

EFFECT OF SEAWEED LIQUID FERTILIZER EXTRACTED FROM DIFFERENT SEAWEEDS ON THE GROWTH OF PADDY PLANT (*Oryza sativa*)

Submitted in partial fulfillment of the requirements for the award of Bachelor of Science in
Biotechnology.

Pratheep . L (40770040)



**DEPARTMENT OF BIOTECHNOLOGY SCHOOL OF BIO AND CHEMICAL
ENGINEERING**

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

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DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bona fide work of Pratheep . L (40770040) who carried out the project entitled EFFECT OF SEAWEED LIQUID FERTILIZER EXTRACTED FROM DIFFERENT SEAWEEDES ON THE GROWTH OF PADDY PLANT (*Oryza sativa*) under my supervision from January to March.

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This is to inform you that **Mr. PRATHEEP. L (Reg. No. 40770040)** pursuing B.Sc. Biotechnology IIIrd Year, batch 2020-2023 at Sathyabhama Institute of Science and Technology is undergoing his final year project at **Bionyme Laboratories Pvt Ltd.,** Chrompet, Chennai - 600 044.



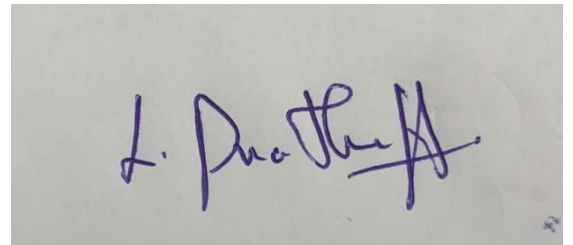

DIRECTOR

DECLARATION

Pratheep.L (40770040) hereby declare that the Project Report entitled EFFECT OF SEAWEED LIQUID FERTILIZER EXTRACTED FROM DIFFERENT SEAWEEDS ON THE GROWTH OF PADDY PLANT (*Oryza sativa*) done by me under the guidance of DR. BAVANI LATHA and DR. LEEBA BALAN at Bionyme laboratories PVT , LTD is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Biotechnology.

DATE:12/3/23

PLACE:Chennai

A photograph of a handwritten signature in blue ink on a light-colored surface. The signature is written in a cursive style and appears to read 'L. Pratheep'.

SIGNATURE OF THE CANDIDATE

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ABSTRACT

Seaweed contains all trace element and growth hormones required by plants. Recently there is a growing concern over using Seaweed liquid fertilizer. There is growing concern over the use of seaweed liquid fertilizer. SLF are very cheap and eco-friendly. And they don't harm the environment and soil. The present study investigates the effect of seaweed extract, from *Enteromorpha species*, *Gracillaria species*, *Padina species* on the seed germination and plant growth of *Oryza sativa*. The extracts and SLF are tested on the paddy plants. The phytochemical analysis showed that alkaloids presents in all the extracts. And there is carbohydrate in *Enteromorpha* extract. All the extracts and SLF were treated on paddy plants, T-4 (SLF *Enteromorpha species*) gave the best yield and growth comparing to other treatments. Quantitative analysis is done for T4 plant sample, Phenol- 0.078 mg/GAE , flavonoids - 0.068 QE. Here by this study shows *Enteromorpha species* are best suited seaweed for the preparation of seaweed liquid fertilizer.

Keywords:

Seaweed, Hormones, Seaweed liquid fertilizer, *Enteromorpha species*, *Gracillaria species*, *Padina species*, *Oryza sativa*

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LIST OF ABBREVIATIONS

LSF – Liquid seaweed fertilizer

UN – United Nations

WHO- World Health Organization

SLF- Seaweed liquid fertilizer

ETC- Etcetera

ML- milliliter

MG- milligram

CM- centimeter

G- grams

i.e. - that is

hrs- hours

min- minute

L- liter

T- treatment

C- celcius

Nm- nanometer

V- volume

RPM- rotation per minute

UL- microliter

TLC- THIN LAYER CHROMATOGRAPHY

UV- ultra violet

RF- retention factor

GAE- Gallic acid equalence

QE- Quercetin equalence

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CHAPTER-1

INTRODUCTION

Seaweed fertilizer is an organic fertilizer made from seaweed that's used in husbandry to increase soil fertility and factory growth. The use of seaweed toxin dates back to age and has a broad array of benefits for soils. Seaweed toxin can be applied in several different forms, including meliorated liquid excerpts and dried, pulverized organic material (Aitken JB and Senn TL 1965).

Through its composition of colorful bioactive motes, seaweed functions as a strong soil conditioner, bio-remediator, and natural pest control, with each seaweed phyla offering colorful benefits to soil and crop health.

These benefits can include increased forbearance to abiotic stressors, bettered soil texture and water retention, and reduced circumstances of conditions. On a broader socio- ecological scale, seaweed monoculture and toxin development have significant places in biogeochemical nutrient cycling through the carbon storehouse and the uptake of nitrogen and phosphorus.

Seaweed toxin operation in soils can also alter the structure and function of microbial communities. Seaweed monoculture has the implicit to yield ecosystem services by furnishing a source of nutrition to mortal communities and a medium for perfecting water quality in natural systems and monoculture operations. (Dawczynski C, Schubert R and Jahreis G 2007)

The rising fashionability of organic husbandry practices is drawing increased attention toward the colorful operations of seaweed-deduced diseases and soil complements. While the seaweed toxin assiduity is still in its immaturity, it holds significant eventuality for sustainable profitable development as well as the reduction of nutrient runoff in littoral systems.

There are still ongoing challenges associated with the use and product of seaweed toxin including the spread of conditions and invasive species, the threat of heavy essence accumulation, and the effectiveness and refinement of product styles.

