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Evaluation the Effects of shade and Humic Acid on the Eco-Physiological Traits of Roselle (*Hibiscus sabdariffa* L.) under Different Irrigation Regimes

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ABSTRACT

Roselle (*Hibiscus sabdariffa* L.) is important due to its versatility and multiple uses, ranging from food and beverages to traditional medicine and cosmetics. The field experiments were conducted at the research farm of Shahed University, and the Agricultural Jihad Department of Abyek City Research Station during 2017-2020. Roselle was grown under 0 and 50% shade, three irrigation regimes (2, 4 and 6 hours per week) and 4 levels of humic acid (0, 3, 6, and 12 kg ha^{-1}) through a split-split experiment based on a randomized complete blocks design with 3 replications. Measured parameters were plant height, stem diameter, number of flowering branches, sepal and capsule weight, number of fruits per plant, 1000-seed weight, plant dry weight, harvest index, fresh fruit length and diameter, inflorescence length, seed biological yield, flower protein (%), essential oil (%), anthocyanin (%), seed oil (%), and relative leaf water content. Results showed that shade treatments affected growth of roselle and significant differences in the most of above parameters were occurred. The highest mean of sepal, seed and biological yields were observed in combination of unshade with 12 kg ha^{-1} humic acid application by 836, 1817 and 25710 kg ha^{-1} respectively. Results indicated that, 2 and 4 hours irrigation reduced seed yield 44 and 49% and biological yield 45 and 48% respectively. Shading caused notable decline of essential oil (44%), anthocyanin (71%), protein (30.5%), and oil content (25%) compared to combinations of unshade and humic acid application treatment. In conclusion, this experiment demonstrated that shading and water deficiency had decreased the morphological traits and biological yield of the roselle plant, and applying humic acid in these conditions mitigated the adverse impacts of the stressors.
Keywords: Roselle, Shade, Humic acid, Medicinal plants

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L., Malvaceae) is an important medicinal plant in tropical and sub-tropical regions [1]. The fleshy calyx of roselle as an economical part has a long history of use as a food, in herbal drinks, in hot and cold beverages, and as a flavoring agent in the food industry among medicinal plants [2].

Roselle extracts showed antibacterial, antioxidant, nephro- and hepato-protective, renal/diuretic, anti-cholesterol, anti-diabetic, and anti-hypertensive effects, among others [3,4]. Currently, there are big international market demand for roselle which can be considered as one of potential crops grown in arid and semi-arid regions [5].

In Iran, roselle are distributed in southern west and east of country where the average annual rainfall and minimum temperature are 125 and 15°C respectively [6]. Although the roselle is a low-expectation plant in terms of nutritional and environmental factors, but the unknown and little information about its high efficiency and ecological limitations have caused a decrease in the cultivated area of this plant in Iran's climatic conditions. In south of Iran, due to water shortage and deficiency, where the growth of other plants is district, roselle is an important cash crop [6]. Increased environmental extremes such as significant rainfall reductions due to climate change are expected that roselle will be considered as a possible future crop. Unfortunately, roselle production challenging many problems such as cultivation poor knowledge, harvesting labour costs, unstable production and low yield potential in Iran [7]. The conventional roselle farming combine with disregarding of cultivation methods in the south of Iran causes the average yield of this plant estimated under 600 kg ha^{-1} [8].

