Effect of seed priming and spraying with melatonin on growth and yield characteristics of soybean (*Glycine max* L.Meer.) grown under salt stress

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Abstract

To mitigate soil salinity resulting from irrigation with well water containing high salt content, which negatively impacts the growth and productivity of soybean crops, a field experiment was conducted at the Soil and Water Resources Research Station of the College of Agriculture, Tikrit University, during 2025 growing season as a factorial experiment with a split-plot arrangement randomized complete block design with three replications. Two types of irrigation water were used: river water (Tigris River) and well water, which represented the first factor (main plots), while the second factor (sub plots) represented melatonin combinations (soaking + spraying). Traits of relative growth rate (RGR), crop growth rate (CGR), net assimilation rate (NAR), leaf chlorophyll content, protein content, and grain yield were studied. The results showed that all studied traits were affected by the irrigation water type. Plants irrigated with well water were more affected by the salinity and exhibited lower values than plants irrigated with river water. Melatonin stimulates plants to tolerate the accumulated salinity in the soil due to irrigation with saline water and mitigates its effects on soybean plants. Treatment of melatonin T8 [100 (soaking) + 200 (spraying) µmol] irrigated with river water was the superior treatment in all studied traits and showed the highest values, reaching 0.1027, 0.0736, and 0.0450 g g^{-1} day^{-1} of RGR, 0.00182, 0.00315, and 0.00643 g cm^{-2} day^{-1} of CGR, and 0.00677, 0.00621, and 0.00395 g cm^{-2} day^{-1}

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of NAR, for measurement periods of 30-60, 60-90, and 90-120 days after planting, respectively. Chlorophyll content, protein content, and grain yield gave the values of 39.522 SPAD,37.826%, and 20.0867 tons ha^{-1}, respectively. Melatonin plays a key role in promoting the growth and productivity of soybean crops, as well as mitigating the effects of salinity on soybean yield.

Keywords: soybean, salt stress, melatonin, irrigation water type, growth rate, chlorophyll content.

Introduction:

Soybean (*Glycine max* (L.)) is an important legume crop used in many industries due to its high oil and protein content [1,2]. The protein content of the seeds ranges from 30-50%, while the oil content 14-24% [3]. Soybean plays a significant role in improving soil properties, as the bacterial nodules present in its roots play an important role in soil fertility by fixing atmospheric nitrogen [4,5]. Abiotic factors have a direct and serious impact on crops and their productivity. Among these factors is salinity, which is considered one of the important factors affecting crop growth and productivity [6]. The harmful effects of salt stress lie in its impact on osmotic pressure, causing it to rise, which inhibits water absorption through the roots and increases the absorption of toxic sodium (Na^+) and chloride (Cl^-) ions by the plant cells [7,8]. Salinity also causes other damage to many crops, including soybean. It leads to a decrease in osmotic potential, specific ion toxicity, ionic disturbance, impaired plant nutrition, and delayed senescence [9,10]. It is estimated that about 25% of arable land is affected by salinity, and these lands lose approximately 5.1 million hectares of productivity due to salinity [11,12]. Plants vary in their ability to adapt to different environmental conditions, such as salinity, and soybean is considered a salinity-sensitive crop [13,14], as salinity negatively affects soybean by delaying plant growth and development [15,16].Plant growth regulators are organic compounds, natural or synthetic, that affect physiological processes within plants at low concentrations. They can be used to stimulate, inhibit, or modify these physiological processes. Growth regulators are used as stimulants to break seed dormancy, break apical dominance, or slow senescence of buds, flowers, or leaves, thereby increasing vegetative growth and leaf area, and consequently increasing grain yield, generally, plant growth regulators improve crop productivity and increase tolerance to salt and drought stresses [17,18]. Among these regulators is melatonin. Melatonin is a powerful antioxidant and contributes to several physiological processes within the plant, such as growth, photosynthetic efficiency, and root development. It also affects leaf senescence, fruit ripening, regulating stomatal opening and closing, and maintaining oxidative balance [19]. Melatonin plays a crucial role in legumes, mitigating the effects of salinity by reducing radicals oxygen species (ROS), electrolyte permeability, and membrane damage, while simultaneously increasing chlorophyll content, plant height, and antioxidant enzymes [20,21]. Melatonin also plays a significant role in enhancing plant tolerance to salt stress through biological processes at the molecular and chemical levels during various stages of plant growth [22]. Due to the significant impact of salinity on the growth and productivity of soybean crop, the aim of this study was to evaluate the feasibility of reducing the impact of salt stress resulting from irrigation water on the growth and yield of soybean by seed priming and foliar spraying with different concentrations of melatonin, determining the optimal concentration, and identifying the best combination to improve soybean growth and productivity.

Materials and Methods:

A field experiment was carried out at the research station of the Department of Soil Science and Water Resources of the College of Agriculture at Tikrit University during 2025 summer season in gypsum soil (Table 1) to evaluate the effect of melatonin on some growth and yield characteristics of soybean crop under the influence of salinity. Experiment land was plowed twice perpendicularly using a disc plow to loosen the topsoil without turning the soil over. It was then divided into three replicates, each replicate containing two main plots (irrigation water treatments), and each main plot was divided into nine subplots (combinations of melatonin soaking and spraying treatments). The area of each experimental unit was 9 m² (3 x 3 m), and the number of experimental units was 54. The experimental land was fertilized with nitrogen fertilizer at a rate of 120 kg N ha^{-1} of urea fertilizer (46% N) in two stages: half was added before planting, and

the other half was added when the plant reached the fourth leaf stage

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(V4). The experimental land was also fertilized with triple superphosphate fertilizer (P_2O_5 46%) at a rate of 80 kg of P_2O_5 ha^{-1} and potassium sulfate fertilizer (K_2O 48%) at a rate of 120 kg K_2O ha^{-1} as a single application before planting. Soybean seeds were sown at a rate of 3-4 seeds per hole at a depth of 5 cm. The variety "Shaimaa" was used, and sowing took place on May 15, 2025, with rows spaced 0.75 m apart and holes spaced 0.10 m apart. Crop management practices included thinning the plants to a single plant when they reached the third trifoliolate stage (V3). Manual hoeing was carried out twice during the growing season, and drip irrigation was used, utilizing both river and well water (Table 1). Irrigation was carried out according to the plant's needs during the growing season. At the end of the season, the electrical conductivity and pH of the soil were measured to assess the cumulative effect of salts in the irrigation water on the soil. This was done by taking three samples from different experimental sites (Table 2).

Table 1: Physical and chemical qualities of the soil and irrigation water used in the experiment.

