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Effect of Urban Wastewater on Soybean Response and Soil CO_2 Emission under Drought Stress Conditions

Saeid Shiukhy Soqanloo*[1], Bahareh Shamgani Mashhadi[1], Saba Ebarhimzadeh[2]

[1]*Department of Water Engineering, Faculty of Agricultural Engineering, Sari Agricultural Sciences and Natural Resources University, Sari, Iran*

[2]*Department of Horticultural Science and Engineering, Faculty of Agricultural Sciences, Sari Agricultural sciences and Natural Resources University, Sari, Iran*

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ABSTRACT

Global warming caused by greenhouse gas (GHG) emissions and the frequency of droughts has made it inevitable to face the challenge of the water resource crisis. The present study was carried out utilizing a split-plot design based on a randomized complete block design with three replications during the growth season of 2022-2023 at Sari University Agricultural Sciences and Natural Resources, Iran. The experimental treatments consisted of drought stress as the main plots, categorized into three levels: non-stress (Field Capacity, FC100), moderate (FC75), and severe stress (FC50). Irrigation management practices were implemented as subplots: Urban Wastewater (UWW) and Well Water (WW). The soil texture of the farm was sandy-loam. Based on the results, the highest CO_2 emission was observed at 60, 75, 90, and 105 days after planting (DAP) in the FC100 treatment, by 535.9, 534.5, 543.6, and 444.5 ppm.min^{-1}, respectively. Also, the highest CO_2 emission was allocated to UWW compared to WW by 536.5, 540.1, 538, and 516.9 ppm.min^{-1}, respectively. The Catalase (CAT) and Peroxidase (POD) enzyme activities, Malondialdehyde (MDA) and proline contents were the highest in FC50 and significantly increased compared to FC75 and FC100. The effect of drought stress on yield and yield components showed that in FC100, a noticeable advantage was observed over FC75 and FC50 conditions. Also, irrigation with UWW compared to WW partially compensated the adverse effects of drought stress.

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1. Introduction

Soybean (*Glycine max* L.) is regarded as a major global oilseed crop, providing approximately 18–22% oil and 35–40% protein, and holds the largest cultivated area among oilseed species worldwide (Shiukhy Soqanloo and Shamgani Mashhadi, 2025; FAO, 1992). Owing to its extensive genetic diversity and broad ecological adaptability, soybean is grown across a wide range of latitudes. Although the average soybean yield in Iran (2.3 t·ha^{-1}) is slightly higher than the global mean (2.1 t·ha^{-1}), more than 90% of the country's demand is still supplied through imports (Majidian *et al*., 2024). In Iran, about 110,000 ha are planted with soybean annually, producing approximately 260,000 tons. In Mazandaran province, the cultivated area includes 87.8 ha under irrigated conditions and 121.7 ha in dryland systems, resulting in yields of 122.3 and 157.2 t·ha^{-1}, respectively (Kiani, 2019).

Increasing concentrations of GHGs and ongoing climate change have substantially disrupted the stability of the Earth's climate system. Global warming is widely recognized as the most significant consequence of these alterations during the 21st century (Buckles, 2018). By the end of the century, temperature increases are projected to exceed 5.4°C under the most pessimistic emission scenarios and approximately 1.1°C under the most optimistic ones (Ghazi *et al*., 2023). Over recent decades, rising emissions of GHGs—particularly CO_2—from soils into the atmosphere have been identified as major drivers of

* Corresponding author.
E-mail addresses: saeid.shiukhy@gmail.com; S.shiukhy@sanru.ac.ir

global warming. Soils contribute nearly 15% of total global GHG emissions, with CO_2 accounting for about 74% of this share. Human activities, including agricultural practices and land-use changes, have intensified these emissions and further accelerated climate change (Kopittke *et al*., 2024).

Findings from numerous studies indicate that CO_2 is the dominant greenhouse gas in soils, with emission levels roughly one hundred times higher than those of nitrogen- and methane-based gases (Chen *et al*., 2024). Iyke-Ofoedu et al. (2024) examined the relationship between carbon sequestration and CO_2 emissions from agricultural activities in South Africa. Their analysis revealed that a one-unit rise in CO_2 emissions results in a 0.5% reduction in carbon sequestration capacity.

Seasonal climatic fluctuations combined with broader climate change trends exert substantial influence on agricultural productivity. Shifts in temperature can disrupt crop performance by moving conditions away from the optimal thresholds required for efficient photosynthesis and plant development. Plants rely on a well-defined temperature range to maximize photosynthetic processes and sustain healthy growth. When temperatures fall outside these optimal limits, key physiological functions become impaired, ultimately lowering production efficiency (Scafaro *et al*., 2023). For instance, extreme heat events can decrease cereal yields by 9–10%, underscoring the high sensitivity of crops to thermal variations (Knight *et al*., 2023). Similarly, Singh et al. (2024) reported that each one-unit increase in the global average temperature leads to an estimated 1.3% decline in soybean yield.

Water resource management has become increasingly challenging over the past two decades due to recurring droughts and the growing competition for water between agricultural and domestic sectors. At the same time, environmental stresses—particularly drought intensified by climate change—have placed additional pressure on agricultural systems, highlighting the need for accurate evaluation and effective management strategies (Sardar Shahraki and Ghasemi, 2023).

