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Impact of biofertiliser and zinc nanoparticles on enzymatic, biochemical, and agronomic properties of sugar beet under different irrigation regimes

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Abstract

To investigate the enzymatic, biochemical, and agronomic response of sugar beet (*Beta vulgaris* L.) to biofertilisers and zinc nanoparticles under different irrigation regimes, an experiment was conducted at the Agricultural and Natural Resources Research Station of Miandoab, West Azerbaijan Province, Iran. Three irrigation levels: normal, mild stress, and severe water stress, were applied (control – without irrigation) in the main plots, and three fertilisation treatments: seed inoculation with arbuscular mycorrhizal fungi (AMF), zinc nano-oxide nanoparticles (ZnO NPs), and AMF + ZnO NPs, were applied (control – without fertilisers) in the sub-plots in two years. The severe water stress reduced the content of chlorophyll *a*, *b*, and carotenoids by 14.53, 8.09, and 22.11 %, respectively, and increased the content of leaf flavonoids and superoxide dismutase (SOD) by 34.17% and 33.14%, respectively, compared to normal irrigation. The AMF + ZnO NPs treatment increased the content of chlorophyll *a*, chlorophyll *b*, carotenoids, and SOD by 36.76, 12.61, 10.36, and 10.18 %, respectively, compared to the control treatment. The highest root yield, sugar yield, and white sugar yield provided the AMF + ZnO NPs treatment under normal irrigation. The highest proline content, total phenolic content (TPC), catalase (CAT) activity, sugar content, and white sugar content were found in sugar beets treated with AMF + ZnO NPs under severe water stress. The AMF + ZnO NPs treatment under normal irrigation and mild water stress and ZnO NPs one under severe water stress increased the white sugar yield by 42.67, 55.69, and 20.11 %, compared to the control treatment. Thus, treating with AMF + ZnO NPs under normal and mild water stress and applying of ZnO NPs under severe water stress can be a solution to increase the economic yield of sugar beet.

Keywords: *Beta vulgaris*, antioxidant, sugar content, mycorrhiza, water deficit.

Introduction

Water deficit has a major impact on crop production. Droughts harm the growth and development of plants and pose a threat to global agriculture by limiting food production (Verma, Deepti, 2016). One of the most important industrial plants is sugar beet (*Beta vulgaris* L.), which is used in the sugar industry and is the main source of sugar production after sugarcane (Monteiro et al., 2018). The annual production of sugar beet roots is about 278 million tons (FAO, 2021). Depending on the climatic conditions, sugar beet requires from 8000 to 1000 m^3 of water. Drought can slow growth, wilt leaves, reduce photosynthesis, and lower water content in beets, and also damage the membrane and reduce white sugar yield (Islam et al., 2020).

The deficiency of zinc (Zn) in the soil is a major agricultural problem in arid and semi-arid regions (Saboor et al., 2021). The optimum amount of Zn in the soil is from 10 to 300 mg kg^{-1}. Most of the Zn content in the soil is in the form of complexes with organic matter, so the amount of Zn available for agricultural plants is usually a small fraction (Saboor et al., 2021).

One of the reasons of declining Zn availability of plants with the optimal amounts in arid and semi-arid regions is a high soil pH (Saboor et al., 2021). Additionally, the uneven distribution of phosphorus in the soil can lead to the formation of Zn-P compound. This compound is insoluble in water and can interfere the uptake of Zn by plants (Bibi et al., 2020). The proper absorption of Zn

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incerases crop yield and also improves overall quality. Photosynthesis, carbohydrate metabolism, protein metabolism, membrane integrity, pollen formation, and auxin metabolism are plant processes influenced by Zn (Hassan et al., 2020). Physiological processes may be disrupted in plants due to Zn deficiency, but this problem can be solved by developing symbiotic relationships, i.e., developing the relationship between plant roots and beneficial microorganisms (Jabborova et al., 2020). The foliar application of micronutrients in plants is found to be more effective and economical. Foliar application can significantly increase crop yields by reducing the loss of nutrients, compared to soil application (Zajaczkowska et al., 2020).

For this purpose, the seed inoculation with arbuscular mycorrhizal fungi is highly recommended (Saboor et al., 2021). Microorganisms facilitate the uptake of water and nutrients by the plants (El-Gizawy et al., 2014). Due to their long period in the field, sugar beet requires a lot of water and inputs. Thus, finding a solution to improve the economic yield of this plant by reducing the amount of water and chemical fertilisers can save water and reduce environmental pollution.

This study aimed to evaluate the effects of sugar beet seed inoculation with arbuscular mycorrhizal fungi and foliar application of zinc nano-oxide nanoparticles on the biochemical and agronomic properties of sugar beet under different irrigation regimes.