Soil traits	Value	Measurement units
pH	$^{+}7.01$	
EC meter^{-1}	2.57	Desi Sims
CEC soil $^{-1}$	9	Senti mol kg
O.M	9.1	g Kg^{-1}
$CaCO_3$	117	g Kg^{-1}
$CaSO_4.2H_2O$	55.92	g Kg^{-1}
Soil Texture	Sandy Clay Loam	
Clay	541	g Kg^{-1}
Loam	284	
Sand	163	
Positive and negative dissolved ions in the soil		
Ca^{++}	17.70	mm L^{-1}
Mg^{++}	13.02	

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Na^{+}	6.74	
K+	0.453	
Cl^{-1}	2.97	
HCO_3^{-1}	5.11	
CO_3^{-2}	Nil	
pH and electrical conductivity of the two types of irrigation water		
Well water	pH	6.50
	EC	2.73
River water	pH	7.13
	EC	0.44
*Soil and water analyses were conducted at the Department of Soil Science and Water resources at the Agriculture College, Tikrit University		

Table 2: Electrical conductivity of the experiment soil at the end of the season

The Samples	The EC
The soil irrigated with well water	3.98
The soil irrigated with river water	2.11

Experimental Design:

The experiment was conducted as a factorial experiment with a split-plot arrangement randomized complete block design (RCBD), with three replications. The main plots represent the irrigation water type parameters, while the subplots represent the melatonin soaking and spraying parameters.

Experimental Factors: Two factors were used in the experiment:

• Irrigation Water Type: Two types of irrigation water were used: the first was water from the Tigris River, and the second was water from a well within the station. To control the irrigation water, the main plots were separated in two directions, one for each type, to prevent the irrigation water from mixing.

• Soaking and Spraying with the Growth Regulator: The seeds were soaked in three levels of melatonin (0, 50, and 100 µmol) for 12 hrs then air dried in the dark.

The leaves were sprayed with three levels of melatonin (0, 100, and 200 µmol) at two intervals: the first was

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when the plant reached the third trifoliolate stage (V3), and the second was at the beginning of pod formation (R3). According to the following combinations:

T0 - Control treatment (soaking and spraying with distilled water)
T1 – Spraying with 100 µmol
T2 – Spraying with 200 µmol
T3 - Soaking with 50 µmol
T4 - Soaking 50 + spraying 100 µmol
T5 - Soaking 50 + spraying 200 µmol
T6 - Soaking 100 µM
T7 - Soaking 100 + spraying 100 µmol
T8 - Soaking 100 + spraying 200 µmol

Characteristics Studied:

Growth Analysis:

A growth analysis was conducted on the vegetative and reproductive growth phases to assess the growth characteristics of soybean under gypsum soil conditions. Three plants were taken from the middle rows of each experimental plot every 30 days across four stages. Growth analysis was calculated using leaf area and dry weight at 30, 60, 90, and 120 days after planting.

• **Leaf Area (cm^2):** Leaf area was estimated using the gravimetric method [23].

• **Dry Weight (g $plant^{-1}$):** Plants were air-dried until a constant weight was reached, and then all plants were weighed to determine the average dry weight of the plant.

Relative Growth Rate (g g^{-1} day^{-1}): was measured according to the following equation [24]:

RGR = (Lnw2 - Lnw1) / (t2 - t1)

where: RGR = relative growth rate, Lnw2 = natural logarithm of dry weight at the subsequent stage (g), Lnw1 = natural logarithm of dry weight at the previous stage (g), T1 = first measurement period (day), T2 = second measurement period (day).

Crop Growth Rate (g cm^{-2} day^{-1}): The crop growth rate was calculated using the following equation [24]:

CGR = 1/GA * (w2 - w1) / (t2 - t1)

where: CGR = crop growth rate, GA = area occupied by the plant, W2 = dry weight at the subsequent stage (g), W1 = dry weight at the previous stage (g), T1 = first measurement period (day), T2 = second measurement period (day).

Net Assimilation Rate (g cm^{-2} day^{-1}):

It was calculated from the following equation [24]:

NAR = (w2-w1)/(T2-T1)*(LNLA2-LNLA1)/(LA2-LA1)

where: NAR = net photosynthesis, W2 = dry weight at the subsequent stage (g), W1 = dry weight at the previous stage (g), T1 = first measurement period (day), T2 = second measurement period (day), LNLA2 = natural logarithm of leaf area at the subsequent period (cm^2), LNLA1 = natural logarithm of leaf area at the previous period (cm^2), LA2

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= leaf area at the subsequent period (cm²), LA1 = leaf area at the previous period (cm²).

Chlorophyll content:

When the plants reached 50 days of age, the chlorophyll content of the leaves was measured using a SPAD device. Five plants were selected from the midlines, and three readings was taken for each plant.

Protein percentage (%):

The protein percentage was measured according to the method of C. C. A.A. (1998) using a Perten Inframatic Protein Analyzers device, which uses Near Infrared Reflection (NIR) [25].

Grain Yield (tons h^{-1}):

At the end of the season, 10 plants were harvested from each experimental unit. The yield per plant was calculated and then multiplied by the plant density per unit area, thus converting the area to hectares. **Statistical analysis:** The data were statistically analyzed as a factorial experiment according to the randomized complete block design (RCBD) with the split plot arrangement using SAS software [26]. Differences between means were compared using Duncan's multi-range test at a probability level of 0.05.

Results and Discussion:

The analysis of variance (Table 3) shows that irrigation water type exhibited highly significant differences ($P < 0.0001$) in all studied traits. This indicates the significant impact of irrigation water type on soybean growth and yield characteristics. Well water with high salinity negatively affected soybean plant growth traits, resulting in lower values for these traits compared to plants irrigated with river water. The results also showed highly significant differences ($P < 0.0001$) among melatonin concentration in all studied traits of soybean. This high significance indicates the role of melatonin in mitigating the effects of soil salinity (Table 2) resulting from irrigation with well water that has a high salt concentration (Table 1). However, the interaction between irrigation water type and melatonin combinations was not significant for any of the studied traits.

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Table 3: Analysis of variance of two types of water irrigation (river, well), nine combinations of melatonin (soaking + spraying) for studied traits of Shaimaa soybean variety.

