



Enhancing growth, nutrient uptake and flowering of *Tagetes patula* plants through the application of suspensions of *Chlorella vulgaris*

João Vieira^{1,2,3} · Moisés Saque² · Catarina Viana³ · Maria Helena Rodrigues² · Luísa Coelho^{1,3} · Florinda Gama^{3,4} · João Varela^{2,3,4} · Hugo Pereira^{3,4} · Mário Reis^{1,2} · Maribela Pestana^{1,2} · Pedro José Correia^{1,2}

Received: 31 July 2024 / Revised: 18 December 2024 / Accepted: 19 December 2024
© The Author(s), under exclusive licence to Springer Nature B.V. 2025

Abstract

Tagetes patula L. is a prominent plant that is frequently used for its ornamental value. Marigolds are sources of various compounds of great interest to agricultural, pharmaceutical, and nutritional industries. Microalgae can be applied as a biostimulant as they have multifunctional properties in agriculture, namely facilitating nutrient uptake, improving crop performance and physiological status, and thus increasing tolerance to abiotic stress. This study evaluated the effect of applying *Chlorella vulgaris* as a plant biostimulant on the growth and flowering of *T. patula* plants. Microalgae suspensions were applied to the substrate at concentrations of 0.5, 2.0 and 5.0 g dry weight (DW) L⁻¹. A set of plants without the application of microalgae was used as control. The use of microalgae had a positive impact on plant growth. Specifically, *C. vulgaris* application resulted in greater height, while the highest concentration (5.0 g DW L⁻¹) resulted in higher fresh weight. Microalgae application significantly promoted the earlier flowering of marigolds. The macronutrient and micronutrient values were statistically similar between the control plants and those treated with microalgae. When considering only the treated plants, there was a significant correlation between the concentration of *C. vulgaris* and the concentrations of N, P, Mn and Cu in the shoots of *T. patula* plants. Plants receiving higher concentrations of *C. vulgaris* exhibited higher levels of these nutrients. These results highlight the potential use of *Chlorella vulgaris* as an effective biostimulant for enhancing the growth, flowering, and nutrient uptake of *T. patula*, offering valuable applications for improving ornamental plant production and, ultimately, agricultural sustainability.

Keywords Biostimulant · Flowering · Marigold · Microalgae · Ornamental plant

Introduction

In recent years the use of biostimulants in agriculture, including horticulture, has seen a notable increase. This trend is driven by the growing demand for commercial products based on natural substances that enhance crop productivity and quality (Kapoor et al. 2021). Plant biostimulants, which include substances or microorganisms applied to plants, aim to increase nutritional efficiency, tolerance to abiotic stress, and crop quality, regardless of their nutritional content (du Jardin 2015; Ma et al. 2022; Viana et al. 2024). This growing interest reflects a broader shift towards sustainable agricultural practices that prioritize environmentally healthy practices.

Among the various types of biostimulants, algae-based products have gained significant attention. Microalgae, which are photosynthetic microorganisms that can be

✉ Pedro José Correia
pcorreia@ualg.pt

¹ MED-Instituto Mediterrâneo Para a Agricultura, Ambiente e Desenvolvimento & CHANGE – Global Change and Sustainability Institute, Universidade Do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
² Faculty of Science and Technology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
³ GreenColab - Associação Oceano Verde, Universidade Do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
⁴ Centro de Ciências Do Mar, Universidade Do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

unicellular or colonial, contain high levels of nutrients, bioactive compounds and growth-promoting substances (Kusvuran 2021). These organisms produce a diverse array of metabolites and have applications in multiple fields, including biofuels, aquaculture, animal feeds, bioremediation, nutrition, pharmaceuticals and cosmetics (Chanda et al. 2019). The estimated number of microalgae species ranges from 50,000 to 1 million (Chiaiese et al. 2018), with commercially significant species including *Isochrysis*, *Chaetoceros*, *Chlorella*, and *Dunaliella*, as well as the cyanobacterium *Arthrospira* (*Spirulina*) (Bayona-Morcillo et al. 2022).

Chlorella vulgaris, a freshwater green microalga, is recognized for its potential as biostimulants in agriculture (Chiaiese et al. 2018). Its biotechnological potential stems from its rich biochemical composition, which includes high content of proteins, lipids, carbohydrates, and pigments such as carotenoids and chlorophylls (Niccolai, 2019). Studies have demonstrated that *C. vulgaris* extracts can stimulate germination, seedling growth, and biomass production in various crops, including lettuce, “Red Russian” kale, soybean, cucumber, tomato, barley and sugar beet (Faheed and Abd-El Fattah 2008; Barone et al. 2017; Morillas-España et al. 2022; Alling et al. 2023). The utilization of *C. vulgaris* not only enhances crop performance but also supports more sustainable agricultural practices by potentially reducing the need for chemical fertilizers (Kusvuran 2021).

Tagetes patula L., commonly known as “marigold” or “French marigold”, are annual plants from the family Asteraceae, native to Mexico and Central America. Known for their ornamental value, marigolds enhance the aesthetic appeal of gardens, flower beds, and decorative arrangements with their vibrant and colourful flowers (Jamal et al. 2023). Economically significant due to their high production and widespread use in ornamental horticulture, marigolds are also valued for their aromatic properties and are used in producing essential oils with larvicidal, insecticidal, herbicidal, and antibacterial properties. These plants are rich sources of various chemical and biochemical compounds, making them of great interest to the agricultural, pharmaceutical, and nutritional industries (Santos et al. 2015, 2022).