Material and methods

This study was carried out as a split-plot experiment designed on a randomised complete block with three replications. This study was conducted at the Agricultural and Natural Resources Research Station of Miandoab (37°44″18′ N, 45°10″53′ E, 1338 m a. s. l.), West Azerbaijan Province, Iran, during the two harvest years of 2020–2021.

The average temperature and amount of precipitation and soil characteristics of the experimental site are presented in Figure and Table 1.

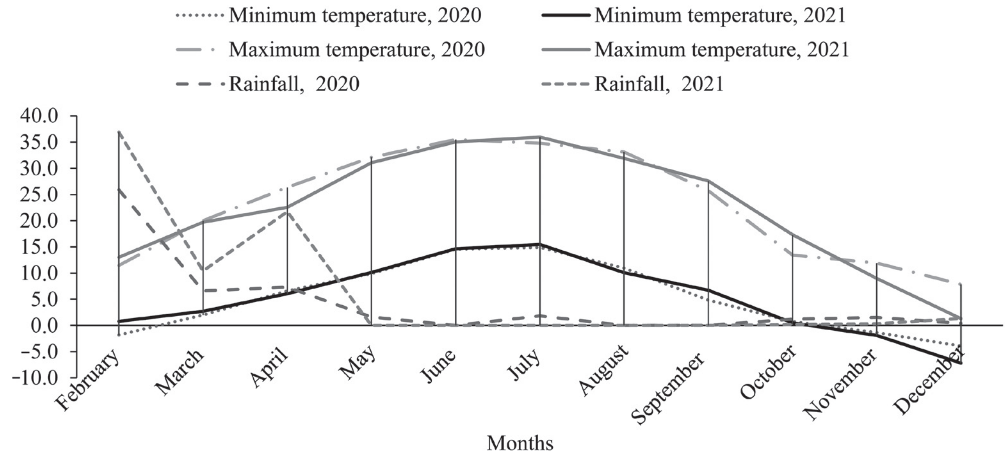

Figure. Rainfall (mm) and minimum and maximum temperature (°C) of the experimental site during 2020–2021

Table 1. Physical and chemical characteristics of the soil of the experimental site

EC dSm^{1}	pH	Texture	Clay	Silt	Sand	CaCO$_3$		Saturation percentage
1.38	7.79	clay loam	41%	36%	23%	15.71%		54%
N		organic carbon	Mn	B	Zn	Fe	K	P
%			mg kg^{-1}					
0.03		1.16	11.2	0.28	1.1	8.11	282	9.02

In this experiment, three irrigation levels: moistening after 60, 90, and 120 mm of evaporation from the class A evaporation pan, were applied (control – without irrigation) in the main plots, and three fertilisation treatments: arbuscular mycorrhizal fungi (AMF), zinc nano-oxide nanoparticles (ZnO NPs), and mycorrhizal fungus + zinc nano-oxide (AMF + ZnO NPs), were carried out (control – without fertilisers) in the sub-plots. Irrigation after 60, 90, and 120 mm of evaporation was considered as normal irrigation and mild and severe water stress, respectively.

Since sugar beet is sensitive to water stress at the germination and beginning of growth, during the germination stage, until the complete establishment of the plant (8-leaf stage), sufficient irrigation was performed (once a week) for all treatments. Irrigation was carried out using a pressure system, a hose, and a meter.

The soil containing mycorrhiza (*Glomus intraradices* L.) obtained from Green Biotechn Company (Symbiagro S.r.l., Italy) was applied under the seeds at the rate of 40 grams per plant (each gram of the sample contained about 300 live spores). Foliar application of ZnO was used according to the manufacturer's instructions (Knowledge Foundation Isdar Ahrar Sharq, registration No. 203369) at a concentration of 1.5 per thousand at two stages after the water defect stress and at the 12-leaf and 20-leaf stages. To prevent the leaves from burning, the foliar application was performed at sunset. To eliminate the effects of foliar application in the control treatment, leaves were simultaneously sprayed with water.

To prepare the planting bed, deep ploughing was done in the autumn. Field preparation operations in the spring included soil ploughing, discing, levelling, line drawing, and planting rows preparation (with a chipper). The required fertilisers were distributed based on the results of soil analysis (Table 1). During three times, 200 kg per hectare of urea fertiliser was applied to the field; in addition, 135 and 110 kg of triple superphosphate ($Ca(H_2PO_4)_2$) and potassium sulphate (K_2SO_4) fertilisers were applied to the field at the same time as the autumn ploughing. Each plot consisted of four planting rows with a length of 5 meters; the distance between the planting rows was 50 cm and between the plants in the row was 20 cm.

