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# Effect of Melatonin on Concentrations of Some Elements and Solubility Substance in *Vigna radiata* (L.) R. Wilczek Plant Affected by Drought Stress

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**Abstract**

Melatonin, a well-known plant growth regulator, has gained attention for its multifaceted role in enhancing plant resilience to environmental stresses. A field experiment was conducted during the 2021–2022 growing season in the Botanical Garden of the Department of Biology at the College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, to investigate the effects of foliar spray with varying concentrations of melatonin (0, 50, 100, 150, and 200 ppm) on mung bean (*Vigna radiata* L.) under drought stress condition. Drought conditions were simulated through three irrigation intervals: every 5 days (control), 10 days, and 15 days. The results indicated that extending the irrigation interval significantly reduced levels of critical plant characteristics, including nutrient and carbohydrate content. However, foliar application of melatonin at increased concentrations effectively mitigated drought stress, enhancing the rates of nitrogen, potassium, phosphorus, protein, and carbohydrate contents in mung beans. Melatonin at 150 ppm proved most effective, leading to notable increases of 75.19% in soluble protein, 47.1% in carbohydrate content, 8.3% in nitrogen content, and 41.4% in potassium content compared to control plants. In contrast, drought stress reduced potassium, protein, and carbohydrate levels by 41.3%, 42.2%, and 11.0%, respectively. These findings highlight melatonin's substantial mitigating effects on nutrient losses and stress-related declines in plant health.



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## 1. INTRODUCTION

Mung Bean, a legume of the family Fabaceae, is one of the most essential edible legume crops. It is a branched annual herbaceous plant. The stem is up to 125 cm long, with three leaflets roughly triangular to ovate. Its flowers are yellow to green and gathered inside racemes inflorescences containing several small flowers. The legume fruits, borne in pendulous whorls, are long and straight, and they turn dark at maturity; each pod holds up to 20 tiny seeds, covered with a glabrous yellow color, which turns brown at maturity. It is cultivated for its edible seed used as animal feed and as fertilizer that improves the properties of the soil, and its taproots bear bacterial nodes [1]. Agriculturally, it maintains soil fertility by providing nitrogen, and its leaves provide approximately 37-40 kg of nitrogen per hectare of soil [2]. Mung bean is highly nutritious and economical because its seeds contain carbohydrates of up to 65% in starch and sugars [3] and high protein concentrations of up to 29% in addition to amino acids [4]. The decrease in rainfall in recent years has led to an increase in the problem of drought in large areas of the world, which has led to a decrease in the water content in the soil and made plants suffer from severe water deficits [5]. Drought is a harmful stress that affects plant growth and development and causes significant productivity losses because it leads to oxidative stress and excessive release of free radicals, leading to significant damage to cells and their death [6].

Melatonin is an indole compound derived from the serotonin of the amino acid tryptophan and is chemically known as N-acytyl-5-methoxytryptoamin. It was discovered in plants in 1995 and is considered a direct antioxidant that scavenges free radicals with high efficiency [7]. Treatment with melatonin has been observed to modulate antioxidant enzymes by increasing the regulation of both levels of transcription and activity, and the regeneration of these antioxidants such as glutathione (GSH) and vitamins E, C and treatment with melatonin has been observed to suppress the peroxide root $H_2O_2$ [8, 9].

Melatonin, a well-known plant growth regulator, has gained attention for its multifaceted role in enhancing plant resilience to environmental stresses. So, this study aims to highlight more evidence about the effectiveness of melatonin foliar spray in increasing the resistance of mung bean plants affected by drought stress. By examining melatonin's impact on critical biochemical parameters—such as macro-nutrients, protein, and carbohydrate levels—this research seeks to elucidate melatonin's potential to optimize nutrient content and metabolic resilience in mung beans to modulate abiotic impact and contribute to sustainable agricultural practices that improve crop productivity in drought-prone regions.

