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An investigation of the effect of locally grown freshwater microalgae suspensions on the germination and growth of wheat (*Triticum aestivum* L)

Abstract

Biological fertilizers are capable of supplying unavailable nutrients in a form that is available to plants during biological processes, thus providing favorable conditions for germination and growth. In pot culture conditions, micro algae were tested for their efficacy as biofertilizers of wheat (*Triticum aestivum* L.) cultivation. Four treatments were performed: control treatment (P2C), inorganic fertilizer treatment (P2F), Azolla powder treatment (P2AZ) and freshwater algae treatment (P2A). Freshwater microalgae (P2A) promoted wheat growth more effectively than Azolla powder (P2AZ) and inorganic fertilizer (P2F). As compared to the other three treatments, P2A significantly increased shoot fresh weight by 0.6 g and root dry weight by 0.016 g. With microalgal-treated soil, all other parameters increased including seeds germination, shoot and root elongation, and fresh and dry weight. As well, when compared with Azolla powder treatment, fertilizer treatment, and control treatments, microalgae treatments led to the highest increases in soil nutrients. There was a significant increase in N-NO3 for 98%, P for 90%, and the Na/Mg ratio for 40% in that microalgae-based soil. As a result, soil treated with freshwater microalgae retained more major nutrients (NPK) than controls and other treatments. In general, this study showed that freshwater microalgae, primarily *Chlorella* sp. and *Pseudococcomyxa* sp., could improve soil properties and promote wheat growth. Therefore, microalgae can be used as biofertilizers to reduce chemical fertilizers and promote sustainable agriculture.

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Introduction

By 2050, it is predicted that global crop demand will rise 60–110%, resulting in huge environmental impacts.[1-5] Chemical fertilizers have been used by farmers for a long time to increase food production; they increase input costs, reduce soil fertilizer utilization, cause soil agglomeration, and decrease biodiversity. The development of agricultural production in a sustainable and stable manner will be seriously compromised as a result.[6-8] Globally, the consequences are already evident. Furthermore, fertilizer accumulation in soil can cause large-scale soil and water pollution through runoff and leaching, making human health and the environment at risk.[9,10] Therefore, preventing food shortages without accelerating environmental pollution and ecological degradation will be a fundamental challenge.[6,11-13]

Several researchers[14-16] agree that brown algae are generally higher in potassium, and commonly used in agriculture, including *Turbinaria* spp., *Laminaria* spp., Fucus spp., and Sargassum spp. The aquatic pteridophyte Azolla is one of the best biofertilizers and green manures of freshwater owing to its high biomass production and wide distribution.[17,18] Both tropical and temperate climates support Azolla growth in freshwater ponds, paddy fields, and ditches.[19] This fern reproduces rapidly and sustains its biomass primarily by vegetative reproduction.[20] Many countries use Azolla as green manure and animal food, including China[21] India[22] Bangladesh[23] Vietnam[24] Tanzania,[25] Niger[26] and Kenya[27] as biofertilizers for improving rice yields.

Due to their autotrophic metabolism, microalgae produce fat, protein, pigment, and polysaccharides, which lead to fast photosynthesis, rapid reproduction, and strong environmental adaptation. A growing number of researchers believe they are able to solve major practical problems including food shortages, greenhouse effects, environmental pollution, and energy shortages.[28-30] Although they are widely used in fuels, medicine, cosmetics, animal feed, and sewage purification,[30-33] their use in agriculture is rarely acknowledged.[6,34,35] Despite the fact that many studies have focused on microalgal resources in water, such as saltwater and freshwater, few have explored microalgal resources in more complex and diverse soil environments. In agriculture, many microalgal resources are still in the early stages of development. Microalgae have been shown to enhance plant yields and quality when they improve soil structure and fertility, promote beneficial soil microorganisms, and balance the soil micro ecosystem.[36-37]

A particular objective of this study was to investigate whether locally grown freshwater microalgae are suitable for replacing or supplementing chemical fertilizers in agricultural production by using them as a bio-fertilizer for wheat (*Triticum aestivum*). The study focused on determining how algae inoculation affects wheat germination and growth, including the weights and lengths of plants, as well as soil properties.

