International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.09 pp 285-293, 2016

Effect of Seaweed Extract (*Sargassum tenerrimum*) on Seed Germination and growth of Tomato Plant (*Solanum lycopersicum*)

M.Sasikala, E.Indumathi, S.Radhika, R.Sasireka*

[1]Department of Biotechnology, Kamaraj College of Engineering and Technology, Virudhunagar, India

Abstract : Agriculture is the backbone of our country. Now-a-days synthetic fertilizers were mostly used in agriculture when compared with bio-fertilizers. Prolonged usage results in diminishing soil fertility, soil erosion, health threads to human, livestock and also microbial community present in the soil. To overcome this problem and to increase the efficiency of plant cultivation, seaweed extracts can be used as fertilizers in sustainable agriculture. Seaweed extracts act as bio stimulants mainly due to the presence of plant hormones. The phyto-hormones identified in seaweed extracts are auxins, cytokinins, gibberellins, abscisic acid and ethylene. When compared to other marine algae Phaeophyceae (brown algae) shows better results than Chlorophyceae (green) and Rhodophyceae (red). Efficiency of the Seaweed Liquid Extract was observed by performing the experiments at different concentration such as 0.2%, 0.4%, 0.6%, 0.8% and 1%. Seaweed extractwas applied to plant in three different ways such as soil treatment, foliar spray and Seed Treatment. By observing the germination percentage, number of leaves, leaf area, shoot length, root length, wet weight, and dry weight of the plants; efficiency of the Seaweeds Liquid Fertilizer can be determined. The objective of this study is to increase the soil fertility using algal extract (*Sargassum tenerrimum*) as a fertilizer and also to improve the seed germination, growth, yield as well as quality for better production and process.

Keywords : Phytohormones, Seaweed, *Sargassum tenerrimum*, *Solanum lycopersicum.*

Introduction:

India is mainly known for its agricultural productivity. In order to get a better harvest and to enhance the plant growth, farmers use fertilizer in the soil. The fertilizers can be Chemical fertilizers or Bio fertilizers[1]. Among these two, Bio fertilizers play a crucial role in maintaining soil fertility and essential components that are needed for organic farming. Nowadays, Seaweed extracts have been replaced in agriculture to enhance the plant growth because it acts as a bio stimulant and bio fertilizer[2]. It contains promoters, plant growth regulators, hormones while other macronutrients and micronutrients which promote faster seed germination and increase in yield[3]. Brown Seaweeds are the second most abundant algae in coastal areas. The extracts obtained from Seaweeds are known as Seaweed Liquid Fertilizer (SLF). Seaweed extract is a natural organic fertilizer which promotes faster seed germination and is highly nutritious to plants. The Seaweed extract contains regulators, plant growth hormones, carbohydrates, auxins, gibberellins and vitamins[4] and helps to maintain soil fertility. It is cost effective and eco-friendly for sustainable agriculture. The fertilizer obtained from Seaweed extract is biodegradable, non-polluting, non-toxic and non-hazardous to humans, animals and birds[5].

Seaweed extract has its wide applications as soil amendment in pest control[6] and in plant disease management. The application of seaweed extracts in plants as foliar spray, seed treatment or soil treatment are mainly focused in many research aspects. Treatment with seaweed extract increases nutrient uptake of soil and makes them resistant to environmental stress. The most promising and advantageous of Seaweed-derived-fertilizers in agriculture is to enhance growth rate, nutrient uptake, shoot and root development and to make the plants resistant to climate and pests[7]. The commercially available seaweed are Maxicorp (seaborn), Sea spray, Goemar GA 14, Algifert (marinure), Seasol, Sea crop 16, Cylex and SM3[8].

The mode of application of SLF (Seaweed Liquid Fertilizer) can be of any of the following methods. It can be Seed treatment (dipping of seeds in seaweed liquid manure before cultivation) or Soil treatment (treating soil with seaweed liquid manure) [9] or Foliar spray application (Spraying seaweed liquid manure to crops)[10]. Most of the research works that has done so far concentrated on above methods either in one mode of application or any of the two methods in combination. Here, combination of all these 3 methods was done to get better efficiency and faster growth rate to get increased yield.

