

# Effects of Melatonin and Citric Acid Foliar Application and their Interaction on Growth and Yield of Local Barley *Hordeum Vulgare L.*

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**Abstract:** A field experiment was conducted during the 2025-2026 winter growing season in the Sumer district to determine the effect of foliar spraying of melatonin and citric acid, and their interaction, on some growth and yield attributes of local barley varieties. The experiment was conducted as a factorial experiment with two factors and three replications. The first factor was melatonin spraying (M0: no spray, M1: 25, M2: 50) mg/L<sup>-1</sup>, while the second factor was citric acid spraying (C0: no spray, C1: 1, C2: 2) g/L<sup>-1</sup>. The results of statistical analysis showed that the melatonin and citric acid spraying treatments, each separately, recorded significant differences of all studied attributes, such as plant height, spike length, leaf area, number of spikes per plant, weight of 1000-grain, grain yield, straw yield, and biological yield. As for the interaction between melatonin and citric

acid spraying M2C2 treatment was one of the best experimental treatments, as it significantly exceeded on them and recorded the highest means of all the mentioned attributes. Hence we conclude that spraying with melatonin and citric acid had a best effect on the growth performance and yield of the local barley crop.

**Keywords:** Melatonin, Citric acid, Barley crop, growth, yield.

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## Introduction

Barley is one of the most important cereal crops widely cultivated throughout the world. It is among the most important crops globally, ranking fourth after wheat, maize, and rice, and is classified among the top ten agricultural crops worldwide (Gadisa and Mekonnen, 2023). Its production is estimated at approximately 157 million tons (FAOSTAT, 2022). It is a cereal crop belonging to the grass family and is a grass crop. It is considered the fourth most important crop in the world after wheat, maize, and rice, and is not grown in the warm, humid climate of tropical regions (Goswami and Pandey, 2018). It is an important cereal crop in Iraq and is primarily used in most countries of the world as a forage crop, either as green fodder or as an ingredient in concentrated feed mixtures. It is characterized by its rapid growth, high resistance to salinity, and drought tolerance. Therefore, most areas of Iraq are suitable for its cultivation (Al-Baldawi *et al.*, 2014). The crop requires less water and is more tolerant of salinity and alkalinity than other winter grains (Singh, 2017). Barley is used as green fodder for animals, in addition to its grains, which are used in some industries (Al-Atabi, 2011). Melatonin is a chemical compound that acts in plants as a plant growth regulator (phytohormone) and a powerful antioxidant. It affects basic physiological processes such as enhancing antioxidant molecules like POD, CAT, and SOD, regulating hormonal balance within the plant, improving photosynthesis, promoting root growth and leaf elongation, and reducing oxidative stress caused by environmental conditions (Gamalero and Glick, 2025). It also plays an important role in regulating hormonal balance within the plant, as it interacts with auxins, cytokinins, and gibberellins, thus promoting cell division and elongation and stimulating root growth and leaf elongation (Taiz *et al.*, 2015). Furthermore, external treatment with melatonin has been shown to increase chlorophyll content and improve photosynthetic efficiency, thereby increasing the production of carbohydrates necessary for growth and grain formation (Arnao and Hernández-Ruiz, 2015). Citric acid is an important organic acid in plants, acting as a chelating agent for metal ions, thus increasing the availability of micronutrients such as iron, manganese, and zinc to the plant (Güneş *et al.*, 2007; Mengel, 2002). When applied as a foliar spray, it contributes to improved absorption of vital nutrients, enhancing the activity of enzymes associated with growth and photosynthesis. In addition, citric acid plays a role in enhancing plant physiological efficiency by improving photosynthesis and increasing chlorophyll content. It also helps balance ions within cells, reducing the negative effects of environmental stresses such as nutrient deficiencies or water stress (Ali, 2024). Citric acid was also observed to reduce the impact of environmental stress on nutrient uptake and enhance nutrient efficiency, which positively affected vegetative characteristics and yield components (Touhami *et al.*, 2017). This study aims to determine the effect of foliar spraying with melatonin and citric acid on some growth and yield characteristics of barley.