Materials and Methods

Algae collection and preparation of Azolla powder

An algal culture vessel of 100 litres is located at Melbourne Polytechnic's Epping Campus and is used to grow freshwater algae in an open environment. Stock cultures of algae are naturally grown with organic manure, predominantly cow dung. A total of 7 litres of algal suspension were collected from this culture vessel. At Melbourne Polytechnic's Aquaculture Training and Research Centre (ATARC), the suspension was further cultured at 22°C with 12/12 cycles of light and dark (2500 Lux) for two weeks. In order to confirm exponential growth, a Nauber haematocytometer was used to determine the cell number of the algal suspension when it reached its peak concentration (Figure 1).

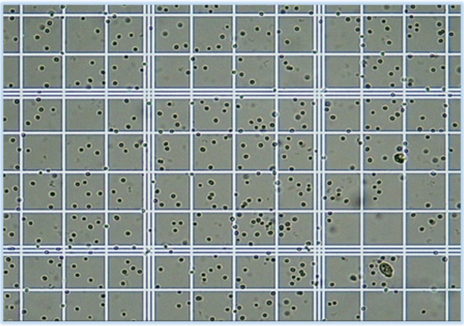

Figure 1 Algae counting using nauber haematocytometer.

The species of algal cells were then identified using light microscopy in accordance with Bellinger and Sigee, Entwisle et al., Prescott.[38-40] The suspension contained *Cosmarium* sp., *Clamydomonas* sp., *Haematococcus* sp., *Chlorella* sp., *Pseudococcomyxa* sp., *Chroococcus* sp., and *Microcystis* sp. *Chlorella* sp. and *Pseudococcomyxa* sp. were the dominant species detected in this study (Figure 2). A low-speed centrifuge (1500 g) was used to collect microalgal biomass, which was then re-suspended in sterilized distilled water to remove the growth medium.[41] Afterwards, the suspension was again passed through GF/C filters[42] and kept in an oven at 60°C temperature for 48 hours to obtain dry powder.[43,44]

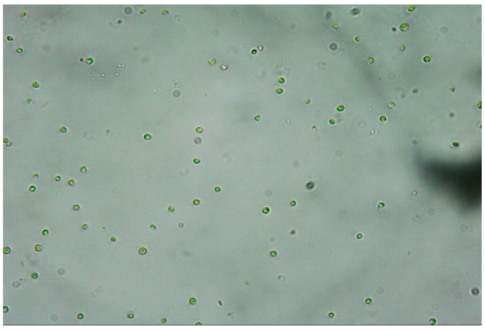

Figure 2 The dominating *Chlorella* sp:

According to Jumadi et al.,[45] *Azolla filiculoides* was collected and prepared using a manual method. It was thoroughly washed with distilled water, then dried in an oven at 60°C until the moisture content was stable at around 10%. In the final step, the dried Azolla biomass was crushed and passed through a 2-millimeter sieve.

Wheat experimental cultivation

The main experiments (P2) were conducted in a glasshouse on the Melbourne Polytechnic Epping Campus with a heater under the benches to maintain an ambient temperature of about 20°C for 80 days. Wheat seeds were collected from La Trobe University Centre for AgriBiosciences. A total of eight culture vessels with dimensions of 34.5 cm x 25.5 cm x 14.5 cm were used in the experiment. Two for the control soil (P2C), two for inorganic fertiliser-treated soil (P2F), two for Azolla-treated soil (P2AZ), and two for microalgae-treated soil (P2A). Figure 3 shows the experimental design for this experiment. A similar experiment was conducted by Sido et al.,[46] For each P2F, 2g.kg^{-1} soil multipurpose inorganic fertilizer (NPK) was used according to Khair et al.,[47] Each P2A included 3g.kg^{-1} soil g microalgae powder.[48-49] The Azolla powder was mixed in two other vessels with 3.2 grams per kilogram of soil.[50] Nothing was mixed in two control vessels. The pots were left for seven weeks, including the control and treatment.

