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Efficacy of microalgal extracts as biostimulants through seed treatment and foliar spray for tomato cultivation

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ABSTRACT

A probable strategy for increasing the economic sustainability of algal technology would involve the utilization of microalgal biomass as biofertilizer by off-setting the high production costs. The present study focusses on the utilization of mixed algal consortia as biofertilizer for analysing the growth rate of tomato plant. Algal extracts (20-100%) in the form of seed primer and foliar spray were used as biostimulants for the growth of tomato plant. Characterization of algal consortium showed the presence of 40.90% carbohydrates and 26.18% proteins that could potentially act as precursors for bioactive compounds to stimulate plant growth. Faster germination percentage was found with extract concentrations ranging from 20-60% in 3 days compared to the untreated seeds. Seeds treated with cellular extracts of 40% concentration also showed faster plant growth rate after sowing in terms of increase in shoot length 19.86 ± 0.51 cm and root length of 14.87 ± 0.63 cm with a fresh and dry weight of 3.47 ± 0.04 g and 0.389 ± 0.036 g respectively after 20 days. Foliar spraying of 60% algal extracts resulted in total plant height of 7.98 ± 0.19 cm with root length of 5.8 ± 0.16 cm, 46% higher compared to the control. 11 ± 0.35 leaves with chlorophyll content of 13.45 ± 0.307 mg g^{-1} were also obtained after 20 days, with fresh and dry biomass content of 0.416 ± 0.015 g and 0.062 ± 0.005 g respectively. Thus, microalgal cellular extracts could act as an environmental-friendly and economical alternative to synthetic liquid fertilizer for promoting sustainable agriculture.

1. Introduction

Owing to the increasing population and demand for food, it is essential to address the food requirements without degrading the environment further (Ronga et al., 2019). In agriculture, due to the increasing demand for sustainability, there is a need to opt for natural or biological fertilizers to substitute the synthetic fertilizers (Tauler and Baraza, 2015; Tahami et al., 2017). In this context, biostimulants, including polysaccharides, phytohormones, vitamins, amino acids, etc., are receiving attention as natural substances that can promote plant growth (Chiaiese et al., 2018). In recent years, microalgae have become a promising source of biofertilizer and biostimulant in agriculture for improving crop production and for producing healthy plants (Michalak et al., 2017).

Microalgae are photosynthetic microorganisms grown in marine, fresh or wastewaters with different nutrient sources and used in fuel, agricultural, food, pharmaceutical and animal sectors (Behera et al., 2019). As microalgae are found to contain various plant growth-promoting substances such as polysaccharides, lipids, proteins and phytohormones as reported by Kumar and Sahoo (2011); Godleweska et al., (2016) and Uthirapandi et al. (2018), their intracellular extracts can be considered for enhancing the plant yield (Elarroussia et al., 2016; Garcia-Gonzalez and Sommerfeld, 2016). Water-soluble extracts of algae containing bioactive compounds could be extracted via physical, mechanical and enzymatic methods (Chiaiese et al., 2018) for use during seed pretreatment or foliar spray (Ronga et al., 2019).

Seed pretreatment increases the rate of imbibition, and the inflow of water-soluble metabolites, softens the testa and regulates the pre-germinative and biochemical processes to initiate growth and protrusion of radicle (Barone et al., 2018). Studies have shown that the seeds pretreated with microalgal cellular extracts have higher germination and plant growth rate compared to the control along with higher soluble carbohydrate, protein and free amino acid content (Kumar and Sahoo, 2011; Hernández-Herrera et al., 2014; Ibrahim, 2016). Garcia-Gonzalez and Sommerfeld, (2016) reported that extracts of *Acutodesmus sp.* with more than 50% concentration showed faster germination of tomato seeds in 2 days earlier than the untreated seeds. Barone et al. (2018) reported that the algal extracts of *C. vulgaris* and *S. quadricauda* resulted in upregulation of genes related to primary and secondary metabolism associated with nutrient uptake, thereby resulting in more fine and

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lengthy roots in sugar beet.

Often due to the physicochemical problems associated with the soil structure, essential nutrients in biofertilizer applied directly to soil might not reach the plants (Ronga et al., 2019). Thus, foliar spraying involving the absorption of biostimulants through stomata is considered as an essential alternative (Battacharyya et al., 2015). This process is usually efficient during the day time when the plant receives maximum sunlight and the stomata and cuticle pores remain open for the maximal uptake of nutrients (Ronga et al., 2019). Several researchers have explored the foliar spraying of microalgal extracts derived from *Spirulina plantensis* over pepper (Aly and Esawy, 2008), egg plant (Dias et al., 2016), and winter wheat (Michalak and Chojnacka, 2014). Application of *Arthrospira sp.* extracts at 3 g L^{-1} resulted in an increment in the fresh and dry weight of red beet along with higher hypocotyl length (de Oliveira et al., 2013). Elarroussia et al. (2016) reported that foliar spray application of 1.5 g L^{-1} and 3 g L^{-1} of *Arthrospira sp.* extracts resulted in 30% increase in the size of tomato plants, along with an increase in root length and root nodes. Foliar application of *Scenedesmus sp.* extracts at a concentration of 10 g L^{-1} have been reported to stimulate the growth of Petunia plants in terms of accelerated shoot and root development, and early incidence of flowering (Plaza et al., 2018).

Tomatoes grow practically in all soils from light sandy to heavy clay in any season of the year depending on the irrigation facilities available unlike other crops such as paddy or wheat. With rich content of vitamins, minerals, lycopene, β-carotene and anti-cancerous agents, it is considered as the vegetable of wide economic value and a major contributor towards food security. Due to its ease of propagation and short life-cycle, it has been utilized as a model crop for several plant growth studies (Delian et al., 2017). India is the second-largest producer of tomato after China. Tomato is the third most important vegetable in India, sharing 8.5% of total vegetable production (Horticulture Statistics Division Report, 2020). According to the statistics, India produced a total of 194.96 lakh tonnes of tomatoes in 2018-19 with a chemical fertilizer consumption of 128 kg ha^{-1} (Horticulture Statistics Division Report, 2020). Thus, it is essential to analyze the efficacy of algal extracts as a biostimulant for tomato in order to meet the growing demand without degrading the environmental standards.

The objective of the present study is to investigate the stimulating potential of cellular extracts from dried biomass of a robust microalgal consortium cultivated in 6.5% (v/v) diluted human urine, as a seed primer and foliar spray for tomato plants. Algal extracts of varying concentrations (20-100%) were characterized for the presence of biochemical constituents and later utilized in seed treatment for enhancing the germination rate, and the plant growth rate through foliar spray. Plant growth rate was determined in terms of increase in biomass, shoot length and number of leaves over the period of growth. Also, the chlorophyll, carotenoid and the elemental content of the plant biomass were measured after harvest. Such studies would aid in harnessing the untapped potential of algal extracts as an environmental friendly biofertilizer for promoting the growth of tomato plants.

2. Materials and Methods

2.1. Cultivation and characterization of microalgal biomass

2.1.1. Microalgae cultivation and harvesting

A mixed consortium of microalgae consisting mainly of *Chlorella sp., Scenedesmus sp., Spirulina sp., and Synechocystis sp.,* were obtained from the wastewater ponds of National Institute of Technology (NIT) Rourkela. It was enriched with 6.5% (v/v) of diluted human urine and was grown at an ambient temperature of (30 ± 5) ºC with a light intensity of 205 μmol photons m^{-2} s^{-1} for 12:12 light: dark cycle. The algal strains were identified morphologically and consortium composition was routinely checked through microscopic analysis. The rationale behind the use of the microalgal consortium as a source of biostimulant is based on the fact that similar algal strains have also been previously reported to show significant influence over the crop yield and plant growth rate (Shaaban, (2001); Shaaban et al. (2010); de Oliveira et al. (2013); Elarroussia et al. (2016); Plaza et al. (2018)). Also, the studies by Hernandez Melchor et al. (2016) and Win et al. (2018) have projected that a consortium functions as biofertilizer more efficiently since, it increases the availability of metabolites better than individual strains.

