Journal of Plant Process and Function, Vol. 14, No. 70, Year 2026 **page: 33-42**

Research Article

Impact of *Arthrospira platensis* biostimulant on the cell biomass and antioxidant defense system of *Matricaria chamomilla* cell culture

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Abstract

Algal biostimulants are a promising strategy to improve crop yield and plant bioactive compounds. *Arthrospira platensis* (Spirulina) alga has received attention due to its low cost of cultivation and nontoxicity to induce plant growth and development. Optimizing cell culture mediums using biostimulants can be applied to scale up cell biomass in medicinal plants. This study examined the impact of various concentrations of Spirulina extract (0, 20, 40, and 80 mg L^{-1}) on *Matricaria chamomilla* cell growth and antioxidant defense system after three weeks. The algal extract markedly increased the fresh and dry weights of *M. chamomilla*, and the maximum contents were obtained at 40 mg L^{-1} by a 2.7 and 2.3-fold raise compared to the control, respectively. Protein and carbohydrate contents increased by raising the extract concentrations, and the highest contents were observed in cells treated with 80 mg L^{-1} extract. H_2O_2 content decreased significantly in cells treated with 40 and 80 mg L^{-1} extract due to enhanced antioxidant enzymes of superoxide dismutase, catalase, and peroxidase activities. Moreover, the algal extract (80 mg L^{-1}) maximized total phenolics (1282.7 mg GAE g^{-1} DW), flavonoids (790.6 mg g^{-1} DW), and DPPH scavenging activity (89.5%) in *M. chamomilla* cells. These findings demonstrate that *A. platensis* extract at 80 mg L^{-1} is a suitable concentration to scale up the cell biomass and secondary metabolite production in *M. chamomilla* cells.

Keywords: Antioxidant enzymes, Algal extract, Cell culture, Chamomile, Flavonoid

Introduction

Matricaria chamomilla L. (chamomile) is a medicinal plant from the family Asteraceae. It is distributed in Europe and Western Asia and has grown spontaneously in various parts of Iran (Lim, 2012; Hassanpour *et al*., 2020). It is widely used in the pharmaceutical, cosmetic, and food industries (Saeedi *et al*., 2024). Chamomile extract is composed of organic acids and phenolics, flavonoids, sterols, and terpene compounds and exhibits various pharmacological properties, including anticancer, anti-inflammatory, antioxidant, antimicrobial, antiallergic, antidiabetic, and neuroprotective effects (Dai *et al*., 2022; Xu *et al*., 2020; Hassanpour and Niknam, 2020; Wan *et al*., 2019). Plant cell tissue and organ culture provide a valuable method to produce secondary metabolites and propagate medicinal plants. Secondary metabolites are usually produced in low amounts in specific tissues of plants, but they can be induced by cell suspension culture under controlled conditions. An alteration in the culture medium composition could promote metabolite production in plant cells (Bapat *et al*., 2023). Biostimulants act as inducers of plant growth and elicit secondary metabolite production by activating the plant defense system. These compounds have different types, including protein hydrolysate, humic substances, and extracts of plants, algae, fungi, and bacteria (Du Jardin, 2015). Seaweed extract could affect plant growth, nutritional quality, and bioactive compound production (Stirk *et al*., 2020). The positive influence of microalgae

Received: Jan. 06, 2024; Revised: Apr. 08, 2025; Accepted: Apr. 22, 2025; Published Online: Feb. 28, 2026
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and cyanobacteria extract on plant growth and development was identified through their impacts on enzyme activities, gene expressions, and antioxidant defense systems (Prisa and Spagnuolo, 2023; Stirk *et al*., 2020; Van Oosten *et al*., 2017). There is little data about the impact of algal extract on callus induction and growth (Amin *et al*., 2009), but no information exists on the plant cell suspension culture. So, applying algal extract into the cell medium is so beneficial to improve valuable secondary metabolites.

Arthrospira platensis (commonly named *Spirulina platensis*) is a cyanobacteria used as a dietary supplement for humans and applied as a biofertilizer in agriculture (Arahou *et al*., 2023). It has been reported that the Spirulina extract is composed of proteins (60-70%) and essential amino acids (47% of the total protein weight), chlorophylls, carbohydrates (15-20%), lipids (5-8%), phytohormones (indole acetic acid, gibberellin, abscisic acid, jasmonic acid, and benzyl adenine), minerals, vitamins (C, B1, B2, B5, and B6), and organic acids (Arahou *et al*., 2023; Hassanpour *et al*., 2024; Lupatini *et al*., 2017; Amin *et al*., 2009; Silva *et al*., 2020). Proteins and phytohormones are involved in various physiological processes, including cell division, elongation, cellular metabolism, and differentiation (Shedeed *et al*., 2022). Moreover, *Spirulina* produces extracellular polymeric substances, and its polysaccharide moiety can help with nutrient absorption (Kapoore *et al*., 2021; El-Sayed, 2018). Recent studies have shown that using algal extract is a sustainable and promising technique. These extracts can enhance crop yield and promote agricultural sustainability (Gharib *et al*., 2024). For instance, *Nannochloropsis salina* extract of up to 1% improved the root and shoot length, number of leaves, and flowers in common beans by increasing growth regulators and antioxidant defense systems (Gharib and Ahmed, 2023). The *S. platensis* and *Chlorella vulgaris* extracts increased tomato tolerance against salinity by promoting chlorophyll synthesis and micronutrient absorption (Mostafa *et al*., 2023). In *Cyamopsis tetragonoloba*, *Chlorella vulgaris* extract stimulates phenolic compounds and photosynthetic rate (Kusvuran, 2020). There is no study about the effect of Spirulina extract on *M. chamomilla* cell culture. Enhancing cell biomass under Spirulina extract can help to enhance pharmaceutical raw materials and sustainable production of bioactive compounds in *M. chamomilla*. So, this research was conducted on the impact of Spirulina extract on the growth and antioxidant defense system by investigating the dynamic responses of H_2O_2, antioxidant enzyme activities, and antioxidant metabolites. Moreover, the regulatory impact of spirulina extract on the protein and carbohydrate contents in chamomile cells was examined for the first time.

