AMERICAN UNIVERSITY OF BEIRUT

RESPONSE OF CROP GROWTH TO MICROALGAE IN IRRIGATION WATER

by
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Microalgae synthesize numerous bioactive compounds which are used in various industries. There are numerous successful applications of microalgae in the agriculture industry and it is well documented that microalgae promote crop growth as a bio-fertilizer. Recently, companies are marketing microalgae products as both a bio-fertilizer and plant bio-stimulant that promote and optimize crop growth. However, there are no empirical data that document the plant bio-stimulant effects of microalgae on crop growth. The objective of this study was to assess whether microalgae stimulated crop growth and flowering parameters and determine if it acts as a bio-fertilizer and/or plant bio-stimulant. Two crops were used, Radish (*Raphinus sativus*) and French marigold (*tagetes sp.*). In the radish experiment five treatments were tested: a control (DW) and four different microalgae treatments (T1, T2, T3 and T4). In the French marigold experiment ten treatments were tested: control (DW), four microalgae treatments (T1, T2, T3 and T4), synthetic fertilizer (150ppm N, P, and K respectively), and four combination treatments of synthetic fertilizer with each microalgae treatment (T6, T7, T8 and T9). Results showed that the microalgae treatments stimulated radish and French marigold growth parameters, but statistical analysis did not show any significant differences among plants treated by the various microalgae treatments. Similarly, analysis did not show any significant difference between marigold plants treated with synthetic fertilizers and plants treated by the combination treatments. The obtained results do not support claims by companies that microalgae promote crop growth as a plant bio-stimulant. However, when applied to the soil, the microalgae stimulated crop growth parameters as a bio-fertilizer.

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ABBREVIATIONS

BGA	Blue-Green Algae
N	Nitrogen
P	Phosphorus
K	Potassium
Zn	Zinc
Se	Selenium
Mn	Manganese
USDA	United States Department of Agriculture
SW	Shoot weight
RW	Root weight
PB	Plant biomass
AGB	Above ground plant biomass
FC	Flower count
FW	Flower weight
SH	Shoot height
DW	Distilled Water
T1	Treatment 1
T2	Treatment 2
T3	Treatment 3
T4	Treatment 4

T5	Treatment 5
T6	Treatment 6
T7	Treatment 7
T8	Treatment 8
T9	Treatment 9
G	Gram
µS	Micro-siemens
M	Meter
Cm	Centimeter
Ml	Milliliters
L	Liter
ppm	Parts per million
%	Percent
°C	Degrees Celsius
SE	Standard error
p	Significant difference
N	Number of replicates

CHAPTER 1

INTRODUCTION

Microalgae are a diverse group of photoautotrophic microorganisms which could be found in soils, rock (Sahu et al., 2012) and any water bodies (fresh or saline) (Lindsey, 2010). They are unicellular organisms with a size of 1-50 µm (Cooper and Smith, 2015). Cyanobacteria (BGA) and phytoplankton have different structures, but both contain at least one type of chlorophyll (a) and therefore fall under the category microalgae. Microalgae are a rich source of carbohydrates, lipids, proteins, pigments, oils, plant bio-stimulants, and other growth promoting substances such as vitamins, amino acids and sugars (Stirk et al., 2002; Mishra and Pabbi, 2004; Borowitzka, 2013). Accordingly, they have become a staple in various industries such as renewable energy (biofuel), nutraceutical, food & beverage industry, and pharmaceuticals. Currently, there are numerous successful applications of microalgae in the agriculture industry. Microalgae are used to enhance the nutritional value in animal feed (owing to their chemical composition) and play a vital role in the rearing of aquatic animals like shrimp and fish (Priyadarshani and Rath, 2012). In crop production, the most prominent feature of microalgae is its ability to fix atmospheric nitrogen and convert it into a bioavailable source of ammonium required for plants (Malik et al., 2001). Microalgae are also a potential source of various bio-active compounds (auxin, gibberellin and cytokinin) (Rodríguez et al., 2006) and several companies have recently started to commercialize microalgae as plant bio-stimulants (van der Voort et al., 2015) and/or as bio-fertilizers.

Numerous compounds active in improving crop yield are synthesized by microalgae (Tarakhovskaya et al., 2007). The elemental composition of various microalgae species includes macronutrients and trace elements such as nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), selenium (Se) and Manganese (Mn) necessary for crop growth (Campanella et al., 1998; Volkman and Brown, 2006; Tibbetts et al., 2015). Growth promoting substances and various bioactive compounds have been identified in cellular extracts and growth media of several microalgae species (Tarakhovskaya et al., 2007). For instance, Stirk et al., (2013) identified the phytohormones auxin, gibberellin and cytokinin in the axenic cultures of twenty-four species of microalgae belonging to four classes: *Ulvophyceae*, *Trebouxiophyceae*, *Chlorophyceae* and *Charophyceae*.

Response of crops to microalgae as a bio-fertilizer has been documented since the 1960's, when Gupta and Lata (1964) reported the positive influence of three species of microalgae, *Fisherlla mucicolo*, *Scytonema hofmanni* and *Nostoc sp*. on a rice paddy field. The microalgae accelerated germination, promoted seedling growth, increased grain yield and protein content of the seed. Bio-fertilizers are substances containing living organisms, which when applied to seed, plant, or soil promote crop growth by supplying essential nutrients (Vessey, 2003). Mandal et al., (1999) and Jha and Prasad (2006) showed that in submerged soils, microalgae increase aeration by releasing oxygen through photosynthesis, act as a cementing agent reducing soil erosion, and finally add organic matter as they decompose. Song et al., (2005) showed the important role of microalgae in the maintenance and build-up of soil fertility, consequently increasing rice growth and yield as a natural fertilizer. Thajuddin and Subramanian (2005) displayed the ability of microalgae to increase soil water holding capacity due to their ‘jelly’ structure and prevent weed growth. Research on the agriculture applications of microalgae have focused on rice production for various reasons, primarily because most paddy soils contain a natural population of microalgae (Mishra and Pabbi, 2004). Recently, few studies have evaluated crops other than rice. For instance, Garcia-Gonzalez and Sommerfeld (2016) recorded the positive effects of aqueous extract and dry application of *Acutodesmus dimorphus (*microalgae) on tomato plant growth. It is well documented that microalgae enhance crop growth as a bio-fertilizer, but its plant bio-stimulant properties are still not fully understood.

Microalgae have been represented to act as a plant bio-stimulant by several commercial companies and even been suggested by some researchers (Kulik, 1995; Adam, 1999; Rodríguez et al., 2006). A plant bio-stimulant is an organic substance that when applied in small quantities, enhances nutrition efficiency, abiotic stress tolerance, growth and development, such that the response cannot be attributed to application of traditional plant nutrients. Bio-stimulants could be ‘hormone-containing-products’, ‘humic substances’, and ‘amino acid containing products (du Jardin, 2015). Kulik, (1995) and Adam, (1999) suggested that the growth promotion of rice in response to the application of microalgae, *Nostoc muscorum,* may be attributed to the nitrogenase as well as nitrate reductase activities of the microalgae associated with the plants, or that the amino acids and peptides produced in the microalgae filtrate and/or other compounds that stimulated growth of plants. Rodríguez et al., (2006) reported that, *Scytonema hofmanni*, microalgae may counteract hormone disturbance of rice seedlings under saline conditions by synthesizing and liberating growth regulators which act like gibberellin. Research concerning microalgae produced bio-stimulants is a recent phenomenon (van der Voort et al., 2015). The market-size of microalgae produced bio-stimulants is relatively small as few suppliers have been found on the internet, such as AgroValley Inc. (USA), Agroplasma S.A and AlgaEnergy S.A (Spain) and Soley Biotech (India). These companies produce several microalgal products and would therefore be used for crop growth as organic fertilizers and bio-stimulants. They are based on commonly cultivated microalgae like *Spirulina*, *Scenedesmus*, *Chlorella sp*. and *Nannochloropsis sp*. For these applications, microalgae are grown in freshwater to avoid salt accumulation on agricultural fields or pots. Companies are advertising their freshwater microalgae as 100% natural products that optimize crop growth as a bio-fertilizer and bio-stimulant. AlgaEnergy S.A state that "farmers all over the world already rely on our bio-stimulants (AgriAlgae®) to take care of their crops and maximize their yields". Agroplasma S.A market their (Ferticell) product stating that it "stimulates the plant systems, resulting in more root mass, increased leaf area, increased number of flowers and fruit and early maturation". However, there are no empirical data on the plant bio-stimulant property of freshwater microalgae. Accordingly, the present study was performed to investigate whether freshwater microalgae stimulates crop growth and flowering parameters and whether the response of crops to microalgae was because of a plant bio-stimulant and/or bio-fertilizer. French marigold (*Marigold tagetes sp.*) and radish (*Raphinus sativus)* were selected.

