Effects of Nitrogen and Micronutrients on *Spirulina platensis* Physiology

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ABSTRACT

Spirulina platensis, a filamentous cyanobacterium, is extensively cultivated for its nutritional value and bioactive compounds. Environmental stressors significantly affect its growth and biochemical composition. This study examines the impact of nitrogen ($NaNO_3$) and micronutrient ($MgSO_4$, $CaCl_2$) stress on biomass production, specific growth rate, and chlorophyll a content in S. platensis. Cultures were grown in modified Zarrouk's medium with fivefold increased nutrient concentrations. Growth was monitored spectrophotometrically at 565 nm (biomass), 680 nm (chlorophyll-a), and 750 nm (growth rate). Results revealed that $NaNO_3$ stress reduced growth and pigment accumulation, while $MgSO_4$ and $CaCl_2$ stress enhanced both. The highest biomass (0.8851 g/L) was recorded under $CaCl_2$ stress, and the lowest (0.2754 g/L) under $NaNO_3$ stress. These findings highlight the potential of micronutrient enrichment in optimizing Spirulina cultivation.

Keywords: Spirulina platensis, nitrogen stress, micronutrient stress, biomass production, chlorophyll-a

INTRODUCTION

Cyanobacteria, such as Spirulina platensis, are oxygenic photosynthetic bacteria that have oxygenated Earth for over 3 billion years (Rasmussen et al., 2008). These microorganisms are very common in freshwater and marine ecosystems, where they tend to bloom and colonize (Whitton & Potts, 2012). Arthrospira (Spirulina) platensis is a helical, filamentous, multicellular, blue-green microalga that grows in hot temperatures, strong sunlight, and alkaline conditions (Habib et al., 2008). Spirulina is highly valued for its rich nutritional content, including up to 70% protein on a dry weight basis, along with essential amino acids, fatty acids, minerals (e.g., iron, copper, zinc), vitamins (notably B12), antioxidant pigments (e.g., phycobiliproteins, carotenoids, and chlorophyll-a), and polysaccharides (Vonshak, 1997). Its commercial uses are expanding, including applications in human dietary supplements, animal feeds (terrestrial, freshwater, and marine), and pharmaceuticals. In aquaculture, Spirulina is typically used as a growth performance enhancer, pigmentation promoter, and probiotic agent in feed (Ghaeni et al., 2011; Ansarifard et al., 2018).

Microalgae such as Spirulina are heavily dependent on environmental conditions including light intensity, temperature, pH, and available nutrients for their growth and biochemical composition (Abd El-Baky et al., 2009). Here, among all, nutrient stress in terms of limitation or even excess can induce dramatic physiological changes in growth as well as pigment synthesis (Sukenik et al., 1991). Nitrogen is an essential macronutrient needed for protein and photosynthetic pigment synthesis. Micronutrients such as magnesium and calcium are essential as cofactors in the enzymatic reactions of photosynthesis (Behrenfeld & Falkowski, 1997). Furthermore, trace metals like iron (Fe), manganese (Mn), cobalt (Co), zinc (Zn), copper (Cu), and nickel (Ni)

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play crucial roles in a variety of metabolic processes supporting cellular function and growth (Bruland et al., 1991; Rastar et al., 2018). Specifically, iron has been found to increase cyanobacterial growth and enhance photosynthesis as well as nitrogen fixation (Rueter & Petersen, 1987).

This research seeks to determine the physiological behaviour of S. platensis under different concentrations of nitrogen ($NaNO_3$) and chosen micronutrients ($MgSO_4$ and $CaCl_2$). By establishing biomass yield, growth rate at specific time, and chlorophyll-a content under these stress levels, this research hopes to guide the strategies for the optimization of Spirulina large-scale cultivation in biotechnological and aquaculture practices (Bavatharny Thevarajah et al., 2022).

MATERIALS AND METHODS

The strain of *S. platensis* was obtained from the Centre for Conservation and Utilisation of Blue Green Algae, IARI, New Delhi

Media preparation: In the batch culture cultivation, the modified Zarrouk's medium (Soni et al., 2019) was used with three variations of the initial nitrogen concentration and micronutrient concentration ($NaNO_3$, $MgSO_4$ & $CaCl_2$): 1.25g, 0.30g and 0.060g respectively represented in Table 1.

Experimental Setup: Culture conditions and growth The alga growing apparatus consists of a horizontal glass surface on the Erlenmeyer flasks had been placed. Erlenmeyer flasks of 100 ml capacity have prepared containing S. platensis (10%) with initial optical density 0.019 (Biomass concentration of 0.002 g L -1 dry weight) and 200 ml Zarrouk media (Zarrouk, 1966) at temperature 32 ˚C, pH 8.7, salinity 20 ppt with an illumination of 2500 lux light intensity, with a light/dark cycle of 12/12 h (Nhu et al., 2014). Fresh air was pumped into the solution through plastic tubes to avoid the generation of alga film layer on the wall of the flasks for 42 days, with samples collected every three days. Each stress condition was tested in triplicate.

Table. 1 Composition of modified Zarrouk Medium

Stress	Component	Normal Conc.	Stress Conc.
1st	$NaNO_3$	0.75g	1.25g
2nd	$MgSO_4$	0.06g	0.30g
3rd	$CaCl_2$	0.012g	0.060g

Growth Parameter Analysis

Biomass Determination

Daily biomass concentration was assessed by measuring the optical density (OD) at 565 nm using a UV-Vis spectrophotometer. A standard curve correlating OD_{565} readings with dry weight (g/L) was established by preparing serial dilutions of *S. platensis* cultures. This curve facilitated the estimation of biomass concentrations in experimental samples based on their OD_{565} values.