Plants exhibit a broad spectrum of responses to drought stress, encompassing morphological, physiological, and biochemical alterations. Moreover, the application of hydrogen peroxide (H_2O_2) has been shown to influence plant responses under such conditions (Zali *et al*., 2020). According to Ma et al. (2020), despite the complex nature of plant reactions to drought, several factors—including the duration and intensity of stress, plant species, age, size, and genotype—play crucial roles in determining how plants cope with water deficit.

Soybean is highly sensitive to drought stress, which triggers a variety of adaptive morphological, physiological, and biochemical mechanisms essential for maintaining growth and productivity under limited water availability. Under drought conditions, soybean plants typically exhibit enhanced antioxidant enzyme activity that mitigates oxidative damage (Xu *et al*., 2023). Additionally, drought stress leads to the accumulation of protective metabolites such as proline and flavonoids, which contribute to osmotic adjustment and stress tolerance. These conditions can also stimulate increases in chlorophyll content and overall plant vigor, particularly in drought-tolerant genotypes (Basal *et al*., 2024).

Inefficient management practices related to the unsustainable extraction of groundwater and inadequate recharge have led to a severe water crisis in Iran, making the use of unconventional water resources increasingly necessary (Noori *et al*., 2023). Utilizing wastewater not only helps conserve freshwater supplies and enhance soil fertility but also reduces dependence on chemical fertilizers and substantially lowers irrigation expenses (Esteve *et al*., 2024). The nutrient content present in wastewater, acting as a soluble fertilizer, ensures adequate nutrient availability for plants and contributes significantly to improving soil fertility (Śniatała *et al*., 2024). Swain et al. (2020) reported that the application of sewage sludge increased plant height and improved leaf greenness in spinach compared with the control. However, wastewater can also act as a major pollutant of rivers and natural environments, posing serious ecological risks; therefore, continuous monitoring of its contamination levels is crucial.

Given Iran's climatic characteristics—with extensive arid and semi-arid regions—the scarcity of freshwater resources and the growing water crisis are unavoidable challenges (Bashara and Qaderi, 2024). Consequently, the adoption of unconventional water resources is highly important. Furthermore, making effective management decisions and developing strategies for reducing future CO_2 emissions in the agricultural sector require an understanding of how

different irrigation water sources influence GHG emissions, particularly CO_2. Therefore, the aim of this study was to assess soil CO_2 emissions under drought stress conditions and irrigation with urban wastewater in soybean cultivation.

2. Materials and methods

2.1. Experimental site

This research was carried out at Sari University of Agricultural Sciences and Natural Resources (SANRU), Mazandaran, Iran. It has a latitude (36° 33′ N) and longitude (53° 00′ E), an elevation (14 m above sea level), an average temperature (17.9°C), and an average total rainfall (650 mm) during the growth season 2022-2023 Fig. 1.

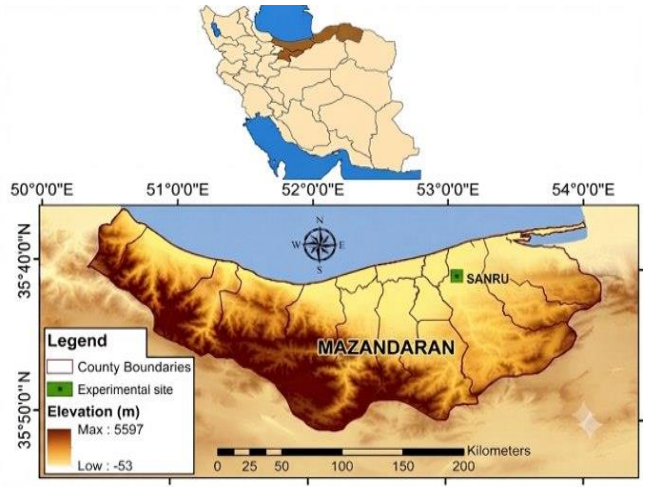


Figure 1. The geographical location of the study area

This region has a humid climate, which is classified based on the De Martonne climate classification system that includes long-term precipitation and temperature data (Shamgani Mashhadi *et al*., 2024). Other information related to the weather conditions of the experimental site during the growth season of the soybean was presented in Table 1.

Table 1. The weather conditions of the experimental site (Iran Meteorological Organization data portal).

Month	T.min	T.max	T.average	P*	E**
	(ºC)			(mm)	
June	23.4	33.6	27.4	22.1	159.9
July	28.7	37.8	32.7	14.8	178.2
August	22.8	35.1	28.6	2.9	205.8
September	19.3	30.1	23.9	36.1	135.8
October	14.4	26.1	19.5	145	67
Average	21.7	32.5	26.4	220.9	746.7

P* and E**: Total monthly Precipitation and evaporation, respectively. https://data.irimo.ir

The present study was conducted utilizing a split-plot design following a randomized complete block design with three replications during the growth season of 2022-2023 at Sari Agricultural Sciences and Natural Resources University (SANRU). The experimental treatments consisted of drought stress conditions as the main plots, categorized into three levels: non-stress (control - FC100), moderate stress (Field Capacity 75% - FC75), and severe stress (Field Capacity 50% - FC50). Soil water content (SWC) was monitored during the growing season using a tensiometer, and plants were re-watered when the SWC dropped to 80 % of soil field capacity. In addition, irrigation management practices were implemented as subplots, encompassing two levels: Urban Wastewater (UWW) and Well Water (WW). The treated wastewater necessary for irrigation was sourced from the Alivak treatment plant. To assess the physical and chemical characteristics of the wastewater, well water, and soil, samples were collected and sent to laboratories specializing in water and soil quality analysis. The findings are summarized in Table 2.

