Effect of Seaweed Extract on Plant Growth and Microbial Activity

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I hereby declare that the data presented in this Dissertation report entitled, "Effect of Seaweed Extract on Plant Growth and Microbial Activity" is based on the results of investigations carried out by me in the Marine Microbiology at the School of Earth, Ocean and Atmospheric Sciences, Goa University under the Supervision of Dr. Nikita P. Lotlikar and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given in the dissertation.

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ABBREVATIONS USED

Entity	Abbreviation
Seaweed liquid fertilizer	SLF
Cold seaweed extract	CSE
Hot seaweed extract	HSE
Low-temperature seaweed extract	LSE
Yeast Mannitol Agar	YMA
Nutrient Agar	NA
Pikovskyas Agar	PA

PREFACE

The research was carried out for the dissertation titled **"Effect of seaweed extract on plant growth and microbial activity"**. Chemical fertilizers pose a significant threat to the environment leading to soil and water pollution and decrease in soil fertility, in addition to the dangerous impact on human health, thus there is a need to switch to organic fertilizers. These are not only affordable but also environmentally friendly, for instance, seaweed, often regarded as waste, is a suitable organic fertilizer. Seaweed is not only a functional food ingredient but also has the potential as an organic fertilizer due to the wide variety of trace metals (Fe, B, Ca, Cu, Cl, K, Mg, and Mn) contained, as well as the presence of growth regulators (PGR), including auxins, cytokinins, and gibberellins, with the ability to stimulate growth and increase plant production. Therefore, the use of seaweed as fertilizer or additional fertilizer is a presumed alternative solution to environmental problems. This application is safe for soil and plant microbes and increases seaweed's economic value. In this study, the addition of seaweed extract is expected to increase microbial activity thereby increasing yield and quality in *Vigna radiata l*. plant Therefore, the study aims to know the effect of various seaweed concentrations on seed germination, phytochemical analysis, and growth of *Vigna radiata l*. plant in fertile as well as infertile soil.

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Gayatri A. Kerkar

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ABBREVIATIONS USED

Entity	Abbreviation
Agricultural field soil	AFS
Agricultural field soil control	AFSC
Amplicon sequence variations	ASVs
Cold seaweed extract	CSE
Garden soil	GS
Garden soil control	GSC
Hot seaweed extract	HSE
Low-temperature seaweed extract	LSE
Mining soil	MS
Mining soil control	MSC
Nutrient Agar	NA
Pikovskya's Agar	PA
Seaweed liquid fertilizer	SLF
Seed treatment	ST
Tobacco mosaic virus	TMV
Vigour index	VI
Yeast Mannitol Agar	YMA

ABSTRACT

Growth-promoting and stress-resistant qualities of seaweeds have gained immense popularity in the field of agriculture in the form of bio-stimulants in crop management. The current study aimed to determine whether extracts of seaweed species found in Goa could be utilized as a biofertilizer. Three types of extracts from *Ulva intestinalis* and *Sargassum sp.* were prepared and evaluated based on the presence of various phytochemical compounds (alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones, and glycosides), and seed germination test. Efficacy of the seaweed liquid fertilizer was also evaluated using germination percentage, foliage count, vigour index and shoot/root length. Germination percentage of 100, 98, and 92% was achieved at 1, 0.5, and 0.1% concentration of the cold seaweed extract respectively, and also had the highest foliage count in comparison to the other extracts. Additionally, it was observed that the hot seaweed extract turned the leaves and stems black. Further, the best extract was selected and applied to plant in three different ways such as soil treatment, foliar spray, and seed treatment. The microbial activity of three different types of soils ie. Agricultural, Garden and Mining soil was checked on Day 0 and Day 7 with 4 different types of media: YMA, PA, Sulphate API and NA to check the number of beneficial and viable microbes with and without seed treatment respectively. The bacterial activity was strongly influenced by the SLF. Overall, it could be concluded that the utilization of seaweed liquid fertilizer may enhance the number of beneficial microorganisms thereby increasing the yield of crops, which could be suggested as a suitable alternative to chemical fertilizers.

Keywords: Seaweed, biofertilizer, seed germination, growth enhancement, microbial activity

CHAPTER 1: INTRODUCTION

1.1 Background

Seaweeds are benthic macroscopic algae that grow on rocky shorelines of shallow marine coastal waters (Dhargalkar et al., 2004). These wonderful photosynthetic aquatic plants that have been commonly known as the "Medical Food of the Twenty-First Century", are often found along the coastline in the subtidal zone up to a depth where there is 0.01% of accessible light for photosynthetic activity (Dhargalkar et al., 2004). Seaweed distribution and variety are influenced by a multitude of environmental factors including plant pigments, light exposure, depth, temperature, tides, and coast characteristics. Macroalgae, though resemble highly vascular plants, they differ in structural and functional components (Dhargalkar et al., 2004). These aquatic flora comprise a hold fast, stipe, and blade (together known as the thallus) and lack true roots, stems, and leaves. Although the holdfast resembles the root of the higher plants, its purpose is attachment, rather than food uptake. The stipe primarily supports the blade during photosynthesis and absorbs nutrients from the surrounding seawater. The blade may have different shapes (smooth, perforated, segmented) and imitate the leaves of higher plants. Its crucial functions include photosynthesis and food absorption (Nasmia et al., 2021). Seaweed plays a variety of ecological roles. Only the tips of the sea spray moisten them at the surface, while certain species may adhere to a substrate that is several meters below the surface (Campbell, 2022) Littoral seaweed colonies can stretch kilometers out to sea in certain places. Seaweed in this environment has to endure abrupt changes in salinity and temperature as well as sporadic drying (Lewis et al., 1964)

Seaweed habitats are crucial to the marine environment as they primarily contribute to global primary production and offer a diversity of creatures with food and shelter. Certain seaweed species, like kelps, safeguard food sources by acting as key nursery habitats for fisheries and other marine creatures. Other seaweed species, such as planktonic algae, are crucial for absorbing carbon dioxide and generating at least half of the oxygen on Earth (Harley et al., 2021). The surface of seaweeds provides bacteria with sheltered and nutrient-rich conditions

for development (Ren et al., 2022) leading to rich microbial diversity. Epiphytic bacterial communities have been observed to be particularly important for the morphological development of seaweeds. Certain bacterial species have bactericidal ability against particular diseases and host specificity; these specificities include intricate metabolic interactions between bacteria and seaweed (Egan et al., 2013)

1.1.1 Classification of Seaweeds

Seaweeds can be classified into three broad groups:

1) Green (Chlorophyta) Pigments: Chlorophyll a & b Reserved food: Starch Examples: *Ulva*, *Acetabularia*		They are found in marine and fresh habitats. Green algae have chromatophores, a unique cell type that houses photosynthetic pigments like chlorophyll a and b. The chloroplast's shape and size could differ. It possesses a double membrane envelope and no endoplasmic reticulum. The principal locations of starch manufacture, chloroplasts, include pyrenoids in a variety of forms. The pyrenoids of green algae are alternatively regarded as significant protein

		stores and as distinctive cell organelles (Sahu et al., 2020).
2) Brown (Phaeophyta) Pigments: Chlorophyll a & c, Fucoxanthin Reserved food: Mannital, Laminaria Examples: *Padina*, *Sargassum*		They are found in marine habitats. The color of the brown algae ranges from olive-yellow to deep brown. The color is caused by the carotenoid pigment and fucoxanthin. The amount of fucoxanthin in different species of brown algae varies. The majority of the brown algae in the littoral zone are high in xanthophylls and fucoxanthin. Fucoxanthin-rich algae have a much higher rate of photosynthesis in blue light than fucoxanthin-deficient algae. The other photosynthetic pigments of brown algae are Chlorophyll a and c, Beta-carotene and

		xanthophylls (Coelho et al., 2011)
3) Red (Rhodophyta) Pigments: Chlorophyll a, d, carotene, phycoerythrin, phycocyanin Reserved food: Floridean starch Examples: *Gracilaria*, *Gelidium*	 (Google Images)	They are found in marine habitats, except for a few species. Red phycoerythrin and blue phycocyanin, two water-soluble pigments, are also responsible for Rhodophyta's color. Chlorophyll a and b, carotene and other colors are also present. Phycoerythrin pigment is found in greater abundance in deeper water and freely illuminated forms, which also have a higher phycoerythrin to chlorophyll ratio. The red algae appear to perform more photosynthesis in low light than brown and green algae (Dhargalkar et al., 2004).

Fig 1.1: Three broad groups of Seaweeds

1.1.2 Microbiota of seaweeds

Seaweed's surface is a great place for microbes to colonize, and it secretes a variety of organic compounds that serve as nutrients for bacterial growth and the development of microbial biofilms (Steinberg et al., 2002). The extremely intricate and dynamic microbial communities that exist on top of seaweed are made up of a range of microorganisms, such as bacteria, fungi, diatoms, protozoa, spores, and larvae of marine invertebrates (Lachnit et al., 2009). Pervasive bacteria are found to be detected in the cytoplasm of living host cells (*Herbaspirillum* sp. in *Caulerpa taxifolia*) or on the surface of seaweed (Lachnit et al., 2009). Quorum sensing (QS) signaling molecules are produced by gram-negative bacterial strains, and are known to regulate spore liberation in *Acrochaetium* and *Gracilaria* species (Singh et al., 2015) species as well as zoospore settling in *Ulva* species (Joint et al., 2002). Additionally, it has been discovered that the induction of morphogenesis and growth in marine macroalgae are caused by the bacterial metabolite thallusin and nitrogen-fixing bacteria linked to seaweeds (Chisholm et al., 1996).

The structure of intertidal communities is heavily influenced by macroalgae, which are also recognized as ecosystem engineers (Jones et al., 1994). Water-soluble monosaccharides such as rhamnose, xylose, glucose, mannose, and galactose are included in some algal polysaccharides, which make up a component of the cell wall and the remaining storage material (Popper et al., 2011). Numerous marine bacteria that manufacture certain compounds, which in turn promote seaweed-bacterial relationships, may use these algal polysaccharides as a source of carbon and energy (Lachnit et al., 2013). As a result, numerous academics from all over the world have been fascinated by and interested in these interactions between seaweed and bacteria.

The study of the structure, succession, and dynamics of bacterial communities associated with seaweeds in relation to the ecology of bacterial-seaweed interactions has significantly increased in recent years. There aren't many studies that examine all bacterial communities on algal surfaces in detail. Denaturing gradient gel electrophoresis (DGGE) fingerprinting and 16S

rRNA gene sequencing, based on the available data, have demonstrated differences between bacterial communities associated with algal and planktonic environments (Goecke et al., 2013). Members of the Gammaproteobacteria and Alphaproteobacteria can be found in oceanic and coastal waters all over the world (Rusch et al., 2007). The Bacteroidetes, Actinobacteria, Planctomycetes, and Chloroflexi are other marine species that are frequently observed (Burke et al., 2011).

On the other hand, bacterial communities connected to seaweed exhibit both temporal oscillations and species-level variation. Bacteria not only live epiphytically on algal surfaces, but also within the thallus or cells. Algal thalli can be damaged by seaweed grazers or epiphytic bacteria that can break down algal cell walls, opening an entry point for pathogenic and opportunistic bacteria (Wang et al., 2008). In the event that these latter bacteria are able to penetrate the algal tissue and aid in the continued breakdown of the host, thallus rupture may eventually result (Goecke et al., 2010). In addition to these pathogenic relationships, non-harmful endophytic bacteria that are connected with seaweed are also described. More than 20 species of red and brown macroalgae have been found to have algal galls, which are aberrant tissue growths of seaweeds. Endophytic bacteria in the red seaweed *Prionitis* cause galls by overproducing the phytohormone indole-3-acetic acid (IAA), which also provides a favorable microhabitat for the growth of the bacteria themselves (Ashen et al., 2008). Despite the fact that these endophytic bacteria have been linked to detoxification, nitrogen fixation, and photosynthetic activities (Chisholm et al., 2013), the true physiological makeup of these endobiotic siphonous seaweed-bacterial symbioses is still unknown.

