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# Role of Ulothrix Prescott as a Biofertilizer on Growth of Tomato

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**Abstract**

The effects of *Ulothrix cylindricum* extract (UCE), an algal liquid fertilizer, on tomato (*Solanum lycopersicum*) seedlings were examined in an in vitro experiment conducted at the Institute of Botany, University of the Punjab, Lahore. NPK, 5%, 10%, and 15% UCE, as well as a control group, were applied to the seedlings. The others died, and only the control group sprouted. As the plants developed, a later pot experiment evaluated other morphological parameters.

With respect to chlorophyll content (31.559 ± 1.049), sodium (92.467 ± 2.326), potassium (4293.3 ± 192.2), phosphorus (380.00 ± 10.97), nitrogen (4166.7 ± 111.3), magnesium (10.133 ± 1.187), calcium (0.249 ± 0.017), and iron, the 15% UCE group outperformed the control group, according to the results. There were notable variations between 15% UCE and the other treatments, with the control having the lowest quantities of these nutrients. In comparison to NPK (25.003 ± 1.056) and the control (11.070 ± 1.169), 15% UCE had a significantly greater chlorophyll content (31.559 ±1.049). Furthermore, the fruit vitamin C content was higher in the 15% UCE group (5.473 ± 0.408) than in the control. According to the biochemical analysis, greater UCE concentrations enhanced fruit quality, raised nutrient levels, and encouraged plant development without having an adverse effect on ecological balance. These findings imply that UCE may improve overall plant health and tomato yield.

**Keywords:** Algal extract, bio-fertilizer, UCE, fruit mineral analysis, growth, phyco-chemical testing, Solanaceae, *Solanum lycopersicum*

## Introduction

Algae are a broad and varied group of microorganisms that can absorb sunlight and carry out photosynthesis. Due to their ability to maintain soil and function as biofertilizers, algae are important in agriculture. Compared to livestock dung, algae especially seaweeds are used as fertilizers and release less phosphate and nitrogen. Consequently, this enhances the quality of water that enters rivers and oceans. These organisms are cultivated all over the world and added to food. Additionally, they can provide a clean, carbon-neutral food that can be grown on desert areas that have been abandoned and have little to no fresh water [1]. Having more than 15,000 species and more than 500 genera, green algae are the largest group of algae [2]. In contrast to the bulk of other orders (such as Ulvales and Cladophorales), which are marine, the Charales and Oedogoniales orders are virtually exclusively found in freshwater. More chemical fertilizer is needed by agricultural industries as the world's food demand rises. Among the many elements found in inorganic fertilizers are potassium, phosphorus, and nitrogen. In agriculture, the overuse

of chemical fertilizers is costly and has several negative effects on the environment and living things. For instance, rainwater can carry residual pollutants into aquatic bodies, causing eutrophication. Additionally, it might lessen soil fertility, nutrient inequality, and water-holding capacity. Gastric cancer, goitre, metabolic disorders, birth defects, hypertension, and livestock poisoning are other consequences of groundwater contamination [3, 4]. The use of biofertilizers and organic fertilizers has become popular. In sustainable agriculture, biofertilizers can be used in place of or in addition to chemical fertilizers since they are inexpensive, renewable, and ecologically friendly sources of plant nutrients. In order to fix nitrogen, mineralize phosphate and potassium, release plant growth stimulants, produce antibiotics, and break down organic detritus in soil, biofertilizers employ microorganisms. Long-term, continuous use of biofertilizers makes parental inoculums sufficient for further multiplication [4], enabling them to take part in nutrient cycling and boost crop output [5]. Compared to chemical or other manure fertilizers, algae produce less mineral runoff, making them an excellent source of fertilizer [6]. Algae growth and crop productivity are influenced by the chemical and biological characteristics of the soil, which also affect its texture, pH, EC, organic matter, and minerals. Soil chemical properties are crucial indicators of nutrient availability, organic matter content, and soil suitability for long-term fruit and agricultural production [7]. The pH of the soil affects the variety of algal communities. One ingredient that limits crop development in agriculture is nitrogen. Because of this, farmers usually use chemical fertilizers or livestock manure to increase output [8, 9, 10]. There is a substantial correlation between the microbial communities, organic matter, and the physical and chemical characteristics of soil [11]. The soil's physical and chemical properties impact the growth of algae. The breakdown of algae improves organic matter and releases micro and macronutrients into the soil. The dangers of chemical fertilizers have previously been established. On the one hand, they exacerbate land degradation, water pollution, and health issues for people. This study was therefore started in order to assess how well algae work as a natural fertilizer. Cheap nutrient sources, microelement suppliers, organic matter suppliers, growth hormone secretion, counteracting the negative effects of chemical fertilizers, no negative effects on the ecosystem, and a longer shelf life are the main advantages of biofertilizers. In biofertilizers, the most commonly used microorganisms that support plant growth include *Azotobacter*, *Azospirillium*, cyanobacteria, *Azolla*, phosphate-soluble microorganisms, mycorrhizae, *Sinorhizobium* and *Rhizobacteria*. Growth regulators, polyamines, natural enzymes, carbohydrates, proteins, amino acids, vitamins, and macro- and micronutrients are all abundant in algae biomass, which promotes vegetative growth [12, 13, 14]. Moreover, the presence of vitamins, auxins, and gibberellins in algal biomass boosts yield. The two types of algae that are most frequently researched as biofertilizers are green algae (Chlorophyta) and blue-green algae (Cyanophyta). According to numerous studies, green algae can increase the amount of organic matter in the soil, produce and release vitamins, amino acids, and auxins, decrease the amount of oxidizable matter, supply oxygen to the submerged rhizosphere, improve salinity and buffer the pH, solubilize phosphate, and increase the efficiency of crop plant fertilizer use [14, 15]. Algae are important soil biofertilizers in sustainable organic farming. Warm weather and moisture promote algal bloom, which raises soil fertility and structure and increases crop output. Algae and other organisms enhance airflow, organic matter, and soil texture [16]. According to [6, 17] algae help in plant succession, fix nitrogen, and increase soil fertility. Additionally, it has been found that algae on soil improve and stabilize nitrogen and organic matter, increasing fertility. This promotes microorganism cultivation and seed germination. Certain species of Cyanobacteria fix elemental nitrogen. Others improve the organic matter, nitrogen, and mineral content of the soil after it has been broken down. It is ironic that there is no improvement in farmer income despite the rise in fertilizer prices and the various