Table 2. Physico-chemical characteristics of soil, source of irrigation and maximum permissible limits for wastewater application in agriculture

Characteristics	pH	NTU	TDS	TSS	EC	TP	COD	N	Ca	Na	Mg
			(mg L^{-1})		(ds m^{-1})	(mg L^{-1})					
UWW	8.1	2.9	663	27	1.06	4.9	17	0.5	100	58	18.1
WW	7.2	9.9	629.3	16	0.78	2.2	11.1	0.1	60	69	16.1
IEPO, 2021	6-8.5	50	-	100	-	6	200	-	-	-	100
WHO, 2006	6-8.5	-	450	-	0.7	4	-	-	-	-	-
FAO, 2006	6-8.5	-	450	-	0.7	4	-	-	-	-	-

Soil	Depth	Texture	pH	EC (ds m^{-1})	OC (%)	P	K	N (%)
						(mg kg^{-1})		
	0-30 cm	Sand-loam	7.6	0.55	0.52	4	146	0.1

NTU: Nephelometric Turbidity unit; TDS: Total Dissolved Solids; TSS: Total Suspended Solids; EC: Electrical Conductivity; TP: Total Phosphorus; COD: Chemical oxygen demand; N: Nitrogen; Ca: Calcium; Na: Sodium; and Mg: Magnesium.

William's soybean seeds were manually sown on June 13 in experimental plots arranged in a 2×3 layout, each consisting of four rows. Rows were spaced 10 cm apart, with 30 cm between adjacent rows, and a 2 m buffer zone was maintained between blocks. Irrigation was applied immediately after sowing. Drought stress treatments were imposed once the plants developed 6–8 leaves, following each irrigation cycle. To prevent rainfall from affecting the experimental conditions, a rain shelter was installed over the plots. Surface irrigation was used throughout the study, and soil water content (SWC) was monitored during the growing season using a tensiometer. Plants were re-watered whenever the SWC decreased to 80% of field capacity.

Prior to planting, the soil was amended with necessary fertilizers according to field soil analysis results. Additional fertilization during the growth period was performed using a 20-20-20 NPK solution (containing 6.5% nitrate nitrogen, 0.4% ammonium nitrogen, 4.10% urea, 0.20% phosphate as P_2O_5, and 0.20% potassium as K_2O) supplemented with micronutrients including manganese (0.03%), boron (0.02%), and molybdenum (0.001%) in the form of EDTA.

2.2. Soil CO_2 emission measurement

To quantify soil carbon dioxide (CO_2) emissions, stationary cylindrical chambers (10 cm in diameter and 30 cm in height) were positioned between crop rows in each plot, with a soil insertion depth of 20 cm. Measurements were conducted at multiple intervals—15, 30, 45, 60, 75, 90, and 105 days after planting (DAP)—continuing until the end of the growth period. CO_2 concentrations were recorded using a CO_2-port-Messwert company GmbH-Gottingen device based on the infrared method (Fig. 2). To minimize potential errors caused by daily temperature fluctuations, all measurements were performed in the morning between 8:00 and 11:00 (Shimizu *et al*., 2009).



Figure 2. The soil CO_2 emission measurement during the growth season.

2.3. Yield and yield components

To evaluate the yield and its components, five plants were randomly selected from the two central rows of each plot and taken to the laboratory for measurement of the specified traits. The plant traits studied included plant height, number of pods per plant and number of seeds per pod. The 100- seeds weight, yield and total dry matter measurements were performed by weighing the samples (Digital scale with an accuracy of 0.01 g) (Yadav *et al*., 2024).

2.4. The antioxidant enzyme activity, MDA and proline contents

Catalase (CAT) activity was determined by monitoring the decline in absorbance of H_2O_2 at 240 nm. Measurements were performed immediately after preparation of the enzyme extract. The reaction mixture (3 ml total volume) consisted of 2950 µl of H_2O_2 solution prepared using 2800 µl of 50 mM sodium–potassium phosphate buffer (pH 7) and 150 µl of H_2O_2 adjusted to an initial absorbance of 0.5, along with 50 µl of filtered enzyme extract. The reaction began upon adding the extract to the H_2O_2 solution. The reduction in absorbance at 240 nm was recorded over two minutes at 20-second intervals. Catalase activity was calculated based on the difference between the initial and final absorbance values.

