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RESEARCH ARTICLE

Effect of mineral phosphates (K_2HPO_4) on mat formation, growth and biochemical responses in *Tolypothrix* sp. KJE1

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Abstract

Phosphate availability plays a crucial role in shaping microbial communities and influencing their structure and growth. In the present study, the impact of higher concentrations of K_2HPO_4 treatment on *Tolypothrix* sp. KJE1, was investigated. The results revealed that increased phosphate concentrations enhanced overall cellular growth and biomass productivity through improved photosynthetic performance and efficient carbon assimilation. Higher phosphate levels resulted in increased chlorophyll-a content, which is crucial for photosynthesis, as well as augmented carotenoid and scytonemin contents, which act as photoprotective pigments. Moreover, the treatment influenced the synthesis of phycobiliproteins, including phycocyanin, phycoerythrin, and allophycocyanin, which are responsible for capturing light energy. Phosphate availability also influenced chlorophyll fluorescence and exopolysaccharide production in *Tolypothrix* sp. KJE1. The treatment facilitated optimal photosynthetic performance and increased exopolysaccharide biosynthesis, which contributed to cell aggregation and protection against environmental stressors. Furthermore, the higher phosphate availability stimulated lipid biosynthesis and storage in *Tolypothrix* sp. KJE1. Phosphorus, an essential component of lipid, increased cell lipid content, while phosphate regulation of lipid metabolism and enzyme activity played a role in lipid accumulation. However, the elevated phosphate concentrations disrupted the nutrient balance, leading to decreased protein and carbohydrate content as resources were diverted towards phosphate uptake and storage. Overall, this study highlights a significant influence of phosphate availability on *Tolypothrix* sp. KJE1, impacting mat structure, pigment synthesis, photosynthetic efficiency, exopolysaccharide production, and lipid content. Understanding these responses enhance our knowledge of the intricate relationships between nutrient availability, microbial physiology, and ecosystem dynamics.

Keywords: Cyanobacteria; K_2HPO_4; Mat; Phosphate; *Tolypothrix*.

1. Introduction

Cyanobacteria are a group of photosynthetic bacteria capable of converting sunlight into chemical energy through photosynthesis. *Tolypothrix* sp. KJE1 is a specific strain of cyanobacteria which is known for its ability to form two unique structures such as heterocysts and akinetes. The heterocysts are involved explicitly in fixing atmospheric nitrogen, which makes nitrogen available in a form which is utilised by various aquatic organisms and ecosystems, including plants (Sharma et al., 2014). Conversely, akinetes are specialised resting cells that are explicitly involved in improving the resilience of *Tolypothrix* under climate extremes such as desiccation, high temperature and nutrient scarcity. The akinetes not only enable *Tolypothrix* to thrive under stress conditions but also serve as a mean of dispersal and also forms mat-like structures in aquatic environments (Zehr and Kudela, 2011). These mats are often observed as dense, filamentous aggregations that can cover submerged surfaces such as rocks, sediments, or other substrates (Herdman and Carr, 2012). Several studies have also shown that the mat formation by *Tolypothrix* instigates various ecological functions, i.e., mats can create a micro-environment providing shelter/protection to different marine organisms such as bacteria, algae and small vertebrates. Mat formation is a complex process which regulated at genetic and physiological levels, which are also capable of influencing the surrounding ecosystem by altering water flow patterns and sedimentation (Zakhary and Sherif, 2018).

Phosphorus is an essential mineral phosphate required by all living organisms, and cyanobacteria are believed to play a significant role in its cycling and availability in aquatic ecosystems (Bunce et al., 2018). Among all the cyanobacteria, *Tolypothrix* sp. KJE1 possesses specific transporters and enzymes to modulate the availability and assimilation of mineral phosphates when present in the environment. Mineral phosphate acts as the primary source of phosphorus for *Tolypothrix* sp. KJE1, which stimulates its growth and biomass production leading to a dynamic increase in its population size, thus outcompeting other microorganisms for phosphorus availability. This competitive advantage of *Tolypothrix* sp. KJE1 for phosphorus assimilation, when abundantly present, can drastically affect the structure and dynamics of other microbial communities within the ecosystem (Sharma et al., 2014). *Tolypothrix* sp. KJE1 also play a unique role in alleviating phosphorus limitation in environments where phosphorus availability is limited. *Tolypothrix* sp. KJE1, in conjunction with other phosphate-utilising cyanobacteria, efficiently utilises mineral phosphates, thus enhancing the ecosystem's overall productivity and nutrient cycling. Furthermore, researchers have also connotated that the presence of excessive mineral phosphates due to anthropogenic activities and agricultural runoff has exaggerated the cause of eutrophication. Excessive eutrophication has dramatically influenced nutrient availability leading to uncontrolled growth of algae and cyanobacteria, resulting in algal blooms that negatively impact the ecosystem by depleting dissolved oxygen and production of toxins (Smith, 2003).

