Plant, Algae, and Environment, Vol. 10, Issue 2, June 2026

Improving Biomass and Phycobiliproteins Production in *Arthrospira platensis* by Ethephon Elicitation

Hadizadeh Mahnaz[1*], Sheykhinejad Ali[1]

Received: 2026-02-25 Accepted: 2026-05-08

Abstract

Arthrospira platensis is the most commercially cultivated microalgae with high nutritional value and various health benefits. It is a rich source of valuable protein pigments known as phycobiliproteins, containing antioxidant and anti-inflammatory properties. Investigation of the factors that enhance the production of phycobiliproteins in photosynthetic organisms is essential due to their potential in various industries, including food, cosmetics, and biomedicine. The Phytohormones play critical roles in regulating the growth and development of plants, as well as responses to environmental stresses. The present study aimed to investigate the effect of the phytohormone ethephon (2-chloroethylphosphonic acid), a commonly used plant growth regulator, on the growth, protein content, and the phycobiliproteins production in *Arthrospira platensis*. In this respect, algae cultures were treated with various concentrations of ethephon for 14 days. Phycobiliproteins were then extracted by multiple freeze-thaw cycles of dried *A. platensis* biomass in phosphate buffer, and the contents were determined spectrophotometrically. Total soluble protein content was also measured by the Bradford method. Ethephon promoted growth at concentrations of 0.005-0.05 mg/L but inhibited growth at higher concentrations (0.1 and 0.15 mg/L). While all ethephon concentrations increased total soluble protein content, the greatest increase was observed at 0.01 and 0.05 mg/L. Ethephon application also enhanced the accumulation of phycocyanin, allophycocyanin, and phycoerythrin in *Arthrospira platensis* at all concentrations except at 0.1 and 0.15 mg/L. Notably, 0.005 mg/L ethephon was the most effective treatment, resulting in an approximately twofold increase in phycocyanin content after 14 days of cultivation. Overall, our results demonstrate that ethephon treatment represents an efficient and cost-effective strategy for enhancing phycobiliprotein production in *Arthrospira platensis*.

Key words: *Arthrospira platensis*, Pigment, Phycocyanin, Ethylene, Antioxidant

Introduction

The growing global demand for food and functional foods together with the limitations of the conventional agriculture, has

1-Department of Biotechnology, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran
*Corresponding author email address: hadizadehmahnaz@gmail.com
Doi: 10.48308/pae.2026.244058.1140

developed the blue-green algae cultivation. As a result, the exploitation of bioactive compounds from them have attracted considerable attention in recent years. Among the bioactive compounds synthesized by algae, natural pigments, such as phycobiliproteins and carotenoids can serve as sustainable alternatives to synthetic colorants in the food, cosmetic, and healthcare industries, as well as dietary supplements for livestock, poultry, and aquatic animals. Their growing application is primarily attributed to their non-toxicity, minimal environmental impact, and antioxidant and medicinal properties (Fernández-Rojas, 2014).

Phycobiliproteins are photosynthetic antenna pigments found in cyanobacteria, red algae, and cryptophytes. They efficiently capture light energy and transfer it to chlorophyll during photosynthesis. Structurally, these proteins are intensely coloured, water-soluble, and highly fluorescent proteins assemble into oligomeric complexes, predominantly hexamers, which constitute the fundamental building blocks of phycobilisomes within the extramembrane light-harvesting antenna complexes associated with photosystem II (Stadnichuk, 2023). The major phycobiliproteins include phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE), which are distinguished by their characterized absorption and emission spectra (García-Gómez, 2025). Beyond their role as photosynthetic pigments, phycobiliproteins, especially phycocyanin, exhibit a broad range of biological activities, including antibacterial, antifungal, antiviral, and antidiabetic effects. Accordingly, these proteins have attracted considerable interest for pharmaceutical, biotechnological, and medical applications (Fabre, 2022; Silva, 2018; Munawaroh, 2024).

Global demand for the natural blue pigment phycocyanin has increased substantially since its approval by USA Food and Drugs Administration (FDA) in 2013, particularly in the Americas and Europe. The global phycocyanin market is expected to grow at a compounded annual growth rate (CAGR) of approximately 7%, reaching an estimated value of US$333 million by 2032 (de Morais, 2018). Natural sources of phycocyanin include cyanobacteria (blue-green algae), and two groups of eukaryotic algae, including Rhodophyta (red algae), and Cryptophyte (Pozzuoli, 2026). Among blue-green microalgae, *Arthrospira platensis* is the primary commercial source of phycocyanin. Commonly known as spirulina, this filamentous photosynthetic cyanobacterium is one of the most extensively cultivated microorganisms owing to its high nutritional value and diverse health-promoting properties. *A. platensis* is rich in proteins, vitamins, minerals, essential fatty acids such as gamma-linoleic acid, and various pigments. Proteins account for approximately 40–60% of its dry biomass, with phycocyanin representing nearly 20% of the total protein contents (García**,** 2021).

Various strategies have been proposed to enhance protein productivity in *A. platensis*, including the optimization of cultivation conditions (Aowtrakool, 2025), genetic engineering (Ji, 2023), and the application of environmental stresses and various eliciting stimuli. (Yu, 2024). Among these strategies, the utilization of plant growth regulators

(PRGs), also known as phytohormones has emerged as a promising approach (Sandybayeva, 2022; Contreras-Ropero, 2024).