CHAPTER II

MATERIALS AND METHODS

A. Study Area

The present work was performed at the plant research facility of the Faculty of Agriculture and Food Sciences at the American University of Beirut (AUB). Two pot experiments were designed to study the effect of freshwater microalgae on crop growth and flowering parameters. Different treatments were prepared to determine whether microalgae act as a bio-fertilizer and/or plant bio-stimulant. The first pot experiment was performed on a taproot vegetable (*Raphinus Sativus)* during the period September 25, 2017 to November 15, 2017. A taproot vegetable was chosen to represent the effects of microalgae on root growth. Radish plants are harvested when the radish bulb (taproot) fully develops before flowering. In the market, farmers sell the whole plant since both root (radish bulb) and leaves are edible. Radish was chosen because it's highly cultivated in Lebanon, has a short life cycle and is easy to cultivate. The second pot experiment was performed on an ornamental crop (*Marigold Tagetes sp.)* between April 05, 2018 and Jun 20, 2018. An ornamental plant was chosen to investigate whether microalgae stimulates flower growth as marketed by various microalgae suppliers. French marigold was chosen because it has a short life-cycle, is widely available in the Lebanese market and is easy to cultivate.

Three tons of soil were ordered from a local supplier and used in both pot experiments. Soil analysis was performed in the laboratory and was classified as sandy loam, non-saline, slightly alkaline and contained low amounts of nutrients. In general, the soil was adequate for crop growth (Table.1).

Table 1. Physical and chemical properties of the soil used in both experiments

Test	Soil Sample
%Sand	66
%Silt	30
%Clay	4
Bulk Density (g/cm^3)	1.27
Texture (USDA)	Sandy Loam
pH (soil/water 1:2)	8.08
EC (μS/m) (soil/water 1:2)	535
Available P (ppm)	11.7
Available K (ppm)	110

B. Preparation of Microalgae Treatments

The same procedures were performed in preparing the microalgae treatments used in both radish and French marigold pot experiments. Distilled water was the control, and four different freshwater microalgae treatments were prepared. The microalgae treatments used were: green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4).

For both experiments, all treatments were derived from the following process. Microalgae were cultivated in a freshwater open tank ($0.8m^3$) system containing a freshwater fish (*Chilcha* species), commonly known as tilapias, at the aquaculture research facility of the Faculty of Arts and Sciences at AUB. The freshwater turned dark green in color as the microalgae population grew. When the microalgae biomass reached a concentration of 10g/l (wet weight), the aquaculture freshwater containing microalgae was used as treatment 1 (T1). The aquaculture freshwater containing microalgae biomass (10g/l) was T1. Treatment 1 was used to prepare all the other treatments.

Treatment 2 and 3 were prepared as follows. Five hundred-ml aliquots of green aquaculture freshwater were centrifuged at 5000 RPM for six minutes to extract the microalgae. After extracting the microalgae, the supernatant aqueous extract that remained was used as treatment 2 while the extracted microalgae were used as treatment 3.

Treatment 3 was in turn used to prepare the mineralized microalgae treatment (T4) by the acid-digestion of freshwater microalgae using the Digesdahl Digestion Apparatus (HACH©). Ten-gram samples of wet microalgae were digested with 6-ml of concentrated sulfuric acid at 440ºC for six minutes. Afterwards, calcium hydroxide was added to neutralize the solution (pH range, between 6.5 and 7.5) and distilled water was poured until the neutral solution reached a volume of 100-ml. Calcium-hydroxide was used instead of sodium-hydroxide to reduce salt accumulation. The resulting 100-ml solution was Treatment 4.

The various microalgae treatments were prepared such that each liter of green aquaculture freshwater (T1) supplied was equivalent to a liter of supernatant aqueous extract (T2), 10-grams of wet microalgae (T3) and 100-ml solution of mineralized microalgae (T4). On the same day treatment 1 was collected, all the treatments were prepared and stored in a refrigerated room to be used throughout the experiment.

C. Radish *(Raphinus sativus)* Pot Experiment:

The first experiment, performed during September 25 to November 15, 2017, assessed the effects of various freshwater microalgae treatments on the growth parameters of the radish plant. Measurements of the shoot weight (SW), root weight (RW) and plant biomass (PB) were performed.

Five treatments were tested using radish plants in pots. There were nine replicates per treatment, resulting in a total of forty-five pots, distributed in the greenhouse in a completely randomized design. Pots were labeled DW, T1, T2, T3 and T4 and assigned to distilled water, green aquaculture freshwater, mineralized microalgae, supernatant aqueous extract and wet microalgae, respectively. All the pots were irrigated manually using a graduated cylinder. Irrigation was scheduled at a rate of 0.2 liter/event per pot three times a week. The rate was calculated based on the water holding capacity of the soil to avoid deep percolation.

Pots (21-liters) were filled with air-dried soil and peat moss at a volumetric ratio of 1:1. Radish seeds were sown on September 25 and irrigated with distilled water. Seedlings were thinned out after the emergence to three radish plants per pot. Treatments were applied during the period October 3 till October 26. Pots labeled DW were watered with 0.2 liters of distilled water each irrigation (control). T1 pots were watered with 0.2 liters of green aquaculture freshwater for 10 irrigations and afterwards watering was continued with distilled water. T2 pots were watered with 0.2 liters of supernatant aqueous extract for 10 irrigations and afterwards watering was continued with distilled water. Twenty grams of wet microalgae were poured on the soil surface of pots labeled T3 on October 3 and were watered with 0.2 liters distilled water. Succeeding irrigations were continued with distilled water. T4 pots were supplied with 0.2 liters of mineralized microalgae solution on October 3, and succeeding irrigations were continued with distilled water.

Table 2. Treatments applied after seedlings emerged during Oct. 3 till Oct. 24, 2017

Abbreviation	Treatment applied
DW	irrigated with distilled water
T1	10-irrigations of green aquaculture freshwater
T2	10-irrigations of supernatant aqueous extract
T3	20-grams of wet microalgae (once on Oct. 3)
T4	200-ml mineralized microalgae solution (once on Oct. 3)

The crop was harvested on November 15, 2017 and the growth parameters shoot weight, root weight and total biomass of each radish plant were measured. Afterwards, the plants were oven dried at 60°C for 72 hours and dry weight measurements were recorded. Statistical analysis of the growth parameters (fresh and dry) were performed using JMP 11 Software package.

D. French Marigold (*Marigold tagetes)* Pot Experiment:

The second experiment, performed during April 05 to June 20, 2018, assessed the effects of freshwater microalgae treatments on different crop growth and flowering parameters of French marigold. Measurements of the shoot height (SH), flower count (FC), shoot weight (SW), flower weight (FW) and above ground plant biomass (AGB) were performed. The shoot height and weight measurements indicated the vegetative growth while flower growth was evaluated by flower count and flower weight measurements. Yield was represented by the above-ground biomass at harvest.

Ten treatments were tested using a single French marigold plant per pot. There were fifteen replicates per treatment, resulting in a total of one hundred and fifty pots, distributed in the greenhouse in a completely randomized design. Irrigation was scheduled at a rate of 0.1 liter/event per pot three times a week. The rate was calculated based on the water holding capacity of the soil to avoid deep percolation.

Pots (3-liters) were filled by air-dried soil and peat moss at a ratio of 1:1. Pots labeled DW, T1, T2, T3, and T4 were assigned to distilled water (control), green aquaculture freshwater, supernatant aqueous extract, wet microalgae and mineralized microalgae respectively. Unlike the first experiment, five additional treatments were tested. Traditional fertilizers at a recommended dose were incorporated in pots labeled T5 and pots T6, T7, T8 and T9 were a combination of the fertilizer treatment (T5) with the different microalgae treatments.

Marigold seeds were sown April 05, 2018 in trays containing a mix of peat moss and perlite. The trays were placed in a controlled environment (hood) with optimum temperature (20°C) and growth conditions for germination. On April 20, seedlings were transplanted into the pots.