Abiotic stress, caused by non-living factors like light, extreme temperatures, salinity and drought seriously affects plant growth, leading to significant crop yield losses in agriculture [9,10]. Drought stress is particularly prominent and is exacerbated by water scarcity and high temperatures [11]. Drought is a pervasive abiotic stressor with significant consequences for crop productivity. Water scarcity limits a plant's ability to perform critical processes like photosynthesis, nutrient uptake, and transpiration [12]. It also increases the concentration of salts in the soil, further exacerbating stress. Developing drought-tolerant crop varieties and implementing efficient irrigation methods are essential for mitigating drought impacts [13]. Crops may exhibit wilting, reduced growth, and diminished yields in response to drought stress [14,15]. It was shown that drought stress significantly decreased growth parameters and relative water content while increasing roselle's ion leakage, antioxidant activity, and total phenolic content [1,16].
Light is another abiotic factor that regulate plant growth and providing energy for photosynthesis and induces different physiological responses in agricultural production [17,18,19]. Previous experiments [13,20] have illustrated the effect of light intensities on plant physiological parameters such as photosynthesis and morphological growth. The morphological pattern and photosynthesis activities of plants varies under different light intensities. Plants for suitable growth need an adequate light intensity which higher or lower than it will inhibit the photosynthesis and/or normal activities. Unlike the optimum light intensity, crops generally suffer from shade conditions by blocking the irradiance from the main source [20,21]. Low light stress by shading influences plants from seedling to maturity stages [22,23]. The accumulation of photosynthesis dry matter of the most annual crops sharply declines under the 40% low light conditions [24,25]. Previous studies [26,27] indicated that plant morphology and physiology under low light intensities causes many changes such as plant height and photosynthetic inhibition. The highest relative growth rate (1.81 cm cm^{-1} week^{-1}) and stem diameter (1.79 cm) of roselle were obtained at 8 hours light, while the maximum height (128.20 cm) was measured at 10 hours day length [28]. This experiment showed that the roselle optimal grow as a short day length was determined at under 12 hours of day length. Also, manipulate light quantity by shading reduced 20 g per plant roselle dry matter accumulation compare with unshaded treatments. [29]. Another experiment revealed that the highest leaf, gel, peel fresh weights and biosynthesis of secondary metabolites of *Aloe vera* were measured at low light intensity and shortage irrigation [30].
Humic acid is indeed known to act as a biostimulant in agriculture by providing several benefits to plants [31,32]. It is a natural organic compound resulting from the decomposition of organic matter, so it is rich in carbon. Humic acid can enhance a plant's resistance to various stresses, including drought, disease, and environmental stress. It can stimulate the plant's natural defense mechanisms and help it cope with adverse conditions more effectively [33]. The application of humic acid increased the levels of osmotically active solutes, endogenous hormones, water content, and nutrient availability in the soil. This, in turn, resulted in enhanced photosynthesis and biomass production, ultimately bolstering the drought resistance of crops [34]. Also, soil amendment treatments significantly increased chlorophyll content index, leaf area, leaf water potential, relative water content, antioxidant activity, and total phenolic content compared to control under mild and severe stress conditions [34, 35]. A study noted that organic amendments could eliminate the adverse effects of several abiotic stresses (drought and salinity), the main factors affecting plant yield [11,35]. A limited number of researches on roselle have been conducted. Therefore, it is essential to combine the humic acid factor to mitigated the adverse effects of stress induced by climatic factors such as light and irrigation on the agro-physiological traits of roselle medicinal plants grown under field conditions.
As the little known about the responses of roselle to low light conditions, the study on the light requirement of this plant, is crucial for expanding its agricultural area and finding the best light intensity to optimize plant growth. Therefore, the objective of this study was to evaluate the effects of humic acid as a biostimulant on different light and irrigation levels on yield and yield components of the roselle medicinal plants grown in the field.

MATERIALS AND METHODS

Field Location

The experiment was carried out at two locations within the research farm of medicinal plants of the Shahed University, Tehran and the Agricultural Jihad Department of Abyek City research farm in Ghazvin Province, Iran, during 2017-2020. Soil physico-chemical characteristics and the weather condition of experimental sites are

	Abyek Research Farm					Shahed University Farm				
Months	Mean Low (°C)	Mean Max (°C)	Mean (°C)	Mean Rainfall (mm)	Mean humidity (%)	Mean Low (°C)	Mean Max (°C)	Mean (°C)	Mean Rainfall (mm)	Mean humidity (%)
January	-2.5	6.1	1.8	30.8	67	1	8.7	4.9	35	65
February	-0.7	9	4.15	32.1	60	2.7	11.4	7	35	56
March	3.2	14.2	8.7	45.4	53	6.9	16.5	11.7	35	48
April	8.4	20.7	14.55	39.1	48	12.2	22.4	17.3	30	41
May	12.2	26.2	19.2	19.5	43	17.4	28.3	22.8	15	33
June	16.5	32.6	24.55	2.7	34	22.2	34.3	28.2	3	25
July	19	35.2	27.1	3	35	25	36.9	31	3	26
August	19.1	34.5	26.8	1.2	34	24.5	35.8	30.2	1	26
September	15.3	30.4	22.85	1.6	36	20.5	31.6	26.1	1	27
October	10.8	23.5	17.15	15.1	44	14.6	24.8	19.7	15	36
November	4.8	15.1	9.95	27.7	56	7.5	15.9	11.7	20	49
December	0.3	8.9	4.6	33.5	66	3	10.4	6.7	35	62
Yearly Mean	8.86	21.7	15.11	20.98	48	13.2	23.2	18.15	230	41

