



## Effect of Melatonin and Glutathione on the Vegetative Characteristics of (*Capsicum annuum* L) hot pepper. Grown Under Conditions of Cadmium Pollution

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### Abstract

This current study was conducted an agricultural nursery in the Al-Muqdadiya district which is in the north of Baqubah, the center of Diyala governorate and lies 90 km north east of the capital Baghdad. The experiment was done in autumn on *capsicum annuum* L, Hybrid Barbarian F1, the Indian origin. It aimed to investigate the impact of treatment with melatonin and glutathione on the Vegetative features of *capsicum annuum* L grown under polluted conditions with cadmium. The experiment was carried out according to Randomized Complete Block Design (R.C.B.D.) in three replicates. It includes three transactions, these were cadmium which was added to soil with the two concentrations 0 and 15 mg.kg soil<sup>-1</sup>, whereas melatonin and glutathione were added spreading on the leaves with the concentrations 0, 50, and 100 mg.l<sup>-1</sup>. The results showed a significant decrement in plant height, number of leaves, and leaf area as a result of treating the agricultural soil with cadmium at a concentration of 15 mg.kg soil<sup>-1</sup>, compared to the treatment without the addition of cadmium, i.e. the control treatment. It was also shown from the results obtained that there was a significant increase in all the studied traits as a result of spraying the plants with melatonin and that the highest average for each of the plant height, number of leaves,



and leaf area was obtained as a result of spraying the plants with melatonin at a concentration of 100 mg. l<sup>-1</sup>. Significant increases were also obtained in all the traits under study as a result of spraying plants with glutathione at a concentration of 100 mg.l<sup>-1</sup>.

**Keywords:** Melatonin, Glutathione, Cadimium, Vegetative Characteristics, pollution, *Capsicum annuum* L.

## Introduction

Heavy metals are known as elements with specific characteristics. Their atomic number is larger than 20 and their dense larger than 5g.c<sup>-3</sup> [1]. Some of the most common polluting heavy metals are cadmium (CD), chromium (Cr), copper (Cu), mercury (Hg), and lead (Pb) [2]. These are environmentally polluting elements that are conveyed to human beings through water, air and food. In recent years pollution via heavy elements has attracted largely researchers' attention. This is because of their direct impact on the growth of plants. Pollution with heavy metals impedes greatly agricultural production and its indirect impact on human health because these elements are transmitted to human via the food chain [3]. Cadmium (Cd) is one of the most damaging heavy metals and it has a dangerous effect on living beings. It occurs naturally a polluting element in the environment, it is derived from agricultural and industrial sources. Cadmium arrives in human beings through having food and water that are polluted by this element [4]. Cd influences a group of chemical, biological, morphological, physiological, and molecular processes that are important in plants, this leads to the plant losing its green colour and ceasing regular growth [5].

Melatonin (N-acetyl-5-methoxytryptamine, MT) is a biologically active molecule having multiple functions, It is found in all animals and plants, Melatonin was identified for the first time in 1995 [6]. It plays a basic role in confining the activity of the free radicals the kind of reactive nitrogen species (RNS), and reactive oxygen species (ROS), improving the antioxidant ability, and preventing oxidative stress in the cells, tissues, and parts. Melatonin works as a first defence line against any dangerous circumstances facing the living being [7].

Glutathione (GSH) which is  $\gamma$ -Lglutamyl-Lcysteinyl- glycine consists of three amino acids- these are Glutamine (Glu), Cysteine (Cys), and Glycerine (Gly), it is dissolvable in water and has a low molecular weight [8]. Glutathione is considered one of the most non-enzyme



antioxidants and it has multiple roles in plants, It takes part directly in preserving the plant cells from damage caused by free radicals, This is achieved via three possible paths; the direct scavenging of ROS in the AsA-GSH cycle removing the heavy metal through creating phytochelatin or conjugating the catalyzed heavy metals by glutathione-S transfers [9, 10, 11]. *Capsicum annuum* L. is one member of the Solanaceae which includes several vegetable crops and the most important ones are tomato, eggplant, potato, and tobacco [12]. It is used widely in all countries [13] because it's fruits are characterized by delicious taste and a high nutrient value [14]. Hot pepper is rich with basic nutrients such as Potassium (K), Phosphorus (P), Magnesium (Mg), Calcium (Ca), Sodium (Na), Iron (Fe), Manganese (Mn ), Boron(B), Selenium (Se), Copper (CU), and Zinc (Zn). The content of these elements depends on different variables like the fruit type, growth stage, influencing environmental factors, and various agricultural practices [15]. The present study aims to know the impact of different concentrations of Melatonin and Glutathione on the vegetative characteristics of the hot Pepper *Capsicum annuum* L. under the conditions of pollution with Cadmium.