Dry Weight Estimation

For dry weight determination, 15 mL of culture was filtered through pre-weighed Whatman GF/C filter papers (pore size 1.2 µm). Post-filtration, filters were rinsed twice with distilled water to remove residual salts. The filters were then dried at 80°C for 4 hours in a hot air oven and cooled in a desiccator before reweighing. The increase in filter weight corresponded to the dry biomass, expressed in g/L.

Chlorophyll-a Content Estimation

Chlorophyll-a (Chl-a) content was determined following pigment extraction using 90% acetone. A 10 mL aliquot of culture was centrifuged at 4000 rpm for 10 minutes to pellet the cells. The pellet was resuspended in 5 mL of 90% acetone and incubated in the dark at 4°C for 24 hours to facilitate pigment extraction. Post-incubation, samples were centrifuged at 5000 rpm for 15 minutes, and the supernatant was collected.

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Absorbance readings were taken at 630 nm, 645 nm, and 665 nm against a 90% acetone blank. Chl-a concentration was calculated using the equation:

Chl-a (mg/L) = 11.6 × A_{665} − 1.31 × A_{645} − 0.14 × A_{630}

Specific Growth Rate (μ) Calculation

The specific growth rate (μ) was calculated during the exponential growth phase using the formula:

$$\mu = (\ln X_2 - \ln X_1) / (t_2 - t_1)$$

Where:

X_1 and X_2 are the biomass concentrations (g/L) at times t_1 and t_2, respectively.

RESULTS

Morphological Observations

Under all treatment conditions, Spirulina platensis retained its characteristic spiral morphology. However, visual changes indicated physiological stress in certain treatments. Cultures subjected to $NaNO_3$ stress turned yellowish-green by day 35 and became whitish by day 42, signifying progressive stress and possible pigment degradation. In contrast, cultures treated with $MgSO_4$ and $CaCl_2$ remained green and appeared healthy throughout the experimental duration.

Biomass Concentration

Biomass accumulation was significantly affected by the type of nutrient stress in Figures 1–3. $NaNO_3$ stress resulted in delayed growth and a reduced final biomass concentration of 0.2754 g/L, compared to the control (0.4423 g/L) in Table 2. In contrast, $MgSO_4$ and $CaCl_2$ stress enhanced biomass production to 0.8175 g/L and 0.8851 g/L, respectively. This suggests that excess nitrogen may inhibit biomass synthesis, possibly due to metabolic imbalance, whereas elevated magnesium and calcium concentrations promote growth by supporting essential enzymatic and structural cellular functions.

