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Research Article

The growth and biochemical responses of pot marigold (*Calendula officinalis* L.) to wastewater types and nutrient application

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Abstract

The research aimed to examine the effects of wastewater and both organic and inorganic fertilizers on the growth and biochemical traits of pot marigolds (*Calendula officinalis*). The study was conducted in a greenhouse at the Faculty of Agriculture, University of Hormozgan. Using a split-plot experiment arranged within a completely randomized block design with three replications, the study considered three types of irrigation water as the main factor: Tap water (control), treated municipal wastewater from Roudan city, and dormitory wastewater (treated wastewater from a local treatment plant established at the university's dormitory complex). The secondary factor included various fertilizers: Humic acid (2000 ppm), seaweed extract (3000 ppm), iron (Fe) (2000 ppm), zinc (Zn) (2000 ppm), and a control group without fertilizer. The results showed that the type of irrigation water significantly affected multiple traits, including the number of branches, plant height, number of flowers per plant, dry flower yield, and total phenol and flavonoid contents (TPC and TFC, respectively). The highest number of branches (7.08) and TPC (3.45 mg GAE g^{-1} DW) were observed with dormitory wastewater irrigation. The maximum plant height (49.6 cm) and the greatest number of flowers per plant (11.27) were achieved with municipal wastewater irrigation. Additionally, the type of fertilizer significantly influenced all traits except flower diameter. The highest number of branches (6.68), plant height (49.55 cm), and flower diameter (4.28 cm) were obtained with seaweed fertilizer. The longest flowering period (35.00 days), number of flowers (11.53), and dry weight of a single flower (38.00 g) were recorded with Fe spraying. Furthermore, the highest levels of chlorophyll a, chlorophyll b, total chlorophyll, and TPC were observed with Fe fertilizer application, at 3.07, 1.23, 4.29 mg g^{-1} FW, and 3.43 mg GAE g^{-1} DW, respectively. The highest carotenoid content (0.291 mg g^{-1} FW) and anthocyanin content (0.215 mg g^{-1} FW) also occurred with Fe fertilizer application. The interaction between irrigation water and fertilizer significantly influenced dry flower yield per unit area and TFC. Municipal wastewater outperformed the control group across all measured traits, indicating its beneficial effects. Furthermore, applying fertilizers enhanced all characteristics compared to the control, with the highest dry flower yield achieved using Fe and Zn fertilizers. Utilizing municipal wastewater helps meet the water requirements of pot marigolds and provides their necessary nutrients. When combined with micronutrient fertilizers, municipal wastewater plays a vital role in improving the growth and biochemical properties of pot marigolds.

Keywords: Humic acid, Iron, Municipal wastewater, Seaweed fertilizer, Zinc

Introduction

Calendula officinalis, also known as pot marigold, belongs to the Asteraceae family and has traditionally been used to treat inflammation, skin issues, digestive problems, and nervous system conditions. Scientific studies confirm its anti-inflammatory, antimicrobial, wound-healing, and antioxidant effects, with clinical trials verifying its safety and effectiveness (Zournatzis *et al.*, 2025). Its key active compounds include flavonoids, glucosides, carotenoids, and terpene-based essential oils. Traditionally, it has been used in foods, natural dyes, cosmetics, and as a flavoring and coloring

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agent (Zhangabay and Berillo, 2023; Lekshmi *et al.*, 2023). The pot marigold is a winter annual herb valued for its decorative and medicinal flowers, which bloom during winter and spring, featuring two rows of hairy bracts. It has simple leaves, a long root system with secondary roots, and a branching stem that reaches 70–90 cm in height (Jan *et al.*, 2017). Commonly, it is used in landscape gardening as an ornamental plant, with its vibrant yellow-to-orange flowers popular as cut flowers (Barut and Tansi, 2024).

Water is essential for life; However, climate change, global warming, and industrial development are increasingly exhausting available water resources amid rising demand. The growth in population and food needs has intensified water scarcity worldwide (Arain *et al.*, 2019). Drought, the most severe environmental stress affecting ornamental plants globally, is worsening with rising temperatures, especially in arid and semi-arid regions where drought impacts nearly 50% of the world's agricultural lands and affects a large part of the global population (FAO, 2021).

As arid lands expand, water security and agricultural productivity face major challenges (FAO, 2021; Zaman *et al.*, 2017). In regions like Iran, situated within the desert belt, water scarcity severely restricts agricultural growth (FAO, 2021). To address these urgent issues, enhancing water use efficiency has become crucial, with methods such as utilizing municipal wastewater emerging as promising solutions (Mircea *et al.*, 2025). Municipal wastewater, rich in minerals and organic matter, can improve soil fertility and serve as a cost-effective water source for irrigation and urban green spaces, thus easing pressure on freshwater supplies (Banitalebi *et al.*, 2016; Tabatabaei *et al.*, 2020). Exploring such alternative resources significantly contributes to sustainable water management, supporting both agricultural productivity and environmental resilience amid ongoing climate challenges.

