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Development of Biofertilizer from Local Qatari Cyanobacteria Strains for Enhancement of Bell Pepper (*Capsicum* annuum L.) Growth, Yield, and Abiotic Stress Tolerance under Hydroponic System

BY

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ABSTRACT

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Title:_Development of Biofertilizer from Local Qatari Cyanobacteria Strains for Enhancement of Bell Pepper (*Capsicum annuum* L.) Growth, Yield, and Abiotic Stress Tolerance under Hydroponic System

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The beneficial cyanobacteria strain (blue-green algae) *Roholtiella* sp. QUCCCM97 has been found to be the origin of an eco-friendly and environmentally safe biofertilizer whose extract and biomass were used to increase the productivity of bell pepper (*Capsicum annunm* L.) in hydroponics. From the collection of hundreds of Qatari cyanobacteria strains, three strains QUCCCM97, 99, and 112 were characterized and classified according to morphological and molecular identification. Then, a preliminary investigation showed that the three strains were effective growth promoters to bell pepper seedlings with QUCCCM97 being the most efficient. Consequently, a scale-up experiment (large scale/mini commercial scale) was conducted to further study the effect of its extract and water re-suspended biomass as biofertilizers through foliar application under a hydroponic system. Besides, the extract was also investigated to treat salinity stress on bell pepper seedlings at different concentrations via a foliar application under a soilless technique. Summarily, QUCCCM97 was found to significantly increase the vegetative parameters, biochemical/nutritional constituent, and crop yield with respect to conventional fertilization alone. Also, QUCCCM97 significantly mitigated the effect of salinity at different concentrations (0, 50, 100, 150, and 200 mM) on vegetative growth parameters and biochemical constituents. Based on our findings, we assume tentatively that the QUCCCM97 strain could be a novel species as no prior identification is found in the GenBank.

DEDICATION

This thesis is dedicated to my late parents Alhaji Bello Olayiwola Atanda and Madam Bello Rafat Asake (my biological parents), Alhaji Abdul Rauf Bello and Alhaja Samiat Bello (my foster parents) my family, and my supervisor Dr. Radhouane Ben Hamadou. *Thanks to them for their support, academically, morally, spiritually, and financially that shape me to be who I am today.*

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CHAPTER 1: INTRODUCTION

General Background

The state of Qatar is undergoing tremendous economic and population growth leading to higher demand for food commodities as well as speedy growth in the agricultural industry to decrease dependency on imported food (Richer, 2014; Ben Hassen et al., 2020). Thus, the application of prokaryotic cyanobacteria high-value product extract (HVPE) and water re-suspended biomass (WRB) as biofertilizers under the hydroponic systems should be considered the sustainable options in agriculture production in Qatar to increase food availability because of their eco-friendly nature. The hydroponic system is an encouraging, rewarding, and environmentally friendly agricultural production technique (AlShrouf, 2017) that has the potential of addressing most of the food security limitations in Qatar, i.e. enhancing water use efficiency thereby reducing agricultural water consumption and equally enhancing the use of biofertilizer extract, consequently reducing the environmental footprint. Moreover, the hydroponic system is getting costly to operate due to the cost of inputs (seed/seedlings, water, pesticides, inorganic fertilizer), particularly inorganic hydroponic fertilizer. Hence, it is imperative to investigate, address, and evaluate the optimization of production by using a sustainable alternative biofertilizer (HVPE and WRB) in growing the selected crop/vegetable (bell pepper). The proposed research is intended to optimize the currently used system/existing system of the Al Sulaiteen Agricultural Industrial Complex (SAIC, Qatar) as a case study. The formulated objectives of this research will be as follow:

Objectives

1. To investigate the bell pepper (BP) growth using blue-green algae high-value product extract and water re-suspended biomass (cyanobacteria – QUCCCM97, 99, and 112) as a growth promoter and to screen for the most effective among them.
2. To investigate the effect of biofertilizer (cyanobacteria: Roholtiella sp. high-value product extract and water re-suspended biomass) under hydroponic system on the yield and quality of bell pepper (Scale-up experiment)
3. To investigate the effectiveness of the cyanobacteria high-value product extract as a salt stress alleviator in bell pepper (salinity experiment)
4. To investigate the biochemical responses of bell pepper production under abiotic stress (extreme/critical temperature)

This research was conducted in two stages: 1. On-site (greenhouse and field) and 2. Extensive laboratory experiments, planting of crops in the greenhouse using a soilless system, while the scale-up crop production was carried out under hydroponic systems at the Al Sulaiteen Agricultural Industrial Complex (SAIC). The alternative source of nutrients, cyanobacteria high-value product extracts (HVPEs), and water re-suspended biomass (WRB) were investigated to know how effective and sustainable they could be. The response of the crop yield in terms of quantity, root, shoot, and other qualitative parameters to various dilution levels of bio-fertilizer (HVPE and WRB) was investigated as growth promoters. The optimization studies were carried out on bell pepper using commercial seed/seedling provided by SAIC (Qatar) and investigating their growth under different dilution levels.

In the long run, there should be an answer to several questions and tackle the challenges associated with optimal production, when the research is completed will equally open

doors in terms of opportunity for the utilization of bio-fertilizer as a better alternative to conventional chemical inorganic fertilizer to enhance intensive food production, mitigate the possible environmental pollution from the using of chemical fertilizers.

Sustainable bell pepper production practices are so consequential as far as food security coupled with social sustainability is nowadays a concern and in the nearest future since bell pepper is of high nutritional value, multiple health benefits, and high commercial value. These advantages make them among the rewarding crops that globally lessen human hunger and poverty as a result of their possible high yield if adequately cultivated. Meanwhile, Qatar is one of the countries in the Arabian Peninsula (AP) where producing locally food commodities has been a major issue, not because of climatic factors only but with other vital parameters, especially the lack of arable lands, agricultural production inputs and techniques. However, bell pepper has been successfully cultivated in AP but is not yet fully sustainable, namely in Qatar. Also, is selected as a model because Qatar has a comparative advantage in its cultivation aside from economic and multiple health benefits. Optimization of BP production is going to be facilitated using an alternative source of nutrients; bio-fertilizer as it enhances yield and quality respectively.

Agriculture in Gulf Cooperation Council (GCC) Countries.

The Gulf Cooperation Council (GCC) countries, including Qatar, lie in the arid region, which is generally known for high temperature, high rates of evaporation as well as shortage and low annual rainfall. The state of Qatar is known to be a country located in an arid region. It is characterized by their peculiar climatic factors as the hot temperature in the summertime that often exceeded 40°C and little rainfall estimated to be around 46.72 mm on the average annually as recorded within the time frame of 2008 - 2013 according to data from the Ministry of Development Planning and Statistics

(MDPS). The evaporation rate recorded annually is high, measured to be approximately 2200 mm. However, considering the harsh climatic condition and fragility associated with the environment coupled with a shortage in renewable energy and water resources, thus the country falls into the same category of countries that have been established to have extremely high-water risk as found in the Aqueduct Water Risk Atlas (Water Resource Institute). Agricultural activities are mostly dependent on irrigation and it was established that it utilized the largest quantity of water compared with other sectors, estimated to be about 78% averagely of total summation of water utilized in the entire GCC countries (Barghouti, 2010; Saif et al., 2014). Virtually all agricultural water (85%) comes from groundwater, which is not renewable (Bazza, 2005) since abstraction rates far exceed recharge rates. The irrigation system in the GCC region has suffered a great setback due to the rapid fall in underground water availability. Generally, water use efficiency (WUE) is very low concerning activities carried out under the field parameters in the entire region (Al Ajmi et al., 2009a). Nevertheless, different techniques have been put in place to upgrade water production to enhance irrigation, inclusive is adequate water resource management (Wang et al., 2002).

Sustainable Agricultural System in Qatar.

The state of Qatar is located on a total area of approximately 11,000 Km^2 with a few numbers offshore islands inclusive. By length, it attains 180 Km at a maximum that spans in the direction of the north-south axis, while is 85 Km in width along the east-west and it has been established to be its widest point so far (Moustafa, 2010). The highest elevation point as found in Qatar is situated in the south, measured roughly 100m in height above the sea level, and contrarily, in the north is where the lowest point of 50 m is found. Qatar biome like most GCC countries is desert, characterized to be rocky with oases scattered all over formed from separate depressions amounted to a

whopping 850, and the depths of soil range between 30 and 150 cm. The population of Qatar is around 2.88 million according to Planning and Statistics Authority in Qatar. The trend in the population increase has been persistent (a yearly growth rate of 1.81%) as far back as 20 years ago and there is variation in the population pattern of Qatar as the concentration of people is mostly in the capital city of Doha (Karanisa et al., 2021).

Food Security

Food security, according to the international organization has been defined differently but in this research, the 1996 World Food Summit (WFS) definition will be adopted as "All people, at all times, should have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life". The issues of food security, as well as food production, are considered of major relevance in the Arabian Peninsula (AP) generally and Qatar is not an exception. More attention has been given to food security as a result of food crises that lead to price rises and the availability of food in the international market. Also, the recent blockade against Qatar from food importation from some countries in the peninsula has posed a threat to the food security in Qatar. Most countries within Arabian Peninsula particularly GCC countries are heavily dependent on food importation to augment their local production. According to the WFS of 1995, it was envisaged that dependency on importation of food in Arabian Peninsula region, Qatar inclusive was considered to be part of the highest globally which attained over 70% (Moustafa, 2010).

Agriculture and Availability of Water in Qatar

In Qatar, agriculture is the sector that consumes more water compared to others economic sectors. In the year 2005, it was documented that total withdrawal water stood at around 444 million m^3, and approximately 59% (262 million m^3) of this total withdrawal was consumed through agricultural activities, municipal purposes used

39%, while just 2% was used for industrial purposes. Qatar water resources are mostly obtained from the groundwater, through the process of seawater desalination as well as sewage effluent treatment and the latter are regarded as non-conventional water sources in Qatar. In the 1990, the total water production from these three resources was 200 million m^3 which increased to 912 million m^3 in 2016 and by 2019 it has attained 1038.16 milliom m^3 (Water, 2021)

Hydroponics and Blue-Green Algae (Cyanobacteria) as a Sustainable Option

The hydroponics system of growing crops as a sustainable option to mitigate the problem of water shortages improves WUE thereby minimizing the quantity of water loss either through evaporation or drainage (Bailey, 1996). This technique has been used successfully in this region to produce different crops and vegetables, a good example is fodder that is produced within the shortest periods of approximately 7 to 10 days, and it has been equally established that is both financially and environmentally reasonable (Rotar 2004). Most studies have proved that under the system agricultural input costs are approximately 10 times lower compared with growing in open field conditions (Mooney, 2005). The higher level of WUE was seen to be the major merit associated with this system which conserves nearly 95 - 97% of water utilized when compared with the normal agricultural system of tilling a relatively small piece of land (Al Ajmi et al., 2009b, 2009a). Also, the different systems of farming are encountering challenges viz. chemical fertilizer cost and pollution, as well as greenhouse gases, have necessitated an increasing need in the agronomic application of cyanobacteria extract as biofertilizer, especially regarding the application of cyanobacteria extracts and biomass as an optional source of nutrients (Zahra et al., 2020) and several studies have established that cyanobacteria extracts contain a high concentration of bioactive nutrients. Also, cyanobacteria biomass and extract can potentially be used to improve

soil productivity, especially on the agricultural land, thus, resulting to soil physical and chemical properties improvement (Zahra et al., 2020; Mutale-joan et al., 2021; Shah et al., 2021)

CHAPTER 2: LITERATURE REVIEW

Elements Uptake by Plants

Two routes have been identified by which plants obtain their nutrients which could be through leaves or roots depending on the plants in question. Hence, the nutrient moves through the plant system internally where it is demanded. Often, the elements usually move through the roots by absorbing the nutrients from the particular growing media usually facilitated by a process understood to be cation exchange, where hydrogen cations are released from the roots to the surrounding media *(Sonneveld and Voogt 2009).*

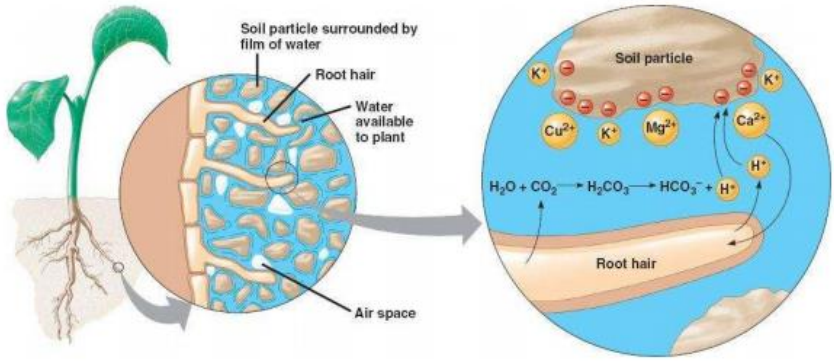


Figure 1. Exchange of cation between roots system and soil.

Source: http://bio1152.nicerweb.com/Locked/media/ch37/soil_availability.html

In return, more cations such as calcium and potassium are released from the soil particles, permitting their take up through the root system as demonstrated in Figure 1. Carbon dioxide is also secreted in the roots of the plant which subsequently dissolves in water to form an acidic compound called carbonic acid (H_2CO_3). This acid further undergoes dissociation, thereby more hydrogen ions are released into the media enhancing the ion's availability to the plant.

The elements are absorbed from the nutrient in form of ions which are bioavailable only in this form to the plant. The most noticeable factor while any nutrient is uptake through the roots system is the potential of water within the soil and surrounding air of the plant. A process known as transpiration is peculiar with plants, in which plants lose water through pores known as stomata on the leaves. There is a linear relationship between transpiration and temperature, as temperature increases rate of transpiration equally increases while humidity remains low around the leaves; this is a process designed to maintain the cooling effect on the plant. During the transpiration, a siphoning effect occurs around the plant, creating negativity in water potential in the roots system, pupping nutrient and water solution into the plant through the roots system Figure 2

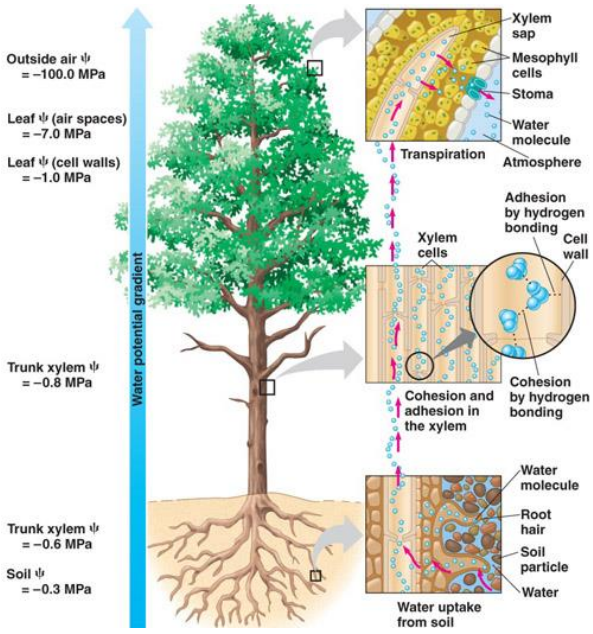


Figure 2. Water movement in plants during transpiration.

Source: https://www.topperlearning.com

Normally, nutrients can be taken up through the roots system in three different ways as described and shown in Figure 3 below. Firstly, through simple diffusion which encompasses nonpolar molecules moving through the epidermis layer taken into consideration the concentration gradient. Usually, it involves the movement of small or minute, hydrophobic, neutral,, or uncharged molecules, including elements such as nitrogen (N), oxygen (o), carbon (IV) oxide CO_2 and water. Facilitated diffusion is the second method that is characterized by the involvement of the element gluing with transport molecules, which are predominantly a protein or humic acid, and their movement into the plant is facilitated through concentration gradient via proteins channel berried within the membrane.

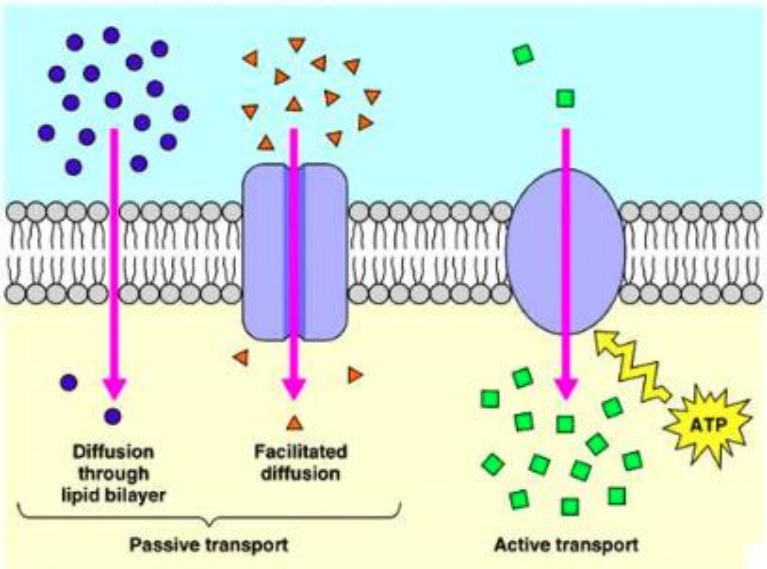


Figure 3. A comparison of passive and active transport

Source: Campbell's Biology, Fourth Edition

Active transport is the third method that involves the utilization of pumps in the membrane of roots, and the mechanism requires energy known as adenosine triphosphate (ATP) to actively move the element through into the plant.

The cortex of the root is the passage that facilitates the movement of the nutrient and once it passes it can further be transported to an area that needed nutrients such as the plant's aerial parts through either the phloem which transport food materials or the xylem that solely responsible for the transportation of liquid. Nevertheless, water and dissolved ions are expected to move via xylem in bulk, through the transpiration stream path and the rate of transpiration will determine the magnitude of what flows through the xylem. Few areas such as fruit in the plant do encounter low transpiration rate and as such the redistribution of ions as well as other molecules are through the phloem to these areas to mitigate any deficiencies. Nutrient bioavailability to the plant can be facilitated by microorganisms which include rhizobacteria, mycorrhizal fungus as well as Trichoderma fungus, which are known for their ability to establish a symbiotic association with the plant. Nitrifying bacteria are inclusive with the ability to convert nitrite to nitrates *(Ranger et al., 2015; Wallander et al., 1997; Tikhonovich and Provorov, 2011*).

Inorganic fertilizer can equally be applied directly by spraying onto the surface of the leaves attached to the plant and is a common method of fertigation and correcting deficiencies in agriculture. Foliar absorption occurs through penetration on the leaf's associated cuticular membrane, in addition, absorption could be witnessed also through the stomata of the leaf *(Bukovac and Wittwer 1957; Seshadri Kannan and Charnel 1986).* The nutrient is transported to the other parts once it entered the leaf through the phloem *(S Kannan, sciences, and 1986).*

Nutrient Sources

Fertilizer (Chemical/Organic).

By definition, fertilizer is known as a material that supplies to the plant one or more important elements when applied to the plant for the healthier growth of the plant. Fertilizers have different types based on the particular nutrients they supply and are commercially available. They can be categorized into two synthetic/inorganic fertilizers which are obtained from natural minerals extracted and further subjected to a series of chemical processes or treatments as well as refinement whereas organic fertilizers, are known to have generally originated from plants and materials, a good example is a compost or manure. Furthermore, classification could be based on the number of primary macronutrients in terms of quantity (N, P, and K) they supply. A straight fertilizer usually contains a single nutrient to supply as a macronutrient. There are various examples such as ammonium nitrate (NH_4NO_3) as well as calcium nitrate ($Ca(NO_3)_2$). Compound /complex / multi-nutrient fertilizer on the other hand contains two or several of the primary micronutrients which include potassium phosphate (KH_2PO_4). Fertilizer comes in various physical forms either powdery or solid (pelleted) form. When in the powdery form it is mostly applied directly through the growing media or sometimes insolubilized in an aqueous solution mostly water and applied directly through the roots system or onto leaves through spraying.

The adoption of nanoparticles is gaining recognition in recent years for nutrient delivery to plants with the likes of particles commonly available which include silver, zinc, copper, and iron all being examined *(R. Nair et al. 2010; Kumar and Yadav 2009).* These elements tend to be molecules like amino acids carriers through electrostatic forces resulting in enough bioavailability to the plant. The peculiar merit associated with Nano is that it increased elements uptake, increase in the use of nutrients efficiency, increased outputs, food security improvement coupled with appreciable

economical fertilizer (*Nair et al. 2010; Dietz, Herth 2011.; Sastry et al. 2011; Coles, Frewer 2013 .; Khot et al. 2012.; Chaudhry et al. 2008*).

There is tremendous popularity in biofertilizers as well as organic farming over the last 10 years. The public has developed overwhelmed interest and shown more concern about improving commercial farming methods. People are nowadays more curious to know what has been applied as a source of nutrients to their various types of food being vegetables, cereals, and fruits before consuming them. By definition, a biofertilizer is an organic-based material or substance obtained from either plant residue or animal waste and has not been subjected to an extensive process of purification. The class of these types of biofertilizers includes manure, compost in the biomass form as well as compost extract *(Sonneveld and Voogt 2009; Tikhonovich and Provorov 2011; Dhabbe et al. 2012).*

Seed Coating.

A seed coat is known as a slightly thick chemical solution prepared solely to serve as coverage and be held by a seed before planting. It is often comprised of a polymer coating as well as inert carriers, which include pumice, for fertilizer, promoters of growth, antifungal, antimicrobial as well as chemicals that are insecticidal to bind to. The most commonly utilized fertilizer for this method is micronutrients because concentration requirements must be lesser coupled with its reasonable method of delivery economically. Several studies using seed coats affirmed it to be a reliable and successful technique suitable for fertilization as well as biofortification of different types of crops *(Scott et al. 1985, 1987; Karaguzel et al. 2004.; Mašauskas et al. 2008; Peltonen-Sainio et al 2006; Rebafka, Bationo, and Marschner 1993; Sekiya and Yano 2010)*

Priming of Seed.

Priming of seed is the utilization of a prepared solution to treat the seed before planting. Usually, the seed is soaked in a prepared solution and thereafter dehydrated until attaining the required moisture content level. Mostly the solution constitutes the nutrients, hormones, or additives that are beneficial to plants, such as mycorrhiza fungus, fulvic acid, and seaweeds, however, very often just ordinary water is utilized. Once the seed is hydrated, the germination process commences leading to the start-up of essential metabolic pathways. There are several merits about this method for the farmers in particular which include convenience to use, seed preparation could be done at any time, nutrient use efficiency is enhanced and waste reduction (as an excess nutrient that is not absorbed is not flushed down the drain). Several studies have established that an increase in germination rate had been facilitated through seed priming, leading to the production of uniformity in germination, incremental of seed vigor as well as plant productivity *(Mašauskas et al. 2008; Khaliq et al. 2015; Ajouri, Asgedom, and Becker 2004).*

Foliar Sprays/Application of Fertilizer (Chemical/Organic).

The process whereby nutrient is applied to the plant through spraying of the leaves. Different factors need to be considered *viz* application time during the day, the prevailing temperature during the application, humidity as well as chemical burns for better results. In this regard, most fertilizer application in field agriculture or hydroponics is through sprayers, hence the fertilizer is delivered through leaves. Is one of the most efficient ways of fertilizer delivery as there is no need for energy essential for active transport associated withF elements' movement from roots to other parts of the plant.

Hydroponics

Introduction.

By 2050, the global population is projected to attain 9.7 billion by the demographers. Additionally, it was predicted that half of the fertile land worldwide will be unusable for farming *(Pardey et al., 2014).* Thus, the production of food should be increased by as much as 110% to take care of the high demand by the populace. Approximately on the daily basis, it has been recommended that minimum consumption of 400g of both vegetables and fruits is expected for healthy living per day according to the World Health Organization (WHO) and the Food and Agriculture Organization FAO *(Badami et al., 2015).* As established by the United Nations (UN), presently several countries are encountering food shortages, particularly in Africa. However, if there is a failure in meeting the demand, it is expected that the food crisis will extend to 2050 *(Pardey et al., 2014).* The crisis occurred due to the unfavorable climatic conditions resulting in drought or floods as they occur frequently. Another reason is the persistent rise in the world population which has resulted into shift in the demand for food to be on increase. As forecasted that the World's population will attain 9.5 billion inhabitants come the year 2050, no doubt this will lead to complicated issues in term of environment and economic in order to meet energy demand for the rising population. However, water reduction in agricultural practices just to achieve or enhancing economic productivity is a serious setback in the countries of arid as well as semiarid regions. The Arabian Peninsula (AP) which comprises of Gulf Cooperation Council; the GCC (Kingdom of Saudi Arabia; KSA, Oman, United Arab Emirates; UAE, Kuwait, Qatar, Bahrain) *(Pirani et al., 2016),* and Yemen. From the global map, AP countries were clock wisely positioned from the north to the south. Bahrain, Kuwait, Qatar, and UAE are found within the eastern part, on the southeast is Oman, and to the south is Yemen while KSA occupies the center as shown in Figure 4

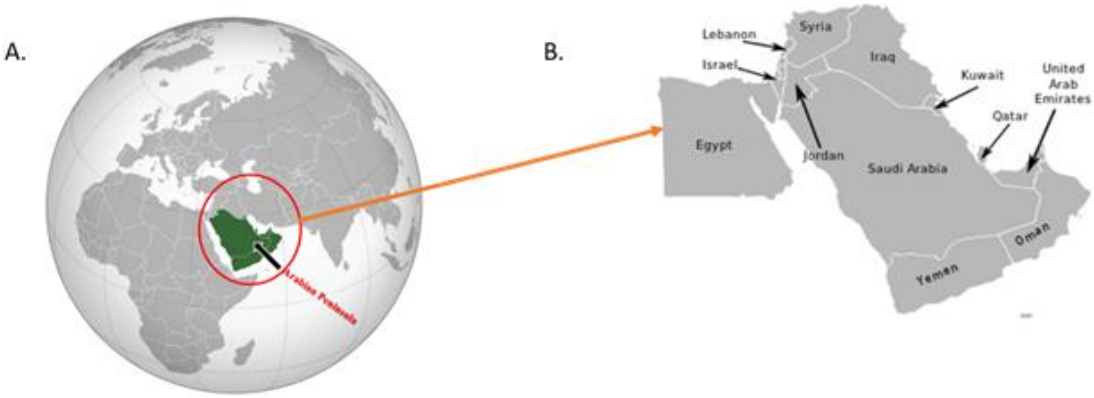


Figure 4. (A) The World map and (B) the Arabian Peninsula countries alongside neighboring countries

All the countries shared peculiar characteristics as they are satiated in the desert with peculiar climatic parameters. The climatic conditions are not suitable for agriculture in these countries as a result of harsh and persistent high temperatures, a high rate of evaporation, little rainfall, and the non-availability of arable land for cultivation.

Water is important for crop production and food security, which is regarded as the lifeblood of various ecosystems. However, the freshwater resources are diminishing at a critical rate and agriculture is majorly responsible for water scarcity. The frequent climate change forecasts show rises in the frequency and degree of drought periods in the arid and semi-arid regions. As indicated in Figure 5, the Middle East and North Africa are more stressed which was expected to have occurred below 1,700 cubic meters (m^3) per capita per year because of the prevailing harsh climatic conditions in these regions; hence, the agricultural production heavily depends on irrigation. Summarily, adequate irrigation management is essential to improve water use efficiency. In addition, as indicated in Figure 6, the highest percentage of freshwater withdrawal was accountable for agriculture compared with other sectors in all the

regions except Europe and Central Asia where industries are dominant because they are highly industrialized. There is an urgent need to utilize our endowed natural resources judiciously most especially water resources which form part of our existence.

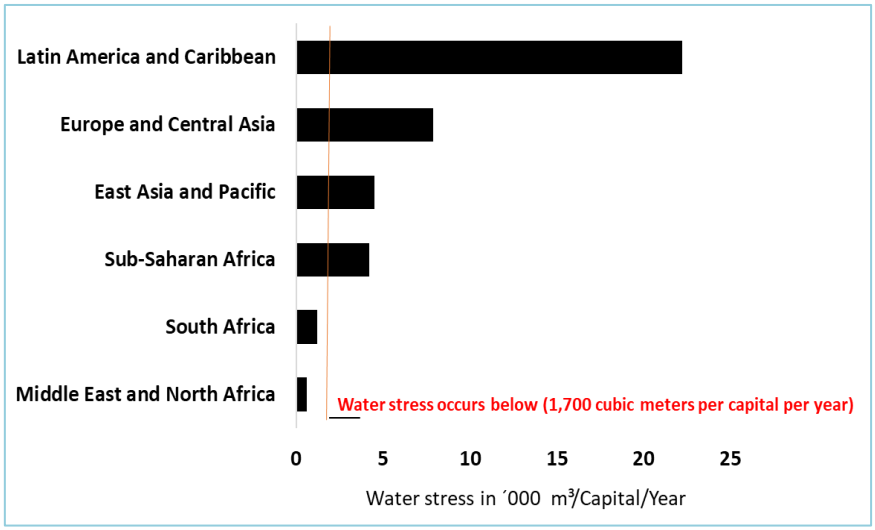


Figure 5. Water stress region. (Water stress occurs below 1,700 cubic meters (m3) per capita per year). Source**:** World Development Indicator (WDI)

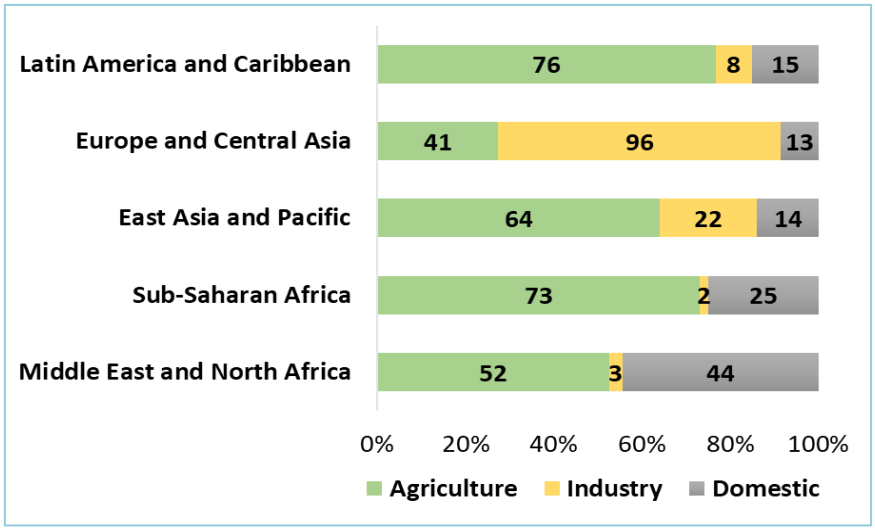


Figure 6. Share of freshwater withdrawals by sectors (%). Source: World Development Indicator (WDI)

Hence, they heavily relied on irrigation agriculture which is responsible for the largest proportion of water consumption amounted to roughly 78 percent of total water utilized in the entire GCC countries on average *(Al Ajmi et al., 2009b)*. Agricultural water is mostly (85%) groundwater which is not renewable *(Al Ajmi et al., 2009b)*.

A steady sharp decrease in underground water availability for irrigation has been a major concern in this region. Generally, water use efficiency (WUE) is considerably low in GCC regions under conventional methods *(Al Ajmi et al., 2009b)*. This issue of scarce water resources has contributed a major setback to agriculture as the need for water resources keeps increasing in these countries to meet regular demand for both agricultural activities and nonagricultural purposes *(Al-Karaki et al., 2012a)*.

Meanwhile, with conventional agriculture practices, other environmental issues such as pesticides' effect on runoff water and groundwater shortage which include soil-borne diseases, non-arable soil, deteriorated physical components of the soil, and water quality is of great concern. Over the past 5 decades, the use of pesticides has increased by nearly 42 times with the current utilization in the amount of 2.5 million tons annually *(BeVier, 2012)*. Also, these constraints are responsible for the reasons why only approximately 36% of the global land is suitable for crop production *(FAO, 2016b)*. Growing agricultural production employing hydroponic systems is one of the numerous systems available to achieve sustainability in agriculture in this region. There are several advantages obtainable by growing agricultural produce hydroponically. Such benefits include but are not limited to efficient water utilization, reduction in pesticides, increased yields, and unrestricted production of food compared to conventional agricultural methods all year round (Barbosa et al., 2015). The key determinants to achieve better productivity in hydroponics are satisfactory production site, easy management, viable varieties, and the ready market must be considered as illustrated in

Figure 7.

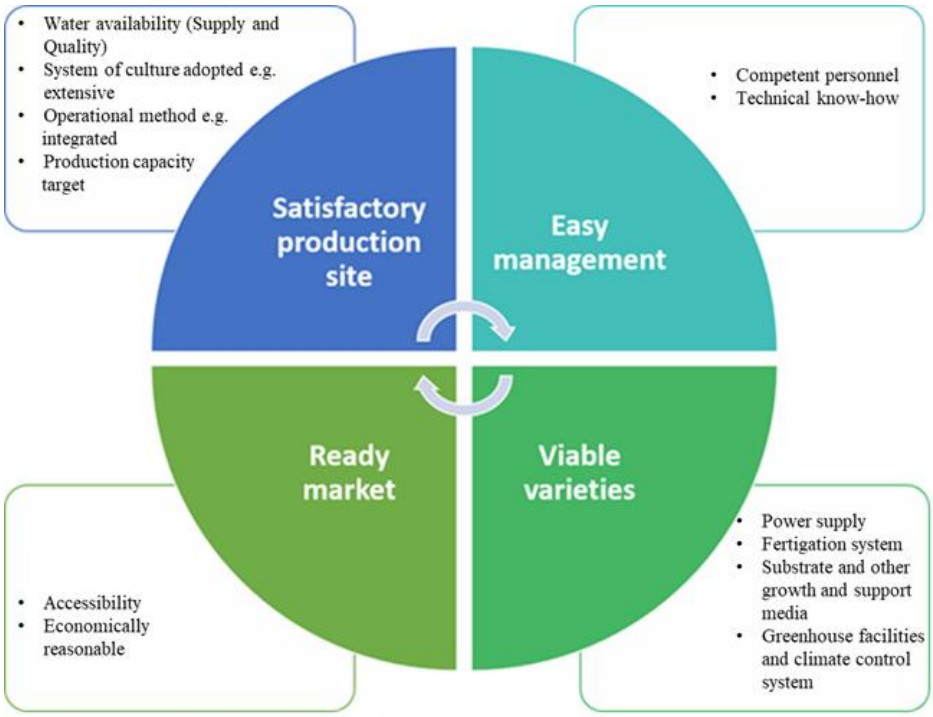


Figure 7. Key determinants in a hydroponic system

The hydroponic system has considerably expanded in the most recent decade as it adds to the escalation of plant production and gives high harvest yields even in zones with unfriendly growing conditions like the Arabian Peninsula countries. In this study, we aim to discuss: 1. the merits of hydroponics compared with the conventional farming method. 2. The probable limitations that affect the hydroponic system. 3. Nutritional value of phytochemicals accumulation of hydroponic compared to soil grown crops, and 4. The potential future of the hydroponic system compared to the conventional method in the Arabian Peninsula.

Hydroponic System.

Hydroponic is defined as a method of growing in a soilless condition using liquid chemical fertilizer as a source of nutrients *(Biebel, 1960).* Most terrestrial crops are

grown with their roots submerged directly in the nutrient solution only or an inert growth media such as mineral wool, gravel, and perlite *(Maharana et al., 2011; Resh, 2016).*

Existing Growing Methods.

Growing crops under soilless culture (hydroponics) can be placed into two different categories, namely a) liquid culture and b) aggregate culture as summarized the Figure 8.

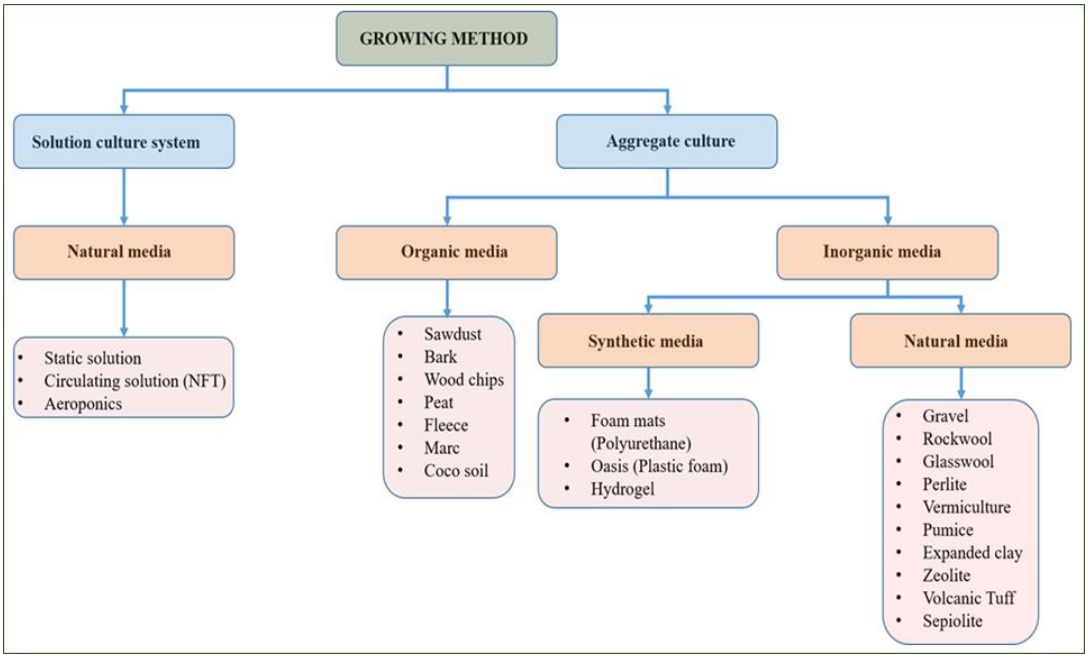


Figure 8. Growing methods in hydroponics as a function of the culture system and used media.

solution/liquid culture. This growing method is equally known to be the "true Hydroponics" because plants nutrient requirement is supplied in solution form in a constant circular motion after ensuring re-aeration and adjustment of all the necessary parameters such as pH, EC, as well as levels of the nutrient, a good example is nutrient film technique NFT (Mamta D. Sardare, 2013).

aggregate culture. In this case, the required nutrient solution is supplied through specific irrigation designed system to the plants supported in a media that is either organic or inert. Mostly, the unused solution is left to run out as waste if the system is open or recirculated if it is a closed system type. Examples of media often used are rock wool, perlite, pumice, etc (Mamta D. Sardare, 2013).

The hydroponic system exists in a few different types such as wick system, ebb, and flow system, drip system, deep water culture system, and nutrient filter technique (NTF) system.

Nevertheless, some factors or parameters must be carefully examined while selecting any of the existing techniques which include:

- Space availability and other available resources
- The expected productivity level
- Growing medium, suitable for growth, must be available
- The quality expectation of the output or product in terms of appearance, colour, size, freedom from infestation from pests; etc.

Different Hydroponics Techniques in Use.

The prevailing hydroponic system for growing crops is classified based on the adopted techniques. A hydroponic technique simply means the adopted style to supply liquid nutrients to the root systems of the plant. The different hydroponic systems in use are shown in Figure 9.

aeroponic. This system (Figure 9A) is the most complex and advanced technology in terms of operations and maintenance. The grown plant is placed in special trays in which they are suspended. The nutrient solution is supplied through the spraying method at every minute as regulated by a timer targeting the roots system, which gives a thin layer of nutrients. The system is constantly monitored to avoid

malfunctioning the pump at any time.

wick technique. In this system (Figure 9B) the parts are immobile. The nutrient solution is stored in a reservoir where the oxygen level is maintained by using an air stone placed at the bottom of the reservoir. The nutrient mixture is drawn into the growing media with a wick (plastic tubing). The root system of the crop is embedded in the growing medium (Ernst et al., 2009).

drip technique. The drip system (Figure 9C) utilization surpasses that of all other systems of hydroponic. The drip system task is somewhat clear - a clock controls a submersed siphon. The siphon supplies water/supplement through a trickle line that is situated over the plant base and growing medium. Water/supplement trickles to the base of every plant supplies the root systems with a supplement, and channels through the growing medium once more into a store where it will be used again (Ernst et al., 2009).

ebb and flow technique. The other name for the ebb and flow technique is the "flood and drain" (Figure 9D) technique due to the continuous and orbital rotation of pumping the nutrient solution into the tray that housed the plant and it draining back to the tank (Jones Jr, 2016). The simple framework of the ebb and flow system is made up of a tray that housed the plant containing the growing medium placed above the tank of nutrient solution. A timer is essential to regulate the submerged pump, where nutrient solution is forcefully trickled into the tray and recycled back into the tank. The process repeats itself continuously at regulated time intervals.

water culture. System (deep system) (Figure 9E) originally, virtually all the hydroponic systems emanated from the deep system (Harris, 1988). This system is well simplified and comprises of tank/reservoir, an air stone placed at bottom of the reservoir, a timer, a tubing system, an air pump to facilitate air movement, as well a

floating platform (Hoagland et al., 1950). With the progress of air circulation strategies to keep disintegrated oxygen, a profound deep system was developed, thus plants can be cultivated with roots continually suspended in the nutrient solution. To attain optimum growth conditions, it is very crucial to constantly monitor the oxygen, the concentration of nutrients, salinity as well as pH level (Domingues, 2012).

nutrient film technique system (NFT). The nutrient film technique (NTF) (Figure 9F) came into existence to improve on the weakness associated with ebb and flow systems in the 1960s and still remain the universally accepted system among horticulturalists. The nutrient solution in a tank flows around the whole system via pumping into the plant tray every second, thus constantly circulates root systems (Domingues, 2012). The collection of the solution back in the reservoir facilitates reused, however, the water volume is regulated by the slant position of the tray as well as the intensity of the water pump. Furthermore, due to the permanent contact of the root systems with water, they are highly susceptible to fungal infestation (Thinggaard et al., 1989).

