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ARTICLE

Effect of humic acid application and CPPU spraying on growth and flowering of Marigold

Efeito da aplicação de ácido húmico e da pulverização com CPPU no crescimento e florescimento de tagetes

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Abstract
This study investigated the effects of humic acid (HA) and forchlorfenuron (CPPU) on the growth, flowering, and chemical characteristics of marigold (*Tagetes ereta* L.) during the 2025 growing season under protected cultivation. The experiment was conducted using a factorial arrangement with two factors: three concentrations of HA (0, 2, and 4 mL L^{-1}) and four concentrations of CPPU (0, 4, 8, and 12 mg L^{-1}). Growth parameters, flowering traits, and biochemical contents were measured. Application of HA significantly improved all evaluated traits, with the highest values obtained at 4 mL L^{-1}, resulting in plant height (37.67 cm), number of branches (6.84 $plant^{-1}$), number of flowers (7.88 $plant^{-1}$), flower diameter (6.68 cm), fresh flower weight (0.83 g), total chlorophyll (1.54 mg g^{-1}), carbohydrates (6.38%), nitrogen (1.65%), phosphorus (0.37%), and potassium (1.79%). Similarly, CPPU application markedly enhanced plant performance. The concentration of 8 mg L^{-1} provided the highest plant height (36.91 cm), flower number (7.35 $plant^{-1}$), fresh flower weight (0.87 g), total chlorophyll (1.48 mg g^{-1}), and nitrogen (1.66%), while 12 mg L^{-1} resulted in superior branch number (6.69 $plant^{-1}$), flower diameter (6.53 cm), carbohydrate content (6.31%), phosphorus (0.34%), and potassium (1.78%). In conclusion, the combined use of humic acid and CPPU enhanced vegetative growth, flowering performance, and biochemical composition of marigold, with the highest doses (4 mL L^{-1} HA and 8 - 12 mg L^{-1} CPPU) showing the most beneficial effects. These findings highlight the potential of HA and CPPU as effective biostimulant and growth regulator strategies for improving ornamental plant production.
Keywords: biostimulants, cytokinin, forchlorfenuron, improvement of flowering, *Tagetes erecta*.

Resumo
Este estudo investigou os efeitos do ácido húmico (HA) e do forclorfenurão (CPPU) sobre o crescimento, floração e características químicas da calêndula (*Tagetes ereta* L.) durante a estação de crescimento de 2025 sob cultivo protegido. O experimento foi conduzido utilizando um arranjo fatorial com dois fatores: três concentrações de HA (0, 2 e 4 mL L^{-1}) e quatro concentrações de CPPU (0, 4, 8 e 12 mg L^{-1}). Foram medidos parâmetros de crescimento, características de floração e conteúdo bioquímico. A aplicação de HA melhorou significativamente todas as características avaliadas, com os maiores valores obtidos em 4 mL L^{-1}, resultando em altura da planta (37,67 cm), número de ramos (6,84 $planta^{-1}$), número de flores (7,88 $planta^{-1}$), diâmetro da flor (6,68 cm), peso da flor fresca (0,83 g), clorofila total (1,54 mg g^{-1}), carboidratos (6,38%), nitrogênio (1,65%), fósforo (0,37%) e potássio (1,79%). A aplicação de CPPU também melhorou significativamente o desempenho da planta. A concentração de 8 mg L^{-1} proporcionou a maior altura de planta (36,91 cm), número de flores (7,35 $plantas^{-1}$), peso de flores frescas (0,87 g), clorofila total (1,48 mg g^{-1}) e nitrogênio (1,66%), enquanto 12 mg L^{-1} resultaram em maior número de ramos (6,69 $plantas^{-1}$), diâmetro da flor (6,53 cm), teor de carboidratos (6,31%), fósforo (0,34%) e potássio (1,78%). Em conclusão, o uso combinado de ácido húmico e CPPU melhorou o crescimento vegetativo, o desempenho da floração e a composição bioquímica da calêndula, com as doses mais altas (4 mL L^{-1} HA e 8–12 mg L^{-1} CPPU) mostrando os efeitos mais benéficos. Estes resultados destacam o potencial do HA e da CPPU como estratégias eficazes de bioestimulantes e reguladores de crescimento para melhorar a produção de plantas ornamentais.
Palavras-chave: bioestimulantes, citocinina, forclorfenurão, melhoria da floração, *Tagetes ereta*.