Irrigation using treated wastewater has become an essential practice, mainly supporting agriculture and landscaping, especially in water-scarce areas. Reusing wastewater not only conserves freshwater resources but also reintroduces vital nutrients, including nitrogen and phosphorus, into the soil, decreasing reliance on mineral fertilizers (Santos *et al.*, 2023). However, it requires careful analysis to determine the right nutrient levels and identify any extra supplements needed for optimal crop growth. Research by Urbano *et al.* (2017) confirms that wastewater alone may not provide enough micronutrients such as copper, Fe, and Zn, which are crucial for healthy plant development. Ensuring an adequate supply of these key nutrients is essential for plant survival, growth, and productivity. To meet these needs, various chemical and organic fertilizers, including seaweed extracts, humic acids, and micronutrient supplements, are used to supply vital nutrients and encourage healthy plant growth (Chaubey *et al.*, 2023).

Seaweed fertilizer, characterized by its organic composition that includes macro and microelements, amino acids, and plant growth regulators, has gained popularity in recent years for improving soil fertility and boosting the growth and yield of various plants (Margal *et al.*, 2023). Some studies have shown the positive effects of seaweed fertilizer on the vegetative, physiological, and reproductive traits of pot marigold plants (Emam *et al.*, 2016).

Humic acid, an organic fertilizer derived from the decomposition of organic matter, plays a vital role in improving nutrient absorption through chelation. When used with various mineral fertilizers, humic acid has been shown to encourage plant growth (Suman *et al.*, 2017). Studies have reported that humic acid can enhance morphological traits, nutrient uptake, dry flower yield, flower number, and the lifespan of pot marigold plants (Allahvirdizadeh and Nazari Deljou, 2014).

Micronutrients like Fe and Zn are essential for plant processes, including photosynthesis, growth, and development (Cakmak *et al.*, 2023), and their deficiency is common in arid and semi-arid regions, which hampers plant growth. Therefore, applying these nutrients in such areas is recommended to enhance plant growth (Bolan *et al.*, 2023).

Some studies indicate a positive growth response of pot marigolds to micronutrients, including Fe and Zn (Yadegari, 2017; Yadegari *et al.*, 2016; Yadegari and Alayian, 2012). These studies showed that in treated plants, the content of flavonoids, carotenoids, and phenols, as well as the dry weight of flowers and the number of flowers (Yadegari, 2016), increased significantly. Furthermore, the study by Yadegari and Alayian (2012) demonstrated that Fe and Zn, when applied at a concentration of 2000 ppm, had a significant effect on the examined characteristics of this plant.

This study investigates the combined effects of municipal and dormitory wastewater, along with micronutrient fertilizers (Fe and Zn), on the growth and biochemical properties of pot marigold. Given Iran's limited freshwater resources, utilizing wastewater for irrigation presents a sustainable solution; However, research on its interaction with micronutrients remains limited. This pioneering approach examines how integrating wastewater reuse with micronutrient supplementation can enhance ornamental plant development, offering new insights for sustainable landscaping in arid regions.

Materials and methods

Site of study description and experimental treatment details: To investigate the effects of various fertilizers and two wastewater sources on the quantitative and qualitative traits of the pot marigold plant, a split-plot experiment was conducted using a completely randomized block design with three replications in the research greenhouse of the University of Hormozgan.

Table 1. Chemical characteristics of humic acid fertilizer

Humic Acid	Humic Acid	K	Organic C	N	Ca	Mg	Fe	Folic acid	
	%								
	60	12	36	1	1	0.15	0.05	5	
Seaweed	N	P	K	Ca	Mg	Fe	Zn	Cu	Mg
	%					ppm			
	0.18	0.48	1.89	0.11	0.01	256	11.9	15.6	13.1

Table 2. Selected soil properties of the greenhouse at a soil depth of 0–30 cm

Physicochemical properties	
Texture	Sandy Loam
pH	7.86
Total C (%)	0.35
Total N (%)	0.03
Electrical conductivity (EC) (dS/m)	1.21
Exchangeable Potassium (mg kg^{-1})	519.33
Exchangeable Phosphorus (mg kg^{-1})	6.20
Fe (mg kg^{-1})	7.9
Zn (mg kg^{-1})	0.8
Cu (mg kg^{-1})	0.9
Mn (mg kg^{-1})	6.1

Table 3. Chemical characteristics of municipal and dormitory wastewaters

Wastewater types	pH	Electrical Conductivity	N	P	Detergents
		ds/m	mg L^{-1}		
Municipal	7.62	1.99	17.96	1.55	0.25
Dormitory	7.64	1.50	21.33	2.06	0.19

Greenhouse conditions were maintained with maximum temperatures ranging from 30 to 35°C and minimum temperatures between 15 and 20°C. Relative humidity levels varied from 30% to 50%, providing a controlled environment conducive to plant growth. Additionally, the plants received at least 9 to 10 hours of sunlight daily, ensuring adequate light for photosynthesis and development.

The main factor included three types of irrigation water: Tap water (control), municipal wastewater from Roudan city, and dormitory wastewater (treated wastewater from a local treatment plant established in the dormitory complex of the University of Hormozgan). The fertilizer levels were considered as the sub-factor and included the control, seaweed extract (3000 ppm), humic acid (2000 ppm), iron (Fe) (2000 ppm), and zinc (Zn) (2000 ppm), and the fertilizer solutions were prepared according to the manufacturer's instructions by mixing 3000, 2000, 2000, and 2000 milligrams of the commercial sample in 1000 cc of water, respectively. The chemical characteristics of humic acid and seaweed extract fertilizers are shown in Table 1.

To assess the soil's physical and chemical properties before planting, soil samples were collected from a depth of 0-30 cm of the field soil and analyzed for their physical and chemical characteristics (Table 2).