Despite the recognized benefits of *C. vulgaris*, there is a notable gap in research regarding its specific effects on ornamental plants like *T. patula*. This study aims to assess the impact of varying concentrations of *C. vulgaris* suspensions on the growth, nutritional, and physiological performance of marigold plants. By addressing this gap, the present study intends to contribute valuable insights into the broader application of microalgae-based biostimulants in horticulture, whose findings might impact significantly on the sustainability and productivity of ornamental plant cultivation.

Materials and methods

Cultivation of *Chlorella vulgaris*

The experiment employed *Chlorella vulgaris* that was cultivated by Allmicroalgae S.A. (Pataias, Portugal) using 10 m³ tubular photobioreactors. To keep pH below 8, CO₂ was injected and a water spray system was used to prevent the culture temperature from exceeding 30°C. At the late exponential growth phase, the culture was harvested and concentrated by tangential filtration system, with 0.2 µm abs. polyvinylidene fluoride (PVDF) membranes. The concentrated paste was obtained by centrifugation and then dried by spray drying, resulting in a final product in powder form. The mineral composition of *C. vulgaris* suspensions was determined using the methods described below for plant material.

Plant material and growth

The experiment was conducted in a greenhouse at the University of Algarve, Portugal (latitude: 37°04'35''N, longitude: 7°97'51'' W). French marigold (*Tagetes patula* L.) seeds were germinated and cultivated in a substrate of vermiculite. After 23 days, the seedlings were transferred to 1 L plastic pots filled with COMPOSANA® universal substrate, a commercial mixture manufactured by Compo (N: 200–450 mg L⁻¹, P₂O₅: 200–500 mg L⁻¹ and K₂O: 300–550 mg L⁻¹). Irrigation was automatic via sprinklers. The microalgal suspensions were applied to the substrate at concentrations of 0.5, 2.0, and 5.0 g DW L⁻¹. Control plants had no microalgae application. Each pot was irrigated with 67 mL of microalgae suspension at each concentration on a weekly basis for a period of 54 days. For the negative control (C: 0.0 g L⁻¹), the plants were irrigated with only water by applying the same volume as that used for other treatments. A total of 20 pots was utilised for each treatment. The water used in the experiment originated from a borehole at the Gambelas campus of the University of Algarve. The experiment was conducted in June under natural photoperiod conditions, with an air temperature below 34 °C and an average relative humidity of 70%. The pH and electrical conductivity (EC) of the microalgae suspensions were measured using a pH and electric conductivity meter (HANNA Instruments HI 9813–5, Romania). The pH ranged from 6.5 for control suspensions (C) to 6.7 for *C. vulgaris* suspensions (CV0.5, CV2 and CV5). The EC of the *C. vulgaris* suspensions were 0.30, 0.39, 0.45, and 0.63 dS m⁻¹, as measured on the control, CV0.5, CV2, and CV5 treatments, respectively.

The number of flower buds and flowers was recorded throughout the experiment. At the end of the experiment

(54 days), the height of the plants was measured for each treatment. The fresh weight (FW) of each plant material was determined for at least three plants, and the dry weight (DW) was subsequently estimated following the drying of the samples at 60°C until constant weight.

Mineral composition

The shoots (stems with leaves, without flowers) were collected from at least three plants per treatment and initially washed with tap water. This was followed by a second wash with deionized water containing a non-ionic detergent (0.1%) to remove surface contamination. The samples were then washed with 0.01 M HCl, and three rinses with distilled water. The FW and DW of the shoots were determined for each treatment following the drying of the samples at 60°C until constant weight.

Subsequently, the dried plant samples and the biomass of *C. vulgaris* underwent milling (1 mm; IKA Werke MF 10 basic, Germany), followed by digestion in an acidic solution consisting of nitric acid and peroxide oxygen. The total nitrogen (N) content was determined by catarametry for the *C. vulgaris* biomass and by the Kjeldahl method (Selecta, micro-Kjeldahl-6014206, Spain) for the plant material. The concentration of K, Ca, Mg, P, S, Fe, Cu, Zn and Mn was determined by inductively coupled plasma optical emission spectroscopy ICP-OES (7000 series, Agilent Technology, U.S.A.). Macronutrients were expressed in g kg⁻¹ while micronutrients were expressed in mg kg⁻¹ DW.

Leaf chlorophyll

During the experimental period, leaf chlorophyll (Chl) concentration in young and mature leaves was evaluated non-destructively using the portable SPAD-502 apparatus (Minolta Corp., Japan). A minimum of three leaves per plant were evaluated, with a total of five readings recorded per leaf. In a previous experiment, a calibration curve was established by measuring chlorophyll (Chl) in leaf disks exhibiting varying degrees of Fe chlorosis using the SPAD-502 (Osório et al. 2014). The same leaf area was ground in a mortar and pestle using 5 mL of 100% acetone in the presence of Na ascorbate. This process was conducted in cold conditions and in the dark to prevent pigment degradation (Abadía and Abadía 1993). The absorbance of the extracts was then measured at 662 and 645 nm. The leaf concentrations of pigments were calculated using the equations of Lichtenthaler (1987):

$$\text{Chl } a = 11.24 A_{662} - 2.04 A_{645}$$

$$\text{Chl } b = 20.13 A_{645} - 4.19 A_{662}$$

$$\text{Chl total} = \text{Chl } a + \text{Chl } b$$

The SPAD-502 values were subsequently converted to total leaf Chl ($\mu\text{mol m}^{-2}$) using the equation for marigold plants.

Fluorescence parameters

At the end of the experiment, the chlorophyll fluorescence parameters F_0 (basal fluorescence), F_m (maximum fluorescence) and F_v (variable fluorescence = $F_m - F_0$) were measured in the first fully developed leaf and in the second mature leaves of each plant using a portable fluorimeter (Plant Efficiency Analyzer, PEA, Hansatech Instruments Ltd., UK). Following at least 30 min dark adaptation the leaves were illuminated with a saturating pulse of 3000 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ for 5 s to induce fluorescence. The F_v/F_m ratio was calculated, reflecting the maximum quantum yield of photosynthetic activity Fig. 1.