The Iranian sugar beet (*Beta vulgaris* L.) seeds of the cultivar 'Shokofa' (mono-germ, resistant to rhizomania and nematode) were obtained from the Sugar Beet Seed Institute (SBSI), Karaj, Iran. Planting was done in late April. To measure traits, samples were taken from the fourth and fifth leaves of each experimental treatment at the end of the growing period (October), before harvesting. The samples prepared from each treatment were immediately wrapped in aluminum foil. The sample number was written, and the samples were placed in liquid nitrogen to freeze (−196°C). The frozen samples were transferred to a freezer with a temperature of −40°C. The irrigation was applied according to the maximum allowable depletion (MAD) by the adopted method of Allen (2000). Changes in soil moisture were measured by Theta probes SM300 (Royal Eijkelkamp, The Netherlands). Two months after the drought stress treatment, the amount of photosynthetic pigments, the content of total phenols, flavonoids, and malondialdehyde (MDA) were determined.

Measurement of physiological and enzymatic characteristics. The amount of chlorophyll *a*, *b*, and carotenoids was measured using the Arnon's (1972) technique. To determine the amount of chlorophyll *a*, *b*, and carotenoids in the extracts, a spectrophotometer was used to measure their absorbance at 663, 645, and 480 nm, respectively. The activities of catalase (CAT) and superoxide dismutase (SOD) and the MDA content were measured by the methods of Bowler et al. (1991), Britton and Mehley (1995), and Madhavara Rao and Sresty (2000), respectively. The total phenolic content (TPC) of the extract was determined using the method of Bates etal. (1973). The crude extract was mixed with the Folin-Ciocalteu reagent followed by the addition of sodium carbonate (Na_2CO_3). After 30 min of incubation, the absorbance was measured at 750 nm. The total flavonoid content was determined according to the aluminum chloride colorimetric assay (Arvouet-Grand et al., 1994). The extract was mixed with the aluminum chloride ($AlCl_3$) solution and sodium acetate ($C_2H_3NaO_2$). After 15 min of incubation, the absorbance was measured at 415 nm.

Measurement of quantitative and qualitative characteristics. Sugar beet was harvested approximately 180 days after seeding, at the BBCH 49 growth stage according to the BBCH-scale. At the harvesting time, all plants of each treatment were harvested, counted, and weighed. The roots were washed, and pulp samples were prepared randomly from the roots. Quantitative and qualitative characteristics of the pulp samples were analysed at the Sugar Technology Laboratory of SBSI located in Karaj, Iran. The frozen samples were thawed and then mixed with 177 ml of lead(II) hydroxide acetate for three minutes. The filtered solution became a clear liquid that was used in Betalysis, an automated sugar beet quality analysis system. The percentage of sugar as well as the content of sodium, alpha-amino nitrogen, and potassium were measured. After determining the content of sugar, nitrogen (alpha-amino N), sodium (Na), and potassium (K), the other studied traits were estimated as follows (Cooke, Scott, 1993):

$$MS = 0.0343(K + Na) + 0.094\ (\text{alpha-amino N}) - 0.31,\ WSC = SC - (MS + 0.6),$$

$$ALC = (K + Na) / (\text{alpha-amino N}),\ WSY = WSC \times RY,$$

where MS is the percentage of molasses sugar, WSC is the white sugar content, and SC is the sugar content; ALC is the alkalinity, WSY is the white sugar yield (t ha^{-1}), and RY is the root yield (t ha^{-1}).

Statistical analysis. The analysis of variance (ANOVA) and the mean comparison (the least significant ranges (LSR) at 5% probability level) of the experiment were performed by the software SAS, version 9.4 (SAS Institute Inc., USA).

Results

The results of ANOVA showed that the difference between the irrigation regimes was significant in terms of the effects on all traits except the coefficient of sugar extraction. The effect of fertilisation treatments was also significant on all the studied traits except the root alkalinity. The interaction of irrigation and fertilisation treatments on the proline content, TPC, CAT activity, MDA content, sugar content, white sugar content, and alkalinity, and the coefficient of sugar extraction, root yield, sugar yield, white sugar yield, and molasses sugar percentage was significant (Table 2).

Photosynthetic pigments. The severe water stress treatment reduced the chlorophyll *a*, *b*, and carotenoid content by 14.53, 8.09, and 22.11 %, respectively, compared to the normal irrigation (Table 3). In addition, the AMF + ZnO NPs treatment increased the content of chlorophyll *a*, *b*, and carotenoids by 36.76, 12.61, and 10.36 %, respectively, compared to the control treatment.

