Interactive Effects of Methanol and Melatonin on Growth and Flowering of China Aster

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Abstract

A greenhouse experiment was conducted during the 2025–2026 growing season at the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Diyala, Iraq, to evaluate the effects of foliar application of methanol and melatonin on the vegetative growth and flowering performance of China aster. The experiment was arranged as a 3 × 3 factorial in a randomized complete block design (RCBD) with three replications. Methanol was applied at 0, 15, and 30%, while melatonin was applied at 0, 50, and 100 mg L^{-1}. Foliar application of 15% methanol significantly enhanced plant height, primary branch number, and flower number per plant, whereas 30% methanol increased leaf nitrogen content, flower stalk diameter, and flower longevity. Melatonin at 50 mg L^{-1} improved leaf nitrogen content, while 100 mg L^{-1} produced the highest leaf fresh weight, flower dry weight, flower longevity, and delayed the appearance of the first flower bud. Significant interaction effects were observed between methanol and melatonin treatments, with M15 × MEL0**,** M0 × MEL50, and M30 × MEL100 providing superior performance for most vegetative and flowering traits. Overall, foliar application of methanol and melatonin effectively improved the growth and flowering characteristics of China aster, demonstrating their potential as practical biostimulants for enhancing ornamental plant quality

Introduction

Callistephus chinensis L. (China aster), a member of the family Asteraceae, is one of the most important annual ornamental flowering plants cultivated worldwide for landscape decoration and the commercial cut-flower industry. It is characterized by remarkable diversity in plant architecture, The intensive use of synthetic chemicals and plant growth regulators to maximize crop productivity has raised increasing environmental and health concerns. Consequently, environmentally friendly biostimulants have gained considerable attention as sustainable alternatives for improving plant growth and productivity [2]. flower size, shape, and color, making it highly valuable for ornamental horticulture. The genus *Callistephus* comprises a single species native to China and Japan, from which all cultivated cultivars have been developed [1].

Among these compounds, methanol has been widely investigated because of its ability to enhance plant growth and yield. Previous studies have demonstrated that foliar application of methanol can improve crop productivity, accelerate plant development, enhance drought tolerance, and reduce water requirements in several

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agricultural crops [3]; [4]. Methanol is naturally produced in plant tissues during cell wall pectin demethylation and influences several physiological processes, including stomatal conductance, leaf expansion, photosynthetic efficiency, glucose metabolism, transpiration, and cell enlargement [5], [6].

Melatonin (N-acetyl-5-methoxytryptamine), an indoleamine derived from tryptophan, has recently emerged as an important plant biostimulant involved in the regulation of numerous physiological and developmental processes [7]. It enhances plant performance by improving osmotic adjustment, maintaining membrane stability, and strengthening antioxidant defense systems, particularly under abiotic stress conditions [8]. Furthermore, melatonin regulates seed germination, vegetative growth, flowering, photosynthesis, senescence, postharvest quality, and stress tolerance while acting as a potent antioxidant and reactive oxygen species scavenger [9]; [10] .

Despite the increasing interest in methanol and melatonin as eco-friendly plant biostimulants, limited information is available regarding their combined effects on the growth and flowering of ornamental plants, particularly *C. chinensis*. Therefore, the present study aimed to evaluate the individual and interactive effects of foliar application of methanol and melatonin on the vegetative growth and flowering characteristics of China aster.

Materials and Methods

The experiment was conducted during the 2025–2026 autumn growing season in a plastic greenhouse at the Horticulture and Landscape Engineering Research Station, College of Agriculture, University of Diyala, Iraq, from 22 October 2025 to 25 May 2026.

Seeds of Chinese aster obtained from **SAKATA** (Japan) were sown in peat moss-filled seedling trays on 22 October 2025. Uniform seedlings (approximately 6 cm in height) were transplanted on 4 December 2025 into plastic pots (26 cm diameter × 26 cm height), three pots for each experimental unit with one seedling per pot. The pots were filled with a peat moss-based growing medium (3:1, v/v), and irrigation was supplied through a drip irrigation system throughout the experiment. To minimize soil-borne diseases, the growing medium was treated with the fungicide **Uno** (containing metalaxyl and chlorothalonil) according to the manufacturer's recommendations. Plants were fertilized with a balanced water-soluble NPK fertilizer (20:20:20) at 1 g L^{-1} as a foliar spray, beginning at the 4–5 true-leaf stage and repeated at two-week intervals until the end of the experiment. Routine cultural practices, including weeding and soil cultivation, were carried out whenever necessary. Before planting, representative samples of the growing medium were collected and analyzed for selected physical and chemical properties at the Central Laboratory for Soil, Water and Plant Analysis, College of Agricultural Engineering Sciences, University of Baghdad (Table 1).