financial losses brought on by overuse of fertilizer. The negative consequences of using too much inorganic fertilizer are therefore being strategically addressed by attempts to enhance nutrient uptake by spraying extracts of natural materials containing stimulants. Prior studies have demonstrated that certain liquid fertilizer formulations derived from raw seaweeds can enhance nutrient absorption, hence promoting the development, growth, and yield of a wide range of agricultural crop species [18, 19]. Due to its considerable buffering capacity, chemical fertilizer has a mild effect on soil. But over time, the pH and natural balance of soil elements are disturbed, and the buildup of hazardous substances causes food chain poisoning. Fertilizers that are high in potassium and salt are to blame. Liming, which is brought on by acidic fertilization, causes symbiotic systems to have imbalances in nitrogen fixation. Additionally, excessive fertilizer use can harm the agricultural field's ability to absorb nutrients and cause nutrient imbalances. Worms and soil mites are among the organisms that are killed by the effects. The main components of nonorganic fertilizers are potassium, ammonium, phosphates, and nitrates. However, these fertilizers contain natural radionuclides including 238U, 232Th, and 210Po, as well as heavy metals like Hg, Cd, As, Pb, Cu, Ni, and Cu [20, 21]. Because of these substances, there are significant environmental dangers. These heavy metals can contaminate water, soil, and air by building up in the soil and plant system and moving up the food chain. Carcinogens such nitrosamines in leafy plants like spinach, an increase in phosphates due to surface flow transfer, and an increase in nitrates in rivers and drinking water are examples of general dangers. They have the ability to eliminate beneficial bacteria and insects from the soil, and most of the measures' highest values were recorded during the monsoon wet season [22]. The majority of biofertilizers are composed of living microorganisms that supply nutrients to plants via their root systems. To provide plants with nutrients, the bacteria in these fertilizers employ a number of strategies. They can mobilize and solubilize phosphate, fix nitrogen, and support rhizobacteria [23]. The preparation includes specific bacteria that could help the soil. The packaging is made to last a long time and to be safe for both users and the environment [24]. Because of their high nutritional content and minimal environmental impact, biofertilizers are becoming more and more popular [25]. Additionally, they are a renewable resource that can take the place of chemical fertilizers. Also, they require less processing, which often includes neutralization, pulverization, sterilization, packing, and shipment. Microorganisms and nutrients that may be lacking or insufficient in soil are added by biofertilizers. These fertilizers then provide sufficient and balanced amounts to the soil and plants while also accelerating the microbial activity process. Once they are on the field, they are easier and more complete to use. By reducing soil-borne diseases and improving the use of chemical fertilizers, biofertilizers boost output. The market for biofertilizers is predicted to grow to USD 10.2 million by 2018 as a result of these characteristics. According to projections, 34% of the world's need will come from the Asia-Pacific region, and chemical fertilizer laws will force use of these fertilizers to shift to Europe and Latin America as well. According to Ajmal *et al*. (2018), bio-pesticides are being created from natural ingredients to fight plant insects by biological means [26]. Pest-resistant cyanobacteria can fix nitrogen even in the presence of synthetic nitrogen sources, and the presence of cyanobacteria in the crop environment can increase the crop's capacity to fix nitrogen. Therefore, with ethical consent, study on these cyanobacteria through genetic engineering or mutational analysis may be a feasible long-term strategy for agricultural enhancement. Thanks to marker selection in plant genetic studies, herbicide-resistant algae can play an important role in the battle against harmful chemicals and herbicides that persist in the environment for years, even after numerous years of spreading. The environment and human health may be covertly threatened by such leftovers. Soil is a potential treatment because algal strains can break down xenobiotic

substances present in agricultural runoff. *Anabaena* sp. and *Nostoc ellipsosoru* strains of Cyanobacteria have effectively destroyed lindane, a long-used insecticide that poses a health risk to humans. The following strains are genetically altered to efficiently degrade pesticides. By introducing it into a bacterium, the lindane dechlorination operon was successfully found and altered. These two types of algae use atmospheric carbon for their activities, are inexpensive, and are simple to grow. *Phymatolithon* spp., *Ecklonia* spp., and *Ascophyllum nodosum* algal biomass, on the other hand, has been used as fertilizer in horticulture. *Solanum lycopersicum*, or tomatoes, are among the most widely consumed vegetables worldwide. Western South America is said to be its birthplace, while Central America is where it was domesticated. The development of tomatoes to increase fruit quality, productivity, and resistance to biotic and abiotic stresses is a result of its culinary significance. Both for culinary and scientific uses, tomatoes are employed extensively. The tomato plant differs from other model plants (such rice and *Arabidopsis*) in several ways, such as meaty fruit, a sympodial stem, and compound leaves. The majority of these traits have agronomic significance and cannot be studied in other model plant systems. Thirteen wild tomato species have been identified; these species can be bred with cultivated tomatoes and have a range of traits. It is beneficial to breed these wild tomatoes, acquire desirable traits, and conduct evolutionary research. Important information for tomato research has been obtained from the ongoing tomato genome sequencing project. Tomatoes are also members of the vast Solanaceae family, which also includes a number of commercially important plants like tobacco, eggplant, peppers, potatoes, and petunias. Studies on tomatoes yield information that is readily applicable to these plants, making them an important research tool. Tomatoes are utilized as model organisms for the Solanaceae family because of these characteristics, especially plants that have meaty fruits [27]. Globally, *Solanum lycopersicum* are becoming more and more important for research into the basic concepts of plant growth and development, as well as for consumption as a fresh crop and a major ingredient in many prepared cuisines. *S. lycopersicum* species are resilient to a range of dietary and environmental stressors. A few of the species have been crossed to create a wide range of cultivars that are meant to produce a field crop for a single harvest or, under protection, a series of fruits for the fresh market over a long period of time. Together, plant breeders and agricultural engineers have developed a method for mechanically harvesting field tomatoes, which allows for the economical production and processing of large quantities of tomatoes. Many different types of canned, frozen, preserved, and dried foods use the goods. Tomato fruit has been the focus of much molecular study over the past ten years because of its economic significance and suitability for molecular biology and genetic engineering techniques [28]. This study aims to investigate the potential use of the filamentous green alga Ulothrix Prescott as a biofertilizer to aid tomato plant growth. Ulothrix is well-known for its capacity to improve microbial activity in the rhizosphere and for releasing organic matter and vital nutrients during its decomposition, both of which can increase soil fertility. The purpose of this study is to evaluate how Ulothrix application affects important tomato plant growth metrics, including fruit yield, plant height, leaf development, and seed germination. By assessing these impacts, the research aims to ascertain whether Ulothrix Prescott may assist better plant development and soil health in tomato farming by acting as an environmentally responsible and sustainable substitute for traditional chemical fertilizers.

