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The effect of *Chlorella variabilis* as a foliar spray utility in Sabaski

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Abstract
The leafy vegetables requires biofertilizers for cultivation, faster and better growth. The utilization of beneficial microbes as a biofertilizer has become major source of nutrient supply for the sustainable crop production. *Chlorella variabilis*, a unicellular photosynthetic green algae, is an intracellular photobiont isolated from UAS, Raichur in BG II media by providing enriched 10% of carbon dioxide and grown under 12h light and dark period under room temperature. On spray to Sabaski as soon as three leaf stage of germination at different dosage (control, 10 ml/l,15 ml/l, 20 ml/l, 25 ml/l, 30 ml/l, 35 ml/l, 40 ml/l and Nyntinol 2 g/L) to know the growth and other biochemical parameters were recorded at harvest. The results showed highest plant height (25.6 to 30.2), chlorophyll content (28.6 to 53.5%), number of leaves (25 to 32) and weight of the plant (6.9 to 8.0 g), and the highest moisture content was 85.06 and 87.46, ash content 2.72 and 3.05, crude fat content 0.85 and 0.96, crude fiber 2.05 and 2.43, crude protein 2.86 and 3.04 and carbohydrates content 7.83 and 8.42 for the treatments T_1 (control), T_6 (30 ml/L) and T_8 (40 ml/L) respectively was recorded. The study showed that fast growing culture (ACR 5) with high contents of Lysine (2.49%), Tryptophan (0.98%), Iron (66.73 mg/kg), Zinc (60.22 mg/kg) on sabaski at T_6 (30ml/L), T_8 (40 ml/L) show increase in production by 20% physiological growth at harvest. Hence the treatment of 30 ml/L can be effectively utilized for the field spray of microalagal culture (SCP) to obtain higher vegetative growth.

Keywords: *Chlorella variabilis,* Sabaski, photobiont, single cell protein, microalgal cultures, seaweed, BG II

1. Introduction
Microalgae are microscopic heterotrophic, autotrophic and photosynthesizing organisms that are capable of utilizing solar energy to combine water with carbon dioxide to create biomass. Microalgae are present in all existing earth ecosystems, both aquatic and terrestrial, and can flourish under a wide range of environmental conditions, including freshwater, brackish water, seawater, and even wastewater. Microalgae have been suggested as good candidates for fuel production because of their higher photosynthetic efficiency, higher biomass production and faster growth compared to those of other energy crops. Microalgae utilize far less water than traditional oilseed crops. For these reasons, microalgae are capable of producing more oil per unit area of land compared to terrestrial oilseed crops (Chisti *et al*., 2008) [6].
Algal bio-fertilizers might be a best option of alternative source of nitrogen to the nitrogenous chemical fertilizers. Algal bio-fertilizers are eco-friendly, fuel independent, cost effective and easily available one. Blue green algae fix nitrogen under anaerobic conditions in specialized cells called heterocyst, which comprises 5–10% of cells in a filament (Flemming *et al.,* 1993) [9]. Biofertilizers are products (Carrier or liquid based) containing living or dormant microbes (bacteria, actinomycetes, fungi, algae) alone or in combination, which help in fixing atmospheric nitrogen or solubilizers soil nutrients in addition to the secretion of growth promoting substances for enhancing crop growth and yield. The cost of chemical fertilizers is increasing continuously and these chemicals are responsible for reducing the environmental health. As a result, microalgae species are recommended to be used as biofertilizers instead of using the expensive industrial chemical fertilizers. Microalgae are best as a cheap source of N_2, which does not cause soil and water pollution.
The present research was aimed to analyze the impact of algal–biofertilizers on sabaski growth and productivity under various dosages of algae (*Chlorella variabilis*) upon spray as a to determine the potentiality of bio-fertilizer application in order to have maximum vegetative frowth by harvest.

2. Materials and method

2.1. Microalgal cultures and preparation

The microalgal culture preparation carried out in Department of Molecular biology and Agricultural Biotechnology College of Agriculture, UAS, Raichur. BG 11 media was used for isolation, enrichment and cultivation of microalgae. The BG-11 medium was prepared according to **(**Stanier *et al*., 1972**)** [15] using distilled water and pH adjusts between 7-7.4. Microalgal cultures were grown by adding 10% of initial inoculum into a growth medium.

