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SCIENTIFIC ARTICLE

Alleviative effects of chitosan or humic acid on *Vitex trifolia* 'Purpurea' grown under salinity stress

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Abstract

Pots experiment was conducted to investigate the effect of bio-stimulators chitosan (CHT) or humic acid (HA) on *Vitex trifolia* 'Purpurea' exposed to salinity stress. Salinity stress was imposed by irrigation with saline water at concentration of 1000, 2500 and 5000 ppm, in addition to control (280 ppm), plants exposed to salinity were sprayed every 4 weeks with either CHT at concentrations of 30, 60 and 90 ppm or HA at concentrations of 1000, 1500 and 2000 ppm, while control plants sprayed only with tap water. The results showed that, with increasing salinity stress all vegetative growth parameters were decreased and chemical constituents including total chlorophylls total carbohydrates, K^+%, Ca^{2+}% and K^+/Na^+ ratio were reduced. In contrast, elevating salinity stress increased contents of proline, total phenolic, Na^+%, Cl^-%. On the other hand, foliar application of either CHT or HA had favorable impact on increasing vegetative traits and chemical compositions, meanwhile reducing accumulation of total phenolic, Na^+ and Cl^-% toxic ions in leaves, HA was generally more effective than CHT. Based on the results, it can be recommended that, CHT or HA at high concentration was the best effective treatments; however, HA was superior and economic treatment recommended for alleviating the adverse impact of salinity stress on *Vitex trifolia* 'Purpurea' plants irrigated with saline water at concentration up to 5000 ppm.
Keywords: Arabian lilac, salt stress, bio-stimulators.

Resumo

Efeitos atenuantes da quitosana ou ácido húmico em *Vitex trifolia* 'Purpurea' cultivada sob estresse salino

O experimento em vasos foi conduzido para investigar o efeito de bioestimulantes quitosana (CHT) ou ácido húmico (HA) em Vitex trifolia 'Purpurea' exposto ao estresse salino. O estresse de salinidade foi imposto pela irrigação com água salina nas concentrações de 1000, 2500 e 5000 ppm, além do controle (280 ppm), as plantas expostas à salinidade foram pulverizadas a cada 4 semanas com CHT nas concentrações de 30, 60 e 90 ppm ou HA nas concentrações de 1000, 1500 e 2000 ppm, enquanto as plantas controle pulverizadas apenas com água da torneira. Os resultados mostraram que, com o aumento do estresse salino, todos os parâmetros de crescimento vegetativo diminuíram e os constituintes químicos, incluindo clorofilas totais, carboidratos totais, K^+ %, $Ca2^+$% e a relação K^+ / Na^+ foram reduzidos. Em contraste, a elevação do estresse salino aumentou os conteúdos de prolina, fenólico total, Na^+%, Cl^-%. Por outro lado, a aplicação foliar de CHT ou HA teve impacto favorável no aumento das características vegetativas e composições químicas, enquanto reduzia o acúmulo de íons fenólicos totais, Na^+ e Cl^-% tóxicos nas folhas, HA foi geralmente mais eficaz do que CHT. Com base nos resultados, pode-se recomendar que, CHT ou HA em alta concentração foram os melhores tratamentos eficazes; entretanto, HA foi superior e um tratamento econômico recomendado para aliviar o impacto adverso do estresse salino em plantas de *Vitex trifolia* 'Purpurea' irrigadas com água salina em concentração de até 5000 ppm.
Palavras-chave: "lilás árabe", estresse salino, bioestimuladores.

Introduction

Vitex trifolia L.is evergreen shrub or small tree belongs to the family of Verbenaceae. It is widespread in Australia, Southeast Asia, East Africa and Micronesia. (Rani and Sharma, 2013). One of the most popular varieties of this species is *V. trifolia* 'Purpurea' which commonly known as Arabian lilac or Fascination. The plant grows to about 5 m tall and width and develops a conical shape with open crown. The leaves are simple, elliptical, arranged oppositely with an entire shape, the color is gray-green on the upper surface to purple underneath. The flowers are single blue-violet at the branch tips and remains on one 18-cm-long panicle, the bloom season runs from May to September. The fruits are green in color, fleshy and berry-like. Furthermore, utilize of Arabian lilac for landscape activates as ornamental

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shrubs, the leaves have been traditionally recommended for treatment of inflammation, sprains, wound healing and rheumatic pains (Dehsheikh et al., 2019).

In arid and semi-arid regions, salinity as one of abiotic stress considers an earnest problem in landscape activities. The harmful influence of salinity is attributed to its impact on osmotic stress, ions toxicity, nutritional disorders and production of reactive oxygen species (ROS). One of the common responses of plants to saline conditions is a growth reduction that starts immediately after exposure of roots to salt; this impact is correlated with an osmotic impediment to water uptake which in turn changes in water relations at a cellular level. Ionic toxicity results from accumulation of certain ions to a level at which inhibit plant growth. Salt stress affects the nutritional status of a plant through a complex net of interactions, including reduction of nutrient uptake and/or transport from root to shoot. Under saline conditions, the accelerated generation of ROS (such as singlet oxygen (O^{2-}), hydrogen peroxide (H_2O_2), superoxide (O^{2-}) and hydroxyl radical (OH^-) which involved in various process in the chloroplasts and other organelles in plant cell such as DNA damage, enzyme inhibition, lipid peroxidation and protein oxidation which is accompanied by damage in chloroplast and reduce in photosynthesis (García-Caparrós and Lao, 2018).

In the last few years, bio- stimulators as biological methods to obviate the application of chemical products and overcome injurious impact of salinity in agriculture have received considerable attention. Among the several categories of bio-stimulators are chitosan and humic acid. Chitosan (CHT) is a natural biopolymer modified from chitins which act as a potential bio stimulant and elicitor in agriculture. It is safe, bioactive, biodegradable and biocompatible that supports potentially widely utilization. Under non-stressed conditions, previous studies evidenced CHT had a favorable influence on some ornamental plants such as increase in growth and flowering traits, chlorophylls content, photosynthesis, and uptake of mineral nutrient (Dzung et al., 2011; Salachna and Zawadzińska, 2014; Salachna et al., 2015; Byczyńska, 2018). Under salt stressed conditions, CHT has the ability to alleviate the adverse effects of abiotic stress like salinity (Jabeen and Ahmad, 2013; Mahdavi, 2013; El-Attar, 2017; Krupa-Małkiewicz and Smolik, 2019). Additionally, it has been reported that salt stress damage could be alleviated by CHT via modifying intracellular ion concentration and promoting the capacity of antioxidant enzyme activities (Safikhan et al., 2018). The positive effect of CHT treatment is stimulates photosynthetic rate, stomatal closure via ABA synthesis, enhances antioxidant enzymes through nitric oxide and hydrogen peroxide signaling pathways, and stimulates production of organic acids, sugars, amino acids and other metabolites that are necessary for the osmotic adjustment, stress signaling, and energy metabolism under stresses (Hidangmayum et al., 2019).