## **Materials and Methodology**

The experiment was done in pots in the plastic house, belonging to one of the agricultural nurseries in Al-Muqdadiya district located north of Baqubah, the center of Diyala governorate which lies 90 km north-east away of the capital Baghdad. This experience was conducted in autumn, 6<sup>th</sup> October 2022 on *capsicum annuum* L, Hybrid Barbarian F1 which is an Indian origin.

### **The Experience Design**

The practical experience was carried out due to Randomized Complete Block Design (R.C.B.D.). It included two concentrations of cadmium ( $\text{CdCl}_2$ ), 0 and 15 mg.kg soil<sup>-1</sup>, and three concentrations of melatonin and glutathione 0, 50, 100 mg.l<sup>-1</sup>. The number of treatments was 18 as shown in Table (1). The treatments were repeated three replicates so the total number of the experimental units was 54 unit. Each experimental unit consisted of 5 pots and one plant in each pot. Accordingly, the total of pots was 270.



## **Polluting the Experiment's Soil**

For polluting the study soil with cadmium in a concentration of  $15 \text{ mg.kg soil}^{-1}$ , the soil was air dried, softened and sifted by passing it through a sieve in which the diameter of its pores was 5mm. Then, the soil was spread on the ground covered with a layer of thick nylon. It was sprayed with cadmium with a purity of 100% dissolved in water by one letter hand sprayer with stirring continuously for good well mixing. Before transferring the soil to plastic pots, it was left to be dry. The pots were prepared 30 days after transferring the hot pepper seedlings to them. It was maintained by watering them regularly.

## **Experimental Treatments**

The hot pepper seedlings (2-leaf stage) were obtained from one of the private agricultural nurseries in Al-Muqdadiya district located 40 km away from north Baghdad. When the plant arrived the 3-leaf stage, the seedlings were transferred to plastic pots—one plant per pot. Melatonin was dissolved in ethanol alcohol at a concentration of 70% and then the volume was completed to 1 liter with distilled water. While glutathione was dissolved in distilled water directly, both melatonin and glutathione were added to foliar in two stages with a 3- day difference between adding the two substances. The first stage was carried out when the plant reached 5-4 leaves stage, whereas the second stage was at the flowering stage. The plants were sprayed first with melatonin and after three days glutathione was sprayed. As a diffuser substance, 2-3 drops of liquid soap were added.

## **Studied characteristics:**

Three plants were randomly selected from each experimental unit and marked for the purpose of studying the following characteristics:

- 1- Plant height(cm)
- 2- The number of leaves per plant (leaf. plant<sup>-1</sup> )
- 3- Leaf area (cm<sup>2</sup>): According to the method of [16].

## **Statistical Analysis**

The Statistical Analysis System (SAS) was adapted for analyzing the data to study the impact of various factors and their intersections with the studied characteristics. This was done according to the Randomized Complete Block Design (R.C.B.D) and it was replicated three



times due to (ANOVA). The significant differences between the means were compared by testing the Least Significant Difference LSD [17].