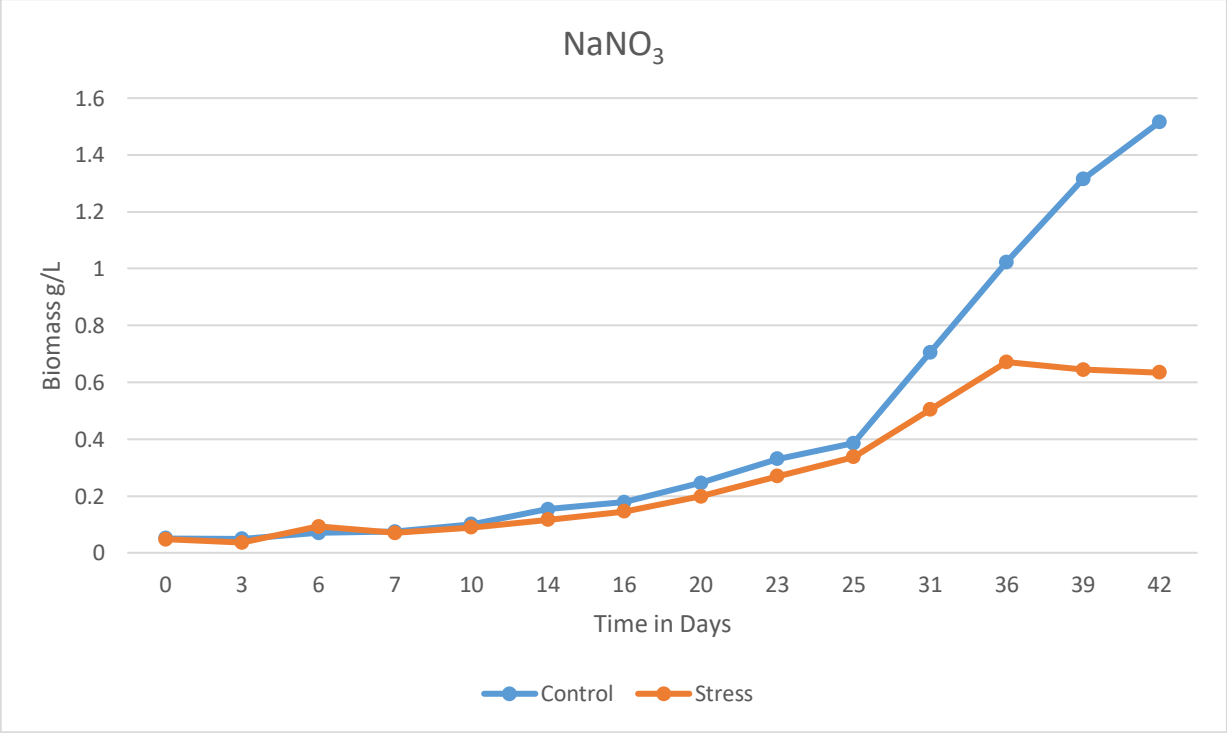

Figure 1. Effect of $NaNO_3$ on Biomass Production Over Time

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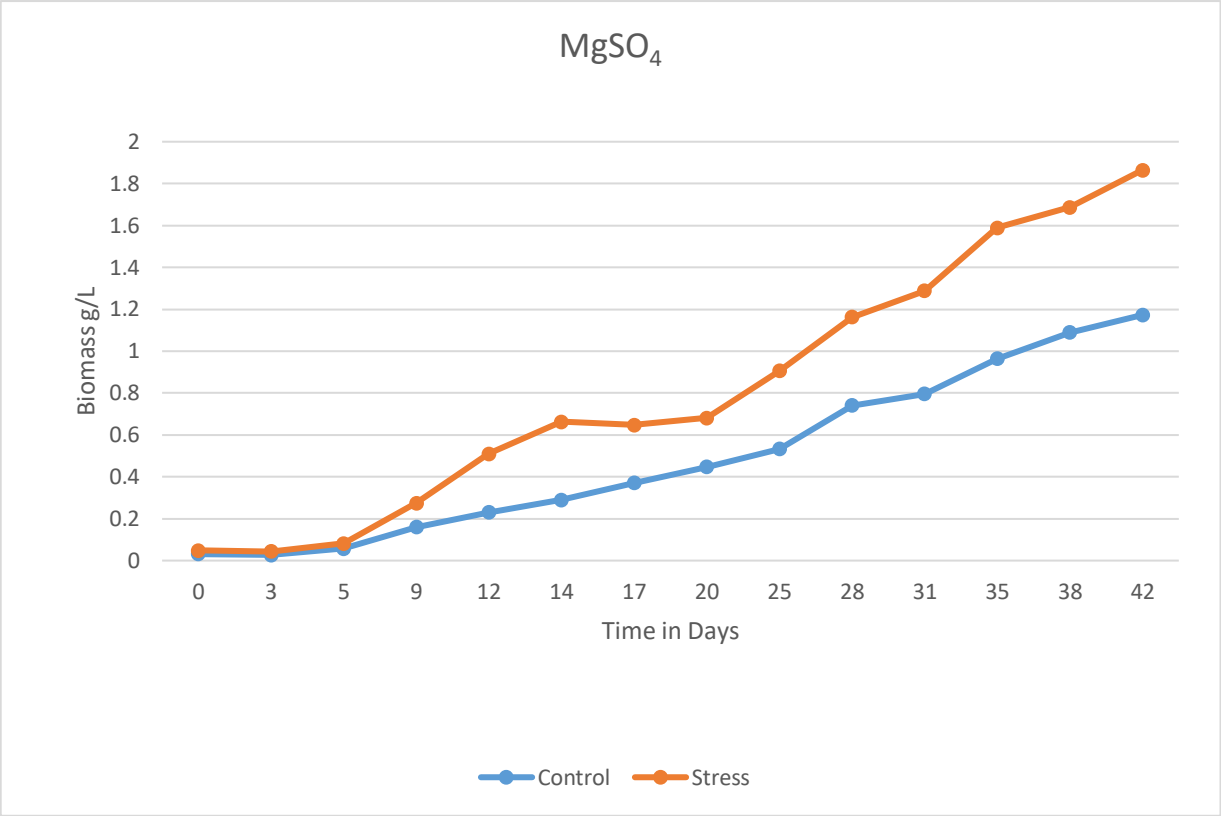

Figure 2. Effect of $MgSO_4$ on Biomass Production Over Time

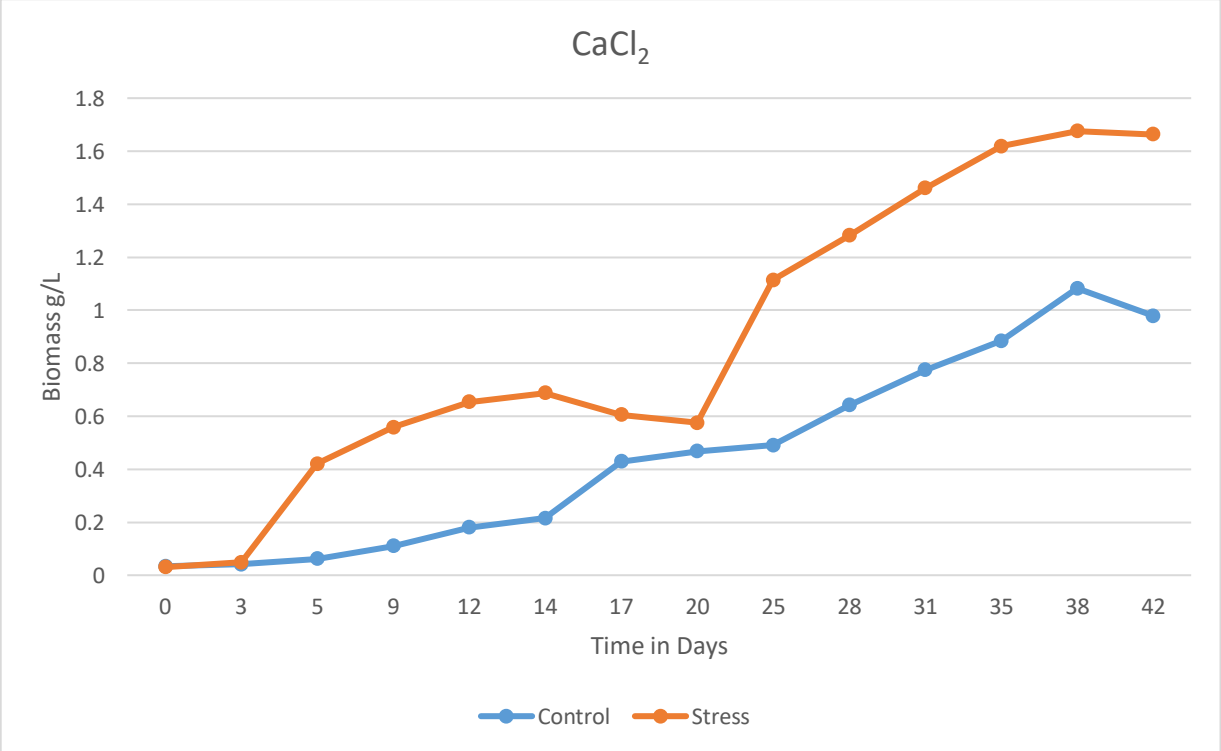

Figure 3. Effect of $CaCl_2$ on Biomass Production Over Time

Table 2. Average Biomass under control and stress conditions

Treatment	**Control (g/l)**	**Stress (g/l)**
$NaNO_3$	0.4423	0.2754
$MgSO_4$	0.4934	0.8175
$CaCl_2$	0.4563	0.8851

