The effect of foliar application of melatonin and carbolizer on some chemical and quantitative traits of strawberry (cv. Albion) under protected cultivation

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Abstract
Strawberry is a high-value fruit crop rich in antioxidants and nutrients, yet its productivity is influenced by various environmental factors, including the effects of organic fertilizers and growth regulators. This study aims to evaluate the effect of foliar application of different concentrations of melatonin and Carbolizer (as a source of organic carbon) on improving the chemical and quantitative traits of strawberry cv. Albion. This experiment was conducted in a plastic greenhouse in Najaf Governorate during the 2023-2024 growing season. A randomized complete block design (RCBD) with three replications and two factors was used: melatonin at concentrations of (0, 1, 2 mg L^{-1}) and Carbolizer at concentrations of (0, 1.5, 3 mL L^{-1}). Plants were sprayed monthly with three applications throughout the experiment. Chemical traits (total chlorophyll content and carbohydrates in leaves) and quantitative traits (fruit number, fruit weight, fruit volume, and marketable fruit percentage) were measured. Results: The results showed a significant effect ($P \leq 0.05$) of both factors individually and their interaction on all studied traits. The interaction between melatonin (2 mg L^{-1}) and Carbolizer (3 mL L^{-1}) recorded the highest values in total chlorophyll content (67.96 mg 100g^{-1} fresh weight), carbohydrates (7.168%), fruit number (23.59 fruit $plant^{-1}$), fruit volume (17.49 cm^3), and marketable fruit percentage (83.04%).
Keywords. Melatonin, Carbolizer, Strawberry, Chlorophyll, Carbohydrates.

Introduction
Strawberry (*Fragaria × ananassa* Duch.) is one of the most valuable small fruit crops globally, owing to its high nutritional value, distinctive flavor, and richness in antioxidants and vitamins [1]. Strawberry cultivation faces numerous environmental and production challenges that negatively affect growth and productivity, necessitating the search for innovative and environmentally friendly solutions to improve production [2].

In recent years, the use of biostimulants has gained significant importance in sustainable agriculture as alternatives or complements to traditional chemical fertilizers. Melatonin (N-acetyl-5-methoxytryptamine) is one of the most important of these compounds, a multifunctional plant hormone that plays a vital role in regulating physiological and biochemical processes in plants [3]. Recent studies have shown that foliar application of melatonin improves photosynthetic pigment content, enhances antioxidant enzyme activity, and increases the productivity and quality of strawberry fruits [1,4]. Carbo Laser is an organic fertilizer solution extracted from natural rocks and processed with modern technology that releases carbon dioxide gas for plants [5].

Carbolizer is an organic fertilizer rich in organic carbon, humic acids, and essential nutrients, and can contribute to improving photosynthesis and increasing carbohydrate content in plants [6].

Several studies have shown that exposing seedlings to different concentrations of carbon dioxide (CO_2) higher than its concentration in the atmosphere is positively associated with the physiological activities of plants, which are reflected in increased stem length and diameter, leaf area, and dry matter [7]. It has become known that CO_2 gas represents the primary raw material for carbohydrate synthesis in plants and the production of organic matter in their tissues, which is later used in human nutrition [8].

In a study conducted by Owainat [9]. on strawberry cv. Ruby Gem using nanotechnology organic fertilizer containing 20% carbon, 2% organic nitrogen, and 29% free organic materials at soil application levels of 0, 2, 3, and 4 mL L^{-1}, results showed increases in fruit number, fresh fruit weight, yield per plant, total yield, and carbohydrate and sugar percentages in fruits.

Due to The low yield and high production costs in Iraq necessitate developing the current production pattern and using modern technologies in agriculture to increase productivity and reduce costs. This also ensures an increase in the quantities offered in the market, an increase in consumption by individuals, and competition with imports. Despite numerous studies on the effect of melatonin or organic acids separately on fruit plants, studies investigating the interactive effect between these two compounds on strawberry remain limited. Therefore, this study aims to evaluate the effect of foliar application of different concentrations of melatonin and Carbolizer, individually and in combination, on some chemical traits (chlorophyll and carbohydrates) and fruit traits (fruit number, fruit volume, fruit weight, and marketable fruit percentage) of strawberry cv. Albion under protected cultivation conditions.

