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EFFECT OF PUTRESCINE AND ARGININE ON PHYSIOLOGICAL CHARACTERISTICS AND CHEMICAL CONSTITUENTS OF *VITEX TRIFOLIA* VAR. PURPUREA PLANT UNDER SALINITY STRESS

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ABSTRACT: Salinity is one of the most crucial variables that limits crop productivity and quality. Pots experiment was conducted in 2019 and 2020 to evaluate the efficiency of foliar application of arginine and putrescine on reducing the harmful effects of saline water on *Vitex trifolia* 'Purpurea' plants. The plants were irrigated with various water salinity levels (2000, 3000 and 4000 ppm), the plants were sprayed with different rates of arginine and putrescine i.e. 0, 50, 100 ppm. Salinity treatments decreased growth characteristics and nutrient contents. Salinity promoted the accumulation of volatile oil content. Foliar application of arginine at a rate of 50 ppm was less effective on growth characteristics, oil content under high salinity levels (3000 and 4000 ppm). However, the foliar application of putrescine at a rate of 100 ppm resulted in positive increases in growth, essential oil and chemical constituents of *V. trifolia* under the different saline irrigation levels, subsequently it reduced the harmful effect of salinity.

Keywords: *Vitex trifolia* 'Purpurea', salinity, arginine, putrescine, essential oil

INTRODUCTION

Vitex trifolia L., a member of the Verbenaceae family, is an evergreen plant that grows as either a flowering shrub or small tree. This species has a broad natural distribution across Australia, Southeast Asia, East Africa, and Micronesia (Rani and Sharma, 2013). Among its cultivated forms, the variety 'Purpurea' stands out as particularly popular, often referred to by the common names Arabian lilac, East Indian wild pepper or fascination.

Numerous active ingredients, including essential oils, diterpenes, and vitetrifolins, are known to be present in this plant. These compounds have a number of pharmacological qualities, including antipyretic, antibacterial, and antiallergic effects. Casticin, beta-sitosterol, beta-sitosterol-3-O-glucoside, rho-hydroxybenzoic acid, and 3,6,7-trimethylquercetagetin are the five compounds that have been isolated from *Vitex trifolia* fruits. The plant's leaves yielded a number of oils that demonstrated strong mosquito-repelling properties. Rotundinal, a cycloterpene aldehyde, was shown to be the active ingredient. Additionally, the plant demonstrated a great deal of promise as a botanical insecticide. Sulforhodamine B, a common tool in traditional Chinese medicine, was utilized to assess the anticancer effects of *Vitex trifolia* extracts on the growth of mammalian cancer cells. (Tiwari and Talreja, 2020).

The most significant issue with water quality in agriculture is salinity. In many parts of the world, water salinity is an environmental stressor that prevents certain crops from growing and producing as much as they could. There is an urgent need for better salt-tolerant plants due to the growing global issue of salinity's impact on crop output. Many scientists worldwide have noted the inhibitory effect of salinity on seed germination, plant development, nutrient uptake, and metabolism. (Ali *et al*., 2011). Since different crops have varying levels of tolerance to salinity, research is required to determine which plants can withstand salinity and to monitor any changes in physiological activity that may occur under saline irrigation.

The universal organic polycations known as polyamines (PAs) are linked to several essential physiological functions of plants, including growth, development, and senescence. (Bais and Ravishankar, 2002).

By modifying osmosis and scavenging ROS from the cell, PAs can also react to abiotic stress (Farooq *et al.,* 2009), controlling the regular physiological functions of plants (Chen *et al.,* 2019). Because it accumulates different ROS and inhibits the activities of antioxidant enzymes in plants, salinity has an impact on morpho-physiological and metabolic processes (Parvin *et al.,* 2014). Exogenous PAs, such as putrescine (Put.), lower Na^+ accumulation, boost antioxidant activity, protect the plasma membrane by regulating MDA and other ROS, and improve the photosynthetic capability of plants impacted by salinity. Putrescine (Put.), the main product of the PA biosynthesis pathway, serves as a synthetic precursor of spermidine (Spd) and spermine (Spm). Plants that receive a controlled foliar application of Put. can produce osmotic adjustment molecules like proline, total soluble sugars, and amino acids, as well as initiate physiological activities (Chen *et al.,* 2019). Additionally, documented were the functions of PAs in DNA, RNA, and protein production, sustaining plant growth and development, preventing aging, and shielding the membrane from oxidative damage by eliminating free radicals in plants (Alcázar *et al.,* 2020).

The primary precursor of polyamines and one of the necessary amino acids is L-arginine, which is decarboxylated by the enzyme arginine decarboxylase to produce putrescine. In higher plants, it has been determined that polyamines and arginine, their precursor, are crucial regulators of several physiological, developmental, and growth processes. Polyamines affect the cell cycle, cell division, morphogenesis in phytochrome and plant hormone-mediated processes, plant senescence regulation, and plant response to different stress stimulus (Abd El-Khalek and Awad Alla, 2023).

Therefore, the main objective of this study was to evaluate the role of arginine and putrescine in mitigating the harmful effects of salinity on growth characteristics and essential oil content, as well as some chemical characteristics of putrescine on *Vitex trifolia* plant.

MATERIALS AND METHODS

The experiment was conducted in the farm of Ornamental Plants Department, Horticulture Research Institute (HRI), ARC, Giza, Egypt, during two successive seasons of 2019 and 2020, in order to evaluate the mitigation effect of putrescine and arginine treatments on growth and active constituents of *Vitex trifolia* plants grown under water salinity.

On 10th February, in both seasons, seedlings of *Vitex trifolia* ‘Purpurea’ plants were obtained from a private nursery with a plant height of 25 cm and 4 branches/plant and planted individually in plastic pots, 30 cm in diameter, which were used in this study. Each pot was filled with 5 kg of sandy soil. Sandy soil was washed with water. The plants were fertilized with 12 g ammonium sulphate (20.5% N) and 4 g of potassium sulphate (48% K_2O_2). Half dose of the nitrogen and potassium fertilizer was applied 45 days after transplanting, while the second one was applied a month after the first one. Calcium superphosphate (15.5%) was mixed with the soil before transplanting. Pots were irrigated with equal amounts of tap water, one liter per pot, and two liters per pot after 15 days from transplanting, tell plants were established.