S.O.V	Df	30-60 day			60-90 day			90-120 day			Chlorophyll content	Protein content	Total Plant yield
		RGR	CGR	NAR	RGR	CGR	NAR	RGR	CGR	NAR			
Block	2	0.00001$^{n.s}$	4.8217$^{n.s}$	0.0000019*	0.000013$^{n.s}$	0.0000005$^{n.s}$	0.00000001$^{n.s}$	0.000009$^{n.s}$	0.0000005$^{n.s}$	0.0000013$^{n.s}$	16.6467$^{n.s}$	2.806^{ns}	0.22$^{n.s}$
A	1	0.00272**	2.7691**	0.000019**	0.001423**	0.000018**	0.00000719**	0.000619**	0.0000248**	0.0000070**	635.764**	68.38**	141.97**
Ea	2	0.00008	1.1035	0.000003	0.000013	0.0000013	0.00000029	0.000069	0.0000015	0.0000016	36.4844	2.630	1.44
B	8	0.00070**	1.1866**	0.000004**	0.001130**	0.0000043**	0.00000820**	0.000473**	0.0000074**	0.0000024**	62.2847**	33.80**	33.77**
AB	8	0.00002$^{n.s}$	6.0494$^{n.s}$	0.0000002$^{n.s}$	0.000045$^{n.s}$	0.0000004$^{n.s}$	0.00000105$^{n.s}$	0.000005$^{n.s}$	0.0000001$^{n.s}$	0.0000001$^{n.s}$	5.4121$^{n.s}$	0.44^{ns}	1.26$^{n.s}$
Eb	32	0.00003	0.00000003	0.0000004	0.000034	0.0000002	0.00000033	0.000045	0.0000008	0.0000003	12.0803	1.95	1.21
total	53												

*And**there were significant differences at the 0.05 and 0.001 levels, respectively.
n.s. No significant differences were found.

Growth Analysis: Analyzing soybean plant growth rates requires a thorough understanding of many factors, such as relative growth rate (RGR), crop growth rate (CRG), and net assimilation rate (NAR). These factors play a crucial role in assessing soybean performance under different growing conditions and are essential for developing and improving cultivation methods. Therefore, conducting growth analysis is a critical step in studying soybean growth during an experiment.

Relative Growth Rate (g g^{-1} day^{-1}):

The relative growth rate (RGR) measures the increase in biomass relative to the total plant mass over a period of time and affects the amount of dry matter produced by the plant during that period. It indicates the plant's efficiency in utilizing available resources (water and nutrients) to promote growth. It has been found

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that RGR of soybean under normal conditions is high in the early stages of growth, while growth slows in the later stages. Figure 1A shows that irrigation water type had a highly significant effect on RGR of soybean during the measurement periods of 30-60, 60-90, and 90-120 days after planting. The initial growth period was characterized by high growth rates, but the accumulation of salts due to well water irrigation hindered plant growth. The salts present in the well water (Table 1) had a clear and significant impact on plant growth. Salt accumulation in the soil impeded water and nutrient absorption by the plant roots, negatively affecting RGR of the crop. Plants irrigated with river water exhibited a higher RGR throughout their life cycle compared to those irrigated with well water. These results are consistent with[27]. Melatonin also had a significant effect on RGR of soybean. Plants treated with melatonin exhibited a higher RGR during the measurement periods compared to plants not treated with melatonin. Melatonin plays an important role in the physiological processes within the plant, as it increases the plant's tolerance to various environmental stresses, including salinity. It is also important in increasing plant growth and productivity, whether under natural conditions or under various stresses [28].

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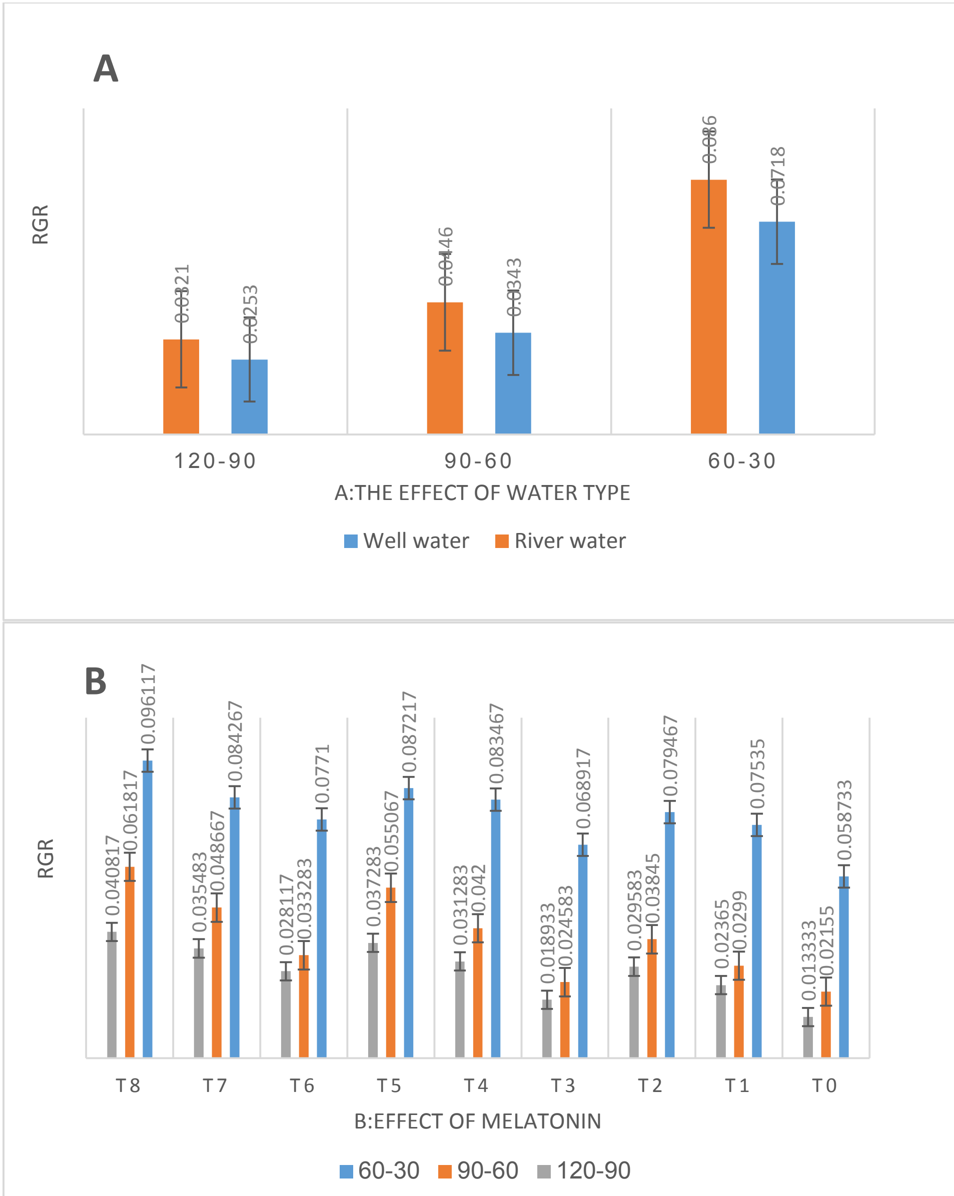

