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Seed Vigor Improvement of Red Chili Seeds (*Capsicum annuum* L.) Using *Spirulina platensis* as Seed Priming Agent

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Abstract. *Red chili is a crucial horticultural crop in Indonesia. The increasing demand for high-quality chili necessitates seeds with optimal germination and growth. Spirulina platensis, a microalgae species, can be cultivated in waste media such as Sugar Mill Effluent (SME). Moreover, Spirulina contains phytohormones that can stimulate seed germination. This study investigated the impact of various concentrations and soaking times of S. platensis solution on red chili seed quality. A factorial Completely Randomized Design (CRD) was employed with 5 levels of microalgae biomass concentration: control (untreated seeds), 0, 30, 45, and 60% and 3 levels of soaking time: 1, 2, and 3 hours. Data analysis involved ANOVA and Bonferroni post-hoc tests at a 5% level using GraphPad Prism version 5.01. The results demonstrated seed priming application using S. platensis significantly enhanced seed vigor. The S. platensis treatment at 60% concentration for 2 hours significantly reduced MGT by 1 day, while 30%-2 hours and 60%-3 hours treatments exhibited the highest germination rate (100%), vigor index (49.17% in 30%-2 hours), seed growth simultaneity (85.56% in 60%-2 hours and 83.33% in 30%-2 hours), and growth rate (11.48% in 60%-3 hours and 11.11% in 30%-2 hours), with the 60%-3 hours treatment showing the longest shoot (2.73 cm) and radicle (8.13 cm) lengths, compared to the control. These findings suggest that S. platensis priming can be a promising approach for improving seed quality and crop establishment.*

Keywords: *biostimulant, chili, seed priming, Spirulina platensis.*

1. Introduction

Red chili (*Capsicum annuum* L.) belongs to the *Solanaceae* family and is a horticultural commodity that is in high demand and has significant economic value in Indonesia (Wijaya *et al*., 2020). According to data from the Central Statistics Agency (BPS), consumption of red chili continues to increase from year to year, namely 596.14 (2021), 636.56 (2022), and 675 (2023) thousand tons, respectively. This increase in consumption has triggered an increase in the need for red chili production. However, the high demand has not been balanced by the availability of high-quality seeds (Sekretariat Jenderal Kementerian Pertanian, 2022). In fact, high-quality seeds are

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important for producing strong, disease-resistant plants that have high productivity (Taghfir *et al*., 2018; Nugrahapsari *et al*., 2021).

The demand for high-quality chili seeds was unfortunately followed by the fact that chili seeds frequently encounter slow and uneven germination (Yadav *et al*., 2011; Barchenger & Bosland, 2016). Therefore, germination enhancement technology such as biostimulants for seed priming technology, is highly demanded. According to Rocha *et al*. (2019), seed priming can maximize germination, increase germination index, seedling strength, shoot length, and radicle length. Seed priming treatment with *Chlorella vulgaris* supernatant at concentrations of 10%, 50%, and 100% has also been shown to shorten germination time by approximately 0.5 days in tomato and 0.25 days in barley (Alling *et al*., 2023). Another research proved that *Chlorella* sp. were capable of improving germination time (3.78%), shoot length (33.88%), root length (60.31%), fresh weight (7.97%), and dry weight (5.79%) in various crops such as radish, spinach, and turnip (Rehmat *et al*., 2021). Similar to *Chlorella* sp., another potential microalgae species for seed priming was *S. platensis* (Parmar *et al*., 2023).

S. platensis, a potential microalgae biostimulant, contains various phytohormones that promote plant germination and growth. *S. platensis* cells are a rich source of nutrients, including carbohydrates, proteins, vitamins, and phenolic compounds. *S. platensis* exceeds the other microalgae species in their amino acid, potassium, and vitamin content (Parmar *et al*., 2023). Moreover, *S. platensis* showed a rapid growth rate, no toxic activity to the environment, and a wide adaptability to various wastewater-based growing mediums (Zapata *et al*., 2021; Deshmane *et al*., 2016), such as the ones used in the present study. They also contain phytohormones like auxins, cytokinins, and gibberellins, as well as beneficial compounds with antibacterial and antifungal properties (Parmar *et al*., 2023; Osman *et al*., 2016). Moreover, *S. platensis* also enhances plant metabolism, seed vigor, and abiotic stress tolerance (Gorka *et al*., 2018; Colla & Rouphael, 2020). The mechanism by which *S. platensis* application affects various plant metabolism, seed vigor, and abiotic stress responses was carried by plants through different physiological and metabolic pathways (Ronga *et al*., 2019). The enhancement of plant metabolism and seed vigor was apparently performed through 3 main plant growth-promoting hormones, such as auxins, cytokinins, and gibberellins that were actively produced by microalgae cells along with various amino acids and vitamins (Parmar *et al*., 2023). This in turn triggers several metabolic responses, including respiration, nutrient uptake, and photosynthesis (Sheheed *et al*., 2022). Meanwhile, under the stress condition, other stress-responsive hormones were produced by microalgal cells, including abscisic acid and ethylene that trigger sets of protective physiological mechanisms (Ferreira *et al*., 2023). Therefore, *S. platensis* biomass has been successfully applied to prime seeds of various crops, including tomatoes, cucumbers, lettuce, wheat, and barley. From those studies, it was observed that tomato has boosted its shoot and root length growth up to 46% higher than the control upon the treatment with *S. platensis*, while in wheat seeds, the increase of germination up to 13% higher than the control was observed in wheat seeds. These facts demonstrate its promising role in promoting seed germination and plant growth rate (Supraja *et al*., 2020; Akgul, 2019).