Treatments were applied during the period April 20 till May 14. DW pots were irrigated with 0.1-liters of distilled water. T1 pots were watered with 0.1-liters of green aquaculture freshwater for ten irrigations, afterwards watering was continued with distilled water. T2 pots were watered with 0.1 liters of supernatant tank water for ten irrigations, afterwards watering was continued with distilled water. Ten-grams of wet microalgae were placed on the soil surface of pots labeled T3 only once on April 20 and watered with 0.1 liters distilled water. Succeeding irrigations were continued with DW. T4 pots were irrigated with 0.1-liters solution of mineralized microalgae only once on April 20 and succeeding irrigations were watered with distilled water. A recommended dose of traditional fertilizers at 150 ppm nitrogen, phosphorus and potassium respectively were incorporated in the soil of pots labeled T5, T6, T7, T8 and T9 on April 20 before transplanting the marigold seedlings. After transplant, T5, T6 T7, T8 and T9 pots followed the same procedures as pots labeled DW, T1, T2, T3 and T4 respectively.

Table 3. Treatments applied after transplant date during April 20 till May 14, 2018

Abbreviation	Treatment applied
DW	Irrigated with distilled water
T1	10-irrigations of green aquaculture freshwater
T2	10-irrigations of supernatant aqueous extract
T3	10-grams of wet microalgae (once on April 20)
T4	100-ml mineralized microalgae solution (once on April 20)
T5	Recommended dose of traditional fertilizer
T6	10-irrigations of green aquaculture freshwater + traditional fertilizer
T7	10-irrigations of supernatant aqueous extract+ traditional fertilizer
T8	10-grams of wet microalgae + traditional fertilizer (once on April 20)
T9	100-ml mineralized microalgae solution + traditional fertilizer (once on April 20)

The crop was harvested on June 20, 2018 by cutting French marigold plants from the base of the shoot (1-cm above the soil surface). The shoot weight, flower weight and above-ground plant biomass were weighed at harvest. Flower count and shoot height were also measured. Afterwards, the plants were oven dried at 60°C for 72 hours and dry weight measurements were recorded. Statistical analysis of the growth parameters (fresh and dry) were performed using JMP 11 Software package.

E. Statistical Analysis

Statistical analysis was performed using JMP 11 Software package for both pot experiments. (Copyright 2012 SAS Institute, Inc., Cary, North Carolina, USA). Significant difference was calculated for all means using Tukey-Kramer HSD multiple range test at $p < 0.05$.

CHAPTER III

RESULTS AND DISCUSSION

A. Results

1. Radish Experiment

The response of radish growth parameters to different microalgae treatments were compared to radish plants irrigated with distilled water (control). The mean results of the crop growth parameters of twenty-seven radish plants per treatment are illustrated in Table 4.

- Fresh root and shoot weight of radish

All the treatments significantly increased the root weight of radish plants compared to the control (DW). However, statistical analysis didn't show any significant difference among the treatments (Table 4). The relative mean root weight of radish plants treated by green aquaculture freshwater, mineralized microalgae, supernatant aqueous extract and wet microalgae was 97%, 87%, 68% and 64% respectively higher than the control (Figure 1). The fresh shoot weight followed a similar trend to the root weight. All the treatments increased the root weight of radish plants compared to the control, but statistical analysis didn't show any significant difference among the microalgae treatments (Table 2). The relative mean shoots weight of radish plants treated by supernatant aqueous extract, mineralized microalgae, wet microalgae and green aquaculture freshwater was 86%, 70%, 67% and 66% respectively higher than the control (Figure 2).

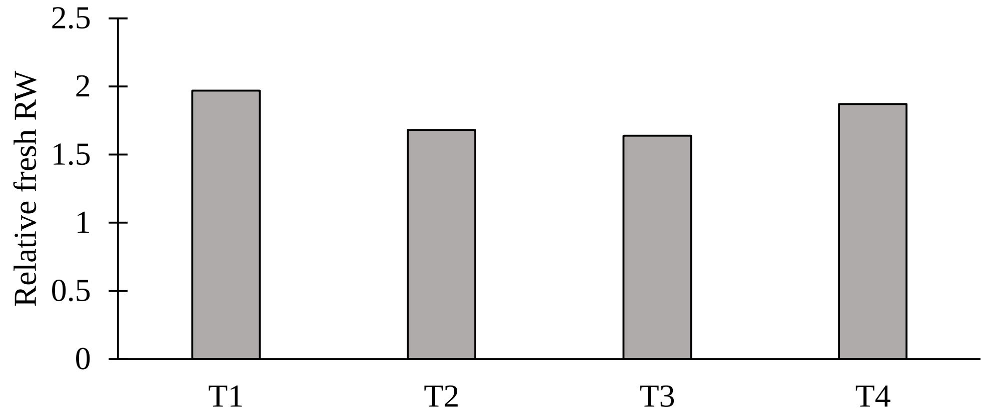

Figure 1. Relative mean root weight (RW) of radish treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

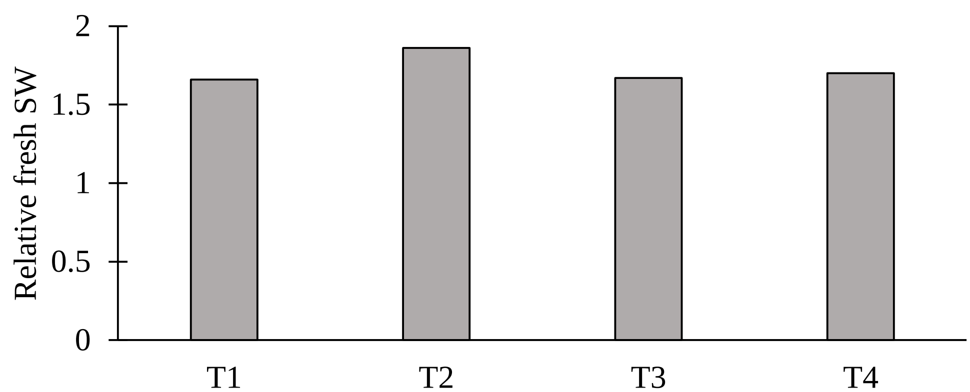

Figure 2. Relative mean shoot weight (SW) of radish treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

- Fresh total biomass of radish

Both root and shoot weight of the different microalgae treatments significantly increased compared to the control, therefore all the treatments significantly increased the yield of radish plants compared to the control (DW). The largest radish plants were observed in pots treated with supernatant aqueous extract (T2). However, statistical analysis didn't show any significant difference among the microalgae treatments (Table 4). The relative yield of radish plants treated by supernatant aqueous extract, green aquaculture freshwater, mineralized microalgae and wet microalgae was 79%, 76%, 75% and 66% respectively higher than the control (Figure 3).

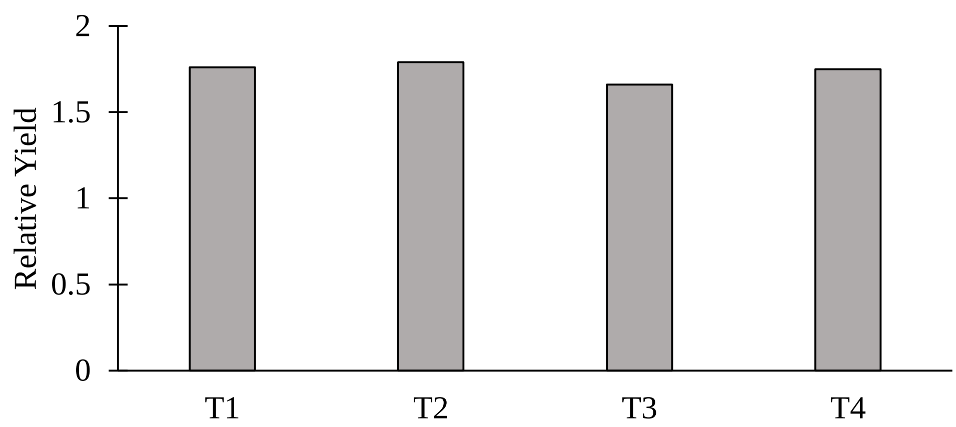

Figure 3. Relative yield of radish treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

- Dry total biomass of radish

All the treatments contained significantly higher amounts of dry matter than those of the control. Radish plants treated with mineralized microalgae accumulated the highest amounts of dry matter. However, statistical analysis didn't show any significant difference among the treatments (Table 4). The relative mean dry total biomass of radish plants treated with mineralized microalgae (T4), green aquaculture freshwater (T1), wet microalgae (T3) and supernatant aqueous extract (T2) was 74%, 59%, 59% and 56% respectively higher than the control (Figure 4).