## **Results and Discussion**

### **1. Plant Height (cm):**

It is evident from the results of the statistical analysis that there are significant differences at the 5% probability level between the averages of the height characteristic of pepper plants as a result of treatment with cadmium. It is clear from the results shown in Table (2) that there is a decrease in the height characteristic of the plant as a result of treatment with cadmium at a concentration of 15 mg.kg.<sup>-1</sup> Soil, where the cadmium addition treatment was recorded at a concentration of 15 mg.kg.<sup>-1</sup> Soil. The minimum average plant Height reached 21.65 cm, with a decrease rate of 45.05% compared to the control treatment of 0 mg.kg.<sup>-1</sup> Soil recorded the highest average, amounting to 39.40 cm. The reason for the decrease in plant height as a result of treatment with cadmium is that cadmium is a toxic element that moves quickly in plant tissues and affects physiological growth through its effect on the absorption of plant nutrients [18], as many previous studies have confirmed that there is an interaction between the absorption of cadmium on the one hand and the absorption of zinc, manganese and copper on the other hand [19,20], and other researchers hypothesize that cadmium stress leads to a decrease in stomatal density, which leads to reduced carbon dioxide absorption [21], thus reducing the rate of photosynthesis and lack of supply of carbohydrates or proteins to the plant which leads to a decrease in various growth characteristics, including plant height, Cadmium also stimulates plants to synthesize a large number of free radicals, reactive oxygen species, which inhibit plant growth as a result of the oxidative stress that results from these radicals [22,23]. These research results are consistent with what was found [24].

It is also clear from the results that there are significant differences between the averages of plant height at the 5% probability level as a result of treating the plant with melatonin. It is clear from the results of Table (2) that the highest average obtained was as a result of the treatment with the concentration of 100 mg /L. <sup>-1</sup> This addition recorded an average of 33.53 cm and an increase of 23.31% and 8.65% compared to the control treatment of 0 mg/ L. <sup>-1</sup> The



concentration is 50 mg.L<sup>-1</sup> respectively, while the control treatment recorded the lowest average, reaching 27.19 cm.

The reason for the increase in plant height as a result of treatment with melatonin may be attributed to the fact that melatonin is synthesized from tryptophan, as is the case with the hormone indole-3-acetic acid (IAA), and for this reason it plays an important role in cell division and increasing their size, and it may also interfere with other plant hormones, which leads to enhanced plant growth, as well as its role in enhancing plants' absorption of various mineral elements [25]. Melatonin also acts as a free radical scavenger and enhances the activities of antioxidant enzymes such as SOD, POD, CAT, and GR, as well as its role in regulating genes associated with stress resistance [26, 27]. These research results are consistent with what was found [28].

Significant differences also at the 5% probability level between the averages of plant height obtained as a result of treating pepper plants with glutathione at concentrations of 0, 50, and 100 mg.l.<sup>-1</sup>. It is clear from the results of the same table that the highest average for this characteristic, which amounted to 32.42 cm, was obtained as a result of treatment with glutathione at a concentration of 100 mg.L.<sup>-1</sup>. Increase rates reached 12.56% and 6.78% compared to the control treatment, 0 mg.L.<sup>-1</sup>. The concentration is 50 mg.L<sup>-1</sup> respectively, while the control treatment recorded the lowest average, reaching 28.80 cm.

The reason for the increase in plant height as a result of treating the plant with glutathione is attributed to its role in increasing cell division and elongation through its role in enhancing the action of the auxin hormone in the plant [29], in addition to being an antioxidant that works to protect cells from collapse as a result of the oxidative activity of free radicals that form in plants as a result of the plant being exposed to various stresses, including heavy metal stress, thus improving its vegetative characteristics, including the plant's height [30].

The results of the statistical analysis indicate that there are significant differences at the 5% probability level between the averages for the plant height trait as a result of the binary interaction between cadmium, melatonin, and glutathione. It is clear from the results of Table (2) that the best averages for the plant height trait were obtained as a result of the combination consisting of cadmium 0. mg.kg<sup>-1</sup> Soil + melatonin 100 mg.l.<sup>-1</sup>. The combination consisting of



cadmium is 0 mg.kg<sup>-1</sup> Soil + glutathione 100 mg.l<sup>-1</sup> The combination consists of melatonin 100 mg.l<sup>-1</sup> + Glutathione 100 mg.l<sup>-1</sup> Their amounts were 44.31 cm, 41.62 cm, and 35.36 cm, respectively. Table 2 also shows significant differences at a probability level of 5% between the averages of the plant height trait obtained as a result of the triple interaction between cadmium, melatonin, and glutathione. The results indicate that the best average for this trait was obtained as a result of the combination consisting of 0 mg cadmium. .kg<sup>-1</sup> Soil + melatonin 100 mg.l<sup>-1</sup> + Glutathione 100 mg.l<sup>-1</sup> Its amount was 46.13 cm, while the mixture consisting of cadmium recorded 15 mg.kg of soil.<sup>-1</sup> + Melatonin 0 mg.L<sup>-1</sup> + Glutathione 0 mg.L<sup>-1</sup> The lowest average was 19.86 cm.

