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# The Effects of Microalgae as a Biostimulant on Seed Germination

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**Summary**

Microalgae have been considered the safe and sustainable new source of biostimulant or soil amendment in organic plant production. As an emerging concept, further research on the effect of different microalgae strains on the production of different crops is needed to develop successful algal biostimulant products. In this study, the effects of microalgae (under different culturing conditions) on seed germination were investigated by treating different plant seeds with microalgae extractions or deionized water. This study included two horticultural crops, basil and tomato, for their fast-growing cycle and evaluation of their nutritional values. Industrial hemp was also included in this study. Seed germination parameters, including daily germination rate, germination index, and seedling growths (root length and shoot length), were evaluated. The results show that the microalgae treatments positively affected the initial seed radicle emergence and final germination percentage of hemp and tomato, respectively. All microalgae treatments had increased the seedling vigor of basil by positively influencing root growth. The results suggest that microalgae have the potential to be used as biostimulants in different crop productions, and further research is required.

## INTRODUCTION

Modern agriculture faces numerous challenges, including increasing global food demand and loss of productivity due to climate change, soil erosion, and other environmental issues such as lack of biodiversity. Meanwhile, better awareness of public food safety has led to the rising demand for high-quality and organically produced agricultural products (Devlet, 2021). To this end, the agriculture industry has been adopting novel and environmentally friendly approaches, such as using plant biostimulants (Colla and Rouphael, 2020). Plant biostimulants are considered as substance(s) or microorganisms that, when applied to plants, can benefit the process of nutrient uptake, nutrient efficiency, and tolerance to abiotic stress, leading to better crop performance independently of its nutrient content (Ricci et al., 2019).

Microalgae, which comprise eukaryotic green microalgae and prokaryotic cyanobacteria (blue-green algae), are praised for their extraordinary capability of production of biomass and various value-added products (Hadipoor et al., 2021), and have been increasingly explored recently as the safe and sustainable alternative source of biostimulant or soil amendment in organic plant production (Colla and Rouphael, 2020). Studies have shown numerous benefits of microalgae, including better seed germination, seedling growth, increased yield, and enhanced tolerance to diseases and environmental stresses (Kim et al., 2018; Martini et al., 2021; Supraja et al., 2020). Although studies have shown that microalgae produce bioactive and signaling molecules such as phytohormones that have biostimulant effects on horticultural and agronomic crops, their targeted applications (e.g., microalgae strains and plant species) and specific mechanism remains unknown or unevaluated (Colla and Rouphael, 2020).

Several important phytohormones in higher plants have also been found within microalgae (Stirk et al., 2014; Stirk et al., 2013). Thus, the biostimulant effects associated with this phytohormone presence detected within microalgae were hypothesized to positively influence the overall crop yields, seed germination and seedling growth, and reduced seedlings diseases such as damping off (commonly found in hemp). Furthermore, the gibberellins (GA) amount was reported to be lower in actively growing cultures compared to slow growing cultures (Stirk et al., 2014). Therefore, this study aims to investigate the differences in effects light conditions have on the endogenous phytohormones within different eukaryotic microalgae strains (*Chlorella* and *Chlamydomonas*) to improve seed germination and seedling growths in different plant species. The objectives of this study are (1) to study microalgae as a source of biostimulant in the seed germination of tomato, basil, and hemp, and (2) to identify the effect of different light conditions on microalgae biostimulant activities by monitoring the seed germination and seedling growth.

## MATERIALS AND METHODS

**Plant material and microalgae strains.** Basil (*Ocimum basilicum* 'Genovese') and tomato (*Solanum lycopersicum*, Homestead 24 red tomato) were included in this study for their fast-growing cycle and evaluation as candidates for organic farming (**Fig. 1 A, B**). Industrial hemp (*Cannabis sativa*) (**Fig. 1C**) was also included in this study due to

its recent legalization and its increased use by Texan farmers. The microalgae strains [*Chlorella Vulgaris* (**Fig. 1D**) and *Chlamydomonas Reinhardtii* (**Fig. 1E**)] used in this study were sourced from the Department of Plant Pathology and Microbiology and the Department of Biology at Texas A&M University.

