BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY
Volume 14, Number 1, April 2025 | Pages: 129-141 | DOI: 10.14421/biomedich.2025.141.129-141
ISSN 2089-6514 (paper)
ISSN 2540-9328 (online)

The Potential of *Spirulina platensis* Biostimulant as a Seed Priming Agent to Enhance the Vigor and Viability of Cucumber Seeds (*Cucumis sativus*)

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Manuscript received: 29 November 2024. Revision accepted: 08 May, 2025. Published: 15 May, 2025.

Abstract

Spirulina platensis is a microalgae that can grow in waste media, including Sugar Mill Effluent (SME). In addition, *Spirulina* contains phytohormones that can be utilized to enhance seed germination. Cultivating microalgae will produce biomass that can be used for priming cucumber seeds. This research aims to determine the effect of *Spirulina* biomass and soaking time on the germination of cucumber seeds. In this research, a factorial Completely Randomized Design (CRD) was used, consisting of 5 levels of microalgae biomass concentration: control (untreated seeds), 0, 30, 45, and 60%, with soaking time of 3 levels: 1, 2, and 3 hours. The results demonstrated that *Spirulina* can grow in SME-based media, as indicated by the increase in size and density of microalgae cells. Analysis of the seed priming test results of 45% algae biomass concentration for 1 and 3 hours showed a significant effect on the parameter of vigor index, seed growth rate, and simultaneous seed growth. In the treatment with a 60% concentration and soaking time of 1, 2, and 3 hours, significant effects were observed on the parameters of MGT, vigor index, seed growth rate, and shoot length. Even though the 45% and 60% treatments showed no significant differences in the few parameters, the priming with *Spirulina platensis* exhibited no decrease in cucumber seed germination, these applications indicated no decrease in seed germination. This demonstrates that priming treatment with *Spirulina platensis* is not toxic to seedling growth.

Keywords: Biostimulant, *Cucumis sativus;* Seed priming, *Spirulina platensis*; Vigor.

Abbreviations: Bold Basal Medium (BBM), Completely Randomized Design (CRD), Laminar Air Flow Cabinet (LAFC), Maximum growth potential (MGP), Mean Germination Time (MGT), Sugar Mill Effluent (SME), Vigor Index (VI).

INTRODUCTION

Spirulina has the synonym name Arthrospira. Species of *Spirulina* include *Spirulina maxima, Spirulina fosiformis, and Spirulina platensis*. *Spirulina platensis* is a type of microalgae that is bluish-green in color, unbranched, filamentous in shape with a spiral (helix) form, measuring 1-12 micrometers, and lives in colonies. *Spirulina* is a microalgae originating from the ocean and can thrive in water with toxic metal pollutants. *Spirulina* is not only found in saltwater, freshwater, and brackish water but also soil, demonstrating its high adaptability (Kamaludin & Holik, 2022). The microalgae *Spirulina*, with its high adaptability, can utilize waste as a growth medium, one of which is Sugar Mill Effluent (SME). SME contains high levels of organic and inorganic compounds, so before being discharged into water bodies, it must meet quality standards to determine the amount of pollutants in the waste water. The organic compounds found in the SME can be utilized by microalgae as a growth medium by utilizing nutrients such as nitrogen and phosphorus in the wastewater. In addition to being known for their high efficiency in wastewater treatment, microalgae can absorb nutrients and metals from wastewater at various stages of treatment (Alling et al., 2023). The cultivation of microalgae produces beneficial biomass that can be used as seed priming agents.

Seed priming or seed soaking is an initial treatment on seeds as one of the invigorating techniques aimed at increasing the germination rate, germination percentage, uniformity of growth, and germination speed in seeds (Andayani et al., 2023). Priming methods include osmopriming, hydropriming, halopriming, and biopriming. Biopriming is a priming treatment combined with applying biological agents such as microbes that can enhance seed germination, produce growth hormones, or increase stress resistance (Kurnia et al., 2017).

Biopriming can be performed using the biomass of the microalgae *Spirulina platensis*. The biomass of *Spirulina platensis* comprises roughly 68% protein with few essential amino acids. *Spirulina platensis* is a microalgae with a structure consisting of amino acids, polysaccharides, phenolics, proteins, carbohydrates, vitamins, and phytohormones (auxin, gibberellin, and cytokinin). The phytohormone content can enhance enzyme activity, cell division, and differentiation, as well as plant growth (Seğmen & Ünlü, 2023). Thus, biomass containing phytohormones can be used as a seed priming agent to enhance germination. The use of microalgae biomass for seed priming can be applied to various types of crop and horticultural plants, including cucumber.

Cucumber is one type of horticultural commodity classified as a vegetable for public consumption, it is rich in nutrients, vitamins, minerals, and beneficial for lowering blood pressure (Oktaviana et al., 2016). Therefore, cucumbers are excellent plants that serve as multi-purpose vegetables starting from food, garnish, cosmetics, and therapeutic substances (Adnan et al., 2024). To achieve good production, appropriate cultivation techniques are necessary. Factors that support successful cucumber cultivation are the use of high-quality seeds. Seed quality encompasses physical, physiological, genetic, and pathological or health quality. a clean, bright, plump appearance and uniform size characterize seeds with high physical quality. Meanwhile, the high physiological quality of seeds can be distinguished from their high viability (germination rate) and vigor (growth speed and simultaneous of seed growth). The genetic quality of seeds is indicated by high genetic uniformity and the absence of other variety contaminants. Meanwhile, for pathological quality, the seeds are assessed based on the absence of seedborne pathogens (Ashari et al., 2024).

The use of low quality seeds can result in a decrease in the quality and productivity of cucumber plants. To improve yield production, the use of high-quality seeds is necessary. Rosita et al. (2022) highlighted seed quality as a key factor in increasing the productivity of apple cucumber plants, which currently remains low at around 27 tons/ha. The decline in seed quality can occur naturally through prolonged storage periods, resulting in decreased seed viability. The decline in seed quality affects the germination and growth of seedlings, leading to suboptimal plant development that could potentially cause losses for farmers. Thus, applying biostimulants in seed priming techniques offers an innovative solution to enhance seed germination and early plant growth in supporting sustainable agricultural practices (García-Locascio et al., 2024). Haerani and Nurdin (2021) reported that biopriming showed a positive response in the parameters of germination rate, vigor index, and simultaneous growth. Cucumber plants are responsive ability to seed priming treatments, making them an ideal subject to test a new potential seed priming agent. This idea was confirmed by Badu et al. (2022) who successfully demonstrated that cucumber seeds induced with seed priming showed a higher germination percentage in response to biopriming compared to untreated seeds.