Phytohormones are chemical messengers that regulate a wide range of physiological and biochemical processes in higher plants, even at extremely low concentrations. They are typically classified into five major groups, including auxins, abscisic acid, cytokinins, gibberellins, and ethylene. Beyond their well-stablished roles in plant growth and development, phytohormones have been reported to promote cyanobacterial growth and stimulate the biosynthesis of bioactive metabolites (Du, 2017).

Ethephon (2-chloroethylphosphonic acid; $C_2H_6ClO_3P$) is a plant growth regulator that is converted to ethylene after metabolism by the plant. Ethylene plays an important role in regulating plant defence responses against pathogens, pests, and abiotic stresses (Abdelsamad, 2019). It has also been reported to induce the formation of defence-related secondary metabolites, such as phytoalexins and phenolic compounds, in some fruit trees (Belhadj, 2008). However, little is known about the functional role and physiological effects of ethylene in microalgae. For example, endogenous ethylene has been shown to regulate cell growth in the microalgae *Chlamydomonas reinhardtii* (Yordanova, 2013). In addition, treatment of *Chlorella vulgaris* with ethephon has been shown to induce extensive changes in metabolite profiles, including alterations in the levels of alcohols, amino acids, organic acids, sugars, fatty acids, and lipids. In particular, ethephon has been shown to significantly increase the production of α-tocopherol and γ-aminobutyric acid in *Chlorella vulgaris* (Kim, 2016). To address this knowledge gap, the present study aimed to evaluate the effects of ethephon or exogenous ethylene on the production of phycobiliproteins in *A. platensis.* Furthermore, it assessed the potential of ethephon application as an effective strategy for enhancing phycobiliprotein production in *Arthrospira platensis*.

Material and methods

Microorganism and cultivation conditions

The microalga *Arthrospira platensis* used in this study was obtained from the Department of Microbiology, Shahid Chamran University of Ahvaz, under accession code 8643. Cultures were maintained in 250 ml Erlenmeyer flasks containing 100 mL of Zarrouk medium at incubated at 28 ± 2 °C, 400 µmol m^{-2} s^{-1} light intensity with 12 h light / 12 h dark photoperiod. The cultures were continuously aerated by shaking at180 rpm and maintained for 18 days (Kaewdam, 2019).

Effect of ethephon on the growth rate of Arthrospira platensis

The effect of ethephon (Agrostym 480 Soluble Concentrate (SL)) at different concentrations (0.005, 0.01, 0.05, 0.1, and 0.15 mg L^{-1}) were evaluated by supplementing of Zarrouk medium. The stock solution of ethephon (2 mg L^{-1}) was prepared in distilled water, sterilized by filteration through a 0.2 µm cellulose acetate membrane filter, and stored at 4 °C until use. Then, the desired concentrations of ethephon (0.005-0.1 mg L^{-1}) were prepared from the stock solution and added individually to the culture medium at the beginning of cultivation (day 0).

The growth of *A. platensis* was determined by measuring the optical density (OD) at 590 nm on days 0, 2, 4, 6, 8, 10, 12, and 14. Each treatment was conducted in triplicate (Liu, 2018).

Measurement of total soluble protein content

The biomass *of A. platensis* was harvested by centrifuging (8000 g, 15 min) in the stationary phase on day 14. The collected biomass was then washed twice with distilled water to minimize the influence of residual culture medium components. The supernatant was discarded and the pellets were dried with a freeze-dryer (RAY 2, GEA, Germany). Subsequently, 50 mg of the dried biomass was dissolved in 100 mL phosphate buffer, pH 6.8. After three freeze-thaw cycles, Cell disruption was achieved by three freeze-thaw cycles, followed by centrifugation at 12000 × *g* for 10 min at 4 °C using a Sigma 3–30KS centrifuge (Sigma Co., USA). The protein content of the resulting supernatant was measured using the Bradford method (Bradford, 1976). Cultures grown without ethephon supplementation served as the untreated control.

Extraction and determination of phycobiliprotein concentration

Phycobiliproteins were extracted from *Arthrospira platensis* using three freeze-thaw cycles following the method described by Coward et al. (2016), with slight modifications. Briefly, 10 mg of freezedried biomass was suspended in 10 mL of 0.1 M phosphate buffer (pH 6.8), mixed vigorously, and frozen at -20 °C for 24 hours. The samples were thawed at room temperature and subjected to homogenization using a vortex (LVOM-A10, UK) with glass beads for 5 min. This process was repeated three times, after which cellular debris was removed by centrifugation at 3000 × *g* for 15 min using a GS-6 centrifuge (Beckman Coulter, USA), The concentrations of the major phycobiliproteins, including PC, PE, and APC were determined by measuring the absorbance of the clarified supernatant using a UV–Vis spectrophotometer (Shimadzu UV mini-1280, Japan) at wavelengths of 474, 562, 615, and 652 nm. Quantification was performed using equations (1) – (3) described by Bennett and Bogard (1973).

$$PC\ (mg\ mL^{-1}) = [A_{615} - 0.474\ (A_{652})] / 5.34 \quad \text{Eq. 1}$$

$$APC\ (mg\ mL^{-1}) = [A_{652} - 0.208\ (A_{615})] / 5.09 \quad \text{Eq. 2}$$

$$PE(mg\ mL^{-1}) = [A_{562} - 2.41(PC) - 0.849(APC)]/9.62 \quad \text{Eq. 3}$$

Statistical Analysis

All experiments were conducted with three biological replicates (n = 3). The results are presented as mean ± standard deviation (SD). Differences among treatment groups (ethephon concentrations) were analyzed using oneway analysis of variance (ANOVA). When a significant effect was detected, Tukey's post hoc test was applied to identify significant differences between groups. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using GraphPad Prism software (version 10.6.1).

