

# Investigating the effect of seaweed extract and humic acid on the quantitative and qualitative performance of sugar beet under water stress

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# Abstract

Water stress is one of the major limitations in crop production and performance stability. This study aimed to investigate the effect of biological stimulants on the quantitative and qualitative performance of sugar beet under drought stress. A field experiment was conducted to evaluate the effect of irrigation regimes (irrigation after 80, 160, 240, and 320 mm of water evaporation from the class A pan) and the application of biological stimulants at 5 levels (control, seaweed extract at 3 and 6 kg ha<sup>-1</sup>, humic acid at 3 and 6 kg ha<sup>-1</sup>). The results showed that the highest gross sugar (15.99%), extraction coefficient (77.31%), and pure sugar (12.37%) were obtained under irrigation conditions after 240 mm of water evaporation, while the highest levels of sodium (16.4%) and nitrogen (3%) were ob tained under irrigation conditions after 320 mm of water evaporation. The use of biological stimulants caused a significant increase in gross sugar and pure sugar and a reduction in sodium and nitrogen compared with the control treatment. The maximum root yield (74.06 t ha<sup>-1</sup>) and pure sugar yield (8.42 t ha<sup>-1</sup>) were obtained in the foliar application of 6 kg ha<sup>-1</sup> of humic acid under irrigation conditions after 80 mm of water evaporation. In general, 6 kg ha<sup>-1</sup> of seaweed extract and 3 kg ha<sup>-1</sup> of humic acid under irrigation conditions after 80 mm of water evaporation. In general, 6 kg ha<sup>-1</sup> of seaweed extract and 3 kg ha<sup>-1</sup> of humic acid under irrigation conditions after 80 mm of water evaporation. In general, 6 kg ha<sup>-1</sup> of seaweed extract and 3 kg ha<sup>-1</sup> of humic acid under moisture stress conditions reduced sugar beet root impurities such as sodium and harmful nitrogen. As a result, the efficiency of root sugar extraction was significantly improved, and the sugar percentage of molasses was reduced.

Keywords: sugar beet, drought stress, root yield, white sugar yield, molasses

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# Introduction

E-mail Address: <u>s.farzaneh@uma.ac.ir</u> Received: June, 2024 Accepted: September, 2024 Sugar beet (*Beta vulgaris* L.), which is cultivated as an annual plant, is a biennial plant belonging to the spinach family. Sugar beet has a wide adaptability to various environmental conditions

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(Heydarzadeh et al., 2021). Soils with medium texture and neutral to slightly alkaline acidity are ideal for sugar beet. Since the beginning of the 19th century, special attention has been directed towards the cultivation of this crop due to its vital role in meeting human food needs. Sugar is the main product of the crop, which supplies vital energy needed by the human body (Pačuta et al., 2023).

The byproducts of sugar beet are molasses and pomace. Statistics show that, on average, about 50% of Iran's sugar consumption comes from imported white sugar or raw sugar, and in recent years, about 52% of domestic sugar production has been obtained from sugar cane, with the rest coming from sugar beet. Although the average per capita consumption of sugar in the world is 23 kg, this indicator in Iran has exceeded the global standard and reached 30 kg (Heydarzadeh et al., 2021). Agricultural production strategies in sugar beet cultivation are designed based on the amount of sugar that can be extracted per unit area. To achieve the maximum sucrose level in sugar beet, two actions should be taken: First, increasing the amount of raw product based on the root yield of sugar beet. Second, increasing product quality by raising the sucrose level and reducing the over-application of potassium, sodium, and nitrogen in the syrup, which reduces its extraction ability by preventing sucrose crystallization (Rašovský et al., 2022). Agricultural and environmental conditions during the vegetative growth period and before sucrose accumulation in the root affect the initial quantitative yield, i.e., the root product. Adjusting crop programs to stop or limit growth by changing source-sink relationships and increasing sucrose synthesis and storage leads to an increase in sugar percentage (Farzaneh et al., 2021).

The increasing incidence of abiotic and biotic stresses affects the productivity of major crops, and this has been observed worldwide (Rahimi et al., 2023). Environmental stresses, especially drought stress, reduce agricultural growth and fertility more than other factors (Heydarzadeh et al., 2023; Ahmed et al., 2023). Drought stress has become a major agricultural challenge in most areas of sugar beet cultivation in Iran. A wide