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Chlorophyll-a Concentration

Chlorophyll-a levels varied notably under different stress conditions (Figures 4–6). Under $NaNO_3$ stress, the pigment concentration decreased slightly to 1.0557 mg/L, while $MgSO_4$ and $CaCl_2$ treatments elevated chlorophyll-a to 1.1212 mg/L and 1.1203 mg/L, respectively (Table 3). Compared to control values (~1.077 mg/L), these findings imply that magnesium and calcium supplementation enhances pigment biosynthesis, possibly through their role as cofactors in chlorophyll and protein synthesis pathways. In contrast, nitrogen excess may suppress pigment formation due to cellular toxicity or imbalance in the nitrogen assimilation process.

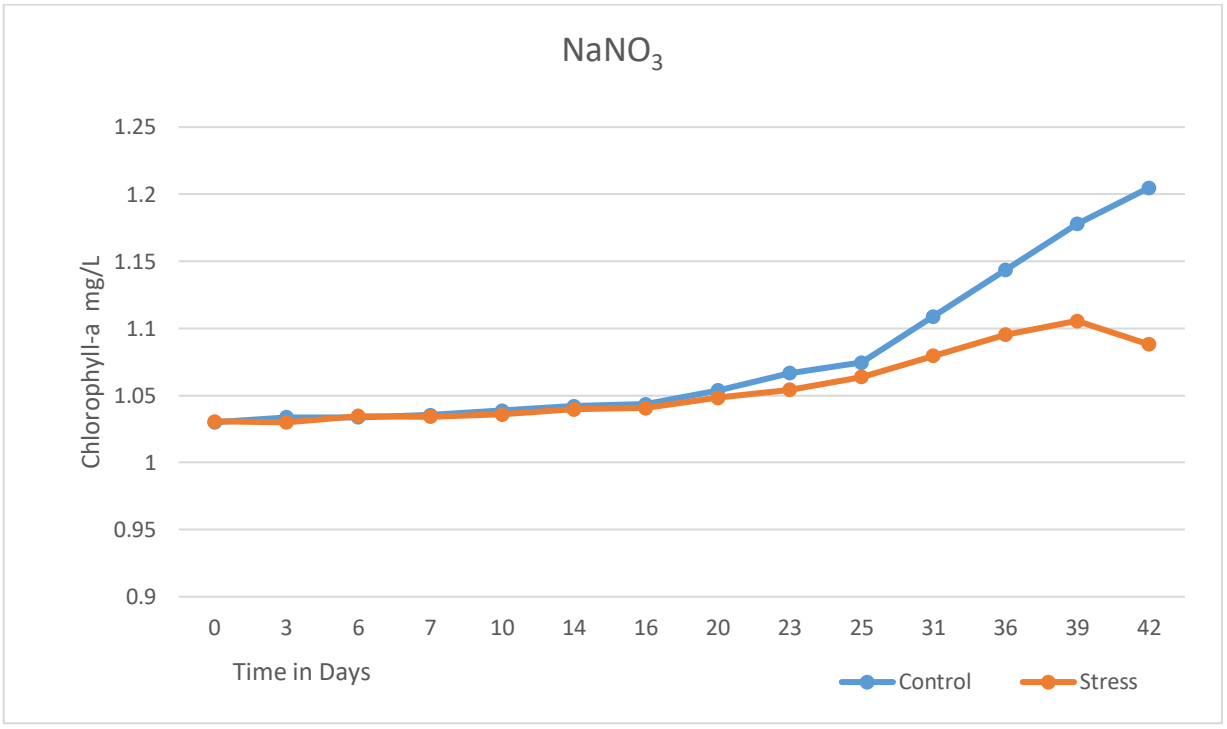

Figure 4: Effect of $NaNO_3$ Stress on Chlorophyll-a Concentration

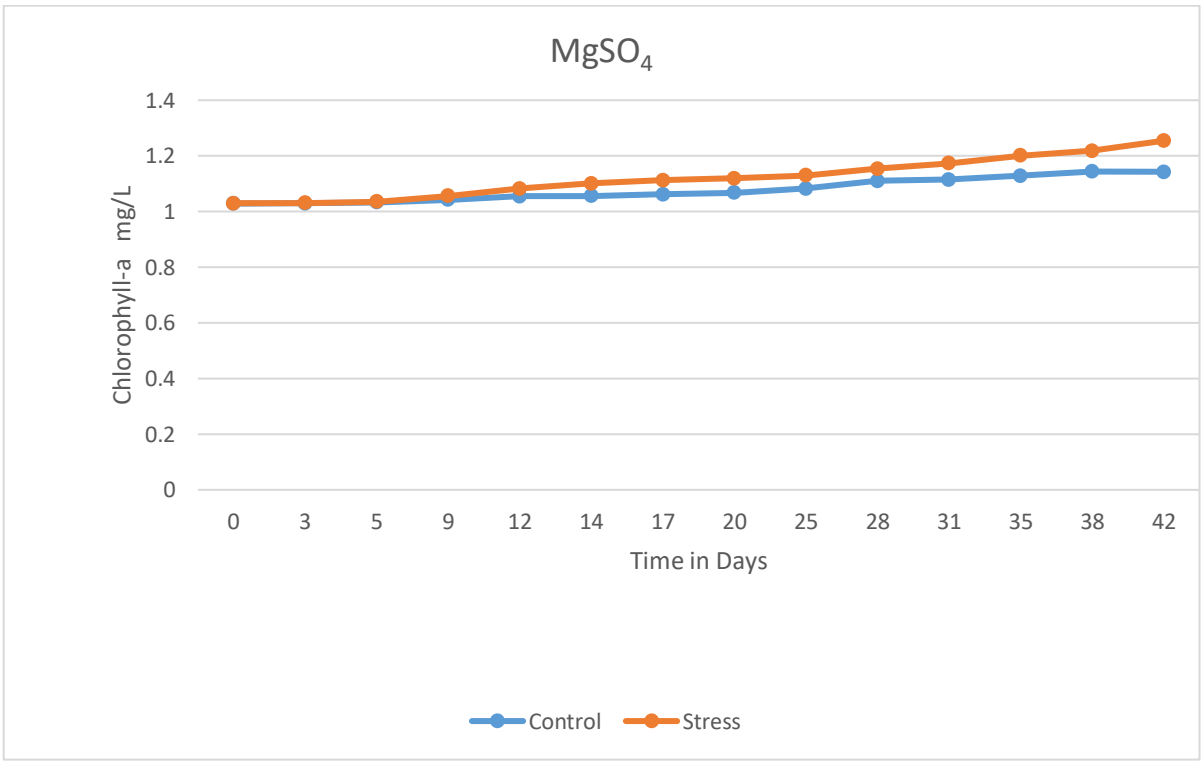

Figure 5: Effect of $MgSO_4$ Stress on Chlorophyll-a Concentration