shown at Tables 1 and 2.

Table 1 Experimental soil physico-chemical characteristics

S.U. Farm: Shahed University Farm; A.R.Farm: Abyek Research Farm.

Table 2 The minimum, maximum and average weather condition of experimental sites

Location	Latitude	Longitude	Elevation (m)	O.C. (%)	T.N.V. (%)	Total N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	EC (ds m^{-1})	pH	Soil Texture
S.U. Faram	51°20'	35°32'	1180	1.45	12.57	1200	26.55	240.31	11.52	7.4	Loam
A.R.Farm	50°51'	36°01'	1008	0.8	21.90	700	20.83	337.91	15.01	7.01	Loam, Clay, Sand

Experimental Design

The 50% (L_{50}) light reduction was provided through the use of neutrally-absorptive polypropylene shade fabrics of variable mesh density the aerial dementias of plots. A wooden frame with a green cover was applied for shading. Mid-day irradiance, photosynthetically active radiation (PAR), light transmittance was measured by a digital illuminance meter (VICTOR 1010D, Shenzhen Yisheng Shengli Technology Co., Ltd., China). The light transmittance was calculated according to the following formula:

$$\text{Light transmittance}=\frac{\text{Photosynthetically active radiation under shade}}{\text{Photosynthetically active radiation under full light}}\times 100$$

Tape irrigation was used with a distance of 30 cm between the droppers and a water output of 2.2 liters per hour. The plots received 528 liters at the first level, the second level was 352 liters, and the third level received 176 liters of water in each stage. Shading treatment (50% reduction) was used to change the temperature and the amount of light. Fertilizer application was according the soil analysis results, using chemical fertilizers of urea, triple superphosphate, and potassium sulfate at the farm level. The experimental plots included four rows 3 meters in length and a distance of 50 cm; the seeds were planted at a 30 cm distance on a row.

Studied Traits

Quantitative traits including plant height, stem diameter, number of flowering branches, sepal weight, capsule weight per plant, plant dry weight, number of fruits per plant, 1000-seed weight, harvest index (the ratio of a harvested product to total plant weight), fresh fruit diameter, fresh fruit length, inflorescence length, seed yield, and biological yield (the total dry matter accumulation of the plant).

The samples were dried at 74 °C for 48 hours to measure the dry weight. The qualitative traits included flower protein percentage, essential oil percentage, anthocyanin percentage, seed oil percentage, and relative leaf water content. Leaf protein and anthocyanin contents were measured by Bradford's [36] and Wagner's methods [2] respectively. According to British Pharmacopeia, the essential oil of shade-dried and pulverized leaves and calyces was extracted by hydrodistillation method in an all-glass Clevenger apparatus for four hours [38]. For relative leaf water content measurement, fully developed leaves were weighed immediately after harvesting, and their fresh weight was recorded. Then, the samples were immersed in distilled water (25 °C). After 16 hours, they were removed from the distilled water, and their wet weight was determined again. The weighed samples were placed in paper envelopes in a fan-assisted oven (70 °C) for 48 hours to dry completely. Finally, the weight of the dried samples was recorded, and the percentage of relative leaf water content was calculated using the below equation [38]:

RWC (%) = [(fresh weight- dry weight)/(turgid weight- dry weight)]*100.