Materials and Methods:

Collection of Seaweeds:

The marine brown seaweed used for the present study was collected from Mandapam, The Gulf of Mannar coast, Rameshwaram, India. The collected sample was washed thoroughly with sea water to remove sand particles, impurities, pebbles and epiphytes[11, 12].

Preparation of Seaweed Liquid Fertilizer:

The samples were washed with tap water to remove salts and it was finally washed with distilled water. The Samples were then shade dried followed by oven drying at 60°C for 5 hours[13]. The dried sample was grounded with blender to get fine powder and it was stored for future use. In this present study, Seaweed liquid fertilizers were prepared by three different methods like boiling method, low temperature method and cold water method[14].

In boiling method, seaweed powder of 10 g was mixed with 100 ml of distilled water and it was then heated for 1 hour at 100ºC. For low temperature method, the same volume of mixture was heated at below 60ºC for 24 hours. For cold water extraction, 10 g of powder was mixed with 100 ml distilled water and incubated at room temperature for 24 hours[15]. Then the contents were filtered through the filter paper. The collected filtrate was stored in refrigerator (0-20ºC). The obtained filtrate was considered as 100%. Five different concentrations of solutions such as 0.2%, 0.4%, 0.6%, 0.8% and 1% were prepared using this 100% extract and were used for the study[16].

Selection of Test Plant:

The test plant selected for the present study was *Solanum lycopersicum* commonly known as tomato which is a simple fleshy fruit. The seeds were collected from JP farms in nearby area and it was stored for future use. Healthy seeds free from visible infection, uniform size, color and weight were selected for this study. The seeds were surface sterilized with 5% sodium hypochlorite and then it was rinsed three times with distilled water[17].

Phytochemical Analysis:

The Seaweed extracts such as Cold extract, Hot water extract and Low temperature extract were subjected to phytochemical analysis to confirm the presence of biomolecules using standard qualitative analysis. It helps to determine the presence of phytochemicals in seaweed extracts which indirectly influences the growth and yield of the selected plant[18].

Biochemical Test:

Bio chemicals like total carbohydrate contents using anthrone test, total protein content by biuret method, total phenolic contents with the help of Folin- Ciocaeltaeu methods were analyzed with standards[19].

FT-IR Analysis:

Raw seaweed powder and the three different extracts were lyophilized and the powdered samples were subjected to FT-IR analysis for identification of functional groups.

Selection of Seaweed Liquid Extract for Present Study:

Among the three different extracts, the extract which showed better results in the above study was Low temperature extract and it was selected for the analysis of growth parameters.

Experimental Design and Treatments:

Healthy Selected Seeds were subjected under investigation to check the growth parameters of tomato plant. 10 seeds were selected and soaked with different concentrations of low temperature seaweed aqueous extract such as 0.2, 0.4, 0.6, 0.8 and 1.0% in the sterilized petriplates. Control plate was also maintained with 10 seeds soaked using distilled water. It was then kept at room temperature. The plates were kept separately with 12 hours of light and 12 hours of dark. The subjected seeds were analyzed for germination and the growth of the plant were analyzed at regular time intervals from the day of treatment[20, 21].

Experimental Design and Treatments (Pot Level Study):

Based on the positive results obtained from previous study, to check the application of SLF as soil treatment and as foliar spray application, here the experiments were designed in such a way to confirm the SLF as bio fertilizer[23, 24, 25].

Vegetative Parameter Analysis:

Germination Test:

Selected seeds were subjected with seed treatment at different concentrations and observed for germination rate[26, 27]. 10 seeds were shown on the pot containing soil and the rate of germination was noted at different concentration at regular time intervals in duplicate.

Root and Shoot Length:

Selected seeds were analyzed for growth measurements in duplicate manner. Shoot length was measured from collar region to the tip of the shoot of tomato plant. Similarly root length was measured from collar region to the tip of the primary root. Total plant height was also measured at different concentrations[28, 29, 30].

Fresh and Dry Weight:

Uprooted plants were washed with distilled water and it was blotted with blotting paper to check the fresh weight of the plant. It was then shade dried to obtain the dry weight of the sample.

Leaf Area:

Fresh leaves were taken plucked from the plant and were subjected for the analysis of leaf area.