range of mitigation and adaptation strategies are needed to address these effects. Efficient management of resources, coupled with the improvement and development of better varieties, can help to some extent in overcoming such stresses (Majeed et al., 2023). However, since these strategies are long-term and costly, there is a need to develop low-cost and simple biological methods for abiotic stress management that can be applied in the short term (Heydarzadeh et al., 2022; Rahimi et al., 2022; Osman et al., 2022). By using the unique characteristics of tolerance in organs, genetic diversity, ubiquity, and their interaction with crop plants, we can develop methods for their successful establishment in agricultural production. Microorganisms, regulators, and growth promoters could play an important role in this field (Bertoldo et al., 2023). A one-sided view of food supply without considering environmental issues and the effect of chemical fertilizers on soil characteristics has led to excessive use of chemical fertilizers. As a result. destructive effects such as reducing soil permeability, increasing density, limiting root growth, and ultimately degrading the soil are common, leading to reduced growth and yield (Rerhou et al., 2023). Therefore, today, the use of organic fertilizers, including humic substances and seaweed, is increasingly gaining attention (Rerhou et al., 2023). Humic substances include a mixture of different organic compounds obtained from plant and animal remains (Helaly et al., 2009). These substances are complex organic macromolecules, formed through chemical and bacterial processes in the soil (Nassar et al., 2023). Humic acid directly affects plant growth, stimulating both the aerial and root growth of plants, with a more prominent effect on the root, where it increases the root volume and enhances the root system's efficiency (Rerhou et al., 2023). The mechanism of humic acid's effect is mainly the formation of complexes between humic acid and mineral ions. It also plays an essential role in respiration and photosynthesis, stimulating nucleic acid metabolism and pseudo-hormone activity (Rahimi et al., 2022). Humic acid increases the absorption of both low- and high-use elements by plants. Its application reduces chlorosis in plants, probably because of humic acid's ability to maintain soil iron in an absorbable and metabolizable form (Makhlouf et al., 2022). This phenomenon is particularly effective in calcareous and alkaline soils, which typically lack absorbable iron and organic matter. The presence of different chemical compounds in seaweed extract, along with their physiological effects, makes them widely used in various industries such as food, cosmetics, and color production (Bertoldo et al., 2023). Seaweed is non-polluting, non-toxic, and environmentally friendly, and due to its growth hormones such as auxins and cytokinins, micronutrients, vitamins, and amino acids, it stimulates root growth, increases plant growth, delays aging, and enhances tolerance to environmental stresses (Pačuta et al., 2023). Given that limited research has been done in the field of agronomy for this plant, and considering the importance of mass cultivation and improving its quantitative and qualitative characteristics in the country, this research aims to examine the effect of biological stimuli on the qualitative and quantitative characteristics of sugar beet under drought stress conditions.

# **Materials and Methods**

This study was executed on the research farm of the University of Mohaghegh Ardabili as a split plot in the form of a completely randomized block design.

To investigate the effect of seaweed extract and humic acid on the quantitative and qualitative yield of sugar beet (Beta vulgaris L.) under drought stress, it was done with four repetitions. The experimental treatments were four (4) irrigation regimes: full irrigation (including irrigation after 80 mm of water evaporation from the class A evaporation pan), irrigation after 160, 240, and 320 mm of water evaporation. Irrigation treatments were applied after the complete establishment of the plant. Foliar spraying was done from the eight-leaf stage in three stages with an interval of 10 days. In the spring season, agricultural operations, including plowing, spreading the required fertilizer based on soil tests, and disc hallowing, were done. Each subplot included 6 cultivation lines with a length of 6 m. The distance between planting lines was 50 cm,

whereas that of inter-plants on the line was set at 20 cm. Therefore, the density of the farm was around 100 thousand plants per hectare. The distance between repetitions was 2m. The agronomic operations included thinning at the stage of 4-6 leaves, with the control of weeds, pests, and diseases being done mechanically and chemically.

During the experiment, chlorophyll a and b, the dry weight of leaves, and the quantum efficiency of photosystem II (Fv/Fm) were recorded. To measure the dry weight of leaves, five plants were selected indiscriminately from each repetition for each treatment, and in each sample, the leaves were first separated from the crown of the plants. They were placed in the oven for 48 hours at a temperature of 70 °C, and then the dry weight of the leaves in grams per plant was measured with a precise digital scale with an accuracy of 0.001. The concentration of chlorophyll a and b was measured using the method of Arnon (1967). This was followed by the gradual crushing of 0.2 g of leaf tissues with 80% acetone for the chlorophyll to get into the acetone solution. Finally, the volume of the solution was increased to 20 ml with 80% acetone. For 10 min, the solution was centrifuged at 400 rpm, and then the optical absorbance of the supernatant solution was read by a spectrophotometer (SP 6-200 Unican QC, Montréal-Est, Quebec, Canada) at wavelengths of 470, 645, and 663 nm.

To assess the influence of different irrigation treatments and biological growth stimulants on the photosynthetic system of the plant, the key parameters of chlorophyll fluorescence were used. Its fluorescence was recorded. Chlorophyll fluorescence was read using an OS-30p chlorophyll fluorometer, and the Fv/Fm index was calculated from the device data. The Fv/Fm index was realized from the relationship (Fm-F0)/Fm (Tadina et al., 2007). In this formula, F0 and Fm denote the minimum and maximum chlorophyll fluorescence of the leaves, respectively. The variable fluorescence, or Fv, was taken as the difference between Fm and F0 (Maxwell and Johnson, 2000).

Some phys	ical and chemica	l characteristics	of the studied so	il sample		
рН	EC	Organic Matter	Total N	Available P	Available K	Soil Texture
	(dS m <sup>-1</sup> )	(%)	(%)	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	
7.86	1.72	0.92	1.01	5.2	372	Silty clay

Table 1Some physical and chemical characteristics of the studied soil sample

At the time of harvest, after removing half a meter from the edges of each plot and the two side rows, the rest of the bushes were harvested on May 15th. Then, 15 kg of samples from each plot were sent to a laboratory for determination of their sugar, Na, K, and N contents. Following the detachment of the roots from the shoots, they were thoroughly rinsed, after which they were placed in a sampling device to prepare them for root pulp extraction. The roots were then decomposed, followed by the determination of the qualitative traits with a flame photometer (Jenway, UK) (Reinefeld et al., 1974). The molasses (MS) content (in percentage) was calculated as indicated in Equation 1 (Dutton and Bowler, 1984). %MS =  $0.17K + 0.13Na + 0.215 (\alpha - amino - N)$  (1) Where Na shows sodium, K denotes potassium, and  $\alpha$ -amino-N signifies nitrogen. The sugar beet (Beta vulgaris L.) Na, K, and  $\alpha$ -amino-N contents were in meg per 100 g of the root. The extractable sugar percentage (WSC), or rather the pure sugar percentage, was calculated as indicated in Equation 2 (Asadi, 2006).

