

EFFECT OF SEAWEED EXTRACT (*SARGASSUM TENERRIMUM*) ON SEED GERMINATION AND GROWTH OF TOMATO SEED (*SOLANUM LYCOPERSICUM*)

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Engineering / technology Degree in Biotechnology

By

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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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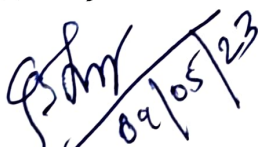
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This is to certify that this project report is the bonafide work of **M. EDWIN VENIS RAJ 39230015** who carried out the project entitled "**EFFECT OF SEAWEED EXTRACT (SARGASSUM TENERRIMUM) ON SEED GERMINATION AND GROWTH OF TOMATO SEED (SOLANUM LYCOPERSICUM) AND CHILLI SEED (CAPSICUM ANNUUM)**" under my / our supervision from

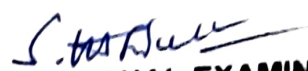

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EDWIN VENIS RAJ M hereby declare that the project report entitled **EFFECT OF SEAWEED EXTRACT (*SARGASSUM TENERRIMUM*) ON SEED GERMINATION AND GROWTH OF TOMATO SEED (*SOLANUM LYCOPERSICUM*) AND CHILLI SEED (*CAPSICUM ANNUUM*)** done by me under the guidance of **Dr. Gracy Jenifer** (Internal) and **Dr. T. Rajasekar** (External) at Centre for Drug Discovery Development is submitted in partial fulfillment of the requirements for the award of bachelor of engineering / technology degree in Biotechnology

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ABSTRACT

Agriculture is the backbone of India. Currently, synthetic fertilizers are mainly used in agriculture compared to biofertilizers. Long-term use of is detrimental to soil fertility reduction, soil erosion, human health, livestock, and the microbial present in the soil. To overcome this problem and increase the efficiency of growing plants, seaweed extract can be used as fertilizer in sustainable agriculture. Seaweed Extract acts as a bio-stimulant primarily due to the presence of plant hormones. The phytohormones identified in seaweed extracts are auxins, cytokinins, gibberellins, ethylene and abscisic acid. When compared to other marine algae Phaeophyceae (brown algae) shows better results than Chlorophyceae (green algae) and Rhodophyceae (red algae). Efficiency of the Seaweed Liquid Extract was observed by performing the experiments at different concentration such as 2%, 4%, 6%, 8% and 10%. Seaweed extract was applied to plant in two different ways such as soil treatment, 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 purpose of this study is to improve production and process technology by using seaweed extract (*Sargassum tenerrimum*) as fertilizer to improve soil fertility and improve seed germination, growth, yield and quality.

Keywords: seaweed (sargassum), agriculture, hydroponics



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LIST OF ABBREVIATION

SLF	-	seaweed liquid fertilizer
SDF	-	seaweed-derived fertilizer
FT-IR	-	fourier transform infrared
CF	-	chemical fertilizer
Ssp	-	sargassum sp.
GR	-	growth rate
CONC	-	concentration
SLE	-	seaweed liquid extract
PGR	-	plant growth regulators
WW	-	wet weight
DW	-	dry weight



CHAPTER - 1

INTRODUCTION

The macroalgae found in marine and salinized lands are called seaweeds. Kelps are categorized as part of the brown group (Phaeophyta), which includes the other two types of seaweed: red (Rhodophyta) and green (Chlorophyta). Spores and vegetative growth, respectively, allow them to reproduce both sexually and asexually. These seaweeds have specialized structures called holdfasts that allow them to adhere to hard objects like rocks and shells. India is now one of the top consumers of seaweeds and related exports and imports, not just in other countries but also globally. The company developed a tractor-like vessel called "Seacombine," which is now used offshore in India and Indonesia to scatter seeds and collect tropical sea plants ("Seaweed for India" 2021).

1.1 AGRICULTURE

The agricultural output of India is primarily renowned. Farmers apply fertilizer to the soil in an effort to increase crop yields and plant development. Fertilizers come in two varieties: chemical and biological. The soil fertility and other vital elements required for organic farming are maintained in large part by bio fertilizers, which are one of these two. Due to its ability to operate as a bio stimulant and bio fertilizer, seaweed extracts have now taken their place in agriculture as a means of promoting plant growth (Sasikala *et al.*,2016). Especially those derived from seaweed extracts, agricultural bio-stimulants are playing a larger role in the global production of food (SWE). (Hussain *et al.*,2021). Every enhancement to the agricultural system that boosts output should lessen agriculture's detrimental effects on the environment and increase the system's sustainability (Rathore *et al.*,2009). Due to their auxin, cytokinins, betanins, macro and micronutrients, which increase crop production, seed germination, and resistance to frost fungal, insect, and disease attacks, seaweeds have been discovered to be developing in the field of modern agriculture over the past ten years (babu *et al.*,2015). Food production has been boosted with the use of chemical fertilizers in modern agriculture, which



is based on human populations. To expand the field of many commercial crops, foliar spray applications of seaweed liquid fertilizers are used because the mode of action is yet unknown despite the extract's presence of several components (Ganapathyselvam *et al.*, 2013) (Vijayakumar *et al.*, 2018).

1.2 HYDROPONICS

The overall area of crops grown utilizing hydroponic systems has increased quickly all over the world. Globally, hydroponic agricultural production has increased significantly in recent years thanks to improved pest and temperature control, more effective water and fertilizer use, and other factors. Due of this demand, closed hydroponic systems have replaced open hydroponic systems. Due to the re-use of water and nutrients, closed hydroponic systems often require less water and fertilizers for plant development (Christie *et al.*, 2014). In order to improve agricultural output in the modern market, soilless culture's most intensive and productive production method—hydroponic cultivation—has grown quickly (Putra *et al.*, 2015). When compared to soil culture, the use of hydroponic systems produces the same amount of productivity without regard to the passing of the seasons and with less land utilization. Nutrition is just as crucial to hydroponic planting success as the planting medium itself, since you cannot grow plants hydroponically without it. For plants to grow, macronutrients and micronutrients must be present. The composition of each form of nutrition varies (Harahap *et al.*, 2020). Substratum hydroponics are hydroponic systems that use material other than soil, such as husk charcoal, or sand, and pure coconut husk powder. For the cultivation of crops and fruits with a high market value, such as lettuce, this hydroponic technology is still utilised (Deng *et al.*, 2014) (Yusuf *et al.*, 2021).

