Effect of foliar spraying of Brassenolide and Melatonin on the growth and productivity of faba beans (Vicia faba L.)

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Abstract

A field experiment was conducted during the winter season (2023-2024) in the Ramadi city of western Iraq. To know the effect of Brassinolide and Melatonin on the growth characteristics and yield of faba bean. A randomized complete block design (RCBD) arranged according to split-plots used with three replicates and 36 experimental units, four concentrations of Brassinolide (0, 2, 4 and 6 mg L^{-1}) were used in Main-plots (B0, B1, B2, B3) respectively, while three concentrations of Melatonin (0, 100 and 200 mg L^{-1}) were used in sub-plots (M0, M1, M2) respectively. The statistical analysis results showed significant differences among the concentrations of the study factors in most of the traits. The results of spraying with Brassinolide were superior at B2 concentration for the following traits: Plant height (85.44 cm), number of branches (11.44 branch $plant^{-1}$), leaf area (4682 cm^2), number of pods (22.98 pod $plant^{-1}$), number of grains per pod (5.13 grain pod^{-1}) weight of 100 grains (137.26 g) and grains yield of the plant (193.6 g). The results of the Melatonin spray treatment showed superiority at the M2 concentration for the following traits: Number of branches (12,17 branch $plant^{-1}$), leaf area (4786 cm^2), number of pods (22.33 pod $plant^{-1}$), number of grains per pod (4.92 grain pod^{-1}), weight of 100 grains (136.58 g) and grains yield of plant (173.7 g). The results of overlap indicated a significant difference in the traits: Number of branches (B2 × M2 13.33 branch $plant^{-1}$), number of pods per plant (B2 × M2 23.53 pod $plant^{-1}$), number of grains per pod (B1 × M0 5.20 grain pod^{-1}), the weight of 100 grains (B1 × M0 137.83 g) and grains yield of the plant (B2 × M1 201 g).

Keywords: Brassinolide, Follicle, Melatonin, Spraying.

1. Introduction

Faba bean (*Vicia faba* L.) is a nutritionally and economically valuable legume, known for its substantial protein content ranging between 25% and 40% [1]. Data indicate that the cultivated area in Iraq amounted to 16,649 dunums, whereas the average crop yield reached 1,858.9 kg per dunum[2] . It also provides around 65% carbohydrates and contains a considerable amount of phytic acid [3]. Faba bean is used as a staple dietary food for human nutrition and in animals; it contains a lot of fiber, vitamins, minerals, and antioxidants [4]. The faba bean has an essential role in improving soil characteristics by fixing atmospheric nitrogen in the root nodules in coexistence with the bacterium Rhizobium [5]. Recently, researchers

ISSN 2072-3857

have turned their attention to employing essential growth stimulants to increase the yield of different plants because these stimulants are free from harmful effects on humans and the environment. It is a chemical and biological agent that enhances the plant's ability to absorb nutrients and other growth factors with high efficiency. The plant makes use of its internal physiological processes and genetic potential to the fullest in carrying out all metabolic activities, which in turn contributes significantly to the improvement of yield components and overall productivity per unit area [6]. Brassinolide is one of the most effective types of stimulants, as it plays an essential role in growth and development, which includes cell division and elongation, resistance to stress, delaying aging, which is reflected in through the assimilation of nitrogen from the atmosphere photosynthesis, increased flowering, growth of pollen tube and stimulation of pollen vitality, which is reflected in an increase crop productivity per unit are [7,8,9]. Melatonin also has an important role, no less critical than Brassinolide, as it is a non-toxic chemical compound classified as a growth regulator. Research indicates that Melatonin activates plant growth and development. Melatonin has various cellular and physiological effects on cells and membrane permeability. It also affects the processes of promoting growth, encouraging rooting, stimulating seed germination and photosynthesis [9]. Enhancing the effectiveness of growth regulators requires applying them at the appropriate time and concentration that effectively influences physiological processes. This study aims to determine the optimal concentration of the plant growth regulator Brassinolide that achieves the highest possible yield per unit area by evaluating crop responses to different application levels under the experimental conditions. Furthermore, The study seeks to analyze the nature of the interaction between Brassinolide and Melatonin, and to identify the most efficient combined treatment in enhancing quantitative productivity per unit land area, as well as improving the qualitative characteristics of the crop. This is undertaken within the framework of understanding the physiological and integrative roles of these two compounds in regulating plant growth and improving the efficiency of vital biological processes, thereby positively reflecting on yield, its components, and overall crop quality.