In addition, studies have also corroborated that the abundance of mineral phosphates can negatively impact the nitrogen-fixing ability of *Tolypothrix* sp. KJE1 (Zehr and Kudela, 2011). In addition, increased levels of phosphorus can result in over-accumulation of phosphorus than is required for normal growth,

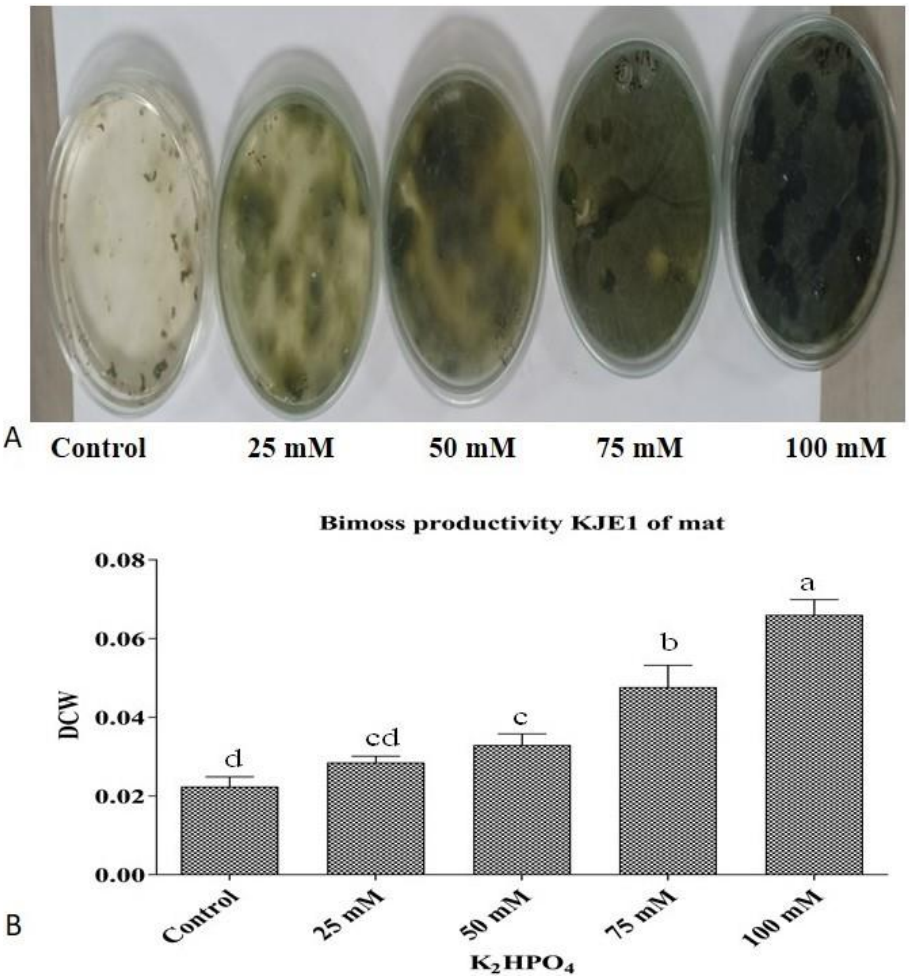

Figure 1. Effect of different concentration of phosphate (K_2HPO_4) on mat formation on petri plate of *Tolypothrix* sp. KJE1 mat morphology (A) and biomass productivity (B).

thus affecting cellular metabolism (Herdman and Carr, 2012). This, in turn, can shift the metabolic priorities by downregulating or inhibiting the expression of genes involved in nitrogen fixation and energy transfer process, thus affecting cellular structures such as DNA, RNA and phospholipids. The present study aims to investigate the role of mineral phosphate (K_2HPO_4) in the growth and mat formation of *Tolypothrix* sp. KJE1 by (i) measuring the growth rate, biomass production, and cellular parameters under varying concentrations of mineral phosphates (ii) analysing the influence of different levels of phosphorus availability on the cellular processes and metabolic activity of *Tolypothrix* sp. KJE1 and (iii) assessing the influence of phosphorus levels on the formation, stability, and morphology of *Tolypothrix* sp. KJE1 mats.

2. Material and method

2.1. Isolation and purification of the cyanobacterial strain

The cultures of *Tolypothrix* sp. KJE1 were collected from a paddy field located at the agriculture experimental farm, B.H.U., Varanasi. The cultures were purified by serial dilution method and were allowed to grow on BG-11N$^-$ (nitrogen-free) medium for two weeks. The cyanobacterial colonies were allowed to grow on agar plates, which were then placed into 100 ml BG-11N$^-$ medium in 500 ml conical flasks. The isolated colonies were subsequently transferred to fresh BG-11N$^-$ plates and incubated at a temperature of 25 ± 2°C under fluorescent tube light with an intensity of 55 µmol photons m^2/s. After purification, the obtained mother cultures were grown in fresh BG-11N (pH 7.4) medium for 25 days under the above-mentioned light and temperature regime with a photoperiod of 14:10 day-night. The medium containing the cyanobacterial cultures was manually shaken 5 times a day to ensure its proper growth and suspension. Finally, 10% of the mother cultures in the exponential growth phase were aseptically transferred to a 2L conical flask containing 1300 mL of BG-11N$^-$ medium. The cultures were then grown under the same conditions as previously described.

2.2. Experimental setup and treatments

To investigate the influence of different concentrations of K_2HPO_4 on *Tolypothrix* sp. KJE1, the cyanobacterial cultures were subjected to treatments with varying levels of K_2HPO_4 (25 mM, 50 mM, 75 mM, and 100 mM). Control cultures without K_2HPO_4 were also included for comparative analysis. Time-specific sampling was conducted to assess the growth parameters, thereby enabling the evaluation of the effects of different K_2HPO_4 concentrations on *Tolypothrix* sp. KJE1. All the experiments were performed in three biological and technical replicates.