Materials and Methods

Experimental Site and Conditions

The experiment was conducted in a plastic greenhouse belonging to the Najaf Governorate Agriculture Directorate, Plant Production Department, during the 2023-2024 growing season. The greenhouse area was 504 m² (9 × 56 meters), covered with transparent polyethylene to provide a suitable protected environment for plant growth.

Plant Material

The strawberry cultivar Albion was used in this study. Albion is a day-neutral cultivar characterized by relatively vigorous vegetative growth, good resistance to bacterial wilt disease, and moderate sensitivity to powdery mildew. The fruits of this cultivar are characterized by attractive red color, elongated shape, acceptable flavor, and a high percentage of marketable fruits [10]. Seedlings were obtained from a specialized nursery in Diyala Governorate and planted in the greenhouse on November 15, 2023.

Physical and Chemical Properties of Soil

Before planting, soil samples were collected and analyzed to determine their physical and chemical properties. Analysis results showed that soil texture was silty loam with sand content of 392 g kg^{-1}, silt 500 g kg^{-1}, and clay 108 g kg^{-1}, with organic matter content of 8.7 g kg^{-1}, pH of 7.55, and electrical conductivity (EC) of 1.3 dS m^{-1}. Regarding available nutrients, the soil recorded 23 mg kg^{-1} nitrogen, 6.82 mg kg^{-1} phosphorus, and 121.75 mg kg^{-1} potassium (Table 1).

Table(1): Physical and chemical properties of greenhouse soil before planting

Property	Value
Sand (g kg^{-1})	392
Silt (g kg^{-1})	500
Clay (g kg^{-1})	108
Soil Texture	Silty loam
Organic Matter (g kg^{-1})	8.7
pH	7.55
EC (dS m^{-1})	1.3
Available N (mg kg^{-1})	23
Available P (mg kg^{-1})	6.82
Available K (mg kg^{-1})	121.75

Land Preparation and Planting

The greenhouse land was prepared through deep plowing and cleaning the soil from weeds and debris. Planting beds were formed and a drip irrigation system was installed along the beds. The beds were covered with black polyethylene to prevent weed growth and maintain soil moisture. Seedlings were planted in three rows with 30 cm spacing between plants and 50 cm between beds, with five plants per experimental unit.

Melatonin Treatment

High-purity melatonin (N-acetyl-5-methoxytryptamine) (American product) was used at three concentrations: 0 (control treatment), 1, and 2 mg L^{-1}.

Carbolizer Treatment

Carbolizer is a liquid organic fertilizer (Egyptian product) containing organic materials rich in carbon and nutrients. Table 2 shows the chemical composition of Carbolizer. Carbolizer was used at three concentrations: 0 (control treatment), 1.5, and 3 mL L^{-1}.

Table (2): Chemical composition of Carbolizer

EC	pH	N	P	K	Carbon	Ca	S
ds\m		(%)	(%)	(%)	(%)	(%)	(%)
43.4	8.60	6.6	0.50	0.50	20	4.5	2

Treatments and Materials Used

Two treatments were used in this study (Table 3).

Table (3): Experimental factors and their levels

Factor	Levels
Melatonin	0, 1, 2 mg L^{-1}
Carbolizer	0, 1.5, 3 mL L^{-1}

Treatment Application

Treatments were applied via foliar spray one month after planting (December 15, 2023), at a rate of one spray per month for each treatment, totaling three sprays during the experiment. Spraying was performed in early morning using a 3-liter hand sprayer, with the addition of a surfactant (Tween 20) at 0.1% to ensure uniform solution distribution on leaves. The first agent was sprayed on December 15th, then the second agent was sprayed on December 19th, and the same date was repeated for January and February. The experiment was completed on April 20th2023- after collecting plant samples and taking field measurements.