Proline content. As water intensity increased, the leaf proline content also increased under normal irrigation and mild water stress. The difference between the fertilisation and the control treatments was not significant, while under severe water stress, all three AMF, ZnO NPs, and AMF + ZnO NPs fertilisation treatments significantly increased the proline content, compared to the control treatment. The AMF + ZnO NPs treatment under severe water stress had the highest proline content, while the lowest proline content was obtained in all three fertilisation treatments under normal irrigation (Table 4).

Phenolic content. Severe water stress and the application of AMF + ZnO NPs induced the highest phenol content in the leaves. The lowest phenol content was related to the normal irrigation and all three fertilisation treatments, and the difference between them was insignificant. The application of Zn as AMF + ZnO NPs significantly increased the leaf phenolic content

Table 2. Commbined analysis of variance of the studied sugar beet traits under different irrigation regimes and fertilisation treatments

Sources of variation (SOV)	df	Chlorophyll		Carotenoids	Proline	TPC	Flavonoids	SOD	CAT	MDA
		a	*b*							
		mean squares								
Year (Y)	1	2.13 ns	1.54 ns	0.29 ns	0.004 ns	3.54 ns	1.15 ns	29.09 ns	5.14 ns	89.15 ns
Replication × (Y)	4	0.39	0.35	0.18	0.002	3.41	0.45	14.17	1.2	21.35
Irrigation regime (IR)	2	1.98*	0.34**	2.71**	0.17**	218.71**	7.88**	281.94**	118.44**	2401.37**
Y × IR	2	1.10 ns	0.03 ns	0.08 ns	0.01 ns	19.41 ns	0.32 ns	3.66 ns	1.31 ns	9.24 ns
Ea	8	0.37	0.01	0.02	0.003	6.03	0.08	1.48	1.18	24.23
Fertiliser (F)	3	0.83*	0.31**	0.21**	0.0009 ns	20.85*	0.20 ns	14.70**	4.50**	313.72*
Y × IR	3	0.3 5ns	0.10 ns	0.05 ns	0.0005 ns	5.14 ns	0.17 ns	2.26 ns	1.02 ns	145.12 ns
IR × F	6	0.33 ns	0.05 ns	0.01 ns	0.40**	20.90**	0.08 ns	1.70 ns	0.95*	324.59**
Y × IR ×F	6	0.16 ns	0.11 ns	0.02 ns	0.0008 ns	12.40 ns	0.20 ns	5.51 ns	0.80 ns	195.36 ns
Eb	24	0.25	0.05	0.02	0.0007	5.18	0.09	1.54	0.36	79.74
CV%	–	5.69	6.64	4.33	4.36	5.84	5.4	6.75	6.97	17.41

SOV	df	Sugar content	White sugar content	Alcalinity	Sugar extraction coefficient	Root yield	Sugar yield	White sugar yield	Molasses sugar
		mean squares							
Year (Y)	1	78.89 ns	13.16 ns	0.57 ns	106.27 ns	297.83 ns	56.78 ns	10.15 ns	0.004 ns
Replication × (Y)	4	54.14	18.58	0.11	52.41	1125.69	22.41	17.15	0.002
Irrigation regime (IR)	2	39.32*	22.92**	0.047*	34.88 ns	4974.42**	80.40**	48.66**	0.011 ns
Y × IR	2	20.16 ns	3.17 ns	0.01 ns	510.55 ns	12.65 ns	10.12 ns	8.26 ns	0.01 ns
Ea	8	6.68	1.71	0.01	177.7	24.99	2.36	2.36	0.005
Fertiliser (F)	3	4.58*	20.01**	0.032 ns	802.11**	310.72**	6.63**	5.65*	0.007*
Y × IR	3	2.20 ns	4.19 ns	0.09*	102.45 ns	27.37 ns	3.20 ns	2.22 ns	0.002 ns
IR × F	6	6.13**	7.42**	0.065**	213.37*	96.49**	4.27*	3.87*	0.051**
Y × IR × F	6	1.42 ns	1.40 ns	0.04 ns	14.59 ns	48.38 ns	1.45 ns	2.15 ns	0.002 ns
Eb	24	1.4	1.83	0.02	67.18	25.91	1.41	1.4	0.002
CV%	–	6.59	9.7	5.58	10.38	9.25	12.21	12.22	5.5

Ea – main error, Eb – sub error; df – degrees of freedom; TPC – total phenolic content, SOD – superoxide dismutase, CAT – catalase, MDA – malondialdehyde; ns, *, and ** – not significant, significant at 5% and 1%

Table 3. Average of the effects of irrigation regimes and fertilisation treatments on the studied sugar beet traits in two years (2020–2021)