## MATERIAL AND METHODS

### Algal collection

The Botanical Garden's water pond at Government College University in Lahore, Pakistan, provided a

sample of freshwater algae. Zip bags and plastic containers were used for the collection of freshwater algae.

**Identification of the Sample**

A compound microscope used to identify the collected algae samples in Phycology Research Lab. According to credible literature, *Ulothrix cylindricum* Prescott was the identified algal species.

**Drying and Grinding of Sample**

The normal shade temperature was used to dry the obtained samples. A grinding machine was used to carefully grind the dried sample. A permanent marker was used to designate the powder, which was collected in a zippered bag.

**Preparation of stock solution**

A 250 g sample of algae was put into a conical flask, dipped in methanol, and then covered with a cotton stopper to seal the flask. To give the algae time to absorb all of the methanol, the soaked algal sample was left for two weeks. The algae extract was filtered and allowed to air dry after two weeks. To create the stock solution, 15 g of algal extract was combined with 150 ml of distilled water. To guarantee homogeneity, the mixture was vigorously shaken using a magnetic stirrer. The stock solution was autoclaved at 15 Psi pressure and 121 °C for 30 minutes. After autoclaving, a pure algal stock solution was the resultant mixture. The stock solution was prepared to create 5%, 10%, and 15% UCE concentrations.

**Prepare 5% UCE solution**

Put 25 ml of the stock solution and 475 ml of purified water in a measuring cylinder.

**Prepare 10% UCE solution**

To get the final volume, put 50 ml of the stock solution and 450 ml of distilled water in a measuring cylinder.

**Prepare 15% UCE solution**

A measuring cylinder was filled with 75 ml of algae extract, and the final volume was raised to 425 ml using distilled water.

**Prepare a 1.5% NPK solution**

A measuring cylinder was filled with 150 g of certified powdered NPK and distilled water to create a final volume of 1000 ml.

**Crop Selection**

Due to its widespread use in Pakistan, *Solanum lycopersicum* (var. Rio Grande) was selected for the experimental investigation.

**Seed Department**

The Federal Seed Certification Department in Lahore provided the seeds of *Solanum lycopersicum* (var. Rio Grande).

**The viability tests**

Poured 500 millilitres of distilled water into a beaker. Give each tomato seed a full day to soak in its own beaker. Those that floated to the top were eliminated, and all that sank to the bottom might be used in experiments.

**Seed Germination Experiment**

Tomato seeds were selected based on size, color, and weight to ensure quality and uniformity. To remove any surface chemicals, the seeds were sterilized by soaking in a 4% hypochlorite solution for ten minutes, followed by three to four rinses with distilled water. Five petri dishes were sterilized and prepared, each

lined with two folds of blotting paper. Five treatment groups were established: control, 5%, 10%, 15% stock solutions, and 15% NPK solution. In each petri dish, five seeds were placed, and 10 milliliters of the appropriate stock solution were added. To prevent fungal infection, all petri plates were properly sealed with cling film. The plates were then incubated in the phycology lab at 25 °C under an 8-hour dark period. Germination was monitored from the start of the experiment for two weeks, during which various parameters such as germination percentage, germination index, germination energy, mean germination time, and seedling vigor index were recorded and analyzed.

**Seed germination in plastic plug tray**

The ideal temperature range for tomato cultivation is between 21° C and 27° C, providing optimal conditions for seed germination and plant growth. Tomato seeds were initially sown in black plastic plug trays measuring 0.3 x 0.6 meters. Once the seeds germinated and developed into young seedlings, they were carefully shifted into pots to continue their growth under controlled conditions (Fig. 1 A, B).

**Pot experiment**

The second part of the investigation involved a cannabis experiment, which required careful management of temperature variability. A total of 65 pots, each measuring 14.5 cm by 16.5 cm, were selected for the study. Using a block design, the experiment was set up with 60 pots assigned to different treatments, each having five replicates, while five pots served as the control group (Fig. 1 C). Each pot was filled with one kilogram of soil, prepared by mixing 80% dirt with 20% natural compost, ensuring that the pots were appropriately sized to accommodate this soil mixture.

**Soil analysis**

George *et al*. (2013) served as the foundation for the soil analysis methodology [29]. Debris and pebbles were picked up by hand. The following characteristics of the soil were assessed after it was passed through a 2 mm sieve. The presence of these elements in soil was tested; Soil texture and organic matter, Electrical conductivity (S/m), pH, Calcium + magnesium ($mg/kg^{-1}$), Potassium (mg/kg), Phosphorus (mg/kg), $CO_3$ (meq/L) and $HCO_3^{-1}$ (meq/L), $Na^+$ (ppm) and Nitrogen (mg/kg).

**Phycochemical Testing**

**Preparation of Solvent Extract**

The following solvents were added to the algal sample distilled water, methanol, chloroform, and n-hexane. The following solvents were used to immerse the algae powder for around two weeks, after which it was allowed to air dry and stored in vials for further testing.

**Reducing sugar tests (Fehling's test)**

**Preparation of Fehling solutions**

**Fehling Solution A**

Inaccurate Resolution 1.75 g copper sulphate and a small amount of water are combined, and then two drops of sulfuric acid are added to create a Fehling solution. The 25 ml total volume produced a blue tinge.

**Solution B**

After dissolving 6g of KOH and 8.65 g of potassium sodium tartrate in a little amount of distilled water, add more distilled water to get the final volume of 25 ml.

**Procedure**

Pour 2 ml of Fehling A and 2 ml of Fehling B into each test tube. The presence of reducing sugar is then indicated by the yellow to brownish red precipitates that are produced when they are heated on a water

bath.

**Test for Alkaloids**

**Mayer's Test**

**Mayer's reagent**

By dissolving 0.675 g of HgCl in 30 ml of distilled water and then adding 2.5 g of KI to 5 ml of distilled water to reach a final volume of 50 ml, fresh Mayer's reagent was made. The presence of alkaloids was shown by the development of a pale-yellow hue in a test tube containing 1 millilitre of extract and a few drops of Mayer's reagent.

**Wagner's test**

After dissolving 2 g of iodine and 6 g of potassium iodide in distilled water, the final volume reached 100 ml.

**Procedure**

Put 1 millilitre of algal extract, 0.2 millilitres of HCl, and 1 millilitre of Wagner's reagent in each test tube. The presence of alkaloids is confirmed by reddish-brown precipitates.

**Test for tannins**

**Get 0.1% $FeCl_3$ ready**

To create a 0.1% ferric chloride solution, dissolve 0.01g of ferric chloride in distilled water. Then, add more distilled water to reach 10 ml.