Experimental Design and Statistical Analysis

The experiment was set up as a split-split plot based on a randomized complete blocks design with 3 replications. Experimental factors consist shading (no shade, 50% shading) as a main factor, irrigation levels (2, 4 and 6 hours per week) as a subfactor and humic acid (0, 3, 6, and 12 kg ha^{-1}) as the sub-subfactor. The range of humic acid were chosen at the recommended doses for the best response in the annual crops [32]. SAS software [39] used for statistical analysis of data, and mean comparisons were done with the Duncan test at the statistical level of $P \leq 0.05$.

The major reasons for using the split plot design are to facilitate the implementation of experiments by arranging treatments into the large plots. In terms of conducting this experiment, shade requires larger plots than irrigation. Therefore, in the form of split-split design, shading had positioned as a main plot, irrigation in the sub-plot, and humic acid in the sub-sub-plots.

RESULTS

Physio-chemical Traits

Significant variations were noted in the impact of the treatment on certain traits studied (Table 3) in terms of physio-chemical properties. Shading resulted in a notable decrease of essential oil (44%), anthocyanin (71%), protein (30.5%), and oil content (25%) when compared to combinations of unshade and humic acid application treatment. Concerning irrigation, it was found that applying irrigation at 4 and 6 hours per week significantly led to the increase of essential oil (5 and 23%) and anthocyanin (55 and 73%) compared to 2 hours per week, respectively. However, these treatments showed decreased RWC (9 and 11%) and oil content (4 and 7%) compared to 2 hours per week irrigation, respectively. Applying humic acid could effectively increase the above traits, so applying 3, 6, and 12 kg^{-1} ha led to 5, 5, and 11% of essential oil compared to the control. Moreover, 12 kg ha^{-1} treatment showed the highest values of anthocyanin (0.28 mg g^{-1} fresh weight), protein (22.94 28 mg g^{-1} fresh weight), RWC (85.3%), and oil content (17.7%). The interaction of treatments revealed that 12 kg ha^{-1} humic acid with 2 hours irrigation per week and no shade had the highest means of essential oil, anthocyanin, and protein content with 12.98%, 0.428 mg g^{-1} fresh weight and 23.9728 mg g^{-1} fresh weight values, respectively (Table 4). The highest means of RWC observed at no shade treatments, 6 hours irrigation per week, and 6 kg ha^{-1} humic acid interaction with 90.15% values (Table 4). With oil content, application of no shade treatments, 4 hours irrigation per week, and 6 kg ha^{-1} humic acid interaction showed the highest mean with 18.99% (Table 4).

Yield and Yields Components

According to the analysis of variance, treatment significantly affected yield and yield components at 1% statistical level (Table 5). Experimental results showed that roselle had better growth in light than shade (Table 6). Yield and yield components of roselle were significantly lower than unshaded treatments (Table 5). Furthermore, the results demonstrated that combinations of light along with acid humic and full irrigation (6 hours per week) increased sepal yield (84%) than control (Table 6).

As seen in table 6, seed and biological yields were reduced in shade treatment compared to control, about 35 and 34%, respectively. Also, 2 and 4 hours irrigation per week reduced seed yield (49 and 44%) and biological yield (48 and 45%), respectively compared to 6 hours irrigation. Under humic acid application, the highest mean of seed and biological yields was obtained by 12 kg ha^{-1} with 1190 and 16817 kg ha^{-1} values, respectively. Interaction between treatments showed the highest mean of seed yield with 1817 kg ha^{-1} value, and biological yield with 25710 kg ha^{-1} was obtained by no shade treatments, no stress, and 12 kg ha^{-1} humic acid (Table 6). Application of shading led to a reduction in sepal yield (56%), plant weight (37%), stem diameter (33%), flower length (8%), and seed weight (8%), but plant height was increased by application shading treatment about 8%. Results showed 2 hours irrigation per week reduced about 43% of sepal yield, 52% of plant weight, 36% of stem diameter, 9% of plant height, and 17% of flower length compared to 6 hours irrigation per week. However, applying 2 hours irrigation per week increased seed weight by about 8%. The increasing of humic acid levels application led to an increase of the above traits, so the highest means of sepal yield (836 kg ha^{-1}), plant weight (306.2gr), stem diameter (27.69 mm), plant height (156.2 cm), flower length (76.11mm) and 1000-seed weight (23.4 gr) were obtained by 12 kg ha^{-1} treatments (Table 6).