Based on the potential application of *Spirulina platensis* in seed priming, this study aimed to determine the effect of the concentration of *Spirulina platensis* microalgae biomass (control, 0%, 30%, 45%, and 60%) with soaking durations (1, 2, and 3 hours) on the seeds of cucumber variety MKGE 701. This research presents a new agricultural innovation by utilizing *Spirulina platensis* as an agent for processing SME for seed priming applications. Consequently, our research aims to contribute to preserving environmental safety, ensuring food security, and promoting a more sustainable agriculture in the future.

MATERIALS AND METHODS

Place and time of research

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

Materials and tools

The equipment used included a centrifuge, Laminar Air Flow Cabinet (LAFC), hemocytometer, aerator, germination rack, germination box, microscope, and autoclave. The materials used consisted of Sugar Mill Effluent (SME), aquades, Bold Basal Medium (BBM) with modifications (Supplementary), 70% alcohol, *Spirulina platensis* isolate from BBPBAP Jepara, label paper, litmus paper, germination paper, seeds of cucumber var. MKGE 701 (CV. Nasienie Indonesia, Jember - Indonesia) has an expiration date of December 2025.

Procedures

This research method used a factorial Completely Randomized Design (CRD) with the factor of microalgae biomass concentration (v/v) consisting of 5 levels: control (untreated seeds), C0 (0%), C1 (30%), C2 (45%), and C3 (60%), and the soaking time consisting of 3 levels: 1, 2, and 3 hours. The total treatment combinations are 15 with 4 repetitions, resulting in 60 experimental units with 30 seeds per unit. The research began with the preparation of a growth medium for *Spirulina platensis*, comprising 25% (v/v) Sugar Mill Effluent (SME) in a modified Bold Basal Medium (BBM) according to UTEX (UTEX, 2019). Subsequently, microalgae were grown in plastic bottles photobioreactors with illumination of 190 lux and aeration for 24 hours at room temperature. The microalgae biomass used for seed priming is obtained from centrifugation at 8000 rpm at 10°C for 10 minutes.

The seed priming experiments were carried out by soaking the seeds with microalgae solutions prepared by diluting pellets with distilled water to achieve the desired concentration. Subsequently, the seeds were germinated on germination paper and placed in a plastic container. The container was then stored at a germination shelf at 28°C, 70% humidity, with an illumination period of 16 hours followed by 8 hours dark period for 8 days.

Analysis of the microalgae test results was observed based on:

Observation of Cells Photos

Visual observation of cells utilizes image processing software (cellSens) integrated with the Olympus BX43 compound microscope.

Analysis of seed priming test results is based on ISTA observation parameters (Fadhilah, 2020):

Germination Rate (%)

Germination observation was conducted on the first count (day 4) and final count (day 8), with the parameter observed being the number of normal seedlings. Normal seedlings demonstrate the ability to grow and develop into normal plant seedlings. The germination percentage can be calculated using the following formula:

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

Mean Germination Time (Days)

MGT was observed based on the number of seeds producing a radicle < 2 mm daily. The measurement of MGT shows the average time required for the seeds to germinate. MGT can be calculated using the following formula:

$$MGT\ (Days) = \frac{\Sigma(n\ x\ t)}{\Sigma numbers\ of\ germinated\ seed}$$

Vigor Index (%)

The vigor index was observed by observing normal seedlings that grew during the first count. The calculation of the vigor index is as follows:

$$VI\ (\%) = \frac{\Sigma Normal\ Germination\ First\ count}{\Sigma numbers\ of\ germinated\ seed} X100\%$$

Seed Growth Rate (%)

Observations were conducted by counting the normal seedlings that grew each day until the final count. The calculation formula is as follows:

$$Seed\ Growth\ Rate\ (\%) = \frac{\%\ Normal\ Seedlings}{etmal}$$

Simultaneous Growth seeds (%)

Observation was conducted by counting the normal seedlings that grow between the first count and the final count day (day 6). Strong normal seedlings are the criteria for normal seedlings that have the appearance of stronger germination than*the average of other normal seedlings. The calculation formula is as follows:

$$Simultaneous\ Growth\ seeds\ (\%) = \frac{\Sigma Strong\ Normal\ Seedlings}{\Sigma Seed\ germinations} X100\%$$

Maximum Growth Potential (%)

Observations were made by calculating the percentage of seeds that grew into normal and abnormal seedlings at the final count. The calculation formula is as follows:

$$Maximum\ Growth\ Potential\ (\%) = \frac{\Sigma Normal + Abnormal\ seedling}{\Sigma numbers\ of\ germinated\ seed} X100\%$$

Shoot Length (cm)

The measurement of the shoot length was taken from the base of the stem to the tip of the bud/growth point of the shoot.

Radicle Length (cm)

The radicle length was measured from the base of the root to the tip of the radicle.

Dry Weight of Normal Seedlings (grams)

This observation was conducted during the final count, to determine seed vigor. Normal seedlings have their cotyledons removed, then placed in paper envelopes, dried using an oven at 60°C for 72 hours, and weighed using an analytical balance. The calculation formula is as follows:

$$Dry\ Weight\ of\ Normal\ Seedlings\ (grams) = \frac{wet\ weight\ - dry\ weight}{wet\ weight}$$

Data Analysis

The research data were analyzed using analysis of variance (ANOVA), and if significant data were found, a Bonferroni post-hoc test was conducted at a 5% significance level. Statistical analysis was performed using GraphPad Prism 5.01 software.

RESULTS AND DISCUSSION

Analysis of Microalgae Growth on SME-based-Bold Basal Medium (SME-BBM)