Results and Discussions:

Phytochemical Analysis of Seaweed Extract:

In the preliminary phytochemical screening, 13 different compounds such as Alkaloids, Phenols, Flavonoids, Protein, Saponins, Steroids, Tannins, Xanthoprotein, Carbohydrates, Terpenoids, Xylose, Amino acids and Glycosides were tested. It was observed that Phenols, Flavonoids, Protein, Carbohydrates and Glycosides were shown positive result in all the extracts but it was relatively high in Low temperature extract.

Biochemical Analysis of Seaweed Extracts:

The total amount of Carbohydrate, Protein and phenols present in the seaweed extract which was prepared in different methods were estimated to identify the extract with high quantity of Biochemical compounds and for future use. It was identified that the amount of bio chemicals was found to be high in low temperature extracts when compared with all other extracts. The amount of phenols was estimated with the help of catechol as a standard and the value was found to be 0.056 mg/ml in low temperature extract. Similarly the amount of carbohydrate and protein content were identified with the help of glucose and BSA as standard. Amount of Carbohydrate and protein was found to be 0.127 mg/ml and0.056 mg/ml (Table 1).

Table 1: Quantitative analysis of Seaweed Extract

S.NO	Biochemicals	Cold Extract	Low Temperature Extract	Boiling Extract
1.	**Carbohydrate (mg/ml)**	0.071	0.127	0.023
2.	**Protein (mg/ml)**	0.612	0.950	0.082
3.	**Phenols (mg/ml)**	0.032	0.056	0.050

Ftir Analysis:

FT-IR was used to analyse the biochemical compounds and its functional groups in the samples. Several indicator bands were formed in the spectrum which represents the functional groups of chemical components or metabolic products (Fig 1-4).Wavelength for each functional group are as follows, OH group (3441 cm^{-1}), C–H stretch (3031–2849 cm^{-1}), allene (1929 cm^{-1}) and C=O, acetate (1732 cm^{-1}).From the Fourier transform infrared (FT-IR) results, the presence of sulphate ester groups were identified with the help of the peak formed at 850 and 1256 cm^{-1} derived from the bending of C–O–S and stretching of S–O of sulphate. FTIR analysis confirms the presence of biochemical compounds by producing peaks which is responsible for amide (3654.12), alkynes (3307.55), alkanes (2918.44), carboxylic acids (2849.92), alkenes (1643.73), aromatics (1454.46), aliphatic amines (1054.13) and alkyl halide compounds (510.34). Spectrum differences indicated the stretching of functional groups of the biochemical compounds. Fourier Transform – Infra Red (FT-IR) spectra of compounds provide unique spectrum at finger print absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. The analytical evaluation of the FT-IR spectra revealed significant differences in band position and absorbance intensities. The stretch of O–H and C=O showed the presence of alcohols. N–H stretch vibration at 3394 cm^{-1} and N–H band at 1620 cm^{-1} and 1581 cm^{-1} indicate the presence of primary amines. The strong band occurring at 2924 cm^{-1}related to C–H stretching and the variable band at 1465 cm^{-1} showed the presence of alkanes**.**Analysis of IR spectra indicates the occurrence of functional groups of primary, secondary amines and amide group and alcohol groups. Peaks formed at 1110 $cm^{-1}$and 1024 cm^{-1}were related to polysaccharides and C-O stretch associated with glycogen. Less intensity peak at 1451 cm^{-1} indicates the presence of methyl groups of proteins which was identified by asymmetric CH_3 bending. Finally FT-IR results were used to analyse that the seaweed sample was suitable for the growth of plants instead of chemical fertilizers.