Materials and methods

Plant material, callus induction, and cell suspension culture: *M. chamomilla* seeds were purchased from Pakan Bazr Isfahan. Seed disinfection and friable callus induction were conducted by the Hassanpour and Niknam (2020) method. The obtained calli were transferred into the liquid MS medium (Murashige and Skoog, 1962) supplied with 1.5 mg L^{-1} 2.4-D and 0.5 mg L^{-1} Kin and put on a rotary shaker (110 rpm, 25°C) in darkness. The cell suspension was subcultured into the fresh MS liquid medium every two weeks for two months, and the cell growth cure was calculated according to our previous study (Hassanpour and Niknam, 2020). Afterward, 2 ml of cell suspension was added to the solid MS medium containing different Spirulina extracts (0, 20, 40, and 80 mg L^{-1}) and was adjusted to pH 5.7. For preparation of the extract, 0.2 g spirulina tablets from Ashbal Chemi Company (www.spiruvit.com) were homogenized in 10 mL sterilized deionized water by an IKA homogenizer (*ULTRA*-TURRAX Disperser, T18, German) and then filtered with a 0.45 µm filter. Afterward, the desired concentrations were added to the autoclaved MS medium at 40°C in a laminar hood. After 3 weeks of cell culture, cells were harvested for physiological and biochemical analysis. For each treatment, four replications were applied in two series of cultures.

Carbohydrates and hydrogen peroxide levels: The total carbohydrate was determined by the phenol-sulfuric method (DuBois *et al*., 1956). Dried cells (0.2 g) were homogenized in 3 mL of distilled water and incubated at room temperature for 24 h. The homogenate was centrifuged at 4500 × g for 5 minutes by a microcentrifuge (Zigma, 1-16K, Germany). The reaction solution was 0.5 ml of extract, 0.5 mL of 5% phenol, and 2 mL of 97% sulfuric acid. The absorbance was recorded at 485 nm, and the total carbohydrate content was determined using a glucose standard curve.

The H_2O_2 content was evaluated using the procedure explained by Alexieva *et al*. (2001). The fresh cell pellets (100 mg) were extracted in trichloroacetic acid 0.1% (2 mL, w/v) in an ice bath. After centrifugation at 12,000 × g for 15 min, the extract (0.5 mL) was added to the reaction mixture containing 100 mM phosphate buffer (0.5 mL, pH 7.4) and 1 M KI (1 mL). The absorption was read at 390 nm, and H_2O_2 content was computed through a standard curve.

Protein level and antioxidant enzyme activities: Fresh cells (0.25 g) were homogenized in Tris-HCl (1 M, pH 6.8) buffer at 4°C, and the supernatant was used for protein and enzyme assays. The Bradford method evaluated the protein content spectrophotometrically at 595 nm (Bradford, 1976).

To evaluate superoxide dismutase (SOD) activity, 200 µL extract was mixed with nitroblue tetrazolium (NBT, 75 µmol), EDTA (0.1 mmol), potassium phosphate buffer (50 mmol), methionine (13 mmol), and riboflavin (75 mmol). After 10 min, the absorbance was measured spectrophotometrically (UNICO UV/Vis, 4802, USA) at 560 nm (Giannopolitis and Ries, 1977). The enzyme activity was presented as units per mg^{-1}

protein, and one unit was considered the amount of enzyme causing 50% inhibition of NBT reduction. The catalase (CAT) enzyme activity was measured at 240 nm using the Aebi method (1984). The reaction mixture was 0.625 mL of 50 mM sodium phosphate buffer (pH 7), 0.075 mL of 3% hydrogen peroxide solution, and the enzyme extract (10 µL). The enzyme activity was 1 µmol of H_2O_2 oxidized per minute per mg protein. The peroxidase (POX) activity was determined using the method of Abeles and Biles (1991). A reaction mixture contained acetate buffer (pH = 4.8, 0.2 M), H_2O_2 (3%), benzidine solution (15 mM), and the enzyme extract (20 µL). The absorbance was recorded at 530 nm. The enzyme activity was presented as µmol min^{-1} mg^{-1} protein.

Total phenolic content: The total phenolic content was measured using a modified Folin-Ciocalteu method (Akkol *et al*., 2008). Dried cells (200 mg) were homogenized in 5 ml of 80% methanol and were centrifuged at 4500 × g for 5 minutes. The reaction mixture was extract (100 µL), 500 µL of Folin-Ciocalteu reagent [previously diluted with water at a ratio of 1:9 (v/v)], and 400 µL of sodium carbonate (20% w/v). After 90 minutes, the absorbance was recorded at 760 nm. The total phenolic content was quantified using a gallic acid calibration curve and expressed as mg gallic acid equivalent.

Flavonoid content: Flavonoid content was estimated using the aluminum chloride colorimetric method with some modifications (Hatamnia *et al*., 2014). In brief, 500 µL of cell extract was combined with 50 µL of sodium nitrate solution (5%), 50 µL of aluminum chloride solution (10%), and 250 µL of sodium hydroxide solution (4%). The mixture was then put at room temperature for 30 minutes. Absorbance was immediately measured at 518 nm. Total flavonoid content was expressed as mg rutin equivalent per 1 g dry weight.

DPPH radical scavenging: The reduction of 2,2-diphenyl-2-picryl hydroxyl (DPPH) solution was assessed following the method described by Patro *et al*. (2005). The 200 µL of the extract solutions were mixed with 300 µL of DPPH solution (0.1 mM DPPH in methanol). The absorbance was measured at 517 nm at room temperature after 30 min incubation. The radical scavenging activity was expressed as the percentage inhibition of the DPPH radical (%) = 100 × (A − B) / A, where A is the absorbance of the control, and B is the absorbance of the sample reaction mixture.

Statistical analysis: Statistical analysis was conducted using one-way analysis of variance (ANOVA) with SPSS Ver 21 software. Duncan's range test was applied to identify statistical differences between the treatments and control. Each experiment was repeated four times at a confidence level of $P \leq 0.5$. Principal component analysis (PCA) was carried out through the XLSTAT 2021.2.2 software.

Results

Effects of algae extract on the cell biomass: A depiction of Chamomilla cells is shown in Fig. 1 and presents a significant difference in cell growth under different concentrations of Spirulina extract. The fresh and dry weights increased significantly with Spirulina extract at concentrations of 40 and 80 mg L^{-1} (Figs. 2a and b). The maximum fresh and dry weights were identified in cells treated with 40 mg L^{-1} by a 2.7 and 2.3-fold rise compared to the control, respectively. The biomass decreased slightly in cells treated with 20 mg L^{-1} extract. However, there was no significant difference compared to the control.

Total carbohydrates and protein: Carbohydrate content increased by applying algal extract into the culture medium (Fig. 3a). Treatments of 40 and 80 mg L^{-1} showed the maximum carbohydrate content with a 62.04 and 45.08.7% rise compared to the control, respectively.