Secondary metabolites are biologically active substances that some endophytes create and exude. Seaweeds are a rich source of bioactive substances, which offer significant health benefits. Phenolic chemicals, halogenated compounds, sterols, terpenes, short peptides, polysaccharides, Polyunsaturated Fatty Acids (PUFAs), proteins, vitamins, and minerals are a few of the bioactive components found in seaweed and algal tissues (Rosa et al., 2019).

According to studies by Debbarama et al., (2016), these chemicals exhibit biological activities and have the potential to be used as medications to treat degenerative disorders such as cancer, tumors, thrombosis, diabetes, inflammation, and others. Secondary metabolites can also assist the host plant in combating bacterial, fungal, and other pest diseases. In contrast to primary metabolites, secondary metabolites are not directly engaged in the development, growth, or reproduction of the organisms (Netzker et al., 2015). The marine environment is a rich source of secondary metabolites, which can be used to develop specialized products.

1.2 Aims and Objectives

Aim: To study the effect of seaweed extract on plant growth and microbial activity.

Objectives:

1. Collection of various seaweeds and seaweed extract preparation
2. To test the effect of seaweed extracts on plant growth and its phytochemical analysis.
3. To study the influence of seaweed extract on microbial activity in various soils.

1.3 Hypothesis

Seaweed extract positively influences plant growth by improving microbial activity which must be in turn acting as a biostimulant.

1.4 Scope

The scope of this study was to create a fertiliser that is affordable, natural, safe for the environment, and simple to make and apply as an alternative to chemical fertilizers. A growing number of people are interested in using seaweeds as fertilisers since they are rich in nutrients, trace minerals, and growth hormones that can encourage healthy growth. Research endeavours may concentrate on refining the process of isolating these precious compounds found in seaweeds and broadening their uses in fields such as medicine, biofuels, and bioplastics. Seaweeds have a bright future as a sustainable fertiliser alternative due to their many benefits and adaptability.

CHAPTER 2: LITERATURE REVIEW

2.1 Seaweed as a fertilizer

The world's population is expected to exceed 10 billion people by 2050, and high crop productivity is needed to feed them. Africa is estimated to receive over half of this projected population growth, or an additional 1.3 billion people, on the continent (Waite et al., 2018). The growing consumer desire in products with strong organoleptic, nutritional, and functional qualities presents another significant issue for modern farmers i.e. to improve their crop quality (Benyouseff et al., 2018). Farmers use a wide range of pesticides and excessive amounts of chemical fertilizers, which can negatively impact agricultural systems' ecology and contaminate soils, water sources, and harvested goods in the process of achieving acceptable yields and high-quality products (Oosterveer et al., 2017). It is still very difficult to increase crop yield and quality without having a detrimental impact on the environment.

Moreover, the use of seaweed fertilizers can increase resistance to abiotic stresses such low moisture, excessive salt, and freezing temperatures, which often impede crop development and output. These benefits of stress tolerance seem to be based on physiological changes that the seaweed causes in crops (Jayaraj et al., 2021). These changes include better energy storage, improved root architecture, and increased metabolic potential, all of which increase the plant's capacity to withstand adverse environments. Extracts from *Kappaphycus alvarezzi* have also led to significant decreases in electrolyte leakage, as well as increased production of water content, carotenoid and chlorophyll (Jayaraj et al., 2021). Seaweed improves soil fertility because it is high in phytohormones, humic acids, and micro- and macronutrients. Furthermore, the polysaccharides, proteins, and fatty acids found in seaweed-derived fertilizers help the soil retain moisture and nutrients, which promotes better crop growth (Jhala et al., 2019). Seaweeds have been utilized to increase plant output and stress tolerance since ancient times. Macroalgae in the form of mulch, compost, and extracts, is often used as a source of organic biofertilizer (Brahmbhatt et al., 2017). Additionally, studies have shown that wheat plants

treated with seaweed extracts have accumulated important osmoprotectants such total protein, proline, and other amino acids (Jayaraj et al., 2021).

It is necessary to move to organic fertilizers because the usage of chemical fertilizers has been shown to have produced serious issues, such as soil and water pollution, decreased soil fertility, and a risk to human health (Sopit, 2006). On the other hand, biofertilizers are not only cost-effective but also eco-friendly. Due to its abundance of trace metals (Fe, B, Ca, Cu, Cl, K, Mg, and Mn) and plant growth regulators (PGR), such as auxins, cytokinins, and gibberellins, which can stimulate plant growth and increase plant production, seaweeds have been investigated as a potential - organic fertilizer (Yusuf et al., 2020).

2.2 Soil conditioning

Seaweed fertilizer functions as a soil conditioner, enhancing the physical properties of soil, including water retention and aeration (Zodape, 2001). The soluble alginates and humic acids prove beneficial to clay soils devoid of organic matter and porosity. Alginates decompose and add organic matter to the soil, increasing its fertility. Particularly brown seaweeds, such as *Sargassum*, are well known for their advantageous effects on soil conditioning. This seaweed contains soluble alginates in addition to alginic acid, which catalyzes the bacterial decomposition of organic waste. This process improves the quality of the soil by multiplying the amount of nitrogen-fixing bacteria and supplementing the soil with additional conditioners through their waste products. (Patel et al., 2019).

2.3 Bioremediation of polluted soils

Seaweed adsorbs dangerous contaminants, which is how it works as a bioremediator. The biosorption of heavy metal ions is fueled by functional groups on the algal surface, including phosphate, sulfhydryl, carbonyl amino, ester, and hydroxyl groups (Kumar et al., 2006). Heavy metals like chromium (III) and (IV), mercury (II), lead (II), and cadmium (II) are effectively

removed from their environment by seaweeds such as *Grateloupia lithophila* and *Gracilaria corticata varcartecala* (Ernest et al., 2013). Furthermore, it has been demonstrated that *Ulva* spp. and *Gelidium* spp. accelerate DDT breakdown in contaminated soils and may lower its bioavailability (Singleton et al., 2024). Seaweed has a great deal of potential to act as a bioremediation for contaminated soils, but additional study is required to completely understand the mechanisms underlying this process in the context of agriculture. In certain situations, crops may absorb heavy metals accumulated by seaweed fertilizer, which could have serious health effects on the general people (Lena et al., 2007).

Infertile soils can also be improved and remedied by applying biochar. The organic matter and nutrient content of the soil can be increased by turning seaweed into biochar (Rocky et al., 2015). It seems that different kinds of seaweed produce different nutrients and characteristics. For instance, biochar produced by red seaweeds has a higher acidity and is richer in potassium and sulfur than biochar produced by brown seaweeds. Although this is a relatively new area of study, data from focused seaweed breeding suggests that the product could be biochar that is suitable for a variety of crops and soil types (Rocky et al., 2015). Chandran., et al 2004 examined the impact of seaweed extracts on plant germination, growth, and toxicity levels to native microorganisms present in the soil. The usage of seaweed liquid fertilizer made from three distinct seaweeds and its effects on the germination and development of Solanum *lycopersicum* and *Abelmoschus esculentus* seeds were the main topics of the paper. The findings demonstrated that while greater quantities of seaweed extract hindered germination, particularly in *Solanum lycopersicum*, lower concentrations encouraged germination and growth. The effect of seaweed extracts on microorganisms was also investigated in this article. The study concluded that the seaweed extracts did not affect the proliferation of bacteria, either gram-positive or gram-negative, meaning that they do not harm the native microorganisms in the soil. According to this, seaweed extracts can increase soil fertility without harming microbes and beneficial bacteria (Chandran et al., 2004)

2.4 Integrated pest management

Seaweed can improve crop health and disease resistance when added to soil. A wide range of bioactive substances, such as steroids, terpenes, acetogenins, and polymers generated from amino acids, are found in seaweeds and can react to diseases such as nematode infestation and pests (Patel et al., 2019). Applying seaweed extracts minimizes the number of dangerous pests, such as insects and nematodes. Although using seaweed can lessen the negative consequences of a nematode infestation, using seaweed in conjunction with a chemical nematocide such as carbofuran appears to be the most successful strategy (Mohammad et al., 2011). Furthermore, a few types of seaweed seem to prevent the early development and growth of a number of harmful insects, such as *Sargassum swartzii, Padina pavonica*, and *Caulerpa denticulata* (Ronaldo et al., 2019).

2.5 Soil microbial response to seaweed fertilizer treatment

It has recently been discovered that treatments with seaweed fertilizer cause changes in the bacterial and fungus communities. The functionality and makeup of the soil microbial community are mostly determined by the abiotic and underlying soil health (Kristina et al., 2020). Numerous omics-based and DNA sequencing techniques, together with greenhouse studies, have been utilized to characterize microbial responses on a range of crops to seaweed fertilizer treatment (Wang et al., 2018). The bacteria in tomato plots treated with a *Sargassum horneri* fermented seaweed fertilizer revealed a significant shift in alpha and beta diversity indices between the untreated and 60-day-treated soils, according to deep 16S ribosomal RNA (rRNA) amplicon sequencing. Tomato yields in treated soils increased 1.48–1.83 times as a result of this change in community composition (Wang et al., 2018). While the predominant bacterial phyla did not differ throughout treatment groups, there were variations in the Bacilli class and Micrococcaceae family abundance. Protease, polyphenol oxidase, dehydrogenase, invertase, and urease activities were also elevated in enzyme assays, and this was assumed to

be caused by changes in the microbial community. The nitrogen turnover and quality in soils treated with fertilizer were found to be improved by each of the microbial and enzymatic results mentioned above (Wang et al., 2018).

Ngoroyemoto et al. 2020 treated *Amaranthus hybridus* with both Kelpak and PGPR to examine the interactions between the plant growth-promoting rhizobacteria (PGPR) and the seaweed-derived extract. The effects on plant growth were then assessed. It was discovered that applying Kelpak® and the microorganisms *Bacillus licheniformis* and *Pseudomonas fluorescens* to plants reduced their reactions to stress and enhanced their yield (Johannes et al., 2020). The study that was previously highlighted discusses the implications for crop advantages when seaweed fertilizer is applied to soils in a way that promotes PGPR growth (Johannes et al., 2020).

When comparing apple seedlings treated with seaweed fertilizer to non-treated control, Wang et al., 2018 discovered significant differences in fungal diversity and species richness. Increase in soil quality and enzyme activity in treated soil groups corroborated these findings, supporting the theory that the fertilizer encouraged the growth of fungus species that are helpful to plants. Renaut et al., 2019 investigated the impact of treating pepper and tomato plants in greenhouses with *Ascophyllum nodosum* extract by using fungal internal transcribed spacer (ITS) sequencing and 16S rRNA (Mohamed et al., 2019) where they discovered that the treatment had an impact on the species makeup and community structures of bacteria and fungi. Increase in plant growth and health were also strongly connected with an increase in the abundance of certain amplicon sequence variations (ASVs). Among these ASVs were various uncultured species as well as fungus belonging to the genus *Mortierella* spp. and family Microascaceae. The same study found that a wide variety of bacterial ASVs, including *Bradyrhizobium, Rhizobium, Sphingomonas*, and *Sphingobium*, positively correlated with growth (Mohamed et al., 2019).

2.6 Resistance to plant pathogens

Applying fertilizer made of seaweed may help strengthen a plant's resistance to plant diseases. Ali et al., 2019 investigated the effects of *Ascophyllum nodosum* extract on tomato and sweet pepper crops in greenhouse samples and discovered that it improved plant health and decreased the prevalence of plant diseases. Further research revealed that the pathogens *Alternaria solani* and *Xanthomonas campestris pv. vesicatoria* were reduced as a result of the over-expression of enzymes linked to pathogen defense. Chen et al., 2020 discovered that treating maize rhizospheres with *Ascophyllum nodosum* had a good effect on the community composition. Because the structure of rhizosphere microbial communities can help plants become resistant to soil-borne diseases, this could have serious effects on plant health (Kelly et al., 2021).