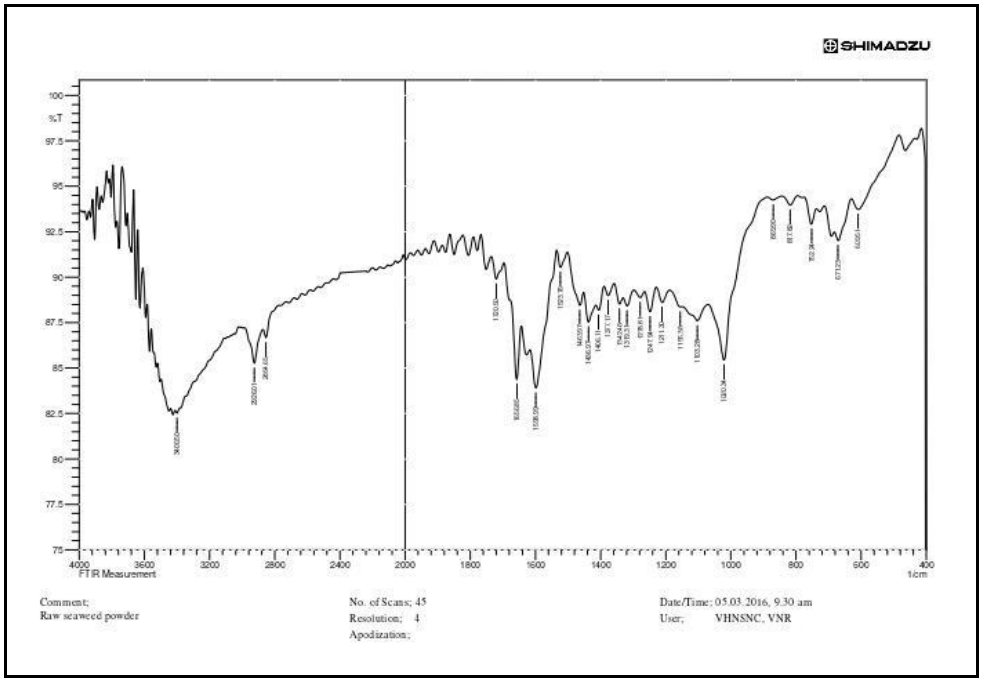

Fig.1: FTIR result of Raw Seaweed powder

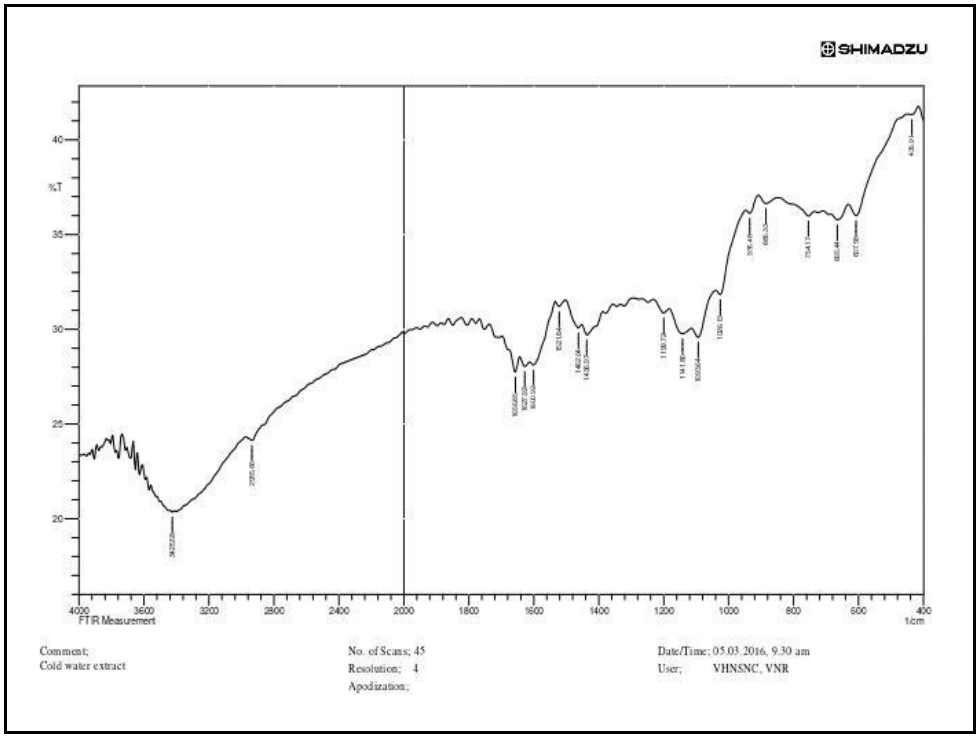

Fig.2: FTIR result of Low Temperature extract

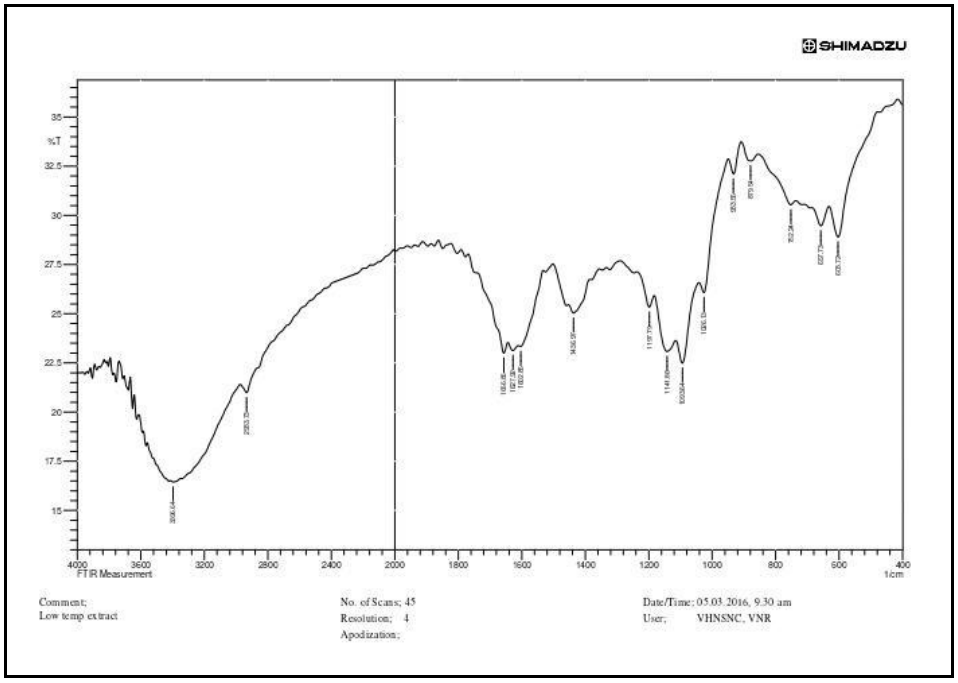

