Potential of *Chlorella* sp. grown in wastewater on the growth and yield of *Amaranthus* sp.

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1. Introduction

Global usage of chemical fertilizer has been increasing over the last three decades to meet the food demand due to the growing population. Consequently, this can lead to soil degradation (Shaaban et al., 2010). Organic fertilizer is a good alternative to inorganic fertilizers as it is an environmentally sound as well as economically viable option. Due to the present covid-19 situation, readily available organic fertilizers are limited. To counteract the current issues, biofertilizer has become the best alternative solution. Biofertilizer, a new tear that's being used widely nowadays to enrich the soil with microalgae, bacteria, and fungi that promote the growth and yield of crops (De Jesus Raposo & De Morais, 2011). Microalgae biomass is one of the sources of biofertilizer. *Chlorella* sp. can be cultivated for biofertilizer production, which provides a high amount of macro and micronutrients, metabolites like protein, carbohydrates, and constituents, and growth-promoting substances such as cytokinin (Dineshkumar et al., 2020).

In Sri Lanka, there is an increasing trend for consuming leafy vegetables to obtain nutrients, especially micronutrients. Dark leafy vegetables are preferred due to the rich sources of vitamins such as A and C; minerals calcium, iron, and phosphorous; antioxidants and phytochemicals. Amaranthus is one of them and is highly cultivated in Sri Lanka (Nadeeshani et al., 2018). As *Chlorella* sp. is an aquatic organism it demands water for its growth. However, wastewater could be used to supplement nutrients as well as to replace freshwater to grow *Chlorella* sp. The objective of this study was to assess the potential of *Chlorella* sp. grown in parboiled effluent on the growth and yield of *Amaranthus* sp.

2. Materials and Methods

Selected physical and chemical properties of soil and the nutrient analysis for both soil and the algae biomass were done to determine the treatment combinations. The microalgae were obtained from a previous study from the Department of Civil Engineering, Faculty of engineering which was grown in parboiled effluent.

Nutrient Analysis

Nutrient contents such as C, N, P, and K were analyzed to ensure enough nutrient availability. Kjeldahl was used to estimate the total nitrogen, Vanadomolybdate method was used to determine the phosphorus content of algae and ammonium molybdate- $SnCl_2$ method was used at the wavelength of 660 nm to determine the phosphorous content in the soil, the flame photometer was used to measure the potassium content and total organic carbon content was estimated by loss on ignition method.

Pot Experiment

The plant was grown in the poly house for two months from December to January. During the growth period, the plant growth parameters such as plant height, number of leaves, and leaf area measurements were recorded. There were eleven treatments with three replicates under CRD (Complete Randomized Design) was practiced as follows: T1 - Control, T2 - *Chlorella* sp. 2g/kg, T3 - *Chlorella* sp. 4g/kg, T4 - *Chlorella* sp. foliar application 100% Live biomass, T5 - *Chlorella* sp. foliar application 0.2g Dry biomass/ Plant, T6 - cattle manure 20 ton/ha, T7 - 50% cattle manure + *Chlorella* sp. 1g/kg, T8 - 50% cattle manure + *Chlorella* sp. 2g/kg, T9 - 50% cattle manure + *Chlorella* sp. foliar application 50% Live biomass, T10 - 50% cattle manure + *Chlorella* sp. foliar application 0.1g Dry biomass/ Plant, T11 - 50% cattle manure.

Each pot was prepared by adding 1kg of soil with the nutrient content of nitrogen, phosphorous, and potassium respectively 20.3 ppm, 27.6 ppm, and 57.9 ppm and for treatments having cattle manure with the nutrient content of nitrogen, phosphorous, and potassium respectively 1.42 ppm, 0.31 ppm, and 1.21ppm, it was added two weeks before seed sowing for better incorporation of nutrients. Fifty seeds were added to each pot and thinning out was done after 10 days of germination. While sowing the seeds, they were mixed with algae dry biomass according to the treatment requirements. The fresh biomass was obtained to produce foliar spray by centrifugation at 5000 rpm for 15 minutes. The foliar spray was prepared and it was given in 4 days intervals for two weeks after thinning out. After a month the plants were harvested the yield weight and dry biomass weight was taken. Data analysis was done by using a statistical analytical system (SAS University version) with Duncan's mean separation at P=0.05.