The pot marigold seeds were purchased from Pakan Bazr Co. in Iran and were sown directly in rows spaced 0.6 meters apart, with 0.2 meters between plants within each row. To support germination and healthy seedling development, the first and second irrigations were thoroughly applied with tap water.

Weekly, municipal wastewater was collected from the Roudan City treatment plant, as well as from the dormitory at the University of Hormozgan. To improve the homogeneity of the nutrient composition in the irrigation water, several samples were collected separately from each wastewater source, mixed, and a uniform sample was used for each irrigation time (Table 3).

The foliar application of Fe and Zn fertilizers was performed at the 5-6 leaf stage, and again, it was repeated 15 days later. To improve accuracy, foliar spraying was done manually in the morning, ensuring even coverage on the plant's aerial parts. Seaweed and humic acid fertilizers were applied during cultivation as fertigation.

Vegetative and reproductive growth traits analysis: During the first week, five pot marigold plants were randomly selected from the middle section of the two central rows in each experimental plot to eliminate border effects. These samples were used to measure various vegetative and floral parameters, including plant height (cm), number of branches, number of flowers per plant, flower diameter (cm), mean flower weight (g), and dry flower yield per unit area. Plant height was measured using a millimeter-centimeter ruler, while flower diameter was measured with a digital caliper. After harvesting the plants, the flowers were separated and weighed using a digital scale with 0.001 g accuracy. The flowers were then oven-dried at 72ºC for 48 hours to estimate the dry flower yield per unit area. The flowering period (days) for each plant was recorded through daily visits, noting the start and end times of flowering.

Biochemical traits analysis: The chlorophyll a, b, total, and carotenoids were measured using the Arnon method (Arnon, 1949). According to this method, one gram of fresh pot marigold leaf was ground in a mortar with 20 ml of 80% acetone. The extract was then centrifuged at 6000 rpm for 10 minutes. Afterwards, 3 ml of the supernatant was transferred to a quartz cuvette, and absorbance was measured at wavelengths of 470, 645, and 663 nm using a spectrophotometer (model CE 2501, Cecil). The chlorophyll a, b, total, and carotenoid contents (mg g^{-1} FW) were then determined.

To measure total phenol content (TPC), 5 g of dried petals from pot marigold flowers were ground in a mortar with 15 mL of 80% ethanol and then centrifuged for 30 minutes. The supernatant was collected, and the Folin-Ciocalteu method using gallic acid (GAE) as a standard was used to determine the phenolic content. For this, 1 mL of extract was mixed with 5.0 mL of Folin-Ciocalteu reagent and 1.5 mL of 20% sodium carbonate solution. The mixture was homogenized for 15 seconds with a vortex mixer and then kept in the dark for 30 minutes. Absorbance was measured at 765 nm using a spectrophotometer. TPC was expressed as milligrams of gallic acid (GAE) per gram of dry extract (Ilic *et al*., 2017).

To assess the total flavonoid content (TFC), an ethanolic petal extract at a concentration of 1.0 mg mL^{-1} was combined with potassium acetate and aluminum chloride solutions. The absorbance was measured at 414 nm using a spectrophotometer, with quercetin serving as the standard. The TFC was expressed as milligrams of quercetin (QE) per gram of dry extract (Ilic *et al*., 2017).

A gram of fresh leaves was ground into a powder using liquid nitrogen to measure anthocyanins. Subsequently, 10 ml of acidified methanol containing 1% hydrochloric acid was added to each sample, which was then stored in the dark at 4ºC for 24 hours. Following centrifugation, the absorbance of the supernatant was measured at 550 nm (Wagner, 1979).

The collected data were analyzed statistically using SAS software (version 9.1). Mean comparisons were performed at a 5% significance level with the Duncan test. Graphs illustrating the results were generated using Excel (version 2016).

Results and discussion

Vegetative growth: The results of the analysis of variance showed that the effect of irrigation types on the number of branches ($P<0.05$) and plant height ($P<0.01$) was significant. While fertilizers significantly affected both of them at 1% levels. The interaction effects of irrigation and fertilizer types on these two vegetative traits were not significant (Table 4).

Both types of wastewater significantly increased the number of branches compared to tap water (control). The highest and lowest values for this trait were observed in plants irrigated with dormitory wastewater (7.08) and tap water (4.66), respectively, showing a significant difference. Consequently, applying dormitory wastewater resulted in a 51.9% increase in the number of branches compared to the control (Figure 1a).

When marigold plants were irrigated with wastewater, they exhibited a significant increase in height compared to control plants (Figure 1b). The tallest plants were found in municipal wastewater (49.6 cm) and dormitory wastewater (49.2 cm) treatments, which were about 20% taller than plants irrigated with tap water (Figure 1b).

These results align with recent studies that highlight the influence of municipal wastewater on the growth of ornamental plants, such as pot marigolds. Municipal wastewater provides essential nutrients and meets the water needs of plants, resulting in increased plant height and branch growth. Additionally, it contains a rich variety of beneficial microorganisms and organic matter that improve plant health and promote branching (Partani and Mahmoudi Mozafar, 2021; Disha *et al*., 2020; Tabatabaei *et al*., 2020).

