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Biochemical and Growth Enhancing Effects of Garlic Clove Extract on *Brachychiton rupestris* Seedling Irrigated with Saline Water: Implications for Bioactive Compound Utilization

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Abstract

This study aimed to test the morphological and chemical responses of *B. rupestris* seedlings subjected to saline water irrigation (SWI) individually or in combination with dry garlic cloves extract (DGCE) which works as eco-friendly bio-stimulant. Seedling of *B. rupestris* were treated with DGCE at concentrations of 0, 5, and 10% under different salinity levels 0, 3000, 6000 and 9000 ppm from the salts mixture consist of 3(NaCl):1[3($CaCl_2$) +1 ($MgCl_2$)]. The study assessed key growth parameters, including plant height, stem diameter, number of leaves per plant, fresh and dry weight of plant organs, and root development. In addition, biochemical traits such as Photosynthetic pigments, the content of phenols, proline, total flavonoids and potassium and sodium were measured. Also HPLC phenolic profile of aqueous DGCE extract was conducted. The results showed that seedlings irrigated with 3000 ppm SWI combined with 5% DGCE exhibited significantly improved growth parameters; including plants height, stem diameter, leaf number, and fresh and dry biomass, compared to the control. The highest root growth was observed in plants treated with 5% DGCE under tap water irrigation. Additionally, the 5% DGCE treatment at 9000 ppm SWI resulted in the highest proline content. While the 10% DGCE treatment at 9000 ppm SWI led to the highest total phenol and flavonoid levels. The K+/Na+ ratio increased with higher DGCE concentrations which improved salt tolerance.

Keywords: *Brachychiton rupestris*, salinity, garlic extract, total phenols and total flavonoids, non-enzymatic antioxidant.

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Introduction

Brachychiton rupestris commonly known as the narrow-leaved bottle tree or the Queensland bottle tree is a Malvaceae tree native to Queensland, Australia. Malvaceae is a flowering plant family with over 200 genera and 2300 species [1,2]. *B. rupestris* bark is dark grey, with superficial tessellation and deeper cracks. Immature tree trunks and smaller branches are either light green or grey, while the mature trees have a thick, robust trunk that helps store water during dry spells [3]. The leaves, resembling those of every species of the genus, alternate along the stems [4]. *Brachychiton sp*. includes a variety of chemical components, including sterols, triterpenes, flavonoids, phenolic acids, coumarins, and alkaloids [5,6]. Aboriginal people exploited the trees by eating the roots of young plants and consuming fluids from the trunk caused by wounds; the leaves were also used in feeding the animals. During drought situations, whole trees are cut for feedstock, and the soft edible pulp inside the trunk can be observed by removing the bark, where the pulp is energy-rich but protein-poor [7].

Salinity is a global concern, and the region that is impacted is steadily expanding, where it is considered a major abiotic stressor severely restricting plant growth and yield. It negatively impacts every stage, from seed germination and seedling development to flowering and fruit formation, consequently lowering both quantity and quality of produce.

[8, 9]. Soil salinization affects almost 50% of irrigated croplands globally, leading to decreased plant growth, development, and survival [10]. The presence of sodium chloride (NaCl) in soil and irrigation water significantly hinders plant growth. The presence of salt stress affects a range of physiological and biochemical processes [11]. The presence of high salinity levels led to a decrease in the amount of water in the plants and also caused toxicity due to excessive ions, resulting in an imbalance of ions. Aside from the osmotic and toxic consequences, salt stress also causes oxidative stress in plants. These processes collectively contribute to the detrimental effects of salinity [12]. The use of bio-stimulants, which are substances or materials that can be used to regulate physiological processes in plants while encouraging their development, has grown over the past decade [13]. Plant extracts are becoming increasingly used as bio-stimulants in vegetable production also different plant extracts have a promoting effect in resisting environmental stress [14,15,16]. Garlic (*Allium sativum* L.) is one of the world's most significant crops, with 1.437.690 ha of harvested land and an annual yield of 24.255.303 tons of dry bulbs [17]. Garlic is important since it is used not only in cooking but also for therapeutic and medicinal purposes in both traditional and modern medicine. It is ingested as a raw vegetable (fresh leaves or dried cloves) or after processing in the form of garlic oil, garlic extracts, and garlic powder, with variations in chemical makeup and bioactive ingredient concentration. Garlic extract contains carbohydrates, fibbers, lipids, manganese, potassium, calcium, sulphur, phosphorus, magnesium, sodium, vitamin B 6, arginine, vitamin C, aspartic acid, glutamic acid, leucine, and lysine [18]. It has a strong antimicrobial potential against a variety of bacteria, fungi and viruses [19,20,21]. Moreover, the antioxidant potential of garlic was reported by Chan *et al*. [22], so Hayat *et al*. [23] suggested using aqueous garlic extract as a natural stimulator for plant growth.

Our study aimed to test the morphological and chemical responses of *B. rupestris* seedlings subjected to saline water irrigation (SWI) individually or in combination with dry garlic cloves extract (DGCE) which works as eco-friendly bio-stimulant.

2. Materials and Methods

2.1. Study Area

The experiment was carried out in the greenhouse of the Ornamental Horticulture Dept., Faculty of Agriculture, Cairo University, Egypt. During 2018 and 2019 seasons, each season started in March and ended in November. The chemical estimates were conducted in Ornamental Plants and Woody Trees Dept., National Research Centre (NRC), Egypt.

2.2. Plant Material

One-year-old seedlings of *Brachychiton rupestris* with traits average 15-20 cm height, 0.4-0.6 cm diameter, and 5-8 leaves/ plant were obtained from private nursery in Qalyubia Governorate.