Based on its potential application in seed priming, this study aims to observe the effect of varying concentrations of *S. platensis* biomass and soaking time on red chili seeds of the Jukan F1 variety. This study has great potential to increase the productivity of red chili farming in the future. By optimizing the seed priming technique using *S. platensis* biomass, we aim for having the chili seeds with improved seed vigor, faster germination, and more uniform plant growth. This has implications for increasing crop yields, fruit quality, and efficient use of seed resources. Thus, this study can contribute to meeting the increasing need for food, protecting environmental sustainability, supporting agricultural sustainability, and maintaining food security. Moreover, *S. platensis* microalgae cultured using sugar mill effluent (SME) can contribute to the development of innovative solutions in waste processing methods.

2. Materials and Methods

2.1. Place and Time of Research

This research was conducted from May to August 2024 at the Integrated Bioscience Laboratory and Seed Technology Laboratory of Jember State Polytechnic.

2.2. Materials

The equipment used in this study was a simple photobioreactor consisting of c.a 0.5% sodium hypochlorite-disinfected plastic bottle, laminar air flow cabinet (LAFC), centrifuge, falcon tube, nursery rack, plastic box, sprayer, shaker, electric timer, tweezers, hygrometer, thermometer, and camera. The materials used were chili seeds of the JUKAN F1 variety (CV. Nasienie Indonesia, Jember, Indonesia) with an expiration date of December 2025. *S. platensis* isolate obtained from BBPBAP Jepara (Jepara, Central Java, Indonesia) Sugar Mill Effluent (SME), the medium used in this study was a Bold Basal Medium (BBM) with modifications comprised of $NaNO_3$ 0,25; $CaCl_2$ $2H_2O$ 0,025; $MgSO_4$ $7H_2O$ 0,075; K_2HPO_4 0,075; KH_2PO_4 0,175; NaCl 0,025; Stok EDTA (EDTA-Na2 0,0025 & KOH 0,00155); Stock Iron ($FeSO_4.7H_2O$ 0,00025; H_2SO_4 0,0005); Boron Stock H_3BO 0,00055; Bold Trace Stock (H_2SO_4 0,0005; $ZnSO_4.7H_2O$ 0,000441; $MnCl.4H_2O$ 0,000072; $Na_2MoO_4.2H_2O$ 0,0000355; $CuSO_4.5H_2O$ 0,0000785; $Co(NO_3)_2.6H_2O$ 0,0000245) g/L (UTEX, 2019). Germination paper and aquadest were used for maintaining seedling growth.

2.3. Method

This study used a 2-factor completely randomized design. The first factor was the concentration of *S. platensis* (v/v), which consists of four treatments: control, 0%, 30%, 45%, and 60%. The control had no soaking treatments being added, while 0% was water. The concentration series of 30%, 45%, and 60% was water-dissolved *S. platensis* biomass on the basis of volume-to-volume ratio (v/v). The second factor was the duration of soaking, which consisted of three treatments: 1, 2, and 3 hours. The total combination of treatments was 15 with 4 replications, resulting in 60 treatment combination units with 30 seeds per unit. Data were analyzed using analysis of variance and continued with the Bonferroni post-hoc test at the 5% level if there was a significant difference. The analysis was carried out using GraphPad Prism 5.01 software.

This study began with the production of *S. platensis* biomass by culturing microalgae in BBM media added with sugar mill effluent (SME) at a concentration of 25% (v/v) with a total culture volume of 400 ml/bottle. The culture was then placed on a culture rack equipped with lighting (190 lux) and aeration for 24 hours at room temperature 25ºC. For efficient harvesting, microalgae biomass at the late logarithmic phase (around 500.000 cells/mL) was separated by centrifugation at 8000 rpm for 10 minutes at 10°C.

The centrifuged *S. platensis* pellets were then used in the seed priming treatment. The *S. platensis* pellets were then added with 100 ml of distilled water to obtain a concentration of 100%. Then the biomass solution was made by dissolving the *S. platensis* pellets in distilled water according to the concentration level (v/v), namely control (untreated), 0%, 30%, 45%, and 60%. The chili seeds were then shaken at a speed of 100 rpm according to the duration of soaking time to ensure the seeds were evenly soaked in the *S. platensis* solution. Chili seeds that had been given seed priming treatment were then arranged in a germination box as many as 30 seeds per replication. The seeds were placed in a germination box using the top of paper method, which was lined with moistened germination paper and then stored in an incubator with humid conditions (70% RH) and controlled temperature 26ºC for 7-14 days with a light: dark lighting ratio of 16:8 hours. Observations were made every day for 14 days. The average light intensity on the germination rack was measured at 2,160 lux using a luxmeter.