Treatment	Chlorophyll *a* mg g^{-1} FW	Chlorophyll *b* mg g^{-1} FW	Carotenoids mg g^{-1} FW	Flavonoids mg of quercetin g^{-1} DW	SOD U mg^{-1} protein
Normal irrigation	9.56 a	3.83 a	4.25 a	4.74 c	1086.09 b
Mild water stress	8.55 b	3.55 b	3.79 b	5.67 b	1412.04 a
Severe water stress	8.17 b	3.52 b	3.31 c	6.36 a	1446.07 a
Control (without fertilisers)	6.80 b	3.41 b	3.57 b	5.81 a	1247.22 c
AMF	8.88 ab	3.66 a	3.84 a	4.91 a	1363.25 a
ZnO NPs	8.86 ab	3.64 ab	3.78 a	5.45 a	1340.7 b
AMF + ZnO NPs	9.30 a	3.84 a	3.94 a	5.90 a	1374.73 a

Note. Mean in each column followed by a similar letter(s) is not significantly different at the 5% probability level; AMF – arbuscular mycorrhizal fungi, ZnO NPs – zinc nano-oxide nanoparticles; FW – fresh weight, DW – dry weight; SOD – superoxide dismutase, U mg^{-1} protein – micromole per minute mg of protein.

under severe water stress, compared to the untreated plants (Table 4).

Flavonoid content in leaves. The flavonoid content of plants increased by 16.40% and 25.47% under mild and severe water stress, respectively, compared to the normal irrigation. The highest content of flavonoids was determined under severe water stress, and the lowest under normal irrigation (Table 3).

Superoxide dismutase (SOD)activity. Severe water stress increased the SOD activity by 33.14% and 30.01%, respectively, compared to the normal irrigation and mild water stress (Table 3). Treatment of sugar beets with AMF, ZnO NPs, and AMF + ZnO NPs increased the SOD activity by 9.30, 7.49, and 10.22 %, respectively, compared to the control treatment (Table 4).

Table 4. Comparison of the interaction of irrigation regimes and fertilisation treatments of the studied sugar beet traits in two years (2020–2021)

Irrigation regime	Treatment	Proline mg g^{-1} FW	TPC mg gallic acid g^{-1} DW	CAT U mg^{-1} protein	MDA nm mg^{-1} protein	Sugar content %	Root yield t ha^{-1}
Normal irrigation	control	0.45 d	32.68 d	65.04 e	42.41 d	16.31 b-e	61.15 c
	AMF	0.49 d	34.83 cd	64.64 e	37.19 d	14.34 e	71.27 ab
	ZnO NPs	0.49 d	34.90 cd	66.88 e	21.85 e	15.37 de	70.21 b
	AMF + ZnO NPs	0.48 d	34.19 d	67.24 e	41.05 d	16.09 cde	76.46 a
Mild water stress	control	0.66 bc	40.38 b	82.12 d	60.26 bc	17.13 b-e	41.68 e
	AMF	0.63 c	42.30 b	91.28 ab	42.92 d	17.68 abc	53.5 d
	ZnO NPs	0.69 bc	40.66 b	84.64 b	60.45 bc	18.53 ab	44.6 e
	AMF + ZnO NPs	0.63 c	36.31 bc	89.76 ab	47.07 cd	17.24 bcd	53.41 d
Severe water stress	control	0.63 c	38.61 bc	83.72 cd	75.67 a	19.52 a	39.62 e
	AMF	0.70 b	41.13 b	88.48 bc	71.86 ab	17.15 b-e	44.76 e
	ZnO NPs	0.71 b	41.11 b	87.64 bc	66.59 ab	17.01 b-e	43.16 e
	AMF + ZnO NPs	0.78 a	47.91 a	93.8 a	48.02 cd	18.66 ab	41.62 e

Irrigation regime	Treatment	Sugar yield t ha^{-1}	White sugar yield t ha^{-1}	White sugar content %	Alkalinity %	Coefficient of sugar extraction %	Molasses sugar %
Normal irrigation	control	9.97 bcd	6.35 cd	10.93 c	2.83 cd	63.70 d	1.02 b
	AMF	10.22 ab	8.84 b	12.4 bc	3.00 bc	86.47 a	0.96 bc
	ZnO NPs	10.79 ab	8.07 b	11.5 c	3.21 ab	74.82 b	0.85 d
	AMF + ZnO NPs	12.30 a	9.06 a	11.58 c	3.30 a	73.65 b	0.81 d
Mild water stress	control	7.14 ef	4.92 f	11.18 c	2.40 e	68.94 bc	1.02 b
	AMF	9.46 cd	7.68 bc	14.53 a	2.66 d	81.17 ab	0.87 cd
	ZnO NPs	8.26 d	5.71 g	11.06 c	3.18 ab	62.60 d	1.01 b
	AMF + ZnO NPs	9.21 cd	7.66 bc	14.43 a	3.03 bc	83.18 a	0.87 cd
Severe water stress	control	7.73 f	5.17 g	14.14 a	2.35 e	73.82 b	1.19 a
	AMF	7.68 de	5.00 gf	11.71 c	2.36 e	65.13 cd	0.98 b
	ZnO NPs	7.34 ef	6.21 cd	14.83 a	2.37 e	84.54 a	1.00 b
	AMF + ZnO NPs	7.77 de	5.48 g	13.61 ab	2.83 cd	70.53 bc	1.05 b