2.3. Saline water preparation

The saline water used was prepared by different salts of NaCl, $CaCl_2$, and $MgCl_2$ according to [24] as follows: 3(NaCl):1{3($CaCl_2$) +1 ($MgCl_2$)}and prepared the concentration 3000, 6000 and 9000ppm by dissolving 3, 6 and 9g of the salts mixture in 1 liter of tap water, in addition to the control treatment (irrigation with tap water).

2.4. Dry garlic cloves extract (DGCE) preparation

The pure powder of dry garlic cloves was obtained from Hort. Pharma Co., Egypt, and was prepared by adding 5 and 10g of garlic powder to 100ml distilled to prepare 5 and 10% rates and left for 24 hours to complete the extraction, and then filtered to be ready to the application.

2.5. HPLC analysis of phenolic compound of DGCE

The HPLC analysis of phenolic compound of DGCE (Table 1) was carried out using an Agilent 1260 series. The separation was carried out using Zorbax Eclipse plus C8 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–1 min (82% A); 1-11 min (75% A); 11-18 min (60% A); 18-22 min (82% A); 22-24 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40 °C.

2.6. Procedures

The seedlings were planted in 30 cm plastic pots (one seedling /pot) filled with 10 Kg of sandy loam soil. Chemical and physical analysis of the soil samples were carried out according to the standard measures that declared by [25,26] inserted in Tables (2& 3). The seedlings treated with saline water irrigation (SWI) with all tested concentrations started to apply after 4 weeks from transplanting until the end of the experiment; tap water was used for control, where the irrigation level is maintained at 60% of the field capacity. Application of DGCE at all rates was conducted twice as foliar application, the first application after 4 weeks from transplanting and the second after two months from the first application. The control plant was sprayed with water. All plants that received kristalon (NPK 19:19:19) through this experimental work were used at the rate of 3g. pot-1 in four doses (after 4,8,16 and 20 weeks from the planting date). The samples were collected to record the vegetative growth measurements and conducted the chemical determinations in November of each season. The vegetative growth characteristics were measured: Plant height (cm), stem diameter (cm), number of branches/plant, root length (cm), and fresh and dry weight of leaves, stems, and roots (g. plant-1). Chemical determinations: Photosynthetic pigments including chlorophyll a, b, and carotenoids (mg. g-1 FW) were determined according to [27], total phenols content (mg. g-1 FW) was determined according to the method stated by [28], proline content (mg. g-1 FW) was determined according to [29], total flavonoids (mg. g-1 FW) were determined

according to [30], Potassium and Sodium (%) were determined according to the method described by [31]. K and Na ratio was calculated by dived K value to Na value for each treatment.

2.7. Experiment Layout

The layout of the experiment was a factorial experiment in complete block design, including 12 treatments, which was the combination of four treatments of SWI and three different levels of DGCE, each treatment was replicated three times for each season during the study.

2.8. Statistical analysis

The obtained data were subjected to statistical analysis variance according to the method described by [32] using least significant analysis at 5%, correlation coefficient analysis was calculated by Microsoft Excel 2010.