Experimental Design and Statistical Analysis

The experiment was conducted according to a randomized complete block design (RCBD) with three replications and a factorial arrangement with two factors (melatonin and Carbolizer), totaling 27 experimental units (3 melatonin concentrations × 3 Carbolizer concentrations × 3 replications). Data were statistically analyzed using GenStat Discovery Edition 4 software, and the Least Significant Difference (LSD) test at a probability level of 0.05 was used for mean comparisons.

Studied Traits

Chemical Traits

Total Chlorophyll Content (mg $100g^{-1}$ fresh weight)

Total chlorophyll content in leaves was determined following the method of [11].(the samples were taken one week after the last third spray treatment), using a spectrophotometer (Model UV-1800, Shimadzu, Japan) at wavelengths of 645 and 663 nm. Total chlorophyll was calculated using the following equation:

$$Total\ Chlorophyll = [20.2 \times D(645) + 8.02 \times D(663)] \times (V/W \times 1000) \times 100$$

Where: D = optical absorbance, V = total extract volume (mL), W = sample weight (g)

Carbohydrate Content in Leaves (%)

Total carbohydrate content in leaves was determined using the phenol-sulfuric acid method as described by Dubois et al. (1956) [12]. Optical absorbance was measured at a wavelength of 490 nm using a spectrophotometer. Carbohydrate percentage was calculated by reference to a previously prepared standard curve using standard glucose solution.

Fruit Traits

Fruit Number (fruit plant^{-1})

Average fruit number per plant was calculated by collecting ripe fruits weekly from each experimental unit throughout the production period, then dividing the total fruit number by the number of plants in the experimental unit (5 plants).

Fruit Volume (cm^3)

A number of fruits were placed in a graduated cylinder with a volume of one liter filled with water, then the displaced water volume was calculated according to the equation:

$$Fruit\ Volume = Displaced\ Water\ Volume\ /\ Fruit\ Number$$

Fruit Weight (g fruit^{-1})

Fresh fruits were weighed using a sensitive balance (Model: XB-220A, Precisa, Switzerland) with an accuracy of 0.01 g. Average fruit weight was calculated by dividing total fruit weight by their total number for each experimental unit.

Marketable Fruit Percentage (%)

Marketable fruit percentage was calculated by sorting fruits according to quality standards (size, shape, color, and absence of defects or deformities), then dividing the number of sound marketable fruits by the total fruit number and multiplying by 100.

Results and Discussion

Effect of Treatments on Total Chlorophyll Content

The results shown in Table 4 indicate a significant effect of melatonin, Carbolizer, and their interaction on total chlorophyll content in strawberry leaves. The results show significant differences in leaf chlorophyll content when plants were sprayed with melatonin at a concentration of 2 mg L^{-1}, achieving the highest content of 67.11 mg 100 g^{-1} fresh weight compared to control plants which gave 65.30 mg 100 g^{-1} fresh weight, respectively. Leaf chlorophyll content also increased in plants sprayed with Carbolizer, where the Carbolizer treatment at a concentration of 3 mL L^{-1} significantly exceeded other treatments, giving 66.81 mg $100g^{-1}$ fresh weight, respectively, compared to the control treatment which gave 65.67 mg $100g^{-1}$ fresh weight, respectively.

Table (4): Effect of melatonin, Carbolizer, and their interaction on total chlorophyll content (mg $100g^{-1}$ fresh weight) in strawberry leaves

Melatonin (mg L^{-1})	Carbolizer (ml. l^{-1})			Mean
	0	1.5	3	
0	64.95	64.21	66.75	65.30
1	65.55	65.20	65.73	65.49
2	66.50	66.86	67.96	67.11
Mean	65.67	65.42	66.81	
$LSD_{0.05}$	Carb = 0.34 Me =0.34 Carb x Me = 0.60			

It was observed that the interaction between melatonin and Carbolizer significantly affected this trait, where the treatment of spraying with melatonin at a concentration of 2 mg L^{-1} and Carbolizer at a concentration of 3 mL L^{-1} achieved the highest total chlorophyll content of 67.96 mg $100g^{-1}$ fresh weight, while the control treatment (0 melatonin with 1.5 mL Carbolizer) recorded the lowest value of 64.21 mg 100 g^{-1} fresh weight.