Protein content changed differently under various extract concentrations. Treatments of 40 and 80 mg L^{-1} promoted protein content in cells with about a 1.7-fold rise compared to the control (Fig. 3b). However, its content did not change significantly in the treatment of 20 mg L^{-1} extract compared to the control.

Antioxidant enzymes and H_2O_2 level: Antioxidant enzyme activities changed markedly under Spirulina extract in *M. chamomilla* cells (Fig. 4). SOD activity was more induced in *M. chamomilla* cells treated with 40 and 80 mg L^{-1} of extracts, with a 64.1% and 50% rise compared to the control, respectively (Fig. 4a). There was no significant difference between cells treated with 20 mg L^{-1} extract and the control. POX activity displayed the maximum activity at 20 and 80 mg L^{-1} extract by a 54.9 and 63.9% rise compared to control, respectively (Fig. 4b). Treatment of 40 mg L^{-1} also caused a 37.4% induction in POX activity. CAT activity increased in cells treated with Spirulina extract at all concentrations, and the maximum activity was obtained at 40 and 80 mg L^{-1} with a 38.1 and 30.2% rise compared to the control, respectively (Fig. 4c). Treatments of 20 mg L^{-1} also caused an insignificant rise (17.1%) in enzyme activity.

The H_2O_2 level showed a different response under Spirulina extracts (Fig. 4d). As its activity displayed a 26.9% rise in cells treated with 20 mg L^{-1} extract, while its content decreased in 40 and 80 mg L^{-1} treatments. The algal extract at 40 mg L^{-1} caused a 52.9% decline in H_2O_2 level in *M. chamomilla* cells.

Total phenolics, flavonoids, and DPPH scavenging activity: Spirulina extracts changed antioxidant metabolites in *M. chamomilla* cells (Table 1). The maximum phenolic content (1282.7 mg g^{-1} DW) obtained in cells treated with 80 mg L^{-1} extract was a 2.18-fold rise compared to the control, followed by treatments of 20 and 40 mg L^{-1}. Flavonoid content increased under algal extract, and the high content was identified in cells treated with 40 and 80 mg L^{-1} by a 1.41 and 1.55-fold rise compared to the control. There was no significant difference between the 20 mg L^{-1}

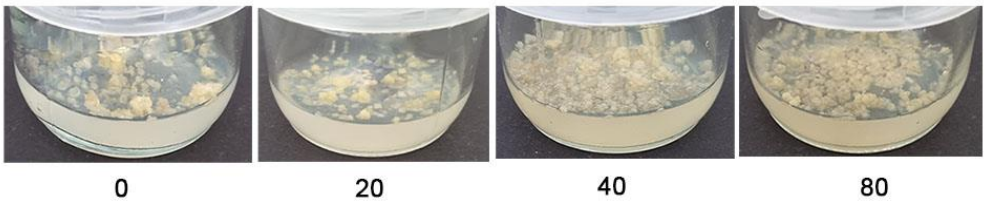

Figure 1. Depiction of *M. chamomilla* cell culture on solid MS medium supplied with different concentrations of Spirulina extract (0, 20, 40, and 80 mg L^{-1}) (a). The scale bar was 10 cm.

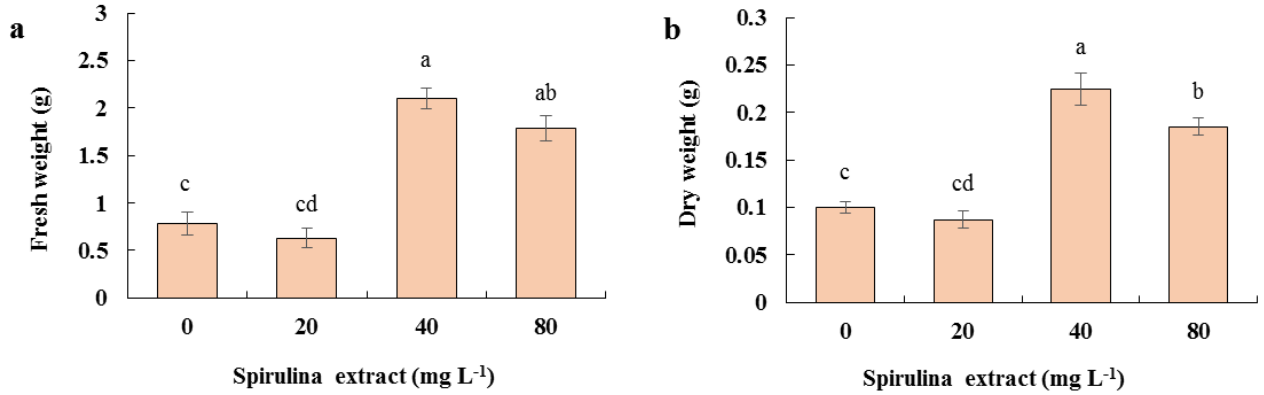

Figure 2. The fresh (b) and dry (c) weights of *M. chamomilla* cells under algal extracts. Different letters indicate significant differences based on the Duncans test at a significance level of P ≤0.05.

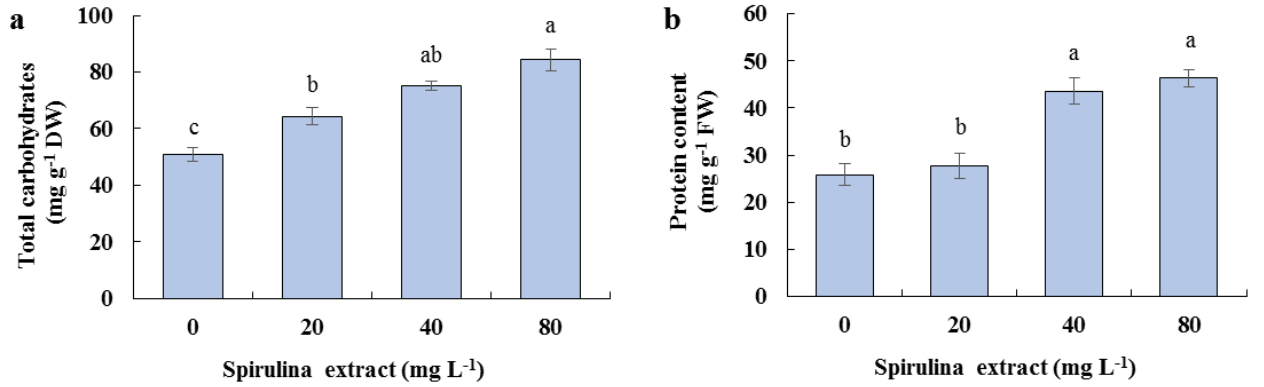

Figure 3. Effect of different extract concentrations on the total carbohydrate (a) and protein (b) contents of *M. chamomilla* cells. Different letters indicate significant differences based on the Duncans test at a significance level of P ≤0.05.