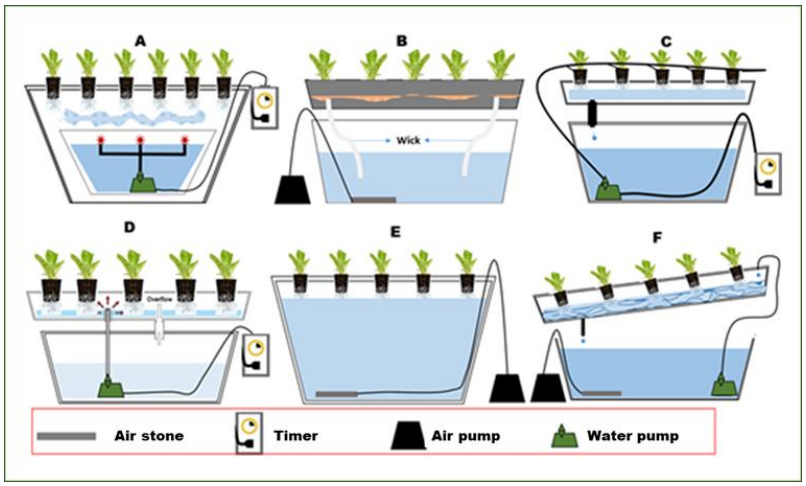


Figure 9. Different types of hydroponics techniques: A. Aeroponic; B. Wick technique; C. Drip technique; D. Ebb and flow technique; E. Water Culture; F. Nutrient film technique system (NFT)

The Nutrient Solution for Hydroponic System

Crops require essential micro and macro elements for their healthy growth and development. The deficiency of any of the nutrients in crops would affect their life cycles. Adequate nutrient management is very important for the soil-less grower to maximize production.

Managing Nutrient Solution

Achieving an optimum in the hydroponic system is very easy; however, inappropriate nutrient solution management can result in poor growth of the plant. Good performance or failure of hydroponic production is, therefore, the function of the adequate nutrient management plan. The adjustment of the pH level, the temperature and electrical conductivity of the nutrient solution, and prompt replacement when required, will surely result in optimum production.

The pH level of nutrient solution

The alkalinity and acidity of the solution are measured on the pH scales that range from 1 to 14. Mostly, the optimum pH of the nutrient solution falls between 5.8 and 6.5 respectively. When the pH is too high or low against the recommended range for a particular crop, the nutrient will promptly exhibit toxicity symptoms on the crop. Table 1 show different hydroponic crops with different pH values.

Electrical Conductivity of the Nutrient Solution

The electrical conductivity (EC) measures the salinity of the solution. This is achievable with a simple measuring instrument; an EC meter. The major setback of EC is that the concentration of the individual nutrient constituents cannot be measured separately but rather the total concentration of the solution. The optimum EC range for most crops is between 1.5 and 2.5 dSm^{-1}. Table 1 show different hydroponic crops with different EC values.

Table 1. The Optimum Range of EC and PH Values for Different Crops under Various Hydroponic Techniques

System	Crop	EC (dSm-1)	pH	Water use efficiency	Substrate	Country	Reference
Aeroponic	Potato	2 - 2.5	5.5 - 6.5	High	Rockwool	Germany	(Abdullateef et al., 2010)
	Potato	2 - 2.5	5.5 - 6.5	High	Perlite	Spain	(Farran et al., 2006)
	Tomato	2 - 2.5	5.5 - 6.5	High	NR	India	(Gopinath et al., 2017)
	Lettuce	0.8 - 1.2	5.5 - 6.5	High	NR	India	(Gopinath et al., 2017)
Wick technique	Lettuce	0.3	5.6	High	Coconut coir substrate	Brazil	(Ferrarezi et al., 2016)
	Kalanchoe blossfeldiana	1.6	6.5	High	Peat moss + Perlite	South Korea	(Son et al., 2006)
Drip technique	vegetable crops	NR	NR	High	NR	USA	(Hartz, 1996)
Ebb and flow technique	Pak choi	NR	NR	High	peat + perlite + organic fertilizer	China	(Liet al., 2018)
Nutrient film technique	Tomato	2 - 2.5	5.5 - 6.5	High	Rock wool	Switzerland	(Schmautz et al., 2016)

NR – Not Reported

Commercially Grown Crops Using Hydroponics

Virtually all crops can be practically grown under the hydroponic system. However, vegetables, fruits, and ornamental crops are mostly cultivated using these various techniques. Nevertheless, other plants can be cultivated under the system depending on the available resources. Different type of crops ranging from vegetables, fruits, fodders, cereals as well as medicinal plants has been successfully cultivated under the hydroponic system at indoor and commercial level. Table 2 shows a list of crops grown commercially.

Table 2 Some Economic Crops Cultivated Hydroponically as Obtainable from Different Literature.

Crop Classification	Crop(s) Name	Countries	Reference(s)
Cereals	Maize *(Zea mays),*	Italy	*(Trevisan et al., 2019)*
	Rice *(Oryza sativa)*	India	*(Lima et al., 2015)*
	Buckwheat (*Fagopyrum esculentum)*	Japan	*(Matsuura et al., 2005)*
	Millets *(Panicum miliaceum)*	Indonesia	*(Karjunita et al., 2019)*
	Sorghum *(Sorghum bicolor)*	China	*(Zou et al., 2019)*
Vegetables	Tomato *(Lycopersicon esculentum)*	Spain	*(Baghour et al., 2019)*
	Bell pepper *(Capsicum annum)*	Brazil	*(Lima et al., 2017)*
	Cucumbers *(Cucumis sativus)*	Iran	*(Azarmi et al., 2018)*
	Melons *(Cucumis melo)*	Indonesia	*(Christy et al., 2018)*
	Radish *(Raphanus sativus)*	China	*(Hong et al., 2018)*
Leafy Vegetable	Lettuce *(Lactuca sativa)*	China	*(Yang et al., 2019)*
	Spinach	Japan	*(Watanabe et al., 2018)*
Fruits	Strawberry *(Fragaria ananassa)*	Spain	*(Sánchez-Rodas et al., 2016)*
	Raspberries	Portugal	*(Ramos et al., 2019)*
Flower/ Ornamental crops	Carnations *(Dianthus caryophyllus)*	Turkey	*(Aydinsakir et al., 2011)*
	Sunflower *(Helianthus annuus L.)*	Brazil	*(Santos et al., 2016)*
Medicinal crops	Coleus *(Solenostemon scutellarioides)*	India	*(Rachappanavar et al., 2019)*
	Indian Aloe *(Aloe vera)*	India	*(Rachappanavar et al., 2019)*
Fodder crops	Alfalfa *(Medicago sativa L.)*	China	*(Cong et al., 2018)*
	Barley *(Hordeum vulgare L.)*	China	*(Wang et al., 2017)*
Leguminous crops	Lentil *(Lens culinaris)*	Pakistan	*(Aslam et al., 2017)*
	Cowpea *(Vigna unguiculata L.)*	Greece	*(Savvas et al., 2017)*
	Chickpea *(Cicer arietinum L.)*	Australia	*(Amalraj et al., 2019)*

Merits of Hydroponics Compared with Conventional Farming Methods

Growing plants under hydroponic systems exhibit many advantages over-cultivation under soil-based culture in several ways *(Savvas, 2003).* Hence there are several merits of the hydroponic system of growing crop over soil-grown crops which include:

It Enhances Productivity

It is obvious that nutrition control in plants grown under hydroponics is very accurate that mostly leads to high yields with good quality but that does not mean that yield from well-managed plants grown conventionally will produce less yield and inferior quality *(Stoughton, 1969).* Also, it is very understandable that when soil encountered some problems such as soil toxicities, high soil salinity, and acidity, in that case, hydroponics will produce crops with high yield and better quality. Several studies had been carried out with outcomes favoring hydroponics as the best in terms of yield and quality with little effort when compared with the conventional method (*Silberbush et al., 2001*). However, it was achievable majorly because growth conditions are more uniform and controllable but this is not controllable in most experiments carried out in the soil. Since the hydroponic system enhances plant growth by providing optimal conditions, hence higher yields are obtainable compared to the soil cultivated as illustrated in Figure 10

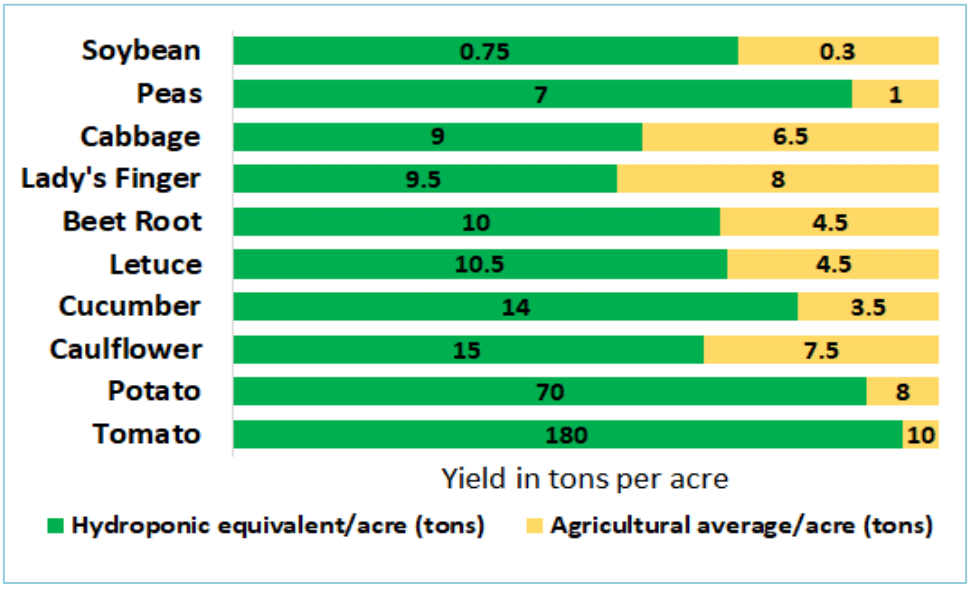


Figure 10. The comparison of yields of different crops under hydroponics and soil culture.

Plant Nutrition Control

The ability to control plant nutrition accurately is one of the advantages of hydroponics compared to soil-grown crops. This is achievable from different angles:

- The concentration control point of view where the applied concentration is regulated to suit different crops' need, different environmental factors, plant growth stages, etc. Also, toxic elements such as Mn, B, Z, Cu, Pb, etc. to plants when exceeding the normal limit can be maintained within concentrations considered safe enough.
- Also, the supply of required nutrient elements uniformly is a guarantee, knowing well which nutrients need to be supplied to the substrate. This is very peculiar to water culture techniques being very advanced in technology compared to aggregate cultures, particularly the drip irrigation system.
- The manager has absolute control over the number of nutrients to be provided when using either water cultures or aggregate cultures with a neutral substrate. Contrarily, this is not achievable under the soil cultures as nutrients could be found in excess within the soils which could lead to high salinity or acidity.
- pH and EC of nutrient solutions are very controllable under hydroponics grown plants to conform to the nutrient requirement of such crop coupled with environmental factors. However, to achieve the same feet in soil-grown crops might be practically impossible, and if otherwise, it could be highly expensive.

Water-Use Efficiency and Control

Water is one of the most essential resources for bountiful crop production. Cultivation under-protected system demands a quite large quantity of water for irrigation due to the lack of enough rainfall for crops good growth performance is needed especially in hot, arid, and semi-arid regions of the globe, water has been considered as a major limiting

factor to crop production in terms of availability (quantity), quality as well as cost.

The convenient irrigation in hydroponic is a great advantage most especially with some hydroponic systems such as NFT and another related system where there is direct contact of roots with the nutrient solution as well as sub-irrigated substrate culture. However, other systems that use organic and inorganic substrates may be deprived of this opportunity as most of the substrates exhibited low water holding capacity when compared to soil.

Considering water use efficiency, some hydroponic systems, particularly those operating under closed systems where nutrients solution is recirculated constantly, the water-saving is undisputedly high as drainage and evaporation is properly checked from the surface based on the operation method and designing of the system (NFT -"closed system").

Manpower Requirement Reduction

In hydroponics, the headache of normal cultural practices prevalent with soil-grown crops such as weeding, soil sterilization, mulching, etc. that requires intensive labour input is mitigated / drastically reduced. However, due to the degree of automation and the kind of substrate used, crop population per substrate have a great influence on the quantity of labour that will be required, the more automation the less labour requirement in term of quantity. In a null shell irrespective of the type of hydroponics system in use, there is still a considerable saving in terms of labour input used in general.

Cultivation All the Year-Round

The number of harvested crops annually is increased as a result of a lack of growing techniques, several operations such as soil cultivation, sterilization of soil, etc. as applicable in a chosen area of production since the interval in duration between crops is practically negligible.

Unproductive Soil

A good alternative is provided by hydroponics in terms of ideal crop to soil culture, particularly in the situation where the soil availability is practically zero, not fertile enough to support cultivation, water lodged soil with poor aeration soil, saline soil (with high salinity), the soil is considered toxic as a result of the accumulation of heavy metals (eg. lead, mercury, cadmium, etc) and high infestation of different soil pathogens resulted from accumulation in the soil.

Root System Environment Well Control

The beauty of accurately controlling the temperature around the root, root oxygen supply are essential factors that are relatively achievable with ease in hydroponic systems.

Setback Associated with Hydroponics System

High Capital Investment Cost

The initial cost of investment in a hydroponics system could be enormous as acquiring inputs, setup cost (construction), system maintenance when compared with conventional and primitive soil cultivation. The hydroponics system in use will determine the rate at which input increases and equally the level of automation in terms of accuracy in control measure adopted by any system, i.e. it is costlier to set up NFT compared to the other system such as rook wool system, but on the long run, the running cost per annum is considerably lower in NFT than in the rook wool system.

Depending on the types of used materials, the NFT system that comprises metal trays and is supported with stands is far costlier than the one in which corrugated asbestos sheets for the production of lettuce under the NFT system, etc. The different countries' economies play a vital role in determining the actual cost of materials as this may vary from country to country, for example, the cost of perlite is relatively cheap in Greece

but on contrary very expensive in the UK.

Technical Know-How Demands

The successful operation of the hydroponic system greatly depends on the fast knowledge and ability to learn the best way to cultivate various crops, physiology of the plant, basic elementary chemical processes, quick detection of disease symptoms as well as a good understanding of the control system, etc. Due to the sophistication of hydroponics, certainly, its operation is not quite easy. Also, with the support and assistance of technical experts, researchers, entrepreneurs dealing with installation and different accessories, government institutions should be sorted from time to time.

Crops cultivation under controlled volume demands a skillful management system. Rewarding commercial hydroponics requires prompt attention from professional staff with good management skills. Hence, the handlers must be versatile with deep knowledge and several skills to give him the edge to handle multiple tasks such as preparation and regulating the nutrient solution when needed, monitoring and control electro - computer devices, understanding plant physiology, ability to detect and control prevailing diseases.

More attention needs to be paid to nutrient solution composition and supply, pH, EC as a slight mistake could be detrimental to crops in hydroponics *(Leonhardt, 1914; Resh, 2016)*. Power and water supply are expected to be constant, any failure in supply could be injurious or total loss of the crops *(Jensen, 2013)*.

Disease Risk

Hydroponically grown crops are prone to disease infections if extra care is not taken. In the "open system," disease infection risk is considerably low because nutrient solution and water drain away freely living the crop rootless prone to disease attack *(Jensen, 1999)* However, in contrary, crops grown under a "closed system" are prone

to disease infections because excess drain continuously flows through the roots of the plant in a circular pattern, once there are any traces of disease causative pathogen in the system, the whole plants may suffer terrific infection attack *(Jensen, 2013).*

Nutritional Value of Phytochemicals Accumulation of Hydroponic Compared to Soil-Grown Crops

The conventional system of agriculture, soil health variation, and fluctuation in environmental factors are among the limitations in the soil cultivation systems. Such factors as water type, temperature variation as well as humidity are stressors that can affect crops by causing possible alteration in their phytochemical makeup irrespective of the cultivation method adopted. Due to these variabilities, several studies have been conducted to compare the nutritional contents of crop cultivated hydroponically to soil cultivated with various results, while some studies show a significant difference between the two methods, others show hydroponic systems is better than soil cultivated crops or vice versa and some show no distinctive differences between the two in the tested nutrient levels. However, it should be noted that the experimental outcomes could be greatly influenced by the experimental design as well as variability in various conditions hence affecting the outcome of the studies.

Hydroponically grown crops particularly vegetables have been established by several studies to produce high-quality products when compared to conventionally produced ones *(Maboko et al., 2008; Palermo et al., 2011).* According to *(Rouphael et al., 2005; Murphy et al., 2011; Treftz et al., 2015),* the conducted studies have shown no significant differences between hydroponic and soil cultivated vegetables in terms of their qualities. Summarily, the majority of the authors are of opinion that hydroponic systems of cultivation are one of the best options in the extreme environment or arid regions where fertile soil is not available or the available types may not support the healthy growth of the crop.

Nevertheless, despite the different and divergent views, the researchers tend to support the general opinion that a possibly hydroponic system can elevate the content of various phytochemical compounds in crops. The hydroponic systems have produced high-quality fresh vegetables showing higher nutritional value as a result of a high build-up of phytochemical compounds as recently established by different studies, as clearly indicated in the Table.3.

A study conducted by Premuzic et al. *(Premuzic Z, 1998)* discovered that hydroponically cultivated tomatoes have an incremental of antioxidants as well as macro-and micronutrients, compared to soil cultivated tomatoes. Also, in Achillea millefolium it was established that the build-up of flavonoid is higher in the hydroponically cultivated plants (0.43% dry weight) compared to the soil-based production 0.38% dry weight) *(Pedneault et al., 2002)*. In addition, flavonoids accumulation in the cultivated plants under hydroponic systems was found to exhibit tremendous improvement in the activity of the antioxidant of aqueous as well as lipids extracts, not only that, ascorbic acid, vitamin E, lipoic acid, total phenols, and rosmarinsic contents are increasing steadily. Furthermore, more findings in various studies revealed the accumulation of phytochemical compounds under the soilless culture method as shown in the Table 3

Table 3. Nutritional Value of Phytochemicals Accumulation of Hydroponic Compared to Soil-Grown Crops.

Crop(s)	**Phytochemicals**	**Hydroponically grown crop(s)**	**Soil grown crop(s)**	**Reference**
Tomato	Lycopene	No significant differences was observed (average content 36.15 µg)	No significant differences was observed (average content 36.25 µg)	*H. Simitchiev, 1983*
	Ascorbic acid	The ascorbic acid	The recorded was lesser	*Alan et al.*

Crop(s)	Phytochemicals	Hydroponically grown crop(s)\	Soil grown crop(s)	Reference
		value recorded was higher (13.3 mg/100 ml)	(0.693 g/100 ml)	*1993*
Onion	Total flavonoids	No significant difference observed	No significant difference observed	*Thompson et al. 2005*
Basil	Ascorbic acid Vitamin E Lipoic acid Total phenol Rosmarinic acid	All of these phytochemicals were more improved in content under the hydroponic	The contents of the phytochemicals were less improved	*Sgherri et al 2010*
	Alpha-tocopherol	The levels were higher	The levels were lower	*Buchanan et al 2013*
Lettuce	Lutein Beta-carotene Violaxanthin Neoxanthin	These were lesser as a result of less exposure to the sunlight	The exposure to sunlight is very high which contributed greatly to the higher deposition of these phytochemicals when compared to the hydroponic	*kimura 2003*
Red paprika	Carotenoids	Was higher in terms of dry weight (4.5 mg/100 g dry weight)	It was relatively lesser (2.81 mg / 100 g dry weight	*Ji-Sun Kim 2016*
	Capsanthin	Was higher in terms of dry weight (46.74 mg/100 g dry weight)	It was relatively lesser (29.57 mg / 100 g dry weight	
Strawberries	Ascorbic acid	The content was higher	The content was lesser	*Traftz et al 2015*
Raspberries		The content was higher	The content was lesser	*Traftz et al 2015*
Sweet potato	Ascorbic acid Carotene Thiamin Oxalic Tannic acids Chymotrypsin	The content of the phytochemicals was found to be relatively higher.	The content of the phytochemicals was found to be relatively lesser compared to hydroponically grown sweet potatoes.	S. Ajlouni, 2001

The Future Potential of Hydroponics in the Arabian Peninsula

The advanced technology of the hydroponic system is growing at an alarming rate in the agricultural sector across all the Arabia peninsula countries, and no doubt it has the potential to dominate future food production (Moustafa et al., 2011). The population keeps growing and the land availability declines because of the pressure exerted from the construction of infrastructures coupled with the uncultivable of the arid lands. Due to these facts, the best alternative is to employ new technologies such as hydroponics

to enhance food production (Ouled Belgacem, 2017). However, to better understand the hydroponic potential in the future is important to critically examine the earlier users of this technology (Sardare et al., 2013). A good example is the adoption of the technology in Tokyo, Japan where the availability of arable land is relatively scarce because of the high population but opted for the hydroponic system in rice production to feed the growing population and to preserve the valuable land (De Kreij et al., 2003). Also, the technology was successfully adopted in the food production in Israel that is characterized by a dry climate. The huge initial capital outlay of hydroponic systems will soon vanish as it is peculiar with most technologies when newly invented in the long run, thereby creating easy accessibility to the technology. Well-constructed and managed hydroponics has the potential to take care of crop production to feed the growing population of Arabian Peninsula countries that are characterized by impoverishing soil and water scarcity.

Cyanobacteria

Introduction

Cyanobacteria are blue-green algae that belong to the domain Bacteria. They are prokaryotic autotrophs organisms capable of manufacturing their food through photosynthesis. They are considered to be very essential ecologically and evolutionary. Their existence on earth has been noticeable as back as 3.5×10^9 years (Summons et al., 1999; Cavalier-Smith, 2006). Afterward, their evolution resulted in generic variation and morphological forms as well as inhabitation in almost ecosystems of our planet. However, without controversy, cyanobacteria are known to be the first microorganisms on earth for the invention of oxygenic photosynthesis, an important bioenergy process associated with different environments such as freshwater, marine, oceans, terrestrial soils, and bare rock (Whitton et al., 2012; Schopf, 2014; Schirrmeister et al., 2015; Zahra et al., 2020).

Cyanobacteria can be found in a different form, as individual cells, filamentous, or colonies. Despite these organisms being microscopic because of their sizes, it is still possible to view them as they thrived as colonies and a good example is as blooms or crusts (Catherine et al., 2013). These organisms are characterized by their easy adaptability to the persistent change in the environment and ability to grow fast at a reasonable speed to a dense population because they possess cellular mechanisms to support this situation (Zahra et al., 2020). Nevertheless, different factors/parameters such as biotic factors, nutrient level variation, and global warming play a vital role in enhancing the fast growth rate (Paerl et al., 2012; Sukenik et al., 2012). Globally, cyanobacteria have gained recognition and acceptance as useful resources in the field of science because of their several characteristics, thus researchers and scientists are carrying out several studies on it to optimize its usefulness in different areas of human endeavor. For instance, cyanobacteria played significant roles in the formation of an important part of the lithosphere because of calcium Fcarbonate deposition in the stromatolites form (Altermann et al., 2006). Also, during oxygenic photosynthesis, cyanobacteria have the potential of generating molecular oxygen as waste or byproducts. In addition, due to this uniqueness in characteristics, they can be successfully grown in almost every kind of water resource viz marine water, brackish water, freshwater, and industrial wastewater because of their adaptability potential (Costa et al., 2018).

Growth Parameters Required for Cyanobacteria Production

The production of cyanobacteria follows the same pattern as microalgae which greatly influemnced by different cultivation conditions. Thus, the following favorable conditions must be present for a successful cultivation of cyanobacteria which include: energy sources, carbon sources, suitability of the reactors, cost involved, taking note of

the issues that are related to the large-scale application, biomass productivity ($g \cdot L^{-1}$), selection of highly productive cyanobacteria species, coupled with its chemical constituents as well as luminous intensity (Grewe et al., 2012; Hossain et al., 2019). The different species responded to each parameter differently, so it is vital to determine their specific optimal growth parameters (Lavens et al., 1996; Grewe et al., 2012; Richmond et al., 2013). However, the photosynthetic activities, cell biomass production, pathway, pattern, and cellular metabolism activities are greatly influenced by the environmental conditions/parameters, such as optimal temperature (25 – 30 °C), sunshine intensity, air temperature during the day, and photoperiod, as well as the pH – 7.5 (Grewe et al., 2012; FAO, 2013; Richmond et al., 2013; Hossain et al., 2019). Generally, most cyanobacteria and microalgae grow under various conditions of light that exhibit a different range from dark (heterotrophic) to light/luminous conditions (phototrophic or mixotrophic). They thrive in saline water (seawater) and brackish water, as well as freshwater. Aside from these factors, other parameters such as the availability of nutrients and aeration are essential for healthy and optimal growth (Enzing et al., 2014). The general parameters for the optimal cultivation of these are organisms (FAO, 2013).

The Biochemical Constituent of Cyanobacteria

Cyanobacteria biomass contains three main biochemical constituents, namely carbohydrates, proteins, and lipids (Garlapati et al., 2019) is tabulated in Table 4.

Table 4. Protein, Carbohydrate, and Lipid Constituents of Selected Cyanobacteria.

Cyanobacteria	Carbohydrate (%)	Lipid (%)	Protein (%)	References
Spirulina platensis	8–20	4– 9	49-65	(Roy et al., 2015; Zabed et al., 2020)
Synechococcus sp.	9–17	14–55	10–63	(Demirbas et al., 2011; Roy et al., 2015)
Anabaena species	25–30	9–14	24–29	(Demirbas et al., 2011; Satyanarayana et al., 2011;

Cyanobacteria	Carbohydrate (%)	Lipid (%)	Protein (%)	References
				Zabed et al., 2020)
Spirulina maxima	13–13	6–7	60–71	(Roy et al., 2015; Zabed et al., 2020)
Arthrospira sp.	15–25	6–8	58–73	(Schreckenbach et al., 2001; Garlapati et al., 2019)
Nostoc sp.	56–57	5–6	10–23	(Danxiang et al., 2004; Garlapati et al., 2019)
Aphanizomenon flos-aquae	20–30	2–8	60–75	(Capelli et al., 2010; Garlapati et al., 2019)

There is variation in the percentage of different constituents among the various cyanobacteria even among the same species as found in microalgae species (Zabed et al., 2020). Considering the highly beneficial potential of fatty acids, they are a good basic material in the production of bio-diesel. However, recently it was discovered that the benefits of cyanobacteria are far greater than being a good raw material in the production of bioenergy (Garlapati et al., 2019; Zahra et al., 2020). Furthermore, aside from the biochemical composition, cyanobacteria like microalgae contain different molecules viz. amino acid compounds, pigments (e.g., chlorophylls, carotenoids, and anthocyanin), vitamins, hormones, and secondary metabolites which are valuable products that have good potential as raw material in the cosmetic, food, and pharmaceutical industries (Chew et al., 2017; Show et al., 2017; Petruk et al., 2018; Garlapati et al., 2019; Zahra et al., 2020). However, three cyanobacterial genera namely *Arthrospira* sp., *Nostoc* sp., and *Aphanizomenon flos-aquae* respectively are known to be most economically relevant in the application as the different product (Grewe et al., 2012). Own to this fact they have been put into large-scale production of cyanobacterial biomass with production in increasing order from *Nostoc* sp. to *Aphanizomenon flos-aquae* to the highest *Arthrospira* sp.

Cyanobacteria Production Schemes

Cyanobacteria is one of the diverse group of microalgae that are prokaryotic and considered as one of the best organisms for recombinant protein production, and are

suitable for fine chemical production, pharmaceutical products, animal (e.g., poultry) feeds, feedstock, and essential basic material for the production of biofuels (biodiesels, bioethanol, hydrogen, as well as methane (CH_4)). The cultivation of this organism is relatively simple, with important growth conditions such as free water (blackish, fresh, sea), cheap nitrogen (N), and phosphorus (P), with required light intensity to enhance the rate of growth (Grewe et al., 2012; Enzing et al., 2014; Dasan et al., 2019; Zahra et al., 2020). Cyanobacteria and microalgae are regarded as a potential feedstock for both feed and food production (Zahra et al., 2020). Nevertheless, technology has yet to be fully developed to overcome the bottleneck for their optimal production. The cultivation using human-made open ponds is technologically easy but is not considered to be cheap because of the enormous processing cost required (Yaakob et al., 2021). However, obtaining higher productivity and limiting production to monocultures resulted in the invention of enclosed tubular and flat-plate photobioreactors (Grewe et al., 2012; Enzing et al., 2014; Garlapati et al., 2019).

The higher biomass production and adequate regulation of culture factors under this system have not proved to be better than open pond cultures in terms of volumetric productivity or purity of biomass; however, the installation and operation cost of these systems is considered higher compared to open pond systems (Grewe et al., 2012; Enzing et al., 2014; Godlewska et al., 2019; Yaakob et al., 2021), along with the production cost. Unlike open pond cultures, photobioreactors are further hindered by the technical problems in decontaminating or purifying their components—therefore, their application is minimized in the production of high-value products such as pharmaceutical products (Lee, 2001). Additionally, solar light availability is another common limitation, especially when the phototrophic culture method is used (Lee, 2001; Enzing et al., 2014; Moreno-Garcia et al., 2017). Recently, research, workshops,

and training have been carried out and are still ongoing to develop optimum productive methodologies in the systems of production (Grewe et al., 2012; Enzing et al., 2014; Garlapati et al., 2019). It is very necessary to develop a more reliable and ecofriendly technology to boost the production level, putting into cognizance the factors of production. The range of bioprocesses will enhance large-scale production after the careful selection of cyanobacteria species. When the production targets are produced on a commercial or industrial scale, there are critical factors that need to be considered when developing the appropriate cyanobacteria and microalgae culture system. The factors include but are not limited to, a high productivity area, high volumetric productivity, cost feasibility, and ability to control the environmental factors (temperature, carbon dioxide, turbidity, and pH), low energy demand, and sustainability (Olaizola, 2003; Grewe et al., 2012; Junying et al., 2013; Garlapati et al., 2019).

In this regard, cyanobacteria like microalgae cultivation are mostly associated with different systems of cultivation, ranging from outdoors to indoors. Practically, the dominant cultivation systems include the open raceway or racetrack ponds and closed bioreactors (Grewe et al., 2012). These systems are operated often on a large and commercial scale, as enumerated graphically in Figure 11. Generally, cyanobacteria are found to be better than algae and plants in terms of photosynthesis efficiency.

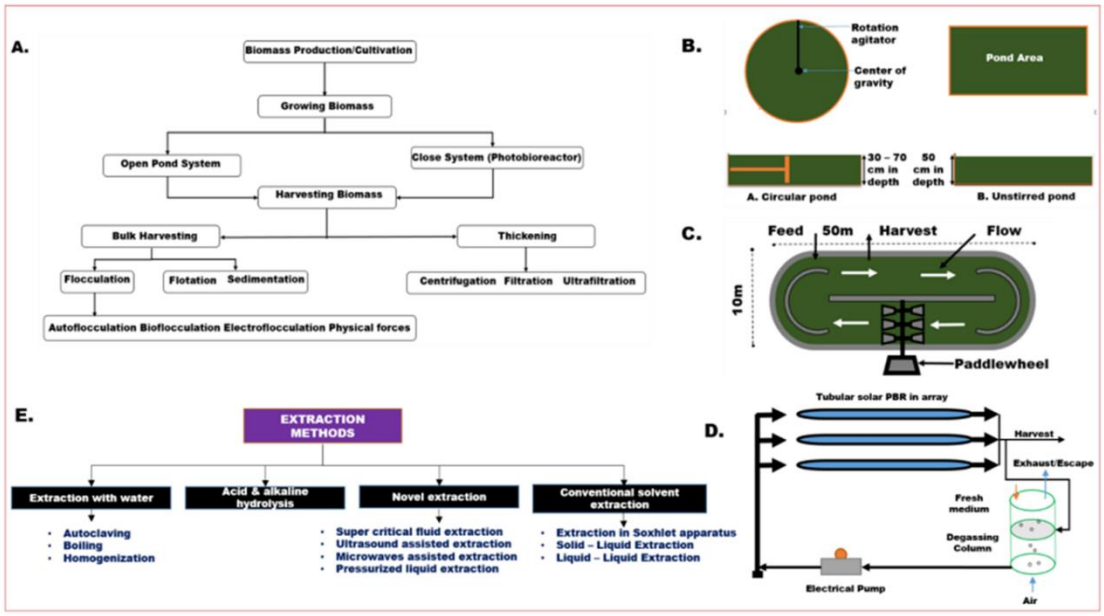


Figure 11. (A) Schematic of cyanobacteria biomass production/cultivation; (B) types of open pond systems; (C) schematic raceway pond for cyanobacteria cultivation; (D) schematic of solar-powered tubular PBR; (E) several techniques of production of cyanobacteria extracts

Open Pond Systems

Ponds are regarded as the most common open system for the industrial-scale production of cyanobacteria and microalgae (Figure 11B). Furthermore, the depth of such ponds, which might come in different shapes, generally does not exceed 35cm (Grewe et al., 2012). The nutrient and water circulation within the ponds is performed mechanically using an arm that rotates in a clockwise direction, which is particular to circular ponds, while a paddle is often used for stirring in other types of pond, e.g., raceway (Carlsson et al., 2007; Grewe et al., 2012; Enzing et al., 2014; Costa et al., 2019). There are two categories of this system, namely unstirred and circular ponds (Figure 11B). The unstirred open system is particular to a natural water source, and as a system, it lacks a stirred point. The cost is relatively cheap on the commercial scale, but mixing is very

poor, which might lead to lower output in the long run. Plastic films can be used to cover the surface water to regulate temperature, as reported in previous studies (Vonshak et al., 1988; Razzak et al., 2013; Costa et al., 2019). In the case of circular ponds, they are predominantly employed in culturing the genera *Chlorella, Spirulina, and Dunaliella* in most countries in Asia (Lee, 2001; Costa et al., 2019; Qin et al., 2019). Unlike unstirred ponds, this type of pond has a long arm that rotates clockwise for proper mixing; the function of this arm is similar to that of the paddlewheels in the raceway pond. The output can range from 8.5 to 21 gm-2d-1 (Benemann et al., 1994; Singh et al., 2011). However, there are a few limitations associated with these systems; the controlling of temperature is almost impossible, which necessitates the need for an alternative source of heat supply. Additionally, predators, parasitic algae, as well as other strains with high viability can invade the pond, thereby dominating the wanted or needed species (Enzing et al., 2014).

Racetrack System

The racetrack cultivation system is the most commonly adopted type of open system being used extensively and commercialized to produce cyanobacteria and microalgae on a large scale (Figure 11C) because it is easy to construct (Grewe et al., 2012; Enzing et al., 2014). Some cyanobacteria and microalgae species showed high productivities using this system, such as *Chlorella* species, *Dunaliella species*, *Haematococ-cus pluvialis*, *Arthrospira platensis*. Usually, the racetrack pond is constructed with various dimensions in terms of breadth and length, but with a depth of approximately 15 to 50 cm, and comprises either a single channel or a collection of channels. However, the ratio of the length to breadth is an important factor, as an extensive width may cause redundancy in the current speed, while an extensive length will cause the usage of a large land area (Ting et al., 2017; Chew et al., 2018). However, paddlewheels are

among the most important parts of raceway ponds, being essential for the controlling of liquid flow, meaning that the mixing of algae cells is homogenized and maximized to avoid unnecessary sedimentation in the configuration. The advantages of this configuration over other open types are that the entire production activities are very effective and easy, which makes it the first choice in large-scale commercial production using the outdoor type of production. In this system, the biomass outputs can be as much as 60-100 mg $L^{-1}d^{-1}$ (Tredici, 2004).

Closed System (Photobioreactor)

The photobioreactor (PBRs) is a closed system that prevents the enclosed microalgae from coming in contact with the prevailing environment (Fig. 1D). The PBRs can be found outdoor sometimes; however, usually, they are located in the greenhouse, where the environmental factors can be regulated to maximize production (Pulz, 2001; Chisti, 2007; Grewe et al., 2012; Singh et al., 2012; Qin et al., 2019). The advancement in PBRs recently has led to the mass production of cyanobacteria and microalgae, and this is necessary because they must be produced free from any form of pollutant, including toxic metals and pathogenic microorganisms. This is important to meet the suitability requirements for the production of valuable products used as raw materials in the agricultural feed, pharmaceutical, and cosmetic industries (Janssen et al., 2003; Tredici, 2004; Grewe et al., 2012; Singh et al., 2012). The rate of evaporation is low, and CO_2 emissions to the atmosphere are much lower with PBRs. There are different configurations associated with this system according to (Brennan et al., 2010; Singh et al., 2012; Vasumathi et al., 2012; Wang et al., 2012), such as (i) vertical column reactors (bubble columns or airlift); (ii) tubular reactors; and (iii) flat-plate reactors. Different studies described the efficacy of PBRs in the large-scale cultivation of cyanobacteria and microalgae at an optimal level (Carlsson et al., 2007; Brennan et al., 2010;

Aishvarya et al., 2012). However, the different types of PBR systems were compared considering the essential factors that influenced the level of biomass productivity, as shown in Table 5.

Table 5. The Comparison of Characteristics of the Operating System under the Open and Close Method.

Characteristics	Open system (Raceway)	Close system (Photobioreactor)			Reference(s)
	Paddlewheel	Stirred tank reactor	Tubular reactor	Column reactor	
Light use efficiency	Good	Good	Best	Good	(Singh et al., 2012; Slegers et al., 2013)
Transfer of gas	Normal	Lower - higher	Lower - higher	Higher	(Harun et al., 2010; Mata et al., 2010)
Mixing potential	Partial uniformity	Nearly uniformity	Perfect/absolute mixing	Partial mixing	(Barbosa et al., 2003; Chisti, 2007)
Control of species	Nil	Best	Good	Good	(Singh et al., 2012; Pires et al., 2017)
Loss through evaporation	High	Moderate	Nil	Nil	(Eriksen, 2008; Slegers et al., 2013)
Quality of biomass	Variable	Reproducible	Reproducible	Reproducible	(Chisti, 2007; Brennan et al., 2010)
Energy demand for mixing	Low	High	High	High	(Das et al., 2011; Suali et al., 2012; Pires et al., 2017)
Maintenance	Easy	Difficult	Difficult	Difficult	(Harun et al., 2010; Suali et al., 2012; Pires et al., 2017)
Required space	Large area	Moderate	Moderate	Moderate	(Barbosa et al., 2003; Chisti, 2007; Brennan et al., 2010)
Type of operation	Batch	Batch	Batch	Batch	(Chisti, 2007; Eriksen, 2008; Suali et al., 2012)
Setup capital	Low	High	High	High	(Suali et al., 2012; Pires et al., 2017)

Characteristics	Open system (Raceway)	Close system (Photobioreactor)			Reference(s)
	Paddlewheel	Stirred tank reactor	Tubular reactor	Column reactor	
Limitations	Requires a huge area of land	Requires large setup capital.	Possible formation of fouling/scale along the bend regions.	High maintenance cost.	(Das et al., 2011; Suali et al., 2012; Pires et al., 2017)

Cyanobacteria and Microalgae-Derived Extracts (Bioactive Compound and High-Value Product)

The derived extracts (bioactive and high-value products) from cyanobacteria and microalgae are categorized based on their physicochemical properties and their bioactivities (e.g., antifungal, antibacterial, antiviral, anti-inflammatory, etc.). The extraction method and the nature of the solvent's influence are strictly dependent on the nature and the quality of the bioactive molecule, presenting an impact on its associated application. Cyanobacteria have several beneficial properties aside from being a source of biogas (Bayona-Morcillo et al., 2020; Zahra et al., 2020). They have gained wide acceptance for agricultural applications because of their embedded bioactive compounds that enhance plant productivity. Such bioactive compounds include carbohydrates, minerals, and trace elements, growth hormones (cytokinins, auxins, and auxin-like compounds), betaines, and sterols (Khan et al., 2009; Michalak et al., 2016; Bayona-Morcillo et al., 2020). Additionally, they are widely gaining global acceptance as a raw material in the production of animal feed additives, cosmetics, pharmaceuticals, biofuels, plant growth promoters, and medicines, and for mitigating abiotic stress and preventing pollution (Spolaore et al., 2006; Mata et al., 2010; Gupta et al., 2011a, 2011b; Priyadarshani et al., 2012; Garlapati et al., 2019; Bayona-Morcillo et al., 2020; Lafarga, 2020; Subashini et al., 2020; Zahra et al., 2020).