Figure 1: (A) Effect of irrigation water type, (B) Effect of melatonin, on the relative growth rate of soybean

The RGR was affected by the irrigation water type, and its effect was highly significant for the 30-60 and 60-90 days after planting measurement periods. River water had a greater effect than well water, exceeding it by 0.0860 and 0.044, respectively (Appendices 1, 2, and 3). However, well water had a lesser effect than the river water for the same period, reaching 0.0718 and 0.0343, respectively. In the 90-120 days measurement period, the effects of river and well water were was

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similar, at 0.0321 and 0.0253, respectively. Melatonin had a clear effect on RGR, with treatment T8 outperforming all other treatments in all measurement periods (30-60, 60-90, and 90-120 days after planting), reaching 0.0961, 0.0618, and 0.0408, respectively. This demonstrates the effect of melatonin on RGR and its ability to make plants more tolerant to environmental stresses, including salinity. Melatonin is now being used by researchers to mitigate the effects of various stresses, as it promotes plant growth at different stages [29]. Regarding the interaction of melatonin combinations with irrigation water type, the effect was significant. Treatment T8 with river water was superior to all other treatments for both river and well water, across all measurement periods (30-60, 60-90, and 90-120 days), reaching 0.012, 0.0736, and 0.0540, respectively. The control treatment, T0, showed the lowest interaction percentage between melatonin and river water across all measurement periods, reaching 0.0707, 0.0261, and 0.0156, respectively. In well water, which had a clear effect on RGR, melatonin interaction demonstrated a clear reduction in its impact on RGR of soybean crops. Treatment T8 was the most effective treatment compared to all other well water treatments across all three measurement phases (30-60, 60-90, and 90-120 days after planting), with values of 0.0718, 0.0500, and 0.0366, respectively. The data (Appendices 1, 2, and 3) indicate that the salinity in the well water (Table 2) led to salt accumulation in the soil, negatively impacting RGR during the plant growth phases. The control treatment T0 in well water had the lowest values across all measurement phases (30-60, 60-90, and 90-120 days), with values of 0.0467, 0.0169, and 0.0110, respectively.

Crop Growth Rate (g cm^{-2} d^{-1}):

Crop growth rate (CGR) expresses the increase in crop biomass per unit area over a specific time period. The CGR is a great importance parameter for estimating plant productivity in agricultural fields. It is a key metric for understanding how efficiently plants utilize available resources to maximize their growth. Analysis of variance (Table 3) shows that irrigation water has a highly significant effect on crop growth rate. Plants irrigated with river water exhibited higher CGRs than those irrigated with well water for the measurement periods of 30-60, 60-90, and 90-120 days after planting (Figure 2A). High soil salinity (Table 2), resulting from irrigation with highly saline water, affects crop growth rates, including soybean, which are a salt-sensitive crop. The use of melatonin affected CGR, with

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melatonin-treated plants exhibiting higher growth rates than untreated plants at all measurement intervals: 30-60, 60-90, and 90-120 days after planting (Figure 2B). Melatonin is considered a compound that supports plant tolerance to various environmental stresses [15, 16, 17].

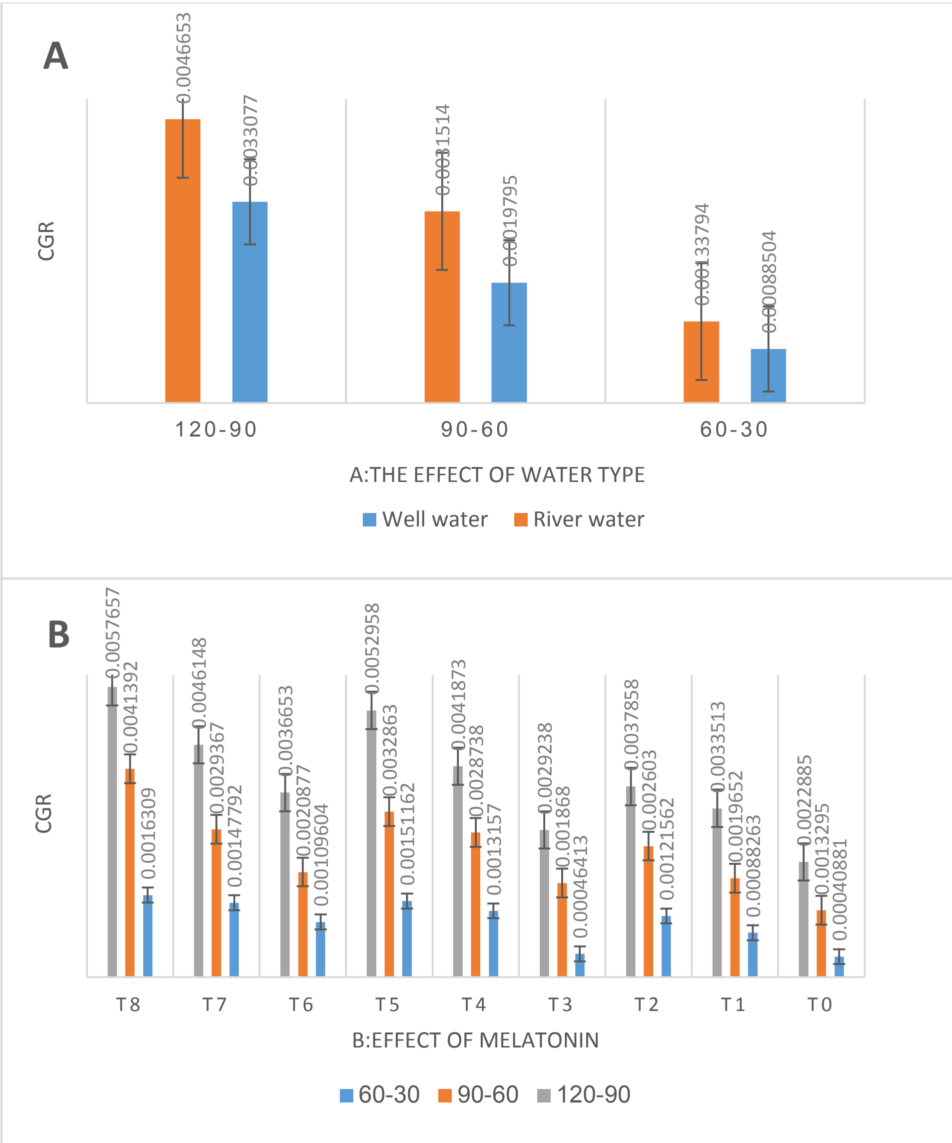

Figure 2: (a) Effect of irrigation water type, (b) Effect of melatonin, on the crop growth rate of soybean