According to Jackson (1973), the chemical characteristics of the soil mixture used in the study were assessed at the Soil, Water and Environment Research Institute, Agriculture Research Centre (ARC), Giza, Egypt. The findings are shown in Table (1).

In both seasons, plants were irrigated twice/week using saline water at concentrations of 2000, 3000, and 4000 ppm, in addition to the control (tap water at 300 ppm). The plants irrigated with salinity levels were foliar-sprayed every 4 weeks with either arginine (A) or putrescine (P) at concentrations of 50 and 100 ppm, while the control plants sprayed only with tap water.

Experimental layout:

This experiment was designed using a split-plot design, with 20 treatment combinations (5 amino acid treatments × 4 salinity treatments, including the control). The salinity treatments were assigned to the main plots in a randomized complete blocks design with three replicates, while amino acid treatments were assigned to the sub-plots.

The recorded data:

After five months, the experiment was completed on July 15th, and the following morphological and physiological traits were recorded for both seasons: Plant height (cm), number of branches per plant, root length (cm), root weight (g), and the plant's fresh and dry weights (g).

Chemical constituents of leaves:

Chlorophyll content was assayed according to the method of Ferruzzi and Schwartz (2001). The Kjeldahl method (Pirie, 1955) was used to determine the total nitrogen levels. The method described by Chapman and Pratt (1982) was used to determine the amounts of the nutrients K, Na, Cl, and P in leaves. Proline content was determined colorimetrically according to (Bates *et al.,* 1973).

Essential oil content was determined according to the method of ASTA (1985). To assess GC/MS analysis of essential oil, the GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) according to Adams (2007).

Statistical analysis of data:

Data recorded on growth, oil content and chemical constituents were statistically analysed, and separation of means were performed using the Least Significant Difference (L.S.D.) test at the 5% level, as described by Little and Hills (1978).

RESULTS AND DISCUSSION

Effect of salinity levels and arginine and putrescine rates on growth characters of *Vitex trifolia* 'Purpurea' plants:

The findings in Tables (2 and 3) show that increasing saline water irrigation during both seasons resulted in a proportionate decline in all growth characteristics, including plant height, shoot number, fresh and dry weights of the plant, and root growth (root length and fresh weight). Irrigating plants with the lowest salinity level (2000 ppm) in the first and second seasons had the highest value of plant height (78.32 and 79.96 cm), shoots number (14.61 and 19.69), fresh weight of plant (236.48 and 201.49 g), dry weight/plant (66.60 and 56.28 g), root length (22.87 and 20.36 cm), and fresh weight of root (76.40 and 69.83 g), respectively. The highest reduction of plant growth parameters was recorded with salinity level at 4000 ppm (S3) compared to salinity level at 2000 ppm (S1). The highest salinity level S3 (4000 ppm) resulted in the lowest values of plant height (55.68 and 50.65 cm), shoots number (9.63

Table 1. Chemical analysis of the soil mixture.

Total nutrient contents (ppm)			E.C	pH	Soluble cations (meq/l)				Soluble anions (meq/l)		
N	P	K	(ds/m)		Na^+	K^+	Ca^{++}	Mg^{++}	HCO_3^-	$C1^-$	SO_4^-
71	23	107	1.51	7.44	0.54	0.32	1.12	0.27	1.03	0.41	0.69

Table 2. Effect of salinity levels and arginine and putrescine rates on vegetative growth of *Vitex trifolia* 'Purpurea' throughout first and second seasons.

Amino Acids (A)	First season (2019)					Second season (2020)				
	Salinity levels (ppm) (S)					Salinity levels (ppm) (S)				
	Control	S1	S2	S3	Mean	Control	S1	S2	S3	Mean
					Plant height (cm)					
AA0	56.05	57.35	50.57	45.49	52.36	61.22	58.18	51.95	40.94	53.07
A1 (50 ppm)	64.85	64.48	55.76	48.40	58.37	70.85	65.41	57.28	43.57	59.28
A2 (100 ppm)	84.72	80.14	65.78	53.25	70.97	92.56	81.30	67.57	47.92	72.34
P1 (50 ppm)	96.99	92.95	89.02	57.20	84.04	105.97	94.30	86.98	51.47	84.68
P2 (100 ppm)	106.05	96.65	93.84	74.08	92.65	114.77	100.61	93.45	69.33	94.54
Mean	81.73	78.32	70.99	55.68		89.07	79.96	71.45	50.65	
LSD (0.05)	S=3.24	A=2.11	S×A= 5.89			S=4.31	A=2.56	S×A=7.24		
					Number of shoots					
AA0	10.27	9.75	8.46	7.44	8.98	14.99	13.12	11.40	9.19	12.18
A1 (50 ppm)	12.74	12.80	8.95	8.65	10.79	18.57	17.24	12.06	10.67	14.63
A2 (100 ppm)	15.35	14.83	10.18	9.08	12.36	22.38	19.98	13.72	11.19	16.82
P1 (50 ppm)	16.41	15.61	13.11	10.04	13.79	23.93	21.02	18.55	12.39	18.97
P2 (100 ppm)	21.45	20.07	17.43	12.93	17.97	29.55	27.08	21.74	14.24	23.15
Mean	15.24	14.61	11.63	9.63		21.88	19.69	15.49	11.54	
LSD (0.05)	S=2.76	A=1.33	S×A= 3.18			S=4.63	A=3.12	S×A= 6.55		
					Fresh weight/plant (g)					
AA0	124.52	116.02	104.04	100.37	111.24	100.06	95.67	91.37	89.68	94.20
A1 (50 ppm)	193.38	151.82	128.15	112.87	146.56	155.39	131.38	110.89	99.99	124.41
A2 (100 ppm)	277.15	209.75	186.37	152.88	206.54	222.71	181.52	161.29	135.42	175.24
P1 (50 ppm)	344.62	286.50	255.44	167.28	263.46	276.93	247.94	209.76	148.18	220.70
P2 (100 ppm)	469.66	418.31	331.81	257.48	369.32	367.40	350.93	321.48	139.02	294.71
Mean	281.87	236.48	201.16	158.18		224.50	201.49	178.96	122.46	
LSD (0.05)	S=23.11	A=12.71	S×A=32.02			S=14.16	A=11.47	S×A=20.33		
					Dry weight/plant (g)					
AA0	31.91	29.22	27.95	26.24	28.83	26.04	25.67	24.56	23.60	24.97
A1 (50 ppm)	50.53	39.67	33.49	29.49	38.29	41.10	34.75	29.33	26.45	32.91
A2 (100 ppm)	76.58	57.95	51.50	42.24	57.07	60.38	49.21	43.73	36.71	47.51
P1 (50 ppm)	96.60	80.31	71.60	46.89	73.85	78.18	69.99	59.22	41.83	62.30
P2 (100 ppm)	134.84	125.84	117.08	73.92	112.92	102.21	101.79	92.46	39.98	84.11
Mean	78.09	66.60	60.32	43.76		61.58	56.28	49.86	33.71	
LSD (0.05)	S=4.17	A=3.52	S×A=8.26			S=6.28	A=4.63	S×A= 11.59		