Though there has been less research examining the effect of seaweed fertilizer treatment on plant resistance to viral infections, some encouraging findings have been shown (Jayaraj et al., 2021). It has been demonstrated that the polysaccharides found in green, brown, and red seaweeds cause plants to initiate pathogen response pathways, enhancing defenses against bacteria, fungi, and viruses (Vera et al., 2011). Viral defense is specifically caused by the activation of defense enzymes, such as lipoxygenase and phenylalanine ammonia-lyase. It has been demonstrated that ethanolic and aqueous extracts of the brown alga *Durvillaea antarctica* reduce the pathological signs of tobacco mosaic virus (TMV) in tobacco leaves (Hugo et al., 2011). Sulfated fucan oligosaccharides, which are derived from brown algae, have been shown in another investigation on tobacco plants to provide both systemic and locally acquired resistance to TMV (Bernard et al., 2003). The aforementioned findings support the notion that applying seaweed fertilizers can benefit agricultural crops broadly and increase their resilience to plant diseases caused by bacteria, fungi, and viruses.

CHAPTER 3: METHODOLOGY

3. Materials and Methods

3.1 Collection of Seaweeds

The seaweeds were collected by hand at low tide from the coastal areas of Goa. The different sampling sites were Anjuna (15°34'24.06"N,73°44'27.52"E) and Bogmalo (15°22'11.29"N, 73°50'1.09"E) (Fig.2). The collected samples were washed thoroughly with seawater to remove sand particles, pebbles, impurities, and epiphytes. Samples were transported to the laboratory in sterile plastic bags placed in an ice box. It was then washed with tap water to remove surface salt and shadow-dried and identified using an identification chart (www.seaweeds.uib.no/key/, Seaweeds identification key). Depending on the quantity of different types of seaweeds collected, most abundantly found seaweeds were selected, air-dried for 4-5 hours, and then oven-dried for 72 h at 60 °C. The dried sample was then powdered using a mortar and pestle and stored in air-tight glass containers for further use.

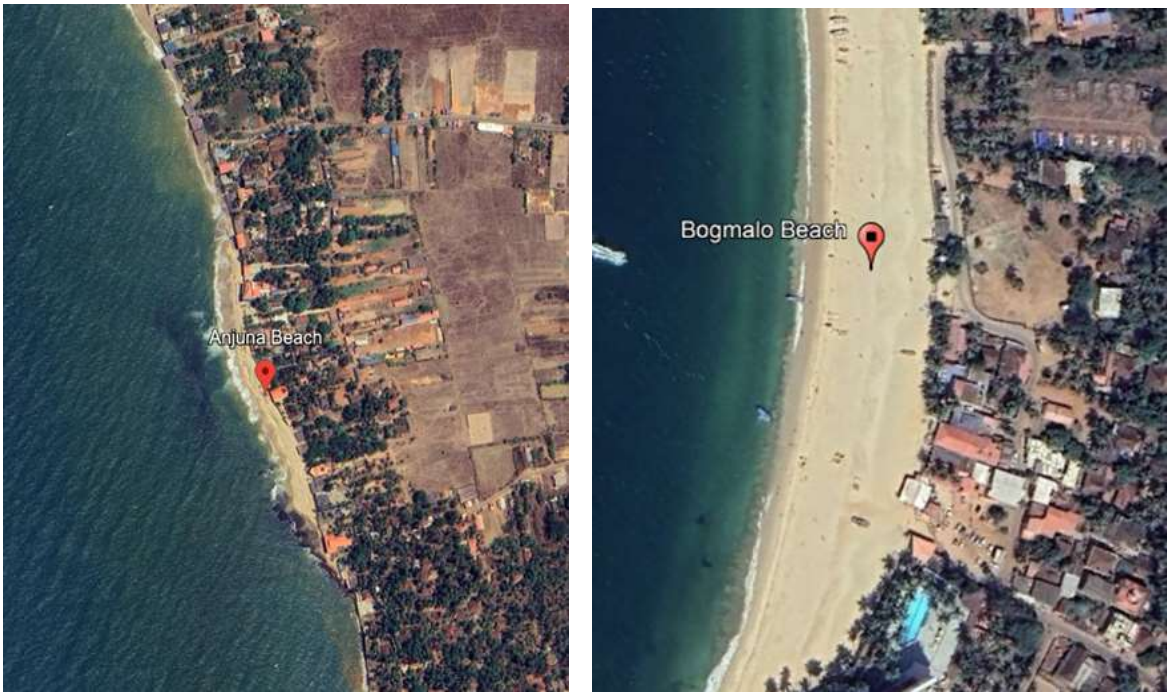


Fig 3.1: Sampling sites for collection of seaweeds (Google images)

3.2 Preparation of Seaweed Liquid Fertilizer (SLF)

Three distinct techniques were used to make the seaweed liquid fertilizers like boiling method, low-temperature method, and cold-water method (Indumathi et al., 2016). For the boiling

method, 10 g of seaweed powder and 100 ml of distilled water were mixed and boiled to 100ºC for an hour. The same volume of mixture was heated for 24 hours at a temperature below 60ºC for the low-temperature approach. For the cold water extraction method , 10 g of powder and 100 ml of distilled water were vortexed for thorough mixing, then allowed to sit at room temperature for a whole day. The filter paper was then used to filter the contents. The collected filtrate was stored in the refrigerator (4ºC). The filtrate that was obtained was regarded as 100%. Using this 100% extract, five distinct concentrations of solutions, including 0.1%, 0.5%, and 1%, were made and used for further study. The boiling method extract was named hot seaweed extract (HSE), low temperature method extract as low temperature seaweed extract (LSE) and cold water extraction method extract as cold seaweed extract (CSE).

3.3 Phytochemical Analysis

The phytochemical analysis of seaweed extracts, including methanol, ethanol, acetone and the aqueous: cold, hot, and low-temperature extracts, was assessed to confirm the presence of biomolecules using the standard method as described by Savithramma et. al. (2011). The primary natural chemical groups, including alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones, and glycosides, were identified by phytochemical screening. The general reactions in these studies indicated whether these chemicals were present in the examined algal extracts or not which is known to indirectly influence the growth and yield of any plant on application of the extracts.

3.4 Seed Germination Test

Mung (*Vigna radiata l.*), chilly (*Capsicum annuum*) and finger millet (*Eleusine coracana*) seeds were chosen for seed germination and subsequent seedling growth. Healthy seeds free from visible infection of uniform size were selected for this study. The seeds were surface sterilized with 5% sodium hypochlorite and then were rinsed three times with tap water. Ten

seeds each of mung, chilly and finger millet were selected and soaked with different concentrations of HSE, LSE and CSE such as 0.1,0.5 and 1% of the respective seaweed species overnight. Control was also maintained with 10 seeds soaked using tap water. Germination was carried out on sterile moistened absorbent cotton and incubated at 28 ± 2ºC for 7 days. The number of seeds germinated was counted after 24 and 48 hours. Germination percentage, speed, and the mean germination time were further calculated using the following formula:

Germination (%) = (Number of seeds germinated/Total number of seeds in Petri plate) × 100

The foliage count and root and shoot length were also analyzed on day 7 and the Vigour Index was calculated using the following formula:

Vigour Index = (Average root length + Average hypocotyl length) × germination percentage.

3.5 Selection of seed and seaweed liquid extract for further analysis

Among the three different extracts and seedlings, the one that gave the best results in the above study was selected for further analysis.

3.6 Effect of seaweed extract on plant growth and microbial activity in soil

To check the effect of seaweed extract on the microbial activity in soil a pot-level study was done in which three different types of soil were used such as Agricultural field soil (AFS) (15°27'27.83"N,74° 0'13.92"E), Garden soil (GS) (15°27'37.59"N, 73°49'58.08"E), and Mining soil (MS) (15°33'15.63"N,74° 0'53.63"E). The soil samples were put in clean 500 mL plastic containers and labeled accordingly. A set of control soil samples was also maintained. Two different sets were made before putting the seeds in the soil samples, one in which the seeds were subjected to seed treatment (seeds soaked overnight in 5 ml of extract and further germinated on sterile moistened absorbent cotton) and the other without seed treatment. For the seed treatment set, control was maintained by using tap water. After putting the seeds in the soil 5 ml of extract was sprayed on each soil sample while tap water was sprayed on the control

soil samples. Soil samples were collected in clean sampling plastic bags and serially diluted till 10^{-2} (except for mining soil for which 10^{0} dilution was preferred) and 100 µL of suspension was then plated out in triplicates on four different types of media: Yeast Mannitol Agar (YMA – for check for nitrate reducers), Pikovaskya's Agar (PA - phosphate solubilizers), Sulfate API medium (sulfate reducers) and Nutrient Agar (NA) to estimate Viable count of the soil samples. The plates were incubated at room temperature and checked for growth after 24 hours. The total number of colonies in each plate was counted and the average viable count was calculated. This was considered as the day 0 estimation of microbial activity in the soil. This procedure was repeated after 7 days. For the second set, without the seed treatment, the seeds were directly put into the soil sample, and the same method was further followed for day 0 and day 7 respectively.

CHAPTER 4: ANALYSIS AND CONCLUSIONS

4. Analysis

4.1 Collection of Seaweeds

A total of 8 samples of seaweed were collected (Fig.2-6). Some of the samples were identified with the help of an identification chart (www.seaweeds.uib.no/key/, Seaweeds identification key).

Fig 4.1: ***Ulva intestinalis***

Fig 4.2: ***Padina tetrasomatica***

Fig 4.3: ***Dictyota dichotoma***

Fig 4.4: ***Sargassum sp.***

Fig 4.5: Different types of ***Sargassum species***

4.2 Preparation of Seaweed Liquid Fertilizer (SLF)

For the present study 3 algal species one green, *Ulva intestinalis,* and two brown, *Sargassum sp.* seaweeds collected in bulk were selected and further processed for the preparation of SLF (Fig.8).

a.  **b.**  **c.** 

Fig 4.6: SLF of *Ulva intestinalis* (a.), *Sargassum sp. I* (b.) and *Sargassum sp. II* (c.) along with processed seaweed powder stored in air tight container.

4.3 Phytochemical Analysis

In the preliminary phytochemical screening of ten different chemical compounds (alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones, and glycosides) were tested in four different extracts (Table No. 1-3). Among the aqueous extracts, it was observed that terpenoids and steroids showed positive results in all extracts. Quinones were exclusive to extracts of *Ulva intestinalis* and the presence of Saponins was noted only in the hot extract of *Ulva intestinalis*, *Sargassum sp. I* and *Sargassum sp. II*. For the methanol, ethanol, and acetone extracts, it was noted that terpenoids and steroids were found to be present in all extracts. Meanwhile, the presence of quinones was found only in the acetone extracts of *Ulva intestinalis*, *Sargassum sp. I* and *Sargassum sp. II* respectively. But in a study by Deyab et al., methanol and acetone extracts of *Dictyota dichotoma* showed the presence of alkaloids, terpenoids, steroids, tannins, flavonoids, phenols, coumarins, quinones, and glycosides. This could be because different species of seaweed might contain different types of phytochemicals. Another study has also shown that hexane could effectively extract flavonoids from *Kappaphycus alvarezii*, a member of red seaweed, followed by chloroform, ethyl acetate and methanol (Lalopua et al., 2011), which is in contrast to the findings from my study. The presence or absence of phytochemicals also depends on the solvent medium used for extraction and since some of the solvents used were different it could be one of the reasons for the absence of certain phytochemicals.