The observation parameters in the analysis of seed priming test results followed ISTA (2020) rules:

a. Mean Germination Time (days)

The calculation formula was as follows (1):

$$Mean\ Germination\ Time\ (days) = \frac{\Sigma(n \times t)}{N} \quad (1)$$

where, n = Number of seeds germinated on each day; t = Number of days from the beginning of the test; N = Total number of seeds germinated at the termination of the experiment

b. Vigor Index (%)

The calculation formula was as follows (2):

$$Vigor\ Index\ (\%) = \frac{\Sigma(Normal\ Germination\ First\ count)}{\Sigma\ numbers\ of\ germinated\ seed} X\ 100\% \quad (2)$$

c. Germination Rate (%)

The calculation formula was as follows (3):

$$Germination\ Rate(\%) = \frac{\Sigma\ Normal\ Germination\ First\ count +\ Final\ count}{\Sigma\ numbers\ of\ germinated\ seed} X100\% \quad (3)$$

d. Seed Growth Simultaneity (%)

The calculation formula was as follows (4):

$$Seed\ Growth\ Simultaneity(\%) = \frac{\Sigma\ normal\ seedling\ day\ 11}{\Sigma\ number\ of\ germination\ seed} x100\% \quad (4)$$

e. Seed Growth Rate (%)

The calculation formula was as follows (5):

$$Seed\ Growth\ Rate(\%) = \frac{\frac{\Sigma normal\ germination}{etmal\ (day\ to - i)}}{\Sigma number\ of\ germination\ seed} x100\% \quad (5)$$

f. Maximum Growth Potential (%)

The calculation formula was as follows (6):

$$Maximum\ Growth\ Potential\ (\%) = \frac{\Sigma\ Normal + abnormal\ seedling}{\Sigma\ number\ of\ germination\ seed} x100\% \quad (6)$$

g. Shoot Length (cm)

Shoot length measurements were carried out on 10 normal/replicate seedling samples.

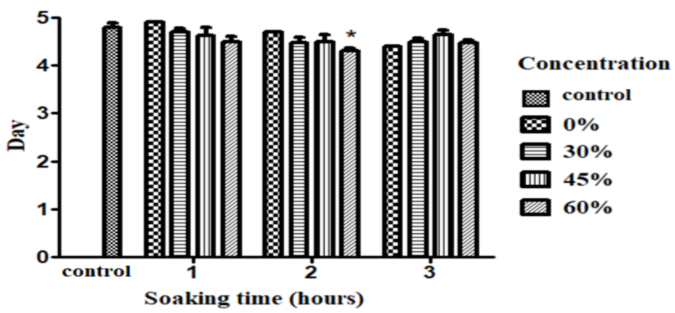
h. Radicle Length (cm)

Radicle length measurements were carried out on 10 normal/replicated seedling samples.

3. Results and Discussion

3.1 Mean Germination Time (days)

Mean Germination Time (MGT) is a parameter in seed vigor testing that is used to indicate the average time of seed germination from the beginning of the emergence of the radicle (Noeryanti *et al*., 2022). Seeds are considered to have germinated once a radicle with a length of at least 2 mm has emerged (ISTA, 2021). Based on Figure 1, soaking chili seeds with *S. platensis* for 2 hours at a concentration of 60% significantly accelerated the germination time by an average of almost 0.5 days faster (4.29 days) than the control (4.78 days). Short germination time indicates that the seeds have high germination potential. This phenomenon can be attributed to the presence of growth hormones, including auxins, cytokinins, and gibberellins in *S. platensis.* The presence of these hormones may promote stimulation for cell division (Wang *et al*., 2022). Although other treatments showed no significant effect, seeds immersion at concentrations of 30%, 45%, and 60% with a soaking time of 1-3 hours enabled a faster germination (4.29-4.72 days) than those of water (4.41-4.90 days) and control (4.78 days). In the current study, we observed that within 1 and 2 hours of immersion time, there was a tendency that the increasing concentration of seed priming from 0% to 60% caused a slightly faster germination, although the significance was only found in 60% during 2 hours of soaking chili seeds. This illustrates that, at least within 1 and 2 hours constraints of time, there was a positive correlation between concentration and reduction of mean germination time in a concentration-dependent fashion. Meanwhile, in the context of soaking time, our current study observed that immersing chili seeds with *S. platensis* biomass consistently showed a faster germination across all concentrations (30% (4.72 to 4.38 days), 45% (4.63 to 4.50 days), and 60% (4.48 to 4.29 days). This germination acceleration was likely attributed to the hormone content in *S. platensis* (Jafarlou *et al*., 2022). A faster germination process guarantees a quicker transplanting process from seedling to field, allowing farmers an earlier downstream plant production and cultivation process.