Fig.3: FTIR result of Cold water extract

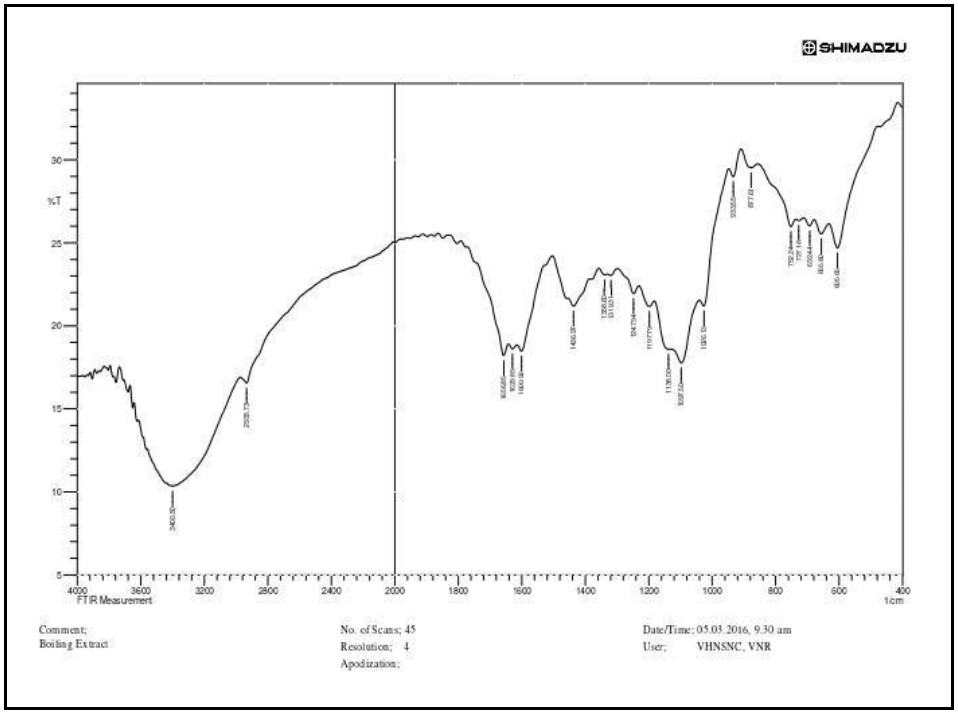

Fig.4: FTIR result of Boiling water extract

Table 2: Effect of SLF on the growth of *Solanumlycopersicum* (Lab Level Study)