The above concentration of urine has been selected based on the previous studies done by authors (Behera et al., 2020). The media (human urine) had a pH varying from 6.2-6.5 with (0.03 ± 0.02) mg ml^{-1} of ammonium ions, (23.68 ± 2.12) mg L^{-1} phosphate ions, and salts like sodium (251 ± 3.53) mg ml^{-1}, potassium (10.65 ± 1.90) mg ml^{-1}, calcium (72.75 ± 5.10) mg ml^{-1} with total dissolved solids at concentration of 2.95 ± 0.07 mg ml^{-1}. The influence of inhibition on microalgal growth rate due to batch variation in salts is expected to be avoided by repeated subcultures over a period of two years. The algal biomass after growth was processed to remove any residual salts and contaminants to avoid its interference in plant growth. The biomass was concentrated, washed with distilled water thrice to remove the residual salts and contaminants and then dried, before being utilized for extraction of organic and inorganic water-soluble constituents to be used as biostimulants. Drying was carried in hot air oven (Labotech) at 60 °C to obtain the dried microalgal biomass.

2.1.2. Characterization of microalgal biomass

The dried microalgal biomass obtained after the harvesting was subjected to biochemical characterization to evaluate the percentages of carbohydrate through colourimetric assay using dinitrosalicylic acid (DNS) method (Miller, 1959), protein and lipid content present using Bradford method (Bradford, 1976) and Bligh and Dyer method as provided in Behera et al. (2020) respectively. Also, elemental characterization was performed to calculate the percentage of Carbon (*C*), Hydrogen (*H*), Nitrogen (*N*), Sulphur (*S*) and Oxygen (*O*) contents present in the dried microalgal biomass using *CHNS* elemental analyzer.

2.2. Preparation and characterization of microalgal cellular extracts

2.2.1. Microalgal cellular extracts preparation

Dried microalgal biomass was dissolved in deionized water and crushed using motor and pestle (10 g of dried microalgal biomass dissolved in 100 ml deionized water considered as 100% extract). The crushed biomass was exposed to ultrasonic waves using a probe ultrasonicator at a frequency of 20 kHz and a pulse rate of 10 s for 60 s. The sonicated sample was centrifuged at 5000 rpm for 20 min to obtain the microalgal cellular extracts. Supernatant collected after the centrifugation was considered as 100% extract. It was used further for the preparation of 20, 40, 60, and 80% of the microalgal cellular extracts with deionized water. Deionized water was considered as control with 0% extract. The prepared microalgal cellular extracts were used for seed pretreatment and foliar spray.

2.2.2. Characterization of the prepared microalgal cellular extracts

Physiochemical characterization of the algal extracts were done to analyze the biochemical content and physical properties. pH of the extracts was measured using a pH meter (Labman Scientific Instruments). Electrical conductivity, salinity and total dissolved solids (TDS) present in the extracts were measured using a multi-parameter water quality meter (Labman Scientific Instruments) with appropriate standards for each measurement. Protein content was measured by performing Bradford assay given by Bradford (1976) and represented as mg ml^{-1}. The concentrations of sodium (*Na*), potassium (*K*) and calcium (*Ca*) present in the microalgal cellular extracts were measured by using a microprocessor-based flame photometer (model 1385) calibrated with the appropriate standards and represented in ppm (parts per million).

2.3. Physiochemical characterization of soil

Soil was collected from organic farm of NIT Rourkela at a latitude of 22.25 °N and a longitude of 84.91 °E from a depth of 20 to 30 m. The soil collected was dried under room temperature until the moisture content reached 4%. Soil moisture content was measured by placing the soil in a crucible at 105 ºC for 24 h in a muffle furnace and calculating the loss in weight of the soil. Eq. (1) was used to measure the moisture content present in the soil.

$$Moisture\ content\,(\%) = \frac{loss\ in\ weight\ of\ soil\ (g)}{initial\ weight\ of\ soil\ (g)} \times 100 \quad (1)$$

Soil pH was determined by mixing the soil in water (1:20 w/v) and measured using a pH meter. Electrical conductivity, salinity and total dissolved solids present in the soil were determined using a multi-parameter water quality meter (Labman Scientific Instruments) with appropriate standards for each measurement as given by Richards (1954). Percentage of organic matter and organic carbon present in the soil was determined using the method given by Davies (1974). Soil bulk and particle density (g cc^{-1}) was measured following the procedure given by Daddow and Warrington (1983). Pore space and solid space present in the soil were calculated using the formula given in Eq. (2) and Eq. (3) respectively.

$$Pore\ space\ (\%) = \frac{Particle\ density\left(\frac{g}{cc}\right) - Bulk\ density\left(\frac{g}{cc}\right)}{Particle\ density\left(\frac{g}{cc}\right)} \times 100 \quad (2)$$

$$Solid\ space\ (\%) = 100 - Pore\ space\,(\%) \quad (3)$$

Cation exchange capacity (CEC) of the soil was determined by the method given by Richards (1954) and performing the experiment by using flame photometer with appropriate standards. The concentration of *Na* and *K* in the soil was measured using flame photometer using the procedure given by Toth and Prince (1949).

2.4. Seed pretreatment and foliar spraying with microalgal cellular extracts

2.4.1. Seed pretreatment process

Tomato (*Solanum lycopersicum*) seeds were procured from Hi-Tech Genetic Crop Science Pvt. Ltd., (India) and soaked in 20 ml of 5% sodium hypochlorite solution for 10 min. The soaked seeds were washed thrice with deionized water and dried on 70 mm Whatman filter paper for 24 h at room temperature. These dried seeds were distributed into 50 mm petri plates with 10 seeds per plate and treated with 10 ml of appropriate concentrations of microalgal cellular extracts. Four replicates per treatment were taken and incubation for 24 h at room temperature. After the incubation period, extracts were removed and the seeds were kept for drying on 70 mm Whatman filter paper for 24 h at room temperature. Seeds without any treatment as well as 0% treatment (only distilled water) served as a control for the seed treatment process. The dried seeds were transferred to pots containing coco peat for germination and placed in environmental conditions with a temperature of 25-28 ºC day/ 15-19 ºC night, humidity of 48-52%, atmospheric pressure of 740-780 mm Hg and light intensity of 230-250 W m^{-2}.

Germination percentage is an estimate of the viability of a population of seeds and calculated with the formula given by Djavanshir and Pourbeik (1976) (Eq. 4).

$$Germination\ percentage = \frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds} \times 100 \quad (4)$$

2.4.2. Foliar spraying process

Tomato seeds were germinated in coco peat to obtain plantlets of two-leaf stage. The plantlets were transferred into pots containing 500 g of soil with a single plantlet per pot. The experiment consisted of five different microalgal cellular extract concentrations (20, 40, 60, 80 and 100%) and control containing 0% extract concentration (distilled water) with four replicates per treatment. The experimental conditions were same as that of the conditions used during seed treatment. Each plant received foliar spraying every 5 days for a period of 20 days. Before the spraying process, the surface of potted soil was completely covered with aluminium foil to avoid leaching of spray into the soil that could be potentially taken up by the roots. Since the rate of absorption and cuticular penetration of the active biostimulants in the extract is dependent over the solar irradiance (Ronga et al., 2019), the spraying of microalgal cellular extracts was conducted during the noon time, when the plant receives maximum possible sunlight resulting in wider stomatal openings. Higher resultant water pressure during noon allows greater penetration of the extracts into the leaf through the stomata. All the plants were watered according to the requirement except after foliar spraying where they were not watered for 24 h.