2.2. Experimental Conditions

This experiment was carried out at College of Agriculture, UAS, Raichur in the horticulture garden by spraying the microalgal culture at appearance of 3 leaf stage in Randomized Block Design with three replications. It consisted of nine treatments which were formed by the combinations of different graded levels and varied concentrations of foliar spray 10, 15, 20, 25, 30, 35, 40 mL/lit, and using recommended dose of fertilizer (RDF) as a control treatment and T_1 as a control treatment with water spray.

The experimental plot was prepared to a fine tilth with bed size of 3.5 x 1.0 m. Seeds were sown in beds at a spacing of 20 × 15 cm in last week of April 2022. Nursery beds were watered daily. Plants are thinned at 20-25 days after sowing and the thinned seedlings are used as greens. One pinching at a height of about 4' will encourage branching. Weeding is done as and when necessary. FYM 20 - 25 t/ha and N, P, K at 30:25:40 kg/ha according to the treatments was applied in the form of urea, Diammonium Phosphate (DAP) and Muriate of Potash (MOP). At the time of transplanting, half of the dose of N and full dose of $P_2O_5$and K_2O were applied in circular band.

2.3 Collection of experimental data

Sampling procedure: For recording biometric observations 25 plants were tagged randomly in each net plot for the purpose of recording observations on growth.

Observations recorded

Plant height and weight of the plant (cm)

The height and weight of 25 random plants was recorded and measured in centimetres from the soil surface up to the terminal top portion of the plant with the help of a meter scale at harvest and their mean was calculated.

Number of leaves per plant

The Number of leaves per plant produced from the whole plant was counted at harvest and their mean was calculated for 25 random plants.

Chlorophyll content of leaves (SPAD)

The chlorophyll content of the leaves was measured using SPAD metre at harvest and their mean was calculated for 25 random plants.

Proximate analysis

The proximate analysis of sabaski was done by AOAC 2006 method.

2.4 Statistical analysis

The data obtained from growth studies was subjected to analysis of variance using randomized block design (Sundararaj *et al*., 1972) and treatment means were separated by Duncan's Multiple Range Test (DMRT) (Little and Hills, 1978). The significant level was set at $p< 0.05$.

3. Result and discussion

The foliar spray of microalgal culture shows increasing growth the average 25 plants in each treatment the different parameters are as follows:

3.1 Plant height

Plant height of sabaski is showing higher with increasing from 25.6 to 30.2 cm from different growing stages. The increasing treatment is T_6 to T_8 with 30 to 40 ml/L sprayed after 20 days of sowing interval. T_6 (30 ml/L) treatment sprayed started to showing increasing growth rate and this treatment shows good affect in sabaski plant. As measured for 25 plants, T_1 is control treatment showing 15 cm, T_2 of treatment of 10 ml/l with 16.5 cm height, T_3 treatment of 15ml/L with 20 cm height, T_4 treatment of 20ml/L with 23.2 cm height, T_5 treatment of 25 ml/L with 23.6 cm in height, T_6 treatment of 30 ml/L with 25.6 cm which recorded increasing in treatment, T_7 treatment of 35 ml/L with 28.6 cm in height, T_8 treatment of 40 ml/L with 30.2 cm in height, T_9 treatment is control spraying with fertilizer recommended dose with 25 cm in height.

3.2 Number of leaves per plant

Number of leaves was increased from 25 to 32 while passing through different growth stage. As measured 25 plants, T_1 is control treatment showing 18 leaves, T_2 is treatment of 10 ml/L with 20 leaves, T_3 treatment is 15ml/L with 21 leaves, T_4 treatment is 20ml/L with 22 leaves, T_5 treatment is 25 ml/L with 24 leaves, T_6 treatment is 30 ml/L with 25 leaves which recorded increasing in treatment, T_7 treatment is 35 ml/L with 26 leaves, T_8 treatment is 40 ml/L with 32 leaves, T_9 treatment is control spraying with fertilizer recommended dose with 24 leaves. The sabaski is broad leaves which is freshly green after 30 days interval with increasing growth from T_6, T_7 to T_8 treatment showing large number of leaves and got positive effect of the natural fertilisers.