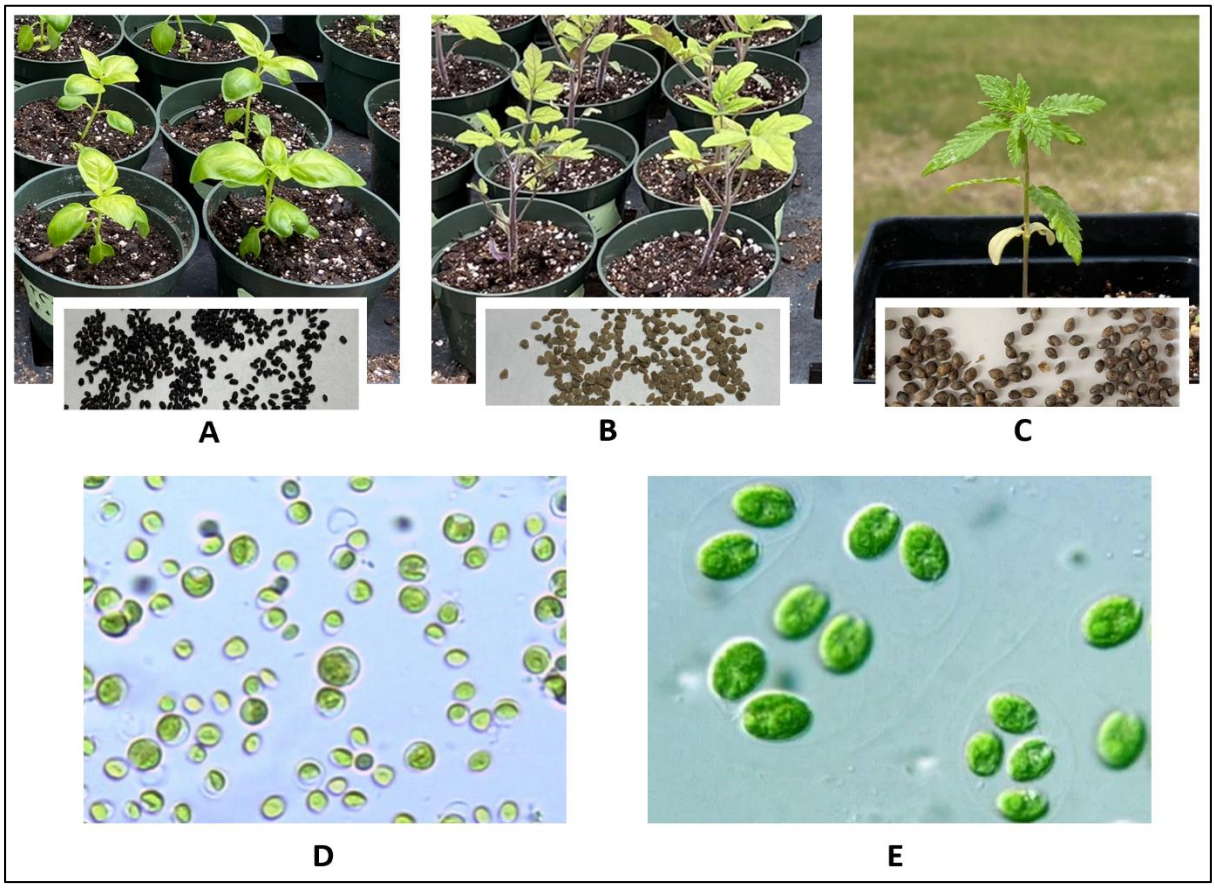


**Figure 1.** Plant material (seeds): (A) basil, (B) tomato, and (C) hemp; and microalgae strains: (D) *Chlorella Vulgaris* and (E) *Chlamydomonas Reinhardtii* used in this study.

**Microalgae culturing and biomass harvesting.** Stock microalgal cell cultures were grown in 1 L TAP (Tris Acetate Phosphate) solutions in a climatic chamber with the following variables controlled: continuous light with a light intensity of (120 µmol $m^{-2}$ $s^{-1}$ PPFD), a temperature of 22 °C and a cell concentration of 1 x $10^{\wedge 7}$ cells/M (**Fig. 2 A, B**). The cultures were then subcultured into 4 L flasks after 96 hours following a one to ten dilutions (**Fig. 2C**). Once the subculture reaches a concentration of 1 x $10^{\wedge 7}$ cells/mL (after 96 hours), they are moved into two lighting conditions: (1) a continuous light (CL) condition (same as described above) and (2) a continuous dark (CD) condition for two days before harvesting.