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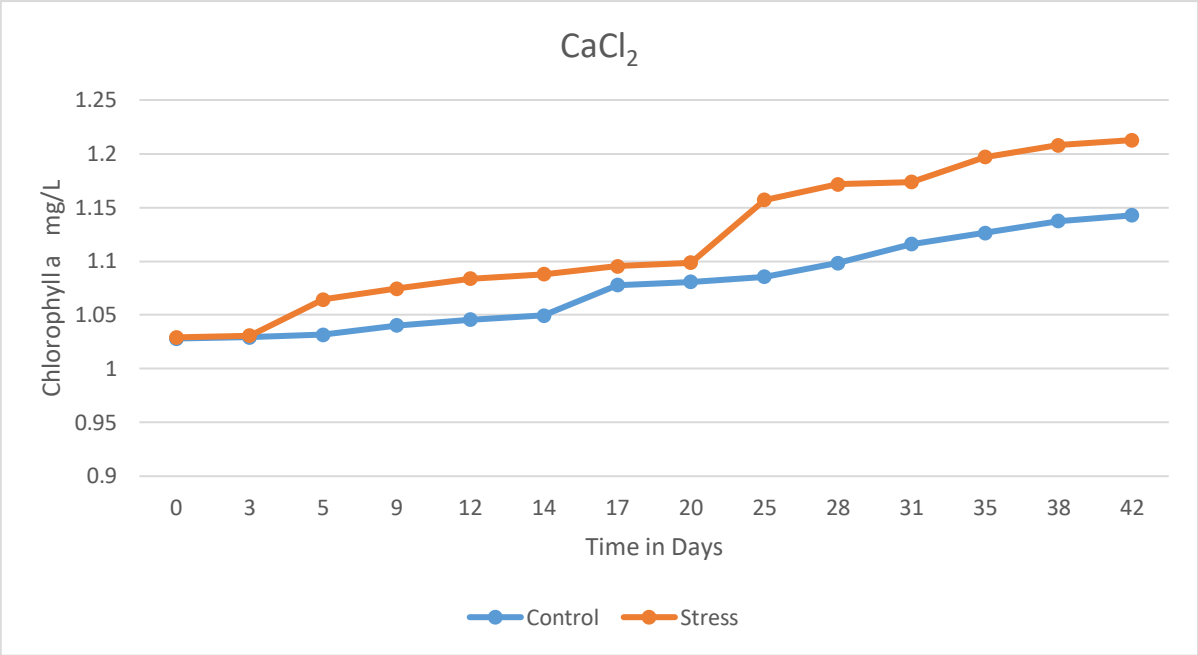

Figure 6: Effect of $NaNO_3$ Stress on Chlorophyll-a Concentration

Table 3. Average Chlorophyll-a Concentration

Treatment	Control (mg/L)	Stress (mg/L)
$NaNO_3$	1.0776	1.0557
$MgSO_4$	1.0781	1.1212
$CaCl_2$	1.0778	1.1203

Specific Growth Rate

Specific growth rate (μ) analysis further emphasized the differential effects of nutrient stress (Figures 7–9). The lowest growth rate was recorded under $NaNO_3$ stress (μ = 0.0055 day^{-1}), accompanied by an extended generation time of 107 hours. In contrast, $MgSO_4$ significantly improved growth (μ = 0.0228 day^{-1}; generation time = 44 hours), followed by $CaCl_2$ (μ = 0.0136 day^{-1}; generation time = 282 hours) (Table 4). These results confirm that magnesium and calcium not only enhance biomass but also support faster population doubling, possibly due to their involvement in photosynthesis and structural stability.

