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Growth and Germination of Barley Seedlings (*Hordeum vulgare*) using local Freshwater Algae

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A B S T R A C T

While it is true that chemical fertilizers are effective when it comes to increasing production and compensating for a lack of resources, they do result in soil, water, and biological resource depletion and destruction, and the search for alternatives is a considerable focus of much research around the globe. It has become increasingly important to explore the use of beneficial biological organisms, including microbes (which microalgae are generally accepted as being classified as) in industrial agriculture as agents that may assist with fertilization, since they can also contribute to sustainable crop production and potential improvements in food safety. In this study, the potential for freshwater microalgae to act as biofertilizers was explored with a view to improving yield quality and productivity whilst minimizing environmental pollution. The purpose of this study was to determine whether freshwater algae (*Pseudo coccomyxa* sp and *Chlorella* sp) could be used to increase barley (*Hordeum vulgare*) seed germination rates and plant growth through the process of biofertilization. A comparison of barley seed growth with algae applied as a fertilizer before sowing was conducted in three control vessels and three treatment vessels. Weekly monitoring and watering schedules were followed while the barley germinated and grew. There was a significant difference in height, root length, and fresh and dry root, leaf, and stem weights between the experimental and control plants. After six weeks, barley seedlings treated with freshwater algae grew the fastest and gained the most weight. Treatment plants averaged 48 cm in length, while control plants averaged 37 cm. As a result of the treatment, the total fresh weights of the plants were heavier (0.37g) than those of the control plants. In addition, microalgae treated soil retained essential nutrients even after plants were removed at the end of the experiment.

Introduction

It is estimated that there will be 9.7 billion people on Earth by 2050 (Adam, 2021; Calicioglu *et al.,* 2019; DESA UN, 2015; FAO, 2016; FAO, 2017), making food security a crucial issue in the near future. It was suggested by the World Health Organization that global food production should double by 2050, whereas the United Nations recommends doubling it by as soon as 2030 (Wudil *et al.,* 2022). As a result of growing human populations, agricultural sectors are increasingly using chemical fertilizers to produce food.

In agriculture, fertilizers are crucial in increasing yields and market value through large-scale production at low cost, with nitrogen, phosphorus, and potassium playing the most important roles in supporting plant physiology, and therefore growth and function (Dineshkumar, 2018; Barłóg *et al.,* 2022; Morari *et al.,* 2011; Penuelas *et al.,* 2023).

A number of problems and harmful side effects may arise with chemical fertilizers, especially when they are overused (Lin, *et al.,* 2019; Santos *et al.,* 2012; Ramankutty *et al.,* 2018). For terrestrial environments, this can include water retention capacity, soil fertility, and soil nutrient disparities, negatively affecting soil microorganisms (Naidu *et al.,* 2021; Rodriguez-Eugenio *et al.,* 2018). In aquatic environments, chemical residues cause eutrophication, which can result in problem algal blooms and consequent declines in dissolved oxygen concentration, leading to hypoxia, and contributing to poor water quality, the loss of biota and the compromise of entire ecosystems (Bashir *et al.,* 2020). This has led to the development of bio-fertilizers and organic fertilizers, which are recognized as being more environmentally friendly and more economical than chemical fertilizers (Morari *et al.,* 2011; Kumar *et al.,* 2022). In addition, there has been a growing awareness of the harmful effects of chemical products on the environment and the health risks associated with chemical residues in plant tissues in recent years (Naidu *et al.,* 2021), and it is therefore likely that biofertilizers could well gain increased acceptance amongst the general public in the near future (Daniel *et al.,* 2022; Ibáñez *et al.,* 2023).