%WSC = %SC - %MS (2)

Where MS is molasses content, and SC is sugar content.

The gross sugar percentage, which is simply known as the sugar content, is composed of the sugar content of molasses and the extractable sugar content. To measure the quality of the roots, lead subacetate and root pulp were mixed at a ratio of 177.7 cm<sup>3</sup> lead subacetate + 26 g pulp with an automatic mixer. This was followed by infiltration through a filter paper (Whatman 42) to separate the extract, which was determined by polarimetry (Clarke et al., 1991).

By employing the Polak Equation (Eq. 3) (Asadi, 2006), the alkalinity coefficient of the samples was calculated.

Alkalinity = (Sodium + Potassium)/Nitrogen (3)

The K and Na contents of the root pulp samples were determined by a flame photometer. Additionally, the  $\alpha$ -amino-N content was measured by a Cooper reagent and a betalyzer, as described by Flavy and Vukou (1977). Data analysis was done using SAS 9.1 software, with the comparison of trait averages done by the LSD test at 1% and 5% levels.

The sugar beet variety that was planted in this study was Marinia, which is a single-bud variety and has a ZN growth type. This variety has a growth period of 150-180 days; hence, it was preferred for its early maturity and high sugar content. This variety has high resistance to rhizomania, nematode, cyst, and rust diseases, besides having relative resistance to bolting. The Marinia variety is produced by Linseed Company from England. Sugar beet planting was done on October 20th. Before planting, a soil sample was taken for laboratory analysis, after which fertilizer treatments were applied to the desired plots and mixed with the soil. Table 1 displays the characteristics of the soil in the experimental field (sampling was done from a depth of 0–30 cm).

# Results

From the analysis of the variance table, it was indicated that there was an interaction effect of spraying treatments with growth stimulants and irrigation on chlorophyll a and b, leaf dry weight, total chlorophyll, and quantum efficiency of photosystem II (Fv/Fm) that was significant at the 1% probability level (Table 2).

Analysis of variance of leaf dry weight, chlorophyll a, chlorophyll b, total chlorophyll, and photosystem II quantum efficiency (Fv/Fm) in sugar beet under the influence of irrigation regime and biological stimulants.

Source of variation	df	Leaf dry weight	Chlorophyll a	Chlorophyll b	Total chlorophyll	Photosystem II quantum efficiency (Fv/Fm)
Rep (Y)	2	22	0.13	0.22	0.27	0.01
Irrigation regime (Irr)	3	1861**	3**	1.20**	7.75**	0.13**
Main error	6	16.86	0.05	0.09	0.25	0.01
Spraying treatment (Spr)	4	110.31**	0.74**	0.02**	0.85**	0.01**
Irr× Spr	12	22.23**	0.12**	0.008**	0.15**	0.004*
Sub- error	32	3.19	0.03	0.002	0.04	0.002
CV (%)		5.97	6.89	3.95	5.55	6.12

\*\*, \*, and ns, significant at 1% and 5% levels of probability, non-significant, respectively.

Table 3

Results of the mean comparison of leaf dry weight, chlorophyll a, chlorophyll b, total chlorophyll, and photosystem II quantum efficiency (Fv/Fm) in sugar beet under the influence of irrigation regime and biological stimulants.

Irrigation regime	Spraying treatment	Leaf dry weight	Chlorophyll a	Chlorophyll b	Total chlorophyll	Photosystem II quantum efficiency (Fv/Fm)
80 mm of water	Seaweed extract of 3 kg ha-1	40.42cd	2.97cd	1.55bc	4.52cd	0.761bcd
evaporation	Seaweed extract of 6 kg ha <sup>-1</sup>	43.45bc	3.16bc	1.61ab	4.81bc	0.829abc
from the pan	Humic acid of 3 kg ha <sup>-1</sup>	46.28ab	3.46ab	1.61ab	5.07ab	0.862ab
	Humic acid of 6 kg ha <sup>-1</sup>	48.90a	3.72a	1.79a	5.48a	0.914a
	Control	38.26de	2.75de	1.46bc	4.21de	0.764bcd
160 mm of	Seaweed extract of 3 kg ha <sup>-1</sup>	32.61fgh	2.63ef	1.43bc	4.07def	0.783bcd
water	Seaweed extract of 6 kg ha <sup>-1</sup>	34.96ef	2.73de	1.45bc	4.18de	0.809a-d
evaporation	Humic acid of 3 kg ha <sup>-1</sup>	32.70fgh	2.73de	1.45bc	4.18de	0.791bcd
from the pan	Humic acid of 6 kg ha <sup>-1</sup>	33.66fg	3.23bc	1.48bc	4.71bc	0.859ab
	Control	31.06gh	2.40fg	1.39c	3.79efg	0.769bcd
240 mm of	Seaweed extract of 3 kg ha <sup>-1</sup>	29.28hi	2.34fg	1.11de	3.44g-j	0.765bcd
water	Seaweed extract of 6 kg ha <sup>-1</sup>	30.66gh	2.74de	1.17d	3.85efg	0.791bcd
evaporation	Humic acid of 3 kg ha <sup>-1</sup>	24.47ij	2.34fg	1.09de	3.44g-j	0.790bcd
from the pan	Humic acid of 6 kg ha <sup>-1</sup>	26.43i	2.54ef	1.10de	3.69fgh	0.824abc
	Control	18.21	2.18g	1.06de	3.26hij	0.736cde
320 mm of	Seaweed extract of 3 kg ha <sup>-1</sup>	19.18kl	2.14gh	0.98de	3.13ij	0.653efg
water	Seaweed extract of 6 kg ha-1	22.08jk	2.63ef	1.01de	3.60f-i	0.708def
evaporation	Humic acid of 3 kg ha <sup>-1</sup>	14.31mn	2.11gh	0.98de	3.09j	0.575gh
from the pan	Humic acid of 6 kg ha <sup>-1</sup>	17.80lm	2.20g	0.97de	3.22hij	0.622fgh
	Control	12.14n	1.81h	0.94e	3.06j	0.546h
LSD (0.05%)		3.82	0.32	0.21	0.47	0.10