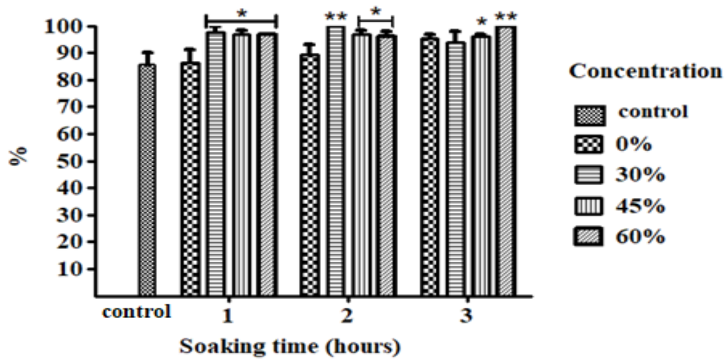
Observation data were taken daily with the condition that the radicle length ≤ 2 mm. The data above represent the mean value with SEM error bars from the ANOVA analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk were considered non-significant. Significance: * (P ≤ 0.05), ** (P ≤ 0.01).

Figure 1. Mean Germination Time (MGT) of Chili Seed.

3.2 Germination Rate (%)

Seed germination rate is an indicator of germination used to determine seed quality. Good seed quality is characterized by a high germination rate. A high germination rate indicates that many seedlings grow normally under optimal environmental conditions. In the current study, the treatment of soaking chili seeds at various concentrations and soaking times with *S. platensis* was intended to increase chili seed viability.

Based on Figure 2, soaking chili seeds in *S. platensis* solution at concentrations of 30% (94,44-100%), 45% (96,67-97,78%), and 60% (95,83-100%) significantly increased the germination percentage compared to the control (85,83%) or water (86,67-95%). The 1-hour soaking treatment at all concentrations showed a significant increase, with the germination percentage reaching 97% compared to the control of 86%. These results indicated that a short soaking time can stimulate the physiological processes needed for chili seeds to germinate. The treatment of seed soaking time has a significant effect on the percentage of germination rate (Syaiful *et al*., 2021). Adding a soaking time of up to 2 hours at all concentrations provided good results, with a germination percentage approaching 100%. This showed that soaking red chili seeds within the 1-2 hour range was effective in increasing germination rate. Meanwhile, the effect of soaking red chili seeds for 3 hours varied among different concentrations. A 30% concentration did not show a significant increase (94,44% germination rate), but at concentrations of 45% and 60%, the treatment of seeds using *Spirulina* biomass resulted in germination rates of 97% and 100%, respectively. The best treatment was obtained by soaking for 2 hours at a concentration of 30% and 3 hours at 60%, resulting in a germination rate of 100%. These results indicated that a short soaking duration of chili seeds with *S. platensis* can stimulate the physiological processes required for germination. The results of this study were in line with the findings of Akgul (2019) that priming treatment of wheat seeds with *S. platensis* at certain concentrations can increase the percentage of germination. This shows that priming treatment with certain concentrations at a short soaking time can be an effective strategy to increase seed vigor of various types of plants, including chili.



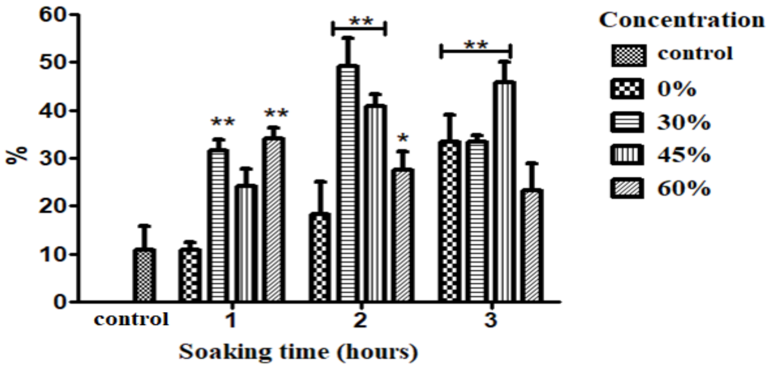
Observation data were taken on the 7th day (first count) and the 14th day (final count) of the normal seedlings criteria. The data above represent the mean value with SEM error bars from the ANOVA analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk were considered non-significant. Significance: * ($P \leq 0.05$), ** ($P \leq 0.01$).

Figure 2. Germination Rate of Chili Seeds

3.3 Vigor Index (%)

The vigor index is one of the seed's quality indicators used to determine the ability of seeds to grow in optimum or suboptimal environmental conditions. The vigor index value is obtained based on the percentage of normal germination at the first count (Day 7th) in chili. The vigor index provides an overview of how quickly and strongly seeds can germinate and grow into healthy plants. The higher the vigor index value, the better the quality of the seeds (Ridho *et al*., 2019).

Consequently, the results of the vigor index test are considered sensitive and can accurately reflect the potential for growth in the field.



Observation data were taken on the 7th day (first count) with the criterion of observing normal seedlings. The data above represent the mean values with SEM error bars from the ANOVA analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk were considered non-significant. Significance: * ($P \leq 0.05$), ** ($P \leq 0.01$).