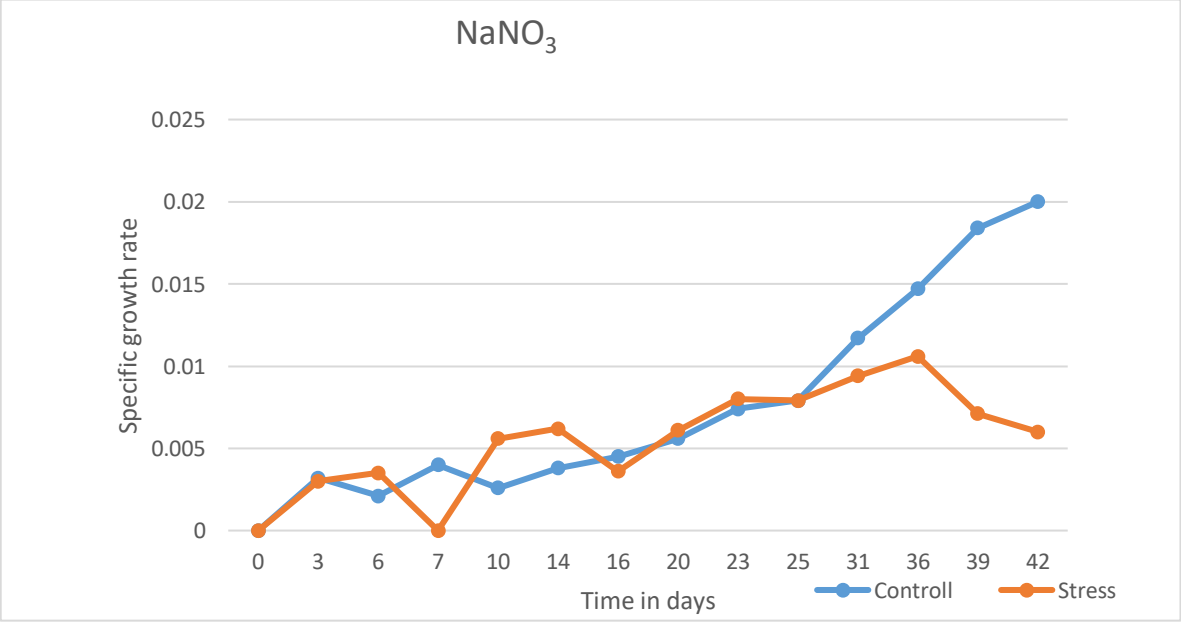

Figure 7. Effect of $NaNO_3$ Stress on Specific Growth Rate

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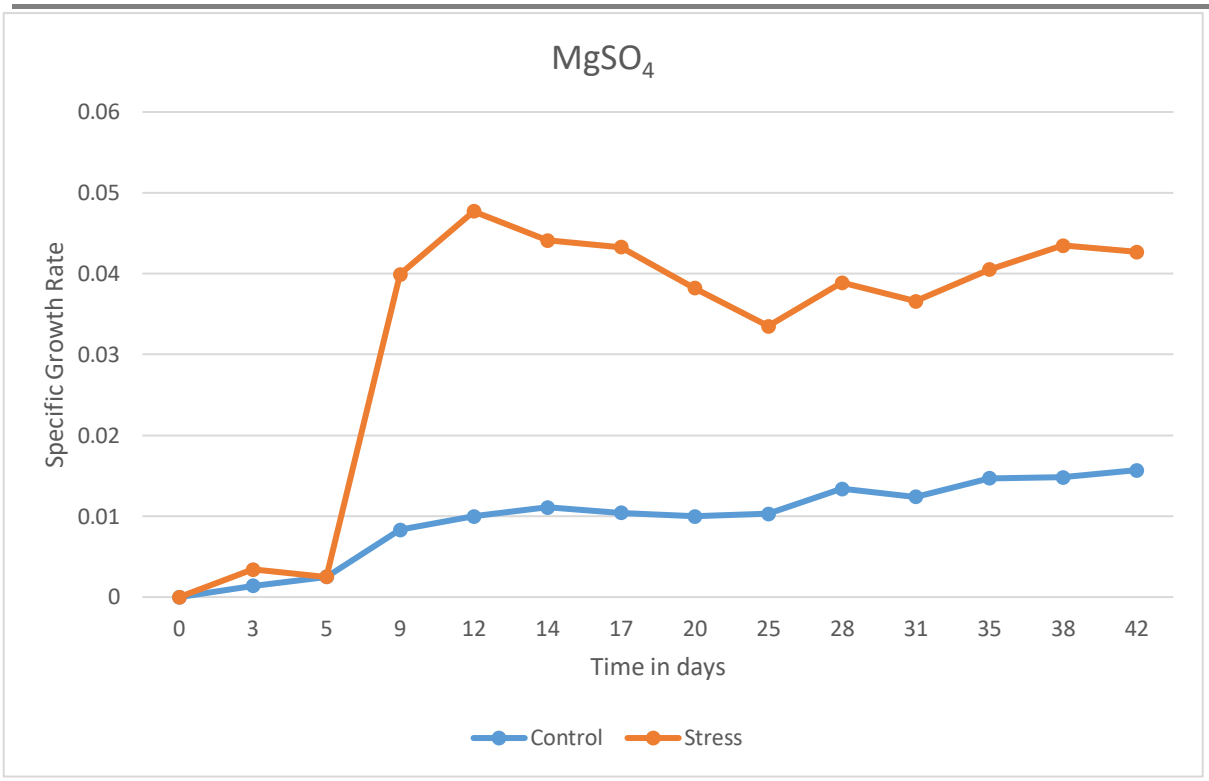