Table 3 Analysis of variances (mean square) for physio-chemical traits in response to studied treatments.

Source of variation	d.f.	Essential oil	Anthocyanin	Protein	RWC	oil content
Location	1	3.376 **	0.072 **	8.935	108.264 **	43.099 **
Replication (Location)	4	0.015	0.001	10.641*	31.613	2.118
Shading	1	7.807 *	0.227 **	98.456 **	62.964	36.744 **
Location*Shading	1	0.054	0.001	9.677	4.877	0.548
Error a	4	0.082	0.001	3.049	10.67	1.832
Irrigation	2	3.294 **	0.208 **	5.637	1256.749 **	20.802 **
Location*Irrigation	2	0.097	0.004 *	3.799	2.264	1.909
Shading*Irrigation	2	0.126	0.002	9.538	13.704	0.35
Location*Shading*Irrigation	2	0.149	0.001	1.558	0.23	2.09
Error b	16	0.061	0.001	3.581	15.879	1.879
Humic acid	3	0.362 **	0.043 **	61.752 **	189.204**	35.798 **
Location*humic acid	3	0.165 *	0.003	8.813	7.073	7.751 *
Shading*Humic acid	3	0.032	0.002	19.255 **	8.624	0.595
Location*Shading*Humic acid	3	0.051	0.001	2.812	16.791	0.074
Irrigation*Humic acid	6	0.333 **	0.001	5.646	35.551 *	5.585 *
Location*Irrigation*Humic acid	6	0.204 **	0.001	4.262	5.382	0.691
Shading*Irrigation*Humic acid	6	0.102	0.001	3.709	5.861	2.567
Location*Shading*Irrigation*Humic acid	6	0.065	0.002	4.086	3.505	2.491
Error	72	0.053	0.001	4.23	15.634	2.104
Cv%		9.61	14.22	9.42	5.9	8.59

* and **: the statistical significance levels at 5% and 1%, respectively.

Table 4 Mean comparison for physio-chemical traits in response to studied treatment interaction.

Shading	Irrigation	Humic acid (kg ha^{-1})	Essential oil (mg g^{-1} FW)	Anthocyanin (mg g^{-1} FW)	Protein (mg g^{-1} FW)	RWC (%)	Oil content (%)
control	I1	0	2.503 cde	0.1617 lm	20.73 bcd	86.76 abc	17.9 abcd
		3	2.413 def	0.205 ijk	22.32 ab	89.4 ab	18.39 abcd
		6	2.497 cde	0.215 hij	23.15 ab	90.15 a	17.51 abcd
		12	2.522 cde	0.247 fgh	22.95 ab	89.11 ab	18.84 ab
	I2	0	2.385 def	0.267 efg	20.73 bcd	76.32 fg	14.97 fg
		3	2.42 def	0.302 cde	23.09 ab	81.07 def	16.91 bcde
		6	2.557 bcde	0.333 bc	23.05 ab	78.98 efg	18.99 a
		12	2.663 bcd	0.358 b	22.53 ab	84.29 bcd	18.54 abc
	I3	0	2.76 abc	0.282 def	24.18 a	76.92 efg	14.54 g
		3	3.023 a	0.317 cd	21.88 abc	77.07 efg	17.13 abcde
		6	2.82 ab	0.355 b	23.25 ab	81.93 cde	17.4 abcde
		12	2.982 a	0.400 a	23.97 a	84.18 bcd	17.63 abcd
Shading	I1	0	1.93 gh	0.123 n	16.80 e	87.49 ab	16.49 def
		3	1.687 h	0.117 n	21.49 abc	87.07 abc	16.98 bcde
		6	1.803 h	0.143 mn	21.50 abc	87.21 ab	17.51 abcd
		12	2.178 fg	0.173 klm	22.51 ab	88.58 ab	16.94 bcde
	I2	0	1.787 h	0.188 jkl	19.38 cd	77.67 efg	14 .33 g
		3	1.97 gh	0.213 hij	21.47 abc	78.06 efg	16.77 cdef
		6	2.257 ef	0.233 ghi	22.54 ab	79.31 defg	16.84 bcdef
		12	2.4 def	0.258 fg	23.07 ab	84.5 bcd	17.41 abcde
	I3	0	2.34 ef	0.218 hij	18.15 de	74.60 g	14.60 g
		3	2.833 ab	0.258 fg	20.55 bcd	75.50 g	15.47 efg
		6	2.35 def	0.282 def	21.88 abc	78.96 efg	16.45 def
		12	2.422 def	0.283 def	22.63 ab	81.36 def	16.83 bcdef

The means with a common alphabet at each column show no significant differences.