Extraction Methods of Cyanobacteria and Microalgae Extract

The different methods often used in the cyanobacteria and microalgae extract were explained extensively in several studies and the literature (Samarasinghe et al., 2012; Michalak et al., 2016; Garlapati et al., 2019). The important first step in the extraction is the rupture of a cell by wall extraction methods to release the bioactive substances (Samarasinghe et al., 2012; Chiaiese et al., 2018; Garlapati et al., 2019).

Extraction with water is a mechanical or physical method using such techniques as autoclaving, boiling, and homogenization to disrupt the cell wall of the organism as a pretreatment to release the bioactive compounds in the liquid medium. These types of techniques are considered to be among the traditional, less expensive methods, but require more energy. Acid and alkaline hydrolysis is a chemical method that uses different types of chemicals to disrupt cyanobacteria cell walls. The most prominent chemicals in use are sodium hydroxide (NaOH), hydrochloric acid (HCl), hydrogen tetraoxosulphate (VI) acid (H_2SO_4), nitrous acid (HNO_2), On the other hand, conventional solvent extraction is considered a traditional method that operates in three different mediums viz. the Soxhlet apparatus, the solid-liquid, and the liquid-liquid extraction method. However, hydrophobic solvents such as petroleum ether, aromatic compounds, hexane, cyclohexane, chloroform, acetone, dichloromethane, and alcohols, e.g., ethanol, methanol, etc., have been the most commonly used solvents in most outstanding extraction methods (Michalak et al., 2014, 2015). Nevertheless, the Soxhlet apparatus has taken over as the most reliable method and is often used in extraction processes because of its advantages of easy operation, safety, and scale-up being possible at all times (Ramluckan et al., 2014). Using solvent methods of extraction in the extraction of the bioactive compounds requires the use of greater volumes of solvents, consumed in longer extraction processes. Not only that, but the output is also

considerably low. However, the existing novel extraction techniques (NET) viz. supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), and pressurized liquid extraction (PLE) provide an improvement over the other extraction methods and as a better substitute because of their various advantages. NET is more efficient, less time-consuming, cost-efficient, and environmentally safe (Grewe et al., 2012; Ibañez et al., 2012; Kadam et al., 2013).

Chemical Constituents of Cyanobacteria and Microalgae Extracts

Cyanobacteria and microalgae extract can generate a reasonable quantity of biologically active primary and secondary metabolites. In the work of Puglisi et al., 2018, it was reported that the extraction methods or techniques applied as well as the species used in the extraction process may have a great influence on the output and the quality of bioactive compounds obtainable from the extract (Puglisi et al., 2018). The primary bioactive metabolites are made up of carbohydrates, proteins, lipids, and vitamins. However, the quantities of these important biochemical components significantly vary among the cyanobacteria and microalga species as well as within the same species. In particular, these variations are likely to be connected with the impacts of numerous biotic and abiotic parameters viz. cultivation under favorable as well as optimal conditions, seasonal variability, nutrient availability, and so forth. Carbohydrates constitute the most essential component of cyanobacteria and microalgae extract. The composition of carbohydrates ranges between 3% and 40% or more. In the common species, the composition of carbohydrates found is as follows, in *Chlorella* spp. (9.42–15.50%), *Chlamydomonas* sp. (3.28%), *Dunaliell*a sp. (21.69%) and *Arthrospira* sp. (12–30.21%), *Cladophora glomerata* (34.70%), *Schizochytrium limacinum* (24%) (Shuping et al., 2010; Maddi et al., 2011; Anand et al., 2017; Li et

al., 2017; Andrade et al., 2018; Yang et al., 2019). Meanwhile, lipids may account for as much as 50% of the dry weight (DW) of the cyanobacteria and microalgae extract, as found in *Chlorella* spp. 2.5%, *Chlamydomonas* spp. (12.19%), *Dunaliella* sp. (2.87%) and *Arthrospira* sp. (10.30%), *Cladophora glomerata* (5.80%), *Schizochytrium limacinum* (51.00%). Furthermore, proteins constitute approximately 18–46 % (DW) or even higher in some cases in various species of cyanobacteria and microalgae-generated extract (Becker, 2013). Among the various amino acid classes, tryptophan present in the extracts, as well as arginine, exhibited significantly high potential enhancement characteristics on the cultivated plant growth, development, and output, as these two amino acids play vital roles, being antecedent to essential signal molecules known mostly as secretory and non-secretory peptides (Colla et al., 2013; Colla et al., 2014; Colla et al., 2015). Among the amino acids, tryptophan plays a key role in plant metabolism activities, as it is responsible for protein formation, and is the forbearer of plant hormones viz. auxin, gibberellin, salicylic, as well as arene secondary compounds that have different biological functions (Colla et al., 2015; Chiaiese et al., 2018; Bayona-Morcillo et al., 2020).

Cyanobacteria and Microalgae Extract as Biostimulant and Biofertilizer

Cyanobacteria and microalgae extracts are derivative products with beneficial potential in modern agriculture, ranging from nutrient uptake enhancement to crop efficiency improvement, nutrient loss prevention, physiological status improvement, and abiotic stress alleviator (Coppens et al., 2016; Renuka et al., 2018; Rocha et al., 2020). Furthermore, cyanobacteria and microalgae's potential has not been fully exploited by plant scientists in the field of agronomy and crop science, despite their ability to produce biologically active substances that have enhancement properties on crop production (de Morais et al., 2015; Borowitzka, 2016; Coppens et al., 2016; Rocha et

al., 2020; Zahra et al., 2020). Experimental studies were conducted to test the impact of cyanobacteria and microalgae extracts as a biostimulant and biofertilizer under different cultivation conditions viz. open field, greenhouse, and hydroponics on different crops such as cereals, vegetables, medicinal crops, etc., exhibiting positive impacts. They displayed the ability to sustain agricultural productivity and minimize environmental degradation (Paudel et al., 2012; Shalaby et al., 2014; Tarraf et al., 2015; Garcia-Gonzalez et al., 2016; Bayona-Morcillo et al., 2020; Lee et al., 2020; Zahra et al., 2020).

As of late, exploratory investigations testing the activity of cyanobacteria and microalgae extracts under open-field cultivation, growth chamber, and greenhouse conditions have exhibited their potential to invigorate germination and the development of seedlings, shoots, and root systems in vegetable and cereals, etc. (Garcia-Gonzalez et al., 2016; Zhang et al., 2017; Arroussi et al., 2018; Zahra et al., 2020), as shown in Table 6. Such crops include, but are not limited to, radish, cabbage, lettuce, red amaranth, pack Choi, tomato, pepper, wheat, and rice (Faheed et al., 2008; Zhang et al., 2017; Arroussi et al., 2018; Barone et al., 2018). Table 6 revealed several studies testing the morphological and molecular responses resulting from the application of microalgae extracts from various species on different crops such as lettuce, tomato, pepper (*Capsicum annuum* L.), pack Choi, red amaranth, and other crops.

Lettuce (*Lactuca sativa* L.) grown in the soil inside a greenhouse was fertilized twice using a fresh and dried extract of *Chlorella vulgaris*. Doses of 0.5, 1, 2, and 3 g fresh and dried algal cells were applied per 1 kg of soil. The factors (agronomic and physiological responses) measured, including chlorophyll a, b, carotenoids, and growth factors (root dry weight and length), displayed positive results compared with the control at the various doses. The most significant results were obtained at the higher

treatments of 2 and 3 g of dry biomass per 1 kg of soil, respectively (Faheed et al., 2008). In a similar study, extracts from *Chlorella vulgaris* and *Scenedesmus quadricanda* were applied to sugar beet (*Betavulgaris* L. sp. *vulgaris*) to investigate its morphological and molecular responses to different treatments. Sugar beet seedlings were cultivated hydroponically using Hoagland solution in a regulated environment. The application of extracts from *Chlorella vulgaris* and *Scenedesmus quadricanda* were applied at two different doses of 2 and 4 mL L^{-1} after five days (Barone et al., 2018). After 36 h, the morphological response was positive, as the treated seedlings displayed greater root length, root surface, and the number of root tips when compared with the control. The molecular analysis revealed the upregulation of some genes related to biological pathways and activities, with primary and secondary metabolism and nutrient movement within the cells, particularly relating to root traits that have to do with nutrient absorption (Barone et al., 2018).

In addition, Garcia-Gonzalez et al., 2016 studied the effect of *Acutodesmus dimorphus* aqueous cell extract as a biofertilizer on tomato (*Solanum lycopersicum* L.) under greenhouse conditions using Petri dishes. The treatments were carried out as seed primer and foliar applications at various concentrations (0, 0.75, 1.875, 3.75, 5.625, and 7.5 g mL^{-1}) of aqueous cell extracts. The treated seeds exhibited a higher germination rate, significant plant growth, and floral production compared to the negative control (Garcia-Gonzalez et al., 2016). In the study conducted by Shariatmadari et al., 2013, the effect of *Anabaena vaginicola* ISC90 and *Nostoc calcicola* ISC89 extracts in potted plants under greenhouse conditions was tested to investigate their effects on the morphological parameters of vegetable crops viz. *Cucurbita maxima Duch*. ex *Lam.* (Squash: UG 5206 F1), *Cucumis sativus* L. (Cucumber: E 32.15720 F1), and *Solanum lycopersicum* L. (Tomato: E 26.32365 F1). Spraying the extract on the soil at 7-day

intervals with extract of *Anabaena vaginicola* ISC90 and *Nostoc calcicola* ISC89 enhanced the plant height, root length, dry weight, fresh weight, and the number of leaves for tomato after 40 days of experiments (Shariatmadari et al., 2013). Dmytryk, 2014 studied the effect of *Arthrospira plantensis* extract treatments on wheat seeds in Petri plates at different concentrations. The seeds were coated with three doses (8, 14, and 20 µl/1g of seeds, respectively) and were compared with untreated control. The treated and control seeds were grown in a cotton base in nine replicates of each sample for 11 days. The seeds coated with the extract exhibited an increase in biomass yield of nearly 13% compared to the untreated seeds. However, the seeds coated with 8.0 µL/1g gave the best results (Dmytryk et al., 2014).

The study conducted by Michalak et al., 2016 on the field trial of the effect of fluid extraction and whole biomass of *Spirulina plantensis* on wheat showed a positive response. It was found that the number of grains per ear and shank length was highest compared to the control group at a dose of 1.5 L/ha (Michalak et al., 2016). In a similar study, Mahmoud A. Saman, 2015 reported that the application of *Laurencia obtuse* and *Corallina* elongate powder (biomass) enhanced the antioxidant and phytochemical constituents of maize (Zea mays. L) (Al-Saman et al., 2015). There was a tremendous improvement in the root, polyphenolic, and antioxidant contents. With the application of Janiarubens (3 g powder/kg soil), the nitrogen content and protein content of the whole plant increased by 129.2%, while the application of *Coralline* elongates at the same dose gave the best results in increasing the polyphenolic and antioxidant contents of the shoot, as well as the tannic acid content of the root (Al-Saman et al., 2015). El-Eslamboly et al., 2019 recorded the extracts of *Spirulina plantensis* and *Amphora cofeaeformis* as being valuable applications, as they boosted/enhanced vegetative growth, yield, fruit quality, and nematode control in cucumber. There was a 2.5 and

2.69 double increment in marketable output compared with the control group when treated with *Amphora cofeaeformis* (El-Eslamboly et al., 2019). Additionally, Figure 12 shows the importance of the final products from the extraction process, as they enhance nutrient intake improvement, increase the quality of the product, and improve abiotic stresses tolerance.

Table 6. The Morphological and Molecular Responses Resulting from the Application of Cyanobacteria/Microalgae in High-Value Products and Whole Biomass from Various Species

Crop	Green house	Species	Extraction/process method	Conc. of Extract	Parameters	Reference
Lettuce	Soil	Chlorella Vulgaris	Fresh and dried algal were applied in the field to vegetables	Biofertilizer —1/2, 1, 2 and 3 g of fresh algal and dry algal cells / 1kg soil Biomass	Chlorophyll a, b and carotenoids. Plant growth (root dry wt. and length)	(Faheed et al., 2008)
Tomato	Petri plates	Acutodesmus dimorphus	1 kg of biomass freeze dried submerged in distilled water, DW (Conc. 150 g L-1) = the suspension + micro fluidizer (M-110EH-30) = intracellular extract. Intracellular extract + centrifugation (8989 x g / 10 mins / 22 ℃). The collected supernatant in a flask covered with foil paper to reduce potential degradation was stored at 4 ℃	Seed primers— different concs. (0, 1, 5, 10, 25, 50, 75, and 100 %) of aqueous cell extracts from DW OR 10 mL, 0.1/9.9 mL, 0.5/9.5 mL, 1/9 mL, 2.5/7.5 mL, 5/7.5 mL, 7.5/2.5 mL, 10 mL	Seed germination, germination energy, lateral root development, flower development	(Garcia-Gonzalez et al., 2016)

Crop	Green house	Species	Extraction/process method	Conc. of Extract	Parameters	Reference
3 types of vegetable — Chinese Cabbage, Chinese broccoli, and Protea White Crown.	Tissue towel	Spirulina platensis	A desirable quantity of microalgae suspension (50 mL) was removed from growing flasks and then allowed to pass through centrifugation for a maximum of 10 minutes. The collected supernatants were examined to determine the level of ammonia, nitrate, and nitrite	Biofertilizer—seed germination study—Spirulina biomass. T1 to T5, T0 (tap water only). (2, 4, 6, 8 and 10 g/L, respectively) biomass	Rate of germination, root and shoot length, vigor index as well as dry weight of 100 seedlings	(Wuang et al., 2016)
Arugula, Bayam Red, and Pak Choy plants	Potted plants experiment	Spirulina platensis	A desirable quantity of microalgae suspension (50 mL) was removed from growing flasks. Then, it was allowed to pass through centrifugation for a maximum of 10 minutes. The collected supernatants were examined to determine the level of ammonia, nitrate, and nitrite	Biofertilizer—potted plants and control—Spirulina platensis (5g / 500 g soil), inorganic fertilizer—Triple Pro 15/15/15 (3 x 10-1 g / 500 g soil / week). Additionally, Spirulina platensis + inorganic fertilizer (3 x 10-1 g / pot / week) biomass	Weekly measurement of plant growth (plant height and number of leaf per plant). After the completion of the experiment, parameters such as the number of leaves, the height of the plant, chlorophyll content, length of root, fresh as well dry weights were determined.	(Wuang et al., 2016)
Tomato	Potted plants experiment	Anabaena vaginicola ISC90 and Nostoc calcicola ISC89	Harvested biomass—DW was used to wash the cells. The cell extraction was carried out by grinding algae with a pestle and blender in DW. The final extract made up of 5.0 g fresh algae as the raw material submerged in 500 mL of DW is assumed to be a 1% extract	The final extract application was conducted by spraying the potted treated soil while the control was irrigated with water every 7 days. The arrangement of pots was a complete randomized design in a fully controlled	The morphological parameters measured after 40 days of the experiment include plant height, root length, dry and fresh weight of plant as well as the number of leaves	(Shariatmadari et al., 2013)

Crop	Green house	Species	Extraction/process method	Conc. of Extract	Parameters	Reference
				experimental greenhouse. 1% extract / spray		
Radish	Petri plates	BGA - Spirulina platensis extract	Commercial dried biomass of SP used. Homogenate + centrifugation = supernatant considered to be 100% algal filtrate (1:10)	Foliar spray (5, 7, 10, 15, 20 and 25%, v/v). Seed soaking—dose of 100, 300, 500, 700 µL per 1.5 g of seed	The longest and heaviest plant was observed at a dose of 300 µL / 1.5 g seeds and 15 % of filtrate as a foliar application. The chlorophyll content was higher at 100 µL / 1.5 g seeds as well as 5 % of filtrate as a foliar application.	(Godlewska et al., 2019)
Rice	Potted plants experiment	BGA—Spirulina maxima extract	Extracts obtained from three types of solvent viz. DW, methanol, and hexane at 0, 2.5, 3.5, 4.5, and 5 g L^{-1} of biomass/solvent	The potted plants were treated with extracts at three different stages of seed development, the dry stage, the radicle emergence stage, and the vegetative growth stage	DW, methanol, and extracts affect the germination of seed while hexane reveals no impact on seed germination.	(Sornchai et al., 2014)
Wheat seeds	Petri plates	BGA—Spirulina platensis extract	The seeds treated with extract were sown in a cotton base for the next 11 days, with nine replicates of each sample.	The coated seeds in three different doses (8, 14, and 20 µL per 1 g of seeds) of formulation were used. Seed coated with 8 µL gave the best result	Seeds coated with the extract resulted in an increase in biomass yield by approx.. 13%	(Dmytryk et al., 2014)

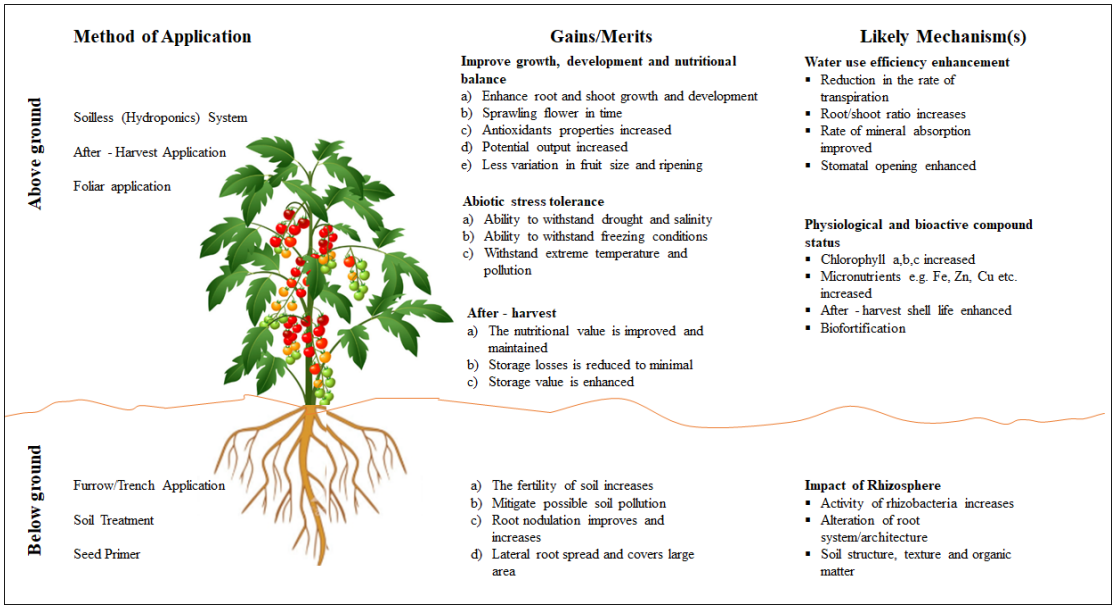


Figure 12. Illustrative diagram showing various application methods of cyanobacteria/microalgae extract, and their impacts and respective mechanisms of operation on the plant.

Application Methods of Cyanobacteria and Microalgae Extracts.

There are several methods by which cyanobacteria and microalgae extracts (high-value products) are applied to crops, either as biostimulants or as biofertilizers, which are understudied. Such methods include, but are not limited to, foliar spray or application, which entails direct fertilization through a plant's leaves, contrary to applying through the soil. Additionally, soil fertilization is the most common method of applying fertilizers/nutrients to plants through the soil to improve the soil's fertility, thus enhancing the growth performance of such plants, while the hydroponics system is a method of applying nutrients in the form of fertilizer to the crop without soil. Cyanobacteria and microalgae extracts are used extensively as bionutrient products on agronomic, ornamental, and horticultural crops, existing in two forms, namely in liquid/aqueous form or in liquid-soluble powder form (Bhalamurugan et al., 2018; Godlewska et al., 2019). The extracts could be applied in powder form as biomass for soil amendment. In another way, the liquid extract is sometimes applied directly to the targeted root system of the plant, as the mixture is prepared by thoroughly mixing the required dose of the extract into irrigation water using different types of irrigation system, e.g., a drip system to crops (Chiaiese et al., 2018). Cyanobacteria and microalgae extracts are mostly used as a foliar spray on different cereal crops, vegetables, a variety of flowers, and tree species viz. aubergine (*Solanum melongena* L.), garlic (*Allium sativum*), pepper (*Capsicum* sp.), tomato (*Solanum lycopersicum* L) and petunia (*Petunia* × *atkinsiana*) (Oancea et al., 2013; Dias et al., 2016; Garcia-Gonzalez et al., 2016; Plaza et al., 2018). As with any other crop, foliar application of cyanobacteria and microalgae extracts was found to exhibit higher performance when applied during the morning, as the stomata of the leaves are wide open, and when relative humidity conditions are high, as the product uptake and permeability rise (Berry

et al., 2019)

As Alleviator of Abiotic Stress

World climatic changes have contributed immensely to the effect of abiotic stresses on crops, which invariably hinder the growth, development, and output of crops and finally reduce world agricultural productivity (Meena et al., 2017). Abiotic stresses viz. drought (irregular and erratic rainfall), salinity, excessive heat/extreme temperatures, and waterlogging are peculiar factors responsible for the poor productivity of most crops (Suzuki, 2016; Bilal et al., 2020). In recent years, the incidence of abiotic stresses has increased, mainly because of climate change, which has resulted in an unusual rise in severe weather conditions and incidents. Abiotic stresses are responsible for substantial losses of crops around the globe (Lamaoui et al., 2018). For instance, climatic change has a negative impact on agricultural production, leading to the losses of nearly USD 220 billion in North America, precisely the USA, as a result of the combination of extreme heat and irregular rainfall (drought) stresses on crops (Lamaoui et al., 2018). In Europe, it was estimated that recent annual economic losses as a result of climate change (drought) are amounted to be approximately nine billion euros (EUR 9 billion) for the European Union (EU) and the United Kingdom (UK), respectively. Interestingly, from these losses, between 39 and 60% were accounted for by agriculture (Cammalleri et al., 2020). Similarly, the situation of the negative impact on agriculture is not different in Asia, Latin America, and the Caribbean (LAC), as well as Sub-Saharan Africa, as economic losses were recorded to the tune of USD 42.934 billion, USD 10.023 billion, and USD 14.374 billion, respectively (FAO, 2016a). Biologically active compounds present in the biostimulants enhance the activities and performances of plants suffering from abiotic stresses. The plant output increases, coupled with the correctional measures on the earlier impairments resulting from adverse climatic

conditions (Calvo et al., 2014b; Van Oosten et al., 2017a; Ronga et al., 2019). To achieve an optimal result from using biostimulants as abiotic stress alleviator, several conditions must be put in place viz. when to apply the biostimulant on the affected crop (pre, during, and after) and the dosage (concentrations) that needs to be applied most efficiently, as it can pose a dual impact on crop performance (Vernieri et al., 2005). In similar experimental testing, cyanobacteria and microalgae extract application as a biostimulant mitigates high salinity stress in wheat (*Triticum aestivum* L.) cultivation. The application of extracts from *Arthrospira* sp. and *Chlorella* sp. significantly enhances the survival of wheat (*Triticum aestivum* L.) under salt stress conditions. An improvement in the whole grains' antioxidant capacity along with protein content was attained due to the anti-salinity potential exhibited by cyanobacteria and microalgae extracts compared to the control (Abd et al., 2010).

In the work of Renuka et al., 2018, it was reported that cyanobacteria and microalgae activities may have a great influence either directly or indirectly on the plant improvement in terms of immunity, health, and the potential to withstand any probable negative impacts of the combination of abiotic and biotic stresses (Renuka et al., 2018). Thus, cyanobacteria and microalgae species that are characterized by numerous applications to agricultural productivity can be seen as bio-alternatives to promote agricultural sustainability. Similarly, in a study conducted by El Arrousi et al., 2018, it was indicated that *D. salina* exopolysaccharide reduces the negative impact of multiple levels of salinity in *Solanum lycopersicum* (tomato) through the incremental increase in the activity of antioxidant enzymes, phenolic compounds, and essential metabolites viz. neophytadiene, tocopherol, stigmasterol as well as 2,4-ditert-butylphenol, which are regarded as constituents of the major influencer against oxidative stress (Arroussi et al., 2018). Additionally, for instance, Oancea et al., 2013 reported that Nannochloris

mitigates the impact of water stress on *Solanum lycopersicum* (Oancea et al., 2013).

Future Direction

Significant developments have been recorded in research in the action mechanisms of cyanobacteria and microalgae extract-elicited physiological responses, achievable courtesy of advancement in various tools such as "omics" available to modern researchers. Nevertheless, there are several bordering concerns and questions that need to be answered to achieve the best use of cyanobacteria and microalgae products as well as their respective extracts in crop cultivation. Such questions include, but are not limited to, the following: The difficulty in determining the exact stage of the crops when the extracts should be supplied to obtain the maximum positive result. Additionally, it is very challenging to determine the accurate timing and frequency required for the application coupled with the concentration levels to obtain the expected result. As it is, this would call for a more accurate protocol on extract application, either through the soil, foliage, or via other areas of the crop. Furthermore, systematic studies have never been embarked upon to unravel the possible disparity in the physiological response exhibited by the crop at different stages during development. What is the duration of the effect that the cyanobacteria and microalgae extract has on the crop after the application at the required concentrations? The ability to establish how long the physiological effect can persist will invariably assist in determining and planning the rate of cyanobacteria and microalgae extract applications. Several experimental tests have revealed that different crops respond in a dissimilar way to the concentration and rate of extract application. Consequently, it is essential to establish a more research-oriented plan tailored to specific crops in terms of extract application optimization and invariably obtain a highly significant result. Although there are numerous studies on the construction and management of ponds for the sustainable production of

cyanobacteria and microalgae, one of the many questions that surround the attainment of optimal production is the impact of pond failures, which are yet to be completely understood and thoroughly resolved. Nonetheless, to understand the underlying mechanisms that are perhaps responsible for this, there is a need for research to unravel and subsequently find a preventive measure against it. Additionally, locally isolated strains from wastewater ponds seem/tend to be more effective from strains obtained from the culture collection, and this should be diligently clarified in future research. Lastly, future research is important to disclose the composition, occurrence, location, and distribution of the target bioactive compounds in cyanobacteria and microalgae cells, and how to establish a more resilient population by employing "omic" technologies which are primarily concerned with detecting genes (genomics), messenger RNA, mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a particular biological sample.

What Crop?

Introduction

In this study, bell pepper (*Capsicum annuum* L.) is selected as a model because Qatar has a comparative advantage in its cultivation aside from economic and health benefits. Bell or sweet peppers are claiming a remarkable position among vegetables globally. The consumption of bell pepper(BP) on daily basis is growing tremendously due to the discovery of its nutritional and health benefits. Interestingly, the global production of chili and pepper has been on increase. Between the years 2000 and 2010, it has grown from 20.8 $x10^6$ tons to 27.5 $x10^6$ (FAOSTAT, 2016). This gives a good signal for efficient and effective cultivation because of this positive economic indicator. In terms of nutritional values, bell pepper is very rich in ascorbic acid, an antioxidant. However, based on BP nutritional and health benefits globally, the demand has been on the rise which necessitates the need to improve on the production to be able to cope with this

increasing demand. Nevertheless, a sustainable method of cultivation has been the only efficient and reliable method to increase the yield as well as the quality of the product with a consistent income flow to the farmers all things being equal.

Biology and Cultivation of Bell Pepper (Capsicum annum L.)

The production of pepper, genus Capsicum and consumption have increased tremendously globally all along the 20th century because they are good as a vegetable as well as spices. Like tomatoes and potatoes, they have become an essential component of most cuisines globally. Countries within the northern latitude which include Canada and Holland as well as Mediterranean countries like Spain have developed protected cultures to meet the increasing demand (Shaw et al., 2003). The persistent increase in demand and competition has resulted in efficient production methods and good quality peppers are released to the farmers and other markets. Additionally, apart from being essential as a source of food, it has been scientifically established to be of health benefits to humans. Such benefits are but are not limited to carotenoids, flavonoids, ascorbic acid, phenolic compounds as well as pungent capsaicinoids *(Bosland and Votava, 2012; Crosby et al. 2005c)*. It has also been documented to have the preventative potential of particular types of tumors found in cancer *(Fery, Dukes, (USA) 1986)*. Also, they have industrial benefits such as the ability to attack pests associated crops, some kind of wood products as well as cable coatings *("Use Chili Peppers to Control Pests - Barza ScriptsBarza Scripts" 1994)*. Bell pepper was first found in the North America of Mexica, Central America as well as northern area of South America. By 1493, bell pepper has spread to the rest of the world by different agents of propagation viz. wind, bird, human, and animals. The rear attribute of bell pepper to survive in different types of climatic conditions is responsible for its spread across temperate and tropical climates. In addition, their acceptance into different local and continental dishes has

been on the rise globally. There are different varieties of bell pepper ranging from the wild chili to the bigger-sized sweet ones and all these constituted the *Capsicum annuum* species. Interestingly, the sweet and spice paprika varieties are the most sorted varieties among all the cultivars of bell pepper. Bell pepper is equally endowed to come in a different colour, size, and shape depending on the particular cultivar cultivated. The different representatives of such cultivars are shown in Figure 13. In terms of colour variation, bell pepper fruit can appear as green, yellow, purple, orange, and red depending on the cultivated type. As per size, a mature fruit attains the size range of 1 cm to 25 cm depending on the species while their flavor also varies depending on the bell pepper type Figure 14 As per nutritional value, bell pepper is a source of vitamin C and natural antioxidants (Salunkhe et al., 1991; Al-Harbi et al., 2020). Most of these natural antioxidants are known for their effective neutralization of free radicals that are injurious to the body cells (Knekt et al., 2002). Different factors such as bell pepper cultivar, growing conditions, and maturation stages play a vital role in the amount of ascorbic acid (vitamin C) in bell peppers. As shown in Table 7, the consumption of 100g of bell pepper will supply the percentage requirement of vitamin C, vitamin A, and vitamin K of the reference daily intake (RDI). Bell pepper's health benefits include her anticancer property because of the embedded Vitamin C, beta-carotene, and folic acid that when consumed can minimize cancer risk (Mateljan, 2007). All these attributes have contributed greatly to the increase in the cultivation of bell pepper globally

Figure 13. Pepper cultivars

Figure 14. Bell pepper with different colour

Table 7. Nutritional Contents of Green Pepper (*Capsicum annuum* L.)

The Nutrition value of Bell peppers (*Capsicum annuum* L.), green, raw, per 100 g (Source: USDA National Nutrient database)		
Contents	Nutrient Quantity	%RDI
Energy	84 kJ (20 kcal)	1.50%
Carbohydrates	4.64 g	4%
Sugars	2.4 g	2%
Dietary fiber	1.8 g	1%
Fat	0.17 g	0%
Protein	0.86 g	5.50%
Vitamins		
Vitamin A equiv.	18 µg	2%
beta-Carotene	208 µg	2%
lutein zeaxanthin	341 µg	
Thiamine (B1)	0.057 mg	5%
Riboflavin (B2)	0.028 mg	2%
Niacin (B3)	0.48 mg	3%
Pantothenicacid(B5)	0.099 mg	2%
Vitamin B6	0.224 mg	17%
Folate (B9)	10 µg	3%
Vitamin C	80.4 mg	97%
Vitamin E	0.37 mg	2%
Vitamin K	7.4 µg	7%
Minerals		
Calcium	10 mg	1%
Iron	0.34 mg	3%
Magnesium	10 mg	3%
Manganese	0.122 mg	6%
Phosphorus	20 mg	3%
Potassium	175 mg	4%
Sodium	3 mg	0%
Zinc	0.13 mg	1%
Other constituents		
Water	93.9 g	

CHAPTER 3. ENHANCEMENT IN BELL PEPPER (*capsicum annuum* L.) PLANTS WITH APPLICATION OF *Roholtiella* sp. (Nostocales) UNDER SOILLESS CULTIVATION

Introduction

The persistent excessive use of chemical fertilizers leads to an increase in crop output but not without adverse/detrimental effects on the environment such as a rapid decrease in soil quality and fertility (Savci, 2012; Lee et al., 2019). Also, it can cause biodiversity decline, eutrophication, and global ecological degradation (Hallmann et al., 2014; Chagnon et al., 2015; Van der Sluijs et al., 2015; Agegnehu et al., 2016; Srivastava et al., 2016). In this regard, several fertilizing alternatives have been proposed to reduce the cost and environmental impacts of chemical fertilizers. Algae are preferred substitutes for chemical fertilizers and can be used as biofertilizers due to their potential of enhancing the general health conditions of plants with the ability to enhance soil fertility and productivity (Abinandan et al., 2019; Chittora et al., 2020; Kheirfam et al., 2020). Aside from being eco-friendly, biofertilizers have other beneficial characteristics such as their sustainability in cultivated soils. Besides, the application of biofertilizers mitigates the possible accumulation of different levels of chemical contaminants within the soil (Saadaoui et al., 2019).

Cyanobacteria or blue-green algae (BGA) constitute the largest group of photosynthetic prokaryotes with a wider spread and huge diversity globally, mostly in the terrestrial and aquatic ecosystems (Jaiswal et al., 2005; Shariatmadari et al., 2013; Dunker, 2014; Dorina et al., 2020). Their presence in different ecosystems, particularly terrestrial, makes them an essential part of the soil microflora with the potential of increasing the productivity of soil either directly or indirectly (Vaishampayan et al., 2001; Mishra et al., 2004; Shariatmadari et al., 2015). The increasing BGA applications in the various

sectors such as a source of food, animal feed, biofuel, biofertilizers, biostimulants, colorants, etc. is a result of their ability to synthesize high value-added products such as vitamins, pharmaceuticals, enzymes, and pharmacological probes in addition to proteins, lipids, and carbohydrates (Garlapati et al., 2019; Jaeschke et al., 2019; Chittora et al., 2020).

Species of terrestrial cyanobacteria are predominantly found in the soil, living symbiotically by enhancing the soil productivity through atmospheric nitrogen fixation, decomposition of organic by-products and residues, heavy metal detoxification, pathogenic microorganism suppression, and moisture content maintenance, and to certain extent soil erosion prevention (Singh et al., 2016). Additionally, terrestrial cyanobacteria facilitate the increment of trace elements in soil, which are vital for plant development and ion uptake, improve nitrogen content within the peripheral of soil (Singh et al., 2016). They equally enhance the production of plant growth-promoting compounds/substances (e.g. phytohormones) (Misra et al., 1989a, 1989b; Karthikeyan et al., 2007; Obana et al., 2007; Kumar et al., 2015; Abinandan et al., 2019). The aqueous extracts were considered as we targeted to establish that phycobiliproteins are plant growth enhancers. However, our goal is to utilize cyanobacteria biomass as growth promoters because they are relatively cheap, sustainable, and cost-effective for crop production when compared with the costly phycobiliprotein "(it costs more than the bell pepper when used as an input)".

Soilless methods for growing seedlings, as a sustainable option to mitigate the problem of water shortages, improve water use efficiency (WUE) thereby minimizing the quantity of water loss either through evaporation or drainage, and improve food production in arid regions (AR). Soilless growing of fruits, vegetables, and other crops in arid regions is a fast-developing agricultural practice, which has been enhancing food

productivity and water use efficiency, in regions with limited extension of arable lands. A study conducted by (AlShrouf, 2017) indicated that farming under the soilless system is likely to be the most sustainable alternative that will provide different types of crops with the utilization of less water, fewer fertilizers as well as little space, which will result in yield increase per unit area. (Al-Karaki et al., 2012b) reported that green forage has the potential to be successfully produced within 8 days from sowing to harvesting using the soilless method. The highest values for fresh green yields are recorded coupled with adequate water utilization efficiency under the hydroponic system. This technique has been used successfully in the arid region.

Several studies have revealed the beneficial impacts of cyanobacteria as a source of biofertilizers to different crops such as cereals e.g. rice (*Oryza sativa L.*); wheat (*Triticum aestivum L.*) (Karthikeyan et al., 2007); maize (*Zea mays* L.) (Maqubela et al., 2010), and radish (*Raphanus sativus* L.) (Godlewska et al., 2019). However, much is yet to be investigated on the effect of cyanobacteria on other important crops such as medicinal plants, legumes, and vegetables. Accordingly, little has been done on investigating the effect of cyanobacteria as a growth promoter on most *Capsicum* species. *Capsicum annuum* L. has been widely cultivated because of its well-recognized health benefits to humans. Such benefits are but not limited to carotenoids, flavonoids, ascorbic acid, phenolic compounds as well as the pungent capsaicinoids (Bosland et al., 2012). *Capsicum annuum* L. in this study was a model, a methodology to testing plant growth promotion, but not the main goal of the study.

The aim of this study was therefore to investigate the effect of extracts and biomasses of three cyanobacteria species isolated from Qatari soils, namely *Roholtiella* sp. (QUCCCM97), *Nostoc ellipsosporum* (QUCCCM99), and *Desmonostoc danxiaense* (QUCCCM112) as potential growth promoters on the growth parameters and spad

index of *C. annuum* seedlings in Hoagland solution.

Material and Methods

Plant Material and Culture Condition

The vegetable crop species selected for the current research study was bell pepper (*Capsicum annuum* L.) belonging to the family, Solanaceae as a model for the study. The seedlings produced from certified bell pepper (BP) hybrid seeds "Lorca" were obtained from a commercial nursery in Qatar (Al Sulaiteen Agricultural & Industrial Complex; SAIC). Vigorous seedlings with relative uniformity in weight and morphological appearances were selected as the propagating materials to kick-start the experiment. The seedlings of 20 days old were grown in a 4 x 4 x 13 cm flower vase in a hydroponic Hoagland solution (Hoagland et al., 1950). Sixteen vases are arranged in a box of 17 x 27 x 10 cm to form a block, which constitutes nine replicates (n=9) Figure 15. Transplanted seedlings displaying four true leaves were selected in the entire case to maintain homogeneity

Figure 15. Treated and control seedlings in a block design (n = 9).

Cyanobacteria Strain Cultivation and Growth Conditions

Three freshwater filamentous and N-fixing cyanobacteria namely, *Roholtiella* sp. (QUCCC97), *Nostoc ellipsosporum* (QUCCCM99), and *Desmonostoc danxiaense*

(QUCCCM112) were selected for the current study based on their ability to produce different phycobiliproteins i.e. phycoerythrin; phycocyanin, and phycobilin. These strains, isolated from the Qatar desert, belong to the Qatar University Culture Collection of Cyanobacteria and Microalgae (QUCCCM) (Saadaoui et al., 2016) and were provided by the center for sustainable development (CSD). The selection of these three strains was based on their different pigments content. One single colony of the cyanobacteria strain was used to inoculate a 5 ml volume of BG11 growth medium (Stanier et al., 1971). Subsequently incubated for 7 days at 30°C, a photon flux density of 100 µmol photons m^{-2} s^{-1} and a 12:12 h dark: light cycle with 150 rpm agitation using an illuminated shaker (Innova 44R, New Brunswick Scientific, USA). Then, the culture was gradually scaled up to 500 ml and incubated under the previously described conditions. Finally, an adequate volume was used to inoculate a DASGIP parallel 1L bioreactor system for phototrophic cultivation (#76DG08PBBB, Eppendorf, USA). This culture was grown at 30°C, pH 8, under 300 rpm agitation to avoid settling of the cyanobacteria isolates, with 100 µmol photons m^{-2} s^{-1}, a 12:12 h dark:light regime and 5% CO_2 during the light phase (Saadaoui et al., 2018). After 15 days of incubation, the biomass from each species was harvested by centrifugation then freeze-dried. All cultures were performed in duplicate.

Preparation of Cyanobacteria Extracts

The freeze-dried biomass obtained after 15 days of cultivation as described previously was divided into two parts. The first one (biomass) was used directly as a biofertilizer denoted as *Roholtiella* sp. QUCCCM97$_{\mathbf{bio}}$, *Nostoc ellipsosporum* QUCCCM99$_{\mathbf{bio}}$, and *Desmonostoc danxiaense* QUCCCM112$_{\mathbf{bio}}$ while the second fraction was subjected to aqueous extraction. To this end, 100 mg of dry biomass of each cyanobacteria strain was first washed with sterile distilled water then dissolved into 12.5 ml phosphate

buffer (0.1 M pH 6.0) before sonication for 10 min (5 s pulses of 8 W over 30 s, on ice, Sonics VCX 130 Ultrasonic processor). The phosphate buffer solution was used to maintain the pH of the system and was not considered to play any role in the growth of the seedlings. Subsequently, extraction tubes were incubated at 4°C for 24 hrs. After centrifugation at 13000 rpm for 10 min, aqueous extracts were collected and stored again at 4°C in the dark and the residual biomass was subjected to repeated cycles of extraction until obtaining colorless biomass. Ultimately, aqueous extracts collected from the different cycles of extraction were mixed and stored in the dark at 4°C until future use. In this case, the cyanobacteria extract stock solutions were denoted as *Roholtiella* sp. QUCCCM97$_{\mathbf{extr}}$, *Nostoc ellipsosporum* QUCCCM99$_{\mathbf{extr}}$, and *Desmonostoc danxiaense* QUCCCM112$_{\mathbf{extr}}$ respectively. The total time of the extraction for *Roholtiella* sp. QUCCCM97$_{\mathbf{extr}}$, *Nostoc ellipsosporum* QUCCCM99$_{\mathbf{extr}}$, and *Desmonostoc danxiaense* QUCCCM112$_{\mathbf{extr}}$ (from cell break up to the analysis of pigments) did not exceed 30 hrs.