Figure 8. Effect of $MgSO_4$ Stress on Specific Growth Rate

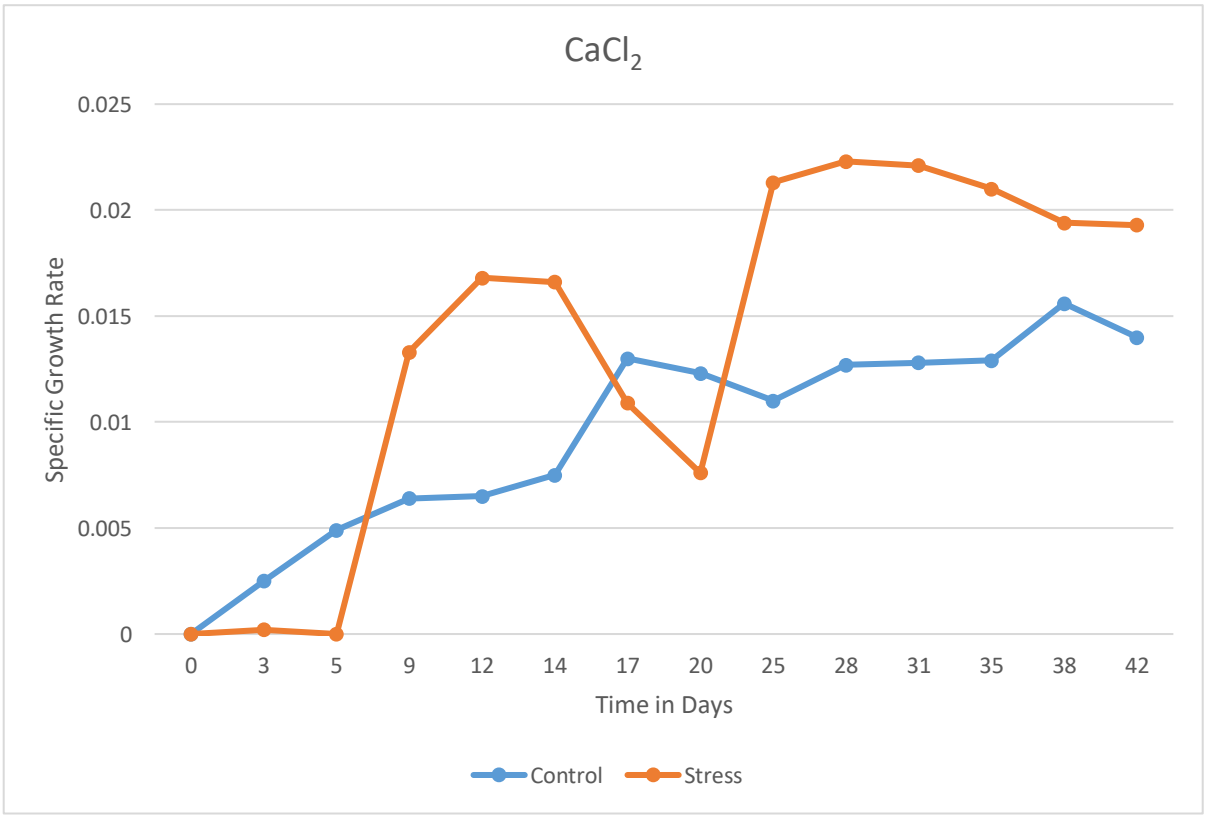

Figure 9. Effect of $CaCl_2$ Stress on Specific Growth Rate

Table 5. Growth Parameters under Stress Conditions

Parameter	**$NaNO_3$**	**$MgSO_4$**	**$CaCl_2$**
Avg Biomass (g/L)	0.2754	0.8175	0.8851
Specific Growth Rate μ	0.0055	0.0228	0.0136
Generation Time (h^{-1})	107	44	282

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Statistical Analysis

Standard deviation analysis (Figure 10) for both biomass and chlorophyll-a measurements under control and stress conditions revealed low variability across all treatments, reflecting high reproducibility. Among the stress treatments, $MgSO_4$ and $CaCl_2$ exhibited lower standard deviation values compared to $NaNO_3$, indicating more stable growth and pigment profiles under these conditions.

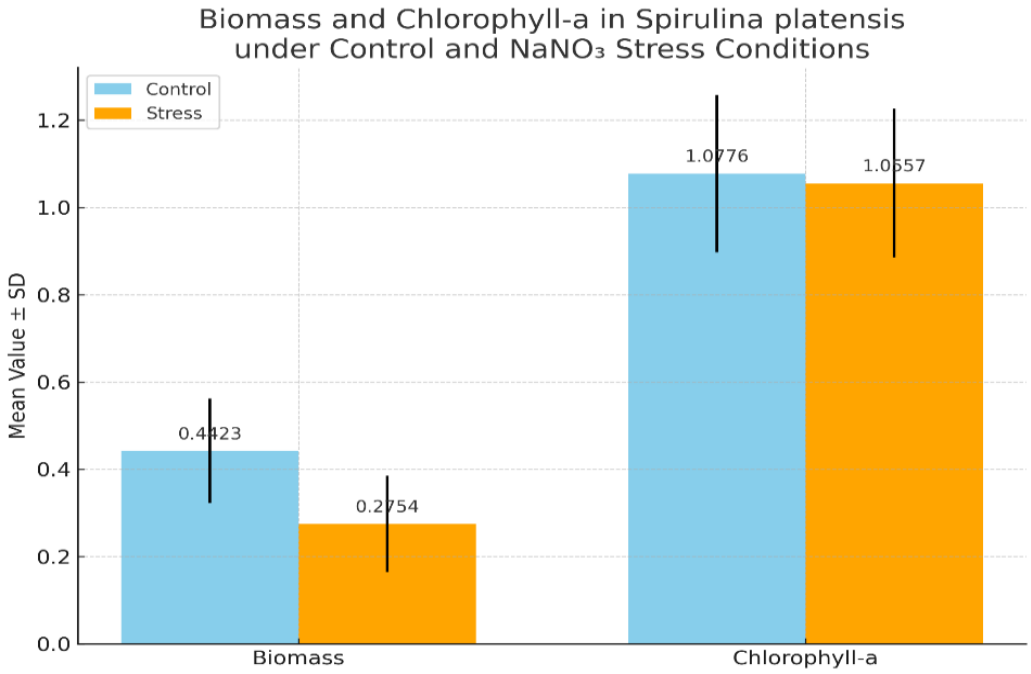

Figure 10. Standard Deviation of Biomass and Chlorophyll-a in Spirulina platensis Under Control and $NaNO_3$ Stress Conditions