WHO- UN in 2021 reported that India has the 8th loftiest per hectare use of fungicides and the alternate loftiest chemical toxin per hectare in the world. Moment soil has critically deteriorated with the inordinate jilting of chemical toxin and agrochemicals. This situation is steadily aggravating as further and further chemical toxin is used for factory growth while further agrochemicals are demanded to cover them from pests and other conditions. It's a

growing trend to concentrate on factory nutrients as an indispensable supplement to chemical toxin. (Khotimchenko YS, Kovalev VV, Savchenko OV and Ziganshina OA 2001)

multitudinous exploration sweats have been made to estimate the feasibility and efficacy of organic sources adding factory growth and soil productivity. Seaweed is one of the implicit sources available around the Indian seacoast but moment it isn't duly employed in India. This resource is available in South East and South West littoral belts in India. About 7411 species belonging to different families are distributed in shallow littoral waters of ocean, arms, and backwoods. Seaweed excerpts contain trace rudiments and factory growth hormones IAA and IBA growth stimulators similar as auxin, gibberellins, and cytokinin needed by shops.

It's also reported that seaweed excerpts are rich in vitamins, amino acids, and trace rudiments. Seaweed excerpts have nitrogen, phosphorus, and a advanced quantum of water-answerable potash, other minerals, and trace rudiments in a readily absorbable by shops. They control insufficiency conditions. Moment is being used of low attention of seaweed excerpts are in combination with marketable toxin. It has been used more effectively in the world in promoting factory growth. (Fortman JL, Chhabra S, Mukhopadhyay A, Chou H, Lee TS, Steen E and Keasling JD 2008)

Diseases weren't only due to nitrogen, phosphorus, and potash content but also because of the presence of trace rudiments and metabolites. An advanced quantum of water-answerable potash, other minerals, and trace rudiments are present in seaweeds and are readily absorbed by shops and they control insufficiency conditions

The carbohydrates and other organic matter present in seaweeds alter the nature of the soil and ameliorate its humidity-retaining capacity. In the present-day world, seaweed diseases are frequently set up to be more successful than chemical diseases. In India, a large volume of macroscopic marine algae has been employed directly as ordure or in the form of compost by littoral peoples.

Besides their operation as Farm Yard Manure, Liquid fertilizer attained from seaweeds popularly known as SLF/ LSF has lately gained important interest as a foliar spray for converting faster growth and yield in cereal crops, vegetables, fruits, vineyards, and horticultural shops. India is an agrarian country; nearly 70 of the population thrives in pastoral areas, engaged in husbandry making the backbone of our frugality. (Hu JF, Geng MY, Zhang JT and Jiang HD 2001)

The fast-growing population is mounting tremendous pressure on food products in the country. To meet this added demand, growers use chemical diseases to enhance crop products. The poisonous chemicals (arsenic and cadmium) from the chemical diseases accumulate in factory products causing health problems in humans through biomagnifications.

Practice among the Romans and also rehearsed in Britain, France, Spain, Japan, and China. The use of marine macro algae as toxins in crop products has a long tradition in littoral areas all over the world. Seaweed cast continued to be so precious to growers, indeed in the early 1900s.

In numerous countries seaweed and sand cast are still used in both husbandry and horticulture. Numerous studies in the once three decades have set up wide operations in ultramodern husbandry for the use of marine macro algae as toxins.

They're used as whole or finely diced pulverized algal ordure or waterless excerpts. In recent times the use of these marine macro algae in ultramodern husbandry has been delved into by numerous. Seaweeds contain all the trace rudiments and factory growth hormones needed by shops.

It was also reported that seaweed ordure is rich in potassium but poor in nitrogen and phosphorus also the ranch ordure. There are numerous factory growth hormones, controllers, and promoters available to enhance yield attributes. (Dawczynski C, Schubert R and Jahreis G 2007)

Seaweed helps to keep soil moist therefore reducing your need to water-soak the theater. An operation of seaweed will reduce how frequently you need to weed. It contains no seeds that could conceivably turn into weeds as dinghy mulch occasionally does. Recently I have come apprehensive that dinghy mulch can be a fire peril because it's dry and acts like igniting. Seaweed presents no similar peril.

Slugs especially detest seaweed because of its sharp edges and swab. Catcalls and other theater pests dislike it for the same reasons. Seaweed helps aerate the soil just like peat moss does, but it has the added benefit of delivering nutrients and minerals. Prevents fungus and complaint. Seaweed helps you to grow strong, healthy shops, and healthy shops repel fungus and complaints. (Neish I 2003)

The high-quality seaweed toxin uses advanced technology to break the cell wall of seaweed, release the contents, and concentrate to form seaweed excerpt, therefore retaining the rich minerals and trace rudiments in seaweed, and also contains a certain quantum of Polyphenol composites and seaweed. Polysaccharides and a large number of growth controllers similar to cytokinins, auxins, cytokinin factors, cytokines, abscisic acid, gibberellins, ethylene, betaine, polyamines, trans- N6- isoprene Bismuth and its derivations, aminocyclopropanecarboxylic acid, ethylene precursor, indole acetic acid, hydrazine emulsion, etc., greatly retain the pure natural active constituents, and also contain a large quantum of potassium, calcium, magnesium, iron, zinc, iodine further than dozens of minerals and rich vitamins, integrating nutrients, anti-biomass, and natural biostimulants.

Rich in a variety of natural growth- suchlike substances. similar to auxin, gibberellin, cell- suchlike division, polyphenolic composites, etc., have high natural exertion, can stimulate the product of-specific active factors in shops and regulate the balance of endogenous hormones, and promote factory photosynthesis, The crops grow and develop in a their coordinated manner, perfecting vitality and resistance to conditions similar as conditions, insects, famines, diminutives, and low temperatures. (Luescher-Mattli M 2003)

Compared with chemical diseases, it has inimitable advantages in terms of yield increase, stress resistance, lightheartedness and-toxic side goods. Compared with conventional splint toxins, in addition to significantly promoting crop growth, root development, enhanced photosynthesis, and strong shops, pure natural seaweed excerpt is also rich in protein, amino acids, carbohydrates, inorganic mariners, vitamins, factory endogenous hormones, and more. Bioactive substances similar to phenol and polysaccharide can greatly ameliorate crop quality, enhance complaint resistance, cold resistance, and failure resistance, promote fruit growing, and increase profitable value.