Results and Discussion

Effects of ethephon on cell growth

The growth of *A. platensis* under different concentrations of ethephon was evaluated and compared with that of the control group. As shown in Fig. 1, all treatments exhibited a similar growth pattern charac-

terized by three distinct phases: lag, exponential, and stationary phases. The results of this study showed that the effects of ethephon on the growth of *A. platensis* were concentration-dependent. Low concentrations of ethephon (0.005, 0.01, and 0.05 mg L^{-1}) stimulated growth, whereas higher concentrations (0.10 and 0.15 mg L^{-1}) significantly inhibited it. The greatest stimulatory effect was observed at of 0.01 mg L^{-1}, resulting in a 16.6% increase in growth compared with the control ($p < 0.01$). while the lowest stimulatory effect (5.5%) occurred at 0.005 mg L^{-1} ($p < 0.05$). In contrast, higher concentrations of ethephon (0.10 and 0.15 mg L^{-1}) led to significant reductions in growth rate after 14 days of treatment, with decreases of 16.4% and 24.5%, respectively, compared to the control ($p < 0.01$).

Ethephon is metabolized to ethylene following being absorbed by plants. Ethylene, a gaseous plant hormone, functions as a regulatory signalling molecule that modulates numerous physiological processes and mediates growth responses to various environmental stresses in higher plants and microalgae. Although ethylene is generally known as a growth inhibitor in higher plants, particularly at elevated concentrations, some species, especially semi-aquatic plants such as *Romex palustris*, exhibit ethylene-induced growth promotion (Wang, 2025).

The dual nature of ethylene's effects on growth depends on several factors, including its concentration, tissue type, plant species, and developmental stage (Pierik, 2006). Kim et al (2016), treated *Chlorella vulgaris* with ethephon and observed that only slightly increased growth in this microalga, even at very high concentrations (2.9 g L^{-1}). Du et al (2017) also reported that 12 days after treatment of the microalga *Chlorella pyrenoidosa* with ethephon concentrations of 0.10, 0.15, and 0.20 mg L^{-1}, the growth of this microalga enhanced by 4 to 6fold. These findings are in contrast with the growth inhi-

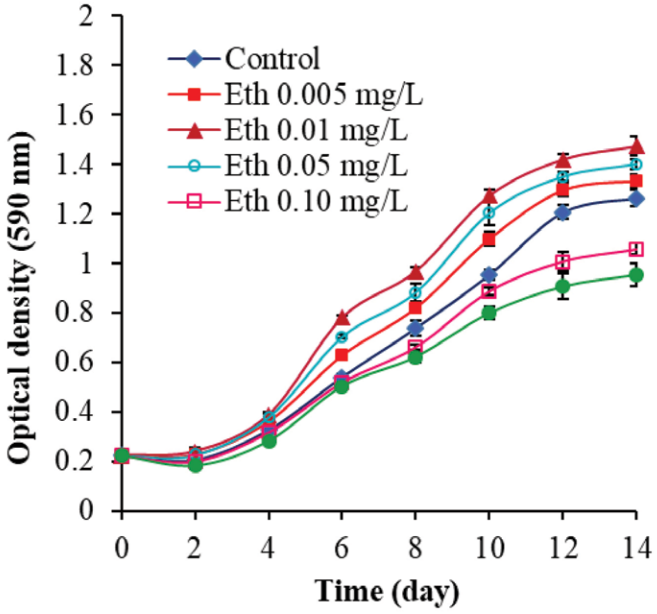

Fig. 1. Growth curve of *Arthrospira platensis* under the influence of differentconcentrations of ethephon

bition observed at similar concentrations in the present study, highlighting speciesspecific differences in ethylene sensitivity and response mechanisms.

Effect of ethephon on total soluble protein content

The content of total soluble proteins of *Arthrospira platensis* after 14 days of treatment with different concentrations of ethephon is shown in Figure 2.

The results showed that ethephon, at all concentrations testedsignificantlyincreased ($p < 0.01$) the total soluble protein content of *Arthrospira platensis* at all concentrations tested. However, lower ethephon concentrations showed a stronger stimulatory effect compared with higher concentrations. Specifically, ethephon at concentrations of 0.005, 0.01, 0.05, 0.1, and 0.15 mg L^{-1} increased the total soluble protein content by 1.23-, 1.30-, 1.47-, 1.14-, and 1.19-fold, respectively, relative to the control (442 ± 17 mg gDW^{-1}).

The highest protein content was observed in cultures treated with 0.05 mg L^{-1} ethephon (650.62 ± 19.50 mg gDW^{-1}), while the lowest was observed at 0.10 mg L^{-1} ethephon (502.11 ± 25.00 mg gDW^{-1}). The observed enhancement in soluble protein content may be attributed to the upregulation of target genes involved in protein synthesis or stressresponse pathways following ethephon treatment (Li, 2022).