## Materials and Methods

A field experiment was conducted during the 2025-2026 winter growing season in the Sumer district, located 18 kilometers northeast of AL-Diwaniyah Governorate (approximately 40 kilometers away). The experiment aimed to determine the growth and yield response of local barley to foliar spraying with melatonin (Focus Force brand, USA origin) and citric acid (anhydrous C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>, manufactured by TTCA Co., Ltd., China origin), and to investigate the interaction between these two agents on certain growth and yield characteristics of the local barley variety. The experiment was conducted as a two-factor factorial experiment. The first factor was melatonin spraying (M0: no spray, M1: 25, M2: 50) mg/L, and the second factor was citric acid spraying (C0: no spray, C1: 1, C2: 2) g/L. The interaction between these two agents on certain growth, yield, and yield components of the local barley variety was also investigated. In a single-treatment replicate, the soil was prepared by tilling, harrowing, and amending. Random samples were then taken to measure certain physical and chemical properties, based on the methods described by Page *et al.* (1982) and Black (1965), as shown in Table (1). The field was divided into 2 x 3 m plots with a 50 cm spacing between plots. Each plot was then divided into 15 rows, each 2 m long and 20 cm apart. Seeds were sown at a depth of 6 cm at a rate of 100 kg ha<sup>-1</sup>. Phosphate fertilizer was added at planting at a rate of 80 kg ha<sup>-1</sup> in the form of triple superphosphate (46% P<sub>2</sub>O<sub>5</sub>). Nitrogen fertilizer was added as urea (46% N) at a rate of 120 kg ha<sup>-1</sup> in two applications: the first 15 days after planting (germination stage) and the second 45 days after the first application. Hoe and weed as needed until the end of the season.

**Table (1) some physical and chemical properties of the soil at the experimental site.**

Attributes	Value	
E.C ) ds/m <sup>-1</sup> )	5.22	
pH	7.6	
Available potassium (mg.kg <sup>-1</sup> )	10.51	
Available phosphor (mg.kg <sup>-1</sup> )	3.00	
Available nitrogen (mg.kg <sup>-1</sup> )	0.05	
Soil separators ( <sup>l</sup> -g.kg)	clay	44.31
	silt	39.21
	sand	25.02
Soil texture	Silt clay loam	

## The Studied Traits

### Plant Height

The height of the plants was measured using a metric ruler from the soil surface to the end of the spike for ten plants from each experimental unit and randomly upon reaching the flowering stage.

### Leaf area (cm<sup>2</sup>)

The leaf area was calculated after flowering was completed according to the equation mentioned by Liang *et al.*, (1973). Leaf area = leaf length \* leaf width \* 0.75

### Spike Length

It was estimated by calculating the distance from the base of the spike to the end of the terminal spike, excluding the terminal spike as an average for ten randomly selected plants.

### Number of spikes (spike.m<sup>-2</sup>)

A square meter of each experimental unit was harvested randomly from the guarded lines at the stage of full maturity of the crop and according to the number of spikes.

### **Weight of 1000 grains (g)**

It was estimated by counting a thousand grains randomly using a seed counter from the yield of a square meter, and then each sample from each experimental unit was weighed.

### **Grain yield (tons ha<sup>-1</sup>)**

A manual study was conducted for the square meter harvested from each experimental unit. After isolating the straw from the grains, the grains were weighed, adding to it the weight of the grains used to estimate the weight of 1000 grains for the same treatment. Then it was converted from gm.m<sup>-2</sup> to ton.ha<sup>-1</sup> based on 14% moisture.

### **Biological yield (tons ha<sup>-1</sup>)**

It was calculated from the harvested plants to estimate the grain yield. The entire plants were weighed (grains + straw) and then converted from gm.m<sup>-2</sup> to ton.ha<sup>-1</sup>.