Humic acid (HA) is a natural organic compound used for enhances early growth and flowering, promotes root and nutrition efficiency owing to its action on physiological and metabolic processes. Under salt stressed conditions, the beneficial role of HA is attributed to its indirect actions on improving physical, chemical and microbiological soil properties and its direct actions on physiological, biochemical processes and hormone-like activity. (Canellas et al., 2015). Furthermore, the effect of HA on amelioration salinity stress is related to its role on osmotic adjust by maintaining water uptake and cell turgor, inducing antioxidant enzymes that scavenging reactive oxygen species (ROS), enhancing levels of endogenous proline and decreasing membrane leakage that consider indicators of better adaptation to saline (Van Oosten et al., 2017). In this respect, on some species of ornamental plants subjected to salinity such as *Chrysanthemum indicum* (Mazhar et al., 2012), *Duranta plumieri* (EL Sayed et al., 2017) and *Acalypha wilkesiana* (Abd-El-Hady et al., 2019) application of HA showed valuable role on ameliorating the adverse effect of salinity and authors attributed positively responses to reduction in accumulation of Na^+ and Cl^- toxic ions in plant organs.

V. trifolia 'Purpurea' is one of the popular shrubs used in landscape activates of touristic villages where relatively saline water is used. However, the available data on mitigating the adverse impact of salinity stress by bio-stimulators has rarely been reported. Thus, the objective of this work was to evaluate the response of plants irrigated with various levels of salinity to foliar application of chitosan or humic acid. According to our knowledge, this is the first study to describe the influence of chitosan or humic acid on morphological features of *Vitex trifolia* 'Purpurea' exposed to salt stress.

Material and Methods

This study was conducted at the experimental nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the two successive seasons of 2018 and 2019. This research was aimed to investigate response of *Vitex trifolia* 'Purpurea' irrigated with different levels of saline water to foliar application of different concentrations of chitosan or humic acid**.**

Plant material

On 15th February, in both seasons, seedlings of *Vitex trifolia* 'Purpurea' plants were obtained from a private nursery with an average plant height of 25-28 cm and 2 branches/plant and planted individually in plastic pots (30 cm in length and 30 cm inner diameter) filled with 10 kg of the mixture of clay + sand (2:1: v/v). The physical and chemical properties of soil mixture used in the study was done at Soil, Water and Environment Research Institute, Agriculture Research Centre A.R.C according to (Jackson, 1973), the results are presented in Table 1.

Table 1. Some physical and chemical properties of the investigated soil used for growing *Vitex trifolia* 'Purpurea' during 2018 and 2019 seasons

Physical properties								
Field capacity (% V)			Clay (%)		Coarse sand (%)	Fine sand (%)	Silt (%)	Soil texture
46.2			40.7		7.71	18.5	33.5	Clay loam
Chemical properties								
Macro-nutrients (ppm)								
N	P	K	Mg	PH	Organic matter (%)	EC (dS m^{-1})	CEC (meq/100 g)	$CaCO_3$ (%)
45.32	11.77	295.14	33.98	7.53	1.45	1.72	30.64	1.54

N: Nitrogen; P: phosphorous; K: potassium; Mg: magnesium; EC: Electrical conductivity; pH: soil acidity; CEC: cation exchange capacity; OM: organic matter; $CaCO_3$: calcium carbonate.

Experimental procedures

On 15th of March the plants were irrigated twice/ week using saline water at concentration of 1000, 2500, and 5000 ppm, in addition to the control (tap water, 280 ppm). The different concentrations of saline water were prepared by mixing salts of NaCl and $CaCl_2$ at the ratio of 1:1 (w w^{-1}) and applied at one liter/ pot. In both seasons, plants irrigated with salinity levels were foliar sprayed every 4 weeks with either chitosan (CHT) at concentrations of 30, 60 and 90 ppm or humic acid (HA) at concentrations of 1000, 1500 or 2000 ppm, while the control plants sprayed only with tap water. Tween 20 as wetting agent was added to bio-solution at concentration of 1 mL L^{-1} and the plants foliage were sprayed using automatic atomizer until run off point (70 ml of bio-solution $plant^{-1}$).

All the plants were fertilized monthly with kristalon (NPK 19:19:19) at a rate of 2.5 g/pot, hand picking of weeds, resistance of diseases, pests were also performed.

Experimental layout

The layout of the experiment was randomized complete blocks design with 28 treatments [4 salt concentrations (including the control) x 7 plant bio -stimulators (including the control)] each treatment consisting of 12 pots arranged in 4 replicates, each replicate containing 84 pots (3 pots from each treatment).

The data recorded

On 15th November, in two seasons (after 9 months), the experiment was finished and morphological, physiological characteristics were registered.

Morphological parameter**;** plant height (cm), number of branches/plant, stem diameter (mm, at 5 cm above soil surface), root length (cm), additionally fresh and dry weights of leaves, stems and roots/plant were also determined. Dry weight/plant was estimated by drying plant at 70 °C until constant weight.

Chemical constituents of leaves

1. Total chlorophylls were determined in fresh leaf samples by using chlorophyll meter Model SPAD 502 (Netto et al., 2005);
2. The total carbohydrates concentration (% of dry matter) was estimated in dried leaves samples as described by Dubois et al. (1956);
3. The proline content in fresh leaves (µ moles /g fresh matter of leaves) was determined using the method of Bates et al. (1973);
4. Mineral constituents: 100 mg of dried leaves samples were digested and the content of K^+, Ca^{2+} and Na^+ were determined according to by Karla (1998). Chloride content were determined using the method described by Gavlak et al. (1994);
5. Total phenolic content was determined spectrophotometrically according to the Folin Ciocalteau's reagent colorimetric method and expressed as milligram gallic acid equivalent per gram of leaves dry weight extract (mg GAE/g DW) (John et al., 2014).

Statistical analysis

The obtained results were subjected to two-ways analysis of variance (ANOVA), and the data means of the two seasons were compared using the "Least Significant Difference (LSD)" test at the 0.05 level (Steel and Torrie, 1997).