2.5. Determination of plant growth rate and productivity

2.5.1. Plant physiological growth analysis

The growth rate of plant was analyzed in terms of an increase in shoot length and number of leaves over a period of 20 days from the day of transfer into the potted soil. The plant growth rate was measured in control (untreated and 0% treatment) as well as in all the treatments (20, 40, 60, 80 and 100%) for every 5 days and a comparison was made to determine the treatment that gave the highest plant growth rate.

2.5.2. Post-harvest analysis

After the seed pre-treatment process and foliar spraying, the plants were allowed to grow for 20 days and harvested for fresh and dry weight analysis. Final shoot and root length of the plants were measured after separating it from the plant during the harvesting process. Fresh weight of the harvested plant was noted. Plant biomass was placed in a hot air oven (Labotech, India) for drying at 60 ºC for 48 h, and dry weight was measured. Chlorophyll a (*Chl a*), chlorophyll b (*Chl b*) and total chlorophyll content (total *Chl*) present in the dried leaves were measured according to the method of Arnon, (1949). Total carotenoid content present in the plant biomass was measured according to Sumanta et al. (2014).

2.6. Statistical analysis

The mean and standard error of mean of the data were calculated using XL STAT incorporated in Microsoft Excel 2016, and OriginPro 2016 64-bit was used to calculate one-way ANOVA along with Tukey test. The means and the differences were considered as significant with probability value (alpha < 0.05).

3. Results and Discussion

3.1. Physiochemical characterization of microalgal biomass

The algal consortium consisting mainly of *Chlorella sp.*, along with strains of *Spirulina sp.*, *Scenedesmus sp.*, and *Synechocystis sp was grown* in 6.5% (v/v) of diluted human urine, resulting in assimilation of the nutrients into the biomass. A thorough processing of algal biomass has been done in order to avoid any interference of residual salts and contaminants from urine, the nutrients metabolized from urine into algae during the growth played an essential role in influencing its biochemical content. The elemental composition of microalgae and the biochemical content has been provided in Table 1. Algal biomass showed appreciable amount of carbohydrates (40.90%), proteins (26.18%) and lipids (27.20%). These wide array of biochemical content has been reported to serve as the precursor compounds of phytohormones, which are utilized in the signalling pathways of plants, thus increasing the tolerance of plants to abiotic stress (Battacharyya et al.,

Table 1
Biochemical and elemental composition of the microalgal consortium. Data are means ± standard error at n = 3.

Biochemical Composition	
Carbohydrates	(40.90 ± 0.35) %
Protein	(26.18 ± 0.28) %
Lipids	(27.20 ± 0.22) %
Elemental Composition	
Carbon	(30.3 ± 0.205) %
Hydrogen	(4.08 ± 0.336) %
Nitrogen	(3.94 ± 0.367) %
Oxygen	(60.74 ± 0.356) %
Sulphur	(0.94 ± 0.53) %

2015). Michalak et al. (2017) reported that algal polysaccharides often trigger signalling cascade for activating the plant defence response against salt stress and provide resistance against pathogens. Proteins composed of essential amino acids like tryptophan and arginine, act as key precursors of phytohormones and secondary aromatic compounds like polyamines, thereby controlling embryogenesis, organogenesis as well as protection against osmotic stress (Chiaiese et al., 2018). Elemental analysis showed the dominance of mainly oxygen (60.74 ± 0.356) % and carbon (30.3 ± 0.205) % with minor quantities of nitrogen, hydrogen and sulphur. The significantly high carbon and oxygen content indicates the presence of carbohydrates which could act as precursors for β-glucan, chrysolaminarin, floridean and myxophycean starch that aids in promoting plant growth (Ronga et al., 2019). Carbohydrates upto 46% and 18-46% protein content have also been reported in *Chlorella sp.*, *Dunaliela sp.*, *Spirulina sp.*, *Chlamydomonas sp.*, mostly used for biofertilizer applications (Chiaiese et al., 2018). The inherent biochemical composition indicated the suitability of microalgae to be used as a potential nutrient source for plant growth. Elansary et al. (2016) reported that the macro and microelements as well as the carbohydrate contents of *A. nodusum* were responsible for the biostimulant effects over mint and basil plants. Chiaiese et al. (2018) and Ronga et al. (2019) projected that 26% proteins in microalgae could act as metabolic precursors for phytohormones that play essential role in embryogenesis, organogenesis and protection against osmotic stress, thus promote plant growth.

3.2. Physiochemical characterization of microalgal cellular extracts

Algal biomass was processed finely to restrict the role of urine till microalgal growth and assimilation of nutrients, and the metabolites accumulated were physically extracted to be utilized as a biostimulant. To confirm the suitability of the algal extracts to be used as nutritional supplement, the physiochemical characterization of the extracts was done to know their physical properties and chemical composition (Table 2). pH measurement showed all the extracts to be in neutral range. Godlewska et al. (2016) reported that biostimulants work best at neutral pH. The range of electrical conductivity, salinity and TDS for the algal extracts was found to be 1.07-5.41 µS, 630-3290 mg L^{-1} and 0.64-3.12 ppt respectively. Shariatmadari et al., 2013) reported an electrical conductivity of 17 µS and 63 µS for extracts of *Nostoc sp.* and *Anabaena sp.,* respectively.

Protein concentration of 0.718 mg L^{-1} was found in 100% algal extract. Micro-elemental composition consisted of 1.64 mg L^{-1}, 0.35 mg L^{-1} and 0.28 mg L^{-1} of *Na*, *K* and *Ca* respectively. Ramya et al. (2015) reported a *Na* concentration of 5.77 mg L^{-1} and *K* concentration of 1.07 mg L^{-1} in the cellular extract of brown marine alga *S. marginatum*. Uthirapandi et al. (2018) also reported *Na* to be present in the range of 0.069- 0.098 mg L^{-1} and *K* in the range of 0.098- 0.17 mg L^{-1} for the marine extracts of *S. weightii*, *C. racemora* and *T. Ornate*. The content varies depending on the species used and the extraction procedure implemented. The use of microalgal extracts for biofertilizer applications with their biochemical characterization are limited with few studies by Shaaban (2001); (2010) and Garcia-Gonzalez and Sommerfeld, (2016). However, the micro and macro elemental composition also profoundly influences the plant metabolism. Michalak et al. (2017) reported that micro elements along with the polysaccharides form hydrocolloids enhance the gelling and wetting capacity and also the cuticular penetration when utilized as foliar spray agent. Microalgal cellular extracts from blue green algae have also been postulated to release growth promoting substances and inorganic elements improving the microbial consortia in the soil and also provide resistance against diseases (Koffi et al., 2018).