When bio-fertilizers have been steadily used for many years, parental inoculabe come sufficient to sustain further fertilizer development once they have reached critical mass (Youseff and Eissa, 2014), which can then bolster the cycling of nutrients and the production of crops (Singh *et al.,* 2011). In addition to being cheap fertilizers, they also contain microelements and organic matter, counteracting the negative effects of chemical fertilizers, and also release growth hormones (Gaur, 2010; Daniel *et al.,* 2022). The benefits of this approach are virtually no adverse effects on ecosystems, and the added benefit of producing crops that may actually have a longer shelf life (Khan *et al.,* 2018). As well as fixing nitrogen, solubilizing phosphates, and mineralizing potassium, microorganisms in bio-fertilizers release plant growth-promoting substances, produce antibiotics, and biodegrade organic matter in the soil to provide micro- and macro-elements (Goel *et al.,* 1999; Sinha *et al.,* 2010; Dasgupta *et al.,* 2021). Through incorporation of bio-fertilizers as living microorganisms, soil can be improved biologically and chemically (Dasgupta *et al.,* 2021; Daniel *et al.,* 2022). As reported by Jaiswal *et al.,* (2021), *Rhizobia* spp induce plant growth parameters when inoculated. Ammar *et al.,* (2022) found that nitrogen-fixing algae extracts contain auxins andcytokinins (which both cause root hairs to grow and elongate) and gibberellins. Additionally, these hormones play a crucial role in the proliferation of root hairs, thus leading to a general improvement in plant health. According to Zang *et al.,* (2016), algae application increased vine growth and gibberellins stimulated lateral growth. According to Castro-Camba (2022), algae extracts increased palm and orange tree leaf area and improved N_2 content. The of algae also leads to a reduction in chemical pollution, is an inexpensive source of nitrogen, and is environmentally friendly (Osorio-Reyes *et al.,* 2023). A biofertilizer based on microalgae (rather than continuing to rely on industrial chemical fertilizers) has therefore been recommended by numerous authorities (Osorio-Reyes *et al.,* 2023; Mutum *et al.,* 2022; Parmar *et al.,* 2023; Chaudhury *et al.,* 2022).

Microalgae are a diverse group of microorganisms that stabilize soil, fertilize it, reduce nitrogen and phosphorus runoff, and improve water quality. Furthermore, nitrogen-fixation, photosynthesis, and the provision of sufficient nutrients can be achieved using renewable natural fertilizers (Hedge *et al.,* 1999; Venkataraman, 2020; Walsh *et al.,* 2020). According to Ammar *et al.,* (2022) using bio-fertilizers like cyanobacteria and green micro-algae increases soil nutrient content and allows higher crop yields through nitrogen fixation. The use of algae as a fertilizer for rice plants has been examined in many studies (e.g., Saadatnia & Riahi, 2009; Dineshkumar *et al.,* 2018; Kumar *et al.,* 2017).

To date, however, there have been very few studies conducted into the use of microalgae as a biofertilizer on barley. A cereal grass, barley has the fourth largest global production tonnages globally, with 147 million metric tons produced at last count (Shahbandeh, 2022), putting it behind only maize, wheat and rice (Vasanthan and Hoover, 2009). Australia produces approximately 9-10 million tonnes per year, grown over four million hectares in the southern grain belt (AEGIC, 2022). By providing high stubble levels, breaking disease and weed cycles, improving soil structure, and increasing soil organic matter, barley is an important rotational crop. With shallow furrows and long, smooth, sharp pointed auricles, barley has a light tan-yellow colour and tapering spindle-shape. There are three types of soil groups used to grow barley, namely Sandy Loam, Loam, and Medium/Heavy Black Soils.

In Indo-Gangetic plains, sandy to moderately heavy loam soils with neutral to saline reactions and medium fertility are most suitable for barley cultivation. It can, however, grow on saline, sodic, and lighter soil types. In general, acidic soils are not suitable for barley cultivation (Awad *et al.,* 2021; Cope *et al.,* 2022). An additional reason for choosing barley for these experiments was because barley grows rapidly and responds quickly to nutrient supply (e.g., nitrogen) compared to other cereals (Delogu *et al.,* 1998), making it an ideal candidate for the experiments that were conducted as part of this study, which featured a relatively compressed timeframe.

The key aim of this study was to determine if freshwater algae can be used as a sustainable biofertilizer in barley production to improve yield quality and productivity.

Materials and Methods

Soil samples for the experiments outlined were collected at a depth of 0-5 cm from a backyard garden in Noble Park, Victoria, Australia (37°58'0.01"S, 145°10'0.01"E). The freshwater algae used in this experiment originated from the Aquaculture Training and Applied Research Centre of Melbourne Polytechnic's Epping Campus, where a culture vessel that can grow a number of different varieties of freshwater algae is located.

Traditionally, algae are grown by using organic manure (primarily cow dung) as their sole fertilizer. Algae samples were collected from this culture vessel once the desired cultures had colonized the culture structure and had stabilized. It was found that *Pseudo coccomyxa* sp and *Chlorella* sp were the most dominant algae species within the culture vessel utilized in this study.