**Table 2:** Effect of cadmium, melatonin, and glutathione and their interaction on plant height (cm).

C Mg.Kg <sup>-1</sup> soil	M Mg.L <sup>-1</sup>	G Mg.L <sup>-1</sup>			C+M	Average of C Effect
		G0	G1	G2		
C1	M0	32.06	32.60	35.70	33.45	39.40
	M1	37.43	40.86	43.03	40.44	
	M2	41.53	45.26	46.13	44.31	
C2	M0	19.86	20.06	22.86	20.93	21.65
	M1	20.46	21.20	22.20	21.28	
	M2	21.46	22.20	24.60	22.75	
G+M+C		LSD: C+M+G = 2.41 *			LSD: C+M=2.05*	LSD: C= 0.803 *
G+C						
C1		37.01	39.57	41.62	LSD: C+G = 2.05 *	
C2		20.60	21.15	23.22		
M+G					Average of M Effect	
M0		25.96	26.33	29.28	27.19	
M1		28.95	31.03	32.62	30.86	
M2		31.50	33.73	35.36	33.53	
LSD value		LSD: M+G = 2.09 *			LSD: M = 0.984 *	
Average of G Effect		28.80	30.36	32.42	---	
LSD value		LSD: G = 0.984 *				

Note: C= Cadmium, C1= 0 Mg.Kg<sup>-1</sup> soil, C2= 15 Mg.Kg<sup>-1</sup> soil, M= Melatonin, M0= 0 Mg.L<sup>-1</sup>, M1= 50 Mg.L<sup>-1</sup>, M2=100 Mg.L<sup>-1</sup>, G= Glutathione, G0= 0 Mg.L<sup>-1</sup>, G1= 50 Mg.L<sup>-1</sup>, G2= 100 Mg.L<sup>-1</sup>, \*= Significant, N.S.= Non-Significant.





## 2. The number of leaves per plant (leaf . plant<sup>-1</sup>):

The results of Table (3) indicate significant differences at the 5% probability level between the averages of the leaf number trait as a result of treating the pepper plant with cadmium at two concentrations of 0 mg.kg.<sup>-1</sup>Soil and 15 mg.kg.<sup>-1</sup>Soil, it is also clear from the results of the same table that the lowest average for the number of leaves, which amounted to 26.59 leaves, was obtained from the 15 mg.kg treatment.<sup>-1</sup>Soil, with a reduction rate of 53.67% compared to the treatment without adding cadmium, 0 mg.kg.<sup>-1</sup>Soil had the highest average, amounting to 57.40 sheets.

The reason for the decrease in this characteristic may be due to treatment with cadmiumTo the harmful effect of this element on both plant height, Table (2) and leaf area, Table (4), which consequently led to a decrease in the rate of photosynthesis, a decrease in its products, and then a general decline in various growth characteristics, including the number of leaves. These research results are consistent with what he found [31]

Significant differences at the 5% probability level were obtained between the averages of leaf number as a result of treatment with melatonin at concentrations of 0, 50, and 100 mg.L.<sup>-1</sup>It is clear from the results of Table (3) that the highest average for this trait was obtained from the treatment of adding melatonin at a concentration of 100 mg.L.<sup>-1</sup> It amounted to 51.72 leaves, with an increase of 53.38% and 27.54% compared to the control treatment of 0 mg.l.<sup>-1</sup> The concentration is 50 mg.L.<sup>-1</sup> Respectively, the results show that the lowest average was from the treatment without adding melatonin, 0 mg.L.<sup>-1</sup> Its amount was 33.72 pages.