Statistical analysis

The experiment was completely randomized design with 20 replicates (pots) for each treatment and 80 replicates (pots) in whole experiment. Means were compared using a one-way analysis of variance (ANOVA) F and the Duncan multiple range test at a significance level of $P \leq 0.05$. Nonlinear regression ($Y = aX^2 + bX + c$) models were employed to establish a relationship between SPAD values and leaf Chl concentrations. Linear regression was used to assess the relationship between the mineral composition of *Tagetes* plants and the doses of *C. vulgaris* applied. All statistical analyses were conducted using IBM SPSS software version 29.

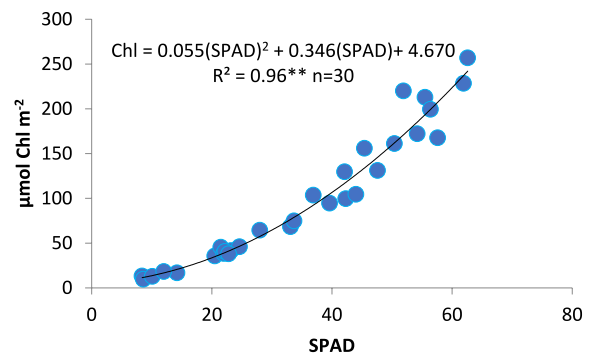


Fig. 1 Relationship between total leaf chlorophyll (Chl) concentration ($\mu\text{mol m}^{-2}$) and SPAD readings for French marigold (*Tagetes patula*) plants. ** significant at $P < 0.001$

Table 1 The mineral composition of *C. vulgaris* biomass. Macronutrients were expressed in g kg^{-1} and micronutrients in mg kg^{-1} . The data are presented as the mean of each nutrient \pm standard error

Macronutrients g kg^{-1}		Micronutrients mg kg^{-1}	
N	80.5 ± 0.4	Fe	3529 ± 180
P	12.2 ± 0.8	Mn	89 ± 8
K	13.8 ± 0.1	B	5.1*
Ca	10.5 ± 0.5	Cu	29 ± 3
Mg	2.5 ± 0.2	Zn	143 ± 10
S	4.6 ± 0.5	Mo	11 ± 1
Na	4.4 ± 0.3	Al	24 ± 1

*single value

Results

The microalgae biomass exhibits elevated levels of N and Fe, with similar values for P, K, and Ca (Table 1). The remaining nutrients display relatively low concentrations, particularly Mg, S, and Na. The Zn concentration was high.

At the beginning of the experiment, the young leaves had lower Chl concentrations with a tendency to increase over time (Fig. 2A). Concurrently, the mature leaves exhibited highest values at the start of the experiment, with a tendency to decrease over time (Fig. 2B). The Chl values were similar across the different treatments, both in young leaves and mature leaves. There were no significant differences between treatments in these physiological parameters.

At the beginning of the experiment, all marigold plants exhibited similar height. At the end of experiment the control plants, which did not receive the microalgae treatment, were significantly smaller than those in the *C. vulgaris* treatments (Fig. 3C).

In the case of total fresh weight (FW), the only significant difference in biomass was between the CV5 and the CV0.5 treatments (Fig. 4A). The total dry weight (DW) showed minimal fluctuations across the experimental treatments, with the highest value observed in the control plants and the lowest in those treated with CV2 (Fig. 4B). There were no significant differences between treatments in the weight of the flowers of each plant (Fig. 4C and D).

Fig. 2 Total leaf chlorophyll concentration (Chl, $\mu\text{mol m}^{-2}$) of young **A)** and mature **B)** leaves for the different treatments during the experimental period. **C, CV0.5, CV2 and CV5:** *Chlorella vulgaris* at 0.0 g L^{-1} , 0.5 g L^{-1} , 2.0 g L^{-1} and 5.0 g L^{-1} , respectively

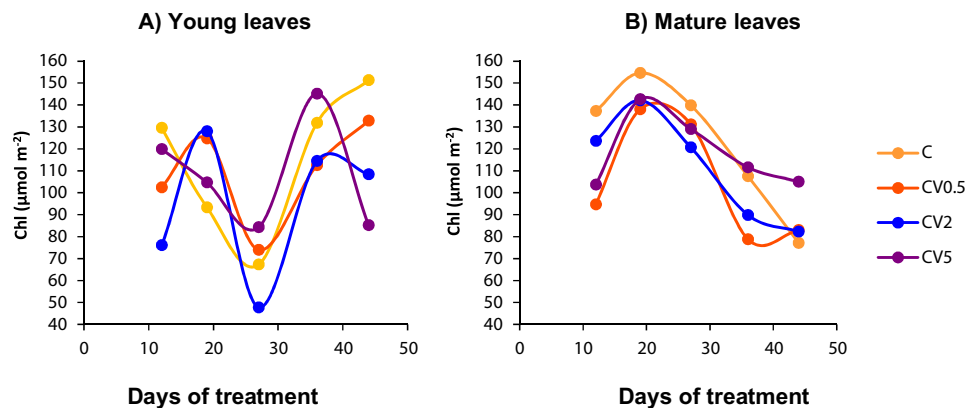


Fig. 3 **A)** Height of plants (in cm) and **B)** Plants at the end of experiment. **C, CV0.5, CV2 and CV5:** *Chlorella vulgaris* at 0.0 g L^{-1} , 0.5 g L^{-1} , 2.0 g L^{-1} and 5.0 g L^{-1} , respectively. For each parameter analysed, different letters represent significant differences between samples ($P \leq 0.05$). Error bars represent 95% confidence intervals