**Procedure**

In a test tube, put 0.25 g of plant extract to a boil and add a few drops of $FeCl_3$. The presence of tannin was indicated by the formation of blackish crimson precipitates.

**Test for lead acetate**

**Lead acetate 10% solution**

After adding 5 g of lead acetate and mixing it with a modest amount of distilled water, the final volume was increased to 50 m using distilled water.

**Procedure**

In a test tube, mix 2 millilitres of algae extract with a few drops of lead acetate. The milky white precipitates confirm the presence of tannins.

**Test for Terpenoids**

**The Salkowski test**

Put 0.5 g, 2 ml of chloroform, and 3ml of strong sulfuric acid in a test tube to make the extract. The characteristic reddish-brown color of terpenoids helps to identify them.

**Anathraquinone test**

**The Borntrager test**

Boil and filter 0.5 g of algal extract with 10ml of diluted sulfuric acid to produce a noticeable organic layer. Next, shake in 5 ml of chloroform. One millilitre of 10% ammonium hydroxide is added to a layer of chloroform that has been pipetted into a test tube. The gradual change in color to violet, red, and pink is a characteristic of anthraquinones.

**Study of Crop Plants**

Following the experiment, the plants were carefully uprooted, the soil and debris were manually removed, and tap water was used to rinse them. measured the fresh weight, dried weight, shoot length, shot area, and leaf area index after separating the root, shoot, and leaves. Using a scale, GP manually counts the quantity of leaves, leaflets, flowers, and fruits. The Spectrophotometer method of [30] was used to determine the amount of chlorophyll, and [5] were used to estimate the leaf area.

The mineral studies of plant material are being carried out along with chlorophyll measurement

**Chlorophyll measurement**

A pestle and mortar were used to completely crush 0.1g of fresh leaves. An 80% acetone solution was added while the material was being crushed. After that, the homogenate was filtered and rinsed two or three times. The final volume was increased to 25 ml by adding acetone. A spectrophotometer operating at wavelengths of 663 and 645 nm was used to measure the concentration of total chlorophyll.

**Tomatoes mineral content determination**

Mineral content of the following elements was performed:

**Sodium**

The following literature was used to calculate the salt content of plants [31].

According to the guidelines of George *et al.* (2013), the amount of phosphorus was determined [29]

**Procedure**

To create greyish white ash, plant material was put in a porcelain crucible and heated to 300 °C to 450 °C for 15 minutes in a muffle furnace. The temperature was then raised to 550 °C. After letting the material cool, add 10 millilitres of 6 N HCl. The mixture was filtered through acid-washed filter paper in the flask after the material was evaporated on a hot plate and 5 ml of 6 N HCI was added. To make it 100 ml, it was diluted.

**Potassium**

Potassium levels were measured using a flame photometer. A series of 40 ppm and 100 ppm KCl standard solutions were made. Using a flame photometer, the filtered plant material was measured and recorded in parts per million.

**Phosphorus**

**Chemicals**

Two solutions were used to make the vanadomolybdate reagent.

**Solution A**

Ammonium molybdate of 25 g was dissolved in 400 ml of warm distilled water and cooled.

**Solution B**

After gradually adding 1.25 g of ammonium metavandate to 300ml of boiling water and cooling it, it was added to 250ml of concentrated HNO and allowed to cool. After adding solution A to a 1-liter volumetric flask, pour solution B into it. After mixing well, dilute with 1 litre of phosphate.

**Standard solution**

Dissolved 0.2195 g of KHPO and diluted to 1 L.

The solution comprised 50 parts per million.

**Procedure**

Fill a 50 ml volumetric flask with 5 ml of ash solution, 10 ml of sodium molybdate, and distilled water. Measure the yellow's intensity at 450 nm using blue light.

**Calculation**

Weight of plant stuff = 1 gram
Volume of plant digest = V mL
Volume of aliquot taken = V1 ml.
The final volume created is 50 mL.
Spectrophotometer transmittance = T, standard curve P (ppm) = Y, and plant material
P (ppm) = Y x 50/1 x V/V1.

**Nitrogen**

The Kjeldahl method is used to determine the nitrogen concentration in the plant samples.

**Procedure**

**Digestion**

Apply sulfuric acid to a material or sample to heat it. Ammonium sulphate, which is reduced nitrogen, is released when the organic molecule is oxidized by the acid. In order to increase the boiling point of the medium, potassium sulphate is frequently added. Digestion also makes use of catalysts like copper, selenium, mercury, and copper ions. The substance is known to be fully decomposed when a clear, colorless solution is obtained.

$Organic\ compound + H_2SO_4 \rightarrow [digest]_2 + (NH_4)_2SO_4$

**Distillation**

The ammonium salt is now converted to ammonia by adding a little amount of sodium hydroxide after the solution has been distilled. After that, the distilled vapours are trapped in a particular trapping solution that contains water and HCl (hydrochloric acid).

$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2H_2O + 2NH_3$

**Titration**

The amount of nitrogen or ammonia in the sample is ascertained using back titration. Some HCl is neutralized as the ammonia dissolves in the acid trapping solution. A standard base solution, like NaOH or another basic, can be used to titrate the remaining acid once more.

$(OH)_2+H_2O+Na_2CO_3 \rightarrow NaHCO_3+CO_2+H_2O$

$NH_3+HCl \rightarrow NH_4Cl$

**Formula for Calculation**

The following formula can be used to determine the percentage of nitrogen:

$Percentage\ of nitrogen\ in\ the\ sample = 1.4V \text{ x } NW$

where,

V = acid used in titration (mL)
N = normality of standard acid
W = sample weight in grams

**Fruit Analysis**

As part of the fruit analysis process, we examined the fresh weight, dry weight, breadth, length, inorganic mineral content, and vitamin C content of each tomato that was picked from each plant.

**Determination of fruit inorganic mineral content**

**Collection of Samples**

To get rid of clay, sand, mud, and other particles, the collected samples were thoroughly cleaned with tape

water and then rinsed with distilled water. An electric balance with peel and shell was used to measure the mass of the collected, fresh, clean fruit samples. The fruit samples were cleaned and stored at the appropriate temperature in the refrigerator. In the Phycology Laboratory at the Institute of Botany, University of the Punjab, Lahore, the ash and mineral content of selected fruit samples were ascertained.

**Determining the Total Ash Content**

Ranganna's approach was used to gravimetrically measure the amount of ash in fruit samples [32]. In the muffle furnace, a fixed mass of fruit was cooked for a few hours at 300 ˚C, overnight at 420 ˚C, or for five to seven hours at 550 ˚C. After cooling in desiccators, the sample's ash was weighed. Until a steady mass was reached, heating, cooling, and weighing were repeated.

