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Biofertilizer and Biostimulants Properties of the Green Microalgae Chlorella Vulgaris on Tomato (Lycopersicon esculentum mill L.)

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Abstract

The tomato, worldwide consumed food for its health effects and taste possess a major demand due to the increasing population and diminishing of cultivable lands. In addition, The increasing demand of agricultural products not only urges to find out the way to increase the yield in huge quantities but also strictly affirms the nature of the product yielded should be devoid of chemical fertilizers as it imparts hazardous effect to its agricultural field to which it was directly applied and as well as the consumers as minute proportions of these still remains inside the edible portion and causes a health defect over a period of consumption. Hence an effort was made to find out the feasibility of yielding high quantity of tomato by applying the non-hazardous microalgal based biofertilizer to augment the chemical usages. The tomato plant was treated with three different kinds of microalgal treatments to find out the most suitable method of application. All the three different techniques employed yielded a maximum growth when compared with the control plants, Among which the treatment three, the combined treatment of microalgae C. vulgaris and cow dung gave constructive results followed by the, soil drench method and foliar spray method. Thus the microalgae were evidenced for its biofertilizing potential and the same can be better utilized to explore the promising fertilizing agent as an eco-friendly and non-hazardous agent.

Key Words: Tomato; Microalgae; C. vulgaris: Biofertilizer; Eco-friendly

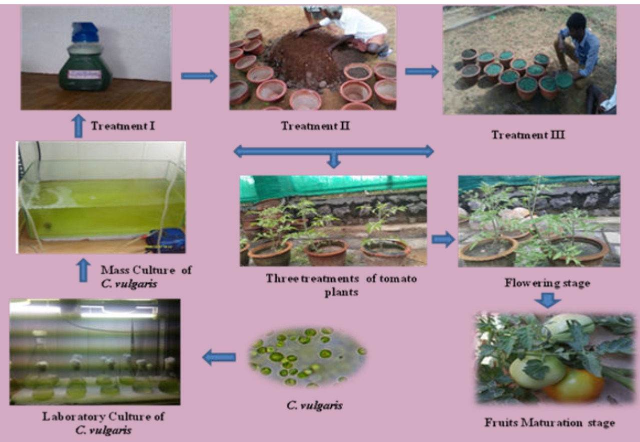

Figure 1: Proposed model of the present work

Statement of novelty

1. The marine source will play a substantial role on agricultural development in the future

2. Microalgae are cheap, renewable, easily available and cultural organisms.

3. Microalgae contain all the essential nutrients needed for plant growth.

4. Microalgae will be the suitable, safe, eco-friendly product that can serve well than the currently used chemical fertilizers.

Introduction

Increase in human population has resulted in increased pressure on land and soil resources that lead to the increased cultivation of crops to meet the ever growing food demand. Increase in pressure on the soil from intensive tillage coupled with unsustainable methods of farming such as continuous cropping results in higher outflow of nutrients which could lead to depletion of soil fertility. Soil-nutrient capital is gradually depleted when farmers are unable to sufficiently compensate losses by returning nutrients to the soil via crop residues, manures and mineral fertilizers. Depletion in soil fertility results in low production of food which could be a threat to the food security of the nation and a drawback to the attainment of the Millennium development goal one; eradicate extreme poverty and hunger by the year 2015.

One of the world's most important vegetable crop in the world is Tomato is Lycopersicon esculentum (L.) Mill belonging to the family Solanaceae. Tomato plays a very important role in day to day life and diet as it is consumed fresh and as paste by mankind (Alofe and Somade, 1982). One of the major percent of vitamin c in human diets comes 90% from vegetables and fruits especially from Tomato (Vallejo et al., 2002). Ascorbic acid and lycopene is present highly in tomato. (Tindall, 1983). Watermelon and tomato containing red colour is imparted by an antioxidant called lycopene. It is cultivated in a huge amount due to their protection on human health against cardiovascular disease as it is highly efficient oxygen radical scavenger and also protects against several cancers especially the prostate cancer.

Biofertilizers are considered as an eco-friendly, sustainable and cost-effective alternative to commercial synthetic fertilizers, as it not only enhances the agricultural production but also reduces the risk of environmental pollution (Kawalekar 2013). Biofertilizers are containing living microorganisms or natural compounds derived from micro-organisms including bacteria, fungi and algae that enhances soil biological and chemical properties, stimulates the plant growth, and aids in restoration of soil fertility (Abdel-Raouf, et al. 2012).

Microalgae has been proven to be a suitable fertilizer due to its high level of micro and macro nutrients essential for plant growth, higher crop yield, greater nutrient uptake and high biomass accumulation. Microalgal biomass can be produced and used in an eco friendly way in a moderate expense and be an alternate to the non eco friendly expensive chemical fertilizers. (Faheed and Abd-El Fattah 2008). The objectives of this study were to investigate the potential agricultural applications of the green microalgae Chlorella vulgaris as a foliar fertilizer, and soil amendment or biofertilizer and assess its effects on plant growth, yield, quality and shelf life of tomato under greenhouse conditions.

Materials and Methods

Collection, Cultivation and harvesting of C.vulgaris

The microalgae C.vulgaris was isolated from the Vellar estuary, Parangipettai, South east coast of India, Tamil Nadu, cultivated out door in 100 liter production glass tank using standard Conway algal culture medium (Lananan et al., 2013). The growth parameters such as temperature, pH, salinity and cell-counts were analysed. The biomass was harvested by filtration (Edzwald, 1993) at day 14 of cultivation and was then frozen until used. The frozen biomass was thawed in a cold room at 4oC for 24 h. Once thawed, the biomass was spread onto then metal trays at a thickness of 1.5 cm and placed inside a freeze-dryer at 4oC to freeze dry for approximately 48 h. The dried biomass was then collected and stored in a cold room at 4oC.

