Chapter III

Results and Discussion

A. Survey of Study Area

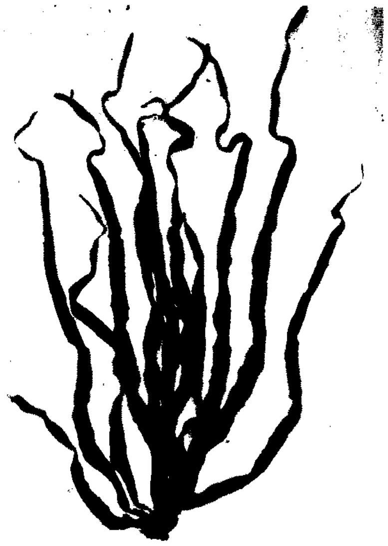
A survey of study area was carried out in order to know the diversity of macroalgae occurring in the coastal areas of Malvan and Kunkeshwar. The rocky and sandy beaches of this area were frequently visited from pre-monsoon period upto April and progress in algal vegetation was recorded critically. Seaweed species such as *Sargassum ilicifolium*, *Hypnea musciformis*, *Gracilaria corticata*, *Padina tetrastromatica* etc. were luxuriantly growing in this region. Six species of genus *Caulerpa* were recorded in the study area during February and March months. Malvan is the only locality along the Maharashtra coast, where the maximum number of *Caulerpa* species were observed. The species are *Sargassum* and *Hypnea particularly* were abundant during post monsoon period.

The seaweed species recorded during the present investigation have been listed in Table 2. More than 35 species were found in the study area. A few dominant seaweeds occurring along the coast of Malvan and Kunkeshwar are displayed in plates 4, 5 & 6.

Table 2. Seaweed species recorded from coastal region of Malvan & Kunkeshwar.

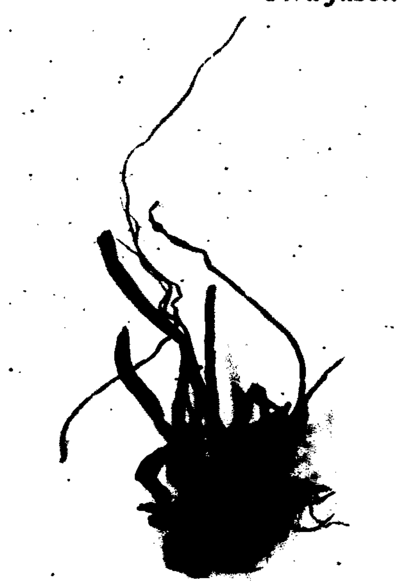
Sr. No.	seaweeds	Malvan	Kunkeshwar	Season
	CHLOROPHYCEAE			
1	*Chaetomorpha media*	✓	✓	Oct.-Feb.
2	*Cladophora fascicularis*	✓	✓	Oct.-Feb.
3	*Cladophora prolifera*	✓	✓	Oct.-Feb.
4	*Caulerpa sertularioides*	✓	✓	Oct.-Feb.
5	*Caulerpa peltata*	✓	✓	March-May
6	*Caulerpa recemosa*	✓	✓	March-May
7	*Caulerpa verticillata*	✓	✓	March-May
8	*Caulerpa taxifoia*	✓	✓	March-May
9	*Caulerpa scalpeliformis*	✓	✓	March-May
10	*Enteromorpha compressa*	✓	✓	March-May
11	*Enteromorpha intestinalis*	✓	✓	Oct.-Feb.
12	*Enteromorpha flexousa*	✓	✓	Oct.-Feb.
13	*Enteromorpha clathrata*	✓	✓	Oct.-Feb.
14	*Ulva fasciata*	✓	✓	Oct.-Feb.
15	*Ulva lactuca*	✓	✓	Oct.-Feb.
16	*Ulva reticulate*	✓	✓	Oct.-Feb.
	PHEOPHYCEAE			
17	*Dictyota dichotoma*	✓	✓	March-May
18	*Dictyota speices*	✓	✓	March-May
19	*Spatoglossum asperum*	✓	✓	Nov.-Dec.
20	*Sphacelaria furcigera*	✓	✓	Nov.-Dec.
21	*Stoechospermum marginatum*	✓	X	March-May
22	*Dictyopteris australis*	✓	✓	Oct.-Feb
23	*Padina tetrastromatica*	✓	✓	Oct.-Feb
24	*Padina gomerahiza*	✓	✓	Oct.-Feb
25	*Sargassum cinereum*	✓	✓	Oct.-Feb
26	*Sargassum ilicifolium*	✓	✓	Oct.-Feb
	RHODOPHYCEAE			
27	*Porphyra vietnamensis*	✓	✓	August- oct.
28	*Porphyra indica*	✓	✓	August- oct.
29	*Jania adhaerens*	✓	X	Nov.-Dec.
30	*Gracillaria corticata*	✓	✓	Oct.-May
31	*Gracillaria verrucosa*	✓	✓	Oct.-May
32	*Hypnea musciformis*	✓	✓	Oct.-May
33	*Grateloupia filicina*	✓	✓	Nov.-Dec.
34	*Grateloupia lithophila*	✓	✓	Nov.-Dec.
35	*Gelidium micropterum*	✓	✓	March-May

Plate IV : Green seaweed species from West coast of Maharashtra

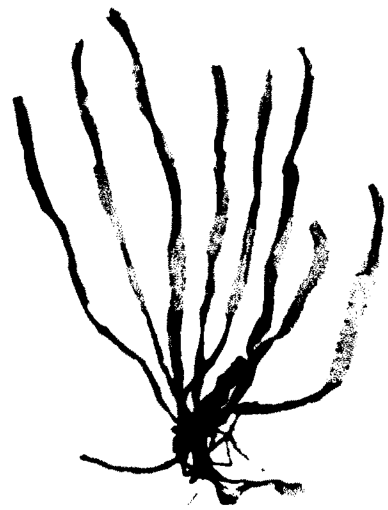


Ulva fasciata Delile.

Ulva lactuca Linnaeus.