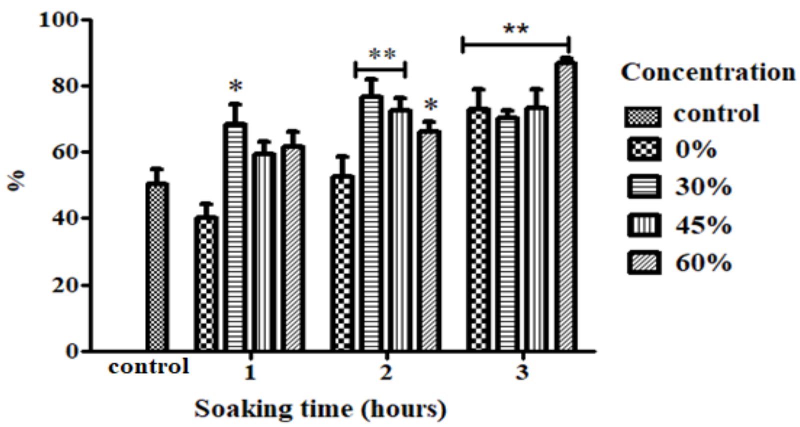
Figure 3. Vigor Index of Chili Seed

Based on Figure 3, the treatment of various concentrations and soaking times with *S. platensis* affected the vigor index of red chili seeds. Several treatments had a significant effect on increasing the seed vigor index compared to the control group. Significant results were shown in the treatment of soaking times of 1 hour at a concentration of 30% (31.67%) and 60% (34.17%), 2 hours at a concentration of 30% (49.17%), 45% (40.83%), 60% (27.50%), and 3 hours of soaking at a concentration of 30% (33.33%) and 45% (45.83%) compared to the control (10.83%) and soaking with water for 1 and 2 hours (10.83% and 18.33%). The highest increase in vigor index was observed at a concentration of 30% at 2 hours of soaking time, reaching a vigor index of 49.17%. Therefore, overall our experiment demonstrates that the use of *S. platensis* for priming chili seeds successfully elevates seed vigor. Although not statistically significant in terms of control, the 45%-1 hour treatment (24.27%) and the 60%-3 hour treatment (23.33%) showed comparatively higher vigor index values than the control (10.83%). Consequently, the utilization of *S. platensis* with the appropriate concentration and soaking duration may enhance seed metabolic activity, thereby increasing germination rate and early plant growth. Our current study was in line with Basavaraja *et al.* (2023) research, which demonstrated that *S. platensis* extract exhibited a stimulating effect on seed germination and vigor index in the early stages of plant growth. Although the physiological and molecular mechanisms of *S. platensis* mediated growth stimulation were still under exploration, there was much evidence in various crops that *S. platensis* contains biologically active biostimulant compounds from phytohormones, polyamine groups, exopolysaccharides (EPS), amino acids, C-phycocyanin (CPC), riboflavin, niacin, and folic acid, which are known to enhance growth and improve plant defense against biotic and abiotic stresses (Zapata *et al*, 2021; Parmar *et al*., 2023; Ferreira *et al*, 2023).

3.4 Seed Growth Simultaneity (%)

Seed growth simultaneity is one of the indicators in seed vigor testing. Growth simultaneity is measured based on the percentage of strong normal seedlings. Seeds that have high growth simultaneity indicate that the seeds have high growth vigor because they show uniform and strong growth and germination (Ningsih *et al*., 2018). This is due to the presence of sufficient food reserves, active metabolism, and healthy physiological conditions of the seeds. Conversely, seeds with a lower vigor tend to germinate unevenly, slowly, or even fail to germinate.

Figure 4 showed the effect of chili seed soaking treatment in *S. platensis* solution with various concentrations and soaking times on growth simultaneity. The value of seed growth simultaneity for chili seeds shows the following variations: control 54.44%; soaking in water only (1 hour 43.33%, 2 hours 47.78%, 3 hours 77.78%); soaking with *Spirulina platensis* biomass 30% (1 hour 73.33%, 2 hours 83.33%, 3 hours 70%), 45% (1 hour 56.67%, 2 hours 68.69%, 3 hours 78.89%), and 60% (1 hour 65.56%, 2 hours 65.83%, 3 hours 85.56%). The results of the analysis showed that treatment with *S. platensis* significantly increased the percentage of growth simultaneity compared to the control. In the treatment of soaking time for 1 hour, the application of biomass solution with a concentration of 30% showed a significant effect on chili growth simultaneity with a percentage of 73%. Meanwhile, concentrations of 45% and 60% of biomass application did not show a significant effect (57% and 66%, respectively) and there was an observable slight decrease in growth simultaneity. Nevertheless, this concentration apparently still outperformed the control (54.44%) and 0% (43%).