Furthermore, all fertilizers significantly increased the number of branches and plant height compared to the control (Table 4). The highest values for branches (6.68) and plant height (49.55 cm) were observed in plants treated with seaweed fertilizer, while the lowest values for branches (5.0) and plant height (39.67 cm) were in the control group (Table 5).

Recent research strongly supports the use of seaweed-based fertilizers to promote plant growth, highlighting their superior effectiveness over synthetic fertilizers due to their bioactive, non-toxic, and environmentally friendly properties (Mukherjee and Patel, 2020). Seaweed extracts are rich in essential nutrients, enhancing the uptake of nitrogen, phosphorus, potassium, and iron. They also contain plant hormones like cytokinin, auxin, and gibberellin, which play vital roles in promoting plant height and branching across various species, including pot marigolds (Kularathne *et al*., 2021; Mukherjee and Patel, 2020; Jadhao *et al*., 2015; Nofal *et al*., 2015).

Reproductive growth, Length of flowering period: The length of the flowering period varied significantly depending on the fertilizer used ($P<0.01$). Neither the irrigation type nor the interaction between irrigation type and fertilizer had a significant effect on this trait (Table 4). The longest flowering periods were observed in plants treated with Fe, Zn, and seaweed fertilizers, with mean durations of 35.00, 34.67, and 32.00 days, respectively. These represented increases of 32.3%, 31.1%, and 21.03% in this trait compared to the control (Table 5).

Supporting these findings, previous studies have shown that foliar application of Fe can promote early bud formation in various ornamental plants, including pot marigolds. The positive effects of Fe and Zn on flowering may stem from their roles as cofactors in

Table 4. Results of variance analysis of the effect of irrigation and fertilizer types on vegetative and reproductive growth of pot marigold

Source of variations	df	Mean of squares						
		Number of branches per plant	Plant height	Length of flowering period	Number of flowers per plant	Flower diameter	Single flower dry weight	Dry flower yield
Block	2	4.54^{**}	298.8^{**}	7.355^{ns}	33.36^{**}	0.152^{ns}	0.0002^{ns}	2592.09^{*}
Irrigation type (I)	2	23.25^{*}	336.8^{**}	15.08^{ns}	71.75^{**}	0.878^{ns}	0.0127^{ns}	348715^{**}
Error a	4	3.32	8.066	4.355	1.822	0.184	0.0042	2407.97
Fertilizer type (F)	4	3.55^{**}	415.2^{**}	112.3^{**}	32.22^{**}	3.136^{*}	0.0213^{**}	228674^{**}
I×F	8	0.91^{ns}	17.24^{ns}	5.7^{ns}	1.639^{ns}	0.177^{ns}	0.002^{ns}	11221^{**}
Error b	24	0.487	17.97	11.21	2.286	0.092	0.002	2588.76
CV		11.53	9.08	10.58	15.46	7.374	15.98	28.89

*** and **: Significant at 5 and 1% probability levels, respectively. ns: non-significant**

Table 5. Effect of fertilizer types on vegetative and reproductive growth of pot marigold

Fertilizer type	Number of branches per plant	Plant height (cm)	Length of flowering period (days)	Number of flowers per plant	Flower diameter (cm)	Single flower dry weight (g)
Control	5.00^{b}	39.67^{b}	26.44^{c}	7.13^{d}	3.86^{b}	0.25^{c}
Humic acid	6.31^{a}	48.88^{a}	30.11^{b}	8.65^{c}	4.11^{a}	0.32^{b}
Seaweed	6.68^{a}	49.55^{a}	32.00^{ab}	10.08^{bc}	4.28^{a}	0.34^{ab}
Fe	6.18^{a}	48.11^{a}	35.00^{a}	11.53^{a}	4.18^{a}	0.38^{a}
Zn	6.07^{a}	47.11^{a}	34.67^{a}	11.47^{ab}	4.06^{ab}	0.36^{ab}

In each column, means with at least one common letter do not have a significant difference from each other (P<0.05)

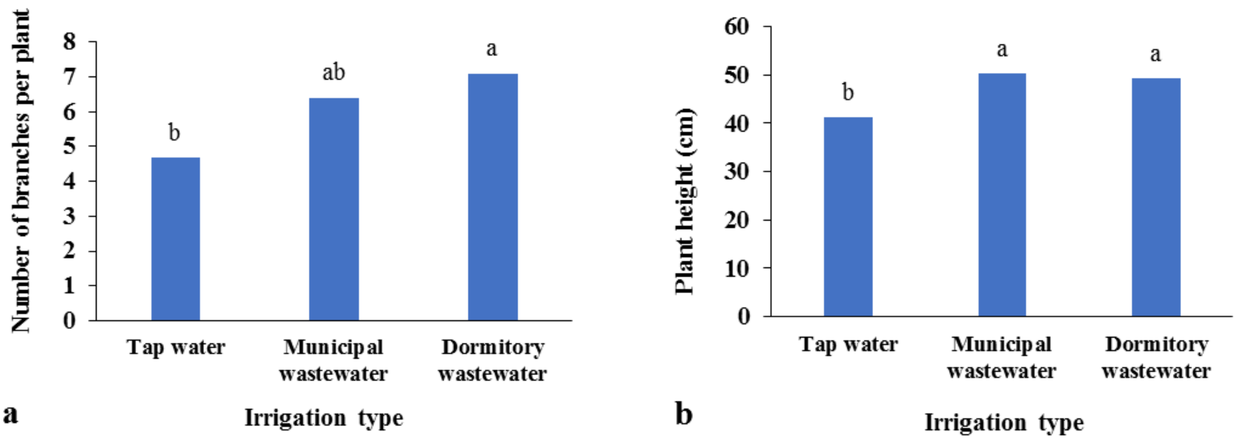

Figure 1. Effect of irrigation types on a) number of branches per plant and b) plant height (cm) of pot marigolds. Means with at least one common letter do not have a significant difference from each other (P<0.05).