Cell extracts

One kilogram of the freeze-dried biomass was suspended in distilled (DI) water at a concentration of 150 g L−1. The suspension was stirred on a stirring plate for 10 min to allow the biomass to dissociate. The suspension was then processed through a Microfluidizer (M-110EH-30) a mobile high-shear fluid processor at a flow rate of 450 mL min−1 at 172 mPa to disrupt the cell wall and obtain the intracellular extracts. The resulting extract was then centrifuged at 8983×g for 10 min at 22 °C to separate the cell extracts from the biomass residue. To minimize potential degradation, the resulting extract supernatant was collected in a flask covered with aluminum foil and stored in a cold room at 4 °C. The biomass residue was also stored in the cold room for potential future use (Jesus Garcia-Gonzalez and Milton Sommerfeld 2016).

Experimental site

The experiment was conducted during January 2018 at the experimental field of CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Tamil Nadu India. The soil samples from 0 to 30 cm depth were collected randomly from different plots of the experimental field prior to fertilizer application to determine the textural class and fertility status of the field soil.

Experimental design

Soil was collected from farmer's field which is located in Aalapakkam, Cuddalore. The collected soil was filled in 5 kg capacity soil pots. The pots were arranged in completely randomized design in three treatments, four different concentrations and three replication. The seed of tomato (Solanum lycopersicum) were procured from the Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Before sowing, seeds were washed in running tap water and about five seed where sown per pots. (For 1 kg of soil 3gram of microalgae is used) Watering was done regularly upto 15 days until the plants were grown to average height. After that, watering was done at 5 days interval.

Foliar spray experiment

The experiment was performed under greenhouse conditions at approximately 28±2 °C and 85 % relative humidity; treatments were arranged in a complete randomized block design. The experiment consisted of five treatments at various extract concentrations (0, 25, 50, 75, and 100 %) diluted in distilled water (DI). Each treatment consisted of three replicates, one seedling per replicate. Each plant received two foliar applications; the first, at 50 mL, was applied at the time of transplant and the second, at 100 mL, 4 weeks later. During foliar treatment applications, the soil surface was covered with aluminum foil to prevent spray runoff from coming in contact with the potting soil and being potentially available to be taken up by the roots. The sprays were conducted in the morning when the stomata were open due to water pressure, thus enabling greater foliar penetration. All plants were watered as needed throughout the experiment, except after foliar application when they were not watered for 24 h (Dineshkumar et al., 2018).

Treatments were as follows:

*For 50-mL spray treatments, the concentrations below were reduced in half to total 50 mL volume

Treatment I

- Control, 0 % extract, 100 mL DI water
- 25% (v/v) 25 mL extract in 75 mL DI water
- 50% (v/v) 50 mL extract in 50 mL DI water
- 75% (v/v) 75 mL extract in 25 mL DI water
- 100 % (v/v) 100 mL extract

Bio fertilizer experiment

The biofertilizer experiment was conducted under greenhouse conditions at approximately 28±2 °C, in 85 % relative humidity, from January through March 2018. Tomato (S. lycopersicum) seeds were grown in sterilized potting soil, a mixture of vermiculite and peat moss. Two biofertilizer treatments of C.vulgaris dry biomass as soil drench method and C.vulgaris + cowdung manure mixed treatments and dry algal biomass were applied 15 days prior to seedling transplant into pots containing potting soil (peat moss:vermiculite:perlite), mixed thoroughly, and watered once a week for 3 weeks prior to seedling transplant. Each treatment had three replicates and was set up in a completely randomized block design. Plants were grown for a total of 8 weeks and were hand watered as needed.

Treatment II

The dry algal biomass was given at four different concentrations. The maximum concentration of 3 g was fixed based on the earlier report of possible application of microalgae as biofertilizer in rice cultivation (Dineshkumar et al., 2018) and it was fixed as 100% for easy interpretation of results. To1kg soil 3gm dry biomass is added which is 100% and 75% depicts 2.5 gm and finally 25% means 0.75gm of biomass.

- Control (no biofertilizer)
- 25 % (0.75 g) Chlorella vulgaris dry algal biomass as soil drench
- 50 % (1.5 g) Chlorella vulgaris dry algal biomass as soil drench
- 75 % (2.25 g) Chlorella vulgaris dry algal biomass as soil drench
- 100 % (3 g) Chlorella vulgaris dry algal biomass as soil drench

Treatment III

The soil was treated with cow dung manure and green microalgae C. vulgaris separately at the maximum dose 3g dry powder per kg soil before planting. The maximum concentration of 3g was fixed based on the earlier report of possible application of microalgae as biofertilizer in maize and onion (Dineshkumar et al., 2017 &2018).

- Control (no biofertilizer)
- Cow dung manure
- Chlorella vulgaris
- 5 g Cow dung + 3 g Chlorella vulgaris
- 10 g Cow dung + 3 g Chlorella vulgaris

Weed Control

Weeding was done after transplanting to prevent competition between the tomato plants and weeds. Weeding was done with a hoe three times during the study period. The first weeding was carried out four weeks after transplanting and the second and third weeding were carried on six and eight weeks respectively after transplanting.

Growth data

Plant growth can be defined as the progressive development of the plant. Growth is expressed as the amount of biomass in the plant or plant part. Data on growth were taken six weeks after application of treatment. Growth parameters measured included plant height, number of stem branches, number of leaves, leaf length and root length \cm were measured at two weeks intervals.

Yield data

The yield of a crop is determined by the total biomass production, biomass partitioning and fruit dry matter content. Data on yield were taken five weeks after transplanting and when fruits had reached maturity they were ready for harvesting. Parameters measured were number of flowers per plant, number of fruits per plant, total weight of 10 fruits (10kg), total fruits yield per one treatment plant at harvest and fruit length, fruit diameter, number of seeds per fruit and seed weighed fruit (g).