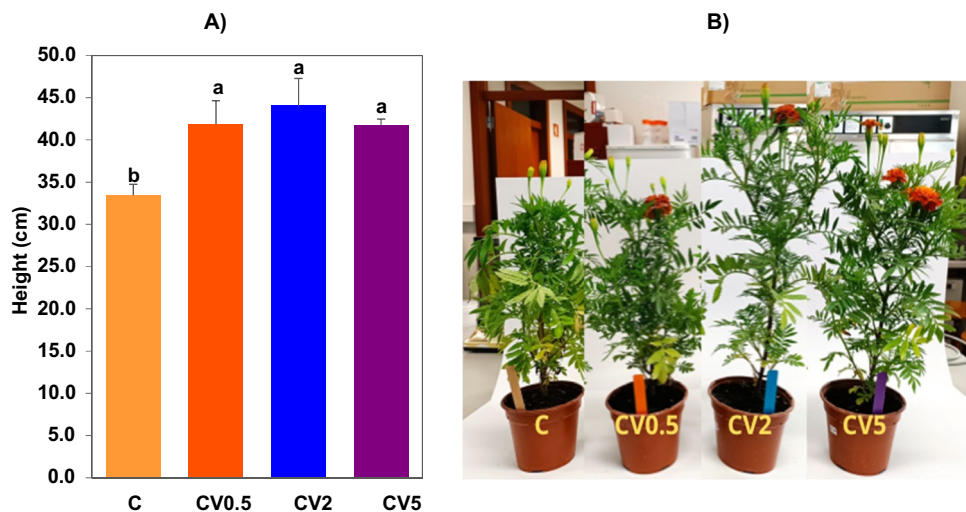
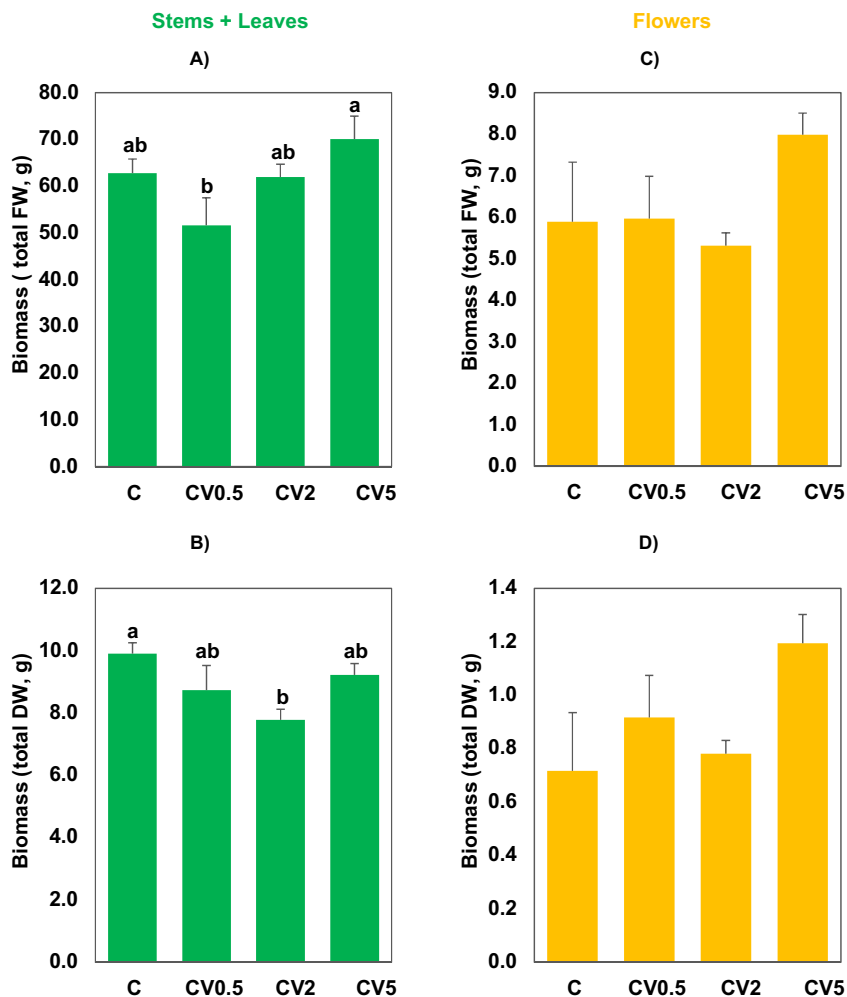


Fig. 4 Biomass parameters at the end of the experiment. Total fresh weight (FW) in stems + leaves (A), and in flowers (C). Total dry weight (DW) in stems + leaves (B), and in flowers (D). C, CV0.5, CV2 and CV5: *Chlorella vulgaris* at 0.0 g L⁻¹, 0.5 g L⁻¹, 2.0 g L⁻¹ and 5.0 g L⁻¹, respectively. For each parameter analysed, different letters represent significant differences between samples ($P \leq 0.05$). Error bars represent 95% confidence intervals



The appearance of flower buds was first observed on day 28 in the CV0.5 and CV5 treatments. From day 37 until the end of the experiment, no significant differences were observed between the treatments. Conversely, the flowers began to emerge on day 44, exclusively in the CV5 treatment. At day 48, the sole discernible disparity in the number of flowers was between the control and the CV5 treatments. At the end of the experiment (day

54), the control plants exhibited a lower number of flowers, while the CV5 plants triggered higher number of flowers, representing a 46,9% increase in comparison to the control plants.

The chlorophyll fluorescence values in both young and mature leaves were comparable across the different treatment groups and there were no significant differences between treatments (Table 2).