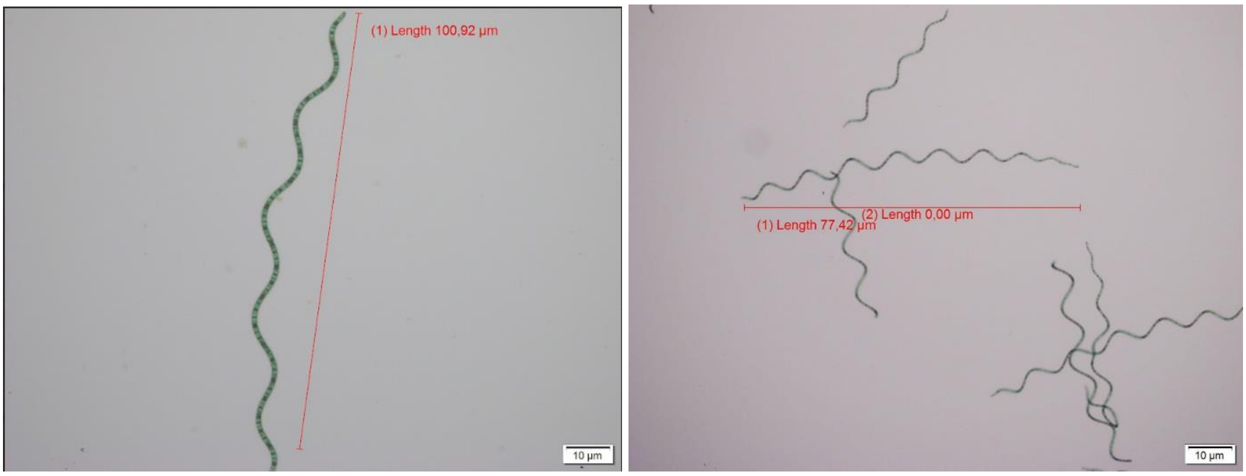

Figure 1. Photograph of *Spirulina platensis* Microalgae Cells Grown on SME-based Bold Basal Medium.

Microalgae are a biomass source that contains important components such as fatty acids, pigments, carbohydrates, and proteins, depending on the growth medium used, the environment, and the drying method. The observation of the microalgae *Spirulina platensis* grown on SME-BBM media was marked by a change in the culture color from light green to a more intense shade. These findings were supported by the research of Widawati et al. (2022), which shows that the growth of the microalga *Spirulina platensis* is marked by a color change in the culture from clear green to dark green. This color change occurs due to the increase in biomass. The growth of microalgae is accompanied by an increase in cell size and an increase in the density of microalgal cells (Lebeharia et al., 2016). The growth of microalgae is divided into several phases, namely the lag phase, exponential phase, stationary phase, and death phase. In the exponential phase (log phase), their growth is marked by an increase in the number and length of microalgae cells. The diameter of microalgal cells ranges from 1 to 12 µm, their body shape resembles threads, and they have a spiral shape with thin cell walls. At the end of the exponential phase, microalgae produce phycocyanin compounds as high antioxidants (Kamaludin & Holik, 2022). Additionally, the research of Chiaiese et al. (2018) found that microalgae biologically produce extracellular compounds in the form of phytohormones, amino acids, polypeptides, and vitamins. Thus, microalgae can be utilized as plant biostimulants to enhance plant growth and development.

Analysis of Seed Priming Result on Cucumber Seeds

Germination Rate (%)

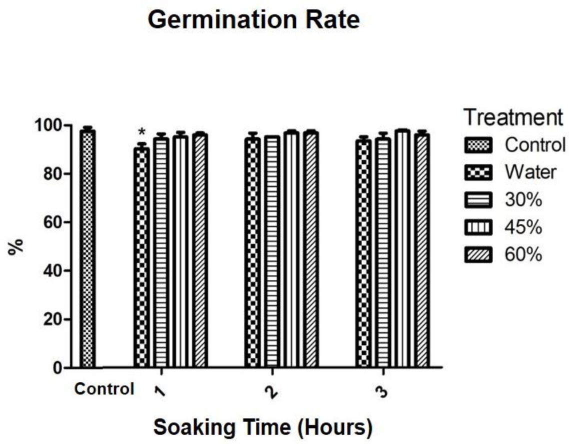

Figure 2. Germination Rate of Cucumber Seeds Under Various Biomass Concentrations and Soaking Time. Observation data were taken on the 4th day (first count) and the 8th day (final count) of the normal seedlings criteria. The data above represent the mean value with SEM error bars from the Anova analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk are considered non-significance. Significant: * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$).

The germination rate of seeds is a parameter that serves as a benchmark for seed viability. Seed viability is defined as the ability of a seed to grow into a normal seedling (Ashari et al., 2024). The germination rate is an important factor that determines plant formation and subsequent production. Based on Figure 2, the results of the analysis of variance showed that the treatment with concentrations of *Spirulina platensis* biomass at 30%, 45%, and 60%, and soaking times of 1, 2, and 3 hours on cucumber seeds have a non-significant effect on the germination rate parameter. The germination rate in the sample without treatment (control) was 97,50%. Meanwhile, the water treatment for 1, 2, and 3 hours showed germination rate values of 91,67; 95,83; and 95,00%. On the other hand, the seed priming application with 30% *Spirulina platensis* biomass for 1, 2, and 3 hours showed germination rate values of 95,83; 96,67; and 95,83%. The 45% concentration showed germination rate values of 96,67; 98,33; and 99.17%. The 60% concentration with soaking times of 1, 2, and 3 hours showed germination rate values of 97,50; 98,33; and 97,50%. Thus, although the application of *Spirulina platensis* biomass showed a non-significant effect, no decrease in germination was found in the seed germination rate parameter.

Based on the results analysis, the seed priming treatment with 45% concentration of *Spirulina platensis* biomass for 2 and 3 hours of soaking time, and 60% concentration for 2 hours of soaking time, showed germination rates of 98,33; 99,17; and 98.33%, respectively. Although all three treatments showed no significant results, these applications provided higher germination effects compared to the control (97,50%). Furthermore, the application of *Spirulina platensis* biomass did not cause a decrease in germination rate. This indicates that treating *Spirulina platensis* biomass concentration with soaking time does not have a harmful or toxic effect on seedling growth. The research of Ribeiro et al. (2019) reported that the supernatant from *Chlorella sorokiniana* culture showed no significant effect on lettuce seed germination and did not cause toxicity to the seeds.

Mean Germination Time (MGT)

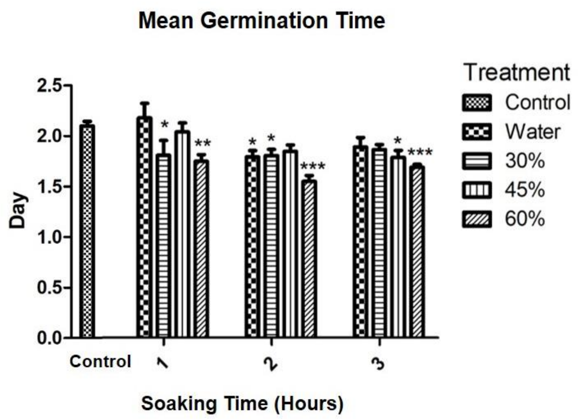

Figure 3. Mean Germination Time (MGT) of Cucumber Seed Under Various Biomass Concentrations and Soaking Time. Observation data were taken daily with the condition that the radicle length ≤ 2 mm. The data above represent the mean value with SEM error bars from the Anova analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk are considered non-significance. Significant: * (P ≤ 0.05), ** (P ≤ 0.01), *** (P ≤ 0.001).