Appendices 4, 5, and 6 show that irrigation water type significantly affected soybean growth rates at all measurement periods (30-60, 60-90, and 90-120) days after planting. Plants irrigated with river water

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exhibited higher growth rates than those irrigated with well water, indicating that increased salinity negatively impacts plant growth, hindering germination and development. Appendices 4, 5, and 6 also demonstrate that melatonin significantly impacted soybean growth rates, with a marked increase in yield at all measurement periods (30-60, 60-90, and 90-120) days after planting, recording the highest values of 0.00163, 0.00413, and 0.00576, respectively. Through Appendices (4, 5, 6), it was shown that the interaction of melatonin with the type of irrigation water was influential on the CGR at all measurement stages: 30-60, 60-90, and 90-120 days after planting. Melatonin played an important role in enhancing the plant tolerance to soil salinity (Table 2) resulting from irrigation with well water containing a high percentage of salts (Table 1). Treatment T8 was the superior treatment and gave the highest value among all treatments irrigated with river water in all measurement stages 30-60, 60-90 and 90-120 days after planting, and its values reached 0.00182, 0.00529 and 0.00643, respectively. The values showed that CGR gradually increased during growing season. This means that melatonin regulated processes within the plant, which led to increased growth. As for the control treatment T0 in river water, it had the lowest value among the river water treatments in all growth measurement stages 30-60, 60-90 and 90-120, and its values over the growing season reached 0.000461, 0.00149 and 0.00260, respectively. As for the plants irrigated with well water, CGR was lower than that of the plants irrigated with river water due to soil salinity (Table 2). Treatment T8 gave the highest value among the plants irrigated with well water in all measurement stages 30-60, 60-90 and 90-120 days after planting, where its value reached 0.00143, 0.0298 and 0.00509, respectively. It is noted that salinity affected the growth rate, but melatonin enhanced the plant tolerance to salinity and reduced its effect on plant growth. As for the T0 control treatment, it gave the lowest value among all plants irrigated with well and river water for all measurement stages 30-60, 60-90 and 90-120 days after planting, where its value reached 0.000355, 0.00116 and 0.00196, respectively. Despite the high salinity, melatonin contributed to plant tolerance and increased growth rate.

Net Assimilation Rate (g cm^{-2} d^{-1}):

Net assimilation rate (NAR) is used to estimate the rate of photosynthesis per unit leaf area and is a measure of a crop's efficiency in converting solar energy into chemical energy used by the plant for growth. NAR is an

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important parameter that contributes to plant growth and development. It is expressed as the difference between the amount of light energy absorbed by the plant and the amount of energy used in respiration. The results showed that the type of irrigation water clearly affected NAR, as plants irrigated with river water outperformed plants irrigated with well water in the measurement periods of 30-60, 60-90, and 90-120 days after planting (Figure 3A). It is noted from the results that the accumulation of salinity in the soil irrigated with well water, which contains a high percentage of salts, led to a decrease in the NAR in the period 90-120 days after planting compared to the first measurement periods of 30-60 and 60-90 days after planting. The salinity affected crop growth, as the accumulation of salts hindered the absorption of elements and nutrients from the soil by the roots, thus causing damage to the plant. The use of melatonin during the growth stages led to an increased plant tolerance to soil salinity, as the NAR gradually increased during the measurement stages of 30-60, 60-90 and 90-120 days after planting, with the period of 30-60 days after planting outperformed subsequent growth stages. This means that melatonin plays an important role in the crop tolerance to salinity (Figure 3B).

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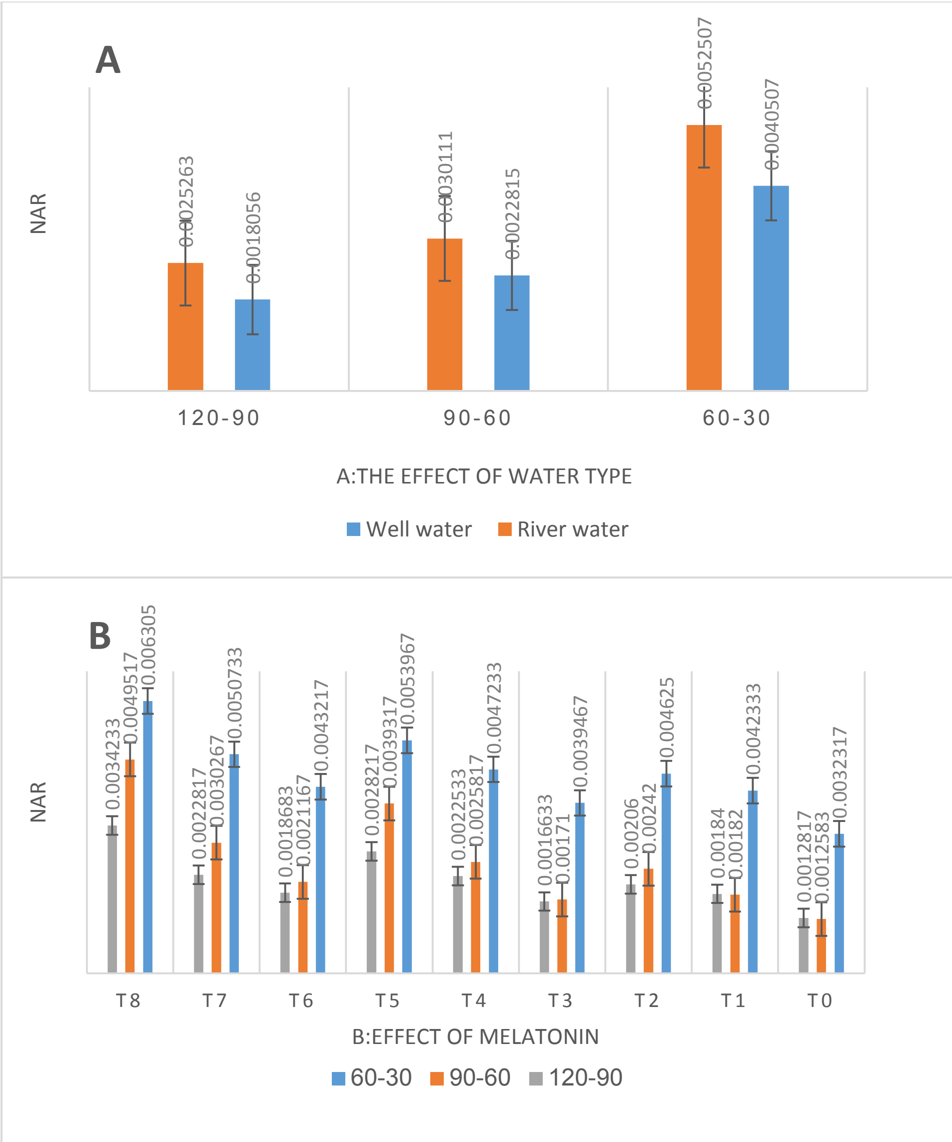

Figure 3: (a) Effect of irrigation water type, (b) Effect of melatonin, on net assimilation rate of soybean

From Appendices (7, 8, 9), it was observed that the type of irrigation water significantly affected NAR. Plants irrigated with river water outperformed those irrigated with well water, thus impacting NAR. The effect of irrigation water type was significant on soybean plants at all measurement periods: 30-60, 60-90, and 90-120 days after planting. Melatonin also had a significant effect, plants treated with melatonin exhibiting higher growth than untreated plants during the measurement periods of 30-60, 60-90, and 90-120 days after planting.

