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**COUPLING *SPIRULINA* WITH HYDROPONICS: STUDIES ON GROWTH OF
VIGNA RADIATA AND *CICER AIRETINUM***

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ABSTRACT

Present-day world is constantly emerging with new technologies in the field of agriculture and hydroponics is amongst one such easily scalable and sustainable technique in the field of agriculture. In hydroponics, plants are grown by just immersing their roots in an aqueous nutrient solution without the usage of soil. Biofertilizers are already proved to be eco-friendly contributors to efficient agriculture that provide required micronutrients and macronutrients to the plants. In search of an economically viable, sustainable, and natural hydroponics system, experiments were conducted to check the effects of the phycocyanin-rich extract of *Spirulina* (PRES) and *Spirulina* residue as biofertilizers on the growth of plants like *Vigna radiata* and *Cicer arietinum* when grown hydroponically. Later, the hydroponically grown plants were tested for numerous growth parameters like leaf area, root length, shoot length chlorophyll content, dry weight, and fresh weight, and data obtained was then compared with control grown with bio compost. In brief, studies concluded that both the plants, showed the overall highest plant growth parameters in *Spirulina* residue hydroponics systems, higher in PRES, and the least in controls with bio compost system after a period of 20 days.

Keywords: hydroponics, *Spirulina*, biofertilizer, *Vigna radiata*, *Cicer arietinum*

INTRODUCTION

With elevated environmental pollution, climate changes, global warming, and rapid urbanization and industrialization, food

security challenges are posed to increase in the future. As a result of all this, the interest of government and agriculturalists has

shifted to developing new sustainable and environmentally friendly systems that would require less space to grow and produce more yield [1]. Hydroponics is one such emerged farming technique where plants are grown by just immersing their roots into aqueous nutrient solution without the usage of soil as substrate [2].

Current studies couple the usage of totally organic and eco-friendly *Spirulina* extract and PRES as a source of nutrient in hydroponics from the initial stages as optimal access to nutrients and careful cultivar selection increases the likelihood of getting closer to the highest production levels in presence of optimal lighting condition throughout the experiment period [3]. Biofertilizer is a single or group of live microorganisms when applied to plant promote the overall growth and help in increasing the yield [4]. Algae are an important part of aquatic biodiversity that grow in habitats like seawater, freshwater, brackish water, etc. Over few years, they have become commercially important microbes with applications in fields like nutraceuticals, pharmaceuticals, aquaculture, agriculture, bioremediation, and cosmetics [5].

It is subjected to research throughout the world due to the unique blend of compounds present in it which makes it the most nutritious concentrated whole food source found in nature. It contains highly

digestible protein which is a major component of about 60-70% of it with all essential amino acids. *Spirulina* also contains a variety of phytonutrients that are essential for the human body. Along with that, it is a very rich source of amino acids, minerals, vitamins, proteins, lipids, carbohydrates, carotenoids, and fibre, etc. [6].

The application of microalgae liquid fertilizer for the hydroponic system is an economic and environmentally friendly option that increases the growth of the plant and provides higher yield. Microalgae are efficient biofertilizers due to variety of roles it performs in the growth media like nitrogen fixation, the release of oxygen, production of plant hormones, increased water holding capacity, and production of plant growth metabolites as well as it thrives in different types of water environments [7, 8]. Algal fertilizers are the most commonly used biofertilizers as well as soil conditioners applied to soils [9]. The algal biofertilizers can be applied in various forms, but there are numerous advantages of liquid algal fertilizers over other forms as they possess longer shelf life for a duration of 12-24 months. The chances of contamination in liquid biofertilizer are rare as the algae grow at high pH and temperature conditions. Algal fertilizers provide protection against a range of organisms like fungi, pests, bacteria, and