various enzymes and their stimulation of plant hormone biosynthesis, such as gibberellins (GA) and auxin, as well as chlorophyll production. Conversely, the flowering promotion caused by seaweed fertilizer is likely due to the presence of cytokinins and auxins in the fertilizer (Srivastava *et al*., 2022; Vanlalruati *et al*., 2019; Pal *et al*., 2016; Kaur *et al*., 2023; Shao *et al*., 2023; Fahad *et al*., 2014; Kularathne *et al*., 2021).

Number of flowers per plant: The number of flowers per plant was significantly affected by both the type of irrigation and fertilizer (Table 4), with municipal and dormitory wastewater leading to a 55% and 48% increase, respectively, compared to tap water (Figure 2). This agrees with Bozdogan's 2014 suggestion that treated wastewater can serve as an alternative irrigation source for marigolds, resulting in the highest number of flowers per plant. The higher concentration of essential nutrients, such as nitrogen, phosphorus, and potassium, in wastewater is identified as a key factor promoting better plant growth aspects, including the number of branches per plant and, consequently, the number of flowers (Partani and Mahmoudi Mozafar, 2021; Disha *et al*., 2020; Tabatabaei *et al*., 2020).

All fertilizers significantly increased the number of flowers per plant compared to the control group. However, pot marigold plants treated with humic acid had significantly fewer flowers than those treated with Zn or Fe. The highest flower numbers were observed with Fe, Zn, and seaweed fertilizer, reaching 11.53, 11.47, and 10.08 flowers per plant, respectively, representing a 61.7%, 60.9%, and 41.4% increase over the control (Table 5). Numerous reports support these findings, highlighting the significant impact of micronutrient fertilizers on flower bud formation and increasing flower numbers in various ornamental plants (Srivastava *et al*., 2022; Hussain *et al*., 2020; Izadi *et*

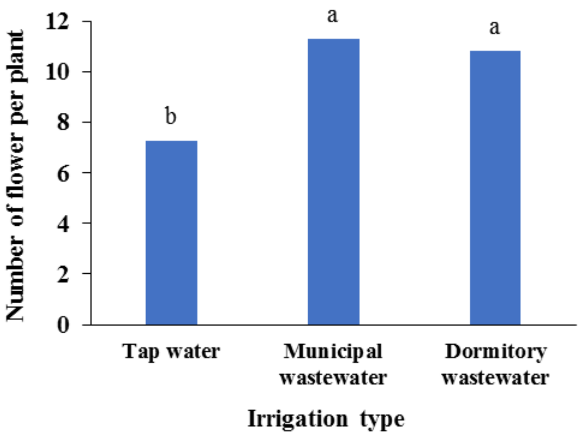

Figure 2. Effect of irrigation types on the number of flowers per plant of pot marigolds. Means with at least one common letter do not have a significant difference from each other (P<0.05).

al., 2020; Vanlalruati *et al*., 2019; Patel *et al*., 2017; Pal *et al*., 2016; Fahad *et al*., 2014). Fe was noted for alleviating chlorophyll biosynthesis, resulting in healthy green leaves and increased assimilate synthesis, which led to better flower production (Aras *et al*., 2022). Similarly, Zn played a role in activating enzymes, participating in chlorophyll synthesis, and supporting physiological processes that promote plant growth, ultimately affecting flower number (Patel *et al*., 2017). The positive effects of seaweed-based fertilizers on flower production were also observed (Al-Hamzawi, 2019), with seaweed extract containing various beneficial components, vitamins, macronutrients, and micronutrients that contribute to improved vegetative growth and, consequently, more flowers (Kularathne *et al*., 2021).

Flower diameter: According to the results in Table 4, only the type of fertilizer significantly affected flower diameter (P<0.05). All fertilizers, except Zn, caused a significant increase in flower diameter compared to the control, with no notable difference between Zn fertilizer and the control (Table 5). The largest flower diameter (4.28 cm) was observed with seaweed fertilizer, while the smallest (3.86 cm) was in the control. Some studies also support the positive effect of seaweed fertilizers on the flower diameter of ornamental plants, including pot marigolds (Al-Hamzawi, 2019; Tartil *et al*., 2016). Seaweed extract used as a fertilizer provides a natural source of plant growth regulators, organic matter, essential macro and microelements, and vitamins, all contributing to improved flower size (Kularathne *et al*., 2021).

Single flower dry weight: According to Table 4, the type of fertilizer significantly affected the single flower dry weight (P<0.01), while the effects of irrigation water and fertilizer on flower diameter were not significant. All types of fertilizers caused a significant increase in this trait compared to the control. The highest single flower dry weight was observed with Fe fertilizer, which was 52% higher than the control (Table 5). Fe is important for flower formation and the development of ornamental flowers, and using Fe fertilizer increases the flower weight of pot marigold (Izadi *et al*., 2020).