Mean Germination Time (MGT) is one of the methods used to measure seed vigor through seedling growth testing. Mean Germination Time (MGT) is an observational parameter that serves as a benchmark to indicate the average time required for seeds to develop a radicle (Soltani et al., 2015). Mean germination time is correlated with seed vigor, where a shorter germination time indicates a faster and more uniform radicle growth potential. Based on Figure 3., the analysis of variance results showed that the treatment with a 30% concentration of *Spirulina platensis* biomass with soaking times of 1, 2, and 3 hours significantly effected the MGT parameter compared to the control. This is evidenced by the emergence time of the radicle without treatment (control) on the 2^{nd} day with an MGT value of 2.10 days. Contrastly, in the 30% treatment for 1, 2, and 3 hours, the radicle appeared before the 2^{nd} day with values of 1,81 and 1,87 days. Although statistically significant results were only found in the 30% treatment for 1 and 2 hours, the 3 hour treatment also showed a faster germination compared to the control with an MGT value of 1,87 days.

The treatment of *Spirulina platensis* biomass at a concentration of 45% for 1, 2, and 3 hours exhibited the MGT values of 2,04; 1,85; and 1,79 days, respectively. Based on the statistical test results, the 45% treatment with a soaking time of 3 hours significantly effected MGT. The emergence of the radicle was faster with a soaking time of 3 hours compared to the control, 1, and 2 hours. Although the soaking times of 1 and 2 hours did not show significant results, the average MGT required to grow the radicle was faster, than the control. Considering the obtained findings, there was a trend that the longer the soaking time, the faster the seeds develop radicles. The current research's outcomes were in line with the research by Shakuntala et al. (2020), which found that the longer the soaking time, the more it affects the quality of cucumber seeds, namely germination, vigor index, germination rate, germination speed, shoot length, and root length, which are all higher.

Subsequently, the treatment with 60% *Spirulina platensis* biomass and soaking times of 1, 2, and 3 hours showed a significant effect on the MGT parameter. Compared to other treatments, the 60% treatment for 1, 2, and 3 hours provided the highest average MGT times of 1,75; 1,55; and 1.69 days, respectively. This indicates that the higher the concentration of *Spirulina platensis* biomass used for seed priming, the more it can accelerate the average time required for the radicle to grow. Overall, the observation analysis showed that treating cucumber seeds with *Spirulina platensis* biomass concentrations of 30%, 45%, and 60% with soaking times of 1, 2, and 3 hours significantly affected the MGT parameter compared to the control. These findings suggest that the varying concentration and soaking time of *Spirulina platensis* can have a significant impact on MGT parameters. Our research aligned with the study of Akgül (2019) that confirmed the use of *Spirulina* *platensis* biomass to improve the germination time of wheat and barley.

Vigor Index (VI)

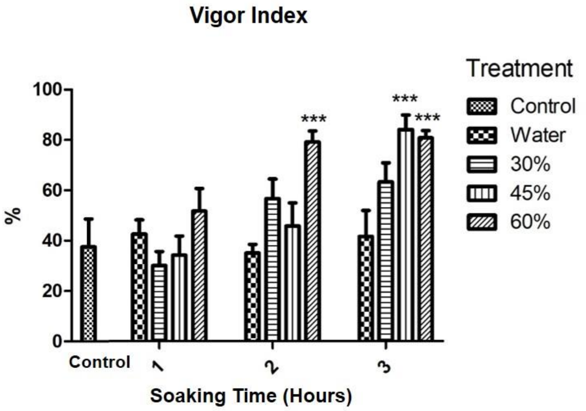

Figure 4. Vigor Index of Cucumber Seed Under Various Biomass Concentrations and Soaking Time. Observation data were taken on the 4th day (first count) to observe normal seedlings. The data above represent the mean value with SEM error bars from the Anova analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk are considered non-significance. Significant: * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$).

Seed vigor index (VI) is the ability of seeds to grow into normal plants under sub-optimal environmental conditions. The vigor index compares the number of normal seedlings at the first count and the number of seeds germinated. The vigor index test is a more accurate approach to assessing the growth potential of seedlings in the field compared to germination power testing (Kartina et al., 2021). Figure 4 showed that results of the analysis of variance showed that the water treatment and soaking durations of 1, 2, and 3 hours did not significantly affect the vigor index parameter compared to the control with each VI percentage values of 42,50; 35; and 41,67%, respectively. Although the water treatment with soaking durations of 1 and 3 hours showed no effect on the VI parameter, these applications positively correlated with the increase in vigor index values compared to the control (37,50%). This indicates that priming treatment with the hydropriming method can enhance seed germination.

In the treatment of *Spirulina platensis* biomass at a concentration of 30% with soaking times of 1, 2, and 3 hours, the vigor index percentages were 30; 56,67; and 63,33%, respectively, compared to the control at 37,50%. Although the results were not significant, the application of biomass at a concentration of 30% for 2 and 3 hours showed higher vigor index percentages compared to the control. Based on the obtained results, there is a tendency that the longer the soaking time, the higher the percentage of vigor index value in the seeds.

Subsequently, the treatment with 45% concentration of *Spirulina platensis* biomass with soaking times of 1, 2, and 3 hours showed VI values of 34,17; 45,83; and 84,17%, respectively. Based on these VI values, the 45% treatment with a soaking time of 3 hours showed a significant effect on the increase of the vigor index parameter. The treatment of cucumber seeds with 45% biomass within 3 hours showed the highest VI value of 84,17% compared to the other treatments. On the other hand, although the 45% treatment for 2 hours showed no significant results, the application of the biomass demonstrated a higher percentage of VI value compared to the control. The elevated vigor index of seeds treated with *Spirulina platensis* biomass indicates a significant development potential in the field, particularly under sub-optimal environmental circumstances. Based on the research by Alam et al. (2023), priming cucumber seeds can protect plants from excessive water loss and make them more tolerant to drought stress. Basavaraja (2024) also suggested that the increase in germination percentage and vigor index using *Spirulina platensis* extract positively affects germination rate and seedling growth strength. Furthermore, algae extracts can enhance the growth of early-stage plants.

Applying a 60% concentration of *Spirulina platensis* biomass treatment with soaking times of 1, 2, and 3 hours showed vigor indices (VI) of 51,67; 79,17; and 80,83%, respectively. Based on these vigor index values, the 60% treatment with soaking times of 2 and 3 hours significantly effected the vigor index parameter. Although the 1 hour soaking at 60% concentration showed no significant results, the application of biomass at this concentration demonstrated a higher vigor index percentage than the control. Based on the obtained results, there is a tendency that the longer the soaking time, the more it affects the increase in the vigor index rate of the seeds. This is evident at a concentration of 60% with a vigor index of 51,67% at 1 hour and reaching 80,83% at 3 hours of soaking.