Guaiacol peroxidase (POD) activity was quantified using a reaction mixture containing 2700 µl of 25 mM potassium phosphate buffer, 100 µl of 5 mM guaiacol, 100 µl of enzyme extract, and 100 µl of H_2O_2 solution, reaching a final volume of 3 ml. Formation of tetraguaiacol was monitored by measuring the increase in absorbance at 470 nm for two minutes at 30-second intervals (Bates *et al*., 1973).

For malondialdehyde (MDA) determination, 100 µl of TBA solution was added to 500 µl of each sample's supernatant, after which the mixture was incubated in a Bain-Marie at 95 °C for 30 minutes. Samples were then rapidly cooled in ice for 30 minutes and centrifuged at 10,000 rpm for 10 minutes. Absorbance was measured at 532 nm and 600 nm, and MDA concentration was calculated using the difference between these absorbance values along with an extinction coefficient of 1155 $mM^{-1}cm^{-1}$.

Proline concentration was assessed following the method of Chen et al. (2023). A 0.5 g leaf sample was homogenized with 10 ml of 3% sulfosalicylic acid and

kept on ice. The homogenate was centrifuged at 15,000 rpm for 10–15 minutes at 4°C. Two milliliters of the supernatant were mixed with 2 mL ninhydrin reagent and 2 mL glacial acetic acid, then incubated in a hot water bath at 100 °C and subsequently cooled on ice for one hour. Following the addition of 4 ml toluene and vigorous shaking for 20 seconds, the absorbance of the upper organic phase was recorded at 520 nm (Chen *et al*., 2023). Finally, the data obtained were analyzed using ANOVA in SAS ver 9.2 software and the Duncan post hoc test was employed to compare treatment means.

3. Results and discussion

3.1. Soil CO_2 emission

The results of the analysis of variance of the effects of drought stress and irrigation with urban wastewater on soil CO_2 emission are presented in Table 3. Based on the results, in the initial stages of sampling, the soil CO_2 emission was not affected by drought stress and irrigation with urban wastewater, but it was significant in the subsequent stages of sampling. Therefore, the effect of drought stress and irrigation with urban wastewater on the rate of soil CO_2 emission was significant ($P \leq 0.01$). The findings also indicated that the interaction effect of drought stress and irrigation with urban wastewater on the rate of soil CO_2 emission was not significant. Based on the results, the effect of drought stress in the early stages of growth and development (15, 30, and 45 DAP) did not have a significant difference on the rate of soil CO_2 emission, although this was expected. In fact, in the first stage of sampling (15 DAP), very small seedlings appeared whose root system had not yet developed in the soil and, due to the weak structure of the seedlings, drought stress had not been applied. However, after passing the first stage and entering the second and third stages of sampling (30 and 45 DAP), with the increase in the growth of aerial organs and the development of the root system in the soil, the rate of soil CO_2 emission also increased. While no significant difference was observed between the treatments. At 60, 75, and 90 DAP, the trend of increasing soil CO_2 emission continued. In the fourth sampling stage (60 DAP), the highest soil CO_2 emission rate was observed at 535.9 ppm min^{-1} under non-stress conditions, and the lowest rate was observed under FC50 by 505.3 ppm min^{-1}. Also, in the fifth and sixth sampling stages (75 and 90 DAP), the highest soil CO_2 emission was observed in non-stressed conditions (FC100) by 534.5 ppm min^{-1} and 543.6 ppm min^{-1}, respectively. While the lowest rate was observed in FC50 by 499.8 and 506.5 ppm min^{-1}, respectively. In the seventh sampling stage (105 DAP), the trend of soil carbon dioxide emission was decreasing. In this stage, the highest CO_2 emission rate was observed at 444.5 ppm min^{-1} under non-stress conditions and the lowest rate was observed under FC50 by 409.8 ppm.min^{-1} Fig. 3.

Table 3. The results of variance analysis of drought stress and irrigation management effects on the soil Co_2 emissions during the soybean growth season

S.O.V	df	Mean of square						
		15	30	45	60	75	90	105
Block (B)	2	0.537^{ns}	11.9858^{ns}	2.5477^{ns}	61.7751^{ns}	5.9461^{ns}	3.5379^{ns}	0.1954^{ns}
Drought Stress (Ds)	2	5.4726^{ns}	3.8841^{ns}	3.2963^{ns}	2513.723^{**}	5125.838^{**}	6153.835^{**}	2293.0198^{**}
Block × Ds	4	0.8967^{ns}	7.4867^{ns}	0.3520^{ns}	23.2844^{ns}	1.4245^{ns}	7.4768^{ns}	7.417^{ns}
Source of Irrigation (SI)	1	1.736^{ns}	1.8561^{ns}	0.2713^{ns}	1926.549^{**}	742.5373^{**}	404.5116^{**}	163.142^{**}
Ds × SI	2	0.5706^{ns}	0.3520^{ns}	0.3001^{ns}	6.733^{ns}	3.9183^{ns}	21.1613^{ns}	7.2496^{ns}
Error	6	4.1058	2.9934	1.3771	12.5569	16.67533	1.7795	7.4619
Total	17	-	-	-	-	-	-	-
C.V. (%)	-	1.5	0.4	0.2	0.9	1.7	0.9	0.6

* Sampling based on the day after planting (DAP); ** and ^{ns}: They indicate significance at 1% and non-significance levels, respectively.