Results and Discussion

Growth parameters

Effect of salinity stress

It is evident from data in Table 2, 3, and 4 that, salinity stress had a harmful impact on the different growth characteristics of *Vitex trifolia* 'Purpurea' plants. In both seasons, raising salinity levels from 1000 to 2500 or 5000 ppm caused steady significant reduction in all of tested growth parameters compared to the control plants. These results are similar to findings of various studies (Farahat, et al., 2013; Breś et al., 2016; Ashour and El-Attar 2017; Ashour and Abdel Wahab, 2017; García-Caparrós and Lao, 2018; Hooks and Niu, 2019; García-Caparrós, et al. 2020) who reported reductions in growth traits of ornamental plants due to negative impact of salt stress.

Table 2. Plant height, number of branches/plant, stem diameter and root length of *Vitex trifolia* 'Purpurea' as affected by water salinity and bio- stimulators treatments and their interactions during the 2018 and 2019 seasons.

Treatments		Plant height (cm)		Number of brances/plant		Stem diameter (mm)		Root length (cm)	
		2018	2019	2018	2019	2018	2019	2018	2019
Mean of salinity (S), ppm									
0 (control)		68.59	67.00	8.93	10.07	10.96	11.76	26.32	27.31
1000		64.79	62.25	8.14	9.13	10.20	11.07	25.12	25.98
2500		62.56	62.20	7.71	9.00	9.85	10.90	24.07	25.38
5000		57.64	59.90	7.11	7.70	10.20	11.26	23.11	23.55
Mean of *bio-stimulators (B), ppm									
0 (Control)		47.51	48.65	4.81	5.86	7.65	8.77	19.25	21.95
CHT at 30		53.66	53.55	7.10	8.52	9.78	10.74	23.87	24.21
CHT at 60		55.83	59.44	8.01	9.13	10.41	11.13	24.43	24.48
CHT at 90		66.94	62.43	7.89	9.12	10.58	11.85	25.74	25.04
HA at 1000		70.05	70.83	9.09	10.07	10.27	11.87	25.91	26.60
HA at 1500		75.07	72.01	8.63	9.38	10.79	11.98	25.85	27.42
HA at 2000		74.70	72.94	10.29	10.76	12.64	12.38	27.54	29.17
Mean of interaction, (ppm)									
S 0	**0**	53.45	59.96	5.17	6.67	8.56	9.63	22.33	25.09
	CHT at 30	58.89	57.65	8.45	9.76	9.51	11.28	25.50	25.15
	CHT at 60	62.90	59.10	8.95	10.09	11.20	11.29	26.28	26.22
	CHT at 90	69.72	64.78	8.87	10.46	12.31	12.13	27.86	27.89
	HA at 1000	76.49	71.20	10.36	11.34	10.36	12.29	26.96	27.36
	HA at 1500	78.66	72.13	9.92	10.59	11.47	12.65	26.05	28.97
	HA at 2000	80.02	84.16	10.81	11.57	13.34	13.02	29.31	30.47
S 1000	**0**	49.20	51.10	4.58	5.92	7.51	8.85	18.86	22.03
	CHT at 30	55.31	58.21	7.47	8.67	10.03	10.83	24.08	24.92
	CHT at 60	58.79	59.85	8.32	9.51	10.03	11.09	25.11	25.14
	CHT at 90	70.77	62.35	8.44	9.09	10.21	11.20	25.85	25.26
	HA at 1000	67.34	66.20	9.17	9.97	9.97	11.67	26.33	27.54
	HA at 1500	77.02	69.29	8.92	9.22	10.83	11.55	27.54	27.99
	HA at 2000	75.09	68.73	10.07	11.55	12.80	12.28	28.09	28.99
S 2500	**0**	48.97	45.74	5.09	5.67	7.08	8.21	18.47	21.84
	CHT at 30	57.54	53.64	6.85	9.00	9.69	10.31	23.73	24.51
	CHT at 60	44.92	65.92	8.11	9.17	10.15	10.81	24.01	24.21
	CHT at 90	65.41	63.76	7.18	8.00	9.58	11.88	25.68	23.92
	HA at 1000	72.92	71.47	9.33	10.59	10.10	11.49	25.57	26.70
	HA at 1500	75.09	72.52	7.50	9.40	10.27	11.64	24.18	27.25
	HA at 2000	73.09	62.40	9.95	11.20	12.08	11.97	26.88	29.25
S 5000	**0**	38.44	37.81	4.42	5.17	7.45	8.41	17.35	18.85
	CHT at 30	42.92	44.72	5.63	6.67	9.90	10.53	22.17	22.27
	CHT at 60	56.70	52.89	6.65	7.75	10.25	11.35	22.34	22.38
	CHT at 90	61.87	58.84	7.08	8.92	10.23	12.20	23.56	23.09
	HA at 1000	63.45	74.46	7.53	8.39	10.65	12.03	24.79	24.82
	HA at 1500	69.52	74.11	8.17	8.30	10.59	12.09	25.66	25.48
	HA at 2000	70.59	76.46	10.34	8.72	12.35	12.26	25.90	27.98
L.S.D. (0.05)									
Salinity (S)		3.06	3.72	0.54	0.68	0.54	0.65	0.97	0.93
Bio- stimulators (B)		4.04	4.92	0.72	0.89	0.71	0.85	1.28	1.22
SX B		8.09	9.84	1.44	1.79	1.42	1.71	2.56	2.45

*CHT= Chitosan HA = Humic acid

Table 3. Fresh and dry weights of leaves and stems of *Vitex trifolia* 'Purpurea' as affected by water salinity and bio-stimulators treatments and their interactions during the 2018 and 2019 seasons.