Cyanobacteria and its characterization

Morphological Characterization

The cyanobacterial strains *Roholtiella* sp. (QUCCCM97), *Nostoc ellipsosporum* (QUCCCM99), and *Desmonostoc danxiaense* (QUCCCM112) were used in the present study. These strains of cyanobacteria are among severals isolated from various freshwater, marine and terrestrial environments in Qatar that led to the establishment of the Qatar University Culture Collection of Cyanobacteria and Microalgae (QUCCCM). However, the QUCCCM has been created, developed and curated by the Centre of Sustainable Development CSD at the College of Arts and Sciences, Qatar University since 2011 (Imen Saadaoui 2016). The three strains QUCCCM97, 99, and 112 underwent morphological observations and the morphology analysis of each of the

three strains was examined by light microscopy under the magnification of 100x Figure 16. Also, the characterization of the aqueous extracts was conducted by spectrophotometric analysis. Analyses were executed using a fluorescence spectrophotometer (Synergy H4 Hybrid multi-mode microplate Reader. Bio Tek Instruments, Inc., USA). Steady and unbroken spectra of absorbance denoted as λ (300 to 800 nm) were amassed (Figure 17).

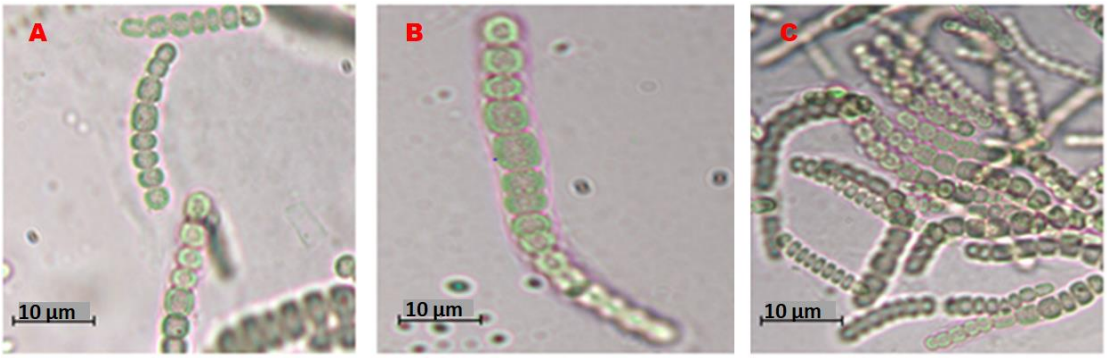


Figure 16. Microscopic observation of the three filamentous cyanobacteria species used in this study: A: Roholtiella sp. (QUCCCM97), B: Nostoc ellipsosporum (QUCCCM99), C: Desmonostoc danxiaense (QUCCCM112

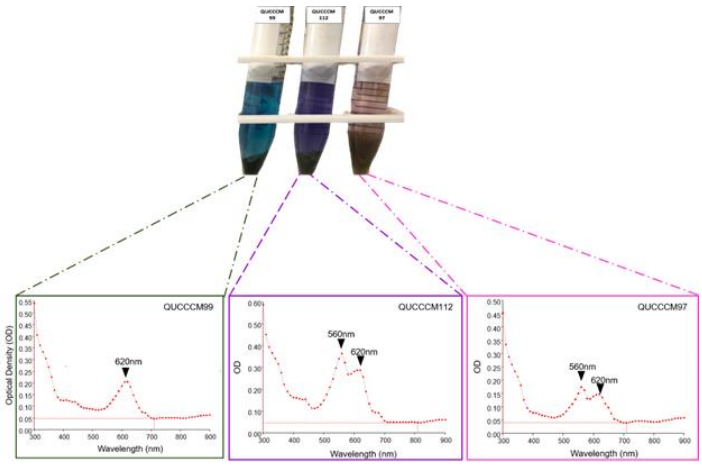


Figure 17. Aqueous extracts and the correspondent spectral scan of the strains *Nostoc ellipsosporum* (QUCCCM99), *Desmonostoc danxiaense* (QUCCCM112), and *Roholtiella* sp. (QUCCC97). (QUCCCM99) presented a high absorbance at 620nm

corresponding to maximum absorbance of the Phycocyanin while QUCCCM97 and QUCCCM112 presented 2 peaks of absorbance at 560 and 620nm. These peaks correspond to the λ_{max} of phycoerythrin and phycocyanin, respectively

DNA Extraction and Gene Sequencing

For further identification, the molecular analysis of each cyanobacteria strain was examined through the sequencing of the 16rDNA of three of our strains isolated from the desert environment namely QUCCCM97, 99, and 112 respectively. The cultured DNA was harvested for analysis during the rapid growth stage and subsequently concentrated via centrifugation. Total genomic DNA was isolated by applying a method previously used with slight modifications (Pitcher et al., 1989; Kiselev et al., 2015). The amplification of 16S rDNA genes was conducted by utilizing both primers BSIF (5‘ GATCCTKGCTCAGGATKAACGCTGGC 3’) and 920R (5‘ TTTGCGGCCGCTCTGTGTGCC 3’). The PCR amplification was performed using the SuperFiTM PCR Master Mix, (Thermo Fisher Scientific, Waltham MA). The purification of the PCR products was performed using ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix, Santa Clara, California, USA). The DNA concentration was determined by NanoDrop 2000c/2000 UV–Vis Spectrophotometer (Thermo Scientific, Wilmington, USA). The sequencing of the purified DNA fragment was carried out by Genetic Analyzer 3500 (Applied Biosystems, California, USA), using the same primers used for the PCR amplification in addition to two other internal primers, i.e., BSL4F (5’GYAACGAGCGCAACCC 3’) and BSL8R (5’AAGGAGGTGATCCAGCCGCA 3’). Assembled sequences were submitted to GenBank and the accession numbers are provided in Table 8

Table 8. Genbank Accession Numbers and Molecular Classification of The Strains

Strain /Isolate	GenBank Accession Number	Molecular Classification
QUCCCM97	MW791421	*Roholtiella* sp
QUCCCM99	MW791422	*Nostoc ellipsosporum*
QUCCCM112	MW791423	*Desmonostoc danxiaense*

[National Center for Biotechnology Information (ncbi.nlm.nih.gov)]

Phylogenetic Analysis

The 16S rDNA sequences of the three cyanobacteria strains were locally aligned using Basic Local Alignment Search Tool (BlastN). Type strains and strains with sequenced genomes were downloaded for analyses. Alignment was performed by MUSCLE (Edgar, 2004) implemented in MEGA X software. Phylogenetic and molecular evolutionary analyses were conducted using MEGA X (Kumar et al., 2018; Stecher et al., 2020).

Experimental Design

The bell pepper growth experiment was conducted at Qatar University, Department of Biological and Environmental Sciences, in January-February 2020. The study utilized the revamped deep-water culture (DWC) hydroponic system and cyanobacteria supplied by the center for sustainable development (CSD). This method was preferred because it allows a continuous access to nutrients to enhance a faster growth of the plants. However, a modification of the construction of the system was essential to minimize the utilization of the nutrient solution. The nutrient solution replenishment was conducted every 7 days of the total 21-day experiment. The shoot length of the plant was measured at T_i (initial) and T_f (final), and other measured parameters include shoot length, root length, spad index, biomass (fresh and dry weight), growth rate, and the number of leaves. Cyanobacteria biomass, its aqueous extract, and Hoagland solution were used as growth media. The modified Hoagland prescription was as

follows: $Ca(NO_3)_2 \cdot 4H_2O$, 1.250 g L^{-1}; KNO_3, 0.410 g L^{-1}; $NH_4H_2PO_4$, 0.280 g L^{-1}; $MgCl_2 \cdot 6H_2O$, 0.624 g L^{-1}; $FeSO_4 \cdot 7H_2O$, 0.060 g L^{-1}; EDTA-Na_2, 0.080 g L^{-1}; H_3BO_3, 0.006 g L^{-1}; $MnCl_2 \cdot 4H_2O$, 0.04 g L^{-1}; $ZnSO_4 \cdot 7H_2O$, 410^{-5} g L^{-1}, and $CuSO_4 \cdot 5H_2O$, $4 \cdot 10^{-5}$ g L^{-1} (Hoagland et al., 1950). The acidity of the solution was adjusted to a pH of 6.0 ± 0.5 (Taiz et al., 2010). The control treatment, a modified Hoagland nutrient solution (Hoagland et al., 1950) was denoted as Tr_0. While the other treatments using cyanobacteria extracts and biomass were named Tr_1, Tr_2, Tr_3, Tr_4, and Tr_5 respectively. Each treatment was replicated nine times, under nine replicate blocks among the arranged containers/reservoirs.

The summary of the treatments is as follows:

Tr_0- Control – 0% extract and biomass (1 L Hoagland solution)

High value product extracts from QUCCCM X

Tr_1 – (2 ml L^{-1}) - 0.2% concentration (2 mL extract of X in 1 L Hoagland solution)

Tr_2 – (4 ml L^{-1}) - 0.4% concentration (4 mL extract of X in 1 L Hoagland solution)

Tr_3 – (6 ml L^{-1}) - 0.6% concentration (6 mL extract of X in 1 L Hoagland solution)

Water re-suspended biomass from QUCCCM X

Tr_4 – (1 mg L^{-1}) - 0.1% concentration (1 mg biomass of X in 1 L Hoagland solution)

Tr_5 – (2 mg L^{-1}) - 0.2% concentration (2 mg biomass of X in 1 L Hoagland solution)

Where X refers to 97, 99, or 112 for the strains *Roholtiella* sp. (QUCCCM97), *Nostoc ellipsosporum* (QUCCCM99), and *Desmonostoc danxiaense* (QUCCCM112) respectively; Tr_1 – Tr_5 are different treatments with different concentrations. Tr_1 – Tr_3 – for extract, while Tr_4 – Tr_5 – for biomass.

Growth Parameter and Spad Index

As previously mentioned, this study was conducted to investigate the effects of microalgae extracts and biomass as growth promoters on bell pepper seedlings by following vegetative growth parameters: shoot length, root length, fresh weight, dry

weight, spad index, number of leaves, and growth rate.

Shoot Length (cm) - SL

The shoot length of bell pepper was taken from the top of support assumed to be the base up to the topmost part where the leaves are fully opened by using a measuring scale, and then the average was recorded. The measurement was carried out at the commencement and completion of the experiment respectively.

Root Length (cm) - RL

The bell pepper root length was measured from the point/base beneath the soil down to the tip of the fully developed root (longest) with the use of a measuring scale and the average was recorded. The RL was measured at 19 days of the experiment.

Fresh Weight (g) - FW

The fresh weight of the whole plants was taken/recorded using an analytical scale for accuracy and the average was taken. The FW was measured on the expiration of the experiment on the 21st day.

Dry Weight (g) - DW

The harvested plants were oven-dried at 60°C (Genlab Drying Cabinet, Genlab Limited. Cheshire, UK) until the weight was found constant. Thereafter, an analytical balance was used to measure the weight for accuracy, and the average was calculated. The DW was taken days after 21 days of the experiment when the weight was found to be constant.

Spad Index - SI

The spad index of bell pepper leaf was ascertained employing SPAD – 02, chlorophyll meter (Konica Minolta, Japan). The spad index measurement was carried before the harvest/termination of the experiment 19 days after the experiment.

Number of Leaves Per Plant - NL

The number of leaves in every plant was counted manually before the end of the experiment, just 19 days after the experiment.

Growth Rate - GR

This was obtained by measuring the height of the seedling at two different times, at the commencement and end of the experiment while the experimental duration lasted 21 days. The initial and final height stage is assumed to be H_i and H_f.

$$\boldsymbol{GR = \frac{Hf - Hi}{Tf - Ti}} \qquad (\mathbf{1})$$

GR – Growth rate (cm day^{-1}), H_f – Final height, H_i – Initial height, T_f - T_i – Duration of experimentation.

Data Analysis

Data were analyzed following the procedure of analysis of variance (ANOVA) based on randomized complete block design (RCBD) using Minitab® software. Mean differences were compared through Tukey's *post hoc* test and were used to analyze the level of significant differences between treatments at a 5% significance level ($P \leq 0.05$).

Results

Cyanobacteria Characterization, Pigment, and Molecular Identification

The morphology of each strain was observed under a microscope revealing their filamentous trichomes features as shown in Figure 2. Furthermore, the aqueous extracts of the strains *Roholtiella* sp. (QUCCCM97), *Nostoc ellipsosporum* (QUCCCM99) and *Desmonostoc danxiaense* (QUCCCM112) were characterized by Optical measurements of the spectral absorption within the range of 300-800 nm. The absorbance band or spectra of the phycocyanin and phycoerythrin in the targeted compounds are shown in Figure 17. The obtained spectrophotometric bands or signatures of phycocyanin and phycoerythrin vary in the shape, value of λ_{max}, and position. The spectra showed the variation in their corresponding bands is related to the absorbance level. This could be further supported by spectra bands exhibited by the same pigments as narrated in a relevant study (Hsieh-Lo et al., 2019). The aqueous extracts of *Nostoc ellipsosporum* QUCCCM99$_{extr}$, *Desmonostoc danxiaense* QUCCCM112$_{extr}$, and *Roholtiella* sp. QUCCCM97$_{extr}$ revealed three different colors of blue-green, purple, and pink, respectively. The investigation of the nature of these pigments based on their λ_{max} of absorbance via spectral scan proved that *Nostoc ellipsosporum* (QUCCCM99) presented phycocyanin with λ_{max} = 620nm. However, *Desmonostoc danxiaense* (QUCCCM112), and *Roholtiella* sp. (QUCCCM97) presented an additional peak at λ_{max} = 560nm, corresponding to the phycoerythrin. Results proved that the absorbance at 620nm of *Desmonostoc danxiaense* (QUCCCM112) is higher than the other strain. Hence, this explains the dark color of the aqueous extract.

However, considering the nature of phycoerythrin and phycocyanin in this study, Phycoerythrin exhibited higher sensitivity with the absorbance technique compared to the phycocyanin in the various extracts analyzed in this study. Overall, the two strong

signals exhibited by *Roholtiella* sp. QUCCCM97$_{extr}$ and *Desmonostoc danxiaense* QUCCCM112$_{extr}$ compared with *Nostoc ellipsosporum* QUCCCM99$_{extr}$ further established the difference in the sensitivity of the strains investigated (Figure 17). Furthermore, the blast search results indicated that the sequence of the 16S rDNA gene in the three strains QUCCCM97 (GenBank accession number - MW791421), QUCCCM99 (accession number - MW791422), and QUCCCM112 (accession number - MW791423) is significantly like the corresponding species of *Roholtiella, Nostoc*, and *Desmonostoc* respectively as illustrated in a tabular form (Table 7). Phylogenetic analyses (Figure 18) drew on 16S rDNA sequences of 47 specimens of the selected major groups of genera employed as outgroups upon which our strains were placed for identification. Our strains were found to belong to the *Roholtialla*, *Nostoc*, and *Desmonostoc* generic clade respectively (Figure 18).

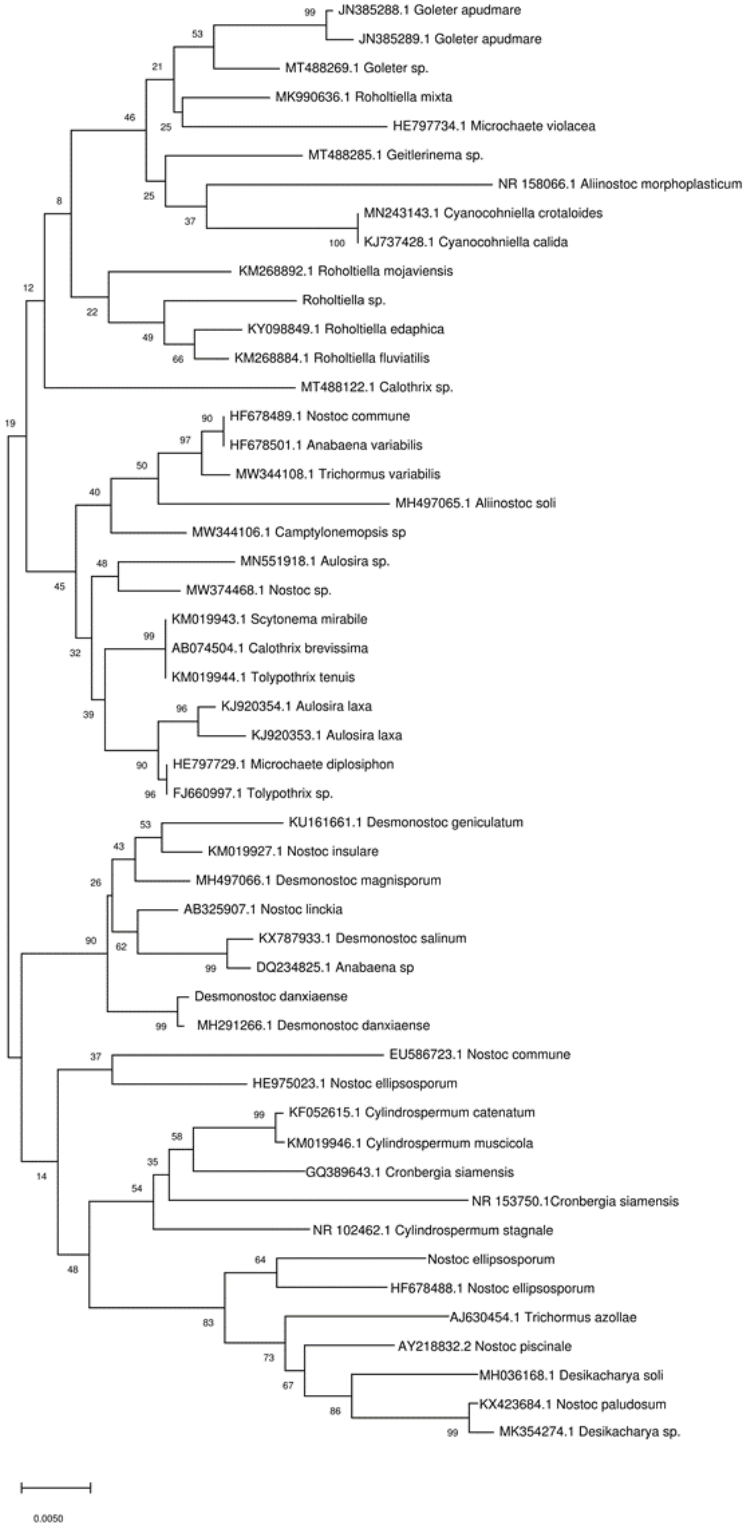
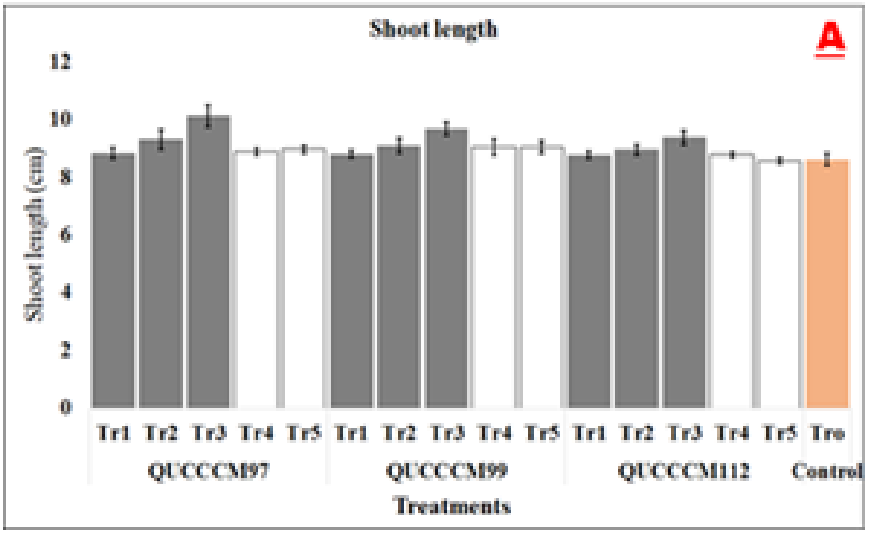


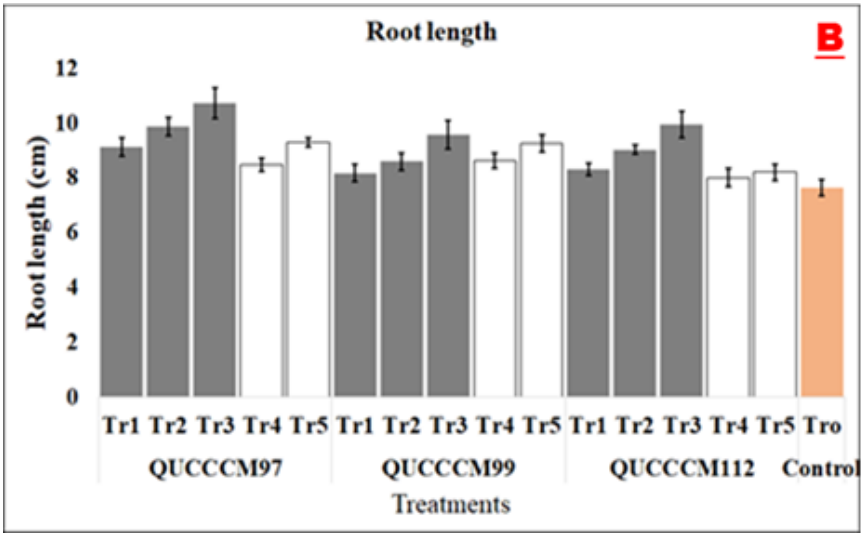
Figure 18. Phylogenetic tree based on neighbour-joining method using MEGA X software. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

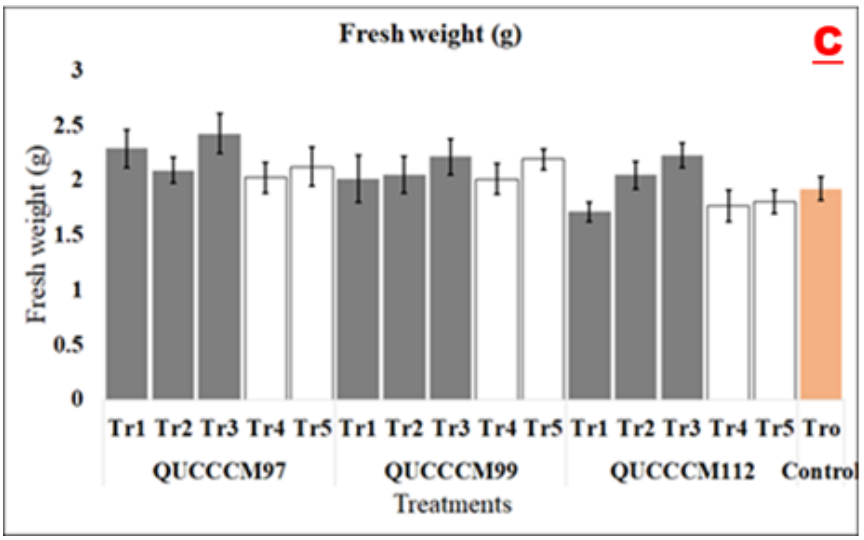
Growth Parameters of Seedlings and Spad Index at the End of the Experimental Period

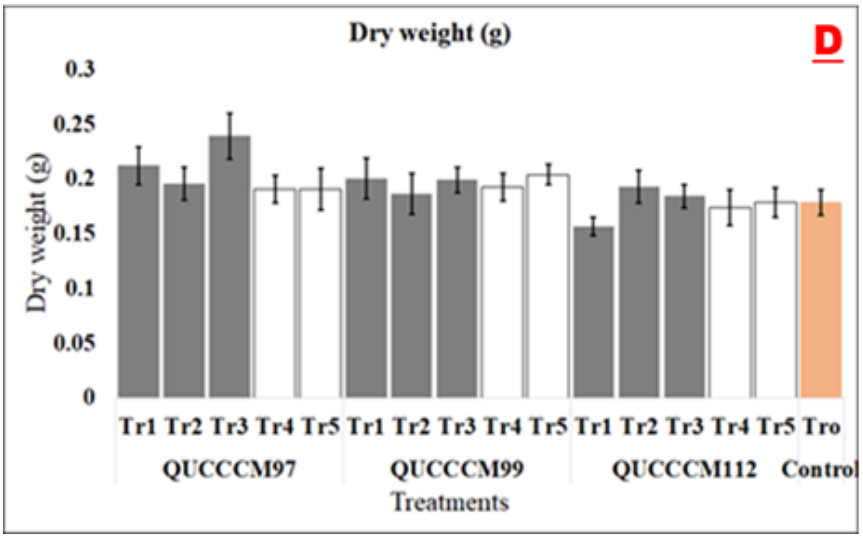
Efficacy of Roholtiella sp. *Extracts, and Biomass as a Growth Enhancer*

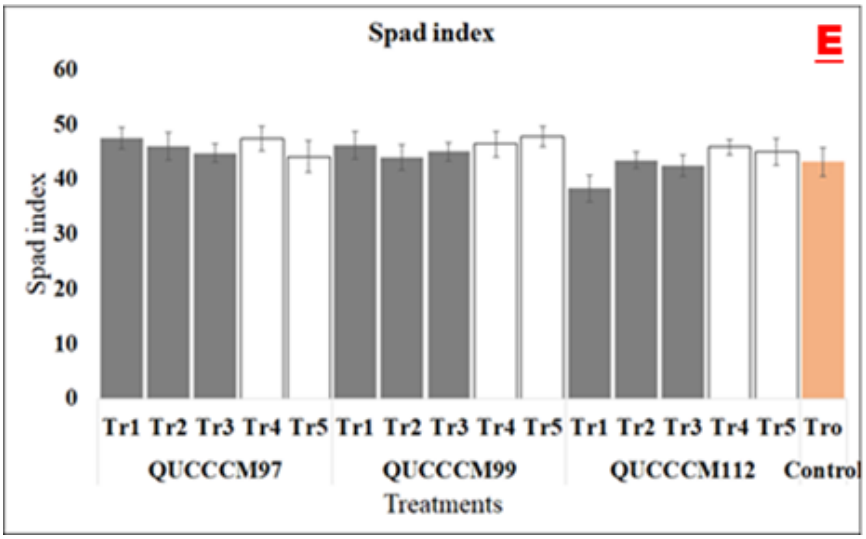
As illustrated in Figure 19A-G, the efficacies/effectiveness of *Roholtiella* sp. (QUCCCM97) extract and biomass in enhancing the growth parameters and spad index of seedlings were very prominent at different concentrations. For seedlings treated with 0.2% or 2 ml L^{-1} (Tr_1), 0.4% or 4 ml L^{-1} (Tr_2), and 0.6% or 6 ml L^{-1} (Tr_3) extract concentrations showed increased shoot length by 2.4%, 8.0%, and 17.5% respectively. In addition, biomass concentrations of 0.1% or 1 mg L^{-1} (Tr_4) and 0.2% or 2 mg L^{-1} (Tr_5) increased by 3.0% and 4.1% respectively compared with the control group. Similarly, treatments 0.2% or 2 ml L^{-1} (Tr_1), 0.4% or 4 ml L^{-1} (Tr_2), 0.6% or 6 ml L^{-1} (Tr_3), 0.1% or 1 mg L^{-1} (Tr_4), and 0.2% or 2 mg L^{-1} (Tr_5) showed increased root length by 19.5%, 29.3%, 40.3%, 10.9%, and 21.6% than the control group. The treatments trend showed higher fresh and dry weights by 19.3%, 8.9%, 26.0%, 5.2%, 10.4%,16.7%, 11.1%, 33.3%, and 5.6% respectively. Also, at the same concentrations, BPseedlings showed a higher spad index, number of leaves per plant, and growth rate by 10.1%, 6.7%, 3.7%, 9.9%, and 2.2% for the spad index; 7.8%. 10.8%, 21.6%, 6.2%, and 7.8% for the number of leaves per plant; 27.3%, 10.5%, 22.8%, 33.3%, and 37.9% for the growth rate respectively than the control group.

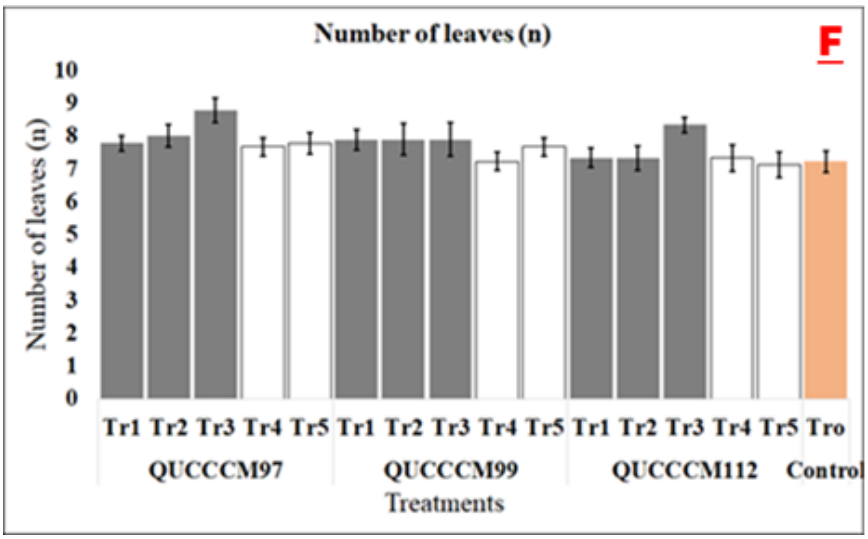

Shoot length
A
Shoot length (cm)
12
10
8
6
4
2
0
Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tro
QUCCCM97
QUCCCM99
QUCCCM112
Control
Treatments


Root length
B
Root length (cm)
12
10
8
6
4
2
0
Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tro
QUCCCM97
QUCCCM99
QUCCCM112
Control
Treatments


Fresh weight (g)
C
Fresh weight (g)
3
2.5
2
1.5
1
0.5
0
Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tro
QUCCCM97
QUCCCM99
QUCCCM112
Control
Treatments


Dry weight (g)
D
Dry weight (g)
0.3
0.25
0.2
0.15
0.1
0.05
0
Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tro
QUCCCM97
QUCCCM99
QUCCCM112
Control
Treatments


Spad index
E
Spad index
60
50
40
30
20
10
0
Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tro
QUCCCM97
QUCCCM99
QUCCCM112
Control
Treatments


Number of leaves (n)
F
Number of leaves (n)
10
9
8
7
6
5
4
3
2
1
0
Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tr1 Tr2 Tr3 Tr4 Tr5 Tro
QUCCCM97
QUCCCM99
QUCCCM112
Control
Treatments

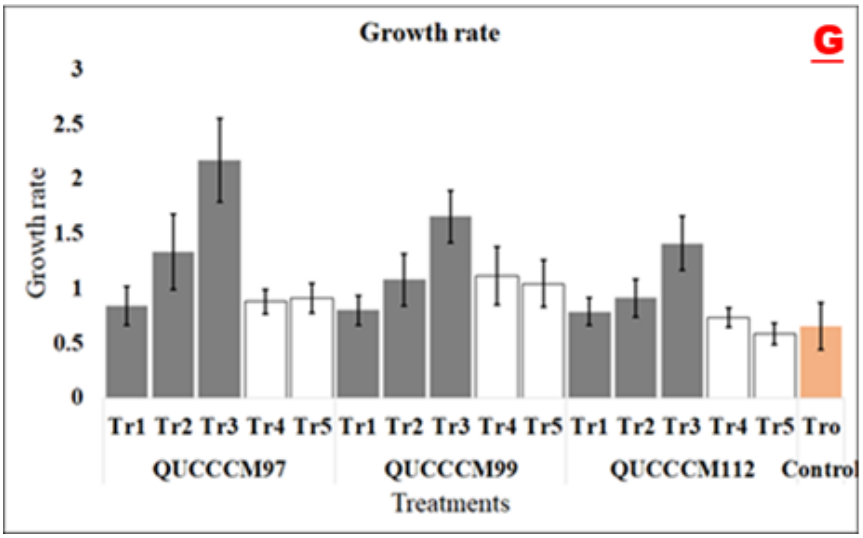


Figure 19. **(A-G)** Shoot length, root length, fresh weight, dry weight, spad index, number of leaves, and growth rate of bell pepper untreated seedlings (Tr_o), treated with 0.2% or 2 ml L^{-1} (Tr_1), 0.4% or 4 ml L^{-1} (Tr_2), and 0.6% or 6 ml L^{-1} (Tr_3) of the three microalgae extracts. Also, with 0.1% or 1 mg L^{-1} (Tr_4) and 0.2% or 2 mg L^{-1} (Tr_5) of the three microalgae biomass.

Efficacy af Nostoc Ellipsosporum Extracts and Biomass as A Growth Enhancer

Similarly, when compared to the control group, *Nostoc ellipsosporum* (QUCCCM99) extracts and biomass exhibited increased growth parameters and spad index. Seedlings treated with 0.2% or 2 ml L^{-1} (Tr_1), 0.4% or 4 ml L^{-1} (Tr_2), 0.6% or 6 ml L^{-1} (Tr_3) extract concentrations showed increased seedling shoot length (by 2.1%, 5.5%, and 12.3%). With biomass concentration of 0.1% or 1 mg L^{-1} (Tr_4) and 0.2% or 2 mg L^{-1} (Tr_5) increased by 5.1% and 4.8% respectively. Equally, seedlings treatments 0.2% or 2 ml L^{-1} (Tr_1), 0.4% or 4 ml L^{-1} (Tr_2), 0.6% or 6 ml L^{-1} (Tr_3), 0.1% or 1 mg L^{-1} (Tr_4), and 0.2% or 2 mg L^{-1} (Tr_5) showed increased seedling root length by 7.1%, 12.4%, 25.3%, 12.8%, and 21.2% than the control group. The treatments trend showed higher fresh and dry weights by 4.7%, 5.3%, 15.1%, 4.7%, 14.1%, and 11.1%, 5.6%, 33.3%, 11.1%, and 11.1% respectively. Also, at the same concentrations, the seedlings showed a higher

spad index, the number of leaves per plant, and growth rate by 7.1%, 2.0%, 4.4%, 7.6%, and 10.8% for the spad index; 9.3%. 9.3%, 9.3%, 6.2%, and 0.0% for the number of leaves per plant; 21.2%, 63.64%, 51.8%, 68.2%, and 57.6% for the growth rate respectively than the control group (Table 3).

Efficacy of Desmonostoc Danxiaense Extracts and Biomass as A Growth Enhancer

Likewise, when compared to the control group, *Desmonostoc danxiaense* (QUCCCM112) extracts and biomass exhibited increased growth parameters and spad index. Seedlings treated with 0.2% or 2 ml L^{-1} (Tr_1), 0.4% or 4 ml L^{-1} (Tr_2), 0.6% or 6 ml L^{-1} (Tr_3) extract concentrations showed increased seedling shoot length (by 1.7%, 3.9%, and 8.7% respectively). With biomass concentrations of 0.1% or 1 mg L^{-1} (Tr_4) and 0.2% or 2 mg L^{-1} (Tr_5) is increased (by 1.9% and -0.7%) respectively. Equally, seedlings treatments 0.2% or 2 ml L^{-1} (Tr_1), 0.4% or 4 ml L^{-1} (Tr_2), 0.6% or 6 ml L^{-1} (Tr_3), 0.1% or 1 mg L^{-1} (Tr_4), and 0.2% or 2 mg L^{-1} (Tr_5) showed increased seedling root length (by 8.8%, 18.2%, 30.1%, 4.8%, and 7.3%) than the control group. The treatment trend showed higher fresh and dry weights at (Tr_2) 6.3%, (Tr_3) 15.6%, for the fresh weigh and (Tr_2) 5.6% for the dry weight than the control group respectively. Likewise, at the same concentrations, the seedlings showed a higher spad index, the number of leaves per plant, and growth rate by 0.9% (Tr_2), 6.3% (Tr_4), and 4.2% (Tr_5) for the spad index. Similarly, 1.5% (Tr_1), 1.5% (Tr_2), 5.4% (Tr_3), and 1.5% (Tr_4) for the number of leaves per plant; 20.0% (Tr_1), 37.9% (Tr_2), 48.6% (Tr_3), and 10.6% (Tr_4) for the growth rate respectively than the control group. Interestingly, even with these positive impacts, the *Desmonostoc danxiaense* (QUCCCM112) extracts and biomass did not enhance growth parameters and spad index when compared with the control group at some concentrations. At Tr_1, Tr_4, and Tr_5 for fresh weight; Tr_1 and Tr_4 for dry weight; Tr_1 and Tr_3 for the spad index; at Tr_5 for both the number of leaves per plant

and growth rate respectively. All showed opposite/no effect when compared with control.

Strain Nature, Pigment, Dose, and Effect Comparison

There is tremendous variation in the nature of the strains investigated that characterized the different spectra/bands and positions as displaced in Figure 17. However, in particular, *Roholtiella* sp. QUCCCM97extr had a significant impact on the shoot length, growth rate, root length, fresh weight, dry weight, spad index, and the number of leaves even at a minimal dose (Tr_1) *Roholtiella* sp. QUCCCM97extr because of the embedded phycoerythrin Figure 5. Nevertheless, noticeable differences were obvious in these parameters studied at this maximum dose (Tr_3) with *Nostoc ellipsosporum* QUCCCM99extr and *Desmonostoc danxiaense* QUCCCM112extr which is an indication that *Roholtiella sp.* QUCCCM97extr has an impact that is more positive on the growth parameters investigated even at minimal dose, a significant difference in these parameters was achievable.

The statistical analysis revealed a clear indication of the significance of various treatments on the growth factors of bell pepper as shown in Table 9. Since *p*-value = 0.001 shoot length (SL), 0.001 root length, 0.02 fresh weight, 0.007 number of leaves, 0.001 growth rate is less than 0.05 then we reject the null hypothesis and we conclude that there is a significant difference in these treatments at 0.05 significance level. But in the case of dry weight, and spad index, there is no significant difference though the trend shows that quantitatively all treatments have a positive effect on growth parameters (Figure 19A-G).

Table 9. Analysis of Variance Showing the Statistical Significance Effect of Various Treatments on the Growth Parameters Shoot Length (SL), Root Length (RL), Fresh Weight (FW), Dry Weight (DW), Spad Index (CC), Number of Leaves (NL), and Growth Rate (GR) of Seedlings. ** Indicates Highly Significant Differences at 0.05 (5%) Level, Ns = Not Significant.

Source of Variation	DF	Mean square (MS)						
		SL	RL	FW	DW	CC	NL	GR
Block	8	0.979	3.8925	1.1712	0.0065	242.3572	5.7153	0.9544
Treatment	15	1.485^{**}	6.1416^{**}	0.3328^{**}	0.0029^{ns}	50.0803^{ns}	1.7884^{**}	1.5226^{**}
Error	120	0.386	0.8399	0.1245	0.00173	30.22	0.7801	0.3815
Total	143							

Furthermore, the comparative analysis revealed that there is a significant difference between a treated bell pepper and control for vegetative growth parameters viz. shoot length, root length, fresh weight, dry weight, spad index, number of leaves, and GL ($p < 0.05$) as shown in Table 10. Also, extracts and biomass from cyanobacteria strain *Roholtiella* sp. QUCCCM97, *Nostoc ellipsosporum* QUCCCM99, and *Desmonostoc danxiaense* QUCCCM112 tested on bell pepper growth parameters further showed a significant growth difference with the control seedlings as shown in Table 10. The effectiveness of various cyanobacteria extracts and biomass treatments could also be compared as displayed in Tukey pairwise comparisons (Table 10).

Means that do not share a letter affirmed to be significantly different while contrarily those that share a letter are not significantly different at $p < 0.05$ in terms of their effect on the measured parameters.

However, the biomass and extracts with the concentration of respectively 3 mg L-1/6 mL-1 gave the highest result while the application at lower concentrations of 1 mg L-1/2 mL-1 or 2 mg L-1 /4 mL-1 yielded lesser growth when compared with the 6 mL-1

for biomass and 3 mg L-1 for extract concentrations.