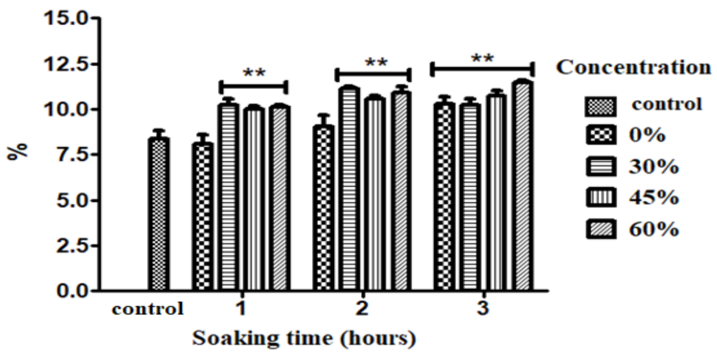
Observation data were taken once between the first count and final count on the 11th day under the criteria for normal seedling. The data above represent the mean value with SEM error bars from the ANOVA analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk were considered non-significant. Significance: * ($P \leq 0.05$), ** ($P \leq 0.01$).

Figure 4. Simultaneous Growth of Seed of Chili Seed

In the treatment of soaking times of 2 hours and 3 hours, the application of biomass at concentrations (30%, 45%, and 60%) showed a significant effect. The treatment with the best simultaneity percentage was obtained at a concentration of 60% for 3 hours with a percentage of 85.56% and at a treatment of 30% for 2 hours with 83.33%, while the lowest average growth simultaneity was found in the control treatment for 1 hour (54.44%) and 1 hour of water (43.33%). This indicated that the combination of optimal concentration and soaking time can maximize the positive effects of *S. platensis* in stimulating uniform germination. Our current study aligns with the results of Dzakwan *et al.* (2023) that observed a significant effect of concentration and length of soaking time with phytohormones on the growth simultaneity of chili seeds. Since microalgae in nature are well known for producing some plant growth regulators (Parmar *et al*., 2023), the utilization of *S. platensis* demonstrated a promising biostimulant for accelerating growth and simultaneous development of chili seeds and seedlings.

3.5 Seed Growth Rate (%/etmal)

The growth rate is a measure of seeds that indicates that the seeds have high vigor. Seeds with higher vigor tend to have a higher growth rate as well. This shows that the seeds have the potential to grow into healthy and productive plants. The percentage of seed growth rate was obtained from the number of seedlings that grow each day until the final count. The faster the seeds grow generally indicates a better physiological quality of the seeds (Harsono *et al*., 2021). The graph below showed how the soaking time and concentration of *S. platensis* affect the growth rate of chili seeds.



Observation data were taken daily according to the criteria for normal seedling. The data above represent the mean value with SEM error bars from the ANOVA analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk were considered non-significant. Significance: * ($P \leq 0.05$), ** ($P \leq 0.01$).

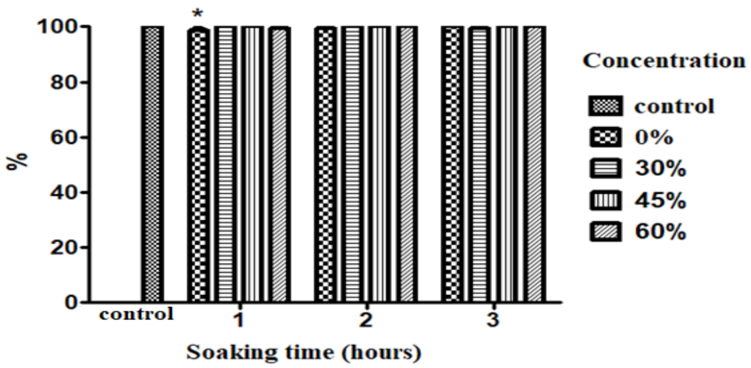
Figure 5. Seed Growth Rate of Chili Seed

Based on Figure 5, the treatment of chili seeds soaking with *S. platensis* at various concentrations (30%, 45%, and 60%) and soaking times (1, 2, and 3 hours) significantly increased the seed growth rate compared to the control. The percentage of growth rate at 1 hour with 30% treatment was 10.23%, while with 45% and 60% treatments the percentage of growth rate reached 10%. Thus it was higher than the control which is only 8%. At 2 hours of soaking time there was an increase in growth rate of 30% (11.11%), 45% (10.56%) and 60% (10.88%). At 3 hours of soaking at a concentration of 30% the percentage of growth rate reached (10.25%) while at concentrations of 45% (10.75%) and 60% (11.48%). The best treatment was obtained at a concentration of 30% for 2 hours and 60% for 3 hours, which produced a growth rate of 11.11%

and 11.48%, higher than other treatments. The present study revealed that both *S. platensis* biomass concentrations and soaking durations have a positive correlation with the seed growth rate of chili seeds. The increase of biomass concentrations and soaking durations simultaneously improves chili seed growth rate. This result might occur and stem from the fact that *S. platensis* contains phytohormones that can have the potential as plant growth biostimulants. *S. platensis* is rich in phytohormones (cytokinins, gibberellins, and auxins) and nutrients (Rady *et al*., 2023). In the study of Liana *et al*. (2022), the treatment of invigorating chili seeds with natural hormones extracted from shallots and banana stems (containing auxins, gibberellins, and cytokinins) showed a very significant effect on increasing seed growth rate. These results indicated that these natural hormones play an important role in stimulating the early growth of chili seeds.