Different lower-case letters indicated after means signify significant differences by LSD test at P< 0.05.

#### Leaf dry weight

According to the results of comparing the average data, with the increase in water limitation, the sugar beet leaves had their dry weights **decrease** significantly, such that the maximum amount of dry weight of sugar beet leaves (48.90 g per plant) was obtained under humic acid foliar spraying at a

rate of 6 kg ha-1 at the level of irrigation of 80 mm water evaporation from the pan. The lowest dry weight (12.14 g per plant) was recorded **for** the control treatment at irrigation levels of 320 mm (Table 3).

# Chlorophyll a, b, total chlorophyll, and quantum performance of photosystem II

Table 4
Analysis of variance of quantitative and qualitative traits of sugar beet under the influence of irrigation regime and biological
stimulants.

Source of variation	df	Root yield	Gross sugar	α- amino- N	Na <sup>+</sup>	K+	Alkalinity	Molasses	Extraction coefficient	Pure sugar	Pure sugar yield
Rep (Y)	2	13.60	0.05	0.68	1.23	0.007	2.65	0.10	6.29	0.26	0.02
Irrigation regime (Irr)	3	2570**	2.41**	1.64**	9.96**	2.75**	5.89*	0.31**	33.58**	4.12**	27.35**
Main error	6	105.29	0.71	0.24	1.86	0.07	1.61	0.19	12.35	1.18	1.99
Spraying treatment (Spr)	4	261.06**	1.69**	0.04 <sup>ns</sup>	2.42**	0.06 <sup>ns</sup>	0.32 <sup>ns</sup>	0.36**	30.53**	3.20**	6.44**
Irr× Spr	12	45.98*	0.21 <sup>ns</sup>	0.11 <sup>ns</sup>	0.45 <sup>ns</sup>	0.13**	0.29 <sup>ns</sup>	0.07 <sup>ns</sup>	3.42 <sup>ns</sup>	0.30 <sup>ns</sup>	0.85**
Sub- error	32	18.31	0.21	0.08	0.46	0.04	0.25	0.05	2.95	0.26	0.25
CV (%)		8.27	2.94	11.32	21.38	3.79	14.63	6.83	2.28	4.38	8.37

\*\*, \*, and ns, significant at 1% and 5% levels of probability, non-significant, respectively.

#### Table 5

Results of the mean comparison of root yield, pure sugar yield, and potassium of sugar beet under the influence of irrigation regime and biological stimulants.

Irrigation regime	Biological stimulants	Root yield	Pure sugar yield	Potassium
		(t ha <sup>-1</sup> )	(t ha-1)	(meq)
80 mm of water	Seaweed extract of 3 kg ha <sup>-1</sup>	65.68abc	7.26ab	5.38ghi
evaporation from the pan	Seaweed extract of 6 kg ha <sup>-1</sup>	72.03ab	8.28a	5.19gh
	Humic acid of 3 kg ha <sup>-1</sup>	65.05abc	7.41ab	4.83i
	Humic acid of 6 kg ha <sup>-1</sup>	74.06a	8.42a	5.20gh
	Control	58.89cde	6.33b-e	5.21fgh
160 mm of water	Seaweed extract of 3 kg ha <sup>-1</sup>	52.02d-h	6.03cde	5.24gh
evaporation from the pan	Seaweed extract of 6 kg ha <sup>-1</sup>	56.90c-f	6.75bcd	5.40e-h
	Humic acid of 3 kg ha <sup>-1</sup>	59.39cd	7.26ab	5.47d-g
	Humic acid of 6 kg ha <sup>-1</sup>	63.39bc	7.22abc	5.46d-g
	Control	49.30fgh	5.15efg	5.04hi
240 mm of water	Seaweed extract of 3 kg ha-1	49.93e-h	6.32b-e	5.54c-g
evaporation from the pan	Seaweed extract of 6 kg ha-1	53.04d-g	6.82bcd	5.69c-f
	Humic acid of 3 kg ha <sup>-1</sup>	42.69ghi	5.31efg	5.80c-f
	Humic acid of 6 kg ha-1	47.52ghi	5.82def	5.72cde
	Control	43.47hij	4.71fgh	5.75cd
320 mm of water	seaweed extract of 3 kg ha-1	38.41ijk	4.81fg	5.84bc
evaporation from the pan	Seaweed extract of 3 kg ha-1	46.41ghi	5.54ef	5.86bc
	Seaweed extract of 6 kg ha <sup>-1</sup>	30.28k	3.57hi	6.17ab
	Humic acid of 3 kg ha <sup>-1</sup>	36.51gk	4.26ghi	6.39a
	Humic acid of 6 kg ha <sup>-1</sup>	30.03k	3.39i	6.36a
LSD (0.05%)	-	Control	1.20	0.36

Different lower-case letters indicated after means signify significant differences by LSD test at P< 0.05.