Enteromorpha clathrata



Enteromorpha compressa

Enteromorpha intestinalis

Enteromorpha tubulosa

Chaetomorpha media

Chaetomorpha linum

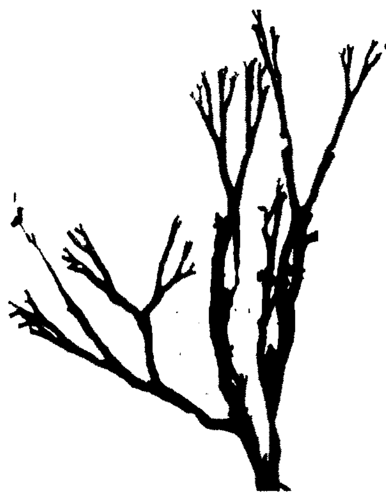
Plate V : Brown seaweed species from West coast of Maharashtra

Sargassum cinereum

Sargassum ilicifolium

Sphacelaria furcigera Kuetz

Dictyota bartayresiana



Dictyota dichotoma

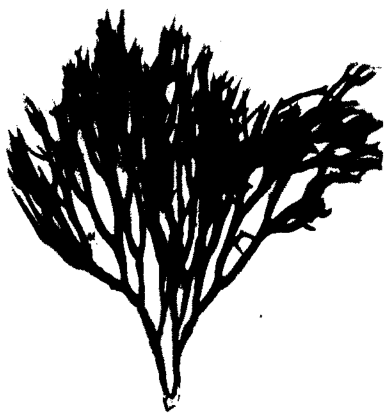
Stoechospermum marginatum

Padina tetrastromatica **Hauck**

Spatoglossum asperum

Gracillaria verrucosa (Red)

Plate VI : Red seaweed species from West coast of Maharashtra



Gracillaria corticata

Porphyra indica

Porphyra vietnamensis

Gelidium micropterum

Jania rubens

Grateloupia indica Borgesen

Grateloupia filicina

Grateloupia lithophila

Hypnea musciformis

B. Analysis of seaweeds

1. Physical parameters

a. Colour

Colour of freshly collected seaweed samples and the colour after their grinding is recorded in the Table 3. The colour of seaweed is always influenced by the pigments present and mostly a similar colour is observed after its homogenization. Thus green alga *Ulva* exhibited a green colour which appeared slightly faded when extract was prepared. *Sargassum* is a brown alga so both the extract and thallus had the same colour. *Gracillaria* being red algae the extract also had a reddish colour. As no solvent (organic/ aqueous) was used for extraction, the pigments present in seaweeds were not totally released in the extract which affected the colour.

b. pH

Hydrogen ion concentration of seaweed liquid fertilizer of *Ulva fasciata*, *Sargassum ilicifolium* and *Gracillaria corticata* were recorded on a pH meter and are presented in Table 3. The pH of all the seaweed liquid extracts was near to 7 i.e. neutral. In *Sargassum* and *Gracillaria* it was inclined towards acidic range and in *Ulva* it was slightly above the neutral. The pH of the fertilizers indicate moor or less a balanced proportion of positive and negative nutrient ions.

Table 3. Physico-chemical analysis of seaweeds.

Sr. No.	Parameters	*Ulva fasicata*	*Sargassum ilicifolium*	*Gracillaria corticata*
A. Physical parameters				
1	Colour of thallus	Green	Brown	Red
2	Extract Colour	Light Green	Brown	Reddish
3	pH	7.12	6.26	6.15
B. Biochemical parameters				
1	Total Carbohydrates (%)	5.92	6.72	4.20
2	Total Proteins (%)	3.45	3.86	3.30
3	Total Lipids (%)	6.3	5.7	4.2
4	Total Minerals (%)	7.5	15.9	10.8

2. Organic constituents

Seaweed contains a number of phytochemicals and nutrients which are released out in the extract during the preparation of fertilizers. These chemicals are absorbed by the crops and used for the growth when the fertilizer is applied to them. Content of these nutrients varies in different species and also as per the method of preparation and the solvent used for extraction. Analysis of fresh and dry samples of *Ulva, Sargassum* and *Gracillaria* was made in the present study before they were extracted for application.

a. Total Carbohydrate content

Total carbohydrate content in the three macroalgae is recorded in Table 3. The content of sugars varied from 4.20 to 6.72g 100^{-1}g dry weight the maximum being recorded in *Sargassum* a brown alga and minimum was found in *Gracillaria,* a member of Rhodophyceae.

Carbohydrate is the most important component for metabolism as it supplies the energy needed for respiration and other metabolic processes. According to Arasaki (1983) carbohydrates comprise 50– 60% of the dry weight of seaweeds. Kennish and Williams (1997) reported 8.1–33.7% soluble carbohydrates in *Enteromorpha, Ulva* and *Porphyra*. Manivannan *et al.*, (2009) studied biochemical composition of twelve species belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae from Mandapam Coastal Regions along Southeast Coast of India and found that the carbohydrate concentration of seaweeds varied from 20 - 24%. Reed (1907) also reported non-fiber carbohydrate content (computed by subtraction) of three Hawaiian seaweeds, which was more than 50%.

Dhargalkar (1979) has reported that the decrease in carbohydrates may be observed due to an extensive growth of thallus of algae. Pattama and Chirapart (2006) reported a high carbohydrate level in green seaweeds *Caulerpa lentillifera* and *Ulva reticulata*. According to McDermid and Stuercke (2003) members of the Rhodophyta contained a low level of soluble carbohydrates (16.0–33.2%); and E. *flexuosa* (Chlorophyta) exhibited the greatest carbohydrate content (39.9%). Mcdermid *et al.,* (2007) have reported somewhat lower values of carbohydrate in *Ulva fasciata*, *Sargassum echinocarpum* and *Gracillaria salicornia* (20, 10 and 24% approx.). According to Haroon, (2000) water temperature during summer months with high solar radiation, can enhance photosynthetic rate leading to an increase in the carbohydrate concentration.

The values of carbohydrates recorded in the present study are found somewhat at a lower level than in other regions. A further confirmation in this matter is highly essential.

b. Total Proteins

Total protein content in three species of marine algae is depicted in Table 3. The content did not vary much in different algal species and ranged from 3.3 to 3.8 $g100^{-1}g$ dry weight.

Protein content in the marine algae has been estimated by several workers (Neela, 1956; Pillai, 1957; Sitakara Rao and Tipnis, 1967). A protein content of less than 10% has been reported from the species of *Sargassum*, *Turbinaria* and *Gracilaria* by Chidambaram and Unny (1953) and *Gelidium folifera* by Manivannam (2009).