3.3. Physiochemical characterization of soil

It is essential to characterize the soil in order to access its suitability for plant growth. The properties of soil used for the experiments have been given in Table 3. pH of soil was found to be nearly neutral and has an electrical conductivity of 69.3 µS. Soil bulk density is an indirect measure of the total pore space present for root penetration. Bulk density of 1.38 g cm^{-3} (less than 1.6 g cm^{-3}), with 47.92% pores space indicated that it is suitable for percolation of nutrients and water (Agegnehu et al., 2015). Elemental analysis showed 0.66 and 0.145 mg of *Na* and *K* per g of soil. Cation exchange capacity (CEC) of the soil was observed to be 13.1 meq g^{-1}, thus presence of exchangeable cations for plant uptake (Richards, 1954; Agegnehu et al., 2015). Hence, the soil taken for the present study can support plant growth, withhold the root firmly and allow water to flow through the roots. It is noteworthy to mention that tomatoes, unlike other crops like paddy can grow in a diverse variety of soil ranging from light sandy to heavy clay in any season based on the available irrigation facilities. However, sometimes due to erratic climatic conditions, unavailability of water, excessive utilization of chemical fertilizers the physicochemical properties of soil changes, making the availability of nutrient uptake by plants difficult (Ronga et al., 2019). This results in increased seed dormancy, reduced germination rate and stunted plant growth due to various biotic and abiotic stress factors. To circumvent these effects and to increase the growth rate and crop yield, it is essential to complement the plant growth system via seed treatment and foliar spray, without modulating the soil properties.

Table 2
Physicochemical characterization of microalgal cellular extracts of 0, 20, 40, 60, 80 and 100% concentrations. Data are means ± Standard error at n = 4.

Characteristics	0%	20%	40%	60%	80%	100%
pH	7.0 ± 0.1	6.9 ± 0.2	7.0 ± 0.1	7.0 ± 0.2	6.9 ± 0.1	6.9 ± 0.2
Electrical conductivity (µS)	0	1.07 ± 0.14	2.27 ± 0.09	3.4 ± 0.16	4.47 ± 0.24	5.41 ± 0.2
Salinity (PSU)	0	0.63 ± 0.23	1.27 ± 0.36	2.05 ± 0.42	2.70 ± 0.34	3.29 ± 0.28
Total Dissolved Solids (ppt)	0	0.64 ± 0.16	1.26 ± 0.28	1.96 ± 0.3	2.60 ± 0.34	3.12 ± 0.26
Protein (mg ml^{-1})	0	0.11 ± 0.05	0.28 ± 0.05	0.42 ± 0.07	0.61 ± 0.06	0.72 ± 0.04
Na (mg ml^{-1})	0	0.16 ± 0.08	0.48 ± 0.07	0.82 ± 0.06	1.11 ± 0.11	1.64 ± 0.08
K (mg ml^{-1})	0	0.052 ± 0.016	0.122 ± 0.05	0.187 ± 0.04	0.271 ± 0.06	0.345 ± 0.08
Ca (mg ml^{-1})	0	0.093 ± 0.015	0.151 ± 0.09	0.201 ± 0.012	0.247 ± 0.009	0.279 ± 0.013

Table 3
Physicochemical characteristics of soil taken for the study. Data are means ± Standard error at n = 4.

Parameter	Value
pH (25 °C)	6.8 ± 0.2
Electrical conductivity (μS)	69.3 ± 1.7
Total Dissolved Solids (ppm)	34.6 ± 1.5
Salinity (PSU)	0.04 ± 0.005
Organic Matter (%)	0.21 ± 0.04
Organic Carbon (%)	0.12 ± 0.02
Bulk Density (g cm^{-3})	1.38 ± 0.24
Particle density (g cm^{-3})	2.65 ± 0.52
Pore space (%)	47.92 ± 1.8
Solid space (%)	52.08 ± 1.8
Moisture content (%)	4.00 ± 0.5
Cation exchange capacity (meq g^{-1} soil)	13.10 ± 1.2
P (mg g^{-1} soil)	0.01 ± 0.007
Na (mg g^{-1} soil)	0.66 ± 3.5
K (mg g^{-1} soil)	0.145 ± 2.4

3.4. Seed pretreatment with microalgal cellular extracts

3.4.1. Determination of seed germination rate

The ability of a seed to germinate uniformly and rapidly at different environmental conditions is an essential characteristic required for most of the plants, including tomato. Germination percentage gives the viability of the number of seeds germinated per treatment. The average percentage of seed germination in tomato is 70-85% for an incubation period of 4-5 days as reported by the **Central Seed Certification Board, Government of India (2020)**. Unfavourable conditions such as inadequate availability of seed nutrients, poor water supply and improper environmental conditions may results in increased seed dormancy and reduce the percentage of seed germination. Conventional method for alleviating the problem includes treating the seeds with chemicals, which causes a detrimental effect over the environment. Thus, to achieve maximal germination without deteriorating the environment, seed pretreatment with biostimulants could be used as an alternative as suggested in the present study. The metabolites/ bioactive compounds imbibed during the course of seed pretreatment, will act as precursors for biochemical pathways to help in early protrusion of radicle (Barone et al., 2018). These are also postulated to protect the plant against abiotic stress in later course of growth providing better yields. All the seeds treated with different concentrations of algal extracts showed positive results of seed germination (**Fig. 1**). The seeds treated with microalgal cellular concentrations of 20, 40 and 60% gave the highest germination rate of 100% after 3 days (higher than the mean germination rate of tomato) compared with the control and other treatments. It was also observed that the plantlets from the treated seeds were healthier than those from the untreated and control ones. From the box plot data, it could be seen that 60% extract showed a more consistent and dependable percentage of germination. The carbohydrates, proteins and other micro and macro-elements extracted from the algal biomass, has been absorbed by the seed testa and thereby increased the rate of imbibition and provided precursors which influenced the biochemical pathways aiding in faster protrusion of radicle during germination (Barone et al., 2018).

Kumar and Sahoo (2011) reported a 100% germination rate with 20% *S. wightii* extracts for wheat. Similar to the above study, Garcia-Gonzalez and Sommerfeld, (2016) also reported highest gemination rate obtained with *Acutodesmus* extracts of 50 and 75%. Ibrahim, (2016) also reported a 12-25% increase in germination percentage in seeds pretreated with marine algal extracts compared to the control (treated only with water). The enhanced germination percentage is due to the presence of carbohydrates, proteins and other microelements that acts as precursor of elicitor compounds accelerating the protrusion of radicle (Hernández-Herrera et al., 2014). Also, seed treatment with microalgal extracts rich in phytohormones and plant growth promoters reduces the seed dormancy, promoting efficient germination.

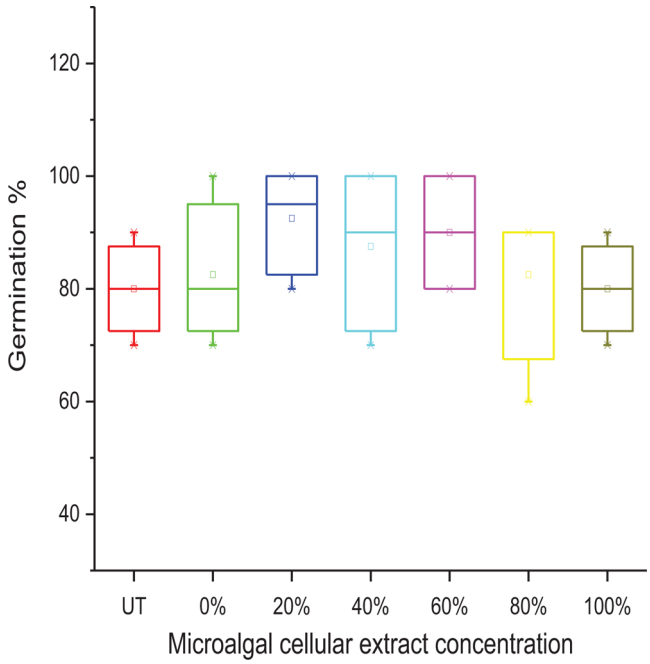

Fig. 1. Germination percentage of seeds pretreated with the concentrations of 0, 20, 40, 60, 80 and 100% of microalgal cellular extracts. Data are means ± Standard error at n = 4 and $p < 0.05$