2.3. Determination of pigments

To analyze the presence of lipid-soluble pigments like chlorophyll-a, carotenoids, and scytonemin in cyanobacterial mats. A sample of 20 mg of the mats was treated with 80% acetone. The mixture was then refrigerated at 4°C overnight to ensure total extraction of the pigments. The concentrations of chlorophyll and other compounds were quantified by measuring the optical density (OD) at specific wavelengths: 665 nm for chlorophyll-a, 461 nm for carotenoids, and 384 nm for scytonemin. To account for light scattering, the absorbance at 750 nm was subtracted from the measurements. The remaining cell pellets were resuspended in DDW (double-distilled water) and subjected to the freeze-thaw method to extract water-soluble phycobiliproteins. The supernatant obtained after centrifugation was used for further analysis. The optical densities of different phycobiliproteins—phycocyanin (PC), phycoerythrin

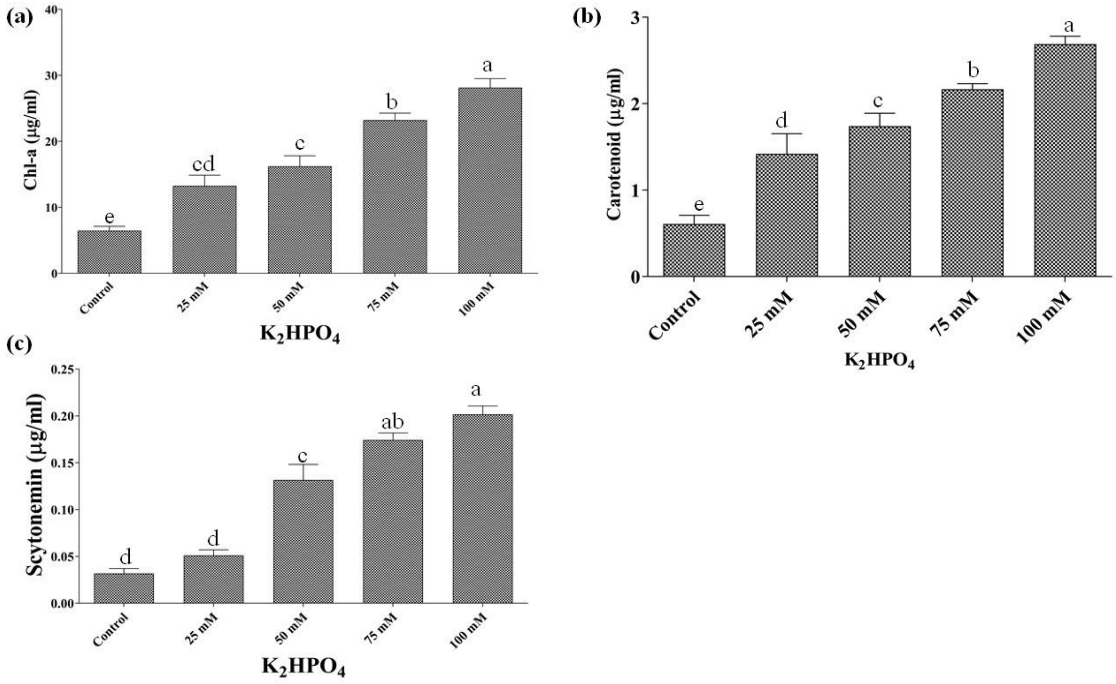

Figure 2. Effect of different concentration of K_2HPO_4 on *Chl-a*, carotenoids and scytonemin

(PE), and allophycocyanin (APC) were measured spectrophotometrically at wavelengths of 562 nm, 615 nm, and 652 nm, respectively. The quantification of all pigments was carried out using standard formulas.

$$\text{Chl} - \text{a}\ (\mu\text{g/mg}) = (A_{665}\text{nm} - A_{750}\text{nm}) \times 13.9$$

$$\text{Carotenoid}\ (\mu\text{g/mg}) = (A_{461}\text{nm} - A_{750}\text{nm}) - 0.046 \times (A_{665}\text{nm} - A_{750}\text{nm}) \times 4$$

$$\text{Scytonemin}\ (\mu\text{g/mg}) = (1.04\, A_{384}\text{nm} - 0.79\, A_{663}\text{nm} - 0.27\, A_{490}\text{nm}) \times V$$

$$\text{PE}\ (\mu\text{g/mg}) = \{A_{562}\text{nm} - (2.41 - \text{PC}) - (0.849 - \text{APC})\}/9.62$$

$$\text{PC}\ (\mu\text{g/mg}) = (A_{615}\text{nm} - 0.474 \times A_{652}\text{nm})/5.34$$

$$\text{APC}\ (\mu\text{g/mg}) = (A_{652}\text{nm} - 0.208 \times A_{615}\text{nm})/5.09$$

2.4. Chlorophyll fluorescence measurement

To assess the effects of K_2HPO_4 on photosystem II (PSII) and the redox potential of the electron transport chain involved in photosynthesis, ChlF parameters were studied using pulse-amplitude modulation (PAM-2500, Walz, Germany) in vivo. ChlF, a non-destructive indicator of photosynthetic activity, was utilized along with various photosynthetic metrics to evaluate the health of dehydrated cells. Fluorescence levels of dehydrated cyanobacterial mats were recorded using the data collection program PamWin-3. This software calculates several parameters including the minimum fluorescence intensity of dark-adapted mats (Fo), maximum fluorescence intensity of dark-adapted mats (Fm), maximum electron transport rate (ETRmax), maximum photosynthetic quantum yield of PSII (Fv/Fm), effective photochemical quantum yield (Y (II)), quantum yield of non-regulated energy dissipation (Y(NO)), quantum yield of regulated energy dissipation (Y(NPQ)), and non-photochemical fluorescence quenching (NPQ). To prevent energy-dependent quenching, the dry up cyanobacterial mats were placed in the dark for 30 minutes prior to observation. Additionally, quantum yield measurements were analyzed by progressively increasing the intensity of actinic light (AC) from 3 to 1469 µmol photons $m^{-2}s^{-1}$.