Enhance the capability of shops to absorb water, retain water, repel failure, and repel cold. It can be combined with chemical diseases to form organic and inorganic emulsion diseases, enhance toxin effectiveness, ameliorate soil structure, increase soil permeability and water retention capacity, and help soil deterioration. The mannitol contained can greatly increase the water immersion capacity and chlorophyll content of crops, and the splint area increases by further than 10. Absciscic acid regulates the stomatal check of the leaves, reduces the water dissipation of the crops, and can reduce the lozenge of toxin and water by about one- third.

Forming a complex with fungicides through physical and chemical relations is a good fungicide dilution synergist. Suitable for crops similar to vegetables, fruits, flowers, tea, tobacco, grain, oil painting, and gardening. Due to the presence of polysaccharides in seaweed toxin, it has strong adhesion when mixed with utmost fungicides(except strong alkaline fungicides), which can significantly ameliorate the efficacy and protract the efficacy period. (Kotiya AS, Gunalan B, Jetani KJ, Solanki JB and Kumaran R 2011)

Enteromorpha species

Habit & habitat - Long and 1 cell thick and 1-7 mm. wide. Habitat: Typically found in saltwater or brackish environments rather than freshwater but seem to particularly thrive in areas where freshwater run-offs occur. Also found in estuaries and saltmarshes, and is able to withstand low salinities.

Taxonomical classification-

Division:	Chlorophyta
Class:	Ulvophyceae
Order:	Ulvaes
Family:	Ulvaceae
Genus:	<i>Ulva</i>

Uses- The sea lettuces comprise the genus *Ulva*, a group of edible green algae that is widely distributed along the coasts of the world's oceans.

Gracilaria species

Habit & habitat- Gracilaria is a genus of red algae (Rhodophyta) notable for its economic importance as an agarophyte, as well as its use as a food for humans and various species of shellfish. Various species in the genus are cultivated among Asia, South America, Africa and Oceania.

Taxonomical classification

Division:	Rhodophyta
Class:	Florideophyceae
Order:	Gracilariales
Family:	Gracilariaceae
Genus:	<i>Gracilaria</i>

Uses - its economic importance as an agarophyte, as well as its use as a food for humans and various species of shellfish.

Padina species

Habit & habitat- *Padina* inhabits tropical regions, although the genus can also be found in cooler temperate waters from South America to Southeast Asia. The genus is distinguishable because of its characteristic shape resembling a peacock tail structure.

Taxonomical classification

Class: [Phaeophyceae](#)

Order: [Dictyotales](#)

Family: [Dictyotaceae](#)

Genus: *Padina*

Uses- *Padina* can be utilized as food, fodder, plant growth promoter and bio-fertilizer. The brown alga is well known and being utilized for its antimicrobial, insecticidal, antioxidants, antibiotics, anti-inflammatory, hypo-allergenic, hepatoprotective and antidiabetic activities.

The present study intends to probe the effect of foliar operation of seaweed liquid fertilizer (SLF) uprooted from seaweed species *Enteromorpha species*, *Gracilaria species*, and *Padina species* on the growth of paddy.

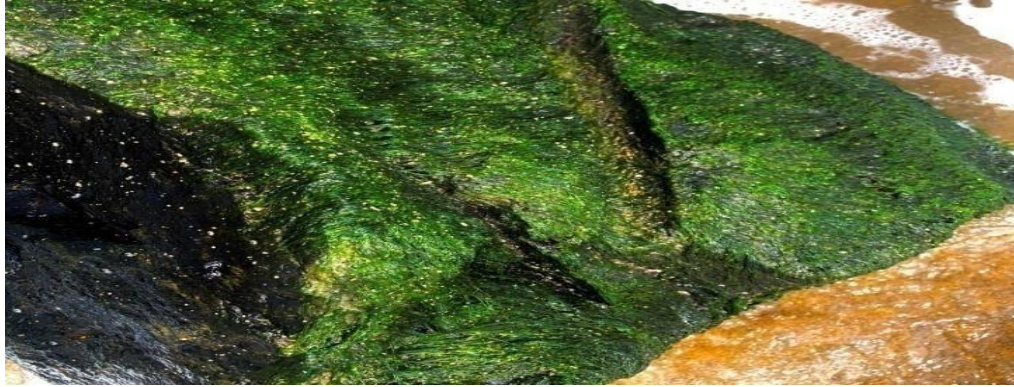


Figure 1.1.1 *Enteromorpha* Species



Figure 1.1.2 *Gracillaria* Species



Figure 1.1.3 *Padina* Species

CHAPTER-2

LITERATURE SURVEY

Seaweed contains all the trace rudiments and growth hormones needed by shops. lately there's a growing concern over the use of seaweed liquid fertilizer (SLF). moment, there's a high demand for terrain friendly husbandry for product of quality and healthy food to nourish the adding population. The present study investigates the effect of seaweed excerpt, from *Ulvalactuca*, *Sargusum wightti*, *Kapphaphycus alvarezii* and *Gracilaria verrucosa* on the seed germination and factory growth of *Capsicum annum*. The Seaweed Liquid Fertilizer was tested both with and without chemical diseases. The trial was arranged in randomized Complete Block design with nine treatments four replications. Once a week a different Seaweed Liquid collectively and different SLF attention in combination with Recommended rate of Chemical Fertilizer were applied to shops after germination and broadcasting. Their performance was recorded formerly in two weeks. The average maximum fresh shoot height(15 cm) and the dry weight(0.856 g) were observed in operation of foliar spray SLF 75 plus RCF uprooted from *Sargusum wightti* and *Kapphaphycus alvarezii* independently. Seaweed excerpt 75 SLF with RCF increased significantly to root dry weight, number of leaves, number of flowers, number of capsules, cover length over the control independently in seaweed excerpts from *Sargusum wightti*, *Gracilaria verrucosa*, *Sargusum wightti* and *Ulvalactuca*. The seaweed liquid toxin of each species with 100 SLF only) foliar operation was less effective on increase in below parameters than combined diseases(SLF plus CRF), but also has significant effect on increase in shoot height and other growth parameters when compared to control and recommended rate of chemical Toxin(RCF) only. thus, it could be concluded that concerted effect in(SLF 75 plus CRF) foliar operation could have met the demand of micro nutrients in chili crop than SLF collectively. The SLF-75 plus CRF foliar operation of *Sargusum wightti* significantly increased several growth parameters similar as shoot weight, root weight, number of leaves and number of capsules when compared to the excerpts medication from other species. SLF operations in combination with CRF are proposed to be used for enhance the growth, yield and quality of *Capsicum annum*. **Jayasinghe PS, Pahalawattaarachchi V, Ranaweera KKDS(1 april 2016).**