Also, the effect of irrigation with urban wastewater showed similar results as drought stress. As can be seen in Fig. 4, in the early stages of growth and development, irrigation with wastewater and well water did not have a significant difference in the rate of soil CO_2 emission. Gradually, as the plant growth and development stages passed and irrigation continued, in the fourth to seventh sampling stages, irrigation with urban wastewater increased the rate of soil CO_2 emission. In these stages, the highest rate of soil CO_2 emission was observed in urban wastewater irrigation conditions by 536.5, 540.1, 538.0 and 516.9 ppm min^{-1}, respectively. While the lowest rate of soil CO_2 emission was related to well water irrigation conditions by 515.8, 527.3, 528.5 and 510.9 ppm min^{-1}, respectively Fig. 4.

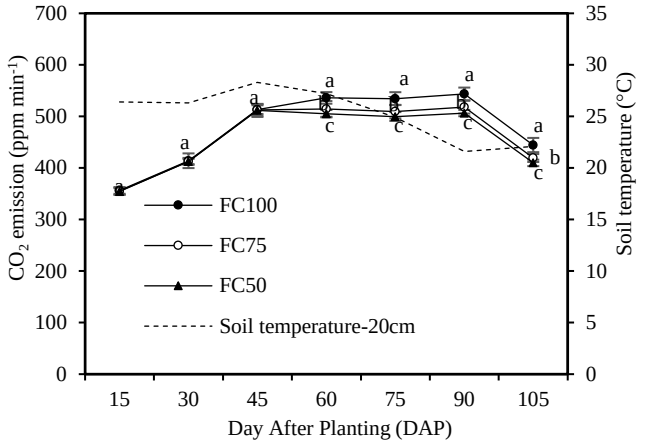


Figure 3. The effect of water stress on the soil CO_2 emission during the soybean growth season. In each of the sampling stages, the same letters indicate non-significant at the 0.01% level (LSD≤0.01)

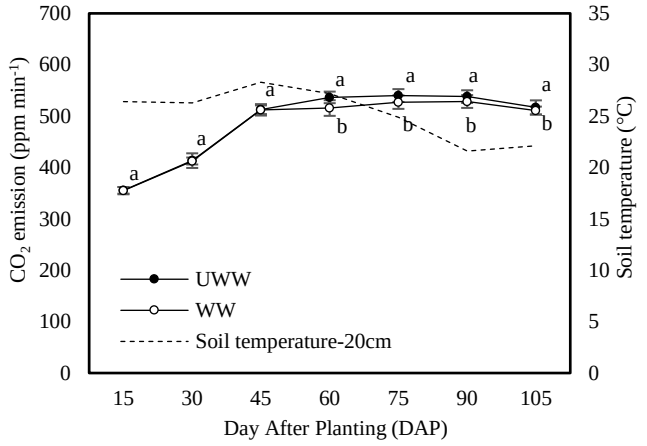


Figure 4. The effect of irrigation management on the soil CO_2 emission during the soybean growth season. In each of the sampling stages, the same letters indicate the non-significant at the 0.01% level (LSD≤0.01).

As shown in Figs. 3 and 4, during the early sampling stages (15–45 DAP), soybean plants were in their initial growth phase, characterized by a relatively small root system and low biomass. At this stage, soil temperature was higher than in later stages, and soil respiration was primarily driven by microbial activity rather than root respiration. Consequently, CO_2 emission rates from the soil were lower in the early stages compared to the middle and late growth phases. As the plants developed and approached seed maturity, CO_2 emission rates increased in response to rising temperatures, root system expansion, enhanced root respiration, and elevated microbial activity. Favorable soil moisture conditions, particularly under FC100 and FC75 treatments with urban wastewater irrigation, further stimulated soil CO_2 emissions. Compared with well water, urban wastewater enhanced microbial activity and supported plant growth, thereby increasing CO_2 release. Under FC100 and FC75, soil pores were largely saturated with water, disrupting the air–water balance and promoting higher CO_2 efflux.

At 105 DAP, corresponding to seed maturity and the harvest stage, plant growth and root development had largely ceased, reducing root respiration. At this stage, soil CO_2 emissions were primarily influenced by microbial activity and soil temperature. Overall, CO_2 emission rates during the final growth stages were lower than those observed in the middle stages, but still higher than in the early stages.

Soil CO_2 emission and carbon mineralization are strongly regulated by soil temperature and moisture through their effects on microbial activity. These factors interact to control microbial processes that govern carbon dynamics, thereby influencing carbon release and storage. La Scala et al. (2006) reported a strong positive correlation between soil temperature and CO_2 emissions, noting higher emissions under summer and elevated temperature conditions. Conversely, low temperatures reduced both root respiration and microbial activity, resulting in decreased soil respiration. Soil nutrients, such as nitrogen and phosphorus, also modulate microbial activity; variations in nutrient availability can increase or suppress microbial-driven CO_2 release. Farooq et al. (2017) similarly indicated that higher temperatures and adequate soil moisture enhance root respiration, subsequently elevating soil respiration and CO_2 emissions. The findings of previous studies align with the results of this research, demonstrating that CO_2 emission rates were higher under favorable conditions, including irrigation with urban wastewater and non-stressed (FC100) or moderately stressed (FC75) treatments, compared with well-water irrigation and severe drought stress (FC50).