1.3 SEAWEED EXTRACT

A natural organic fertilizer known as seaweed extract is very nutrient-rich for plants and encourages quicker seed germination. The regulators, plant growth hormones, sugars, auxins, gibberellins, and vitamins (Erulan *et al.*, 2009). found in seaweed extract support the preservation of soil fertility. For sustainable agriculture, it is economical and environmentally friendly. The fertilizer made from

seaweed extract is biodegradable, not dangerous to humans, animals, or birds, and it is also not polluting (Vijayanand *et al.*, 2014). The use of seaweed extract as a soil addition for insect control and plant disease management is very widespread. The primary emphasis of many research areas is the utilization of seaweed extracts in plants as foliar sprays, seed treatments, or soil treatments. Increased nutrient uptake and resistance to environmental stress are two benefits of seaweed extract treatment for soil (Sasireka *et al.*, 2016). (Arioli *et al.*, 2015) and (Shukla *et al.*, 2019) found that bio-stimulants derived from seaweeds have qualities that enhance plant growth, yield, and quality as well as increase tolerance to abiotic and biotic challenges and enhance nutrient usage (Hussian *et al.*, 2021). Seaweed extracts also contain major and minor nutrients, amino acids, vitamins, cytokinin, auxin. Although the exact mechanism of action is yet unknown, the positive effect of applying seaweed extract is due to a variety of components that may operate synergistically at various dosages (Rathore *et al.*, 2009). Due to the large variety of trace metals (Fe, B, Ca, Cu, Cl, K, Mg, and Mn) and plant growth regulators (PGR), such as auxins, cytokinins, and gibberellins, that are present and have the ability to stimulate growth and increase plant production, seaweed has potential as an organic fertilizer in addition to its use as a functional food ingredient (Yusuf *et al.*, 2020) (Vijayanand *et al.*, 2014). Many seaweeds have specialized tissues and growth forms, making them much more complex organisms than land plants. There are approximately 10,000 species of seaweed, of which 6500 are red algae, 2000 are brown algae, and 1500 are green algae. There are 800 species of Bryopsidophyceae, 50 species of Cyanophyceae, 400 Siphonocladophyceae, and 250 marine Ulvophyceae. Seaweed must produce some amazing adhesives because quite small holdfasts seem to be sufficient for quite large plants.



CHAPTER - 2

REVIEW OF LITERATURE

Sasikala *et al.*, (2016) says that our nation's economy is based on agriculture. In contrast to bio-fertilizers, synthetic fertilizers are now utilised in agriculture more frequently. Long-term use reduces soil fertility, causes soil erosion, affects human and animal health, and also has microbiological effects. a community that exists in the soil. Seaweed extracts can be utilised as fertilizers in sustainable agriculture to solve this issue and boost the productivity of plant production. Mostly because plant hormones are present, seaweed extracts work as bio stimulants. Auxins, cytokinins, gibberellins, abscisic acid, and ethylene are the phytohormones found in seaweed extracts. In comparison to other marine algae, Phaeophyceae (brown algae) outperforms Rhodophyceae and Chlorophyceae (red). The effectiveness of the seaweed liquid extract was evaluated by trials at concentrations of 0.2%, 0.4%, 0.6%, 0.8%, and 1%. Three distinct applications of seaweed extract were made to plants: soil treatment, foliar spray, and seed treatment. It is possible to gauge the effectiveness of the seaweeds liquid fertilizer by looking at the plants' germination percentage, number of leaves, leaf area, shoot length, root length, wet weight, and dry weight. They evaluated improve seed germination, growth, yield, and quality for improved production and process, as well as to boost soil fertility by employing *Sargassum tenerrimum*.

Hashmath *et al.*, (2021) reported that the brown algae *Durvillaea potatorum* and *Ascophyllum nodosum*, which are used to make seaweed extract (SWE), affect soil and plant growth. Tomato plants growing in soil responded to SWE treatment with two different outcomes. With regard to flower clusters, flower number, fruit number, root length, root and shoot dry weight, and SPAD, SWE significantly enhanced tomato plant development and raised plant production (yield and quality). Similar to this, SWE application had an impact on soil biology at the soil



root zone by raising the total bacterial count, increasing the amount of nitrogen in the soil, and changing the composition of the bacterial community by increasing some bacterial families that are important for maintaining healthy soil. A deeper comprehension of SWE's impacts on the plant-soil ecology could result in the development of ground-breaking methods for the production of sustainable food.

Rathore *et al.*, (2009) reviewed that in 2006, a field experiment was carried out in India's rainy (kharif) season to examine the effects of foliar applications of various concentrations of seaweed extract (made from *Kappaphycus alvarezii*) on soybean [*Glycine max* (L.) Merr.] grown under rainfed conditions without the use of chemical fertilizers. Two times, the foliar spray was used with seaweed extract in seven different concentrations (0, 2.5, 5, 7.5, 10, 12.5 and 15% v/v). Applications of seaweed extract topically greatly improved yield metrics. With applications of 15% seaweed extract, the maximum grain yield was seen. This was followed by applications of 12.5% seaweed extract, which led to increases of 57% and 46%, respectively, over the control. With a 15% seaweed extract treatment, the highest straw yield was likewise attained. Applications of seaweed extract also led to improved nutrient absorption (N, P, K, and S). In order to increase yield in rainfed soybean cultivation, foliar treatments of seaweed extracts appear to be a promising strategy.

Abou El-Yazied *et al.*, (2012) was summarised that on cultivars of the snap bean (*Phaseolus vulgaris* L.), the study was conducted in the 2009 and 2010 growing seasons. At Brnashat hamlet in the Egyptian governorate of Giza, a fine-type Bronco. to research the effects of compost treatments and seaweed extract. The study examined the effects of four different seaweed compost levels (0, 1, 2, and 3 m³ seaweed compost/feddan) and four concentrations of seaweed extract applied topically (0, 250, 500, and 750 ppm) on vegetative growth, blooming traits, yield parameters, and chemical composition. Findings showed that spraying plants with seaweed extract at a greater rate significantly increased the number of leaves per plant, the average leaf area, the weight of the fresh leaves and stems per plant, and the average number of leaves per plant. Compared to the control, the amount



of fruit set, leaf and stem dry weight per plant, and percentage. Comparing the yield of pods produced by seaweed extract at 750 ppm tested concentrations to those produced by untreated check and other treatments, this concentration showed the highest significant yield. Higher rates of seaweed extract spraying resulted in higher values of photosynthetic pigments, N, P, K, and Mg content in leaves, whereas higher rates of seaweed extract spraying (500 and 750 ppm) resulted in higher values with significance over 250 ppm and control plants for protein content in pods, free amino acid percentage in leaves, and carbohydrates in leaves and pods. Yet, there is no discernible difference in fibre percentages between the treatment and control groups. In experiments utilising seaweed compost, it was discovered that employing 3 or 2 m³ per feddan provided the best results for vegetative development, flowering features, yield parameters, and chemical composition (minerals and biochemical values). It was determined that 3m³ or 2m³ seaweed compost in combination with foliar application of 750 ppm seaweed extract had positive effects on snap bean reproductive, pod yield characteristics, and chemical composition.