Parameters	SLF Concentration					
	Control	**0.2%**	**0.4%**	**0.6%**	**0.8%**	**1%**
Shoot Length (cm)	7 ± 0.07	8 ± 0.14	9 ± 0.07	11 ± 0.07	10 ± 0.14	8 ± 0.07
Number of Leaves	9.5 ± 0.01	12 ± 0.07	13 ± 0.07	17 ± 0.14	19 ± 0.03	14 ± 0.07
Root Length (cm)	1.5 ± 0.01	1.7 ± 0.03	1.8 ± 0.05	2 ± 0.24	2.1 ± 0.05	1.7 ± 0.05
Total Plant Height (cm)	9.5 ± 0.02	9.7 ± 0.01	10.8 ± 0.07	13 ± 0.35	12.1 ± 0.14	9.7 ± 0.05

Vegetative Growth Parameters of Tomato Plant:

Before pot level study to check the efficacy of SLF, initially the experiments were done to monitor the germination rate, root length, shoot length and number of leaves on petri dishes under proper conditions. Tomato seeds were initially subjected to seed treatment and the efficacy of SLF was analysed. Growth parameters like germination rate, number of leaves, root length, shoot length and total plant height was observed. It was found that germination rate was high at 0.8%. Likewise number of leaves and root length was high at 0.8% concentration. But shoot length and total plant height was observed to be high at 0.6% concentration. (Table 2)

Vegetative Growth Parameters of Tomato Plant (Pot Study):

Seed Germination:

Germination test determines the percentage of seeds that are alive in any Seed lot. While the rate of germination varies slightly across varieties, seeds should absorb moisture within 2 days and produce a root and the first leaf within 4 days. In *Solanumlycopersicum***,**100% Seed Germination was found at 0.8%. When compared with other concentrations as well as control it was high. 90% germination rate was observed at 0.6%.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds on a pot}} \times 100$$

Growth Parameters:

The physical parameters like total plant height, shoot and root height (cm), number of leaves, dry weight (g), wet weight (g) and leaf area (cm^2) were also recorded. In this present study, SLF prepared from brown seaweed *(Sargassum tenerrimum)* was applied in three different methods such as Soil treatment, Seed treatment and foliar application to tomato plant.

Table 3: Effect of SLF on the growth of *Solanum lycopersicum* under pot study on 40th day

Parameters	SLF Concentration					
	Control	**0.2%**	**0.4%**	**0.6%**	**0.8%**	**1%**
Shoot Length (cm)	16 ± 0.33	18 ± 0.07	19 ± 0.33	22 ± 0.33	21 ± 0.04	18 ± 0.33
Number of Leaves	19 ± 0.21	24 ± 0.07	26 ± 0.07	23 ± 0.28	37 ± 0.03	28 ± 0.07
Leaf Area(cm^2)	1.2 ± 0.01	1.15 ± 0.01	2.1 ± 0.01	1.5 ± 0.01	3.2 ± 0.03	1.8 ± 0.02
Root Length (cm)	3.6 ± 0.03	3.5 ± 0.03	4.2 ± 0.05	4 ± 0.24	4.8 ± 0.05	4.5 ± 0.05
Total Plant Height (cm)	19.6 ± 0.03	21.5 ± 0.33	23.2 ± 0.22	26 ± 0.14	25.8 ± 0.03	22.3 ± 0.14
Dry Weight (g)	0.009 ± 0.0007	0.021 ±0.0007	0.019 ±0.0007	0.035 ± 0.0007	0.039 ±0.0007	0.028 ±0.0007
Wet Weight (g)	0.114 ± 0.003	0.137 ± 0.0007	0.319 ± 0.0004	0.514 ± 0.0007	0.472 ±0.0007	0.352 ±0.0007

Plants treated with different concentrations of SLF and seaweed powder were monitored at an interval of 10 days. Based on the observation, 0.6% concentration resulted in maximum shoot length as 22 ± 0.33 cm as on 40th day. The Number of leaves was also observed at an interval of 10 days. At 0.8% concentration, maximum number of leaves was observed as 37 ± 0.03 followed by 1% with 28 ± 0.07 leaves. Moderate level of leaves was present in other concentrations as well as control plant. Increase in number of leaves will result more amount of light absorption thus promotes the photosynthesis process which in turn further increase in yield of the plant.