Number of flowers per plant

The number of flowers on the tagged plants was counted. Total number of flowers per experimental treatment of tagged plants was recorded. This was done at 2, 5, 7 and 10 weeks after fertilizer application.

Number of fruits per plant

The number of fruits harvested from five sampled plants was counted from which total number of fruits per plant was calculated.

Fruit weight per plant

Fruits from the five randomly tagged plants were weighed with the help of a weighing scale from which the total weight of fruits per plant was calculated. The average weight of fruits from the experimental plot was estimated by dividing the total weight by the number of fruits.

Fruits quality

Measurement of tomato fruits quality like total soluble solids, total soluble sugars, L-Ascorbic acid (vitamin C), total protein and water content were determined AOAC (1984) method. The atomic absorption spectrometer was used to determine Ca, K and Fe. Phosphorus (P) was determined using the colorimetric molybdenum-blue procedure (Murphy and Riley, 1962).

Shelf life data

Shelf life is calculated as the period of time between harvesting and period of start of rotting of fruits. Parameters measured were number of days for fruits to wrinkle and number of days to watery. The harvested ripe fruits were placed on a clean table in a store at room temperature whiles observing the changes critically daily. Signs of wrinkleness were observed and the number of days it took to wrinkle. The number of days it took to become watery was also noted for each treatment samples taken.

Statistical Analysis

The values reported are the means and standard deviations (mean ± SD) of three replicates.

Results

Chlorella vulgaris was used to study their influence by three different treatments viz. foliar spray (Treatment I), Soil drench method (Treatment II) and cow dung treatment + C.vulgaris (Biofertilizer treatment III).

Growth parameters of tomato as influenced by three treatments of C. vulgaris Bio fertilizers and bio stimulants

The growth parameters including plant height (35.6±0.30, 35.4± 0.55 and 38.7±0.90 cm), number of stem branches (17±0.16, 17±0.25 and 19±0.12), number of leaves (18±0.65, 19±0.14 and 23±0.91), leaves length (7.6±0.28, 7.8±0.22 and 8.3±0.42 cm) and foot length (13.5±0.19, 13.8±0.61 and 14.7±0.84 cm) were found to be higher at a concentration of 100% followed by 75%, 50%, 25% and control plants in treatment I, treatment II and treatment III respectively. Growth of tomato plants was significantly affected by the various treatments: the plant height, number of stem branches, number of leaves, leaves length and root length increased with increase in the level of foliar spray, soil drench, cowdung mixed with C.vulgaris (table.1)

Treatments	Different concentration	Plant height (cm)	Number of stem branches	Number of leaves	Leaves length (cm)	Root length (cm)
Control	---	32.1 ±0.25	13.0 ±0.15	16.0 ±0.15	5.2 ±0.47	11.3 ±0.35
Treatments I Foliar spray experiments	25% extract	32.8 ±0.18	15.0 ±0.45	15.0 ±0.87	6.8 ±0.25	12.1 ±0.31
	50% extract	33.3 ±0.25	15.0 ±0.67	17.0 ±0.36	7.4 ±0.63	12.5 ±0.15
	75% extract	34.2 ±0.15	16.0 ±0.20	17.0 ±0.71	7.5 ±0.19	12.9 ±0.24
	100% extract	35.6 ±0.30	17.0 ±0.16	18.0 ±0.65	7.6 ±0.28	13.5 ±0.19
Treatment II Biofertilizer experiments	25% dry algal biomass	33.5 ±0.57	15.0 ±0.57	16.0 ±0.50	7.3 ±0.54	12.8 ±0.28
	50% dry algal biomass	34.0 ±0.41	16.0 ±0.78	15.0 ±0.35	7.5 ±0.39	13.1 ±0.47
	75% dry algal biomass	34.6 ±0.65	17.0 ±0.65	17.0 ±0.27	7.6 ±0.12	13.5 ±0.55
	100% algal dry algal biomass	35.4 ±0.55	17.0 ±0.25	19.0 ±0.14	7.8 ±0.22	13.8 ±0.61
Treatment III Biofertilizer experiment	Cow dung manure	35.8 ±0.86	15.0 ±0.95	18.0 ±0.19	7.5 ±0.0.9	13.2 ±0.35
	Chlorella vulgaris	36.4 ±0.72	16.0 ±0.84	20.0 ±0.34	7.8 ±0.25	14.0 ±0.40
	50 % Cow dung + Chlorella vulgaris	37.2 ±0.15	17.0 ±0.35	21.0 ±0.55	8.0 ±0.78	14.2 ±0.61
	100 % Cow dung + Chlorella vulgaris	38.7 ±0.90	19.0 ±0.12	23.0 ±0.91	8.3 ±0.42	14.7 ±0.84

Table 1: growth of tomato as influenced by three treatments of microalgae biofertilizer and biostimulants

Yield parameters

Results on yield of tomato from the three treatments are presented in table 2. The yields of tomato plants at different concentrations were recorded. The yield parameters such as average number of flowers per plant (14.0±0.33, 19.0±0.51 and 21.0±055), average fruits no of per plant (14.0±0.82, 17.0±0.26 and 19.0±0.20) weight of 10 fruits (732±0.08, 765±0.65 and 820±0.20 g) and total fruits yield (1.35±0.41, 1.59±0.77 and 1.74±0.17 kg) were found to be maximum at a concentration of 100% followed by 75%, 50%, 25% and control plants in treatment I, treatment II and treatment III respectively.