Table No 4.1: Qualitative analyses of phytochemical substances in different extracts of *Ulva intestinalis*

Sr No.	Phytochemical parameters	Methanol	Ethanol	Acetone	Aqueous		
					CSE	LSE	HSE
1	Alkaloids	-	-	-	-	-	-
2	Terpenoids	+	+	+	+	+	+
3	Steroids	+	+	+	+	+	+
4	Tannins	-	-	-	-	-	-
5	Saponins	-	-	-	-	-	+
6	Flavonoids	-	-	-	-	-	-
7	Phenols	-	-	-	-	-	-
8	Coumarins	-	-	-	-	-	-
9	Quinones	-	-	+	+	+	+
10	Glycosides	-	-	-	-	-	-

Table No 4.2: Qualitative analysis of phytochemical substances in different extracts of *Sargassum sp.* I

Sr No.	Phytochemical parameters	Methanol	Ethanol	Acetone	Aqueous		
					CSE	LSE	HSE
1	Alkaloids	-	-	-	-	-	-
2	Terpenoids	+	+	+	+	+	+
3	Steroids	+	+	+	+	+	+
4	Tannins	-	-	-	-	-	-
5	Saponins	-	-	-	-	-	+
6	Flavonoids	-	-	-	-	-	-
7	Phenols	-	-	-	-	-	-
8	Coumarins	-	-	-	-	-	-
9	Quinones	-	-	+	-	-	-
10	Glycosides	-	-	-	-	-	-

Table No 4.3: Qualitative analyses of phytochemical substances in different extracts of *Sargassum sp.* II

Sr No.	Phytochemical parameters	Methanol	Ethanol	Acetone	Aqueous		
					CSE	LSE	HSE
1	Alkaloids	-	-	-	-	-	-
2	Terpenoids	+	+	+	+	+	+
3	Steroids	+	+	+	+	+	+
4	Tannins	-	-	-	-	-	-
5	Saponins	-	-	-	-	-	+
6	Flavonoids	-	-	-	-	-	-
7	Phenols	-	-	-	-	-	-
8	Coumarins	-	-	-	-	-	-
9	Quinones	-	-	+	-	-	-
10	Glycosides	-	-	-	-	-	-

4.4 Seed Germination Test

The selected seeds were first subjected to seed treatment (Fig.9). In *Vigna radiata l.*, 100% Seed Germination was found on Day 1 and Day 2 when subjected to 1% and 0.5% CSE of *Ulva intestinalis* as compared with other concentrations as well as control it was high (Table No.4). In *Eleusine coracana*, seed germination was found to be only 10% from day 5 onwards when subjected to 1% CSE and 10 % on Day 7 when subjected to 0.5% CSE (Table No.5) (Fig.11). Whereas, no seed germination was found in *Capsicum annuum* till the end of the incubation period. Since *Capsicum annuum* and *Eleusine coracana* seeds showed low germination percentage and negative results of seed germination, only *Vigna radiata l.* seeds were used further to test the effect of other seaweed species extract on seed germination.

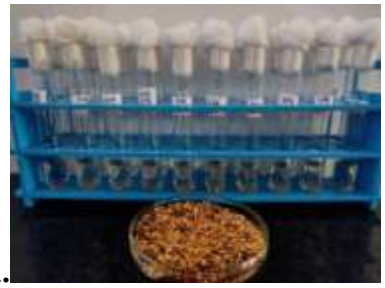
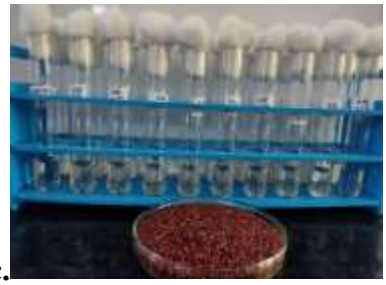
a.  **b.**  **c.** 

Fig 4.1: a. *Capsicum annuum* seeds b. *Vigna radiata l.* seeds and c. *Eleusine coracana* seeds subjected to seed treatment for 24 hours at different concentrations of *Ulva intestinalis* SLF

Table No 4.4: Effect of *Ulva intestinalis* SLF on seed germination of *Vigna radiata l.* seeds

SLF	Conc. of SLF (%)	% of Seeds Germinated					Average %
		D1	D2	D3	D4	D5	
HSE	0.1	90	90	90	100	100	94
	0.5	60	90	90	100	100	88
	1	80	90	100	100	100	94
CSE	0.1	70	90	100	100	100	92
	0.5	90	100	100	100	100	98
	1	100	100	100	100	100	100
LSE	0.1	80	90	90	90	100	90
	0.5	80	90	90	90	100	90
	1	90	100	100	100	100	98
Control (tap water)	-	60	80	90	90	90	82

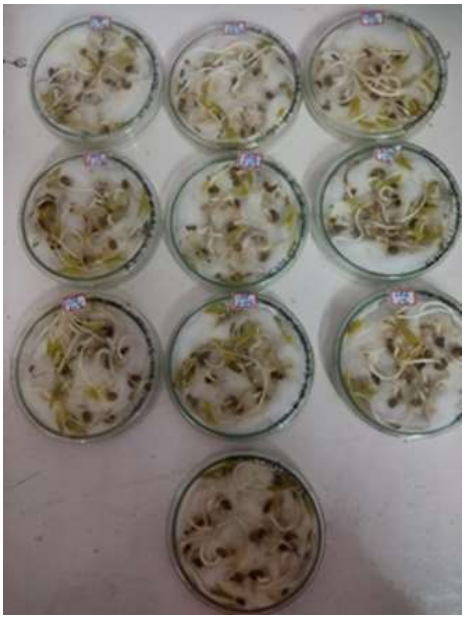
a.  b. 

Fig 4.2: Effect of *Ulva intestinalis* SLF on seed germination of *Vigna radiata l. seeds* a. Day 1, b. Day 7.

Table No 4.5: Effect of *Ulva intestinalis* SLF on seed germination of *Eleusine coracana* seeds

SLF	Conc. of SLF (%)	% of Seeds Germinated										Average %
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	
HSE	0.1	0	0	0	0	0	0	0	0	0	0	0
	0.5	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0
CSE	0.1	0	0	0	0	0	0	0	0	0	0	0
	0.5	0	0	0	0	0	0	10	10	10	10	30
	1	0	0	0	0	10	10	10	10	10	10	60
LSE	0.1	0	0	0	0	0	0	0	0	0	0	0
	0.5	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0
Control (tap water)	-	0	0	0	0	0	0	0	0	0	0	0

Fig 4.3: Effect of *Ulva intestinalis* SLF on seed germination of finger millet seeds a. Day 1, b. Day 10 and c. positive results obtained only at CSE 0.5% and 1% (red circle).

The highest foliage count was obtained at CSE 1% and 0.5% on Day 7 whereas the lowest foliage count was obtained at LSE 0.1% on Day 3. The HSE had a negative effect on the foliage count resulting in blackened shoots and leaves. (Fig No.5). This may be the result of the HSE's lack of microbes due to its preparation at 100ºC, which destroyed them. On the other hand, less heating is required for the CSE and LSE extracts, allowing the microorganisms to thrive and potentially contribute to increased leaf count and overall development.

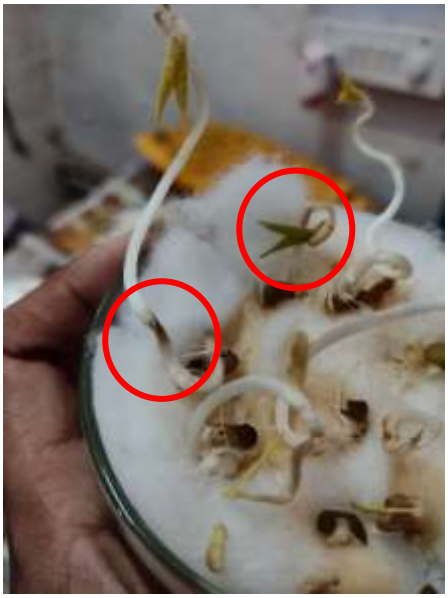


Fig 4.4: Negative effect of HSE resulting in blackened shoots and leaves

Table No 4.6: Effect of *Ulva intestinalis* SLF on foliage count of *Vigna radiata l.* seeds

SLF	Conc. of SLF %	Number of Days						
		D1	D2	D3	D4	D5	D6	D7
CSE	0.1%	0	0	5	8	9	9	9
	0.5%	0	0	5	6	7	8	10
	1%	0	0	3	4	9	9	10
LSE	0.1%	0	0	3	8	9	9	10
	0.5%	0	0	3	5	7	8	9
	1%	0	0	4	6	8	8	8
HSE	0.1%	0	0	4	5	6	7	10
	0.5%	0	0	4	6	8	8	10
	1%	0	0	5	7	8	8	9
Control(dw)	-	0	0	6	7	7	8	9

CSE 0.1, 0.5 and 1% showed maximum shoot length in comparison to the other extract concentrations whereas root length was found to be maximum at CSE 0.5 and 1% and even at HSE 1%. (Table No.6-7). Carbohydrates, proteins and auxin hormones present in the seaweed may have helped in root development after being subjected to seed treatment (Fig.13).

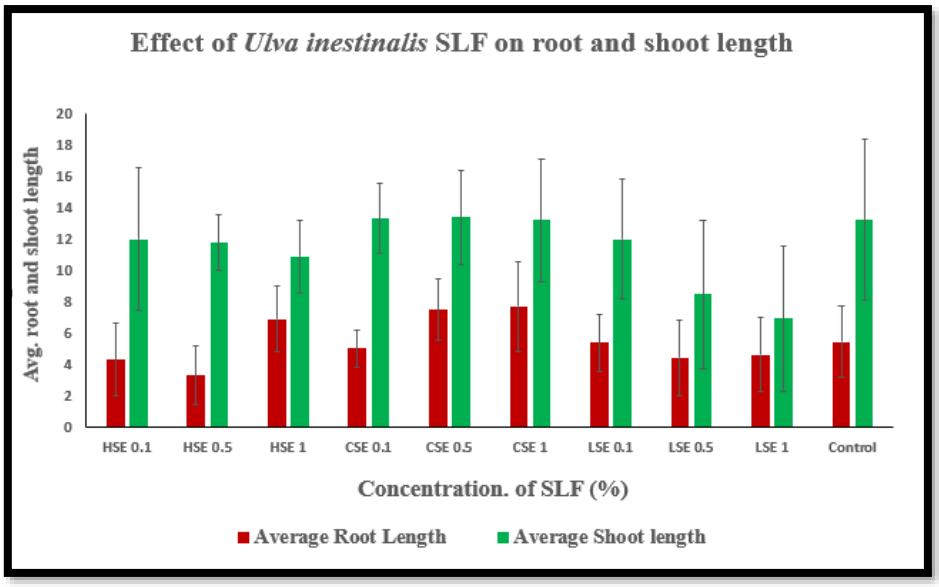


Fig 4.5: Bar graph depicting the root and shoot length of *Vigna radiata l.* seeds.

In *Ulva intestinalis* the Vigour index was highest at CSE 1% and lowest at LSE 1%,

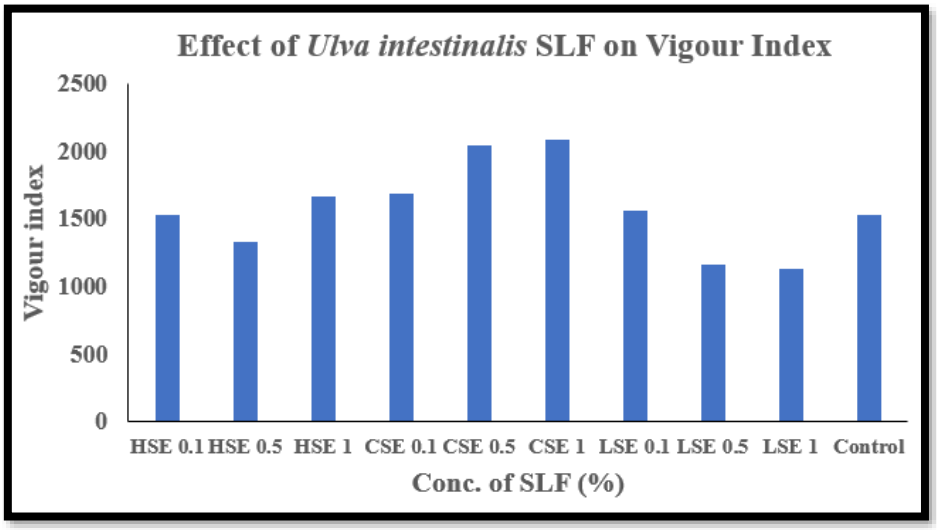


Fig 4.6: Bar graph depicting the effect of *Ulva intestinalis* SLF on Vigour Index

In *Sargassum sp.* I seed germination percentage was found highest at CSE 0.5 and 1% (Table No.10).