insects. Algal fertilizers are safe when applied to plants and increases the germination rate and yield of crops. Cyanobacterial fertilizer can be easily applied to crops by farmers and has high export value as well as commercial potential. The hydroponic system requires only 10% of water compared to traditional farming techniques [10]. The algal fertilizers help in providing and maintaining nutrients in hydroponic systems [11]. Sharon et.al reviewed the effect of algal biofertilizer on the cultivation of *Mentha piperita*, also commonly known as peppermint. Leonard Lerer *et al* conducted experiments for studying the effect of phycocyanin-rich *Spirulina* extract on the growth of vertically cultivated lettuce where PRSE was applied as a bio stimulant. Application of PRSE reduced the harvesting time by six days and the crop yield by 12.5% with enhanced taste, colour, texture, nutritional levels, and shelf life was observed [12]. Not much work is done in the field of combining *Spirulina* biofertilizers with hydroponics, as hydroponics is an emerging technique and researchers have focused more on the exploitation of *Spirulina* in the field of nutraceuticals compared to hydroponics in agriculture. The main objective of the study is to check the effect of *Spirulina* and its crude phycocyanin extract on the overall

growth of two hydroponics plants *Vigna radiata* and *Cicer arietinum*.

MATERIALS AND METHODS

Materials

Three brown coloured trays with dimensions 10 x 9.5 x 4 cm were used. Three green coloured PVC mat with holes at 2.5 cm apart were used to cover the tray and make the system opaque. Circular plastic containers with diameter 4.5 cm and equal number of holes (approx. 30) in its bottom, were fitted into the mat. Cocopeat was used to hold plants well in systems. Seeds of *Vigna radiata* and *Cicer arietinum* were collected from local grocery shop in Karelibaug area, Vadodara, Gujarat, India. Dried powder of *Spirulina* gifted from Algallio Biotech Limited, Vadodara weighing 11 grams was used for the study. Chemicals used includes 0.1 M sodium phosphate buffer. All the experiments were performed in microbiology laboratory at Department of Microbiology, Parul University, Vadodara Gujarat.

Methodology

Preparation of allophycocyanin rich extract from *Spirulina*.

Allophycocyanin was extracted from *Spirulina* using repeated freezing and thawing (RFT) method. Dry powder of *Spirulina* weighing 11 grams was taken and suspended into 165ml of 0.1 M sodium phosphate buffer with ratio of 1:15 w/v in conical flask. The pH of the solution was

checked and set to 7. The solution was frozen at 18 degrees Celsius for 3 hours and later thawed at 25 degrees Celsius for 1 hour. Repeated freeze thaw cycles were operational for 2 consecutive days until dark blue colour due to presence of allophycocyanin after cell lysis was observed in the flask. The solution was further filtered to separate the lysed cell debris in the form of green cake and clear blue coloured allophycocyanin rich filtrate was obtained as shown in **Figure 2**. As the allophycocyanin is light sensitive, cake and filtrate both were stored in refrigerator appropriately for further use.

Germination of *Vigna radiata* and *Cicer arietinum*

The seeds bought from local grocery store were thoroughly washed and soaked in water for about 2 hours. Later the seeds were transferred on clean autoclaved petri plates with wet tissue covered on its inner surface to provide moist conditions suitable for germination. The plates were incubated in dark conditions at room temperature for 2 days for complete germination of seeds as shown in **Figure 2**.

The germinated seeds were later transferred to the hydroponics set up and allowed to grow under optimal sunlight and temperature conditions in the lab. The design of experiment is explained in **Table 1**.

Each of three trays had the capacity of 1000 ml and the water was changed after every two days to prevent the water from fungal contamination. The trays were further washed and equal amount of PRSE and *Spirulina* residue were added freshly to the water. The plants were allowed to grow for a period of 20 days

The experimental set up of hydroponics system is shown in **Figure 3**.

The plants were allowed to grow for 20 days under constant monitoring of water for contamination and after 20 days they were harvested from the trays. The different plant growth parameters checked after harvesting are listed below.