Table 1. Effect of spirulina extract on total phenolics, flavonoids, and DPPH scavenging activity of *M. chamomilla* cells

Parameters	Spirulina extract (mg L^{-1})			
	0	20	40	80
Total phenolics (mg GAE g^{-1} DW)	587.4 ± 22.3 ^{d}	993.1 ± 34.9 ^{b}	863.5 ± 63.1 ^{bc}	1282.7 ± 52.7 ^{a}
Total Flavonoids (mg g^{-1} DW)	509.1± 29.4 ^{c}	519.2 ± 31.7 ^{c}	718.5 ± 40.6 ^{ab}	790.6 ± 32.8 ^{b}
DPPH scavenging activity (%)	53.1± 2.35 ^{b}	60.8± 3.12 ^{b}	83.1± 2.93 ^{a}	89.5± 3.89 ^{a}

Different letters indicate significant differences based on the Duncans test at a significance level of P ≤0.05.

treatment and the control regarding flavonoid production.

DPPH activity increased by applying algal extract in the medium. Treatments of 80 mg L^{-1} and 40 mg L^{-1} significantly enhanced DPPH activity compared to the control, which was in agreement with the results of antioxidant metabolites. The maximum activity obtained in cells treated with 80 mg L extract was a 68.5% rise compared to the control.

Relationships between physiological and biochemical parameters: The correlation between growth parameters and antioxidant capacity in the cells treated with algal extract and control was exhibited by the PCA graph in Fig. 5. Principal component 1 (F1) presented 77.42% of the total variation, and principal component 2 (F2) presented 18.99%. As presented in the PCA graph, the results from growth, SOD and CAT enzymes, protein, flavonoids, and DPPH are closely loaded to F1 axis and are positively related together.

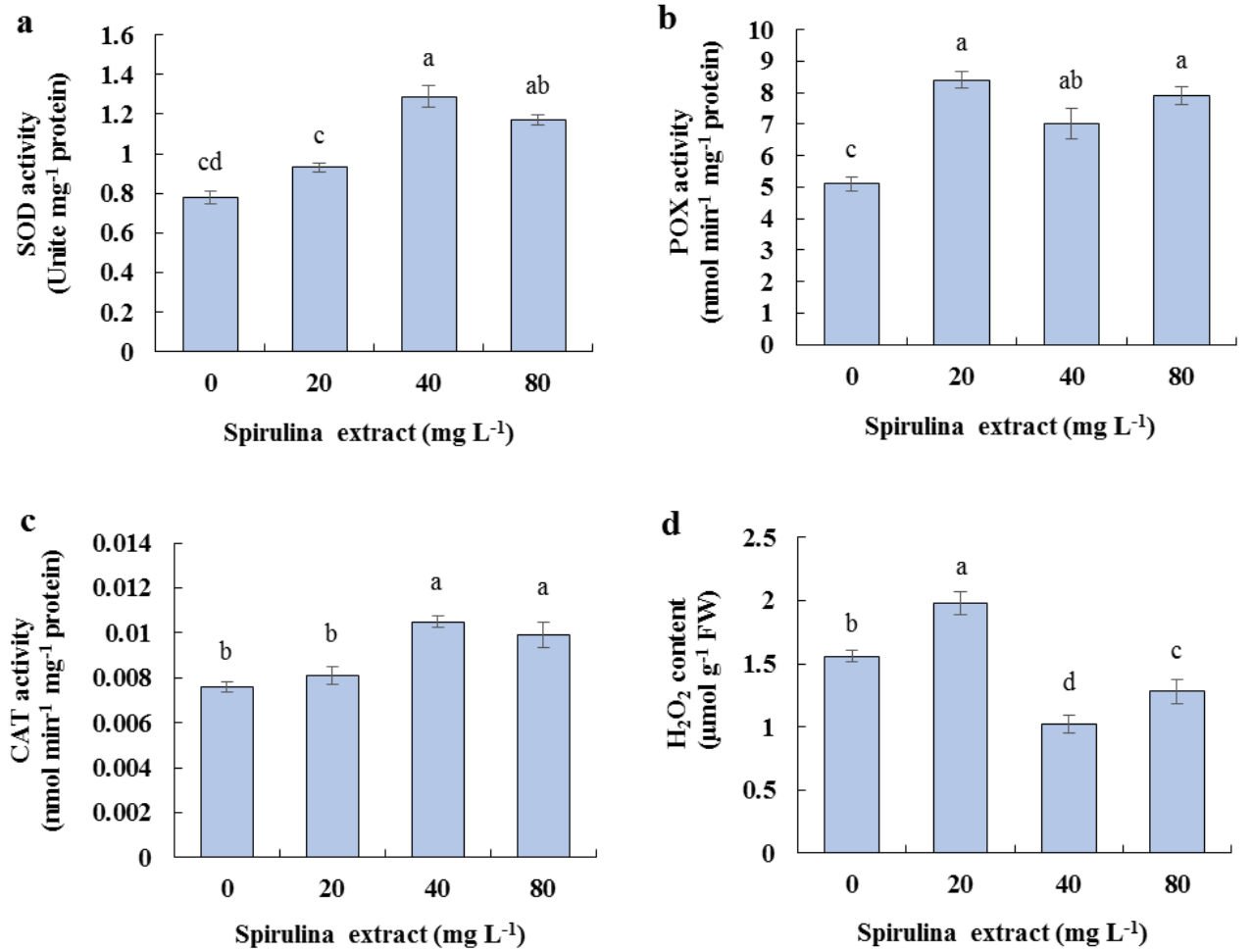

Figure 4. Effect of different extract concentrations on the SOD (a), POX (b), CAT (c), and H_2O_2 (d) contents of *M. chamomilla* cells. Different letters indicate significant differences based on the Duncan's test at a significance level of P ≤0.05.