## 2. METHOD

### 2.1 The experimental design:

The experiment was designed according to the Randomized Complete Block Design (RCBD) with three replicates, i.e., a total number of 45 experimental units, noting that the treatment of one experimental unit is 1.8 $m^2$ and dimensions of 1.20×1.50 m. Healthy uniform seeds were sown on 1/7/2021. The drought stress was conducted in three irrigation intervals (5 [control], 10, 15 days) between irrigation.

### 2.2 Melatonin Application:

The melatonin concentrations were prepared after preparing the standard solution by dissolving 1 g of melatonin (USA origin) in a liter of distilled water according to the dilution formula. Plants were well-foliar sprayed with melatonin (0, 50, 100, 150, and 200 ppm) on 15/07/2021 early morning [10].

### 2.3 Biochemical measurements:

Three random plant samples were harvested on 20/09/2021. The samples were oven-dried and digested [11]; after that, the samples were obtained on an acidic extract ready for determination. Total nitrogen content (mg/plant) was measured using the Kjeldahl method, which involves digesting the sample with sulfuric acid to convert nitrogen into ammonia. The ammonia is then distilled and quantified by titration with a standard acid solution [12]. Phosphorus content (mg/plant) was determined by adding molybdate reagent to the sample, followed by the addition of ascorbic acid to form a blue complex. The absorbance of the solution is measured at 880 nm and compared to a calibration curve to calculate the phosphorus content. [13]. Potassium content (mg/plant) was determined using a flame photometer, extracting the sample with a neutral ammonium acetate solution [14]. Protein content (%) was determined using the Kjeldahl method, which involves digesting the sample with sulfuric acid to release nitrogen compounds and then measuring the total nitrogen content. The protein content is estimated by multiplying the total nitrogen by a factor "6.25" [15]. The anthrone method determined carbohydrate content (%), where the sample is first hydrolyzed to break down the carbohydrates into simpler sugars. The resulting sugars react with anthrone reagent in the presence of concentrated sulfuric acid to form a greenish-blue complex, which is then measured spectrophotometrically at 620 nm [16].

### 2.4 Statistical Analysis:

The results of this study were analyzed statistically using SPSS V.23. The significant differences between the mean were compared using the least significant difference (LSD) test under the probability level of $P < 0.05$ [17].

## 3. RESULTS AND DISCUSSION

The results of Table 1 indicate that drought stress did not have any significant results on the means of nitrogen content. As for melatonin, the table results show a significant effect on increasing nitrogen content. It was observed that when the concentration of melatonin increased from zero ppm to 150 ppm, the rate of nitrogen content increased from 4.06% to 5.91%, with an increase of ratio of 45.66% compared to the control treatment, because melatonin may be attributed to its role in enhancing and regulating the activities of nitrogen absorption and enzymes associated with metabolism [18]. This is consistent with results obtained from the winter wheat [19].

Table 1. Effect of melatonin on mung bean plant's nitrogen content affected by drought stress.

| Melatonin mean | Irrigation interval (day) | | | Melatonin Mean |
|---|---|---|---|---|
| | 5 | 10 | 15 | |
| 0 | 4.72±0.7 | 4.14±1.15 | 3.32±0.58 | 4.06±1.76 |
| 50 | 4.84±0.85 | 4.20±0.58 | 4.26±1.01 | 4.43±1.35 |
| 100 | 5.25±0.6 | 4.38±0.88 | 4.31±1.17 | 4.65±1.17 |
| 150 | 5.42±0.7 | 4.37±0.9 | 5.46±0.82 | 5.08±2.13 |
| 200 | 6.30±1.07 | 6.12±1.1 | 5.31±0.89 | 5.91±1.46 |
| Irrigation Mean | 5.31±0.56 | 4.64±0.76 | 4.53±0.66 | |
| LSD 0.05 | Melatonin 0.73 | Irrigation N.S | Interaction 1.27 | |
| SE = 0.76 | | SD = 1.08 | | |

The results of the same table indicate that the interaction between drought stress and melatonin was significant, and melatonin mitigated the severity of drought and increased the value of nitrogen content in the mung bean plant. The highest value was observed when irrigation was done every 5 days, and the concentration of melatonin was 200 ppm, which amounted to 6.30%, while the lowest nitrogen content was when irrigation was done every 15 days and without melatonin, which amounted to 3.32%.