2. Materials and Methods

A field experiment was conducted during the 2023–2024 winter season. Sowing was carried out on November 23 in Ramadi, located at longitude (43.2992° E) and latitude (33.4258° N) in western Iraq, with the objective of evaluating the effects of Brassinolide and Melatonin treatments on the growth and yield of a Spanish cultivar of broad bean (Vicia faba L.). The cultivar Aquadulce, supplied by OVO American Seeds, was used in this study. The experiment followed a cluster-based randomized complete block design (RCBD) with a split-plot arrangement. It included three replications and 36 treatment units. Four concentrations of Brassinolide (0, 2, 4, and 6 mg/L^{-1}) were allocated to the main plots (B0, B1, B2, and B3) and applied at the beginning of the flowering stage. One week later, Melatonin treatments at three levels (0, 100, and 200 mg L^{-1}) were administered to the sub-plots (M0,M1, and M3).

The field was tilled in two perpendicular directions, then leveled and smoothed. Ridges were formed at a length of 2 meters, with 0.60 meters between ridges and 0.30 meters between plants, resulting in a planting density of 55,555 plants per hectare. Phosphorus fertilizer was applied at a rate of 150 kg P_2O_5 ha^{-1} at the time of sowing. Nitrogen was supplied in the form of urea (46% N) at a total rate of 60 kg N ha^{-1}, divided into two applications: the first one week after germination, and the second at the flowering stage [11].

The measured traits were evaluated based on individual plants selected from the midpoint of each treatment unit to ensure accuracy. Stem length (cm) was recorded from the soil level to the apex of the main stem. The number of shoots per individual was determined by counting the branches emerging from the crown area, using the average of five randomly selected plants. Additionally, five medium-sized plants were selected. The number of leaves on each was counted, and the area of each leaf (cm^2) was measured at the pod formation stage. Leaf area was estimated using the equation: LA = 0.624 + 0.723 × (length × width) [12].

The leaf area was determined by the equation LA = 0.624 + 0.723 × (length × width), where LA is the leaf area measured in cm^2, L represents the leaf length (cm), and W is the maximum width of the leaf measured in cm. Total leaf area per plant was then calculated by multiplying the average leaf size by the total number of leaves. Chlorophyll content in these leaves at the stage of flowering (SPAD) was then measured using SPAD readings. Three readings were taken and the average of five plants per experimental unit was calculated. Other crop components were evaluated, such as the number of pods per plant, the number of seeds per pod, the mass of 100 seeds (g), and the grain yield per plant (g). The collected data were subjected to statistical analysis, and the Least Significant Difference (LSD) test was applied at the 0.05 probability level to assess the significance of differences among treatment means [13].

3. Results and Discussion

The results presented in Table (1) indicate that foliar application of the plant growth regulator Brassinolide resulted in significant differences in plant height. The treatment B2 (at a concentration of 4 mg L^{-1}) recorded the highest mean plant height, reaching 85.44 cm. In contrast, Melatonin treatments did not show any significant differences for this trait, and no significant interaction was observed between Brassinolide and Melatonin. The observed positive effect is attributed to the physiological role of Brassinolide in stimulating meristematic

ISSN 2072-3857

activity, which enhances cell division and elongation, thereby promoting increased plant height [8, 9]

Table 1: Effect of Brassinolide and Melatonin in plant height (cm) of faba bean.

Brassinolide / Melatonin	B0	B1	B2	B3	**Mean**
M0	82.67	79.17	88.67	71.00	80.38
M1	82.67	80.33	80.67	73.00	79.17
M2	81.67	80.67	87.00	74.33	80.92
Mean	82.33	80.06	85.44	72.78	
LSD 0.05	Brassinolide = 6.03		Melatonin = NS		
	B×M =NS				

NS= no significant

"The results of the analysis of variance presented in Table (2) indicate that different foliar application levels of Brassinolide caused significant variation in the number of branches in the bean plant. The B3 treatment recorded the highest mean number of branches, reaching 11.44 branches per plant. Likewise, Melatonin concentration M2 showed a significant effect, recording the highest value of 12.17 branches per plant. The results also revealed a significant interaction between the two factors, where the combination of B3 and M2 achieved the highest mean number of branches per plant (13.33). This increase in branch number is attributed to the physiological role of both Brassinolide and Melatonin in stimulating and activating dormant basal buds. This effect is likely associated with enhanced meristematic cell division, which promotes the development of both the root system and the vegetative growth of the plant [14]."

Table 2: Effect of Brassinolide and Melatonin in the number of branches (branch plant^{-1}) of faba bean.