Discussion

Spirulina is found to be a bio-fertilizer supporting plant growth, and its composition is environmentally friendly and nontoxic for adding to the culture medium. It can be used as a low-cost alternative compared to traditional fertilizers. This study was established to clarify the underlying mechanisms of Spirulina extract on biomass and secondary metabolite production in *M. chamomilla*. The findings of this research displayed that Spirulina extracts at 40 mgL^{-1} markedly promoted the fresh and dry weights of *M. chamomilla* cells after 3 weeks, and this concentration can be used as a suitable tool for promoting cell growth in culture medium. The PCA graph displayed the results from protein, antioxidant enzymes of CAT and SOD; fresh and dry weights are closely loaded to F1 axis under 40 mg L^{-1} extract and are positively related together (Fig. 5).

The impact of spirulina extract on growth promotion is more studied in intact plants, and less research has been done on the cell culture. For instance, Amin *et al.* (2009) reported that Spirulina extract improved callus and cell biomass yield in *Sisymbrium irio*, which was due to the promotive impact of phytohormones (cytokinin and auxins) and vitamins (C and B) existed in the extract on the cell division. In rosemary, Spirulina extract promoted the growth parameters under cadmium and lead stress by improving photosynthetic pigments, nutrient absorption, and activating the antioxidant defense system (Gharib and Ahmed, 2023). Moreover, the potential impact of seaweed extracts on the growth and protein content has been identified in *Phaseolus vulgaris* L. (Kocira *et al*., 2020) and *Spinacia oleracea* L. (Rouphael *et al*., 2018). Spirulina extract contains various bioactive molecules (polysaccharides, amino acids, organic acids, minerals, etc.), which can be considered as potent chelating agents (Arahou *et al*., 2023) and help to increase absorption and availability of nutrients into cells and following cell growth (Mostafa *et al*., 2023). On the other hand, Enan *et al*. (2016) stated that the considerable impact of algae extract on the growth and protein content may be due to its influence on enhancing cell membrane permeability and plant capability in improving nutrient absorption.

Carbohydrates are an important supplier of carbon and energy in plant metabolism, are involved in the plant defense system as a signaling molecule, and act as osmotic adjustments under stress conditions (Khornti, 2023). In this study, Spirulina extracts gradually

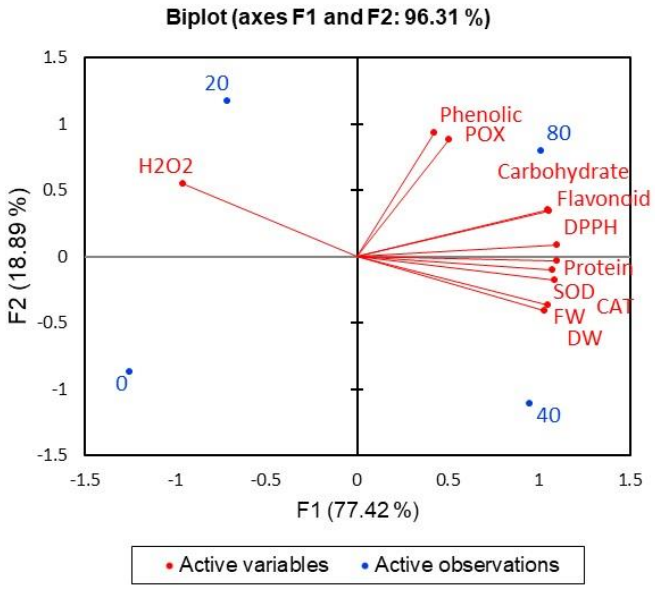

Figure 5. PCA analysis of the investigated parameters of *M. chamomilla* cells under Spirulina extract. CAT-catalase, DW-dry weight, FW-fresh weight, POX-peroxidase, SOD-superoxide dismutase

enhanced carbohydrate content in *M. Chamomilla* cells by raising the extract concentrations. Hamouda *et al*. (2022) reported that Spirulina extracts increased carbohydrate content in *Triticum aestivum*, which was related to inducing photosynthetic capacity (Aziz *et al*., 2011). Puglisi *et al*. (2020) reported that microalgal extracts enhanced carbohydrates, proteins, and photosynthetic activity in lettuce by stimulating nitrogen and carbon metabolism. On the other hand, increasing carbohydrates can boost the enzymatic and metabolic antioxidative defense system against oxidative stress (Camejo *et al*., 2012). The PCA graph showed a positive relation between antioxidant metabolites and carbohydrates to scavenge ROS under 80 mg L^{-1} (Fig. 5), suggesting a protective role of carbohydrates against ROS and oxidative damage in cells.

Biostimulants can promote cell biomass by enhancing the antioxidant capacity to regulate ROS production and level in plants (Hassanpour, 2024). In this research, spirulina extract at 40 and 80 mg L^{-1} boosted the efficiency of redox processes by decreasing the H_2O_2 level and stimulating enzymes linked to the defense system. H_2O_2 at a suitable level acts as a signaling molecule involved in many physiological processes by regulating gene expressions (Niu and Liao, 2016), which is in accordance with the results of H_2O_2 levels in cells treated with 40 and 80 mg L^{-1} extract. Similarly, Spirulina extract at 1% concentration significantly decreased H_2O_2, malondialdehyde, and electrolyte leakage in rosemary leaves (Gharib and Ahmed, 2023). Vignaud *et al*. (2025) reported that Spirulina, particularly its phycocyanin content, activates the Nrf2 pathway to regulate ROS levels. The Nrf2 factor regulates the expression of antioxidant genes and is negatively regulated by binding to Kelch-like ECH-associated protein (Keap1) in the cytosol. Upon oxidative stress, sensor cysteines in Keap1 are modified by ROS, leading to Nrf2 stabilization and translocation to the nucleus and activating the transcription of antioxidant genes. On the other hand, Spirulina extract at a low level (20 mg L^{-1}) increased the H_2O_2 level, suggesting the metabolic balance of free radical regulation was disrupted in cells and caused oxidative damage and lipid peroxidation (Camejo *et al*., 2012). Similarly, the foliar spray of *Chlorella vulgaris*, *Nannochloropsis salina*, and *S. platensis* extracts on the bean leaf showed that the lower extract concentration (0.25%) of these extracts boosted the H_2O_2 level more than that of higher extract concentrations (0.5 and 1%), which was due to lower induction of antioxidant enzyme activities of glutathione reductase, ascorbate peroxidase, and SOD (Gharib *et al*., 2024). Findings showed that the extract at low concentration could not activate signaling pathways linked to ROS scavenging.