Treatments		Leaves fresh weight (g/ plant)		Leaves dry weight (g/ plant)		Stems fresh weight (g/ plant)		Stems dry weight (g/ plant)	
		2018	2019	2018	2019	2018	2019	2018	2019
Mean of salinity (S), ppm									
0 (control)		99.06	101.48	44.57	42.77	121.29	120.90	67.43	62.27
1000		93.29	96.52	42.78	41.29	114.76	114.71	64.88	60.54
2500		91.81	97.01	40.82	41.34	109.33	110.68	63.95	59.79
5000		86.95	92.95	39.25	37.84	103.54	106.22	62.11	58.54
Mean of *bio-stimulators (B), ppm									
0 (Control)		76.79	81.03	37.22	35.32	94.37	93.18	55.71	51.31
CHT at 30		85.30	89.33	42.36	38.97	98.36	97.79	57.96	52.99
CHT at 60		86.65	93.39	41.79	40.77	104.23	101.76	60.78	56.17
CHT at 90		90.41	92.67	40.99	40.95	119.12	122.16	64.87	63.28
HA at 1000		101.69	105.33	43.12	43.17	124.31	125.04	64.72	65.38
HA at 1500		104.15	106.15	42.92	42.38	117.35	122.74	68.07	67.16
HA at 2000		104.48	111.02	44.59	44.11	127.86	129.22	80.02	65.69
Mean of interaction, (ppm)									
S 0	**0**	87.29	89.68	40.08	37.78	100.84	97.28	57.78	54.56
	CHT at 30	91.29	93.91	45.02	42.37	104.96	101.12	60.01	54.60
	CHT at 60	95.91	101.85	47.51	40.18	112.99	107.28	59.88	56.64
	CHT at 90	95.33	96.97	41.96	45.36	130.90	130.97	69.42	64.24
	HA at 1000	107.32	107.37	45.71	44.42	135.24	136.69	67.61	69.09
	HA at 1500	107.44	107.21	43.96	43.25	124.03	131.43	73.84	70.58
	HA at 2000	108.86	113.39	47.80	46.02	140.08	141.54	83.46	66.16
S 1000	**0**	76.60	81.76	38.97	35.92	97.06	95.45	56.34	51.37
	CHT at 30	84.91	88.57	42.35	36.49	104.74	103.61	59.34	53.33
	CHT at 60	84.85	92.37	42.11	42.69	107.42	102.82	61.89	56.66
	CHT at 90	92.16	93.73	42.76	43.71	121.79	123.94	65.21	64.47
	HA at 1000	102.17	106.28	45.44	43.70	124.65	126.02	64.66	65.29
	HA at 1500	103.59	104.15	43.40	43.68	121.72	123.84	68.41	66.90
	HA at 2000	108.79	108.77	44.46	42.88	125.93	127.30	78.34	65.76
S 2500	**0**	72.56	79.79	35.83	34.28	95.17	96.26	54.24	51.32
	CHT at 30	84.58	90.88	43.09	42.72	95.99	94.89	56.38	52.41
	CHT at 60	83.80	91.96	38.30	42.84	103.10	103.28	63.51	56.98
	CHT at 90	89.15	95.00	40.36	38.66	113.38	119.11	63.66	63.77
	HA at 1000	103.24	106.62	41.12	44.40	121.03	121.82	63.74	64.89
	HA at 1500	105.54	103.70	42.75	42.87	112.52	114.50	66.78	66.02
	HA at 2000	103.79	111.14	44.26	43.61	124.11	124.90	79.33	63.14
S 5000	**0**	70.72	72.92	34.01	33.30	84.42	83.73	54.49	47.99
	CHT at 30	80.40	83.97	38.98	34.31	87.77	91.53	56.12	51.64
	CHT at 60	82.05	87.37	39.26	37.36	93.39	93.68	57.86	54.41
	CHT at 90	84.99	84.99	38.88	36.09	110.41	114.62	61.21	60.66
	HA at 1000	94.04	101.06	40.22	40.17	116.30	115.63	62.87	62.27
	HA at 1500	100.02	109.53	41.55	39.72	111.14	121.21	63.24	65.13
	HA at 2000	96.47	110.79	41.87	43.93	121.33	123.17	78.96	67.71
L.S.D. (0.05)									
Salinity (S)		3.53	4.45	1.3	1.34	3.01	2.72	1.72	1.22
Bio- stimulators (B)		4.67	5.88	1.72	1.77	3.98	3.60	2.28	1.61
SX B		9.35	11.76	3.44	3.55	7.97	7.20	4.55	3.22

*CHT= Chitosan HA = Humic acid

Table 4. Fresh and dry weights of roots of *Vitex trifolia* 'Purpurea' as affected by water salinity and bio- stimulators treatments and their interactions during the 2018 and 2019 seasons.

Treatments		Roots fresh weight (g/plant)		Roots dry weight (g/plant)	
		2018	2019	2018	2019
Mean of salinity (S), ppm					
0 (control)		107.72	104.88	53.42	55.81
1000		102.90	103.26	51.82	53.89
2500		103.41	99.51	50.68	53.64
5000		98.54	99.10	49.51	52.46
Mean of *bio-stimulators (B), ppm					
0 (Control)		87.06	91.37	47.72	49.13
CHT at 30		100.99	95.53	49.92	52.16
CHT at 60		101.00	98.28	50.58	51.78
CHT at 90		99.41	99.57	50.60	52.44
HA at 1000		115.82	105.55	53.52	56.27
HA at 1500		98.91	109.06	53.53	57.27
HA at 2000		118.80	112.44	53.61	58.60
Mean of interaction, (ppm)					
S 0	**0**	91.54	94.66	50.66	53.06
	CHT at 30	108.33	94.74	53.51	54.62
	CHT at 60	107.88	102.68	53.87	54.10
	CHT at 90	102.95	103.86	54.03	55.97
	HA at 1000	117.14	110.50	54.13	57.47
	HA at 1500	100.17	109.20	53.33	55.77
	HA at 2000	126.04	118.55	54.42	59.72
S 1000	**0**	88.31	92.03	49.75	50.25
	CHT at 30	100.26	100.44	51.26	53.45
	CHT at 60	103.27	99.96	51.00	51.95
	CHT at 90	102.38	100.76	50.69	51.02
	HA at 1000	115.54	108.82	53.32	56.96
	HA at 1500	92.65	109.54	52.49	54.89
	HA at 2000	117.90	111.26	54.21	58.70
S 2500	**0**	85.42	90.01	46.57	47.79
	CHT at 30	100.27	94.96	48.47	51.17
	CHT at 60	100.18	97.89	50.19	52.13
	CHT at 90	101.02	98.57	50.23	52.35
	HA at 1000	118.53	104.17	52.48	55.77
	HA at 1500	100.45	99.10	54.15	59.72
	HA at 2000	117.99	111.89	52.65	56.52
S 5000	**0**	82.98	88.77	43.90	45.43
	CHT at 30	95.10	91.99	46.44	49.41
	CHT at 60	92.65	92.61	47.29	48.96
	CHT at 90	91.31	95.10	47.46	50.41
	HA at 1000	112.06	98.72	54.15	54.89
	HA at 1500	102.39	118.42	54.16	58.70
	HA at 2000	113.29	108.07	53.17	59.46
L.S.D. (0.05)					
Salinity (S)		4.84	3.31	0.97	1.28
Bio- stimulators (B)		5.93	4.38	1.28	1.70
SX B		11.85	8.76	2.56	3.40