The experiment consisted of two factors arranged in a factorial design. The first factor was foliar application of methanol at

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0, 15, and 30% (v/v), designated as M0, M1, and M2, respectively. The second factor was foliar application of melatonin at 0, 50, and 100 mg L^{-1}, designated as MEL0, MEL1, and MEL2, respectively. Melatonin was purchased from **NOW Foods** (USA), whereas methanol was obtained from RCL Labscan (Thailand) through local chemical suppliers. The plants were sprayed with methanol and melatonin twice after the emergence of 10–12 pairs of true leaves, with a two-week interval between applications. A volume of 0.1 mL L^{-1} of liquid soap was added as a surfactant to the spray solution.

Table 1. physical and chemical properties of the growing medium.

Measured trait	Value	Unit
PH	7.48	-
EC	2.10	Ds m^{-1}
Available Nitrogen	51	mg kg^{-1}
Available Phosphorus	7.2	mg kg^{-1}
Available Potassium	106	mg kg^{-1}
Organic matter	0.97	%
Calcium carbonate (CaCO3)	28.2	g kg^{-1}
Soil texture	Loamy	-
sand	43	%
clay	20	%
Silt	37	%

Measured Parameters

Measurements were recorded from five randomly selected plants per experimental unit in each replicate.

Vegetative growth traits

1. **Plant height (cm):** Plant height was measured from the growing medium surface to the highest floral apex.
2. **Number of leaves (plant^{-1}):** The total number of leaves per plant was recorded at the end of the vegetative growth stage.
3. **Number of primary branches (plant^{-1}):** The number of primary branches per plant was counted.
4. **Leaf fresh weight (g):** Leaf fresh weight was estimated from 20 randomly selected leaves (four leaves per plant) using an analytical balance according to [11].
5. **Leaf nitrogen concentration (%):** Nitrogen concentration was determined using the Kjeldahl method after wet digestion of dried leaf samples, and the percentage of nitrogen was calculated according to [11].

ISSN 2072-3857

Flowering traits

6. **Days to first flower bud emergence:** The number of days from transplanting to the appearance of the first visible flower bud was recorded.
7. **Number of flowers (plant^{-1}):** The total number of fully opened flowers per plant was counted.
8. **Flower stem diameter (mm):** Flower stem diameter was measured using a digital Vernier caliper at 1 cm above its attachment to the main stem.
9. **Flower dry weight (g):** Flower dry weight was determined from ten fully opened flowers per experimental unit after air-drying to constant weight.
10. **Flower longevity (days):** Flower longevity was recorded as the period from the appearance of the first flower bud until flower senescence on the plant.

Results and Discussion

Vegetative growth traits

1- Plant height (cm)

The results presented in table 2 showed that methanol significantly increased plant height, with 15% producing the highest mean (40.84 cm), which was comparable to 30% but significantly higher than the control. In contrast, melatonin had no significant effect on this trait. The methanol × melatonin interaction significantly affected plant height. The treatment M15 × MEL0 recorded the highest mean (42.34 cm), which did not differ significantly from the other interaction treatments but was significantly higher than the control treatment (M0 × MEL0), which produced the lowest mean (33.21 cm).

2- Number of leaves (plant^{-1})

Neither methanol nor melatonin applied individually had a significant effect on leaf number. However, their interaction significantly influenced this trait, with M15 × MEL0 producing the highest mean (80.13 leaves plant^{-1}), whereas M0 × MEL0 recorded the lowest value (68.27 leaves plant^{-1}).

3- Number of primary branches (plant^{-1})

Methanol significantly increased number of primary branches, with 15% producing highest mean (13.95 branches plant^{-1}), comparable to 30% but significantly higher than the control (12.33 branches plant^{-1}). In contrast, melatonin had no significant effect on this trait. The methanol × melatonin interaction was significant, with M15 × MEL0 recording highest value (15.00 branches plant^{-1}), whereas M0 × MEL0 produced lowest (10.28 branches plant^{-1}).

4- Leaf fresh weight (g)

Methanol had no significant effect on leaf fresh weight, whereas melatonin significantly increased this trait, with 100 mg L^{-1} producing the highest mean (21.64 g), comparable to 50 mg L^{-1} but significantly higher than the control (18.41 g). The methanol × melatonin interaction was significant, with M0 × MEL50 recording the highest leaf fresh weight (23.34 g),

ISSN 2072-3857

while M15 × MEL50 and M0 × MEL0 produced the lowest values (18.55 and 14.22 g, respectively). Methanol significantly increased leaf nitrogen content, with 30% producing the highest value (4.12%), significantly exceeding both 15% methanol and the control, which did not differ significantly from each other.