- Root length
- Shoot length
- Chlorophyll Estimation
- Fresh Weight
- Wilting time
- Dry weight



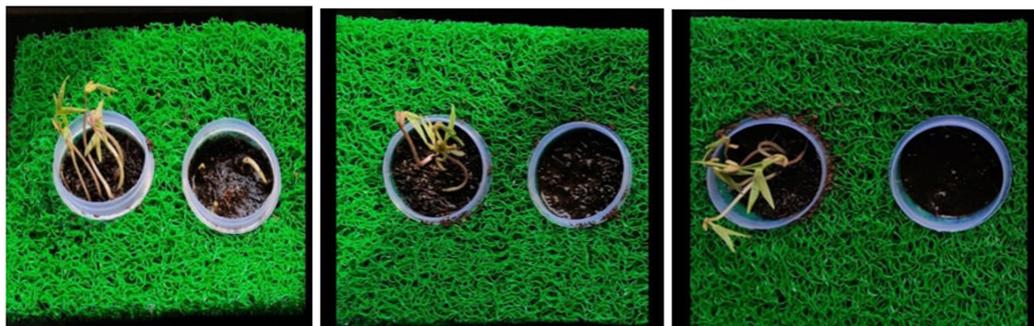
Figure 1: Filtration of Phycocyanin rich *Spirulina* extract and *Spirulina* residue cake



Figure 2: Germination of *Vigna radiata* and *Cicer arietinum*

Table 1: Treatment given in trays for hydroponics

Sr. No	Tray type	Treatment for <i>Vigna radiata</i>	Treatment for <i>Cicer arietinum</i>
1.	Control (1000 ml Drinking water)	5 germinated seeds + Cocopeat + 1 gm Bio-compost.	5 germinated seeds Cocopeat + 1 gm Bio- compost.
2.	Phycocyanin rich <i>Spirulina</i> extract. (1000 ml Drinking water)	5 germinated seeds + Cocopeat + 5 ml Phycocyanin extract	5 germinated seeds + Cocopeat + 5 ml Phycocyanin extract
3.	<i>Spirulina</i> cake (1000 ml Drinking water)	5 germinated seeds + Cocopeat + 150 mg of <i>Spirulina</i> residue.	5 germinated seeds + Cocopeat + 150 mg of <i>Spirulina</i> residue.



TRAY 1- Control

TRAY 2- PRSE

TRAY 3- *Spirulina* residue.

Figure 3: Experimental setup of hydroponics system

Measurement of leaf area

Leaf area of plant is one of the important plant growth parameters that helps in understanding the photosynthesis, light interception, water and nutrient use and crop growth. Leaf area is also considered as the indicator of conditions in which the plants are grown, their adaptation and response to the environment. The non-destructive method of direct measurement was used for calculating the leaf area. Different leaves from each shoot were taken respectively and measurement of their length and width was done. Further, total leaf area was calculated by given formula for each leaf, where L is the length of leaf and w corresponds to width of leaf.

$$\text{Total leaf area (LF)} = L \times w$$

Measurement of root length

Root length and surface area of roots plays a major role in nutrient uptake as well as water uptake. From agricultural point of view 1% change in root size corresponds to 2% change in the overall crop yield. The measurement of root length is important parameter is important for determining the growth of the plant as well as response of plants to various components added for plant growth and biotic and abiotic stress present in the surrounding. The plants with roots were removed from the hydroponic system and washed thoroughly in distilled water to remove all the cocopeat associated with it. The excess water on roots was

blotted on blotting paper, further the plants were spread on white paper and measured for root length using centimetre scale.

Measurement of shoot length

The shoot is considered as major production centre of plants which is necessary for development of all different organs systems of like stems, leaves and flowers in the plant. The shoot system performs major important functions like photosynthesis and reproduction. The shoots have tendency to grow more in length to provide plant maximum exposure to sunlight. Measuring the shoot length helps in determining the overall growth of plant as well as development of vascular tissues like xylem and phloem in the plants which are responsible for transport of water, minerals and food developed by the process of photosynthesis. The measurement of shoot length follows the same approach as root length measurement.