According to the results, drought stress significantly reduced the amount of chlorophyll a, b, total chlorophyll, and quantum performance of sugar beet photosystem II. Generally, the highest amount of chlorophyll a, b, total chlorophyll, and photosystem II quantum performance of sugar beet with 3.72, 1.79, 5.48 mg g-1 FW, and 0.914%, respectively, were observed under humic acid

foliar spraying in the amount of 6 kg ha-1 at irrigation levels of 80 mm water evaporation from the pan. On the other hand, the respective lowest amounts were 1.81, 0.941, and 3.06 mg g-1 FW, and 0.546%, as noted in the control treatment at irrigation levels of 320 mm water evaporation from the pan (Table 3).

#### Root yield

The simple effect of irrigation treatments on the percentage of gross sugar, the amount of sodium, harmful nitrogen, alkalinity coefficient, molasses sugar, extraction coefficient, or percentage of extractable or pure sugar was significant at the 1% probability level (Table 4). The main effect of growth stimulants on the percentage of gross sugar, harmful nitrogen, the amount of sodium, the coefficient or percentage of extraction, the percentage of extractable or pure sugar, and the performance of pure or extractable sugar at the probability level of 1% and on molasses sugar was significant at the 5% probability level (Table 4). Also, the interaction effect of irrigation treatment × foliar spraying of growth stimulants on root performance, potassium content, and pure or extractable sugar performance was significant at the 1% probability level (Table 4).

The results of comparing the average data showed that the highest yield of sugar beet roots (74.06 t ha<sup>-1</sup>) was obtained under humic acid foliar spraying with an amount of 6 kg ha-1 at irrigation levels of 80 mm of water evaporation from the pan. The lowest amount of 30.03 t ha<sup>-1</sup> was recorded in the control treatment and irrigation levels of 320 mm of water evaporation from the pan, which did not show a statistically significant difference with the treatment of humic acid foliar spraying in the amount of 3 kg ha<sup>-1</sup> at the irrigation levels of 320 mm of water evaporation from the pan, which did not show a statistically significant difference with the treatment of humic acid foliar spraying in the amount of 3 kg ha<sup>-1</sup> at the irrigation levels of 320 mm of water evaporation from the pan (Table 5).

# Pure sugar yield

The comparison of the average treatments in terms of pure sugar yield showed that in the treatment where growth stimulants were not used, the amount of pure sugar yield decreased with increasing water limitation, so that the highest yield of pure sugar beet (8.42 t ha<sup>-1</sup>) was obtained under humic foliar application (6 kg ha<sup>-1</sup>) at the irrigation level of 80 mm of water evaporation from the pan, while the lowest amount (3.39 t ha<sup>-1</sup>) was recorded in the control treatment at the irrigation level of 320 mm of water evaporation from the pan (Table 5).

# Gross sugar

The average comparison (Table 3) showed that the percentage of gross sugar (sugar content) in irrigation treatments after 80, 160, 240, and 320 mm of evaporation from the class A evaporation pan was 15.09, 15.49, 15.99, and 15.86%, respectively. Thus, the restriction on irrigation significantly increased the percentage of gross sugar. Even though the difference between the irrigation treatments after 160, 240, and 320 mm of evaporation from the pan was not significant in terms of gross sugar, nevertheless, the highest percentage of gross sugar, with 15.99 and 15.86%, respectively, was obtained in irrigation treatments of 240 and 320 mm of evaporation from the class A evaporation pan (Table 6).

The results of the foliar application of biological stimulants showed that the percentage of gross sugar in the root was significantly higher in all foliar treatments with growth stimulants compared with the control treatment. Thus, the highest percentage of gross sugar (15.92%) was obtained under seaweed extract foliar application (6 kg ha<sup>-1</sup>), while the lowest amount was 14.96% in the control treatment (Table 7).

# Potassium

According to the results, the highest amount of potassium (6.39 mmol per 100 g of root) was obtained in the treatment with no growth stimulants at the irrigation level of 320 mm water evaporation from the pan, while the lowest amount of potassium (4.83 mmol per 100 g of pulp) was obtained under foliar spraying of humic acid in the amount of 3 kg ha<sup>-1</sup> at irrigation levels of 80 mm water evaporation from the pan (Table 5).

# Sodium and Nitrogen

The results of the average comparison indicate that with the increase in water limitation, the amount of harmful sodium and nitrogen increased. Thus, the highest amounts of harmful sodium and nitrogen were achieved with 4.16 and 3 mmol per 100 g of root pulp, respectively, at irrigation levels of 320 mm evaporation from the pan. In comparison, their lowest amount (2.53 and

Irrigation regime	Gross sugar (%)	α-amino-N (meq)	Na⁺ (meq)	Alkalinity (%)	Molasses (%)	Extraction coefficient (%)	Pure sugar (%)
80 mm of water evaporation from the pan	15.09b	2.49bc	2.53bc	3.61a	3.49ab	74.15b	11.20b
160 mm of water evaporation from the pan	15.49ab	2.24c	2.45c	3.67a	3.29b	74.27ab	11.52ab
240 mm of water evaporation from the pan	15.99a	2.73ab	3.62ab	3.31ab	3.61ab	77.31a	12.37a
320 mm of water evaporation from the pan	15.86a	3.00a	4.16a	3.11b	3.77a	75.91ab	12.04ab
LSD (0.05%)	0.75	0.43	1.14	0.41	0.42	3.14	0.97

Table 6Results of the mean comparison of quantitative and qualitative traits under the influence of irrigation regime.