CONTROL P2C1	CONTROL P2C2	FERTILIZER P2F1	FERTILIZER P2F1
AZOLLA P2AZ1	AZOLLA P2AZ2	ALGAE P2A1	ALGAE P2A2

Figure 3 Design and layout of the experiment.

Soil testing

To establish a baseline, soil samples were collected from each treatment and control before seeds were inoculated. At Nutrient Advantage in Werribee, Victoria, a NATA (National Association of Testing Authorities)-accredited laboratory, soil tests were conducted before and after the experiment.

Seed sowing and germination

In each pot, 30 healthy seeds were sown 1 cm deep. A total of 240 seeds were used. A germination rate was calculated for each pot after the seed germinated completely. After the seeds germinated and the seedlings were 2-3 cm long, fertilizer, Azolla, and algae powder were added a second time. A control group did not receive any additional supplements.

Growth study

For the weekly growth study, six randomly selected plants were measured using the method described by Dineshkumar et al.[51] Following the method described by Dineshkumar et al.,[51] fresh and dried shoot and root weights were measured at the end of the experiment. Data were analyzed using Excel spreadsheets to determine the mean, standard error, and growth performance.

Statistical analysis

The data are described using the mean with standard deviation. An analysis of variance was conducted using R commander, a statistical analysis software, version 4.1.0 (2021-05-18) with p 0.05 significance level and letters based on Tukey's test. Plotting was done using Origin 2021 software.

Results

Changes in soil properties

Soil chemical qualities include the levels and availability of nutritious mineral components for plants, as well as soil chemical factors related to their repair or availability. The nitrogen and phosphorus as well as other important component values are illustrated in detail in Table 1.

Seeds germination in different treatment

Seed germination began on the sixth day after cultivation, and germinated seeds were counted and recorded on that same day. The germination efficiency of seeds was determined by Abbasi et al.[52] by calculating the germination percentage (Table 2).

Table 1 Changes in physical and chemical characteristics of soil.

Parameters	Pre-experiment				Post experiment			
	Control	Fertilizer	Microalgae	Azolla	Control	Fertilizer	Microalgae	Azolla
available Potassium (mg/kg)	570	730	630	640	520	800	1000	740
pH (1:5 $CaCl_2$)	6.8	6.5	7.2	6.8	6.8	5.7	6.1	6.8
Nitrate Nitrogen (mg/kg)	43	320	55	74	40	320	460	100
Ammonium Nitrogen (mg/kg)	4.5	84	9.2	5.7	2.3	2.5	31	2.6
Sulphur (KCl 40) (mg/kg)	49	92	27	61	47	57	110	85
Phosphorus (Colwell) (mg/kg)	210	340	260	250	220	350	490	290
Phosphorus Buffer Index (PBI-Col)	73	82	130	78	77	70	110	83
Potassium (Amm-acet.) (cmol(+)/kg)	1.5	1.9	1.6	1.6	1.3	2.0	2.6	1.9
Calcium (Amm-acet.) (cmol(+)/kg)	14	12	13	14	12	11	12	13
Magnesium (Amm-acet.) (cmol(+)/kg)	3.9	3.6	3.9	3.8	3.4	3.1	4.1	3.6
Sodium (Amm-acet.) (cmol(+)/kg)	0.53	0.43	0.73	0..50	0.49	0.37	1.1	0.57
Aluminium (KCl) (mg/kg)	<9.0	<9.0	12	9.3	9.5	15	12	<9.0
Aluminium (KCl) (cmol(+)/kg)	<0.10	<0.10	0.13	0.10	0.11	0.16	0.14	<0.10
Cation Exchange Capacity (Amm-acet.) (cmol(+)/kg)	19.4	18.1	19.4	19.9	17.8	16.9	19.9	18.8
Sodium % of cations (%)	2.7	2.4	3.8	2.5	2.8	2.2	5.6	3.0
Aluminium % of cations (%)	<1	<1	0.66	0.52	0.59	0.96	0.69	<1
Calcium/Magnesium Ratio	3.6	3.3	3.3	3.7	3.5	3.5	2.9	3.6
pH (1:5 Water)	7.4	6.8	7.9	7.4	7.4	6.1	6.5	7.2
Electrical Conductivity (1:5 Water) (dS/m)	0.26	0.71	0.24	0.33	0.29	0.74	1.19	0.47
Chloride (mg/kg)	52	69	110	77	47	41	220	94