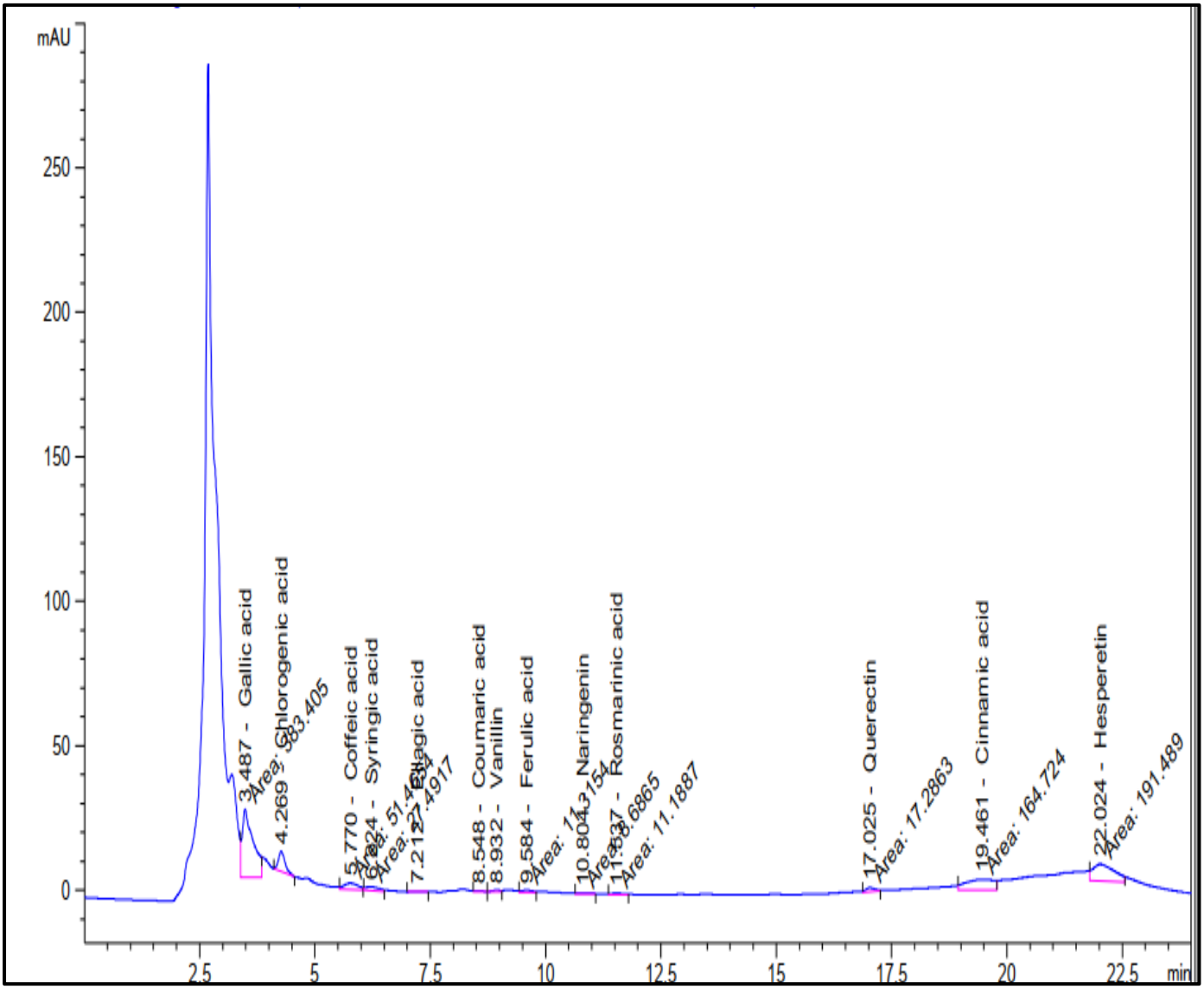

Fig. 1. HPLC profile for aqueous solution of dry garlic cloves extract (DGCE)

Table 1. HPLC phenolic profile of DGCE

Components Conc. (µg. ml-1)	Garlic extract
Gallic acid	28.07
Chlorogenic acid	9.55
Coffeic acid	2.64
Syringic acid	1.62
Ellagic acid	0.20
Coumaric acid	0.13
Vanillin	0.23
Ferulic acid	0.66
Naringenin	0.80
Rosmarinic acid	1.09
Querectin	2.15
Cinnamic acid	3.19
Hesperetin	8.97

Table 2. Soil chemical analysis

PH 1:2.5	EC (ds/m)	Sp	Anion (ppm)				Cation (ppm)			
			$SO4^{--}$	Cl	$HCO3^{-}$	$CO3^{---}$	Ca^{++}	Mg^{++}	Na^{+}	K^{+}
7.5	2.85	25.0	2.39	25.1	0.51	_	7.9	3.6	16.0	0.5

Table 3. Soil physical analysis

Physical analysis				Texture
Coarse sand	Fine sand	Silt	Loam	
40.3	43.0	12.5	4.2	Sandy loam

3. Results

3.1. Vegetative growth parameters

Data on morphological parameters of vegetative growth of *Brachychiton rupestris* seedlings were affected by SWI stress and DGCE in the two successive seasons are given in Tables (4&5). The studied parameters of vegetative growth included plant height, stem diameter, number of leaves/plant, root length, and fresh and dry weight of all plant organs (leaves, stems, and roots/plant in both successive seasons. It is realized that the concentrations of 6000 and 9000 ppm of SWI induced decrements in all investigated growth parameters in both seasons. The highest values for all these parameters were obtained due to the low salinity level of 3000 ppm which gave 86.00 and 85.83 cm for plant height, 1.29 and 1.35 cm for stem diameter, 40.44 and 43.00 for No. of leaves/plant, 33.51 and 39.50 g/plant for leaves fresh weight, 25.15 and 25.27 g/plant for stem fresh weight, 22.66 and 26.89 g/plant for leaves dry weight and 14.57 and 14.80 g/plant for stem dry weight, respectively, in the first and second seasons. The achieved results showed that all tested levels of SWI in the irrigation water decreased root parameters (length, fresh and dry weight) in the two seasons. The major decrease was recorded on SWI concentrations at 6000 and 9000 ppm. It was revealed that increasing the concentration of SWI significantly delayed these parameters and reached its maximum at SWI (9000 ppm) concentration in both seasons being (26.90 and 20.65%) for root length, (39.65 and 36.43%) for root fresh weight and (51.54 and 44.68 %) for root dry weight, respectively, in the first and second seasons compared with the control plants.

Table 4: Effect of dry garlic cloves extract (DGCE) on vegetative growth of *B. rupestris* under saline water irrigation (SWI) during 2018 and 2019 seasons

Treatment		Plant height (cm)		Stem diameter (cm)		No. of leaves/ plant		Root length (cm)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control		79.31	77.83	1.15	1.23	34.55	38.11	35.95	37.33
SWI 3000ppm		86.00	85.83	1.29	1.35	40.44	43.00	33.78	35.92
SWI 6000ppm		74.00	74.61	1.05	1.15	31.11	34.33	29.72	33.01
SWI 9000ppm		61.62	64.67	0.87	0.98	26.56	26.78	26.28	29.62
$LSD_{5\%}$		3.11	2.53	0.05	0.05	2.58	2.61	2.73	2.46
Control		65.32	64.17	0.90	0.95	26.25	28.59	28.64	31.62
DGCE 5%		83.08	86.38	1.29	1.40	40.34	41.83	34.13	37.66
DGCE 10%		77.20	76.66	1.08	1.18	32.92	36.25	31.54	32.63
$LSD_{5\%}$		2.70	2.92	0.04	0.04	2.23	2.26	2.37	2.13
Control	Control	68.27	65.00	0.97	0.99	27.00	31.67	33.36	35.33
	DGCE 5%	88.00	91.00	1.36	1.50	44.33	45.00	38.00	40.67
	DGCE 10%	81.67	77.50	1.12	1.21	32.33	37.67	36.50	36.00
SWI 3000ppm	Control	71.00	70.00	1.00	1.06	30.33	32.00	30.67	34.12
	DGCE 5%	96.33	97.50	1.53	1.58	49.67	50.33	37.00	39.30
	DGCE 10%	90.67	90.00	1.35	1.40	41.33	46.67	33.67	34.33
SWI 6000ppm	Control	62.67	61.67	0.86	0.90	25.00	26.67	26.33	30.36
	DGCE 5%	83.00	86.67	1.24	1.36	36.67	41.00	32.50	36.67
	DGCE 10%	76.33	75.48	1.03	1.18	31.67	35.33	30.33	32.00
SWI 9000ppm	Control	59.76	60.00	0.78	0.86	22.67	24.00	24.18	26.67
	DGCE 5%	65.00	70.33	1.02	1.15	30.67	31.00	29.00	34.00
	DGCE 10%	60.12	63.67	0.82	0.94	26.33	25.33	25.67	28.18
$LSD_{5\%}$		5.54	5.19	0.08	0.08	4.59	4.64	4.73	4.38