The reason for the increase in the number of leaves as a result of treatment with melatonin To the efficiency of melatonin in alleviating damage resulting from various environmental stresses, including heavy metals, increasing gene expression, which includes cell division and elongation, and increasing the efficiency of photosynthesis and cellular metabolism, as well as hormonal balance and thus a significant increase in various growth characteristics, including the number of leaves [7, 32, 33].

The results of the statistical analysis indicate significant differences at the 5% probability level between the averages of the leaf number trait as a result of treating pepper plants with glutathione at concentrations of 0, 50, and 100 mg.L.<sup>-1</sup> It is clear from Table (3) that the highest average for this trait, which amounted to 46.33 leaves, was obtained as a result of





treating the plants with a concentration of  $100 \text{ mg.L}^{-1}$ . Increase rates reached 23.94% and 9.60% compared to the concentration of  $0 \text{ mg.L}^{-1}$ . The concentration is  $50 \text{ mg.L}^{-1}$ . Respectively, it is clear from the values displayed in the same table that the lowest average was obtained from the treatment without adding glutathione,  $0 \text{ mg.L}^{-1}$ . Its amount was 37.38 pages.

The reason for the increase in the number of leaves as a result of treatment with glutathione may be due to its role in enhancing the plants' tolerance to various stresses through its role in suppressing free radicals, increasing the activity of antioxidants, and raising the plant's content of photosynthetic pigments, thus raising the efficiency of the photosynthesis process and improving the characteristics of the plant. Vegetative growth of the plant, including the number of leaves [34]. The results of this research are consistent with what was found [35,36].

Significant differences at the 5% probability level between the averages of the number of pepper plant leaves were obtained as a result of the binary interactions between cadmium, melatonin, and glutathione. It is clear from Table (3) that the highest averages for this trait were obtained from the combinations consisting of  $0 \text{ mg cadmium. kg}^{-1}\text{Soil} + \text{melatonin } 100 \text{ mg.l}^{-1}$  and  $0 \text{ cadmium mg.kg}^{-1}\text{Soil} + \text{glutathione } 100 \text{ mg.l}^{-1}$ . And melatonin  $100 \text{ mg.l}^{-1} + \text{Glutathione } 100 \text{ mg.L}^{-1}$ . These averages reached 71.44, 61.66, and 59.33 sheets, respectively, while the blends consisting of cadmium recorded  $15 \text{ mg.kg}^{-1}\text{Soil} + \text{melatonin } 0 \text{ mg.L}^{-1}$ . And  $15 \text{ cadmium mg.kg}^{-1}\text{Soil} + \text{glutathione } 0 \text{ mg.l}^{-1}$ . And melatonin  $0 \text{ mg.L}^{-1} + \text{Glutathione } 0 \text{ mg.L}^{-1}$ . The lowest averages were 22.22, 23.22, and 30.83 sheets, respectively.

It is clear from the results of Table (3) that there are significant differences at the 5% probability level between the means of this trait as a result of the triple interaction between cadmium, melatonin and glutathione. It is clear from the results that the highest mean was the result of the combination consisting of  $\text{cadmium } 0 \text{ mg.kg}^{-1}\text{Soil} + \text{melatonin } 100 \text{ mg.l}^{-1} + \text{Glutathione } 100 \text{ mg.L}^{-1}$ . Its amount was 77.33 sheets, while the mixture consisting of  $\text{cadmium recorded } 15 \text{ mg.kg}^{-1}\text{Soil} + \text{melatonin } 0 \text{ mg.l}^{-1} + \text{Glutathione } 0 \text{ mg.L}^{-1}$ . The lowest average value was 20.33 papers.