Different lower-case letters indicated after means signify significant differences by LSD test at P< 0.05.

Table 7

Results of the mean comparison of quantitative and qualitative traits of sugar beet under the influence of biological stimulants.

Biological stimulants	Gross sugar (%)	α-amino-N (meq)	Na⁺ (meq)	Molasses (%)	Extraction coefficient (%)	Pure sugar (%)
Seaweed extract of 3 kg ha-1	15.67a	2.53b	2.79c	3.38c	76.51a	12.00a
Seaweed extract of 6 kg ha-1	15.92a	2.54b	2.99bc	3.47bc	76.33a	12.17a
Humic acid of 3 kg ha <sup>-1</sup>	15.76a	2.54b	2.82c	3.42c	76.53a	12.07a
Humic acid of 6 kg ha <sup>-1</sup>	15.72a	2.58b	3.51ab	3.67ab	74.83b	11.76a
Control	14.96b	2.88a	3.84a	3.78a	72.85c	10.90b
LSD (0.05%)	0.38	0.24	0.56	0.20	1.43	0.43

Different lower-case letters indicated after means signify significant differences by LSD test at P< 0.05.

2.49 mmol per 100 g of root pulp, respectively) was obtained at 80 mm irrigation levels by evaporation from the pan (Table 6). The results of the comparison of averages show that with foliar application of biological stimulators, the amount of harmful sodium and nitrogen in the root pulp sap was significantly lower in all foliar treatments with growth stimulants compared with the control treatment. In this case, the highest amount of sodium and nitrogen (2.79 and 2.53 mmol per 100 g of root pulp, respectively) were noted in the control treatment, while their lowest values (2.79 and 2.53 mmol per 100 g of root pulp, respectively) were obtained under seaweed extract foliar application of 3 kg ha<sup>-1</sup> (Table 7).

#### **Coefficient of Alkalinity**

Although the alkalinity coefficient decreased with the increase in water limitation, the highest alkalinity coefficient (3.67%) was obtained at the irrigation levels of 160 mm of evaporation from the pan, which did not vary significantly from the irrigation levels of 80 mm of evaporation from the pan. While, the least amount (3.11%) was obtained at irrigation levels of 320 mm evaporation from the pan (Table 6).

#### Molasses

The mean results of molasses sugar showed that with the increase in water limitation, molasses sugar increased such that the highest (3.77%) amount was obtained at irrigation levels of 320 mm evaporation from the pan, while the lowest (3.29%) was obtained at irrigation levels of 160 mm evaporation from the pan (Table 6). The mean results of biological stimulant foliar application showed that the amount of molasses sugar in root pulp sap was significantly lower in all foliar application treatments with growth stimulants compared with the control. Thus, the highest amount of molasses sugar (3.78%) was observed in the control treatment, while the lowest one of 3.38% was obtained in the foliar spraying of seaweed extract in the amount of 3 kg ha<sup>-1</sup> (Table 7).

# **Extractable Coefficient and Pure Sugar**

The mean results of extractable coefficient and pure sugar showed that the highest extractable coefficient and pure sugar of sugar beet (77.31 and 12.37%, respectively) were obtained at irrigation levels of 240 mm evaporation from the pan, while the lowest ones (74.15% and 11.20%, respectively) were achieved at irrigation levels of 80 mm evaporation from the pan (Table 6). The results of biological stimulant application showed that the extractable coefficient and pure sugar in all treatments of foliar spraying with growth stimulants were significantly higher than the control treatment. Thus, the highest extractable coefficient and pure sugar of sugar beet (76.53 and 12.17%, respectively) were observed under humic acid foliar application at the rate of 3 kg ha<sup>-</sup> <sup>1</sup> and seaweed extract at the rate of 6 kg ha<sup>-1</sup>, while the lowest (72.85 and 10.90%, respectively) were obtained in the control treatment (Table 7).

# Discussion

The results of various research studies have shown that the plant leaf is the first plant organ that is affected by drought stress, so plant leaves are very sensitive to lack of water, and the reduction of their growth due to water limitation can be seen earlier (Sadeghi-Shoae et al., 2013; Goher et al., 2023). It has also been stated that the lack of water causes a series of morphological and physiological changes in the sugar beet, such as reducing the leaf surface, the number of leaves, and photosynthesis (Helaly et al., 2009). Under drought stress conditions, the yellowing and aging of sugar beet leaves may be accelerated; as a result, the life span of the leaf decreases, which causes a decrease in the leaves' dry weight (El-Hassanin et al., 2016). So, foliar spraying of biological stimulants was effective in significantly increasing the dry weight of leaves in sugar beet. It has been reported that seaweed extract reduces a range of abiotic stresses, such as salinity,

drought, and extreme temperature (Heydarzadeh et al., 2021; Rahimi et al., 2020). Seaweed extract also increases the internal concentration of stressrelated molecules, including proline, antioxidants, antioxidant enzymes, and cytokinins, in treated plants (Pačuta et al., 2023), which increases the dry weight of sugar beet leaves.