DISCUSSION

Nitrogen plays a central role in the metabolism of *Spirulina platensis*, directly influencing biomass accumulation, pigment synthesis, and enzymatic activity. While earlier studies suggested that increased nitrogen enhances protein, phycocyanin, and lipid synthesis but represses carotenoid production due to a metabolic shift (Kand & Nagarajan, 2013), our study observed that an excess of $NaNO_3$ actually suppressed biomass, chlorophyll-a levels, and growth rate. This may be attributed to nitrogen toxicity, which causes metabolic imbalance, oxidative stress, and potential ammonia accumulation, negatively affecting cellular functions (Yadav et al., 2021). On the other hand, supplementation with micronutrients such as $MgSO_4$ and $CaCl_2$ significantly improved growth parameters, indicating their beneficial roles. Magnesium, as the central atom of chlorophyll and a cofactor for RuBisCO and ATPase, enhances photosynthetic efficiency and energy metabolism (Hu et al., 2020), while calcium stabilizes membrane integrity, modulates ion channels, and acts as a secondary messenger in stress signaling (Singh et al., 2022). These ions not only support pigment biosynthesis and protein function but also help mitigate oxidative damage under stressful conditions. Our findings align with recent studies that demonstrate how optimizing nutrient stoichiometry (particularly N:Mg:Ca ratios) enhances microalgal resilience and metabolic output (Li et al., 2023). Therefore, careful modulation of nutrient levels—rather than excess—offers a practical route to maximize *S. platensis* productivity for biotechnology and aquaculture applications.

Nitrogen starvation usually results in physiological responses including reduced phycocyanin production and enhanced carotenoid and exopolysaccharide production, possibly as defense mechanisms against stress (Solovchenko et al., 2008). Micronutrients like iron, magnesium, calcium, and zinc are responsible for regulating these stress reactions through the provision of enzymatic functions and biosynthesis of pigments. For instance, iron is required for chlorophyll synthesis and redox processes in photosynthesis; its deprivation can lead to serious growth inhibition (Panyakampol et al., 2016).

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In our work, elevated levels of $NaNO_3$ in Zarrouk's medium resulted in repressed growth with a maximal biomass concentration as low as 0.2754 g/L. In both $MgSO_4$ and $CaCl_2$ treatments, significantly enhanced biomass concentrations were obtained, viz., 0.8175 g/L and 0.8851 g/L respectively. These findings attest to the degradative influences of excessive nitrogen and the enhancing growth advantage from magnesium and calcium supplementation. Interestingly, there were no morphological alterations among Spirulina filaments throughout the treatments, showing that physiological rather than structural parameters were affected mainly by stress conditions.

Chlorophyll-a content followed the same trends as biomass data. In the $NaNO_3$ stress condition, chlorophyll-a decreased slightly to 1.0557 mg/L, while $MgSO_4$ and $CaCl_2$ treatments increased pigment levels to 1.1212 mg/L and 1.1203 mg/L, respectively—about double the initial values.

Specific growth rate (μ) analysis also corroborated these findings. $NaNO_3$-stressed cultures had the lowest growth rate (0.0055 day^{-1}), whereas $MgSO_4$-stressed cultures had the highest (0.0228 day^{-1}). $CaCl_2$ also stimulated growth (0.0136 day^{-1}), albeit less so. Generation time s(doubling time) was shortest in $MgSO_4$ stress (44 hours) and longest in $CaCl_2$ stress (282 hours), indicating variations in metabolic efficiency.

In general, the data show that controlled enrichment with micronutrients, as opposed to nitrogen increase, is a superior method for maximizing Spirulina growth and pigment yield. These results indicate that accurate management of nutrients can have a major impact on increasing the productivity of Spirulina culture systems under normal conditions (33 °C, 1900 cd·sr/m^2 light intensity, 18:6 light/dark cycle, and pH 8.7).

Statistically, the variability between treatments was moderate, confirming the stability of the experimental design. Yet, future research needs to try to minimize control condition variability further and investigate molecular mechanisms behind stress responses in Spirulina in order to tailor cultivation strategies even more precisely.

Limitations and future study: While this study provides valuable insights into the individual effects of nitrogen and micronutrient stress, it is limited by its laboratory-scale design and single-stressor focus. Natural environments present more complex conditions involving multifactorial stresses such as light fluctuations, temperature shifts, and nutrient interactions. Future research should explore combined stressors, particularly interactions between macronutrients and micronutrients, and validate findings in semi-field or outdoor cultivation systems. Molecular-level investigations into stress-responsive gene expression could also yield a deeper understanding of *S. platensis* adaptability.

CONCLUSION

This work proves that micronutrient supplementation $MgSO_4$ and $CaCl_2$ increases S. platensis biomass and chlorophyll-a content, whereas a surplus of nitrogen ($NaNO_3$) inhibits growth. This information is important for maximizing large-scale cultivation practices in aquaculture and biotechnology. Spirulina platensis stress physiology is highly dependent on the availability of nitrogen and micronutrient levels with important consequences for growth, biochemical structure, and pigment accumulation. Nitrogen stress regulates protein, phycocyanin, and carotenoid synthesis, whereas micronutrient stress affects enzyme function, fatty acid composition, and carbohydrate production. Optimizing growth conditions requires knowledge of these responses to augment the production of valuable compounds like phycocyanin and carotenoids. Future research must investigate combined effects of multiple micronutrients and light regimens to maximize Spirulina productivity.

Summary table:

Treatment	Avg. Biomass (g/L)	Chlorophyll-a (mg/L)	Growth Rate μ (day^{-1})	Generation Time (h)
Control	~0.45	~1.08	—	—
$NaNO_3$	0.2754	1.0557	0.0055	107
$MgSO_4$	0.8175	1.1212	0.0228	44
$CaCl_2$	0.8851	1.1203	0.0136	282

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