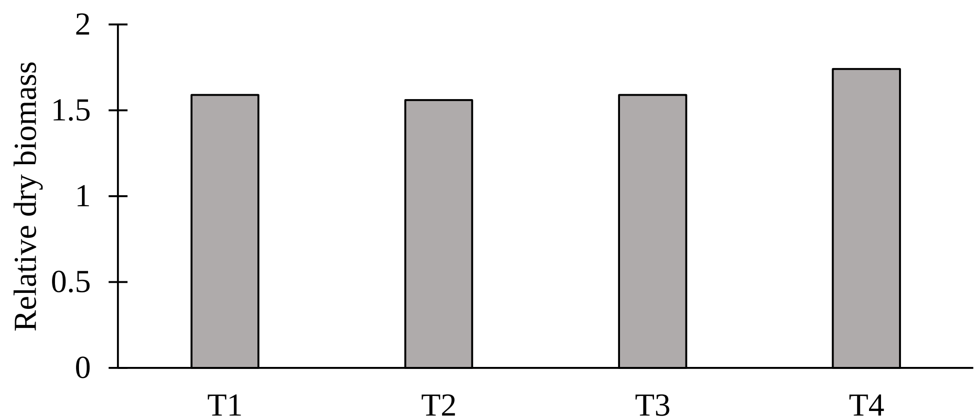

Figure 4. Relative mean dry biomass of radish treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

- Dry shoot weight of radish

All the treatments contained significantly higher amounts of shoot dry matter compared to the control (DW). However, statistical analysis did not show any significant difference among the microalgae treatments (Table 4). The relative mean dry shoot weight of radish plants treated with green aquaculture freshwater, supernatant aqueous extract, mineralized microalgae and wet microalgae (T3) was 55%, 55%, 48% and 45% respectively higher than the control (Figure 5).

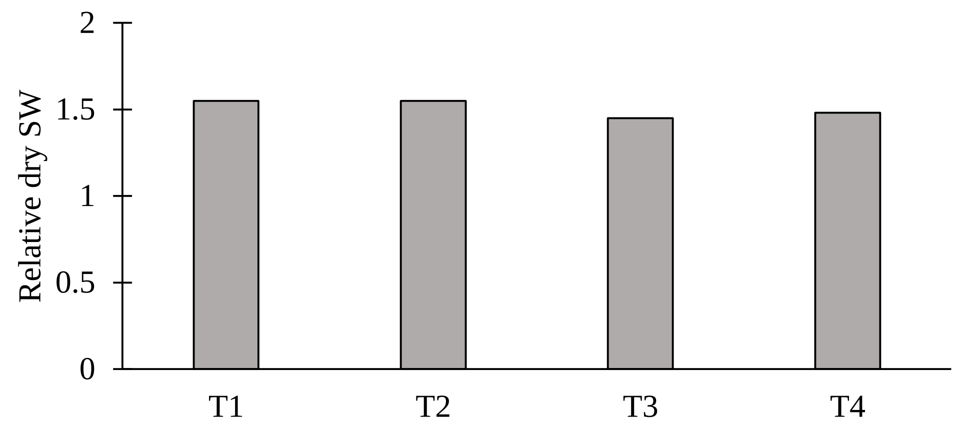

Figure 5. Relative mean dry shoot weight (SW) of radish treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

- Dry root weight of radish

According to Table 4, all the treatments contained significantly higher amounts of root dry matter compared to the control (DW). The mean dry root weight of radish plants treated with mineralized microalgae was significantly higher than green aquaculture freshwater and supernatant aqueous extract but was not different than the wet microalgae. Plants treated with supernatant aqueous extract accumulated the lowest amounts of root dry matter but were not significantly different to plants treated with green aquaculture freshwater and wet microalgae. The relative mean dry root weight of radish plants treated with mineralized microalgae, wet microalgae, green aquaculture freshwater and supernatant aqueous extract was 150%, 110%, 80% and 60% respectively higher than the control (Figure 6).

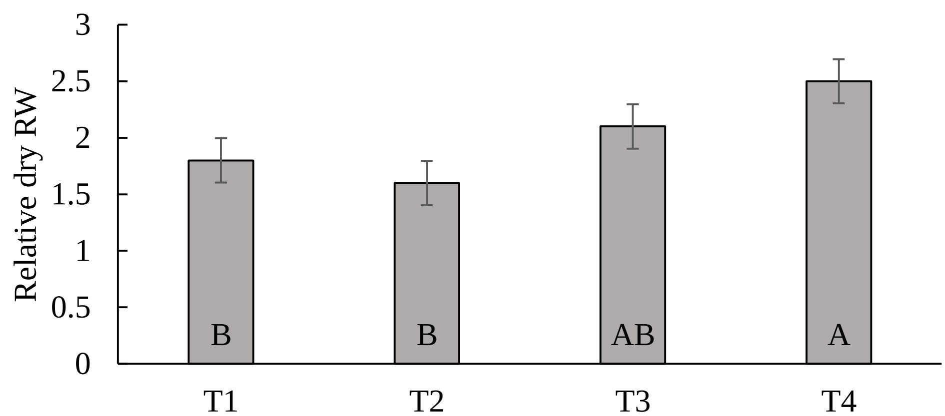

Figure 6. Relative mean dry root weight of radish treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control. (Bars with different letters are significantly different at $P < 0.05$).

Table 4. Response of radish growth parameters to various microalgae treatments (Values with different letters down the column indicate significant difference at $P < 0.05$) *(n = 27)* SE is the standard error of the means (g/plant)

Treatment	Fresh Root Weight (g/plant)	Fresh Shoot Weight (g/plant)	Fresh Total Biomass (g/plant)	Dry Root Weight (g/plant)	Dry Shoot Weight (g/plant)	Dry Total Biomass (g/plant)
DW	1.49^{B}	2.77^{B}	4.27^{B}	0.10^{C}	0.29^{B}	0.39^{B}
T1	2.93^{A}	4.6^{A}	7.54^{A}	0.18^{B}	0.45^{A}	0.62^{A}
T2	2.51^{A}	5.14^{A}	7.65^{A}	0.16^{B}	0.45^{A}	0.61^{A}
T3	2.45^{A}	4.63^{A}	7.08^{A}	0.21^{AB}	0.42^{A}	0.62^{A}
T4	2.78^{A}	4.71^{A}	7.49^{A}	0.25^{A}	0.43^{A}	0.68^{A}
± SE	0.23	0.43	0.64	0.02	0.04	0.06

*Control (DW), Green aquaculture freshwater (T1), Supernatant tank water (T2), Wet microalgae (T3), Mineralized microalgae (T4)

2. French Marigold Experiment

- Microalgae treatments compared to the control

French marigold plants treated by the different microalgae treatments were compared to plants irrigated by distilled water (control). The mean results of the shoot weight (SW), flower weight (FW), above-ground plant biomass (AGB), flower count and shoot height of fifteen marigold plants per treatment are illustrated in Table 5.

– Fresh flower weight of French marigold

The mean fresh flower weights of the different microalgae treatments were significantly larger than the control (DW). The largest mean fresh flower weight was recorded for plants treated with green aquaculture freshwater. However, statistical analysis didn’t show any significant difference among the microalgae treatments (Table 5). The relative mean fresh flower weight of green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) increased by 224%, 203%, 198% and 154% respectively (Figure 7).