The stimulatory effect of low concentrations of ethephon on soluble protein accumulation have also been reported in other microalgae species. As an ethylene-releasing precursor, ethephon plays a role in regulating microalgae cell growth and protein production in a concentration-dependent manner. For example, Du et al (2017) reported that, among eight phytohormones examined, ethephon was the most effective phytohormone in enhancing protein content in the microal-

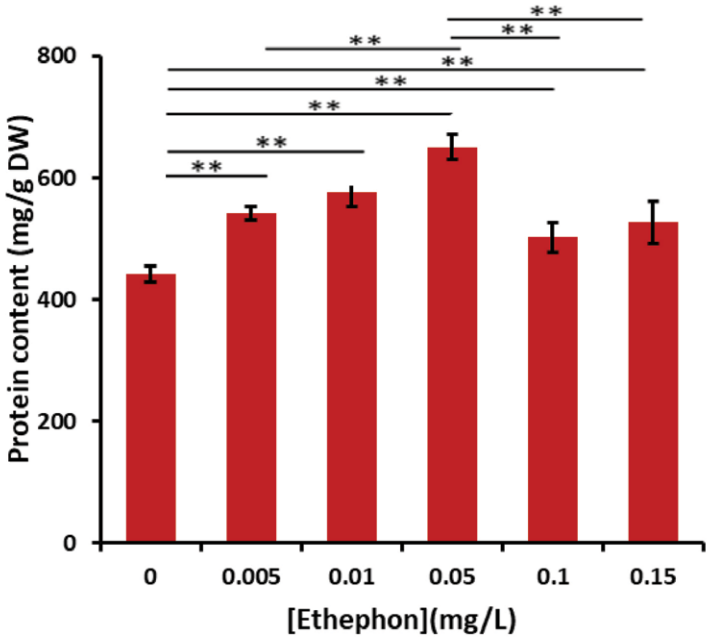

Fig. 2. Effect of different concentrations of ethephon on the total soluble protein content of *Arthrospira platensis*.

**Significant at the level of 0.01; DF (degrees of freedom) = (5, 12)

ga *Chlorella pyrenoidosa*. Ethephon at a concentration of 0.005 mg L^{-1} resulted in a 3.5-fold increase in the protein content of *C. vulgaris*, with the total soluble protein content increasing from 28 mg L^{-1} in the control to 99 mg L^{-1}. In contrast, Xie et al (2023) investigated the effect of different ethephon concentrations (1-10 mg L^{-1}) on the protein production rate in *Chlorella vulgaris*. It is noteworthy that ethephon concentrations evaluated in their study were 10 to 100 times higher than the concentrations used in the present study. These findings suggest that the physiological effects of ethephon are strongly centration-dependent and that identifying an optimal concentration is critical for maximizing both growth and protein accumulation in microalgae. Therefore, selecting the appropriate ethephon concentration should be considered a crucial factor for optimizing both growth and protein production in microalgae.

Effect of ethephon on the production rate of phycobiloproteins

The effect of different concentrations of the phytohormone ethephon (0.005, 0.01, 0.05, 0.1, and 0.15 mg L^{-1}) on the concentration of major phycobiloproteins, including phycocyanin, allophycocyanin, and phycoerythrin in *Arthrospira platensis* were investigated. Following the harvesting of microalgae on the 14th day of cultivation, the concentration of phycocyanin in the crude extract the untreated control was 0.27 mg mL^{1}, which was 3.8-fold higher than that of phycoerythrin and 1.42 times higher than that of allophycocyanin. After treating the microalgae *Arthrospira platensis* with varying concentrations of ethephon, the most increase (approximately a 2-fold increase) in phycobiliproteins was observed on the 14th day of cultivation, influenced by 0.005 mg mL^{-1} ethephon. As the concentration of ethephon increased from 0.005 mg mL^{-1} to 0.01 mg mL^{-1} and 0.05 mg mL^{-1}, the amount of all phycobiliproteins in *Arthrospira platensis* remained higher than the control, which did not receive phytohormone treatment. Although, these two higher concentrations of ethephon caused a lesser increase in all three phycobiliproteins compared to the 0.005 mg mL^{-1} ethephon concentration. Among the three phycobiliproteins, phycocyanin showed the greatest response, exhibiting an approximately 1.8-fold compared with the control following treatment with 0.005 mg mL^{-1} ethephon (Table 1).

Higher concentrations of ethephon (0.1 and 0.15 mg L^{-1}) significantly reduced concentrations of phycobiliproteins in *Arthrospira platensis* ($p < 0.05$). Specifically, the phycocyanin concentration decreased from 0.27 mg mL^{-1} in the untreated control to 0.22 and 0.23 mg mL^{-1} following treatment with 0.10 and 0.15 mg L^{-1} ethephon, respectively. Similar reductions were also observed in concentrations of phycoerythrin and allophycocyanin at both ethephon concentrations (Table 1).

he phycobiliprotein contents of *A. platensis* biomass, expressed as mg g^{-1} dry weight (DW), are presented .in Figure 3.