*CHT= Chitosan HA = Humic acid

Effect of bio-stimulators treatments

Data in Table 2, 3, and 4 also revealed that vegetative growth attributes of *Vitex trifolia* 'Purpurea' plants were dramatically affected by foliar application of the bio-stimulators treatments. In both seasons, spraying plants with any concentration of two tested bio-stimulators (CHT or HA) resulted in significant increase in most of growth parameters compared to control plants. The only one exception to the obtained trend were observed in the second season with plants sprayed with lowest concentration of CHT (30 ppm) which caused insignificant increase in plant height compared to control plants. Although, the recorded mean values in most cases were increased steadily as result of raising the concentration of CHT or HA compared to control, however HA was superior in its effect than CHT and among the different concentrations, the highest on (2000 ppm) was the most effective one for increasing of studied parameter. The obtained increases in vegetative growth parameters due to CHT treatments are is in agreement with reports of several researches (Ohta, et al., 1999; Salachna et al., 2015; Salachna et al., 2017; Pirbalouti et al., 2017; Byczyńska, 2018; El-Khateeb et al., 2018). Whereas the valuable pronounced increase in vegetative growth attributes due to HA treatments are is in harmony with the finding of numerous studies (Zhang et al., 2014; Esringü et al., 2015; El-Sayed et al., 2016; Ibrahim et al., 2016; Abou Dahab et al., 2017; Dorria et al., 2018; Noor El-Deen and El-Ashwah, 2019).

The Interaction effects between of salinity levels and bio-stimulators treatments data listed in Table (2 and 4) exhibited that, within each level of salinity, in most cases plants foliar sprayed with any concentration of two bio-stimulators (CHT or HA) had significantly higher values for the most of studied parameters than those recorded with the control plants (plants irrigated with salinity level and not received any bio-stimulators treatments). The data also cleared that under the same level of salinity; HA treatments gave higher values than those recorded with chitosan. In both seasons, in most cases, the highest values of the tested growth attributes were obtained from spraying plants irrigated with tap water with the highest concentration of HA (2000 ppm). On contrary, the lowest values were resulted from plants irrigated with the highest level of salinity (5000 ppm) and sprayed with tap water. In this concern previous authors reported increases in growth characters of plants subjected to salt stress as result of CHT treatment (Mahdavi, 2013; Yahyaabadi et al., 2016; El-Attar, 2017; Safikhan et al., 2018; Krupa-Małkiewicz and Smolik, 2019) or HA treatment (Mazhar et al., 2012; EL Sayed et al., 2017; Abd-El-Hady, 2019). The Superior effect of HA compared to CHT one may attribute to HA role on osmotic adjustment by stimulating the production of proline levels, reducing membrane leakage and enhancing antioxidant enzymes which scavenging reactive oxygen species under salt stresses (Van Oosten et al., 2017).

Chemical constituents

Chlorophylls content and total carbohydrates, K^+ % and Ca^{2+} %

It is clear from data in Table 5 that accumulation of total chlorophylls and total carbohydrates, K% and Ca^{2+} % in leaves was negatively affected by salinity stress. Generally, in both seasons, the tested parameters were reduced significantly in response to raising salinity levels from 1000 to 5000 ppm compared to the control. the lowest salinity levels (1000 ppm) was the only one exception to the observed trend since resulted in insignificant reduction in total chlorophylls content and K% in the second season compared to control.

Table 5. Total chlorophylls, total carbohydrates, K and Ca% of *Vitex trifolia* 'Purpurea' as affected by water salinity and bio- stimulators treatments and their interactions during the 2018 and 2019 seasons.

Treatments		Total chlorophylls content (SPAD)		Total carbohydrates (% DW)		K (% DW)		Ca (% DW)	
		2018	2019	2018	2019	2018	2019	2018	2019
Mean of salinity (S), ppm									
0 (control)		49.53	49.27	18.99	17.71	2.32	2.12	1.12	1.13
1000		47.68	47.51	17.73	16.93	2.27	2.09	1.05	1.04
2500		47.56	47.35	17.00	16.55	2.23	2.04	1.03	1.06
5000		45.29	45.32	15.94	15.47	2.20	2.01	1.03	1.04
Mean of *bio- stimulators (B), ppm									
0 (Control)		39.23	39.21	13.01	12.61	2.02	1.75	0.79	0.92
CHT at 30		44.70	42.28	15.72	15.06	2.31	2.05	1.02	1.08
CHT at 60		44.82	45.41	16.83	16.22	2.20	2.15	1.05	1.07
CHT at 90		47.12	45.32	17.04	17.05	2.21	2.13	1.19	1.11
HA at 1000		52.04	50.15	18.55	18.04	2.37	2.05	1.01	1.05
HA at 1500		51.61	51.37	19.01	18.65	2.31	2.10	1.09	1.03
HA at 2000		53.08	57.80	21.75	19.02	2.38	2.22	1.26	1.20
Mean of interaction, (ppm)									
S 0	**0**	42.78	44.62	14.01	14.13	2.09	1.93	0.81	0.98
	CHT at 30	46.36	43.76	16.51	15.07	2.36	2.16	1.13	1.12
	CHT at 60	46.48	46.76	17.57	16.42	2.30	2.23	1.15	1.07
	CHT at 90	49.44	45.59	19.47	18.40	2.29	2.14	1.25	1.18
	HA at 1000	53.77	51.72	21.07	19.58	2.38	1.99	1.07	1.13
	HA at 1500	54.59	53.07	21.62	19.42	2.39	2.19	1.14	1.18
	HA at 2000	53.28	59.40	22.68	20.92	2.45	2.21	1.33	1.29
S 1000	**0**	37.66	39.52	14.03	13.37	2.02	1.86	0.82	0.93
	CHT at 30	45.55	41.60	16.44	14.78	2.44	2.02	0.98	1.06
	CHT at 60	45.28	46.76	17.20	16.94	2.19	2.21	1.11	1.13
	CHT at 90	46.58	45.66	16.64	16.98	2.19	2.15	1.16	1.11
	HA at 1000	53.53	50.37	18.10	17.86	2.36	2.07	0.94	1.05
	HA at 1500	51.86	50.44	19.13	19.29	2.31	2.05	1.12	0.91
	HA at 2000	53.32	58.27	22.58	19.29	2.40	2.27	1.25	1.07
S 2500	**0**	39.86	37.25	13.19	12.37	2.05	1.74	0.78	0.88
	CHT at 30	46.13	43.24	15.47	15.71	2.27	1.98	1.03	1.05
	CHT at 60	45.63	45.91	17.04	16.34	2.13	2.09	1.00	1.05
	CHT at 90	46.24	44.25	16.31	16.49	2.21	2.11	1.12	1.15
	HA at 1000	51.78	50.89	17.38	17.15	2.35	2.09	1.01	1.03
	HA at 1500	50.57	53.09	18.61	18.88	2.26	2.10	1.03	1.03
	HA at 2000	52.75	56.82	21.05	18.88	2.35	2.21	1.25	1.23
S 5000	**0**	36.63	35.48	10.81	10.59	1.93	1.49	0.74	0.88
	CHT at 30	40.78	40.53	14.47	14.69	2.16	2.03	0.95	1.11
	CHT at 60	41.90	42.23	15.51	15.16	2.19	2.06	0.97	1.04
	CHT at 90	46.21	45.77	15.75	16.32	2.17	2.13	1.24	1.00
	HA at 1000	49.09	47.64	17.65	17.58	2.38	2.06	1.03	0.99
	HA at 1500	49.42	48.90	16.70	17.00	2.28	2.08	1.07	1.02
	HA at 2000	52.99	56.72	20.71	17.00	2.32	2.19	1.22	1.21
L.S.D. (0.05)									
Salinity (S)		1.23	1.80	0.73	0.57	0.04	0.07	0.05	0.04
Bio- stimulators (B)		1.62	2.38	0.97	0.75	0.05	0.10	0.08	0.06
SX B		3.25	4.75	1.94	1.50	0.10	0.19	0.16	0.11