Similarly the maximum leaf area was obtained at 0.8% concentration as 3.2 ± 0.03 cm^2. It was significantly high when compared to other SLF treated plants and control and it was followed by 0.4%. Larger leaf area will contribute to the enzyme synthesis and pigment production which will result in increased plant growth.Based on the observations of root length, 0.8% concentration showed maximum root length as 4.8 ± 0.05 cm. When compared to this concentration, root length of other concentrations was low. It was inferred that the application of soil treatment and seed treatment increased the root length of the plant. Carbohydrates, proteins and auxin hormones present in the seaweed helped in root development.

The hormones were important compounds for cell size and cell division enhancement. Seaweed derived-fertilizers increased the hormone production and help to enhance the plant growth and development. The total height of the plant was continuously monitored for 40 days. At 0.6% concentration of SLF, growth was moderately high as 26 $\pm$ 0.14 cm and it was followed by 0.8%. Plants treated with remaining concentrations were slightly higher than the control plant.

While focussing on wet weight, at 0.6% concentration wet weight of the plant is high. When compared to these concentrations, others were found to be low. Dry weight was found to be slightly higher at 0.8% concentration than 0.6%.

Conclusion:

Based on the experimental results, it was clear that maximum growth and yield of the tomato plant can be achieved at 0.6% concentration. Even though the number of leaves, leaf area, and root length was high in 0.8 % it doesn't deviate much from 0.6%. If higher concentration is preferred, sometimes it may inhibit the growth and yield of the tomato plant. 0.6% itself is enough to act as bio fertilizer.

References:

1. Mishra P. and Dash D. (2014). Rejuvenation of Biofertiliser for Sustainable Agriculture Economic Development (SAED), Consilience: The Journal of Sustainable Development , 2014, 11, 41-61.
2. Hurtado A.Q. Yunque D.A. Tibubos K. Critchley A.T. Use of Acadian marine plant extract powder from *Ascophyllum nodosum* in tissue culture of Kappaphycus varieties, Journal of Applied Phycology, 2009, 21, 6, 633-639.
3. Sathya B. Indu H. Seenivasan R. Geetha S. Influence of seaweed liquid fertilizer on the growth and biochemical composition of legume crop, *Cajanus cajan* (L.) Mill sp, Journal of Phytology, 2010, 2,5.
4. Erulan V. Soundarapandian P. Thirumaran G. Ananthan G. Studies on the effect of *Sargassum polycystum* (C. Agardh, 1824) extract on the growth and biochemical composition of *Cajanus cajan* (L.) Mill Sp, American-Eurasian J. Agric. & Environ. Sci, 2009, 6, 4, 392-399.
5. Vijayanand N. Ramya S.S. Rathinavel S. Potential of liquid extracts of *Sargassum wightii* on growth, biochemical and yield parameters of cluster bean plant, Asian Pacific Journal of Reproduction, 2014, 3, 2, 150-155.
6. Hong D. Hien H Son P. Seaweeds from Vietnam used for functional food, medicine and biofertilizer, Journal of Applied Phycology, 2007, 19, 6, 817-826.
7. Kuwada K. Kuramoto M. Utamura M. Matsushita I. Ishii T. Isolation and structural elucidation of a growth stimulant for arbuscular mycorrhizal fungus from *Laminaria japonica Areschoug*, Journal of Applied Phycology, 2006, 18,6, 795-800.
8. Jeannin I. Lescure J. Morot-Gaudry J.-F. The effects of aqueous seaweed sprays on the growth of maize, Botanica marina, 1991, 34, 6, 469-474.
9. Sharma P. Gupta K. C. Analysis and optimized design of single feed circularly polarized microstrip antennas, Antennas and Propagation, IEEE Transactions on, 1983, 31,6, 949-955.
10. Ganapathy-selvam G. Sivakumar K. Influence of seaweed extract as an organic fertilizer on the growth and yield of *Arachis hypogaea* L. and their elemental composition using SEM-energy dispersive spectroscopic analysis, Asian Pacific J. Reprod, 2014, 3, 1, 18-22.
11. Abdel-Raouf N. Al-Homaidan A. and Ibraheem I. "Agricultural importance of algae", *African Journal of Biotechnology*, 2016, vol 11(54), pp11648-11658.
12. Aggani S.L."Development of Bio-Fertilizers and its Future Perspective", *Scholars Academic Journal of Pharmacy*, 2013, *vol 2*(4), pp327-332.
13. Anand M and Suresh S. "Marine seaweed *Sargassum wightii* extract as a low-cost sensitizer for ZnO photoanode based dye-sensitized solar cell", *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 2015, *vol 6*(3), pp 035008.
14. Christobel G.J. Effect of seaweed (*Sargassum weightii* L.) on the germination and growth of green gram (Phaseolus aureus, L.). *J Basic Appl Biol*, 2008, *2*(1), 105-108.
15. Devi J.A.I. Sathiya Balan G. & Periyanayaga K. Pharmacognostical study and phytochemical evaluation of brown seaweed Sargassum wightii. *J Coastal Life Med*, 2013, *1*(3), 199-194.