3.3 Chlorophyll content

The increase in chlorophyll may be due to the availability of the required nutrients in sufficient amount that improved the chlorophyll biosynthesis and chlorophyll contents in vegetative growth. Such increase might be useful for photosynthetic activity due to absorption of available nutrients, which cause an increase in growth and photosynthesis efficiency.

Chlorophyll content was increased from 28.6 to 53.5% while passing through different growth stage. As measured 25 plants, T_1 is control treatment showing 15.9, T_2 is treatment of 10 ml/L with 12.67, T_3 treatment is 15ml/L with 16.5, T_4 treatment is 20ml/L with 25.6, T_5 treatment is 25 ml/l with 28, T_6 treatment is 30 ml with 28.6 which recorded increasing in treatment, T_7 treatment is 35 ml/L with 30.2, T_8 treatment is 40 ml/L with 53.5, T_9 treatment is control spraying with fertilizer recommended dose with 15.8 % content of chlorophyll. The sabaski has broad leaves which is freshly green after 30 days interval with increasing growth from T_6, T_7 to T_8 treatment showing large number of leaves and got positive affect of the natural fertilisers.

3.4 Weight of plant sabaski measured 25 plants

Weight of sabaski was increased from 5.76 to 8.01 g while passing through different growth stages. As measured 25 plants, T_1 is control treatment showing 5.76 g, T_2 is treatment of 10 ml/L with 6.51 g, T_3 treatment is 15ml/L with 6.19 g, T_4 treatment is 20ml/L with 6.65 g, T_5 treatment is 25 ml/l with 6.78 g, T_6 treatment is 30 ml with 6.91 g which recorded increasing in treatment, T_7 treatment is 35 ml/L with 7.64 g, T_8 treatment is 40 ml/L with 8.01 g, T_9 treatment is control spraying with fertilizer recommended dose with 6.28g. The spinach has broad leaves which is freshly green after 30 days interval with increasing growth from T_6, T_7 to T_8 treatment showing large number of leaves and got positive effect of the natural fertilisers.

Increase in length and diameter of the plant might be the reason for increasing the weight of the plants shoot and root weight. Increased uptake of nitrogen showed positive impact on cell division and enlargement which leads to more accumulation which resulted in increased 25 plants weight from T6 to T8 treatment with 6.9 to 8.0 g.

Similar results were obtained by Mahmoud and Amara (2000), in which all treatments significantly increased plant growth parameters compared to untreated plant. Moreover, enhancement in the growth parameter leads to improved crop productivity. The stimulatory effects of alga as bio-fertilizer on some growth parameters of lettuce was obtained in the study results by Rani and Sathiamoorthy (1997) [13].

Table 1: Sabaski growth parameter average of 25 plants

Treatments	Weight (gm) (25 plants)	Number of leaves (25 plants)	Chlorophyll content (%)	Height (cm)
T1 (Control)	5.76	18	15.9	15
T2(10ml/L)	6.51	20	12.6	16.5
T3(15ml/L)	6.19	21	16.5	20
T4(20ml/L)	6.65	22	25.6	25.2
T5(25ml/L)	6.78	24	28.1	23.5
T6(30ml/L)	6.91	25	28.6	25.6
T7(35ml/L)	7.64	26	30.2	28.6
T8(40ml/L)	8.01	32	53.5	30.2
T9 (Nyntinol 2gm/L)	6.28	24	15.8	25
Mean	6.74	23.5	25.2	23.2
CD(5%)	0.318	1.087	1.211	0.99
SEm ±	0.105	0.36	0.401	0.328

Table 2: Proximate analysis of sabaski.