Introduction

Tagetes, an annual herbaceous plant that grows up to 50 cm during the summer, belongs to the family Asteraceae. Although wild marigolds grow in several countries under diverse environmental conditions, their original habitat is southern Europe and North Africa (Dahal et al., 2021). The species has ornamental significance, being widely used in bouquets and garden decoration. Beyond its aesthetic value, marigold flowers are also harvested for their medicinal and industrial applications (Rime et al., 2025).

Marigold is tolerant to saline, clayey, and acidic soils and has considerable economic value because of its bioactive compounds, particularly flavonoids and carotenoids, which are widely utilized in the food and pharmaceutical industries as antioxidants and natural coloring agents (Domínguez-Niño et al., 2025). Its essential oil, rich in terpinolene and limonene, has positioned India as the leading producer for various applications, including chemicals, agro-industrial materials, fragrances, and cosmetics (Mishra et al., 2024). With 56 species worldwide-27 annuals and 29 perennials-the genus Tagetes is considered one of the most significant medicinal plant families, distinguished by its profuse summer and autumn blooms and the strong fragrance emitted by its leaves and petals. The species is propagated by seeds that germinate within 3 - 5 days at 24 – 27 °C (Zhang et al., 2020). In addition, marigold tissues contain several bioactive compounds such as linalool, salicylaldehyde, tagetone, ocimene, 1,8-cineole, myrcene, pinene, and limonene, which are used in treating liver disorders, stomach pain, and intestinal parasites (Salehi et al., 2018). Marigold cultivation is also important in orchards and ornamental fields because its roots exude allelopathic compounds that control soil nematodes and act as insect repellents (Gökalp, 2023).

Among soil amendments, both natural and synthetic organic fertilizers have demonstrated high efficiency in promoting plant growth and development while being environmentally safe (Piccolo and Drosos, 2025). Humic acid (HA), due to its complex composition of organic components, amino acids, and minerals, is one of the most effective organic amendments, with proven potential to improve plant productivity (Ampong et al., 2022). HA enhances soil chemical, biological, and physical properties, facilitates nutrient absorption, increases membrane permeability, and promotes enzymatic activity, carbohydrate accumulation, and chlorophyll biosynthesis (Bera et al., 2024; Ren et al., 2022). It also enhances root and shoot development, accelerates cell division, improves plant resistance to stress, and ensures the continuity of essential physiological functions. Previous studies have confirmed that

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HA application increases vegetative growth by enhancing photosynthetic efficiency and stimulating protein and carbohydrate metabolism (Khudair and Albbas, 2021; Nazarova et al., 2022).

In addition to biostimulants, plant growth regulators play an important role in agriculture by stimulating vegetative growth, modifying morphological traits, and improving yield (Aremu et al., 2020). Forchlorfenuron (CPPU; N-(2-chloro-4-pyridyl)-N-phenylurea), also known as KT-30, is a synthetic cytokinin with far greater efficiency than benzyladenine, having a longer active life due to its slow metabolic degradation (Harhash et al., 2023; Omar and Al-Dulaimy, 2023). Physiologically, CPPU stimulates cell division and expansion, breaks apical dominance to promote lateral bud growth, and enhances both the quality and quantity of several horticultural crops (Emery and Kisiala, 2020).

It is hypothesized that the combined application of humic acid (HA) and CPPU will exert a synergistic effect, leading to significant improvements in vegetative growth, flowering performance, and biochemical attributes of marigold plants relative to untreated controls. In line with this hypothesis, the present study was undertaken to: (i) elucidate the individual effects of varying concentrations of HA on growth dynamics, flowering behavior, and key biochemical parameters; (ii) determine the influence of CPPU concentrations on plant development and flower quality; and (iii) identify the optimal HA–CPPU combination capable of maximizing both the ornamental value and physiological performance of marigold.