### **Straw yield (tons ha<sup>-1</sup>)**

Straw yield was calculated by subtracting the weight of the grains from the weight of the total biomass.

## **Results and Discussion**

The results of the statistical analysis in Table (2) show that spray treatments with different levels of melatonin significantly affected ( $P \leq 0.05$ ) all studied traits. Level M2, in particular, showed a significant advantage, recording the highest values and a significant difference for all studied traits at level M0. This superiority may be attributed to melatonin's ability to stimulate the secretion of auxin-synthesizing enzymes and enhance the cells' response to auxins by activating proteins that increase plant height and internodal length (AUX and IAA). This, in turn, increases the activity of cell wall-releasing enzymes, leading to cell division in the meristematic tissue and consequently increased cell elongation and internodal length, thus increasing plant height (Wang *et al.*, 2022). Furthermore, melatonin, acting as a growth promoter with hormone-like effects, increases physiological nutrition and vegetative growth. It stimulates gene expression associated with cell division and works synergistically with auxins, leading to... Melatonin increases internode elongation, plant height, and leaf area (Wang *et al.*, 2016). It also regulates the balance between auxin and cytokinin, leading to increased lateral bud formation and consequently, increased tiller formation, spike length, and spike count (Ahmed *et al.*, 2019). Regarding the 1000-grain weight characteristic, Zhang *et al.*, (2020) indicated that melatonin increases the activity of starch-synthetic enzymes such as ADP-glucose pyrophosphorylase and starch synthase, thus promoting the flow of synthesized nutrients, especially sugars, from the leaves to the grains. Furthermore, it reduces ABA levels during grain filling, prolonging the filling period and increasing the grain filling rate and individual grain weight. On the other hand, it increases the chlorophyll content in the plant by regulating chlorophyll biosynthesis, reducing the effect of free radicals within chloroplasts, and increasing the activity of the Rubisco enzyme, thus increasing the efficiency of photosynthesis. This leads to increased sugar synthesis and accumulation in vegetative tissues, resulting in an increase in the dry weight of the stem and leaves, and consequently, an increase in straw yield (Zhang *et al.*, 2015). This was observed in all application treatments in this experiment, and was particularly evident in treatment M2. Regarding grain yield and biological yield, it was noted that the application treatments, especially treatment M2, which performed best, recorded the highest values for the two aforementioned parameters. This is attributed to improved photosynthetic efficiency, improved nitrogen utilization efficiency, and reduced oxidative energy loss, thus improving carbohydrate storage efficiency in grains. This resulted in the high values recorded for the two aforementioned parameters (Wang *et al.*, 2022). These results are consistent with those indicated by Arnao and Hernández-Ruiz (2019) and Lie *et al.*, (2018) demonstrated a high response of barley crops to melatonin and increased photosynthetic efficiency, which is reflected in increased nutrient accumulation within barley crop tissues.

**Table (2) Effect of melatonin spraying on some growth and yield characteristics of local barley crops**

Treatment	Plant Height (cm)	Leaf area (cm <sup>2</sup> )	Spike length (cm)	Spikes number per plant	Weight of 1000 grain (g)	Grain yield (ton/ha <sup>-1</sup> )	Straw yield (ton/ha <sup>-1</sup> )	Biological yield (ton/ha <sup>-1</sup> )
M0	70.53±0.65 c	12.69±0.23 c	7.12±0.18 c	3.44±0.14 c	16.14±0.20 c	1.68±0.03 c	0.92±0.04 c	2.05±0.01 c
M1	74.09±0.13 b	15.03±0.50 b	8.52±0.20 b	4.62±0.20 b	21.82±0.68 b	1.91±0.01 b	1.75±0.04 b	2.53±0.07 b
M2	77.11±0.50 a	19.03±0.47 a	10.40±0.31 a	6.42±0.22 a	27.36±0.64 a	2.37±0.08 a	2.14±0.07 a	3.12±0.09 a

- (M0: No spray, M1: 25 mg/L<sup>-1</sup>, M2: 50) mg/L<sup>-1</sup>
- Different letters indicate significant differences between experimental treatments within the same column according to Duncan's multiple range test (Duncan, 1955).