The algae were cultured for two weeks at 25°C with 12/12 cycles of light and dark, with light intensities in the region of 2000-2500 Lux. Algae were identified through light microscopy, and cells were measured using a Neubauer haematocytometer following on from the methodologies identified in such studies as Desikachary (1959); Prescott (1970) and Wehr *et al.,* (2014).

Barley seeds were purchased locally and grown organically for sprouting and the production of microgreens (young vegetable greens that are approximately 2.5–7.5 cm tall). This was conducted because a growing number of ready-to-eat markets and dietary supplement companies have shown increasing interest in microscale vegetables in recent years, which have become increasingly popular for homemade food preparations. With simple equipment and supplies, sprouts and microgreens can be produced quickly, easily, and cost-effectively. The developmental process varies from a few days (sprouts) to approximately two weeks (microgreens). Three control cultures (which consisted of soil only, with no fertilizer or microalgae) and three treatment cultures each received 50 seeds sown 1 cm deep. A combined cell count of 5.6×10^{6} cells/mL was achieved when algae cells were incubated with treated soil (Galieni *et al.,* 2020).

The main experiments were conducted at Melbourne Polytechnic's Epping Campus in a glasshouse with a heated bench that maintained a temperature of approximately 25°C. In the experiment, six culture vessels were used, which consisted of three for the control group and three for the treatment group. A total of 3kg of soil was placed in each container. This research methodology was chosen due to similar studies that have been conducted previously, as outlined earlier in this study. The effects of minerals and biofertilizers on barley growth were studied in a pot experiment, where three replicates were grown in a greenhouse using a Randomized Complete Block Design, and roots were measured (both freshly and dried) following the methodology of Mustafa *et al.,* (2007).

Azimi *et al.,* (2015) also conducted the same type of experiment, although it was conducted in the field. In order to meet university deadlines for assessment due dates, this project was somewhat limited in scope and scale, but it was envisioned that the results obtained from such a study could still be relatively conclusive if significant differences in controls and treatments were observed (which they ultimately were).

A total of 500 mL of cultured algae was inoculated into three treatment vessels and thoroughly mixed. After two weeks and six weeks, soil samples from both the control and treatment vessels were analysed. A second inoculation of algae was performed after the seedlings germinated as a rejuvenation measure to ensure that algae was available in abundance, and so that the treatment vessels were not running low. A total of 500mL of no less than 7.9×10^6 cells/mL of algae samples were used for inoculations of each treatment vessel.

A statistical analysis of fresh and dry weight data of the barley was carried out to determine growth rates and germination rates. Drying of the samples was performed using aSelbys Scientific 30L Laboratory Vacuum Oven at 65°C for 24 hours. To calculate the mean, growth performance, and standard error, a Microsoft Excel (Seattle, Washington) spreadsheet was used.

Scientific soil tests were conducted at week 1 and week 6 of the experiment at Nutrient Advantage in Werribee, Victoria (a NATA (National Association of Testing Authorities) -accredited laboratory).

Results and Discussion

Comparisons of crop growth performances in the three control and three treatment culture vessels over six weeks in the Epping Glasshouse yielded some interesting results. Data revealed that the treatment vessels had higher NO_3^--N, K, and P values, with all other values relatively similar, however NH_4^+-N levels in the week 6 treatment were significantly higher (Table 1).

In Table 2, the effect of algae on seed germination is shown, with the treatment vessels having the highest seed germination rates at 91% and the control vessels at 75%.

The average fresh lengths and weights of six randomly selected barley seedlings are listed in Table 3 as means ± SE. Weights were recorded for the shoot, root, leaves, and whole plant, and lengths were recorded for the shoot, root, and overall plant, with the treatment seedlings having the highest averages.

Table 4 shows the average dry weights of six random barley seedlings with means and standard errors. Shoots, roots, leaves, and the entire plant were weighed, with the treatment seedlings having the highest average dry weights.

The barley growth rate performance over 6 weeks in the glasshouse, expressed as average plant heights in cm, is shown in Figure 1. The average length of the plants in the final treatment 3 vessel was 48 cm, the longest of the three treatments. Control 3 had an average final length of 37 cm, which was the smallest vessel plant height on average. There was a significant difference in growth performance between the treatment vessels that received algae fertilizer and those that did not between 3-4 weeks after treatment. In figure 2, total fresh and dry weights were heavier for the treatment plants than the controls ((1.24 g vs 0.37 g and 0.18 g vs 0.25 g, respectively). In comparing the standard error fresh weights between the control and treatment vessels, there is no overlap, indicating that a significant difference might exist, but the dry weights did not differ statistically. The treatment dry weight SE bars show a wide range of data, indicating that the figures are somewhat less reliable, while the fresh and dry control SE bars demonstrate more concentrated data, signalling the plotted average is more likely.