Asha Prajapati *et al.*, (2015) has reported that the impact of seaweed extracts from *Kappaphycus alvarezii* (K sap) and *Gracilaria edulis* (G sap) on potato development and yield was examined over the course of two years in field studies. The results showed that the application of 10% K sap + recommended dose of fertilizer and 10% G sap + recommended dose of fertilizer significantly increased the growth features of the potato crop over the control, including plant height, number of stems, and tubers per hill. Potato marketable and total tuber yield increased by 19.81% and 17.18%, 11.04% and 6.90%, respectively, over the control when 10% G sap + recommended dose of fertilizer and 10% K sap + recommended dose of fertilizer were applied. In comparison to control, the relative reduction in nonmarketable tuber and damage tuber yields under the influence of 10% G sap + RDF and 10% K sap + RDF was 39.94%, 34.37%, 35.50%, and 34.91%, respectively.

Dogra *et al.*, (2011) revealed that during the 2009 Rabi growing season, a field experiment was carried out to examine the effects of soil applications of various



concentrations of seaweed extract (made from *Ascophyllum nodosum*) on onion growth, yield, and the severity of downy mildew. The basal dose of seaweed granules was used (1.5, 2.0, 2.5, 3.0, and 3.5 g/m²). Application of seaweed extract to the soil significantly increased plant growth and yield. The application of 2.5g/m² yielded the highest yield, which was followed by 3.0g/m² yields, which produced increases of 120.8% and 102.5%, respectively, in comparison to the control. Application of 2.5 g/m² seaweed extract resulted in the greatest increase in shoot height and number per plant as well as the least amount of disease severity. Applying seaweed extract to the soil in Himachal Pradesh could therefore be a promising strategy for increasing onion yields due to the subtropical climate and low hills there.

Yusuf *et al.*, (2021) says that the goal of this study is to determine how different seaweed extract concentrations affect the growth and yield of *brassica juncea* L. mustard greens. A randomised block design (RBD) with six treatments was used for the study, which was carried out at the Faculty of Agriculture Screen House, University Tadulako Palu. These included a seaweed extract concentration of 0% (E0), 20% (E1), 40% (E2), 60% (E3), 80% (E4), and 100% (E5). There were 24 experimental units as a result of the experiment being run four times. After that, analysis of variance and honest real difference at level 5% were used to analyse the data that had been collected. The findings showed that different concentrations of seaweed extract had a significant impact on plant height, leaf number, leaf area, fresh weight, and dry weight.

Darwin H. Pangaribuan *et al.*, (2022) concluded that although the cost of nutrient solution is rising, it is crucial to the growth and quality of hydroponic plants. Along tropical coastlines, seaweed is widely accessible. The goal of the study was to identify the ideal mixture of brown seaweed extract (*Sargassum* sp.) combined with coconut husk, lamtoro leaves, moringa leaves, or African leaves that fit to the nutritional quality of AB mix as hydroponically fed mustard plants. The University of Lampung's Faculty of Agriculture's greenhouse served as the site of this study's operations. Six replications of a completely randomised design were used. The



treatments were broken down into six different categories: AB mix nutrition, brown seaweed, seaweed and lamtoro leaf, seaweed and coconut fibre, seaweed and moringa leaf, and seaweed and African leaf. The findings demonstrated that the organic nutrient solution derived from the combination of brown seaweed with lamtoro leaf, coconut fibre, moringa leaf, or African leaf was unable to match the AB mix nutrition quality for mustard plants in the hydroponic wick system. The treatment combining brown seaweed and lamtoro leaf, which had the best fresh weight of mustard and was 51.08% higher than the total fresh weight from the control AB mix, was found to be the best among those treatments.

Ashok Pal *et al.*, (2015) has reported that in order to investigate the effects of seaweed saps on the growth, yield, nutrient uptake, and economics of maize (sweet corn) in Matasi soil of Chhattisgarh, a field experiment was carried out during the rabi season of 2012–2013 at Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh). Three times, at various intervals during the crop, foliar sprays of two distinct species—Kappaphycus and Gracilaria—were applied with varying concentrations of seaweed extracts (0, 2.5, 5.0, 7.5, 10.0, and 15% v/v). The growth, yield, nutrient uptake, and B:C ratio parameters were all significantly improved by foliar applications of seaweed extract. The highest yields of green corn (189.97 q ha⁻¹) and fodder (345.19 q ha⁻¹) were obtained from treatment (T8) 15% G Sap + recommended dose of fertiliser (RDF), which was significantly similar to treatment (15%) K Sap + RDF (185.24 q ha⁻¹) in terms of green corn yield. Green maize and fodder absorbed the most N, P, and K when 15% G Sap + RDF (T8) was present. The treatment 15% K Sap + RDF (T4) had the next highest net return (Rs. 1,33,199 ha⁻¹) and B:C ratio (1.95), followed by the treatment 15% G Sap + RDF (T8), which had the highest gross return (2,07,230 ha⁻¹), net return (1,38,756 ha⁻¹), and B:C ratio (2.0). In comparison to the treatment 15% G Sap + RDF (T9), treatment T8 produced a yield of Rs. 45,996 ha⁻¹ more.

Priyanka nayak *et al.*, (2020) summarized that in order to assess the effectiveness of seaweed extracts on the growth, yield, and economics of rice, a field experiment was conducted at Instructional Farm, BCKV, West Bengal, India during the kharif



(rainy) season of 2018. The experiment was framed in RBD comprising 7 treatments (T1- 100% RDF, T2 -100% RDF + Biozyme granule @15 kg/ha, T3 -100% RDF +Amaze-x granule @ 10 kg/ha, T4 -75% RDF + Biozyme granule @ 15kg/ha, T5 -75% RDF +Amaze-x granule @ 10 kg/ha, T6 75% RDF + Biozyme granule @ 15 kg/ha + Proventus DS legacy spray @ 625ml/ha, T7 -75% RDF +Amaze-x granule @10 kg/ha + Proventus DS legacy spray @ 625ml/ha), replicated thrice. According to experimental results, the conditions that resulted in the highest growth and yield characteristics for rice were 75% RDF + Amaze-x granules @ 10 kg/ha + Proventus DS legacy spray @ 625 ml/ha (T7). Under treatment T7, the highest rice grain (3.90 t/ha) and straw (5.94 t/ha) yields as well as high nutrient uptake (N: 91.45 kg/ha, P2O5: 11.49 kg/ha, and K2O: 190.74 kg/ha) were also made possible. Although T7 was still the best option, the second-best option was 75% RDF + Biozyme granules @ 15 kg/ha + Proventus DS legacy spray @ 625 ml/ha (T6). Furthermore, both the treatment T6 (BCR: 1.59) and T7 (BCR: 1.58) demonstrated high economic viability. Therefore, for the best growth, yield, and economics of kharif rice, the application of 75% RDF and Proventus DS legacy spray at 625ml/ha along with either Biozyme granule at 15 kg/ha or Amaze-x granule at 10 kg/ha can be advised.