Twenty nine genera comprising forty two species of red seaweed from Gujarat coast have been analysed for protein content by Dave *et al.* (1987). Dave and Parekh (1975) studied eight genera of green algae of Saurashtra coast and found a significant variation in protein in the same species of algae growing at different localities and different periods.

Mabeau and Fleurence (1993) reported a high protein content in red seaweeds *Porphyra tenera* and *Palmaria palmate* (47 and 35 % respectively). Nishizawa *et al.*, (1987) observed 5 to 10% proteins in brown seaweeds and 18 to 26% proteins in green algae belonging to genus *Ulva*. On the contrary Dhargalkar (1986) and Chakraborthy and Santra (2008) recorded about 3-11% of protein in species of *Ulva*. Wong and Cheung, (2000) also reported a high Protein content in *Hypnea musciformis Hypnea spinella* and *Gracillaria* (>20%). According to Fleurence (1999) protein content of red seaweed ranged from 10-47%.

Yada (2004) demonstrated that the protein content of marine algae differs according to species, and is low for brown sea weeds (3-15 %), moderate in green algae (9-26%) and high for red seaweeds (up to 47%).

Thus a great variation is observed in the content of protein and several factors are responsible for this fluctuation. According to Zaragoza *et al.*, (2002) the changes in protein content are related to latitudinal temperature differences and local environmental conditions. For most of seaweeds a large part of amino acid fraction is comprised of aspartic and glutamic acids in proteins from brown sea weeds (Yada, 2004). In the present study we notices higher levels of protein in *Sargassum ilicifolium* than *Ulva fasciata* and *Gracillaria corticata*

c. Total Lipids

Total lipid content of *Ulva, Sargassum* and *Gracillaria* from west coast of Maharashtra ranged from 4.2 to 6.3 %, (Table 3) highest being in green alga *Ulva* followed by brown alga *Sargassum.*

According to Jurkovi *et al*., (1995) lipid content in sea vegetables is very low, ranging from 1–5% of dry matter. Esteves *et al.,* (2000) studied seasonal variation in lipid content in species of *Derbesia, Ulva, Gracillaria, Hypnea, Enteromorpha, Cladophora,* which ranged from 4 to 82 $mg.g^{-1}$ dry weight. Haroon *et al*., (2000) also reported a low lipid content in *Enteromorpha* species which remained almost stable throughout the year, ranging from 3 to 4% of dry weight. Several workers have analyzed lipid content in different seaweeds (Banaimoon, 1992; Mercer *et al.,* 1993; McDermid and Stuercke, 2003; Vierra *et al*., 2005; Manivannan, *et al.,* 2009; Chakraborthy and Santra, 2008). As per the studies made by Herbetreau *et al*., (1997) generally the lipid content of sea weed is less than 4%. The differences may occur due to factors such as climate and geography of development of the seaweed.

Temperature has a characteristic effect on many plant lipids in that it increases the level of unsaturation of acyl chains, which slows down both metabolism and transport (Jones & Harwood 1993). The lowering of lipid content in seaweeds of west coast of Maharashtra is might be due to high temperature of both climate and sea. Lipid content in the seaweeds under investigations was found within the recorded range.

3. Inorganic constituents

a. Total Ash

Three seaweeds *Ulva, Sargassum* and *Gracillaria* were evaluated for their ash content. The results are depicted in Table 3. Total Content of ash in green algae was higher as compared to brown and red seaweeds. *Sargassum ilicifolium* exhibited a higher ash content (15.9%) than *Ulva fasciata* (7.5%) and *Gracillaria corticata* (10.8 %).

Ash is the most abundant component of dried material in all species. Studies on the chemical composition of seaweeds have shown that ash is a good source of minerals and trace elements (Rao and Tipnis, 1967).

McDermid and Stuercke (2003) studied ash content of algal species from Chlorophyceae, Phaeophyceae and Rhodophyceae species. They recorded a greater range in ash values in the green algae (22- 64.3%) than in brown (28-

Table 4. Inorganic constituents in seaweeds.

Element*	*Ulva*	*Sargassum*	*Gracillaria*
Macroelements			
N	14350	9140	9080
P	3125	3500	5500
K	3125	4640	2819
Ca	3940	4320	4337
Mg	3478	3348	3568
Microelements			
Fe	05.38	18.34	13.76
Mn	02.30	09.62	03.64
Cu	01.16	01.20	00.92
Zn	04.52	06.40	0.96
Ni	oo.74	01.20	01.02

All values in mg/100g.dry weight basis

32%). Pattama and Chirapart (2006) also reported high ash content in *Ulva* and *Caulerpa*, the green algal species.

It is usually noticed that the ash content of seaweeds is much higher than those of terrestrial vegetables other than spinach (Ruperze et al., 2002; Sanchez-Machado *et al.,* 2004). A great variation in ash content has been reported by different workers in a variety of seaweed species (Wong and Cheung, 2000; *Zubia et al.* 2003; Vieraa *et al.*, 2005; Vega- 2006; Marinho *et al.*, 2007 and McDermid *et al.,* 2007).

b. *Macroelements*

Essential mineral elements are usually classified as macronutrients and micronutrients, according to their relative concentration in plant tissue. In some cases, the differences in tissue content of macronutrients and micronutrients are variable. Many elements often are present in concentrations greater than the plant's minimum requirement.

Minerals composition of the three seaweeds from west coast of Maharashtra is depicted in Table 4.

i. Total Nitrogen

Total nitrogen content in three seaweed species varied from 9.08 to 14.35% dry weight, being maximum in *Ulva fasciata.*

With the expectation of carbon, hydrogen and oxygen, N is the most prevalent, element in the living organism. It is invariably found in such essential compounds as proteins, nucleic acids, some of the plant growth regulators (IAA and Cytokinins) and in many of the vitamins. As a compound of these and many other compounds, nitrogen is involved in most of the biochemical reactions. Since nitrogen insufficiency is probably a single major factor limiting crop growth and yields, all our fertilizer practices are ultimately based on proper and reasonable application of theses elements in various forms.

Lourenço *et al,* (2006) analysed nitrogen content in ten species of seaweeds (6 green and 4 red algae) and reported a high N content in *Bostrychia radicans* and *Grateloupia doryphora* (red algae) and lower in *Cladophora rupestris* and *Codium decorticatum* (green algae). Nitrogen contents surveyed in seaweed species from Hiroshima Bay, Inland Sea ranged from 0.71-3.5% (Yoshida, 2001).

ii. Phosphorus

In the present study a high phosphorus content (5.5%) was noticed in *Gracillaria corticata* (red algae) as compared to that in brown and green algae (Table 4).