3.4.2. Plant growth rate analysis

Growth of plant was analyzed in terms of changes in shoot length and the number of leaves, following the transfer of plantlets into the soil over the period of 20 days. Fig. 2**a and 2b** shows the results of shoot length and number of leaves obtained over a period of 20 days for all the treated plants. It was seen that the increase in shoot length and that of the number of leaves for the plants treated with 60% and 40% cellular extracts of algal consortium dominated mainly with *Chlorella sp.,* along with strains of *Scenedesmus sp., Synechocystis sp.,* and *Spirulina sp.,* were almost the same until 10^{th} day. However, a rapid increase in shoot length and leaf number were seen over the last 10 days with 40% cellular extracts. Even though the germination percentage was highest for seeds treated with 20, 40 and 60% extracts, the plant growth rate was found to be maximum with 40% extract with 19.86 ± 0.51 cm shoot with 34.6 ± 1.9 leaves. Kumar and Sahoo, (2011) also reported that *S. wightii* extract concentrations greater than 20% resulted in shorter shoot lengths, smaller root lengths, lower lateral roots of wheat plants. Hernández-Herrera et al., (2014) reported that 1% extracts of *U. lactuca* and *P. gymnospora* resulted in highest germination rate as well as maximum plumule and radicle length of tomato plants. Algal extracts increases the uptake of nutrients by the radicle, thereby enhancing their overall growth (Ghaderiardakani et al., 2019). The bioactive compounds of carbohydrates and proteins along with microelements like *Na, K* and *Ca* can promote the growth only at an optimum concentration, beyond which it is found to have inhibitory effects. The optimum concentration of algal extract (biostimulants) showing maximal results depends on the species and biochemical content of microalgae used, environmental conditions and the plant targetted.

3.4.3. Post-harvest analysis

The final root and shoot length along with the fresh and dry weight of the plants obtained post-harvest (after 20 days) provides an estimate of the overall growth of plant. Fig. 3**a** represents the shoot and root length of plants obtained after the harvest. From the graph, it was

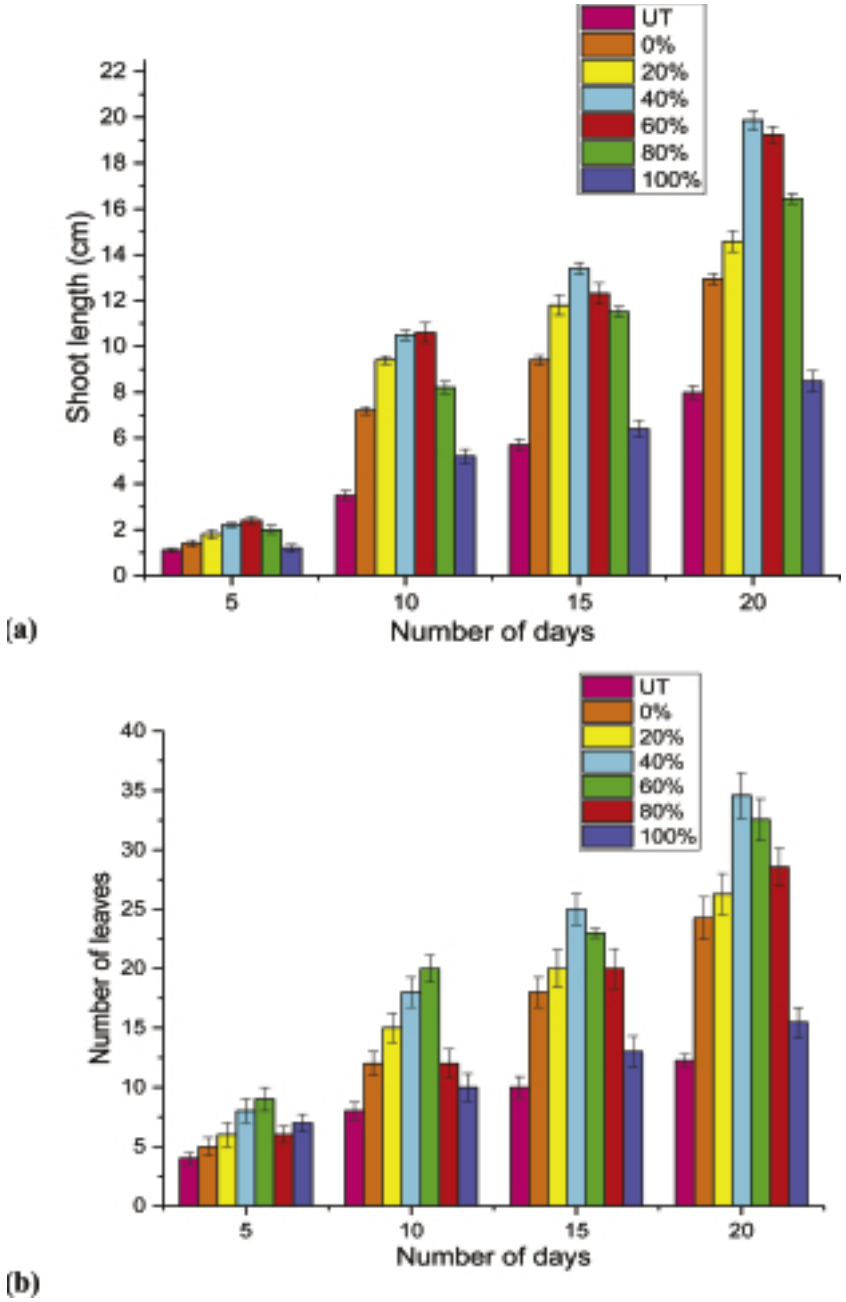

Fig. 2. (a) Shoot length and (b) Number of leaves in tomato plants over a period of time obtained after seed pretreatment with concentration of 0, 20, 40, 60, 80 and 100% microalgal cellular extracts. Data are means ± Standard error at n = 4 and $p < 0.05$

observed that the seeds treated with 40% microalgal cellular extract gave the highest shoot length of 19.86 ± 0.51 cm and root length of 14.87 ± 0.63 cm compared to other pre-treatments. Kumar and Sahoo, (2011) reported a shoot length of 25 cm and root length of 10 cm with *S, wightii* extracts after 25 days of sowing. Hernández-Herrera et al., (2014) reported a maximum radicle length of 7.3 m and plumule length of 8.3 m, with *U. lactuca* and *P. gymnospora* extracts respectively after 8 days of sowing with 1% cellular extracts. Fig. 3**b** shows the fresh and dry weight of plants obtained after the harvest. From the graph, it was observed that the plants treated with 40% microalgal cellular extracts gave the highest productivity with fresh weight of 3.471 ± 0.04 g and dry weight of 0.389 ± 0.036 g. A minimum productivity was obtained from the plants whose seeds were untreated with a fresh weight of 0.181 ± 0.023 g and dry weight of 0.03 ± 0.006 g. An increase in micro-elemental composition was also obtained with the seed treatment compared to the untreated sample as shown in Table 4. Maximum dry weight of 360 mg of tomato plant after 8 days was reported with 1% cellular extracts of *U. lactuca* and *P. gymnospora* (Hernández-Herrera et al., 2014). At post-germination stage, the rate of increase in shoot and root length and dry weight has been reported to be efficient at low algal extracts, while higher concentration showed inhibitory effect (Ganapathy Selvam et al., 2013).