Radames Trejo Valencia *et al.*, (2018) clarified that the study's goal was to assess how cucumber (*Cucumis sativus*) fruit production and phytochemical quality reacted to foliar applications of various seaweed extracts. The Instituto Tecnológico de Torreón in Torreón, Coahuila, Mexico, conducted this study there in the autumn-winter agricultural cycle while using a shadow mesh. The experimental setup consisted of six treatments with each treatment receiving six repetitions. *Ascophyllum nodosum*, *Grammatophora* spp., *Macrocystis integrifolia*, *Bryothamnion triquetrum*, *Macrocystis pyrifera*, and an inorganic fertilization control treatment were the treatments that were examined. Sand and vermicompost were combined to create the substrate. We assessed the fruit's yield, commercial size, and phytochemical composition. The yield using Steiner solution (6.75 kg m²) was higher than that with *Bryothamnion triquetrum* algae (6.07 kg m²), according to the results. The *Macrocystis pyrifera* and *Macrocystis integrifolia* extracts were statistically equal, with values of 47.37 and 43.73 mg equiv. of Ac. Gallic per 100 g fresh weight, respectively. The extracts outperformed



the control treatment in terms of phenolic content. The use of the *Macrocystis pyrifera* algae treatment with 149.4 and 454.1 M equivalent. Trolox/100 g fresh base, respectively, increased the antioxidant capacity by the ABTS+ and DPPH+ methods. With 5.07 mg/100 g of fresh base, this treatment also had the highest level of vitamin C, outpacing the control treatment by 27%. By having the highest antioxidant capacity, algae extract enhanced the quality of the fruits, making their use a viable alternative to limiting the use of conventional fertilizers, thereby attenuating the effects on the environment and enhancing population health.

Gayathri *et al.*, (2014) says that a sizeable amount of plant growth hormones and micronutrients are found in seaweeds, which aid in germination and plant growth. It becomes toxic as its concentration rises and inhibits plant growth. Seaweed extract's impact on plant germination, toxicity, and the potential for use as bio fertiliser are all covered in the current study. Three different types of seaweed were combined to create the liquid fertiliser. Seaweed extracts prepared in a 1:10 W/V ratio were applied to the seeds of *Abelmoschus esculentus* and *Solanum lycopersicum*. It is used to determine the effectiveness of seaweed extracts on seed germination and plant growth inhibiting (toxic) concentrations. The length of the roots, the length of the shoots, and the quantity of lateral roots are some of the parameters that are measured in the seeds that have received various concentrations of seaweed extract. The results showed increased germination and growth at low concentrations, but as the concentration rises, *Solanum lycopersicum* toxicity prevents germination from occurring as quickly. The optimal concentration ranges for all three seaweed extracts in *Abelmoschus esculentus* were between 40 and 60%, and higher concentrations resulted in toxicity. Against five different bacterial species, none of the three seaweed extracts demonstrated any antibacterial activity.

Senad *et al.*, (2018) has reported that the growth, yield, and quality of cherry tomatoes under stress and non-stress conditions were the subjects of an experiment to ascertain the impact of foliar application of seaweed extract (0.2%). A randomised block design with four treatments and three replications was used



to set up the greenhouse experiment. The following treatments were used: V1 – seedlings treated with seaweed extract and exposed to drought; V2 – seedlings treated with seaweed extract and regularly watered; V3 – non-treated seedlings exposed to drought; V4 – non-treated seedlings regularly watered. In comparison to untreated seedlings, cherry tomato seedlings treated with seaweed extract had higher leaf water potential and a lower proline content. This suggests that using this fertilizer helps cherry tomato seedlings adapt to stress better. In comparison to untreated plants under the same conditions, the treatment with seaweed extract also improved cherry tomato yield and quality (total soluble solids, vitamin C, and lycopene). Due to its unique composition and the cherry tomato plants' capacity to use the bioactive substances in seaweed extracts for their own growth and development, seaweed extract has a positive impact on the growth and quality of cherry tomatoes.

Vijayakumar *et al.*, (2018) concluded that the effect of seaweed liquid *fertilizer* (SLF) from *Codium decorticatum* on *Capsicum annum* seed germination yield, biochemical composition, and pigment characteristics was thoroughly investigated in the current study, both in pots and under laboratory conditions. Distilled water was used to prepare SLF in various concentrations, including 10%, 20%, 30%, 40%, and 50%. The seeds were soaked for 10 hours for each SLF concentration before being put in different Petri plates. As controls, seeds that had been soaked in water were employed. application of SLF at a lesser concentration (20%) Maximum seed germination, fresh weight, dry weight, root and shoot length, branch count, leaf area, number of pods, and content of total chlorophyll, chlorophyll a, and chlorophyll b, protein, carbohydrate, and lipids were all noted. Therefore, the findings of the present study suggested that the SLF of *C. decortianum* might be used as an alternative bio-fertilizer because it is economical, eco-friendly, and provides farmers with significant economic and environmental benefits.



CHAPTER - 3

AIM AND OBJECTIVES

3.1 AIM

Organic fertilizer made from seaweed is known as seaweed fertilizer (sf) and is used in agriculture to improve soil fertility and plant growth. Seaweed has long been used as a fertilizer and provides a variety of advantages for soils.

3.2 OBJECTIVES

The biochemical components of plants, such as carbohydrates, lipids, proteins, fibres, ash, phenol, and dietary fibre, are enhanced by seaweed, which might be considered a possible source of bio-fertilizer in dried or fresh form. The seaweed is a good source of both the micro and macronutrients needed by plants.



CHAPTER - 4

MATERIALS AND METHODS

4.1 Collection of seaweeds:

The marine brown algae 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, salt content, impurities, pebbles, epiphytes and other waste.



Fig 4.1: collection of seaweed sargassum sp.