Overall, our current study suggested a positive correlation between Spirulina platensis biomass concentration and the rate of vigor index of cucumber seeds. Indeed, this is evident at concentrations of 30%, 45%, and 60%, as shown by the increase in the percentage of vigor index values from low to high concentrations, ranging from 30% to 80,83%. This increase might occur due to the use of increasingly concentrated *Spirulina platensis* biomass, which enhances the hormone content in the biomass, thereby improving the germination rate, particularly in the vigor index parameter. The current work was in line with the research by Basavaraja (2024), which demonstrated that higher concentrations of *Spirulina platensis* can enhance seed germination and seedling vigor index in various plant species, even at a concentration of 100%.

Seed priming on cucumber seeds induces pre-germination metabolic conditions to increase germination rates and enable seeds to germinate under sub-optimal conditions. Cucumber seeds have a responsive ability to seed priming treatment. This idea was in line with the research by Badu et al. (2022), cucumber seeds induced with seed priming showed a higher germination percentage in response to biopriming due to changes in physiological activity, seed metabolism, and enzyme stimulation compared to untreated seeds.

Seed Growth Rate (%/etmal)

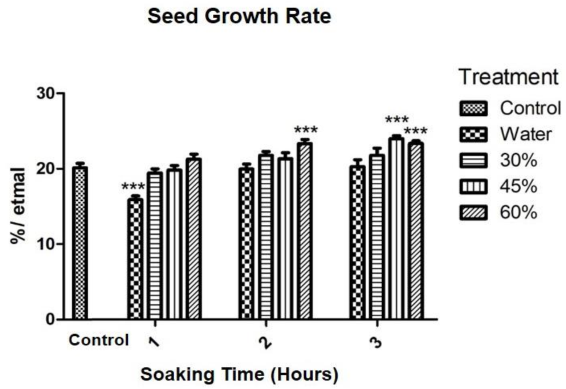

Figure 5. Seed Growth Rate of Cucumber Seed Under Various Biomass Concentrations and Soaking Time. Observation data were taken daily according to the criteria for normal seedling. The data above represent the mean value with SEM error bars from the Anova analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk are considered non-significance. Significant: * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$).

The seed growth rate can indicate vigor and growth strength (Nurwiati & Budiman, 2023). The growth rate can be observed using a shorter germination process (Kolo & Tefa, 2016). A high growth rate commonly indicates higher seed vigor. This reflects that the higher the seed growth rate, the greater the seed's ability to grow under suboptimal conditions (Syaban et al., 2023). Based on Figure 5, the results of the analysis of variance showed that the treatment with *Spirulina platensis* biomass at a concentration of 30% and soaking durations of 1, 2, and 3 hours resulted in growth rates of 19,43; 21,76; and 21,77%, compared to the control at 20,13%. Although the results were not significant, the application of biomass at a concentration of 30% for 2 and 3 hours showed higher growth rates compared to the control.

In the treatment with a concentration of 45% and soaking times of 1, 2, and 3 hours, the growth rate percentages were 19,80; 21,34; and 23,99%, compared to the control at 20,13%. Applying seed priming with a 45% concentration for 3 hours showed significant results on the growth rate parameter. Although the 2 hour soaking at 45% concentration showed no significant results, the biomass application demonstrated a higher growth rate percentage than the control. Based on the results, our observation demonstrated a tendency for the longer the soaking time, the more it affects the increase in seed growth rate. This is evident at a concentration of 45% with a growth rate of 19.80% at 1 hour and 23.99% at 3 hours. The result made the application of seed priming at a concentration of 45% for 3 hours provide the best growth rate compared to other treatments (Figure 5). It can be inferred that *Spirulina platensis* biomass has an effect of accelerating seed germination by promoting higher initial growth. The current study was in line with Akgül (2019) research, where the supernatant of *Spirulina platensis* has a plant biostimulant effect capable of accelerating the germination of wheat and barley seeds.

In the treatment with a concentration of 60% and soaking times of 1, 2, and 3 hours, there was an increase in the percentage of seed growth rate (21,27; 23,34; and 23,37%). The 60% concentration treatment for 2 and 3 hours showed significant results. The 60% concentration treatment for 1 hour showed no significant results, but the seed priming application had a higher growth rate than the control. This indicates that seed priming with *Spirulina platensis* biomass provides a better growth rate compared to untreated groups. According to the research by Nurwiati and Budiman (2023), a reasonable seed growth rate ranges from 25-30%. Although in this study the seed growth rate percentage did not reach the ideal target, the increased growth rate in seeds treated with *Spirulina platensis* biomass opens up opportunities for further development in using *Spirulina platensis* as biopriming.

The results of the current study suggest that there is a tendency for a positive correlation between biomass concentration and the rate of increase in growth speed in the seeds. The increase in biomass concentration correlates with a significant rise in the percentage of seed growth rate, from 19,43% to 23,37%. This increase occurred due to using a more concentrated biomass of *Spirulina platensis*. *Spirulina* has a structure of amino acids, polysaccharides, phenolics, proteins, carbohydrates, vitamins, and phytohormones (auxin, gibberellin, and cytokinin). Therefore, the cellular content of *Spirulina* is capable of enhancing plant growth (Singh, 2014).

Simultaneous Growth of Seeds (%)

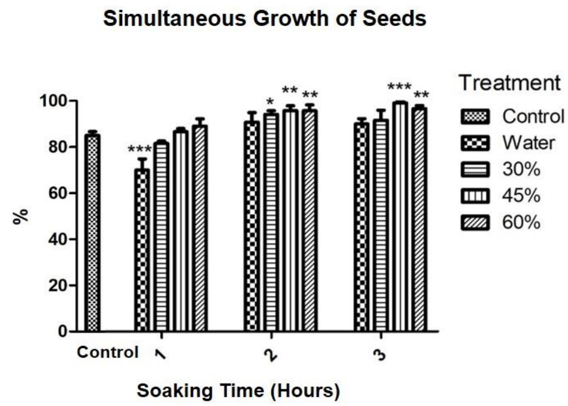

Figure 6. Simultaneous Growth of Seed of Cucumber Seed Under Various Biomass Concentrations and Soaking Time. Observation data were taken once between the first count and final count on the 6th day under the criteria for normal seedling. The data above represent the mean value with SEM error bars from the Anova analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk are considered non-significance. Significant: * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$).