*CHT= Chitosan HA = Humic acid

The reductions in total chlorophylls and total carbohydrates content a result of raising salinity stress are similar to those reported by various studies (Farahat et al., 2013; Ashour and Abdel Wahab, 2017; García-Caparrós and Lao, 2018). In recent study (García-Caparrós et al., 2020) reported decrease in total carbohydrates content in response to salt stress. Further, the reduction in K^+ and Ca^{2+} % is in harmony with that recorded by García-Caparrós and Lao (2018); Hooks and Niu (2019). In the present study the obtained reduction of K^+ and Ca^{+2} % in leaves as a result of salt stress may be due to physical and chemical similarities between K^+ and Na^+ and the tendency of Na^+ to compete with K^+ for major binding sites, including control of enzymatic activity which occurs at unfavorable cytosolic K^+/Na^+ ratios. The inhibition in Ca^{+2} uptake is due to the opposite effect between Ca^{2+} and Na^+ ions, that affects membrane properties due to displacement of membrane-associated Ca^{2+} by Na^+ which leading to degradation of membrane integrity and selectivity (García-Caparrós and Lao, 2018; Hooks and Niu, 2019).

Data presented in Table 5 also displayed that, application of bio-stimulators treatments had a positive influence on accumulation of total chlorophylls and total carbohydrates, K% and Ca% in leaves. In both seasons, foliar application of any concentration of the two types of bio-stimulators (CHT or HA) caused significant augmentation in the values compared to control. Generally, application of HA was superior in its effect than CHT one, especially at highest concentration (2000 ppm) since giving the highest values for tested traits in both seasons. These results in accordance with findings of earlier studies which reported that application of CHT caused increase in total chlorophylls (Dzung et al., 2011; Salachna et al., 2015; Byczyńska, 2018; El-Khateeb et al., 2018), carbohydrates content (Bistgani et al., 2017; Shafiei-Masouleh, 2019) and K, Ca% (Dzung et al., 2011). Whereas, the marked increase in tested components due to HA treatments are in conformity with prior studies that reported stimulatory influence of HA in augmentation of chlorophylls and carbohydrates content (Farahat et al., 2012; El Sayed et al., 2016; Abou Dahab et al., 2017; Noor El-Deen and El-Ashwah, 2019), increase K% (Zhang et al., 2014; Ibrahim et al., 2016; Noroozisharaf and Kaviani, 2018) and Ca% (Nikbakht et al., 2008; Dorria et al., 2018).

The increase in chlorophyll contents as a result of HA treatments may be attributed to its action on activation the plasma membrane H^+-ATP as enzyme, acidifying the rhizospheric region and increasing the NH_4^+ and NO_3^- uptake capacity which contributing to increase chlorophyll synthesis (Zandonadi et al., 2007, Canellas and Olivares, 2014). The augmentation in chlorophylls contents and photosynthetic activity could indirectly lead to increase in carbohydrates percentage.

As for the effect of interaction between salinity levels and bio-stimulators treatments the data point out that, In both seasons the lowest values of the tested traits were gained from spraying plants irrigated with the highest level of salinity (5000 ppm) with tap water. On the other hand, in most cases, the highest values were produced as a result of spraying plants irrigated with tap water with the highest concentration of HA (2000 ppm). The data also clarified that, within each level of salinity, in most cases using any concentration of the tested bio-stimulators (CHT or HA) caused significant increase in the recorded values compared to the control plants. Under the same level of salinity, HA treatments appeared to be more effective than CHT one. Among the different concentration of HA, the highest on (2000 ppm) was the most effective for increasing of studied parameter. In this regard prior researchers (El-Attar, 2017) declared that application of CHT resulted in augmentation in the content of chlorophylls, carbohydrates and K% in plants exposed to salt stress. Increasing chlorophylls content and K% in salt stressed plants due to HA treatment are similar to those obtained by earlier authors (El Sayed et al., 2017: Abd-El-Hady, 2019). Meanwhile, the present augmentation in carbohydrates content is supported by the results of Mazhar et al. (2012) and Karimian et al. (2019) who reported that foliar application of HA caused increase in total carbohydrates of in salt stressed ornamental plants compared to control plants.

Proline content

It is obvious from data in Table 6 that proline content in leaves was augmented linearly with increasing salinity stress compared to control plants.

In both seasons, the increments in proline content were insignificant with the lowest salinity level (1000 ppm), while higher levels (2500- 5000 ppm) caused significant increases in recorded mean values compared to control plants. Similar results of increasing proline content due to salinity stress were reported by many prior researchers (Bhatt et al., 2008; Farahat et al., 2013; Breś et al., 2016; Ashour and Abdel Wahab, 2017; García-Caparrós and Lao, 2018; García-Caparrós et al., 2020). They attributed proline augmentations in salt stress conditions to its role as osmotic adjustment, acting as a reservoir of energy and nitrogen for utilization, protection of enzymes and membranes. Additionally, proline accumulation under salt stress has been shown to protect plants against free radical-induced damage (Hayat et al., 2012).