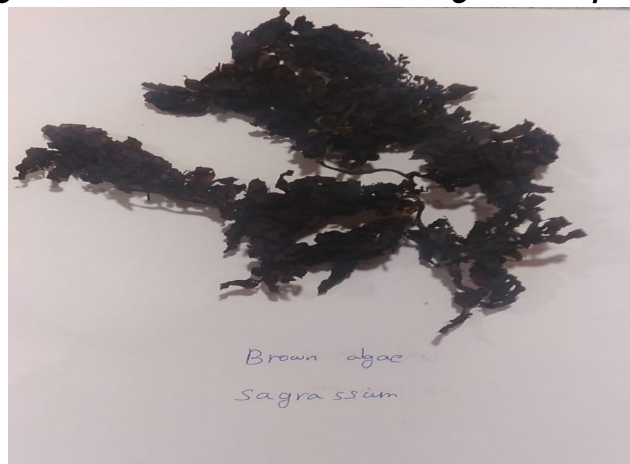


Fig 4.2: washed seaweed sargassum

4.2 Preparation of seaweed liquid fertilizer:

The sample was washed with tap water to remove salt and finally washed with distilled water. The samples were then dried in the shade and then oven dried at 60 °C for 5 hrs. The dried samples were ground with a blender until a fine powder was obtained and stored for later use. In this study, liquid seaweed fertilizer was prepared by three methods: a boiling method, a low temperature method, and a coldwater method.



Fig 4.3: 50 gram of seaweed powder

Add 500ml of distilled water to 50g of seaweed powder, boil it, and then heat at 100°C for 1 hour. For the low temperature method, an equal volume of the mixture was heated below 60°C for 8 hours. The contents were then filtered through filter paper. The collected filtrate was stored in a refrigerator (0-20°C). The resulting filtrate was taken as 100%. 5 different concentrations of solutions such as 2%, 4%, 6%, 8% and 10% were prepared using this 100% extract and used in the study.

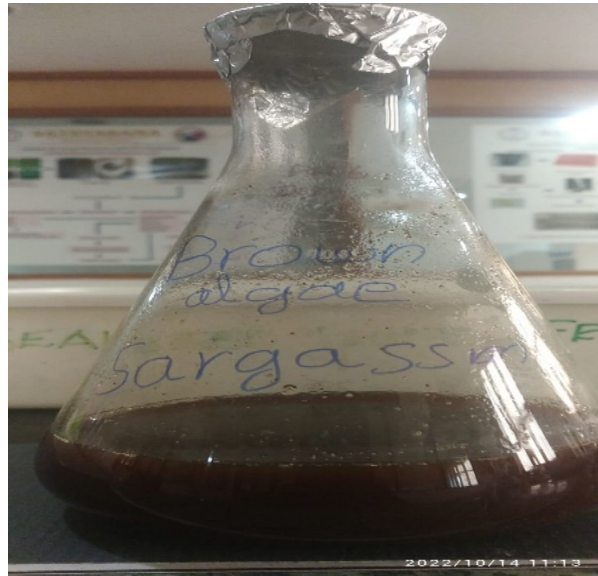


Fig 4.4: extraction of seaweed



Fig 4.5: dilution of seaweed

The percentage of seeds in any seed lot that are alive is determined by the germination test. Though the speed of germination varies a little depending on the variety, seeds should start absorbing moisture in 2 days and create a root and their first leaf in 4 days. The percentage of 100% seed germination in *Solanum lycopersicum* was 0.8%. When it was high when compared to control and other concentrations. At 0.6%, a germination rate of 90% was noted. Physical characteristics such as the total plant height, shoot and root heights (cm), number of leaves, dry weight (g), wet weight (g), and leaf area (cm²) were also noted. SLF made from brown seaweed (*Sargassum tenerrimum*) was used in this study in three different ways: foliar spray to tomato plants, soil treatment, and seed treatment.

4.3 Selection of test plant(seed):

The test plant selected for this study. Seeds were collected from nearby areas and stored for future use. Healthy seeds with no visible infection and uniform in size, weight and colour were selected for study. The seed surface was sterilized with 5% sodium hypochlorite solution and washed three times with distilled water.



Fig 4.6: selection of seed (tomato and chilli)

4.4 Phytochemicals test:

In the initial phytochemical screening, 13 distinct substances including alkaloids, phenols, flavonoids, protein, saponins, steroids, tannins, xanthoprotein, carbohydrates, terpenoids, xylose, amino acids, and glycosides were examined. Phenols, Flavonoids, Protein, Carbohydrates, and Glycosides were seen to have favourable results in all of the extracts, although they were particularly high in the low temperature extract. In hot water extracts were subjected to phytochemical analysis is confirmed the presence of saponins and terpenoids in sargassum sp.

4.1.1. Test for Alkaloids

For Alkaloid identification, 2 mL of concentrated Hydrochloric acid (HCl) was added to 2 mL algal extract. Then few drops Mayer's reagent was added. Presence of green color or white precipitate indicates the presence of alkaloids.

4.1.2. Test for Terpenoids

For Terpenoids identification, 2 mL of chloroform along with concentrated Sulphuric acid were added to 0.5 ml of the algal extract. Formation of reddish-brown color at the interface indicates the presence of Terpenoids

4.1.3. Test for Steroids

For steroids identification, 2 mL of chloroform and 1 mL of sulphuric acid (H₂SO₄) were added to 0.5 mL of the algal extract. Formation of reddish-brown ring at interface indicates the presence of steroids.

4.1.4. Test for Tannins

For tannins identification, one mL of ferric chloride (5% FeCl₃) was added to 1 mL of the algal extract. Formation of dark blue or greenish black color indicates the presence of tannins.

4.1.5. Test for Saponins

For saponins identification, 2 mL of distilled water was added to 2 mL algal extract and shaken in graduated cylinder for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins.

4.1.6. Test for Flavonoids

For flavonoids identification, 1 mL of 2N sodium hydroxide (NaOH) was added to 2 mL of algal extract. Formation of yellow color indicates the presence of flavonoids.

4.1.7. Test for Phenols

For phenols identification, 2 mL of distilled water followed by few drops of 10 % ferric chloride was added to 1 mL of the algal extract. Formation of blue / green color indicates the presence of phenols

4.1.8. Test for Coumarins

For coumarins identification, 1 mL of 10 % NaOH was added to 1 mL of algal extract. Formation of yellow color indicates the presence of coumarins.

4.1.9. Test for Quinones

For Quinone identification, 1 mL of concentrated sulphuric acid (H_2SO_4) was added to 1 mL algal extract. Formation of red color indicates the presence of quinones.

4.1.10. Test for Glycosides

For glycosides identification, 3 mL of chloroform and 10% ammonium solution was added to 2 mL of the algal extract. Formation of pink color indicates the presence of glycosides.

4.5 FT-IR Analysis:

Raw seaweed powder and three different extracts were lyophilized and powder samples were subjected to FT-IR analysis to confirm functional groups.

4.6 Selection of Seaweed Liquid Extract for Present Study:

Among the three different extracts, the extract that performed best in the above study was hot water extract and was selected for analysis of growth parameters.