The results of the statistical analysis in Table (3) showed that spraying barley plants with different levels of citric acid resulted in significant differences between the experimental treatments for all studied traits. This effect was particularly evident in the C2 treatment compared to the control C0 treatment, including plant height, spike length, leaf area, number of spikes per plant, 1000-grain weight, grain yield, straw yield, and biological yield. The increase in these traits in the treatment treatments, especially C2, can be attributed to the physiological and molecular mechanisms associated with citric acid's role as an active organic compound in plant metabolism. Through its physiological role, citric acid acts as a chelating agent for mineral elements such as iron, zinc, and manganese, increasing their availability for root uptake and enhancing chlorophyll synthesis and photosynthetic efficiency (Gunes *et al.*, 2007; Marschner, 2012). Increased photosynthetic efficiency supports carbohydrate accumulation in the leaves and stems, resulting in increased plant height and spike length. Leaf area was increased in treatments M1 and M2, particularly in the latter, compared to the control treatment (M0). Regarding the number of spikes, 1000-grain weight, and grain yield, citric acid enhances nitrogen utilization efficiency and stimulates metabolic pathways for energy production (ATP) via the TCA cycle, thus supporting cell division and elongation and improving grain filling (Foy *et al.*, 1978; Cakmak, 2002). Furthermore, increased photosynthesis and available energy lead to improved carbohydrate distribution in the grain, which increases individual grain weight and total yield. For vegetative mass traits such as straw yield and biological yield, citric acid promotes dry matter accumulation by increasing leaf and stem growth and improving photosynthetic efficiency in leaves, in addition to supporting root metabolic activity and nutrient uptake (Abd Elhamid *et al.*, 2014). The results of this study are consistent with numerous published studies demonstrating that applying citric acid, whether through foliar spraying or in nutrient solutions, leads to significant improvements in growth characteristics and yield in cereal crops such as barley *Hordeum vulgare* and wheat *Triticum aestivum*. Abdel Hamid *et al.*, (2014) found that foliar spraying with citric acid enhanced chlorophyll synthesis and increased iron content in wheat plants, which in turn improved photosynthesis and increased biomass. These results also align with those of Marschner (2012), who stated that citric acid use increases the availability of essential mineral elements, enhances photosynthetic activity, and leads to increased plant height and leaf area. This is because elements such as Fe and Mn are essential for the formation of photosynthetic compounds and for activating the Rubisco enzyme, which is responsible for carbon fixation. Furthermore, these results are consistent with those of Güneş *et al.*, (2007), who reported that citric acid applications act as a chelating agent, increasing micronutrient uptake and thus improving the activity of growth-related enzymes. Finally, these results are consistent with those summarized by Foy *et al.*, (1978) Organic acids such as citric acid are involved in the intracellular TCA metabolic cycle, increasing the production of energy (ATP) necessary for vegetative growth processes and regulating mineral

nutrient channel signaling, thus increasing individual grain weight and grain yield. Mengel (2002) also indicated that increasing nutrient availability through organic chelates enhances nitrogen utilization efficiency, which positively impacts root and leaf growth, thereby increasing biological and total yield.

**Table (3) Effect of citric acid spraying on some growth and yield characteristics of local barley crop**

Treatment	Plant Height (cm)	Leaf area (cm <sup>2</sup> )	Spike length (cm)	Spikes number per plant	Weight of 1000 grain (g)	Grain yield (ton/ha <sup>-1</sup> )	Straw yield (ton/ha <sup>-1</sup> )	Biological yield (ton/ha <sup>-1</sup> )
C0	72.60±1.0 6 c	14.36±0.7 9 c	8.07±0.4 2 c	4.54±0.4 0 b	20.15±1.4 1 c	1.81±0.0 7 c	1.44±0.1 6 c	2.38±0.1 3 c
C1	73.79±1.0 0 b	15.58±1.0 0 b	8.76±0.4 4 b	4.69±0.4 7 b	21.71±1.5 8 b	2.03±0.1 1 b	1.58±0.1 7 b	2.53±0.1 4 b
C2	75.34±0.8 9 a	16.80±1.0 4 a	9.21±0.6 2 a	5.26±0.5 0 a	23.47±1.8 8 a	2.12±0.1 2 a	1.80±0.1 9 a	2.78±0.1 9 a