After determining the total ash content, the following minerals content including Iron, Calcium, Magnesium, Chloride and Vitamin C then can be calculated from it.

## RESULTS

**Phycochemical tests of algal extract**

Different phycochemicals analysis were being performed to analyze the data at different levels of concentration. Tests for secondary metabolites like carbohydrates, alkaloids, terpenoids and anthraquinones were performed and different results were obtained (Table 1).

**Table 1. Phyochemical analysis of methanolic extract of *Ulothrix cylindricum*.**

| Phycochemicals | Phycochemical Tests | n-hexane | Chloroform | Methanol | Distilled water |
|---|---|---|---|---|---|
| **Reducing sugars** | Felhing's test | + | + | - | - |
| **Alkaloids** | Mayer's test | + | + | + | + |
| | Wagner's test | + | + | + | + |
| **Tannins** | Ferric chloride test | + | - | + | + |
| | Lead acetate test | + | - | + | + |
| **Terpenoids** | Salkowski test | - | + | + | + |
| **Anyhraquinones** | Borntrager's test | - | - | - | - |

**+ = Presence of Phycochemical, - = Absence of phycochemical**

**Analysis of soil**

Analysis of soil was being performed that was being used in the whole experiment just for understanding the nature of soil, nutrients present in soil, pH and electrical conductivity (EC) of the soil (Table 2).

**Table 2. Analysis of soil used for Pot experiment.**

| Sr. No. | Parameters | Soil |
|---|---|---|
| 1 | Soil texture | Sandy loam |
| 2 | pH | 7.44 |
| 3 | EC | 1.72mScm$^{-1}$ |
| 4 | Ca : Mg | 2 : 8 meq/L |

| | | |
|---|---|---|
| 5 | CO3 | 0 |
| 6 | $HCO_3$ | 1.6 meq/L |
| 7 | Na | 180ppm |
| 8 | N | 0.01% |
| 9 | P | 10.7 mg $kg^{-1}$ |
| 10 | K | 104 mg $kg^{-1}$ |
| 11 | Saturation percentage | 38 % |
| 12 | Organic matter | 0.63 |

**Where; EC = Electrical conductivity, Ca = Calcium, Mg = Magnesium, CO3 = Carbonates, HCO3 =Bicarbonates, Na = Sodium, N = Nitrogen, P = Phosphorus, K = Potassium**

**Effect of algal extract on seed germination**

Some of the factors have been studied during the process of germination and they are following: Germination percentage, Germination Index, Germination energy, Mean Germination time, Seedling vigor index.

**Effect of algal extract on seeds of *Solanum lycopersicum* (var. Rio grande) in petri plates**

After two weeks of sowing the seeds the process of germination was being observed in 20 percent seeds. Only control group shows growth. Seeds that were being treated with algal extract of different concentration and NPK shows no growth, this is probably due to the stress caused by mineral content present in algal extract and NPK.

**Pot experiment**

Vegetative growth was being observed in pots experiment. All the pots were arranged in rectangular pattern and labeled along with their concentrations. On daily basis their shoot length, leaf number and chlorophyll content were observed and recorded. After three month they showed maximum results and removed and recorded their calculations (Fig. 1D-F).

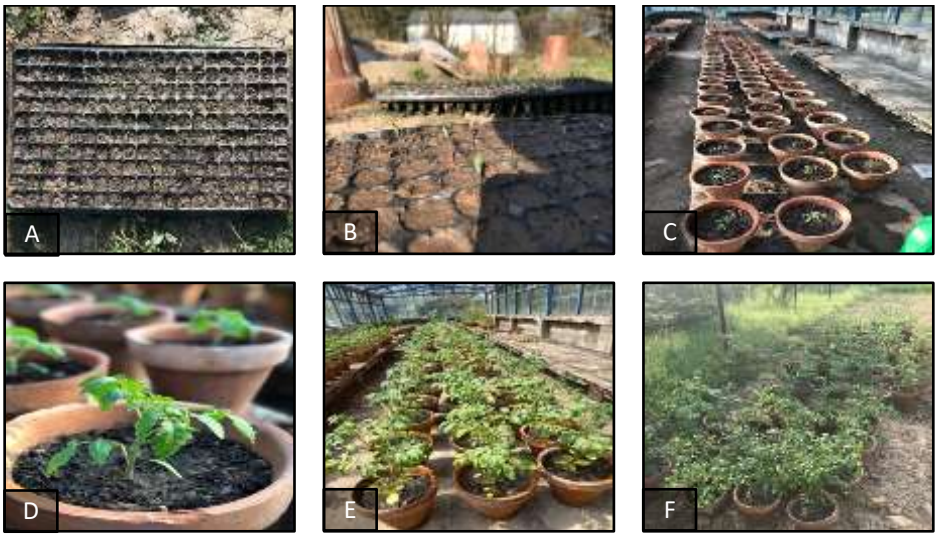


**Figure 1. Some photographs during field experiment (A) Seedling growth in plastic plug tray (B) Growth of seedlings in tray (Zoom view) (C) Transfer the seedlings in pot arranged in rectangular manner (D) Healthy growing tomato plants in pots (E) Tomato plants during flowering (F) Tomato plants during Fruiting in different concentrations.**

**Growth Pattern for tomato plants (var. Rio grande)**

**Germination Percentage (GP)**

The plants that were grow in pots showed different GP as compared to petri plates experiment. The maximum and minimum percentage was 100%, all the plants able to grow within the limited time of requirement. No difference were observed in their GP rate that showed that pots experiment have positive results as compared to Petri plates experiment that were in vitro.

GP = Total no of Seeds germinated / Total number of seeds x 100

**Germination Index (GI)**

Plants that were grow in pots showed equal percentage of germination index as compared to Petri plates experiment which showed a lot of variability (Table 3a). But in pot experiment control 5% and 2% have nearly equal values and other concentrations have somehow higher GI values.

$GI = \sum (Gt)/(Dt)$

where:

Gt: number of germinated seeds on day

Dt: time to germinate

Germination energy, Mean germination time, Seedling vigor index were observed and their readings were mentioned in (Table 3a).