The data shown in Table 6 visualized also that in most cases; plants sprayed with any concentration of CHT or HA had significantly higher values of proline content, except for in the case of spraying the lowest concentration of CHT (30 ppm) which caused insignificant increase in mean values compared to control. Similar increases in proline due to application of CHT treatments been reported by prior researches (Bistgani et al., 2017).

As for the effect of interactions between salinity levels and bio-stimulators treatments The data in Table 6 elucidated that, within each level of salinity, proline content in leaves of plants sprayed with any concentration of CHT or HA was higher than those of control plants, with superiority of HA. In both seasons, the highest values (6.88 and 7.01 µ moles/g fresh matter in the first and second seasons, respectively) were resulted from spraying plants irrigated with the highest level of salinity (5000 ppm) with HA at 2000 ppm. On contrary, the lowest values (3.04 and 2.46 µ moles/g fresh matter in the two seasons, respectively) were obtained from plants irrigated and sprayed with tap water (control). Under salt stress condition**,** although previous studies (El-Attar, 2017) stated that application of CHT reduced proline content in snapdragon salt stressed plants. However, recent authors (Jabeen and Ahmad, 2013; Safikhan et al., 2018; Krupa-Małkiewicz and Smolik, 2019) reported increase in accumulation of proline salt stressed plants due to CHT treatments which supported ours results. According to pervious researcher (Mazhar et al., 2012; Farahat et al., 2012) foliar application of HA caused reduction in proline content. However, in the present study proline content was increased with HA application which confirmed the reports of EL Sayed et al., 2017; Karimian et al., 2019; Abd-El-Hady, 2019.

Total phenolic content, Na^+ and Cl^- %

The data shown in Table 6 exhibited that, in most cases accumulation of total phenolic content, Na^+ and Cl^- % in leaves were augmented significantly with raising salinity levels compared to control. The only one exception to the obtained trend were recorded with the lowest salinity level (1000 ppm) which caused insignificant increase in total phenolic content in the first season seasons as well as Na^+ and Cl^- % in both seasons compared to control. the results of increasing total phenolic content in leaves of salt stressed plants are in good accordance with those elicited by Farahat et al., 2013; Karimian et al., 2019. While increase in Na^+% and Cl^-% are in agreement with findings of many previous studies (Breś et al., 2016; Ashour and Abdel Wahab, 2017, Hooks and Niu, 2019).

The data in Table 6 also disclosed that, total phenolic content, Na^+ and Cl^- % were reduced significantly as a result of foliar application of any concentration of the two types of bio-stimulators (CHT or HA) compared to the control. The data also evinced that when the two types of bio-stimulators sprayed at different concentration, HA appeared to be more effective than CHT. in most cases, the highest concentration of HA was the most effective for reducing the accumulation of total phenolic content, Na^+ and Cl^- % in leaves of *Vitix* plants. Although previous study revealed increase in total phenolic content due to CHT (Pirbalouti et al., 2017), and due to HA treatments (Abou Dahab et al., 2017) However, under the present study phenolic content was reduced in response to application of HA which support the results of El-Sayed et al., 2016 on Cycas Plant. Further, the reduction in Na^+% due to foliar application of HA is in accordance with findings of Farahat et al. (2012).

Concerning the interaction affects between two studied factors the data in Table 6 manifestly that**,** within each level of salinity spraying plants with any concentration of CHT or HA resulted in lower values for total phenolic content, Na^+ and Cl^- than that registered with the control plants. In most cases such reduction was significant compared to the control. Under the same level of salinity, HA treatments was preferable in reducing total phenolic content, Na^+ and Cl^- values than those CHT treatments. In both seasons, the highest values of three tested parameter were obtained from spraying plants irrigated with highest salinity level with tap water, whereas, the lowest values were resulted from plants irrigated with tap water and sprayed with the highest concentration of HA (2000 ppm). Under salt stress condition**,** earlier study (El-Attar, 2017) reported that application of CHT reduced the accumulation of Na^+ and Cl^- % in leaves of snapdragon salt stressed plants. While the noticeable reduction in accumulation of Na^+ and Cl^- % in plants exposed to salt stresses and treated with HA confirmed the reports of EL Sayed et al. (2017), Abd-El-Hady (2019).

K^+/Na^+ ratio

As shown in Figure 1 (a and b) the data revealed that, in both seasons K^+/Na^+ ratio in leaves was reduced progressively with raising salinity levels from 1000-5000 ppm compared to control. The obtained results of reduced K^+/Na^+ ratio have been demonstrated in previous studies (Bhatt et al., 2008; Hooks and Niu, 2019).

Table 6. Proline content, total phenolic, Na and Cl% of *Vitex trifolia* 'Purpurea' of *Vitex trifolia* 'Purpurea' as affected by water salinity and bio- stimulators treatments and their interactions during the 2018 and 2019 seasons.