The magpie use of agrochemicals since green revolution redounded in adverse effect on the soil fertility, crop productivity, quality of yield and more specifically on the terrain system. The current script under similar situation forcefully emphasizes the need to borrow eco-friendly

agrarian practices for food product by considering the sustainability of soil and terrain. The use of Seaweed is considered as low cost input for factory growth and development due to presence of numerous nutrients as well as factory growth hormones which encourage the growth of the shops. **Shikha Singh, Dhananjay Tiwari, Satyendra Singh Gautam, M. K. Singh and S. K. Pal November 12TH (2019).**

Seaweeds or marine macro-algae are important renewable factory coffers being in the swell, brackish waters and lagoons across the globe. Operation of liquid excerpt from these organisms as foliar spray and/ or seed treatment showed positive result on improvement of vegetative growth and yield of several crops. In addition they increase the biochemical ingredients of shops and retain environmental stress mollifying eventuality. Correction of seaweed liquid fertilizer (SLF) to soils improves the soil health by enhancing the micronutrient volume and quality, and microbial exertion. The exploration work carried out so far on the salutary part of SLF in husbandry has been reviewed. The seaweed coffers of the Indian seacoast and the styles so far followed for medication of SLF and their mode of operation to crops is presented. **Debabrata Mohanty, S.P. Adhikary, G.N. Chatopadhyay January (2013).**

Biofertilizers offer a new eco-friendly technology would overcome short comings of the conventional chemical based farming. Biofertilizers showed positive influence on both soil sustainability and plant growth. Biofertilizers are not only the alternative to chemical fertilizers but also tend to increase the soil fertility and plant productivity which discuss in present review study. Seaweed plays an important and vital role in the marine ecosystem and growing in large amount in the sea. Seaweed can be regarded as a potential source of bio-fertilizer in of dried or fresh form; it helps to enhance biochemical constituents like carbohydrates, lipids, proteins, fibers, ash, phenol, dietary fiber etc in plant. The seaweed also good source of micro & macro elements required for plant nutrition. Seaweed extract is effective for improves the quality of produce and soil conditioner. This technology can be implemented in form of organic farming for sustainable agriculture which is better solution for eco friendly approach. **Rinku V. Patel, Krishna Y. Pandya, Dr. R.T. Jasrai and Dr. Nayana Brahmhatt July 2017.**

Seaweed is a multicellular marine algae, it's veritably important for the marine living renewable source, seaweeds hectically used for gelatinizing and stabilizing agents for numerous food and medicinal diligence. Soil fertility it depends upon the toxin demand of the factory or crop, now a day's lot of chemical diseases commercially available but the growth, yield and fertility of the soil are veritably much reduced damaged. Recent studies proved that

seaweed toxin veritably much better than other toxin. The seaweed diseases contained a lot of nutrients similar as nitrogen, phosphorus, potash and factory growth hormones and trace element. This seaweed toxin is used to enhance the seed germination and factory growth/ crop yield. **Ruban P and C.Govindasamy October 2018.**

In Istanbul, which is girdled by the ocean on 3 sides, thousands of tons of seaweed that have formed naturally every time are washed ashore. In this study, the usability of these seaweeds which are land filling formerly in toxin product was banded. Liquid toxin product was carried out using 3 different physical and 4 different natural styles, and the produced diseases were adulterated in 5 different rates (1, 10, 25, 50, and 100) and applied to cress seed. The effect of each toxin and its attention on seed germination, factory length, number of leaves, and soil humidity- holding capacity was studied. The data attained were anatomized using Response face Methodology. The results showed that if seaweed was instigated with anaerobic seed sludge for 15 days and applied to the factory by lacing it to 15 – 25, factory growth will be supported at an optimum position. It has also been shown that if the seaweed was instigated with incentive culture for 18 days and fed with a attention of > 90, the humidity- holding capacity of the soil could be increased by over to 27. **panelHulya Civelek Yoruklu, Bestami Ozkaya, Ahmet Demir (January 2022).**

Ferocious examinations were made on the effectiveness of Liquid Seaweed Fertilizer(LSF) attained from the brown seaweed *Sargassum wightii* Grev. on the germination, growth and biochemical ingredients of green gram(*Vigna radiata*(L.)R. Wilczek) under laboratory conditions and in pots. The seeds that are soaked for 6 hrs duration at lower attention similar as 0.5 and 1.0 showed briskly germination compared with the seeds that are soaked at advanced attention(2.0) for long durations (12 hrs and 24 hrs). In pot studies, both the shoot length and root length were set up to be increased as the attention of LSF increases, but at high attention i.e. at 2.0 attention the growth was slightly reduced. Of the two different types of operations i.e. foliar and root operations, the foliar operation was set up to be more effective in determining the growth of the factory than the root operation. shops entered with 1.0 attention of LSF as foliar spray showed further shoot and root length, early flowering and further number of capsules. The biochemical analysis of the experimental shops showed that the foliar treated shops showed more photosynthetic colors compared to root

treated shops, whereas the accumulation of total protein, total carbohydrate and total lipid content was set up outside in root treated shops than the foliar treated shops. **N. Ashok-Kumar , B. Vanlalzarzova , Sekaran Sridhar, Murugesan Baluswami (2012).**