Phosphorus content in seaweeds of *Undaria pinnatifida* and *Aminaria digitata* has been reported by Kolb *et al,* (2004). According to Fuller and Roger (1952) plants respond well to phosphorus applied as algal fertilizer than to inorganic phosphates. Vicente *et al.* (1980) reported values for phosphorus in *Thallassia testudinum* that were similar to the levels of these minerals in *Hypnea decipiens* and *Hypnea hawaiiana.*

Phosphorus is an integral component of important compounds of plant cells, including DNA, RNA, sugar–phosphate intermediates of respiration and photosynthesis, and the phospholipids that make up plant membranes.

iii. Potassium

Potassium content was maximum in *Sargassum lilicifolium* (4.6%) as compared to *Gracillaria corticata and Ulva fasciata* (2.8 to 3.1% respectively) (Table 4). The observed concentrations were higher than in other seaweeds (1.5 to 3.9%) such as *Caulerpa lantillifra, Gracillaria parpispora, Monostroma oxysperum, Enteromorpha flexuosa* and *P. vietnamensis* (Subba Rao *et al.*, 2007). Several seasonal, environmental and physiological factors including the seaweed species, oceanic residence time, geographical place of harvest, wave exposure and types of processing, method of mineralization etc. affect the concentration of K (Honya *et al.*, 1993).

Vicente *et al.* (1980) reported values for potassium in *Thallasia testudinum* that were similar to the levels of these minerals in *Hypnea decipiens* and *Hypnea hawaiiana.* Joko *et al,* 2006 found a high K concentration in brown algae *Kappaphycus alvarezii*, *Turbinaria conoides*, and *Sargassum polycystum*. A large variation (32-115mg/g) was found in K- content of edible brown and red algae in Japan and Spain (Ruperez, 2002).

Among different mineral nutrients the indispensability of potassium for growth and metabolism is indisputable. K is the major plant nutrient which plays biophysical role in cellular water relation and biochemical role in a variety of metabolic processes such as protein synthesis (Hsiao and Lauchli, 1986), enzyme activities (Evans and Sorger, 1966), photoreduction and photophosphorylation (Pfiuger and Mengle, 1972), starch formation (Hawker *et* *al.*, 1974). potassium plays a key role in the process of stomatal behaviour and assimilate translocation.

iv. Calcium

Calcium content in *Gracillaria corticata* was marginally higher (4.34%) than in the other two species of seaweeds.

According to Ruperez, (2002) Ca values in different edible algae ranged from 0.4-10%. Calcium is of fundamental importance for membrane functioning and the maintance of cell integrity. It is important for synthesis of pectin in the middle lamella of the cell wall. It plays an important role in the regulation of membrane permeability to various ions, in particular to inorganic cations (Van Steveninck, 1965). According to Clarkson and Hanson (1980) a major role of calcium appears to be its binding with proteins, nucleic acids, lipids and enzyme.

v. Magnesium

Magnesium content in *Gracillaria corticata* (3.5%) and *Ulva fasciata* (3.4%) was slightly more than that in *Sargassum ilicifolium* (3.3%).

In plant cells, magnesium ions (Mg^{2+}) have a specific role in the activation of enzymes involved in respiration, photosynthesis, and the synthesis of DNA and RNA. The most well known role of Mg is its contribution to the center of the chlorophyll molecule. Magnesium stabilizes the ribosomal particles in the configuration necessary for protein synthesis and it is belived to have a similar stabilizing effect in the matrix of the nucleus (Menel and Kirkby, 1982). Mg is highly essential in many enzyme reactions (Clark, 1984).

vi. Iron

Iron content was found to be highest in *Sargassum* as compared to *Ulva fasciata* and *Gracillaria corticata*, in the present study.

Iron composition of edible seaweeds *Porphyra vietnamensis, Undaria pinnatifida* and *Aminaria digitata* varied from 1.54 to 13.7 mg/ 100g. (Kolb *et al.*, 2004 and Rao, 2006). It was also reported that Fe content in *Caulerpa racemosa*, was higher. Compared with the Japanese seaweeds *Hizikia fusiformis*, *Porphyra yezoensis* and *Enteromorpha intestinalis* the Indonesian seaweed samples had slightly lower Fe content (Yoshie *et al.* 1999).

Furthermore, Fe contents of brown algae (*Fucus vesiculosus, Laminaria digitata*, and *Undaria pinnatifida*) and red algae (*Chondrus cryspus* and *Porphyra tennera*) grown in Spain were almost the same, with ranges of 0.33 – 10.3 mg/100g dry weight, respectively (Ruperez, 2002).

According to El-Baroty (2007) the alga *Asparagopsis taxiformis* was found to be rich in iron (1.500 mg/100g). Similarly it was reported to be highest (9.6-11 mg/100g) in *Gracillaria* (Joko *et al*, 2006).

Among the various microelements iron has perhaps the greatest biological significance. This is due to its association with proteins. According to Sandmann and Boger (1983), there are two groups of well defined iron-containing proteins: hemoprotiens and iron sulfur proteins. The role of iron in the biosynthesis of chlorophyll is well established. Iron is required for the formation of protochlorophyllide from Mg-protoporphyrin (Machold and Stephan, 1969).

vii. Manganese

In the present study we noticed that manganese content (9.62 mg/100g dry weight) in *S. lilicifolium* was higher than in red and green algae.

Manganese composition of edible seaweed *Porphyra vietnamensis* is 9mg/100g dry wt. as reported by Rao (2006). According to Kolb *et al,* (2004) manganese content in of *Undaria pinnatifida* and *Aminaria digitata* varied from 0.332 to 29.4mg/100g dry weight. El-Baroty (2007) reported 1.4mg/100g manganese in *Asparagopsis taxiformis..*

Manganese ions (Mn^{2+}) activate several enzymes in plant cells. In particular, decarboxylases and dehydrogenases involved in the tricarboxylic acid (Krebs) cycle are specifically activated by manganese. The best defined function of manganese is in the photosynthetic reaction through which oxygen is produced from water (Marschner 1995).