Dry flower yield: The results of the ANOVA (Table 4) show that dry flower yield was significantly affected by irrigation water, fertilizer type, and their interaction (P<0.01). Across various irrigation levels, fertilizer generally increased dry flower yield, except when using humic acid in tap water conditions. Although humic acid and seaweed fertilizers improved dry flower yield, their effectiveness was lower than that of Fe and Zn fertilizers. The greatest improvement was observed with Fe and Zn application (Figure 3). Results from Table 4 indicate that Fe and Zn fertilizers significantly increased dry flower yield by extending the flowering period, increasing single flower dry weight, and enhancing the number of flowers per plant.

These improvements in floral traits promoted strong vegetative growth and lush green leaf development, leading to increased assimilate production. This, in turn, supports better bud and flower development, ultimately resulting in higher dry flower yields in pot marigolds (Jena *et al*., 2023; Aras *et al*., 2022; Srivastava *et al*., 2022; Hussain *et al*., 2020; Izadi *et al*., 2020; Pal *et al*., 2016). Many studies have examined the effects of seaweed or humic acid fertilizers on growth characteristics and floral indices of ornamental plants, including pot marigolds, independent of micronutrient applications such as Fe and Zn (El-Nashar, 2022; Karimi *et al*., 2020; Kularathne *et al*., 2021; Al-Hamzawi, 2019). However, the limited influence of seaweed and humic acid fertilizers on improving dry flower yield may be due to their lower nutrient availability over a short period (Ampong *et al*., 2022; Kularathne *et al*., 2021).

Municipal and dormitory wastewater significantly increased dry flower yield compared to tap water, with municipal wastewater generally being more effective than dormitory wastewater in enhancing this trait. The highest dry flower yield (847.3 kg ha^{-1}) was achieved in plants irrigated with municipal wastewater and treated with Fe fertilizer. The lowest yield (193.6 kg ha^{-1}) was observed in pot marigold plants with tap water and no

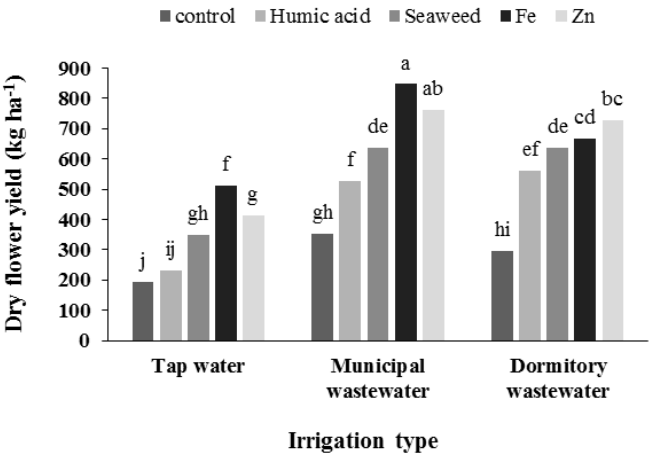

Figure 3. Effect of different fertilizers on dry flower yield of pot marigold under different irrigation type conditions. Means with at least one common letter do not have a significant difference from each other (P<0.05). Fe and Zn are iron and zinc fertilizers, respectively.

fertilizer application. This result indicates a 4.4-fold increase in dry flower yield under these conditions (Figure 3).

In line with these findings, Mann *et al*. (2023) investigated the impact of treated and untreated municipal wastewater on marigold growth, demonstrating that wastewater irrigation enhanced reproductive traits, including dry flower yield. Municipal and dormitory wastewater was found to boost the number of flowers per plant (Figure 2), supporting recent research that highlights the positive effect of municipal wastewater on ornamental reproductive growth, such as flower yield (Partani and Mahmoudi Mozafar, 2021; Disha *et al*., 2020; Tabatabaei *et al*., 2020; Bozdogan, 2014). Municipal wastewater provides essential nutrients, helps fulfill plant water needs, and contains beneficial microorganisms and organic matter, which enhance plant health and branching (Tabatabaei *et al*., 2020).

Biochemical traits, Leaf pigments content: Table 6 shows that the fertilizer has a significant effect on chlorophyll a, b, total carotenoids, and anthocyanins. However, neither the type of irrigation nor the interaction between irrigation and fertilizer had significant effects on these traits.

Statistically, both Fe and seaweed fertilizers similarly resulted in a significant increase in chlorophyll a, b, and total content compared to the control. The highest chlorophyll content (3.07 mg g^{-1} FW) was observed in pot marigold plants treated with Fe fertilizer, and this was the only significant difference compared to other fertilizers, aside from seaweed. Overall, Fe fertilizer resulted in a 27.4% increase in chlorophyll content compared to the control (Table 7). The patterns of chlorophyll b and total chlorophyll content changes in response to different fertilizer levels were similar to those of chlorophyll a. Applying all fertilizers, except humic acid, increased chlorophyll b and total chlorophyll compared to the control. At the same time, there was no significant difference between humic acid and the control. The highest chlorophyll b (1.23 mg g^{-1} FW) and total chlorophyll (4.29 mg g^{-1} FW) levels in plants were achieved when sprayed with Fe fertilizer, showing increases of 36.7% and 30.4%, respectively, compared to the control (Table 7).