Simultaneous seed growth represents the ability of seeds to form normal seedlings. Simultaneous growth of seed is one of the tests of growth vigor that can be seen from the ability of seeds to grow uniformly. The greater the seed's growth strength, the more uniformly the seeds will be germinating (Septirosya et al., 2024). The simultaneous growth of seeds was calculated based on the number of normal seedlings growing between the first and final count. The seedlings with complete and healthy root, shoot, and normal leaves and growth were considered normal. The simultaneous growth of seed indicates the vigor of a seed lot. Low vigor and viability cause low seedling uniformity (Andayani et al., 2023). Based on Figure 6, the analysis of variance showed that the water treatment and soaking time of 1, 2, and 3 hours resulted in simultaneous growth of seed percentages of 70; 90,83; and 90%, while the control group had a percentage of 85%. Although the water application with soaking time of 2 and 3 hours showed no significant results, these applications demonstrated higher simultaneous growth of seed percentages compared to the control. This indicates that priming treatment with the hydropriming method can enhance germination, particularly in the uniformity of seedling growth. Water plays a crucial role in seed germination, as it is necessary to provide dissolved oxygen for the embryo, soften the seed coat, and increase seed permeability. This finding was in line with the research by Shakuntala et al. (2020) that soaking cucumber seeds using the hydropriming method positively effects seed germination, germination speed, and germination rate.

In the treatment of *Spirulina platensis* biomass at a concentration of 30% with soaking times of 1, 2, and 3 hours, the percentage values of simultaneous growth of seed were 81,67; 94,17; and 91,67%, respectively, with a control value of 85%. Based on the statistical test results, *Spirulina platensis* biomass at a concentration of 30% for 2 hours significantly effected the uniformity of seed growth. Although the 30% concentration for 3 hours had a non-significant effect, this application showed a higher simultaneous seed value growth than the control. This indicated that priming conducted with *Spirulina platensis* biomass can enhance the uniformity of seed germination. Haerani and Nurdin (2021) further asserted that biopriming exhibited a positive response in the parameters of germination power, vigor index, and growth synchrony.

The treatment of *Spirulina platensis* biomass at a concentration of 45% with soaking times of 1, 2, and 3 hours exhibited a simultaneous simultaneous growth of seed percentage values of 86,67; 95,83; and 99,17%, respectively, compared to the control at 85%. Based on the statistical test results, the treatment of 45% biomass with soaking times of 2 and 3 hours showed significant results. Despite being statistically insignificant at 1 hour, the 45% biomass application still demonstrated better simultaneous growth than the control. Furthermore, seed priming at a concentration of 45% for 3 hours provided the best simultaneous growth of seed value compared to other treatments. This indicated that *Spirulina platensis* biomass effects improving seed uniformity by promoting the growth of normal seedlings. The current result was in line with the research by Xie et al. (2022) microalgae have the potential as plant biostimulants, as evidenced by the positive response of microalgae treatment on the parameters of radicle emergence, seed vigor, and seed germination. Additionally, there was a tendency for longer soaking times to show higher values of uniformity in growth. This was proven by applying *Spirulina platensis* biomass at a concentration of 45% with soaking times of 1 – 3 hours, resulting in a simultaneous growth of seed percentage from 86 to 99,17%.

The application of *Spirulina platensis* biomass at a concentration of 60% with soaking times of 1, 2, and 3 hours showed simultaneous growth of seed percentages of 89,17; 95,83; and 96,67%, respectively, compared to a control of 85%. Based on these statistical test results, the 60% concentration for 2 and 3 hours displayed a significant effect on the simultaneous growth of the seed parameter. Although the 60% concentration for 1 hour showed no significant results, it produced better seed germination simultaneity than the control. In addition, there was a tendency that the higher the concentration of biomass used, the higher the seed uniformity value for growing into normal seedlings. This is because the compounds found in the microalgae extract can trigger the simultaneous growth of cucumber seeds. The current study was in line with Susanti's (2014) reports that seeds treated with osmoconditioning using high-concentration solutions can reactivate growth enzymes within the seeds, thereby breaking seed dormancy. In addition, there was a tendency that longer soaking time in seeds can influence plant growth, especially in the parameter of uniformity of growth, as evidenced by the simultaneous growth of seeds value from the lowest concentration of 30% at 81,67% and the 60% concentration reaching 96,67%.

Maximum Growth Potential (MGP)

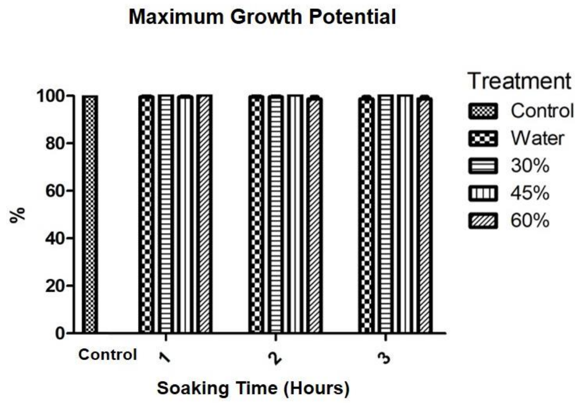

Figure 7. Maximum Growth Potential of Cucumber Seed Under Various Biomass Concentrations and Soaking Time. Observation data were taken on day 8 (final count) stipulating the percentage of normal and abnormal sprouts. The data above represent the mean value with SEM error bars from the Anova analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk are considered non-significance. Significant: * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$).

Maximum growth potential (MGP) is the ability of seeds to grow into normal or abnormal seedlings under optimal conditions. Maximum growth potential describes seed viability, closely related to the seed's ability to germinate and grow at its optimal capacity. Maximum growth potential is influenced by environmental factors such as light, temperature, humidity, and internal factors. A high number of maximum growth potential results from high seed viability conditions (Harsono et al., 2021). Based on Figure 7, the results of the analysis of variance showed that the treatment with 30%, 45%, and 60% concentrations of *Spirulina platensis* biomass and soaking times of 1, 2, and 3 hours on cucumber seeds had a non-significant effect on the MGP parameter compared with the untreated seeds (control) with 100% MGP value. The water treatment for 1, 2, and 3 hours showed MGP values of 99,17; 99,17; and 98,33%. Meanwhile, the seed priming application with a 30% concentration of *Spirulina platensis* biomass for 1, 2, and 3 hours showed MGP values of 100%; 99,17; and 100%. The 45% concentration for 1, 2, and 3 hours showed maximum growth potential values of 99,17; 100; and 100%. Additionally, the 60% concentration with soaking times of 1, 2, and 3 hours showed maximum growth potential values of 100; 98,33; and 98,33%. Although it showed no significant effect, there was no decrease in germination in the maximum growth potential parameter. This indicated that the treatment of *Spirulina platensis* for cucumber seed priming was not toxic and did not have an adverse effect on seedling growth, as evidenced by the absence of a decrease in the percentage of maximum growth potential in each treatment. Seeds are very sensitive or responsive to environmental changes, including the presence of toxic substances. Therefore, seeds have great potential to be used as bioindicators of toxicity (Luo et al., 2018). The response triggered by the seeds is often manifested by a decrease in germination percentage and abnormalities in the seedlings. Our current study was in line with the research by Ribeiro et al. (2019) the supernatant from the culture of *Chlorella sorokiniana* showed no significant difference in seed germination and did not cause toxicity in lettuce seeds.