Sl. No.	Parameters	T_1 (Control)	T_6 (35ml/L)	T_8 (40ml/L)
1	Moisture (%)	71.2	85.06	87.46
2	Ash (%)	1.32	2.72	3.05
3	Crude Fat (%)	0.71	0.85	0.96
4	Crude Fibre (%)	1.64	2.05	2.43
5	Crude Protein (%)	2.06	2.86	3.04
6	CARBOHYDRATES (%)	5.16	7.83	8.42

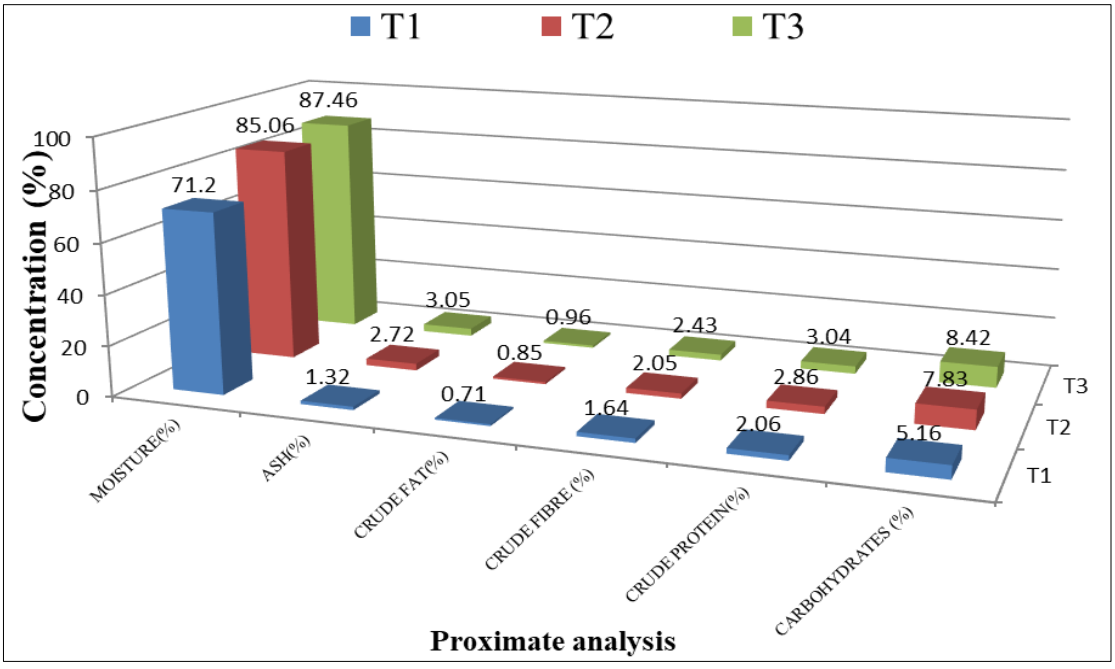
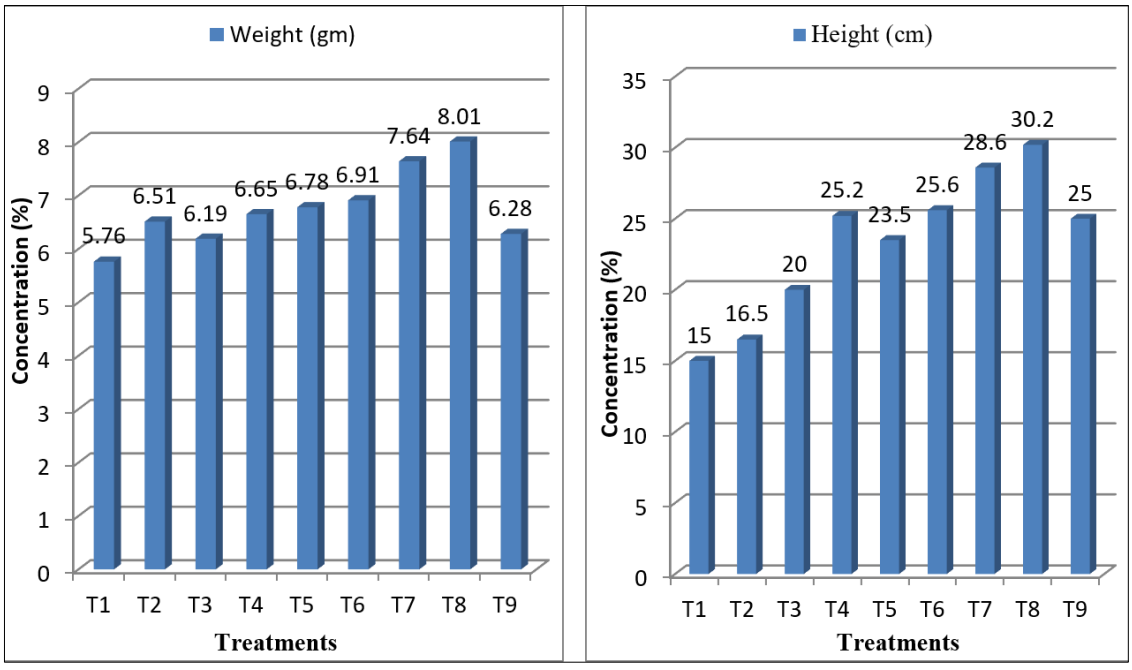