Brassinolide / Melatonin	B0	B1	B2	B3	**Mean**
M0	7.33	8.67	8.67	8.33	8.28
M1	9.00	9.00	12.33	9.00	9.83
M2	11.67	11.67	13.33	12.00	12.17
Mean	9.33	9.78	11.44	9.78	
LSD 0.05	Brassinolide = 1.40		Melatonin = 0.61		
	B×M =1.58				

ISSN 2072-3857

The analysis of variance presented in Table (3) shows that foliar application of Brassinolide and Melatonin resulted in a significant increase in the number of leaves per plant. The B2 treatment recorded the highest mean number of leaves, reaching 183 leaves per plant. Significant differences were also observed among Melatonin treatments, where M2 achieved the highest mean value of 194.1 leaves per plant. Furthermore, a significant interaction between the two factors was recorded, as the combination B2 × M2 produced the highest mean number of leaves, reaching 228 leaves per plant. This increase in leaf number is attributed to the role of Brassinolide in enhancing cell division and elongation, in addition to its effect on plant height (Table 1), which collectively contributed to an increase in leaf production per plant. These findings are in agreement with Sivakumar and Surendar [15], who reported that increasing Brassinolide concentrations significantly improved leaf number

Table 3: Effect of Brassinolide and Melatonin in the number of leaves (leaf plant^{-1}) of faba bean.

Brassinolide / Melatonin	B0	B1	B2	B3	**Mean**
M0	119.7	133.3	126.0	141.0	130.0
M1	155.7	156.7	195.0	145.7	163.2
M2	181.0	177.0	228.0	190.3	194.1
Mean	152.1	155.7	183.0	159.0	
LSD 0.05	Brassinolide = 17.65		Melatonin = 11.91		
	B×M =24.29				

The relatively increased leaf area reflects the plant’s enhanced capacity for assimilate production, resulting from improved photosynthetic efficiency and the accumulation of photosynthates, which are subsequently translocated to storage organs (i.e., grains), thereby contributing to yield improvement. The analysis of variance presented in Table (4) indicates that foliar application of brassinolide had a significant effect on leaf area, with treatment B2 recording the highest mean value of 4682 cm². Similarly, significant differences were observed among melatonin concentrations, where treatment M2 achieved the highest mean value of this trait, reaching 4786 cm². No significant interaction effect between brassinolide and melatonin treatments was detected.The superiority of the higher concentrations of both brassinolide (B2) and melatonin (M2) in increasing leaf area may be attributed to their stimulatory role in enhancing vegetative growth, as evidenced by the increased leaf formation (as shown in Table 3), as well as their ability to promote cell division and cell elongation, ultimately leading to an expansion of leaf surface

ISSN 2072-3857

area. These findings are in agreement with those reported by [15, 16, 17, 18].

Table 4: Effect of Brassinolide and Melatonin in Leaf area (cm^2) of faba bean.

Brassinolide / Melatonin	B0	B1	B2	B3	**Mean**
M0	3365	3766	3454	3959	3636
M1	3187	3733	5031	3099	3763
M2	4240	4603	5562	4740	4786
Mean	3597	4034	4682	3933	
LSD 0.05	Brassinolide = 492.6		Melatonin = 562.3		
	B×M =NS				

NS= no significant

The analysis of variance presented in Table (5) indicates that foliar application of brassinolide resulted in significant differences in chlorophyll content, where treatment B3 recorded the highest chlorophyll value in leaves, reaching 52.44 SPAD units. This reflects the positive role of brassinolide in enhancing this trait as the concentration increased from 0 to 4 mg L^{-1} compared with the untreated control. In contrast, no significant differences were observed in the melatonin treatments, and no significant interaction effect between brassinolide and melatonin was detected.

The beneficial effect of brassinolide on chlorophyll maintenance can be attributed to its role in stabilizing chloroplast membranes and preserving their ultrastructural integrity [19]. The observed increase in chlorophyll levels may also be explained by the ability of brassinolide to regulate chlorophyll biosynthesis processes and reduce its degradation within leaf tissues, thereby enhancing photosynthetic efficiency [20].

Table 5: Effect of Brassinolide and Melatonin in Chlorophyll content (spad) of faba bean.

Brassinolide / Melatonin	B0	B1	B2	B3	**Mean**
M0	44.40	49.70	47.17	54.77	49.01
M1	47.70	43.37	48.40	52.90	48.09
M2	49.57	40.83	45.37	49.67	46.36
Mean	47.22	44.63	46.98	52.44	
LSD 0.05	Brassinolide = 4.408		Melatonin = NS		
	B×M =NS				

NS= no significant

ISSN 2072-3857

The analysis of variance results shown in Table 6 reveal that Brassinolide spraying led to significant variation in the number of pods, with B2 treatment achieving the highest recorded value of 22.98 (pod plant^{-1}). The second concentration of Melatonin (M2) with a value of 22.33 (pod plant^{-1}), regarding the interaction, the trait reached its maximum value (23.53 pod plant^{-1}) under the B2 × M2 combination. Brassinolide causes an2 5 increase in pod number by enhancing pod formation per fertile node [21]. Melatonin increases the number of soybean pods compared to controls [22].