**Morphological Parameters**

All the seeds that were sown in pots were germinated with excellent results with minute or few exceptions of variability. In pot experiment control plants continuously shown the least amount of development, whereas plants treated with a 15% concentration of UCE performed the best across all assessed growth and yield criteria. The highest root length, shoot length, and overall plant height attained by this concentration demonstrated improved vegetative growth (Fig. 2 A-C). Additionally, it produced the highest plant fresh and dry weights, indicating better biomass accumulation. Increased photosynthetic capacity was supported by noticeably higher leaf-related characteristics, including leaf area, number of leaves, and leaf area index (LAI). Significant improvements were also seen in reproductive characteristics, such as the quantity of flowers and fruits, as well as the diameter, length, and fresh and dry weights of the fruits (Fig. 3 A-E). Table 3a and 3b illustrates that, in contrast, all of these metrics were lowest in the control group. These findings imply that the optimal concentration of UCE for fostering overall plant growth and productivity is 15%.


**Figure 2. Measurement of plant parameters (A) Measurement of root length (B) Measurement of shoot length (C) Comparative analysis of different concentrations of tomato plants.**


**Figure 3. Comparative analysis of fruits (A) Fruiting in control group (B) Fruiting in 5% UCE (C) Fruiting in 10% UCE (D) Fruiting in 15% UCE (E) Fruiting in 1.5% NPK**

**Table 3a. Fisher's Means comparison analysis on the effect of applied treatments on morphological and growth attributes of *Solanum lycopersicum.***

| Treatments | Germination Index | Germination energy | MGT | SVI | Root length (cm) | Shoot length (cm) | Total plant height (cm) | Plant fresh weight (g) | Plant Dry weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| **Control** | 0.082 ± 0.013 e | 42.68 ± 3.39 e | 156.48 ± 4.23 e | 1886.6 ± 51.0d | 10.708 ± 1.788 d | 22.660 ± 0.756 d | 32.888 ± 1.394 e | 63.20 ± 10.66 e | 6.000 ± 1.000 e |
| **5% UCE** | 0.118 ± 0.009 d | 49.246 ± 3.39 d | 167.753 ± 2.218 d | 2832.2 ± 84.9c | 16.597 ± 1.284 c | 30.461 ± 1.319 c | 50.524 ± 2.128 d | 118.33 ± 7.36 d | 19.267 ± 2.187 d |
| **10% UCE** | 0.150 ± 0.014 c | 56.351 ± 2.578 c | 175.023 ± 2.096 c | 3216.7 ± 219.1b | 16.673 ± 1.257 c | 35.527 ± 3.649 b | 55.979 ± 2.196 c | 182.80 ± 12.45 c | 25.667 ± 3.638 c |
| **15% UCE** | 0.299 ± 0.025 a | 78.162 ± 1.495 a | 188.15 ± 4.62 a | 3792.4 ± 112.5 a | 22.574 ± 1.329 a | 47.727 ± 0.855 a | 69.768 ± 1.932 a | 271.67 ± 9.98 a | 35.400 ± 1.920 a |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **NPK** | 0.238 ± 0.033 b | 60.43 ± 1.881 b | 178.962 ± 2.283 e | 3298.1 ± 177.7b | 18.169 ± 1.271b | 36.970 ± 1.653b | 62.847 ± 1.375 b | 228.47 ± 15.81 b | 29.400 ± 1.549 b |

**(UCE = Ulothrix cylindricum extract, MGT= Mean germination time, SVI= seedling vigor index, ± = Standard deviation, Alphabet shows level maximum to minimum values observed).**

**Table 3b. Fisher's Means comparison analysis on the effect of applied treatments on morphological and growth attributes of *Solanum lycopersicum*.**

| **Treat-ment** | **No. of Leaves** | **Leaf area ($cm^2$)** | **Leaf area index ($cm^2$)** | **No. of flowers** | **No. of Fruits** | **Diame-ter of fruits (cm)** | **Length of fruits (cm)** | **Fresh weight of Fruits (g)** | **Dry weight of fruits (g)** |
|---|---|---|---|---|---|---|---|---|---|
| **Con-trol** | 22.600 ± 1.140 e | 21.200 ± 1.643 e | 2.6800 ± 0.1643 e | 5.000 ± 0.707 e | 3.800 ± 0.837 e | 5.052 ± 0.218 e | 2.8900 ± 0.0583 e | 29.200 ± 1.304 e | 7.000 ± 1.000 e |
| **5% UCE** | 28.067 ± 1.223 d | 28.733 ± 1.486d | 3.7800 ± 0.1320 d | 9.000 ± 1.000 d | 6.733 ± 0.884 d | 8.172 ± 0.305 d | 3.7000 ± 0.2094 d | 38.733 ± 1.486 d | 14.200 ± 0.862 d |
| **10% UCE** | 36.667 ± 3.244 c | 35.133 ± 1.552 c | 4.6733 ± 0.3807 c | 11.553 ± 0.990c | 8.800 ± 0.775 c | 9.711 ± 0.444 c | 4.0127 ± 0.0570 c | 45.667 ± 2.610 c | 25.600 ± 2.098 c |
| **15% UCE** | 63.267 ± 1.870 a | 50.000 ± 2.299 a | 6.7333 ± 0.3036 a | 16.333 ± 1.113a | 12.667 ± 1.543 a | 14.986 ± 0.338 a | 6.0786 ± 0.0297 a | 74.200 ± 3.234 a | 33.933 ± 1.668 a |
| **NPK** | 51.667 ± 2.469 b | 39.933 ± 2.520 b | 5.413 ± 0.431 b | 12.667 ± 1.345 b | 9.800 ± 0.862b | 11.399 ± 0.702 b | 4.3920 ± 0.1701 b | 60.533 ± 2.066b | 27.667 ± 3.658 b |

**(UCE = Ulothrix cylindricum extract, MGT= Mean germination time, SVI= seedling vigor index, ± = Standard deviation, Alphabet shows level maximum to minimum values observed).**

**Table 3c. Analysis of variance of different parameters.**

| **No.** | **Parameters** | **P- value** |
|---|---|---|
| 1 | Root length | 0.000 |
| 2 | Shoot length | 0.000 |
| 3 | Total plant height | 0.000 |
| 4 | Plant fresh weight | 0.000 |

| | | |
|---|---|---|
| 5 | Plant dry weight | 0.000 |
| 6 | Number of leaves | 0.000 |
| 7 | Leaf area | 0.000 |
| 8 | Leaf area index | 0.000 |
| 9 | Number of flowers | 0.000 |
| 10 | Number of fruits | 0.000 |
| 11 | Diameter of fruits | 0.000 |
| 12 | Length of fruits | 0.000 |
| 13 | Fresh weight of fruits | 0.000 |
| 14 | Dry weight of fruits | 0.000 |
| 15 | Chlorophyll a | 0.000 |
| 16 | Chlorophyll b | 0.000 |
| 17 | Total chlorophyll | 0.000 |
| 18 | Carotenoid | 0.000 |
| 19 | Sodium | 0.000 |
| 20 | Potassium | 0.000 |
| 21 | Phosphorus | 0.000 |
| 22 | Nitrogen | 0.000 |
| 23 | Chloride | 0.000 |
| 24 | Magnesium | 0.000 |
| 25 | Calcium | 0.000 |
| 26 | Iron | 0.011 |
| 27 | Vitamin C | 0.000 |