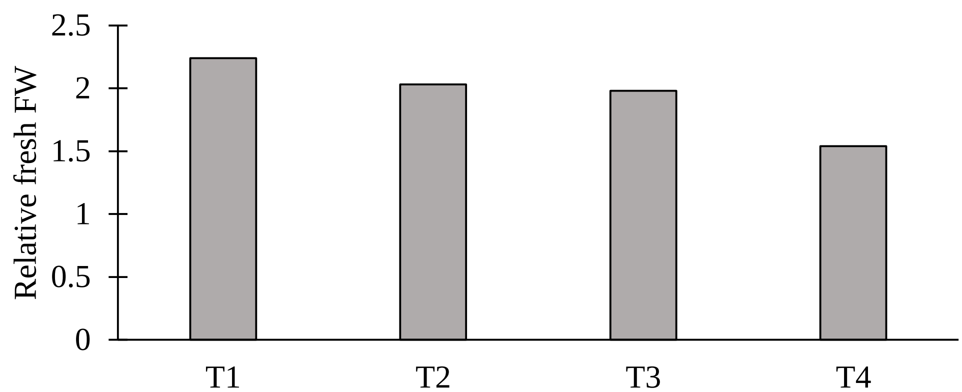

Figure 7. Relative mean fresh flower weight (FW) of marigold treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

- Fresh shoot weight of French marigold

According to Table 5, the average shoot weights of the various microalgae treatments were significantly larger than the control (DW). The largest shoots were observed French marigolds treated with wet microalgae. However, statistical analysis didn’t show any significant difference among the microalgae treatments. The relative mean shoots weight of wet microalgae (T3), green aquaculture freshwater (T1), supernatant tank water (T2) and mineralized microalgae (T4) was 121%, 105%, 91% and 81% respectively higher than the control (Figure 8).

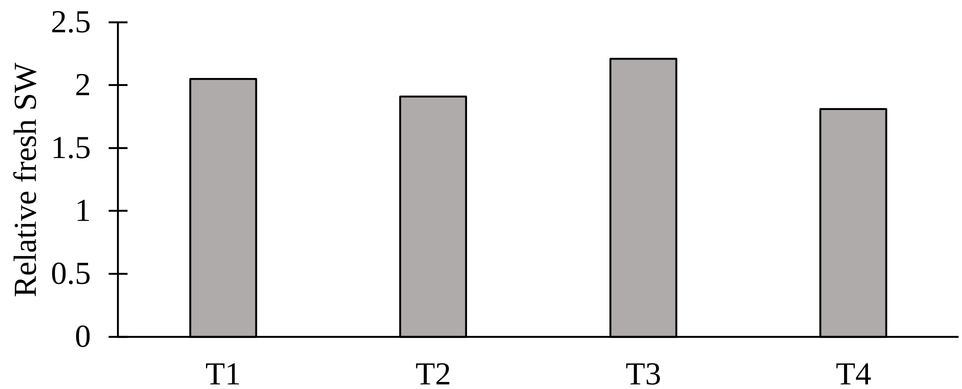

Figure 8. Relative mean shoot weight (SW) of marigold treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

- Fresh above-ground plant biomass of French marigold

Both root and shoot weights of the different microalgae treatments increased significantly, therefore, the mean above-ground plant biomass (AGB) were significantly larger than plants of the control (DW). The largest French marigolds were observed in plants treated with wet microalgae (T3). However, statistical analysis didn't show any significant difference among the microalgae treatments (Table 5). The relative mean above-ground plant biomass of wet microalgae (T3), green aquaculture freshwater (T1), supernatant tank water (T2) and mineralized microalgae (T4) was 149%, 143%, 126% and 100% respectively higher than the control (Figure 9).

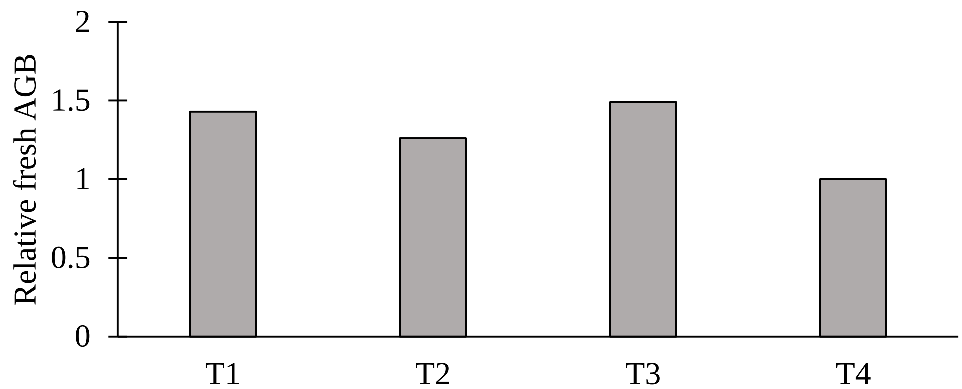

Figure 9. Relative mean above-ground biomass (AGB) of marigold treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

– Flower count of French marigold

The average flower count of the different microalgae treatments were significantly greater in number than the control (DW). Plants treated with green aquaculture freshwater recorded the highest number of flowers per marigold plant. However, statistical analysis didn't show any significant difference among the microalgae treatments (Table 5). The relative mean flower count of green aquaculture freshwater (T1) supernatant tank water (T2), mineralized microalgae (T4) and wet microalgae (T3) was 121%, 111%, 111% and 110% respectively higher than the control (Figure 10).

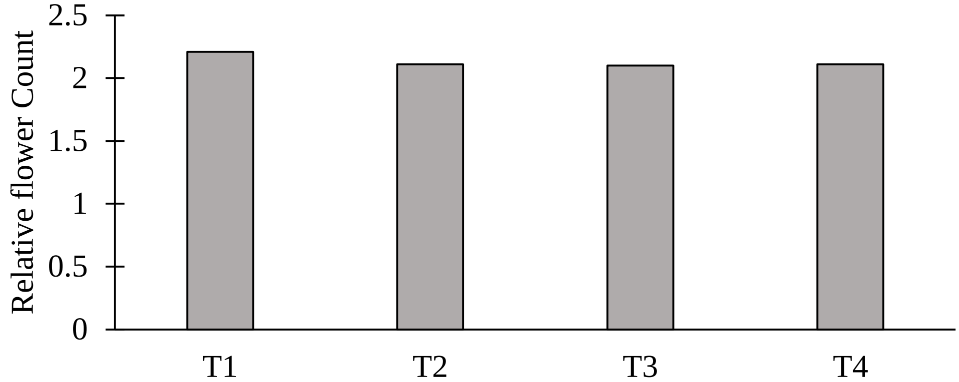

Figure 10. Relative mean flower count of marigold treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

– Shoot height of French marigold

According to Table 5, French marigold plants treated by the various microalgae treatments were significantly taller than the control (DW). The tallest French marigold plants were in pots treated with green aquaculture freshwater. However, statistical analysis did not show any significant differences among the treatments. The relative mean shoot height of green aquaculture freshwater (T1), mineralized microalgae (T4), supernatant aqueous extract (T2) and wet microalgae (T3) was 33%, 26%, 21% and 20% respectively higher than the control (Figure 11).

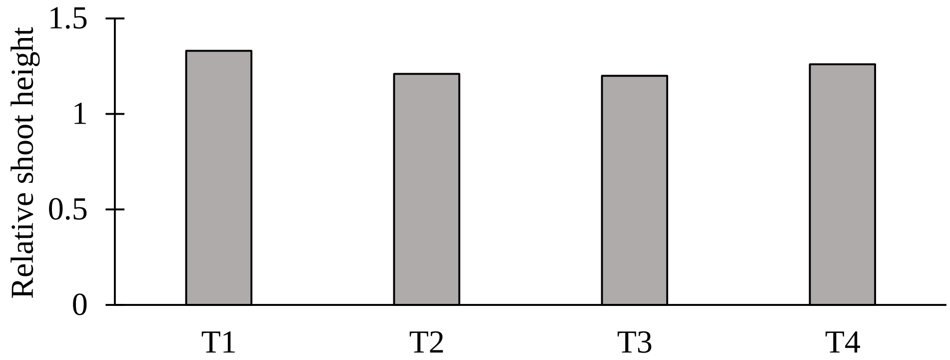

Figure 11. Relative mean shoot height of marigold treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

– Dry above-ground biomass of French marigold

French marigold plants treated with the different microalgae treatments contained significantly greater amounts of dry matter content than plants irrigated with distilled water (control). According to Table 5, French marigold plants treated with green aquaculture freshwater accumulated the highest amounts of dry matter content. However statistical analysis didn't show any significant difference among the microalgae treatments. The relative mean dry matter content of French marigold plants treated with green aquaculture freshwater (T1), wet microalgae (T3), supernatant aqueous extract (T2) and mineralized microalgae (T4) was 138%, 125%, 120% and 93% respectively higher than the control.