The results show that Fe most significantly increases leaf chlorophyll content, supporting reports that state Fe is essential for chlorophyll synthesis and chloroplast health (Jena *et al*., 2023; Kaur *et al*., 2023; Vanlalruati *et al*., 2019; Fahad *et al*., 2014) and helps prevent physiological issues like chlorosis and resetting (Kaur *et al*., 2023; Rout and Sahoo, 2015). Fe and Zn are crucial for photosynthesis, primarily functioning within chloroplasts as cofactors for key protein complexes involved in electron transport. Adequate levels of these elements enhance chlorophyll synthesis and photosynthetic efficiency by regulating gene expression and acting as signaling molecules (Gao *et al*., 2024; Wang *et al*., 2024). Similar positive effects on chlorophyll levels in ornamental plants have been observed with seaweed fertilizer application (Kularathne *et al.,* 2021) due to the cytokinins in seaweed that promote chlorophyll production (Kularathne *et al*., 2021) and improve nutrient uptake (Nofal *et al*., 2015; Jadhao *et al*., 2015).

Furthermore, the application of humic acid, seaweed extract, Fe, and Zn fertilizers significantly increased carotenoid content by 31.6%, 38.8%, 34.0%, and 31.6%, respectively. Similarly, anthocyanin content increased by 27.6%, 48.3%, 36.6%, and 38.6%, respectively, compared to the control. (Table 7). The highest carotenoid content (0.291 mg g^{-1} FW) and anthocyanin (0.215 mg g^{-1} FW) were observed with seaweed fertilizer application, while the lowest (0.209 mg g^{-1} FW) was in the control. These findings align with previous research, which has shown that seaweed foliar application significantly increases carotenoid content in pot marigold flowers (Emam *et al*., 2016). Similar results have been reported by other researchers, highlighting the positive impact of seaweed on

Table 6. Results of variance analysis of the effect of irrigation and fertilizer types on biochemical traits of pot marigold

Source of variations	df	Means of squares						
		Chl a	Chl b	Chl total	Carotenoid	Anthocyanin	TPC	TFC
Block	2	0.296^{ns}	0.03^{ns}	0.515^{ns}	0.008*	0.001^{ns}	0.132^{ns}	0.017^{ns}
Irrigation type (I)	2	0.65^{ns}	0. 018^{ns}	0.134^{ns}	0.0011^{ns}	0.002^{ns}	1.45**	0.258**
Error a	4	0.062	0.005	0.104	0.0009	0.0004	0.18	0.001
Fertilizer type (F)	4	0.579**	0.146**	1.305**	0.01**	0.025**	0.69**	0.096*
I×F	8	0.02^{ns}	0.004^{ns}	0.038^{ns}	0.001^{ns}	0.0015^{ns}	0.22^{ns}	0.051*
Error b	24	0.106	0.01	0.182	0.002	0.0002	0.132	0.025
CV		11.98	9.78	11.32	17.42	18.55	11.16	13.89

*** and **: Significant at 5 and 1% probability levels, respectively. ns: non-significant. TPC: total phenolic content; TFC: total flavonoid content.**

Table 7. Effect of fertilizer types on biochemical traits of pot marigold

Fertilizer type	Chl a	Chl b	Chl total	Carotenoid	Anthocyanin	TPC
			(mg g^{-1} FW)			(mg GAE g^{-1} DW)
Control	2.41^{c}	0.90^{c}	3.29^{d}	0.209^{b}	0.145^{b}	2.78^{b}
Humic acid	2.55^{bc}	0.99^{c}	3.54^{cd}	0.276^{a}	0.185^{a}	3.21^{a}
Seaweed	2.85^{ab}	1.13^{ab}	3.98^{ab}	0.291^{a}	0.215^{a}	3.41^{a}
Fe	3.07^{a}	1.23^{a}	4.29^{a}	0.286^{a}	0.198^{a}	3.43^{a}
Zn	2.71^{bc}	1.03^{bc}	3.74^{bc}	0.275^{a}	0.201^{a}	3.42^{a}
LSD	0.31	0.10	0.41	0.045	0.035	0.35

In each column, means with at least one common letter do not have a significant difference from each other (P<0.05). TPC: total phenolic content

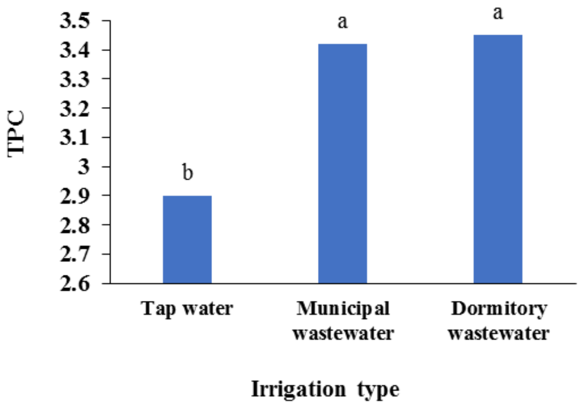

Figure 4. Effect of irrigation types on total phenolic content (TPC (mg GAE g^{-1} DW)) of pot marigolds. Means with at least one common letter do not have a significant difference from each other (P<0.05).

carotenoid levels in plants (Machado *et al.*, 2014). Previous research indicates that organic fertilizers can enhance anthocyanin synthesis in pot marigold (Zaferanchi *et al.*, 2019). Applying seaweed fertilizer has been shown to positively influence pigment levels in ornamental plants, primarily due to the presence of cytokinins that promote pigment synthesis (Kularathne *et al.*, 2021). Additionally, seaweed extracts improve nutrient uptake, further supporting pigment development (Nofal *et al.*, 2015; Jadhao *et al.*, 2015). Furthermore, increased anthocyanin levels are associated with higher soluble sugars and alpha-amylase activity, often as a stress response in organic systems, promoting the synthesis of protective compounds (Zhao *et al.*, 2025).