Materials and Methods

Experiment Site

This study examined the effects of humic acid (H), CPPU (C), and their interaction on the growth, flowering, and chemical parameters of marigold cv. Double Eagle (Its seeds are produced by Mr. Fothergill's Seeds, Kentford, Newmarket, CB7QB,UK Company). The experiment was carried out in the lath house that is made of wood that allows approximately 50% of the light to pass through of the Horticulture Department at the College of Agriculture, University of Anbar located in Ramadi city, Al-Anbar Governorate, Iraq, at approximately 33.35°N latitude and 43.79°E longitude. The area features a hot and arid desert climate, characterized by long, extremely hot summers, short winters with occasional cold spells, and limited rainfall mainly occurring during the winter months.

The pots were filled with a growth medium composed of river sand and peat moss at a ratio of 2:1, which was sterilized with 4% formalin and left for seven days before transplanting the seedlings, and 1.9 kg of culture medium was put inside each pot. A total of 82 pots were used in the experiment.

The NPK fertilizer (20:20:20) was applied at a rate of 6 grams per pot. It was divided into two portions: the first portion was applied two weeks after planting (early vegetative stage), and the second portion was applied two months later (early flowering stage). Drip irrigation method was used to irrigate plants.

Treatments

Adding with the humic acid (HA) at three concentrations: HA0 (Using distilled water only), HA1 2 mL and HA2 4 mL. This was done after diluting the last two concentrations in one liter of distilled water.

Spraying with the CPPU (C) at four concentrations: C0 (Using distilled water only), C1 4 mg, C2 8 mg and C3 12 mg (This was done after diluting the last two concentrations in one liter of distilled water).

Plants were sprayed to full coverage using a 2 L sprayer. Tween 20 was added as a dispersant at a rate of 0.1 mL L^{-1} to reduce the surface tension of the spray solution and facilitate maximum absorption by the plants. After the appearance of four to five pairs of true leaves, humic acid (HA) was added to the growth medium, and the plants were sprayed with CPPU twice, with a two-day interval between applications. The second spraying was conducted one month later.

Experimental Design

A two-factor experiment (3 × 4), comprising a total of twelve treatments and three blocks, with five plants in each experimental unit with one plant per pot, resulting in a total of 180 plants used in the experiment, were carried out using a randomized complete block design (RCBD). The least significant difference (LSD) test was employed to compare means at the 5% level of significance following statistical analysis. Data were analyzed using the Genstat program (Al-Mohammedi and Al-Mohammedi, 2012).

The following parameters were evaluated after 15 weeks of planting:

1. Plant height (cm): Measured from the base of the stem to the highest point of the plant using a measuring ruler.

2. Number of branches (branches plant^{-1}): The lateral branches formed on each plant were counted.

3. Number of flowers (flowers plant^{-1}): The number of flowers that opened on each plant was recorded from the onset of flowering until the end of the experiment.

4. Flower diameter (cm): Measured at the widest point of the flower using a digital vernier caliper. Measurements were taken on five flowers per plant, and the mean value was recorded.

5. Fresh flower weight (g): The weight of the whole flower, including the stalk, was measured using a sensitive electronic balance with an accuracy of 0.001 g. Five flowers per plant were measured, and the mean weight was recorded.

6. Total chlorophyll (mg g^{-1}): Approximately 0.1 g of fresh leaf tissue was ground in a ceramic mortar with a suitable amount of 80% acetone until a light green extract was obtained, following the method described by (Goodwin, 1976). The extract was filtered, and the volume adjusted to 10 mL. Absorbance was measured at 645 and 663 nm using a spectrophotometer. Total chlorophyll was calculated as:

Total chlorophyll = (20.2 × A645) + (8.02 × A663).