2.5. Estimation of exopolysaccharides (EPS)

The estimation of exopolysaccharides (EPS) in *Tolypothrix* sp. KJE1 was performed using a colorimetric assay (Angelis et al., 2012). Cultures of cyanobacteria were grown under suitable conditions, and the cells were harvested during the stationary growth phase. EPS extraction was done using an appropriate method, such as sonication. The extracted EPS was then quantified using a carbohydrate-specific assay, such as the phenol-sulfuric acid oranthrone-sulfuric acid method. A standard curve prepared with known concentrations of a reference glucose was used to calculate the EPS content in the cyanobacterial samples.

2.6. Carbohydrate quantification

The total carbohydrate content was determined by the use of anthrone method. In summary, 10 mg of cyanobacterial mats from each treatment were combined with 1 mL of 1N NaOH and heated in a boiling water bath for 25 minutes. The resulting mixture was grounded with a mortar and pestle to produce a crude homogenate, which was subsequently centrifuged at 3158 g for 10 minutes. Subsequently, 100 µL from every supernatant was utilized to evaluate the total carbohydrate content. The anthrone reagent was formulated by dissolving 400 mg of anthrone in 200 mL of chilled 95% H_2SO_4. Then, 1 mL of the sample (consisting of 100 µL of cyanobacterial supernatant and 900 µL of double-distilled water) was mixed with 4 mL of freshly prepared anthrone reagent and allowed to incubate at room temperature for 15 minutes. The reaction mixture was then placed in a preheated water bath for 15 minutes, followed by rapid cooling on ice for 5 minutes to stop the reaction. The absorbance of every sample was recorded at a wavelength of 625 nm. A glucose-based standard curve was generated and used to determine the carbohydrate content in the mats.

2.7. Protein quantification

The total protein content of the algal mats was determined using the traditional colorimetric method following the standard protocol. At first, 10 mg of cyanobacterial mats were given treatment of 1 ml of reagent-A (0.1 N NaOH) and were then placed in a water bath for 30 minutes. Further, the mixture was setup with 2 ml of reagent-B (2M Na_2CO_3 and 0.5 M $CuSO_4.5H_2O$ in 1M sodium potassium tartrate) at room temperature for 30 minutes. Afterward, an additional 0.5 ml of 1N Folin-Ciocalteu reagent (FCR) was added and allowed to incubate at room temperature for 20 minutes. Evolution of a blue color established the presence of proteins, and their concentration was determined by measuring the optical density (O.D.) at 650 nm. To establish a standard curve for protein estimation, bovine serum albumin (BSA) was utilized.