Table 10. Effect of QUCCCM 97, 99, 112 Extracts and Biomass on Growth Parameters and Spad Index of Seedlings (Mean ± Standard Error). Means that Do Not Share a Letter are Significantly Different Based on Tukey Pairwise Comparisons of Treatments. This Implies that Different Letters (A–E) Indicate Significantly Different Values in Response to the Different Treatments (Nine Replicates Per Treatment)

Strains	Treatments	Con. (in Hoagland) ml L^{-1}	**Growth parameters**						
			Shoot length (cm)	**Root length (cm)**	**Fresh weight (g)**	**Dry weight (g)**	**Spad index**	**Number of leaves/plant**	**Growth rate**
Roholtiella sp. (QUCCCM 97)	Tr_1	**2 ml L^{-1}**	8.83±0.19 (bc)	9.13±0.33 (bcde)	2.29±0.17 (ab)	0.21±0.02 (ab)	47.48a±1.96	7.78±0.22 (ab)	0.84±0.18 (bc)
	Tr_2	**4 ml L^{-1}**	9.319±0.34 (abc)	9.88±0.32 (ab)	2.09±0.12 (ab)	0.20±0.02 (ab)	46.01±2.50 (ab)	8.00b±0.33	1.33±0.34 (abc)
	Tr_3	**6 ml L^{-1}**	10.13a±0.38	10.72a±0.55	2.42a±0.18	0.24a±0.02	44.72±1.65 (ab)	8.78a±0.36	2.17a±0.38
	Tr_4	**2 ml L^{-1}**	8.88±0.12 (bc)	8.47±0.24 (bcde)	2.02±0.14 (ab)	0.19±0.01 (ab)	47.39a±2.31	7.67±0.29 (ab)	0.88±0.11 (bc)
	Tr_5	**4 ml L^{-1}**	8.97±0.14 (bc)	9.29±0.16 (abcd)	2.12±0.18 (ab)	0.19±0.02 (ab)	44.06±2.89 (ab)	7.78±0.32 (ab)	0.91±0.14 (bc)
Nostoc ellipsosporum (QUCCCM 99)	Tr_1	**2 ml L^{-1}**	8.80±0.13 (bc)	8.18±0.30 (cde)	2.01±0.21 (ab)	0.20±0.02 (ab)	46.19±2.54 (ab)	7.89±0.31 (ab)	0.80±0.13 (bc)
	Tr_2	**4 ml L^{-1}**	9.09±0.24 (bc)	8.59±0.30 (bcde)	2.04±0.17 (ab)	0.19±0.02 (ab)	43.98±2.27 (ab)	7.89±0.484 (ab)	1.08±0.23 (bc)
	Tr_3	**6 ml L^{-1}**	9.68a±0.23	9.57±0.52 (abc)	2.21±0.17 (ab)	0.20±0.01 (ab)	45.01±1.64 (ab)	7.89±0.51 (ab)	1.66±0.24 (ab)
	Tr_4	**2 ml L^{-1}**	9.06±0.27 (bc)	8.62±0.29 (bcde)	2.01±0.14 (ab)	0.19±0.01 (ab)	46.38±2.37 (ab)	7.22b±0.28	1.11±0.27 (bc)
	Tr_5	**4 ml L^{-1}**	9.03±0.21 (bc)	9.26±0.30 (abcd)	2.19±0.10 (ab)	0.20±0.01 (ab)	47.76a±1.94	7.67±0.29 (ab)	1.04±0.22 (bc)
Desmonostoc danxiaense (QUCCCM 112)	Tr_1	**2 ml L^{-1}**	8.77±0.13 (bc)	8.31±0.22 (cde)	1.71b±0.09	0.16b±0.01	38.28b±2.44	7.33±0.29 (ab)	0.79±0.13 (bc)
	Tr_2	**4 ml L^{-1}**	8.96±0.17 (bc)	9.03±0.16 (bcde)	2.04±0.13 (ab)	0.19±0.01 (ab)	43.48±1.56 (ab)	7.33±0.37 (ab)	0.91±0.17 (bc)
	Tr_3	**6 ml L^{-1}**	9.37±0.25a	9.94±0.48 (ab)	2.22±0.11 (ab)	0.18±0.01 (ab)	42.46±2.03 (ab)	8.33±0.24 (ab)	1.41±0.25 (abc)
	Tr_4	**2 ml L^{-1}**	8.78±0.10c	8.01±0.34 (de)	1.77b±0.15	0.17±0.02 (ab)	45.82±1.42 (ab)	7.33±0.41 (ab)	0.73±0.10 (bc)
	Tr_5	**4 ml L^{-1}**	8.56±0.11c	8.20±0.30 (cde)	1.80b±0.11	0.18±0.01 (ab)	44.92±2.41 (ab)	7.11b±0.39	0.59c±0.10
Control	T_O	Hoagland	8.62±0.21c	7.64e±0.29	1.92b±0.11	0.18±0.01 (ab)	43.11±2.62 (ab)	7.22b±0.32	0.66±0.21 (bc)

CHAPTER 4. EVALUATION OF *Roholtiella* sp. EXTRACT ON BELL PEPPER (*Capsicum annuum* L.) YIELD AND QUALITY IN A HYDROPONIC GREENHOUSE SYSTEM

Introduction

The persistent rise in global industrialization, urbanization, and technological advancement is among the reasons why the global population is on the rise; it is forecasted that the population will rise beyond 9.7 billion by 2050 (Abdelaal et al., 2020a). Thus, the population increase coupled with the ecological implications of climatic change make agriculture liable to several constraints such as abiotic stresses, causing negative impacts on crop production globally (Soliman et al., 2018; Mutale-joan et al., 2021). Generally, the conventional methods of agriculture viz irrigation farming, mechanized farming, and application of chemical fertilizer (urea, NPK, etc.) to maximize plant productivity and the attainment of the optimum plant's growing conditions are in practice (Elkeilsh et al., 2019). However, these conventional techniques have been practiced for decades but are seen to pose some threat to the environment and the existing ecosystem as a whole (Carvalho, 2017; Rahman et al., 2018). Consequently, to reduce these effects, there is a need for practicing modern agriculture as an alternative eco-friendly and sustainable technique. Furthermore, the discovery of green technologies that make use of bioresources such as biofertilizers and biostimulants are widely accepted as innovative options to improve crop growth and productivity (Calvo et al., 2014a; Elzaawely et al., 2017; Van Oosten et al., 2017b; Yakhin et al., 2017; Desoky et al., 2018; Bello et al., 2021a; Bello et al., 2021b).

Capsicum annuum L. is an essential vegetable that belongs to the Solanaceae family. It remains one of the most important and universally cultivated vegetables globally owing to its high economic and health benefits (Abdelaal et al., 2020a). Bell pepper is

unarguably one of the richest vegetables in ascorbic acid content (Rao et al., 2011; Domínguez-Martínez et al., 2014; Endo et al., 2019; Papathanasiou et al., 2021; Rehman et al., 2021). Interestingly, it has been established that one fruit of bell pepper weighing 70 g or more can supply a daily necessary amount of vitamin C required for metabolic activity by humans (Marhoon et al., 2015). Also, a good reasonable amount of essential vitamin A, B1 and other vitamins suitable for growth are embedded in bell pepper (Marhoon et al., 2015; Awad-Allah et al., 2021; Mohamed et al., 2021). Synthetic chemical fertilizer and most inorganic commercial hydroponic nutrients are among the most productive and effective practices to manage crop production for a better yield. However, their potential adverse effects on the environment coupled with decrease in their efficiency after excessive and continuous applications have necessitated the need to restrict or drastically limit the use of chemical fertilizers and inorganic nutrients to come up with better, sustainable, and eco-friendly alternatives. Thus, organic fertilizers and eco-friendly practices for crop production and management viz. fertigation, pest control, etc. are the best options (Nair et al., 2020; Shah et al., 2021). The blue-green algae (BGA) such as cyanobacteria form essential constituents of the soil microflora that increase soil nutrient composition and productivity either directly or indirectly (Vaishampayan et al., 2001; Mishra et al., 2004; Shariatmadari et al., 2015). Different research studies have shown that the application of microalgae and cyanobacteria either as biomass (particle form) or extract (liquid form) contributes immensely in increasing nitrogen composition of the soil as well as growth-enhancing substances such as phytohormones, amino acids, phenolic compounds, and important trace elements found to be essential for plant health, development, and ion distribution (Obana et al., 2007; Cuellar-Bermudez et al., 2015; de Morais et al., 2015; Shariatmadari et al., 2015; Renuka et al., 2018). Also, the use of

algae and microalgae extract as biofertilizers through the foliar application will supply organic and natural plant hormones namely, auxin, auxin compounds, cytokinins, vitamins, and numerous macro and microelements that enhance plant development (El-Eslamboly et al., 2019). Naturally, cyanobacteria extract and biomass are seen as one of the novel sources used to attain sustainability and increase agricultural production owning to by gradually shifting away from using inorganic chemical fertilizers that are characterized by their numerous environmental issues. Furthermore, many studies have established the importance of cyanobacteria to improve the release of essential metabolites in plants (Saker et al., 2000; Shanab, 2001). Interestingly, the positive effects of microalgae and cyanobacteria extracts and biomass have been widely studied by numerous researchers/scholars as biostimulants and biofertilizer suitable for the production crops viz rice, maize, wheat, and many more (Barone et al., 2018; Ronga et al., 2019; Carillo et al., 2020; Colla et al., 2020), but more attention needs to be paid to the application and effect of cyanobacteria on vegetable particularly bell pepper considering its benefits to human. Thus, this study aims to investigate the effect of *Roholtiella* sp. as a bionutrient on bell pepper growth performance as well as on fruit yield and quality including nutrient composition under greenhouse large-scale hydroponic production system.

Material and Methods

Plant Material

Bell pepper (*Capsicum annuum* L.) seeds were first planted in the germination trays of seven by twelve cells making 84 cells. Each of the cells was filled with pot soil to raise the seedlings. They were irrigated with a commercial nutrient solution. The 30 days old seedlings were transplanted to a greenhouse under a hydroponic system (drip technique). The agricultural practices such as weeding, pruning, pest management, and fertigation, conformed with the commercially standardized practices and were executed as recommended by the local authorities for bell pepper cultivation

Cyanobacteria Strains Cultivation and Growth Conditions

Roholtiella sp. (QUCCC97) was obtained from Qatar University's Culture Collection of Cyanobacteria and Microalgae (QUCCCM), Doha, Qatar, and was originally isolated from Qatar soil (Saadaoui et al., 2016). The selection of the strain was based on its positive impacts on the seedlings of bell pepper and its pigments composition based on a previous study (Bello et al., 2021a). One single colony of the cyanobacteria strain was used to inoculate a 5 ml volume of BG11 growth medium (Stanier et al., 1971). It was subsequently incubated for 12 days at 30°C under the following conditions: a photon flux density of 100 μmol photons m^{-2} s^{-1} and a 12:12 h dark:light cycle with 150 rpm agitation using an illuminated shaker (Innova 44R, New Brunswick Scientific, USA). Then, the culture was gradually scaled up to 500 ml and incubated under the previously described conditions. Then, this culture was scaled up to 2 L then 10 L under room temperature, air bubbling, and the illumination of 400 μmol photons m^{-2} s^{-1} prior to being used to inoculate an open raceway pond of 200 L capacity. This pilot-scale cultivation of *Roholtiella* sp. was performed outdoor under Qatar climate. Environmental parameters from winter to spring were recorded for the duration of the experiment. After 12 days of outdoor incubation, the biomass was harvested by

centrifugation then freeze-dried. All cultures were performed in duplicate.

Preparation of Cyanobacteria (Roholtiella sp.*) Extract*

The preparation of cyanobacterial extract followed the method described previously by Bello et al. (2021). Briefly, the freeze-dried biomass gotten after cultivation explained earlier was divided equally into two portions. One part was re-suspended in water (WRB) which was used directly as a biofertilizer, and the second part underwent aqueous extraction to obtain the phycobiliproteins, a high-value product extract (HVPE). The methodology has been fully described in our earlier study conducted on the efficacy of cyanobacteria extract and biomass on bell pepper production (Bello et al., 2021a).

Analysis of Chemical Compositions of Cyanobacteria Extract and Biomass

In our previous study, cyanobacteria (*Roholtiella* sp.) was characterized morphologically, also through optical measurement of pigments, and molecular identification (Bello et al., 2021a). Furthermore, the chemical composition of this cyanobacterium (*Roholtiella* sp.) HVPE and WRB were analyzed using the ion chromatography method at the Central Laboratory Unit, Qatar University, Qatar. The analyzed chemical includes Sodium (Na^{+}), Ammonium (NH_4^{+}), Potassium (K^{+}), Calcium (Ca^{++}), Magnesium (Mg^{++}), Fluoride (F^{-}), Chloride (Cl^{-}), Nitrate (NO_3^{-}), Phosphate (PO_4^{---}), Sulphate (SO_4^{--}). This is considered necessary to further establish the presence of a considerable amount of mineral nutrients in both HVPE and WRB respectively.

Greenhouse Hydroponics Experiment and Growth Conditions

The experiment was carried out for approximately 180 days from seed germination to fruiting/harvesting. The seedlings were transplanted into white containers (25 x 25 x 30 cm), each per container a month after planting the seeds. The container is filled with

cocopeat as the substrate for plant support and provision of structure for root development. The temperature was maintained at a range of 21-26°C respectively in the greenhouse at Al Sulaiteen Agricultural & Industrial Complex, Umm Salal Ali, Qatar. The greenhouse hydroponic system is a drip technique that is fully automated with the adoption of a cooling system. The drip system's usefulness/importance/uniqueness exceeds that of other types of hydroponic systems because of its simplicity to operate, water use efficiency, and control (Bello et al., 2019). Thus, healthy seedlings with similar physical appearances and relatively same weight were considered for the propagation to commence the experiment and to ensure uniformity.

Treatments

Seven treatments were executed during the growing period. Tr1 to Tr3 (foliar application with Roholtiella sp. HVPE), Tr4 to Tr6 (foliar application with *Roholtiella* sp WRB), and Tr0 (foliar application with water as a control). Foliar spraying was done every week at the rate of 0.576 ml stroke^{-1} leaf^{-1} and thus, increased to two strokes as the leaves area increases which lasted for six weeks as summarized/enumerated in Table 11 below:

Table 11. The Concentrations of *Roholtiella* sp. Extracts (HVPE) and Water Re-Suspended Biomass (WRB)

Strain	Treatments/concentration
HVPE from *Roholtiella* sp.	Tr1— (2 mL L-1)—0.2% concentration (2 mL HVPE of *Roholtiella* sp.)
	Tr2— (4 mL L-1)—0.4% concentration (4 mL HVPE of *Roholtiella* sp.)
	Tr3— (6 mL L-1)—0.6% concentration (6 mL HVPE of *Roholtiella* sp.)
WRB from *Roholtiella* sp.	Tr4— (2 mL L-1)—0.2% concentration (2 mL WRB of *Roholtiella* sp.)
	Tr5— (4 mL L-1)—0.4% concentration (4 mL WRB of *Roholtiella* sp.)
	Tr6— (6 mL L-1)—0.6% concentration (6 mL WRB of *Roholtiella* sp.)
Control	Tr0- water

Vegetative/Physical Growth Parameters and Spad Index

Physical growth parameters were recorded at different times after transplanting. Randomly selected plants in each plot were considered and evaluated as follows: plant height (cm), number of leaves per plant, plant leaf length (cm), plant leaf width (cm), the diameter of the shoot, and SPAD index

Plant Height (cm) SL

The plant height of bell pepper was taken from the top of support assumed to be the base up to the topmost part where the leaves are fully opened by using a measuring scale, and then the average was recorded. The measurement was carried out at the commencement and completion of the experiment respectively.

Number of Leaves Per Plant - NL

The number of leaves in every plant was counted manually before the end of the experiment, just 20 days of the experiment

Plant Leaf Length and Width (cm)

The length of the leaf and width were measured using a measuring ruler. Fully expanded leaves were considered, the measuring runs from the base of the leaf to the tip. The average length and width were taken, thus, later entered into the spreadsheet.

Stem Diameter (mm)

The width of the stem was taken to determine the diameter of the plants. Stem width was measured (cm) manually with an electronic digital vernier caliper (Clarke CM145 Digital Vernier Caliper) at the 6th and 8th weeks after transplanting. The measurement was taken at a distance of 11 inch from the base to maintain uniformity

Spad Index - SI

The SPAD index of bell pepper leaf was measured using SPAD – 02, chlorophyll meter (Konica Minolta Optics, Osaka, Japan). The SPAD index measurement was carried out

before the harvest commences/termination of the experiment at the second week of the experiment when the leaves were very green and healthy.

Fruit Parameters

Length of Fruit (mm)

The length of the fruit was manually determined using an electronic digital vernier caliper (Clarke CM145 Digital Vernier Caliper) at every harvest for twelve weeks the harvest lasted, and the values were recorded at all times for further uses.

Diameter/Width of Fruit (mm)

Similarly, the width of the fruits was recorded concurrently in the same way as the fruit length using an electronic digital vernier caliper (Clarke CM145 Digital Vernier Caliper).

Output and its constituents

The output (yield) and its constituents are considered as mentioned below:

Total Yield Per Plant

The total weight of the fruits harvested was determined at the end of the experiment at the 12th week of cultivation using a measuring standard laboratory scale which was the summation of every harvest for the entire harvesting period. The summation of the weekly harvest was recorded in grams per fruit and this amounted to the total yield per plant.

Total Number of Fruits Per Plant

The counting of the fruit harvested per plant at every harvest (weekly number of fruits per plant) was manually counted and recorded in a spreadsheet. After the cultivation, the summation of the recorded values (weekly number of fruits per plant) was obtained as the total number of fruits per plant obtained based on the various treatments.

Average Fruit Weight (g) Plant^{-1}

This is computed by dividing the total weight of fruits (g) by the total number of fruits per plant

$$Average\ fruit\ weight\ (g) = \frac{\text{Total weight (g) of fruits plant}^{-1}}{\text{Total number of fruits plant}^{-1}} \quad (2)$$

Total Yield (Kg/Treatment)

The summation of the total yield of fruits per plant per treatment was recorded as the total yield in grams and converted to a kilogram per treatment.

Chemical Component Analyses/Fruit Nutritional Value

Total Soluble Solids (TSS in °Brix)

TSS is regarded as an index of soluble solids concentration in the fruit. Thus, each whole bell pepper was ground using Kenwood blender/mixer (Blend-X Compact Blender) until a homogeneous mixture was produced. Thereafter, about 45 g of subsample was extracted and centrifuged for 20 min at 1700 x g. To determine the TSS, the filtrate was then taken and placed on the handheld digital refractometer (ATAGO, USA Inc. Kirkland, WA, USA). Generally, the results were expressed as °Brix (AOAC, 2016; Continella et al., 2018; Cortés-Estrada et al., 2020).

Ascorbic Acid Determination (Xylene Assay)

Ascorbic acid (Vitamin C) was extracted through the xylene extraction method (Ranganna, 1986). The concentration was estimated from the absorbance measured at 520nm using a Jenway 6715 UV/Visible Scanning Spectrophotometer, 1.5-nm Bandwidth.

Standard curve: Six test tubes were prepared with an ascorbic standard solution of 0.0, 0.5, 0.75, 1, 1.5, and 2 mL in 3% Orthophosphoric acid H_3PO_3 (100 µg mL^{-1}) and

makeup to 2 ml of 3% H_3PO_3 solution. Thereafter, 2 ml of acetate buffer (pH 4) was added followed by adding 3 ml of 3,6, dichlorophenol indophenol reagent, and 15 ml xylene solution simultaneously in quick succession. The capped tubes were vortexed for 10-15 s to create phase separation. The xylene topmost phase was extracted and absorbance was measured spectrophotometrically at a wavelength of 520 nm using xylene as blank (Domínguez-Martínez et al., 2014). Sample analysis/extraction procedure: Bell pepper (100g) was macerated in 3% H_3PO_3 and the volume make up to 2000 µl. Followed by the filtration of the solution, transferring 2 ml of aliquot to a test tube, adding one after the other 2 ml of acetate buffer (pH 4), 3 ml of 2,6 dichlorophenol indophenol (7×10^{-4} M), and 15 ml xylene in quick succession. Thereafter, the capped tube was vortexed for 10-15 s. The topmost xylene phase was extracted, and absorbance was taken at 520 nm. The sample absorbance was read against the blank prepared in a similar way above but substituting 2,6 dichlorophenol indophenol with distilled water instead. The ascorbic acid content was denoted as mg acid 100 g^{-1} sample (Deepa et al., 2007; Beltrán-Orozco et al., 2009).

Total Phenolic Compound

Standard curve: Solutions containing 0, 20, 40, 60, 80, and 100 mg L^{-1} gallic acid standard were prepared respectively. 0.1 ml were transferred to each of the test tubes adding 0.1 ml deionized water, 1 ml Folin-Ciocalteau reagent, and 0.8 ml of 7.5% soda crystals (Na_2CO_3). The tubes were vortexed, and the final solution is incubated for 30 min in the dark at room temperature. The absorbance of the solution was measured at 765 nm spectrophotometrically (Jenway 6715 UV/Visible Scanning Spectrophotometer, 1.5-nm Bandwidth) against blank prepared in the same way but replacing Folin-Ciocalteau with distilled water (Domínguez-Martínez et al., 2014)

Sample analysis/extraction procedure: Bell pepper (2.0 g) was macerated in and

extracted with 80 % methanol, vortex for 50 to 60 secs 3 times, then centrifuged for 15 mins at 500 rpm. Thereafter, the final mixture was separated by simple decantation. The supernatant/solvent of the combined extract was concentrated in a flash evaporator to obtain a final volume of 10 ml (Domínguez-Martínez et al., 2014).

Determination of Phenolic compound: To determine the total phenolic compound, 2 ml of the extract was pipetted into the test tube, adding 1 ml Folin-Ciocalteau reagent, 2 ml Na_2CO_3 (20%), and 2.5 ml distilled water. The final solution is incubated for 30 min in the dark at room temperature. Subsequently, the absorbance of the solution was measured at 765 nm spectrophotometrically (Jenway 6715 UV/Visible Scanning Spectrophotometer, 1.5-nm Bandwidth) against blank prepared in the same way but substituting Folin-Ciocalteau with distilled water. The total phenolic compound was computed through the phenolic standard curve $(y = aX + C)$. The results were presented in gallic acid equivalents (GAE) per gram of fresh weight (µg GAE g^{-1} fw)

Experimental Design and Data Analysis

A randomized complete block design (RCBD) experiments' layout was put in place, with seven blocks. Each block is a replicate and each replicate is equivalent to the seven plots at 5 plants per plot. The analysis of variance (ANOVA) was used to analyze the data using a statistical tool. Additionally, Tukey's Honest Significant Difference test was conducted to establish the range distribution as a post-doc test, thus comparing the means within and among treatments. Also, Pearson's correlation matrix was calculated to determine the interaction or relationship between plant vegetative growth factors, biochemical parameters, yield, and its components, phenolic compound, and ascorbic acid contents of bell pepper cultivated in hydroponic systems under *Roholtiella* sp. HVPE and WRB treatment respectively as a growth enhancer/promoter (biofertilizer)

Results

Blue-Green Algae (Cyanobacteria - Roholtiella sp.*)*

Cyanobacteria Chemical Characterization

Roholtiella sp. is one of three promising cyanobacteria strains previously investigated aimed to evaluate their growth-enhancing abilities (Bello et al., 2021a). Among them, *Roholtiella* sp. was selected for further analysis based on its better performance. However, the elemental composition of *Roholtiella* sp extract and biomass evidence the availability of a considerable amount of both anions and cations respectively as shown in Table 12. Interestingly, these results are supported by those of Saadaoui et al. (2019), who reported the chemical composition of algae.

Table 12. Chemical Content of Cyanobacteria *Roholtiella* sp. Extracts (HVPE) and Biomass (WRB). Mean that Shared the Same Letter(s) are Significantly Different at 5% Significant Level

Anions/Cations (ppm)	Analytical method	HVPE	WRB
Sodium (Na+),	Ion chromatography	2.379	9.501
Ammonium NH4+),		0.674	0.641
Potassium (K+),		8.533	0.541
Calcium (Ca++),		1.777	0.594
Magnesium (Mg++),		3.483	0.255
Fluoride (F-),		0.0191	0.02
Chloride (Cl-),		3.168	1.192
Nitrate (NO3-),		5.247	8.071
Phosphate (PO43-),		13.67	3.266
Sulphate (SO42-).		0.212	0.12

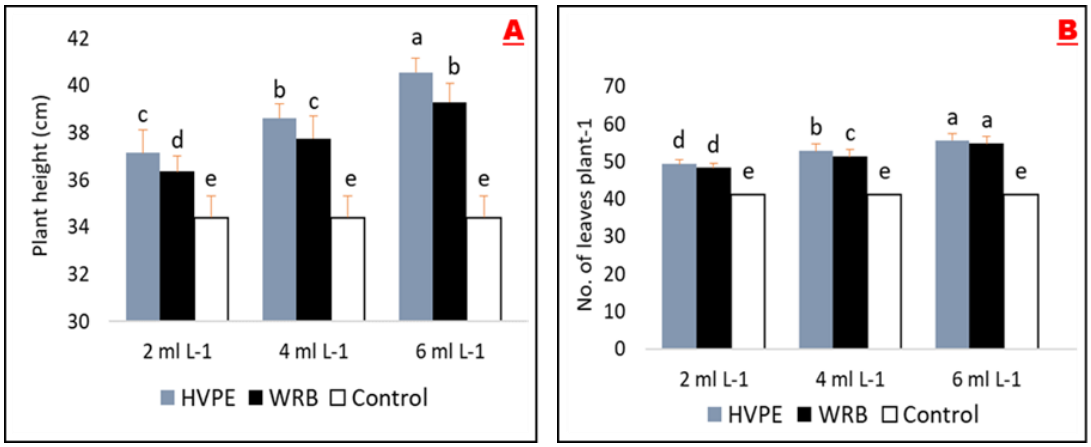
Impacts of Roholtiella sp. *HVPE and WRB Treatments on the Biometric/Vegetative/Physical Growth Parameters and Spad Index.*

As shown in Figures 20 A - F and Table 13 that the bell pepper plant height, the number of leaves, stem diameter, and SPAD index are significantly increased compared to the characteristics of the control group in the cultivation period/grown season. All the treatments applied exhibited a significant difference in all the vegetative parameters and SPAD index. The *Roholtiella* sp HVPE at 6 ml L^{-1} (Tr3) recorded the highest plant height with an increase of 15.15%. Also, bell pepper treated with 4 ml L^{-1} (Tr2) and 2 ml L^{-1} (Tr1) HVPE concentrations showed increased plant height by 10.93%, and 7.37% respectively compared with the control group. Similarly, the various concentrations of WRB, 6 ml L^{-1} (Tr3), 4 ml L^{-1} (Tr2), and 2 ml L^{-1} (Tr1) exhibited a significant increase in shoot length by 12.43%, 8.87%, and 5.38 % respectively when compared to the control. Furthermore, HVPE treatments 6 ml L^{-1} (Tr3), 4 ml L^{-1} (Tr2), and 2 ml L^{-1} (Tr1) exhibited a significant increase in the number of leaves by 26.09%, 22.16%, and 16.68%, respectively when compared to the control groups. In the same vein, WTB concentrations of 6 ml L^{-1} (Tr3), 4 ml L^{-1} (Tr2), and 2 ml L^{-1} (Tr1) showed a significant increase in the number of leaves by 25.00%, 19.78%, and 14.79% respectively when compared with control. Likewise, as compared to the control group, *Roholtiella* sp HVPE and WRB exhibited a significant increase in the leaf length and width respectively. Bell pepper treated with 6 mL L^{-1} (Tr3), 4 mL L^{-1} (Tr2), 2 mL L^{-1} (Tr1) HVPE concentrations showed a significant increase plant leaf length by 24.36%, 15.58%, 4.66% and with the biomass by 23.36%, 11.02%, 4.83%. Similarly, treatments with 6 mL L^{-1} (Tr3), 4 mL L^{-1} (Tr2), 2 mL L^{-1} (Tr1) HVPE concentrations showed a significant increase in plant leaf width by 41.12%, 20.00% and 17.20%, and for WRB treatments at the same concentration by 31.40%, 20.45%, 15.98% respectively. Also,

HVPE treatments followed the same trend by showing a significant increase in the diameter of the shoot by 16.49%, 13.72%, and 12.29%, and spad index by 9.36%, 4.84%, and 4.73% respectively. Correspondingly to WRB as well as the diameter of the shoot increased by 15.33%, 13.90%, and 6.21%, and spad index by 7.40%, 4.69%, and 2.22% when compared with the control group.

Table 13. Effect of *Roholtiella* sp. HYPE and WRB on the Plant Height (cm), Number of Leaves, Length of Leaf (cm), Leaf Width (cm), Stem Diameter (mm), and SPAD Index (Mean).

Treatments		Concentration ml L^{-1}	Groth parameter and Spad Index					
			PH	NL	LL	LW	SD	SI
HVPE	Tr1	2 ml L^{-1}	37.143c	49.381d	8.0619d	4.9c	9.578ab	62.781c
	Tr2	4 ml L^{-1}	38.629b	52.857b	9.1048b	5.0714c	9.737a	62.852c
	Tr3	6 ml L^{-1}	40.548a	55.667a	10.1619a	6.8905a	10.06a	65.986a
WRB	Tr4	2 ml L^{-1}	36.362d	48.286d	8.0762d	4.8286c	8.957bg	61.171d
	Tr5	4 ml L^{-1}	37.752c	51.286c	8.6381c	5.1c	9.757a	62.752c
	Tr6	6 ml L^{-1}	39.29b	54.857a	10.0286a	5.9143b	9.922a	64.59b
Control	Tr0	Water	34.405e	41.143e	7.686e	4.0571d	8.401c	59.81e



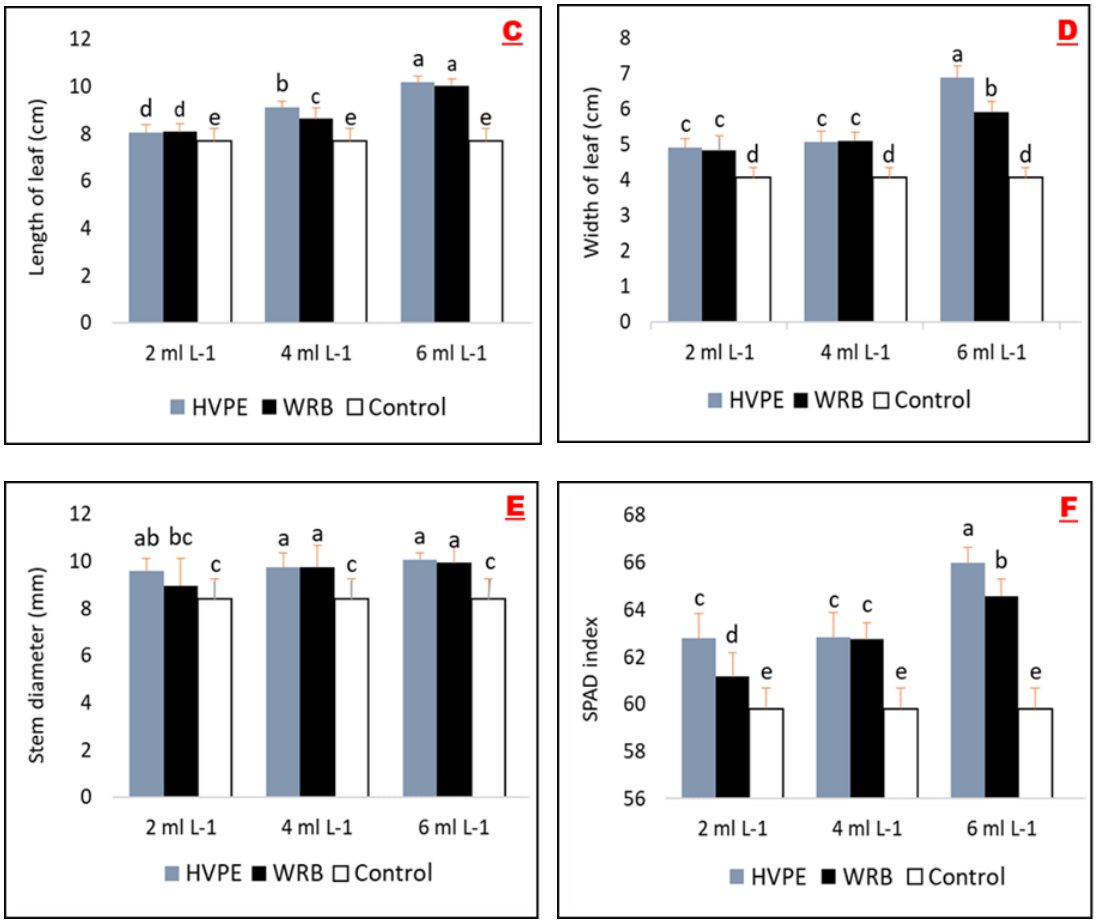


Figure 20. Plant height, number of leaves, length of leaf, the width of leaf, stem diameter, and spad index **(A–F)** of bell pepper untreated plants (Tr0), treated with 2 mL L^{-1} (Tr1), 4 mL L^{-1} (Tr2), 6 mL L^{-1} (Tr3) of the HVPE. Additionally, with 2 mL L^{-1} (Tr4), 4 mL L^{-1} (Tr5), 6 mL L^{-1} (Tr6) of the WRB. Plant height **(A)**, Number of leaves $plant^{-1}$ **(B)**, Length of the leaf **(C),** Width of the leaf **(D**), Stem Diameter **(E)**, SPAD Index **(F)**.

Impacts of Rohotiella sp on HVPE and WRB Treatments on the Fruit Characteristics

Figure 21 (A – B) and Table 14 showed that the fruit length and width (diameter) of bell pepper had significant increases than the characteristics exhibited by the control group with all the treatments.

Table 14. Effect of *Roholtiella s*p. HVPE and WRB on the Fruit Length (mm) and Fruit Width (mm). Mean that Shared the Same Letter(s) are Significantly Different at 5% Significant Level

Treatments		Concentration (mg L-1)	Fruit Characteristics	
			FL	FW
HVPE	Tr1	1 (2 ml L-1)	74.1cd	58.969b
	Tr2	2 (4 ml L-1)	75.179b	60.063b
	Tr3	3 (6 ml L-1)	81.048a	62.287a
WRB	Tr4	1 (2 ml L-1)	70.981e	55.213d
	Tr5	2 (4 ml L-1)	73.114d	59.892c
	Tr6	3 (6 ml L-1)	74.52bc	61.967a
Control	Tr0	Water	61.184f	49.409e

However, the highest fruit length with HVPE treatment was observed with the concentration of 2 ml^{-1} (Tr1), 4 ml^{-1} (Tr2), and 6 ml^{-1} (Tr3) respectively at an increase of 17.43%, 18.62%, and 24.51% when compared with the group control. Interestingly, the trend was similar with the fruit width at the same concentrations of 2 ml^{-1} (Tr1), 4 ml^{-1} (Tr2), and 6 ml^{-1} (Tr3) when compared with the control group. The fruit width is thus increased by 13.08%, 16.32%, and 17.90%. Similarly, the fruit length and width increased by 16.21, 17.74, 20.68%, and 10.51, 17.50, 20.27%, respectively with WRB at the same concentrations as compared with the control group.

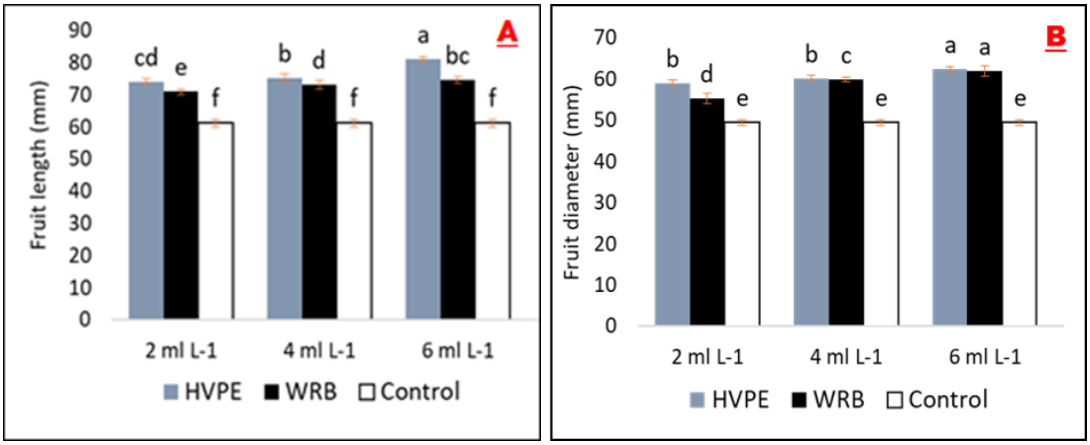


Figure 21. Fruit length and width (diameter) **(A- B)** of bell pepper untreated plants (Tr0), treated with 2 mL L^{-1} (Tr1), 4 mL L^{-1} (Tr2), 6 mL L^{-1} (Tr3) of the HVPE. Additionally, with 2 mL L^{-1} (Tr4), 4 mL L^{-1} (Tr5), 6 mL L^{-1} (Tr6) of the WRB. Fruit Length **(A)** and Fruit Diameter **(B)**

Impacts of Rohotiella sp. *HVPE and WRB Treatments on the Yield Components and Total Yield*

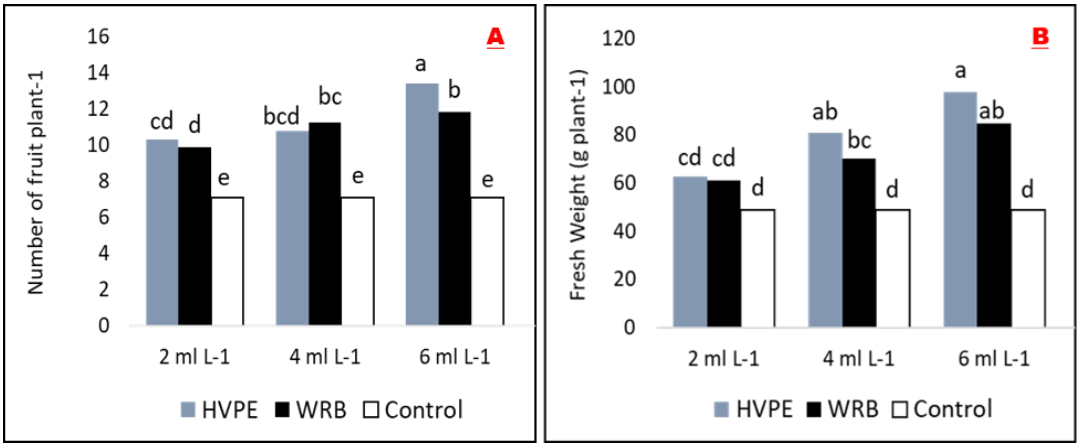
It is clear from the obtained results in Table 15 and Figure 22 (Castro-Puyana et al.) that the number of fruits $plant^{-1}$, fresh weight of fruit $plant^{-1}$, and total yield were significantly increased at all levels of concentration of *Roholtiella* sp HVPE and WRB when compared with the control group. The number of fruits $plant^{-1}$ increased significantly with the various concentrations of *Roholtiella* sp HVPE when compared to the control group. Thus, the concentration of 2 ml^{-1} (Tr1), followed by 4 ml^{-1} (Tr2), and 6 ml^{-1} (Tr3) increased the number of fruits $plant^{-1}$ by 31.34%, 34.37%, and 47.17%, respectively compared to the control group. Equally, these different concentrations of biomass caused significant increases in the number of fruits $plant^{-1}$ by 28.37%, 36.87, and 40.16% compared with the control group.

In a like manner, at the same concentrations of *Roholtiella* sp HVPE, the bell pepper showed a significant increase in the fruit weight $plant^{-1}$ and total yield by 21.60%,

39.23%, 49.82%, and 45.43%, 60.09%, and 73.35%. The treatments showed a similar trend with WRB treatment at the same concentrations for the fruit weight plant^{-1} and total yield by 19.82%, 30.25%, 42.23%, and 42.15%, 55.52%, 64.98% respectively when compared with the control group.