The results of Table 2 indicate that drought stress did not have any significant results in the rate of phosphorus concentration. The results of the same table indicate that the increased melatonin concentrations had no significant effect on the mean phosphorus concentration. The same table showed that the interaction between drought stress and increased melatonin concentrations significantly mitigated the adverse effects of drought and increased the concentration of phosphorus rate. The results indicate that the highest mean of phosphorus was at irrigation every 10 days, and the concentration of melatonin was 150 ppm, which amounted to 0.487% compared to control plants. The lowest phosphorus result was obtained at irrigation every 15 days, and the melatonin concentration was 50 ppm, which was 0.177%.

Table 2. Effect of melatonin on phosphorus content of mung bean affected by drought stress.

| **Melatonin concentration** | **Irrigation interval (day)** | | | **Melatonin Mean** |
|---|---|---|---|---|
| | 5 | 10 | 15 | |
| 0 | 0.267±0.02 | 0.223±0.01 | 0.267±0.01 | **0.252±0.01** |
| 50 | 0.287±0.01 | 0.463±0.13 | 0.177±0.09 | **0.309±0.08** |
| 100 | 0.290±0.00 | 0.243±0.01 | 0.300±0.04 | **0.300±0.02** |
| 150 | 0.317±0.00 | 0.487±0.11 | 0.247±0.003 | **0.350±0.07** |
| 200 | 0.317±0.003 | 0.220±0.00 | 0.297±0.02 | **0.278±0.03** |
| Irrigation Mean | 0.295±0.01 | 0.327±0.06 | 0.257±0.02 | |
| LSD 0.05 | Melatonin N.S | Irrigation N.S | Interaction 0.146 | |
| SE=0.072 | | SD=1.42 | | |

The results of Table 3 show that the drought stress from 5 days to 15 days significantly reduced the mean of potassium content from 4.74% to 2.78%, with a decrease of 41.35% compared to the control treatment. The reason for the decrease may be that drought leads to inhibition of plant growth in general by reducing the absorption of water and nutrients from the soil, which leads to a decrease in the concentration of elements within the plant [20], and these results are consistent with [20] on corn plant *Zea mays* L. Melatonin had a significant effect on increasing the mean of potassium of the mung bean plant under drought stress conditions. It was noted that when the melatonin concentration increased from 0 ppm to 200 ppm, the mean potassium content increased from 2.80% to 3.99%, with an increase of 42.50% compared to control plants. The increase is attributed to melatonin's vital role in plant growth and development during biotic stress, where gene expression analysis revealed that melatonin treatment significantly enhanced the transcription of potassium transport genes under stress conditions [21]. The same results were obtained when melatonin was applied to *Moringa oleifera* L. plant [22].

Table 3. Effect of melatonin on phosphorus content of mung bean affected by drought stress.

| Melatonin concentration | Irrigation interval (day) | | | Melatonin Mean |
|---|---|---|---|---|
| | 5 | 10 | 15 | |
| 0 | 4.76±0.004 | 1.82±0.00 | 1.82±0.04 | **2.80±0.98** |
| 50 | 4.55±0.29 | 3.85±0.26 | 2.69±0.16 | **3.70±0.54** |
| 100 | 4.61±0.19 | 2.64±0.78 | 2.54±0.71 | **3.26±0.67** |
| 150 | 5.11±0.02 | 2.73±0.96 | 2.08±0.15 | **3.31±0.92** |
| 200 | 4.65±0.19 | 2.53±0.80 | 4.80±0.05 | **3.99±0.73** |
| Irrigation Mean | 4.74±0.10 | 2.72±0.33 | 2.78±0.53 | |
| LSD 0.05 | Melatonin 0.96 | Irrigation 0.53 | Interaction 1.20 | |
| SE=0.08 | | SD=0.11 | | |