**Table 3:** Effect of cadmium, melatonin, and glutathione and their interaction on the number of leaves (leaf.plant<sup>-1</sup>)

C	M	G			C+M	Average Of C Effect
		G0	G1	G2		
C1	M0	41.33	46.00	48.33	45.22	57.40
	M1	51.66	55.66	59.33	55.55	
	M2	61.66	75.33	77.33	71.44	
C2	M0	20.33	22.33	24.00	22.22	26.59
	M1	23.33	25.66	27.66	25.55	
	M2	26.00	28.66	41.33	32.00	
C+M+G		LSD: C+M+G = 5.69 *			LSD: C+M=4.72*	LSD: C= 0.898 *
G + C						---
C1		51.55	59.00	61.66	LSD: C+G = 4.72 *	
C2		23.22	25.55	31.00		
G + M					Average of M Effect	
M0		30.83	34.17	36.17	33.72	
M1		37.50	49.67	43.50	40.55	
M2		43.83	52.00	59.33	51.72	
LSD value		LSD: M*G = 5.55			LSD: M = 1.100 *	
Averge of G Effect		37.38	42.27	46.33	---	
LSD value		LSD: G = 1.100 *				

Note: C= Cadmium, C1= 0 Mg.Kg<sup>-1</sup> soil, C2= 15 Mg.Kg<sup>-1</sup> soil, M= Melatonin, M0= 0 Mg.L<sup>-1</sup>, M1=50 Mg.L<sup>-1</sup>, M2=100Mg.L<sup>-1</sup>, G= Glutathione, G0= 0 Mg.L<sup>-1</sup>, G1=50 Mg.L<sup>-1</sup>, G2= 100 Mg.L<sup>-1</sup>, \*= Significant, N.S.= Non-Significant.

### 3. Leaf Area (cm<sup>2</sup>)

It is clear from the results of Table (4) that there are significant differences at the 5% probability level between the averages of the leaf area characteristic of the pepper plant as a result of its treatment with cadmium. It is clear from the results shown in the table that there is a decrease in the leaf area characteristic as a result of treatment with cadmium at a concentration of 15 mg.kg.<sup>-1</sup>Soil, where a cadmium addition treatment was recorded at a concentration of 15 mg.kg.<sup>-1</sup>Soil average minimum 14.38 cm<sup>2</sup> A decrease rate of 55.91% compared to the control treatment of 0 mg.kg.<sup>-1</sup>Soil recorded the highest average amounting to 32.62 cm<sup>2</sup>. The reason for the decrease in the leaf area of the plant as a result of its treatment with cadmium is that this element is toxic element. Its high concentrations cause a decrease in the rate of photosynthesis, enzymatic activity, and meristematic cell division, absorption of water and various nutrients, and cell expansion. Thus, these factors together lead to a decrease in the paper space [37, 38].



There are also significant differences between the averages of leaf area at the 5% probability level obtained as a result of treating the plant with melatonin. It is clear from the results of Table (4) that the highest average obtained was as a result of the treatment with the concentration of 50 mg.l.<sup>-1</sup>. This addition recorded an average of 24.86 cm<sup>2</sup>. An increase of 18.94% and 0.48% compared to the control treatment of 0 mg.L.<sup>-1</sup>. The concentration is 100 mg.L.<sup>-1</sup> respectively, while the control treatment recorded the lowest average, amounting to 20.90 cm<sup>2</sup>. The reason for the increase in leaf area of pepper plants treated with melatonin is attributed to the role of melatonin in increasing the efficiency of the photosynthesis process [39, 40], in addition to its action similar to that of auxin in plants, as it increases the use of melatonin of leaf area by increasing the number and size of cells [41]. The hormone melatonin also works to enhance plant tolerance to heavy metal stress, as melatonin acts as a powerful antioxidant and biostimulant, and has been identified by many studies as a growth regulator for plants [42].

It is evident from the results of Table (4) that there are significant differences at the 5% probability level between the average leaf area of the plant as a result of treating the pepper plant with glutathione at concentrations of 0, 50, and 100 mg.l.<sup>-1</sup>. It is clear from the results of the same table that the highest average for this characteristic is 26.46 cm<sup>2</sup>. The result of treatment with glutathione at a concentration of 100 mg.L was obtained with increased rates of 24.69% and 15.95% compared to the control treatment, 0 mg.L.<sup>-1</sup>. The concentration is 50 mg.L.<sup>-1</sup> respectively, while the lowest average for this trait was the result of the treatment without adding glutathione and amounted to 21.22 cm<sup>2</sup>. The reason for the increase in leaf area is due to treating the plant with glutathione to the role of glutathione in increasing the plant's tolerance to various abiotic stresses, such as drought, salinity, and the toxicity of heavy metals, including cadmium, which cause oxidative stress as a result of the formation of free radicals, and because glutathione is one of the antioxidants that work to suppress the activity of free radicals, which consequently leads to improving the various growth characteristics of the plant. Including the characteristics of paper space [43].