Treatments	Different concentration	Number of flowers per plant	Fruits No of plant	Weight of 10 fruits (g)	Total fruits yield per one concentration (kg)
Control	---	9.0±0.36	8.0±0.22	480±0.41	0.80±0.36

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Treatments I Foliar spray experiments	25% extract	11.0±012	9.0±0.29	530±0.26	1.15±0.31
	50% extract	11.0±0.05	9.0±0.35	625±0.19	1.27±0.47
	75% extract	13.0±0.54	11.0±0.21	690±0.15	1.30±0.25
	100% extract	14.0±0.33	14.0±0.82	732±0.08	1.35±0.41
Treatment II Biofertilizer experiments	25% dry algal biomass	10.0±0.23	8.0±0.47	615±0.11	1.20±0.55
	50% dry algal biomass	12.0±0.12	10.0±0.36	674±0.28	1.33±0.51
	75% dry algal biomass	15.0±0.35	12.0±0.11	736±0.97	1.40±0.68
	100% algal dry algal biomass	19.0±0.51	17.0±0.26	765±0.65	1.59±0.77
Treatment III Biofertilizer experiment	Cow dung manure	12.0±0.65	13.0±0.74	654±0.16	1.17±0.29
	Chlorella vulgaris	14.0±0.95	15.0±0.75	715±0.84	1.25±0.14
	50 % Cow dung + Chlorella vulgaris	18.0±0.86	18.0±0.64	770±0.60	1.58±0.18
	100 % Cow dung + Chlorella vulgaris	21.0±0.55	19.0±0.20	820±0.20	1.74±0.17

Table 2: Yield of tomato as influenced by three treatments of microalgae biofertilizer and bio stimulants

Fruits and Seed Parameters

The main and interactive influences of biofertilizer treatments on tomato fruit and seed parameters are presented in table 3. Fruit length and diameter, number of seeds per fruit and seed weight / fruit were significantly affected by the applied treatment. Fruits and seed parameters of tomato treatment I foliar spray treatment with C.vulgaris cell extract at different concentration the fruit and seed parameters such as fruit length (8.8±0.36, 9.5±0.61 and 10.7±0.35 cm), fruit diameter (11.3±0.73,13.2±0.24 and 14.7±0.31 cm), number of Seed/Fruit (89±0.45, 118±0.98 and 153±0.96 g) and seed weight/fruit (3.7±0.14, 4.2±0.19 and 5.3±0.28 g) were found to be maximum at a concentration of 100% followed by 75%, 50%, 25% and control plants in treatment I, treatment II and treatment III respectively.

Treatments	**Different concentration**	**Fruit length (cm)**	**Fruit diameter (cm)**	**Number of seeds/one fruit (g)**	**Total Seed weight/ fruits (g)**
Control	---	5.4±0.14	6.5±0.84	28±0.16	1.2±0.23
Treatments I Foliar spray experiments	25% extract	6.3±0.18	8.4±0.72	40±0.19	1.8±0.29
	50% extract	6.7±0.24	9.1±0.94	58±0.23	2.3±0.32
	75% extract	7.5±0.31	10.2±0.61	72±0.27	2.9±0.17
	100% extract	8.8±0.36	11.3±0.73	89±0.45	3.7±0.14
Treatment II Biofertilizer experiments	25% dry algal biomass	6.7±0.28	9.2±0.52	41±0.33	1.8±0.36
	50% dry algal biomass	7.5±0.17	10.4±0.31	73±0.75	2.5±0.25
	75% dry algal biomass	8.3±0.54	11.5±0.23	95±0.91	3.3±0.21
	100% algal dry algal biomass	9.5±0.61	13.2±0.24	118±0.98	4.2±0.19
Treatment III Biofertilizer experiment	Cow dung manure	7.0±0.27	10.2±0.15	66±0.84	2.1± 0.47
	Chlorella vulgaris	7.5±0.19	11.1±0.30	98±0.82	2.5±0.21
	50 % Cow dung + Chlorella vulgaris	8.9±0.28	12.6±0.45	126±0.97	3.8±0.26
	100 % Cow dung + Chlorella vulgaris	10.7±0.35	14.7±0.31	153±0.96	5.3±0.28

Table 3: Fruit and seed parameters of tomato as influenced by three treatments of microalgae biofertilizer and biostimulants

Fruits quality of tomato

Data presented in table 4. Show the effect of biofertilizer on chemical constituents. Results indicate the treatments favored the total soluble solid (3.78±0.39, 4.27±0.92 and 4.09±0.75 g/100g), total soluble sugar (2.30±0.76, 2.37±0.57 and 2.40±0.90 g/100g), L-Ascorbic acid (13.6±0.86, 13.9±0.93 and 14.23±0.69 g/100g), total protein (1.88±0.36, 1.82±0.88 and 2.37±0.23 g/100g) and moisture (92.1±0.81, 92.5±055 and 91.5±0.42 g/100g) were found to be higher in treatment I, II and III at a concentration of 100% extract follower by 75%, 50%, 25%, and control tomato plants.

Treatments	**Different concentration**	**Total Soluble solid (g/ 100g)**	**Total soluble sugar (g/ 100g)**	**L-Ascorbic acid/ 1000g fresh (g/ 100g)**	**Total protein (g/ 100g)**	**Moisture (g/ 100g)**