Effect of Treatments on Carbohydrate Content in Leaves

Table 5 shows the significant effect of melatonin, Carbolizer, and their interaction on carbohydrate content in strawberry leaves. Spraying plants with melatonin at a concentration of 2 mg L^{-1} significantly exceeded other treatments in leaf carbohydrate content, reaching 6.999% compared to the control treatment which reached 6.835%. As for the effect of spraying with Carbolizer, plants sprayed at a concentration of 3 mL L^{-1} significantly exceeded plants that were not sprayed, reaching 7.048%.

The interaction between the two spray factors melatonin and Carbolizer showed a significant effect, as all spray treatments with melatonin at a concentration of(0) and Carbolizer at a concentration of 3 mL L^{-1}) significantly exceeded in leaf carbohydrate content, reaching 7.262 %, and the lowest carbohydrate content in leaves was 6.571% in control treatment plants.

Table (5): Effect of melatonin, Carbolizer, and their interaction on carbohydrate content (%) in strawberry leaves

Melatonin (mg L^{-1})	Carbolizer (ml l^{-1})			Mean
	0	1.5	3	
0	6.571	6.672	7.262	6.835
1	7.001	6.826	6.714	6.847
2	6.973	6.857	7.168	6.999
Mean	6.849	6.785	7.048	
$LSD_{0.05}$	carb =0.085 Me=0.085 Carb x Me= 0.148			

Effect of Treatments on Fruit Traits

Fruit Number (fruit plant⁻¹)

The data presented in Table 6 indicate a significant effect of melatonin, Carbolizer, and their interaction on average fruit number per plant. The concentration of 2 mg L^{-1} melatonin showed the highest average fruit number (22.73 fruit $plant^{-1}$), followed by 1 mg L^{-1} (22.32 fruit $plant^{-1}$), then the control treatment (21.37 fruit $plant^{-1}$). For Carbolizer, the concentration of 3 mL L^{-1} recorded the highest average fruit number (22.73 fruit $plant^{-1}$), followed by 1.5 mL L^{-1} (21.11 fruit $plant^{-1}$), then the control treatment (21.57 fruit $plant^{-1}$). The interaction between melatonin (2 mg L^{-1}) and Carbolizer (3 mL L^{-1}) achieved the highest significant value for fruit number (23.59 fruit $plant^{-1}$), while the control treatment (0 melatonin + 0 Carbolizer) recorded the lowest value (20.72 fruit $plant^{-1}$).

Table (6): Effect of melatonin, Carbolizer, and their interaction on fruit number (fruit $plant^{-1}$) in strawberry

Melatonin (mg L^{-1})	Carbolizer (ml l^{-1})			Mean
	0	1.5	3	
0	20.72	21.67	21.71	21.37
1	21.900	22.16	22.89	22.32
2	22.09	22.50	23.59	22.73
Mean	21.57	21.11	22.73	
$LSD_{0.05}$	Carb = 0.37 Me =0.37 Carb x Me=0.65			

Fruit Volume (cm^3)

Table 7 shows that foliar spray with melatonin had a significant effect on fruit volume, as plants sprayed with melatonin at 2 mg L^{-1} significantly exceeded other spray treatments in fruit volume. Spraying plants with Carbolizer at 3 mL L^{-1} resulted in the largest fruit volume, which significantly exceeded the control treatment. The interaction between melatonin and Carbolizer spray had a significant effect on fruit volume, where the largest fruit volume in plants sprayed with melatonin at 2 mg L^{-1} and Carbolizer at 3 mL L^{-1} was 17.49 cm^3 compared to the control treatment which was 15.68 cm^3.