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Melatonin treatment T8 gave the highest values during all measurement periods, which were 0.00630, 0.00495, and 0.00342, respectively. The interaction of melatonin with irrigation water type showed a clear effect on NAR. Plants irrigated with river water and treated with melatonin exhibited the highest NAR at all measurement stages. Treatment T8 was the superior treatment compared to all other treatments irrigated with river or well water at all measurement stages (30-60, 60-90, and 90-120 days after planting), with values of 0.00677, 0.00621, and 0.00395, respectively. Although melatonin was used to mitigate the effects of salinity, this did not preclude its negative impact on crop growth. The control treatment T0 gave the lowest values among the river-irrigated treatments at all measurement stages (30-60, 60-90, and 90-120 days after planting), with values of 0.00343, 0.00133, and 0.00162, respectively. Using well water for irrigation negatively impacted overall crop growth and net photosynthesis in particular. Excessive soil salinity hindered solar absorption due to its effect on the plant's leaf area, thus reducing light interception. Treatment T8 exhibited the highest values among all well-water irrigated treatments in the measurement periods 30-60, 60-90, and 90-120 days after planting, reaching 0.00583, 0.00369, and 0.00289, respectively. The control treatment, T0, gave the lowest values among all river or well-water irrigated treatments in all measurement periods 30-60, 60-90, and 90-120 days after planting, reaching 0.00302, 0.00118, and 0.000936, respectively. The effect of soil salinity on NAR was observed during crop growth, but the use of melatonin mitigated this effect.

Chlorophyll content (SPAD):

The amount of solar energy absorbed by a plant is an indicator of leaf chlorophyll content [30]. Thus, chlorophyll content is linked to crop productivity and photosynthesis [31]. Therefore, analyzing changes in chlorophyll content helps confirm the plant's response to various stress factors [32,33]. Chlorophyll is an important compound in plants that converts absorbed light energy into chemical energy that the plant uses in its vital processes. Irrigation water type is important in determining a plant's ability to absorb solar radiation, as salinity negatively affects leaf chlorophyll content (Table 1). Table 4 shows that irrigation water type significantly affected leaf chlorophyll content; plants irrigated with well water were more affected than those irrigated with river water. Plants irrigated with river water showed higher chlorophyll content than those irrigated with well water. This indicates that the chlorophyll content in soybean leaves is affected by soil salinity (Table 2) resulting from irrigation with well water containing a high concentration of salts (Table 1). Table 4 shows that melatonin had a significant effect on the chlorophyll content of soybean leaves. Plants treated with melatonin had higher chlorophyll content than untreated plants, melatonin treatment T8 gave the highest value, reaching 37.015, while the control treatment T0 gave the lowest value 26.814. The interaction between melatonin and irrigation water type was also significant, as plants irrigated with river water and treated with melatonin showed higher chlorophyll content than plants irrigated with well water, due to the effect of accumulated salinity in the soil resulting from irrigation with highly saline water. Treatments T8, T7, and T5 gave the highest chlorophyll content among all treatments irrigated with river and well water, reaching 39.522, 38.516, and 39.216, respectively. Treatment T0 had the lowest chlorophyll content among river-irrigated plants, at 28.178. Well water negatively impacted leaf chlorophyll content, resulting in lower levels compared to river-irrigated plants. Treatment T8 showed the highest chlorophyll content among well-irrigated plants, at 34.507, while treatment T0 had the lowest value among all well and river-irrigated treatments, at 25.450. This is attributed to salt accumulation resulting from irrigation with well water, which has a high salt content. The superiority of T8 treatment in RGR, CGR , and NAR traits (Figures 1, 2, and 3) led to a positive effect of this treatment on the chlorophyll content of the treated plants.

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Table 4: Effect of irrigation water type and melatonin treatment on chlorophyll content (SPAD) of soybean leaves

Melatonin	Irrigation water type		Mean
	Well	River	
T0	25.450 e	28.178 dec	26.814 e
T1	26.784 de	33.292 b-c	30.038 dec
T2	27.555 de	36.451 ba	32.003 bdc
T3	25.713 e	33.123 b-c	29.418 de
T4	28.564 dec	36.630 ba	32.597 b-c
T5	31.790 b-c	39.216 a	35.503 ba
T6	26.876 de	34.558 bac	30.717 dec
T7	30.487 b-c	38.516 a	34.501 bac
T8	34.507 bac	39.522 a	37.015 a
Mean	28.636 a	35.499 a	
*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.			

Protein percentage (%):

Table 5 shows that irrigation water type significantly affected protein percentage, plants irrigated with river water gave a higher protein percentage than those irrigated with well water. Table 5 also shows that melatonin significantly affected protein percentage, treatment T8 of melatonin gave the highest value reaching 36.610%, while control treatment T0 gave the lowest value 29.325%, confirming the significant effect of melatonin in increasing the protein percentage in soybean seeds. The interaction between irrigation water type and melatonin was significant, as melatonin helped the plants to tolerate the salinity present in the well water used for irrigation. Plants irrigated with river water and supplemented with melatonin had the highest protein percentage among the other treatments. Treatment T8 had the highest protein percentage among all treatments irrigated with river and well water, reaching 37.827%, while control treatment T0 had the lowest

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protein percentage among the river-irrigated plants, at 30.780%. Well water had an effect on reducing the protein percentage of soybean seeds due to the salinity present in the water (Table 1), which led to the accumulation of salts in the soil, resulting in a decrease in the protein percentage compared to other plants. Treatment T8 had the highest protein percentage among the treatments irrigated with well water, reaching 35.393%, while the control treatment T0 irrigated with well water had the lowest percentage among all treatments irrigated with well and river water, reaching 27.870%.