Enzymatic antioxidants, such as SOD, CAT, and POX, play a crucial role in balancing cellular ROS levels. SOD plays an essential role in converting superoxide radicals to oxygen and H_2O_2 (Mohammadi *et al*., 2019), which is more activated in 40 mg L^{-1} of extract, followed by 80 mg L^{-1} (Fig. 3). POX and CAT enzymes reduce H_2O_2 into water and oxygen (Chen *et al*., 2013). Similar to SOD, CAT activity increased significantly under 40 and 80 mg L^{-1} of extract in *M. chamomilla* cells. POX activity was more heightened in cells treated to 20 mg L^{-1} and followed by 80 mg L^{-1} compared to the control. The PCA graph also confirmed the negative relation between antioxidant enzymes and H_2O_2 level (Fig. 5). These results suggest the regulatory impact of extract concentrations on the response of these enzymes. SOD, CAT, and POX enzymes showed a central role in scavenging H_2O_2 at 40 and 80 mg L^{-1} extract, while only POX activity was heightened at 20 mg L^{-1} extract, oxidative damage increased, and then the cell growth decreased. Other research has shown that

seaweed extract could promote antioxidant enzymes in rosemary (Gharib and Ahmed, 2023), pistachio (Nikoogoftar-Sedghi, 2023), etc., in plants due to the inducing effect of compounds existed in extract on the enzymatic and non-enzymatic antioxidant defense system (Morillas-Espana *et al*., 2022). Spirulina polysaccharides (SPPs) have been found to have the potential to scavenge ROS radicals (Finamore *et al*., 2017). SPPs (100 µg/kg) could downregulate the MDA level by promoting the activity of SOD, CAT, and GPX enzymes and eliminating hydroxyl radicals in rats (Elsonbaty and Ismail, 2020). SPPs could increase cytokines and signal transduction pathways of MAPKs and NF-kB factors to scavenge free radicals and immunomodulatory activity (Ai *et al*., 2023).

Spirulina extract in the medium culture improved phenolic and flavonoid metabolites and DPPH scavenging activity in *M. chamomilla* cells, and the maximum antioxidant metabolites were obtained in cells treated with 80 mg L^{-1} compared to the control. Battacharyya *et al*. (2015) have reported that algae-based extracts are able to promote the biosynthesis pathway of defense metabolites such as phenylpropanoids and flavonoids by changing primary and secondary metabolism. Applying A. platensis extract in onion increased total phenolic and flavonoid compounds (Gharib and Ahmed, 2023). Cyanobacterial (*Calothrix elenkinii*) inoculation enhanced the activity of phenylalanine ammonia-lyase (PAL), POX, and APX in the roots and shoots of rice seedlings (Priya *et al*., 2015). The PAL enzyme plays a vital role in upregulating the phenylpropanoid pathway and producing phenolic compounds. On the other hand, Amin *et al*. (2009) pointed out that Spirulina extract stimulated antioxidant metabolites (total phenolics and flavonoids) and antioxidant capacity in the callus and cell suspension culture of *Sisymbrium irio*, which was found due to the elicitor function of algal extract. In addition, Spirulina extract contains amino acids, which can act as precursors for secondary metabolite synthesis and ameliorate the formation of reactive oxygen (Arahou *et al*., 2023).

Conclusion

The present study exhibits that organic materials like Spirulina are highly efficient in promoting the cell biomass yield and antioxidant capacity in chamomile cells. Spirulina at 40 and 80 mg L^{-1} can be applied as suitable concentrations to induce cell biomass and antioxidant defense systems. The algal extract (40 and 80 mg L^{-1}) prevented the generation and accumulation of ROS algal extract by raising SOD, CAT, and POX activities and antioxidant metabolite production (phenolics and flavonoids). Moreover, increasing cell biomass in cells treated with 80 mg L^{-1} was in accordance with more carbohydrate accumulation, phenolics, flavonoids, and DPPH scavenging activity. This research shows the possibility of utilizing spirulina extract as an effective method to scale up the raw materials for the pharmaceutical industry and sustainable production of bioactive compounds in chamomile cells. More details should be addressed in future studies using microscopic studies and patterns of gene expressions related to the metabolic pathways in extract-treated cells.

References

Abeles, F. B, & Biles, C. L. (1991). Cellulase activity in developing apple fruits. *Scientia Horticulturae*, *47*(1-2), 77-87. https://doi.org/10.1016/0304-4238(91)90029-X

Aebi, H. (1984). Methods in Enzymology. Academic Press. https://doi.org/10.1016/S0076-6879(84)05016-3

Ai, X., Yu, P., Li, X., Lai, X., Yang, M., Liu, F., Luan, F., & Meng, X. (2023). Polysaccharides from *Spirulina platensis*: Extraction methods, structural features and bioactivities diversity. *International Journal of Biological Macromolecules*, *231*, 123211. https://doi.org/10.1016/j.ijbiomac.2023.123211

Akkol, E. K., Goger, F., Kosar, M., & Baser, K. H. (2008). Phenolic composition and biological activities of *Salvia halophila* and *Salvia virgata* from Turkey. *Food Chemistry*, *108*, 942-949. https://doi.org/10.1016/j.foodchem.2007.11.071

Alexieva, V., Sergiev, I., Mapelli, S., & Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell and Environment*, *24*(12), 1337-1344. https://doi.org/10.1046/j.1365-3040.2001.00778.x

Amin, G. H., Al-Gendy, A. A., Yassin, M. E., & Abdel-Motteleb, A. (2009). Effect of Spirulina platensis extract on growth, phenolic compounds and antioxidant activities of *Sisymbrium irio* callus and cell suspension cultures. *Australian Journal of Basic and Applied Sciences*, *3*, 2097-110.

Arahou, F., Lijassi, I., Wahby, A., Rhazi, L., Arahou, M., & Wahby, I. (2023). Spirulina-based biostimulants for sustainable agriculture: Yield improvement and market trends. *BioEnergy Research*, *16*(3), 1401-1416. https://doi.org/10.1007/s12155-022-10537-8

Aziz, N. G. A., Mahgoub, M. H., & Siam, H. S. (2011). Growth, flowering and chemical constituents performence of *Amaranthus tricolor* plants as influenced by seaweed (*Ascophyllum nodosum*) extract application under salt stress conditions. *Journal of Applied Science Research*, *11*, 1472-84.