Chlorophyll estimation

The plants require all the important macro and micronutrients to achieve important physiological functions. Deficiencies in nutrients like, nitrogen, calcium, sulphur, magnesium, iron can lead to reduction in overall chlorophyll content of plants. Reduction in chlorophyll content reduces the amount of solar radiation that can be trapped into leaves which decreases the photosynthetic capacity of plants. Estimation of chlorophyll gives information

of photosynthetic capacity, biotic stress on plants and acquisition of nutrients by plants.

Freshly cut leaves weighing 0.8 gram for each plant, i.e. control, PRSE treated, and *Spirulina* treated were grounded with 20 ml of 80% freshly prepared acetone in chilled mortar and pestle. The sample was transferred into centrifuge tubes and centrifuged at 4000 rpm for 10 mins. The green supernatant was transferred to fresh tube and procedure was repeated until the pellet becomes colourless. The absorbance of supernatant was taken at 645nm and 663 nm respectively and content of chlorophyll a, chlorophyll b and total chlorophyll content was calculated using formula stated below.

$$\text{Total Chlorophyll: } 20.2 (A_{645}) + 8.02 (A_{663})$$

$$\text{Chlorophyll a: } 12.7 (A_{663}) - 2.69 (A_{645})$$

$$\text{Chlorophyll b: } 22.9 (A_{645}) - 4.68 (A_{663})$$

Estimation of fresh weight

Fresh weight of plant is important parameter for determining the commercial value of any plant. The water holding capacity of plant tissues and photosynthetic capacity can also be determined by fresh weight. Measuring the fresh weight gives information on addition of organic materials to the plant's cells directly. The plants with roots which were removed from hydroponic system were weighed on

weighing balance directly after cleaning and blotting on paper properly.

Estimation of wilting time

Wilting time is important for measuring the shelf life of plants, transpiration and overall water activity throughout vascular bundles of tissues. After estimation of leaf area, root length, shoot length, fresh weight and chlorophyll content, the plants were kept in petri dishes for drying and exposed to sunlight for few days. The time duration taken by the plants to start wilting before they are completely dried is termed as wilting time.

Estimation of dry weight

Fresh water of plant includes major part of water present in the plant which may vary according to amount of water present in the surrounding environmental as well as season in which the plant is growing, as seasonal moisture will affect the growth of the plant. Dry weight of plant is more reliable as compared fresh weight as all such problems are eliminated and accurate measurement of growth can be done by estimating the weight of dry biomass directly. The dry weight of plant is independent of moisture content in the surrounding as well as the type of season in which the plant is grown. The plants were kept in petri dishes for drying and exposed to sunlight for few days. After complete drying the plants were weighed for dry weight.

RESULTS

The overall results for plant growth in hydroponics after 20 days of period can be shown in **Figure 4** for *Cicer arietinum*.

Results of leaf area estimation of *Cicer arietinum* (Table 2).

Results of root length measurement of *Cicer arietinum* (Table 3).

Results of shoot length measurement of *Cicer arietinum* (Table 4).

Results of chlorophyll estimation of *Cicer arietinum* (Table 5).

Results of fresh weight of *Cicer arietinum* (Table 6).

Results of wilting time of *Cicer arietinum* (Table 7).

Results of dry weight of *Cicer arietinum* (Table 8).

The overall results for plant growth in hydroponics after period of 20 days can be shown in **Figure 6** for *Vigna radiata*.

Results of leaf area estimation of *Vigna radiata* (Table 9).

Results of root length measurement of *Vigna radiata* (Table 10).

Results of shoot length measurement of *Vigna radiata* (Table 11).

Results of chlorophyll estimation of *Vigna radiata* (Table 12).