Table 15. Effect of *Roholtiella* sp. HVPE and WRB on the Number of Fruit Plant^{-1}, Fresh Weight Fruit^{-1}, and Total Fruit Yield in Kg Per Treatment (Mean). Mean that Do Not Shared the Same Letter(s) are Significantly Different at 5% Significant Level

Treatments		Concentration ml L^{-1}	Fruit yield and its components		
			NF plant^{-1}	TFW fruit^{-1}	TFY (Kg/treatment)
HVPE	Tr1	2 ml L^{-1}	13.429cd	62.45 cd	0.642c
	Tr2	4 ml L^{-1}	10.81bcd	80.56 ab	0.878b
	Tr3	6 ml L^{-1}	13.429a	97.56 a	1.3149a
WRB	Tr4	2 ml L^{-1}	9.905d	61.06 cd	0.6056c
	Tr5	4 ml L^{-1}	11.238bc	70.19 bc	0.7877bc
	Tr6	6 ml L^{-1}	11.857b	84.75 ab	1.0005b
Control	Tr0	Water	7.095e	48.96 d	0.3504d



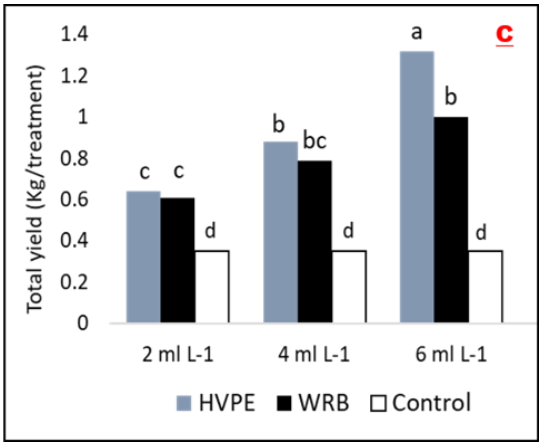


Figure 22. The number of fruits plant^{-1}, fresh weight of fruit plant^{-1}, and the total yield **(A–C)** of bell pepper untreated plants (Tr0), treated with 2 mL L^{-1} (Tr1), 4 mL L^{-1} (Tr2), 6 mL L^{-1} (Tr3) of the HVPE. Additionally, with 2 mL L^{-1} (Tr4), 4 mL L^{-1} (Tr5), 6 mL L^{-1} (Tr6) of the WRB. Number of fruit plant $^{-1}$ **(A)**; Fresh Weight **(B),** and Total yield **(C)**

increased the number of fruits plant^{-1} by 31.34%, 34.37%, and 47.17%, respectively compared to the control group. Equally, these different concentrations of biomass caused significant increases in the number of fruits plant^{-1} by 28.37%, 36.87, and 40.16% compared with the control group.

In a like manner, at the same concentrations of *Roholtiella* sp HVPE, the bell pepper showed a significant increase in the fruit weight plant^{-1} and total yield by 21.60%, 39.23%, 49.82%, and 45.43%, 60.09%, and 73.35%. The treatments showed a similar trend with WRB treatment at the same concentrations for the fruit weight plant^{-1} and total yield by 19.82%, 30.25%, 42.23%, and 42.15%, 55.52%, 64.98% respectively when compared with the control group.

Impacts of Rohotiella sp. *HVPE and WRB Treatments on Chemical Component Analyses/Fruit Nutritional Value*

As shown in Figure 23 A-C and Table 16, the fruit nutritional value viz the total soluble solids (TSS), ascorbic acid, and total phenolic acid significantly increased with the treatments compared to the control group. From our results, the different concentrations of *Roholtiella* sp. HVPE and WRB 2 mL L^{-1} (Tr1), 4 mL L^{-1} (Tr2), 6 mL L^{-1} (Tr3) have a significant effect on the TSS content of bell pepper fruit except at the minimum concentrations for both HVPE and WRB [2 mL L^{-1} (Tr1)] respectively that the change was not significant. The total soluble solids significantly increased with the various concentrations of *Roholtiella* sp HVPE compared to the control group. Consequently, the concentration of 2 ml L^{-1} (Tr1), followed by 4 ml L^{-1} (Tr2), and 6 ml L^{-1} (Tr3) respectively increased the total soluble solids of fruits by12.20% (3.459 °Brix), 18.54% (3.728 °Brix), and 25.20% (4.06 °Brix) compared to the control group (3.037 °Brix). The pattern is similar with the biomass treatment as TSS increased by11.33% (3.425 °Brix), 20.70% (3.83 °Brix), and 22.33% (3.91 °Brix) compared with the control group (3.037 °Brix) as shown in Figure 23A and Table 16

Ascorbic Acid

From our results, as shown in Figure 21B and Table 18, the ascorbic acid content in the fruits of bell pepper studies under different treatments indicates a significant difference from the control group. The ascorbic content (vitamin c) in the fruits treated with 2 ml^{-1} (Tr1) *Rohotiella* sp. HVPE (133.74 mg 100 g^{-1} fw), per 100 mg fresh weight, accounts for a significant increase of 0.89% compared to the control group. Similarly, at the concentrations of 4 mL L^{-1} (141.42 mg 100 g^{-1} fw) and 6 mL L^{-1} (145.10 mg 100 g^{-1} fw) there is a significant increase in the ascorbic acid content by 6.27% and 8.65% respectively compared to the control group (132.55 mg 100 g^{-1} fw). In a like manner, the trend followed the same pattern with the WRB treatments with a significant increase

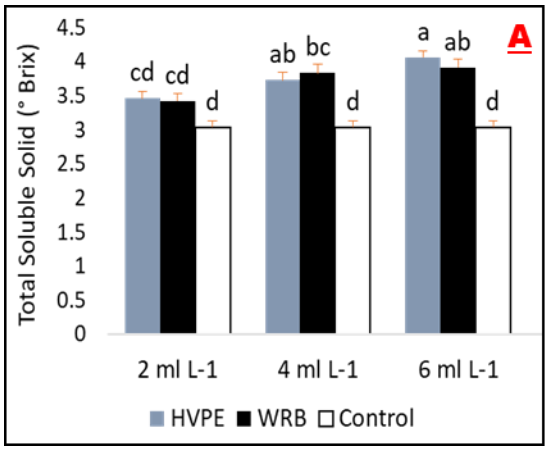
of 0.37% with 2 mL L^{-1} (133.04 mg 100 g^{-1} fw), 4.73% with 4 mL L^{-1} (139.12 mg 100 g^{-1} fw), and 8.53% with 6 mL L^{-1} (144.90 mg 100 g^{-1} fw) compared with the control group (132.55 mg 100 g^{-1} fw). However, fresh bell peppers are a good source of ascorbic acid and they are so rich in this content irrespective of their pigments (green, red, or orange) ranging from 76 - 243 mg 100 g^{-1} fresh weight (Howard et al., 1994; Deepa et al., 2007).

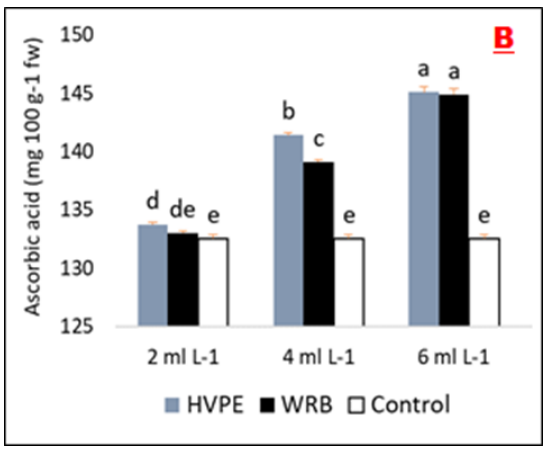
Total Phenolic Compounds

The findings on the total phenolic contents of bell pepper are shown in Figure 21C. There is variation in the total phenolic concentration in the bell pepper as influenced by different treatments. At the highest concentration of 2 mL L^{-1} (1286.96 µg GAE g^{-1} fw)of *Roholtiella* sp HVPE, there is a significant increase of 0.26%, 4 mL L^{-1} (1301 µg GAE g^{-1} fw) by 1.33%- and 6 mL L^{-1} (1321.23 µg GAE g^{-1} fw) by 2.85% respectively. Furthermore, the trend is the same with treatments using WRB at the same concentration (1284.06 µg GAE g^{-1} fw), (1292.34 µg GAE g^{-1} fw), and (1313.46 µg GAE g^{-1} fw) as the phenolic concentration increased by 0.03%, 0.67%, and 2.27% compared with the group control (1283.64 µg GAE g^{-1} fw).

Table 16. Effect of *Roholtiella* sp. HVPE and WRB on the Total Soluble Solids (TSS), Ascorbic Acid (AA), and Phenolic Content (PC) of Bell Pepper Fruits (Mean ± Standard Deviation). Means that Do Not Share a Letter are Significantly Different Based on Tukey Pairwise Comparisons of Treatments. this Implies that Different Letters (A–E) Indicate Significantly Different Values in Response to the Different Treatments (Seven Replicates Per Treatment)

. Strains	Treatments	Con. ml L^{-1}	TSS (°Brix)	Ascorbic (mg 100 g-1 fw)	Phenolic (µg GAE g-1 FW)
Roholtiella sp	HVPE	2 ml L^{-1}	34.59±0.98 cd	133.742±0.17 d	1286.96±2.15 c
		4 ml L^{-1}	37.28±1.22 ab	141.415±0.17 b	1301±0 bc
		6 ml L^{-1}	40.6±0.91 a	145.102±0.46 a	1321.33±7.17 a
	WRB	2 ml L^{-1}	34.25±1.11 cd	133.044±0.17 de	1284.06±7.17 c
		4 ml L^{-1}	38.3±1.29 bc	139.123±0.17 3 c	1292.34±7.17 c
		6 ml L^{-1}	39.1±1.31 a	144.903±0.52 a	1313.46±11.8 1 ab
	Control	Water	30.37±0.94 d	132.546±0.35 e	1283.64±6.84 c





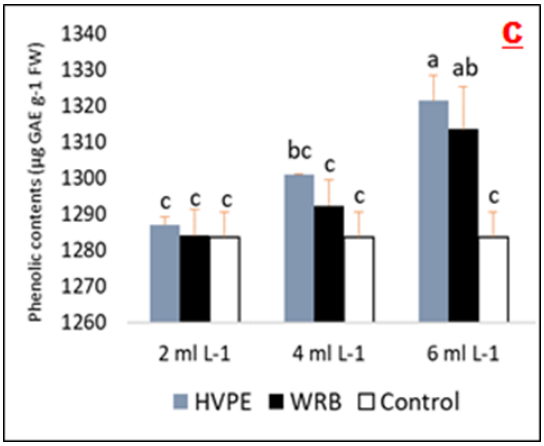


Figure 23. Total soluble solids, ascorbic acid, and the total phenolic content **(A-C)** of bell pepper untreated plants (Tr0), treated with 2 mL L^{-1} (Tr1), 4 mL L^{-1} (Tr2), 6 mL L^{-1} (Tr3) of the *Roholtiella* sp HVPE. Additionally, with 2 mL L^{-1} (Tr1), 4 mL L^{-1} (Tr2), 6 mL L^{-1} (Tr3) of the *Roholtiella* sp WRB. Total Soluble Solid **(A)**; Ascorbic Acid **(B)**; Phenolic Content **(C)**.

Pearson's Correlation Studies.

In the present work as shown in Table 17 chlorophyll content (spad index) showed a positive and significant correlation with shoot height (r = 0.954), number of leaves (r = 0.918), width of leaves (r = 0.892), and fruits weight (r = 0.892) as well. In a like manner, the relationship trend among the growth vegetative parameter chlorophyll content and fruit weight $plant^{-1}$, as well as the total soluble solids, are positive and significantly correlated; fruit weight $plant^{-1}$ (r = 0.991), and total soluble solids (r = 0.874) respectively. Also, a positive correlation was recorded between the phenolic compound and SH = Shoot heigh (r = 0.983), NL = Number of leaves (r = 0.981), SI = Spad index (r = 0.946), SD = Shoot diameter (r = 0.82). Furthermore, the ascorbic acid exhibited a highly positive correlation with FW = Fruit weight (r = 0.898), LL = Leaf length (r = 0.973), LW = Leaf width (r = 0.985), and Total fruit yield Kg per treatment, (r = 0.906). Among all the studies, a very similar values of correlation were recorded

Table 17. Pearson's Correlation Coefficient of Plant Vegetative Growth Factors, Biochemical Parameter, Yield, and its Components, Phenolic Compound, and Ascorbic Acid Contents of Bell Pepper (*Capsicum annuum* L) Foliar Sprayed with Cyanobacteria (*Roholtiella* sp.) HVPE and WRB under Hydroponic Systems.

Traits	SH	NL	SI	SD	FL	FW	LL	LW	NFP	FWP	TFY	TSS	PA	AS
SH	1													
NL	0.981*	1												
SI	0.954*	0.918*	1											
SD	0.883*	0.885*	0.881*	1										
FL	0.897*	0.795	0.902*	0.771	1									
FW	0.908*	0.921*	0.908*	0.991*	0.763	1								
LL	0.959*	0.984*	0.905*	0.803	0.754	0.857*	1							
LW	0.959*	0.989*	0.892*	0.814*	0.744	0.864*	0.998*	1						
NFP	0.506	0.402	0.699	0.63	0.697	0.613	0.344	0.317	1					
FWP	0.99*	0.972*	0.916*	0.817*	0.878*	0.846*	0.963*	0.964*	0.402	1				
TFY	0.981*	0.944*	0.945*	0.809	0.915*	0.832*	0.94*	0.934*	0.481	0.985*	1			
TSS	0.925*	0.94*	0.874*	0.874*	0.75	0.884*	0.916*	0.925*	0.351	0.915*	0.921*	1		
PA	0.983*	0.981*	0.946*	0.82*	0.841*	0.866*	0.989*	0.983*	0.447	0.983*	0.972*	0.906*	1	
AS	0.947*	0.989*	0.858*	0.862*	0.712	0.898*	0.973*	0.985*	0.282	0.946*	0.906*	0.949*	0.95*	1

SH = Shoot height, NL = Number of leaves, SI = Spad index, SD = Shoot diameter, FL

= Fruit length, FW = Fruit weight, LL = Leaf length, LW = Leaf weight, NFP = Number of fruit plant^{-1}, FWP = Fruit weight plant^{-1}, TFY = Total fuit yield kg treatment^{-1}, TSS = Total soluble solids, PA = Phenolic compound, AS = Ascorbic acid. * Correlation is significant at the $p < 0.05$ (5% significant level)

CHAPTER 5 APPLICATION OF CYANOBACTERIA (*Roholtiella* sp.) LIQUID EXTRACT FOR THE ALLEVIATION OF SALT STRESS IN BELL PEPPER (*Capsicum annuum* L.) PLANTS GROWN IN A SOILLESS SYSTEM

Introduction

Several abiotic stresses viz drought (water stress), excessive water accumulation (waterlogging), extreme temperatures (cold, frost, and heat), and salinity, etc. are often responsible for poor crop production (Ronga et al., 2019). Thus, salinity is one of the serious and increasing challenges mitigating optimum crop production, particularly the quality and quantity (yield) of the crop in arid and semi-arid countries (Zörb et al., 2019; Bayona-Morcillo et al., 2020). Globally, over 9.0×10^2 million hectares of land are affected by salination, which constitutes one-fifth of total cultivable land (Arroussi et al., 2018; Ronga et al., 2019). Consequently, there is an urgent need for proactive measures to improve plant productivity and crop output under salination conditions to solve the problem of increasing food demand of the world population (Lynch, 2019; Pereira et al., 2020; Ding et al., 2021; Hafez et al., 2021). The incidence of abiotic stress has been on increase lately because of global warming leading to the persistent rise in unfavorable weather conditions (Battacharyya et al., 2015). However, several studies have established a deleterious effect of the increasing rate of soil salinity on crop productivity globally (Kumar et al., 2020a; Kumar et al., 2020b). Accumulation of salt may lead to an alteration in physiological, molecular, metabolic activities, nutrient deficiency, ion toxicity, and water deficit or water potential reduction (Wang et al., 2018b; Bistgani et al., 2019; Zörb et al., 2019; Kumar et al., 2020a; Kumar et al., 2020b; Kumar et al., 2021). Also, in other studies, it has been demonstrated that the impact of toxicity due to higher concentration of salt in soil may be regulated or conciliated through the production of antioxidants as well as hampering or obstructing the

generation of reactive oxygen species (ROS) (Abbas et al., 2019; Kumar et al., 2021). Naturally, the sensitivity of plants to salination is general and predominant in respective of the type of plant/crop simply because there is a likelihood of disruption in the interaction of the natural microbes in soil and inhibit the growth of microbial in their natural ecosystem (Barnawal et al., 2014; Pereira et al., 2020).

Microalgae, viz. cyanobacteria, and eukaryotic algae can manufacture their foods using radiant energy, thus they are photoautotrophic microorganisms. The application of microalgae as biostimulants and biofertilizers is gaining popularity as a potential and sustainable alternative to the inorganic fertilizer with a wider acceptance by farmers and agrochemical industries (Bello et al., 2021a). Interestingly, several studies have established that microalgae exhibits biostimulants properties that enhance the resistance of crops to the deleterious impacts of abiotic stress mostly salt stress (Ronga et al., 2019; Bello et al., 2021b). Similarly, wastewater may be used as a source of raw material in microalgae production because of its richness in organic nutrients, consequently, minimizing the usual huge cost of production (Acién et al., 2016; Bayona-Morcillo et al., 2020). Naturally, algae extracts may have a positive impact to alleviate abiotic stresses in plants by concentrating/targeting different pathways (Van Oosten et al., 2017b).

Bell pepper (*Capsicum annuum* L.) is an essential vegetable crop because of its economic value and health benefits, thus, it is cultivated across all the continents of the world. Bell pepper (*Capsicum annuum* L.) fruits contain ascorbic acid as well as lycopene, a worthy or treasured compound containing anti-oxidant and anti-cancer characteristics. Thus, its cultivation, usefulness, and consumption are rising every year (Deepa et al., 2007; Almadhoun, 2021). However, bell pepper is believed to have an adaptable potential to harsh climatic change, still, rising soil and water salination hinder

the development, output, and fruit quality of fruit, thus leading to a huge reduction in productivity (Soliman et al., 2018; Elkeilsh et al., 2019; Abdelaal et al., 2020a). In an attempt to mitigate the negative effects engendered by salinity stresses researchers have undertaken different measures such as using marine resources such as macro and microalgae as elicitors to enhance crop production (Sadasivam et al., 2017)

This study is an advancement on the previous study (Bello et al., 2021a) conducted to screen three unidentified cyanobacteria strains provided by the center for sustainable development (CSD) in which the most effective strain *Roholtiella* sp was selected to further investigate its potential benefit as a stress alleviator. However, up till now, little research has been conducted to describe the impact of the application of cyanobacteria extract on plant performance, productivity, and salt stress alleviation, thus, to the best of our understanding, this study was never carried out before in bell pepper (*Capsicum annuum L.*) under a salt-affected soilless system using *Roholtiella* spp strain. Therefore, the novelty of this investigation was to establish the positive impact of *Roholtiella* spp extract (foliar application/spraying) alleviation of bell pepper plant under salinity stress. Nonetheless, the significant discovery/result could be extended to other crops as a model and for agricultural farming.

Material and Methods

Plant Material, Experiment Preparation of the Soilless System, and Salt Stress Application

The hybrid bell pepper (*Capsicum annuum* L.) was procured from the certified commercial seed supplier Technical Agricultural Company, Doha, Qatar. Surface sterilization of the seeds was facilitated with 5% NaClO (Sodium hypochlorite) for 5 min and subsequently thoroughly rinsed with deionized water. However, the seeds were first germinated in the germination boxes inside the greenhouse at the Department of Biological and Environmental, Qatar University. After 30 days, healthy seedlings were transferred into a 192 mL glass vase containing Hoagland solution as media in a deep-water culture system hydroponically (soilless culture) Figure 24.

Standard nutrient solution with concentration in g L^{-1} was prepared from $Ca(NO_3)_2 \cdot 4H_2O$, 1.250 g L^{-1} ; KNO_3, 0.410 g L^{-1} ; $NH_4H_2PO_4$, 0.280 g L^{-1} ; $MgCl_2 \cdot 6H_2O$, 0.624 g L^{-1} ; $FeSO_4 \cdot 7H_2O$, 0.060 g L^{-1} ; EDTA-Na_2, 0.080 g L^{-1} ; H_3BO_3, 0.006 g L^{-1} ; $MnCl_2 \cdot 4H_2O$, 0.04 g L^{-1}; $ZnSO_4 \cdot 7H_2O$, 4×10^{-5} g L^{-1} , and $CuSO_4 \cdot 5H_2O$, 4×10^{-5} g L^{-1}. The pH of the final solution was adjusted to 6.0±0.5 (Kumar et al., 2021). After 30 days, healthy seedlings were transferred into a 192 mL glass vase (one plant per vase) containing the final solution as media in a deep-water culture system (soilless culture) Fig 24.

Figure 24. The experimental design and morphological variation of induced NaCl bell pepper plants **1.** Water sprayed, and **2.** *Roholtiella* sp extract sprayed induced plants after 10 days a: 0 Mm induced plant, b: 50 mM NaCl induced plants, c: 100 mM NaCl induced plants, d: 150 mM NaCl induced plants, and e: 200 mM NaCl induced plants

Subsequently, sodium chloride NaCl was added at different concentrations from 50, 100, 150, and 200 mM and 0 mM (Control) 2 days after transplanting to allow acclimatization. The experiment was maintained under optimal conditions with natural light conditions of day length of 12 h and a controlled light environment of 12 h (12h light / 12h dark photoperiod) in the greenhouse.

Cyanobacteria Strain Cultivation and Growth Conditions

A freshwater filamentous and N-fixing cyanobacteria namely, *Roholtiella* sp. (QUCCCM97) was selected for this study based on the enhancement potential earlier established on bell pepper (*Capsicum annuum* L.) in a previous study conducted (Bello et al., 2021a). The strain, isolated from the Qatar desert, belongs to the Qatar University

Culture Collection of Cyanobacteria and Microalgae (QUCCCM) (Saadaoui et al., 2016). The cultivation of *Roholtiella* sp. (QUCCCM97) was performed as described by Bello et al (Bello et al., 2021a). One single colony of the cyanobacteria strain was used to inoculate a 5 ml volume of BG11 growth medium (Stanier et al., 1971). Thereafter, incubated for 7 days at 30°C, a photon flux density of 1.0 x 10^{-4} mol photons m^{-2} s^{-1} and a 12:12 h dark: light cycle with 150 rpm agitation using an illuminated shaker (Innova 44R, New Brunswick Scientific, USA). However, the scale-up of the culture to 500 ml was gradually attained and incubated under the previously described conditions. Furthermore, an adequate volume was used to inoculate a DASGIP parallel 1L bioreactor system for phototrophic cultivation (#76DG08PBBB, Eppendorf, USA). This culture was grown at 30°C, pH 8, under 300 rpm agitation to avoid settling of the cyanobacteria isolates, with 100 µmol photons m^{-2} s^{-1}, a 12:12 h dark: light and 5% CO_2 during the light phase (Saadaoui et al., 2018). After 15 days of incubation, the biomass from *Roholtiella* sp. (QUCCCM97) species was harvested by centrifugation then freeze-dried. All cultures were performed in duplicate (Bello et al., 2021a).

Preparation of Cyanobacteria Extracts

The *Roholtellia* sp freeze-dried biomass obtained after 15 days of cultivation previously described (Bello et al., 2021a) was divided into two parts to maximize utilization. The first part was kept at -80°C and the second fraction was subjected to aqueous extraction. To this end, 100 mg of dry biomass of *Roholtellia* sp strain was first washed with sterile distilled water then dissolved into 12.5 ml phosphate buffer (0.1 M pH6.0) before sonication for 10 min (5 s pulses of 8 W over 30 s, on ice, Sonics VCX 130 Ultrasonic processor). The phosphate buffer solution was used to stabilize and maintain the pH of the system and was not necessarily considered to have any influence on the nutrient composition of the extract and the subsequent growth-enhancing potential of the

seedlings. Furthermore, extraction tubes were incubated at 4°C for 24 h. After centrifugation at 13000 rpm for 10 min, aqueous extracts were collected and freeze-dried. In this case, the cyanobacteria extract stock solutions were denoted as *Roholtiella* sp. QUCCCM97extr. In reality, the total time of the extraction for Roholtiella sp. QUCCCM97extr (from cell break up to the analysis of pigments) did not exceed 30 hrs (Bello et al., 2021a).

Nutrient Composition of Cyanobacteria Extract Analysis

The chemical composition of Roholtiella sp extract was determined by Ion Chromatography in the Central Laboratory Unit of the Qatar University, Qatar. The chemical analyzed are Sodium (Na^+), Ammonium (NH_4^+), Potassium (K^+), Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Fluoride (F^-), Chloride (Cl^-), Nitrate (NO_3^-), Phosphate (PO43-), Sulphate (SO_4^{2-}). Also, the targeted compounds were previously determined through the spectral scan of the strain (Bello et al., 2021a).

Experimental Design and Treatments with Cyanobacteria Extracts

A factorial experiment with two factors (Foliar spraying application and salt treatments) of plant ceramic vase (one plant per vase) designed based on Completely Randomized Design (CRD) was conducted with four replications (Figure 24). Salt-stressed bell pepper *(Capsicum annuum L.)* plants were treated with cyanobacteria extract and distilled water through foliar spraying respectively. The salt stress treatments that were applied to bell pepper seedlings (32 days old seedlings) at different concentration levels were 0, 50, 100, 150, and 200 mM of Sodium Chloride (NaCl) respectively and the solutions were checked every 3 days and refreshed if necessary. However, the sequel to the optimal cyanobacteria extracts previously reported when three strains QUCCCM97 *Roholtiella* sp., QUCCCM99 *Nostoc ellipsosporum*, QUCCCM112 *Desmonostoc danxiaense* were screened (Bello et al., 2021a) Thus, the extract was

tested at 6 ml L^{-1} and was sprayed at the rate of 0.576 ml/stroke/leaf and subsequently increased to two strokes as the leaves expanded. The application of the extract and water on salt-stressed seedlings was carried out for the first time 5 days after the salt-stress induction (at day 37) and the foliar extract and water application to the salt-stressed continue every 5 days interval for 28 days Figure 25. The plant treatment lasted for thirty days (after transplanting) under regulated environmental conditions at the Biological and Environmental Sciences Department greenhouse facility at the optimal conditions. After the experiment at 35 days old, the final sampling was conducted and plants were harvested, bagged with frozen bags individually, and stored at -80°C. Prior to the harvesting, the analysis of the randomly sampled bell pepper seedlings for the different vegetative parameters viz. the shoot height, root length, number of leaves, total fresh weight, dry weight, as well as relative water content was conducted. In addition, the biochemical analysis was conducted as the pigments assay (Chlorophyll a, b, and total chlorophyll) and antioxidants assays (ABTS and catalase assay). Analysis of the total proline content in the salt-stressed (0-200 mM NaCl) plants, as well as treated plants (stress-induced 0-200 mM NaCl), was carried out after the treatments. These biochemical analyses were limited to the two most homogeneous replicates in each treatment and the leaves were randomly sampled for the different analyses. However, the entire experiments were conducted in quadruplicates and maintained at optimal conditions in the greenhouse.

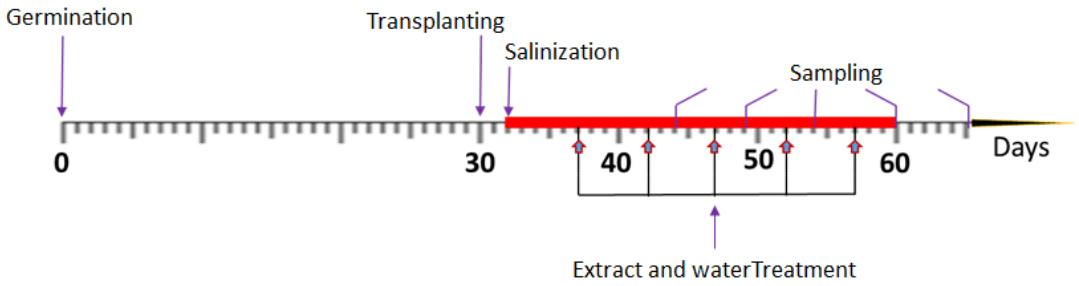


Figure 25. Scheme of treatment: Adapted from (Todorova et al., 2016) and modified as deemed fit. *Note.* Salinity treatment was started on day 32 to day 60, and *Roholtiella* sp extract and water treatment commenced at day 37 and continued at every five days intervals. Sampling was carried out on different days for the vegetative and biochemical analysis.

Vegetative/Growth Characteristics

Shoot Length (cm) – SL

Plant shoot length (cm) was measured twice, commencing ten days after transplanting and at the end of the experiment, using a steel measuring tape (STANLEY 8 m/26´, Tylon). The entire plants population was measured i.e. four plants per treatment. Thus, the average length was recorded

Root Length (cm) – R*L*

The bell pepper root length (cm) was taken at the end of the experiment using a steel metal tape (STANLEY 8 m/26´, Tylon) from the tip of the well-developed root to the point where the shoot emerges and the average value was taken.

Number of Leaves Per Plant – NL

This measurement was taken manually by counting the number of visible leaves at 20 days of the experiment, long before the end of the experiment.

Fresh Weight (g) – FW

The weight of the whole bell pepper plant was recorded at the termination of the experiment on the last day of the experiment with a precision weight balance and the

average weight was taken.

Dry Weight (g) – DW

The fully grown seedlings were harvested after the experiment and oven-dried (Genlab Drying Cabinet, Genlab Limited. Cheshire, UK) at 70°C until the weight attained constancy. Subsequently, the dried samples were weighed with the precision weight and the average weight recorded for further analysis.

Biochemical Analyses

Relative Water Content (RWC)

The relative water content (RWC) was determined to estimate the amount of moisture present in the leaf which was conducted by measuring five fresh leaf discs. The fresh weight (FW) of 1-cm discs (Nepomuceno et al., 1998; Abdelaal et al., 2020b) was determined with "high precision weight balance" and subsequently submerged in Petri dishes containing deionized water for 24 hours and the weight was taken again to determine a complete turgid weight (TW). Subsequently, the leaf disc was oven-dried at 80^{O}C until the attainment of stable weight, and the final dry weight (DW) was recorded (Sadasivam et al., 2017). Thus, RWC was measured as follows:

$$\mathrm{RWC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \quad (3)$$

Determination of Chlorophyll Concentrations (N, N-Dimethyl Formamide Assay)

The freshly cut bell pepper leaf discs (~100 mg) were placed in a test tube containing N, N-dimethyl formamide (DMF) (Abdelaal et al., 2020b). Subsequently, the mixture was kept overnight (24 h) in the refrigerator at 4°C. Thereafter, an analytical procedure was conducted with a spectrophotometer (Jenway 6715 UV/Visible Scanning Spectrophotometer, 1.5-nm Bandwidth) to determine the absorbance of the greenish

supernatant at wavelengths 647 and 666 nm respectively (Inskeep et al., 1985). Equations used to determine the values are shown below:

$$\text{Chl a } (\text{mg g}^{-1}\text{fw}) = \frac{12.7(A_{664.5}) - 2.79(A_{647})}{1000 \times W \times a} \times V \quad (4)$$

$$\text{Chl b } (\text{mg g}^{-1}\text{fw}) = \frac{20.7(A_{647}) - 4.62(A_{664.5})}{1000 \times W \times a} \times V \quad (5)$$

$$\text{Chl a + b } (\text{mg g}^{-1}\text{fw}) = \frac{17.9(A_{647}) + 8.08(A_{664.5})}{1000 \times W \times a} \times V \quad (6)$$

Where, A =absorbance, a = length of the light path in the cell (1 cm - constant), V = volume of the extract in ml. and W = fresh weight of the sample in g.

Proline Assay

The plant material of approximately 0.5 g was smatched/grounded in 10 ml of 3% sulfosalicylic acid which should be freshly prepared at all times. The mixture was filtered through filter paper and the residue was discarded. Thereafter, 2 milliliters of supernatant was reacted with 2 ml ninhydrin and subsequently, 2 ml of glacial acetic was added to the mixture in a glass test tube. Interestingly, the final mixture was placed in a water bath for 1 hr at 90 - 100 °C, thereafter the mixture was submerged in ice to stop the reaction and incubated for 5 mins. Furthermore, the extract was obtained from the mixture after adding 4 ml of toluene and vortex strenuously for ~ 20 seconds. The topmost layer of the mixture was collected and the absorbance was recorded spectrophotometrically (Jenway 6715 UV/Visible Scanning Spectrophotometer, 1.5-nm Bandwidth) at 520 nm wavelength with toluene as a blank (Bates et al., 1973) and proline accumulation was computed from the standard curve using the below equation as µmole g-1 fresh weight FW. Finally, the proline standard curve was prepared by using proline standard or L-Proline, (S)-Pyrrolidine-2-carboxylic acid.

$$\text{PC } (\mu\text{moles g}^{-1}\text{FW}) = \frac{(\mu\text{g proline ml}^{-1} \times \text{ml toluene})}{(115.5)} \text{ x } \frac{(5)}{(\text{g sample})} \quad (7)$$

Where PC is the proline content

Antioxidant/Enzymatic Activity

The antioxidant assay was carried out using two different procedures to measure antioxidant capacity and activity respectively, as one procedure might not be enough to precisely predict the antioxidant patterns. 2,2´-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay measures the antioxidant capacity and catalase assay measures the antioxidant activity respectively.

Plant Extraction

For the investigation of catalase 500 mg of fresh leaf tissue was frozen in liquid nitrogen, subsequently ground in a 5 ml extraction buffer prepared from 0.1 M phosphate buffer (7 - 7.5 ph), 0.0005 M EDTA, and 0.1% polyvinyl pyridine (PVP) respectively. The mixture was centrifuged at 15000x g for 20 mins at 4°C. The collected supernatant was used for different assays as enumerated below. The temperature of enzyme preparation and activity was maintained at 4°C throughout. The protein content was determined from the aliquot obtained from the extract by using the Bradford method (Bradford, 1976) while the standard curve was generated from bovine serum albumin (BSA) with the equation

2,2´ azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay

The concept of ABTS assay is the production of ferryl myoglobin radical from metmyoglobin and H_2O_2, thus, leading to the oxidation of ABTS to form radical cations $ABTS^{+}$, a greenish chromagen with high solubility potential and is measurable spectrophotometrically at a wavelength of 405 nm. The total antioxidant capacity of

leaf tissues was determined instantly at the expiration of the exposure period. Fresh leaves were collected randomly from the four replicates as designed. The description and procedure explained in the assay kit (Antioxidant assay CS0790, Sigma-Aldrich Co. LLC) were followed to carry out the extraction of the enzyme and subsequently, the capacity measurement. Summarily, ~100 mg leaf tissue was frozen in liquid nitrogen, grinded, and subsequently homogenized in 0.5 ml 1x Assay Buffer, then centrifuged at 12,000 g at 4°C for 15 minutes. Thereafter, the assay was conducted/carried out/prepared in the 96 well plates. The first 1 - 12 wells contained the synthetic Trolox employed to generate the standard curve that was subsequently used for antioxidant activities quantification, 10 µl of a Trolox standard solution, and 20 µl of Myoglobin working solution were added. Subsequently, in the wells for the test samples 1 – 12 wells, 10 µl of leaf tissue samples (supernatant - ~100 mg/0.5 ml of 1x Assay Buffer) and 20 µl of Myoglobin were added. To each well, 150 µl of ABTS substrate working solution (addition of 25 µl of Hydrogen Peroxide; H_2O_2 to 10 µl of ABTS Substrate Solution/Master solution) was added. The homogenates were incubated for 5 minutes at room temperature and thereafter, 100 µl stop solution was added to each well to terminate the reaction. However, before adding the stop solution, it was warmed to room temperature and mixed thoroughly until homogeneous. The plate reader was used to read the endpoint absorbance at 405 nm wavelength. The antioxidant capacity results were revealed as coequal of mM Trolox Equivalent (TE) per g of fresh weight of the samples (mM of TE g^{-1} FW) using the below equation 5

$$\mathrm{Z(mM)} = \frac{\mathrm{y\ (A_{405})} - \mathrm{Intercept}}{\mathrm{Slope}}\ \mathrm{x\ dilution\ factor} \quad (8)$$

Where, Z (mM) – Antioxidant concentration [(mM) relative to the Trolox standard curve concentration], y (A_{405}) – the average absorbance of the leaf tissue samples at 405 nm, Intercept – stands for the intercept of the Y-axis by the standard curve, the dilution factor is used only if the sample should be diluted before adding to the well. It is the fold dilution of the original sample.

Catalase Activity Assay

The activity of catalase was measured and estimated according to Aebi et al. 1984 (Aebi, 1984; Todorova et al., 2016). The constituent of the reaction mixture contained 0.1 ml enzyme extract, 9.9. ml 0.1 M phosphate buffer (pH 7.0) and 0.5 ml 0.03 M H_2O_2 adding up to final volume of 1.5 ml. The addition of H_2O_2 occurs lastly and absorbance was measured spectrophotometrically at a wavelength of 240 nm. The enzymatic breakdown and subsequent disappearance of the substrate (H_2O_2) were monitored as absorbance decreased for 30 minutes as further illustrated in the equatn

$$\mathrm{H_2O_2} \xrightarrow{\textbf{catalase}} \mathrm{2H_2O + O_2} \quad (9)$$

Catalytic activity to decompose H_2O_2 to produce H_2O and O_2 respectively

While the computation of the catalase activity is shown in equation 7

$$\mathrm{Catalase\ activity\ (unit\ mg\ protein^{-1}\ min^{-1})} = \mathbf{\Delta A_{240}} \frac{(\mathbf{1000})}{(\boldsymbol{\varepsilon_I})} * \mathbf{PC} \quad (\mathbf{10})$$

Where $\Delta\mathbf{A_{240}}$ is the change in catalase absorbance at 240 wavelengths, $\boldsymbol{\varepsilon_i}$ is extinction coefficient (40 mM^{-1} cm^{-1}) for H_2O_2 (Velikova et al., 2000), and **PC** is the protein content respectively

Statistical Analysis

The statistical data analysis was determined by the analysis of variance (two-way ANOVA) using a Minitab version 19. The data reported are mean values ± SD. The mean comparison of values was conducted by Tukey's post hoc test of Pairwise Comparisons while differences were considered significant at p-Value less than 0.05 ($p < 0.05$) as represented with different letters

Results

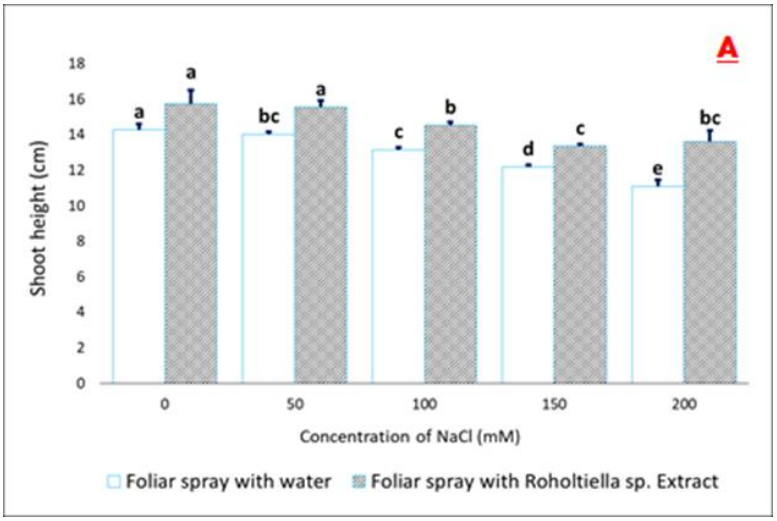
Cyanobacteria Nutrient Composition

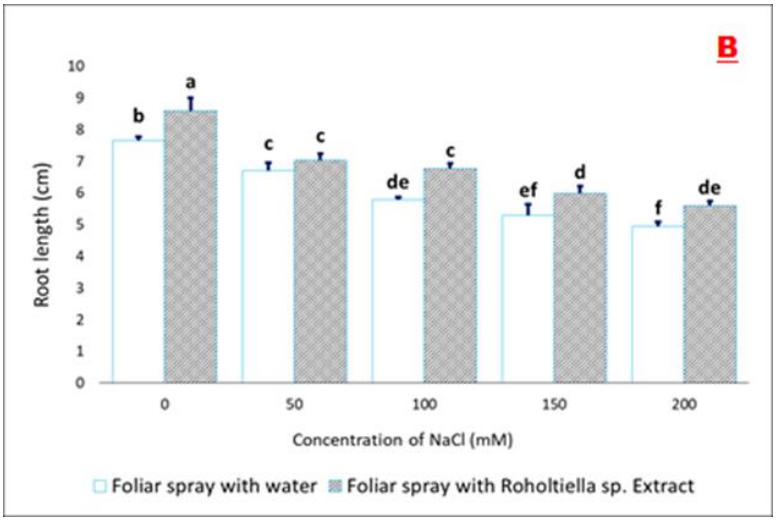
Nutrient composition of Roholtiella sp extract in part per million (ppm) contained considerable amount of Sodium (Na^{+}) 2.379, Ammonium (NH_4^{+}) 0.674, Potassium (K^{+}) 8.533, Calcium (Ca^{2+}) 1.777, Magnesium (Mg^{2+}) 3.483, Fluoride (F^{-}) 0.0191, Chloride (Cl^{-}) 3.168, Nitrate (NO_3^{-}) 5.247, Phosphate (PO_4^{3-}) 13.67, Sulphate (SO_4^{2-}) 0.212. In a like manner, the spectra scan of this strain showed that it contains a reasonable amount of phycoerythrin and phycocyanin (Bello et al., 2021a).