Chlorophyll is the green coloured pigment present in plants which is essential for carrying out the photosynthesis, whereby plants derive energy for their growth, metabolism and reproductive processes, thus is a measure of plant overall growth (Gaikwad et al., 2012). Higher the chlorophyll content, greater is the plant photosynthesis and growth rate. As seen in Fig. 3c, total chlorophyll content present in the plants with 40% treatment was highest with 12.86 ± 0.417 mg g^{-1} having a *Chl a* content of 5.36 ± 0.15 mg g^{-1} and *Chl b* content of 7.5 ± 0.26 mg

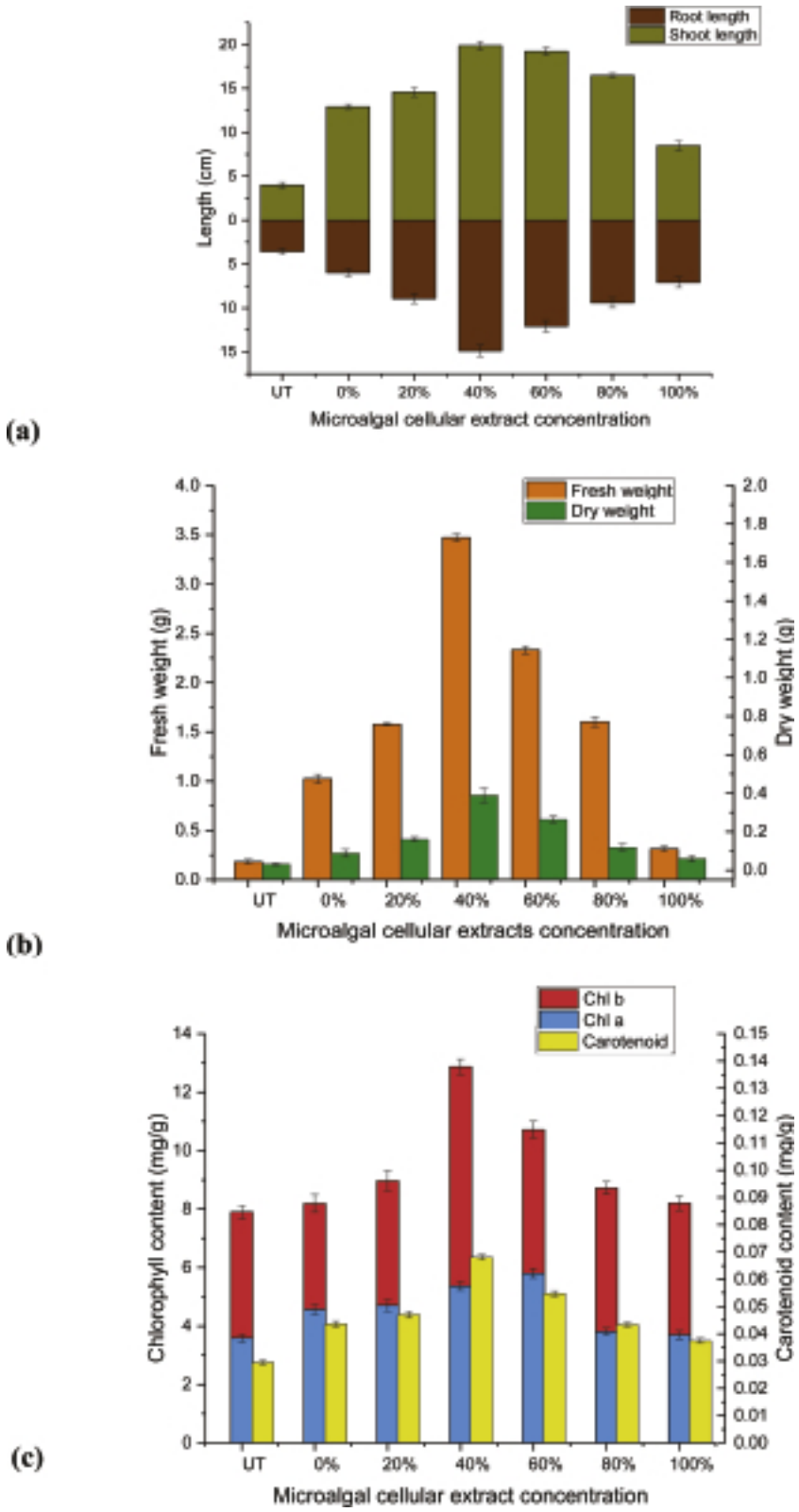

Fig. 3. (a) Shoot and root length (b) Fresh and dry weight (c) Chlorophyll and carotenoid content present in the tomato plants at the end of study due to the seed pretreatment. Data are means ± Standard error at n = 4 and $p < 0.05$

g^{-1}. 2.5% of seaweed extracts resulted in 26.4 mg L^{-1} of *Chl a* and 9.55 mg L^{-1} *Chl b* in Garden cress after 7 days (Godlewska et al., 2016). Carotenoids are the important antioxidant pigments present in plant that play essential role in photosynthesis and plant defence against pathogens. 40% treatment gave the highest carotenoid content of 0.07 ± 0.001 mg g^{-1} compared with the other treatments and control. Gireesh et al. (2011) and Gaikwad et al. (2012) reported that lower concentration of *Ulva* extracts enhanced the pigment concentration while increasing the concentration to even 20% decreased the pigment content in *Vigna unguiculata* and *Solanum melongena*. Thus, the influence

Table 4
Physicochemical properties of tomatoes grown after seed pretreatment with different concentrations of microalgal cellular extracts. Data are means ± Standard error at n = 4

Seed Treatment	Moisture content (%)	P (mg g^{-1})	K (mg g^{-1})	Na (mg g^{-1})	Ca (mg g^{-1})
Untreated	83.6 ± 2.3	0.12 ± 0.02	1.06 ± 0.04	0.12 ± 0.02	0.08 ± 0.005
0%	91.25 ± 3.23	0.15 ± 0.08	1.07 ± 0.06	0.13 ± 0.03	0.08 ± 0.004
20%	89.82 ± 1.2	0.31 ± 0.06	1.09 ± 0.04	0.14 ± 0.02	0.09 ± 0.034
40%	88.81 ± 1.29	0.85 ± 0.11	1.11 ± 0.03	0.16 ± 0.04	0.1 ± 0.042
60%	88.72 ± 0.73	0.72 ± 0.09	1.08 ± 0.05	0.15 ± 0.03	0.09 ± 0.028
80%	92.7 ± 1.3	0.46 ± 0.06	1.07 ± 0.04	0.13 ± 0.02	0.08 ± 0.036
100%	81.58 ± 4.8	0.38 ± 0.05	1.08 ± 0.03	0.14 ± 0.03	0.08 ± 0.038