Table 5 Analysis of variances (mean square) for yield and yield components in response to studied treatments.

	df	Seed yield	Biological yield	Harvest index	Sepal yield	Plant weight	Stem diameter	Plant height	Flower length	1000 Seed weight
Location	1	1886857 **	408346898 **	0.146	425781 **	96034 **	113.0 **	707.560 **	928 **	8.935
Replication (Location)	4	5852	2258000	0.055	3682	994.7	2.118	6.399	23.629	10.641 *
Shading	1	5728152 **	1097679296 **	0.343	3137330 **	207874 **	1868 **	3816 **	1276 **	109.046 **
Location*Shading	1	938	2127897	0.517	151049 **	3.8	0.548	0.533	5.111	9.677
Error a	4	2415	1829189	0.266	4114	738.7	1.832	6.881	12.604	3.049
Irrigation	2	6399501 **	1241405598 **	0.25	758834 **	229362 **	868.8 **	2187 **	1867**	34.357 **
Location*Irrigation	2	32118 **	2379969	0.159	4322	119.8	1.909	2.191	83.249*	3.799
Shading*Irrigation	2	3738	1455920	0.139	90891 **	74.1	0.350	0.294	13.677	14.430 *
Location*Shading*Irrigation	2	4778	1984708	0.029	25567 **	249.5	2.090	1.842	0.276	1.558
Error b	16	4420	1079168	0.187	3496	660.1	1.879	8.291	13.901	3.581
Humic acid	3	2291680 **	412302456 **	1.249	331966 **	75960 **	175.4 **	603.835 **	462.544 **	61.752 **
Location*humic acid	3	130688 **	23220591 **	0.051	105202 **	7577 **	1.690	1.640	6.912	8.813
Shading*Humic acid	3	14863	1166390	0.183	32468 **	580.0	18.29 **	90.649 **	49.201 *	19.255 **
Location*Shading*Humic acid	3	18788	2887652	0.584	38745 **	1511	0.074	0.065	17.150	2.812
Irrigation*Humic acid	6	15732	3815735	0.264	4456	612.2	5.585 *	5.072	35.327 *	5.646
Location*Irrigation*Humic acid	6	68134 **	12386832 **	0.043	41634 **	1697	0.691	0.770	5.105	4.262
Shading*Irrigation*Humic acid	6	12895	4387134	0.441	9250 **	1699	2.567	2.516	6.193	3.709
Location*Shading*Irrigation*Humic acid	6	15360	1778001	0.561	18271 **	857.8	2.491	2.263	3.689	4.086
Error	72	14442	3427872	0.552	2583	847.8	2.104	9.532	16.544	4.230
CV%		12.9	14.1	10.58	13.34	17.60	7.800	22.200	5.890	9.600

* and **: the statistical significance levels at 5% and 1%, respectively.

Table 6 Mean comparisons for yield and yield components in response to studied treatments interaction.