***Control (tap water= 300 ppm), S1(2000 ppm), S2 (3000 ppm) and S3 (4000 ppm), AA0= without arginine or putrescine, A1= arginine at rate 50 ppm, A2=arginine at rate 100 ppm, P1= putrescine at rate 50 ppm, P2= putrescine at rate 100 ppm**

Table 3. Effect of salinity levels and arginine and putrescine rates on root growth of *Vitex trifolia* 'Purpurea' throughout first and second seasons.

Amino Acids (A)	First season (2019) Salinity levels (ppm) (S)					Second season (2020) Salinity levels (ppm) (S)				
	Control	S1	S2	S3	Mean	Control	S1	S2	S3	Mean
	Root length (cm)									
AA0	17.48	14.65	14.52	11.37	14.51	15.56	13.04	12.92	10.12	12.91
A1 (50 ppm)	21.03	22.64	19.32	14.26	19.31	18.71	20.15	17.19	12.70	17.19
A2 (100 ppm)	24.41	22.77	20.00	16.94	21.03	21.72	20.26	17.80	15.07	18.71
P1 (50 ppm)	28.72	24.67	24.50	19.26	24.29	25.56	21.95	21.81	17.14	21.62
P2 (100 ppm)	32.91	29.66	26.85	21.83	27.81	29.29	26.39	23.90	19.43	24.75
Mean	24.91	22.87	21.04	16.73		22.17	20.36	18.73	14.89	
LSD (0.05)	S=1.89	A=1.11	S×A= 3.78			S=1.67	A=1.32	S×A=3.25		
	Fresh weigh of root (g)									
AA0	58.40	48.93	48.50	37.96	48.45	53.38	44.73	44.33	34.70	44.28
A1 (50 ppm)	70.23	75.61	64.53	47.64	64.50	64.19	69.10	58.98	43.55	58.95
A2 (100 ppm)	81.52	76.04	66.79	56.57	70.23	74.51	69.50	61.04	51.70	64.19
P1 (50 ppm)	95.93	82.38	81.84	64.31	81.12	87.68	75.30	74.80	58.78	74.14
P2 (100 ppm)	109.91	99.05	89.70	72.92	92.89	100.46	90.53	81.98	66.65	84.90
Mean	83.20	76.40	70.27	55.88		76.04	69.83	64.23	51.08	
LSD (0.05)	S=5.03	A=3.18	S×A= 8.23			S=4.72	A=3.29	S×A= 7.52		

***Control (tap water= 300 ppm), S1(2000 ppm), S2 (3000 ppm) and S3 (4000 ppm), AA0= without arginine or putrescine, A1= arginine at rate 50 ppm, A2=arginine at rate 100 ppm, P1= putrescine at rate 50 ppm, P2= putrescine at rate 100 ppm**

and 11.54), fresh weight (158.18 and 122.46 g), dry weight (43.76 and 33.71 g), root length (16.73 and 14.89 cm), and fresh weight of root (55.88 and 51.08 g), indicating a significant decline in all growth characteristics and root growth. The reduction in vegetative growth caused by the highest salinity level is in accordance with prior investigators (Mohamedin *et al.,* 2006 on sunflower, Mousa *et al.,* 2020 and Safwat and Abdel Salam, 2022 on basil plants, Said and Mohammed, 2023 on cumin plant and Abd El-Khalek and Awad Alla, 2023 on anise plant).

Data in Tables (2 and 3) demonstrate the effectiveness of putrescine and arginine in mitigating the detrimental effects of saline irrigation water on growth parameters. Plant height (58.37 and 59.28 cm), shoots number (10.79 and 14.63), fresh weight (146.56 and 124.41 g), dry weight (38.29 and 32.91 g), root length (19.31 and 17.19 cm), and fresh weight of root (64.50 and 58.95 g) were among the growth characteristics that were least affected by arginine spraying at a rate of 50 ppm in the first and second seasons, respectively. In contrast, plants treated with P2 (100 ppm) produced the highest values of plant height (92.65 and 94.54 cm), number of shoots (17.97 and 23.15 shoots/plant), fresh weight/plant (369.32 and 294.71 g/plant), dry weight of plant (112.92 and 84.11 g), root length (27.81 and 24.75 cm), and fresh weight (92.89 and 84.90 g) in the two seasons, respectively, when compared to the control and the other two rates of arginine A2 and A1 in the two seasons. According to the findings in Tables 2 and 3, *Vitex trifolia* plants sprayed with arginine and putrescine under various saline irrigation water levels showed a promotion response in growth parameters. Spraying arginine at rate of 50 ppm was less effective on growth characteristics and root growth under saline irrigation at the high level (4000 ppm) with values of plant height (48.40 and 43.57 cm), shoots number (8.65 and 10.67), fresh weight of plant (112.87 and 99.99 g), dry weight of plant (29.49 and 26.45