Microalgal culture (1 x 10^7 cells/mL or more) was harvested by centrifugation at 2000×g (**Fig. 2**); the collected biomass was washed and freeze-dried (**Fig. 2E**). The dried powder (algae biomass) can be used for long-term storage and re-suspended in DI water (0.5 mg/mL) right before use. The algae suspension was treated with sonication for 3 min (e.g., Branson sonicator 150, amplitude 40%, 3 min) to disrupt the cell walls.

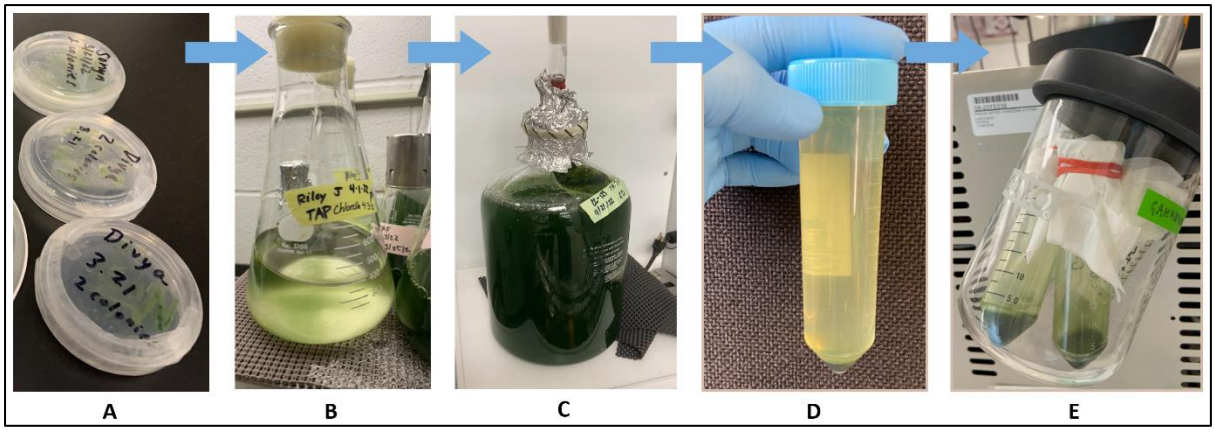


**Figure 2.** Procedure of microalgae culturing and harvesting of biomass: (A) microalgae colonies developed from TAP plates, (B) inoculation in liquid medium, (C) increasing cell density in 4 L container, (D) harvesting by centrifuging, and (E) dried-freezing.

**Seed germination experiments**. Seeds of different plant species were soaked for 3 min in 5% aqueous NaClO for sterilization and thereafter washed three times with DI water. The sterilized seeds [10 seeds per plate (5 replications)] were placed on filter paper in Petri dishes and soaked with 4 mL of microalgae solution or deionized water, respectively. The Petri dishes with treated seeds were then sealed with parafilm and placed in a growth chamber under a temperature of 25 °C. The number of germinated seeds was monitored and recorded daily to determine the germination percentage, and the root and shoot length were recorded at the end of the experiment.

Seed germination data were analyzed using JMP software (JMP Pro16, Statistical Analysis System, Cary, NC, USA). One-way analysis of variance (ANOVA) was used to analyze the number of germinated seeds, root length, and shoot length under different microalgae treatments. The difference in germination percentages was tested using the Adjusted Wald Test for comparing proportions. The multiple means under the treatment groups were compared to the control using Dunnett's test.

## RESULTS AND DISCUSSION

**The effect of microalgae on seed germination**. The germination of seeds in different plant species showed different responses to the microalgae treatments. For basil, the radicle emergence of seeds was observed on day two of the experiments, and the seeds under Chlamydomonas-CD treatment showed a higher response/emergence compared to the control group ($p = 0.0023$) (**Fig. 3A**). Overall, the germination rate of basil seeds was high (>90%) across all treatments and the control group, and most seeds germinated after day three of the experiment (**Fig. 3A**). Microalgae treatments did not show any adverse effects on basil seed germination.

For the tomato seed germination, the highest germination rate was reached approximately after day six of the experiment, while significant differences in germination rates between microalgae and the control group (according to Dunnett's test) were detected on days three and four (**Fig. 3B**).