Figure 3 indicates that the fresh and dry weights of the treatment plants (0.75 g and 0.07 g respectively) was higher than those for the control plants (0.21 g and 0.04 g respectively). The level of standard error encountered was relatively tight for all, except for the dry weights of the treatment plants.

When considering root growth, it was evident that treatment plants had the highest fresh and dry weights (0.08 g and 0.05 g respectively) when compared to the controls (0.06 g and 0.05 g respectively), though standard errors indicated that the differences may not have been statistically significant ($p>0.306$).

According to the barley leaf growth performances (figure 5), the treatment plants once again had the highest fresh and dry weights (0.38 g and 0.13 g respectively) weights compared to the control plants (0.10 g and 0.083 g respectively).

In glasshouse conditions, freshwater algae was applied as a potentially ecofriendly organic fertilizer that significantly increased barley seedling growth. Results showed that treatment seedlings had heavier shoots, roots, and leaves, and overall, had the longest lengths, compared to controls that had no fertilizer applied. Similar results were obtained by Sido *et al.,* (2022), who applied *Chlamydomonas applanata* and *Chlorella vulgaris* for the promotion of wheat growth, and who found that the algae treatments were superior to control and urea-based fertilizer treatments (resulting in enhanced shoot fresh weight, root dry weight, leaf length, root length, chlorophyll *a* and chlorophyll *b* contents). In this study, the organic algae fertilizer improved the plants' water retention capacity and overall nutrient availability, as demonstrated by the dramatic decline in dry weights of the controls because of the large total average treatment weights being observed in the treatments. It has been proven that the application of microalgae and cyanobacteria to plants or their derived formulations (biomass, extracts, hydrolysates) produces a wide range of beneficial effects that are often interconnected. The most common effect observed is an increase in yield in leafy vegetables (lettuce, spinach, rocket) and herbs (mint, basil) (Alvarez *et al.,* 2021; Santini *et al.,* 2021; Parmer *et al.,* 2023). It has been reported that plants treated with microalgal extracts had increased plant growth and fresh weight due to a stimulation of nitrogen and carbon metabolism, which resulted in increased leaf, protein, carbohydrate, and photosynthetic pigment (chlorophyll and carotenoid) content (Puglisi *et al.,* 2020). As a result of using *Chlorella vulgaris* and *Scenedesmus quadricauda* on beetroot, Puglisi *et al.,* (2020) observed increased root length and lateral roots, resulting in an increase in root surface area for the uptake of nutrients. When applied directly to the leaves, the biostimulant resulted in considerable growth of the roots and the basal part of the plant, as well as the tissue of the plant itself, and simultaneously induced macro- and micronutrient increases (Prisa and Spagnuolo, 2023; Santini *et al.,* 2021; Singh *et al.,* 2016; Ronga *et al.,* 2019).

As a result of the high potassium and NO_3^- levels in the treatment vessels, the barley seeds were able to use water more efficiently and were therefore potentially more drought tolerant, which is a critical consideration for cultivating crops under Australian conditions (Kebede *et al.,* 2019). The stark contrast that was evident in the controls, where plants could not properly retain water, presumably means that the overall physiology of the plant would have been affected, and which may even have caused detrimental effects in terms of fundamental processes such as photosynthesis.

The relatively high NO_3-N content in the treatment soil was clear evidence of the beneficial biofertilization capacity of the green microalgae being tested during this experiment. Researchers have shown that microalgae are an important source of NO_3^-, which improves soil quality and plant nutrition (Gonçalves *et al.,* 2023; Song *et al.,* 2022; Mücksche *et al.,* 2023).

Both the treatment and control soil tests produced relatively low PBI (Phosphorus Buffering Index) levels, binding minimal amounts of phosphorus. When considered in concert (as is standard practice) with a very low Colwell P, the suggestion appears to be that the control vessels in this experiment would have resulted in relatively minimal phosphorus being available for plant uptake, which is another potential advantage of utilizing microalgae-based biofertilizers (Osorio-Reyes *et al.,* 2023; Gonçalves *et al.,* 2023; Parmar *et al.,* 2023).