The results were given in mg per g fresh weight.

7. Total carbohydrate (%): Approximately 0.5 g of dried, ground leaf sample (oven-dried at 65 °C) was extracted with 100 mL distilled water, stirred for 24 hours, and filtered. A 1 mL aliquot of the extract was mixed with 5 mL of 5% phenol solution and 5 mL of concentrated H_2SO4, then placed in a boiling water bath for 30 minutes. Absorbance was measured at 485 nm using a spectrophotometer, and carbohydrate content was determined using a glucose standard calibration curve (Herbert et al., 1971).

8. Macroelements content in leaves (N, P, K): Leaf samples were washed with water to remove dust and dried in an oven at 65 °C until constant weight. Samples were ground and sieved through a 0.2 mm mesh. Approximately 0.2 g of dried sample was digested with 5 mL of 98% concentrated sulfuric acid. After obtaining a clear solution, the samples were cooled and diluted to 50 mL with distilled water. Nitrogen was determined using the Semi-Micro Kjeldahl method (Van Dijk and Houba, 2000), phosphorus was measured spectrophotometrically (Chapman and Pratt, 1961), and potassium was determined using a flame photometer (Tandon, 1995).

Results

Plant height (cm)

According to the results, humic acid (HA) application significantly affected plant height (Table 1). The 4 mL L^{-1} and 2 mL L^{-1} HA treatments produced the highest values, measuring 37.67 cm and 36.70 cm, respectively, whereas the control treatment (0 mL L^{-1}) resulted in the lowest plant height of 33.65 cm. Similarly, foliar spraying with CPPU had a significant effect, with the 8 mg L^{-1} treatment yielding the highest height (36.91 cm) and the 0 mg L^{-1} treatment the lowest (34.97 cm).

A significant two-way interaction between HA and CPPU was also observed. The combination of 4 mL L^{-1} HA and 8 mg L^{-1} CPPU produced the tallest plants at 39.54 cm, while the control combination (0 mL L^{-1} HA + 0 mg L^{-1} CPPU) resulted in the shortest plants at 32.04 cm (Table 2).

Table 1. Effect of adding humic acid (HA) and spraying with CPPU (C) on growth and flowering traits of Marigold.

Treatments		Plant height (cm)	Branch numbers (branch plant^{-1})	Flowers number (flower plant^{-1})	Flower diameter (cm)	Fresh flower weight (g)
Humic acid (HA)	HA0 (0 ml L^{-1})	33.65	5.73	5.97	5.86	0.72
	HA1 (2 ml L^{-1})	36.70	6.04	7.05	6.17	0.80
	HA2 (4 ml L^{-1})	37.67	6.84	7.88	6.68	0.83
LSD 5%		0.98	0.64	0.47	0.40	0.06
CPPU (C)	C0 (0 mg L^{-1})	34.97	5.76	6.40	5.97	0.69
	C0 (4 mg L^{-1})	35.33	5.82	6.96	6.42	0.81
	C0 (8 mg L^{-1})	36.91	6.56	7.35	6.29	0.87
	C0 (12 mg L^{-1})	36.82	6.69	7.15	6.53	0.76
LSD 5%		1.14	0.73	0.92	0.46	0.07

Table 2. Effect of interactions between adding humic acid (HA) and spraying with CPPU (C) on growth and flowering traits of Marigold.