Table 2 Seeds germination rate in different treatments

Treatments	Number of seeds sowed (N)	Number of seeds germinated (Ni)	Percentage of seed germinated (GP= (Ni/N)*100
Control	30	16	53.3
Fertilizer	30	19	63.3
Azolla	30	26	86.6
Microalgae	30	30	100

In order to facilitate data collection, ten random shoot length were measured every week over a period of seven weeks as shown in Figure 4. To quantify the baseline uncertainty between the treatments, the treatments were repeated two times to increase the variable as well as the bounds of standard deviation from this data collection have been employed as precise objectives for routine tracking of composition data and quality evaluation of during production tests as shown in Figure 5.

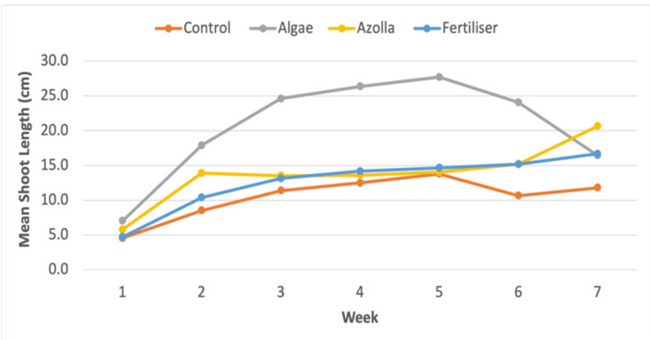

Figure 4 Mean shoot length weekly (cm).

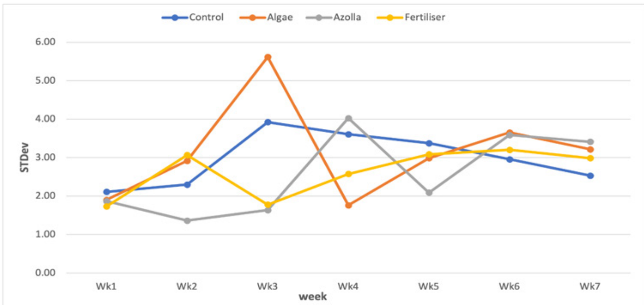

Figure 5 Average amount of variability weekly in four treatments (cm).

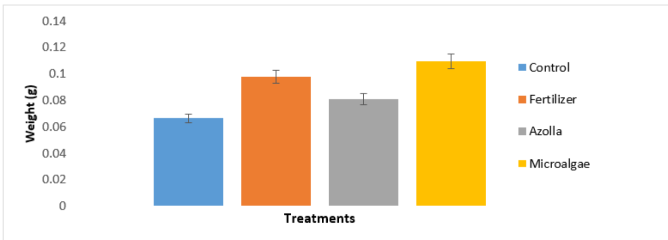

Figure 6 Raw root weight in four treatments.

The data presented in Figure 6 to Figure 11 is a well-established summary of statistics and metrics. Even before censoring, the dataset for algae was more than expected as compared to the Azolla treatment.

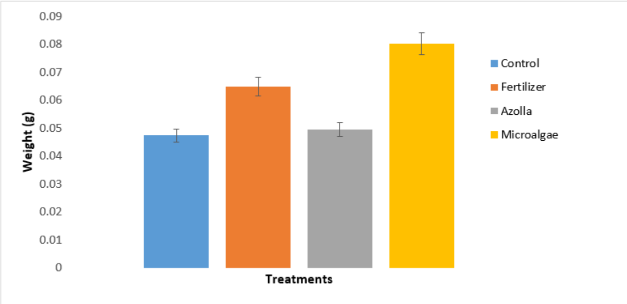

Figure 7 Dry root weight at in four treatments.