16. Hossain M.A. AL-Raqmi K.A.S. AL-Mijizy Z.H. Weli A.M. & Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. *Asian Pacific Journal of Tropical Biomedicine*, 2013, *3*(9), 705-710
17. Isnansetyo A. & Kamei Y. MC21-A, a bactericidal antibiotic produced by a new marine bacterium, Pseudoalteromonas phenolica sp. nov. O-BC30T, against methicillin-resistant Staphylococcus aureus. *Antimicrobial agents and chemotherapy*, 2003, *47*(2), 480-488.
18. Jothinayagi N. & Anbazhagan C.Effect of seaweed liquid fertilizer of Sargassum wightii on the growth and biochemical characteristics of Abelmoschus esculentus (L.) Medikus. *Recent Research in Science and Technology*, 2009, *1*(4).
19. Kamaladhasan N. & Subramanian S. Influence of seaweed liquid fertilizers on legume crop, Red gram. *J. Basic Appl. Biol*, 2009, *3*(1-2), 21-24
20. Naguib N.Y.M. Organic vs chemical fertilization of medicinal plants: a concise review of researches. *Advances in Environmental Biology*, 2011, 394-401
21. Thamaraiselvi P. & Jayanthi P. Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of Eichhornia crassipes (Mart.) Solms. *Asian Journal of Plant Science and Research*, 2012, *2*(2), 115-122.
22. Zhang X. Ervin E. & Schmidt R. Plant growth regulators can enhance the recovery of Kentucky bluegrass sod from heat injury. *Crop Science*, 2003 *43*(3), 952-956.
23. Vulgaris T. Hossain M. Salim K. Raqmi A. Mijizy Z. Weli A. & Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown. *Asian Pac J Trop Biomed*, 2013, *3*, 705-710.
24. Zodape S. Seaweeds as a biofertilizer. *Journal of Scientific and Industrial Research*, 2001,*60*(5), 378-382.
25. Govindasamy C. Arulpriya M. & Ruban P. Nuclear magnetic resonance analysis for antimicrobial compounds from the red seaweed Gracilaria corticata. *Asian Pacific Journal of Tropical Biomedicine*, 2012, *2*(1), S329-S333.
26. Study on the Effect of Pre-harvest Treatments by Seaweed Extract and Amino Acids on Anna Apple Growth, Leaf Mineral Content, Yield, Fruit Quality at Harvest and Storability, International Journal of ChemTech Research, 2016,9(5),pp 161-171.
27. Effect of foliar spraying with licorice root and seaweed extracts on growth and seed production of onion (Allium cepa L.), International Journal of ChemTech Research, 2015,8(11),pp 557-563.
28. Impact of Spraying some Organic Manure Tea, Seaweed Extract and Royal Jelly on Fruiting of Keitte Mango Trees,International Journal of ChemTech Research, 2015,8(4),pp 2131-2141.
29. Studies on Effect of Seaweed Extracts on Crop Plants and Microbes, International Journal of ChemTech Research, 2014,6(9),pp 4235-4240.
30. Phycoremediation of Olive Wastewater for Sustainable Production, International Journal of ChemTech Research, 2016,9(6),pp 567-579.

*****