Treatments		Plant height (cm)	Branch numbers (branch plant^{-1})	Flowers number (flower plant^{-1})	Flower diameter (cm)	Fresh flower weight (g)
HA0 (0 mL L^{-1})	C0 (0 mg L^{-1})	32.04	4.15	5.32	5.13	0.61
	C1 (4 mg L^{-1})	32.73	5.71	6.43	5.79	0.72
	C2 (8 mg L^{-1})	34.17	6.35	6.15	6.46	0.84
	C3 (12 mg L^{-1})	35.65	6.73	5.97	6.54	0.70
HA1 (2 mL L^{-1})	C0 (0 mg L^{-1})	35.75	6.40	6.81	5.21	0.72
	C1 (4 mg L^{-1})	35.66	5.59	7.24	6.64	0.93
	C2 (8 mg L^{-1})	37.02	6.23	6.96	6.07	0.82
	C3 (12 mg L^{-1})	38.36	5.96	7.18	6.16	0.73
HA2 (4 mL L^{-1})	C0 (0 mg L^{-1})	37.12	6.74	7.08	6.67	0.75
	C1 (4 mg L^{-1})	37.60	6.16	7.22	6.82	0.77
	C2 (8 mg L^{-1})	39.54	7.07	8.93	6.35	0.95
	C3 (12 mg L^{-1})	36.43	7.39	8.30	6.88	0.87
LSD 5%		1.97	1.26	0.93	0.80	0.12

Branch numbers (branch plant^{-1})

As shown in Table 1, humic acid (HA) significantly affected the number of branches. The 4 mL L^{-1} HA treatment produced the highest value, reaching 6.84 branches per plant, while the control treatment (0 mL L^{-1}) resulted in the lowest value of 5.73 branches per plant. Foliar spraying with CPPU also had a significant effect, with the 12 mg L^{-1} treatment yielding the highest number of branches (6.69 branches per plant) and the 0 mg L^{-1} treatment the lowest (5.76 branches per plant). A significant two-way interaction between HA and CPPU was observed. The control combination (0 mL L^{-1} HA + 0 mg L^{-1} CPPU) produced the lowest number of branches at 4.15 per plant, whereas the combination of 4 mL L^{-1} HA and 12 mg L^{-1} CPPU resulted in the maximum number of branches at 7.39 per plant (Table 2).

Flowers number (flowers plant^{-1})

The application of humic acid (HA) had a significant effect on the number of flowers, as illustrated in Table 1. The 4 mL L^{-1} HA treatment produced the highest number of flowers, recording 7.88 flowers plant^{-1}, while the control treatment (0 mL L^{-1}) resulted in the lowest number, 5.97 flowers plant^{-1}.

Regarding CPPU foliar spraying, the 8 mg L^{-1} treatment yielded the highest number of flowers at 7.35 per plant, whereas the 0 mg L^{-1} treatment produced the lowest, 6.40 flowers plant^{-1}. A significant two-way interaction between HA and CPPU was observed. The combination of 4 mL L^{-1} HA and 8 mg L^{-1} CPPU produced the highest number of flowers at 8.93 plant^{-1}, in contrast to the control combination (0 mL L^{-1} HA + 0 mg L^{-1} CPPU), which yielded the fewest flowers at 5.32 plant^{-1} (Table 2).

Flower diameter (cm)

The 4 mL L^{-1} HA treatment produced the largest flowers, measuring 6.68 cm, while the control treatment (0 mL L^{-1}) resulted in the smallest flowers, measuring 5.86 cm. CPPU foliar spraying also had a notable effect, with the 12 mg L^{-1} treatment yielding the largest flower diameter at 6.53 cm, and the 0 mg L^{-1} treatment the smallest at 5.67 cm (Table 1). A significant two-way interaction between HA and CPPU was observed; the combination of 4 mL L^{-1} HA and 12 mg L^{-1} CPPU produced the largest flower diameter at 6.88 cm, whereas the control combination (0 mL L^{-1} HA + 0 mg L^{-1} CPPU) resulted in the smallest flowers at 5.13 cm (Table 2).

Fresh flower weight (g)

Humic acid (HA) treatments of 2 mL L^{-1} and 4 mL L^{-1} produced the highest fresh flower weights, measuring 0.83 g and 0.80 g, respectively, while the control treatment (0 mL L^{-1}) showed the lowest weight of 0.72 g (Table 1). Foliar application of HA had a significant effect on flower weight. Similarly, CPPU spraying significantly influenced this trait, with the 8 mg L^{-1} treatment producing the highest weight at 0.87 g, and the 0 mg L^{-1} treatment the lowest at 0.69 g. Furthermore, a significant two-way interaction between HA and CPPU was observed. The combination of 4 mL L^{-11} HA and 8 mg L^{-1} CPPU produced the heaviest flowers at 0.95 g, whereas the control combination (0 mL L^{-1} HA + 0 mg L^{-1} CPPU) resulted in the lowest flower weight at 0.61 g (Table 2).