Table (7): Effect of melatonin, Carbolizer, and their interaction on fruit volume (cm^3) in strawberry

Melatonin (mg L^{-1})	Carbolizer (ml. l^{-1})			Mean
	0	1.5	3	
0	15.68	15.36	15.61	15.55
1	15.15	14.83	15.88	15.29
2	16.02	16.01	17.49	16.51
Mean	15.62	15.44	16.32	
$LSD_{0.05}$	Carb = 0.41 Me =0.41 Carb x Me=0.70			

Fruit Weight (g)

The results in Table 8 indicate that increasing the melatonin concentration added had a significant effect on average fruit weight, where the spray treatment with melatonin at 2 mg L^{-1} significantly exceeded other spray treatments, reaching 17.87 g. Spraying with Carbolizer also had a significant effect on this trait, as plants sprayed with Carbolizer at 3 mL L^{-1} gave the highest average fruit weight of 17.62 g. The same table shows a significant difference for interactions between melatonin and Carbolizer, where plants sprayed with melatonin at 2 mg L^{-1} and Carbolizer at 1.5 mL L^{-1} gave the highest average fruit weight(17.80) , and the lowest average fruit weight was in the control treatment (16.44) .

Table (8): Effect of melatonin, Carbolizer, and their interaction on average fruit weight (g) in strawberry

Melatonin ($mg\ L^{-1}$)	Carbolizer ($ml\ l^{-1}$)			Mean
	0	1.5	3	
0	16.44	17.23	17.25	16.97
1	17.13	17.05	17.79	17.33
2	17.70	18.11	17.80	17.87
Mean	17.09	17.46	17.62	
$LSD_{0.05}$	Carb =0.46 Me =0.46 Carb x Me=0.80			

Marketable Fruit Percentage

Table 9 shows a significant effect of melatonin, Carbolizer, and their interaction on marketable fruit percentage. The concentration of 2 mg L^{-1} melatonin recorded the highest average (82.47%), followed by 1 mg L^{-1} (80.92%), then the control treatment (79.36%). For Carbolizer, the concentration of 3 mL L^{-1} gave the highest average (82.35%), followed by 1.5 mL L^{-1} (81.07%), then the control treatment (79.33%). The interaction between melatonin (2 mg L^{-1}) and Carbolizer (3 mL L^{-1}) achieved the highest marketable fruit percentage (83.04%), while the control treatment (0 melatonin + 0 Carbolizer) recorded the lowest percentage (76.22%).

Table (9): Effect of melatonin, Carbolizer, and their interaction on marketable fruit percentage (%) in strawberry

Melatonin ($mg\ L^{-1}$)	Carbolizer ($mL\ L^{-1}$)			Mean
	0	1.5	3	
0	76.22	79.51	82.35	79.36
1	79.42	80.94	82.40	80.92
2	82.35	82.77	83.04	82.47
Mean	79.33	81.07	82.35	
$LSD_{0.05}$: Me =1.14, Carbo =1.41 Carb x Me=2.32				

The results of the current study showed that foliar application of melatonin and Carbolizer, individually or in combination, led to a significant increase in total chlorophyll content in strawberry leaves. This increase can be explained through several physiological and biochemical mechanisms. For melatonin, numerous studies have shown that this plant hormone plays an important role in protecting chlorophyll molecules from oxidative degradation by activating enzymatic and non-enzymatic antioxidant systems [1]. Melatonin also enhances chlorophyll synthesis by stimulating enzymes in the chlorophyll biosynthetic pathway, particularly δ-aminolevulinic acid dehydratase and protochlorophyllide reductase [2]. Additionally, melatonin maintains the integrity of thylakoid membrane structure in chloroplasts, protecting chlorophyll from photodegradation [13].