\* (C0: No spraying, C1: 1, C2: 2 )g/L

\* Different letters indicate significant differences between experimental treatments within the same column according to Duncan's multiple range test (Duncan,1955).

The results in Table (4) show that the M2C2 intervention treatment was the best, significantly outperforming all other experimental intervention treatments ( $P \leq 0.05$ ) for all studied traits. Specifically, the values recorded for plant height ( $78.74 \pm 0.58$ ) cm, leaf area ( $20.36 \pm 0.35$ ) cm<sup>2</sup>, spike length ( $9.21 \pm 0.62$ ) cm, number of spikes per plant ( $5.26 \pm 0.50$ ), weight of 1000 grains ( $23.47 \pm 1.88$ ) g, grain yield ( $2.12 \pm 0.12$ ) t.ha<sup>-1</sup>, straw yield ( $1.80 \pm 0.19$ ) t.ha<sup>-1</sup>, and biological yield ( $2.78 \pm 0.19$ ) t.ha<sup>-1</sup> were recorded, respectively. The significant superiority of the M2C2 level ( $P \leq 0.05$ ) over the other treatment levels for these traits may be attributed to this performance. The above refers to the combination of melatonin and citric acid at the highest levels, which resulted in the high performance of the barley crop. This was evident in the highest values recorded for the M2H2 treatment for the aforementioned traits, due to the synergistic effect of the two substances. Arnao and Hernández-Ruiz (2023) indicated that the significant increase in plant height and leaf area is linked to melatonin's ability to act as a plant growth regulator and a potent antioxidant. It regulates auxin balance and stimulates cell division and elongation, in addition to its role in enhancing the activity of antioxidant enzymes and reducing oxidative stress. Melatonin has also been shown to increase chlorophyll content and enhance photosynthetic efficiency, thus increasing the rate of dry matter production. On the other hand, citric acid acts as an intermediate organic acid in the Krebs cycle and contributes to improving nutrient absorption through its ability to chelate minerals and increase their availability within plant tissues, in addition to its role in enhancing antioxidant activity (Mallhi *et al.*, 2019). When the two compounds are combined, functional synergy is achieved; citric acid improves nutrient availability, while melatonin enhances the plant's ability to utilize them physiologically, leading to a significant increase in vegetative growth characteristics, particularly in the M2C2 treatment. As for yield characteristics such as grain, straw, and biological yield, the M2C2 treatment, as previously mentioned, recorded the highest values for these traits among the other interaction treatments. This likely reflects the synergistic effects of the two compounds. Their combination improved photosynthetic efficiency, leading to increased accumulation of photosynthetic products, improved carbohydrate translocation to reproductive parts, reduced oxidative stress, enhanced nutrient absorption, and an increase in total biomass. Some scientific studies have demonstrated that melatonin enhances the

efficiency of the photosynthetic system and improves Rubisco activity, thus increasing carbohydrate accumulation (Arnao and Hernández-Ruiz, 2023). Furthermore, citric acid... Citric acid contributes to improving plant nutrition and activating metabolic processes associated with grain formation (Ali, 2024).