Treatments		Proline content (µ moles/g FW)		Total phenolic (mg GAE/g DW)		Na (% DW)		Cl (% DW)	
		2018	2019	2018	2019	2018	2019	2018	2019
Mean of salinity (S), ppm									
0 (control)		4.17	3.95	1.31	1.41	0.86	0.83	0.51	0.57
1000		4.46	4.34	1.32	1.54	0.88	0.88	0.54	0.60
2500		4.51	4.61	1.46	1.61	0.90	0.90	0.57	0.63
5000		4.63	4.73	1.49	1.77	0.90	0.90	0.57	0.64
Mean of *bio- stimulators (B), ppm									
0 (Control)		3.45	2.90	1.63	1.95	1.12	1.24	0.75	0.86
CHT at 30		3.73	3.43	1.47	1.68	0.93	1.04	0.59	0.73
CHT at 60		3.99	3.65	1.45	1.67	0.87	0.89	0.52	0.61
CHT at 90		4.14	4.36	1.41	1.62	0.85	0.76	0.56	0.56
HA at 1000		4.63	4.97	1.42	1.52	0.81	0.77	0.50	0.54
HA at 1500		5.06	5.09	1.27	1.37	0.80	0.79	0.46	0.53
HA at 2000		6.09	6.47	1.10	1.27	0.80	0.65	0.46	0.45
Mean of interaction, (ppm)									
S 0	**0**	3.04	2.46	1.61	1.89	1.13	1.22	0.77	0.73
	CHT at 30	3.41	3.15	1.48	1.61	1.00	0.99	0.60	0.70
	CHT at 60	4.03	3.46	1.36	1.58	0.84	0.87	0.46	0.61
	CHT at 90	3.67	3.89	1.45	1.53	0.82	0.62	0.52	0.60
	HA at 1000	4.52	4.28	1.38	1.30	0.75	0.74	0.45	0.48
	HA at 1500	5.07	4.69	1.10	1.17	0.77	0.76	0.40	0.48
	HA at 2000	5.45	5.73	0.81	0.83	0.73	0.60	0.36	0.43
S 1000	**0**	3.40	2.86	1.63	1.94	1.15	1.25	0.74	0.84
	CHT at 30	3.89	3.35	1.38	1.68	0.97	1.06	0.60	0.76
	CHT at 60	4.25	3.76	1.59	1.66	0.84	0.87	0.47	0.63
	CHT at 90	4.29	4.19	1.29	1.50	0.83	0.83	0.51	0.53
	HA at 1000	4.57	4.95	1.15	1.49	0.83	0.76	0.50	0.49
	HA at 1500	5.30	4.83	1.14	1.35	0.79	0.78	0.50	0.47
	HA at 2000	5.51	6.48	1.06	1.17	0.74	0.63	0.47	0.46
S 2500	**0**	3.54	3.09	1.62	1.95	1.02	1.24	0.68	0.94
	CHT at 30	3.74	3.76	1.40	1.67	0.90	1.05	0.59	0.78
	CHT at 60	4.12	3.80	1.51	1.70	0.90	0.91	0.57	0.61
	CHT at 90	4.28	4.54	1.40	1.65	0.91	0.78	0.65	0.53
	HA at 1000	4.63	5.07	1.52	1.59	0.84	0.81	0.50	0.54
	HA at 1500	4.73	5.36	1.37	1.33	0.85	0.85	0.51	0.59
	HA at 2000	6.54	6.65	1.39	1.42	0.88	0.67	0.51	0.45
S 5000	**0**	3.82	3.19	1.68	2.02	1.20	1.25	0.82	0.95
	CHT at 30	3.88	3.45	1.64	1.76	0.85	1.09	0.55	0.70
	CHT at 60	3.58	3.59	1.35	1.74	0.90	0.89	0.57	0.58
	CHT at 90	4.33	4.81	1.50	1.81	0.86	0.82	0.55	0.58
	HA at 1000	4.78	5.59	1.64	1.71	0.85	0.78	0.55	0.63
	HA at 1500	5.14	5.51	1.47	1.66	0.80	0.78	0.44	0.56
	HA at 2000	6.88	7.01	1.13	1.66	0.87	0.71	0.52	0.45
L.S.D. (0.05)									
Salinity (S)		0.30	0.44	0.12	0.08	0.04	0.06	0.05	0.04
Bio- stimulators (B)		0.40	0.58	0.16	0.11	0.06	0.08	0.06	0.06
SX B		0.80	1.17	0.31	0.22	0.12	0.16	0.13	0.12

*CHT= Chitosan HA = Humic acid

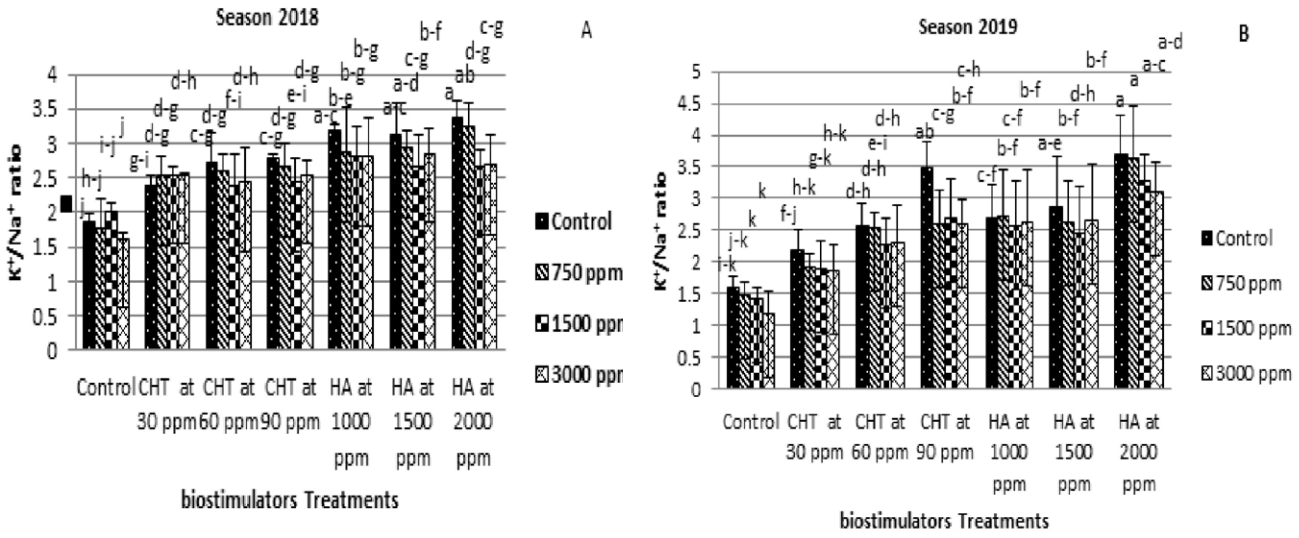

Figure 1. K^+/Na^+ ratio of *Vitex trifolia* 'Purpurea' as affected by water salinity and bio- stimulators during the 2018 and 2019 seasons. Different lower case letters indicate significant differences at 5% level of significance by LSD test.CHT= Chitosan HA = Humic acid

Application of bio-stimulators treatments had a noticeable effect on K+/Na+ ratio. In both seasons, foliar spaying of any concentration of CHT or HA caused increase in K+/Na+ ratio compared to control. In two seasons, HA treatments appeared to be generally more effective than CHT one. In both seasons, the lowest values of K+/Na+ ratio were obtained from plants irrigated with the highest level of salinity (5000 ppm) and sprayed with tap water. On the other hand, the highest values were resulted from plants irrigated with tap water and sprayed with the highest concentration of HA (2000 ppm).

Conclusions

Based on the results, it can be recommended that, CHT or HA at high concentration was the best effective treatments; however, HA was superior and economic treatment recommended for alleviating the adverse impact of salinity stress on *Vitex trifolia* 'Purpurea' plants irrigated with saline water at concentration up to 5000 ppm.

Author Contribution

HAA: implantation of the experiment, monitoring growth and development of plants, data collection, and data evaluation, statistical analysis, preparation and writing of the manuscript**. SEAE**: assistance in implantation of the experiment, chemical analysis, data collection, statistical analysis, preparation and writing of the manuscript. **MSK:** assistance in data collection, chemical analysis, statistical analysis, assistance in the preparation of manuscript.

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