Table 5: Effects of dry garlic cloves extract (DGCE) on fresh and dry weight of (leaves, stems, and roots) of *B. rupestris* under saline water irrigation (SWI) during 2018 and 2019 seasons

Treatment		Leaves F.W. (g/ plant)		Stem F.W. (g/ plant)		Root F.W. (g/ plant)		Leaves D.W. (g/ plant)		Stem D.W. (g/ plant)		Root D.W. (g/ plant)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control		29.71	35.85	23.68	23.51	131.87	137.32	20.38	24.73	13.99	13.98	49.96	51.74
SWI 3000ppm		33.51	39.50	25.15	25.27	114.63	129.22	22.66	26.89	14.57	14.80	41.29	47.65
SWI 6000ppm		26.94	33.24	22.50	22.14	93.14	109.69	18.80	23.07	13.43	13.32	30.62	38.69
SWI 9000ppm		21.46	27.18	18.86	19.93	79.59	87.30	15.44	19.21	11.61	12.19	24.21	28.62
$LSD_{5\%}$		2.03	1.87	0.94	0.85	4.57	4.97	0.93	0.87	0.94	0.79	2.69	2.49
Control		22.09	26.78	19.65	19.48	89.72	101.60	15.57	18.99	12.06	11.99	29.22	35.08
DGCE 5%		32.91	39.88	25.55	26.06	121.58	138.92	22.35	27.13	14.78	15.19	44.64	52.39
DGCE 10%		28.71	35.17	22.53	22.60	103.14	107.13	19.86	24.32	13.37	13.55	35.70	37.56
$LSD_{5\%}$		1.76	1.62	0.81	0.74	3.96	4.30	0.80	0.76	0.81	0.68	2.33	2.16
Control	Control	22.68	28.62	20.87	20.14	112.64	124.46	16.02	20.25	12.68	12.43	40.75	45.77
	DGCE 5%	35.40	42.42	26.79	27.71	155.33	153.79	23.70	28.77	15.35	15.96	61.11	59.87
	DGCE 10%	31.04	36.50	23.68	22.67	127.64	133.70	21.42	25.18	13.95	13.55	48.02	49.57
SWI 3000ppm	Control	23.30	32.23	21.25	21.00	94.72	117.03	16.40	22.60	12.82	12.77	31.04	41.56
	DGCE 5%	41.05	45.28	27.84	28.92	132.92	150.46	27.26	30.24	15.68	16.49	50.59	58.02
	DGCE 10%	36.17	41.00	26.36	25.88	116.25	120.18	24.31	27.84	15.20	15.15	42.25	43.37
SWI 6000ppm	Control	21.84	24.47	19.33	18.50	79.69	92.27	15.63	17.46	11.98	11.51	24.18	30.76
	DGCE 5%	32.75	39.06	25.62	25.83	106.67	139.88	22.55	26.69	14.89	15.18	37.56	52.34
	DGCE 10%	26.22	36.19	22.56	22.09	93.07	96.91	18.23	25.05	13.43	13.27	30.12	32.97
SWI 9000ppm	Control	20.55	21.80	17.13	18.26	71.81	72.64	14.96	15.65	10.74	11.24	20.90	22.21
	DGCE 5%	22.43	32.77	21.96	21.76	91.38	111.53	15.90	22.80	13.19	13.11	29.31	39.34
	DGCE 10%	21.39	26.98	17.50	19.76	75.59	77.72	15.47	19.19	10.91	12.23	22.41	24.31
$LSD_{5\%}$		3.62	3.32	1.66	1.51	7.92	8.61	1.66	1.55	1.67	1.40	4.67	4.32

Table 6: Effects of dry garlic cloves extract (DGCE) on photosynthetic pigments of *Brachychiton rupestris* under saline water irrigation (SWI) during 2018 and 2019 seasons.