Table 5: Effect of irrigation water type and melatonin treatment on protein content (%) of soybean seeds

Melatonin	Irrigation water type		Mean
	Well	River	
T0	27.870 i	30.780 hg	29.3250 f
T1	30.740 hg	33.270 f-g	32.0050 de
T2	32.647 feg	33.990 b-c	33.3183 dc
T3	29.693 ih	32.643 feg	31.1683 e
T4	33.697 f-c	35.413 b-c	34.5550 bc
T5	34.613 b-c	36.627 ba	35.6200 ba
T6	31.117 fhg	33.573 fde	32.3450 de
T7	34.413 b-c	36.317 bac	35.3650 ba
T8	35.393 b-c	37.827 a	36.6100 a
Mean	32.2426 b	34.4933 a	
*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.			

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Grain yield (ton ha^{-1}):

Table 6 shows that irrigation water type significantly affected total seed yield, with plants irrigated with river water outperforming those irrigated with well water in grain yield. Melatonin also had a significant effect on grain yield, plants treated with melatonin showing a higher yield compared to plants not treated with melatonin. Treatment T8 of melatonin gave the highest grain yield, which was 18.270, while control treatment T0 had the lowest value 10.640. These results indicate that melatonin helped increase the grain yield of soybean. As for the interaction of melatonin with the type of irrigation water, its effect was significant. Melatonin helped to reduce the effects of salinity accumulated in the soil as a result of irrigation with well water, which contains a high percentage of salts that hinder physiological processes within the plant. Plants irrigated with river water had a higher grain yield than those irrigated with well water. Treatment T8 had the highest value among all treatments irrigated with river and well water, reaching 20.068, while the control treatment T0 had the lowest value

among all treatments irrigated with river water, reaching 12.426. As for the plants that were irrigated with well water, they had a lower grain yield than those irrigated

with river water as a result of the accumulation of salts in the soil. Treatment T8 gave the highest value of grain yield among the plants irrigated with well water, reaching a value of 16.453, while the control treatment T0 had the lowest value among all plants irrigated with well and river water due to salinity, reaching 8.853. Treating soybean plants with melatonin resulted a significant increase of the RGR (Appendix 1, 2, and 3), CGR (Appendix 4, 5, and 6), and NAR (Appendix 7, 8, and 9). This resulted to increase chlorophyll content (Table 4). All of these led to a significant increase of grain yield. These results confirm the role of melatonin in mitigating the negative effects of salts, particularly (Na) and Cl ions, through complex physiological and biochemical mechanisms. Seed priming and spraying with melatonin enhances plant tolerance to salinity because it acts as a direct antioxidant, stimulates antioxidant enzymes (Catalase, Superoxide Dismutase, Peroxidases), regulates the ionic balance between Na and Cl, improves

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photosynthesis by increasing chlorophyll content, stomatal opening and closing efficiency, and the accumulation of osmotic substances such as proline and soluble sugars. It also regulates plant hormones, such as reducing abscisic acid and increasing cytokinins[34,35].

Table 6: Effect of irrigation water type and melatonin treatment on grain yield (ton ha^{-1}) of soybean

Melatonin	Irrigation water type		Mean
	Well	River	
T0	8.8533 h	12.4267 fg	10.6400 d
T1	12.5867 fg	13.9367 fe	13.2617 c
T2	13.9633 fe	17.3767 bc	15.6700 b
T3	11.6000 g	13.6333 fe	12.6167 c
T4	14.0900 fe	17.8933 bc	15.9917 b
T5	14.7367 de	18.6300 ba	16.6833 b
T6	13.5733 feg	17.0400 bc	15.3067 b
T7	14.4967 fe	18.5167 ba	16.5067 b
T8	16.4533 dc	20.0867 a	18.2700 a
Mean	13.3726 b	16.6156 a	

*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.

Conclusion:

Melatonin is considered one of the important plant growth regulators that contribute to mitigating the effects of harmful environmental stresses, including salinity, which is a problem that hinders germination, vegetative and root growth, and consequently the yield and its components. The studied traits were significantly affected by irrigation water type. Well water negatively impacted the characteristics of the soybean crop, leading to a decrease all studied traits. Adding melatonin (soaking + spraying) improved the studied traits and mitigated the effects of salinit

Treatment T8 (soaking 100 + spraying 200 µmol) outperformed all other treatments for all studied traits, whether for the plants irrigated with river or well water. Melatonin plays a key role in developing physiological processes within the plant, such as seed germination, root elongation, chlorophyll content, photosynthesis, antioxidant enzymes, and reducing the reactive oxygen species (ROS) and membrane damage. These results indicate the role of melatonin in mitigating the effects of salinity on soybean plants, as well as improving crop growth and productivity.

Appendices: Appendix 1: Effect of irrigation, soaking, and melatonin spraying water quality on the relative growth rate of soybeans (g day-1) for the period 30-60 days

Melatonin	Irrigation water type		Mean
	Well	River	
T0	0.046700 i	0.070767 fg	0.058733 g
T1	0.069233 hg	0.081467 ecd	0.075350 fe
T2	0.073833 feg	0.085100 bcd	0.079467 ced
T3	0.060400 h	0.077433 f-d	0.068917 f
T4	0.077800 f-d	0.089133 bc	0.083467 cbd
T5	0.080400 f-d	0.094033 ba	0.087217 b
T6	0.070233 fg	0.083967 b-d	0.077100 ed
T7	0.078600 f-d	0.089933 bc	0.084267 cb
T8	0.089533 bc	0.102700 a	0.096117 a
Mean	0.071859 b	0.086059 a	

*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.

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Appendix 2: Effect of irrigation, soaking, and melatonin spraying water quality on the relative growth rate of soybeans (g day^{-1}) for the period 60-90 days

Melatonin	Irrigation water type		Mean
	Well	River	
T0	0.016967 j	0.026133 g-i	0.021550 h
T1	0.024833 hgi	0.034967 g-e	0.029900 fg
T2	0.036367 gfe	0.040533 de	0.038450 de
T3	0.020967 ji	0.028200 g-i	0.024583 hg
T4	0.038333 fe	0.028200 cde	0.042000 dc
T5	0.049733 cd	0.060400 b	0.055067 ba
T6	0.027433 g-i	0.039133 de	0.033283 fe
T7	0.044467 cde	0.052867 cb	0.048667 bc
T8	0.050000 cd	0.073633 a	0.061817 a
Mean	0.034344 b	0.044615 a	

*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.