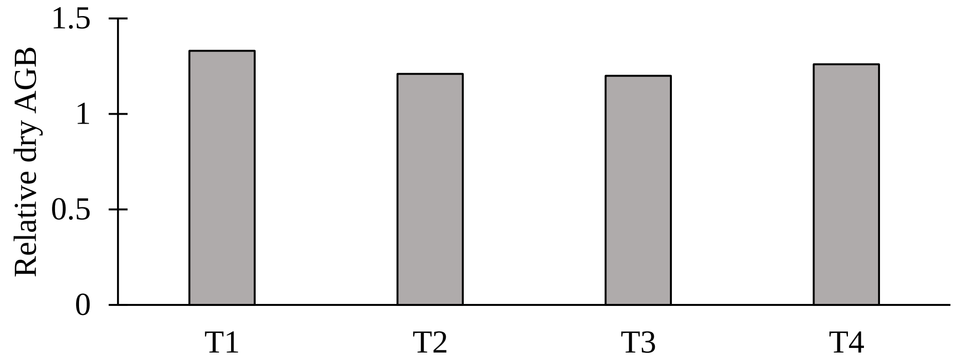

Figure 12. Relative mean dry above-ground biomass (AGB) of marigold treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

– Dry flower weight of French marigold

According to Table 5, flowers of French marigold plants treated with green aquaculture freshwater, supernatant aqueous extract and wet microalgae accumulated significantly higher amounts of dry matter than plants in the control (DW). The flowers of marigold plants treated with green aquaculture freshwater accumulated the highest amounts of dry matter content and therefore recorded the largest mean dry flower weight. Although plants treated with mineralized microalgae were not significantly different to the control, statistical analysis did not show any significant difference among the different microalgae treatments. The relative mean dry flower weight of marigold plants treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2) and wet microalgae (T3) was 240%, 220% and 210% respectively higher than the control (Figure 12).

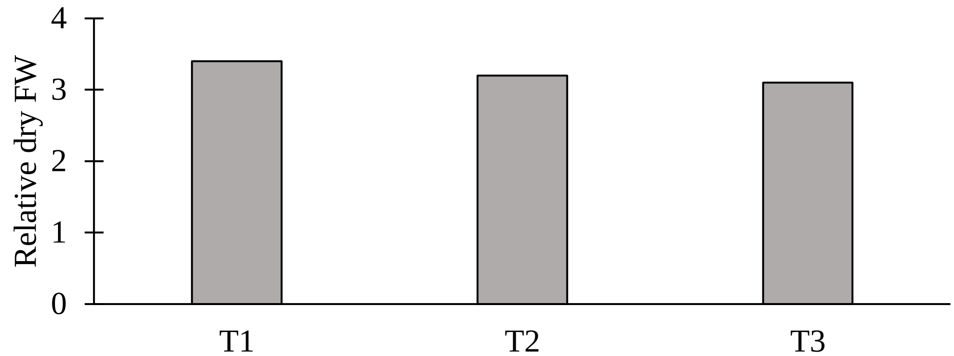

Figure 13. Relative mean dry flower weight (FW) of marigold treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2) and wet microalgae (T3) compared to the control.

– Dry shoot weight of French marigold

According to Table 5, the dry matter content accumulated in the shoots of French marigold plants treated with the different microalgae treatments were significantly higher than the control (DW). The largest mean dry shoot weight was recorded for plants treated with green aquaculture freshwater. However, statistical analysis did not show any significant difference among the microalgae treatments. The relative mean dry shoot weight of green aquaculture freshwater (T1), wet microalgae (T3), supernatant aqueous extract (T2) and mineralized microalgae (T4) was 97%, 90%, 81% and 71% respectively higher than the control (Figure 14).

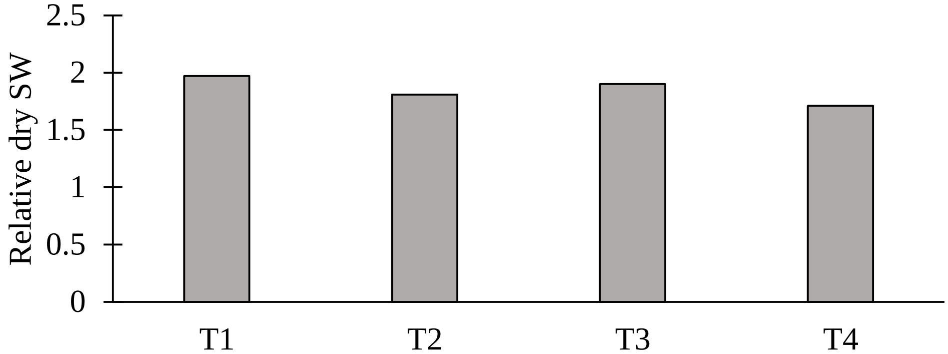

Figure 14. Relative mean dry shoot weight (SW) of marigold treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) compared to the control.

Table 5. Response of French marigold growth parameters to various microalgae treatments (Values with different letters indicate significant difference at level $P < 0.05$) *(n=15)* SE is the standard error of the means (g/plant)

Treatment	Fresh SW g/plant	Fresh FW g/plant	Fresh AGB g/plant	Flower Count	Shoot Height Cm	Dry SW g/plant	Dry FW g/plant	Dry AGB g/plant
DW	1.66^{B}	0.63^{B}	2.24^{B}	1.3^{B}	12.8^{B}	0.31^{B}	0.1^{B}	0.40^{B}
T1	3.40^{A}	2.04^{A}	5.44^{A}	2.8^{A}	17.0^{A}	0.61^{A}	0.34^{A}	0.95^{A}
T2	3.17^{A}	1.88^{A}	5.06^{A}	2.6^{A}	15.5^{A}	0.56^{A}	0.32^{A}	0.88^{A}
T3	3.66^{A}	1.91^{A}	5.57^{A}	2.6^{A}	15.3^{A}	0.59^{A}	0.31^{A}	0.90^{A}
T4	3.00^{A}	1.60^{A}	4.49^{A}	2.6^{A}	16.1^{A}	0.53^{A}	0.26^{AB}	0.77^{A}
± SE	0.61	0.39	0.35	0.45	0.78	0.07	0.06	0.1

*Control (DW), Green aquaculture freshwater (T1), Supernatant aqueous water (T2), wet microalgae (T3), mineralized microalgae (T4).

- Microalgae treatments compared to fertilizer treatment

The response of French marigold growth parameters to the different microalgae treatments were compared to the marigold plants supplied with synthetic fertilizers. As stated previously, the statistical analysis did not show any significant difference between marigold treated by green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4) (Table 4). Analysis of the comparison between the average fresh and dry weight measurements of marigolds (n = 15) supplied with synthetic fertilizers and marigolds treated by the various microalgae treatments are illustrated in Figures 14, 15, 16, 17 and 18. The mean fresh flower weight, flower count and shoot height measurements are not illustrated since they didn't show any significant difference between the fertilizer treatment and the different microalgae treatments.

  - Fresh shoot weight above ground biomass of French marigold

According to Figure 15, the mean above-ground plant biomass (AGB) of fertilizer treatment was significantly larger than green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4). The fertilizer treatment (T5) increased the yield of French marigolds by 57% to 94% when compared to plants in the different microalgae treatments. The shoot weight followed the same trend as the above-ground biomass. The average shoot weight of the fertilizer treatment was 74% to 111% larger than the mean shoot weights of the different microalgae treatments (Figure 16).

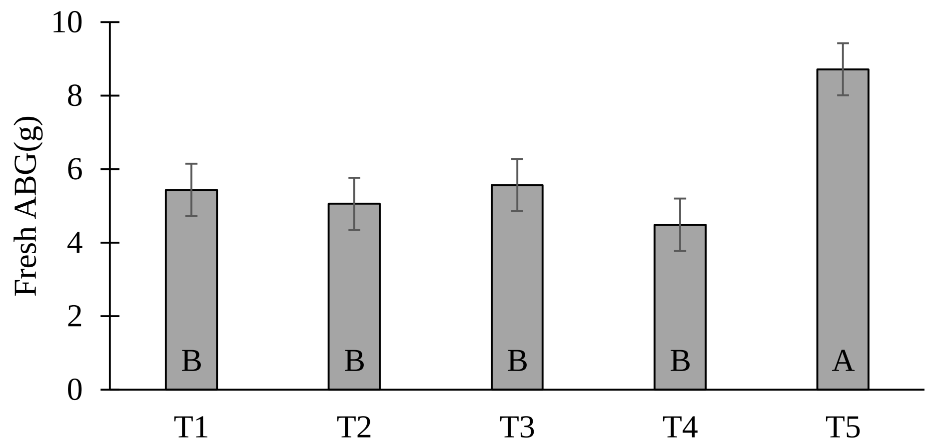

Figure 15. Response of marigold above-ground biomass (AGB) to green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3), mineralized microalgae (T4) and recommended dose of fertilizer (T5). Bars with different letters indicate significant difference at P < 0.05.