Results of fresh weight of *Vigna radiata* (Table 13).

Results of wilting time of *Vigna radiata* (Table 14).

Results of dry weight of *Vigna radiata* (Table 15).

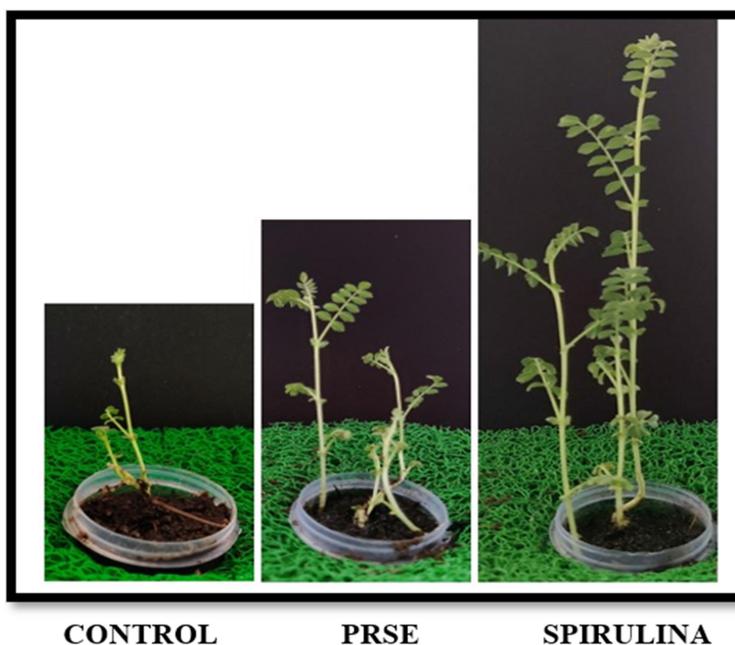


Figure 4: The overall growth of *Cicer arietinum* after 20 days

Table 2: Average results of leaf area in three treatment groups of *Cicer arietinum*

SHOOT NUMBER	CONTROL (l x b) cm	PRSE (l x b) cm	SPIRULINA (l x b) cm
1.	0.5 x 0.3 = 0.15	0.6 x 0.3 = 0.18	0.8 x 0.4 = 0.32
2.	0.5 x 0.4 = 0.20	0.7 x 0.3 = 0.21	0.7 x 0.4 = 0.28
3.	0.6 x 0.3 = 0.18	0.7 x 0.4 = 0.28	0.8 x 0.4 = 0.32
Average	0.17	0.22	0.30

Table 3: Average results of root length in three treatment groups of *Cicer arietinum*

SHOOT NUMBER	CONTROL	PRSE	SPIRULINA
1.	3 cm	4 cm	4 cm
2.	3.5 cm	5 cm	8 cm
3.	3 cm	3.5 cm	5 cm
Average	3.1 cm	4.1 cm	3.6 cm

Table 4: Average results of shoot length in three treatment groups of *Cicer arietinum*

SHOOT NUMBER	CONTROL	PRSE	SPIRULINA
1.	5 cm	12 cm	17 cm
2.	7 cm	11 cm	14 cm
3.	6 cm	11.5 cm	12 cm
Average	6 cm	11.5 cm	14.33 cm

Table 5: Results of chlorophyll a, chlorophyll b, and total chlorophyll content in three treatment groups of *Cicer arietinum*