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Appendix 3: Effect of irrigation, soaking, and melatonin spraying water quality on the relative growth rate of soybeans (g day^{-1}) for the period 90-120 days

Melatonin	Irrigation water type		Mean
	Well	River	
T0	0.011033 h	0.015633 gh	0.013333 f
T1	0.019167 gfh	0.028133 e-c	0.023650 ed
T2	0.027033 e-f	0.032133 e-c	0.029583 bdc
T3	0.016533 gh	0.021333 e-h	0.018933 ef
T4	0.027967 e-c	0.034600 b-c	0.031283 bdc
T5	0.033200 e-c	0.041367 ba	0.037283 ba
T6	0.025600 e-f	0.030633 e-c	0.028117 dc
T7	0.030833 e-c	0.040133 bac	0.035483 bac
T8	0.036633 b-c	0.045000 a	0.040817 a
Mean	0.025333 a	0.032107 a	

*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.

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Appendix 4: Effect of irrigation, soaking, and melatonin spraying water quality on soybean crop growth rate (g cm^{-2} 1 day^{-1}) for the period 30-60 days

Melatonin	Irrigation water type		Mean
	Well	River	
T0	0.0003557 k	0.0004619 jk	0.00040881 f
T1	0.0005907 jk	0.0011746 gfh	0.00088263 e
T2	0.0008988 ih	0.0015325 e-c	0.00121562 dc
T3	0.0003755 k	0.0005528 jk	0.00046413 f
T4	0.0010863 gh	0.0015451 b-c	0.00131570 bc
T5	0.0012633 e-f	0.0017600 ba	0.00151162 ba
T6	0.0007384 ji	0.0014537 e-c	0.00109604 d
T7	0.0012248 egf	0.0017310 bac	0.00147792 ba
T8	0.0014319 e-c	0.0018299 a	0.00163090 a
Mean	0.00088504 a	0.00133794 a	

*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.

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Appendix 5: Effect of irrigation, soaking, and melatonin spraying water quality on soybean crop growth rate (g cm^{-2} 1 day^{-1}) for the period 60-90 days

Melatonin	Irrigation water type		Mean
	Well	River	
T0	0.0011647 g	0.0014943 g	0.0013295 f
T1	0.0013753 g	0.0025550 cde	0.0019652 e
T2	0.0018633 fge	0.0033427 cb	0.0026030 cd
T3	0.0013153 g	0.0024207 fde	0.0018680 e
T4	0.0023870 fde	0.0033607 cb	0.0028738 cb
T5	0.0026010 cde	0.0039717 b	0.0032863 b
T6	0.0016163 fg	0.0025590 cde	0.0020877 ed
T7	0.0025060 de	0.0033673 cb	0.0029367 cb
T8	0.0029867 cd	0.0052917 a	0.0041392 a
Mean	0.0019795 a	0.0031514 a	

*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.

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Appendix 6: Effect of irrigation, soaking, and melatonin spraying water quality on soybean crop growth rate (g cm^{-2} 1 day^{-1}) for the period 90-120 days

Melatonin	Irrigation water type		Mean
	Well	River	
T0	0.0019687 j	0.0026083 h-g	0.0022885 f
T1	0.0024930 hji	0.0042097 e-g	0.0033513 efd
T2	0.0031673 e-g	0.0044043 e-c	0.0037858 ecd
T3	0.0021393 ji	0.0037083 e-g	0.0029238 ef
T4	0.0034627 e-g	0.0049120 e-c	0.0041873 bcd
T5	0.0045957 e-c	0.0059960 ba	0.0052958 ba
T6	0.0030167 h-g	0.0043140 e-g	0.0036653 ecd
T7	0.0038290 e-g	0.0054007 bac	0.0046148 bc
T8	0.0050970 b-c	0.0064343 a	0.0057657 a
Mean	0.0033077 a	0.0046653 a	

*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.

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Appendix 7: Effect of irrigation, soaking, and melatonin spraying water quality on net photosynthesis of soybeans (g cm^{-2} 1 day^{-1}) for the period 30-60 days

Melatonin	Irrigation water type		Mean
	Well	River	
T0	0.0030267 g	0.0034367 fg	0.0032317 e
T1	0.0036667 feg	0.0048000 b-c	0.0042333 cd
T2	0.0037833 feg	0.0054667 bdc	0.0046250 cbd
T3	0.0034200 fg	0.0044733 fde	0.0039467 ed
T4	0.0039067 feg	0.0055400 b-c	0.0047233 cbd
T5	0.0046767 f-c	0.0061167 ba	0.0053967 b
T6	0.0037133 feg	0.0049300 b-c	0.0043217 cd
T7	0.0044267 fde	0.0057200 b-c	0.0050733 cb
T8	0.0058367 bac	0.0067733 a	0.0063050 a
Mean	0.0040507 a	0.0052507 a	

*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.

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Appendix 8: Effect of irrigation, soaking, and melatonin spraying water quality on net photosynthesis of soybeans (g cm^{-2} 1 day^{-1}) for the period 60-90 days

Melatonin	Irrigation water type		Mean
	Well	River	
T0	0.0011800 i	0.0013367 hi	0.0012583 f
T1	0.0017467 h-g	0.0018933 h-g	0.0018200 fe
T2	0.0022200 h-e	0.0026200 d-e	0.0024200 dce
T3	0.0015967 hig	0.0018233 h-g	0.0017100 fe
T4	0.0024067 h-e	0.0027567 d-e	0.0025817 dc
T5	0.0030500 dce	0.0048133 b	0.0039317 b
T6	0.0019133 h-g	0.0023200 h-e	0.0021167 de
T7	0.0027300 d-e	0.0033233 dc	0.0030267 c
T8	0.0036900 c	0.0062133 a	0.0049517 a
Mean	0.0022815 b	0.0030111 a	

*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.

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Appendix 9: Effect of irrigation, soaking, and melatonin spraying water quality on net photosynthesis of soybeans (g cm^{-2} 1 day^{-1}) for the period 90-120 days

Melatonin	Irrigation water type		Mean
	Well	River	
T0	0.0009367 f	0.0016267 efd	0.0012817 d
T1	0.0016733 efd	0.0020067 e-d	0.0018400 cd
T2	0.0018000 e-d	0.0023200 ecd	0.0020600 bcd
T3	0.0013367 ef	0.0019900 e-d	0.0016633 cd
T4	0.0018800 e-d	0.0026267 bcd	0.0022533 bc
T5	0.0020967 e-d	0.0035467 ba	0.0028217 ba
T6	0.0017067 efd	0.0020300 e-d	0.0018683 cd
T7	0.0019300 e-d	0.0026333 bcd	0.0022817 bc
T8	0.0028900 bc	0.0039567 a	0.0034233 a
Mean	0.0018056 a	0.0025263 a	

*Values with the same letters do not differ significantly from each other according to Duncan's multiple range test at a probability level of 0.05.

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