Bapat, V. A., Kavi Kishor, P. B., Jalaja, N., Jain, S. M., & Penna, S. (2023). Plant cell cultures: Biofactories for the production of bioactive compounds. *Agronomy*, *13*(3), 858. https://doi.org/10.3390/agronomy13030858

Battacharyya, D., Babgohari, M. Z., Rathor, P., & Prithiviraj, B. (2015). Seaweed extracts as bio-stimulants in horticulture. *Scientia Horticulturae*, *196*, 39-48. https://doi.org/10.1016/j.scienta.2015.09.012

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-54. https://doi.org/10.1016/0003-2697(76)90527-3

Camejo, D., Marti, M. C., Olmos, E., Torres, W., Sevilla, F., & Jimenez, A. (2012). Oligogalacturonides stimulate antioxidant system in alfalfa roots. *Biologia Plantarum*, *56*, 537-544. https://doi.org/10.1007/s10535-012-0107-1

Chen, Z., Pan, Y. H., An, L. Y., Yang, W. J., Xu, L. G., & Zhu, C. (2013). Heterologous expression of a halophilic archaeon manganese superoxide dismutase enhances salt tolerance in transgenic rice. *Russian Journal of Plant Physiology*, *60*, 359-366. Doi.org/10.1134/S1021443713030059

Dai, Y. L., Li, Y., Wang, Q., Niu, F. J., Li, W., Wang, Y. Y., Wang, J., Zhou, C. Z., & Gao, L. N. (2022). Chamomile: A review of its traditional uses, chemical constituents, pharmacological activities and quality control studies. *Molecules*, *28*(1), 133. https://doi.org/10.3390/molecules28010133

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350-356. https://doi.org/10.1021/ac60111a017

Du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*, *196*, 3-14. https://doi.org/10.1016/j.scienta.2015.09.021

EL-Sayed, A. E, & Mostafa, E. S. (2018). Outdoor cultivation of Spirulina platensis for mass production. *Notulae Scientia Biologicae*, *10*(1), 38-44. https://doi.org/10.15835/nsb10110177

Elsonbaty, S. M, & Ismail, A. F. (2020). Nicotine encourages oxidative stress and impairment of rats' brain mitigated by *Spirulina platensis* lipopolysaccharides and low-dose ionizing radiation. *Archives of Biochemistry and Biophysics*, *689*, 108382. https://doi.org/10.1016/j.abb.2020.108382

Enan, S. A. A. M., El-Saady, A. M., & El-Sayed, A. B. (2016). Impact of foliar feeding with alga extract and boron on yield and quality of sugar beet grown in sandy soil. *Egyptian Journal of Agronomy*, *38*(2), 319-336. https://doi.org/10.21608/agro.2016.622

Finamore, A., Palmery, M., Bensehaila, S., & Peluso, I. (2017). Antioxidant, immunomodulating, and microbial-modulating activities of the sustainable and ecofriendly Spirulina. *Oxidative Medicine and Cellular Longevity*, *2017*(1), 3247528. https://doi.org/10.1155/2017/3247528

Gharib, F. A, & Ahmed, E. Z. (2023). Spirulina platensis improves growth, oil content, and antioxidant activitiy of rosemary plant under cadmium and lead stress. *Scientific Reports*, *13*(1), 8008. https://doi.org/10.1038/s41598-023-35063-1

Gharib, F. A., Osama, K., Sattar, A. M., & Ahmed, E. Z. (2024). Impact of *Chlorella vulgaris*, *Nannochloropsis salina*, and *Arthrospira platensis* as bio-stimulants on common bean plant growth, yield and antioxidant capacity. *Scientific Reports*, *14*(1), 1398. https://doi.org/s41598-023-50040-4

Giannopolitis, C. N, & Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*, *59*(2), 309-14. https://doi.org/10.1104/pp.59.2.309

Hassanpour, H., Niknam, V., Ahmadi-Sakha, S., & Haddadi, B. (2020). Antioxidant activity and flavonoid content of *Matricaria chamomilla* extracts from different populations of Iran. *Journal of Botanical Research*, *2*(2), 8-13. https://doi.org/10.30564/jrb.v2i2.1909

Hassanpour, H, & Niknam, V. (2020). Establishment and assessment of cell suspension cultures of *Matricaria chamomilla* as a possible source of apigenin under static magnetic field. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *142*(3), 583-93. Doi.org/10.1007/s11240-020-01885-4

Hassanpour, H., Mahlouji Rad, M., & Asgarpanah, J. (2024). Growth induction in *Spirulina platensis* under SiO_2 nanoparticles: Function of antioxidant enzymes, phycobiliproteins, and secondary metabolites. *Journal of Plant Process and Function*, *12*(58), 11-20. https://doi.org/10.22034/12.58.11

Hassanpour, H. (2024). ROS regulation in *Dunaliella salina* by fulvic acid: induction of enzymes related to the ascorbate–glutathione pathway and antioxidant metabolites. *Journal of Applied Phycology*, 1-11. https://doi.org/10.1007/s10811-024-03346-3

Hatamnia, A. A., Abbaspour, N., & Darvishzadeh, R. (2014). Antioxidant activity and phenolic profile of different parts of Bene (*Pistacia atlantica* subsp. Kurdica) fruits. *Food Chemistry*, *145*, 306-311. https://doi.org/10.1016/j.foodchem.2013.08.031

Hamouda, R. A., Shehawy, M. A., Mohy, E. L., Din, S. M., Albalwe, F. M., Albalawi, H. M., & Hussein, M. H. (2022). Protective role of *Spirulina platensis* liquid extract against salinity stress effects on *Triticum aestivum* L. Green *Processing and Synthesis*, *11*(1), 648-58. https://doi.org/10.1515/gps-2022-0065

Kapoore, R. V., Wood, E. E., & Llewellyn, C. A. (2021). Algae biostimulants: A critical look at microalgal biostimulants for sustainable agricultural practices. *Biotechnology Advances*, *49*, 107754. https://doi.org/10.1016/j.biotechadv.2021.107754

Khornti, S. (2023). Multifaceted role of carbohydrates in plants. *Journal of Plant Biochemistry and Physiology*, *11*(3), 1000278.

Kocira, A., Lamorska, J., Kornas, R., Nowosad, N., Tomaszewska, M., Leszczynska, D., Kozłowicz, K., & Tabor, S. (2020). Changes in biochemistry and yield in response to biostimulants applied in bean (*Phaseolus vulgaris* L.). *Agronomy*, *10*(2), 189. https://doi.org/10.3390/agronomy10020189

Kusvuran, A, & Can, A. G. (2020). Effects of microalga (*Chlorella vulgaris* beijerinck) on seconder metabolites and antioxidative defense system improve plant growth and salt tolerance in guar [*Cyamopsis tetragonoloba* (L.) taub.]. *Legume Research-An International Journal*, *43*(1), 56-60. https://doi.org/10.18805/LR-492

Lim, T. (2012). *Matricaria chamomilla*. *Edible Medicinal and Non-Medicinal Plants*, *7*, 397-431. https://doi.org/10.1007/978-94-017-9511-1

Lupatini, A. L., de Oliveira Bispo, L., Colla, L. M., Costa, J. A., Canan, C., & Colla, E. (2017). Protein and carbohydrate extraction from *S. platensis* biomass by ultrasound and mechanical agitation. *Food Research International*, *99*, 1028-35.