Manganese is essential in both lower and higher plants for the Hill reaction. In chloroplast larger fraction of Mn is held in a less tightly combined state and seems to be most closely involved in oxygen evolution, whereas the smaller fraction may be more directly involved in thylakoid structure of stability (Takahashi and Asada, 1977). It is directly involved as component of biotin enzyme in the biosynthesis of fatty acids (Marschner, 1986).

b. Microelements (trace metals)

i. Copper

The content of copper was found to be very less in different seaweeds under investigation and ranged from 0.9 – 1.2 mg/100g dry weight (Table 4)

According to Yoshie *et al.* (1999), The Cu content of Indonesian seaweeds varied from 0.25 to 2.51mg/g dry weight. According to El-Baroty (2007), the alga *Asparagopsis taxiformis* was found to be rich in copper.

Kolb *et al.,* (2004) analyzed and reported copper contents in seaweeds *U. pinnatifida* and *A. digitata* (0.185 to 0.247 mg/ 100g dry weight respectively). Copper composition of edible seaweed *P. vietnamensis* is 0.83mg/100g dry wt (Rao 2006).

Copper acts as a cofactor in multiple enzymes, energy conversion etc. It is taken up by the plants in very small quantities. Copper is a component of several metalloenzymes and it appears to act as an intermediate electron acceptor in the direct oxidation of substrate by molecular oxygen (Gupta, 1979). Cu also plays an important role in maintaing membrane structure and thylakoids (Henriques, 1989). Copper is associated with enzymes involved in redox reactions being reversibly oxidized from Cu^{+} to Cu^{2+}.

ii. Zinc

Zinc content was 6.4, 4.52 and 5.96 mg/100g dry weight respectively in *Sargassum*, *Ulva* and *Gracillaria* (Table 4).

Zinc plays very important role as an enzyme catalyst. It enhances the catalytic, structural and regulatory function, stabilizes membranes, hormones and nucleic acids (Norziah and Ching, 2000). Zn is required for the activity of various types of enzymes, including aldolases, isomerases, transphosphorylases and RNA and DNA polymerases (Marschner, 1986).

Compared with the Japanese seaweeds *Hizikia fusiformis, Porphyra yezoensis* and *Enteromorpha intestinalis* the Indonesian seaweed samples had a slightly lower Zn content which ranged from 0.82-5.24 mg/100g dry weight (Yoshie *et al.* 1999).

In brown and red algae (*Fucus vesiculosus, Laminaria digitata, Undaria pinnatifida* and *Chondrus cryspus, Porphyra tennera)* Zn content ranged from 1.7- 07.1 mg/100g dry weight (Ruperez, 2002). According to El-Baroty (2007), the alga *Asparagopsis taxiformis* was rich in zinc (01.20 mg/100g).

iii. Nickel

Nickel metal content varied from 0.74–1.2 mg/100g in *Sargassum*, *Ulva* and *Gracillaria* (Table 4). Nickel contents in *Undaria pinnatifida* and *Aminaria digitata* was 0.265 and 3.25mg/ 100g dry weight, as recorded by Nada Kolb *et al,* 2004. In *Porphyra* it was 0.29mg/100g (Rao, 2006). Ni is a relevant element and is of immediate concern due to their potential toxicity for living organisms.

Urease is the only known nickel-containing enzyme in plants, although nitrogen-fixing micro-organisms require nickel for the enzyme that reprocesses some of the hydrogen gas generated during fixation. Nickel- deficient plants accumulate urea in their leaves and, consequently, show leaf tip necrosis.

In the present study low levels of Ni content is beneficial as far as theses extract are sprayed on vegetables which may reduce the chances of Ni toxicity.

C. Seaweed Application to Trigonella foenum-graecum

1. Germination

Effect of fresh extract, of the three seaweeds viz. *Ulva fasciata, Sargassum lilicifolium* and *Gracillaria corticata* at various concentrations (5 - 100%) on the germination of fenugreek was analysed in a petri plate experiment. It was noticed that the fresh extract up to 50 - 60% promoted germination in fenugreek. At higher concentration slowly the rate of germination declined and was completely inhibited at 100% concentration of all the seaweeds. A cent percent germination was recorded on the third/ fourth day after treatment for the concentrations ranging from 5 to 50% which was not seen in control. This trend was similar to that occurred for the Hoagland treated seeds which also exhibited 100% germination after the third day of treatment (Table 5, 6 and 7).

When extract was prepared by boiling dried seaweed in d.w. and applied to fenugreek seeds a similar influence on germination was observed in the present study. The concentrations which caused a beneficial effect on germination were from 10 to 50% in all the seaweeds. Even at the lower concentration of 5% (*Ulva* and *Sargassum*) the germination percentage was greater than the control seeds.

For *Sargassum* treatment 100% germination was recorded for all the concentration upto 50% whereas in case of other two extracts it was slightly below 100% for lower concentrations.

Table 5: Effect of SWC of *U. fasciata* on seed germination of *T. foenum-graecum*.

Concentration / Days	Fresh extract				Boiled extract				Soaked Extract			
	1	2	3	4	1	2	3	4	1	2	3	4
Control	00	43	67	78	00	43	67	78	00	52	88	100
5%	--	07	70	10	00	57	66	80	00	08	68	90
10%	--	68	76	100	00	77	82	90	19	67	99	100
20%	01	77	96	100	38	69	79	98	31	92	99	100
30%	12	54	100	100	47	70	90	100	29	80	89	100
40%	23	62	100	100	48	70	98	100	30	69	89	100
50%	22	59	100	100	52	67	99	100	29	88	98	100
60%	02	08	38	100	00	00	89	90	10	55	85	89
70%	--	01	03	03	00	00	38	39	00	22	33	51
100%	--	--	01	01	00	00	00	00	00	00	00	00
Hoagland*	48	87	100	100	48	87	100	100	48	87	100	100

Table 6: Effect of SWC of *S. ilicifolium* on seed germination of *T. foenum-graecum*.