Likewise, only limited information is available on the effects of the phytohormone ethephon on the phycobiliprotein content of *Arthrospira platensis*. There is also little information on the effect of exogenous application of other phytohormones on the

Table 1 Concentration of phycobiloproteins extracted from *Arthrospira platensis* after treatment with different concentrations of ethephon

Ethephon (mg L^{-1})	Phycocyanin (mg mL^{-1})	Phycoerythrin (mg mL^{-1})	Allophycocyanin (mg mL^{-1})
0	$0.27 \pm 0.01^{**}$	$0.07 \pm 0.01^{**}$	$0.19 \pm 0.02^{**}$
0.005	$0.49 \pm 0.02^{**}$	$0.14 \pm 0.02^{**}$	$0.43 \pm 0.03^{**}$
0.01	$0.36 \pm 0.01^{**}$	0.12 ± 0.02^{ns}	$0.38 \pm 0.01^{**}$
0.05	$0.36 \pm 0.03^{**}$	0.10 ± 0.01^{ns}	$0.26 \pm 0.01^{**}$
0.10	$0.22 \pm 0.02^{**}$	0.06 ± 0.01^{ns}	$0.20 \pm 0.02^{**}$
0.15	$0.23 \pm 0.02^{**}$	0.07 ± 0.02^{ns}	$0.17 \pm 0.01^{**}$

Significant at the level of 0.05; **Significant at the level of 0.01; ns = non-

phycobiliprotein content of microalgae. In a study conducted by Mansouri and Talebizadeh (2016), the effect of gibberellic acid on the growth and phycobiliprotein content of the microalga *Nostoc linckia* was investigated. The results of the study showed that the wet weight of *Nostoc linckia* was significantly increased by treatments of 10 and 100 µM gibberellic acid. The amount of phycocyanin in 7-day cultures treated with 1 µM gibberellic acid increased twofold; from 0.46 to 0.96 mgg^{-1} wet biomass.

Similar changes were observed for the content of allophycocyanin and phycoerythrin after 7 days of culture. In another study, the effect of the phytohormone indole-3-butyric acid (IBA) on the growth and production of some primary and secondary metabolites in *Nostoc linckia* was investigated. In this regard, algal cultures were treated with 0, 0.01, 0.1, 1, 10, and 100 µM IBA for 14 days. The results showed that the amount of all phycobiliproteins increased under the influence of 10 and 100 µM IBA concentrations. The highest amounts of phycocyanin, allophycocyanin, and phycoerythrin (5.6, 2.2, and 0.25 mg / g wet weight, respectively) were observed in the culture treated with 10 µM IBA (Mansouri and Talebizadeh, 2017).

The increase in phycobiliprotein content along with the improvement in *Arthrospira platensis* biomass production observed in the present study may suggest that a secondary role for phycobiliproteins as intracellular nitrogen storage compounds (Eriksen, 2008).

Determination of Phycobiloprotein Purity

In the present study, the purity index of phycocyanin, allophycocyanin and phycoerythrin extracted from *Arthrospira platensis* treated with different concentrations of ethephon were evaluated as the ratio of the absorbance of each phycobiloprotein (615 nm for (PC), 652 nm for APC, and 63 nm for PE) to the absorbance at 280 nm, representing total soluble proteins. The results showed that phycocyanin was the predominant phycobiliprotein and exhibited a higher purity index than allophycocyanin and phycoerythrin. The purity index of phycocyanin

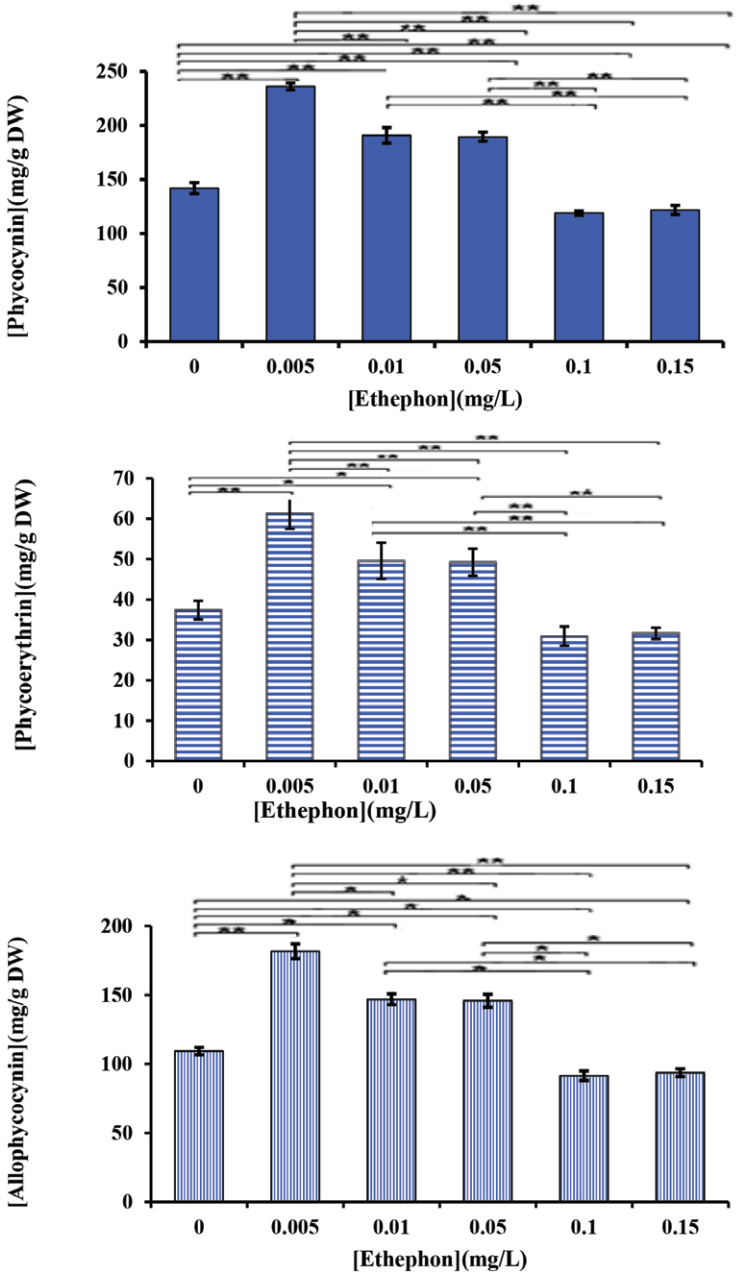

Fig. 3. The effect of different concentrations of Ethephon on a) phycocyanin, b) phycoerythrin, and c) allophycocyanin content of *Arthrospira platensis* after 14 days of cultivation.