g), root length (14.26 and 12.70 cm) and fresh weight of root (47.64 and 43.55 g) in the first and the second seasons, respectively. However, the plants that were irrigated with S1 (2000 ppm) and sprayed with P2 (100 ppm) had the highest values of growth characteristics, giving plant height (96.65 and 100.61 cm), shoot number (20.07 and 27.08), fresh weight of the plant (418.31 and 350.93 g), dry weight of the plant (125.84 and 101.79 g), root length (29.66 and 26.39 cm), and root fresh weight (99.05 and 90.53 g). It was observed that, there was non-significant effect between S1(2000 ppm) and S2(3000 ppm) in most growth characteristics.

The results were consistent with the findings reported by Bijay (1999) and Galston *et al.* (1997), who discovered that polyamines under stress cause a change in hormonal balance that is characterized by an increase in endogenous phytohormones in stressed plants. Exogenous application of putrescine on salt-stressed plants increased plant height, shoots number, fresh and dry weights of plant and root growth (root length and root fresh weight). In addition, Sarita and Mishra (2005) stated that because of their impact on cell division and differentiation, polyamines are currently thought to be regulators of plant growth and development. As polyamines and putrescine has been shown to assist plants deal with salt stress by increasing root growth and cell division, regulating osmotic balance, functioning as antioxidants, preserving ion homeostasis, and improving plant resistance to high salt stress situations (Chen *et al.,* 2019). Putrescine reduces the ABA/GA ratio, which causes the treated plants to grow more and have better morphological characteristics. Putrescine regulates the hormonal balance of plants, according to Mohammadi *et al.* (2024).

Effect of salinity levels and arginine and putrescine rates on oil content of *Vitex trifolia* 'Purpurea' plants:

The obtained results in Table (4) showed that irrigation of *Vitex trifolia* 'Purpurea' plants with different salinity levels recorded significant effects on essential oil percentage and yield per plant. Essential oil percentage was enhanced by increasing salinity levels, this increase was accompanied by a decrease in the essential oil yield per plant due to decreasing the plant fresh weight. Essential oil content was 0.45% and 0.53% at control (300 ppm) and increased to 0.47% and 0.55% at S1 (2000 ppm), 0.49% and 0.57% with S2 (3000 ppm) in the two seasons, respectively. However, the high salinity level S3 caused a maximum decrease, an oil content 0.41% and 0.49% at S3 (4000 ppm) in the two seasons, respectively. The reduction of primary metabolites in salt-stressed plants may be the cause of the rise in oil content, which makes intermediary products available for the synthesis of secondary metabolites (Morales *et al.,* 1993). Additionally, salt stress may have an indirect impact on the accumulation of essential oils by influencing either the net assimilation or the distribution of assimilates among processes of growth and differentiation (Charles *et al.,* 1990). Salinity's impacts on enzyme activity and metabolism may be the cause of its effects on essential oils and their constituents (Burbott and Loomis, 1969). Similar result was obtained by Neffati and Marzouk (2009) on coriander. On the other hand, the oil yield ml/plant has decreased gradually with increased salts in irrigation water. The highest values of oil yield/plant were recorded in control, 1.35 and 1.25 ml/plant, whereas the lowest ones (0.70 and 0.61 ml/plant) were recorded in plants irrigated by the highest level of saline water irrigation S3 (4000 ppm), this reduction may be due to the decrease in plant fresh weight under high salinity level. Similar results of negative effect of salt stress on essential oil yield were found by Tabatabaie and Nazari (2007) and Aziz *et al.* (2008) on peppermint, Belaqziz *et al.* (2009) on *Thymus maroccanus* and Said-Al Ahl and Mahmoud (2010) on basil, and Abd El-Wahab (2006) on fennel. It was also shown that essential oil yield of coriander was stimulated only under low salinity level, while it decreased at the high salinity levels (Neffati and Marzouk, 2008).

Table 4. Effect of salinity levels and arginine and putrescine rates on oil content of *Vitex trifolia* 'Purpurea' throughout first and second seasons.

Amino Acids (A)	First season (2019) Salinity levels (ppm) (S)					Second season (2020) Salinity levels (ppm) (S)				
	Control	S1	S2	S3	Mean	Control	S1	S2	S3	Mean
	Essential oil percentage									
AA0	0.33	0.33	0.35	0.28	0.32	0.39	0.40	0.41	0.36	0.39
A1 (50 ppm)	0.43	0.43	0.45	0.33	0.41	0.45	0.48	0.54	0.46	0.48
A2 (100 ppm)	0.47	0.48	0.52	0.41	0.47	0.55	0.56	0.63	0.52	0.57
P1 (50 ppm)	0.53	0.53	0.55	0.50	0.53	0.59	0.61	0.64	0.53	0.59
P2 (100 ppm)	0.51	0.56	0.60	0.52	0.55	0.63	0.65	0.68	0.57	0.63
Mean	0.45	0.47	0.49	0.41		0.52	0.54	0.58	0.49	
LSD (0.05)	S=0.02	A=0.01	S×A= 0.03			S=0.01	A=0.01	S×A=0.02		
	Essential oil yield (ml/plant)									
AA0	0.41	0.38	0.36	0.28	0.36	0.39	0.38	0.37	0.32	0.37
A1 (50 ppm)	0.83	0.65	0.58	0.37	0.61	0.70	0.63	0.60	0.46	0.60
A2 (100 ppm)	1.30	1.01	0.97	0.63	0.98	1.22	1.02	1.02	0.70	0.99
P1 (50 ppm)	1.83	1.52	1.40	0.84	1.40	1.63	1.51	1.34	0.79	1.32
P2 (100 ppm)	2.40	2.34	1.99	1.34	2.02	2.31	2.28	2.19	0.79	1.89
Mean	1.35	1.18	1.06	0.69		1.25	1.16	1.10	0.61	
LSD (0.05)	S=0.07	A=0.05	S×A= 0.09			S=0.05	A=0.04	S×A= 0.12		