5- Leaf nitrogen concentration (%)

Melatonin also significantly increased leaf nitrogen content, with both MEL100 and MEL50 producing the highest mean (3.76%), whereas the control (MEL0) recorded the lowest value (2.99%). The methanol × melatonin interaction was also significant, with M30 × MEL100 producing the highest nitrogen content (4.50%), significantly exceeding M15 × MEL50 (3.09%) and the control treatment (M0 × MEL0; 2.18%).

Table 2. Effect of methanol and melatonin foliar application and their interaction on vegetative growth traits of China aster **.**

Treatment		Plant height (cm)	Number of leaves (leaves plant^{-1})	Number of primary branches (branches plant^{-1})	Leaf fresh weight (g)	Leaf nitrogen content (%)
Methanol mean						
M 0		38.32 B	75.83 A	12.33 B	20.25 A	3.05 B
M 15		40.84 A	77.35 A	13.95 A	19.83 A	3.35 B
M 30		40.63 A	78.66 A	13.75 A	21.29 A	4.12 A
Melatonin effect						
MEL0		39.01 A	75.78 A	12.83 A	18.41 B	2.99 B
MEL50		40.46 A	76.76 A	13.79 A	21.32 A	3.76 A
MEL100		40.32 A	79.29 A	13.40 A	21.64 A	3.76 A
Methanol × Melatonin interaction						
M0	MEL0	33.21 b	68.27 a	10.28 b	14.22 b	2.18 c
	MEL50	42.33 a	79.90 a	13.82 a	23.34 a	3.70 ab
	MEL100	39.41 a	79.31 a	12.89 a	23.19 a	3.27 abc
M15	MEL0	42.34 a	80.13 a	15.00 a	20.45 ac	3.41 Abc
	MEL50	39.21	72.50	13.63	18.55	3.09

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		a	ab	a	c	bc
	MEL100	40.97 a	79.42 a	13.21 a	20.48 ac	3.53 ab
M30	MEL0	41.49 a	78.95 a	13.21 a	20.56 ac	3.39 abc
	MEL50	39.84 a	77.89 a	13.93 a	22.06 ac	4.49 a
	MEL100	40.57 a	79.13 a	14.10 a	21.24 ac	4.50 a

Flowering traits

6- Days to first flower bud emergence

Regarding flowering traits, the results presented in table 3 showed that methanol foliar application significantly accelerated the appearance of first flower bud. The 15% methanol treatment recorded shortest period to first flower bud emergence (116.61 days), with no significant difference compared with the 30% methanol treatment, whereas both treatments were significantly superior to control, which required longest period (120.57 days). Similarly, melatonin application significantly hastened the appearance of first flower bud. The 100 mg L^{-1} treatment recorded shortest duration (116.22 days), with no significant difference compared with 50 mg L^{-1} treatment, while both treatments significantly outperformed control, which recorded longest period (120.78 days). The interaction treatment M15 × MEL100 resulted in earliest appearance of first flower bud (115.30 days), whereas control treatment recorded longest period (128.07 days).

7- Number of flowers (flower $plant^{-1}$)

Results showed that methanol foliar application significantly increased number of flowers. 15% methanol treatment produced highest mean number of flowers (19.50 flowers $plant^{-1}$), with no significant difference compared with 30% methanol treatment, whereas both treatments were significantly superior to control, which recorded lowest mean (17.26 flowers $plant^{-1}$). In contrast, foliar application of melatonin alone at all tested concentrations had no significant effect on number of flowers. The interaction treatment M0 × MEL50 produced highest mean number of flowers (21.39 flowers $plant^{-1}$), significantly exceeding M0 × MEL100 and M30 × MEL50 interaction treatments, which recorded 17.33 and 17.25 flowers $plant^{-1}$,

8- Flower stem diameter (mm)

The results indicated that methanol foliar application significantly increased flower stem diameter. Treatment 30% methanol recorded highest mean stem diameter (2.63 mm), significantly exceeding both 15% methanol treatment and control, which recorded mean values of 2.45 and 2.42 mm, respectively. In contrast, melatonin application alone at all tested concentrations had no significant effect on flower stem diameter. The interaction between methanol and melatonin significantly affected this trait, with the M30 × MEL50 treatment producing the highest mean stem diameter (2.72 mm), whereas M15 × MEL50 and M0

ISSN 2072-3857

× MEL0 treatments recorded lowest mean values of 2.29 and 2.15 mm, respectively.