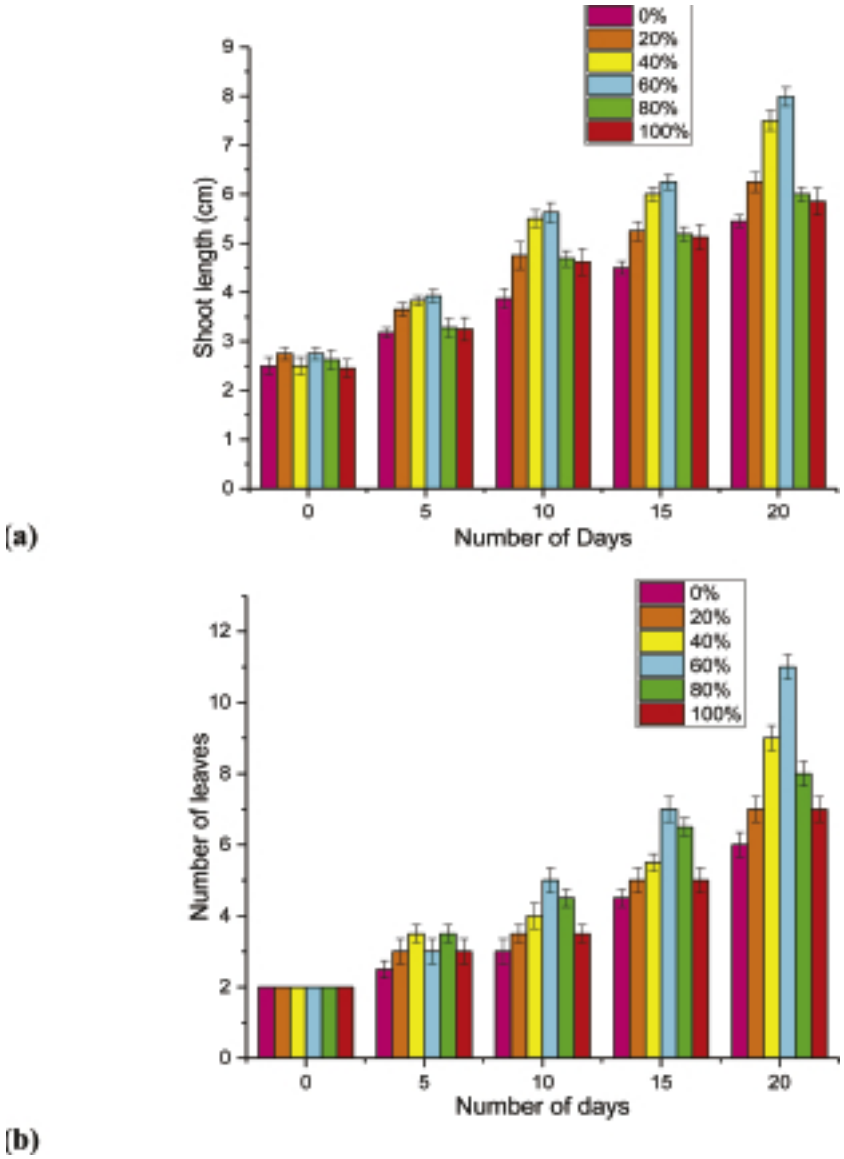

Fig. 4. Increase in (a) shoot length and (b) number of leaves with time after foliar spraying of 0, 20, 40, 60, 80 and 100% microalgal cellular extract concentrations. Data are means ± Standard error at n = 4 and $p < 0.05$.

on the pigment content and the increase in pigment content is specific to the nature of algae and the composition of algal extracts utilized.

3.5. Foliar spraying of microalgal cellular extracts

3.5.1. Plant growth rate analysis

Foliar spraying of nutrients on plants is a common practice followed when plants are deprived of essential nutrients for their growth (Win et al., 2018). A plant can absorb nutrients through its leaves at a faster rate than its roots. Due to the opening and closing of stomatal pores of the leaves, water soluble microalgal cellular metabolites can also pass and disperse along the whole plant through translocation (Ronga et al., 2019). This process of cuticular absorption and penetration is governed by the laws of diffusion and depends on the availability of sunlight. During day time, when the plant exposure to sunlight is the highest, application of foliar spray through the stomatal opening leads to a

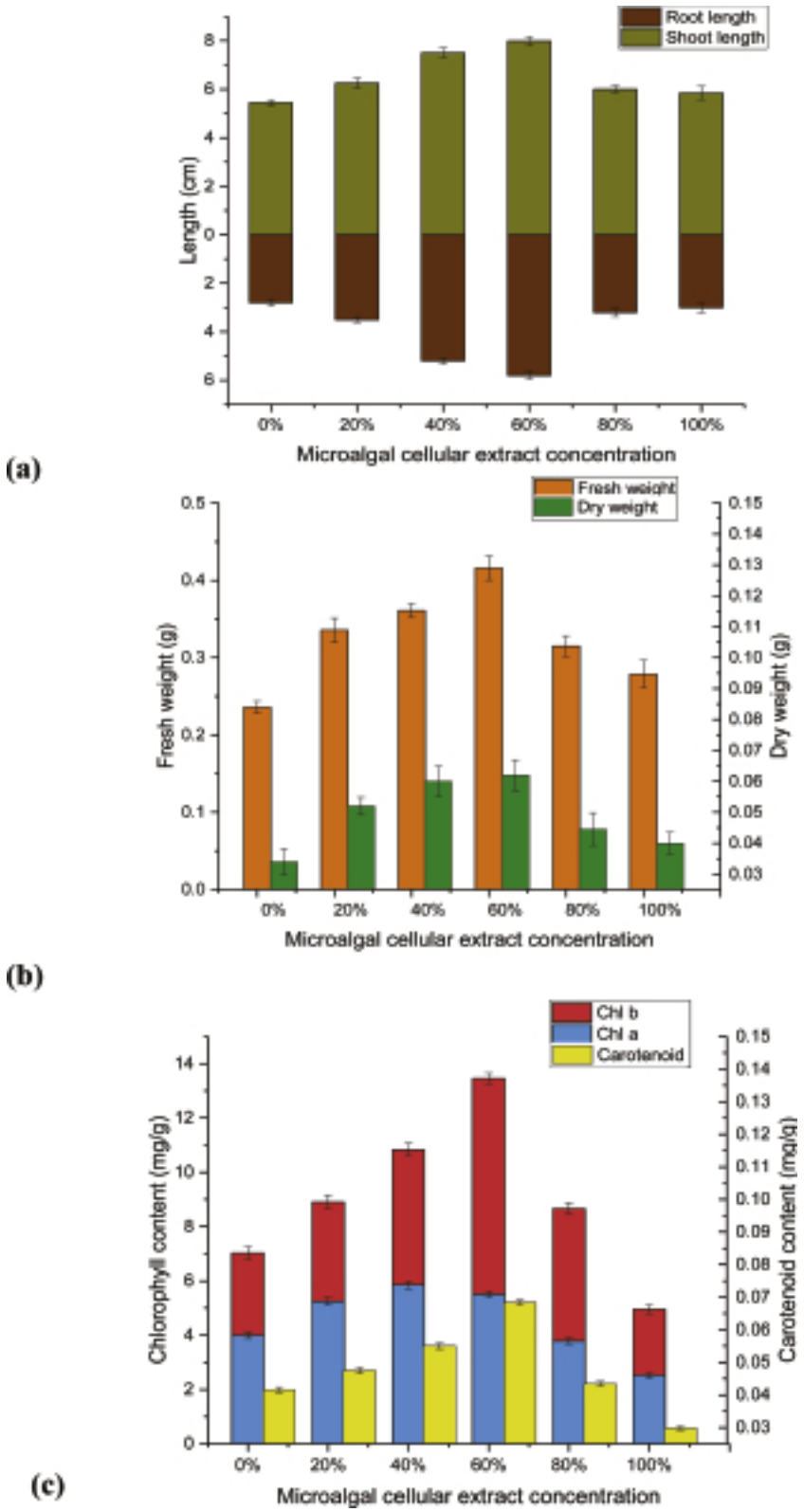

Fig. 5. (a) Shoot and root length (b) Fresh and dry weight (c) Chlorophyll and carotenoid content present in the tomato plants after foliar spraying of microalgal cellular extracts. Data are means ± Standard error at n = 4 and $p < 0.05$

maximum plant nutrient uptake (Battacharyya et al., 2015). Growth of plant was analyzed in terms of changes in shoot length and increase in number of leaves as shown in Fig. 4**a**. The algal extracts from the mixed consortium showed an increase in shoot length and leaves over time. It was observed that the shoot length of plants with 60% treatment was the highest with 7.98 ± 0.19 cm, 31.7% higher compared to the untreated sample. Highest number of leaves 11 ± 0.35 were obtained with 60% foliar spraying compared to the control with 6 ± 0.35 and other treatments (Fig. 4b). Foliar spraying of higher concentrations often inhibited the metabolic process resulting in lesser productivity