Effect of Salt Stress and Foliar Spray on Vegetative Parameters

The obtained results as shown in Figure 26 (A – E) respectively indicated that salt-stressed bell pepper plants exposed to various concentration levels caused a significant decrease in the vegetative parameters measured which are shoot length, root length, fresh weight, dry weight, and the number of leaves $plant^{-1.}$ The shoot length was significantly influenced by the *Roholtellia* sp. extract and the degree of salt concentration level. At the 0 mM salt concentration, there was no significant difference between the control (0 Mm) and foliar treated plants though the shoot length increased by (9.33%) (Figure 1A). Also, at the 50, 100,150, and 200 mM salt concentrations and compared with those plants' foliar sprayed with water, the shoot height increased by 9.77%, 8.68%, 8.28%, and 17.04% respectively. Also, the trend is similar with the root length as there was a significant difference at all the salt treatment levels 0-200 mM. The seedling sprayed with *Roholtiella* sp exhibited a significant increase in root length by 11.05%, 4.63%, 14.76%, 11.67%, and 11.61% compared with the control group sprayed with water. In addition, there was a significant increase in the fresh weight, dry weight, and the number of leaves of the treated stressed seedlings (0-200 mM) with *Roholtiella* sp compared with the control group that was treated with water. For the plant fresh weight, it was significantly increased by 12.16%, 30.13%, 39.15%, 28.13%,

and 27.08%, while for the plant dry weight, the pattern is the same as it significantly increased except at 0 mM by 1.56%, 19.93%, 18.94% 22% and 31.02%. The number of leaves of the stressed plant (0 - 200 mM) significantly increased by 11.11% 26% 23.4% 24.44% and 30.23% respectively.





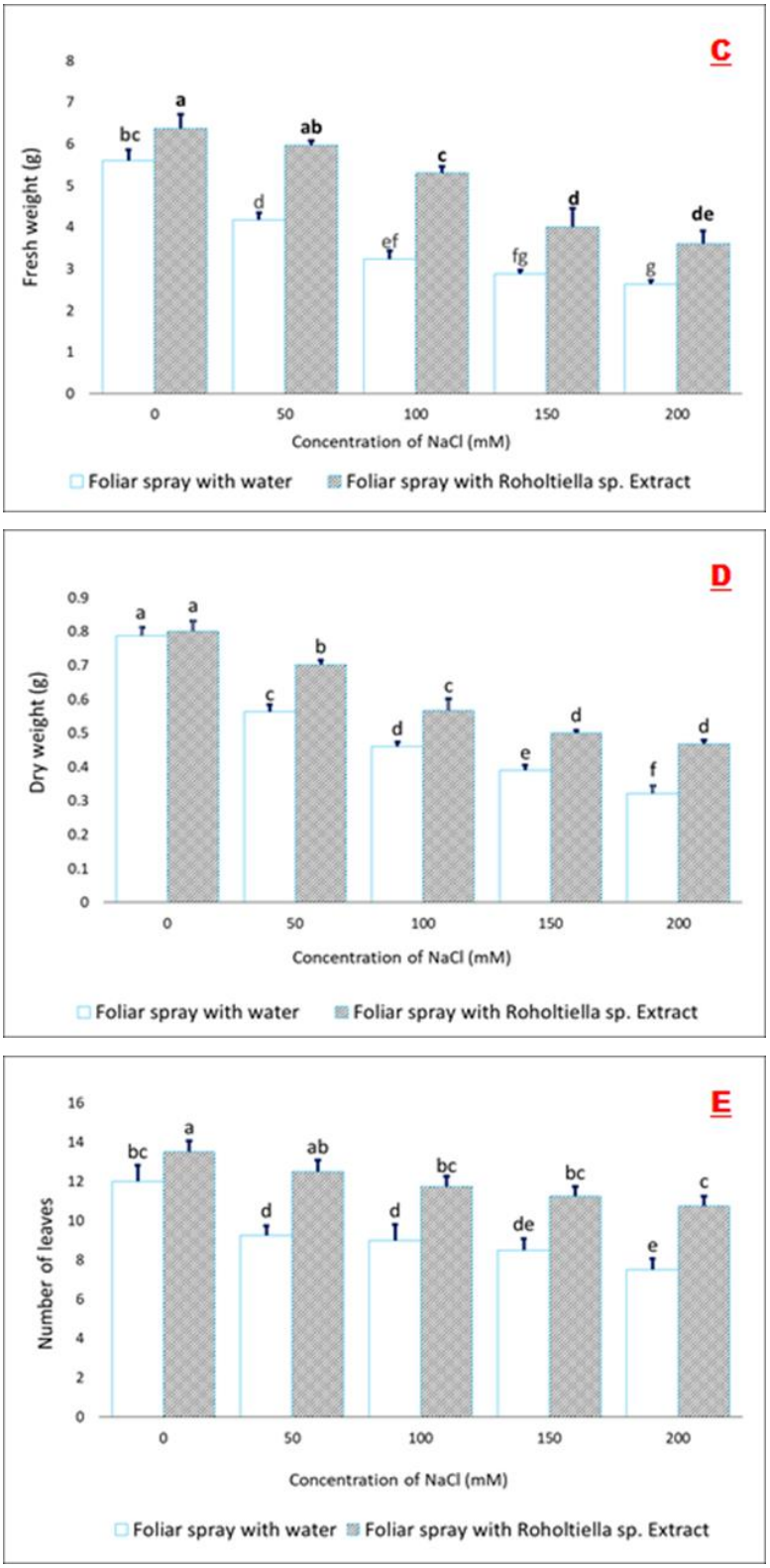


Figure 26. Effect of *Roholtiella* sp. extract on the shoot height (A), root length (B), fresh weight (C), dry weight (D), and (E) the number of leaves in salt-stressed bell pepper seedlings.

Effect of Roholtiella sp. *Extract on Relative Water Content (RWC)*

The obtained results in Figure 27 showed relative water content (RWC) of the stressed bell pepper plants reduced with an increased level of salinity stress (0 - 200 mM). The seedlings foliar sprayed with water showed 91.58%, 80.76%, 64.78% 59.07%, and 50.87% in RWC. In a like manner, the seedlings foliar sprayed with *Roholtiella* sp extract. showed 92.05%, 89.51% 83.39% 69.61% and 65.77% in RWC. Thus, it shows that the seedlings foliar sprayed with *Roholtiella* sp extract exhibited a significant increase in RWC when compared with the seedlings foliar sprayed with water and their respective control (0 mM) groups.

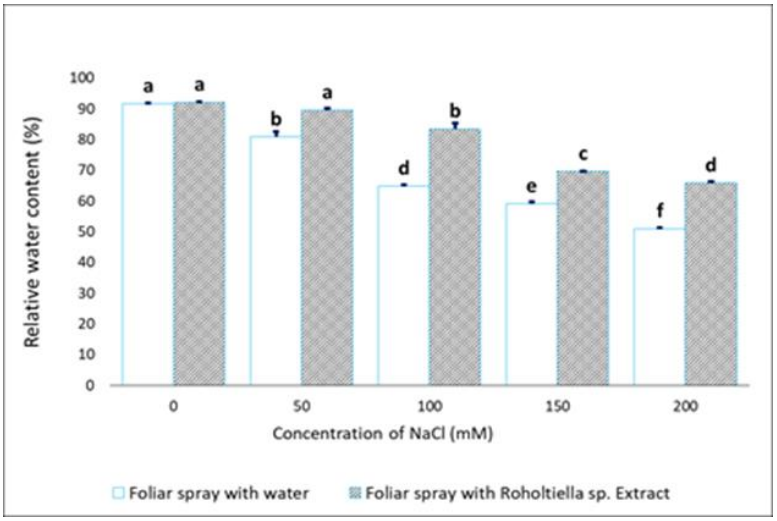
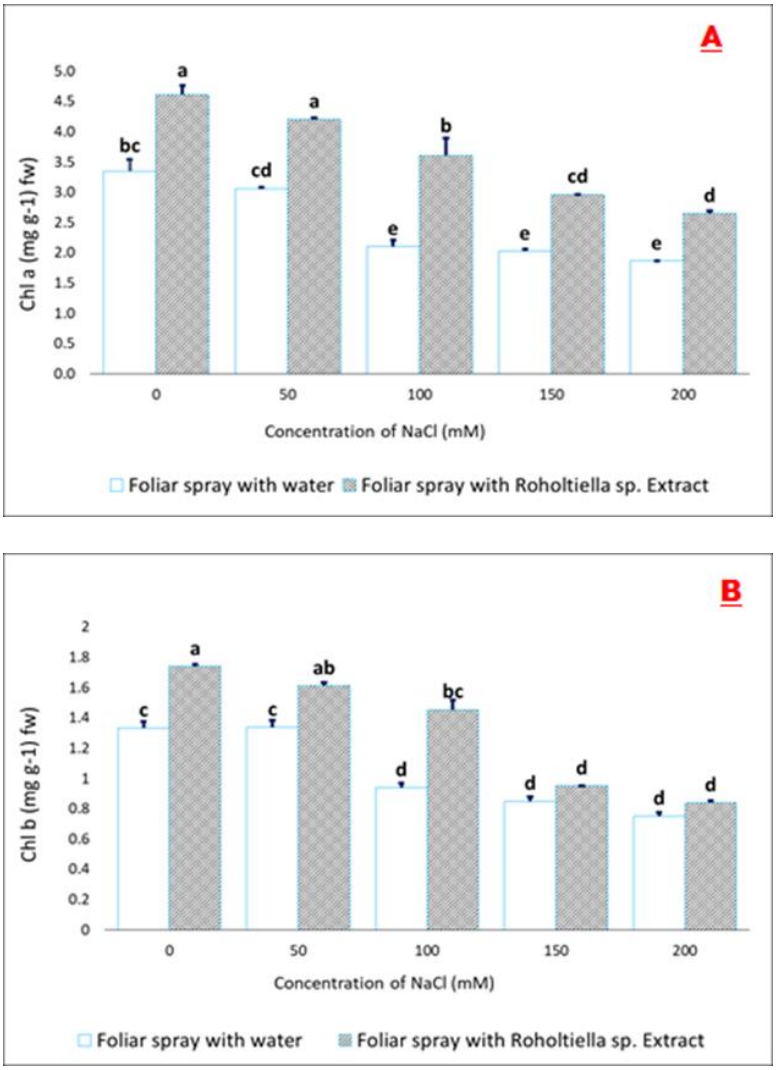


Figure 27. Effect of *Roholtiella* sp extract on the relative water content of leaves in salt-stressed bell pepper seedlings.

Effect of Salt Stress and Foliar Spray on Chlorophyll Pigments and Proline Concentration

It is clear from the obtained results in Figure 28 that there was a significant change in the content of the pigment on the foliar treated plants of bell pepper with *Roholtiella* sp extract compared with the group treated with water. The concentration of chlorophyll-

a (3.34, 3.06, 2.1, 2.02, and 1.86 mg g^{-1} Fw), chlorophyll b (1.33, 1.34, 0.94, 0.85, and 0.75 mg g^{-1} Fw), and total chlorophyll (4.68, 4.4, 3.04, 2.87, and 2.7 mg g^{-1} Fw) significantly declined in plants of bell pepper under all concentrations of salinity (0, 50, 100, 150, and 200 mM) respectively.



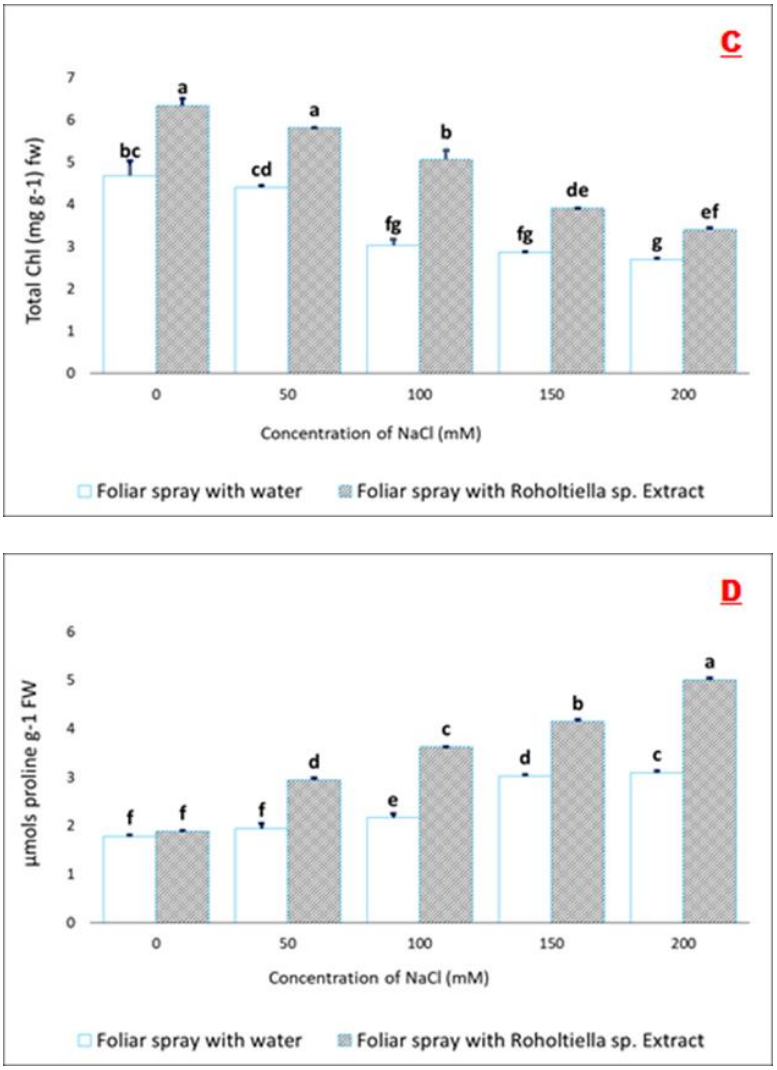


Figure 28. Effect of *Roholtiella* sp extract on contents of chlorophyll-a (A), chlorophyll b (B), total chlorophyll (C), and proline in bell pepper seedlings under salt stress. Bars accompanied with different letters indicate significant differences according to Turkey's test at a significant level of 5% ($p < 0.05$). Salinity concentrations are 0, 50, 100, 150, and 200 mM.

Furthermore, the application of *Roholtiella* sp extract lead to a significant increase of chlorophyll-a (4.61, 4.20, 3.60, 2.96, and 2.65 mg g^{-1} Fw) when compared with those plants foliar sprayed with water (3.49, 3.13, 2.36, 2.23, and 1.82 mg g^{-1} FW) at 0, 50, 100, 150, and 200 mM salinity level respectively. In a like manner, chlorophyll b (1.74,

1.61, 1.46, 0.95, and 0.84 mg g^{-1} Fw) and total chlorophyll (6.35, 5.81, 5.06, 3.91, and 3.40 mg g^{-1} FW) increased significantly when treated with the extract compared to the untreated seedlings (1.33, 1.34, 0.94, 0.85, and 0.75 mg g^{-1} Fw) and (4.68, 4.4, 3.04, 2.87, and 2.7 mg g^{-1} FW) at all the concentration levels (0, 50, 100, 150, and 200 mM) of salt stress. However, even at zero salinity level, there was an incremental in the chlorophyll content level because of the growth enhancer potential of the *Rholtiella* sp extract (Bello et al., 2021a). Generally, the proline accumulation increases faster in stressed plants at different conditions when compared with other amino acids (Sadasivam et al., 2017). The bell pepper seedlings sprayed with Roholtiella sp extract exhibited an increase in the proline accumulation at all the concentration levels of NaCl as compared to those treated with water at the same NaCl level. Thus, proline concentrations (1.79, 1.95, 2.16, 3.03, 3.10µmols proline g^{-1} FW) under salinity concentrations (0, 50, 100, 150, and 200 mM) significantly increased in bell pepper seedlings compared to the group treated with water. However, the accumulation of proline increases under the application of *Roholtiella* sp to 1.88, 2.94, 3.63, 4.15, 5.01 µmols proline g^{-1} FW corresponding to salt-stressed seedlings at 0, 50, 100, 150, and 200 mM respectively. Thus, Bell pepper (*Capsicum annuum* L.) plants treated with the *Roholtiella* sp extract showed significant impacts on the content of proline under the control (0 mM) and all the salinity levels (50, 100, 150, and 200 mM). However, the observed significant percentage increase recorded was 5.18%, 33.89%, 40.32%, 26.96%, and 38.20% in the plants treated with extract compared to those foliar sprayed with water at all the salinity levels.

Effect of Salt Stress and Foliar Spray on the Antioxidant Capacity and Activity

ABTS assay is one of the most available procedures commonly used for evaluating the antioxidant capacity ($ABTS^+$ radical scavenging capacity) of plants (Xia et al., 2017). The recorded values of $ABTS^+$ free radical scavenging potential/ability in the induced plants foliar sprayed with water and *Roholtiella* sp extract exhibited significant differences Figure 4A. In the ABTS procedures, the antioxidant capacity of bell pepper treated with water in control (0 mM) plants was (1.3 mM TE g^{-1} FW). However, in responding to salinity, antioxidant capacity decreases as salinity increases, the highest value (0.88 mM TE g^{-1} FW) was recorded at 50 mM and further reduced significantly (0.86 mM TE g^{-1} FW) at 100 mM. At 150 and 200 mM of salt concentrations, the seedlings exhibited more weakness in antioxidant capacity at 0.76 mM TE g^{-1} FW and 0.73 mM TE g^{-1} FW respectively. Interestingly, when the plants were sprayed with extract, among all the concentrations, 200 mM is the highest, attaining 1.64 mM TE g^{-1} FW followed by 150 mM (1.63 mM TE g^{-1} FW), 100, 50 Mm reaching (1.6 mM TE g^{-1} FW and 1.59 mM TE g^{-1} FW) respectively when compared with the group sprayed with water but there is no significant difference at the concentration of 0mM (1.36 mM TE g^{-1} FW)

In a like manner, the obtained results as shown in Figure 4B indicate Bell pepper plants exposed to salt stress at four concentrations (50, 100, 150, and 200 mM) caused a significant increase in the antioxidant enzymatic activity. Catalase activity significantly increased in the stressed bell pepper plants (5.37, 7.87, 8.48, 9.26, 10.42 unit mg $protein^{-1}$ min^{-1}) foliar sprayed with *Roholtiella* sp extract as compared to salt-stressed and water foliar sprayed bell pepper seedlings (5.39, 5.41, 6.12, 6.89, 7.99 unit mg $protein^{-1}$ min^{-1}) as well as the control (0 Mm) group. Interestingly, as the concentration levels of NaCl gradually increase, the corresponding catalase activity also increases as

observed in Figure 29.

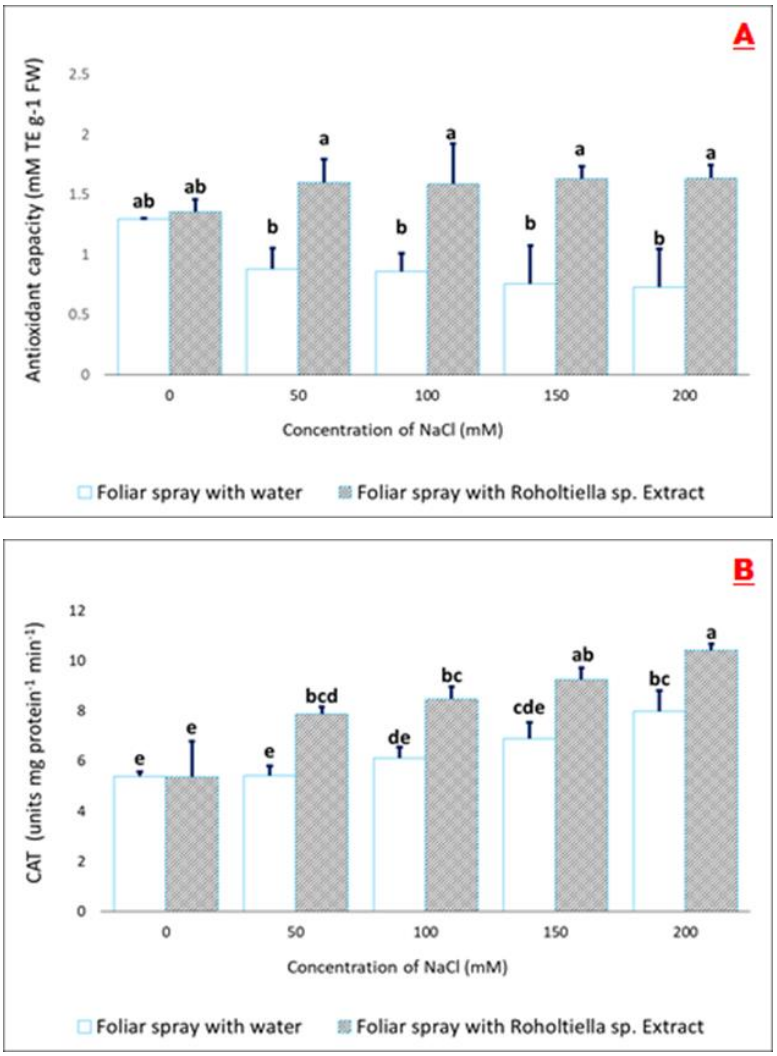


Figure 29. Effect of *Roholtiella* sp extract on the Trolox equivalent antioxidant capacity and activity of catalase (CAT) in induced bell pepper seedlings under salt stress. Bars accompanied with different letters indicate significant differences according to Turkey's test at a significant level of 5% ($p < 0.05$). Salinity concentrations are 0, 50, 100, 150, and 200 mM

CHAPTER 6. INVESTIGATING BELL PEPPERS (C*apsicum annuum* L.) RESPONSE TO HEAT STRESS IN LEAVES VIA BIOCHEMICAL ANALYSIS

Introduction

Plant activities and metabolisms are highly susceptible to heat variation, where excessive heat stress can cause negative impacts on plants' development and survival. Generally, heat stress hinders the biosystematics of thermolabile components, particularly photosystem II (PSII) (Santarius, 1973; Berry et al., 1980; Heckathorn et al., 1998; Heckathorn et al., 2002; Mathur et al., 2014; Lai et al., 2016; Wang et al., 2018a). The constant change in ambient temperature is regarded as one of the most deleterious stresses causing environmental instability globally (Hasanuzzaman et al., 2013). It was predicted that air temperature will rise by 2 x 10^{-1}°C per decade globally, thus leading to a rise in temperature that will exceed the current level by 1.4 - 4.0°C and is anticipated to occur beyond 2100 (Solomon, 2007). No doubt, the forecast has created great concern among scientists, as heat stress is known to harm the life and forms of organisms, coupled with the direct or indirect influences on the environmental compositions. Interestingly, as plants are characterized as being a sessile organism, thus their mobility is practically impossible from unfavorable to favorable environments, thus, high temperature (HT) stress causes D1 protein and antenna destruction, restricts the plant developmental growth and processes (Lobell et al., 2003; Lobell et al., 2007; Hasanuzzaman et al., 2012; Lu et al., 2017; Pan et al., 2018).

The production of reactive oxygen species superoxide ($O2^{-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH)/peroxides, singlet oxygen ($1[O_2]$ or $1O_2$)), and alpha-oxygen (α-O) are normal from the cellular metabolism of plants. However, different environmental stresses such as heat may cause excessive production of ROS, thus leading to oxidative stress in plants (Ergin et al., 2012; Krishnamurthy and

Rathinasabapathi, 2013). Furthermore, ROS hinder enzymes activity and cause deleterious effects on the essential cellular components (Ergin et al., 2012; Krishnamurthy et al., 2013). Interestingly, the most important ROS-scavenging strategy include superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Foyer et al., 2005a, 2005b; Das et al., 2014). From previous studies, any alterations in antioxidant enzymes will enhance the plant's resistance to excessive temperature and other abiotic stresses (Yin et al., 2008; He et al., 2010; Bello et al., 2021b). Also, high-temperature stress inhibiting physiological, biochemical, and molecular modifications in plant activities viz denaturation of protein, liquefaction of lipids, or membrane integrity perturbation (Levitt, 1980; Gulen et al., 2004). Furthermore, high temperature is expected to become a hindering factor for bell pepper cultivation, and many other plants species owing to the persistent increase in global warming, positioning future farming in a jeopardy. Thus, investigating the mechanisms associated with high-temperature stress in plants is inevitable to guarantee food security in the future.

Bell pepper is one of the vegetables that is cultivated all year round under hydroponic systems with higher productivity during the spring in Qatar. Bell pepper is characterized by a high yield during this period when the temperature is mild and moderate. However, slightly elevated temperatures exceeding the optimum temperature during the bell pepper flowering might result in blossom shedding and invariably affect the yield and quality of bell pepper. Nevertheless, these persistent temperature changes and harsh climatic conditions have been the regular circumstances in the country being that is in the arid region. Cultivation under a hydroponic system is a sustainable technology that is expected to mitigate these environmental stress issues during the summer but the result is often contrary. Thus, this study aimed to evaluate the effect of heat stress on

different variables such as pigments content, antioxidant activity, and proline content at different temperatures.

Material and Methods

Plant Materials and Growth Parameters

The hybrid bell pepper (*Capsicum annuum L.*) was procured from the certified commercial seed supplier Technical Agricultural Company, Doha, Qatar. Seeds were first germinated in the germination boxes inside the greenhouse at the Biological and Environmental Sciences Department, Qatar University. After 30 days, healthy seedlings were transferred into a 192 mL glass vase to be grown in a deep-water culture system in Hoagland solution Figure 30. Seedlings were grown in a controlled, illumination, and aerated growth chamber (day/night period: 16 h/8 h, day/night temperatures: adjusted according to the treatments 25, 32, and 40°C respectively), and the nutrient solution was replenished once on the third day.

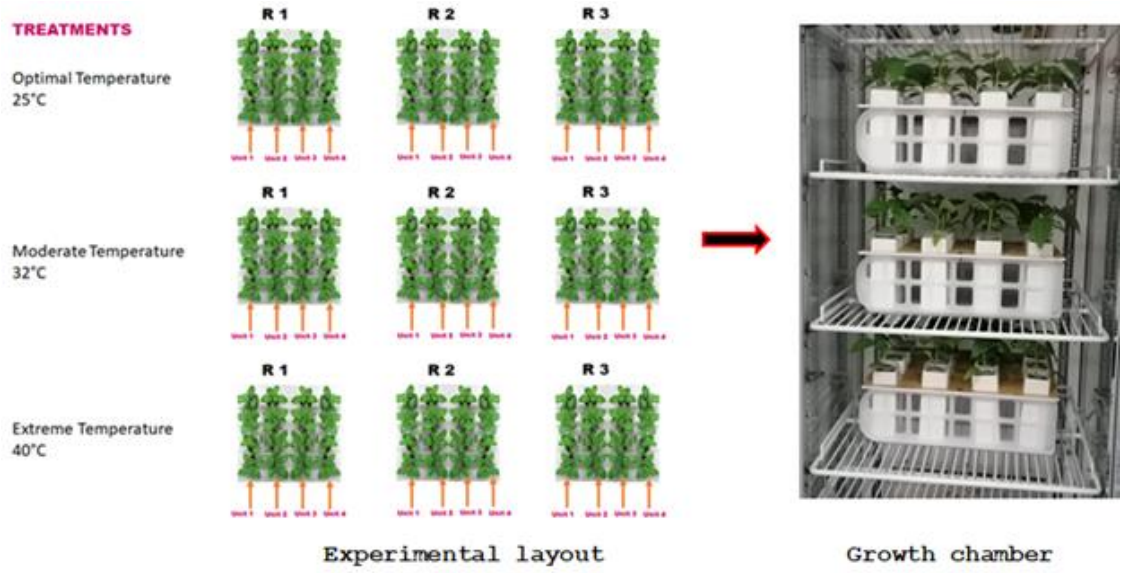


Figure 30. The diagram shows the planting layout and the growth chamber arrangement

Exposure Conditions

Exposure to heat stress was conducted using 30-d grown *Capsicum annuum L* seedlings. For every replicate treatment, 16 seedlings were submerged into the Hoagland nutrient solution (Arnon, 1948) and three replicates were used (48

seedlings/per treatment). Three treatments: optimal/control (25°C), moderate (32°C), and extreme temperatures (40°C) were applied as shown (Figure 30). All treatments were in three replicate/triplicate subjected to three to five day's exposure during which the Hoagland nutrients solutions and the whole systems were continuously monitored. The plant collections/sampling commenced after the third day of the exposure until the end of the exposure period Figure 31. The plants collected were stored at **-80°C** for further analysis.

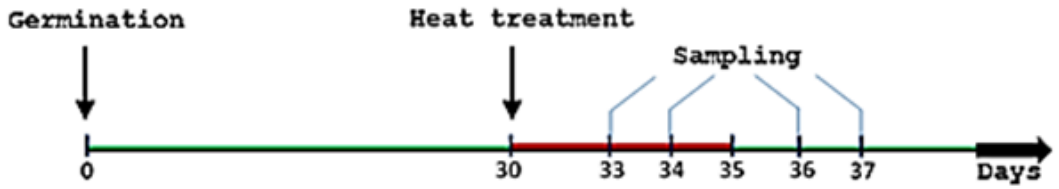


Figure 31. Scheme of germination and heat treatment. Note. Heat treatment was held during days 30–35, and during days 33–37 the plants' sampling was carried out.

Biochemical Analyses

Chlorophyll (Chl.) Content Analysis (Acetone Assay)

During and at the expiration of the exposure time after the completion of the treatments, freshly plucked leaves of approximately 12 to 14 were randomly selected from the plant shoot. The green leaves from *Capsicum annuum* were chop/macerated into a fractional part; approximately 0.5 g of sample was homogenized in 10 ml chilled 80% acetone in mortar and pestle (Azpack Mortar and Pestle, Thermo fisher scientific, UK). The final acetone extract was filtered (Advantec GC-50, Tokyo, Japan) and the final volume was scaled up to 20 ml with 80% acetone. The determination of the Chl. a, Chl. b, and total Chl. content (Chl. a + Chl. b) absorbance values were consequently obtained using a

spectrophotometer (Jenway 6715 UV/Visible Scanning Spectrophotometer, 1.5-nm Bandwidth) at two wavelengths, 663 and 645 nm (Arnon, 1948; Saoussen et al., 2012). with 80% acetone as a reference (Kakade et al., 2020). The concentrations of the chlorophyll contents were finally determined using the following equations (Inskeep et al., 1985; Bello et al., 2022):

$$\text{Chl a } (\text{mg g}^{-1}\text{fw}) = \frac{12.7(A_{664.5}) - 2.79(A_{647})}{1000 \times W \times a} \times V \quad (11)$$

$$\text{Chl b } (\text{mg g}^{-1}\text{fw}) = \frac{20.7(A_{647}) - 4.62(A_{664.5})}{1000 \times W \times a} \times V \quad (12)$$

$$\text{Chl a + b } (\text{mg g}^{-1}\text{fw}) = \frac{17.9(A_{647}) + 8.08(A_{664.5})}{1000 \times W \times a} \times V \quad (13)$$

Where, A =absorbance, a = length of the light path in the cell (1 cm - constant), V = volume of the extract in ml. and W = fresh weight of the sample in g.

Total Carotene Determination

The determination of carotene followed the same pattern with chlorophyll extraction explained above except that the absorbance values were obtained at three different wavelengths. The total carotene was, therefore, determined spectrophotometrically at the wavelengths of 470, 663, and 645 nm respectively. Thereafter, the carotene content was estimated/calculated using the below formula/equation:

$$\text{Total Carotene } (C_{x+c}) = \frac{1000(A_{470}) - 1.82(C_a) - 85.02(C_b)}{198} \quad (14)$$

Where, A = absorbance, C_a is chlorophyll a, and C_b is chlorophyll b respectively

Proline Content Determination Assay/Analysis

The proline content assessments were carried out at the expiration of the experiment according to the procedure/method of (Bates et al., 1973). Extraction was performed

using a sample of approximately 0.5 g fresh expanded leaf material, chopped in 3% sulfosalicylic acid (w/v), and subsequently estimated by applying freshly prepared ninhydrin solution/reagent. The dissolved proline in the solution was separated by fractionation using toluene. The absorbance of the topmost liquid phase (fraction) was determined spectrophotometrically (Jenway 6715 UV/Visible Scanning Spectrophotometer, 1.5-nm Bandwidth) at the wavelength of 520 nm. Afterward, the concentration of proline content was estimated from a calibration curve in the standard unit of µ mol. proline g^{-1} FW (fresh weight) after calculation as µmoles g^{-1} FW against standard proline (Sigma–Aldrich Chemie, Germany) using the following equation:

$$\text{PC } (\mu\text{moles g}^{-1}\text{FW}) = \frac{(\mu\text{g proline ml}^{-1} \times \text{ml toluene})}{(115.5)} \text{ x } \frac{(5)}{(\text{g sample})} \qquad (15)$$

Where PC is the proline content

Antioxidant/Enzymatic Activity

Plant Extraction

For the investigation of catalase activities, 0.5 g of fresh leaf tissue was frozen in liquid nitrogen, subsequently ground in a 5 ml extraction buffer prepared from 0.1 M phosphate buffer (7 - 7.5 ph), 0.0005 M EDTA, and 0.1% polyvinyl pyridine (PVP) respectively. The mixture was centrifuged at 15000x g for 20 mins at 4°C. The collected supernatant was used for catalase assay as enumerated below. The temperature of enzyme preparation and activity was maintained at 4°C throughout.

2,2´ azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay

The total antioxidant capacity of leaf tissues was determined instantly at the expiration of the exposure period. Fresh leaves were collected randomly from the three replicates as designed. The description and procedure explained in the assay kit (Antioxidant

assay CS0790, Sigma-Aldrich Co. LLC) were followed to carry out the extraction of enzyme and subsequently, activity measurement. Summarily, ~100 mg leaf tissue was frozen in liquid nitrogen, grinded, and subsequently homogenized in 0.5 ml 1x Assay Buffer, then centrifuged at 12,000 g at 4°C for 15 minutes. Thereafter, the assay was conducted/carried out/prepared in the 96 well plate. The first 1 - 12 wells contained the synthetic Trolox employed to generate the standard curve that was subsequently used for antioxidant activities quantification, 10 µl of a Trolox standard solution, and 20 µl of Myoglobin working solution were added. Subsequently, in the wells for the test samples 1 – 12 wells, 10 µl of leaf tissue samples (supernatant - ~100 mg/0.5 ml of 1x Assay Buffer) and 20 µl of Myoglobin were added. To each well, 150 µl of ABTS substrate working solution (addition of 25 µl of Hydrogen Peroxide; H_2O_2 to 10 µl of ABTS Substrate Solution/Master solution) was added. The homogenates were incubated for 5 minutes at room temperature and thereafter, 100 µl stop solution was added to each well to terminate the reaction. However, before adding the stop solution, it was warmed to room temperature and mixed thoroughly until homogeneous. The plate reader was used to read the endpoint absorbance at 405 nm wavelength. The formula/equation below was used to determine the antioxidant activity in the leaf tissue samples. The antioxidant activities results were revealed as coequal of Trolox per g of fresh weight of the samples (mM of Trolox/g FW) (Xia et al., 2017)

$$y = aX + b \quad \text{(Linear regression of the standard curve} \tag{16}$$

$$Z(\text{mM}) = \frac{y\ (A_{405}) - \text{Intercept}}{\text{Slope}} \text{ x dilution factor} \tag{17}$$

Where,

Z (mM) – Antioxidant concentration [(mM) relative to the Trolox standard curve concentration], y (A_{405}) – the average absorbance of the leaf tissue samples at 405 nm,

Intercept – stands for the intercept of the Y-axis by the standard curve (**b** in the equation I), while Slope - represent the slope of the standard curve (**a** in the equation II), the dilution factor is used only if the sample should be diluted before adding to the well. It is the fold dilution of the original sample.

Catalase Activity Assay

The activity of catalase was measured and estimated according to Aebi et al. 1984 (Aebi, 1984). The constituent of the reaction mixture contained 0.1 ml enzyme extract, 9.9. ml 0.1 M phosphate buffer (pH 7.0) and 0.5 ml 0.03 M H_2O_2 adding up to final volume of 1.5 ml. The addition of H_2O_2 occurs lastly and absorbance was measured spectrophotometrically at a wavelength of 240 nm. The enzymatic breakdown and subsequent disappearance of the substrate (H_2O_2) were monitored as absorbance decreased for 30 minutes as further illustrated in equation 17

$$H_2O_2 \xrightarrow{\textbf{catalase}} 2H_2O + O_2 \qquad (18)$$

Catalytic metabolism of breaking down H_2O_2 to give H_2O and O_2 respectively

While the catalase activity was computed by using the below equation 19

$$\text{Catalase activity } (\mu\text{mol mg}^{-1} \text{ protein min}^{-1} = \Delta A_{240}(1000|\varepsilon_I * PC) \qquad (19)$$

Where $\Delta\mathbf{A_{240}}$ is the change in catalase absorbance at 240 wavelengths, $\boldsymbol{\varepsilon_i}$ is extinction coefficient, and **PC** is the protein content respectively

Statistical Analysis

Data analysis was carried out using Statistical Package for the Social Sciences (SPSS - IBM SPSS Statistics, version 17, USA) software. The statistical significance of the obtained data was determined by one-way analysis of variance (ANOVA) and reported as mean values ± SD. The comparison of the mean values was obtained by post hoc Tukey HSD test $p \leq 0.05$ (Tukey's test for mean comparisons).

Results

Effects of Heat Stress on Chlorophyll Pigments

Heat stress imposed had shown a significant decrease in chlorophyll a, chlorophyll b, and total chlorophyll content Table 18 The increase in temperatures during the entire period had a negative impact on these contents when compared with the control as visually observed. Under regulated conditions, the *Capsicum annuum* L. had the highest chlorophyll-a (5.383 mg g^{-1} FW), chlorophyll b (2.358 mg g^{-1} FW), and total chlorophyll (6.692 mg g^{-1} FW), at the optimal temperature of 25°C respectively, compared with the moderate temperature of 32°C; chlorophyll a (4.327 mg g^{-1} FW), chlorophyll b (1.710 mg g^{-1} FW), and total chlorophyll (5.202 mg g^{-1} FW) Table 19. However, when *Capsicum annuum* seedlings were susceptible to the extreme temperature of 40°C, a noticeable reduction in chlorophyll a, chlorophyll b, and total chlorophyll content was equally recorded as chlorophyll a (4.705 mg g^{-1} FW), chlorophyll b (1.876 mg g^{-1} FW), and total chlorophyll (5.674 mg g^{-1} FW) respectively. In terms of heat stress impacts, average chlorophyll concentration dropped over the control by 19.62% for chlorophyll a, 27.50% for chlorophyll b, and 22.27% for total chlorophyll respectively.

Table 18. Analysis of Variance Showing the Statistical Significance Effect of Various Treatments on the Pigments (Chlorophyll A, B, Total Chlorophyll, And Carotene) Composition. Significant Statistical Difference at 0.05 (5%) Level, Ns = Not Significant/** Indicate/Show Significance Difference at 5 % Significance Level ($P < 0.05$)

Source of variation	DF	Analysis of variance - Mean Square (MS)			
		Chl a. (mg g-1 fw)	Chl b. (mg g-1 fw)	Total Chl. (mg g-1 fw)	Carotene (mg g-1 fw)
Treatments	2	3.4350*	1.3599*	6.960*	2.0957*

Source of variation	DF	Analysis of variance - Mean Square (MS)			
		Chl a. (mg g-1 fw)	Chl b. (mg g-1 fw)	Total Chl. (mg g-1 fw)	Carotene (mg g-1 fw)
Error	33	0.9540	0.1715	1.410	0.1043
Total	35				
P-Value		0.039	0.02	0.013	0.000

Table 19. Heat Effects on Chlorophyll and Carotenoids of *Capsicum annuum* (Mean ± Standard Error). Mean that Do Not Share a Letter are Significantly Different – Tukey Pairwise Comparisons of Treatment

Treatments	Chl a. (mg g-1 fw)	Chl b. (mg g^{-1} fw)	Total Chl. (mg g-1 fw)	Carotene (mg g-1 fw)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control 25°C	5.383 ± 0.997 a	2.358 ± 0.412 a	6.692 ± 1.195 a	1.092 ± 0.196 b
Moderate 32°C	4.327 ± 1.284 ab	1.710 ± 0.545 b	5.202 ± 1.566 ab	1.817 ± 0.514 a
Extreme 40°C	4.705 ± 0.468 b	1.876 ± 0.217 b	5.674 ± 0.591 b	1.094 ± 0.102 b

The least drop in chlorophyll concentrations was chlorophyll-a while the maximum was chlorophyll b. Interestingly, it is also important to reveal that at the maximum temperature treatment, the *Capsicum annum* did not show any sign of wilting or chlorosis under the moderate or extreme temperature despite the significant drop of chlorophyll a, b, and total chlorophyll respectively.