Fig 4.7: hot water extract of seaweed

4.7 Experimental Design and Treatments:

Healthy selected seeds were tested to determine the growth parameters of tomato plant. Ten seeds were selected and soaked in sterile petri dishes in water extracts of cold seaweed at various concentrations, example 0.2, 0.4, 0.6, 0.8 and 1.0%. The control dish also contained 10 seeds soaked in distilled water. It was then stored at room temperature. Plates were stored separately at 12 hours of light and 12 hours of darkness. Treated seeds were analyzed for germination and plant growth at regular intervals from the day of treatment in hydroponics.



Fig 4.8: treatment of seeds



CHAPTER - 5

RESULTS AND DISCUSSIONS

5.1 Germination Test:

The germination rate was observed by treating the selected seeds with various concentrations. Ten seeds were placed in pots and germination rates were observed in duplicate at different concentrations at regular intervals. The formula for the germination percentage were written below:

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

5.2 Length of Leaves and Stems:

Selected seeds underwent a double-blind analysis of their growth. The length of the tomato plant's shoot was measured from the collar to the tip. From the collar region to the primary root's tip, the root length was also measured. Additionally, total plant height was measured at various doses. In 5th week the length of stem reached upto 16cm in three percentage (3%) and in 6th week the length of leaves reached upto 5cm in four percentage(4%).

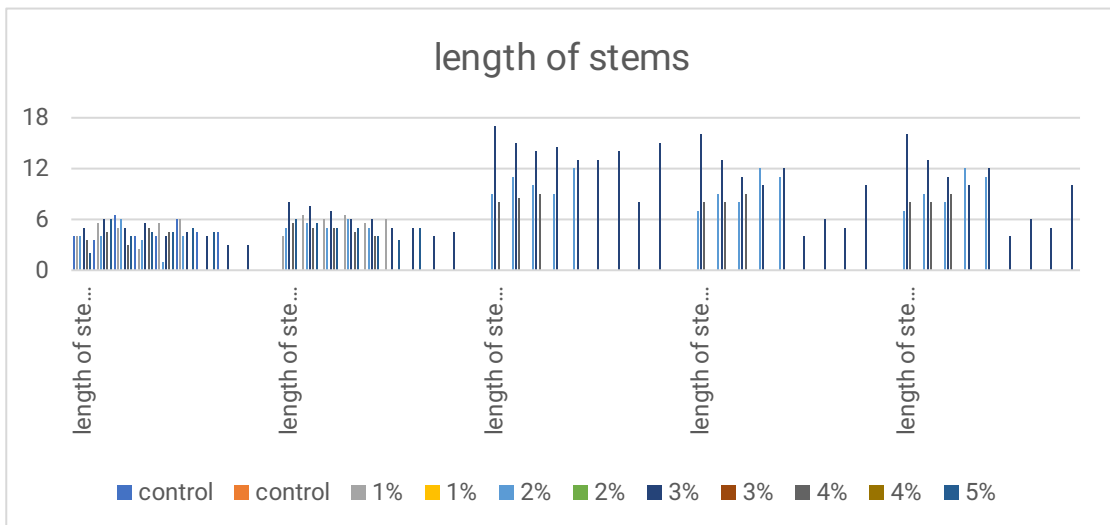


Fig 5.1: length of stem (graph)

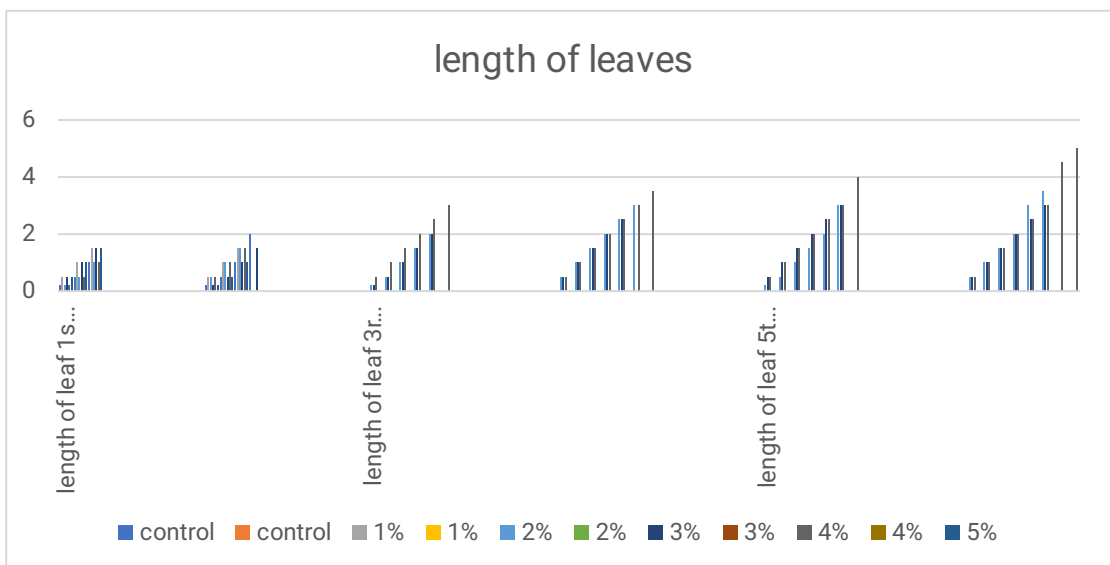


Fig 5.2: length of leaves (graph)

5.3 Fresh leaves(pigment):

We were harvested from the plant and submitted to a study of their leaf area. The leaf area was taken under UV-spectrometry to check pigment of the leaves and its growth toward the usage of seaweed extract obtained from the species sargassum. In chilli plant, observed growth was compared to the control. The result had reached the optimum value compare to others percent but in tomato plant the 4th percent reached upto 0.12 in 663nm more growth.



Fig 5.3: leaves pigment taken under UV-spectrometry

5.4 Number of plants and leaves:

The graph for the number of plants and leaves were shown below. Following the graph it was clear that the number of leaves grown during usage of 3% of seaweed extract in six weeks is more than the other number of leaves grown with various percentage in same six weeks. The result was compared using control and other number of leaves growth. Following the graph it was clear that the number of plants grown during usage of 3% of seaweed extract in six weeks is more than the other number plants grown with various percentage in same six weeks. The result was compared using control and other number plant growth.