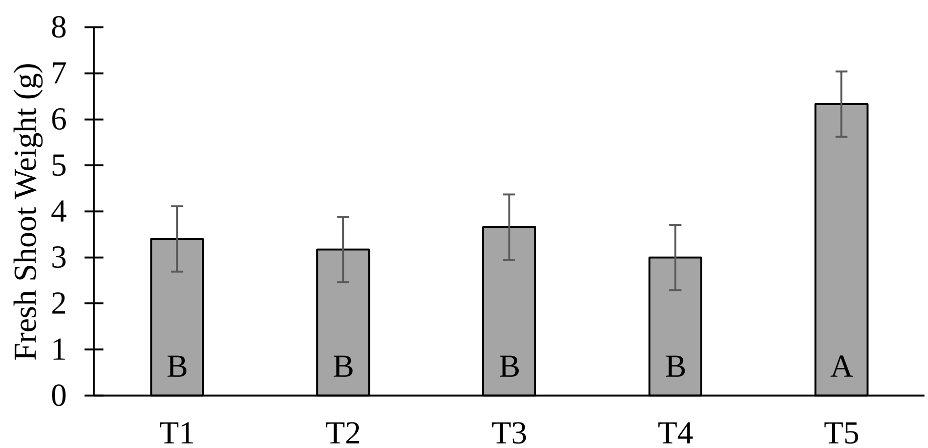

Figure 16. Response of marigold shoot weight to green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3), mineralized microalgae (T4) and recommended dose of fertilizer (T5). Bars with different letters indicate significant difference at P < 0.05.

– Dry weight measurements of French marigold

According to Figures 16, 17 and 18, the shoots of marigold plants in the fertilizer treatment accumulated the highest amount of dry matter content. The mean dry shoot weight of the fertilizer treatment significantly increased by 98% to 128% compared to the different microalgae treatments. Similarly, the mean dry flower weight of the fertilizer treatment was 82% to 139% higher than the different microalgae treatments. Both dry shoot and flower weights significantly increased, accordingly, the dry matter content of the above-ground biomass also increased. French marigold plants in the fertilizer treatment contained higher amounts of dry matter than plants in the green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3) and mineralized microalgae (T4). The mean dry above-ground biomass of the fertilizer treatment was 93% to 138% higher than the different microalgae treatments.

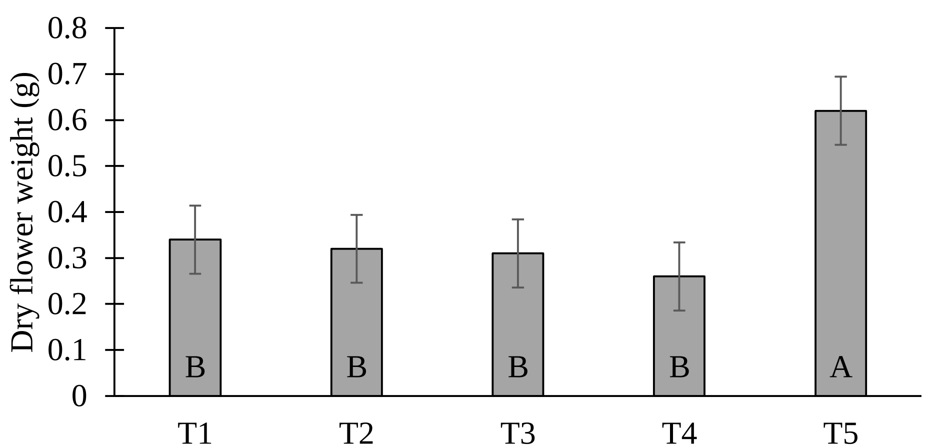

Figure 17. Response of the dry flower weight of French marigold to green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3), mineralized microalgae (T4) and recommended dose of fertilizer (T5). Bars with different letters indicate significant difference at P < 0.05.

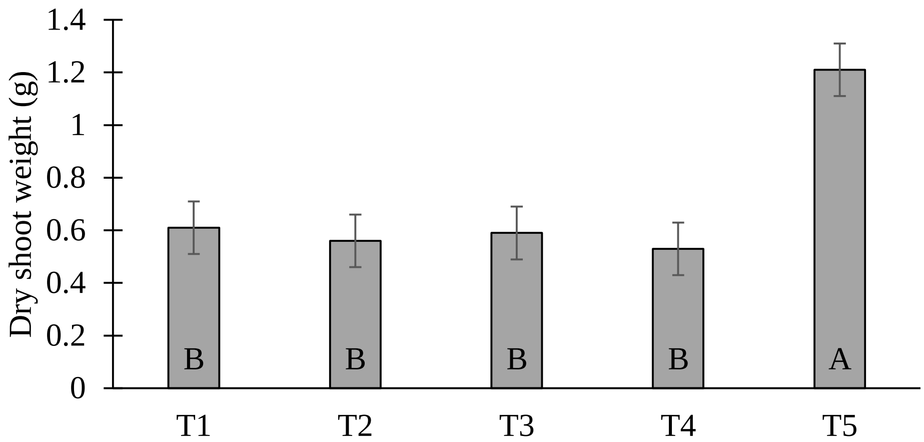

Figure 18. Response of the dry shoot weight of French marigold to green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3), mineralized microalgae (T4) and recommended dose of fertilizer (T5). Bars with different letters indicate significant difference at $P < 0.05$.

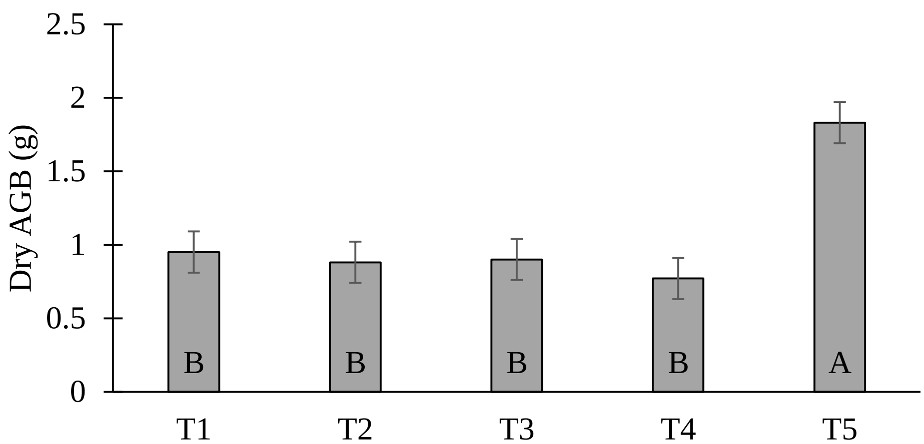

Figure 19. Response of the dry above-ground biomass (AGB) of French marigold to green aquaculture freshwater (T1), supernatant aqueous extract (T2), wet microalgae (T3), mineralized microalgae (T4) and recommended dose of fertilizer (T5). Bars with different letters indicate significant difference at $P < 0.05$.

– Flower count and shoot height of French marigold

The highest number of flowers per marigold plant were observed in the fertilizer treatment. However, statistical analysis of the flower count did not show any significant difference between the fertilizer treatment and different microalgae treatments (T1, T2, T3 and T4). The mean shoot height of French marigolds followed a similar trend as the flower count. The fertilizer treatment contained the tallest marigold plants followed by green aquaculture freshwater, mineralize microalgae, supernatant aqueous extract and wet microalgae. However, statistical analysis of the shoot height did not show any significant difference between the fertilizer treatment and the different microalgae treatments.

- Fertilizer treatment compared to combination treatments:

The response of French marigold growth parameters to the four combination treatments were compared to plants in the fertilizer treatment. The combination treatments T6, T7, T8, and T9 were made by combining the fertilizer treatment (T5) with the different microalgae treatments T1, T2, T3, and T4 respectively. The mean results of the shoot weight (SW), flower weight (FW), above-ground plant biomass (AGB), flower count and shoot height of fifteen marigold plants per treatment are illustrated in Table.4.