Interaction between drought stress and melatonin had a significant effect in mitigating the harmful effects of drought and increasing the value of potassium content in the vegetative part of the mage plant. The results show that the highest value was when irrigation was done every 5 days and the concentration of melatonin was 50 ppm, which amounted to 5.11%, while the lowest rate of concentration of the element was when treating irrigation every 15 days and the concentration of melatonin is zero ppm which amounted to 1.82%. The results of Table 4 indicate that drought stress significantly affected the decrease in the percentage of protein in the vegetative part of the mung bean plant.

Table 4. The effect of melatonin on the protein percentage of the mung bean plant affected by drought stress.

| Melatonin concentration | Irrigation interval (day) | | | Melatonin Mean |
|---|---|---|---|---|
| | 5 | 10 | 15 | |
| 0 | 18.76±0.004 | 17.43±0.23 | 14.82±0.17 | **16.81±1.05** |
| 50 | 19.11±0.02 | 18.84±0.10 | 17.08±0.20 | **18.56±0.54** |
| 100 | 22.61±0.19 | 22.23±0.17 | 19.54±0.13 | **21.57±1.20** |
| 150 | 31.55±0.29 | 36.90±0.17 | 20.69±0.47 | **29.45±4.50** |
| 200 | 27.65±0.19 | 23.80±3.10 | 19.80±0.07 | **23.63±2.35** |
| Irrigation Mean | 23.74±0.10 | 1.09±3.34 | 18.78±1.05 | |
| LSD 0.05 | Melatonin 1.41 | Irrigation 1.09 | Interaction 2.44 | |
| SE=1.46 | | SD=5.74 | | |

When the irrigation intervals increased from 5 to 15 days, the means of protein content decreased from 23.83% to 18.38%, with a decrease of 42.22% compared to the control treatment. The reason may be attributed to the fact that drought stress has led to an increase in the activity of some enzymes that increase the reduction of nucleic acids and the breakdown of ribosomes, for example, the enzyme Lipoxygenase, Protase and RNAse, which caused a decrease in the percentage of protein, in addition to the role of drought in increasing the accumulation of free radicals with a toxic effect to the cell, as well as leading to a disorder in the binding of nucleic acids, which leads to inhibition of protein synthesis [23, 24].

This is consistent with [25] on Wheat *Triticum aestivum* L. The results of the same table indicate that the treatment with melatonin significantly increased the protein concentration in the vegetative part of the mung bean plant. It was noted that the increase was in all concentrations exceeding 150 ppm if the protein rate increased from 16.81% at zero ppm to 29.45% at 150 ppm concentration and an increase of 75.19% compared to the control treatment. This may be because melatonin has an activity similar to auxin [26, 27, 28], which stimulates protein synthesis by increasing the average amino acid synthesis. This is consistent with data obtained from chickpeas [29]. The results of Table 4 indicate that the interaction between drought stress and foliar spray with melatonin was significant. It was noted that the highest percentage of protein concentration in the vegetative part was when treating irrigation every 10 days, and the concentration of melatonin was 150 ppm, which amounted to 36.23%, while the lowest value of protein concentration was when treating irrigation every 15 days and the concentration of melatonin 0 ppm, which amounted to 14.77%.

The results of Table 5 indicate that drought stress had a significant effect in reducing the rate of dissolved carbohydrates in the vegetative part, as the results indicate that the increase in irrigation intervals from 5 to 15 days led to a reduction in the rate of carbohydrates from 38.08 % to 33.93% and a decrease rate of 10.99% compared to control plants. The drought-induced decrease in carbohydrate levels may be due to the accumulation of radical oxygen species (ROS), which increase the production of digestive enzymes such as lipoxygenase that block carbohydrate aggregation [30].