As for Carbolizer, it is an organic fertilizer rich in organic carbon and elements such as nitrogen, phosphorus, and potassium, which contribute to improving nutrient uptake, especially nitrogen, magnesium, and iron, which are essential elements in chlorophyll molecule composition. The high content of nitrogen (6.6%) and organic carbon (20 %) in Carbolizer provides the raw materials necessary for building chlorophyll molecules and their associated proteins. The synergistic effect between melatonin and Carbolizer explains the greater improvement in chlorophyll content when used together. These results are consistent with those of Arabia et al (2024) [3]. on strawberry, who found that foliar application of melatonin at 100 micromolar concentration led to a significant increase in chlorophyll a and b content in leaves.

The study recorded a significant increase in carbohydrate content in strawberry leaves as a result of foliar application of melatonin and Carbolizer. Carbohydrate content is closely related to photosynthesis efficiency and organic matter accumulation in leaves. Melatonin enhances photosynthesis efficiency through several mechanisms, including: improving Photosystem II efficiency, activating Rubisco enzyme which plays a crucial role in carbon dioxide fixation, and improving electron transport chain efficiency in chloroplasts [2]. The organic carbon in carbolizer can also provide an additional carbon source, further promoting carbohydrate accumulation in leaves [6].

A higher carbohydrate content in leaves provides more energy and raw materials for plant growth and fruit development. Studies indicate that plants with high leaf carbohydrate content are more resistant to environmental stresses and produce higher-quality crops [14]. These findings are consistent with those of Fan et al. (2014),[15] who found that treating plants with humic acid significantly increased total carbohydrate and chlorophyll content in chrysanthemum plants.

The results showed that foliar application of melatonin and Carbolizer led to a significant increase in both fruit number and individual fruit weight. This improvement in yield components can be explained by the increase in chlorophyll content in leaves, which enhances photosynthesis efficiency, providing more assimilates that are translocated to developing fruits [1]. This greater availability of carbohydrates supports both increased fruit number and increased size of individual fruits. Melatonin plays an important role in regulating physiological processes related to flowering and fruit set, as studies have shown that melatonin affects the production and balance of other plant hormones, such as auxins and gibberellins, which play a crucial role in flower development and fruit set [4].

On the other hand, carbolizer contributes to improving the balanced nutrition of the plant by providing macro and micronutrients in appropriate proportions. This balanced nutrition is reflected in the quality of the fruits and the regularity of their shape and size. The calcium present in carbolizer (4.5 %) also plays an important role in strengthening the cell walls of the fruit and maintaining its firmness, thus reducing the occurrence of cracks and deformities [5]. In addition, this organic fertilizer provides the plant with the carbon structures of organic molecules, which are the structural support of the plant in general, as carbon, hydrogen and oxygen are converted into organic molecules through the process of photosynthesis [16], and thus an increase in the rate of photosynthesis of the leaves due to the increased concentration of this gas at the sites of the enzyme that fixes this gas [17].

The study recorded a significant increase in marketable fruit percentage as a result of treatment with melatonin and Carbolizer. Melatonin contributes to improving fruit quality by: enhancing sugar and phenolic compound accumulation in fruits, improving their taste and color [1]; protecting fruit cells from oxidative damage, reducing physiological defects and deformities [2]; and improving fruit firmness and tissue cohesion, reducing mechanical damage during growth and harvesting [4]. On the other hand, Carbolizer contributes to improving balanced plant nutrition by providing major and minor nutrients in appropriate proportions. These results are consistent with those of Arabia et al. (2024) [3], who found that melatonin maintains strawberry fruit quality by enhancing antioxidant systems and reducing oxidative stress.

Conclusions

Based on the results obtained from this study, the following conclusions can be drawn:

Foliar application of melatonin led to a significant increase in total chlorophyll content, leaf carbohydrate content, fruit number, fruit weight, and marketable fruit percentage compared to control treatment. Foliar application of Carbolizer led to significant improvement in all studied traits. The interaction between melatonin (2 mg L^{-1}) and Carbolizer (3 mL L^{-1}) achieved the best results in all studied traits, indicating a positive synergistic effect between these two compounds. The combined application of melatonin and Carbolizer represents an effective and environmentally friendly strategy for improving strawberry crop productivity and quality under protected cultivation conditions in Najaf Governorate.

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