3.6 Maximum Seed Growth Potential (%)

Maximum growth potential (MGP) is a parameter in seed viability testing used to measure seed growth ability by counting the number of normal and abnormal seedlings at the final count (Kolo & Tefa, 2016). Maximum growth potential indicates that seeds can grow under optimum conditions. A higher MGP value indicates that seeds have a good germination rate.



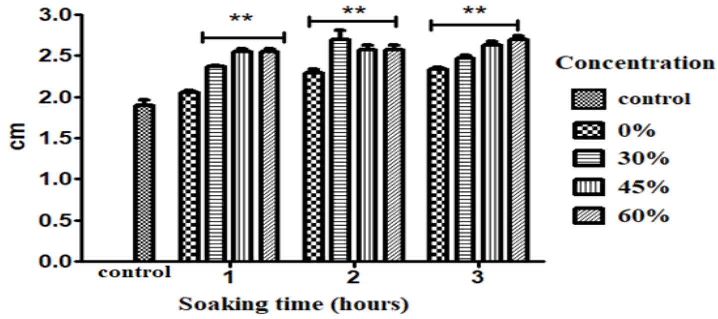
Observation data were taken on day 14 (final count) with the criteria of the percentage of normal and abnormal seedlings. The data above represent the mean value with SEM error bars from the ANOVA analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk were considered non-significant. Significance: * ($P \leq 0.05$), ** ($P \leq 0.01$).

Figure 6. Maximum Growth Potential of Chili

Based on Figure 6, the effect of concentration and duration of immersion on MGP tends to be high in all treatments. In the 60% 1-hour treatment, the average MGP was 99.17%, while the other concentrations and durations of immersion obtained an MGP percentage of 100%. Meanwhile, immersion using water for 1, 2, and 3 hours each obtained an MGP percentage of (98.33%), (99.17%), and (99.17%). This indicates that the varying concentration and duration of soaking time imposed no negative effect on the growth potential of chili seedlings. Similar results were found in the study of (Rahmadina *et al*., 2024) that confirmed that varying the auxin concentration did not exert an adverse effect on Chili seed germination and growth.

3.7 Shoot Length (cm)

Shoot length is an important parameter that describes plant growth. Shoot is part of the seedling that will develop into stems and leaves (Parnidi *et al*., 2022). The longer the shoot generally indicates better vegetative growth potential, such as more branching and wider leaves. In this study, the observation of shoot length aims to determine the effect of seed priming treatment with *S. platensis* on the growth of red chili seedlings. Measuring shoot length provides an indication of the growth strength of young plants (Dharma *et al*., 2015).



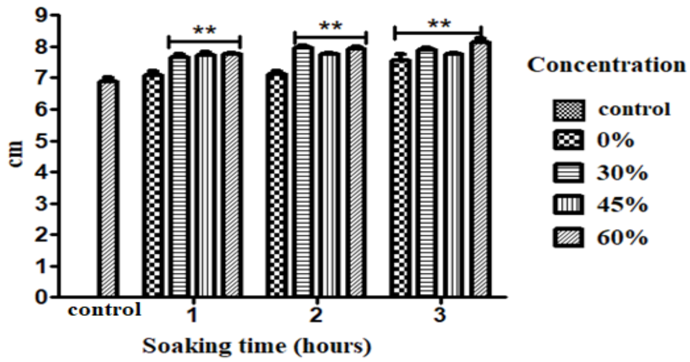
Observation data were taken on day 14 (final count) measuring from the base of the stem to the tip of the shoot bud/growth point in normal seedlings. The data above represent the mean value with SEM error bars from the ANOVA analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk were considered non-significant. Significance: * (P ≤ 0.05), ** (P ≤ 0.01).

Figure 7. Shoot Length of Chili Seed

Figure 7 showed that treatment with *S. platensis* can significantly increase the length of the shoot in red chilies. The average length of the shoot in the 1-hour immersion time at-concentrations of 30, 45, and 60% is 2.37; 2.55; and 2.55 cm, respectively. This result was still higher than the control which reached only 1.90 cm and immersion with water reached only 2.05 cm. Soaking for 2 hours also showed a significant effect with an average shoot length at concentrations of 30, 45, and 60%, namely 2.70; 2.60; and 2.58 cm, respectively. And at 3 hours of soaking period, the application of concentrations of 30, 45, and 60% also had a significant effect, obtaining an average shoot length of 2.48; 2.63; and 2.73 cm. The highest increase in average shoot length was observed at a concentration of 60% with a soaking time of 3 hours, which was 2.8 cm compared to the control (2.2 cm). These results indicated that the administration of *S. platensis* can stimulate the early growth of chili plants through complex mechanisms, including an increase in cell metabolic activity and stimulation of growth hormone production. These results were in line with the study of Akgul (2019) that observed the application of seed priming using *S. platensis* has a positive effect on the length of the shoot in seeds compared to the control.