Significant differences at the 5% probability level between the averages for leaf area were the result of the binary interaction between cadmium, melatonin, and glutathione. It is clear from



the results of Table (4) that the best averages for leaf area were obtained as a result of the combination consisting of cadmium 0 mg.kg.<sup>-1</sup> Soil + melatonin 100 mg.l<sup>-1</sup>. The combination consisting of cadmium is 0 mg.kg.<sup>-1</sup> Soil + glutathione 100 mg.l<sup>-1</sup>. The combination consists of melatonin 100 mg.l<sup>-1</sup> + Glutathione 100 mg.l<sup>-1</sup>. Their amounts reached 34.29, 34.75, and 29.73 cm<sup>2</sup> in a row.

Table (4) shows significant differences at the 5% probability level between the average plant leaf area obtained as a result of the triple interaction between cadmium, melatonin, and glutathione. The results indicate that the best average for this trait was obtained as a result of the combination consisting of cadmium 0 mg.kg.<sup>-1</sup> Soil + melatonin 100 mg.l<sup>-1</sup> + Glutathione 100 mg.l<sup>-1</sup>. Its size was 37.33 cm<sup>2</sup> while the combination consisting of cadmium recorded 15 mg.kg of soil<sup>-1</sup> + Melatonin 100 mg.l<sup>-1</sup> + Glutathione 50 mg.l<sup>-1</sup>. The lowest average was 7.93 cm<sup>2</sup>.

**Table 4:** Effect of cadmium, melatonin, and glutathione and their interaction on leaf area (cm<sup>2</sup>)

C Mg.Kg <sup>-1</sup> soil	M Mg.L <sup>-1</sup>	G Mg.L <sup>-1</sup>			C+M	Average Of C Effect
		G0	G1	G2		
C1	M0	28.42	29.24	33.92	30.53	32.62
	M1	30.86	34.67	33.01	32.84	
	M2	31.10	35.03	37.33	34.49	
C2	M0	8.35	12.11	13.36	11.27	14.38
	M1	13.66	17.96	19.00	16.87	
	M2	14.95	7.93	22.14	15.01	
C+M+G		LSD: C+M+G = 6.988 *			LSD: C+M=4.70*	LSD: C=2.329*
G + C						---
C1		30.13	32.98	34.75	LSD: C+G = 4.701 *	
C2		12.32	12.67	18.17		
G + M					Average of M Effect	
M0		18.38	20.67	23.64	20.90	
M1		22.26	26.31	26.01	24.86	
M2		23.02	21.48	29.73	24.74	
LSD value		LSD: M+G = 6.071 *			LSD: M =2.853	
Averge of G Effect		21.22	22.82	26.46	---	
LSD value		LSD: G = 2.853 *				

Note: C= Cadmium, C1= 0 Mg.Kg<sup>-1</sup> soil, C2= 15 Mg.Kg<sup>-1</sup> soil, M= Melatonin, M0= 0 Mg.L<sup>-1</sup>, M1=50 Mg.L<sup>-1</sup>, M2=100Mg.L<sup>-1</sup>, G= Glutathione, G0= 0 Mg.L<sup>-1</sup>, G1=50 Mg.L<sup>-1</sup>, G2= 100 Mg.L<sup>-1</sup>, \*= Significant, N.S.= Non-Significant.



## Conclusion

Through the results of the research, we conclude that the harmful effect of cadmium on plant growth, represented by a decline in its vegetative characteristics, as well as the positive effect of melatonin and glutathione in suppressing the activity of free radicals such as reactive nitrogen (RNS) and reactive oxygen (ROS) resulting from oxidative stress caused by elemental pollution. Heavy metals, including cadmium, and glutathione are one of the most important non-enzymatic antioxidants, while melatonin acts as a first line of defense against any dangerous conditions to which the organism is exposed, such as exposure to various abiotic stresses, including contamination with heavy metals.

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