The results obtained in this study are consistent with those of numerous studies conducted on cereal crops, particularly barley and wheat, which have confirmed the pivotal role of melatonin and citric acid in improving growth and yield. Studies on wheat found that melatonin treatment led to a significant increase in plant height, leaf area, and yield components due to improved photosynthetic efficiency, regulation of antioxidant balance, and reduction of oxidative stress. This was reflected positively in 1000-grain weight and grain yield (Arnao and Hernández-Ruiz, 2023). Other reports have indicated that melatonin stimulates carbohydrate accumulation in reproductive parts and improves the efficiency of photosynthetic translocation to the grain, which explains the significant increase in grain yield and biological yield. As for citric acid, studies have shown In cereal crops, its application, whether to the soil or as a foliar spray, improved the uptake of macro and micronutrients and increased chlorophyll content, contributing to improved vegetative growth characteristics and yield components, including the number of spikes and grain weight. Physiological research has shown that citric acid acts as a chelating agent, improving the availability of elements such as iron, calcium, and magnesium, thus enhancing metabolic activity associated with dry matter formation (Mallhi *et al.*, 2019). Studies conducted on barley under various stress conditions observed that enhancing the antioxidant system through bio-treatments led to a significant improvement in growth and yield characteristics. This aligns with the results of the current study, which demonstrated the superiority of the interaction between melatonin and citric acid, indicating that combining oxidative stress reduction with improved plant nutrition represents an effective complementary mechanism for increasing productivity.

**Table (4) Effect of the interaction between melatonin and citric acid spraying on some growth and yield characteristics of local barley crop**

Treatment	Plant Height (cm)	Leaf area (cm <sup>2</sup> )	Spike length (cm)	Spikes number per plant	Weight of 1000 grain (g)	Grain yield (ton/ha <sup>-1</sup> )	Straw yield (ton/ha <sup>-1</sup> )	Biological yield (ton/ha <sup>-1</sup> )	
M0	C0	68.51±0.28 g	12.03±0.01 e	6.68±0.33 f	3.43±0.29 fg	15.69±0.34 h	1.54±0.02 g	0.79±0.01 h	2.01±0.06 h
	C1	70.21±0.44 f	12.68±0.34 de	7.57±0.30 de	3.19±0.15 g	16.05±0.02 gh	1.71±0.03 f	0.89±0.02 g	2.02±0.01 h
	C2	72.88±0.18 e	13.35±0.32 d	7.11±0.09 ef	3.70±0.30 efg	16.69±0.34 g	1.79±0.02 e	1.09±0.04 f	2.12±0.10 g
M1	C0	73.76±0.33 de	13.70±0.31 cd	8.02±0.08 d	4.25±0.01 def	19.39±0.34 f	1.87±0.01 d	1.59±0.07 e	2.25±0.01 f
	C1	74.10±0.10 d	14.68±0.34 c	8.35±0.32 d	4.55±0.40 de	22.05±0.02 e	1.90±0.01 d	1.79±0.03 d	2.59±0.04 e
	C2	74.40±0.05 d	16.70±0.67 b	9.19±0.09 c	5.06±0.41 cd	24.03±0.01 d	1.97±0.09 c	1.86±0.06 cd	2.74±0.01 d
M2	C0	75.53±0.03 c	17.35±0.32 b	9.50±0.08 c	5.94±0.46 bc	25.36±0.32 c	2.03±0.06 c	1.95±0.00 c	2.88±0.07 c
	C1	77.04±0.33	19.37±0.34	10.36±0.34	6.32±0.16	27.03±0.03	2.49±0.02	2.04±0.02	2.99±0.02

		b	a	b	ab	b	b	b	b
		78.74±0.	20.36±0.	11.35±0.	7.01±0.2	29.70±0.	2.59±0.0	2.44±0.0	3.49±0.1
	C2	58	35	32	8	35	1	9	1
		a	a	a	a	a	a	a	a

\* (M0: No spray, M1: 25 mg/L<sup>-1</sup>, M2: 50) mg/L<sup>-1</sup>

\* (C0: No spraying, C1: 1, C2: 2 )g/L

\* Different letters indicate significant differences between experimental treatments within the same column according to Duncan's multiple range test (Duncan,1955).

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