*Significant at the level of 0.05; **Significant at the level of 0.01; FD (5,12)

increased from 0.62 in the untreated control to a maximum value of 0.89 following treatment with 0.10 mg L^{-1} ethephon. The purity indices of both phycoerythrin and allophycocyanin were 0.47 in the untreated control.. The highest purity index of phycoerythrin (0.88) was obtained with 0.05 mg L^{-1} ethephon, whereas the maximum purity index of allophycocyanin (0.77) was observed at 0.005 mg L^{-1} ethephon.(Table 2).

The results obtained in the present study, including a phycocyanin concentration of 0.27 mg mL^{-1} and a purity index of 0.62, are in consistent with the results of Hokmollahi

Table 2. The purity index of phycobiliproteins extracted from the *Arthrospira palatensis*

	Purity index		
Phytohormone	PC	APC	PE
Ethephon (0 mg L^{-1})	0.62 ± 0.04	0.46 ± 0.03	0.46 ± 0.08
Ethephon (0.005 mg L^{-1}	0.83 ± 0.08	0.77 ± 0.04	0.71 ± 0.04
Ethephon (0.01 mg L^{-1})	0.62 ± 0.01	0.49 ±0.09	0.85 ± 0.05
Ethephon (0.05 mg L^{-1})	0.62 ±0.04	0.48 ± 0.09	0.87 ± 0.09
Ethephon (0.10 mg L^{-1})	0.88 ± 0.06	0.75 ± 0.07	0.69 ± 0.08
Ethephon (0.15 mg L^{-1})	0.81 ± 0.08	0.71 ± 0.05	0.66 ± 0.06

et al (2025). In this studyIn this study, the saltfree control exhibited a phycocyanin purity index of 0.77 increased with the addition of NaCl, reaching a maximum value of 1.17. Similarly, Athiyappan (2024) reported a maximum phycocyanin purity ratio of 0.62 when extracted using a chloridebased solvent. The purity of phycobioproteins plays a vital role in their commercial applications. Among the various phycobioproteins, phycocyanin is considered the most valuable pigment used in the pharmaceutical and food industries due to its colour, fluorescence, and antioxidant properties (Winayu, 2021). Phycocyanin with a purity index of 0.7 is considered food grade, whereas purity indices of 3.9 and ≥ 4.0 correspond to reactive and analytical grade (Hsieh-Lo, 2019). The results of the present study showed that ethephon treatment did not adversely affect the purity of phycobiliproteins extracted from *Arthrospira platensis*. Moreover, at specific concentrations, ethephon increased the purity indices of phycobiliproteins, indicating its potential to improve phycobiliprotein quality for food-grade applications..

Conclusions

In summary, the results of this study demonstrate that the exogenous application of the phytohormone ethephon during the cultivation of *Arthrospira platensis* enhances not only microalgal growth but also the accumulation of soluble proteins and valuable phycobiliprotein pigments. These effects are strongly concentrationdependent, with stimulatory responses are observed at ethephon concentrations between 0.005 mg L^{-1} and 0.05 mg L^{-1}, whereas higher concentrations (0.1 and 0.15 mg L^{-1}) inhibited the growth and metabolite accumulation. The stimulatory effects of ethephon may be associated with enhanced photosynthetic activity and the upregulation of key enzymes involved in phycobiliprotein biosynthesis. However, further studies are required to elucidate the underlying molecular mechanisms. Given the growing global demand for natural pigments and proteinrich microalgal products in the food, pharmaceutical, and nutraceutical industries, optimizing ethephon application could improve a costeffective strategy to improve production efficiency

and strengthen commercial competitiveness.

Acknowledgment

This research was supported by the Iranian Research Organization for Science and Technology (IROST) under project: 1012101007, entitled Increasing the production of phycocyanin C pigment in *Spirulina platensis* using phytohormones Ethephon and 1-naphthalene acetic acid.

References

Abdelsamad, N. A., MacIntosh, G. C., Leandro, L. F. S., 2019. Induction of ethylene inhibits development of soybean sudden death syndrome by inducing defence-related genes and reducing *fusarium virguliforme* growth. *PLOS One*, 14(5), pp. e0215653. https://doi: 10.1371/journal.pone.0215653.

Aowtrakool, N., Laomettachit, T., walya, N. R., 2025. Optimizing semi-continuous cultivation of *Arthrospira platensis* C1 for phycocyanin production using dynamic modeling. *Algal Research*, 90, pp. 104157. https://doi.org/10.1016/j.algal.2025.104157.

Athiyappan, k. D., Routray, W., Paramasiva, B., 2024. Comprehensive review on cultivation, extraction, purification, and its application in food and allied industries. *Food and Humanity,* 2, pp. 100235. https://doi.org/10.1016/j.foohum.2024.100235

Belhadj, A., Telef, N., Cluzet, S., Bouscaut, J., Corio-Costet, M., Mérillon, J-M., 2008. Ethephon elicits protection against Erysiphe necator in grapevine. *Journal of Agricultural and Food Chemistry*, 56(14), pp. 5781-5787. https://doi.org/10.1021/jf800578c.