Table 2 Fluorescence parameters (F_0 , F_m and F_v/F_m) and Chl values in the young and mature leaves at the conclusion of the experiment

Treatments	Young leaves				Mature leaves			
	F_0	F_m	F_v/F_m	Chl	F_0	F_m	F_v/F_m	Chl
C	482	3575	0.86	122.2	564	3257	0.83	68.6
CV0.5	463	3492	0.87	136.6	453	3631	0.87	86.4
CV2	451	3502	0.87	155.9	408	2877	0.86	93.7
CV5	468	3571	0.87	85.7	448	3460	0.87	103.1

F_0 : basal fluorescence; F_m : maximum fluorescence; F_v : variable fluorescence. Chl – total leaf chlorophyll. C, CV0.5, CV2 and CV5: *Chlorella vulgaris* at 0.0 g L⁻¹, 0.5 g L⁻¹, 2.0 g L⁻¹ and 5.0 g L⁻¹, respectively. For each parameter analysed no significant differences were observed ($P > 0.05$)

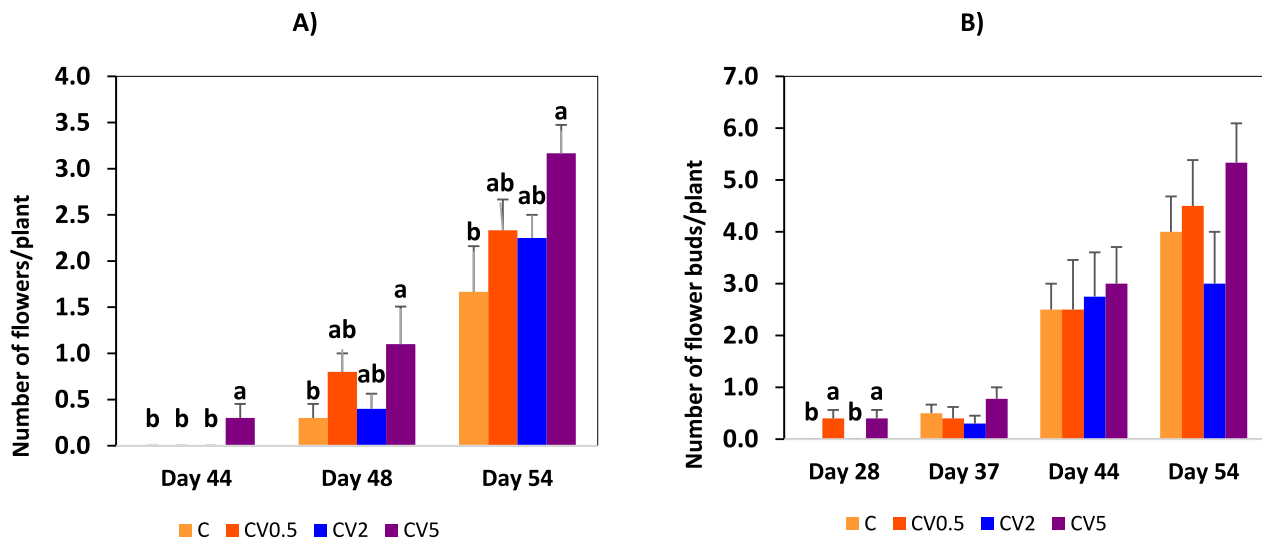


Fig. 5 A) Number of flowers per plant and B) Number of flower buds per plant. C, CV0.5, CV2 and CV5: *Chlorella vulgaris* at 0.0 g L⁻¹, 0.5 g L⁻¹, 2.0 g L⁻¹ and 5.0 g L⁻¹, respectively. For each parameter

analysed, different letters represent significant differences between samples ($P \leq 0.05$). Error bars represent 95% confidence intervals

Table 3 The mean macronutrient (g kg⁻¹ DW) and micronutrient (mg kg⁻¹ DW) concentrations (\pm standard error) at the end of the experiment in shoots (stems plus leaves) of *Tagetes* plants with *Chlorella vulgaris* (CV) application, irrespective of the extract concentration, in contrast to control (C) plants. For each nutrient analysed, the P value represents the level of statistical significance between samples

	Control (C)	+ <i>C. vulgaris</i> (CV)	P value
Macronutrients (g kg⁻¹ DW)			
N	19.0 \pm 1.00	17.6 \pm 1.80	0.667
P	3.7 \pm 0.30	3.3 \pm 0.37	0.591
K	16.3 \pm 0.33	16.7 \pm 1.00	0.857
Ca	36.3 \pm 1.33	38.1 \pm 2.28	0.677
Mg	8.9 \pm 0.54	8.0 \pm 0.62	0.977
Micronutrients (mg kg⁻¹ DW)			
Fe	869.7 \pm 161	515.6 \pm 123.6	0.165
Mn	426.0 \pm 60.5	277.7 \pm 40.1	0.087
Zn	91.0 \pm 9.29	72.4 \pm 11.7	0.406
Cu	17.3 \pm 1.20	15.4 \pm 0.94	0.319

A comparison of the variable fluorescence and maximum fluorescence (F_v/F_m) ratios in young and mature leaves did not reveal significant differences. The Chl concentration of mature leaves used for fluorescence assessment, increased in accordance with the increment of the CV concentrations Fig. 5.

The mineral composition of shoots was initially analysed with a focus on two groups: control plants ($n=3$) and CV plants (in which all plants treated with CV were considered

as one group; $n=9$). As shown in Table 3, macro- and micronutrients were statistically similar between the two groups, although the concentrations of Ca and K were slightly higher in CV plants.