Note. Means in each column followed by a similar letter(s) are not significantly different at the 5% probability level; control – without fertilisers; AMF – arbuscular mycorrhizal fungi, ZnO NPs – zinc nano-oxide nanoparticles; FW – fresh weight, DW – dry weight; TPC – total fenolic content, CAT – catalase, MDA – malondialdehyde; U mg^{-1} protein – micromole per minute mg of protein.

Catalase (CAT) activity. The results showed that the application of AMF + ZnO NPs and AMF under normal irrigation and of AMF, ZnO NPs, and AMF + ZnO NPs under mild water stress significantly increased the CAT activity, compared to the control treatment. AMF + ZnO NPs application had the the greatest effect on the CAT activity under severe water stress, while the lowest CAT activity was found in all three fertilisation treatments under normal irrigation (Table 4).

Malondialdehyde (MDA) content increased with the intensification of water stress, but the foliar application of ZnO NPs under normal irrigation treated with AMF under mild water stress and AMF + ZnO NPs under severe water stress reduced the amount of this substance by 47.48% and 77.28%, respectively, compared to the control treatment (Table 4).

Root yield (RY). Water deficit decreased the RY, but all three fertilisation treatments significantly increased the RY under normal irrigation and mild water stress, compared to the untreated plants. The AMF + ZnO NPs treatment produced the highest RY (76.46 t ha^{-1}) under normal irrigation, while the lowest RY (39.62 t ha^{-1}) was obtained in the control treatment under severe water stress (Table 4).

Sugar content (SC) increased by water deficit and reached the maximum amount (19.52%) in the control treatment without fertilisation and under severe water stress. The difference between the control and AMF and ZnO NPs treatments was statistically similar. The lowest SC (14.34%) was found in the AMF treatment under normal irrigation (Table 4).

Sugar yield (SY). Although the SY was negatively affected by drought stress, the fertilisation treatments, especially AMF and AMF + ZnO NPs, significantly increased the SY at all three irrigation levels, compared to the untreated plants. The application of AMF, ZnO NPs, and AMF + ZnO NPs under normal irrigation (10.22, 10.79, and 12.30 t ha^{-1}, respectively) resulted in the highest SY, while the sugar beets grown under the control treatment and severe water stress (7.73 t ha^{-1}) produced the lowest one (Table 4).

Alkalinity (ALC). Different fertilisation treatments, especially treated with AMF, significantly increased root ALC at all three irrigation levels. The sugar beets treated with ZnO NPs and AMF + ZnO NPs under severe water stress had the highest ALC, while the lowest one was in the AMF, ZnO NPs, and the control treatments under normal irrigation (Table 4).

White sugar content (WSC). As the intensity of water deficit stress increased, the WSC rose. The sugar beets treated with ZnO NPs and AMF + ZnO NPs under severe water stress had the highest WSC (14.83% and 13.61%, respectively). The difference between these treatments and the application of AMF and AMF + ZnO NPs at mild water stress was insignificant. The lowest WSC (10.93%) was found in the control treatment and under normal irrigation (Table 4).

White sugar yield (WSY). Water deficit negatively affected the WSY and reduced it. However, the sugar beets treated with all three fertilisation treatments under normal and mild water stress and the plants treated with ZnO NPs under severe water stress produced significantly higher WSY, compared to the control treatment. The AMF + ZnO NPs treatment under normal irrigation had the highest WSY (9.06 t ha^{-1}), while the lowest one was determined in the control and AMF + ZnO NPs treatments under severe water stress (5.17 and 5.48 t ha^{-1}) and at ZnO NPs application under mild water stress (5.17 t ha^{-1}) (Table 4). Under normal irrigation, WSY increased by 39.21%, 27.08%, and 42.67% when AMF, ZnO NPs, and AMF + ZnO NPs were applied. Under mild water stress, only the AMF and AMF + ZnO NPs treatments increased WSY by 56.09% and 3663%, respectively. Under severe water stress, only the application of ZnO NPs increased WSY by 20.11%.