Effect of Heat Stress on Proline Content

An assessment/investigation of proline level in the present study showed a considerable increase in proline content (µmol g^{-1} FW) in the leaf of the *Capsicum annuum* in response to heat stress. The highest proline content under-regulated condition was seen in the extreme condition at 40°C (4.064 µmol. g-1 FW) followed by the moderate treatment at 32°C (3.261 µmol. g-1 FW) compared with the control at 25°C (0.938 µmol. g-1 FW) respectively. Consequently, the proline accumulation mean fold over

the control (optimum) followed the trend as extreme (76.92%) > moderate (71.24%) respectively. Hence, heat stress significantly increased the production of free proline ($p < 0.05$) as higher accumulation levels of proline occurred in both moderate and extreme treatments but at different concentrations. However, the proline content greatly relies on the age of the plant, leaf, the position of the leaf or leaf part (Chiang et al., 1995; Mafakheri et al., 2010). Generally, at the vegetative stage, heat stress increased the accumulation of proline in multiple folds to mitigate the impact of the stress on the plant. Interestingly, proline accumulation has been considered as a very reliable factor/parameter of consideration for stress resistance (Mafakheri et al., 2010).

Table 20. Heat Effects on the Proline Content, Trolox Equivalent, and Catalase Activity of *Capsicum Annuum.* Values are the Mean of Three Replicates ± Standard Deviation. Different Letters Within Each Column Show Statistically Significant Differences at 5%, $P \leq 0.05$.

Treatments	Proline (µmoles g^{-1} FW)	ABTS (mM of TE g^{-1} FW)	CAT (unit mg $protein^{-1}$ min^{-1})
	Mean ± SD	Mean ± SD	Mean ± SD
Control 25°C	0.9379±0.07 a	1.8118 ± 0.997 a	3.861±0.653 b
Moderate 32°C	3.261±0.71 b	1.6333 ± 0.468 a	5.718±1.289 b
Extreme 40°C	4.064±0.79 c	0.6339 ± 1.284 b	8.339±1.289 a

Table 21. Analysis of Variance Showing the Statistical Significance Effect of Various Treatments on the Proline Content, Trolox Equivalent, and Catalase Activity. * Significant Statistical Difference at 0.05 (5%) Level, Ns = Not Significant Indicate/Show Significance Difference At 5 % Significance Level ($P < 0.05$)

Source of variation	DF	Analysis of variance - Mean Square (MS)		
		Proline (µmoles g^{-1} FW)	ABTS (mM of TE g^{-1} FW)	CAT (unit mg protein^{-1} min^{-1})
Treatments	2	31.6248*	4.83623*	15.1836*
Error	33	0.3769	0.09836	0.8811
Total	35			

Antioxidant Activity

2,2´ azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)

The effect of heat stress on the antioxidant activity of leaves of *Capsicum annuum* L. is shown in Table 20. Following 5 days of heat stress treatments, the activity of antioxidant of the *Capsicum annuum* L. leaves was measured using ABTS assay indicated a tremendous increase compared to the control (optimum - 25°C). The activity is higher with the extreme (40°C) treatment followed by the moderate treatment (32°C) respectively.

Effect on the Activity of Catalase (CAT)

It could be seen from Table 20 the effect of heat stress on the catalase activity of leaves of *Capsicum annuum* L., following 5 days of heat stress treatments caused a significant increase in the catalase activity of the heat-stressed seedlings compared with the control. At the extreme condition (40°C), the CAT activity significantly increases (8.34 units mg of protein^{-1} min^{-1}) and at the moderates condition (32°C) there was a significant increase (5.72 units mg of protein^{-1} min^{-1}) compared to the optimum condition/control (25°C) where the activity was maintained as (3.86 units mg of protein^{-1} min^{-1}) respective.

CHAPTER 7: DISCUSSION

Several studies that address the application of algae as biostimulants and biofertilizers/enhancers have mostly focused on the application of BGA (cyanobacteria) on cereal crops, primarily rice (Sharma et al., 2011; Garcia-Gonzalez et al., 2016). Importantly, their potential to fix atmospheric nitrogen for plant use makes it essential in several agricultural cultivations (Irisarri et al., 2001; Jha et al., 2006; Pereira et al., 2009; Sharma et al., 2011). Several cyanobacteria and microalgae extracts exhibited enhancing characteristics/properties on the development of crops as reported by previous studies (Garcia-Gonzalez et al., 2016). Growth is stimulated because of biochemical/bioregulators such as auxin, gibberellins, and cytokines coupled with a reasonable amount of macro and micronutrients (Tarakhovskaya et al., 2007). Reports on BGA as biostimulants on bell pepper are almost nonexistent, although bell pepper is rich in vitamins and antioxidants, particularly ascorbic acid and different carotenoids. Thus, they may have several therapeutic advantages, for example, improved eyesight and decreased potential risks associated with chronic ailments (Dias, 2012). Several studies have shown that the presence of BGA in soil has the potential to positively influence plant growth. For instance, (Riahi et al., 2011) showed positive impacts of cyanobacteria on vegetative and reproductive parameters of herbaceous plants. The results of our study indicated that the three cyanobacteria strains possessed the ability to significantly enhance several growth parameters when applied in different forms and doses to plants including shoot length, root length, fresh weight, dry weight, spad index, number of leaves, and growth rate parameters. This study showed that the usage of *Roholtiella sp.* QUCCCM97, *Nostoc ellipsosporum* QUCCCM99, and *Desmonostoc danxiaense* QUCCCM112 aqueous extracts and biomass enhanced the growth of seedlings as compared to control group.

Several studies have pointed out the positive impacts of cyanobacteria on plant development, nutrient consumption, and nitrogen fixation, leading to plant growth enhancement (Gantar et al., 1995; Irisarri et al., 2001; Nilsson et al., 2002; Shariatmadari et al., 2013). Our results are in line with these reports because the extracts and biomass of *Roholtiella sp*. QUCCC97, *Nostoc ellipsosporum* QUCCCM99, and *Desmonostoc danxiaense* QUCCCM112 were very rich in total nitrogen, and its bioavailable forms nitrate, ammonium, and nitrite. Consequently, treated seedlings had better and higher performance in the vegetative growth parameters. Also, other bioactive compounds aside from the nitrogenous level of cyanobacterial played vital roles in stimulating the growth of treated seedlings (Michalak et al., 2016). Furthermore, qualitatively and quantitatively, the phycocyanin and phycoerythrin are found in *Roholtiella sp.* QUCCCM97extr, *Nostoc ellipsosporum* QUCCCM99extr, and *Desmonostoc danxiaense* QUCCCM112extr of cultures were similar to study (Sobiechowska-Sasim et al., 2014), Phycocyanin was found in all extracts, appearing more in excess than *Desmonostoc danxiaense*, and *Roholtiella sp*. of three species analyzed. The absorbance spectra of phycobiliproteins (phycoerythrin, phycocyanin) isolated from these strains of cyanobacteria (*Roholtiella sp.* QUCCCM97, *Nostoc ellipsosporum* QUCCCM99, and *Desmonostoc danxiaense* QUCCCM112) were remarkably similar and conform with the high peak wavelength absorbance maxima of phycobiliproteins investigated in similar studies and with slight variation in the range of λ_{max} (Figure 16). Several studies have equally shown that at the spectra peak of 620 nm and 565 nm of the high-value phycobiliproteins (phycoerythrin, phycocyanin) could be found (Hsieh-Lo et al., 2019). This further proved that our used strains contain these pigments as they were eventually responsible for the positive impacts on the investigated growth parameters.

Virtually all concentrations of *Roholtiella sp.* QUCCCM97, *Nostoc ellipsosporum* QUCCCM99, and *Desmonostoc danxiaense* QUCCCM112 HVPEs and WRB influenced statistically the shoot length and other growth factors. The obtained results corroborate the studies conducted previously (Hegazi et al., 2010; Wuang et al., 2016). Also, the study conducted by (Aghofack-Nguemezi et al., 2015) showed that cyanobacteria aqueous extracts affect the growth parameters and performance of tomato plants. Accordingly, there was an increase in the length and diameter of the plant by 19% and 33% respectively, which is in line with current outcomes.

The application of the HVPE and WRB of *Roholtiella* sp. QUCCCM97, *Nostoc ellipsosporum* QUCCCM99, and *Desmonostoc danxiaense* QUCCCM112 enhanced plant growth and parameters compared to the controls in a positive manner. Interestingly, our results could be compared with those of (Kumari et al., 2011) who observed greater growth and development in vegetative parameters (shoot length, root length, and fresh weight) as concentrations of *Sargassum johnstonii* extracts increase. In contrast (Hernández-Herrera et al., 2014) observed greater plant growth factors with decreasing extract concentrations. Furthermore, our study revealed that all BGA strains *Roholtiella sp.* QUCCCM97, *Nostoc ellipsosporum* QUCCCM99, and *Desmonostoc danxiaense* QUCCCM112 positively enhanced the fresh mass of the seedling and this confirms data from earlier studies conducted on cyanobacteria (Hegazi et al., 2010; Tuhy et al., 2015; Wuang et al., 2016; Mógor et al., 2018). For example, *Spirulina plantensis* can influence the fresh weight and fruit biomass of tomato plants by 48% and 43% respectively when compared with untreated plants (Aghofack-Nguemezi et al., 2015). In another study, the effect of this organism extract was observed to be positive on the yield factors of winter wheat (variety Akteur). Applied doses with the highest extract concentrations of 1.5 L ha^{-1} and 1 L ha^{-1} showed significant differences

when compared with the control group (Michalak et al., 2016). In the study conducted by (Mógor et al., 2018) on the potential of *Arthrospira platensis (Spirulina platensis)* as biostimulant, outcomes showed higher fresh weight in lettuce (*L. sativa*) due to the activity of cytokinin, which could be equally embedded in *Roholtiella sp.* QUCCCM97, *Nostoc ellipsosporum* QUCCCM99, and *Desmonostoc danxiaense* QUCCCM112.

However, the beneficial impacts of heterocystous cyanobacteria were previously assessed on the growth of pumpkin *(Cucubita pepo L.)* and cucumber *(Cucumis sativus L.)* seedlings from the application of *Westiellopsis prolifica* extracts (Nanda et al., 1991; Shariatmadari et al., 2013). The observed significant increase in the growth and development of both crops is in line with our results on bell pepper seedlings. A study conducted on the beneficial effects of cyanobacteria extracts on potato (*Solanum tuberosum* L.) established that the increase in the crop yields should not be seen only as of the nitrogen-fixing potential of cyanobacteria. It could also be a result of the endogenous growth-regulating substances synthesized by these cyanobacteria (Shanab et al., 2003; Shariatmadari et al., 2013). This assumption was duly supported because some non-nitrogen fixing cyanobacterial species e.g. *Oscillatoria spp*. and *Phnomedium spp*. improved the growth of different plants such as rice (Shukla et al., 1967; Gupta et al., 1970). In addition, this is connected with the synthesis of growth-promoting compounds and essential vitamins such as folic acid, vit. B12, nicotinic acid as well as pantothenic acid, are likely other reasons for the substantial growth, development, and yield of the treated plants (Mutale-joan et al., 2020) .

The utilization of algae as biostimulants and biofertilizers has been reported by different studies with more emphasis on the application of blue-green algae such as cyanobacteria on cereal crops, vegetables, legumes, etc. (Bello et al., 2021b). Interestingly, they are gaining fast recognition/popularity and acceptance globally in

the field of agriculture and horticulture simply because of their atmospheric nitrogen-fixing potential for the betterment of plants (Youssef et al., 2014; El-Eslamboly et al., 2019). It was obvious from our previous study/results that the evaluated three strains of cyanobacteria namely *Roholtiella* sp. (QUCCCM97), *Nostoc ellipsosporum* (QUCCCM99), and *Desmonostoc danxiaense* (QUCCCM112) extracts and biomass have positive effects on the bell pepper vegetative parameters (Bello et al., 2021a).

Also, enough comprehensive studies on a large scale have not been carried out to investigate the impacts of BGA on bell pepper as a biostimulant and biofertilizers simultaneously. Thus, considering the essentiality of this vegetable which contains vitamins and antioxidants, especially vitamin c and different carotenoids. However, it is clear from the obtained results that the *Roholtiella* sp. HVPE and WRB have shown a significant positive impact on the various estimated parameter/factors viz shoot height, shoot/stem diameter, number of leaves, leaf length, leaf width (growth parameters), chlorophyll index, fruit length, fruit width (fruit parameters), fruit weight plant^{-1}, number of fruit plant^{-1}, and yield (kg/treatment) (yield and its components) investigated. The exhibited significant effects are the same with the fruit nutritional value such as the total soluble solids, ascorbic acid, and phenolic acid. Furthermore, our study has established the positive impacts of the application of *Roholtiella* sp. HVPE and WRB at different doses. Interestingly, our study showed that *Roholtiella* sp. HVPE and WRB have the potential to enhance growth and productivity in treated bell pepper. Getting to know the impacts of HVPE and WRB on the physiological regulation as well as biochemical pathways associated with plant development, nutrient absorption, and metabolic processes can have a huge influence on improving productivity and sustainability in agriculture. To the best of our understanding, our study is the first in Qatar to investigate the effect of *Roholtiella* sp. HVPE and WRB

simultaneously under hydroponic agriculture.

Furthermore, the application of HVPEs and WRBs to bell pepper significantly enhances vegetative parameters such as shoot length, number of leaves, plant leaf length, plant leaf width, and diameter of the shoot. The previous investigation has shown that algae crude extracts and biomass, as well as compounds that are purified, can cause strong physiological responses in plants and growth parameters viz. shoot, root weight (Michalak et al., 2016; Zheng et al., 2016; Yakhin et al., 2017; Ertani et al., 2018; Hamed et al., 2018) which falls in line with our findings. Also, microalgae extract viz polysaccharides have been reported to enhance productivity in crops (Vacca et al., 2004; Obertello et al., 2010; Ding et al., 2018; Jagodzik et al., 2018). In a like manner, the recorded chlorophyll spad index as an indicator for the relative chlorophyll content in this study as a significant increase was found to be statistically significant in all the treatments when compared with the control groups. However, the foliar application of *Roholtiella* sp. HVPEs and WRBs exhibited significantly higher estimates of pigments such as chlorophyll a and b as well as carotenoids. Interestingly, our results are not unlikely to relate to the enhancement potential of cyanobacteria extract and biomass as a result of a reasonable amount of hormones such as cytokinins, gibberellin as well as likewise substances present in them. Therefore, the growth enhancer constituents in cyanobacteria might be responsible for these positive effects in bell pepper (*Capsicum annuum* L.) plants. Our results are in line with previous results reported by (Bello et al., 2021a) on bell pepper seedlings, a study on sugar beet by (Enan et al., 2016), and the evaluation of tomatoes by (El-Sayed et al., 2018). Furthermore, reference to the results in yield components and yield itself, 6 ml L^{-1} HVPE produced the highest number of fruit $plant^{-1}$ and fresh weight in gram $fruit^{-1}$ followed by 4 and 2 ml L^{-1} HVPE respectively. Similarly, the WRB followed the trend in which the highest number of

fruit plant^{-1} and fruit weight in gram fruit^{-1} was produced at the concentration of 6, 4, and 2 ml L^{-1} in that order compared with the control group. Naturally, the number of fruit plant^{-1} and fresh weight in gram fruit^{-1} as the yield components are the function of the total fruit yield, meaning that when these components increase the yield will increase. Though this phenomenon could change when they are not fully independent, that is when one component increases and the other decreases, thus the yield could be affected. Therefore, the obtained results may be connected to the impacts of several growth constituents in the composition of HVPE and WRB viz phytohormones, amino acids, fatty acids as well as amino acids coupled with the facts about the endowing ability of blue-green algae to reserve a quite large amount of mineral nutrient. Similarly, from our results, the ability of HVPE and WRB to positively enhance the photosynthetic pigments has greatly influenced the carbohydrate storage at the optimal level which invariably leads to the greater bell pepper yield and its respective components when compared with the control group. All these results have been supported by the findings reported by (El-Eslamboly et al., 2019) on the impacts of algae extract on the yield characteristics and total yield of cucumber. In the same manner, our results are supported by the findings from a study conducted by (Ahmed et al., 2012) that reported the large composition of amino acid together with the huge amounts of fatty acid, phycobiliproteins, as well as polypeptides hormones in algae extract which plays a major role in the improvement of the vegetative growth, yield and fruit quality of cucumber.

The data reported in this study indicated that HVPE and WRB foliar sprayed bell pepper plants showed a significant increase in all the fruit constituents with the highest or at the maximum concentration of HVPE and WRB compared with the control groups because of their mineral composition such as nitrate (nitrogen), phosphorus, and

potassium. This result could be equally connected with the presence of a huge amount of mineral nutrients in algae both cation and anions as reported by Marrez et al. (2014). Interestingly, we could convincingly submit that the significant increase in vegetative growth, yield components, and the total yield itself is strongly connected with the increased accumulation and increase of these minerals such as potassium, sodium, calcium, potassium, etc as embedded in algae (Enan et al., 2016). Furthermore, data reported in Table 16. indicated that *Roholtiella* sp (foliar sprayed with HVPE and WRB) showed insignificant differences at the minimal concentration of 2 ml L^{-1} with the control group for the total soluble solids (TSS). This result is correlated with those reported on paddy rice by Youssef and Eissa which caused just a 10 - 30% increase in the production (Youssef et al., 2014). However, at a higher concentration of 4 and 6 ml L^{-1} respectively, we obtained contrary results as there is a significant difference in TSS compared to the control groups and this might be connected to a possible increase in the mineral concentrations. Similarly, our findings showed a high positive significant difference between the treatments with phenolic compound accumulation and ascorbic acid content. The Folin-Ciocalteau assay was used to measure the total phenolic content in the bell pepper.

The total phenolic content is often overestimated by this method because of the availability of some hindering compounds viz. ascorbic acid, yet, the method remains the most reliable and only single method to estimate the total phenol content and it is still extensively in use (Deepa et al., 2007). The phenolic and ascorbic accumulation increased with the increase in the concentration of HVPE and WRB as compared with the control group. These results may be connected with the antioxidant and enhancer potential of algae generally. In the present study, Total phenolic contents for the raw bell pepper (*Capsicum annuum* L.) fall in the range of those previously reported (1152.8

- 1344.8 ug GAE g^{-1} fresh tissue or fresh weight) by (Turkmen et al., 2005; de Jesús Ornelas-Paz et al., 2010). In general, all these significant increases of the parameter is a result of the enhancer potential of *Roholtiella* sp HVPE and WRB respectively. However, the positive contribution of phenolic compounds and ascorbic acid on bell pepper vegetative growth parameters, total soluble solids, and yield were established by their strong positive correlations with Shoot height, the number of leaves, length, and width of leaves, fruit weight, total fruit yield, and total soluble solids Table 17.

The HVPE and WRB as a salt stress alleviator, generally, plant production has been estimated to be lower by as much as 50% simply because of the adverse effect of abiotic stresses on plants (Battacharyya et al., 2015). Plants expend most of their energy on essential processes for maintenance, cell rejuvenation, vegetative, and generative growth when grown under non-stressed conditions (Bayona-Morcillo et al., 2020). Once plants are stressed, resources allocation is affected as more of it is required as salinity increases to reduce the purported stress (Zörb et al., 2019). Bell pepper (*Capsicum annuum* L.) exhibited reasonable tolerance to salinity conditions when treated with *Roholtiella* sp extract compared with water-treated stressed seedlings. However, it has been reported that abiotic stress negatively affects plant growth and development, therefore, salinity stress retarding/curbing plant growth and development (ALKahtani et al., 2020). Consequently, by definition, salt/salinity tolerance is the ability of a stressed plant to withstand the deleterious impacts of excessive concentration of salt or increasing salinity without recording any significant adverse impacts viz. growth retardation, output reduction, or foliar salt destruction (Niu et al., 2017). Interestingly, the positive impacts of cyanobacteria and microalgae on flora growth and development have been reported by several studies but not much attention has been given to important vegetables such as bell pepper despite its both economic

and health benefits. Also, in this study, the evaluated vegetative parameters (shoot height, number of leaves, dry weight, fresh weight, and the number of leaves) are significantly affected as salinity levels increase. High salinity concentration caused the decline in the growth parameter as a result of alteration in the bioprocesses of the plant. However, these growth parameters were found to increase in the seedlings treated/foliar sprayed with *Roholtiella* sp extract compared to the control group. The shoot length, root length, and the number of leaves significantly increased compared to the control. This result complies with the report from Plaza et al 2018 study, in which he reported a positive effect from the application of A. *platensis* extract by improving the number of flowers per plant as well as the root dry contents (Plaza et al., 2018). Similarly, the increase in the shoot length complies with the report from the study conducted by Mansori et al (Mansori et al., 2015), in which *phaseolus* plant treated with extract exhibited an increase in the shoot and root length respectively. Generally, from our study, the application of *Roholtiella* sp extract to treat the salt-stressed plants of bell pepper has shown an improvement in their growth performance throughout the experimental period. Furthermore, from our results, it was shown that salinity stress has damaging effects on bell pepper at the concentrations level. The process of photosynthesis greatly depends on chlorophyll a, b, and to some extent the total chlorophyll, a biochemical process that is executed by two reactions which are light and dark sensitive. The first reaction which is light sensitive, there is the formation of Nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) while the second reaction is dark sensitive leading to fixing carbon dioxide (Allakhverdiev et al., 2002; ALKahtani et al., 2020). However, from our results, it is obvious that chlorophyll content decreased significantly under the four salinity concentrations, thus the decremental trend is from the lower concentration (50 mM) to

the higher concentration (200 mM) respectively that is likely to be caused by the deleterious effect of salinity stress on the composition of stomata (El-Banna et al., 2018; Khan et al., 2020b). because it is a known fact that leaf growth rate retardation and subsequent leaf area reduction are the number one plant responses to salt stress. No doubt, there is a positive relationship between the chlorophyll reduction and the relative water content (RWC) reduction as these are influenced by the varying degree of salinity. However, the foliar sprayed of *Roholtiella* sp extract reduces the negative effects of salt stress on the chlorophyll content that subsequently enhanced bell pepper growth and rapid development of plants exposed to stressful environments which conform with an investigation conducted on bell pepper by Al-Kahtani et al (ALKahtani et al., 2020). In addition, the application of *Rohpltiella* sp extract increasing the chlorophyll content may be connected with the availability of amino acid and phycobiliproteins in most blue-green algae which is equally peculiar with cyanobacteria such as *Roholtiella* sp as well. These findings comply with a previous study conducted by Possingham (Possingham, 1980). Also, the significant increase in bell pepper plant growth and pigments content in *Roholtiella* sp extract in the foliar treatment of the stressed seedlings improvement can be connected to the osmotic adjustment as previously reported by Mutale-joan et al. (Mutale-joan et al., 2021). Generally, the proline accumulation increases faster in stressed plants at different conditions when compared with other amino acids (Sadasivam et al., 2017). The bell pepper plants sprayed with *Roholtiella* sp. extract exhibited an increase in the proline accumulation at all the concentration levels of NaCl as compared to those treated with water at the same NaCl levels. Plants are immobile or sessile organism that is often affected by different stresses at every growth stage. Proline like every other inert solute or inactive metabolites such as glycine-betaine are regarded as osmolytes because of

their potential to suppress osmotic stresses emanated from salt stress in particular. Many studies established that proline contributes essentially to the adjustment of osmotic stresses by indemnifying for the osmotic pressure of cations such as Na+ (Acosta-Motos et al., 2017; Yakhin et al., 2017; Chun et al., 2018). From the present study, the accumulation of proline was significantly activated by *Roholtiella* sp. extract in bell pepper seedlings that were put through different NaCl levels (50, 100, 150, and 200 mM) when compared with seedlings sprayed with water. This result correlates with a similar study conducted by Chanda Mutale-joan et al. that exhibited a significant increase in the proline accumulation treated with microalgae extract when compared with the control (Mutale-joan et al., 2021). In a like manner, *Roholtiella* sp. extract increased reactive oxygen species ROS scavenging enzymatic processes of catalase compared to bell pepper-sprayed with water. Tukan in his study expressed that ROS serve as signaling molecules that control bioactivities and ways plant deals with different stresses both the biotic and abiotic (Turkan, 2018). At different salinity conditions, the excessive accumulation of ROS is triggered resulting in a situation capable of destroying the cellular structures of plants (Huang et al., 2019; Khan et al., 2020a; Zhao et al., 2020). However, to counter the possible oxidative stress resulting from excessive accumulation of ROS, thus, RSO scavenging mechanism is triggered by plants (Hanin et al., 2016). Interestingly, in the present work, the activities of CAT in *Roholtiella* sp extract sprayed plants have increased significantly compared to water sprayed plants. This is an indication that the enhanced activity of CAT in the extract sprayed plants can scavenge ROS by decomposing H_2O_2 into H_2O and O_2. This result is in line with several studies that reported that the activities of antioxidant enzymes viz. SOD, POX, and CAT reduce the probable oxidative damage through the ROS decomposition to H_2O_2 or by detoxification (Caverzan et al., 2019; Tahjib-UI-Arif et

al., 2019; Khan et al., 2020a). In a like manner, this result is supported by a report from the study conducted by El-Sharkawy et al. detailing the detoxification potential of CAT activity in two different cultivars of Alfalfa that are salt sensitive and tolerance (El-Sharkawy et al., 2017). Similarly, there is a correlation between this result and that of Mittova et al reported the enhancement of CAT activity mitigating salinity stress in *Lycopersicon pennellii*, a wild salt-tolerant tomato cultivar (Mittova et al., 2003). The comprehensive analysis of results of antioxidant capacity of bell pepper plants subjected to salinity at various concentrations obtained from foliar spraying with water and *Roholtiella* sp extract exhibited that the extract treatment significantly enhanced antioxidant capacity compared with the plant group treated with water.

Finally, the study conducted on the effect of temperature on bell pepper seedling and the biochemical response has shown that there is a significant effect at different treatments compared with the control group. Bell pepper plant is often exposed to abiotic stresses that affect their vegetative growth, physiological development, and productivity negatively. Of all these stresses, drought and heat stress are responsible for the most destruction in plant development. Generally, plant responses to extreme temperature are regulated by their genetic potential to withstand and ability to attain tolerance level to heat stress. In this study, at the extreme temperature of 40°C treatment with the seedlings, the pigment contents (chlorophyll a, b, total chlorophyll, and carotene) significantly decrease, an indication that the chloroplast has suffered from structural damage in bell pepper (*Capsicum annuum* L.) seedling because of the extreme temperature, even at the assumed moderate temperature of 32°C. The decline in chlorophyll as found in the heat stress susceptible bell pepper complies with the study conducted by Jagtap et al, Kumar et al in which they reported a decline in chlorophyll a, b, and total chlorophyll content (Jagtap et al., 1998; Kumar et al., 2012).

In a like manner, the significant increase in proline content accumulation under the moderate and extreme heat stress was recorded in the bell pepper when compared to the control group at 25°C. The accumulation of proline under the heat stress may be connected to the osmoprotectant for cellular composition to fight against/repel the high temperature (Kumar et al., 2012; Gosavi et al., 2014). The cytoplasm serves as the point or location where the synthesis of proline in plants occurs from glutamate via glutamyl-y-semialdehyde as the major pathway Not only that, the accumulation may be a result of a double-step reaction mostly catalyzed by the biofunctional enzymes such as -1-pyrroline-5-carboxylate synthetase (P5CS) (Parida et al., 2008). However, the proline accumulation increase as reported by Kumar et al 2012 that probably contributes toward higher heat stress is in support of this present investigation.

Also, in the present study, exposing bell pepper to different temperatures as heat stress caused the significant increase in the enzymatic activity of catalase (CAT), a probable indication that an antioxidant enzyme could be triggered to scavage or remove reactive oxygen species (ROS) such as O_2^-, H_2O_2, OH^- etc. to countereffect of their harmful actions. The significant increase in the activity of CAT is attributable to the scavenging of hydrogen peroxide H_2O_2. The findings in this study concerning the significant increase in antioxidants enzymatic activity have been supported by several reports (Hameed et al., 2012; Kumar et al., 2012).

CHAPTER 8: CONCLUSION

In conclusion, we closely examined the effect of extracts and biomass of three cyanobacteria strains namely, *Roholtiella* sp. QUCCCM97, *Nostoc ellipsosporum* QUCCCM99, and *Desmonostoc danxiaense* QUCCCM112 on the growth of bell pepper seedlings (*Capsicum annuum* L.). Outcomes showed that the highest treatment dose/concentration Tr3 (i.e., extracts added to a modified Hoagland nutrient solution at 0.6% concentration) led to the highest performance in growth parameters compared with controls and other treatments. The positive effect was dose-dependent with the increase of biomass or extract concentrations. Besides, the soilless experimental results showed that at the highest dose, phycoerythrin exhibited a great positive impact on the bell pepper when compared with the control. Hence, phycoerythrin can be regarded as the plant growth stimulator for bell pepper. In conclusion, all of the three local cyanobacteria strains had the potential of promoting the growth and development of bell pepper with *Roholtiella* sp. QUCCCM97 having the highest potential under the described experimental conditions. Consequently, they can be suitable for the formulation of a biofertilizer based on their beneficial performances. Future experiments should focus on field trials using separate or mixtures of the *Roholtiella* sp. QUCCCM97, *Nostoc ellipsosporum* QUCCCM99, and *Desmonostoc danxiaense* QUCCCM112 extracts and biomass as they showed higher seedling growth performances.

This study clearly demonstrated the foliar application of cyanobacterium (*Roholtiella* sp.) HVPE and WRB at various concentrations positively affect the bell pepper growth parameters, fruits production yields, and quality. Collectively, our results suggest that the strain *Roholtiella* sp. QUCCCM97 isolated locally, can serve as a potential biofertilizer candidate for enhancing vegetative growth characteristics, fruit

components, yield, and fruit quality of bell pepper. Based on this finding, bell pepper could be considered as a template crop and similar results could be expected on similar crops if *Roholtiella* sp. extract (HVPE) and biomass (WRB) are used to promote the production of vegetable and non-vegetable plants.

Also, from the salinity study, we noticed that bell pepper (*Capsicum annuum* L.) exposed to salt stress at various concentrations (50, 100, 150, and 200 mM) was negatively affected and this impact could be minimized by foliar application of *Roholtiella* sp. extract. The extract was advantageous and played a crucial role in attenuating the adverse impacts of salt stress on bell pepper vegetative growth, biochemical characteristics, and enzymatic activity. Accordingly, the application of *Roholtiella* sp. extracts resulted in an increased shoot length, root length, number of leaves, fresh weight, dry weight, chlorophyll content, proline accumulation, and enzymatic activity. However, the obtained results from this study will support the enhancement of bell pepper production under salt stress in large-scale field and hydroponic production systems by the application of *Roholtiella* sp. extracts. Interestingly, to the best of our understanding, this report on the role of cyanobacteria (*Roholtiella* sp.) high-value product extract in salt stress mitigation on sweet pepper (*Capsicum annum* L.) is likely the first study of its kind in Qatar and probably in the entire Gulf Cooperation Council (GCC) countries

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Appendix

Appendix A: Published Article 1

Type of article: Original research article

Title:

Enhancement in Bell Pepper (*Capsicum annuum* L.) Plants with Application of *Roholtiella* sp. (Nostocales) under Soilless Cultivation

Journal: agronomy

Impact factor: 3.417 **(Q1)**

Date of publishing: 16 August 2021

Authors: Adewale Suraj Bello, Imen Saadaoui, Talaat Ahmed, Helmi Hamdi, Maroua Cherif,Tasneem Dalgamouni, Ghamza Al Ghazal, and Radhouane Ben-Hamadou

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agronomy MDPI

Article

Enhancement in Bell Pepper (*Capsicum annuum* L.) Plants with Application of *Roholtiella* sp. (Nostocales) under Soilless Cultivation

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Abstract: Cyanobacteria are found to be renewable and sustainable additives for growth improvement in crops. Extracts and biomass of three nitrogen-fixing cyanobacteria namely, *Roholtiella* sp. (QUCCCM97), *Nostoc ellipsosporum* (QUCCCM99), and *Desmonostoc danxiaense* (QUCCCM112) isolated from Qatar desert environment were tested for their ability to enhance the growth of bell pepper (*Capsicum annuum* L.) seedlings. Soilless cultivation experiments were carried out by applying the biomass and the aqueous extract of the three cyanobacteria separately. Seedlings were transplanted to Hoagland's solution under regulated conditions. In total, 2, 4, and 6 mL L^{-1} of the three microalgae extract as well as 1 and 2 mg L^{-1} of the three microalgae biomasses (as biofertilizer) were added to the Hoagland solution. An assessment of seedling growth parameters such as shoot length, root length, fresh weight, dry weight, spad index, number of leaves per plant, and growth rate was performed. However, among the different doses and concentrations of investigated QUCCCM97, 99, and 112, our findings revealed that shoot length (cm), root length (cm), fresh weight (g), the number of leaves per plant, and growth rate were positively affected and significantly increased at maximum dose/concentration compared to control plants. With QUCCCM97, shoot length, root length, fresh weight, the number of leaves, and the growth rate increased by 17.5%, 40.3%, 26.0%, 21.6%, and 22.8%, respectively, compared to the control. Additionally, with QUCCCM99, the same parameters increased by 12.3%, 25.3%, 15.1%, 9.3%, 51.8%, respectively. While in presence of QUCCCM112, they increased by 8.7%, 30.1%, 15.6%, 5.4%, 48.6%, respectively. Our results demonstrated that extracts and biomass of cyanobacteria strains investigated here, and particularly *Roholtiella* sp. (Nostocales), have an enhancement potential of the seedling growth and could be used in modern agriculture to enhance productivity under the soilless system and ensure sustainability.

Keywords: aqueous extract; *Capsicum annuum*; cyanobacteria biomass; growth enhancer; soilless cultivation system

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Citation: Bello, A.S.; Saadaoui, I.; Ahmed, T.; Hamdi, H.; Cherif, M.; Dalgamouni, T.; Al Ghazal, G.; Ben-Hamadou, R. Enhancement in Bell Pepper (*Capsicum annuum* L.) Plants with Application of *Roholtiella* sp. (Nostocales) under Soilless Cultivation. *Agronomy* **2021**, *11*, 1624. https://doi.org/10.3390/agronomy11081624

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1. Introduction

The persistent excessive use of chemical fertilizers leads to an increase in crop output but not without adverse/detrimental effects on the environment such as a rapid decrease in soil quality and fertility [1,2]. Additionally, it can cause biodiversity decline, eutrophication, and global ecological degradation [3–7]. In this regard, several fertilizing alternatives have been proposed to reduce the cost and environmental impacts of chemical fertilizers. Algae are preferred substitutes for chemical fertilizers and can be used as biofertilizers due to their potential of enhancing the general health conditions of plants with the ability to

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Appendix B: Published Article 2

Type of article: Original research article

Title:

Application of Cyanobacteria (*Roholtiella* sp.) Liquid Extract for the Alleviation of Salt Stress in Bell Pepper (*Capsicum annuum* L.) Plants Grown in a Soilless System

Journal: plants

Impact factor: 3.935 **(Q1)**

Date of publishing: 30 December 2021

Authors: Adewale Suraj Bello, Radhouane Ben-Hamadou, Helmi Hamdi, Imen Saadaoui, and Talaat Ahmed

plants

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Article

Application of Cyanobacteria (*Roholtiella* sp.) Liquid Extract for the Alleviation of Salt Stress in Bell Pepper (*Capsicum annuum* L.) Plants Grown in a Soilless System

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Abstract: Salinity is one of the abiotic stresses that affect crop growth and productivity in arid and semi-arid regions. Unfortunately, there are few known methods to mitigate the deleterious impacts of salt stress on the development and yield of vegetable crops. Blue-green algae (cyanobacteria) are endowed with the potential to curb the negative impacts of salt stress as they are characterized by biostimulant properties. The present work aimed to investigate the effects of *Roholtiella* sp. as a foliar extract on the growth characteristics, physiological and biochemical responses of bell pepper (*Capsicum annuum* L.) plants under varying levels of salinity conditions. A soilless water experiment was carried out in a greenhouse where bell pepper seedlings were grown under five salt concentrations (0, 50, 200, 150, and 200 mM of NaCl). Growth characteristics, pigments content, relative water content, and antioxidant activity (CAT) were determined. Our results showed that growth parameters, relative water content (RWC), chlorophyll a & b concentrations under salinity conditions were negatively affected at the highest concentration (200 mM). Interestingly, the application of *Roholtiella* sp. foliar extract enhanced the plant growth characteristics as shoot length increased by 17.014%, fresh weight by 39.15%, dry and weight by 31.02%, at various salt treatments. Moreover, chlorophyll a and b increased significantly compared with seedlings sprayed with water. Similarly, RWC exhibited a significant increase (92.05%) compared with plants sprayed with water. In addition, antioxidants activities and accumulation of proline were improved in *Roholtella* sp. extract foliar sprayed seedlings compared to the plants foliar sprayed with water. Conclusively, at the expiration of our study, the *Rohotiella* sp. extract-treated plants were found to be more efficient in mitigating the deleterious effects caused by the salinity conditions which is an indication of an enhancement potential of tolerating salt-stressed plants when compared to the control group.

Keywords: *Roholtiella* spp.; salinity stress; Bell pepper (*Capsicum annuum* L.); foliar spray/application

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Citation: Bello, A.S.; Ben-Hamadou, R.; Hamdi, H.; Saadaoui, I.; Ahmed, T. Application of Cyanobacteria (*Roholtiella* sp.) Liquid Extract for the Alleviation of Salt Stress in Bell Pepper (*Capsicum annuum* L.) Plants Grown in a Soilless System. *Plants* **2022**, *11*, 104. https://doi.org/10.3390/plants11010104

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1. Introduction

Several abiotic stresses viz drought (water stress), excessive water accumulation (waterlogging), extreme temperatures (cold, frost, and heat), and salinity, etc. are often responsible for poor crop production [1]. Thus, salinity is one of the serious and increasing challenges mitigating optimum crop production particularly the quality and quantity (yield) of the crop in arid and semi-arid countries [2,3]. Globally, over 9.0×10^2 million hectares of land are affected by salination, which constitutes one-fifth of total cultivable land [1,4]. Consequently, there is an urgent need for proactive measures to improve plant productivity and crop output under salination conditions to solve the problem of increasing food demand of

Appendix C: Submitted Manuscript (Accepted)

Type of article: Original research article

Title:

Evaluation of *Roholtiella* sp. extract on Bell pepper (*Capsicum annuum* L.) yield and quality in a hydroponic greenhouse system

Journal: Frontiers

Impact factor: 5.753 **(Q1)**

Date of publishing: In Press (2022)

Authors: Adewale Suraj Bello, Imen Saadaoui, Talaat Ahmed, Helmi Hamdi, and Radhouane Ben-Hamadou

Investigating bell peppers (*Capsicum annuum* L.) response to heat stress in leaves via biochemical and transcriptomic analysis

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Parameters - Chlorophyll content, carotene, proline, antioxidant, transcriptome.

Abstract - 25/9/21

The effects of heat stress on the chlorophyll content, total carotene, proline, antioxidant enzymatic activity (ABTS assay and catalase), transcriptomic analysis were studied in bell pepper (*Capsicum annuum* L.). Seedlings were planted using potting soil for almost 4 weeks at 25/18 day/night temperature and irrigated every other day with Hoagland nutrient solution. Selected healthy/vigor plants were transferred to a growth chamber at a constant optimum temperature of 25°C for 2 days to acclimate the seedlings. The temperature was adjusted systematically to 32 and 40°C to curb the possibility of heat shock. However, the effects of systematic/gradual heat stress on the parameters evaluated were significant. At the moderate temperature of 25°C, Chlorophyll a (4.327 mg g^{-1} FW), b (1.710 mg g^{-1} FW), total chlorophyll (5.202 mg g^{-1} FW), protein, and total carotene were decreased by heat stress compared to the control at 25°C for chlorophyll-a (5.383 mg g^{-1} FW), chlorophyll b (2.358 mg g^{-1} FW), and total chlorophyll (6.692 mg g^{-1} FW). **Conversely**, antioxidant activity (ABTS) was high in the sample exposed to 32 and 40°C compared to the control group in a response to the moderate and extreme temperature. In the same way, catalase activity significantly increased in response to the high temperature, at extreme conditions 40°C (8.34 units mg of $protein^{-1}$ min^{-1}) and moderates condition 32°C (5.72 units mg of $protein^{-1}$ min^{-1}) compared with the control group (3.86 units mg of $protein^{-1}$ min^{-1}) at a varying temperature (25, 32, and 40°C – optimal, moderate, and extreme). Also, the proline content of the stressed seedlings

Appendix D: Pictures of the Field Experiment (Greenhouse)

SITE PREPARATION

PLANTING OF SEEDLING

GROWING PLANT

FRUITING

LABORATORY EXPERIMENTS

YIELD/FRUIT

Integrated Recirculating Hydroponic Systems designed to investigate the effect of HVPE and WRB on yield of Bell Pepper and laboratory experimentation to determine their biochemical constituents.

Appendix E: Pictures of the Cultivation of Cyanobacteria

Outdoor Cultivation of Cyanobacteria In Large Scale Raceway Pond at the Farm of the Center for Sustainable Development (CSD), Qatar University, Qatar.