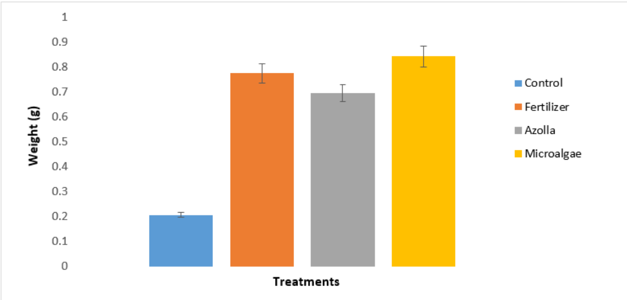

Figure 8 Raw shoot weight in four treatments.

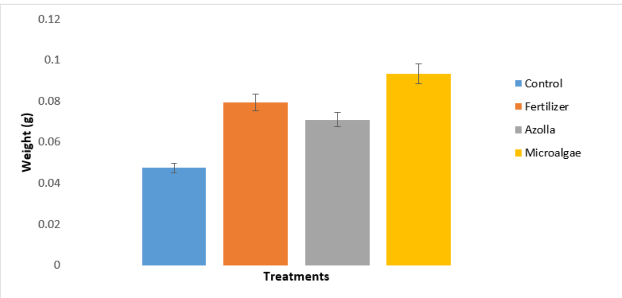

Figure 9 Dry shoot weight in four treatments.

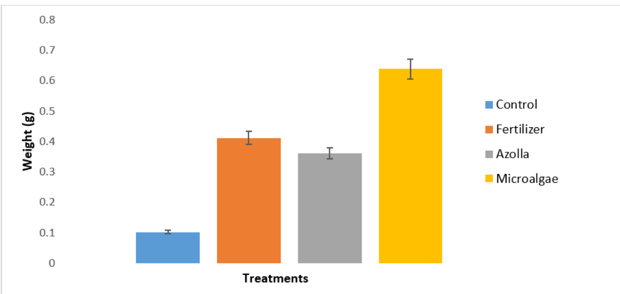

Figure 10 Raw leaves weight in four treatments.

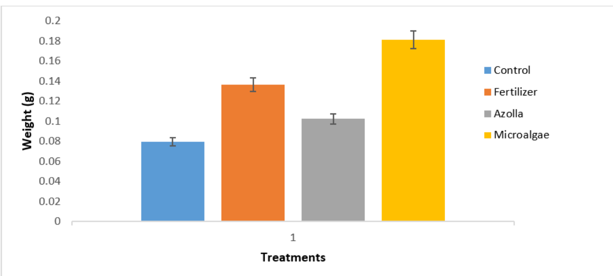

Figure 11 Dry leaves weight in four treatments.

Figure 12 shows the soil nitrate-nitrogen concentrations after the experiment compared to the beginning. As shown in figure 12, the microalgae treatment had lower phosphorus and potassium levels at the beginning than at the end of the experiment.

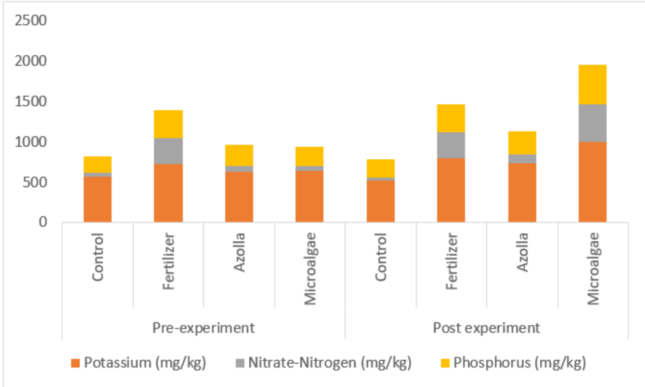

Figure 12 Pre- and post-experiment soil nutrients (NPK).