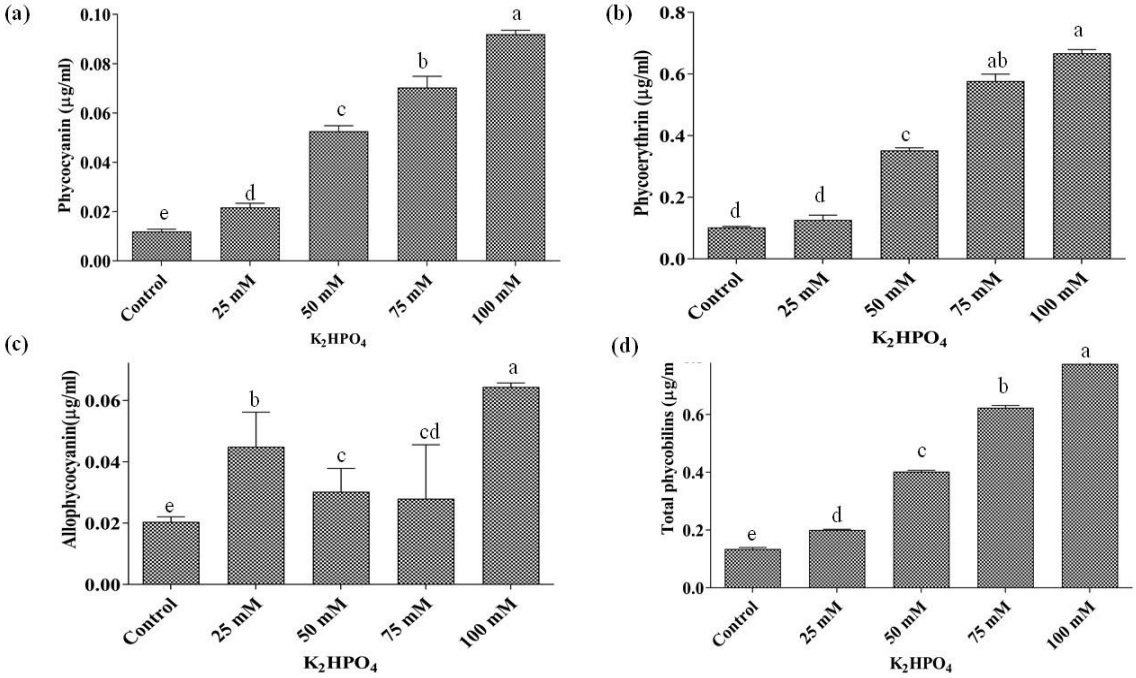

Figure 3. Effect of different concentration of K_2HPO_4 on Phycobiliproteins

2.8. Lipid extraction and quantification

The total lipid content of cyanobacterial mats was extracted using an adapted version of the Bligh and Dyer method. For the extraction of total lipids from dehydrated samples, a mixture of water, methanol, and chloroform in a ratio of 2:1:2 was employed. The cyanobacterial mats were combined with the aforementioned solution and subjected to vortexing for 10 minutes. Subsequently, the mixture was sonicated for 5 minutes with a pulse of 30 seconds on and 30 seconds off with a frequency of 20 kHz. The entire slurry was then centrifuged at 12633 g for 15 minutes, and the bottom organic phase was gently transferred into a tube that had been pre-weighed. The organic phase was removed by evaporating at 40°C using a SpeedVac vacuum concentrator. The overall lipid content in the mats was determined by using a suitable formula.

$$\% \text{ Lipid content} = \text{lipid weight (mg)} \times \frac{100}{\text{dried mat weight (mg)}}$$

2.9. Statistical analysis

Statistical analysis of data obtained was performed using appropriate SPSS (IBM Inc.) statistical software, and significant differences between groups or treatments were determined by performing analysis of variance (ANOVA) and Tukey's test. The significance level (α) was set at 0.05, and p-values were reported to indicate the significance level.

3. Result

3.1. Morphological variation and biomass productivity

As the concentration of K_2HPO_4 increased from 25 mM to 100 mM, distinct changes in mat morphology were observed (Figure 1a). These changes included variations in mat thickness, filament arrangement, and overall mat structure. The highest morphological variation was observed in the 100 mM treatment group, indicating a correlation between phosphate availability and the morphological characteristics of *Tolypothrix* sp. KJE1 mats (Figure 1a). Also, significant biomass productivity was observed in *Tolypothrix* sp. KJE1 with increasing levels of K_2HPO_4 treatment (Figure 1b). As the concentration of K_2HPO_4 increased from 25 mM to 100 mM, biomass productivity exhibited a progressive and dose-dependent increase (2.0-3.0-fold). The 100 mM treatment group observed the highest biomass productivity, indicating a positive correlation between phosphate availability and biomass production in *Tolypothrix* sp. KJE1 (Figure 1b)

3.2. Accumulation of Chl-a, carotenoids and scytonemin

The results showed a significant increase in the chlorophyll-a, carotenoid, and scytonemin contents in *Tolypothrix* sp. KJE1 with increasing levels of K_2HPO_4 treatment (Figure 2). In the control group, the chlorophyll-a content was relatively low compared to the treated groups. However, as the K_2HPO_4 concentration increased, a gradual increase in chlorophyll-a content was observed. The low level of K_2HPO_4 (25mM and 50 mM) showed a moderate increase (0.5 to 1.5-fold), while K_2HPO_4 (75mM and 100 mM) exhibited even higher levels of chlorophyll-a (2.5-3.2-fold). This suggests that K_2HPO_4 treatment positively influences chlorophyll-a synthesis in *Tolypothrix* sp. KJE1 (Figure 2a). Similarly, the carotenoid content showed a similar pattern of increase with increasing K_2HPO_4 treatment. The control group displayed a relatively low carotenoid content. In contrast, the treated groups exhibited a significant enhancement in carotenoid accumulation (Figure 2b). The K_2HPO_4 (75mM and 100 mM) showed higher carotenoid levels (2.5-3.2-fold) compared to the K_2HPO_4 (25mM and 50 mM), indicating a dose-dependent effect of K_2HPO_4 on carotenoid synthesis in *Tolypothrix* sp. KJE1 (0.5 to 1.5-fold). Moreover, the scytonemin content also substantially increased in response to K_2HPO_4 treatment (Figure 2c). The control group had a low scytonemin content. In contrast, the treated groups displayed a significant boost in scytonemin synthesis. The K_2HPO_4 (75mM and 100 mM) exhibited the highest scytonemin content among the treated groups (2.0-2.5-fold), suggesting that higher concentrations of K_2HPO_4 stimulate scytonemin production in *Tolypothrix* sp. KJE1 (Figure 2c).

3.3. Phycobiliproteins contents

The results demonstrated a significant increase in the contents of phycocyanin, phycoerythrin, and allophycocyanin in *Tolypothrix* sp. KJE1 with increasing levels of K_2HPO_4 treatment (Figure 3). As the concentration of K_2HPO_4 increased from 25 mM to 100 mM, the content of phycocyanin showed a gradual and progressive increase (Figure 3a). The highest level of phycocyanin was observed in the 100 mM treatment group (3-4-fold), indicating a dose-dependent effect of K_2HPO_4 on phycocyanin synthesis in *Tolypothrix* sp. KJE1. Similarly, phycoerythrin content exhibited a significant increase with increasing K_2HPO_4 concentrations. The