3. Results and Discussion

The soil texture was Sandy clay loam and the physical and chemical properties were pH 6.9, electrical Conductivity 72 µS/cm, bulk density 1.55 g/cm^{3,} and organic carbon content 0.88 %. The nutrient availability of soil was 20.3 ppm nitrogen, 27.6 ppm phosphorus, and 57.9 ppm of potassium. Comparatively all nutrients were poor and there was a need to be fed by an external source for nutrients.

The nutrient content of *Chlorella* sp. was observed to be 27%, 3.23%, 0.73%, and 38.05% of nitrogen, phosphorous, potassium, and organic carbon respectively. Table 01 shows the plant height, leaf number, leaf area, fresh yield, and dry biomass yield of *Amaranthus* sp at the time of harvest. Plant height was comparatively higher in T7 treatment (50% Cattle manure + *Chlorella* sp. 1g/kg soil) which was 23.8 cm. However, there were no any significant differences (p=0.05) in height among treatments T2 (*Chlorella* sp. 2g/kg), T3 (*Chlorella* sp. 4g/kg), T6 (Cattle manure 100% 20 ton/ha), T7 (50% cattle manure+ *Chlorella* sp. 1g/kg), T8 (50% cattle manure+ *Chlorella* sp. 2g/kg), T9 (50% cattle manure+ *Chlorella* sp. foliar application 50% Live biomass) and T10 (50% cattle manure+ *Chlorella* sp. foliar application 0.1g Dry biomass/ Plant). Treatment T2 (*Chlorella* sp. 2g/kg) recorded the highest number of leaves. Meanwhile there were no any significant differences among the treatments T2 (*Chlorella* sp. 2g/kg), T3 (*Chlorella* sp. 4g/kg), T8 (50% cattle manure+ *Chlorella* sp. 2g/kg), T9 (50% cattle manure+ *Chlorella* sp. foliar application 50% Live biomass) and T10 (50% cattle manure+ *Chlorella* sp. foliar application 0.1g Dry biomass/ Plant). As it is a leafy vegetable, the number of leaves matters a lot on a commercial scale. A large leaf area was observed in the treatment T2 (*Chlorella* sp. 2g/kg) soil application that is significantly highest among the treatments. In the yield comparison, treatment T8 (50% cattle manure + *Chlorella* sp. 2g/kg) had the highest yield among others. However the yield of T2 (*Chlorella* sp. 2g/kg), T3 (*Chlorella* sp. 4g/kg), T6 (100% Cattle manure (20 ton/ha), T7 (50% cattle manure+ *Chlorella* sp. 1g/kg), T9 (50% cattle manure+ *Chlorella* sp. foliar application 50% Live biomass) and T10 (50% cattle manure+ *Chlorella* sp. foliar application 0.1g Dry biomass/ Plant) treatments were not significantly different compared to T8 (50% cattle manure+ *Chlorella* sp. 1g/kg). According to the nutrient uptake the nitrogen, phosphorous and potassium were higher in treatments T8 (50% cattle manure + *Chlorella* sp. *2*g/kg), T10 (50% cattle manure+ *Chlorella* sp. foliar application 0.1g Dry biomass/ Plant) and T11 - 50% Cattle manure (20 ton/ha) respectively. As *Chlorella* is rich in nitrogen content, which is essential to improve the vegetative growth of plants, it improves the yield.