Seaweeds or marine macro-algae are important renewable resources being in the swell, brackish waters and lagoons across the globe. Application of liquid extract from these organisms as foliar spray and/ or seed treatment showed positive result on improvement of vegetative growth and yield of several crops. In addition they increase the biochemical ingredients of crops and retain environmental stress mollifying eventuality. Application of seaweed liquid fertilizer (SLF) to soils improves the soil health by enhancing the micronutrient volume and quality, and microbial exertion. The exploration work carried out so far on the salutary part of SLF in husbandry has been reviewed. The seaweed resources of the Indian seacoast and the styles so far followed for application of SLF and their mode of application to crops is presented. Numerous of them are used as food, application of several artificial products and also as a raw material for toxin for application in the crop fields as liquid toxin or compost for adding productivity. The major uses of seaweeds are (i) product of phytochemicals similar as agar- agar, carrageen and alginate and (ii) as food for mortal consumption as green vegetable, salad and also in the form of jelly, jam, chocolates and pickles. piecemeal from the photochemical, other products similar as mannitol, laminarin and fucoidin are also attained from these marine macro algae. Seaweed product (liquid/ compost) when applied to seed, soil or scattered on crops it increased seed germination chance, nutrient uptake, growth and yield of crops. The adulterated liquid seaweed extract also enhanced the factory's defense against conditions and increases swab and failure forbearance. An acceptable quantum of growth promoting hormones and micronutrients present in seaweed makes them an excellent toxin. Unlike chemical toxin, seaweed extract are biodegradable, non-toxic, non-polluting and non-hazardous to mortal. **Debabrata Mohanty (2015).**

In the present study, ferocious disquisition was made on the effect of seaweed liquid fertilizer (SLF) of *Codium decortatum* on the seed germination yield biochemical and color specific of *Capsium annum* under laboratory conditions and in pots. Different attention similar as 10, 20, 30, 40 and 50 of SLF were prepared using distilled water. The seeds were soaked in 10 hrs for each SLF attention also placed in separate Petri plates. also, water soaked seeds were used as controls. application of a lower attention(20) of SLF Showed maximum seed germination, fresh weight, dry weight, root and shoot length, number of branches, splint area, number of capsules and content of total chlorophyll, chl a, and chl b, protein, carbohydrate and lipids were observed. thus, the results of the present study suggested that the SLF of *C. decortianum* could serve as an indispensable bio-fertilizer as iseco-friendly, cost-effective, deliver substantial profitable and environmental benefits to planter. **S. Vijayakumar , S. Durgadevi , P. Arulmozhi , S. Rajalakshmi , T. Gopalakrishnan , N. Parameswari (2019).**

The effect of seaweed liquid fertilizer(SLFs) made from *Gracilaria salicornia*, *Padina gymnospora*, *Padina boergesenii*, *Gelidiella acerosa* and factory growth controllers(PGRs) were examined on in vitro mass propagation using hypocotyls and splint slice explants of brinjal(*Solanum melongena*L.) cultivar Pusa grandiloquent long. For the germination bioassay, seeds germinated with 20 – 40 SLEs displayed enhanced germination. originally, hypocotyls and splint discs were dressed on Murashige and Skoog(MS) medium containing 6- benzylaminopurine, zeatin and thidiazuron for shoot induction. The stylish responding cytokinin, 6- benzylaminopurine, was employed with different auxins(indole-3-acetic acid, indole-3-butyric acid and 1- naphthaleneacetic acid) for shoot proliferation. In a alternate trial, all the four SLEs(10 – 60) combined with MS medium were studied for shoot propagation. stoked shoots transferred to half- strength MS medium and supplemented with auxins and SLEs(10 – 70) collectively to induce lodging. In these trials high rate of shoot induction(96.2), proliferation(6 cm) and lodging(95.3) was set up with 20 – 40 of SLEs. Well- progressed plantlets were transferred to soil mugs, maintained in a growth chamber for a week to control moisture and also shifted to a hothouse. This study demonstrated that SLEs could serve as an volition to phytohormones as they were easy to prize and gave quick and high- frequency mass propagation. **Satish, L., Rameshkumar, R., Rathinapriya, P., Pandian, S., Rency, A. S., Sunitha, T. and Ramesh,(2015).**

Seaweed fertilizer as natural factory growth goad could be significantly used as a good agrarian practice in sustainable and organic crop product. thus, the current study was carried out at a private ranch in Shibin El- Kom, Monufia Governorate, Egypt during downtime growing seasons of 2017 and 2018. The effect of brown seaweed excerpt used as apre-sowing seed soaking for 12 h at a rate of 3 ml/ l, in addition as foliar scattering formerly at rates of 1, 2 or 3 ml/ l after 20 days from sowing date on vegetative growth, yield and some nutritive values of leaves and roots of red radish shops of Celesta F1 mongrel was assessed. The trial was set in a split plot design with three replicates. The attained results sprucely indicated that seaweed soaked seeds gave significantly the advanced values of all measured characters than water soaked seeds. It was largely apparent that the foliar scattering treatment of the loftiest rate of seaweed excerpt(3 ml/ l) gave significantly the stylish values of all studied characters compared to the other treatments. The commerce had significant goods on all studied parameters, except for leaves and roots dry weight, leaves Iron, and roots Iron and Zinc contents in both seasons as well as for chlorophyll a in the first season and chlorophyll b and carotenoids in the alternate season. The most effective treatment was set up to be seaweed soaked seeds before sowing and foliar scattered seaweed excerpt at 3 ml/ l formerly 20 days after sowing(DAS). Keywords Raphanus sativus, Brown seaweed excerpt, Leaf colors, Phytochemicals, Phenols, Flavonoids,

Anthocyanins, Mineral contents. Sami Hosni , MahmoudAhmed M M , El-tanahyDina ,M. Salama (December 2019)

The main goods of seaweed excerpts(Ascophyllum, Fucus, Sargassum, Saccorhiza, Laminaria, Gelidium and other species), when used as agrarian diseases, are better seed germination and advanced quality fruit product, with longer shelf life; better use of soil nutrients; more productive crops and shops with lesser resistance to inimical environmental conditions. Algae also have a long history of use as beast feed. They've a largely variable composition depending on the species, collection season and niche, and on external conditions similar as water temperature, light intensity and nutrient attention in water. In relation to ruminal turmoil, a high variability of the insipidity values was set up among seaweed species and can not be attributed only to the composition of different nutrients of the algae. The part of marine algae for reduction of methane product is bandied with particular emphasis on new algae- grounded feed strategies that target minimum methane emigrations without affecting the functionality of the microbiota and overall beast productivity. **Bahcevandziev, K. and Pereira, L. (2021).**

CHAPTER - 3

3.1 AIM

To study the effect of Seaweed Liquid Fertilizer extracted from different seaweeds on the growth of paddy plants .