According to Table 6, the largest French marigolds were in response to the combination treatment fertilizer with wet microalgae (T8). The tallest plants were observed in the combination treatment fertilizer with green aquaculture freshwater (T6) and the highest mean flower count of marigold plants was observed in plants treated with the combined fertilizer treatment and supernatant aqueous extract (T7). However, statistical analysis of the French marigold growth parameters did not show any significant difference among any of the combined treatments and fertilizer treatment.

As stated previously, Marigold growth parameters enhanced in response to the fertilizer treatment when compared to the different microalgae treatments. Accordingly, the combined treatments also promoted the growth of French marigold in comparison to the different microalgae treatments.

Table 6. Response of French marigold growth parameters to synthetic fertilizer and combined treatments *(n=15)* SE is the standard error of the means (g/plant) no significant differences were recorded at $P < 0.05$

Treatment	Fresh SW g/plant	Fresh FW g/plant	Fresh AGB g/plant	Flower Count	Shoot Height Cm	Dry SW g/plant	Dry FW g/plant	Dry AGB g/plant
T5	6.33	2.40	8.72	3.3	17.5	1.21	0.62	1.83
T6	6.27	2.87	9.15	3.9	18.2	1.04	0.75	1.79
T7	6.11	2.95	9.06	4.7	17.9	1.06	0.70	1.76
T8	6.68	2.77	9.45	3.9	17.4	1.14	0.54	1.68
T9	5.71	2.55	8.26	3.9	17.5	1.04	0.46	1.50
± SE	0.84	0.37	1.07	0.5	0.71	0.13	0.12	0.2

*Fertilizer (T5), Fertilizer + Green aquaculture freshwater (T6), Fertilizer + Supernatant aqueous extract (T7), Fertilizer + Wet microalgae (T8), Fertilizer + Mineralized microalgae (T9).

B. Discussion

The obtained results do not support claims by numerous companies that microalgae promote crop growth and flowering parameters as a plant bio-stimulant. However, crop growth and flowering parameters were stimulated by nutrients present in the aquaculture effluent and freshwater microalgae. In comparison to the control (DW), higher biomass was observed in radish and French marigold plants treated with green aquaculture freshwater, supernatant aqueous extract, mineralized microalgae and wet microalgae, but analysis did not show any significant difference amongst the four microalgae treatments (at $P < 0.05$).

1. Response of crop growth to various microalgae treatments

Microalgae produce a vast array of bioactive compounds (vitamins, amino acids auxin etc.) in their cellular extracts and growth media (Tarakhovskaya et al., 2007; Stirk et al., 2002; Stirk et al., 2013). This encouraged commercial suppliers to market microalgal products as plant bio-stimulants. The three microalgae treatments (green aquaculture freshwater, supernatant aqueous extract, and wet microalgae) were tested on French marigold and radish plants to show whether the bioactive compounds synthesized by microalgae stimulated crop growth and/or flowering. The mineralized microalgae treatment consisted of nutrients liberated by the acid-digestion of freshwater microalgae. It is well documented that nutrients necessary for crop growth such as nitrogen, phosphorus, and other minerals make up the elemental composition of microalgae (Grobbelaar, 2013). As expected, the mineralized microalgae treatment stimulated the crop growth parameters of radish and French marigold plants. Similarly, the three microalgae treatments (green aquaculture freshwater, wet microalgae, and supernatant aqueous extract) enhanced crop growth, but analysis did not show any significant differences among plants treated by the four microalgae treatments (including the mineralized microalgae). Therefore, it could be deduced that microalgae promoted crop growth as a source of nutrients. This is justified by numerous studies which documented the response of crop growth to microalgae as a fertilizer (Garcia-Gonzalez and Sommerfeld, 2016; Song et al., 2005; Thajuddin and Subramanian, 2005). However, this comparison alone is not enough to indicate whether microalgae acted as a plant bio-stimulant in addition to its bio-fertilizer properties.

2. Response of crop growth to combination treatments

Plant bio-stimulants promote crop growth through different mechanisms than nutrients do. Bio-stimulants improve plant tolerance to abiotic stresses, facilitate nutrient assimilation, increase yield and enhance crop qualities. The three combination treatments of recommended dose of synthetic fertilizers with green aquaculture freshwater, supernatant aqueous extract and wet microalgae respectively (T6, T7 and T8) were tested on French marigolds. We compared growth parameters of marigolds treated with the combination treatments to those treated with only synthetic fertilizers to explicitly show whether bio-active compounds in the cellular extracts and growth media of freshwater microalgae stimulated flowering of marigolds. Marigolds treated with synthetic fertilizers were supplied with the primary macronutrients (N, P, and K). Therefore, if bio-active compounds stimulated flowering of marigolds, then the combination treatments (T6, T7 and T8) would have significantly increased the yield of marigolds compared to plants supplied with synthetic fertilizers. However, statistical analysis didn’t show any significant difference between the growth parameters of marigolds supplied with synthetic fertilizers and marigolds treated with the combination treatments. Therefore, microalgae promoted crop growth explicitly as a natural fertilizer and not as a plant bio-stimulant.

CHAPTER IV

SUMMARY, CONCLUSION & RECOMMENDATIONS

A. Summary

Microalgae were cultivated in an open tank system containing freshwater fish. Two pot experiments were performed to assess the effects of freshwater microalgae on crop growth and flowering parameters. Two crops were tested, French marigold and radish. Both experiments were performed in greenhouses and the microalgae treatments were irrigated manually using a graduated cylinder. The first experiment was performed during the period of September 25 till November 15, 2017 and assessed the effects of microalgae on radish growth parameters. This was the preliminary experiment which was performed to show whether microalgae stimulate crop growth parameters. Four different microalgae treatments were prepared and tested. One of the treatments was prepared by the acid digestion of freshwater microalgae and therefore could have only promoted crop growth as a source of nutrients. The four different treatments enhanced the growth of radish compared to plants irrigated with the control. However, analysis didn't show any significant differences among the growth parameters of radish plants treated with the various microalgae treatments. According to the results of the radish experiment, freshwater microalgae in irrigation water promoted crop growth as an organic fertilizer, but its bio-stimulant properties where not verified. The second experiment was performed during the period of April 04 till June 20, 2018 and it assessed the effects of microalgae on French marigold growth. Unlike the radish experiment, a recommended dose of synthetic fertilizers and combination treatments of microalgae with synthetic fertilizers were used to determine whether microalgae act as a plant bio-stimulant. Statistical analysis did not show any significant differences between the growth and flowering of marigolds treated with recommended dose of synthetic fertilizers and marigolds treated with the combination treatments. The results of the French marigold experiment indicated that freshwater microalgae in irrigation water enhanced the growth and development of marigolds only as an organic fertilizer and not as a plant bio-stimulant.

B. Conclusion

Several companies state that microalgae optimize crop growth and development not just as an organic fertilizer but also as a plant bio-stimulant, however the results of this study do not support these claims. When applied to the soil, freshwater microalgae in irrigation water promotes crop growth and flowering parameters only as an organic fertilizer.

C. Recommendations

Microalgae in irrigation water didn’t act as a plant bio-stimulant and therefore should only be used as an organic fertilizer. If farmers are supplying sufficient amounts of synthetic fertilizers for optimum crop growth then microalgae should not be used. However, if microalgae were supplied, lower amounts of synthetic fertilizer will be required for optimum growth. Future research should address the required quantitative analysis.

Discharges that flow through aquaculture systems contain organic matter, suspended solids, microalgae and nutrients which may have negative impacts on our water resources such as eutrophication in receiving water bodies (Tookwinas, 1996; Boyd and Tucker, 2000). Instead of discharging freshwater aquaculture effluents into receiving water bodies or as waste, it could be collected and used to produce microalgae biomass which in turn could be used as an organic fertilizer in crop production.

Microalgae are small enough to pass through filters of irrigation systems and consequently form aggregates and colonies which may clog up emitters. Emitter problems are common when using surface water that have high concentrations of microalgae. Therefore, it is not recommended to supply aquaculture effluents containing freshwater microalgae when using drip irrigation. Freshwater microalgae can be supplied through irrigation water, using other systems that do not require fine filtration. Future research should try different methods of applications.

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