The agricultural industry needs organic fertilizers to increase plant growth and yields, protect soil fertility, and increase soil aggregation and organic matter. As algae release amino acids, phytohormones, carotenoids, and vitamins, they prevent nutrient losses, while promoting plant growth and performance, as evident in the growth performance of algae-treated plants (Ammar *et al.,* 2022; Kumar *et al.,* 2022; Solomon *et al.,* 2023).

Microalgae mixes significantly increased barley seed germination under glasshouse conditions, and this study indicated that barley plants grew faster, had longer shoots, and had longer roots in soils pre-mixed with algae. The use of microalgal extracts in lettuce, red amaranth, pak choi, tomato, and pepper has been demonstrated to stimulate germination, seedling growth, shoots, and root biomass under greenhouse and open-field conditions in recent years (Faheed and Abd-El Fattah, 2008; Garcia-Gonzalez and Sommerfield, 2016; Barone *et al.,* 2018a, b and El Arroussi *et al.,* 2018).

Faheed and Abd-El Fattah (2008) reported that a *C. vulgaris*-containing medium promoted lettuce growth (on both a fresh and dry weight basis) at early stages of development (at 2 and 3g of dry microalgae extract per kilogram of soil). In a similar study, carotenoid and chlorophyll pigment biosynthesis was stimulated, which led to improved plant growth (e.g., shoot, root, and leaf lengths). *Spirulina platensis* was also known to enhance the growth of rocket, bayam reds, and pak choi plants (Wuang *et al.,* 2016). According to Garcia-Gonzalez and Sommerfield (2016) and El Arroussi *et al.,* (2018), microalgal extracts are beneficial to fruits such as tomatoes and peppers.

Future research regarding the use of microalgae as an eco-friendly organic fertilizer is needed (as has been the urging of many researchers) in order to ensure this will positively impact crop growth on a large scale (Ferreira *et al.,* 2023; Gonçalves *et al.,* 2023; Alvarez *et al.,* 2021; Solomon *et al.,* 2023; Song *et al.,* 2022; Mückschel *et al.,* 2023). It may be necessary to conduct further research on how algae grow on various crops and possibly in the field under normal environmental conditions.

Table.1 Physical and chemical characteristics of the soil.

Culture Vessels	pH (1:5 Water)	pH (1:5 $CaCl_2$)	NO_3-N (mg/kg)	NH_4-N 9mg/kg)	P (Colwell) (mg/kg)	PBI-Col	Available K	Ca (Amm-acet.) %	Mg (Amm-acet.) %	Ca/Mg Ratio
Week 1 (before the seeds sowed)										
Control	7.4	6.8	21.0	5.8	220	73	570	69%	19%	3.5
Treatment	7.4	6.9	60.0	5.0	280	94	700	68%	20%	3.4
Week 6 (after the completion of the experiment)										
Control	7.5	6.9	4.5	6.4	220	98	580	69%	20%	3.5
Treatment	7.6	7.1	650	170	300	110	670	54%	16%	3.2

Table.2 The effect of algae on seeds germination

Recorded parameters	Control	Treatment
Number of seeds sowed	150	150
Number of seeds germinated	113	137
Percentage of seed germination	75.33%	91.33%

Table.3 Average fresh length and weight of barley seedlings (values are means ± SE)

Recorded parameters	Control	Treatment
Shoot (gm)	0.21 ± SE 0.02	0.75 ± SE 0.04
Root (gm)	0.07 ± SE 0.01	0.09 ± SE 0.01
Leaves (gm)	0.10 ± SE 0.01	0.38 ± SE 0.03
Whole plant (gm)	0.37 ± SE 0.03	1.24 ± SE 0.06
Shoot height (cm)	40 ± SE 1.00	43.6 ± SE 1.26
Root length (cm)	12.3 ± SE 0.70	15.0 ± SE 1.10
Total length (cm)	26.13 ± SE 3.41	29.25 ± SE 3.61

* Significant at the 0.05 level

Table.4 Average dry weights of barley seedlings (values are means ± SE)

Recorded parameters	Control	Treatment
Shoot (gm)	0.05 ± SE 0.003	0.08 ± SE 0.003
Root (gm)	0.05 ± SE 0.01	0.05 ± SE 0.004
Leaves (gm)	0.08 ± SE 0.01	0.13 ± SE 0.01
Whole plant (gm)	0.18 ± SE 0.01	0.25 ± SE 0.004

* Significant at the 0.05 level

Figure.1 Barley growth performances over 6 weeks, expressed as average plant heights.