Fig 1: Proximate analysis of Sabaski. T_1 (Control), T_6 (30ml/L) and T_8 (40ml/L)

a) T1 b) T8

Fig 1: Sabaski growth parameters. a) Field layout, b) leaves of sabaski c) Height growth of treatments.



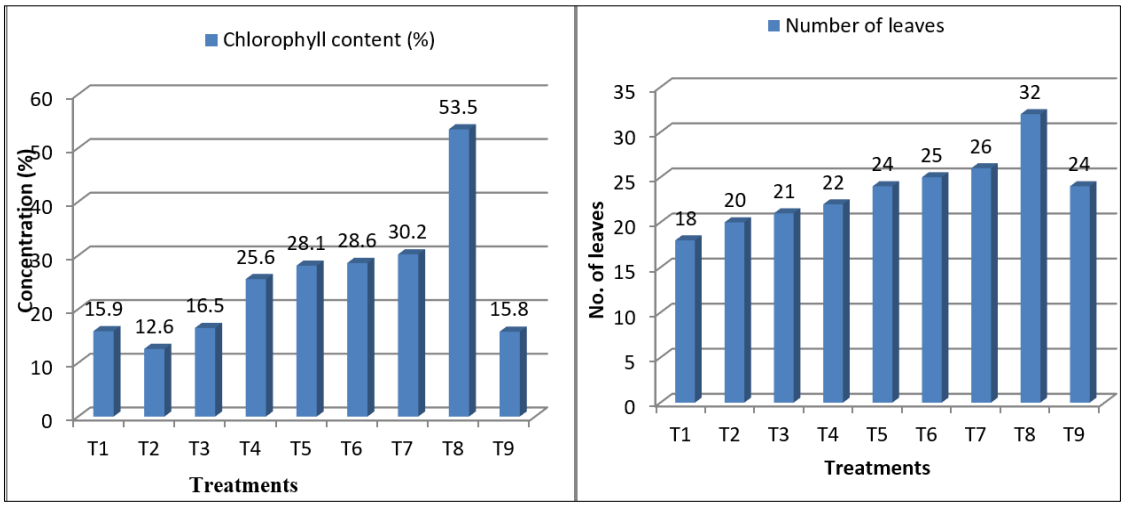


Fig 2: Sabaski growth parameters. a) weight (gm), b) height (cm), c) chlorophyll content (%) and d) numbers of leaves.

Conclusion

In this study, sabaski showed increased growth from T_6 to T_8 treatments sprayed at 30, 35, 40 ml/L at three leaf stage of growth. The highest plant height, chlorophyll content, number of leaves and weight of the plant was recorded for leafy vegetables at 40 ml/L of spray dosage. The treatments of 30, 35 and 40 ml/L showed the effective result in field spray by microalgal cultures. Based on the results obtained in the present study, we can conclude that *Chlorella variabilis* can be utilized for the crop productivity for the enhancement of Sabaski growth as a vegetable. It has enhanced the yield by 15-20% at 30ml/L and above sprays at one time till harvest.

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