Control	---	2.18 ±0.19	1.63 ±0.83	10.3 ±0.66	1.06 ±0.81	92.4 ±0.42
Treatments I Foliar spray experiments	25% extract	2.47 ±0.12	1.85 ±0.86	11.7 ±0.73	1.24 ±0.45	91.3 ±0.54
	50% extract	3.25 ±0.23	2.06 ±0.91	12.3 ±0.76	1.35 ±0.23	91.5 ±0.36
	75% extract	3.50 ±0.26	2.17 ±0.95	12.8 ±0.82	1.57 ±0.29	92.3 ±0.15
	100% extract	3.78 ±0.39	2.30 ±0.76	13.6 ±0.86	1.88 ±0.36	92.1 ±0.81
Treatment II Biofertilizer experiments	25% dry algal biomass	3.23 ±0.74	2.12 ±0.85	12.5 ±0.88	1.35 ±0.28	92.2 ±0.98
	50% dry algal biomass	3.57 ±0.61	2.22 ±0.34	12.7 ±0.90	1.59 ±0.68	91.8 ±0.72
	75% dry algal biomass	4.06 ±0.85	2.30 ±0.29	13.3 ±0.94	1.76 ±0.72	91.9 ±0.64
	100% algal dry algal biomass	4.27 ±0.92	2.37 ±0.57	13.9 ±0.93	1.82 ±0.88	92.5 ±0.55
Treatment III Biofertilizer experiment	Cow dung manure	3.30 ±0.91	2.19 ±0.86	12.7 ±0.99	1.42 ±0.42	91.7 ±0.61
	Chlorella vulgaris	3.65 ±0.88	2.28 ±0.94	13.8 ±0.97	1.76 ±0.65	91.8 ±0.45
	50 % Cow dung + Chlorella vulgaris	3.82 ±0.69	2.35 ±0.99	14.10 ±0.72	1.81 ±0.18	92.3 ±0.57
	100 % Cow dung + Chlorella vulgaris	4.09 ±0.75	2.40 ±0.90	14.23 ±0.69	2.37 ±0.23	91.5 ±0.42

Table 4: Fruits quality of tomato as influenced by three treatments of microalgae bio fertilizer and bio stimulants

Elements contents of tomato

Table 5. Illustrates the data collected on effect of biofertilizer on elemental composition of fruit of three experimental treatments of tomato. The elements contents of tomato such as phosphorus content (19.2±0.94, 20.5±0.15 and 22.3±0.28 g100 g-1), potassium content (5.3±0.40, 5.7±0.14 and 6.2±0.74 g100g-1), calcium content (5.2±0.53, 5.6±0.18 and 6.0±0.55 g100 g-1) and magnesium content (17.9±0.55, 18.5±0.45 and 19.1±0.21 g100 g-1) were found to be maximum at a concentration of 100% followed by 75%, 50%, 25% and control plants in treatment I, treatment II and treatment III respectively.

Treatments	Different concentration	Phosphorus content (g 100 g-1)	Potassium content (g 100 g-1)	Calcium content (g 100 g-1)	Magnesium content (g 100 g-1)
Control	---	15.4 ±0.41	4.2 ±0.11	3.8 ±0.21	15.7 ±0.36
Treatments I Foliar spray experiments	25% extract	16.8 ±0.33	4.5 ±0.09	4.3 ±0.39	16.4 ±0.32
	50% extract	17.5 ±0.18	4.6 ±0.26	4.7 ±0.11	17.0 ±0.15
	75% extract	18.6 ±0.12	4.9 ±0.32	5.1 ±0.39	17.5 ±0.65
	100% extract	19.2 ±0.94	5.3 ±0.40	5.2 ±0.53	17.9 ±0.55
Treatment II Biofertilizer experiments	25% dry algal biomass	16.4 ±0.70	4.4 ±0.21	4.7 ±0.66	17.2 ±0.21
	50% dry algal biomass	17.6 ±0.66	5.1 ±0.26	5.0 ±0.87	17.6 ±0.47
	75% dry algal biomass	18.9 ±0.24	5.4 ±0.35	5.3 ±0.91	18.2 ±0.22
	100% algal dry algal biomass	20.5 ±0.15	5.7 ±0.14	5.6 ±0.18	18.5 ±0.45
Treatment III Biofertilizer experiment	Cow dung manure	17.5 ±0.36	4.8 ±0.32	4.9 ±0.15	17.3 ±0.88
	Chlorella vulgaris	18.4 ±0.53	5.2 ±0.17	5.1 ±0.32	18.2 ±0.91
	50 % Cow dung + Chlorella vulgaris	19.5 ±0.27	5.8 ±0.25	5.7 ±0.12	18.7 ±0.55
	100 % Cow dung + Chlorella vulgaris	22.3 ±0.28	6.2 ±0.74	6.0 ±0.55	19.1 ±0.21

Table 5: Phosphorus, potassium, calcium and magnesium contents

Shelf life in tomato plant

At the levels of application, the average number of days for wrinkle and get watery from three treatments are presented in table 6. The maximum wrinkle (5.0±0.19, 6.0±0.15 and 7.0±0.16 days) and get watery (4.0±0.35, 5.0±0.21 and 6.0±0.45 days) were found to be maximum at a concentration of 100% followed by 75%, 50%, 25% and control plants in treatment I, treatment II and treatment III respectively.

Treatments	Different concentration	Average no of days to wrinkle	Average no of days to get watery
Control	---	4.0 ±0.65	2.0 ±0.24
Treatments I Foliar spray experiments	25% extract	3.0 ±0.29	3.0 ±0.14
	50% extract	4.0 ±0.31	3.0 ±0.26
	75% extract	5.0 ±0.45	4.0 ±0.39
	100% extract	5.0 ±0.19	4.0 ±0.35
Treatment II Biofertilizer experiments	25% dry algal biomass	3.0 ±0.28	3.0 ±0.45
	50% dry algal biomass	4.0 ±0.47	4.0 ±0.27
	75% dry algal biomass	5.0 ±0.42	4.0 ±0.68
	100% algal dry algal biomass	6.0 ±0.55	5.0 ±0.21
Treatment III Biofertilizer experiment	Cow dung manure	3.0 ±0.33	3.0 ±0.84
	Chlorella vulgaris	4.0 ± 0.48	3.0 ±064
	50 % Cow dung + Chlorella vulgaris	5.0 ±0.21	4.0 ±0.77
	100 % Cow dung + Chlorella vulgaris	7.0 ±0.16	6.0 ±0.45

Table 6: Shelf life of tomato as influenced by three treatments of microalgae bio fertilizer and bio stimulants

Discussion

The results of this experiments showed that the treatment of foliar spray experiments, biofertilizer soil trench method and biofertilizer + cowdung manure significantly increased the production of tomato.