CHLOROPHYLL	CONTROL	PRSE	SPIRULINA
Chlorophyll a = 12.7(A663) – 2.69 (A645)(µg/ml)	12.7(1.88) – 2.69 (0.76) = 21.832 (µg/ml)	12.7(2.066) – 2.69 (0.908) = 23.796 (µg/ml)	12.7(2.26) – 2.69 (1.69) = 24.162 (µg/ml)
Chlorophyll b = 22.9(A645) – 4.68 (A663)(µg/ml)	22.9 (0.76) – 4.68 (1.88) = 8.614 (µg/ml)	22.9 (0.908) – 4.68 (2.066) = 11.133 (µg/ml)	22.9 (1.69) – 4.68 (2.26) = 28.131 (µg/ml)
Total chlorophyll = 20.2(A645) + 8.02(A663) (µg/ml)	20.2(0.76) + 8.02(1.88) = 30.427 (µg/ml)	20.2(0.98) + 8.02(2.066) = 36.35 (µg/ml)	20.2(1.69) + 8.02(2.26) = 56.82 (µg/ml)

Table 6: Results of fresh weight in three treatment groups of *Cicer arietinum*

SR. NO	FRESH WEIGHT (grams).
1.	Control 0.9
2.	PRSE 1.2
3.	SPIRULINA 2.4

Table 7: Results of wilting time in three treatment groups of *Cicer arietinum*

SR. NO	WILTING TIME (hours)
1.	Control 1.5 hours
2.	PRSE 2 hours
3.	SPIRULINA 2 hours and 20 minutes

Table 8: Results of dry weight in three treatment groups of *Cicer arietinum*

SR. NO	DRY WEIGHT (grams)
1.	Control 0.40
2.	PRSE 0.54
3.	SPIRULINA 1.09

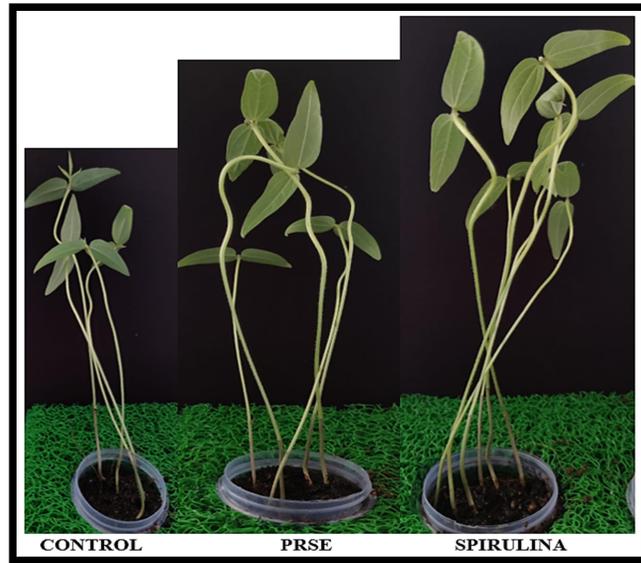


Figure 6: The overall growth of *Vigna radiata* after 20 days

Table 9: Average results of leaf area in three treatment groups of *Vigna radiata*

SHOOT NUMBER	CONTROL (l x b) cm	PRSE (l x b) cm	SPIRULINA (l x b) cm
1.	3 x 1 = 3	3.2 x 1.3 = 4.16	3.5 x 1.3 = 4.55
2.	2.8 x 1 = 2.8	3 x 1.2 = 3.6	3.6 x 1.3 = 4.68
3.	3 x 0.9 = 2.7	3.4 x 1.2 = 4.08	3.5 x 1.28 = 4.48
4.	2.9 x 1 = 2.9	3.2 x 1.1 = 3.52	3.4 x 1.3 = 4.42
Average	2.85	3.84	4.53

Table 10: Average results of root length in three treatment groups of *Vigna radiata*.