Shoot Length (cm)

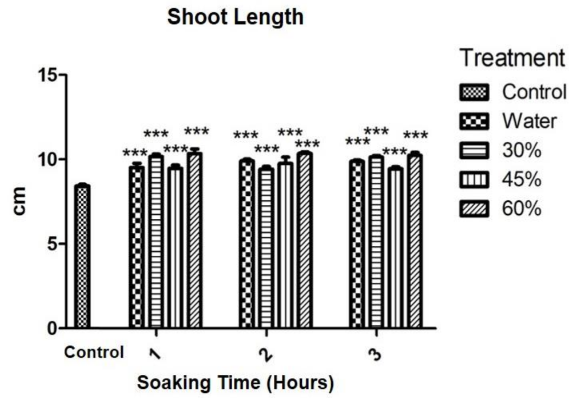

Figure 8. Shoot Length of Cucumber Seed Under Various Biomass Concentrations and Soaking Time. Observation data were taken on day 8 (final count) measuring from the base of the stem to the tip of the plumule bud/growth point in normal seedlings. The data above represent the mean value with SEM error bars from the Anova analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk are considered non-significance. Significant: * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$).

The shoot is the precursor to the stem that grows during the germination phase. Shoot grows and emerges above the ground and is located above the cotyledon. The shoot becomes part of the plant that will develop upwards to form buds and leaves (Parnidi et al., 2022). Based on Figure 8, the results of the analysis of variance generally showed that the treatment with *Spirulina platensis* biomass concentrations of 30%, 45%, and 60% and soaking times of 1, 2, and 3 hours on cucumber seeds significantly affected the shoot length parameter compared to the control. At a concentration of 30%, the application of biomass showed a significant increase at all soaking durations of 1, 2, and 3 hours with the shoot lengths of 10,18; 9,43; and 10.14 cm, respectively. Subsequently, at a concentration of 45%, the application of biomass also showed significance at all soaking durations of 1, 2, and 3 hours, with shoot lengths of 9,48; 9,77; and 9,46 cm, respectively. At a concentration of 60% and soaking durations of 1, 2, and 3 hours, the application of biomass also showed a significant effect on the shoot length parameter, evidenced by shoot lengths of 10,36; 10,37; and 10,24 cm, respectively. Meanwhile, the control only produced a shoot length of 8,43 cm. Based on these results, there was a tendency for a constant increase in shoot length at a concentration of 60% of *Spirulina platensis* biomass over 1, 2, and 3 hours of soaking. This concentration resulted in a more extended shoot compared to the other treatments. This indicated that priming of cucumber seeds using *Spirulina platensis* biomass could potentially increase radicle length. This increase occurred presumably because *Spirulina platensis* contains hormones that can stimulate seed germination. Among the phytohormones processed by *Spirulina platensis* are auxin, gibberellin, and cytokinin. This is in line with the study of Andianingsih et al. (2021) who elaborated that auxin plays a role in stimulating cell elongation processes and gibberellin affects embryo development and germination. The results of this observation are supported by the study of Seğmen and Ünlü (2023), which reported that the phytohormone content of *Spirulina* can enhance cell division and differentiation, enzyme activity, leaf growth, and photosynthetic capacity.

Radicle Length (cm)

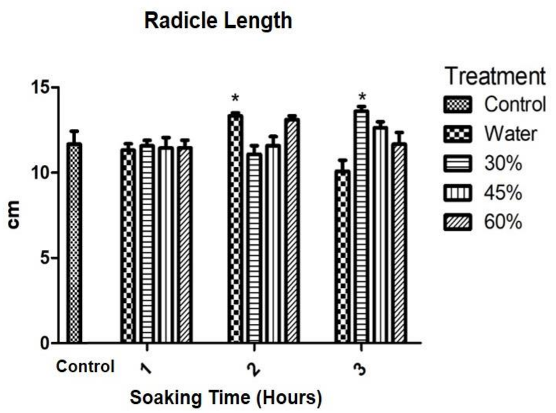

Figure 9. Radicle Length of Cucumber Seed Under Various Biomass Concentrations and Soaking Time. Observation data were taken on day 8 (final count) measuring the root base and the root tip of normal seedlings. The data above represent the mean value with SEM error bars from the Anova analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk are considered non-significance. Significant: * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$).

The radicle is a part of the seed embryo that emerges through the lower part of the microphyll end and will develop into the plant's root. Radicle is the primary root that appears first and will continue to develop to stimulate a complete root system (Dharma et al., 2015). Radicles will develop into the plant's root, which functions to supply nutrients and transport mineral salts from the soil to all parts of the plant. Based on Figure 9, the treatment with a 30% concentration and soaking times of 1, 2, and 3 hours exhibited radicle lengths of 11,57; 11,08; and 13,60 cm, respectively, while a control length displayed radicle length of 11,67 cm. Based on the statistical test results, the 30% treatment with a soaking time of 3 hours significantly effected radicle length compared to the control. Although at the 30% concentration for 1 and 2 hours, there was a slightly observable decrease, the radicle length produced at that concentration did not show a significant decrease. At a concentration of 45% with soaking times of 1, 2, and 3 hours, the radicle lengths were 11,45; 11,58; and 12,64 cm, respectively. Although the seed priming application at a concentration of 45% for 3 hours showed no significant effect, there was a 0,95% increase in radicle length compared to the control. Subsequently, at a concentration level of 60% and soaking durations of 1, 2, and 3 hours, the radicle lengths were 11,46; 13,11; and 11,68 cm, respectively. Although the 60% concentration for 2 and 3 hours indicated no significant effect, this application showed increased radicle length compared to the control. This indicated an influence on the increase in radicle length primed using *Spirulina platensis* biomass. *Spirulina* biomass contains phytohormonal compounds that can enhance plant growth, including auxin. In line with the research by Arahou et al. (2023), it is inferred that microalgae have active molecules in the form of amino acids, polysaccharides, and phytohormones that can promote plant growth and increase tolerance to biotic and abiotic stress.