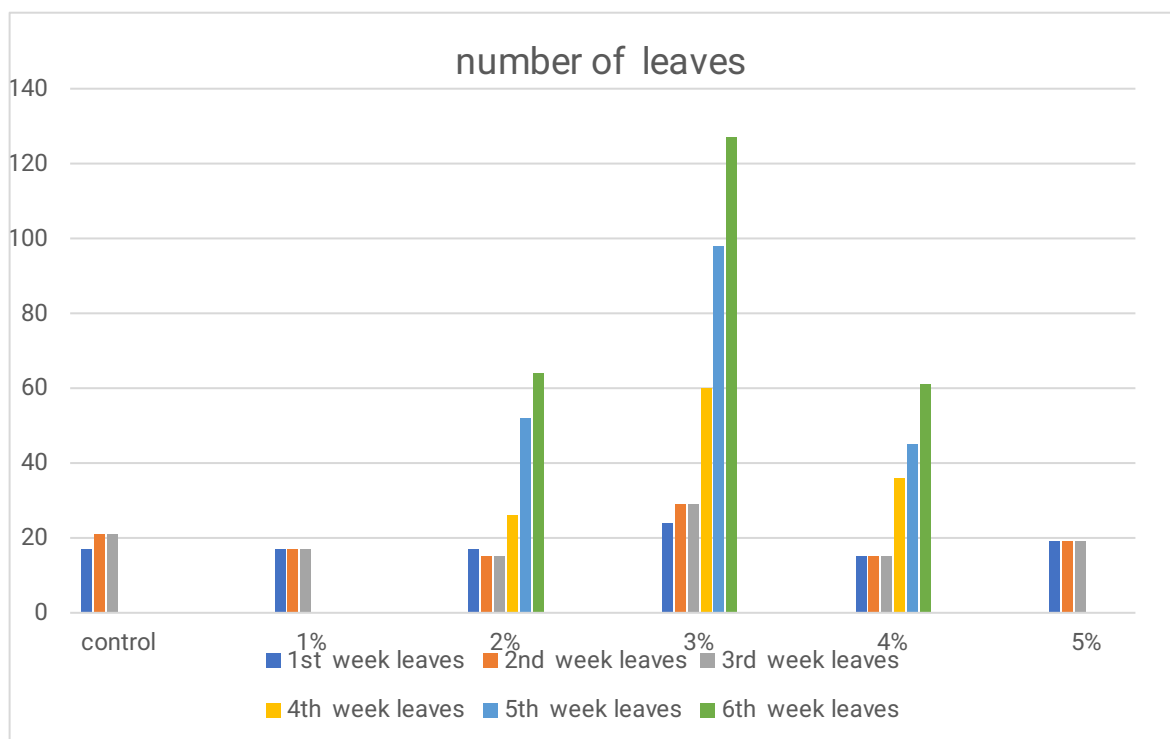


Fig 5.4: number of leaves (graph)

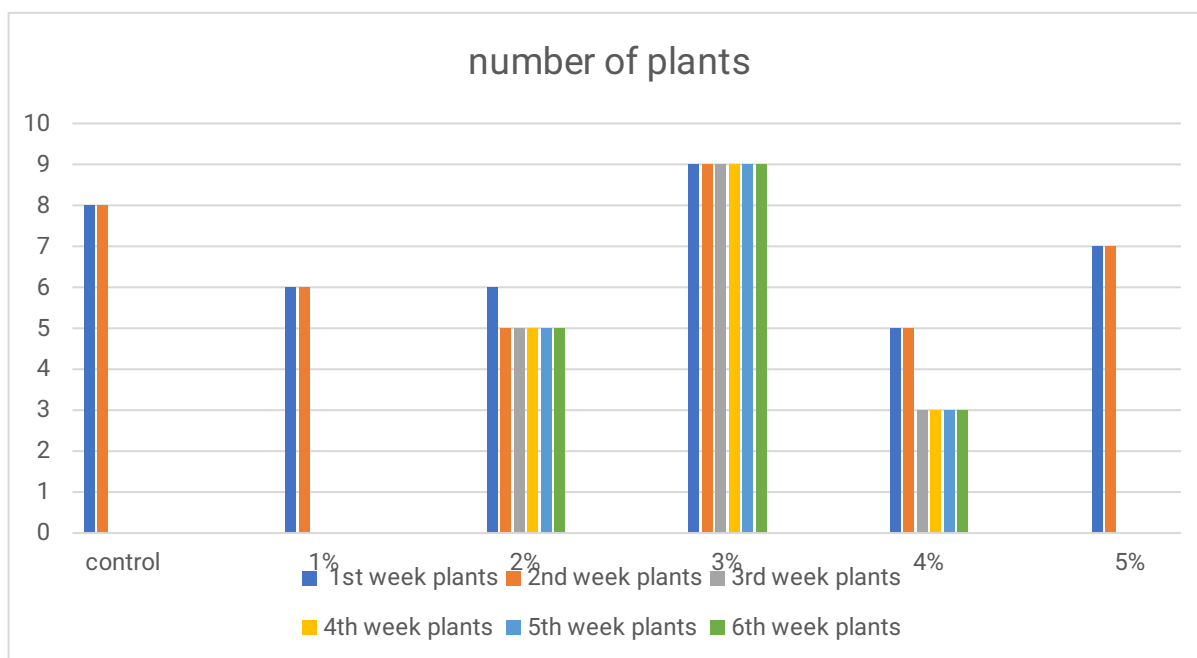


Fig 5.5: number of plants (graph)

5.5 Phytochemical Analysis of Seaweed Extract:

Seaweed extracts such as cold extracts, hot water extracts and low temperature extracts were subjected to phytochemical analysis to confirm the presence of biomolecules using standard qualitative analysis. This helps to determine the presence of phytochemicals in seaweed extracts that indirectly affect the growth and yield of selected plants. In hot water, the seaweed extract was subjected to phytochemical analysis was confirmed the presence of saponins and terpenoids in sargassum sp.



Fig 5.6: phytochemical test of seaweed extract

5.6 FT-IR Analysis of Seaweed Extract:

The seaweed extract was given for FT-IR testing and results were displayed below.