The germinated seeds in the control group were initially more than the ones under microalgae treatment on day three ($p < 0.001$). However, the final germination rate of the control group (70%) was lower than the *Chlorella*-CD ($p = 0.0067$) and *Chlamydomonas*-CD ($p = 0.0291$) according to the Adjusted Wald Test.

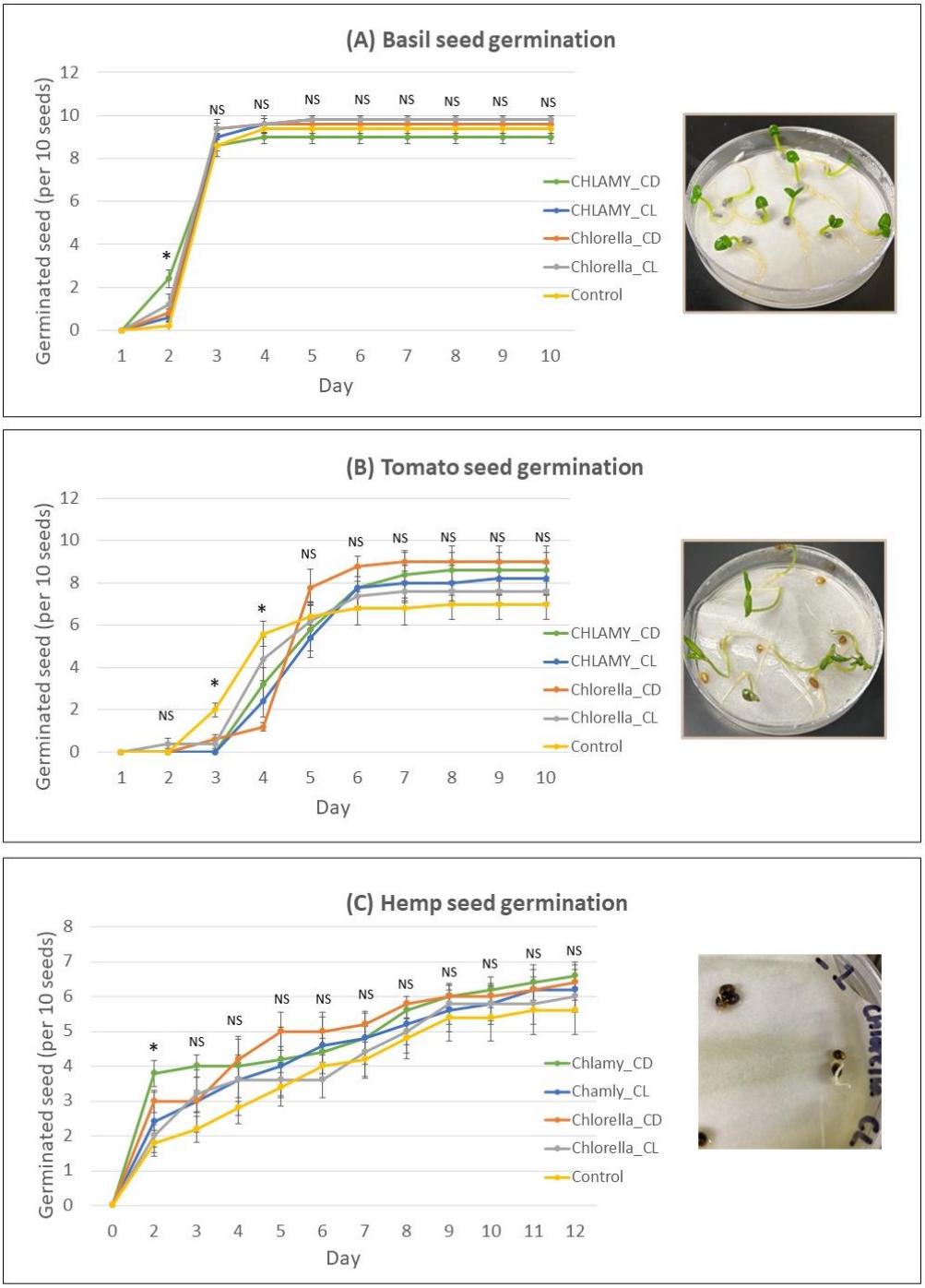


**Figure 3.** Average germinated seed (per 10 seeds) of (A) basil, (B) tomato, and (C) hemp, during the experiment; an asterisk (*) denotes significant differences detected, and 'NS' means no significant difference detected between microalgae treatments and controls at the same day according to the Dunnett's test.