2. OBJECTIVES

- To collect different seaweeds from coastal area.
- Seaweed liquid fertilizer and extracts to be prepared from different seaweed.
- The prepared seaweed liquid fertilizer and extracts to be tested on different paddy plants along with controlled one.
- Plants growth analysis should be done after 30 days.
- To determine the phytoconstituents , phytochemical analysis should be done .
- To identify the total phenolic and flavonoids content , quantitative analysis should be done.
- Thin layer chromatography should be done for all the extracts.

CHAPTER - 4

MATERIALS AND METHOD.

1. Materials required.

- Algae sample - *Enteromorpha species*, *Gracillaria species* and *Padina species*
- Distilled water
- Beaker
- Funnel
- Motor and pestle
- Autoclave
- 0.1% formaldehyde
- Pots
- Soil
- Test tubes
- Test tube stand
- methanol
- Dragendorff reagent
- Fehling solution I and II
- Chloroform
- 10% ammonia solution
- 2% copper sulphate
- 95% ethanol
- Potassium hydroxide pellets
- Ninhydrin solution
- Ferric chloride
- Trichloroacetic acid
- Folin-Ciocalteu phenol reagent
- 20% Sodium carbonate
- 5% Sodium nitrite
- 1 M Sodium hydroxide
- 10% ammonium chloride

- TLC plates
- Ethyl acetate
- Measuring scale
- Micro pipette

2. Preparation of Seaweed Liquid Fertilizer.

- Seaweeds namely *Enteromorpha species*, *Gracillaria species* and *Padina species* were collected from kovalam , Chennai.
- The collected seaweeds were sun-dried and powdered.
- 5 gram of dried powder from each seaweed species were blended at the ratio of (1:20) to distilled water (100ml).
- Then it was blended for 10 minutes using motor and pestle and autoclaved for 30 minutes.
- The autoclaved extracts were strained using funnel and silk cloth .
- The strained extracts were named as T1 - (*Enteromorpha Species*) , T2-(*Gracillaria Species*) and T3- (*Padina Species*).
- For preparation of Seaweed Liquid Fertilizer , 0.1% of formaldehyde solution is added to the extracts in the ratio of 1 : 20 .
- Seaweed Liquid Fertilizer named as as T4 - (*Enteromorpha Species*) , T5-(*Gracillaria Species*) and T6- (*Padina Species*).



Figure 4.2.1
Extracts after autoclaved



Figure 4.2.2
Extracts and SLF of seaweeds

4.3 Pot Study

- The crop plant selected for the present study was *Oryza Sativa*.
- Plastic pots with seeding holes were used for raising the crops .
- The plastic pots were filled with garden soil and seeds were sowed at a depth of 1.5cm.
- Totally 7 plant pots were taken for T1 , T2 , T3 , T4, T5 , T6 and controlled .



FIGURE 4.3.1



FIGURE 4.3.2

**Both SLF and extract treated plant pots with controlled plant pot
(day 0) .**

4.4 Phytochemical Analysis

The different qualitative chemical tests were performed for establishing the profile of the extract for its chemical composition. The following tests were performed on the extract to detect various phytoconstituents present in them.

1.Detection of alkaloids

Dragendorff's test

To a few ml of filtrate, 1 or 2 ml of dragendorff's reagent was added. A prominent yellow precipitate indicated the test as positive.

2.Detection of carbohydrates and glycosides

Fehling's test

One ml of filtrate was boiled on water bath with 1 ml each of Fehling Solutions I and II. A red precipitate indicated the presence of sugar.

3.Detection of glycosides

Borntrager's test

To 2 ml of extract 3 ml of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

4.Detection of saponins

Foam test

The extract (50 mg) was diluted with distilled water and made up to 20 ml . The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of saponins.

5. Detection of proteins and amino acids

A. Biuret test

An aliquot of 2 ml of extract was treated with one drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

B. Ninhydrin test

Two drops of ninhydrin solution were added to two ml of extract . A characteristic purple color
Indicates the presence of amino acids.

6. Detection of phenolic compounds**Ferric chloride test**

The extract (50 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour
indicated the presence of phenolic compounds.

8. Test for terpenoids

To 1 ml of extract, 2 ml of trichloroacetic acid was added and the formation of yellow to red precipitate showed the presence of terpenoids.

4.5 Quantitative Analysis

Quantitative analysis

Determination of total phenolic content .

The different extracts of plant material were then centrifuged at 10,000 rpm for 15 min at 4°C. Twenty μ L of extracts was prepared using the supernatant and made up to 3 ml of distilled water. Then, 0.5 ml of Folin-Ciocalteu's phenol reagent was added to all the tubes. The tubes were then placed in the incubator for 3 min.

Sodium carbonate at 45°C. After 3 min, 2 ml of 20% (Na_2CO_3) was added to all the tubes and kept for incubation after which, its absorbance was measured at 650 nm. The total phenol content in the sample was calculated using the formula,

$$C(\text{GAE}) = C \times V/M$$

where, c = concentration of sample from the curve obtained (mg/ml), V = volume used during the assay (ml) and M = mass of the sample used

during the assay (g)

Determination of total flavonoids

Flavonoid contents were determined by slightly modified spectrophotometry method of Karadeniz et al. (2005). One g of dry powder was weighed and ground with 200 ml of 80 % aqueous methanol in a mortar and pestle. The ground sample was filtered and a clear filtrate was obtained. The aliquot of the sample (0.5 ml) was taken in a test tube add 3 ml of distilled water and 0.3 ml of (5% sodium nitrite) were added. The solution was vortexed and allowed to stand at room temperature for 5 min. and 0.6 ml of 10% aluminum chloride was added to the solution. After 6 min, 2 ml of 1 M Sodium hydroxide was added to the test tube. The solution was made up to 10 ml with distilled water. The absorbance was read at 510 nm. The total flavonoid content was calculated as quercetin equivalent (mg QE/g) using the formula, Remaining - 3.6ml H₂O * make upto 20 ml.

$$X = (A.M_0 / A_0 .M)$$

where, A= absorption of sample, A₀ = absorption of standard (quercetin),

M= weight of sample (mg/ml) and M₀ = weight of quercetin in solution (mg/ml).