Table 5
Physicochemical properties of tomatoes grown with foliar spraying of different concentrations of microalgal cellular extracts. Data are means ± Standard error at n = 4

Seed Treatment	Moisture content (%)	P (mg g^{-1})	K (mg g^{-1})	Na (mg g^{-1})	Ca (mg g^{-1})
0%	85.68 ± 1.87	0.24 ± 0.07	1.02 ± 0.04	0.12 ± 0.02	0.07 ± 0.004
20%	84.51 ± 0.87	0.28 ± 0.08	1.05 ± 0.06	0.13 ± 0.03	0.08 ± 0.025
40%	83.42 ± 1.34	0.64 ± 0.14	1.22 ± 0.06	0.15 ± 0.03	0.09 ± 0.045
60%	85.14 ± 0.89	0.66 ± 0.12	1.23 ± 0.04	0.14 ± 0.04	0.12 ± 0.04
80%	85.96 ± 1.53	0.48 ± 0.07	1.15 ± 0.05	0.12 ± 0.03	0.09 ± 0.038
100%	85.68 ± 0.68	0.37 ± 0.06	1.14 ± 0.06	0.14 ± 0.02	0.08 ± 0.04

due to reduced plant nutrient uptake (Plaza et al., 2018). Similar results were obtained by Hernández-Herrera et al., (2014) where foliar spraying of seaweed extracts greater than 180 mg ml^{-1} for tomato plants resulted in smaller shoot lengths. Also, Garcia-Gonzalez and Sommerfeld, (2016) reported that 50% spray treatment of *Acutodesmus dimorphus* in tomato plant led to the maximum shoot length of 89 cm after 60 days. 10% extracts of green algae *C.racemosa* resulted in 38.1 cm shoot length and 58 leaves in *Ocimum sanctum* after 60 days (Uthirapandi et al., 2018).

3.5.2. Post-harvest analysis

Plants grown with foliar spraying of microalgal cellular extracts were harvested from the pots and its root length was determined as shown in Fig. 5**a.** The root length was found to be highest with 5.8 ± 0.16 cm for plants sprayed with 60% microalgal extract compared to the control and other treatments. Fresh and dry weight of plant biomass was found to be highest with 0.416 ± 0.015 g and 0.062 ± 0.005 g respectively for plants sprayed with 60% microalgal cellular extracts. Along with the increase in dry weight, an accelerated microelemental composition was also observed in the treated samples (Table 5). Similar results were obtained by Garcia-Gonzalez and Sommerfeld, (2016) where tomato plants were sprayed with different concentrations of aqueous extracts of *Acutodesmus dimorphus* and obtained the highest productivity in case of 50% treatment resulting in 89 cm shoot length, 390 g fresh weight of tomato plants after 60 days. Also, Shaaban, (2001) reported that the foliar spraying of wheat plants with extracts of *Chlorella vulgaris* resulted in increased nutrient uptake, grain yield, fresh and dry weight by 60.7% compared to the control. Shaaban et al. (2010) reported that spraying 1 g L^{-1} of *Scenedesmus sp*. extract with 1 g L^{-1} chelated micronutrients resulted in the harvest of 8 g of dry matter accumulation in wheat plants after 66 days. Elansary et al. (2016) reported that foliar spray of 5 and 7 ml per L of *A. Nodusum* extracts has been reported to contain polysacharides, amino acids which act as ellicitor for signaling pathways of phytohormones thus enhancing the leave numbers, root and shoot length as well as fresh and dry weight of mint and basil plants. Root length of 10.46 cm with total plant height of 48.47 cm along with fresh and dry weight of 5.66 g and 1.96 g respectively was reported for *Ocimum sanctum* with 10% *C. racemosa* extracts after 60 days (Uthirapandi et al., 2018).

Foliar spraying usually helps the plant in attaining the deficient nutrients like *N, P, Fe, Mg, Ca* and enable nutrient corrections. Hence, an optimum quantity of nutrients (appropriate concentrations) should be fed to the leaves without damaging them in order to get a healthy plant (Plaza et al., 2018). Also, foliar spraying depends on several other factors such as temperature, intensity of light, rate of application, humidity, etc. All these factors should be properly maintained in order to get a higher plant growth rate and productivity.

Chlorophyll content present in plant biomass is a measure of the plant growth rate, metabolism and productivity (Mohsen et al., 2016). Fig. 5**b** shows the *Chl a, Chl b* and total *Chl* content present in the plant biomass obtained after spraying microalgal cellular extracts. It can be observed that the chlorophyll content was found to be maximum in case of 60% treatment with (13.45 ± 0.307) mg g^{-1} compared to control and other treatments. Mohsen et al., (2016) reported an increase in chlorophyll content of 3.26-3.75 mg mg^{-1} of lettuce plants treated with *Anabaena sp.* and *Nostoc sp.* extracts. The carotenoid content of plants were also increased following foliar spray compared to the control as illustrated in Fig. 5**c**. 60% treatment was found to be highest with 0.069 ± 0.0007 mg g^{-1} compared to the control and other treatments. The increase in pigments during foliar spray indicated an increase in overall specific growth of plants (Gaikwad et al., 2012). Foliar spraying of *Nostoc sp.* extracts (750 ml) resulted in 1.4 mg mg^{-1} of carotenoids in lettuce (Mohsen et al., 2016). Thus foliar spraying of algal extracts have a significant impact over the increase in leaf pigments thereby the photosynthetic growth rate.

4. Conclusion

Seed treatment and foliar spray of extracts from the mixed algal consortium were found to have a positive influence over the seed germination and plant growth rate. 40% treatment of seeds with algal extracts showed maximum germination efficiency resulting in a shoot length of 19.86 ± 0.51 cm and 34.6 ± 1.9 leaves having a chlorophyll content of 12.86 ± 0.417 mg g^{-1}, fresh weight and dry weight of 3.47 ± 0.04 g and 0.389 ± 0.036 g respectively, after 20 days of sowing. In case of foliar spray, maximal growth rate was observed with 60% algal extracts resulting in total plant height of 7.98 ± 0.19 cm and 11 ± 0.35 leaves with 13.45 ± 0.307 mg g^{-1} total chlorophyll content and root length of 5.8 ± 0.16 cm after 20 days. Fresh and dry weight of 0.416 ± 0.015 g and 0.062 ± 0.005 g was also observed. Overall, seed treatment was found to be more effective compared to foliar spray. It was also observed that, similar to the synthetic fertilizers there exists a concentration cut off beyond which the extracts have an inhibitory effect. More studies are needed to gain insight into the actual cellular mechanism involved in stimulating plant growth. Algal extracts as biostimulants possess an extraordinary potential to revolutionize the agriculture sector, thereby reducing the harmful environmental and health issues associated with conventional fertilizers.]

Authors' declaration

PB has initiated the concept of the project. Experiments and acquisition of data were carried out by KVS and BB. KVS, BB and PB have done data analysis and interpretation. KVS has drafted the manuscript and BB has revised. PB has reviewed and finalized the manuscript. All authors read and approved the final manuscript for peer review and possible publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2020.112453.

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