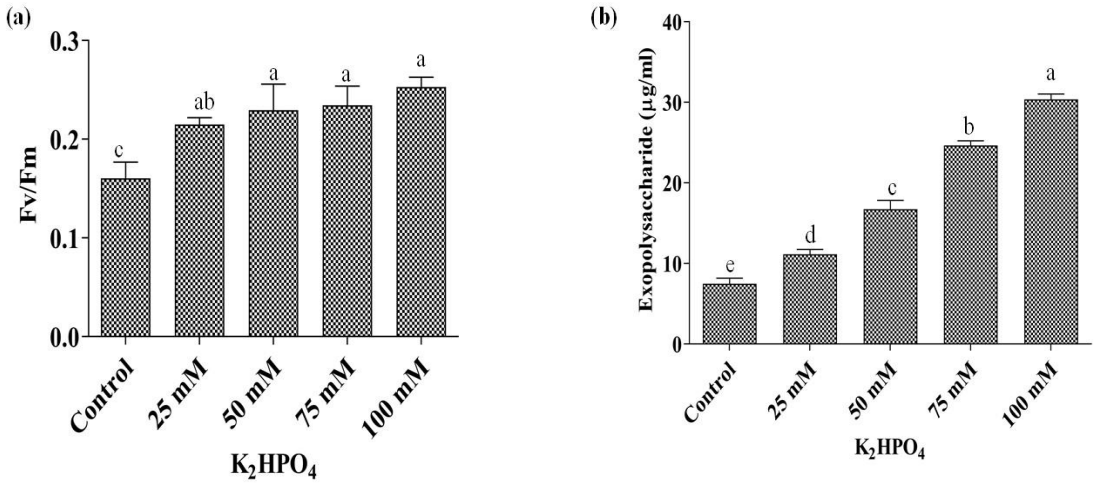

Figure 4. Effect of different concentration of K_2HPO_4 on Chlorophyll fluorescence and exopolysaccharides

25 mM treatment group showed a moderate elevation in phycoerythrin content (0.5-fold), while the 50 mM, 75 mM, and 100 mM treatment groups displayed further enhancements (up to 5-fold). These results suggest that higher concentrations of K_2HPO_4 positively influence phycoerythrin synthesis in *Tolypothrix* sp. KJE1 (Figure 3b). Furthermore, the allophycocyanin content also showed a substantial increase in response to increasing levels of K_2HPO_4 treatment (Figure 3c). The 25 mM treatment group displayed a noticeable increase (2-fold). In comparison, the 50 mM, 75 mM, and 100 mM treatment groups exhibited even lower to higher allophycocyanin contents (0.5-3.0-fold). These findings suggest that *Tolypothrix* sp. KJE1 responds to elevated concentrations of K_2HPO_4 by enhancing allophycocyanin production.

3.4. Chlorophyll fluorescence and exopolysaccharide analysis

Chlorophyll fluorescence and exopolysaccharide content in *Tolypothrix* sp. KJE1 exhibited a differential response with increasing levels of K_2HPO_4 treatment (Figure 4a and Figure 4b). As the concentration of K_2HPO_4 increased from 25 mM to 100 mM, chlorophyll fluorescence exhibited a progressive and dose-dependent increase (0.5-0.7-fold). The highest level of chlorophyll fluorescence was observed in the 100 mM treatment group, indicating enhanced photosynthetic activity in *Tolypothrix* sp. KJE1 under high phosphate conditions (Figure 4a). Similarly, exopolysaccharide content showed a significant increase in response to higher levels of K_2HPO_4 treatment (Figure 4b). The 25 mM treatment group displayed a noticeable increase (0.3-fold). In comparison, the 50 mM, 75 mM, and 100 mM treatment groups exhibited further enhancements in exopolysaccharide production (1.8-3.0-fold). These results suggest that *Tolypothrix* sp. KJE1 responds to elevated phosphate concentrations by stimulating the synthesis and secretion of exopolysaccharides.

3.5. Protein, carbohydrate, and lipid composition

The results demonstrated a significant increase in lipid content in *Tolypothrix* sp. KJE1 with increasing levels of K_2HPO_4 treatment (Figure 5a). As the concentration of K_2HPO_4 increased from 25 mM to 100 mM, the lipid content exhibited a progressive and dose-dependent increase (2.5-fold). The 100 mM treatment group observed the highest lipid content, indicating a positive correlation between phosphate availability and lipid accumulation in *Tolypothrix* sp. KJE1 (Figure 5a). Furthermore, protein and carbohydrate content in *Tolypothrix* sp. KJE1 increased with increasing levels of K_2HPO_4 treatment (Figure 5b and Figure 5c). As the concentration of K_2HPO_4 increased from 25 mM to 100 mM, both protein and carbohydrate content exhibited a progressive and dose-dependent decrease (3.7-4.8-fold). The lowest protein and carbohydrate content levels were observed in the 100 mM treatment group, indicating a negative correlation between phosphate availability and protein/carbohydrate accumulation in *Tolypothrix* sp. KJE1 (Figure 5b and Figure 5c).