Coefficient of sugar extraction. The highest coefficient of sugar extraction had the inoculation with AMF under normal and mild water stress (86.47% and 83.18%, respectively) and the ZnO NPs treatment under severe water stress (84.54%). Its lowest amount was found for the untreated sugar beets under normal irrigation and at ZnO NPs application under mild water stress: 63.70% and 62.60%, respectively (Table 4).

Molasses sugar (MS) percentage increased, as water deficit stress became more severe. The AMF + ZnO NPs treatment reduced the effect of water stress on the increase in the MS percentage. The control treatment without fertilisation under severe water stress had the highest MS percentage, while the treatment with AMF and AMF + ZnO NPs under normal irrigation had the lowest one (Table 4).

Discussion

The content of photosynthetic pigments decreases due to water deficit stress. The content of photosynthetic pigments is considered an indicator of drought tolerance of plants. The reduction of photosynthetic pigments under water stress conditions is associated with the accumulation of oxygen-free radicals such as hydrogen peroxide (H_2O_2). Free radicals damage the thylakoid membrane and its peroxidation, which leads to the destruction of the chlorophyll structure. Under water stress conditions, the absorption of elements such as nitrogen, iron, and magnesium decreases. These elements play a significant role in chlorophyll biosynthesis (Armand et al., 2016).

The highest content of photosynthetic pigments was found in the AMF + ZnO NPs treatment. It has been revealed that mycorrhiza play a major role in the better uptake of water and nutrients by protecting photosystem II (PSII) during photosynthesis. The balanced water uptake regulates the photosynthesis rate by improving the transpiration rate of stomatal conductance (Yang etal., 2015). Zinc plays a significant role in the preservation and stability of the cell membrane, which improves the photosynthesis process (Ma et al., 2017). Carbonic anhydrase is a Zn-containing enzyme that is part of plant photosynthetic machinery (Qiao et al., 2014). In the study on maize, Saboor et al. (2021) reported that photosynthetic pigments responded positively to the application of arbuscular mycorrhizal fungi (AMF) and Zn.

The treatment of sugar beets with AMF + ZnO NPs under severe water stress increased the proline and TPC. The increased levels of these two substances under water deficit stress resulted in the resistance to water stress. In addition, the highest flavonoid content was found under severe water stress. Various metabolites and osmolytes, i.e., soluble sugars, proteins, and proline, are most important in the regulation of plant osmosis.

In a previous experiment (Noreen et al., 2021), the accumulation of proline under water stress conditions was detected. Increasing the antioxidant capacity of plants under water stress is a defence response to reduce the toxic effects of reactive oxygen species (ROS). It is possible that the AMF treatment can increase the plant resistance to water stress by creating an optimal balance of plant hormones (Zhang et al., 2021) and nutrient bioavailability (Kumar et al., 2018). Nanoparticles (NPs) such as ZnO NPs act as nano-fertilisers to ensure bioavailability of suitable nutrients (Dey et al., 2018). Awan et al. (2021) found that the use of microorganisms improves plant metabolism under water deficit stress by increasing compounds such as proline and phenol.

The results showed that severe water stress increased the SOD content, compared to the control treatment. Moreover, the treatment with AMF, ZnO NPs, and AMF + ZnO NPs increased the content of this enzyme, compared to the untreated plants. Fertilisation treatments, especially AMF + ZnO NPs, increased CAT activity under severe and mild water stress. The results of mean comparisons showed that the MDA content increased with the increasing water stress; however, the MDA content significantly reduced the application of ZnO NPs under normal irrigation, of AMF under mild water stress, and of AMF + ZnO NPs under severe water stress. The decrease in the MDA content under fertilisation treatments may be due to the improved activity of antioxidant enzymes and the increased detoxification of ROS elements.

Antioxidant enzymes detoxify the ROS produced under unfavourable conditions. There is a strong relationship between the antioxidant enzymes activity and plants resistance to water stress (Ahanger et al., 2021). The availability of some nutrients such as zinc, manganese, iron, and copper, which can provide the plants with biofertilisers, can play an important role in increasing the activity of antioxidant enzymes (Kumar et al., 2018). Ma et al. (2017) found that Zn enhances the transcription of genes and enzymes contributing to ROS scavenging and improves antioxidant properties. An improvement of the antioxidant defence mechanism due to the Zn foliar application under drought stress was also revealed (Sattar et al., 2022). In the study by Saboor et al. (2021), mycorrhizal inoculated plants showed a significantly higher enzymatic activity at each Zn treatment, compared to the untreated plants.