3.2. Yield and yield components

The results of the analysis of variance of the effects of drought stress and irrigation with urban wastewater on yield and yield components are presented in Table 2. Based on the findings, the effects of drought stress and irrigation with urban wastewater on plant height, number of pods, number of seeds per pod, 100-seed weight, total dry matter and yield were significant (P≤0.01). The findings also indicated that the interaction of drought stress and irrigation with urban wastewater on the studied traits was not significant Table 4.

Table 4. The result of analysis of variance the drought stress and irrigation with urban wastewater affects on yield and yield components of soybean during growth season

S.O.V	df	PH	NP	NSP	100-SW	TDM	Yield
Block (B)	2	13.389^{ns}	4.695^{ns}	2.75^{ns}	0.1088^{ns}	350.8^{ns}	4020.93^{ns}
Drought Stress (Ds)	2	766.723**	8453.46**	3620.9**	3.0838**	83273**	886894.12**
Block × Ds	4	4.89^{ns}	6.433^{ns}	3.37^{ns}	0.1363^{ns}	258.5^{ns}	3981.763^{ns}
Source of Irrigation (SI)	1	34.723**	69.62**	16.69**	3.2088*	1830.1**	69552.06**
Ds × SI	2	7.722^{ns}	6.5116^{ns}	21.02^{ns}	0.44^{ns}	110.92^{ns}	644.276^{ns}
Error	6	3.0556	2.0277	42.50	0.2716	154.96	4472.667
Total	17	-	-	-	-	-	-
C.V. (%)	-	1.6	1.3	1.8	2.8	1.9	10.8

PH: Plant height; NP: Number of Pods per plant; NSP: Number of seeds per pod; 100-SW: 100-Seed weight; TDM: Total dry matter. **, * and ^{ns}: They indicate significance at 1%, 5% and non-significance levels, respectively.

The highest plant height was observed in FC100 at 119 cm. The lowest was related to severe drought stress (FC50) at 96.5 cm. Also, in irrigation with urban wastewater (UWW), the plant height was significantly higher by 109.7 cm compared to irrigation with well water (WW) by 107 cm (Table 5). In fact, the results showed that irrigation with urban wastewater, by providing the plant's nutritional needs, partially compensated for some of the adverse effects of drought stress by expanding the plant's aerial organs and canopy area. The results showed that the highest number of pods and number of seeds per pod were observed in FC100 by 304.8 and 665.1, respectively, and the lowest was observed in FC50 by 173.1 and 351.5, respectively. There was no significant difference in the 100-seed weight in the FC100 and FC75 treatments (18.7 g and 18.2 g, respectively), but a significant difference was observed compared to FC50 (17.3 g). Also, the highest dry matter production of 743.3 kg ha^{-1} was related to FC100 and the lowest occurred in FC50 by 509.8 kg ha^{-1}. The results indicated that the yield under FC75 (419.4 kg ha^{-1}) and FC50 (372 kg ha^{-1}) was reduced compared to FC100 (1060.4 kg ha^{-1}) by 60% and 65%, respectively Table 5.

As can be seen in Table 5, soybean yield and yield components under urban wastewater irrigation showed more favorable results than well water. Thus, under urban wastewater irrigation conditions, the highest number of pods per plant, number of seeds per pod, 100-seed weight, total dry matter and seed yield were observed to be 234.5, 244.1, 18.5 g, 645.7 kg ha^{-1} and 679.5 kg ha^{-1}, respectively.

Table 5. Effect of drought stress and irrigation with urban wastewater on yield and yield components during soybean growth season

Factor	Treatments	PH (cm)	NP	NSP	100-SW (g)	TDM (kg ha^{-1})	Yield (kg ha^{-1})
Drought stress	FC100	119^{a}	304.8^{a}	665.1^{a}	18.7^{a}	743.3^{a}	1060.4^{a}
	FC75	109^{b}	201.8^{b}	452.1^{b}	18.2^{a}	653.7^{b}	419.4^{b}
	FC50	96.5^{c}	173.10^{c}	351.5^{c}	17.3^{b}	509.8^{c}	372^{b}
Source of irrigation	UWW	109.7^{a}	234.5^{a}	244.1^{a}	18.5^{a}	645.7^{a}	679.5^{a}
	WW	107^{b}	218.6^{b}	236.8^{b}	17.7^{b}	625.5^{b}	541.2^{b}

Means with the same letter within each row indicate no significant difference (Duncan, P≤0.01).