Table No 4.7: Effect of *Sargassum sp.* I SLF on seed germination of *Vigna radiata l.* seeds

SLF	Conc. of SLF (%)	% of Seeds Germinated							Average %
		D1	D2	D3	D4	D5	D6	D7	
CSE	0.1	0	70	80	80	80	100	100	78.57
	0.5	0	60	70	80	90	100	100	82.85
	1	10	20	40	70	80	90	100	82.85
LSE	0.1	30	50	90	90	90	100	100	67.1
	0.5	50	60	90	90	90	100	100	50
	1	50	80	80	90	100	100	100	62.85
HSE	0.1	10	50	70	70	80	90	100	72.85
	0.5	10	30	40	40	40	90	100	71.42
	1	20	30	40	70	80	90	100	58.57
Control (tap water)	-	0	70	80	90	100	100	100	77.1

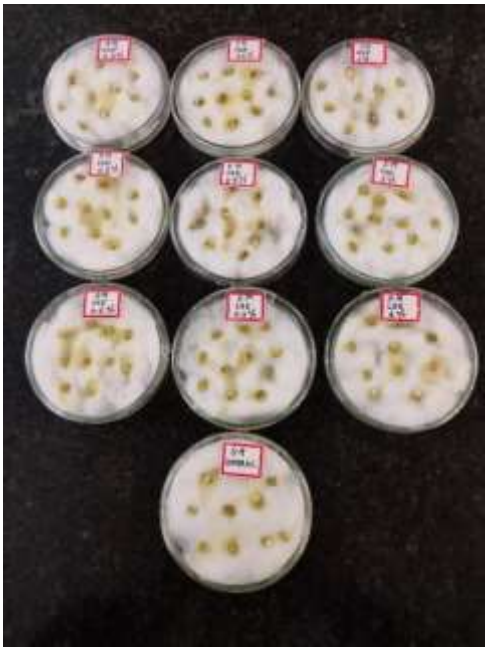
a.  **b.** 

Fig 4.7: Effect of *Sargassum sp.* I on seed germination of *Vigna radiata l.* seeds. a. Day 1 and b. Day 2

The highest foliage count was obtained at CSE 1% whereas the lowest foliage count was obtained at LSE 0.5%, followed by LSE 0.1% and HSE 1% on Day 7 (Table No.11).

Table No 4.8: Effect of *Sargassum sp.* I SLF on foliage count of *Vigna radiata l.* seeds

SLF	Conc. of SLF %	Number of Days						
		D1	D2	D3	D4	D5	D6	D7
CSE	0.1%	0	0	3	3	3	7	8
	0.5%	0	0	2	2	3	6	7
	1%	0	0	1	3	4	5	9
LSE	0.1%	0	0	2	3	4	4	4
	0.5%	0	0	0	0	0	0	0
	1%	0	0	2	3	4	4	5
HSE	0.1%	0	0	6	7	8	8	8
	0.5%	0	0	6	6	7	7	7
	1%	0	0	1	1	2	3	4
Control(dw)	-	0	0	3	4	4	4	5

CSE 0.5 and 1% showed maximum shoot and root length respectively in comparison to the other extract concentrations (Fig.16).

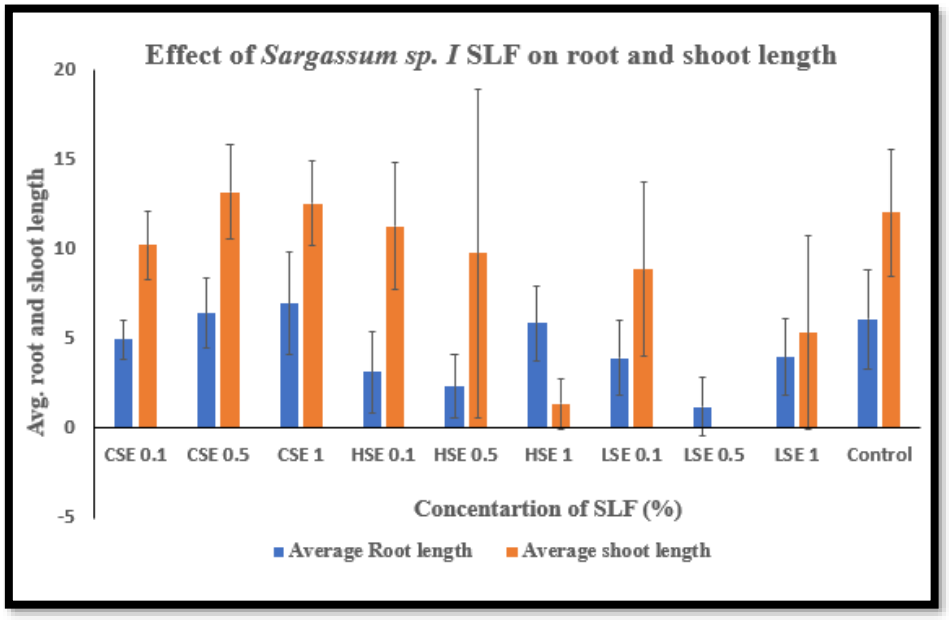


Fig 4.8: Bar graph depicting the effect of *Sargassum sp. I* SLF on shoot and root length of *Vigna radiata l.* seeds

Seed germination results and vigour index were obtained for *Sargassum sp.* I (Fig.17).

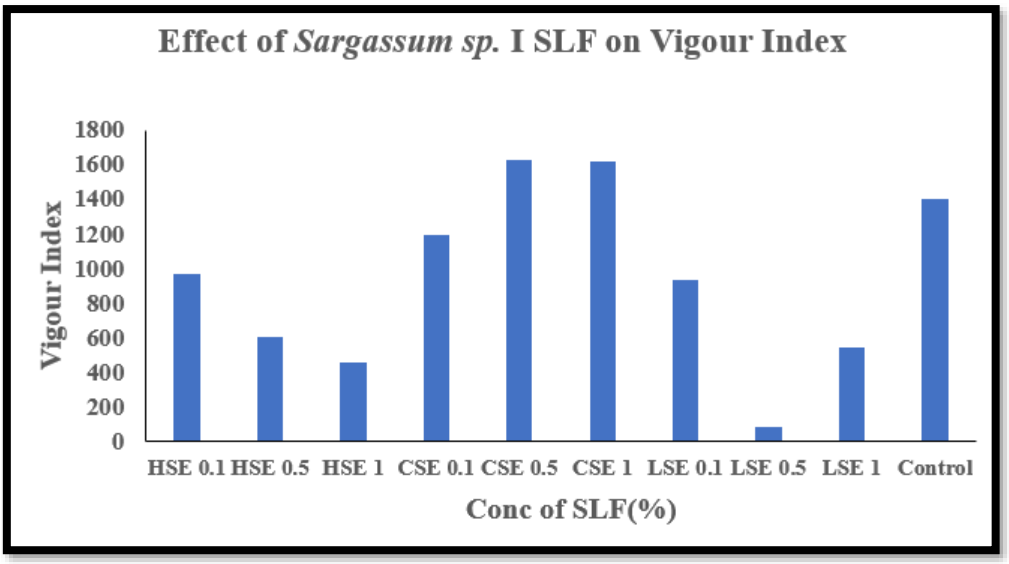


Fig 4.9: Bar graph depicting the effect of *Sargassum sp. I* SLF on Vigour Index

In *Sargassum sp.* II the seed germination percentage was found highest at CSE 1% (Table No.14). Meanwhile in *Sargassum sp. I* and *II* the vigour index was found to be maximum at CSE 1 and 0.5% and least at LSE 0.5 and 1 % respectively.

Table No 4.9: Effect of *Sargassum sp.* II SLF on seed germination of *Vigna radiata l.* seeds

SLF	Conc. of SLF (%)	% of Seeds Germinated							Average %
		D1	D2	D3	D4	D5	D6	D7	
HSE	0.1	50	50	50	60	70	80	90	64.28
	0.5	40	90	90	90	90	90	100	84.28
	1	10	30	30	30	30	90	90	44.28
CSE	0.1	30	60	90	90	100	100	100	81.42
	0.5	40	60	90	90	100	100	100	82.57
	1	60	80	90	90	90	100	100	87.1
LSE	0.1	50	80	80	80	90	90	90	80
	0.5	30	30	30	30	30	30	30	30
	1	0	0	0	0	0	0	0	0
Control (tap water)	-	20	60	60	70	80	90	90	67.14

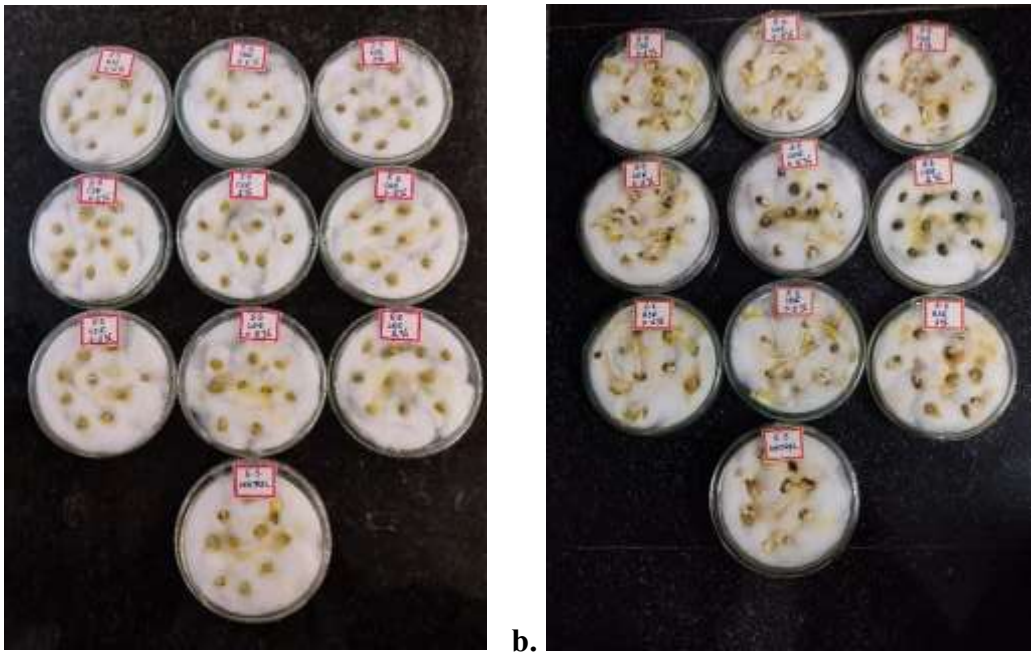


Fig 4.10: Effect of *Sargassum sp.* II on seed germination of *Vigna radiata l.* seeds. a. Day 1 and b. Day 2

Foliage count was highest at CSE 0.1,0.5 and 1% whereas lowest at LSE 0.1% and 0 at HSE 1%, LSE 0.5,1% and control (Table No.15). The CSE might be promoting the biosynthesis of chlorophyll and minimize its breakdown, resulting in an increase in the number of leaves (Kulkarni et al., 2020)

Table No 4.10: Effect of *Sargassum sp.* II SLF on foliage count of *Vigna radiata l.* seeds

SLF	Conc. of SLF %	Number of Days						
		D1	D2	D3	D4	D5	D6	D7
CSE	0.1%	0	0	5	6	7	8	9
	0.5%	0	0	1	3	4	7	9
	1%	0	0	1	2	3	8	9
LSE	0.1%	0	0	3	3	4	4	4
	0.5%	0	0	0	0	0	0	0
	1%	0	0	0	0	0	0	0
HSE	0.1%	0	0	4	4	4	4	5
	0.5%	0	0	3	6	7	7	8
	1%	0	0	0	0	0	0	0
Control(dw)	-	0	0	0	0	0	0	0

The maximum shoot and root length was obtained at HSE 0.5 and 1% respectively (Fig.19).