A second analysis was conducted to examine the macro- and micronutrient composition of stems and leaves (Fig. 6), with only the CV treatments included in the assessment. In general, higher concentrations of *C. vulgaris* suspensions result in higher concentrations of macro- and micronutrients in shoots, with particular significance observed for N, P, Mg and Cu. There were no significant differences between treatments regarding the concentrations of K. There were minor fluctuations in the concentration of calcium (Ca). The highest value was in CV5 plants, while the lowest was in CV0.5 plants. Mg levels displayed only small variations between treatments. The highest value was in the CV5 plants, while the lowest was identified in the CV0.5 plants. There were no significant differences between treatments in Fe and Mn concentrations. There were no significant differences between treatments in Zn contents between treatments. The highest value of Cu was registered in the CV5 plants and the lowest in CV0.5 plants.

The analysis of nutrients revealed a linear and positive correlation between the concentrations of CV suspension and the nutrient concentrations (Fig. 6). As the concentration of CV suspensions increased, so did the concentration of nutrients in the plants. The correlation coefficients (R^2) were highly significant, indicating a strong relationship between these variables.

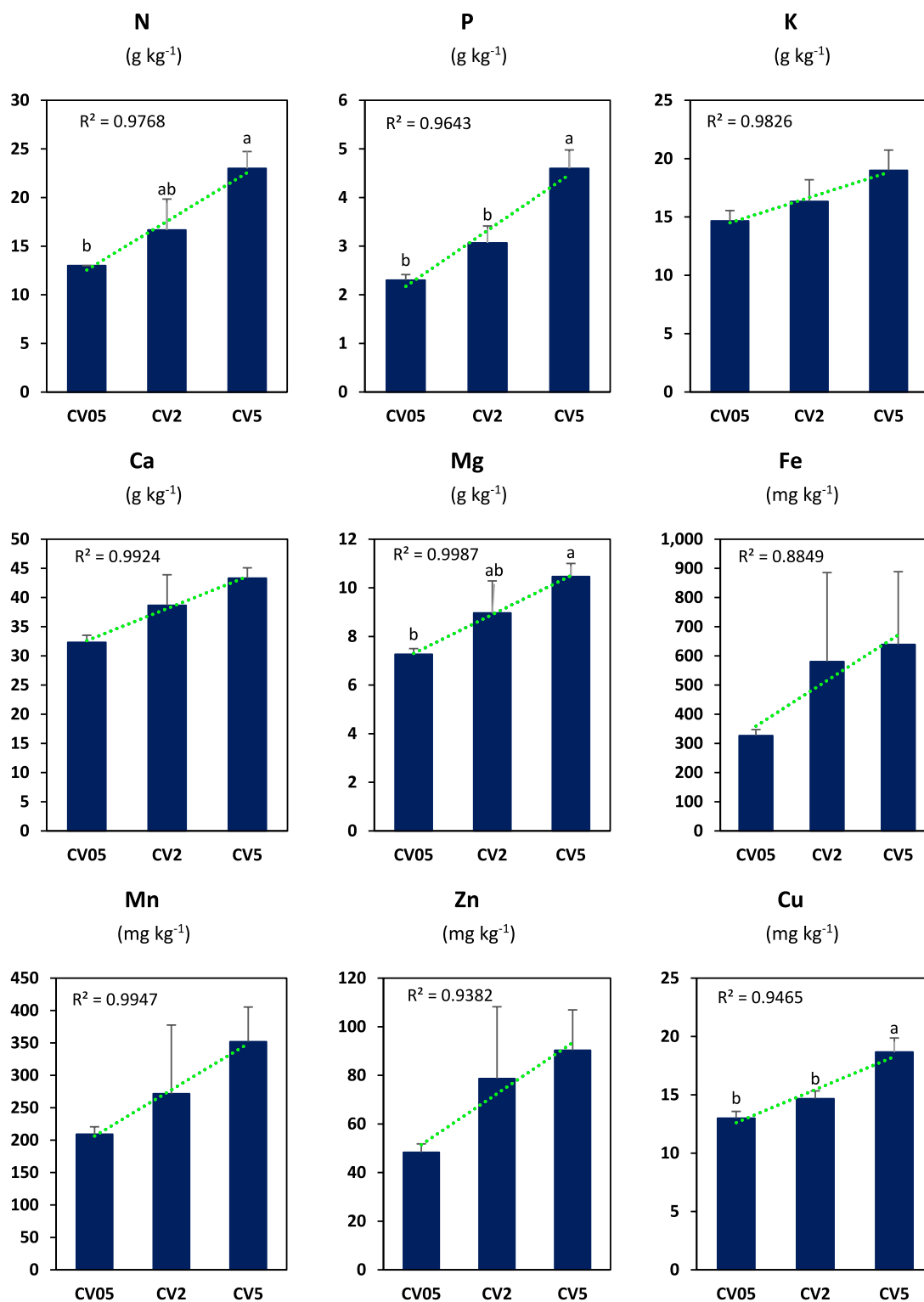


Fig. 6 Mean of macronutrients (g kg⁻¹ DW) and micronutrients concentrations (mg kg⁻¹ DW) at the end of the experiment in shoots (stems plus leaves) of *Tagetes* plants. **CV0.5**, **CV2** and **CV5**: *Chlorella vulgaris* at 0.5 g L⁻¹, 2.0 g L⁻¹ and 5.0 g L⁻¹, respectively. For

each parameter analysed, different letters represent significant differences between samples ($P \leq 0.05$). Error bars represent 95% confidence intervals. R² of the linear models were included

Discussion

The results demonstrated that the application of *C. vulgaris* significantly enhanced plant height, fresh weight, and the timing of flowering, particularly at the highest concentration of 5.0 g L⁻¹. These findings support the hypothesis that *C. vulgaris* can act as an effective biostimulant for ornamental plants, promoting their growth and developmental processes. The positive impact of microalgae on plant growth observed in the present study is consistent with previous findings where enhanced growth parameters occurred in various crops following the application of microalgae (Kapoore et al. 2021; Kusvuran et al. 2021). Specifically, *C. vulgaris* stimulated germination and biomass accumulation in several plant species, including lettuce, kale, soybean, cucumber, tomato, barley, and sugar beet (Faheed and Abd-El Fattah 2008; Park et al. 2022). Microalgal-derived biostimulants offer numerous benefits to various crops, improving morphological traits such as plant height, leaf number, dry and fresh biomass, root mass and root length (Braun and Colla 2023). The results in the present study extend these findings to ornamental plants, demonstrating that *C. vulgaris* can also significantly improve the growth and flowering of *T. patula*. This adds a new stepstone to the existing knowledge on the multifunctional role of microalgae in agriculture.