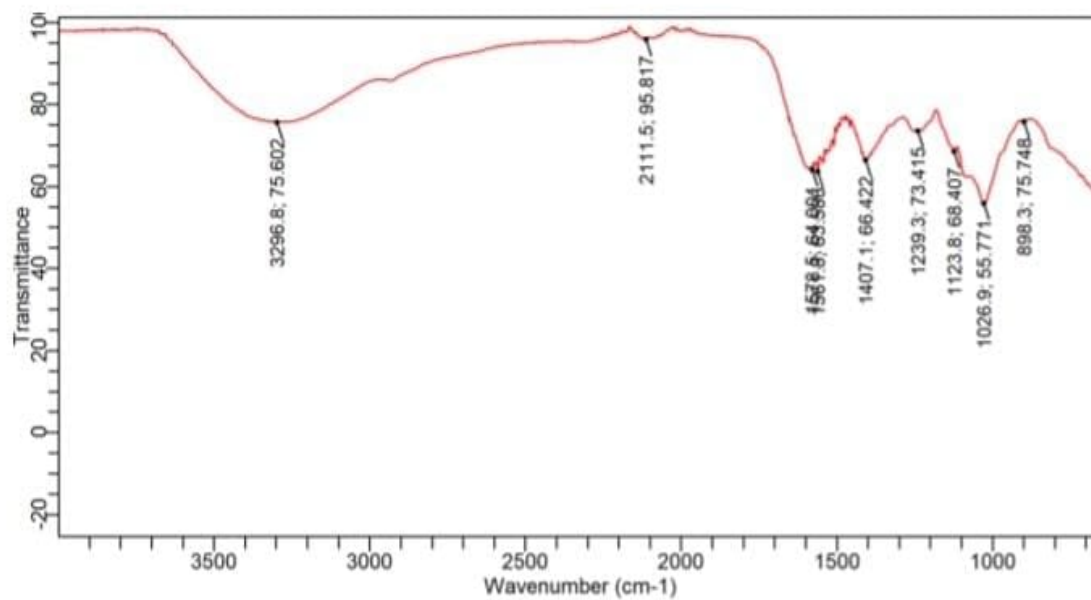


Fig 5.7: FT-IR analysis of seaweed extract (graph)

Peak number	Wavenumber (cm-1)	Intensity
1	898.28842	75.74827
2	1026.88157	55.77147
3	1123.79236	68.40723
4	1239.33983	73.41486
5	1407.07003	66.42234
6	1561.75455	63.58587
7	1578.52757	64.00077
8	2111.53688	95.81724
9	3296.83032	75.60150

Fig 5.8: table of FT-IR value for sargassum sp.

5.7 GCMS Analysis of Seaweed Extract:

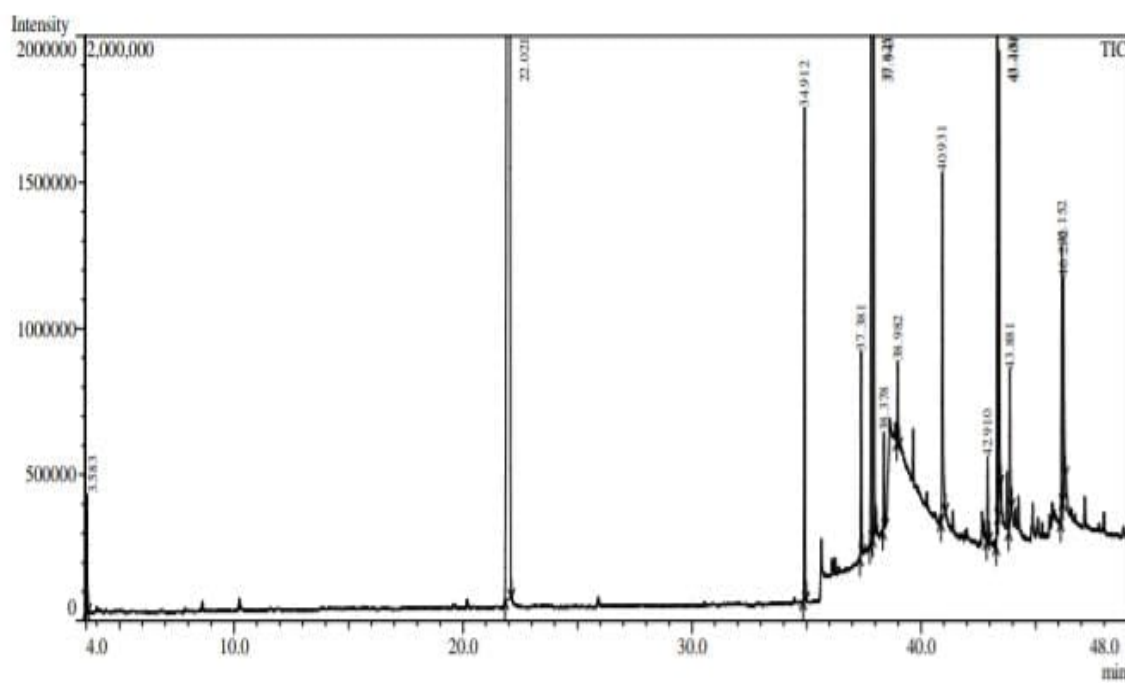


Fig 5.9: GCMS analysis of seaweed sargassum sp.

Peak	R. Time	Area	Area%	Name
1	3.583	712405	0.75	1-UNDECYNE
2	22.021	56531526	59.72	PHENOL, 2,6-BIS (1,1-DIMETHYLETHYL
3	34.912	4245042	4.48	Hexadecenoic acid, methyl ester
4	37.381	1629099	1.72	Methyl 2-octylcyclopropene-1heptanoate
5	37.825	5989613	6.33	9,12-Octadecadienoic acid (Z, Z)-, methyl ester

6	37.943	4443985	4.69	9-Octadecenoic acid, methyl ester, (E)-
7	38.378	720257	0.76	Methyl stearate
8	38.982	704912	0.74	Methyl 2-octylcyclopropene-1octanoate
9	40.931	3306089	3.49	Glycidyl palmitate
10	42.910	702861	0.74	Methyl 2-octylcyclopropene-1heptanoate
11	43.330	5397794	5.70	Linoleyl acetate
12	43.404	3998959	4.22	9-Octadecenoic acid (Z)-, oxiranylmethyl este
13	43.881	1339748	1.42	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)
14	46.152	2650490	2.80	n-Propyl 9,12-octadecadienoate
15	46.212	2293053	2.42	9-Octadecenoic acid (Z)-, 2,3dihydroxypropyl e
		94665833	100.00	

Fig 5.10: table of GCMS value for sargassum sp.

CHAPTER - 6

CONCLUSION

The findings of the experiment made it abundantly evident that a 3% concentration can result in the tomato and chilli plant's optimum growth and output. The number of leaves, leaf area, and root length were all higher in 4%, although they didn't depart significantly from 3%. Sometimes it can prevent the tomato plant and chilli plant from growing and producing as much fruit and vegetable if a higher concentration is preferred. It takes just 3% to function as bio fertilizer. At the first to fifth week after planting (WAP), the application of sargassum sp. seaweed

extract to tomato and chilli plants showed an enhancement effect on plant growth. The extract also lengthened the stem, shoots and increased the number of leaves and the size of the leaves. The current study showed the potential value of using various species of seaweed as biofertilizer to enhance tomato and chilli plant vegetative growth. As a result, tomato and chilli can be thought of as a potential source of bio fertiliser to accelerate the growth of plants in agriculture. This study found that 3% extract of Sargassum was most effective in stimulating the growth of tomato and chilli.

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