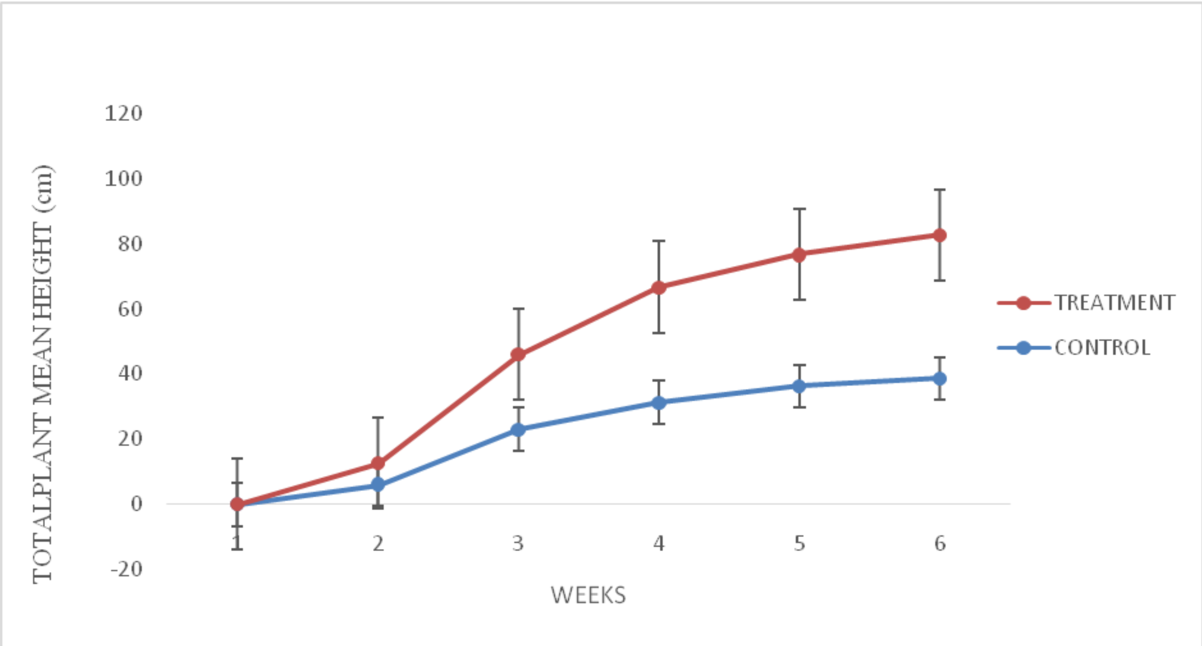


Figure.2 Barley growth performances expressed as average plant fresh and dry weight (g).

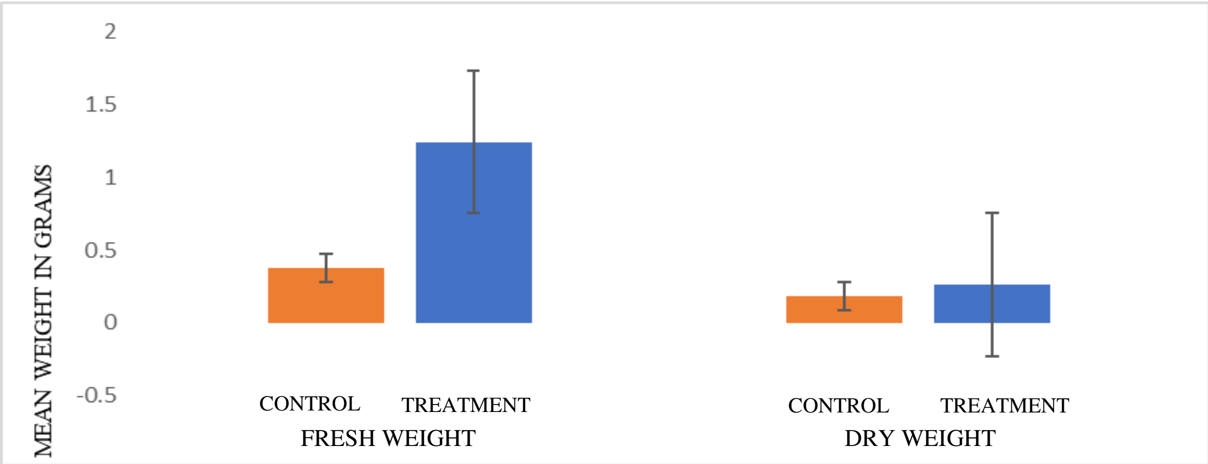


Figure.3 Barley shoot performances expressed as average plant fresh and dry weight (g).