Table 6: Effect of Brassinolide and Melatonin in the number of pods (pod plant^{-1}) of faba bean.

Brassinolide / Melatonin	B0	B1	B2	B3	**Mean**
M0	12.33	23.13	22.93	19.27	19.42
M1	17.40	20.93	22.47	17.27	19.52
M2	21.53	21.33	23.53	22.93	22.33
Mean	17.09	21.80	22.98	19.82	
LSD 0.05	Brassinolide = 2.219		Melatonin = 1.423		
	B×M =2.966				

The results showed (Table 7) that significant differences were observed between the mean number of seeds per pod, with the highest value of the Brassinolide treatment at the B2 concentration (5.13 grain pod^{-1}) and the highest value for the Melatonin effect at the M2 concentration (4.92 grain pod^{-1}). In comparison, the overlap was the highest value of 5.20 grain pod^{-1} at the overlap B1×M0. The positive role of Brassinolide in increasing the number of grains is due to the fertility of flowers [22,23]. The findings of our study were in agreement with what Wei et al. [22] found, where Melatonin affected the increase in grain number within the pod of soybean crop.

Table 7: Effect of Brassinolide and Melatonin in the number of grains per pod (grain pod^{-1}) of faba bean.

Brassinolide / Melatonin	B0	B1	B2	B3	**Mean**
M0	3.70	5.20	5.13	4.50	4.63
M1	4.23	4.77	5.10	4.20	4.58
M2	4.43	4.90	5.17	5.17	4.92
Mean	4.12	4.96	5.13	4.62	
LSD 0.05	Brassinolide = 0.472		Melatonin = 0.291		
	B×M =0.618				

ISSN 2072-3857

The findings presented in Table 8 revealed notable differences among the means of 100-grain weight (g), with the B2 Brassinolide treatment yielding the highest value of 137.26 g. As for the melatonin treatment, the M2 concentration exceeded with a value of 136.58 g per hundred grains, and concerning the overlap, the overlap B1× M0 was superior (137.83 g). Brassinolide has a positive effect on grain size; it increases the size of wheat grains and, as a result, the total grain yield by facilitating the movement of greater amounts of photoassimilates toward the grain [25]. Melatonin increases photosynthesis in soybean plants, improving the enhancement of carbohydrate metabolism [22].

Table 8: Effect of Brassinolide and Melatonin in weight of 100 grains (g) of faba bean.

Brassinolide / Melatonin	B0	B1	B2	B3	**Mean**
M0	129.07	137.83	137.53	133.63	134.52
M1	134.53	135.73	136.80	131.07	134.53
M2	134.33	136.83	137.43	137.73	136.58
Mean	132.64	136.80	137.26	134.14	
LSD 0.05	Brassinolide =1.993		Melatonin = 1.417		
	B×M =2.831				

The data in Table 9 indicated the presence of significant variation in grains yield per plant (g), where the concentrations of Brassinolide and Melatonin B2 and M2 (193.6 and 173.7 g) were superior, respectively, while the B2 × M1 interaction resulted in the highest yield value of 201.0 g. Brassinolide effectively increases the assimilation of photosynthetic CO_2 , provides more carbohydrates to support metabolic activities, and exports them to the sink organs [26]. Melatonin maintains the integrity of chloroplasts and delays leaf senescence, which enhances the photosynthesis process, ultimately contributing to higher crop yield [26].

ISSN 2072-3857

Table 9: Effect of Brassinolide and Melatonin in grains yield of the plant (g) of faba bean.

Brassinolide / Melatonin	B0	B1	B2	B3	**Mean**
M0	68.4	190.4	186.4	134.0	144.8
M1	114.4	156.8	201.0	109.7	145.5
M2	149.6	164.6	193.4	187.3	173.7
Mean	110.8	170.6	193.6	143.7	
LSD 0.05	Brassinolide =36.10		Melatonin =23.80		
	B×M =49.01				

4. Conclusions:

The agronomic performance of the faba bean crop showed clear variation in relation to the untreated control plants due to the difference in the added concentrations of the study factors (Brassinolide and Melatonin). Brassinolide showed a positive behavior in increasing the means of the studied traits (e.g., plant height, branching, leaf count, and leaf area) when the concentration (4–0 mg L⁻ ¹) increased notably. As for the chlorophyll level, the peak value for this trait was observed at 6 mg L⁻ ¹. There is a positive and significant relationship regarding the addition of Melatonin concentrations in some of the studied traits (branching, leaf number, and leaf area). Based on the obtained results, it is recommended to use Brassinolide at 4 mg L⁻ ¹ in combination with Melatonin at 200 mg L⁻ ¹ to achieve improved plant performance."

Acknowledgment: We are grateful to the Desert Studies Center, University of Anbar, for providing the facilities and technical support that made this research possible.

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