The photosynthetic pigment content (known as chlorophyll) is an indicator of drought stress tolerance in plants (Pačuta et al., 2023). The decrease in chlorophyll content caused by water limitation is linked to the disparity in oxygen radicals such as H2O2 in cells and the activity of numerous enzymes. Indeed, free radicals cause peroxidation and damage to the thylakoid membrane, which leads to the destruction of chlorophyll pigments (Enan et al., 2016). It seems that the decrease in chlorophyll content under drought stress is chiefly ascribed to the activity of the chlorophyllase enzyme, resulting in the degradation of chlorophyll (Sadeghi-Shoae et al., 2013). It has also been reported that the absorption of iron and magnesium from the soil decreases under water limitation conditions since magnesium is directly involved in the synthesis of chlorophyll, so by reducing the absorption of magnesium ions, the synthesis of chlorophyll is also limited (Pačuta et al., 2023). Humic acid increases sugar beet root performance through a positive physiological effect, which entails affecting the plant cell metabolism to increase leaf chlorophyll concentration (Enan et al., 2016). The increase in the content of photosynthetic pigments (chlorophyll) may be due to the of nitrogen acceleration absorption, the improvement of nitrogen metabolism, and the synthesis of proteins, which ultimately increase content of photosynthetic pigments the (chlorophyll). The additional probable reason for the increase is other functions of humic acid, such as increasing oxygen absorption, cell membrane permeability, respiration and photosynthesis, phosphorus absorption, and increasing root length (Ghaffari et al., 2022). Seaweed extract contains cytokines that stimulate physiological activities and increase total chlorophyll in plants. This reflects photosynthesis activity and synthesized substances that positively reflect growth characteristics (Beigzadeh et al., 2020). Besides

being rich in potassium, manganese, and phosphorus, seaweed extract contains significant amounts of iron, magnesium, calcium, zinc, and copper; therefore, it increases the absorption and accumulation of such elements in plants. In return, this justifies the significant increase in vegetative growth and yield and its components, as well as the content of phosphorus, nitrogen, and protein in leaves, as well as the content of leaf chlorophyll for the majority of the crops, especially those cultivated in arid and desert areas (Pačuta et al., 2023). The significant effect of seaweed extract can be attributed to its influence on increasing the porousness of the cell membrane and improving the efficiency of the plant in absorbing nutrients like N, which has a direct relationship with the concentration of leaf chlorophyll. In addition, seaweed extract may help in reducing the degradation of chlorophyll through its content of cytokinins, resulting in delayed aging of leaves. Also, seaweed extract is a biological regulator that affects the equilibrium between respiration in plants and processes of photosynthesis (Bertoldo et al., 2023). The Fv/Fm ratio is a parameter to gauge the potential photochemical conversion efficacy of photosystem II. As the level of Fv/Fm decreases, the limitation of photosynthetic efficacy becomes more apparent (Singh and Reddy, 2014). It has also been reported that foliar application of growth stimulants in both favorable and water-limited conditions improved Fv/Fm in sugar beet plants, improved photosynthesis, and increased sugar beet plant yield (Helaly et al., 2009).

As a result of water limitation, the size of the aerial organs of the plant is reduced, resulting in a decrease in yield in plants (Rašovský et al., 2022). In other words, in conditions of water limitation, the growth and development of the leaves decrease. With the reduction in leaf area, the amount of total photosynthesis in the plant also decreases. With the decrease in the production of photosynthetic products under conditions of water limitation, plant growth and, finally, yield will also decrease (Ahmed et al., 2023). In this research, foliar spraying with humic acid and seaweed extract has improved the root performance of sugar beet, and reports have shown that the use of seaweed extract reduces the adverse effects of environmental stress due to the presence of glycine betaine in the cell, which increases the tolerance of plants to water stress (Nassar et al., 2023). Both mannitol and betaine in seaweed extract maintain the osmotic balance of plant cells; these substances also maintain the external structure of the cell (Rerhou et al., 2023). This causes the loss of cell water to take place slowly, and finally, the plant has more tolerance against the stresses caused by drought and dehydration. It has also been reported that the use of seaweed extract at the field level can increase the capacity of sugar beet to deal with environmental stress (Makhlouf et al., 2022). The use of humic acid in plants can positively affect their physiological processes. Such processes include strengthening the plants and helping them to resist various environmental stresses (Rahimi et al., 2023; Sadeghi-Shoae et al., 2013). The operational mechanism of humic acid entails the formation of a complex between inorganic elements and acids. Additionally, it plays a crucial processes such as role in respiration. photosynthesis, and stimulation of nucleic acid metabolism (Majeed et al., 2023; Enan et al., 2016). Humic acid increases root performance, hence upholds photosynthetic tissue activity, and increases leaf area for sunlight interception (Makhlouf et al., 2022). Humic acid increases the performance of sugar beet roots through positive physiological effects, including increasing leaf concentration plant chlorophyll and cell metabolism (Enan et al., 2016). The increase in root function following the use of humic acid may be associated with its influence on hormonal activity (gibberellin, cytokinin, and auxin). In addition, this acid increases access to water and nutrients by the root system, resulting in improved sugar beet root performance (Nassar et al., 2023).

The yield of pure sugar was obtained as the product of the percentage of white sugar in the yield of the root, and the increase in each of these traits increases the yield of pure sugar. It has also been reported that water limitation increased the amount of sugar in sugar beet by about 1–5%, but sugar production decreased by approximately 20% due to the decrease in sugar beet root yield and the increase in sugar beet impurities (Sadeghi-

Shoae et al., 2013). The reports of Bloch et al. (2006) also stated that water stress increases the percentage of pure and gross sugar in sugar beet but significantly decreases the root weight. The increase in the yield of pure sugar is probably because of the application of seaweed extract and humic acid, which create favorable growth conditions and improve the nutrition of sugar beet plants. Humic acid improves the growth of sugar beet roots, which is part of the pure sugar yield component.