The plant growth analysis has been developed over the decades as a discipline related to the ecophysiology and agronomy (Diez, et.al. 2001). Plant growth parameters such as plant height, number of stem branches, and number of leaves, leaves length and root length were better in cowdung mixed C.vulgaris biofertilizer treatment III than the foliar spray and C.vulgaris soil drench method. Among the 100% cowdung mixed C.vulgaris biofertilizer concentration treatment showed the highest response in tomato plants. This has motivated the search for other alternatives substrates like microalgae extract and dry biomass substrate among other (Abad et.al. 1998; Guy et.al. 1989; Harz K.et.al. 1986; Pena, 2005). There are foliar applied as stimulators of plant growth (Pohly. 2000). The effects achieved by C.vulgaris as source of foliar spray, biofertilizers soil drench method, cowdung+ C.vulgaris mixed biofertilizers treatments corroborate the points made by scientists who have devoted serious effects to related to the plant growth, soil biological, physical chemical activity of paddy maize, onion and black gram cultivation (Cassnova et.al, 2003; Dineshkumar, et.al. 2017, & Dineshkumar 2018)

Foliar spray the 75% and 100% C.vulgaris concentration treatments lead to greater plant height, number of stem, number of leaves length and root length (Table. 1). Foliar spray of higher concentration resulted in less plant growth parameters. These results are similar to those obtained by (Hernadez – Herrera et.al. 2013), who observed smaller plant growth on foliar sprays of seaweed extracts (Kumari, kaur & Bhatnagar, 2011). In the biofertilizer experiments evaluating the C.vulgaris dry biomass soil drench method and cowdung+ C.vulgaris both biomass mixed treatments and biofertilizer on organism microalgae biofertilizer the results demonstrated a significant correlation between biofertilizer treatments and greater plant growth compared to the control group for the 50% and 75% biofertilizer soil drench treatments and 100% cowdung + C.vulgaris mixed treatment led to a slightly greater plant growth treatments (Table.1) the finding is consistent with that of Dineshkumar et.al. 2018 & 2017.

All yield parameters measured were higher with microalgae C.vulgaris foliar spray experiments and two different biofertilizer methods. This is an indication that foliar spray treatments resulted in lower yields. Compared to C.vulgaris dry biomass soil drench and cowdung mixed treatments in the production of tomatoes. Foliar sprays provide a more rapid nutrient utilization and enable, correction of efficiencies compared to soil fertilizer applications. The greatest difficult in supplying nutrient via foliar sprays is in adequately applying the right quantity the without burring the yield (Fernadez and Eichart 2009; Kannan & Charnel, 1986)

When comparing the two treatments of biofertilizer effects on both C.vulgaris soil drench and cow dung and C.vulgaris mixed treatments it can be concluded that the two biofertilizer treatments applied to the tomato cultivation experiments. Higher tomato yields (number of flower per plant fruits no of plant, weight of 10 fruits and total fruits. Yield per one concentration) were obtained from the two biofertilizer applications. Although higher crop yields were obtained with the cowdung and C.vulgaris biomass mixed. Biofertilizer treatments our results corroborate with the findings of other investigators (A. A. Agboola and P.A., Unamma, 1991). The increase in yield attributes of tomato due to C.vulgaris biofertilizers and biostimulants and sowing (Table.2) could be attributed to easy dissolution effect and sowing tomato at the C.vulgaris + Cowdung mixed treatment in the soil was adequate which enhanced the released of plant nutrients leading to improved the nutrient and increase yield attributes. The results obtained were in agreement with the findings of (Saider et.al. 2011; Tiamiyu et.al. 2012). Which they reported that higher yield response of crops due to C.vulgaris biofertilizer and biostimulants application could be attributed to improved number of flowers fruit number, weight of ten fruits and total fruits yield resulting in better supply of nutrients to the plants.

The main and interactive effects of C.vulgaris fertilizer types and tomato fruit and seed parameters are presented in Table. 3. Fruit length, fruit diameter, fruit number and seed weight were significantly affected try the applied three treatments. Fruit length (cm) varied from 10.7 cm in 100% cow dung+ C.vulgaris mixed biofertilizer treatment. Treatment II C.vulgaris soil drench methods. Concentration of 100% algal dry biomass gave due 9.5cm, fruit length. This was significantly higher than values obtained with other treatments with the exception of C.vulgaris cell extract foliar spray experiments 100% concentration treatments in case of fruit diameter cow dung+ C.vulgaris mixed biofertilizer treatments had the widest while foliar treatments least. Treatment of 100% cow dung+ C.vulgaris mixed biofertilizer treatments gave the widest fruit diameter (14.7 cm) while the least (11.3 cm) was obtained with the 100% foliar spray treatments per number of fruits and seeds weight varied significantly. Cow dung + C.vulgaris mixed biofertilizer had the highest number of seeds per fruit. The value obtained with this (Table.3) higher than what was observed with C.vulgaris soil drench method biofertilizer and C.vulgaris foliar spray experiment's treatment respectively. As much as seed weight/ fruits are treatment III had the highest (5.3g) followed by 4.2 g and 3.7 g obtained from treatment II and I respectively. They (Adebooye et.al., 2006; Togun et.al. 2003; Alofe and somade 1982) reported. Significant increase in fruit and seed parameters of tomato plants.