Overall, the observed enhancement in plant height and biomass can be attributed to the high nutrient availability and bioactive compounds in *C. vulgaris*. *Tagetes patula* exhibits a high degree of responsiveness to biostimulant application, which has been demonstrated to enhance nutrient uptake and growth (Miceli et al. 2023; Zeljković et al. 2023). In the present study, the significant uptake of N was likely attributable to the elevated concentrations of this nutrient present in the microalgal biomass (80.5 g kg⁻¹), which proved particularly efficacious with respect to reproductive growth. For example, in apple trees, application of N fertiliser can increase the percentage of flowering buds. This effect is enhanced when ammonium is supplied to the roots for a short period of time compared to a continuous supply of nitrate (e.g., Marschner 1999). This suggests that the form and timing of N application are critical in modelling the process of flower initiation. In ornamentals, information on this effect is scarce, but we can hypothesise that rapid uptake and translocation of N to active sinks, such as flower initiation, has occurred. In *Ruellia simplex*, extracts of *Ascophyllum nodosum* (a well-known brown macroalga widely used in biofertilizer formulations) resulted in a higher number of flowers per plant compared to a control treatment, but only in the second growing season (Eldeeb and Hussein 2024).

Another noteworthy outcome is the increase in Cu, which suggests that *Tagetes* may possess the capacity to remediate

Cu-contaminated soils. It is noteworthy that despite the high concentration of Fe in the suspension (3529 mg kg⁻¹), no significant Fe accumulation was observed in the stems and leaves. However, it is plausible that Fe was preferentially accumulated in the roots, as has been reported in several species.

While the overall growth parameters showed significant improvement, the fluorescence parameters (F_v/F_m) did not show significant differences between treatments. This may indicate that although *C. vulgaris* improves growth and plant biomass, it may not have a significant effect on photosystem efficiency under the conditions tested. On the other hand, the addition of mineral fertiliser to the substrate had no effect on the photosynthetic machinery of the control plants. These results suggest that *C. vulgaris* extracts can supplement the mineral nutrient pools present in the substrate, potentially reducing the need for additional chemical fertilisers at certain stages of plant development and contributing to more sustainable agricultural practices.

The aforementioned findings are in accordance with the increasing interest in natural and environmentally friendly agricultural inputs that promote plant health and productivity, whilst simultaneously reducing the adverse environmental impacts associated with synthetic fertilizers. Future research should investigate the long-term effects of *C. vulgaris* on ornamental plants and explore its efficacy under different environmental conditions. Studies focusing on the molecular mechanisms underlying the observed growth promotion will provide deeper insights into how microalgae biostimulants can be optimized for agricultural use. Understanding the specific nutrient interactions and the role of secondary metabolites in plant growth enhancement will further solidify the practical applications of microalgae in sustainable agriculture. It would also be beneficial to evaluate the economic feasibility of large-scale application of *C. vulgaris* in commercial horticulture.

Conclusions

The data presented in this study indicate that the application of *C. vulgaris* suspensions had a positive effect on plant growth. The application of this microalga was observed to result in greater plant height, irrespective of the concentration tested. The application of the microalgae had a significant impact on the early flowering of marigolds.

The highest microalgal concentration had a positive effect on the concentration of nitrogen, phosphorus, calcium, magnesium, and copper. The findings of this study indicate that microalgae could be employed as biostimulants in ornamental plants, thereby facilitating the implementation of

more environmentally sustainable fertilization strategies. The increase in flower production is of paramount importance in ornamental plants; however, the application of *C. vulgaris* suspensions on horticultural crops might open new possibilities for the promotion of flowering performance, fruit set and yield.

Authors contribution The concept was initially proposed by J.Vieira, M.P. and P.J.C., who subsequently devised the experimental design. The experiment was conducted by J.Vieira and M.S., who also collated the data. All authors engaged in the analysis and discussion of the data. The authors responsible for the preparation of the manuscript were J.Vieira, M.S., M.P. and P.J.C.. All authors have read and approved the final manuscript.

Funding This study was funded by the National Projects (UIDB/Multi/05183/2020 and LA/P/0101/2020, and UIDB/04326/2020) from Foundation for Science and Technology (FCT) and from the operational programme CRESC Algarve 2020 European Regional Development Fund and COMPETE 2020 through project EMBRC.PT, ALG-01–0145-FEDER-022121 and ALG-01–0247-FEDER-069961 PERFORMALGAE project.