Total phenolic content (TPC): The results from Table 6 show that the type of irrigation water (P<0.05) and fertilizer (P<0.01) significantly influenced the changes in petal total phenolic content (TPC). Irrigating pot marigold plants with municipal and dormitory wastewater significantly increased TPC compared to the tap water, with increases of 17.9% and 19.0%, respectively (Figure 4). The high levels of phenolic compounds in the wastewater are responsible for this rise, as the plants absorb these compounds (Mohamad Said *et al.*, 2021). Additionally, research indicates that nutrient-rich irrigation waters, such as municipal wastewater, can boost the biosynthesis of phenolic compounds in plants (Tabatabaei *et al.*, 2020; Banitalebi *et al.*, 2016). Likewise, all fertilizers led to a significant increase in TPC compared to the control. The highest TPC (3.43 mg GAE g^{-1} DW) was observed with Fe fertilizer. Although the difference between this fertilizer and others was not significant (Table 7), similar studies show that applying Fe and Zn fertilizers can greatly increase the biosynthesis of phenolic compounds in plants. This is probably because these elements help activate the genes involved in phenolic compound production (Shafiee Adib *et al.*, 2020).

Total flavonoid content (TFC): The results shown

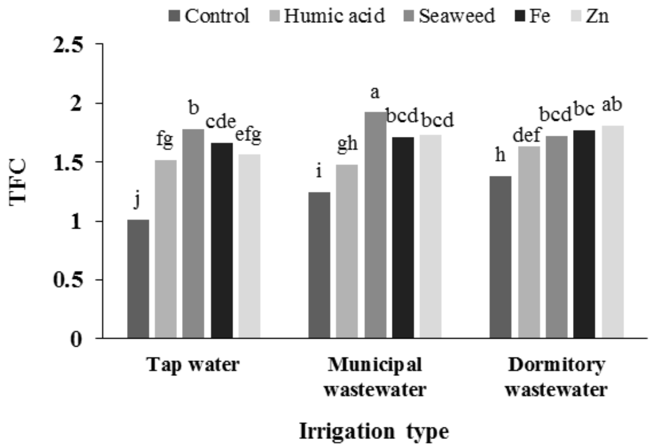

Figure 5. Effect of different fertilizers on total flavonoid content (TFC) (mg QE g^{-1} DW) of pot marigold under different irrigation type conditions. Means with at least one common letter do not have a significant difference from each other (P<0.05). Fe and Zn are iron and zinc fertilizers, respectively.

in Figure 5 indicate that irrigation with municipal wastewater and dormitory wastewater led to an increase in TFC compared to tap water. This increase was significant in unfertilized plants. The high levels of phenolic compounds in wastewater lead to increased absorption by plants (Mohamad Said *et al*., 2021). This supports Bozdogan's (2014) suggestion that treated wastewater can be an effective irrigation source for marigolds, promoting optimal growth. Additionally, elevated nutrients such as nitrogen, phosphorus, and potassium in wastewater play a key role in enhancing plant development (Partani and Mahmoudi Mozafar, 2021; Disha *et al*., 2020; Tabatabaei *et al*., 2020).

For each source of irrigation water, applying fertilizers led to a significant increase in TFC compared to the unfertilized control. In plants irrigated with tap water and municipal water, the largest increases occurred in those fertilized with seaweed, with respective increases of 76.2% and 54.8% compared to the unfertilized control. To support these findings, a recent review study reports that seaweed-based fertilizers promote the synthesis of phenolic compounds, including flavonoids, by activating enzymes involved in their biosynthesis pathway (Sarinana-Aldaco *et al*., 2025). Meanwhile, in plants irrigated with dormitory wastewater, the highest increase (31.2%) was observed in plants treated with Zn. Similar studies indicate that Zn fertilizers significantly boost phenolic compound biosynthesis in plants by activating relevant genes (Shafiee Adib *et al*., 2020).

Conclusion

This study demonstrates that the strategic use of wastewater and targeted fertilization notably enhances the vegetative, reproductive, and biochemical traits of pot marigolds. Specifically, municipal and dormitory wastewater irrigation significantly increased plant height, branch number, and flower production, attributable to their rich nutrient content. Among fertilizers, Fe and seaweed formulations proved most effective in promoting growth parameters, flowering duration, flower size, and pigment synthesis, likely due to their roles in chlorophyll biosynthesis and hormone regulation. The application of these treatments also elevated phenolic and flavonoid contents, indicating improved phytochemical quality and potential health benefits. The synergistic effects observed suggest that integrating wastewater irrigation with specific organic and micronutrient fertilizers can optimize ornamental plant productivity. Nonetheless, careful consideration of environmental safety, particularly concerning heavy metal accumulation, remains essential to ensure sustainable implementation. Overall, the findings support the potential of wastewater and eco-friendly fertilization strategies to enhance ornamental plant cultivation while emphasizing the need for environmental monitoring.

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