In the water treatment and soaking durations of 1, 2, and 3 hours, the radicle lengths were 11,32; 13,34; and 10,09 cm, respectively, while the control was 11,67 cm. Based on the statistical test results, the water treatment with a soaking duration of 2 hours showed a significant effect on radicle length compared to the control. This indicated that the application increases the radicle length in cucumber seeds. Although the application of seed priming with water treatment for 1 hour experienced a slight decrease, the radicle length produced at that concentration did not significantly decrease compared to the control. Priming is performed to hydrate the seeds before germination, aiming to balance the water potential to activate metabolic activities within the seeds. According to Rocha et al. (2019), seed priming can maximize germination, resulting in increased germination index, improved seedling strength, and enhanced shoot and radicle length.

Based on the results of the analysis, the seed priming treatment using *Spirulina platensis* biomass at concentrations of 30% and 45% for a soaking duration of 3 hours and 60% concentration for 2 hours, showed an increase in radicle length of 13,60; 12,63; and 13,11 cm. These applications indicated a higher increase in radicle length compared to the control. Additionally, 30% *Spirulina platensis* biomass seed priming for 3 hours showed the most effective treatment for increasing radicle length compared to other treatments.

Dry Weight of Normal Seedlings

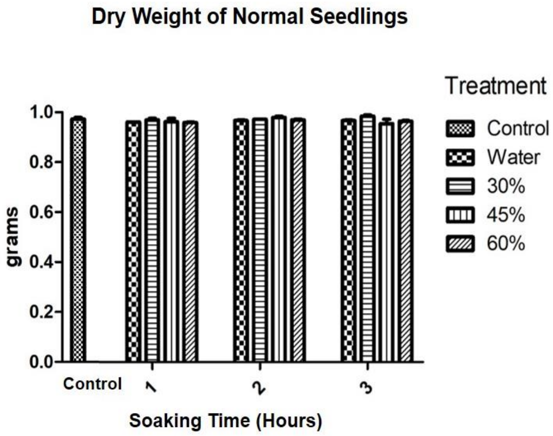

Figure 10. Dry Weight of Normal Cucumber Seedlings Under Various Biomass Concentrations and Soaking Time. Observation data were taken on day 8 (final count) of the dry weight criteria for normal seedlings. The data above represent the mean value with SEM error bars from the Anova analysis with the Bonferroni Post Hoc test compared to the control (untreated group). Values without an asterisk are considered non-significance. Significant: * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$).

The dry weight of normal seedlings is one of the benchmarks used in seed viability testing. Dry weight of normal seedlings reflects an important growth factor that determines crop yield. Dry weight of normal seedlings showed viability that reflects the amount of food reserves available in the seeds (Rolin et al., 2024). The dry weight of normal seedlings is often referred to as the dry weight of seedling cotyledons, which indicates the assimilate content and food reserves. Based on Figure 10, the analysis of variance results showed that the treatment with *Spirulina platensis* biomass at concentrations of 30%, 45%, and 60% and soaking times of 1, 2, and 3 hours on cucumber seeds has no significant effect on the dry weight of normal seedlings parameter. Meanwhile, the control (untreated seeds) showed the dry weight of normal seedlings value of 0,97 grams. The water treatment for 1, 2, and 3 hours showed dry weight of normal seedlings values of 0,96; 0,97; and 0,96 grams, respectively. Meanwhile, the seed priming application with 30% *Spirulina platensis* biomass for 1, 2, and 3 hours showed dry weight of normal seedlings values of 0,96; 0,97; and 0,98 grams, respectively. The 45% concentration for 1, 2, and 3 hours showed dry weight of normal seedlings values of 0,96; 0,98; and 0,95 grams, respectively. Moreover, the 60% concentration with soaking times of 1, 2, and 3 hours showed dry weight of normal seedlings values of 0,96; 0,97; and 0,96 grams, respectively. Although it showed no significant effect, based on these values, no significant decrease in the dry weight of normal seedlings was found between the different treatments. According to the study by Ratnaningtyas et al. (2019), the organ matrix priming treatment with fermented bean seedlings on chili seeds exhibited no significant difference in the dry weight of normal seedlings, indicating that the seedlings were capable of translocating food reserves. In addition, the priming treatment with *Spirulina platensis* indicated no harmful or toxic effects were observed on seedling growth, as evidenced by the absence of a decrease in the dry weight of normal seedlings in each treatment.

CONCLUSIONS

Spirulina platensis can thrive in SME-based media, which was characterized by an increase in cell size and a color change in the cell culture from light to dark green, indicating an increase in microalgal cell density. The treatment of *Spirulina platensis* biomass at a concentration of 45% for 1 and 3 hours increased the vigor index, seed growth rate, and simultaneous growth rate of cucumber seedlings. In the treatment with a 60% concentration and soaking times of 1, 2, and 3 hours, there was an increase in the parameters of mean germination time, vigor index, seed growth rate, and shoot length. Although the 45% and 60% treatments showed no significant differences in the parameters of germination rate, maximum growth potential, and dry weight of normal seedlings, these applications indicated no decrease in seed germination. This indicates that seed priming treatment with *Spirulina platensis* is not toxic to seedling growth, as evidenced by the absence of a decrease in germination rate, maximum growth potential, and normal dry weight of seedlings in each treatment. Additionally, this research explored the potential of microalgae grown on SME-based media and its application to enhance plant growth. The significant improvement in seed growth parameters achieved through *Spirulina platensis*-based priming demonstrates its prospect to enhance crop yield, contribute to global food security, and promote sustainable agricultural practices.

Acknowledgments: The author expresses the most profound gratitude to the supervising lecturer and colleagues who have guided, directed and assisted in the implementation of this research until its completion.

Authors' Contributions: The first author designed the research and prepared the manuscript, the second and third authors conducted the experiments, the fourth and fifth authors interpreted the data, and the last author, as an principal investigator, supervised, directed and guided the experiments.

Competing Interests: The author has no conflicts of interest.

Funding: The author would like to express their heartfelt gratitude to the Ministry of Education, Culture, Research, and Technology (Kemendikbutristek) and Politeknik Negeri Jember for their assistance with facilities and

Student Research Project Funding 2024 entitled "CAPS (Carbon Capture for Seed Priming Using *Spirulina*): The Potential of *Spirulina platensis* Biostimulant as a Sugar Mill Effluent Seed Priming Agent."

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