Treatment		Chlorophyll a (mg/ g F.W.)		Chlorophyll b (mg/ g F.W.)		Carotenoids (mg/ g F.W.)		Proline (mg/ g F.W.)	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control		2.58	2.91	1.00	1.16	1.70	1.77	1.60	1.65
SWI 3000ppm		2.96	3.36	1.13	1.30	1.62	1.69	1.36	1.44
SWI 6000ppm		2.10	2.45	0.84	0.97	1.93	2.00	1.92	1.97
SWI 9000ppm		1.74	1.95	0.62	0.68	1.82	1.90	1.98	2.02
$LSD_{5\%}$		0.06	0.06	0.05	0.05	0.05	0.04	0.05	0.06
Control		2.16	2.53	0.89	0.79	1.76	1.84	1.77	1.82
DGCE 5%		2.82	3.10	1.05	1.15	1.68	1.80	1.65	1.71
DGCE 10%		2.05	2.38	0.76	0.97	1.87	1.89	1.74	1.79
$LSD_{5\%}$		0.07	0.05	0.04	0.05	0.06	0.04	0.04	0.05
Control	Control	2.32	2.65	0.98	1.03	1.66	1.76	1.53	1.59
	DGCE 5%	3.17	3.55	1.14	1.30	1.57	1.74	1.57	1.62
	DGCE 10%	3.17	3.55	1.14	1.30	1.57	1.74	1.57	1.62
SWI 3000ppm	Control	2.77	3.42	1.19	1.26	1.62	1.70	1.44	1.51
	DGCE 5%	2.77	3.42	1.19	1.26	1.62	1.70	1.44	1.51
	DGCE 10%	2.48	2.99	0.92	1.22	1.70	1.73	1.36	1.43
SWI 6000ppm	Control	1.93	2.26	0.80	0.94	1.95	2.00	2.02	2.06
	DGCE 5%	2.41	2.80	1.05	1.11	1.86	1.97	1.82	1.88
	DGCE 10%	1.97	2.30	0.68	0.87	1.98	2.04	1.93	1.96
SWI 9000ppm	Control	1.62	1.79	0.57	0.65	1.80	1.91	2.07	2.11
	DGCE 5%	2.07	2.38	0.74	0.77	1.74	1.85	1.90	1.94
	DGCE 10%	1.52	1.67	0.55	0.62	1.93	1.95	1.98	2.00
$LSD_{5\%}$		0.13	0.11	0.08	0.09	0.01	0.08	0.08	0.10

Concerning the effect of DGCE on the growth characters, data in Tables (3 & 4) revealed that all plant growth criteria studied were significantly increased under the low level of DGCE at 5% compared with other levels in both seasons. However, the most effective treatment which had the highest plant, stem diameter, leaves number, fresh and dry weight of (leaves, stems and roots), when treated with DGCE at 5%, where the increments were (27.19 and 34.61%) for plant height, (43.33 and 47.39%) for stem diameter, (53.68 and 46.31%) for leaves number, (19.17 and 19.10%) for root length, (48.98 and 48.32%) for leaves fresh weight, (30.02 and 33.78%) for stem fresh weight, (35.51 and 36.73%) for root fresh weight, (43.55 and 42.86%) for leaves dry weight, (22.55 and 25.26%) for stem dry weight, in the first and second seasons respectively, over the control plant. Regarding the effect of interaction between SWI and DGCE, data presented in Tables (4&5) indicated that combined treatment of DGCE at 5% with SWI at 3000 ppm gave the highest values of plant height, stem diameter, leaf number, and fresh and dry weight of leaves and stem in both seasons compared with the control. The application of 5% DGCE combined with irrigating the seedlings with tap water (0 ppm salinity) gave the highest values of root parameters.

3.2. Chemical constituents

3.2.1. Photosynthetic pigments

According to the data in Table (6), it is noticed SWI significantly decreased chlorophyll a and chlorophyll b, especially 6000 and 9000 ppm in both seasons, while, saline water at 3000 ppm increased them, the increments were 12.84 and 15.46% for chl. a and 13.0 and 12.07% for chl. b, in the first and second seasons, respectively, in comparison with the other treatments and untreated plants. On the other hand, salinity at 6000 ppm or 9000 ppm increased carotenoid pigment compared with the control. The highest values of carotenoid pigment were obtained by salinity at 6000 ppm which gave (1.93 and 2.00mg/ g F.W.), respectively, in both seasons compared with the untreated plants. Concerning the effect of DGCE, data in Table (6) showed that application of garlic at 5% concentration significantly increased chl.a and chl.b of *B. rupestris* seedlings in the first and second seasons. The increments were (30.56 and 22.53%) for chl.a and (17.98 and 45.57%) for chl. b, respectively, in the two seasons

compared to the control plants; while, garlic at 10% concentration gave the highest values of carotenoids in comparison with the untreated plants, respectively. The increments were (6.25 and 2.72%) for carotenoids, respectively, in both seasons compared with the control. Regarding the effect of interaction between SWI and DGCE on photosynthetic pigments of *B. rupestris* seedlings, data presented in Table (6) indicated that the highest values of chl. a and chl. b were obtained by SWI at 3000 ppm + DGCE at 5% which were (3.64 and 3.68 mg/ g F.W.) for chl.a and (1.28 and 1.42 mg/ g F.W.) for chl.b, in the first and second seasons, respectively compared to the untreated plant; while, SWI at 6000 ppm + DGCE extract at 10% gave the highest carotenoid, which gave (1.98 and 2.04 mg/ g F.W.), respectively, in both seasons compared with the control.

Table 7: Effects of dry garlic cloves extract (DGCE) on chemical constituents of *Brachychiton rupestris* under saline water irrigation (SWI) during 2018 and 2019 seasons