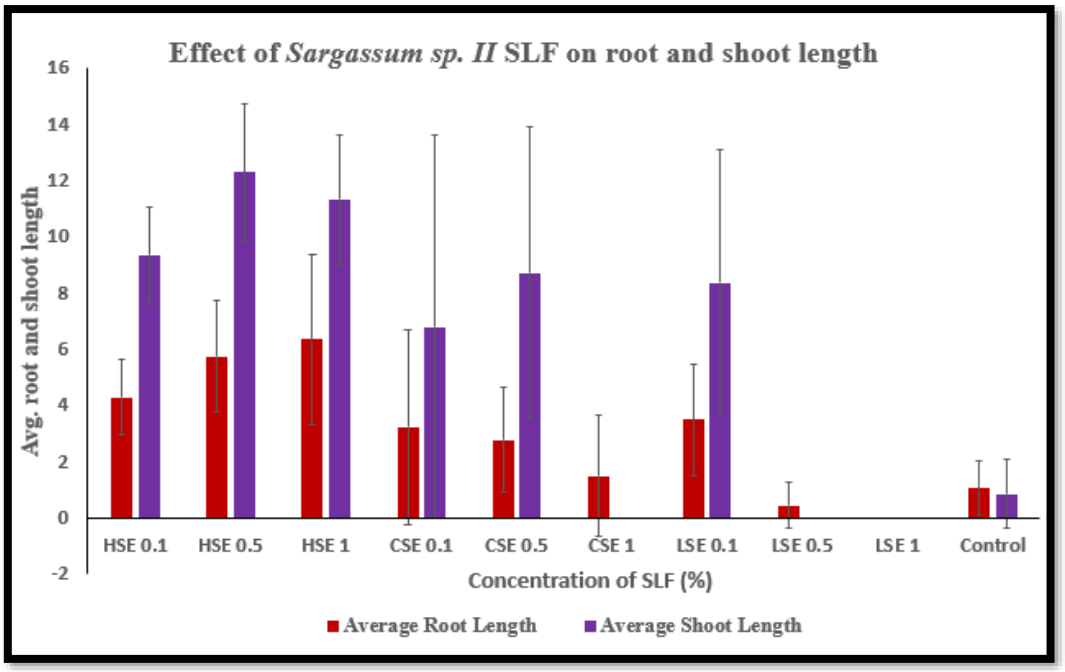


Fig 4.11: Graph depicting the effect of *Sargassum sp. II* SLF on shoot and root length of *Vigna radiata l.* seeds.

Seed germination results and vigour index were obtained for *Sargassum* II (Table No.14) (Fig.20).

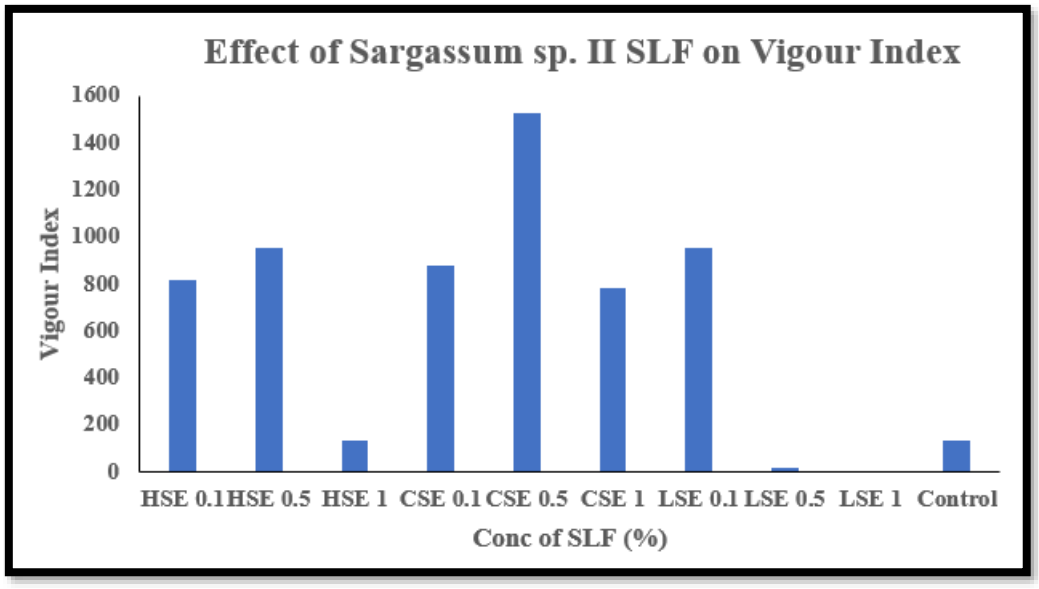


Fig 4.12: Graph depicting the effect of *Sargassum sp. II* on Vigour Index

4.5 Selection of seaweed liquid extract for further analysis

Based on the results obtained from various experiments mentioned above, it was noted that CSE gave the best results for seed germination, foliage count, root/shoot length as well as vigour index, therefore it was chosen from for conducting further experiments. Amongst the three seaweed extracts, performance of *Ulva intestinalis* and Sargassum sp I were chosen for further analysis. Whereas in a study by Sasikala et al., 2016 the extract was chosen based on phytochemical, biochemical and FI-IR analysis.

4.6 Effect of *Ulva intestinalis* SLF on plant growth

Plant growth was maximal in AFS and non-existent in MS and MSC in the absence of seed treatment. (Fig.21-22) However, after seed treatment, some plant development was observed in MS and MSC, suggesting that the application of SLF to the seeds has a good effect on seed germination and promotes plant growth. The growth of plants observed after seed treatment with SLF demonstrates that even infertile and polluted soil can be remediated with further SLF application as MS is a significantly polluted soil that does not support plant growth due to the presence of heavy metal pollution. The microorganisms present in the SLF may also be involved in the promotion of tolerance of heavy metals by transporting them across the cell membrane, accumulation on cell walls (intra and extracellular), redox reactions, and production of complexes (Koza et al., 2022)

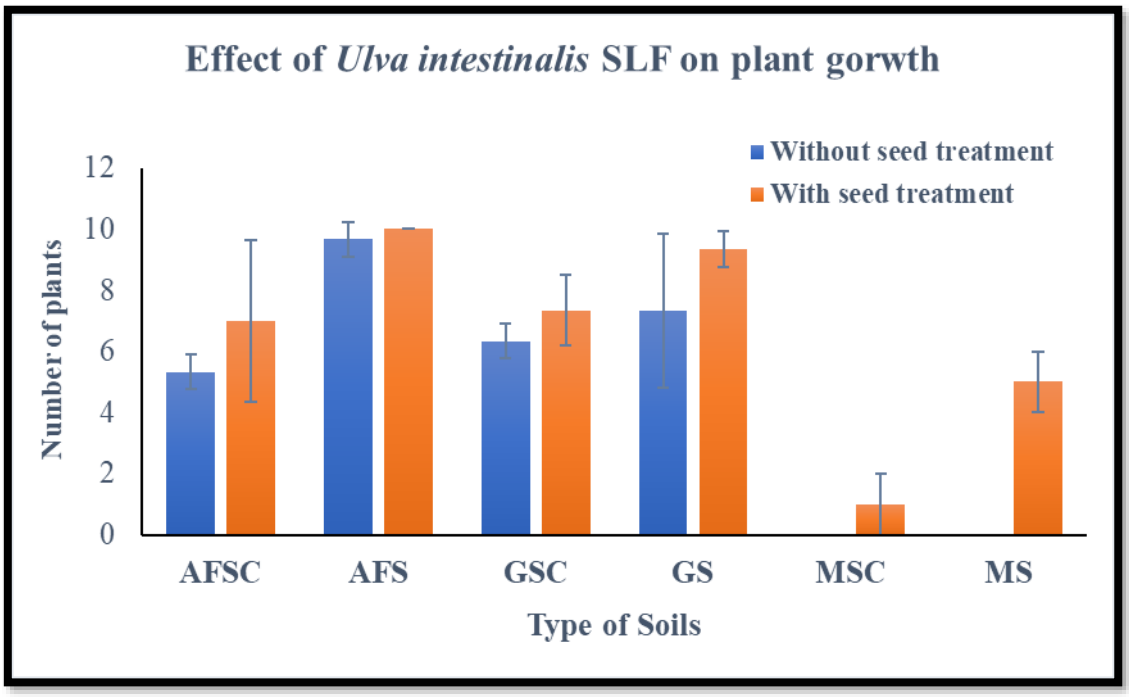


Fig 4.13: Graph depicting the number of plants grown in different soils with/without *Ulva intestinalis* SLF seed treatment.

Fig 4.14: Pot study to check growth of *Vigna radiata l.* subjected to *Ulva intestinalis* SLF [(a) Control AFS without ST; (b) AFS with ST; (c) GS without ST; (d) GS with ST; (e) MS without ST and (f) MS with ST]

4.7 Effect of *Ulva intestinalis* SLF on microbial activity

The number of microorganisms without and with seed treatment on Day 0 was found to be less in number as compared to Day 7 (Fig.23-24).

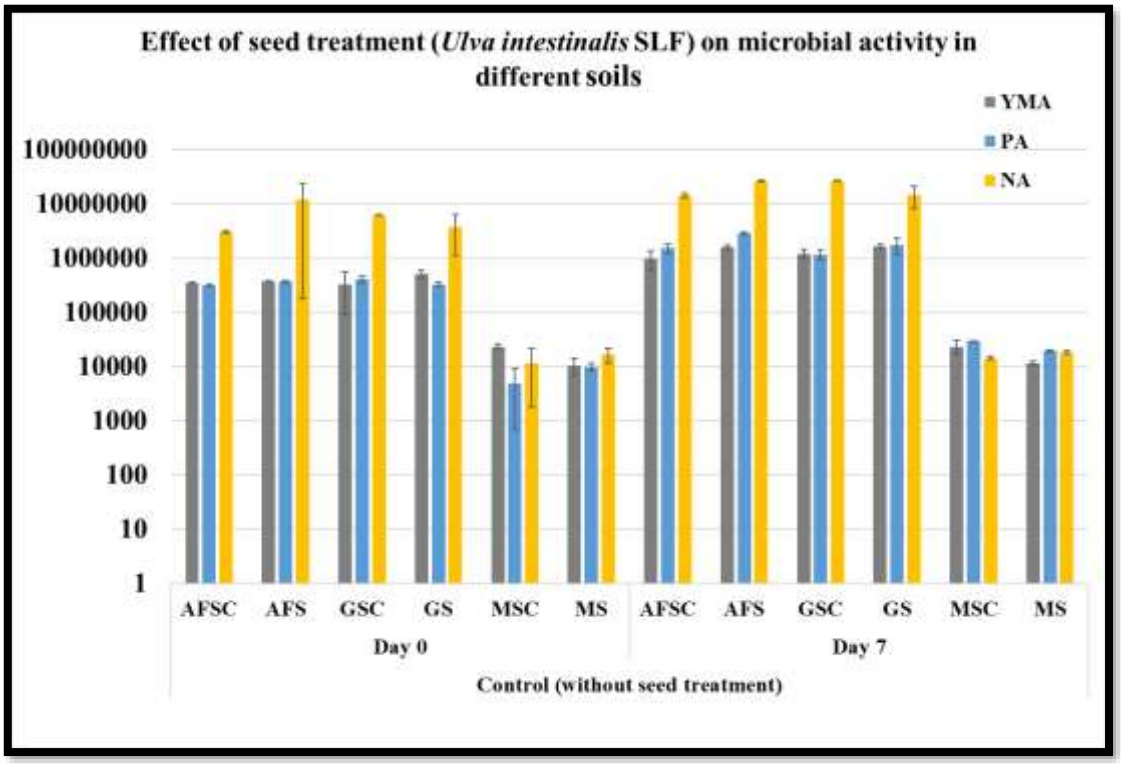


Fig 4.15: Effect *Ulva intestinalis* SLF on microbial activity in different soils without seed treatment.