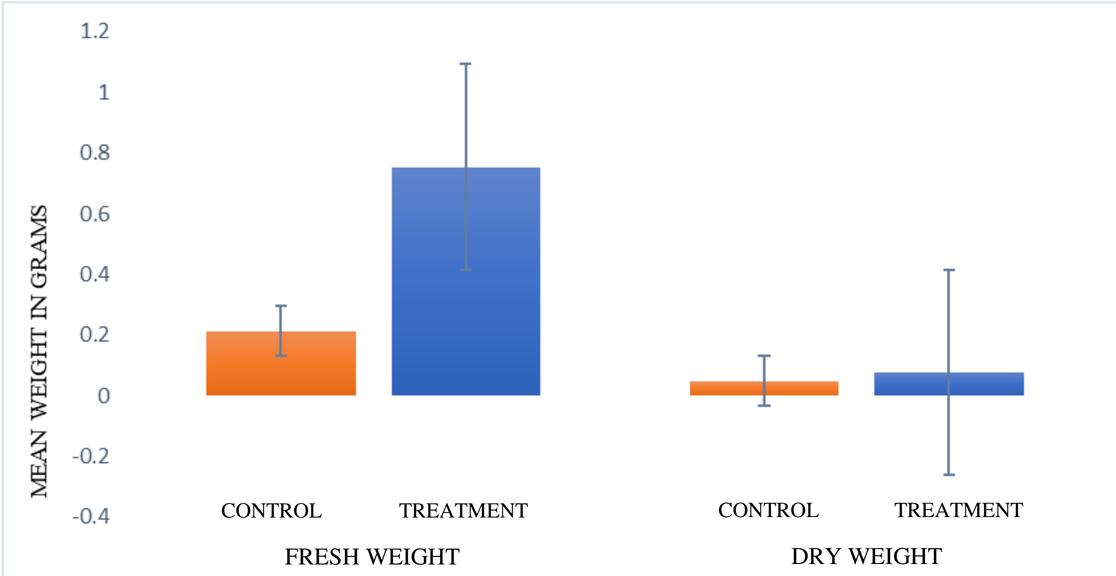


Figure.4 Barley root performances expressed as average plant fresh and dry weight (g).