For the hemp seed germination, the effects of microalgae on the radicle emergence were significant, while the difference between treatments and control decreased as time progressed (**Fig. 3C**). On day three, the seeds treated with *Chlamydomonas*-CD had a significantly higher germination rate compared to the control group ($p = 0.0337$). The hemp seeds treated with deionized water (control) are among the lowest germination levels throughout the entire experiment; however, the effects were not statistically significant at the end of the data collection.

**The effect of microalgae on seedling growth.** Different effects of microalgae treatments on seedling growth were observed and specific to different plant species (**Table 1**).

**Table 1.** The effect of microalgae on seedling growth; the same letter in each column denotes no significance detected according to the student's t-test at 0.05 level.

| | Mean length (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Tomato | | Basil | | Hemp | |
| | Root | Shoot | Root | Shoot | Root | Shoot |
| *Chlorella*_CD | $46.56^{ab}$ | $23.98^{abc}$ | $28.69^{ab}$ | $10.96^{b}$ | $11.3^{a}$ | $6.13^{b}$ |
| *Chlorella*_CL | $58.15^{a}$ | $26.32^{a}$ | $26.60^{b}$ | $10.92^{b}$ | $22.21^{a}$ | $5.94^{b}$ |
| *Chlamy*_CD | $43.53^{b}$ | $20.86^{c}$ | $31.02^{a}$ | $12.13^{a}$ | $13.92^{a}$ | $7.94^{ab}$ |
| *Chlamy*_CL | $57.03^{a}$ | $21.38^{bc}$ | $29.98^{a}$ | $9.79^{c}$ | $29.8^{a}$ | $13.21^{a}$ |
| Control | $50.41^{ab}$ | $24.22^{ab}$ | $21.68^{c}$ | $7.09^{d}$ | $19.61^{a}$ | $5.46^{b}$ |

Among the three tested plant species in this study, basil showed the most apparent and positive responses to all microalgae treatments in terms of higher root and shoot growth. For instance, basil seeds treated with the Chlorella-CD or -CL solution had roots grow 32% or 22% in length, respectively, more than the control. Similarly, basil seeds treated with the Chlamydomonas-CD or -CL solution had a more than 30% increase in root growth compared to the control. The basil seedlings treated with deionized water (control) also exhibited the lowest shoot growth (7.09 mm), while the highest shoot length was found in the Chlamydomonas-CD treatment (**Table 1**).

In the tomato experiment, despite *Chlamydomonas*-CL or *Chlorella*-CL solutions resulting in an increased root length growth of 13% or 15% compared to control, the effects of microalgae on root growth were not statistically significant between treatments and the control. On the other hand, tomato seedlings under microalgae treatments and the control group showed similar root and shoot growth, with the only exception of *Chlamydomonas*-CD treatment, which had a shorter shoot length compared to the control ($p = 0.0487$).

For hemp seedlings' growth, there were no statistical effects detected among the treatments and the control in terms of root development. In contrast, microalgae treatments had equivalent or positive effects on the shoot development compared to the control. For instance, the average shoot length of hemp seedlings was more than doubled when treated with *Chlamydomonas*-CL compared to the control ($p = 0.01$). Overall, no negative effects of microalgae treatments were detected on hemp seed growth.

## CONCLUSION

In this study, the effects of microalgae on seed germination were investigated by treating different plant seeds with microalgae extractions or deionized water. The microalgae treatments positively affected the initial seed radicle emergence and final germination percentage of hemp and tomato, respectively. All microalgae treatments had increased the seedling vigor of basil by positively influencing root growth. Overall, the microalgae treatments were an equivalent or positive influence on seed germination and seedling growth in all tested plant species, except for tomato shoot growth when treated with *Chlamydomonas*-CD.

The results suggest that microalgae have the potential to be used as biostimulants in selected crop production; however, further research is required. Irrigation experiments will be needed to further evaluate microalgae treatments on plant growth and yields. In order to gain insight into how microalgae influence plant growth, it is also crucial to conduct different assays to quantify the factors, such as the phytohormone compounds of microalgae under different conditions.

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