Concentration / Days	Fresh extract				Boiled extract				Soaked Extract			
	1	2	3	4	1	2	3	4	1	2	3	4
Control	00	43	67	78	00	43	67	78	00	52	88	100
5%	00	69	94	100	00	67	89	100	11	68	70	99
10%	19	78	100	100	19	68	99	100	22	66	69	100
20%	29	72	100	100	29	70	90	100	18	36	69	100
30%	27	89	100	100	27	79	92	100	30	68	90	100
40%	38	88	100	100	38	78	98	100	40	88	98	100
50%	29	80	100	100	29	78	100	100	39	66	69	100
60%	00	19	28	42	00	21	39	42	30	50	90	90
70%	00	00	22	32	00	00	32	32	20	20	20	20
100%	00	00	00	00	00	00	00	00	00	00	00	00
Hoagland*	48	87	100	100	48	87	100	100	48	87	100	100

Values represent percent germination
* Full strength

When extract obtained after soaking the seaweed, in d.w. was used for treatment, more or less a similar trend was observed. Germination was influenced upto 50% of concentration and later on a reduction in germination percentage occurred in all the seaweeds used.

A proper establishment of crop stand mainly depends on the successful seed germination. Seed germination thus, represents the most important phase in the life cycle of crop plants and it is regulated by several environmental factors. Various types of chemicals and biofertilizers are used for enhancing germination, growth and yield of crop plants. Hoagland is one of the nutrient media used for growing plants under laboratory condition for studying several physiological aspects.

Wilczek *et al.* (1982) reported that seaweed extracts improved seed germination in table beet. Improved seed germination in faba bean due to seaweed extracts has been reported by El-Sheekh and El-Saled (2000). Hong (2007) demonstrated that the seeds soaked in aqueous extract of *S. wightii* performed better than the water-soaked controls. According to Moller and Smith (1999) under optimal conditions, seaweed extract shows a limitation in seed germination, but under stress condition seed germination is more prominent. A wide range of beneficial effects of seaweed extract applications on plants, including seed germination and establishment have been reported, (Beckett and van Staden 1989; Blunden 1991)

Table 7: Effect of SWC of *Gracillaria corticata* on seed germination of *T. foenum-graecum*.

Concentration / Days	Fresh extract				Boiled extract				Soaked Extract			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Control	00	43	67	78	00	43	67	78	00	52	88	100
5%	01	48	86	100	00	45	66	69	00	71	89	90
10%	02	58	93	100	00	65	70	90	18	68	69	90
20%	13	66	99	100	07	60	78	100	31	79	99	99
30%	16	89	100	100	08	68	90	100	30	96	99	100
40%	26	94	100	100	08	70	79	100	29	84	99	100
50%	20	97	100	100	24	94	99	100	33	73	92	100
60%	18	46	46	46	10	10	20	20	28	60	70	70
70%	07	11	11	11	00	00	00	00	12	22	23	24
100%	00	00	00	01	00	00	00	00	00	00	00	00
Hoagland*	48	87	100	100	48	87	100	100	48	87	100	100

Values represent percent germination
* Full strength

Demir *et al.*, (2006) studied the effect of seaweed suspension of three alage namely *Codium tomentosum*, *Gracillaria gracilis* and *Cystoseria barbata* on seed germination of tomato, pepper and aubergine. They found that pepper seeds treated with green seaweed had higher germination than those of others and the percentage recorded was 52% and 68% at 15°C and 25 °C respectively. Further the germination percentage of aubergine and tomato seeds treated with green seaweeds was much higher at low temperature (91% and 99%, respectively).

According to El-Sheekh and El-Saled (2000) green algae have a higher cytokinin content than red seaweeds while Jennings (1968) reported that green algae and brown algae contain gibberlic acid which play important role in seed germination.

Effect of different seaweed concentrates on the germination and growth was analysed by growing the plants in a mixture of coco-peat and soil (1:1) in plastic trays. A few selected concentrations of seaweed liquid fertilizers were used for this study. Results of germination studies are presented in Tables 8,9 and 10.

In control seeds of fenugreek no germination was observed on the first day. But fresh extracts of *Sargassum* and *Gracillaria* (10 and 25%) and *Ulva*, (25%) gave upto 20% germination on the first day. On the third day a cent percent germination was recorded in all the treated seeds when control seeds had 90% germination.

Table 8: Effect of selected concentrations of *U. fasciata* on seed germination of *T. foenum-graecum.*

Sr. No.	Concentration	Germination % after day											
		Fresh extract				Boiled extract				Soaked Extract			
		1	2	3	4	1	2	3	4	1	2	3	4
1	Control	00	70	90	100	00	40	70	100	00	50	70	100
2	10%	00	80	100	100	10	50	80	100	00	50	80	100
3	25%	10	70	100	100	20	70	90	100	40	60	80	100
4	50%	00	80	100	100	30	60	100	100	30	90	100	100
5	Hoagland	20	09	100	100	40	80	100	100	40	90	100	100

Table 9: Effect of selected concentrations of *S. ilicifolium* SWC on seed germination of *T. foenum-graecum.*

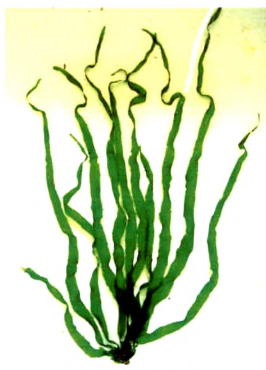
Sr. No.	Concentration	Germination % after day											
		Fresh extract				Boiled extract				Soaked Extract			
		1	2	3	4	1	2	3	4	1	2	3	4
1	Control	00	70	90	100	00	40	70	100	00	50	70	100
2	10%	10	80	90	100	10	80	90	100	00	60	90	100
3	25%	10	80	100	100	30	70	90	100	10	60	100	100
4	50%	00	70	100	100	40	80	100	100	20	80	100	100
5	Hoagland	20	90	100	100	40	80	100	100	40	90	100	100

Table 10: Effect of selected concentrations of *G. corticata* SWC on seed germination *of T. foenum-graecum.*

Sr. No.	Concentration	Germination % after day											
		Fresh extract				Boiled extract				Soaked Extract			
		1	2	3	4	1	2	3	4	1	2	3	4
1	Control	00	80	90	100	00	40	70	100	00	50	70	100
2	10%	10	70	100	100	00	30	70	100	00	70	90	100
3	25%	20	50	100	100	20	60	90	100	10	70	100	100
4	50%	00	60	100	100	30	70	100	100	30	90	100	100
5	Hoagland	20	90	100	100	40	80	100	100	40	90	100	100

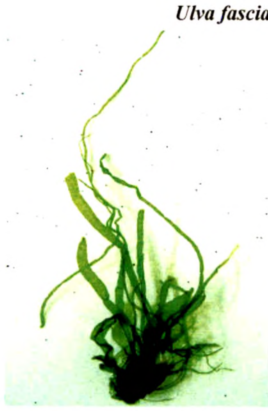
Seeds were sown in coco peat and soil mixture in plastic tray.