**(If P value is 0.000-0.02 the effect of treatment is highly significant, if it is 0.02-0.05 the effect is significant, if it is above 0.05 the effect is not significant).**

**Plant Biochemical analysis**

**Chlorophyll content of leaves**

Plants treated with 15% UCE showed the highest levels of chlorophyll, including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, whereas the control group showed the lowest levels (Table 4). This suggests that UCE has a beneficial effect on pigment accumulation, which could increase photosynthetic efficiency. Analysis of tomato plants (var. Rio Grande) showed that the inorganic mineral content of their leaves increased from the control to the 15% UCE treatment in terms of sodium, potassium, phosphorus, and nitrogen levels. Given that 15% UCE produced the highest mineral content of any treatment, this implies that higher algal extract concentrations lead to greater mineral uptake (Table 4).

**Table 4. Fisher's Means comparison analysis on the effect of applied treatments on chlorophyll content of the leaves of *Solanum lycopersicum*.**

| **Parameters** | **Chlorophyll a ($mg/g^{-1}$)** | **Chlorophyll b ($mg/g^{-1}$)** | **Total chlorophyll ($mg/g^{-1}$)** | **Carotenoid ($mg/g^{-1}$)** |
|---|---|---|---|---|
| **Control** | 2.1423±0.0310e | 8.928±1.149e | 11.070±1.169e | 1.0269±0.0310e |
| **5% UCE** | 2.3368±0.0640d | 12.732±1.318d | 15.069±1.295d | 1.8729±0.1543d |

| | | | | |
|---|---|---|---|---|
| **10% UCE** | 2.6929±0.1275c | 17.757±0.980c | 20.451±0.936c | 2.8112±0.2414c |
| **15%UCE** | 4.4266±0.0962a | 27.133±1.049a | 31.559±1.058a | 4.5964±0.2003a |
| **NPK** | 3.6197±0.1400b | 21.384±1.050b | 25.003±1.056b | 3.8625±0.1610b |

**(UCE= Ulothrix cylindricum extract, ± = standard deviation, Alphabets shows level maximum to minimum values observed).**

**Phosphorus content (P)**

The trend seemed to increase from control to 15% UCE. Which showed that more algal extract, more sodium will be present (Table 4).

**Nitrogen content (N)**

The trend seemed to increase from control to 15% UCE. Which showed that more algal extract, more sodium will be present (Table 4).

Comparative analysis on the effect of applied treatments on mineral content of the leaves of *Solanum lycopersicum* are given below (Table 5).

**Table 5. Comparative analysis on the effect of applied treatments on mineral content of the leaves of Solanum lycopersicum.**

| Treatments | Sodium (Na) (mg/100) | Potassium (K) (mg/100) | Phosphorus (P) (mg/100) | Nitrogen (N) (mg/100) |
|---|---|---|---|---|
| **Control** | 52.600 ± 1.994 e | 1120.0 ± 148.3 e | 141.20 ± 7.40 e | 1037.6 ± 57.1 d |
| **5% UCE** | 69.667 ± 1.447 d | 1713.3 ± 192.2 d | 198.13 ± 9.55 d | 1760.0 ± 209.8 c |
| **10% UCE** | 76.467 ± 1.807 c | 2726.7 ± 143.8 c | 247.93 ± 10.26 c | 2880.0 ± 280.8 b |
| **15% UCE** | 92.467 ± 2.326 a | 4293.3 ± 192.2 a | 380.00 ± 10.97 b | 4166.7 ± 111.3 a |
| **NPK** | 82.733 ± 1.981 b | 3626.7 ± 257.6 b | 401.20 ± 3.46a | 4226.7 ± 138.7 a |

**(UCE= *Ulothrix cylindricum* extract, NPK = Nitrogen, Phosphorus and potassium fertilizer 15% ± = standard deviation, Alphabets shows level maximum to minimum values observed).**

**Biochemical analysis of the fruits**

**Mineral Content of fruit**

As indicated in Table 6, the mineral analysis of tomato fruits showed a progressive increase in the levels of calcium (Ca), magnesium (Mg), iron (Fe), and chloride (Cl) from the control group to plants treated with 15% UCE. This pattern implies that the accumulation of these vital minerals in the fruit is facilitated by increased algal extract concentrations. The greatest amounts of all evaluated minerals were consistently found in the 15% UCE treatment, suggesting that it is beneficial in enhancing the nutritional value of tomato fruits by increasing mineral absorption.

**Table 6. Comparative analysis on the effect of applied treatments on mineral content of fruits of *Solanum lycopersicum*.**

| Treat-ments | Chloride (mg/100 g) | Magnesium (mg/100g) | Calcium (mg/100 g) | Iron (mg/100 g) |
|---|---|---|---|---|
| **Control** | 1.3400 ± 0.114 e | 2.200 ± 0.837 e | 2.600 ± 0.548 d | 0.080 ± 0.015 b |
| **5%** | 1.920 ± 0.156 d | 5.600 ± 0.828 d | 4.800 ± 0.775 c | 0.170 ± 0.202 b |

| | | | | |
|---|---|---|---|---|
| **10%** | 2.686 ± 0.220 c | 6.733 ± 1.100 c | 5.400 ± 0.737 c | 0.148 ± 0.009 b |
| **15%** | 4.226 ± 0.228 a | 10.133 ± 1.187 a | 9.400 ± 2.028 a | 0.249 ± 0.017 a |
| **NPK** | 3.480 ± 0.239 b | 7.533 ± 0.915 b | 7.200 ± 0.862 b | 0.178 ± 0.009 a b |

**(UCE= *Ulothrix cylindricum* extract, NPK = Nitrogen, Phosphorus and potassium fertilizer 15% ± = standard deviation, Alphabets shows level maximum to minimum values observed).**

**Vitamin C content in fruits**

Vitamin C content in fruits seemed to increase from control to 15% UCE. Which showed that more algal extract, more Vitamin C content will be present (Table 7). The treatment (15% UCA) were found to have very low Vitamin C in comparison with fresh tomatoes. This difference might arise as a result of possible Vitamin C loss during the processing procedure.