Shading	Irrigation	Humic acid (kg ha^{-1})	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index%	Sepal yield (kg ha^{-1})	Plant weight (g)	Stem diameter (mm)	Plant height (cm)	Flower length (mm)	1000 Seed weight
control	I1	0	1181 cde	17040 cd	6.933 ab	542 cd	222.4 d	25.97 a	138.5 ef	75.66 abc	20.2 bcd
		3	1452 b	20370 b	7.133 ab	680.1 b	267.6 c	27.29 a	144 cd	78.4 ab	21.79 abc
		6	1774 a	24700 a	7.22 ab	818.5 a	330.3 a	26.45 a	143.1 d	79.36 a	22.61 abc
		12	1817 a	25710 a	7.083 ab	836.5 a	306.2 ab	27.69 a	144.4 cd	78.11 ab	22.42 abc
	I2	0	649.5 jkl	9917 hij	6.568 ab	321.7 fg	124.2 ghi	18.04 fg	127.9 i	68.32 efgh	20.17 bcd
		3	879 gh	12320 g	7.153 ab	460 e	154.9 fg	20.81 cde	134.5 fgh	73.07 bcdef	22.53 abc
		6	1048 ef	14640 ef	7.185 ab	500 de	193.5 de	22.93 b	136.6 fg	70.98 cdef	22.49 abc
		12	1203 cd	16690 cde	7.238 ab	578.2 c	216.9 d	22.39 bc	136.1 fg	76.29 abc	21.98 abc
	I3	0	543.9l	8100 jk	6.752 ab	246.2 hi	95.42 ij	15.61 hi	123.3 j	62.91 ij	23.61 a
		3	810.5 hi	11390 ghi	7.133 ab	373.8 f	141.8 fgh	19.03 ef	130.7 hi	63.08 hij	21.31 abcd
		6	984.5 fg	13500 fg	7.4 ab	455.7 e	171.9 ef	19.33 ef	131 hi	67.93 fghi	22.68 ab
		12	1170 cde	16540 cde	7.017 ab	536 cd	214 d	19.48 ef	131.1 hi	70.18 defg	23.4 a
Shading	I1	0	795 hij	11360 ghi	7.017 ab	218.3 i	141.2 fgh	16.06 hi	147.7 bc	68.19 efghi	15.07 e
		3	1055 def	14930 def	7.083 ab	302.3 gh	190.2 de	19.99 de	149.7 b	70.88 cdef	19.76 cd
		6	1260 c	17350 c	7.283 ab	349.8 fg	225.2 d	20.52 de	156.2 a	73.61 bcde	19.77 cd
		12	1438 b	20490 b	7.067 ab	462.5 e	273.9 bc	21.33 bcd	157 a	75.18 abcd	20.78 abcd
	I2	0	328.1 mn	4927 lm	6.735 ab	134.4 j	49.39 k	8.902 k	137.6 fg	61.37 j	18.82 d
		3	384.6 m	5444 lm	7.133 ab	84.92 j	56.76 k	14.78 i	141.5 de	64.87 ghij	20.91 abcd
		6	687.1 ijkl	9934 hij	6.97 ab	224.6 i	120.9 ghi	14.85 i	147.5 bc	68.71 efg	21.98 abc
		12	805.5 hi	11800 gh	6.82 ab	257.2 hi	147.5 fg	16.8 gh	149.5 b	74.1 abcd	22.51 abc
	I3	0	3311 mn	3603 m	6.47 b	140.8 j	30.48 k	7.173 l	133.8 gh	52.3 k	18.52 d
		3	400.7 m	5988 kl	6.7 ab	146.8 j	64.55 jk	11.48 j	136.1 fg	56.31 k	20.92 abcd
		6	627 kl	9165 ij	6.767 ab	225.1 i	109 hi	12.47 j	143.1 d	62.36 j	22.24 abc
		12	711.5 ijk	9657 hij	7.6 a	254.7 hi	118.1 ghi	14.22 i	144.9 cd	64.96 ghij	23.00 ab

The means with a common alphabet at each column show no significant differences.

DISCUSSION

In this research, the effects of shade and humic acid on the eco-physiological traits of roselle under different irrigation regimes were examined. Results showed that humic acid by compensate the negative effects of light reduction and water deficiency had promoted the roselle calyx yield and yield components. These findings were similar to those of study by [10,40] who reported the water deficiency by reduces the nitrogen and other nutrient availability had affect the roselle yield, yield components, essential oil and secondary metabolites.

Sanjari *et al.* [41] provided evidence that drought stress may led to an increase in the secondary metabolite of roselle, but it caused a reduction in photosynthesis pigments. Fallahi *et al.* [40] reported that red anthocyanins in sepals of roselle are responsible for their brilliantly red color, and their qualitative analysis revealed that its amount in drought stress condition was 12% lower than no stress treatment. Fathi and Bahamin [42] studied three irrigation levels with 100-, 130- and 160-mm evaporation from a class A pan on roselle. The result of irrigation showed that the highest (2.21%) and lowest (1.98%) essential oil yields were recorded in the treatments of 160- and 100-mm evaporation treatment, respectively.