Drought stress exerts substantial negative effects on soybean growth and yield by reducing leaf photosynthetic capacity, accelerating leaf senescence, and inducing oxidative stress, thereby limiting the production of photosynthetic assimilates (Farooq *et al*., 2017). This stress decreases the biomass of stems, roots, and seeds, ultimately reducing seed weight due to lower allocation of biomass to reproductive organs. Additionally, drought diminishes the partitioning of dry matter between soybean seeds and pod walls. Omidi et al. (2021) reported that grain yield declines under drought conditions, with reductions in dry matter accumulation being proportional to the severity of stress. Along with decreases in other yield components, this leads to a significant decline in overall grain yield. The pod formation stage is particularly sensitive to drought; the number of pods formed—and consequently pod weight—responds strongly to both the intensity and duration of water deficit, making it a critical stage for yield determination. Urban wastewater, rich in nutrients, can improve the growth of plant organs depending on its quality by supplying essential elements directly to the plant, ultimately enhancing yield. Its use also improves soil physical

properties and nitrogen availability, providing favorable conditions for increased soybean productivity. By supplying additional nutrients, irrigation with wastewater supports the plant's photosynthetic system, thereby boosting overall crop yield (Mahfooz *et al*., 2020).

3.3. The antioxidant enzyme activity, MDA and proline contents

The results of the analysis of variance of the physiological response of soybean to drought stress and irrigation with urban wastewater are presented in Table 6. Based on the findings, the effect of drought stress on membrane lipid peroxidation (MDA content), osmotic regulation (proline content), and antioxidant enzyme activity (POD and CAT) was significant (P≤0.01). The results also indicated that the effect of irrigation with urban wastewater and the interaction of drought stress and irrigation with urban wastewater on the physiological response of soybean was not significant.

Based on the results, membrane lipid peroxidation had an increasing trend from non-stressed conditions (FC100) to moderate drought stress (FC75) and severe drought stress (FC50). The highest MDA content was observed in FC50, at 2.8 nmol g FW min^{-1}. While the lowest content was observed in FC75 and FC100, at 2.4 and 2.2 nmol g FW min^{-1}, respectively. As can be seen in Fig. 3, the MDA content did not differ significantly between FC75 and FC100. The trend of changes in osmotic regulation was also increasing. The lowest proline content was observed in FC50 and FC75 by 0.03 and 0.04 µg g^{-1}, respectively. While the highest proline content was observed in FC50 by 0.06 µg g^{-1} Fig. 5. The result showed that with increasing drought stress levels, the activity of antioxidant enzymes increased. In this way, the activity of the CAT enzyme in FC100 by 0.79 U g^{-1} FW min^{-1} decreased by 48% compared to moderate drought stress (FC75) and significantly decreased by 115% under severe drought stress (FC50). In fact, as stress levels increased, CAT enzyme activity increased significantly. The findings also showed that the POD enzyme activity also increased under FC75 and FC50 compared to FC100, but it was less intense than that of CAT enzyme activity. Thus, the POD enzyme activity in FC75 and FC100 by 2.38 and 2.33 U g^{-1} FW min^{-1}, respectively, did not differ significantly from each other, but in FC50 by 2.93 U g^{-1} FW min^{-1} showed a significant increase compared to FC75 and FC100 Fig. 5.

Table 6. The result of analysis of variance the drought stress and irrigation with urban wastewater affects on physiological characteristics of soybean at during growth season

S.O.V	df	MDA	Proline	POD	CAT
Block (B)	2	0.035^{ns}	0.00007^{ns}	0.0650^{ns}	0.0037^{ns}
Drought Stress (Ds)	2	0.485^{*}	0.0016^{**}	0.6650^{**}	1.2484^{**}
Block × Ds	4	0.0400^{ns}	0.0001^{ns}	0.0175^{ns}	0.0226^{ns}
Source of Irrigation (SI)	1	0.0005^{ns}	0.00001^{ns}	0.00055^{ns}	0.0151^{ns}
Ds × SI	2	0.0372^{ns}	0.000016^{ns}	0.0072^{ns}	0.0105^{ns}
Error	6	0.0683	0.00027	0.0533	0.0170
Total	17	-	-	-	-
C.V. (%)	-	10.5	11	9.1	10.6

*, * and ns: They indicate significance at 1%, 5% and non-significance levels, respectively.