***Control (tap water= 300 ppm), S1(2000 ppm), S2 (3000 ppm) and S3 (4000 ppm), AA0= without arginine or putrescine, A1= arginine at rate 50 ppm, A2=arginine at rate 100 ppm, P1= putrescine at rate 50 ppm, P2= putrescine at rate 100 ppm**

As for the effect of spraying with arginine and putrescine, the results shown in Table (4) revealed that all the treatments significantly mitigated the harmful effects of the saline irrigation water, an increase of essential oil percentage and yield compared to the control were recorded. P2 (100 ppm) recorded the highest percentage of essential oil (0.55 and 0.63%) compared to untreated plants (0.32 and 0.39%) in the two seasons, respectively. Also, the highest values of oil yield/plant were 2.02 and 1.89 ml/plant compared to 0.36 and 0.37 ml/plant in the untreated plants in the first and the second seasons, respectively. Similar effect had reported by Omer *et al.* (2013) on chamomile, and Velicka *et al.* (2022) on mint plants.

The results of the interaction between the saline irrigation water and arginine and putrescine treatments showed that, the superiority of S2 (3000 ppm) with P2 (100 ppm) treatment which giving the highest percentages of essential oil (0.60 and 0.63%) for both seasons, while the lowest percentages of essential oil were recorded in treatment S3 with A1 (0.33 and 0.46%) in both seasons. However, the highest essential oil yields ml/plant were 2.40 and 2.31 ml/plant which obtained from P3 under control (300 ppm) in two seasons, followed by S1 (2000 ppm) and S2 (3000 ppm) with same rate of putrescine 100 ppm. On the other hand, the lowest values of oil yields were 0.28 and 0.32 ml/plant which recorded in the control (without arginine or putrescine) with S3 (4000 ppm) in the two seasons, respectively. In general, the increase in plant growth characteristics resulting from spraying putrescine can be attributed to its stimulating role for vegetation growth as a stimulating plant hormone. It also works to reduce the effect of the abiotic stress, so it stimulates growth and increases the level of some plant hormones such as auxins and cytokines (Shahin *et al.,* 2010). Amino acid spraying contributes to the stimulation of biochemical and evolutionary processes and the carbohydrate industry by building chlorophyll, improving the properties of the components. Plants can be protected from environmental stress by amino acids. Abd El-Khalek and Awad Alla (2023).

Essential oil components:

Results in Table (5) showed that, the GC-Mass analysis of *Vitex trifolia* oil, 18 components were identified. β-caryophyllene as which represent the main component and others such as 1.8 cineol, sabinene, α-pinene, caryophyllene oxide and spathulenol were identified. It was noticed that, β-caryophyllene content was decreased with increasing salinity level. This may be due to the stress which lowers the amounts of secondary metabolites, which are linked to overall anabolism. Under salinity stress, anabolism is inhibited (Said-Al Ahl and Omer, 2011). β-caryophyllene content (20.33%) was achieved by (control) and decreased gradually with increasing saline water levels, the lowest value of β-caryophyllene content (14.71%) by S3. On the other hand, using Putrescine treatment, led to raise the β-caryophyllene content under saline water stress compared with control (AA0) treatment. The highest value of β-caryophyllene content was obtained with S2 + P2 compared to the other treatments. On the other hand, the highest content of α-Pinene (11.77%) was resulted from S3, while the lowest one (7.32%) was recorded by using control. It could be mentioned that, putrescine treatments led to an increase in the quality of the volatile oil content. The favorable effect of putrescine on the main compounds of essential oil is in agreement with those of Shehata *et al.* (2011) on celeriac plant, Omer *et al.* (2013) on chamomile plant and Saburi *et. al.* (2014) on the basil plant. Similar results of an effect of the maximum level of salinity were also found on lemon balm (Ozturk *et al.,* 2004), chamomile (Razmjoo *et al.,* 2008) and *Salvia officinalis* (Shalan *et al*., 2006). Moreover, Said-Al Ahl and Hussein (2010) on *Origanum vulgare* found that carvacrol content as the main essential oil constituent was decreased under salt stress, while p-cymene and γ-terpinene contents increased under non-salt stress treatments.

Effect of salinity levels and arginine and putrescine rates on total chlorophyll (mg/g F.W.) and total anthocyanin (mg/100 g F.W.) of *Vitex trifolia* 'Purpurea' plants:

The obtained results in Table (6) showed that irrigation of *Vitex* plants with different salinity levels recorded significant effects on the total chlorophyll and total anthocyanin. The salinity level of 2000 ppm recorded the highest values of total chlorophyll (12.70 and 13.97 mg/g) and total anthocyanin (0.75 and 0.74 mg/100g) in the first and second seasons, respectively. The highest reduction of the total chlorophyll and total anthocyanin were recorded with S3 compared to S1 treatments.

The efficacy of arginine in reducing the harmful effect of salinity on pigments are indicated in Table (6). Spraying arginine at a rate of 50 ppm had low efficacy on the pigments content, giving total chlorophyll of 9.44 and 10.57 mg/g and total anthocyanin of 0.63 and 0.65 mg/100 g, in the first and the second seasons, respectively. While plants treated with P2 (100 ppm) produced the highest values of total chlorophyll (14.93 and 16.12 mg/g) and total anthocyanin (0.95 and 0.86 mg/100 g), compared to the control and the other two rates of arginine A2 and A1, in two seasons, respectively.

Spraying arginine at a rate of 50 ppm was less effective on the pigment contents under saline irrigation at a level of 4000 ppm, with values of total chlorophyll of 6.88 and 7.71 mg/g and total anthocyanin of 0.40 and 0.43 mg/100 g in the first and the second seasons, respectively. On the other hand, the highest values of total chlorophyll (19.05 and 20.58 mg/g) and total anthocyanin were recorded in plants irrigated with tap water (300 ppm) and sprayed by P2 (100 ppm), followed by saline irrigation at level (S1) at 2000 ppm with P2 (100 ppm). It was observed that, there was non-significant effect between S1 and S2 on pigments contents.