4. Discussion

Phosphate is a vital nutrient that can influence the structure and growth of microbial communities. Higher concentrations of K_2HPO_4 treatment may have increased phosphate availability, affecting the nutrient balance within the mat. Phosphate availability can influence the expression of these genes, subsequently affecting the structural characteristics of the mat. The higher concentrations of K_2HPO_4 treatment may have triggered changes in gene expression patterns related to biofilm formation in *Tolypothrix* sp. KJE1, leading to variations in mat structure and morphology (Huisman et al., 2018). In addition, phosphate stress caused by increased K_2HPO_4 treatment could trigger physiological responses in *Tolypothrix* sp. KJE1, such as producing extracellular polymeric substances (EPS) or modifying EPS composition (Rossi and De Philippis, 2015). These responses can influence cell adhesion, filament growth, and mat development, resulting in morphological variations in the formed mats. In addition, the increased levels of K_2HPO_4 treatment provided in this study likely facilitated an ample supply of phosphate, which is essential for various metabolic processes, including nucleic acid synthesis, ATP production, and protein synthesis (Li et al., 2016). The availability of an optimal phosphate concentration may have relieved nutrient limitation and promoted overall cellular growth, leading to increased biomass productivity. Higher phosphate concentrations provided by K_2HPO_4 treatment may have enhanced the efficiency of photosynthesis in *Tolypothrix* sp. KJE1. This improvement in photosynthetic performance could result in higher biomass productivity due to increased carbon assimilation and energy generation (Mendez et al., 2016).

The observed increase in chlorophyll-a content in *Tolypothrix* sp. KJE1 with K_2HPO_4 treatment can be attributed to the role of phosphate as an essential nutrient for photosynthesis. Phosphate is a critical component of ATP and NADPH, which are crucial for the light-dependent reactions of photosynthesis (Qu et al., 2019). Increased phosphate availability through K_2HPO_4 treatment likely enhances the production of ATP and NADPH, facilitating higher chlorophyll-a synthesis (Rose et al., 2017). The augmentation of carotenoid content in *Tolypothrix* sp. KJE1 with K_2HPO_4 treatment can be attributed to the role of carotenoids in photoprotection and light-harvesting. Carotenoids act as accessory pigments that absorb excess light energy and protect the photosynthetic machinery from photooxidative damage (Paulo et al., 2018). The increase in carotenoid accumulation in response to K_2HPO_4 treatment suggests that the cyanobacterium is responding to the availability of nutrients by enhancing its photoprotective

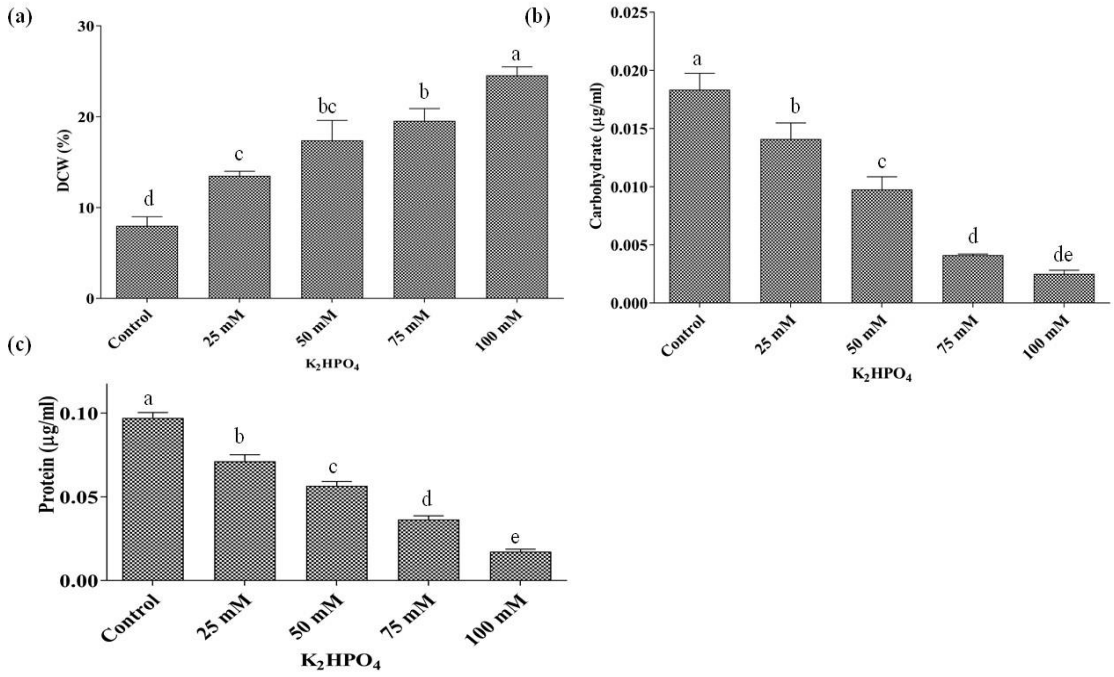

Figure 5. Effect of different concentration of K_2HPO_4 on Lipid, carbohydrate and Protein

capacity. The significant increase in scytonemin content with K_2HPO_4 treatment can be attributed to the role of scytonemin as a sunscreen pigment. Scytonemin is synthesised by cyanobacteria in response to high levels of solar radiation, particularly in the UV range. It acts as a protective shield against harmful UV radiation by absorbing and dissipating UV photons. The increased production of scytonemin in *Tolypothrix* sp. KJE1 treated with K_2HPO_4 indicates that the cyanobacterium perceives the treatment as a stressor and activates its defence mechanism to protect itself from potentially damaging UV radiation (Garlapati et al., 2019).