Discussion

This study found that microalgal biomass was a beneficial biofertilizer for wheat plants, improving their height, root weight, shoot weight, and NPK retention in the soil. Kholssi et al.[53] found significant increases in fresh and dry weights of roots and shoots after adding algal extracts. As a liquid fertilizer, Taha and Youssef[54] obtained similar results on other plants (other than wheat) when applying C *oocystoides* and C *minutissima* as liquid fertilizer to Zea mays plantlets. Using *Chlorella vulgaris* as a germination promoter, Faheed & Abd-el Fattah[44] found that *Lactuca sativa* seedlings germinated more effectively, grew faster, and contained more pigment. Abd et al.,[55] found that the application of seawater with extracts from *C. ellipsoidea* and *S. maxima* a wheat plants generated an increase in Chl-a production between 0.53–0.61 and 0.53–0.84 mg g^{-1} fw, compared to plants irrigated only with seawater, which generated 0.32 mg g^{-1} fw of Chl-a. Grzesik et al.[56] demonstrated that *Chlorella* sp. increased the growth and physiological performance of willow plants when applied foliarly. According to Eifediyi and Remison,[57] inorganic fertilizer and farmyard manure fertilizer were comparable in terms of plant height and shoot weight. As well, a previous study found that Spirulina platensis increased leaf number, plant height, and root length.[58] After reviewing all the results, it was evident that microalgal biomass is a top-notch fertilizer.

In various plants, green algal microalgae, including *Acutodesmus dimorphus*, *C. vulgaris*, *Scenedesmus quadricauda*, *Chlamydomonas reinhardtii*, *Chlorella sorokiniana*, *Asterarcys quadricellulare, Dunaliella salina,* and *Chlorella ellipsoidea*, have been studied for their fertilizer properties.[59-62] Biomass or extracts of green algal algae can positively affect plant growth through phytohormones, exopolysaccharides, and nutrient availability.[61,63]

Rahman and Zhang[64] report that excessive chemical fertilizer use decreases crop yields and significantly increases soil pollution. It is necessary to employ innovative technologies in agriculture to improve yields, minimize inputs, and protect the environment.[6,65,66] Biofertilizers use living microorganisms, natural compounds, or plant-derived substances, such as bacteria, fungi, and algae, to improve soil chemical and biological properties, stimulate plant growth, and restore soil fertility.[37,67] Several reports provide insight into the potential use of microalgae as biofertilizers, considering that microalgae contain biochemical components, bioactive metabolites, micronutrients, and macronutrients, including proteins, carbohydrates, lipids, phytohormones, carotenoids, and vitamins. Plant growth would be benefited by increased nutrient absorption, biomass accumulation, and crop yields.[28,44,68] In addition to being environmentally friendly and economically viable, microalgal biofertilizers have the potential to replace chemical fertilizers. As a result of their use, these plants not only increase agricultural productivity, but also decrease environmental pollution.[6,69] Using microalgal biofertilizers to replace chemical fertilizers improves soil health, stabilizes soil aggregates, retains water better, prevents nutrients from being lost, and sequesters carbon dioxide.[70,71] The use of microalgae in ecological crop production, soil remediation, and adverse conditions of changing climate has recently gained increasing worldwide attention, but microalgae with biofertilizer properties remain largely unknown.[37,56] In this study, freshwater microalgae (mostly *Chlorella* sp. and *Pseudococcomyxa* sp.) promoted crop growth and soil nutrition, thereby promoting the use of microalgae as biofertilizers to reduce chemical fertilizer usage.

Conclusion

Algae contain several bioactive compounds that function as beneficial biofertilizers. Microalgae may also be used to improve soil quality. Due to the use of inorganic fertilizers in Australia, traditional farming and soil have degraded over time. The research examined whether freshwater microalgae can be used as a biofertilizer to boost nutrient accessibility for wheat germination and seedlings growth in pot culture conditions. Wheat seedlings in microalgae-treated soils showed significantly better establishment and vigour when compared to those grown with conventional fertilizer, azolla or without treatment. The project question receives a definitive response, as findings show freshwater microalgae have the dual capacity to adding vital nutrients to improve plant growth and retained the boost up the soil nutrients. Future research needs to expand practical adoption through field-scale validation experiments and optimization of algal species together with their application rates while assessing long-term soil health impacts and economic feasibility. In conclusion, Microalgal biofertilizers serve as a sustainable approach to balance soils nutrients while enhancing crop yields in horticultural practices.

Acknowledgments

None

Conflicts of interest

There is no conflict of interest.

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