Plate IV : Green seaweed species from West coast of Maharashtra



Ulva fasciata Delile.

Ulva lactuca Linnaeus.



Enteromorpha clathrata

Enteromorpha compressa

Enteromorpha intestinalis

Enteromorpha tubulosa

Chaetomorpha media

Chaetomorpha linum

Plate V : Brown seaweed species from West coast of Maharashtra

Sargassum cinereum

Sargassum ilicifolium

Sphacelaria furcigera Kuetz

Dictyota bartayresiana

Dictyota dichotoma

Stoechospermum marginatum

Padina tetrastromatica **Hauck**

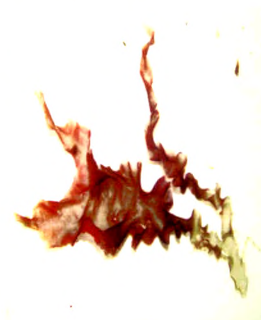
Spatoglossum asperum

Gracillaria verrucosa (Red)

Plate VI : Red seaweed species from West coast of Maharashtra

Gracillaria corticata

Porphyra indica



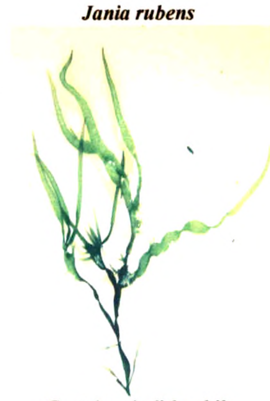
Porphyra vietnamensis

Gelidium micropterum

Jania rubens

Grateloupia indica **Borgesen**

Grateloupia filicina



Grateloupia lithophila

Hypnea musciformis

B. Analysis of seaweeds

1. Physical parameters

a. Colour

Colour of freshly collected seaweed samples and the colour after their grinding is recorded in the Table 3. The colour of seaweed is always influenced by the pigments present and mostly a similar colour is observed after its homogenization. Thus green alga *Ulva* exhibited a green colour which appeared slightly faded when extract was prepared. *Sargassum* is a brown alga so both the extract and thallus had the same colour. *Gracillaria* being red algae the extract also had a reddish colour. As no solvent (organic/ aqueous) was used for extraction, the pigments present in seaweeds were not totally released in the extract which affected the colour.

b. pH

Hydrogen ion concentration of seaweed liquid fertilizer of *Ulva fasciata*, *Sargassum ilicifolium* and *Gracillaria corticata* were recorded on a pH meter and are presented in Table 3. The pH of all the seaweed liquid extracts was near to 7 i.e. neutral. In *Sargassum* and *Gracillaria* it was inclined towards acidic range and in *Ulva* it was slightly above the neutral. The pH of the fertilizers indicate moor or less a balanced proportion of positive and negative nutrient ions.

Table 3. Physico-chemical analysis of seaweeds.

Sr. No.	Parameters	*Ulva fasicata*	*Sargassum ilicifolium*	*Gracillaria corticata*
A. Physical parameters				
1	Colour of thallus	Green	Brown	Red
2	Extract Colour	Light Green	Brown	Reddish
3	pH	7.12	6.26	6.15
B. Biochemical parameters				
1	Total Carbohydrates (%)	5.92	6.72	4.20
2	Total Proteins (%)	3.45	3.86	3.30
3	Total Lipids (%)	6.3	5.7	4.2
4	Total Minerals (%)	7.5	15.9	10.8

2. Organic constituents

Seaweed contains a number of phytochemicals and nutrients which are released out in the extract during the preparation of fertilizers. These chemicals are absorbed by the crops and used for the growth when the fertilizer is applied to them. Content of these nutrients varies in different species and also as per the method of preparation and the solvent used for extraction. Analysis of fresh and dry samples of *Ulva, Sargassum* and *Gracillaria* was made in the present study before they were extracted for application.

a. Total Carbohydrate content

Total carbohydrate content in the three macroalgae is recorded in Table 3. The content of sugars varied from 4.20 to 6.72g 100^{-1}g dry weight the maximum being recorded in *Sargassum* a brown alga and minimum was found in *Gracillaria,* a member of Rhodophyceae.

Carbohydrate is the most important component for metabolism as it supplies the energy needed for respiration and other metabolic processes. According to Arasaki (1983) carbohydrates comprise 50– 60% of the dry weight of seaweeds. Kennish and Williams (1997) reported 8.1–33.7% soluble carbohydrates in *Enteromorpha*, *Ulva* and *Porphyra*. Manivannan *et al.*, (2009) studied biochemical composition of twelve species belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae from Mandapam Coastal Regions along Southeast Coast of India and found that the carbohydrate concentration of seaweeds varied from 20 - 24%. Reed (1907) also reported non-fiber carbohydrate content (computed by subtraction) of three Hawaiian seaweeds, which was more than 50%.

Dhargalkar (1979) has reported that the decrease in carbohydrates may be observed due to an extensive growth of thallus of algae. Pattama and Chirapart (2006) reported a high carbohydrate level in green seaweeds *Caulerpa lentillifera* and *Ulva reticulata*. According to McDermid and Stuercke (2003) members of the Rhodophyta contained a low level of soluble carbohydrates (16.0–33.2%); and E. *flexuosa* (Chlorophyta) exhibited the greatest carbohydrate content (39.9%). Mcdermid *et al.,* (2007) have reported somewhat lower values of carbohydrate in *Ulva fasciata, Sargassum echinocarpum* and *Gracillaria salicornia* (20, 10 and 24% approx.). According to Haroon, (2000) water temperature during summer months with high solar radiation, can enhance photosynthetic rate leading to an increase in the carbohydrate concentration.

The values of carbohydrates recorded in the present study are found somewhat at a lower level than in other regions. A further confirmation in this matter is highly essential.

b. Total Proteins

Total protein content in three species of marine algae is depicted in Table 3. The content did not vary much in different algal species and ranged from 3.3 to 3.8 $g100^{-1}g$ dry weight.

Protein content in the marine algae has been estimated by several workers (Neela, 1956; Pillai, 1957; Sitakara Rao and Tipnis, 1967). A protein content of less than 10% has been reported from the species of *Sargassum*, *Turbinaria* and *Gracilaria* by Chidambaram and Unny (1953) and *Gelidium folifera* by Manivannam (2009).