3.8 Radicle Length

Radicle length is one of the important parameters in measuring the early growth stages of a plant. The radicle is the first embryonic root that emerges from a seed when it germinates. The level of seed metabolic activity during germination can be seen from the length of the radicle formed (Khoirunnissa *et al*., 2022). The longer the radicle produced generally indicates the potential for better plant growth. Radicle length provides an indication of the vigor or growth strength of a seed (Wirianto *et al*., 2024). In this study, radicle length was used as an indicator to assess the effect of soaking time and *S. platensis* concentration on the early growth of red chilies.



Observation data were taken on day 14 (final count) measuring from the root base to the root tip in normal seedlings. The data above represent the mean value with SEM error bars from the ANOVA analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk were considered non-significant. Significance: * ($P \leq 0.05$), ** ($P \leq 0.01$).

Figure 8. Radicle Length of Chili Seed

In Figure 8, the application of seed priming with *S. platensis* biomass concentration and soaking time can significantly increase the length of the radicle in red chili. The average length of the radicle in the treatment of 1 hour soaking time at concentrations of 30, 45, and 60%, namely 7.67; 7.73; and 7.78 cm, showed a significant effect. Subsequently, at a soaking time of 2 hours, concentrations of 30%, 45%, and 60% also had a significant effect, namely 7.98; 7.75; and 7.95 cm. Meanwhile, treatments at a soaking time of 3 hours with a concentration of 30%, 45%, and 60% obtained a fairly high average radicle length of 7.84; 7.75; and 8.13 cm, which had a statistically significant effect. At all concentrations, the average radicle length was higher compared to the control (6.88 cm) and water at 1, 2, and 3 hours, namely 7.08; 7.10; and 7.38 cm. The highest increase in average radicle length was observed at a concentration of 60% with a soaking time of 3 hours, which was 8.13 cm. The significant increase in radicle length in the *S. platensis* treatment might be associated with the nutritional content and bioactive compounds in *S. platensis* (Parmar *et al*., 2023). These beneficial compounds such as vitamins, minerals, and amino acids can increase cell metabolism and stimulate cell division, thereby promoting radicle growth. In addition, bioactive compounds such as plant growth hormones (auxins) found in *S. platensis* can also play a role in stimulating root cell elongation (Akgul, 2019). Microalgae biomass treatment has successfully enhanced plant growth through diverse applications including seed treatment, soil enrichment, and direct fertilizing such as foliar spray (Ferreira *et al.*, 2023; Ronga *et al*., 2023). The current study provides a short elaboration on the effect of *S. platensis* biomass application on chili at seedling stages. Further studies are necessary to examine the impact at more mature stages of plant growth. Additionally, the examination of seed metabolism upon the application of *S. platensis* at a biochemical and molecular level may enrich current knowledge of the mechanism by which microalgae cells provide growth support to plants.

4. Conclusion

Seed priming application using *S. platensis* significantly enhanced seed vigor. The *S. platensis* treatment at 60% concentration for 2 hours significantly reduced MGT by 1 day, while the 30%-2 hours and 60%-3 hours treatments exhibited the highest germination rate (100%), vigor index (49.17% in 30%-2 hours), seed growth simultaneity (85.56% in 60%-2 hours and 83.33% in 30%-2 hours), and growth rate (11.48% in 60%-3 hours and 11.11% in 30%-2 hours), with the 60%-3 hours treatment showing the longest shoot (2.73 cm) and radicle (8.13 cm) lengths compared to the control, although maximum germination potential remained unaffected. This study uncovers the potential role of *S. platensis* in priming chili seeds. Overall, the biomass of *S. platensis* has improved the growth parameters of chili seedlings, especially germination time, vigor index, and growth rate. These findings suggest that *S. platensis* priming can be a promising approach for improving seed quality and crop establishment. Our current understanding of how *S. platensis* supports plant growth could be further improved by exploring its impact on later plant development and by analyzing its biochemical and molecular effects on seed metabolism. This step will allow the implementation of microalgae biomass as a biostimulant or biofertilizer at a larger scale to promote crop productivity and agricultural sustainability.

Abbreviations

SME Sugar Mill Effluent
CRD a Factorial Completely Randomized Design
MGT Mean Germination Time
BBM Bold Basal Medium
RH Relative Humidity
ISTA International Seed Testing Association

Data availability statement

We are willing to provide assistance involving our original images and data if the relevant author is contacted.

CRediT authorship contribution statement

Kariena Samtani: data curation, resources, and initial draft writing. **Fitri Ayu Rahmawati:** methodology, resources, and preparation. **Indah Wahyu Pratiwi:** methodology and draft writing. **Nuning Atuillah:** project administration, **Reza Aris Hidayatullah:** preparation and resources. **Dewi Fatmawaty Sabiku:** grammar checking. **Maria Azizah:** statistical analysis. **Hari Prasetyo:** revision drafting. **Suwardi:** graph preparation. **Nantil Bambang Eko Sulistyono:** graph preparation. **M. Bintoro**: revision writing. **Moch. Rosyadi Adnan:** supervision, conceptualisation.

Declaration of Competing Interest

The authors of this manuscript declare that there are no conflicts of interest or competing interests.

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