Fruits quality of tomato in (Table.4) shows the effect of micro algae, biofertilizer and biostimulants tomatoes chemical constituents. The results indicate the favorable effect of C.vulgaris bio-fertilizer and biostimulants on table soluble solids, total soluble sugar, L-Ascorbic acid, total protein and moisture, compared to control. Cowdung + C.vulgaris mixed biofertilizer C.vulgaris, foliar spray, C.vulgaris dry biomass soil drench method followed by significantly increased fruits quality in (Table.4) respectively. Untreated control gave the lowest values in all tomatoes chemical constituent. The positive effect of chemical composition of quality of fruit from tomato of C.vulgaris biofertilizer and biostimulants had been reported for tomato fruit quality (olaniyi and Ajiboa, 2008) pangaribuan et.al., (2011) also showed the organic fertilizers from tomato fruit quality was effect to microalgal fertilizer usage. Dineshkumar et.al. (2018 and 2019) stated that tomato plant treated with microalgae fertilizer would have higher tomato fruit quality of C.vulgaris accumulation in upper part compared to plant treated with chemical fertilizer Table.5 contained data collected on effect of C.vulgaris biofertilizer and biostimulants types on elemental composition of tomato fruits of three treatments of tomato. The different concentration, C.vulgaris fertilizer types and their interaction are significant on tomato plants nourished with 100% Cowdung + C.vulgaris mixed biofertilizer treatments the highest chemical composition. Treatment I and II of Phosphorus potassium, calcium and magnesium were similar and higher to than higher than the chemical composition. Chlorella biofertilizer and biostimulants or cowdung combination with small doses of biofertilizers has been reported widely (Togu 2003n. et.al.). The microalgal fertilizer has been slowly released to the plants and has nourished the tomato plants with high fruit quality and chemical composition which prevents the leaching and nutrient loss. The quality attributes of high titratable acidity, high soluble carbohydrates, low pH, excellent red colour, low seed content, firm fruits crack resistance and long shelf life are dependent on inherent genetic control and influenced by the type, amount and time of the application of the fertilizer application. (Ogunlela et.al. 2005).

Comparatively, it could be stated that tomato fruit from C.vulgaris biofertilizer and biostimulants treated plants had a longer shelf life than those from C.vulgaris fertilizer (foliar spray, soil drench and cowdung mixed treatment treated plants. This finding is however disputed by other researchers Nyamah et.al. (2011) reported that organic fertilizer tomatoes recorded longer shelf life than poultry manure fertilized ones. The found that fruits harvested from cowdung and C.vulgaris mixed biofertilizers amended fields recorded highest shelf life (Table. 6) followed try fruit harvested from field's amended with C.vulgaris biofertilizer treatment method and C.vulgaris cell extract foliar spray methods and control tomato plants. Further reported that fruit harvesting from fields amended with C.vulgaris cell extract foliar spray treatment recorded the lowest weight loss followed by fruits from fields amended with two biofertilizer treatment and control tomato fruits. (Sharma et.al.1996).

Conclusion

Chlorella vulgaris were used to study their influence by three different treatments viz. foliar spray (Treatment I), Soil drench method (Treatment II) and cow dung treatment + C.vulgaris (Biofertilizer treatment III). Growth parameters of tomato as influenced by three treatments of C. vulgaris Biofertilizers and biostimulant. The growth such as plant height, number of stem branches, number of leaves, leaves length and root length increased with increase in the level of microalgal treatment. The yield parameters including average number of flowers per plant, average fruits no per plant, weight of fruits and total fruits yield were found to be higher than the normal control conditions. Similarly the microalgal treatment not only had a positive effect over the fruit and seed parameters but also increased the seed quality (chemical constituents and elemental composition). The major point to be noted is the shelf life of the tomato fruits had been increased almost 2/3rd of the control treatments. Thus further agricultural field trials will bring out the most suitable microalgal biofertilizer to produce safe and nutrient rich agricultural products.

References

1. Abad, J.M., Pariente, F., Hernandez, L., Abruna, H.D. and Lorenzo, E., (1998). Determination of organophosphorus and carbamate pesticides using a piezoelectric biosensor. Analytical chemistry, 70: 2848-2855.
2. Abdel-Raouf, N., Al-Homaidan, A.A. and Ibraheem, I.B.M., (2012). Microalgae and wastewater treatment. Saudi journal of biological sciences, 19: 257-275.
3. Adebooye, O.C., Adeoye, G.O. and Tijani-Eniola, H., (2006. Quality of fruits of three varieties of tomato (Lycopersicon esculentum (L.) Mill) as affected by phosphorus rates. J. Agron, 5: 396-400.
4. Agboola, A. A. and R. P. A. Unamma (1991). Maintenance of soil fertility under traditional farming systems. Organic fertilizer in the Nigerian Agriculture: present and future. Proceedings of a National Organic Fertilizer Seminar held at Durbar Hotel Kaduna Nigeria march 26-27,

5. Alofe, C.O. and Somade, O., (1982). Influence of spacing on the performance of three cultivars of tomato Nigeria. J. Sci, 16: 119-127.

6. Alofe, C.O. and Somade, O., 1982. Influence of spacing on the performance of three cultivars of tomato Nigeria. J. Sci, 16(1), pp. 119-127.

7. Casanova, E., Zuker, A., Trillas, M.I., Moysset, L. and Vainstein, A., (2003). The role C gene in carnation exhibits cytokinin-and auxin-like activities. Scientia horticulturae, 97: 321-331.

8. Diez, J.A., De la Torre, A.I., Cartagena, M.C., Carballo, M., Vallejo, A. and Muñoz, M.J., (2001). Evaluation of the application of pig slurry to an experimental crop using agronomic and ecotoxicological approaches. Journal of environmental quality, 30: 2165-2172.

9. Dineshkumar, R., Kumaravel, R., Gopalsamy, J., Sikder, M.N.A. and Sampathkumar, P., (2018). Microalgae as Bio-fertilizers for Rice Growth and Seed Yield Productivity, Waste Biomass Valor 9: 793.