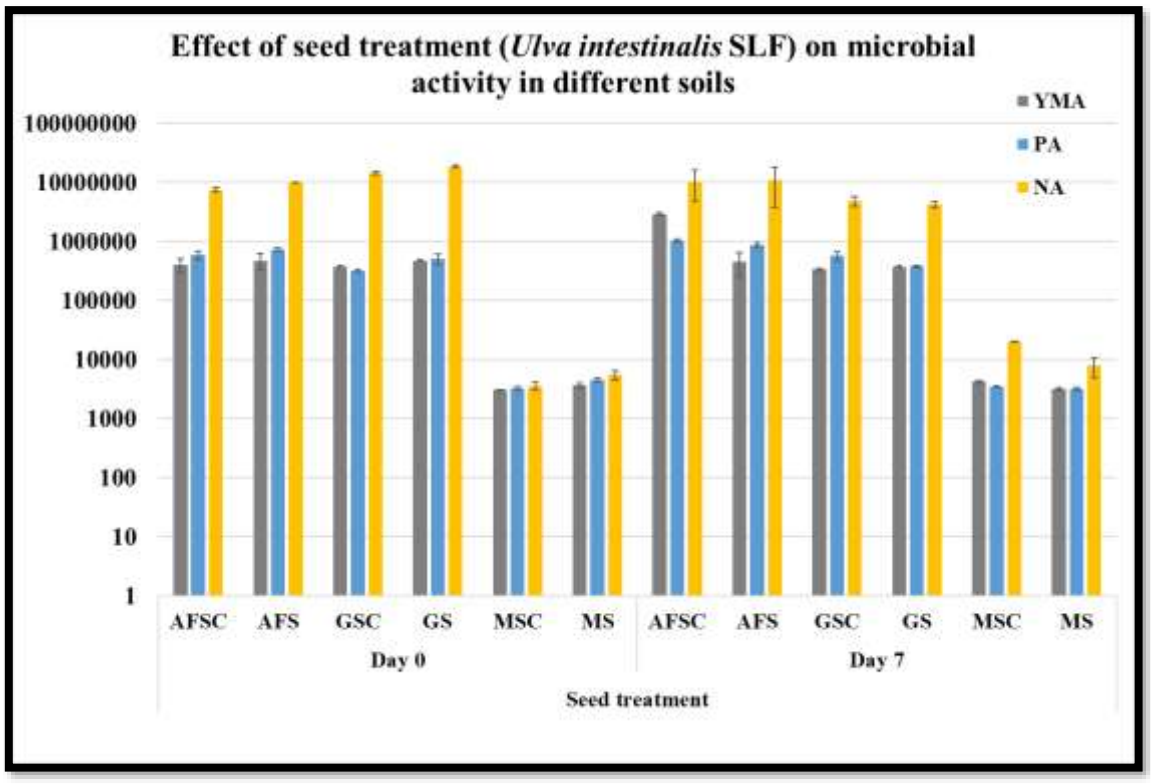


Fig 4.16: Effect *Ulva intestinalis* SLF on microbial activity in different soils with seed treatment.

This may indicate that the increase in the number of beneficiary microorganisms is due to the development of the plants since microorganisms have the potential to improve plant growth under abiotic stress conditions by promoting the production of low-molecular-weight osmolytes, such as glycinebetaine, proline, and other amino acids, mineral phosphate solubilization, nitrogen fixation, organic acids, and producing key enzymes such as ACC-deaminase, chitinase and glucanase (Sharma et al., 2018).

4.8 Effect of *Sargassum sp.* I SLF on plant growth

The greatest plant development was observed in AFS without seed treatment, while the least amount was observed in MSC . It was observed that *Sargassum sp.* I SLF exhibited superior growth in comparison to *Ulva intestinalis* SLF, since growth was observed in MS and MSC even in the absence of seed treatment, while *Ulva intestinalis* SLF showed no growth at all. This may suggest that Sargassum Sp. I SLF is superior to Ulva intestinalis SLF in pot level studies used to monitor *Vigna l.radiata* seed growth.

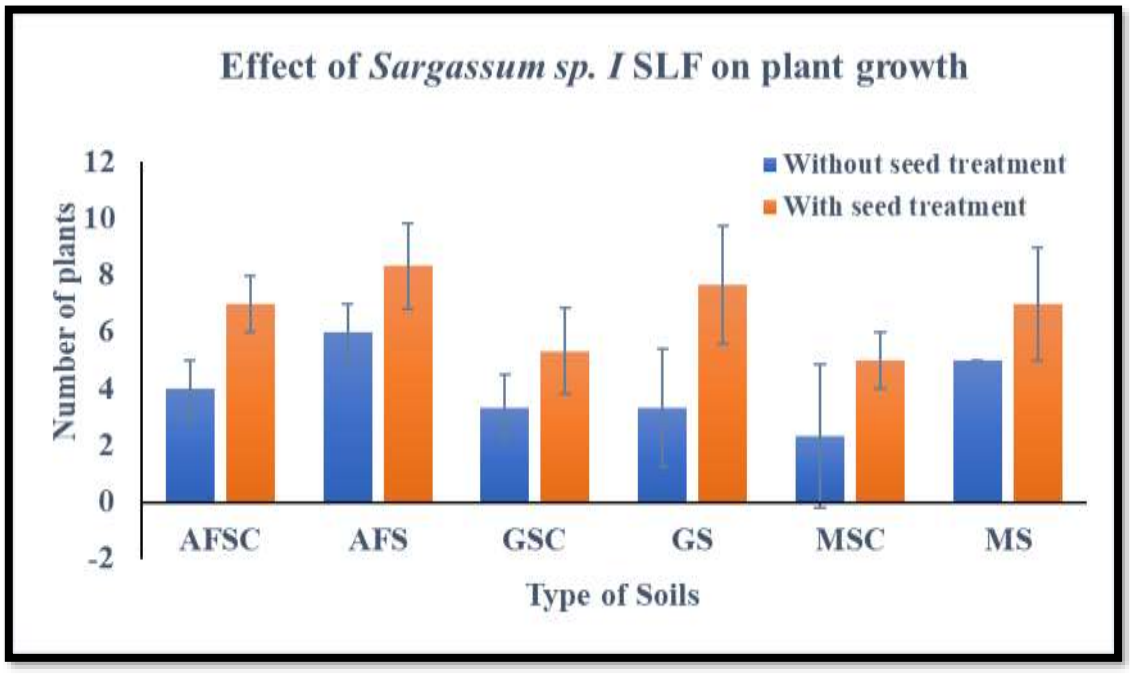


Fig 4.17: Graph depicting the number of plants grown in different soils with/without *Sargassum sp* SLF seed treatment.

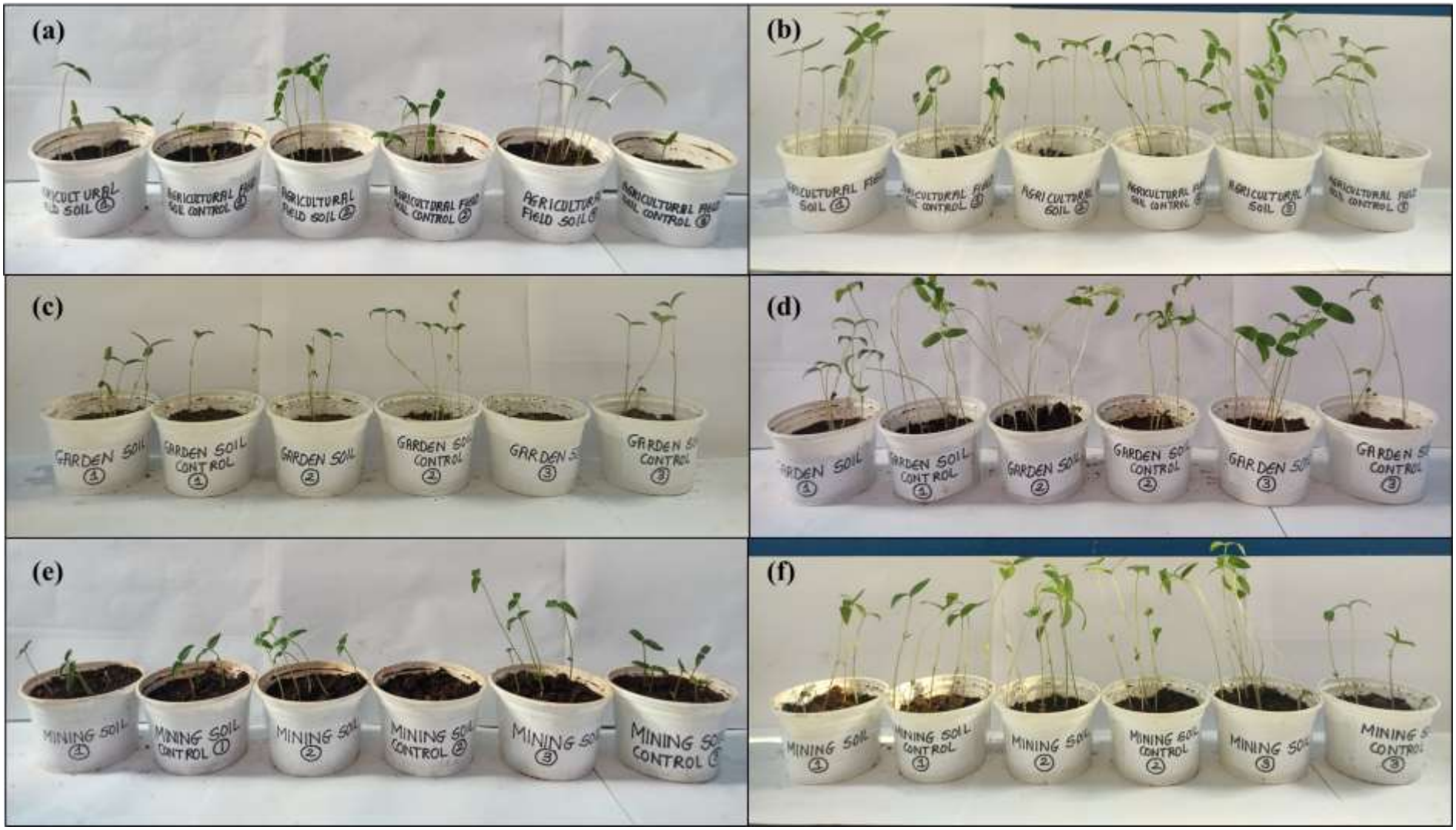


Fig 4.18: Pot study to check the growth of *Vigna radiata l.* subjected to *Sargassum sp. I* SLF [(a) Control AFS without ST; (b) AFS with ST; (c) GS without ST; (d) GS with ST; (e) MS without ST and (f) MS with ST]

4.9 Effect of *Sargassum sp* I SLF on microbial activity

On Day 0 compared to Day 7, there were less microorganisms in the set without seed treatment (Fig. 27).