The data clearly show that as the salinity level increased, there was a decrease in total chlorophyll and total anthocyanin levels compared to the control and putrescine

Table 5. Effect of Salinity levels and arginine and putrescine rates on the essential oil constituents (%) of *Vitex trifolia* 'Purpurea' throughout first season (2019).

Components	Treatments						
	Control	S1	S2	S3	S1P2	S2P2	S3P2
	The essential oil constituents (%)						
α-pinene	7.32	8.11	9.65	11.77	9.73	6.95	12.13
Sabinene	13.65	12.87	11.97	11.73	15.44	15.56	11.50
β- pinene	0.88	0.56	0.78	1.26	0.67	0.80	0.82
Mycene	0.22	0.93	1.11	2.01	1.12	1.12	0.87
α-terpinene	0.75	0.84	1.00	1.72	1.01	1.03	2.06
ρ-cymene	0.32	0.67	0.82	1.57	0.80	0.84	1.88
1.8 cineol	15.99	13.54	11.64	11.30	16.25	13.97	13.55
ɣ- terpinene	3.76	4.87	5.80	5.91	5.84	4.93	7.09
α-terpinolene	0.43	0.54	0.59	1.01	0.65	0.61	1.21
Terpinen-4-ol	0.66	0.98	1.26	2.17	1.18	1.11	2.61
Estragol	3.71	3.09	3.00	2.61	3.71	1.65	3.13
Bronyl acetate	1.82	1.91	2.08	2.25	0.44	2.14	2.70
α- terpinyl acetate	6.02	5.49	5.54	4.66	3.68	5.71	3.54
β-caryophyllene	20.33	19.43	16.90	14.71	20.01	22.45	21.70
α-caryophyllene	1.56	2.21	3.07	3.44	2.65	2.33	2.27
Germacrene	1.14	2.40	2.62	2.93	2.88	2.69	3.52
Caryophyllene oxide	7.11	6.43	5.72	5.38	4.05	4.35	3.44
Spathulenol	6.77	6.15	5.47	5.25	3.87	5.64	3.84

***Control (tap water= 300 ppm), S1(2000 ppm), S2 (3000 ppm) and S3 (4000 ppm), P2= putrescine at rate 100 ppm**

Table 6. Effect of salinity levels and arginine and putrescine rates on total chlorophyll (mg/g) and total anthocyanin (mg/100g f.w.) of *Vitex trifolia* 'Purpurea' throughout first and second seasons.

Amino Acids (A)	First season (2019) Salinity levels (ppm) (S)					Second season (2020) Salinity levels (ppm) (S)				
	Control	S1	S2	S3	Mean	Control	S1	S2	S3	Mean
	Total chlorophyll (mg/g)									
AA0	9.67	8.46	7.35	5.93	7.85	10.83	9.48	8.23	6.64	8.79
A1 (50 ppm)	11.98	11.12	7.78	6.88	9.44	13.42	12.45	8.71	7.71	10.57
A2 (100 ppm)	14.44	12.89	8.85	7.22	10.85	16.17	14.43	9.91	8.08	12.15
P1 (50 ppm)	15.43	13.55	11.96	7.99	12.24	16.67	14.64	12.92	8.63	13.21
P2 (100 ppm)	19.05	17.46	14.02	9.18	14.93	20.58	18.86	15.14	9.92	16.12
Mean	14.11	12.70	9.99	7.44		15.53	13.97	10.98	8.20	
LSD (0.05)	S=1.23	A=1.67	S×A= 2.51			S=1.33	A=1.15	S×A=2.26		
	Total anthocyanin (mg/100 g)									
AA0	0.84	0.55	0.44	0.35	0.55	0.92	0.54	0.45	0.36	0.57
A1 (50 ppm)	0.95	0.70	0.47	0.40	0.63	1.02	0.70	0.48	0.40	0.65
A2 (100 ppm)	0.99	0.75	0.57	0.44	0.69	1.02	0.74	0.58	0.45	0.70
P1 (50 ppm)	1.19	0.83	0.69	0.47	0.80	1.14	0.81	0.71	0.48	0.78
P2 (100 ppm)	1.35	0.93	0.87	0.64	0.95	1.16	0.94	0.77	0.55	0.86
Mean	1.06	0.75	0.61	0.46		1.05	0.74	0.60	0.45	
LSD (0.05)	S=0.15	A=0.11	S×A= 0.21			S=0.19	A=0.09	S×A= 0.18		

***Control (tap water= 300 ppm), S1(2000 ppm), S2 (3000 ppm) and S3 (4000 ppm), AA0= without arginine or putrescine, A1= arginine at rate 50 ppm, A2=arginine at rate 100 ppm, P1= putrescine at rate 50 ppm, P2= putrescine at rate 100 ppm**

treatments. This could be linked to the stress effects on moisture content or the ratio of bound to free water in plant cells, which may impact enzymes activity and protein synthesis, ultimately affecting the production of these pigments (Ma *et al.*, 2022). This outcome was consistent in both seasons. The increase in photosynthetic pigments due to spraying plants with putrescine might be because putrescine acts as a growth regulator, promoting plant development processes (Mohammadi *et al.,* 2024). Additionally, Tyagi *et al*. (2023) found that polyamines help maintain the cell's ionic balance, keep cell membranes intact, and prevent loss of chlorophyll.