It has been stated that the gross sugar percentage of sugar beet increases with an increasing water deficit (Enan et al., 2016). The use of plant growth stimulants, especially humic acid and seaweed extract, for justifiable agricultural purposes can maintain soil fertility besides compensating for food shortages (Nassar et al., 2023). According to various reports, the percentage of sucrose has the greatest effect on the percentage of gross sugar (sugar content) (Osman, 2022). So, considering such results, we can conclude that the application of growth stimulants can upsurge the production of sucrose by enhancing the development of leaf surface and photosynthesis (Nassar et al., 2023). It has also been shown that the use of growth stimulants reduces the amount of potassium in sugar beet (Nassar et al., 2023). Based on the results of this study, increasing the level of humic acid in foliar spraying increased the amount of potassium in sugar beet, as reported by Rahimi et al. (2020). It has also been stated that the content of potassium and nitrogen ( $\alpha$ -aminoN) in beet juice increased significantly as compared with the control treatment due to the application of humic acid (Makhlouf et al., 2022).

It has been reported that with the increase in water limitation, the amount of potassium in the root decreased and the amount of sodium in the root increased (Enan et al., 2016). Some studies also show that sodium decreases with an increasing water deficit (Helaly et al., 2009). Sodium is one of the soluble mineral solutes in sugar beet root, which interferes with the crystallization of sucrose in the process of sugar extraction, and increasing the percentage of sugar in molasses causes an increase in sugar waste and a decrease in the percentage of extractable sugar. Drought stress causes changes in the amount of soluble substances in the sugar beet root sap. Hence, researchers reported that the accumulation and increase of alpha-amino nitrogen compounds in the root under drought stress conditions is due to a decrease in their uptake in the root (Ahmed et al., 2023). Reducing the amount of harmful nitrogen and sodium in the sugar beet root pulp by sprinkling growth stimulants indicates an improvement in root growth. The reduction of harmful nitrogen and sodium due to the use of growth stimulants had been reported earlier (Rašovský et al., 2022). Impurities present in root pulp, including harmful sodium and nitrogen, play a significant role in the processing of sugar from sugar beet. The higher amount of these impurities in root pulp hurts the extraction of sugar from the root and causes a significant decrease in sugar extraction (Pačuta et al., 2023). Rahimi et al. (2019) recounted that foliar application of humic acid reduces harmful nitrogen in sugar beet. It has also been reported that biological stimulants affect the metabolism of carbohydrates while increasing the amount of sugar and starch, causing an increase in dry matter (Makhlouf et al., 2022). So, it is worth noting that the consumption of biological stimulants not only increases yield and improves quality but also reduces the accumulation of nitrates in the roots of sugar beet (Ahmed et al., 2023).

The alkalinity factor is the ratio of the sum of potassium and sodium to the harmful nitrogen present in the sugar beet root. In this case, the increase of sodium and potassium in the sugar beet root pulp under water stress will lead to a decrease in the alkalinity factor, and the higher the amount of harmful nitrogen, the lower the alkalinity coefficient (Rerhou et al., 2023). The threshold value for the alkalinity coefficient is 1.8% (Rerhou et al., 2023). The higher or lower the alkalinity coefficient is, the amount of extractable sucrose (sugar) will decrease. It has been reported that drought stress increases molasses sugar (Enan et al., 2016). The number of photosynthesizing pigments and the amount of leaf chlorophyll decrease under water stress conditions (Nassar et al., 2023). Based on this, the amount of photosynthesis and the rate of carbon dioxide fixation per unit of leaf area are reduced,

and the storage of sugar and starch in roots and leaves is reduced. Under these conditions, the amount of molasses, or root pulp, increases. Therefore, the application of growth stimulants in the form of foliar spraying has reduced the percentage of molasses sugar in sugar beet. Potassium, sodium, and nitrogen constituents of molasses are harmful, and these constituents of molasses are an important criterion for the quality of beet sugar and play a role in reducing sugar yield. In this context, the reports of Osman et al. (2022) show that the use of growth stimulants in most cases increased the amounts of sodium, potassium, and alpha-amino nitrogen in sugar beet pulp.

It has been recounted that the coefficient and percentage of extractable sugar increased in mild water stress and moderate water stress, but with the increase in the level of drought stress (severe stress), the coefficient and percentage of extractable sugar decreased (Ahmed et al., 2023). Previously, it was equally noted that foliar spraying with biological stimulants such as humic acid and seaweed extract significantly increased the coefficient and percentage of extractable sugar (Osman et al., 2022). Foliar spraying with biological stimulants to improve the nutritional

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needs of sugar beet, which increases leaf area and enhances photosynthetic activity, can increase both the coefficient and percentage of extractable sugar in sugar beet (Enan et al., 2016).

#### Conclusion

In the current study, higher levels of moisture stress led to an increase in the percentage of crude sugar, harmful nitrogen, sodium, molasses sugar, extraction coefficient, and extractable sugar. At the same time, moisture stress decreased the content of chlorophyll a, chlorophyll b, and total chlorophyll, as well as leaf dry weight and sugar beet root yield. The foliar application of 6 kg ha-1 humic acid under irrigation conditions, following 80 mm of water evaporation from the pan, resulted in the highest root yield and gross sugar vield. Overall, the use of 6 kg ha-1 seaweed extract and 3 kg ha-1 humic acid significantly increased the percentage of gross sugar, extraction coefficient, and extractable sugar, while reducing harmful sodium and nitrogen compared to the control treatment.

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