Table 01. *Amaranthus* sp. plant height, leaf number, leaf area, fresh yield, and dry biomass yield at the time of harvest

Treatments	Plant height (cm)	Leaf Number	Leaf Area (mm^2)	Fresh yield (g)	Dry biomass Yield (g)
T1	18.7^{b}	12^{bc}	441.75^{e}	4.66^{cd}	0.11^{cd}
T2	23.3^{a}	18^{a}	2898.99^{a}	13.11^{ab}	0.24^{ab}
T3	18.8^{b}	16^{a}	2407.89^{ab}	12.64^{ab}	0.18^{bcd}
T4	19.7^{ab}	12^{bc}	1549.91^{cd}	3.77^{d}	0.02^{d}
T5	17.7^{b}	12^{bc}	1612.16^{bcd}	7.74^{bcd}	0.11^{cd}
T6	18.7^{b}	10^{c}	2048.29^{abcd}	11.09^{ab}	0.17^{bcd}
T7	23.8^{a}	12^{bc}	2212.69^{abcd}	10.20^{abc}	0.18^{bcd}
T8	22.8^{a}	16^{a}	2583.04^{ab}	15.50^{a}	0.34^{a}
T9	20.8^{ab}	15^{a}	2048.09^{abcd}	10.05^{abc}	0.21^{abc}
T10	19.2^{ab}	16^{a}	2001.38^{abcd}	10.11^{abc}	0.12^{cd}
T11	18.2^{b}	10^{c}	1356.03^{d}	8.12^{bcd}	0.11^{cd}

T1 Control (No fertilizer application), T2 *Chlorella* soil application (SA) - 2g/kg soil, T3 *Chlorella* SA - 4g/kg soil, T4 *Chlorella* foliar application (FA) - 100% live biomass, T5 *Chlorella* FA- 0.2g dry biomass/L, T6 100% cattle manure - 20 tons/ha, T7 *Chlorella* SA- 1g/kg soil + 50% cattle manure, T8 *Chlorella* SA 2g/kg soil + 50% cattle manure, T9 *Chlorella* FA 50% live biomass + 50% cattle manure, T10 *Chlorella* FA 0.1g dry biomass/L + 50% cattle manure, T11 50% cattle manure (10 tons/ha).

4. Conclusions

Nutrient analysis of *Chlorella* sp. grown in parboiled effluent indicated that it has a considerable amount of potassium (0.73%), phosphorous (3.23%), nitrogen (27%), and carbon (38.05%). According to the overall observation, *Amaranthus* sp. shows best results in T8 which is 50% cattle manure + *Chlorella* sp. 2g/kg, and T2 which is only the algae biomass 2g/kg. Comparing the yield of *Amaranthus* sp. T8 (50% cattle manure + *Chlorella* sp. *2*g/kg) shows a higher response. However, there were no significant differences among T8, T2, T3, T6, T7, T9, and T10 in yield. Considering the cost-effectiveness, treatment T2 *(Chlorella* sp. 2g/kg) is the best as it uses the lowest amount of *Chlorella* sp. biomass among all the treatments. With the least input, the wastewater-grown biofertilizer also can be obtained which is a win-win situation for a farmer. The present study concluded that the *Chlorella* biofertilizer positively influenced on growth and yield of *Amaranthus* sp.

5. References

De Jesus Raposo, M. F., and De, Morais, R. M. S. C. 2011 "*Chlorella vulgaris* amendment: Influence of encapsulation and enrichment with rhizobacteria", *International Journal of Agriculture and Biology, 13*(5), 719–724.

Nadeeshani, H. et al. 2018. "Evaluation of the Nutritional Value of Selected Leafy Vegetables Grown in Sri Lanka." *Tropical Agricultural Research, 29*(3), 255.

Dineshkumar, R., Subramanian, J. and Sampathkumar, P., 2020. Prospective of Chlorella Vulgaris to augment growth and yield parameters along with superior seed qualities in black gram, Vigna mungo (L.). *Waste and Biomass Valorization*, *11*(4), 1279-1287.

Shaaban, Mahmoud M, Abdel-Karim M El-Saady, Abo El-Khair, and B El-Sayed. 2010. "Green Microalgae Water Extract and Micronutrients Foliar Application as Promoters to Nutrient Balance and Growth of Wheat Plants." *Journal of American Science, 6*(9), 631–636. Retrieved from http://www.americanscience.org.