Effect of salinity levels and arginine and putrescine rates on macro, micro elements and proline (%) of *Vitex trifolia* 'Purpurea' plants:

Data in Tables (7 and 8) showed that N, P and K concentrations *Vitex trifolia* plant were significantly decreased under the different salinity levels of water irrigation compared with control plants (300 ppm). The highest concentration of salinity S3 (4000 ppm) resulted in the minimum values for nitrogen (1.42 and 1.24%), phosphorus (0.13 and 0.16%) and potassium (1.15 and 1.13%) in the first and the second seasons, respectively. While Na, Cl and proline concentrations increased significantly in response to irrigation with all salinity levels as compared to control (300 ppm). The irrigation with saline water at 4000 ppm resulted in the highest concentration of sodium (1.73 and 1.95%), chloride (0.24 and 0.25%) and proline (7.59 and 8.11 mg/100g) in plants. While the control (irrigation with tap water) gave the minimum values of sodium (1.02 and 1.11%), chloride (0.13 and 0.14%) and proline (5.56 and 5.99 mg/100g) in plants. These results are similar to those obtained by Pessarakli (1991) and Al-Rawahy *et al.* (1992), who revealed that salinity could limit plant N accumulation. This might be the result of a rise in Cl absorption coupled with a fall in NO_3 concentration. (Bar *et al.,* 1997) and (Lea-Cox and Syverten, 1993). Phosphate content in anise plants reduced under saline condition due to ionic strength impact that lowers phosphate activity, as well as the fact that sorption processes and the limited solubility of P mineral tightly regulate phosphate concentration in soil solution. So, it makes sense that phosphate content in plants reduced as salinity increased. (Mohamedin *et al.,* 2006). Whereas, Grattan and Grieve (1999) reported that the K content in plants decreased under saline conditions due to high level of external Na interfere with K^+ acquisition by roots and also its effect on root membranes. The obtained results of Na and Cl concentrations are in good harmony with those obtained by Shi and Sheng (2005) on sunflower. Arginine and putrescine applications significantly reduced Na, Cl and proline concentrations, while they increased N, P and K concentrations under all salinity levels compared to control. Foliar application of P2 (100 ppm) significantly decreased the concentrations of Na (1.10 and 1.16%) and Cl (0.13 and 0.14%), in the two seasons. However, the same concentration of P2 significantly increased the concentration of N (1.66 and1.50%), P (0.27 and 0.33%), K (1.53 and 1.45%) and proline (7.75 and 8.37 mg/100g) in the first and the second seasons, respectively. Similar results were found by Sharma *et al.* (1997) they found that applying putrescine, which comes from the breakdown of arginine, to chick pea plants helped them take in more nitrogen, phosphorus, and potassium, but less sodium and chloride. Mansour and Al-Mutawa (1999) and El-Bassiouny and Bekheta (2001) showed that all arginine products, like putrescine, spermidine, and spermine, help salt-stressed plants over time by keeping the balance of positive and negative ions in their tissues. They also protect the plant cells by keeping the cell membranes stable when there is a lot of salt. When looking at how saline water interacts with arginine and putrescine treatments, it was found that the plants sprayed with P2 (100 ppm) under regular tap water (the control) had the highest levels of nitrogen, phosphorus, potassium, and proline. This was followed in the plants treated with

Table 7. Effect of salinity levels and arginine and putrescine rates on macro and micro elements (%) of *Vitex trifolia* 'Purpurea' throughout first and second seasons.

Amino Acids (A)	First season (2019) Salinity levels (ppm) (S)					Second season (2020) Salinity levels (ppm) (S)				
	Control	S1	S2	S3	Mean	Control	S1	S2	S3	Mean
N %										
AA0	1.47	1.40	1.36	1.30	1.38	1.41	1.32	1.26	1.15	1.28
A1 (50 ppm)	1.51	1.44	1.39	1.37	1.43	1.44	1.36	1.29	1.21	1.32
A2 (100 ppm)	1.64	1.46	1.44	1.42	1.49	1.57	1.37	1.35	1.25	1.38
P1 (50 ppm)	1.70	1.54	1.49	1.44	1.54	1.62	1.44	1.38	1.28	1.43
P2 (100 ppm)	1.77	1.67	1.63	1.56	1.66	1.67	1.58	1.40	1.34	1.50
Mean	1.62	1.50	1.46	1.42		1.54	1.41	1.34	1.24	
LSD (0.05)	S=0.03	A=0.02	S×A= 0.06			S=0.06	A=0.03	S×A=0.08		
P %										
AA0	0.24	0.16	0.13	0.10	0.16	0.34	0.20	0.17	0.13	0.21
A1 (50 ppm)	0.27	0.20	0.14	0.11	0.18	0.37	0.25	0.18	0.15	0.24
A2 (100 ppm)	0.29	0.22	0.16	0.13	0.20	0.37	0.27	0.21	0.17	0.26
P1 (50 ppm)	0.34	0.24	0.20	0.14	0.23	0.42	0.30	0.26	0.18	0.29
P2 (100 ppm)	0.39	0.27	0.25	0.18	0.27	0.43	0.40	0.28	0.20	0.33
Mean	0.31	0.22	0.18	0.13		0.39	0.28	0.22	0.16	
LSD (0.05)	S=0.04	A=0.02	S×A= 0.07			S=0.02	A=0.01	S×A= 0.02		
K %										
AA0	1.32	1.27	1.17	1.09	1.21	1.28	1.24	1.15	1.06	1.18
A1 (50 ppm)	1.40	1.32	1.22	1.11	1.26	1.35	1.28	1.20	1.08	1.23
A2 (100 ppm)	1.47	1.39	1.26	1.15	1.32	1.43	1.35	1.24	1.12	1.28
P1 (50 ppm)	1.57	1.46	1.31	1.19	1.38	1.51	1.43	1.29	1.16	1.35
P2 (100 ppm)	1.79	1.66	1.44	1.23	1.53	1.64	1.58	1.34	1.22	1.45
Mean	1.51	1.42	1.28	1.15		1.44	1.38	1.24	1.13	
LSD (0.05)	S=0.05	A=0.04	S×A=0.09			S=0.04	A=0.03	S×A=0.07		
Na %										
AA0	1.11	1.40	1.79	2.10	1.60	1.21	1.60	2.03	2.41	1.81
A1 (50 ppm)	1.08	1.29	1.56	1.92	1.46	1.18	1.48	1.79	2.21	1.66
A2 (100 ppm)	1.00	1.22	1.39	1.76	1.34	1.09	1.40	1.59	2.02	1.52
P1 (50 ppm)	0.97	1.06	1.13	1.52	1.17	1.06	1.23	1.29	1.74	1.33
P2 (100 ppm)	0.94	1.03	1.08	1.34	1.10	1.00	1.11	1.20	1.35	1.16
Mean	1.02	1.20	1.39	1.73		1.11	1.36	1.58	1.95	
LSD (0.05)	S=0.02	A=0.02	S×A=0.04			S=0.04	A=0.03	S×A= 0.09		
Cl %										
AA0	0.17	0.23	0.25	0.27	0.23	0.18	0.24	0.27	0.30	0.25
A1 (50 ppm)	0.15	0.16	0.22	0.25	0.19	0.15	0.16	0.23	0.27	0.20
A2 (100 ppm)	0.14	0.13	0.21	0.25	0.18	0.15	0.13	0.22	0.27	0.19
P1 (50 ppm)	0.11	0.12	0.15	0.23	0.15	0.12	0.12	0.16	0.24	0.16
P2 (100 ppm)	0.10	0.11	0.14	0.19	0.13	0.11	0.12	0.15	0.17	0.14
Mean	0.13	0.15	0.19	0.24		0.14	0.16	0.21	0.25	
LSD (0.05)	S=0.01	A=0.01	S×A=0.02			S=0.01	A=0.02	S×A= 0.02		