Treatment		Total phenols (mg/ g F.W.)		Total flavonoids (mg/ g F.W.)		K content (%)		Na content (%)		K/Na	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control		1.33	1.41	6.56	7.14	0.82	0.79	0.46	0.44	1.80	1.79
SWI 3000ppm		1.66	2.03	5.32	6.07	0.89	0.92	0.61	0.61	1.48	1.54
SWI 6000ppm		2.27	2.72	8.53	8.87	0.71	0.68	0.66	0.66	1.09	1.05
SWI 9000ppm		3.08	3.30	9.28	9.55	0.62	0.55	0.73	0.72	0.85	0.77
$LSD_{5\%}$		0.05	0.04	0.76	0.80	0.04	0.04	0.04	0.03	0.04	0.04
Control		1.76	2.04	8.02	8.45	0.70	0.69	0.68	0.66	1.07	1.09
DGCE 5%		1.88	2.18	6.56	7.14	0.84	0.79	0.63	0.62	1.39	1.34
DGCE 10%		2.62	2.89	7.69	8.13	0.74	0.74	0.53	0.54	1.46	1.44
$LSD_{5\%}$		0.04	0.04	0.66	0.69	0.04	0.03	0.04	0.03	0.03	0.04
Control	Control	1.27	1.31	7.37	7.82	0.75	0.72	0.50	0.48	1.50	1.50
	DGCE 5%	1.32	1.36	5.78	6.26	0.91	0.87	0.47	0.45	1.94	1.93
	DGCE 10%	1.40	1.57	6.53	7.34	0.80	0.78	0.41	0.40	1.95	1.95
SWI 3000ppm	Control	1.44	1.70	6.49	7.10	0.84	0.89	0.70	0.68	1.20	1.31
	DGCE 5%	1.49	1.87	4.36	5.31	0.96	0.94	0.62	0.62	1.55	1.52
	DGCE 10%	2.06	2.53	5.11	5.80	0.87	0.93	0.51	0.52	1.71	1.79
SWI 6000ppm	Control	1.76	2.27	8.67	9.13	0.65	0.63	0.74	0.73	0.88	0.86
	DGCE 5%	1.87	2.42	7.65	8.02	0.78	0.75	0.66	0.67	1.18	1.12
	DGCE 10%	3.17	3.48	9.27	9.45	0.69	0.67	0.57	0.58	1.21	1.16
SWI 9000ppm	Control	2.56	2.86	9.53	9.74	0.55	0.52	0.79	0.76	0.70	0.68
	DGCE 5%	2.83	3.07	8.44	8.97	0.70	0.58	0.77	0.75	0.91	0.77
	DGCE 10%	3.85	3.98	9.86	9.93	0.61	0.56	0.64	0.66	0.95	0.85
$LSD_{5\%}$		0.08	0.08	1.32	1.38	0.07	0.06	0.08	0.05	0.07	0.08

3.2.2. Proline, total phenols, and total flavonoid content

Proline, total phenols, and flavonoid content in the s *B. rupestris* plant, showed increased content with an increased concentration of SWI Table (7). The highest content was found at 9000 ppm (1.98 and 2.02 mg/g F.W.) for proline content, 3.08 and 3.30 mg/g F.W. for total phenol content, and 9.28 and 9.55 for total flavonoids content, respectively, in the first and second seasons.

Concerning the effect of DGCE on proline, total phenol, and total flavonoids, data presented in Table (7) revealed that all concentrations of DGCE decreased total proline and total flavonoids compared with the control in both seasons. DGCE at the lowest levels of 5% gave the lowest values of total proline (1.65 and 1.71 mg/g F.W.) and total flavonoids (6.56 and 7.14 mg/g F.W.) as compared with the untreated seedlings, respectively, in the first and second seasons. On the other hand, total phenols significantly increased by increasing DGCE concentrations. DGCE at the highest level gave the highest values of total phenols which gave 2.62 and 2.89 mg/g F.W. for the control, respectively, in both seasons.

Regarding the interaction between SWI and DGCE on *B. rupestris* seedlings, data presented in Table (7) indicated that SWI at 9000 ppm concentration + DGCE at 5% gave the

highest values of total proline which gave 2.07 and 2.11 mg/g F.W., respectively, compared with the control and other treatments, in both seasons. While, SWI at 9000ppm + DGCE at 10% gave the highest values of total phenols and total flavonoids, which gave 3.85 and 3.98 mg/g F.W. for total phenols and 9.86 and 9.93 mg/g F.W. for total flavonoids, respectively, in comparison with the control and other treatments, in the first and second seasons.

3.2.3. Potassium, Sodium % and K/Na

From the data in Table (7) it can be noticed that ion K, N%, and K/Na ratio, in both seasons were affected under salinity stress. SWI at high levels (6000, 9000 ppm) significantly decreased K% as compared with the control whereas, the highest values of K% (0.39 and 0.92%) resulted in SWI at 3000 ppm, respectively, in the first and second seasons. It was noticed also that Na content was increased by increasing SWI treatment where the highest percentage 0.73 and 0.72%, respectively, in the first and second seasons were obtained from plants irrigated with SWI at a concentration of 9000ppm. On the other hand, the highest ratio of K/Na (1.80 and 1.79 respectively, in the first and second seasons) was produced in control plants.

Concerning the effect of DGCE, it was clear that the highest values of K% (0.84 and 0.79%) were obtained with the DGCE at 5%, respectively, in both seasons, while the highest values of Na% (0.68 – 0.66%) were obtained from control plants, respectively, in both seasons, then decreased by increasing DGCE level. For the K/Na ratio it was found that the highest values (1.46 and 1.44, respectively, in both seasons) showed in plants treated with DGCE at 10%.

Regarding the interaction between salinity concentrations and DGCE levels application on K%, it was evident that SWI at 3000ppm + DGCE at 5% gave the highest K% which gave (0.96 and 0.94%) in the two seasons, respectively, in comparison with the untreated plant and the other treatments; while, DGCE at all levels significantly decreased Na content in both seasons. The interaction between SWI at 9000ppm + DGCE 0% gave the highest Na% (0.79 and 0.76%) in the first and second seasons, respectively, from the data in Table (7) it can be noticed that salinity concentrations significantly decreased K/Na by increasing SWI levels, but K/Na significantly increased by increasing DGCE levels in both seasons. The interaction between (SWI at 0 ppm + DGCE at 10%) gave the highest and the same value of K/Na (1.95) in both seasons.