Bennett A, Bogorad L (1973) Complementary chromatic adaptation in a filamentous blue-green alga. Journal of cell biology 58(2): 419-435. https://doi.org/10.1083/jcb.58.2.419.

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), pp. 248-254. https://doi.org/10.1016/0003-2697(76)90527-3.

Contreras-Ropero, J. E., Barajas-Solano, A. F., García-Martínez, J. B., Barajas-Ferreira, C., Zuorro, A., 2024. Optimization of phycobiliprotein biosynthesis in thermotolerant cyanobacteria through light parameter adjustment. *Results in Engineering,* 23, pp. 102644. https://doi.org/10.1016/j.rineng.2024.102644.

Coward. T., Fuentes-Grunewald, C., Silkina, A., Oatley-Radcliffe, D.L., Llewellyn, G., Lovitt, R. W., 2016. Utilising light-emitting diodes of specific narrow wavelengths for the optimization and co-production of multiple high-value compounds in Porphyridium purpureum. *Bioresource Technology*, 221, pp. 607–615. https://doi.org/10.1016/j.biortech.2016.09.093.

de Morais, M. G., da Fontoura, D. P., Moreira, J. B., Duarte, J. H., Costa, J. A.V., 2018. Phycocyanin from microalgae: properties, extraction and purification, with some recent applications. *Industrial Biotechnology*, 14(1), pp. 30-37. https://doi.org/10.1089/ind.2017.0009.

Du, H., Ahmed, F., Lin, B., Li, Z., Huang, Y., Sun, G., Ding, H., wang, C., meng, C., Gao, Z., 2017. The effects of plant growth regulators on cell growth, protein, carotenoid, PUFAs and lipid production of *chlorella pyrenoidosa* ZF strain. *Energies*, 10(11), pp. 1696. https://doi.org/10.3390/en10111696.

Eriksen, N. T., 2008. Production of phycocyanin—a pigment with applications in biology, biotechnology, foods and medicine. *Applied Microbiology and Biotechnology*, 80(1), pp. 1-14. https://doi.org/10.1007/s00253-008-1542-y.

Fabre, J. F., Niangoran, N. U. F., Gaignard, C., Buso, D., Mouloungui, Z., Valentin, R., 2022. Extraction, purification and stability of Cphycocyanin from *Arthrospira platensis*. *European Food Research and Technology,* 248, pp. 1583–1599. https://doi.org/10.1007/s00217-022-03987-z.

Fernández-Rojas, B., Hernández-Juárez, J., Pedraza-Chaverri, J., 2014. Nutraceutical properties of phycocyanin. *Journal of Functional Foods,* 11(3), pp. 375-392. https://doi.org/10.1016/j.jff.2014.10.011.

García-Gómez, C., Aguirre-Cavazos, D. E., Chávez-Montes, A., Ballesteros-Torres, J. M., Orozco-Flores, A. A., et al., 2025. Phycobilins versatile pigments with wide-ranging applications: exploring their uses, biological activities, extraction methods and future perspectives. *Marine Drugs*, 23(5), pp. 201. https://doi.org/10.3390/md23050201.

García, A. B., Longo, E., Bermejo, R., 2021. The application of a phycocyanin extract obtained from *Arthrospira platensis* as a blue natural colorant in beverages. *Journal of Applied Phycology,* 33, pp. 3059-3070. https://doi.org/10.1007/s10811-021-02522-z.

Hokmollahi1, F., Mirbagheri Firoozabad, M. S., Sodaeizadeh, H., Nateghi, A., 2025. Production of phycocyanin natural blue dye of algal origin and evaluation of different extraction and purification methods. *Plant, Algae, and Environment*, 9(3), p.p. 50-60. https://doi.org/10.48308/pae.2025.240991.1119.

Hsieh-Lo, M., Castillo, G., Ochoa-Becerra, M. A., Mojica, L., 2019. Phycocyanin and phycoerythrin: Strategies to improve production yield and chemical stability. *Algal Research*, 42, pp. 101600. https://doi.org/10.1016/j.algal.2019.101600.

Ji, L., Qiu, S., Wang, Z., Zhao, C., Tang, B., Gao, Z., Fan, J., 2023. Phycobiliproteins from algae: current updates in sustainable production and applications in food and health. *Food Res Int*, 167, pp. 112737. https://doi.org/10.1016/j.foodres.2023.11273.

Kaewdam, S., Jaturonglumlert, S., Varith, J., Nitatwichit, C., Narkprasom, K., 2019. Kinetic models for phycocyanin production by fed -batch cultivation of the *Spirulina platensis. International Journal of GEOMATE* 17, pp. 187-194. Doi:10.21660/2019.61.892 05.

Kim, S-H., Lim, S. R., Hong, S-J., Cho, B-K., Lee, H., lee, C., Choi, H-K., 2016. Effect of ethephon as an ethylene-releasing compound on the metabolic profile of *chlorella vulgaris*. *Journal of Agricultural and Food Chemistry*, 64, pp. 4807−4816. https://doi.org/10.1021/acs.jafc. 6b00 541.