Mohammadi, H., Hazrati, S., & Janmohammadi, M. (2019). Approaches to enhance antioxidant defense in plants. In: Approaches for Enhancing Abiotic Stress Tolerance in Plants. (eds. Hasanuzzaman, M., Na-har, K., Fujita, M., Oku, H., and Islam, M. T.) Pp. 273-298. CRC Press, Taylor & Francis Group, Florida. https://doi.org/10.1201/9781351104722-15

Morillas-Espana, A., Ruiz-Nieto, A., Lafarga, T., Acien, G., Arbib, Z., & Gonzalez-Lopez, C. V. (2022). Biostimulant capacity of *Chlorella* and *Chlamydopodium* species produced using wastewater and centrate. Biology, *11*(7), 1086. https://doi.org/10.3390/biology11071086

Mostafa, M. M., Hammad, D. M., Reda, M. M., & El-Sayed, A. E. (2023). Water extracts of *Spirulina platensis* and *Chlorella vulgaris* enhance tomato (*Solanum lycopersicum* L.) tolerance against saline water irrigation. *Biomass Conversion and Biorefinery*, *14*(17), 21181-91. d https://doi.org/10.1007/s13399-023-04460-x

Murashige, T, & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3).

Nikoogoftar-Sedghi, M., Rabiei, V., Razavi, F., Molaei, S., & Khadivi, A. (2023). The effect of foliar application of *Ascophyllum nodosum* (L.) Le Jol. seaweed extract on biochemical traits related to abiotic stresses in pistachio (*Pistacia vera* L. cv. Kaleh-Ghoochi). *BMC Plant Biology*, *23*(1). https://doi.org/635.10.1186/s12870-023-04654-5

Niu, L, & Liao, W. (2016). Hydrogen peroxide signaling in plant development and abiotic responses: Crosstalk with nitric oxide and calcium. *Frontiers in Plant Science*, *7*, 230. https://doi.org/10.3389/fpls.2016.00230

Patro, B. S., Bauri, A. K., Mishra, S., & Chattopadhyay, S. (2005). Antioxidant activity of *Myristica malabarica* extracts and their constituents. *Journal of Agricultural and Food Chemistry*, *53*, 6912-6918. https://doi.org/10.1021/jf050861x

Prisa, D, & Spagnuolo, D. (2023). Plant production with microalgal biostimulants. *Horticulturae*, *9*(7), 829. https://doi.org/10.3390/horticulturae9070829

Priya, H., Prasanna, R., Ramakrishnan, B., Bidyarani, N., Babu, S., Thapa, S., & Renuka, N. (2015). Influence of cyanobacterial inoculation on the culturable microbiome and growth of rice. *Microbiological Research*, *171*, 78-89. https://doi.org/10.1016/j.micres.2014.12.011

Puglisi, I., la Bella, E., Rovetto, E. I., & lo Piero, A. R. (2020). Baglieri, A. Biostimulant effect and biochemical response in lettuce seedlings treated with a *Scenedesmus quadricauda* extract. Plants, *9*(1), 123. https://doi.org/10.3390/plants9010123

Rouphael, Y., Giordano, M., Cardarelli, M., Cozzolino, E., Mori, M., Kyriacou, M. C., Bonini, P., & Colla, G. (2018). Plant and seaweed-based extracts increase yield but di erentially modulate nutritional quality of greenhouse spinach through biostimulant action. *Agronomy*, *8*(7), 126. https://doi.org/10.3390/agronomy8070126

Saeedi, M., Khanavi, M., & Shahsavari, K. (2024). *Matricaria chamomilla*: An updated review on biological activities of the plant and constituents. *Research Journal of Pharmacognosy*, *11*(1), 109-36. https://doi.org/10.22127/RJP.2023.404256.2145

Silva, N. C., Duarte, C. R., & Barrozo, M. A. (2020) Analysis of the use of a non-conventional rotary drum for dehydration of microalga *Spirulina platensis*. *Bioprocess and Biosystems Engineering*, *43*(8), 1359-67. https://doi.org/10.1007/s00449-020-02329-1

Shedeed, Z. A., Gheda, S., Elsanadily, S., Alharbi, K., & Osman, M. E. (2022). *Spirulina platensis* biofertilization for enhancing growth, photosynthetic capacity and yield of *Lupinus luteus*. *Agriculture*, *12*(6), 781.

Stirk, W. A., Rengasamy, K. R., Kulkarni, M. G., & van Staden, J. (2020). Plant biostimulants from seaweed: An overview. *The Chemical Biology of Plant Biostimulants*, *2*, 31-55. https://doi.org/10.1002/9781119357254.ch2

Van Oosten, M. J., Pepe, O., De Pascale, S., Silletti, S., & Maggio, A. (2017). The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Chemical and Biological Technologies in Agriculture*, *4*, 1-2. https://doi.org/10.1186/s40538-017-0089-5

Vignaud, J., Loiseau, C., Come, M., Martin, I., Rasoanarivo, R., Herault, J., Mayer, C., Lepine, O., & Ulmann, L. (2025). Combined effects of Spirulina liquid extract and endurance training on aerobic performance and muscle metabolism adaptation in wistar rats. *Nutrients*, *17*(2), 283.

Wan, W. T., Song, Y. J., Xu, L. J., Xiao, P. G., & Miao, J. H. (2019). Research review and application prospect analysis of matricaria. *Modern Chinese Medicine*, *21*, 260-265.

Wang, Y., Ying, Y., Chen, J., & Wang, X. (2004). Transgenic Arabidopsis overexpressing Mn-SOD enhanced salt-tolerance. *Plant Science*, *167*(4), 671-677. https://doi.org/10.1016/j.plantsci.2004.03.032

Xu, C., Sheng, S., Dou H., Chen, J., Zhou, K., Lin, Y., & Yang, H. (2020). α-Bisabolol suppresses the inflammatory response and ECM catabolism in advanced glycation end products-treated chondrocytes and attenuates murine osteoarthritis. *International Immunopharmacology*, *84*, 106530. https://doi.org/10.1016/j.intimp.2020.106530