3.3. Correlation analysis

It was observed from correlation coefficient presented in Tables (8 & 9) that all vegetative growth traits had a strong positive correlation and this correlation was also in parallel with the K/Na ratio, chlorophyll a, chlorophyll b and potassium content. On the contrary, an inverse correlation appeared between the direction of vegetative traits and some chemical components, which include carotenoids, proline, total phenols, total flavonoids and sodium content in both seasons.

4. Discussions

The results were obtained during this study showed that the seedlings of *B. rupestris* treated with SWI at concentrations 6000 and 9000ppm showed a significantly reduction in the recorded values of all vegetative growth traits. A large number of researchers have clarified the negative role of salinity on the vegetative growth and flowering characteristics of many plants such as [3,34] they stated that the growth parameters were decreased by increasing salinity concentrations. The depressive effect of salinity on plant height might refer to cell division and enlargement, salinity induced water stress, also causes stomata closure reducing the supply of CO_2 for photosynthesis [35]. [36,37] reported that the decrease in vegetative characteristics after exposure of plants to salinity stress may be due to altered metabolism, producing reactive oxygen species (ROS) in mitochondria and chloroplast, changes in ion balances, mineral nutrition, stomata behaving photosynthetic and ultimately causing a decline in plant growth and electrolyte leakage (EL). In addition to affecting endogenous growth hormones, changes in water states caused by osmotic stress, usually arise from a decrease in the solute potential of the soil solution, which impacts the hydraulic conductivity and is often observed as reducing water and solute uptake [38]. The excess salinity mediates ion toxicity, which results from the increasing accumulation of toxic ions like Na^+ and Ca^{++} leading to an imbalance in their cellular homeostasis, oxidative stress, nutrient deficiency, retarded growth and cell death [39].

Our results are following those reported by [40,41,42] that high salt concentration in rooting media affected growth might be due to the osmotic inhibition of water absorption, specific ion concentration in the saline media, or a combination of both factors. Cell division, enlargement, and differentiation resulted in plant growth; water status of the plant affected all these factors. Moreover, decreasing fresh and dry weight of all the plant organs due to the Cl^- or Na^+ accumulation in leaves might cause injury by interfering with stomata closure causing excessive water loss, and leaf injury symptoms like those of drought and CO_2 fixation might reduce under high level of SWI which led to lower metabolism [35].

To protect the plant cells from the adverse effect of salt stress, the plant produces osmolytes such as proline, which maintain the osmotic strength of cytosol with that of vacuole and external environment [43]. Under salinity conditions, the phenolic compounds play a crucial role in absorbing and neutralizing free radicals, peroxides decomposing. [44] Found the total phenolic content of Thymus vulgaris and *Thymus daenensis* plants, increased by 20% after applying 60 mM NaCl compared with the control plant. [45] showed that phenolic content was increased in *Schizonepeta tenuifolia* plants under mild salinity levels (25 mM) but depressed content under higher salinity concentrations (75 and 100mM).

Table 8: Correlation coefficient analysis for morphological parameters and chemical compositions of *B. rupestris* as affected by dry garlic cloves extract under saline water irrigation stress during 2018 season

	PH	*SD*	*NL*	*RL*	*LFW*	*SFW*	*RFW*	*LDW*	*SDW*	*RDW*	*Chl. a*	*Chl. b*	*Cart.*	*Pr*	*TP*	*TF*	*K*	*Na*	*K/Na*
PH	1																		
SD	0.977	1																	
NL	0.946	0.985	1																
RL	0.875	0.860	0.813	1															
LFW	0.981	0.972	0.959	0.827	1														
SFW	0.966	0.980	0.946	0.883	0.942	1													
RFW	0.842	0.845	0.820	0.972	0.819	0.855	1												
LDW	0.978	0.966	0.951	0.819	0.999	0.937	0.811	1											
SDW	0.956	0.969	0.929	0.882	0.926	0.999	0.851	0.921	1										
RDW	0.841	0.843	0.816	0.970	0.822	0.849	0.999	0.814	0.844	1									
Chl. a	0.747	0.721	0.671	0.910	0.687	0.758	0.887	0.680	0.763	0.883	1								
Chl. b	0.709	0.705	0.646	0.843	0.653	0.725	0.787	0.647	0.729	0.785	0.932	1							
Cart.	-0.508	-0.534	-0.497	-0.733	-0.474	-0.506	-0.742	-0.463	-0.499	-0.745	-0.828	-0.764	1						
Pr	-0.720	-0.705	-0.657	-0.801	-0.645	-0.682	-0.737	-0.633	-0.682	-0.736	-0.794	-0.816	0.804	1					
TP	-0.489	-0.478	-0.390	-0.669	-0.442	-0.502	-0.657	-0.435	-0.514	-0.662	-0.787	-0.867	0.715	0.671	1				
TF	-0.861	-0.875	-0.840	-0.852	-0.837	-0.837	-0.827	-0.826	-0.829	-0.827	-0.837	-0.853	0.782	0.916	0.725	1			
K	0.893	0.914	0.893	0.906	0.851	0.901	0.872	0.839	0.896	0.866	0.871	0.885	-0.718	-0.887	-0.664	-0.957	1		
Na	-0.592	-0.483	-0.437	-0.772	-0.528	-0.524	-0.750	-0.526	-0.528	-0.754	-0.643	-0.479	0.477	0.609	0.3791	0.508	-0.542	1	
K/Na	0.785	0.721	0.677	0.933	0.732	0.745	0.923	0.726	0.746	0.924	0.866	0.727	-0.714	-0.794	-0.599	-0.781	0.798	-0.918	1

Where; PH: plant height, SD: stem diameter, NL: No. of leaves, RL: root length, LFW: leaves fresh weight, SFW: shoot fresh weight, RFW: root fresh weight, LDW: leaves dry weight, SDW: shoot dry weight, RDW: root dry weight, Chl. a: chlorophyll a, Chl. b: chlorophyll b, Cart: carotenoids, Pr: proline, TP: total phenol, TF: total flavonoids, K: potassium content, Na: sodium content, K/Na: potassium sodium ratio.