4.6 THIN-LAYER CHROMATOGRAPHY

Materials required:

1. Readymade TLC plates
2. Solvent system (mobile phase):

100% ethyl acetate

Procedure:

1. Ready-made TLC plate was cut to the required size.
2. Samples were spotted at one end of the TLC plate .
3. The plate was kept in the beaker containing the mobile phase in such a way .
that the end near the sample application should touch the mobile phase .
4. Allow the chromatogram to run for about 1-2 hours. (3/4th level for TLC plate)
5. The plate was dried at RT and viewed under UV light.
6. RF value of the samples can be determined by using the formula .

$$\text{Retention factor} = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

CHAPTER-5 Result and Discussion

5.1 Plant Growth Analysis

Plant growth of different paddy pots were measured after 30 days using measuring scale .

Rice plants were treated with different SLF prepared using three different algae namely *Enteromorpha species*, *Gracillaria species* and *Padina Species*. Among the samples used for the analysis,T4 showed the maximum growth in shoot(22.2 cm),root (13.5 cm),and total plant length(35.7cm).T1 showed the maximum growth in shoot(17.8 cm),root (13.9 cm),and total plant length(31.7cm). T3 showed the maximum growth in shoot(19.4 cm),root (6.2 cm),and total plant length(25.6cm).

Among the different extracts and SLF used T4(SLF prepared from *Enteromorpha species*) showed best results in term of shooting, rooting and growth of whole plant here further studies has to be carried out to formulate the fertilizer.

Table 5.1.1 plant growth analysis

SAMPLE	SHOOT LENGTH	ROOT LENGTH	TOTAL LENGTH OF PLANT
T1	17.8 CM	13.9 CM	31.7 CM
T2	14.9 CM	6 CM	20.9 CM
T3	19.4 CM	6.2 CM	25.6 CM
T4	22.2 CM	13.5 CM	35.7 CM
T5	12.6 CM	1.9 CM	14.5 CM
T6	11.4 CM	4.6 CM	16 CM
CONTROLLED	15.5 CM	12.7 CM	28.2 CM



Figure 5.1.1



Figure 5.1.2

Both SLF and extract treated plant pots with controlled plant pot
(30TH DAY)



Figure 5.1.3

Plants after removed from its pots,

5.2 Phytochemical analysis

Table 5.2.1 Phytochemical analysis

PHYTOCHEMICAL TEST	IDENTIFICATION	T1 <i>Enteromorpha</i> sps	T2 <i>Gracillaria</i> sps	T3 <i>Padina</i> sps
1. Detection of alkaloids Dragendorff's test	Present- yellow precipitate Absent- No yellow precipitate	PRESENT	PRESENT	PRESENT
2. Detection of carbohydrates and glycosides Fehling's test	Present- Red precipitate Absent- No red precipitate	PRESENT	ABSENT	ABSENT
3. Detection of glycosides Borntrager's test	Present- Pink color changes Absent- No pink color changes	ABSENT	ABSENT	ABSENT
4. Detection of saponins Foam test	Present- Foam formed Absent- No foam formed	ABSENT	ABSENT	ABSENT
5. Detection of proteins and amino acids a) Biuret test b) Ninhydrin test	a) Present- Pink layer formed Absent- No pink layer formed b) Present- purple color changes Absent- no purple color changes	a) ABSENT b) ABSENT	a) ABSENT b) ABSENT	a) ABSENT b) ABSENT
6) Detection of phenolic components Ferric chloride test	Present- dark green changes Absent- no dark green changes	ABSENT	ABSENT	ABSENT