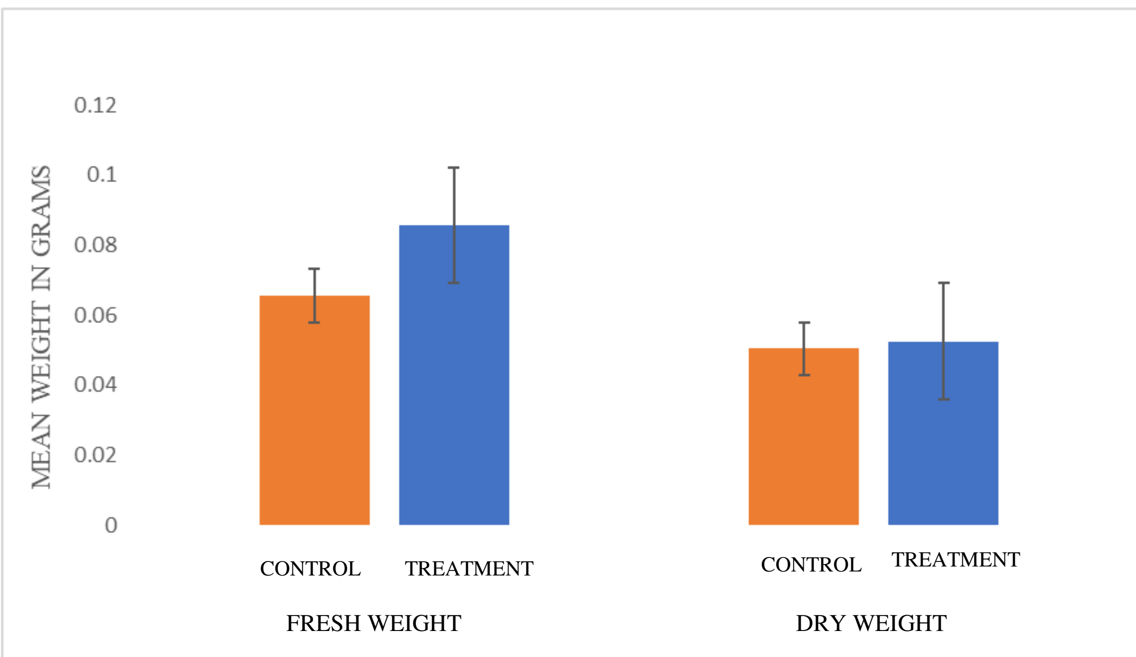
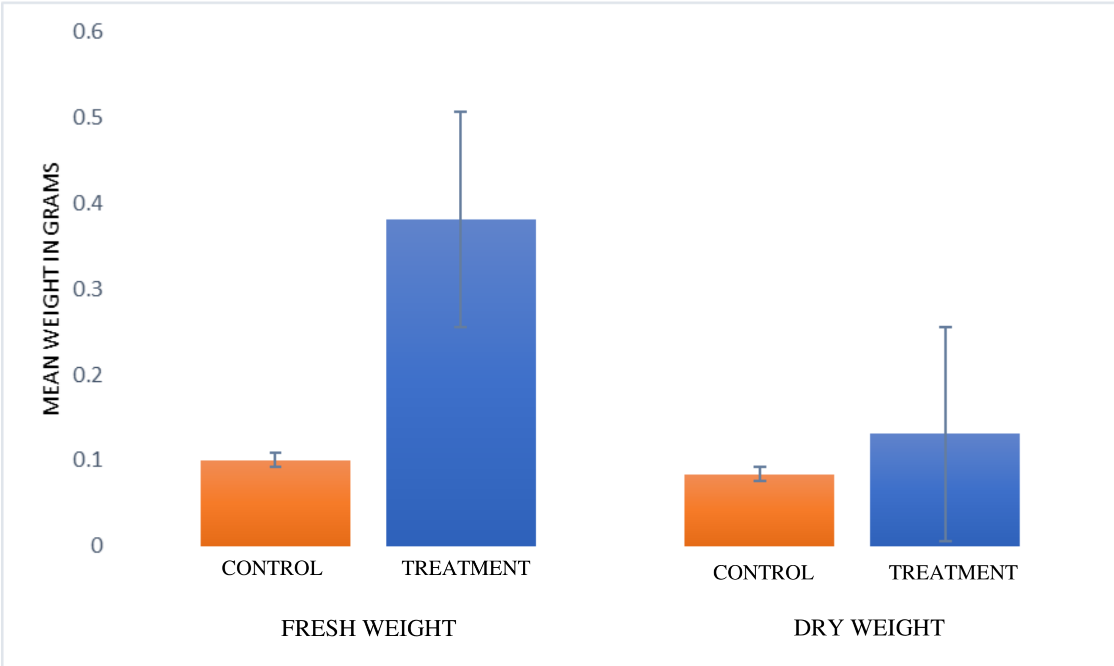


Figure.5 Barley leaves biomass expressed as average plant fresh and dry weight (g).



Algae fertilizer products should be readily available in the future, as they are environmentally friendly, improve soil fertility for future crops, and increase plant growth and yields. While these conclusions make sense for all intents and purposes, there is always the chance that researcher bias can creep in and affect the outcome of studies. For this particular investigation, a significant level of care was taken to ensure that root lengths and weights were not being unfairly impacted by roots breaking off during the removal process, which could have unfairly impacted results. Algae fertilizer was only tested on barley seedlings for a short period of six weeks, instead of until full maturity, which would have provided data on the full spectrum of growth throughout the life of the plant. The authors of this study recognise too that the number of replicates used in this particular experiment were relatively minimal, and would benefit from being considerably expanded and replicated.

From the conduct of this study, it can safely be stated that freshwater microalgae positively affect barley seedling growth, development, and weights to a statistically significant degree, thereby ensuring water retention and drought tolerance. The positive effects and impacts of microalgae-based fertilizer in promoting plant growth and development is surely worthy of further experimentation and investigation. The developments highlighted in this study can potentially be utilised by agronomists and cropping farmers to improve soil fertility, plant growth, and performance for the agricultural cropping industry, and thus represents a fascinating and exciting field of further research.

Author Contributions

M. McCullen: Investigation, formal analysis, writing—original draft. S. Awal: Validation, methodology, writing—reviewing. A. Christie:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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