10. Dineshkumar, R., Subramanian, J. & Sampathkumar, P.(2020) Prospective of Chlorella vulgaris to Augment Growth and Yield Parameters Along with Superior Seed Qualities in Black Gram, Vigna mungo (L.). Waste Biomass Valor 11: 1279–1287

11. Dineshkumar, R., Subramanian, J., Gopalsamy, J., Jayasingam, P., Arumugam, A., Kannadasan, S., Sampathkumar, P. (2019). The Impact of Using Microalgae as Biofertilizer in Maize (Zea mays L.) Waste Biomass Valor 10: 1101.

12. Dineshkumar, R., Subramanian, J., Arumugam, A., Ahamed Rasheeq, A., and
Sampathkumar, P., (2018). "Exploring the Microalgae Biofertilizer Effect on Onion Cultivation by Field Experiment" Waste Biomass Valor.

13. Dineshkumar R., Ahamed Rasheeq A., Arumugam A., Nathiga Nambi K.S.,
Sampathkumar.,P (2019). Marine Microalgal Extracts on cultivable crops as a considerable bio-fertilizer: A review, 18: 249-254.

14. Edzwald, J.K., (1993). Algae, bubbles, coagulants, and dissolved air flotation. Water Science and Technology, 27: 67.

15. Faheed, F., A. and Z. Abd–El Fattah (2008): Effect of Chlorella vulgaris as Bio-fertilizer on growth parameters and metabolic aspects of Lettus plant. J. Agr. and Soc. Sci, 4: 165-169.

16. Fernández, V. and Eichert, T., (2009). Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. Critical Reviews in Plant Sciences, 28: 36-68.

17. Garcia-Gonzalez, J. and Sommerfeld, M., (2016). Biofertilizer and biostimulant properties of the microalga Acutodesmus dimorphus. Journal of applied Phycology, 28: 1051-1061.

18. Guy, R.D., Vanlerberghe, G.C. and Turpin, D.H., (1989). Significance of phosphoenolpyruvate carboxylase during ammonium assimilation: carbon isotope discrimination in photosynthesis and respiration by the N-limited green alga Selenastrum minutum. Plant Physiology, 89: 1150-1157.

19. Hartz, T.K., Mitchell, J.P. and Giannini, C., 2000. Nitrogen and carbon mineralization dynamics of manures and composts. Hort Science, 35(2), pp.209-212.

20. Herrera-Herrera, A.V., Hernández-Borges, J., Afonso, M.M., Palenzuela, J.A. and Rodríguez-Delgado, M.Á., (2013). Comparison between magnetic and non magnetic multi-walled carbon nanotubes-dispersive solid-phase extraction combined with ultra-high performance liquid chromatography for the determination of sulfonamide antibiotics in water samples. Talanta, 116: 695-703.

21. Kannan, S. and Charnel, A., (1986). Foliar absorption and transport of inorganic nutrients. Critical reviews in plant sciences, 4: 341-375.

22. Kawalekar, J.S., (2013). Role of biofertilizers and biopesticides for sustainable agriculture. J Bio Innov, 2: 73-78.

23. Kumari, R., Kaur, I. and Bhatnagar, A.K., (2011). Effect of aqueous extract of Sargassum johnstonii Setchell & Gardner on growth, yield and quality of Lycopersicon esculentum Mill. Journal of Applied Phycology, 23: 623-633.

24. Lananan, F., Jusoh, A., Lam, S.S. and Endut, A., (2013). Effect of Conway Medium and f/2 Medium on the growth of six genera of South China Sea marine microalgae. Bioresource technology, 141: 75-82.

25. Murphy, J.A.M.E.S. and Riley, J.P., (196)2. A modified single solution method for the determination of phosphate in natural waters. Analytical chimica acta, 27: 31-36.

26. Nyamah, E.Y., (2011). Influence of different soil amendments and cultivar types on postharvest performance of three (3) commercial tomatoes (Lycopersicon esculentum, Mill) (Doctoral dissertation).

27. Odoemene, A. (2011). Social Consequences of Environmental Change in the Niger Delta of Nigeria. Journal of Sustainable Development, 4(2).

28. Ogunlela, V.B. and Koomen, I., (2005). Paprika (Capsicum annuum L.) field establishment and crop productivity under varying planting density and ammonium nitrate topdressing. J Food Agric Environ, 3: 185-189.

29. Olaniyi, J.O. and Ajibola, A.T., (2008). Growth and yield performance of Corchorus olitorius varieties as affected by nitrogen and phosphorus fertilizers application. American-Eurasian Journal of Sustainable Agriculture, 2: 234-241.

30. Pangaribuan, D.H. and Monica, W., (2016), September. Growth and yield of sweet corn as affected by paddy straw plant compost and potassium fertilizer. In II Asian Horticultural Congress 1208 (pp. 281-286).

31. Peña-Vázquez, E., Maneiro, E., Pérez-Conde, C., Moreno-Bondi, M.C. and Costas, E., (2009). Microalgae fiber optic biosensors for herbicide monitoring using sol–gel technology. Biosensors and Bioelectronics, 24; 3538-3543.

32. Pohly, L., (2000). The Barbra Streisand companion: a guide to her vocal style and repertoire (p. xvii269). Green wood Press.

33. Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. and Gobi, T.A., (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus, 2: 587.

34. Tiamiyu, R.A., Ahmed, H.G. and Muhammad, A.S., (2012). Effect of sources of organic manure on growth and yields of okra (Abelmoschus esculentus L.) in Sokoto, Nigeria. Nigerian Journal of Basic and Applied Sciences, 20: 213-216.

35. Tindall, H.D., (1983). Vegetables in the tropics. Macmillan Press Ltd.

36. Togun, A.O. and Akanbi, W.B., (2003). Comparative effectiveness of organic-based fertilizer to mineral fertilizer on tomato growth and fruit yield. Compost science & utilization, 11: 337-342.

37. Vallejo, F., Tomás-Barberán, F. and García-Viguera, C., (2002). Glucosinolates and vitamin C content in edible parts of broccoli florets after domestic cooking. European Food Research and Technology, 215: 310-316.