9- Flower dry weight (g)

Results showed that methanol foliar application alone had no significant effect on this trait. In contrast, melatonin application significantly increased flower dry weight, with 100 mg L^{-1} treatment recording highest mean value (5.56 g), which did not differ significantly from 50 mg L^{-1} treatment, but was significantly higher than control, which recorded lowest mean value (4.96 g). The interaction between methanol and melatonin significantly affected flower fresh weight. The M0 × MEL100 treatment produced highest mean value (6.05 g), significantly exceeding all other interaction treatments.

10- Flower longevity (days)

Results indicated that methanol foliar application significantly increased flower longevity on plant. treatment 30% methanol recorded highest mean flower longevity (41.77 days), with no significant difference compared with 15% treatment, whereas both treatments were increased/prolonged flower longevity to control, which recorded lowest mean value (39.13 days). Similarly, melatonin application significantly prolonged flower longevity. Treatment 100 mg L^{-1} recorded highest mean value (42.22 days), without a significant difference from 50 mg L^{-1} treatment, but significantly exceeded control, which recorded lowest mean value (39.70 days). Interaction treatment M30 × MEL100 produced highest mean flower longevity, reaching 43.00 days.

Table 3. Effect of methanol and melatonin foliar application and their interaction on the floral growth traits of China aster.

Treatment	Days to first flower bud emergence	Number of flowers (flower $plant^{-1}$)	Flower stem diameter (mm)	Flower dry weight (g)	Flower longevity (days)
Methanol mean					
M 0	120.57 a	17.26 b	2.42 b	5.18 a	39.13 b
M 15	116.61 a	19.50 a	2.45 b	5.42 a	41.66 a
M 30	117.51 ab	18.81 ab	2.63 a	5.32 a	41.55 a
Melatonin effect					
MEL0	120.78 a	18.15 a	2.42 a	4.96 b	39.70 b
MEL50	117.69 ab	19.24 a	2.53 a	5.39 a	40.65 ab
MEL100	116.22	18.17	2.55	5.56	42.22

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		b	a	a	a	a
Methanol × Melatonin interaction						
M0	MEL0	128.07 b	13.06 c	2.15 c	4.18 c	35.00 b
	MEL50	118.30 b	21.39 a	2.57 ab	5.33 b	40.34 a
	MEL100	115.33 b	17.33 b	2.53 ab	6.05 a	42.05 a
M15	MEL0	117.17 b	20.29 ab	2.53 ab	5.33 b	42.88 a
	MEL50	117.37 b	19.10 ab	2.29 bc	5.45 b	40.50 a
	MEL100	115.30 b	19.12 ab	2.53 ab	5.46 b	41.61 a
M30	MEL0	117.10 b	21.10 a	2.58 ab	5.38 b	41.22 a
	MEL50	117.40 b	17.25 b	2.72 a	5.40 b	41.11 a
	MEL100	118.03 b	18.07 ab	2.60 a	5.17 b	43.00 a

The superior performance of the 15% methanol treatment in several vegetative and floral traits, including plant height, primary branch number, and flower number, can be attributed to the physiological role of methanol in C3 plants. [3] reported that, despite its rapid metabolism, methanol exerts sustained physiological effects by regulating metabolic activities beyond its direct assimilation. Foliar-applied methanol enhances cell expansion, promotes vegetative growth, and accelerates flowering through improving the carbon-to-nitrogen (C/N) balance. Moreover, methanol serves as an additional source of CO_2 following its oxidation within plant tissues, thereby enhancing photosynthetic efficiency, improving carbon assimilation, and ultimately promoting plant growth and productivity under different environmental conditions [12], [13], [14].

Similarly, foliar application of melatonin at 100 mg L^{-1} significantly improved several vegetative and floral traits, including leaf fresh weight, leaf nitrogen content, flower dry weight, time to first flower bud appearance, and flower longevity. These findings agree with those of [15], who reported marked improvements in the growth and flowering of Rosa centifolia following melatonin application. The promotive effects of melatonin are mainly attributed to its powerful antioxidant capacity, which enhances enzymatic and non-enzymatic antioxidant systems, scavenges reactive oxygen species (ROS), and reduces oxidative damage, thereby improving nutrient uptake, maintaining membrane stability, and increasing plant tolerance to abiotic stress [8]; [16]. In addition, melatonin regulates the biosynthesis and signaling of several

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phytohormones, particularly auxin, gibberellins, cytokinins, abscisic acid, and ethylene, resulting in enhanced vegetative growth, delayed senescence, improved photosynthetic efficiency, and prolonged flower longevity [10]; [17].

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