Fallahi *et al.* [41] reported that drought stress reduced the amounts of morphological indices and yield components of the roselle, while humic acid application declines the negative impacts of water deficit on the growth and yield of plants. It has been reported that water deficit irrigation in roselle caused a decrease in relative humidity, chlorophyll and carotenoid contents, and increased proline content [27]. In another study in roselle, the highest calyx yield was obtained from water deficit treatment by providing 75% field capacity [41]. These findings suggest that roselle is a relatively suitable plant for semi dry farming and deficit irrigation. However, applying appropriate nutritional methods such as humic acid is necessary to improve its growth under water deficit conditions. These practices would provide feasible approaches to conserve limited water resources under climate change [12]. Furthermore, the positive effect of humic substances on counteracting the inhibitory effects of drought stress and biomass production has been reported on different crops such as soybean [31], wheat [33], and maize [34]. It was noticed that the use of humic acid on roselle plants caused an increase in plant height, number of branches, stem diameter, fresh and dry biomass of leaves and branches, number of fruits, fresh and dry weights of sepals and seed yield [35, 43].

Therefore, it seems that the resistant plant of roselle somehow escapes from non-pore limiting factors in stress conditions by maintaining RWC values. Since humic acid has a positive role in root expansion and absorption of water and nutrients, this effect can be expected. The results of the previous experiments [40,41,43] showed that the effect of drought stress and humic acid on the amount of proline in roselle was significant. Applying drought stress had increased the amount of proline, so that the highest amount of proline was observed in severe stress treatment.

The use of humic acid on the aerial organs improves the photosynthesis conditions of the plant and increases the resistance to living and vegetative stresses. On the other hand, the results of various researches [41,44,45] have shown that the use of humic acid increases chlorophyll and increases the photosynthetic capacity of the plant. Also, in the conditions of drought stress, humic acid increases the photosynthetic activity of the plant by increasing the activity of Rubisco enzyme.

In stress conditions, the amount of plant carbohydrates increases and the distribution of carbohydrate substances is directly affected by stresses such as water shortage and indirectly by plant hormones [13].

The application of humic acid increases the plant's tolerance to stress due to the increase in photosynthesis and the production of carbon hydrates. The results of various experiments showed that in severe water stress, sugar is converted into proline in the plant [34].

Roselle, like the most plants, shows a physiological response to stress, therefore the amount of chlorophyll decreases with increasing stress. With the progress of severe drought stress, the amount of proline increases, and in moderate stress, the carbohydrate concentration had improved, which is considered a type of adaptation of the plant to stress conditions [44]. The use of humic acid in the conditions of moisture stress led to a decrease in the concentration of proline and an increase in the concentration of soluble carbon hydrates in leaves. This shows that humic acid, both soluble in irrigation water and foliar spraying, can be effective as an organic fertilizer in osmotic regulation under drought stress conditions and can be a suitable substitute for chemical fertilizers, which results in sustainable agriculture [42,45]

CONCLUSION

In general, the results of this study showed that drought stress reduced the yield of roselle in quantitative and qualitative terms. This study revealed that climatic factors significantly influenced the morpho-physiological parameters of roselle. Specifically, no shading (associated with higher temperature) and low moisture levels were found to enhance secondary metabolite production. However, it became evident that these favorable conditions for secondary metabolites were accompanied by a reduction in the plant's morphological and biological yield. Furthermore, the research highlighted the potential of humic acid as a valuable tool for mitigating the adverse impacts of climatic changes on roselle. This finding suggests that humic acid application may offer a practical strategy for optimizing roselle cultivation under varying environmental conditions. Additional research and experimentation are warranted to explore humic acid's precise mechanisms and optimal application methods in this context.

The study emphasized that application of humic acid as soil and foliar application improves the yield attributes, yield and quality of coffee apart from the economic profitability.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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