Total chlorophyll (mg g^{-1})

Table 3 illustrates that humic acid (HA) significantly affects total chlorophyll content. The 4 mL L^{-1} HA treatment produced the highest level at 1.54 mg g^{-1}, while the control treatment (0 mL L^{-1}) had the lowest at 1.36 mg g^{-1}. Similarly, foliar spraying with CPPU had a significant effect on this trait, with the 8 mg L^{-1} treatment yielding the highest value of 1.48 mg g^{-1}, and the 0 mg L^{-1} treatment the lowest at 1.36 mg g^{-1}.

A significant interaction between HA and CPPU was also observed; the combination of 4 mL L^{-1} HA and 8 mg L^{-1} CPPU produced the highest total chlorophyll content at 1.67 mg g^{-1}, whereas the control combination (0 mL L^{-1} HA + 0 mg L^{-1} CPPU) resulted in the lowest value of 1.28 mg g^{-1} (Table 4).

Table 3. Effect of adding humic acid (H) and spraying with CPPU(C) and their interactions on chemical traits of Marigold leaves.

Treatments		Total chlorophyll (mg100 gm^{-1})	Total carbohydrates (%)	Nitrogen (%)	Phosphorous (%)	Potassium (%)
Humic acid (HA)	HA0 (0 ml L^{-1})	1.36	5.77	1.57	0.21	1.66
	HA1 (2 ml L^{-1})	1.40	6.07	1.59	0.30	1.73
	HA2 (4 ml L^{-1})	1.54	6.38	1.65	0.37	1.79
LSD 5%		0.05	0.36	0.04	0.04	0.06
CPPU (C)	C0 (0 mg L^{-1})	1.36	5.69	1.51	0.25	1.68
	C0 (4 mg L^{-1})	1.43	6.12	1.60	0.30	1.69
	C0 (8 mg L^{-1})	1.48	6.18	1.66	0.29	1.76
	C0 (12 mg L^{-1})	1.45	6.31	1.64	0.34	1.78
LSD 5%		0.07	0.41	0.06	0.05	0.07

Table 4. Effect of interactions between adding humic acid (HA) and spraying with CPPU(C) and their interactions on chemical traits of Marigold leaves.

Treatments		Total chlorophyll (mg100 g^{-1})	Total carbohydrates (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)
HA0 (0 mL L^{-1})	C0 (0 mg L^{-1})	1.28	5.24	1.42	0.17	1.62
	C1 (4 mg L^{-1})	1.43	5.67	1.53	0.19	1.64
	C2(8 mg L^{-1})	1.31	6.19	1.70	0.24	1.71
	C3 (12 mg L^{-1})	1.40	5.98	1.63	0.26	1.67
HA1 (2 mL L^{-1})	C0 (0 mg L^{-1})	1.35	5.37	1.55	0.23	1.69
	C1 (4 mg L^{-1})	1.42	6.39	1.63	0.30	1.77
	C2 (8 mg L^{-1})	1.44	6.42	1.67	0.34	1.74
	C3 (12 mg L^{-1})	1.38	6.11	1.54	0.32	1.70
HA2 (4 mL L^{-1})	C0 (0 mg L^{-1})	1.46	6.45	1.58	0.36	1.73
	C1 (4 mg L^{-1})	1.43	6.30	1.65	0.41	1.67
	C2 (8 mg L^{-1})	1.67	5.94	1.62	0.28	1.82
	C3 (12 mg L^{-1})	1.59	6.83	1.75	0.43	1.95
LSD 5%		0.13	0.69	0.09	0.08	0.10

Total carbohydrates (%)