In addition, drought stress reduced chlorophyll and most of the primary growth parameters, leading to reduced photosynthesis and reduced carbohydrate synthesis, and these results are consistent with [29] on the Chickpea plant.

Table 5. Effect of melatonin on mung bean plant carbohydrate content affected by drought stress.

| **Melatonin concentration** | **Irrigation interval (day)** | | | **Melatonin Mean** |
|---|---|---|---|---|
| | 5 | 10 | 15 | |
| 0 | 33.30±0.20 | 30.60±0.50 | 27.53±0.23 | **30.48±1.67** |
| 50 | 34.57±0.23 | 33.50±0.20 | 29.63±0.23 | **32.57±1.5** |
| 100 | 37.10±0.20 | 39.03±0.33 | 33.90±0.30 | **36.68±1.5** |
| 150 | 44.73±0.20 | 48.77±0.33 | 41.03±0.53 | **44.84±2.24** |
| 200 | 40.70±0.63 | 46.47±0.27 | 37.57±0.47 | **41.58±2.61** |
| Irrigation Mean | 38.08±0.40 | 39.67±0.53 | 33.93±2.48 | |
| LSD 0.05 | Melatonin 0.48 | Irrigation 0.37 | Interaction 0.83 | |
| SE=0.49 | | SD=6.13 | | |

The results of the same table indicate that the external treatment with different concentrations of melatonin significantly increased the percentage of dissolved carbohydrates in the vegetative part of the mung bean plant. It was observed that when increasing the concentration of melatonin from zero ppm to 150 ppm, the rate of dissolved carbohydrates increased from 30.45% to 44.84%, with an increase of 47.11% compared to control plants. This may be due to the role of melatonin in promoting chlorophyll, which leads to an increase in the production of total carbohydrates in the plant, accompanied by an improvement in the quality and quantity of the crop [29]. These results are consistent with [29] on chickpea plants. The table indicates that the interaction was significant as melatonin mitigated the effect of drought and increased carbohydrate concentration. It was noted that the highest rate of carbohydrate concentration at the treatment concentration of melatonin 150 ppm and drought 10 days, amounted to 48.77%. The lowest carbohydrate concentration was at dehydration treatment every 15 days, and the concentration of melatonin was zero ppm, with a value of 27.53%.

The results of the same table indicate that the external treatment with different concentrations of melatonin significantly increased the percentage of dissolved carbohydrates in the vegetative part of the mung bean plant. It was observed that when increasing the concentration of melatonin from zero ppm to 150 ppm, the rate of dissolved carbohydrates increased from 30.45% to 44.84%, with an increase of 47.11% compared to control plants. This may be due to the role of melatonin in promoting chlorophyll, which leads to an increase in the production of total carbohydrates in the plant, accompanied by an improvement in the quality and quantity of the crop [29]. These results are consistent with [29] on chickpea plants. The table indicates that the interaction was significant as melatonin mitigated the effect of drought and increased carbohydrate concentration. It was noted that the highest rate of carbohydrate concentration at the treatment concentration of melatonin 150 ppm and drought 10 days, amounted to 48.77%. The lowest carbohydrate concentration was at dehydration treatment every 15 days, and the concentration of melatonin was zero ppm, with a value of 27.53%.

## 4. CONCLUSION

The results showed that *Vigna radiata* (L.) plant is one of the plants with the ability to tolerate moderate and medium water stress periods. It was observed that the spacing of dry periods from 5 to 15 days increased the concentration of dissolved carbohydrates in the vegetative part. The results indicated that drought stress led to the inhibition of plant growth by reducing the absorption of water and nutrients from the soil, which decreased the concentration of potassium elements and protein content. The investigation also concluded that the external treatment of melatonin, especially at concentrations of 150 ppm, mitigated the adverse impact of drought and increased the concentration of potassium, protein, and carbohydrate contents in the vegetative part of the plant. Based on the impact of melatonin on biochemical processes that enhance plant resilience under water.

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