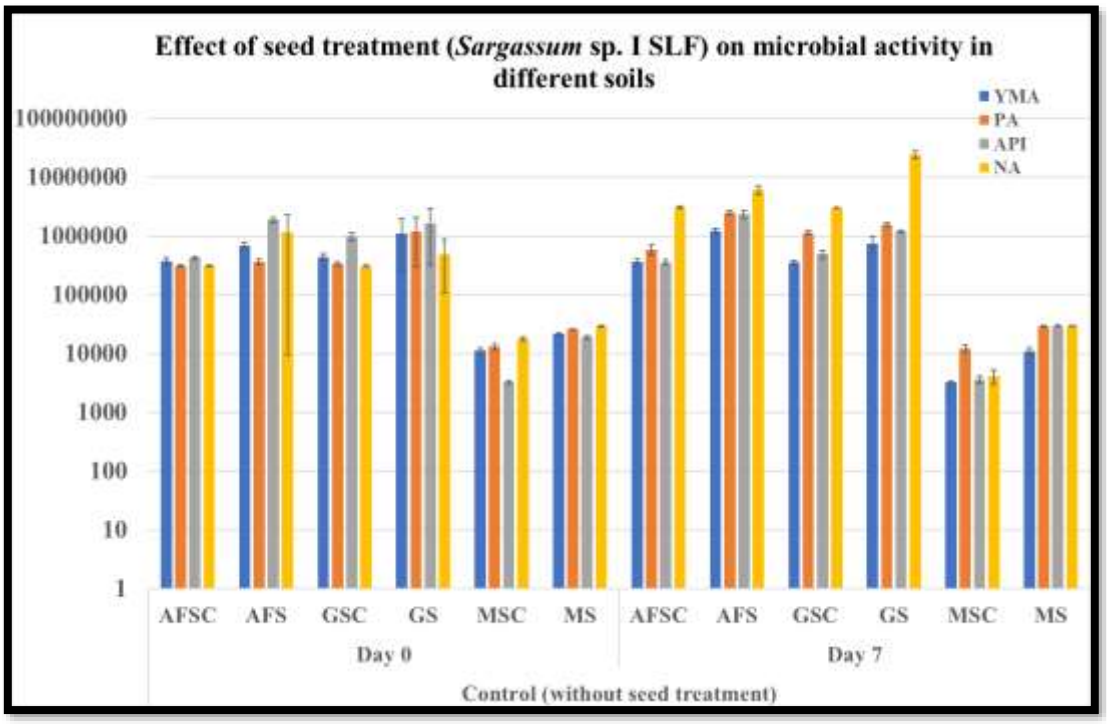


Fig 4.19: Effect of *Sargassum sp.* I SLF on microbial activity in different soils without seed treatment

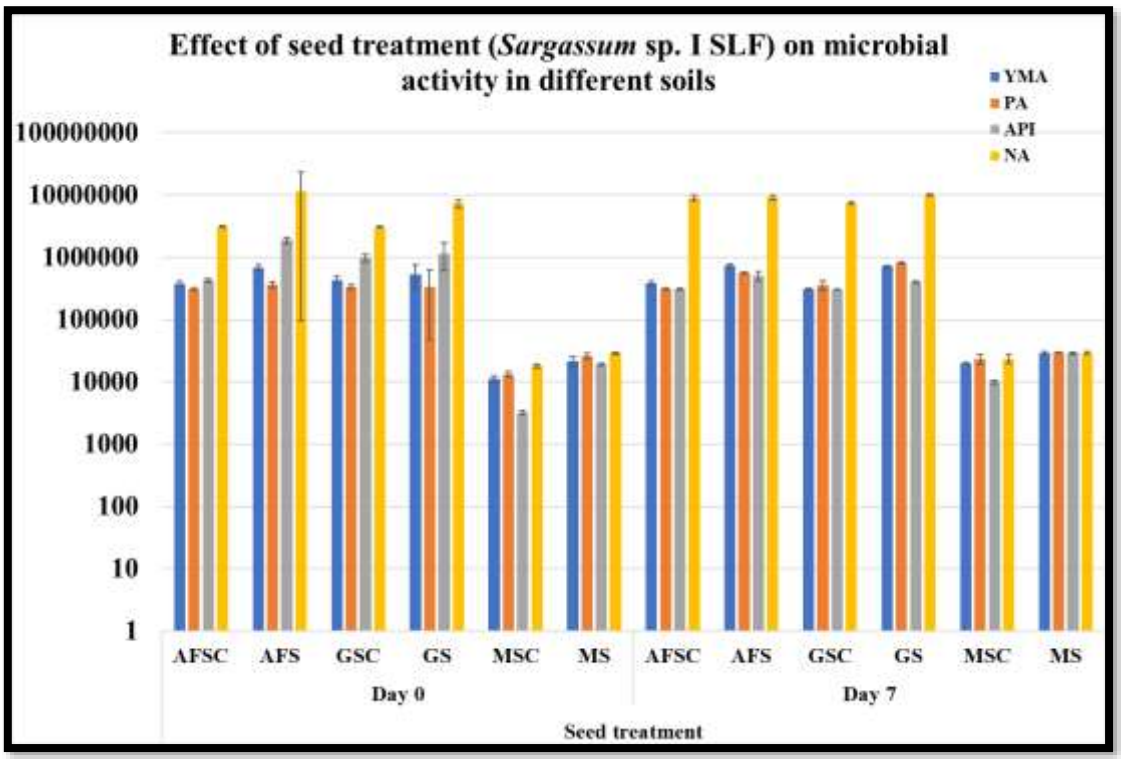


Fig 4.20: Effect of *Sargassum sp.* I SLF on microbial activity in different soils with seed treatment

On Days 0 and 7, the number of microorganisms in the set that received seed treatment was roughly the same (Fig 28). When comparing the two graphs, we can see that the one with seed treatment has higher levels of microbial activity. This might occur from the fact that SLF is applied twice in the seed treatment set compared to the set without seed treatment.

CONCLUSION

In this study seaweed samples were collected from Bogmalo and Anjuna beach and the surrounding ecosystem was examined. A total of 3 seaweed species were used namely: *Ulva intestinalis* and two *Sargassum sp.* Seaweed liquid fertilizers were prepared from all three species and evaluated based on the presence of various phytochemical compounds (alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones, and glycosides). Efficacy of the Seaweed liquid fertilizer was also evaluated using germination percentage, foliage count, vigour index and shoot/root length. Additionally, the impact of seaweed liquid fertiliser was examined on microbial activity in both fertile and infertile soil to determine whether applying seaweed liquid fertiliser to infertile soil would improve soil fertility resulting in plant development. This study concluded that different extract of *Ulva intestinalis*, *Sargassum sp.* I and II possess several chemical compounds including terpenoids, steroids, quinones and saponins. Extraction solvents affect yield of different phytochemicals. Based on the experimental results, it was clear that maximum growth of *Vigna radiata l.* plant can be achieved CSE 1% concentration. Even though the root and shoot length was high at HSE 1% concentration it was negatively affecting the plant causing blackened shoots making it clear that CSE 1% is the most promising, compared to other extracts. The microbial activity in the soil was increasing by Day 7 with the SLF application and even plants with SLF treatment grew better as compared to the plants without SLF treatment. Overall, it could be concluded that the utilization of seaweed liquid fertilizer may enhance the yield of crops, which could be suggested as a suitable alternative to chemical fertilizers.

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APPENDIX

Media Composition

1) Yeast Mannitol Agar

Ingredients	Grams/Litre
Yeast extract	1
Mannitol	10
Dipotassium phosphate	0.5
Magnesium sulphate	0.2
Sodium chloride	0.1
Calcium carbonate	1
Agar	15
Final pH (at 25ºC)	6.8 -7

2) Pikovskyaya's Agar

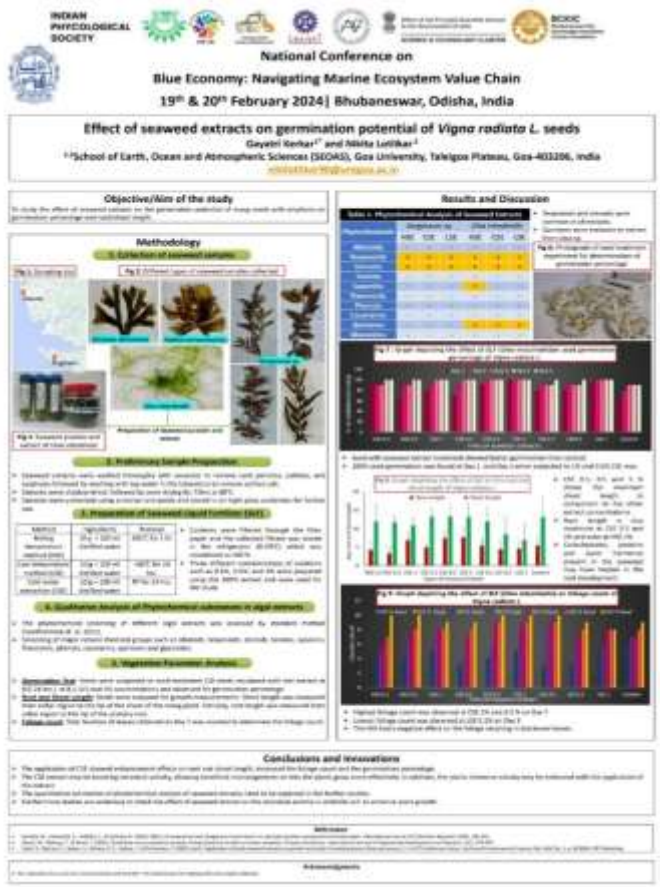
Ingredients	Grams/Litre
Yeast extract	0.5
Dextrose	10
Calcium phosphate	5
Ammonium sulphate	0.5
Potassium chloride	0.2
Magnesium sulphate	0.1
Manganese sulphate	0.0001
Ferrous sulphate	0.0001
Agar	15
Final pH (at 25º)	7.4 -7.8

3) Nutrient Agar

Ingredients	Grams/Litre
Peptone	5
Sodium chloride	5
HM peptone B	1.5
Yeast extract	1.5
Agar	15
Final pH (at 25ºC)	7.4 -7.8

4) Modified Sulphate-reducing API agar

Ingredients	Grams/litre
Yeast extract	1
Dipotassium phosphate	0.01
Citric acid powder	0.1
Sodium chloride	10
Magnesium sulphate	0.2
Ferrous ammonium sulphate	0.1
Glycerol	4
Agar	24
Final pH (at 25 ºC)	7.4 -7.8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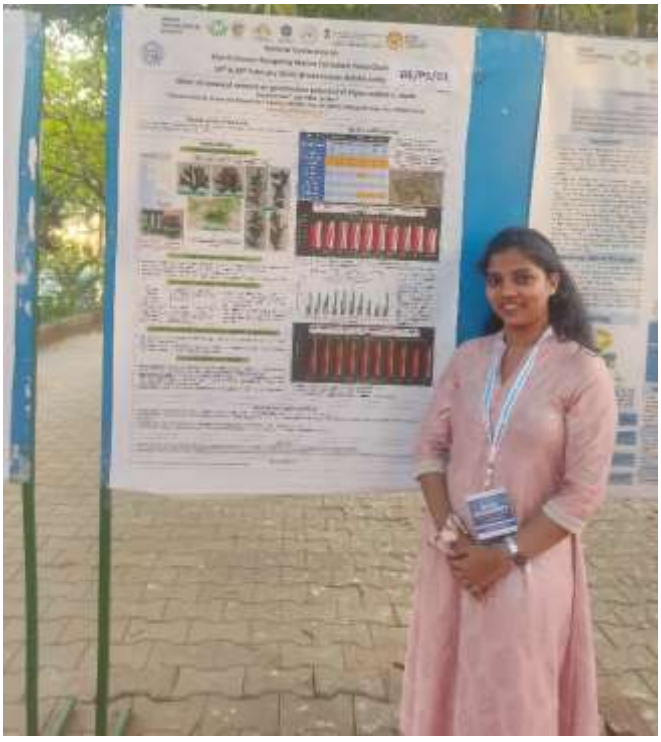
19th & 20th February 2024| Bhubaneswar, Odisha, India
Effect of seaweed extracts on germination potential of Vigna radiata L. seeds
Objective/Aim of the study
Methodology
Results and Discussion
Conclusions and Innovations



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CERTIFICATE
OF PARTICIPATION

Gayatri Abhay Kerkar

has presented Poster in

"NATIONAL CONFERENCE ON BLUE ECONOMY"

conducted on 19th - 20th February 2024 organized by Indian Phycological Society, KIIT Deemed to be University, DBT ILS, CSIR IMMT, CSIR NIO & BCKIC Foundation, O/o PSA to Govt of India.

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for his/her active Participation in

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