**Table 7. Comparative analysis on the effect of applied treatments on mineral content of fruits of *Solanum lycopersicum*.**

| Treatments | Vitamin C (mg/100g) |
|---|---|
| **Control** | 5.473±0.408 d |
| **5% UCE** | 6.813±0.837 c |
| **10% UCE** | 11.293±0.937 a |
| **15% UCE** | 8.477±0.662 b |
| **NPK** | 8.477±0.622 b |

Conclusively, all parameters demonstrated the following order of effectiveness: UCE 15% exhibited the greatest impact, followed by NPK, then UCE 10%, UCE 5%, and finally the control group, which showed the least effect (UCE 15% > NPK > 10% > 5% > Control).

## DISCUSSION

Green algae are the biggest algal category, with over 500 genera and over 15,000 species [2]. The most majority (more than 80%) live in freshwater, with the remaining in environments that include seawater/brackish water, soil, or soilless surfaces. The majority live freely, but some are parasitic or symbiotic. The Charales and Oedogoniales orders are almost entirely limited to fresh water, as opposed to most other orders (Ulvales, Cladophorales), where the majority are marine. As global food demand increases, agricultural sectors require more chemical fertilizer. Inorganic fertilizers contain a variety of substances such as nitrogen, phosphorus, and potassium. Excessive use of chemical fertilizers in agriculture is expensive and has a number of harmful consequences for both living creatures and the environment. For example, leftover contaminants can infiltrate water bodies through rains, creating eutrophication. It may also decrease water-holding capacity, soil fertility, and nutrient inequalities. Furthermore, groundwater contamination can induce gastric cancer, goiter, metabolic diseases, birth deformities, hypertension, and livestock poisoning [3, 4]. Biofertilizers are an environmentally benign, low-cost, and renewable source of plant nutrients that can supplement or replace chemical fertilizers in sustainable agriculture. Algae are a good source of fertilizer, causing less mineral runoff than other manure or chemical fertilizers [7]. Soil chemical and biological properties affect soil texture, pH, EC, organic matter, and minerals, all of which contribute to algae development and crop output. Soil chemical characteristics are essential markers of soil suitability for long-term fruit and agricultural production, nutrient availability, and organic matter concentration [7]. Many studies have shown that green algae can

add organic matter, synthesize and liberate amino acids, vitamins, and auxins, reduce soil oxidizable matter content, provide oxygen to the submerged rhizosphere, improve salinity and buffer pH, solubilize phosphate, and increase crop plant fertilizer use efficiency [14, 15]. The current study describes the algal effect as a bio fertilizer by preparing different algal concentrations as standard values and observing their influence on the Rio Grande tomato crop. On a global scale, *Solanum lycopersicum* (tomato) is becoming increasingly essential for fresh crop consumption, as a key component in many prepared cuisines, and for study into the fundamental principles of plant growth and development. *S. lycopersicum* members can withstand a variety of environmental and nutritional stresses. A handful of the species have been crossed to develop a significant variety of varieties intended at either producing a single-harvest field crop or, when protected, a succession of fruit for the fresh market over an extended period of time. Ironically, the rise in fertilizer costs, along with a variety of economic losses caused by excessive fertilizer application, has not resulted in an increase in farmer earnings. As a result, attempting to improve nutrient absorption by spraying extracts of natural products containing stimulants is a strategic step to mitigate the detrimental effects of applying excessive amounts of inorganic fertilizer. Previous research has shown that some liquid fertilizer formulations derived from raw seaweeds can boost nutrient absorption, hence improving the growth, development, and production of several agricultural crop species [18, 19]. The current study reveals that in the case of *Solanum lycopersicum* variety Rio Grande, 5%, 10%, and 15% UCE algal extract and NPK demonstrate 0% germination percentage, which is possible due to the mineral composition of algal extract and NPK, which suppresses seed germination. However, in the control group, 100% germination was found, as well as maximal GP and GI, indicating that the algal effect is not particularly beneficial to seedlings in vitro. In vivo experimental plants provide different and spectacular results than in vitro plants. *Solanum lycopersicum* (Var. Rio Grande) has a GP rate of 100%, demonstrating that atmospheric temperature, light, humidity, and other variables promote plant development. GI, MGT, and SVI levels were highest in 15% UCE and lowest in control (Table 3). The soil also aids in boosting plant length, shoot length, and root length to maximum height compared to in vitro conditions. Different morphological parameters were noticed, and the data acquired after computations suggest that 15% UCE has the highest values in the data, while the control group has the lowest values (Table 3). The chlorophyll content at varying concentrations of growing plants varies. In a 15% algal extract, *Solanum lycopersicum* (Var. Rio Grande) had the highest chlorophyll concentration (31.559 ± 1.049 a) compared to the control group (Table 4). The current study found that plants with a high concentration of algal extract have higher chlorophyll content than others. Na, K, P, and nitrogen content in leaves also shows higher values in 15% UCE, while plants grown under NPK show higher values of these minerals, and the tendency reduced from NPK to Control (Table 4). Analysis of variance of different parameters are presented in (Table 3c). Thus, this trend appears to match the literature of Mahadik et al. (2021) the biochemical study of fruit also revealed that the mineral content and vitamin C concentration in *Solanum lycopersicum* (var. Rio Grande) increased from control to 15% UCE [34]. NPK falls below 15% UCE. All of these data demonstrate that higher concentrations of algal extract encourage and increase plant development.

## CONCLUSION

The use of algae as biofertilizers is a sustainable and environmentally friendly method of soil fertility management that benefits both agricultural productivity and ecosystem health. Algae as a biofertilizer can dramatically improve tomato crop growth, yield, and quality while also encouraging environmental sustainability in agriculture. Algae-based biofertilizers frequently contain bioactive substances that can

boost plant stress tolerance, allowing tomatoes to tolerate environmental difficulties such as drought, heat and disease strain. Algae nutrients, such as nitrogen, phosphorus, potassium, and micronutrients, are required for tomato plant growth. Furthermore, the organic matter given by algae contributes to soil health, resulting in improved water retention, aeration and microbial activity, all of which are essential for vigorous plant growth. The experiment was designed to test the growth and yield of tomatoes using various concentrations. We observed significantly lower yields in the control group compared to the 5%, 10%, 15%, and NPK groups. The highest excellent yield was observed with 15% concentration. Overall, the successful growing of healthy tomatoes with algae as a biofertilizer indicates the efficacy of this sustainable and environmentally beneficial agricultural method. It not only increases crop output but also improves soil health and ecological sustainability.

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