SHOOT NUMBER	CONTROL	PRSE	SPIRULINA
1.	3 cm	4 cm	4 cm
2.	4 cm	5 cm	8 cm
3.	3.5 cm	3.5 cm	5 cm
4.	2.5 cm	4 cm	4.5 cm
Average	3.25	4.12	5.37

Table 11: Average results of shoot length in three treatment groups of *Vignaradia*

SHOOT NUMBER	CONTROL	PRSE	SPIRULINA
1.	17 cm	18 cm	19 cm
2.	18 cm	18 cm	20.1 cm
3.	17 cm	19 cm	19 cm
4.	16.5 cm	20 cm	18.5 cm
Average	17.125 cm	18.75 cm	19.15 cm

Table 12: Results of chlorophyll a, chlorophyll b, and total chlorophyll content in three treatment groups of *Vigna radiata*

CHLOROPHYLL	CONTROL	PRSE	SPIRULINA
Chlorophyll a = 12.7(A663) – 2.69 (A645) (µg/ml)	12.7(0.804) – 2.69 (0.749) = 8.11 (µg/ml)	12.7(2.066) – 2.69 (2.065) = 20.68 (µg/ml)	12.7(2.55) – 2.69 (2.45) = 25.79 (µg/ml)
Chlorophyll b = 22.9(A645) – 4.68 (A663) (µg/ml)	22.9 (0.749) – 4.68 (0.804) = 13.39 (µg/ml)	22.9 (2.065) – 4.68 (2.066) = 37.62 (µg/ml)	22.9 (2.45) – 4.68 (2.55) = 44.17 (µg/ml)
Total chlorophyll = 20.2(A645) + 8.02(A663) (µg/ml)	20.2(0.749) + 8.02(0.804) = 21.56 (µg/ml)	20.2 (2.065) + 8.02(2.066) = 58.273 (µg/ml)	20.2(2.45) + 8.02(2.55) = 69.94 (µg/ml)

Table 13: Results of fresh weight in three treatment groups of *Vigna radiata*

SR. NO	FRESH WEIGHT(gms)	
1.	Control	1.50
2.	PRSE	1.53
3.	<i>SPIRULINA</i>	2.05

Table 14: Results of wilting time in three treatment groups of *Vigna radiata*

SR. NO	WILTING TIME	
1.	Control	1.5 hours
2.	PRSE	2 hours
3.	<i>SPIRULINA</i>	2 hours and 20 minutes

Table 15: Results of dry weight in three treatment groups of *Vigna radiata*

SR. NO	DRY WEIGHT (grams)	
1.	Control	0.68
2.	PRSE	0.69
3.	<i>SPIRULINA</i>	0.93

DISCUSSION

After 20 days of cultivation in hydroponics system, the data obtained for both the plants *Vigna radiata* and *Cicer arietinum* suggested that those plants which were provided with *Spirulina* residue as nutrient source had the highest leaf area, root length, chlorophyll content, fresh weight and dry weight out of all the three systems. The PRSE treated plants showed the second highest values of all the plant growth parameters with suggested that phycocyanin not only induced the photosynthesis rate in plants but also provided required nutrients to plants. The least values were observed in plants treated with bio compost which were used as controls. Results indicate that *Spirulina* and phycocyanin extracted from *Spirulina* both can be used for formulation of liquid fertilizers for application hydroponics.

CONCLUSION

The current investigation of research mainly emphasizes on use of *Spirulina* algae and compounds extracted from it in the field of hydroponics. Due to unique blend of compounds present in *Spirulina* it acted as wonderful source of nutrients required for growth of *Vigna radiata* and *Cicer arietinum*. The presence of such cost-effective bio stimulants helps in development of hydroponics on larger scales, that has the potential to reap high commercial of crops in the field of agriculture. Further as investigated, phycocyanin extract from *Spirulina* showed remarkable results in overall plant growth as the pigment is known to induce the rate of photosynthesis in plants. Based on the results of plant growth parameters, it can be concluded that plants provided with *Spirulina* residue as nutrient source

possessed better light interception, enhanced water and nutrient use, higher division rate of tissues, enhanced photosynthetic capacities and lower biotic stress. The longer wilting time also suggests high water holding capacities and low transpiration rates in them as compared to controls. Development of such liquid formulations containing *Spirulina* and their application in hydroponics can solve the problem related to food security in areas with low farmland for cultivation and adverse environmental conditions.

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