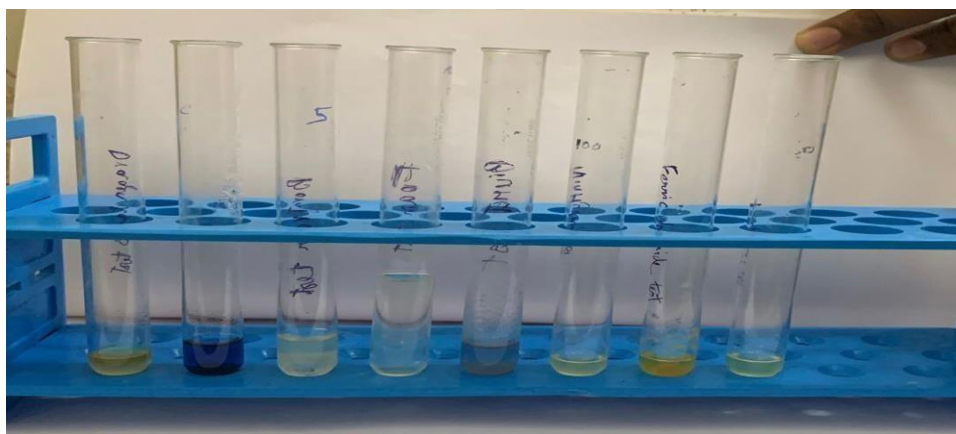


Figure 5.2.1 Phytochemical Analysis result for T1 sample



Figure 5.2.2 Phytochemical Analysis result for T2 sample

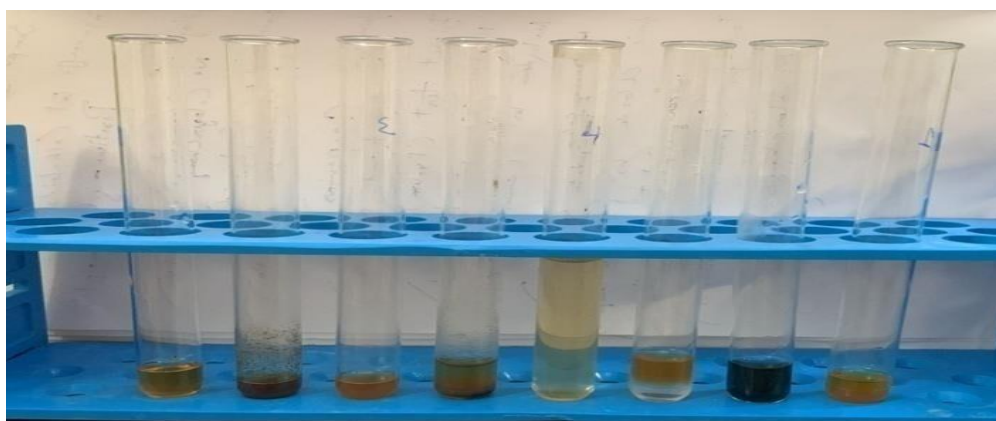


Figure 5.2.3 Phytochemical Analysis result for T3 sample

5.3 Quantitative analysis

Result:

Total phenol content of T4 extract showed 0.078 mg/GAE and total flavonoids content of T4 extract showed 0.068 mg/QE.

- Phenol- 0.078 mg/GAE
- flavonoids - 0.068 mg/QE.

GAE- Gallic acid equivalence

QE- Quercetin equivalence

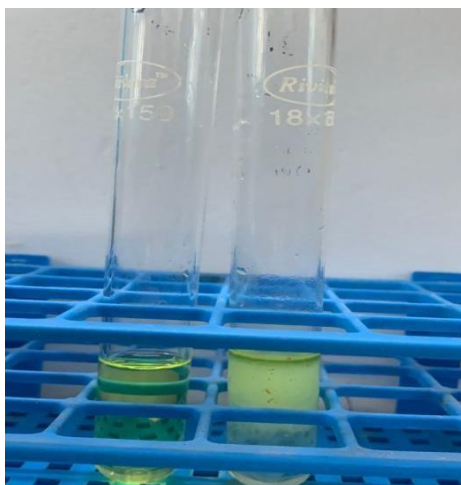


Figure 5.3.1 Quantitative analysis done T4 plant sample (T4- *Enteromorpha Species* seaweed liquid fertilizer)

5.4 Thin layer chromatography

Results :

Thin layer chromatography is done for all the seaweed extracts.

Thin layer chromatography of *Enteromorpha Species* showed RF value at 0.84.

Thin layer chromatography of *Gracillaria Species* showed RF value at 0.74.

Thin layer chromatography of *Padina Species* showed RF value at 0.75.

Table 5.4.1 Thin layer chromatography

Sample	Distance moved by the solute	Distance moved by the solvent	Retention factor (RF)
<i>Enteromorpha Species</i>	5.6	6.6	0.84
<i>Gracillaria Species</i>	4.9	6.6	0.74
<i>Padina Species</i>	5.0	6.6	0.75

Figure 5.4.1 Thin layer chromatography plate.



DISCUSSION :

The collected algae samples from kovalam, Chennai namely *Enteromorpha species*, *Gracillaria species* and *Padina species* were used to prepare SLF and tested on the paddy plants. Among them T4 SLF (*Enteromorpha species*) showed the best plant growth. (Sadhar et al., 2012) states that liquid foliar application of *Enteromorpha species* obtained from study contained maximum concentrations of auxins and cytokinins. Seaweed concentration applied to wheat significantly increased clam diameter.

Phytochemical analysis results showed that alkaloids presents in all the extracts. And there is carbohydrate in *Enteromorpha* extract. The rest of the phytoconstituents were absent on all three extracts. (M. Niranjadevi, K. Sivakumar et al., 2018) states that 39 phytochemical constituents were identified from the methanolic extract of *L. esculentum* .After treatment with SLF. Since the identified phytochemical constituents biologically antimicrobial activity such as, antifungal and antiviral potential candidate.

TLC is done for all three extracts in a single TLC plate and the retention factor of *Enteromorpha species* is 0.84, *Gracillaria species* is 0.74 and *Padina species* is 0.75. Total phenol content of T4 extract showed 0.078 mg/GAE and total flavonoids content of T4 extract showed 0.068 mg/QE. (Abd El-Moniem M. Naguib , Farouk K. El-BazTotal et al ., 2012) states that phenolic content (TPC), and total flavonoid content (TFC) were almost higher in seaweed as a result of organic fertilizer treatment.

CHAPTER -6

SUMMARY AND CONCLUSIONS

Seaweed fertilizer is an organic fertilizer made from seaweed that's used in husbandry to increase soil fertility and factory growth. The use of seaweed toxin dates back to age and has a broad array of benefits for soils. Seaweed toxin can be applied in several different forms, including meliorated liquid excerpts and dried, pulverized organic material.

In this study seaweeds namely *Enteromorpha species*, *Gracillaria species* and *Padina species* were collected from kovalam, Chennai. The extract and SLF were prepared from different seaweeds namely T1, T2, T3, T4, T5 and T6 were studied on paddy plants along with controlled. Phytochemical analysis results showed that alkaloids presents in all the extracts. And there is carbohydrate in *Enteromorpha* extract. The rest of the phytoconstituents were absent on all three extracts.

The extracts and SLF were treated on the different paddy pots and after 30 days the plants were measured using scale. T4 gave best result and growth in paddy plants. So quantitative analysis is done on plant sample of T4 (*Enteromorphasps* SLF), it showed that it contains 0.078 mg/GAE Phenol and 0.068 QE flavonoids. TLC is done for all three extracts in a single TLC plate and the retention factor of *Enteromorpha species is* is 0.84, *Gracillaria species* is 0.74 and *Padina species* is 0.75.

Here by this result shows that SLF prepared from *Enteromorpha sps* has the better growth and seed germination on paddy plants. And also phytochemical analysis shows that *Enteromorpha species has more phytoconstituents comparing to other seaweeds. Hence Enteromorpha species* are the best suitable seaweed for the preparation of SLF.

Among the extract and SLF used, T4 showed the maximum growth in shoot(22.2 cm),root (13.5 cm),and total plant length(35.7cm). Further studies has to be carried out to check the growth and the stability.

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