***Control (tap water= 300 ppm), S1(2000 ppm), S2 (3000 ppm) and S3 (4000 ppm), AA0= without arginine or putrescine, A1= arginine at rate 50 ppm, A2=arginine at rate 100 ppm, P1= putrescine at rate 50 ppm, P2= putrescine at rate 100 ppm**

Table 8. Effect of salinity levels and arginine and putrescine rates on proline (mg/100 g F.W.) of *Vitex trifolia* 'Purpurea' throughout first and second seasons.

Amino Acids (A)	First season (2019) Salinity levels (ppm) (S)					Second season (2020) Salinity levels (ppm) (S)				
	Control	S1	S2	S3	Mean	Control	S1	S2	S3	Mean
	Proline (mg/100 g F.W.)									
AA0	4.88	5.92	6.11	6.48	5.85	5.32	6.45	6.66	7.05	6.37
A1 (50 ppm)	5.26	6.08	6.70	7.09	6.28	5.74	6.63	7.30	7.73	6.85
A2 (100 ppm)	5.28	6.70	6.97	7.69	6.66	5.75	7.30	7.60	8.38	7.26
P1 (50 ppm)	5.93	7.05	7.60	7.50	7.02	6.46	7.69	8.28	8.18	7.65
P2 (100 ppm)	6.48	7.42	7.87	9.21	7.75	6.69	8.69	8.91	9.21	8.37
Mean	5.56	6.63	7.05	7.59		5.99	7.35	7.75	8.11	
LSD (0.05)	S=0.26	A=0.15	S×A= 0.38			S=0.43	A=0.33	S×A=0.76		

***Control (tap water= 300 ppm), S1(2000 ppm), S2 (3000 ppm) and S3 (4000 ppm), AA0= without arginine or putrescine, A1= arginine at rate 50 ppm, A2=arginine at rate 100 ppm, P1= putrescine at rate 50 ppm, P2= putrescine at rate 100 ppm**

S1 (2000 ppm) and S2 (3000 ppm), along with the same rate of putrescine (100 ppm). These treatments also helped lower the sodium and chloride levels in the plants. However, when plants were sprayed with the control solution (without arginine or putrescine) under high saline water (S4), they ended up having the highest levels of sodium and chloride. At the same time, the levels of nitrogen, phosphorus, and potassium dropped in the first and second growing seasons, respectively. Nassar (1997) noted that putrescine was better at increasing proline levels in pea plants. These findings match those reported by Bijay (1999) and Maiti *et al.* (2004).

CONCLUSION

From the results, it is suggested that arginine or putrescine at high levels worked well, but putrescine was the better choice for helping *Vitex trifolia* 'Purpurea' plants deal with the harmful effects of saline irrigation stress at a concentration up to 3000 ppm.

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تأثير البوتريسين والأرجينين على الخصائص الفسيولوجية والمكونات الكيميائية لنبات فيتكس تريفوليا صنف بوربوريا تحت الإجهاد الملحي

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تُعد الملوحة من أهم العوامل التي تُؤثّر سلبًا على إنتاجية وجودة المحاصيل. أجريت تجربة في أصصٍ عامي ٢٠١٩ و٢٠٢٠ لتقييم كفاءة الرش الورقي بالأرجينين والبوتريسين في الحد من الآثار الضارة لمياه الملوحة على نباتات فيتكس تريفوليا 'بوربوريا'. رُويت نباتات فيتكس بمستويات ملوحة مياه مختلفة (٢٠٠٠ ، ٣٠٠٠ و٤٠٠٠ جزء في المليون)، ورشّت النباتات بتركيزات مختلفة من الأرجينين والبوتريسين (٠ ، ٥٠ و١٠٠ جزء في المليون). أدّت مُعاملات الملوحة إلى انخفاض خصائص النموّ ومحتويات العناصر الغذائية، كما عزّزت الملوحة تراكم محتوى الزيت الطيار. كان رشّ الأرجينين بمعدل ٥٠ جزءًا في المليون أقل فعاليةً على خصائص النموّ ومحتوى الزيت في ظل مستويات الملوحة العالية (٣٠٠٠ و٤٠٠٠ جزء في المليون). ومع ذلك، أدى الرش الورقي للبوتريسين بمعدل ١٠٠ جزء في المليون إلى زيادات إيجابية في النمو والزيت العطري والمكونات الكيميائية لنبات الفيتكس تحت المستويات المختلفة من الري الملحي، وبالتالي ادي الي تقليل التأثير الضار للملوحة.