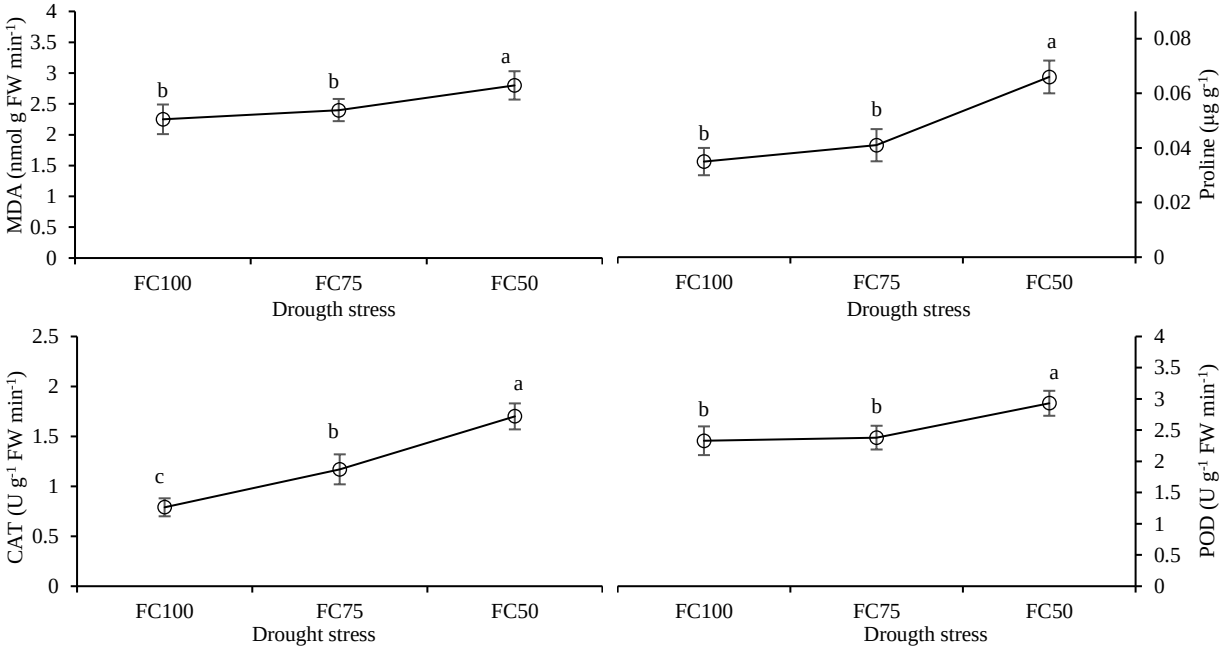


Figure 5. Evaluation of drought stress effects on soybean physiological response at during growth season

Under prolonged water limitation, plants undergo morphological adjustments to mitigate the adverse effects of drought stress (Wang *et al*., 2022a). Malondialdehyde (MDA) content generally increases under stress and is commonly used as a marker of membrane lipid peroxidation, reflecting the plant's capacity to respond to unfavorable conditions such as drought (Morales and Munné-Bosch, 2024). Studies have shown that MDA levels in maize and soybean rise with increasing drought intensity and duration. Wang et al. (2022b) reported that higher drought severity gradually increased membrane permeability and MDA content, while POD and CAT activities initially rose and then declined, likely due to variations in planting methods, cultivars, and environmental conditions. Plants also reduce water loss through transpiration by decreasing leaf area under drought stress. Fahim et al. (2023) demonstrated that MDA content significantly increased in most of the six grape cultivars studied as drought stress intensified. Drought stress can disrupt cell membrane structure, induce membrane damage, increase permeability, and lead to the accumulation of reactive radicals, thereby impairing normal cellular and physiological functions in soybean (Wang *et al*., 2022a).

Antioxidant activity generally rises in response to drought, as reported by Esmaeeli et al. (2024). Wang et al. (2022b) observed that SOD, POD, and CAT activities in soybean leaves initially increased under stress but decreased over time. Osmotically active compounds such as proline help maintain ion concentrations and reduce cell permeability, supporting normal growth under stress. Wang et al. (2014) reported that proline levels significantly increased in soybean under drought, with drought-tolerant cultivars showing higher accumulation than sensitive ones. Similarly, Zhang et al. (2020) found that drought stress elevated proline and soluble sugar levels in maize leaves, with greater stress intensity leading to higher concentrations. Li et al. (2021) also noted that drought can substantially affect root growth and related physiological processes, ultimately impacting soybean yield. The findings of the present study are in agreement with these previous reports.

4. Conclusion

Given the prevalence of drought stress, uneven rainfall distribution during the soybean growth period, and limited water availability in the study area, assessing plant responses to water deficit and evaluating the potential of urban wastewater irrigation to mitigate drought effects has become a research priority. The results indicated that soil CO_2 emission rates were strongly influenced by plant growth and development stages, particularly root system expansion. From the early to late growth stages, as the underground and aerial organs developed and root respiration increased, soil CO_2 emission exhibited a rising trend. Moderate and severe drought stress negatively affected soybean yield and its components. In contrast, irrigation with urban wastewater, rich in nutrients and improving soil conditions, was able to partially offset the detrimental effects of drought compared with well water irrigation.

Soybean leaves mitigated oxidative stress by accumulating osmotically active compounds, enhancing antioxidant enzyme activities, and regulating membrane lipid peroxidation, thereby maintaining cell membrane stability and minimizing drought-induced damage. Overall, these findings highlight the critical need for research on drought resistance in soybean, including advanced predictive tools, early-warning systems, and efficient irrigation management, to ensure sustainable production in drought-prone areas. Implementing wastewater irrigation can improve soil physical properties and fertility, increasing yield while reducing reliance on chemical fertilizers. Consequently, treated urban wastewater represents a promising approach for achieving sustainable crop production and mitigating the environmental impacts associated with excessive fertilizer use.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

Saeid Shiukhy Soqanloo: analysis and interpretation of data, statistical analysis, critical revision of the manuscript for important intellectual content, supervision; Bahareh Shamgani Mashhadi: drafting of the manuscript, analysis and interpretation of data; Saba Ebarhimzadeh: acquisition of data, drafting of the manuscript.

Informed consent

The authors declare not to use any patients in this research.

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Use of artificial intelligence tools

Some sentences in this manuscript were revised with the assistance of artificial intelligence, using language models to improve clarity and readability. All intellectual content, interpretations, and conclusions are solely the responsibility of the authors.

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