo.: 00.48**		**SA: 01.62**	**Ribo.: 01.86**	**SA X Ribo.: 03.21**	
Salinity ppm / Ribo. form	**Total phenols**				**Prolein content (micromole / g f. w.)**			
	0	6000	8000	**Mean**	0	6000	8000	**Mean**
Control	3.67	3.53	3.14	**3.45**	29.62	35.61	43.11	**36.11**
Riboflavin	4.31	4.16	4.01	**4.16**	22.01	26.35	33.11	**27.16**
Ribo. $NH_2.NH_2$	4.61	4.52	4.21	**4.45**	18.11	19.81	26.31	**21.41**
Ribo. $NH_2.OH$	4.80	4.67	4.39	**4.62**	15.01	17.01	21.31	**17.78**
Mean	**4.35**	**4.22**	**3.94**	**4.17**	**21.19**	**24.70**	**30.96**	**25.61**
L.S.D. 0.05:	**SA: 0.02**	**Ribo.: 0.03**	**SA X Ribo.: 0.04**		**SA: 04.47**	**Ribo.: 05.16**	**SA X Ribo.: 08.94**	

This result is due to plants being exposed to stress, soil salinity, irrigation water or high temperatures. Thus, high level of amino acids appeared and caused by protein decomposition or Lack of accumulation resulting from decreasing the use of these acids in the composition of protoplasm, where the increment in proline led to increasing osmosis of the cell, or the role of riboflavin in preservation the water balance of plant cells. In addition, the regulation of antioxidant enzymes or perhaps it is an effective antioxidant by regulating the osmotic and ionic balance, enhancing plant resistance to stress and disposing the plant from the radicals, which promotes better plant growth **Afzal, *et al.*, (2022)**. These results agree with **Mesquita, *et al.*, (2018**. Irrigation salinity at 8000-ppm combined with spraying of riboflavin gave the highest values of proline and phenol contents.

Water content:

It is noted from Table (4) that increasing salinity levels led to decrease the water content, the decrement was (16.66 %). Foliar spraying of riboflavin derivative (2) increased the water

content. The increments were (40.26 %) compared with control plants. Application of riboflavin derivative (2) combined with unsalized water gave the highest value of water content followed by application of riboflavin (NH_2NH_2) combined with unsalinity treatment. The promotional effects of foliar application of vit. B_2 on growth parameters were associated with an improvement of water content. This probably reflects the efficiency of water uptake and utilization or depression excessive loss of water by *Hibsscus sabdriffa* L. seedlings because of vit. B_2 treatment, which can be considered as an adaptive response to salinity. Hence, it can be concluded that the beneficial effect of vit. B_2 on seedling growth has been related to efficiency of their water uptake and utilization, **Kumari, *et al.*, (2012)**. The response of protein content (mg / g D.W.) appeared did not depend on the concentration of applied salt and riboflavin, in the highest treatment as 1000 ppm riboflavin derivative (1) and 0 ppm salinity (control), but the latest treatment as 8000 ppm salinity + control (0 ppm of riboflavin).

This only table shows that riboflavin derivative (1) led to increase in protein content measurement inside the leaves of plant.

Protein content (mg / g D.W.)				
Salinity ppm				
Ribo. Form	0	6000	8000	**Mean**
Control	0.57	0.54	0.15	**0.42**
Riboflavin	0.17	0.17	0.67	**0.51**
Ribo. NH_2.OH	0.69	0.64	0.40	**0.58**
Ribo. NH_2.NH_2	0.76	0.51	0.49	**0.59**
Mean	**0.56**	**0.52**	**0.42**	**0.53**
L.S.D. 0.05:	**SA: 0.022**	**Ribo.: 0.025**	**SA X Ribo.: 0.044**	

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