- MED-UIDB/05183/2020 (doi.org/10.54499/UIDB/05183/2020)
- CHANGE-LA/P/0121/2020 (doi.org/10.54499/LA/P/0121/2020)
- CCMAR-UIDB/04326/2020 (<https://doi.org/10.54499/UIDB/04326/2020>)

Data availability The data are available by request from the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

References

- Abadía J, Abadía A (1993) Iron and pigments. In: Barton LL, Hemming BC (eds) Iron Chelation in Plants and Soil Microorganisms. Academic Press, San Diego, pp 327–343
- Alling T, Funk C, Gentili FG (2023) Nordic microalgae produce biostimulant for the germination of tomato and barley seeds. *Sci Rep* 13:3509
- Barone V, Baglieri A, Stevanato P, Broccanello C, Bertoldo G, Bertaggia M, Cagnin M, Pizzeghello D, Moliterni VMC, Mandolino G, Fornasier F, Squartini A, Nardi S, Concheri G (2017) Root morphological and molecular responses induced by microalgae extracts in sugar beet (*Beta vulgaris* L.). *J Appl Phycol* 30:1061–1071
- Bayona-Morcillo PJ, Gómez-Serrano C, González-López CV, Massa D, Jiménez-Becker S (2022) Effect of the application of hydrolysate of *Chlorella vulgaris* extracted by different techniques on the growth of *Pelargonium × hortorum*. *Plants* 11:2308
- Braun JCA, Colla LM (2023) Use of microalgae for the development of biofertilizers and biostimulants. *BioEnergy Res* 16:289–310
- Chanda M, Merghoub N, EL Arroussi H (2019) Microalgae polysaccharides: the new sustainable bioactive products for the development of plant bio-stimulants? *World J Microbiol Biotechnol* 35:177
- Chiaiese P, Corrado G, Colla G, Kyriacou MC, Roupael Y (2018) Renewable sources of plant biostimulation: microalgae as a sustainable means to improve crop performance. *Front Plant Sci* 9:1782
- du Jardin P (2015) Plant biostimulants: Definition, concept, main categories, and regulation. *Scientia Horticult* 196:3–14
- Eldeeb M, Hussein S (2024) Effect of using algae extracts on growth of *Rutellia simplex* plants grown under salinity stress. *J Hort Sci Ornament Plants* 16:11–21
- Faheed F, Abd-El Fattah Z (2008) Effect of *Chlorella vulgaris* as bio-fertilizer on growth parameters and metabolic aspects of lettuce plant. *J Ag Social Sci* 4:165–169
- Jamal A, Yasin NA, Javad S, Ahmed S, Yasmin A, Chaudhry O, Daoud MS, Gatasheh MK (2023) Investigating the efficacy of tartaric acid and zinc-mediated endogenous melatonin induction for mitigating arsenic stress in *Tagetes patula* L. *Sci Horticult* 322:112399–112399
- Kapoor RV, Wood EE, Llewellyn C (2021) Algae biostimulants: A critical look at microalgal biostimulants for sustainable agricultural practices. *Biotech Adv* 49:107754
- Kusvuran S (2021) Microalgae (*Chlorella vulgaris* Beijerinck) alleviates drought stress of broccoli plants by improving nutrient uptake, secondary metabolites, and antioxidative defense system. *Hort Plant J* 7:221–231
- Kusvuran A, Bilgici M, Kusvuran S, Nazli R (2021) The effect of different organic matters on plant growth regulation and nutritional components under salt stress in sweet sorghum [*Sorghum bicolor* (L.) Moench.]. *Maydica* 66:1–9
- Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Meth Enzymol* 148:350–382
- Ma Y, Freitas H, Dias MC (2022) Strategies and prospects for biostimulants to alleviate abiotic stress in plants. *Front Plant Sci* 13:1024243
- Marschner H (1995) Mineral Nutrition of Higher Plants, 2nd edn. Academic Press, London, p 861
- Miceli A, Moncada A, Vetrano F, Esposito A (2023) Response of *Tagetes patula* L. and *Geranium houstonianum* Mill. to microbial biostimulant inoculation and organic fertilization. *Agronomy* 13: 2522
- Morillas-España A, Ruiz-Nieto Á, Lafarga T, Acién G, Arbib Z, González-López CV (2022) Biostimulant capacity of *Chlorella* and *Chlamydomonium* species produced using wastewater and centrate. *Biology* 11:1086–1086
- Niccolai A, Chini Zittelli G, Rodolfi L, Biondi N, Tredici MR (2019) Microalgae of interest as food source: biochemical composition and digestibility. *Algal Res* 42:101617
- Osório J, Osório ML, Correia PJ, de Varennes A, Pestana M (2014) Chlorophyll fluorescence imaging as a tool for understanding the impact of iron deficiency and resupply on photosynthetic performance of strawberry plants. *Sci Horticult* 165:148–155
- Park YJ, Park J-E, Truong TQ, Koo SY, Choi J-H, Kim SM (2022) Effect of *Chlorella vulgaris* on the growth and phytochemical contents of “Red Russian” Kale (*Brassica napus* var. Pabularia). *Agronomy* 12:2138
- Santos PC, Granero FO, Junior JLB, Pavarini R, Pavarini GMP, Chorilli M, Zambom CR, Silva LP, da Silva RMG (2022) Insecticidal activity of *Tagetes erecta* and *Tagetes patula* extracts and fractions free and microencapsulated. *Biocatal Ag Biotechnol* 45:102511
- Santos PC, Santos VHM, Mecina GF, Andrade AR, Figueiredo PA, Moraes VMO, Silva LP, Silva RMG (2015) Phytotoxicity of *Tagetes erecta* L. and *Tagetes patula* L. on plant germination and growth. *S Afr J Bot* 100:114–121

- Viana C, Genevace M, Gama F, Coelho L, Pereira H, Varela J, Reis M (2024) *Chlorella vulgaris* and *Tetrademus obliquus* protect spinach (*Spinacia oleracea* L.) against *Fusarium oxysporum*. *Plants* 13:1697
- Zeljковић S, Paradiković N, Maksimović I, Teklić T, Kojić MT (2023) Growth and nutrient status of French marigold (*Tagetes patula* L) under biostimulants application. *N Z J Crops Horticult Sci* 51:614–624

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.