The observed increased phycocyanin, phycoerythrin, and allophycocyanin contents in *Tolypothrix* sp. KJE1, with rising levels of K_2HPO_4 treatment, can be attributed to the role of phosphate as a crucial nutrient in pigment synthesis and light harvesting. Phosphate availability influences the expression of genes involved in synthesizing phycobiliproteins, including phycocyanin, phycoerythrin, and allophycocyanin (Stadnichuk et al., 2015). Phycobiliproteins are water-soluble pigments that play a vital role in capturing light energy and transferring it to the photosynthetic reaction centres in cyanobacteria (Kannaujiya et al., 2019). They comprise a protein component known as the apoprotein, which binds chromophores called phycobilins in *Tolypothrix* sp. KJE1, the increase in phycocyanin, phycoerythrin, and allophycocyanin contents in response to K_2HPO_4 treatment can be attributed to several underlying factors. For instance, adequate phosphate levels can upregulate the activity of genes s associated with the biosynthesis of phycobiliproteins, leading to increased production of phycocyanin, phycoerythrin, and allophycocyanin (Lu et al., 2019). The elevated concentrations of K_2HPO_4 provided in this study likely stimulated the expression of these genes, resulting in enhanced pigment synthesis.

The observed increase in chlorophyll fluorescence and exopolysaccharide content in *Tolypothrix*s sp. KJE1 treated with increasing concentrations of K_2HPO_4 can be attributed to phosphate availability is essential for optimal photosynthetic performance in cyanobacteria (Shinde et al., 2020). Phosphorus plays a crucial role in ATP synthesis and electron transport chain components, vital for converting light energy into chemical energy during photosynthesis. The higher levels of K_2HPO_4 treatment provided in this study likely facilitated ATP synthesis and electron transport, leading to increased chlorophyll fluorescence and improved photosynthetic efficiency in *Tolypothrix* sp. KJE1 (Liu, 2016). Phosphate availability can influence carbon metabolism in cyanobacteria, including the production of exopolysaccharides. Exopolysaccharides serve multiple functions, such as cell adhesion, protection against environmental stressors, and nutrient storage (Cruz et al., 2020). The increased exopolysaccharide production in *Tolypothrix* sp. KJE1 treated with higher concentrations of K_2HPO_4 may be a response to the ample phosphate supply, allowing the cyanobacterium to allocate more carbon resources towards exopolysaccharide biosynthesis (Rossi and De Philippis, 2015). The exopolysaccharides serve as a structural component of the extracellular matrix, promoting cell aggregation and protecting against desiccation and predation.

Phosphorus is an essential component of lipids, including phospholipids and glycolipids, major cell membrane constituents. Higher phosphate availability provided by K_2HPO_4 treatment likely increased phosphorus availability for lipid biosynthesis in *Tolypothrix* sp. KJE1. Phosphorus serves as a precursor for synthesising phospholipids and other lipid molecules, increasing cell lipid content (Kumar et al., 2017). Moreover, phosphate availability can influence the regulation of lipid metabolism in microorganisms. Phosphate regulates key enzymes and metabolic pathways associated with lipid synthesis and accumulation (Wang et al., 2016). The higher concentrations of K_2HPO_4 in this study might have stimulated the activity of enzymes involved in lipid biosynthesis and storage, resulting in increased lipid content in *Tolypothrix* sp. KJE1 (Kumar et al., 2017). The elevated phosphate concentrations provided by K_2HPO_4 treatment may disrupt the nutrient balance in *Tolypothrix* sp. KJE1, leading to metabolic shifts. Excessive phosphate levels can alter the carbon-to-nitrogen ratio and disrupt the balance between energy production and biosynthesis (Baer et al., 2017).

As a consequence, the diversion of resources towards phosphate uptake and storage may lead to a reduced allocation of carbon and nitrogen for protein and carbohydrate synthesis, resulting in decreased protein and carbohydrate content (Mendez et al., 2016). Cyanobacteria can undergo metabolic adjustments to adapt to nutrient stress. In response to high phosphate levels, *Tolypothrix* sp. KJE1 may prioritise lipid accumulation as a storage compound to cope with the nutrient imbalance. Carbon reallocation from protein and carbohydrate synthesis to lipid biosynthesis may occur, leading to decreased cell protein and carbohydrate content (Kushwaha et al., 2018).

5. Conclusion

This study explored the effect of different levels of K_2HPO_4 treatment on various aspects of *Tolypothrix* sp. KJE1, including biomass productivity, chlorophyll fluorescence, exopolysaccharide production, lipid content, protein and carbohydrate content, phycobiliprotein content, and morphological variation in mat formation. The availability of an optimal phosphate concentration likely relieved nutrient limitation and promoted overall cellular growth, leading to increased biomass productivity. Furthermore, the increase in chlorophyll fluorescence and exopolysaccharide, biochemical composition, morphological variation and photosynthetic pigment contents suggests the role of phosphate availability in enhancing the photosynthetic performance and extracellular matrix production in *Tolypothrix* sp. KJE1. These findings highlight the importance of phosphate in driving cellular processes associated with energy production, carbon fixation, and structural stability. The findings contribute to our understanding of the metabolic and ecological adaptations of *Tolypothrix* sp. KJE1 in response to varying phosphate conditions. Further research could focus on elucidating the specific molecular mechanisms involved in *Tolypothrix* sp. KJE1 response to phosphate availability and exploring its ecological significance in natural ecosystems.

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Authors' Contribution

Jalaluddin: Methodology, Visualization, Investigation, Data curation, Validation, Writing – original draft. Rajan Kumar Gupta: Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Writing – review and editing.

Declaration of competing interest

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

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