The results indicated that foliar application of humic acid (HA) significantly increased the total carbohydrate content of the leaves, reaching a maximum of 6.38% with the 4 mL L^{-1} treatment, compared to the control (0 mL L^{-1}), which produced the lowest value of 5.77% (Table 3). Similarly, foliar spraying with CPPU significantly affected this trait; the 12 mg L^{-1} treatment yielded the highest carbohydrate content at 6.31%, while the 0 mg L^{-1} treatment produced the lowest at 5.69%. A significant interaction between HA and CPPU was observed, with the combination of 4 mL L^{-1} HA and 12 mg L^{-1} CPPU producing the highest value at 6.83%, whereas the control combination (0 mL L^{-1} HA + 0 mg L^{-1} CPPU) resulted in the lowest value of 5.24% (Table 4).

Percentage of nitrogen

Application of humic acid (HA) at 4 mL L^{-1} significantly increased the nitrogen content of the leaves, reaching a maximum of 1.65%, whereas the 0 mL L^{-1} and 2 mL L^{-1} treatments showed lower values of 1.57% and 1.59%, respectively (Table 3). Similarly, foliar spraying with CPPU significantly influenced this trait. The highest nitrogen contents, 1.66% and 1.64%, were observed in the 8 mg L^{-1} and 12 mg L^{-1} treatments, respectively, while the lowest value of 1.51% was recorded for the 0 mg L^{-1} treatment. A significant interaction between HA and CPPU was also observed (Table 4); the combination of 4 mL L^{-1} HA and 12 mg L^{-1} CPPU produced the highest nitrogen content at 1.75%, whereas the control combination (0 mL L^{-1} HA + 0 mg L^{-1} CPPU) resulted in the lowest value of 1.42%.

Percentage of phosphorus

Application of humic acid (HA) significantly increased the phosphorus content of the leaves, reaching a maximum of 0.37% in the 4 mL L^{-11} treatment, whereas the control treatment (0 mL L^{-1}) had the lowest value of 0.21%, as shown in Table 3. Foliar spraying with CPPU also had a significant effect, with the 12 mg L^{-1} treatment producing the highest phosphorus content at 0.34%, while the 0 mg L^{-1} treatment yielded the lowest value of 0.25%. A significant interaction between HA and CPPU was observed; the control combination (0 mL L^{-1} HA + 0 mg L^{-1} CPPU) resulted in the lowest phosphorus content at 0.17%, whereas the combination of 4 mL L^{-1} HA and 12 mg L^{-1} CPPU achieved the highest leaf phosphorus content at 0.43% (Table 4).

Percentage of potassium

The 4 mL L^{-1} humic acid (HA) treatment had the greatest effect, producing the highest potassium content in the leaves at 1.79%, whereas the control treatment (0 mL L^{-1}) resulted in the lowest value of 1.66%, as shown in Table 3. Foliar spraying with CPPU also had a notable impact on this trait, with the 12 mg L^{-1} and 8 mg L^{-1} treatments yielding the highest potassium contents at 1.78% and 1.76%, respectively. In contrast, the 0 mg L^{-1} and 4 mg L^{-1} treatments produced the lowest values at 1.68% and 1.69%, respectively. A significant interaction between HA and CPPU was observed, with the combination of 4 mL L^{-1} HA and 12 mg L^{-1} CPPU producing the highest leaf potassium content at 1.95%, whereas the control combination (0 mL L^{-1} HA + 0 mg L^{-1} CPPU) resulted in the lowest value of 1.62% (Table 4).