The MDA content is an estimate of the extent of oxidative stress damage to cell membranes. The increase in the MDA content under drought stress is related to an increase in the amount of ROS, such as H_2O_2 and superoxide radicals (O^{2-}) (Ghassemi et al., 2018). An increase in the MDA content and a decrease in cell membrane stability under water deficit stress was observed by Shanazari et al. (2018).

El-Gizawy et al. (2014) revealed that the biofertiliser vermicompost tea (VCT) reduced H_2O_2 production and MDA content in sugar beet leaves by improving the plant defence mechanism. Biofertilisers such as mycorrhiza reduce MDA production and stabilise the cell membrane of treated plants (Mamnabi et al., 2020).

Although in this study, under the intensification of water deficit stress, the sugar content and white sugar content increased, the percentage of sugar extraction decreased. The treatment without the fertilisation (control) under severe water stress had the highest sugar and white sugar contents; however, under the mild water stress, the application of AMF and AMF + ZnO NPs increased the sugar content, white sugar content, and sugar extraction, compared to the control treatment. The sugar and white sugar contents increased under water deficit stress, but the application of ZnO NPs and AMF + ZnO NPs under severe water stress increased only the white sugar content and did not significantly affect the sugar content.

In this study, the highest root yield, sugar yield, and white sugar yield were obtained under AMF + ZnO NPs treatment and normal irrigation. The application of AMF + ZnO NPs increased all these traits under mild water stress, while under severe water stress, increased the sugar yield, while only ZnO NPs increased the white sugar yield, compared to the control treatment.

The nutrient deficiency is another consequence of plant water deficit. AMF hyphae likely expand their surface area and moisture absorption regions of the host plant roots and thus increase water absorption by roots (Gong et al., 2013). Zulfiqar et al. (2019) found that the application of nanoparticle-based fertilisers increased the plant tolerance to abiotic stress. They also concluded that combining nanoparticles with microorganisms can synergistically increase crop production under water deficit stress. A remarkable effect of mycorrhiza is the development of plant roots (El-Gizawy et al., 2014). Biofertilisers significantly improve the growth and accumulation of dry matter, resulting in the increased root sucrose content and decreased impurity parameters. This is the reason for the positive response of sugar content to the biofertiliser application (El-Gizawy et al., 2014).

The combination of AMF + ZnO NPs effectively improved the fresh weight, dry weight, and plant length by retaining more water in cells and thus reducing drought stress (Amjad et al., 2021). In the study of Zewail et al. (2020), the high amount of Zn and molybdenum (Mo) foliar application resulted in the highest sugar content, white sucrose content, sugar yield, and root yield. In another study (Barłóg et al., 2016), the highest biomass, sugar content, and white sugar content were obtained after the application of Zn fertiliser. This phenomenon can be confirmed by the increase of indole-3-acetic-acid and endogenic gibberellin content in the plants treated with Zn (Barker, Eaton, 2015).

In this study, the AMF + ZnO NPs treatment under all three irrigation levels decreased the percentage of molasses sugar and increased the percentage of sugar extraction by reducing root impurities such as Na, K, and alpha-amino N. It can be concluded that the access to sufficient water and nutrients increased the sugar beet growth period, as a result of which elements such as Na, K, and N (which are components of root impurities) are consumed in cell metabolism, and the percentage of root impurities decreases.

It was found that the foliar application of Zn can improve the quality of taproots by increasing the sugar content and decreasing root impurities (Gobarah et al., 2014). In another study (Piskin, 2017), the application of 5 kg ha^{-1} Zn improved the sugar beet quantitative and qualitative characteristics such as root yield, sugar content, white sugar content, sugar yield, and white sugar compared to those of the control.

Conclusion

The effect of foliar application of zinc nano-oxide nanoparticles (ZnO NPs) with arbuscular mycorrhizal fungi (AMF) on sugar beets under water stress was studied. Previous studies focused on macro fertilisers and conventional irrigation.

In this study, the white sugar yield increased the treating with AMF + ZnO NPs under normal and mild water stress and applying of ZnO NPs under severe water stress. This treatment improved the production of photosynthetic pigments, enhanced the protection against water stress by increasing the activity of antioxidant enzymes, the total phenolic content (TPC) and flavonoids as well as compounds such as sugar, and reduced the negative effects of water deficit on the white sugar yield and increased the value of this trait, compared to the control treatment. Thus, the application of AMF + ZnO NPs under normal irrigation and mild water stress and the application of ZnO NPs under severe water stress may be a solution to improve economic yield in the areas where sugar beet experiences different periods of water deficit of varying intensity.

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