Li, X., Li, B., Li, M., Fu, X., Zhao, X., Min, D., Li, F., Zhang, X., 2022. Ethylene pretreatment induces phenolic biosynthesis of fresh-cut pitaya fruit by regulating ethylene signaling pathway. *Postharvest Biology and Technology*,192, p.p. 112028. https://doi.org/10.1016/j.postharvbio.2022.112028.

Liu, H., Chen, H., Wang, S., Liu, Q., Li, S., Song, X., Huang, Wang, X., Jia, L., Optimizing light distribution and controlling biomass concentration by continuously pre-harvesting Spirulina platensis for improving the microalgae production. *Bioresource Technology*, 252, pp. 14-19. https://doi.org/10.1016/j.biortech.2017.12.046.

Mansouri, H., Talebizadeh, B., 2016. Effect of gibberellic acid on the cyanobacterium Nostoc linckia. *Journal of Applied Phycology*, 28(5), pp. 2187–2193. https://doi.org/10.1007/s10811-015-0756-5.

Mansouri, H., Talebizadeh, R., 2017. Effects of indole-3-butyric acid on growth, pigments and UV-screening compounds in Nostoc linckia. *Phycological Research*, 65(3), pp. 212–216. https://doi.org/10.1111/pre.12177.

Munawaroh, H. S. H., Gumilar, G. G., Pratiwi, R. N., Khoiriah, S. F., Ningrum, A., Martha, L., Chew, K. W., Show, P-L., 2024. In silico antiviral properties of *Spirulina platensis* phycobiliprotein and phycobilin as natural inhibitor for SARS-CoV-2. *Algal Research*, 79, pp. 103468. https://doi.org/10.1016/j.algal.2024.103468.

Pierik, R., Tholen, D., Poorter, H., Visser, E. J., Voesenek, L. A., 2006. The Janus face of ethylene: Growth inhibition and stimulation. *Trends in Plant Science*, 11(4), pp. 176−183. https://doi.org/10.1016/j.tplants.2006.02.006.

Pozzuoli, E., Auciello, C., Avilia, S., Marra, L., Iovinella, M., De Stefano, M., Papa, S., Ciniglia, C., 2026. Biological and economic advantages of C-Phycocyanin production from red algae (Rhodophyta), yield optimization and sustainability strategies: a systematic review. *European Food Research and Technology*, 252, pp. 133. https://doi.org/10.1007/s00217-026-05049-0.

Prabuthas, P., Majumdar, S., Srivastav, P. P., Mishra, H. N., 2011. Standardization of rapid and economical method for neutraceuticals extraction from algae. *Journal of Stored Products and Postharvest Research*, 2(25), pp. 93–96.

Sandybayeva, S. K., Kossalbayev, B. D., Zayadan, B. K., Sadvakasova, A. K., Bolatkhan, K., 2022. Prospects of cyanobacterial pigment production: Biotechnological potential and optimization strategies. *Biochemical Engineering Journal*, 187, pp. 108640. https://doi.org/10.1016/j.bej.2022.108640.

Silva, E. F. E., Figueira, F.S., Lettnin, A. P., Carrett-Dias, M., Filgueira, D. M. V. B., 2018. C-phycocyanin: cellular targets, mechanisms of action and multi drug resistance in cancer. *Pharmacological Reports*, 70(1), pp. 75–80. https://doi.org/10.1016/j.pharep.2017.07.018.

Stadnichuk, I. N., Kusnetsov, V. V., 2023. Phycobilisomes and phycobiliproteins in the pigment apparatus of oxygenic photosynthetics: From cyanobacteria to tertia-

ry endosymbiosis. *International Journal of Molecular Sciences*, 24(3), pp. 2290. https://doi.org/10.3390/ijms24032290.

Wang, X., Wen, H., Suprun, A., Zhu, H., 2025. Ethylene signaling in regulating plant growth, development, and stress responses. *Plant Cell Reports*, 32(7), pp. 1099-1109. https://doi.org/10.1007/s00299-013-1421-6.

Winayu, B. N. R., Lai, K.T., Hsueh, H-T., Chu, H., 2021. Production of phycobiliprotein and carotenoid by efficient extraction from Thermosynechococcus sp. CL-1 cultivation in swine wastewater. *Bioresource Technology*, 319, pp. 124125. https://doi.org/10.1016/j.biortech.2020.124125.

Xie, Z., Ma, S, Cao. Y., Peng, S., Zhang, X., 2023. Effects of six phytohormones on the growth behavior and cellular biochemical components of *Chlorella vulgaris*. *Journal of Applied Phycology*, 4, pp. 1589-1602. https://doi.org/10.21203/rs.3.rs-2671883/v1.

Yordanova, Z. P., Iakimova, E. T., Cristescu, S. M., Harren, F. J., Kapchina-Toteva, V. M., Woltering, E. J., 2013. Involvement of ethylene and nitric oxide in cell death in mastoparan-treated unicellular alga *Chlamydomonas reinhardtii*. *Cell Biology International*, 34 (3), pp. 301−308. https://doi.org/10.1042/CBI20090138.

Yu, C., Hu, Y., Zhang, Y., Luo, W., Zhang, J., Xu, P., Qian, J., Li, J., Yu, J., Liu, J., Zhou, W., Shao, S., 2024. Concurrent enhancement of biomass production and phycocyanin content in salt-stressed Arthrospira platensis: A glycine betaine- supplementation approach. *Chemosphere*, 353, PP. 141387. https://doi.org/10.1016/j.chemosphere.2024.141387.