Discussion

The overall improvement in the vegetative growth of marigold plants-including plant height, branch number, and leaf area-as well as flowering-related traits, such as early flowering, flower diameter, and flower number per plant, can be attributed to the physiological and biochemical effects of humic acid (HA) soil application. HA is well recognized for enhancing nutrient uptake efficiency, root absorption capacity, and the translocation of nutrients from the soil to the plant (El-Nashar, 2021). This occurs due to its role as a natural chelating agent, forming stable complexes with essential cationic elements, thereby improving their availability to plants (Taih et al., 2023). Moreover, HA restores soil physical, chemical, and biological properties, including soil structure, porosity, and microbial activity. By disintegrating compact soil particles, it improves water retention and aeration in the rhizosphere (Bhatt and Singh, 2022). These improvements are consistent with experimental results in the present study, where HA-treated marigold plants exhibited higher chlorophyll content, indicating enhanced photosynthesis. This agrees with previous reports showing that HA promotes chlorophyll biosynthesis through its effects on carbonyl groups, amino acids, and photosynthetic enzymes (Hardan and Al-Dulaimy, 2022). Additionally, HA plays a critical role in mitigating salt stress by limiting sodium accumulation, thereby protecting roots from damage and improving their functional efficiency under adverse environmental conditions (Mishra et al., 2022). Similar findings have been reported by Alziyituni (2023) and Kumar et al. (2025).

Similarly, foliar application of the synthetic growth regulator forchlorfenuron (CPPU) significantly enhanced both vegetative growth and reproductive performance of marigold plants. The stimulating effect of CPPU is associated with its auxin-like activity, particularly in promoting shoot elongation, leaf production, and dry matter accumulation. CPPU is reported to disrupt apical dominance, thus stimulating lateral bud outgrowth and resulting in bushier plants (Roopa et al., 2023). In this study, the observed increase in branch number and earlier flowering in CPPU-treated plants corroborates this mechanism. Furthermore, CPPU enhances cell division in the apical meristem, supporting tissue differentiation and organ development. Previous studies have also confirmed that CPPU promotes enzymatic activities related to nucleic acid and protein synthesis, which are essential for organogenesis, photosynthesis, and carbohydrate metabolism (Wu et al., 2021). The higher chlorophyll content in treated plants may also be linked to CPPU's role in stimulating chloroplast development and plastid formation (Al-Dulaimy and Mohammed, 2024). By improving photosynthetic efficiency, CPPU enhances carbohydrate production, which supports immediate energy needs for leaf expansion and is later translocated to stems, roots, and developing flowers as structural and metabolic reserves (Sosnowski et al., 2023). CPPU has also been shown to induce enzymes such as NADP-protochlorophyllide reductase, which plays a central role in chlorophyll biosynthesis and chloroplast maturation (Camas-Reyes et al., 2022). Consequently, treated plants exhibit greater carbohydrate accumulation, biomass production, and dry matter content across multiple organs (Gan et al., 2022; Regi and Acharya, 2023). These results are consistent with earlier findings by Al-Gubouri and Al-Saad (2020), Star et al. (2023), and Awad and Ahmed (2024).

Overall, the improvements in morphological, physiological, and reproductive traits observed in marigold plants under individual or combined applications of HA and CPPU highlight their complementary roles in enhancing nutrient uptake, regulating hormonal balance, promoting growth, mitigating stress, and increasing photosynthetic efficiency.

Conclusions

The study showed that the development, flowering, and chemical traits of marigold plants were considerably impacted by the application of humic acid (HA) and the sprinkling of CPPU. At 4 mL L^{-1} of HA, the greatest of traits were noted, resulting in improved biochemical characteristics, higher plant height, and more branches and flowers. Similarly, 8 mg L^{-1} and 12 mg L^{-1} of CPPU spraying promoted growth, flowering, and chemical content of plants. The most favorable outcomes were achieved when HA and CPPU were applied, demonstrating the complementary effects of various treatments. These results highlight how crucial it is to incorporate organic biostimulants to improve marigold growing in lath house. It is advised that more research be done to examine the long-term effects of these treatments during various growth seasons.

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Author Contribution

AFZA: Conceptualization, Methodology, Formal Analysis, Validation, Writing – Review & Editing. **SHA:** Conceptualization, Methodology, Formal Analysis, Validation, Writing – Review & Editing. **HEA:** Investigation, Data Curation, Writing – Original Draft.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available upon request to the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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