Table 9: Correlation coefficient analysis for morphological parameters and chemical compositions of *B. rupestris* as affected by dry garlic cloves extract under saline water irrigation stress during 2019 season

	PH	*SD*	*NL*	*RL*	*LFW*	*SFW*	*RFW*	*LDW*	*SDW*	*RDW*	*Chl. a*	*Chl. b*	*Cart.*	*Pr*	*TP*	*TF*	*K*	*Na*	*K/Na*
PH	1																		
SD	0.992	1																	
NL	0.985	0.975	1																
RL	0.814	0.852	0.8426	1															
LFW	0.974	0.982	0.967	0.853	1														
SFW	0.991	0.994	0.968	0.846	0.966	1													
RFW	0.819	0.8451	0.8486	0.989	0.842	0.844	1												
LDW	0.968	0.977	0.961	0.855	0.999	0.959	0.843	1											
SDW	0.989	0.992	0.968	0.852	0.969	0.999	0.851	0.963	1										
RDW	0.823	0.849	0.851	0.990	0.842	0.849	0.999	0.842	0.855	1									
Chl. a	0.708	0.716	0.757	0.871	0.751	0.697	0.886	0.755	0.699	0.880	1								
Chl. b	0.714	0.698	0.777	0.834	0.734	0.687	0.862	0.737	0.691	0.855	0.970	1							
Cart.	-0.449	-0.458	-0.525	-0.612	-0.466	-0.462	-0.627	-0.464	-0.459	-0.626	-0.769	-0.705	1						
Pr	-0.628	-0.614	-0.719	-0.718	-0.665	-0.612	-0.732	-0.669	-0.621	-0.726	-0.841	-0.851	0.890	1					
TP	-0.327	-0.335	-0.424	-0.691	-0.323	-0.340	-0.722	-0.323	-0.338	-0.722	-0.795	-0.803	0.704	0.675	1				
TF	-0.822	-0.807	-0.880	-0.821	-0.799	-0.812	-0.839	-0.794	-0.811	-0.839	-0.878	-0.893	0.825	0.914	0.687	1			
K	0.799	0.771	0.854	0.781	0.804	0.772	0.794	0.803	0.776	0.790	0.890	0.928	-0.737	-0.916	-0.636	-0.956	1		
Na	-0.4450	-0.448	-0.5297	-0.581	-0.510	-0.428	-0.578	-0.521	-0.439	-0.582	-0.607	-0.628	0.496	0.643	0.532	0.536	-0.535	1	
K/Na	0.668	0.661	0.746	0.755	0.705	0.645	0.764	0.710	0.651	0.765	0.846	0.867	-0.711	-0.847	-0.685	-0.816	0.814	-0.912	1

Where; PH: plant height, SD: stem diameter, NL: No. of leaves, RL: root length, LFW: leaves fresh weight, SFW: shoot fresh weight, RFW: root fresh weight, LDW: leaves dry weight, SDW: shoot dry weight, RDW: root dry weight, Chl. a: chlorophyll a, Chl. b: chlorophyll b, Cart: carotenoids, Pr: proline, TP: total phenol, TF: total flavonoids, K: potassium content, Na: sodium content, K/Na: potassium sodium ratio.

It was noticed from the mentioned results that the seedlings of *B. rupestris* sprayed with DGCE showed improvement in all vegetative traits and most chemical compounds. Through the HPLC analysis that was conducted on the aqueous solution of DGCE that used during this study, it was found that it contains a large group of phenolic compounds which mentioned in Table (1), Through this analysis, it was found that gallic acid is the dominant compound, as its concentration was found to be higher than the rest of the phenolic compounds in the extract. [46] illustrated that the application of gallic acid under salinity stress reduced sodium absorption and thus encourages potassium absorption which results in a higher ratio of K^+/Na^+ this is consistent with the results obtained through our study. A balanced or high K+/Na+ ratio is vital for photosynthesis, protein synthesis and activation of numerous enzymes, stomatal function, and adjustment of cell osmoregulation [47]. Our results are in line with [23] declared that the aqueous garlic extract the morphological and physiological traits of tomato seedlings. In addition to phenolic compounds, garlic extract contains a group of other compounds such as saponins, carbohydrates, alkaloids, flavonoids, some essential amino acids, and some nutritious minerals (potassium, zinc, phosphorus, manganese, magnesium, calcium, iron) [48,49,50].

Our study also found an increase in the plant's total phenolic content when treated with DGCE under salt stress conditions. Phenolic compounds have been found to play an important role as antioxidants by donating electrons or hydrogen atoms, where it is act as radical scavengers by donating electrons or hydrogen atoms [50]. Our results are in harmony with [46] who stated the foliar application gallic acid significantly mitigated the hazardous effects of salt stress by regulating ion uptake, enhancing the accumulation of phenolic, and chlorophyll content.

5. Conclusions

The results were obtained in our investigation cleared that *Brachychiton rupestris* seedlings well-grown when irrigated with low concentration of saline water (SWI) at (3000ppm) but the rest concentrations (moderate and high) caused decrement in the growth indicators (toxic symptoms). Dry garlic cloves extract (DGCE) at 5% had a promoter effect to all growth and chemical traits under the irrigation with saline water condition at all concentration as compared with control treatment.

Conflict of interest

The authors declare there is no conflict of interest.

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