Twenty nine genera comprising forty two species of red seaweed from Gujarat coast have been analysed for protein content by Dave *et al.* (1987). Dave and Parekh (1975) studied eight genera of green algae of Saurashtra coast and found a significant variation in protein in the same species of algae growing at different localities and different periods.

Mabeau and Fleurence (1993) reported a high protein content in red seaweeds *Porphyra tenera* and *Palmaria palmate* (47 and 35 % respectively). Nishizawa *et al.,* (1987) observed 5 to 10% proteins in brown seaweeds and 18 to 26% proteins in green algae belonging to genus *Ulva*. On the contrary Dhargalkar (1986) and Chakraborthy and Santra (2008) recorded about 3-11% of protein in species of *Ulva*. Wong and Cheung, (2000) also reported a high Protein content in *Hypnea musciformis Hypnea spinella* and *Gracillaria* (>20%). According to Fleurence (1999) protein content of red seaweed ranged from 10-47%.

Yada (2004) demonstrated that the protein content of marine algae differs according to species, and is low for brown sea weeds (3-15 %), moderate in green algae (9-26%) and high for red seaweeds (up to 47%).

Thus a great variation is observed in the content of protein and several factors are responsible for this fluctuation. According to Zaragoza *et al.,* (2002) the changes in protein content are related to latitudinal temperature differences and local environmental conditions. For most of seaweeds a large part of amino acid fraction is comprised of aspartic and glutamic acids in proteins from brown sea weeds (Yada, 2004). In the present study we notices higher levels of protein in *Sargassum ilicifolium* than *Ulva fasciata* and *Gracillaria corticata*

c. Total Lipids

Total lipid content of *Ulva, Sargassum* and *Gracillaria* from west coast of Maharashtra ranged from 4.2 to 6.3 %, (Table 3) highest being in green alga *Ulva* followed by brown alga *Sargassum.*

According to Jurkovi *et al.*, (1995) lipid content in sea vegetables is very low, ranging from 1–5% of dry matter. Esteves *et al.,* (2000) studied seasonal variation in lipid content in species of *Derbesia, Ulva, Gracillaria, Hypnea, Enteromorpha, Cladophora,* which ranged from 4 to 82 $mg.g^{-1}$ dry weight. Haroon *et al.*, (2000) also reported a low lipid content in *Enteromorpha* species which remained almost stable throughout the year, ranging from 3 to 4% of dry weight. Several workers have analyzed lipid content in different seaweeds (Banaimoon, 1992; Mercer *et al.,* 1993; McDermid and Stuercke, 2003; Vierra *et al.*, 2005; Manivannan, *et al.,* 2009; Chakraborthy and Santra, 2008). As per the studies made by Herbetreau *et al.*, (1997) generally the lipid content of sea weed is less than 4%. The differences may occur due to factors such as climate and geography of development of the seaweed.

Temperature has a characteristic effect on many plant lipids in that it increases the level of unsaturation of acyl chains, which slows down both metabolism and transport (Jones & Harwood 1993). The lowering of lipid content in seaweeds of west coast of Maharashtra is might be due to high temperature of both climate and sea. Lipid content in the seaweeds under investigations was found within the recorded range.

3. Inorganic constituents

a. Total Ash

Three seaweeds *Ulva, Sargassum* and *Gracillaria* were evaluated for their ash content. The results are depicted in Table 3. Total Content of ash in green algae was higher as compared to brown and red seaweeds. *Sargassum ilicifolium* exhibited a higher ash content (15.9%) than *Ulva fasciata* (7.5%) and *Gracillaria corticata* (10.8 %).

Ash is the most abundant component of dried material in all species. Studies on the chemical composition of seaweeds have shown that ash is a good source of minerals and trace elements (Rao and Tipnis, 1967).

McDermid and Stuercke (2003) studied ash content of algal species from Chlorophyceae, Phaeophyceae and Rhodophyceae species. They recorded a greater range in ash values in the green algae (22- 64.3%) than in brown (28-

Table 4. Inorganic constituents in seaweeds.

Element*	*Ulva*	*Sargassum*	*Gracillaria*
Macroelements			
N	14350	9140	9080
P	3125	3500	5500
K	3125	4640	2819
Ca	3940	4320	4337
Mg	3478	3348	3568
Microelements			
Fe	05.38	18.34	13.76
Mn	02.30	09.62	03.64
Cu	01.16	01.20	00.92
Zn	04.52	06.40	0.96
Ni	oo.74	01.20	01.02

All values in mg/100g.dry weight basis

32%). Pattama and Chirapart (2006) also reported high ash content in *Ulva* and *Caulerpa*, the green algal species.

It is usually noticed that the ash content of seaweeds is much higher than those of terrestrial vegetables other than spinach (Ruperze et al., 2002; Sanchez-Machado *et al.,* 2004). A great variation in ash content has been reported by different workers in a variety of seaweed species (Wong and Cheung, 2000; *Zubia et al.* 2003; Vieraa *et al.*, 2005; Vega- 2006; Marinho *et al.*, 2007 and McDermid *et al.,* 2007).

b. *Macroelements*

Essential mineral elements are usually classified as macronutrients and micronutrients, according to their relative concentration in plant tissue. In some cases, the differences in tissue content of macronutrients and micronutrients are variable. Many elements often are present in concentrations greater than the plant's minimum requirement.

Minerals composition of the three seaweeds from west coast of Maharashtra is depicted in Table 4.

i. Total Nitrogen

Total nitrogen content in three seaweed species varied from 9.08 to 14.35% dry weight, being maximum in *Ulva fasciata.*

With the expectation of carbon, hydrogen and oxygen, N is the most prevalent, element in the living organism. It is invariably found in such essential compounds as proteins, nucleic acids, some of the plant growth regulators (IAA and Cytokinins) and in many of the vitamins. As a compound of these and many other compounds, nitrogen is involved in most of the biochemical reactions. Since nitrogen insufficiency is probably a single major factor limiting crop growth and yields, all our fertilizer practices are ultimately based on proper and reasonable application of theses elements in various forms.

Lourenço *et al,* (2006) analysed nitrogen content in ten species of seaweeds (6 green and 4 red algae) and reported a high N content in *Bostrychia radicans* and *Grateloupia doryphora* (red algae) and lower in *Cladophora rupestris* and *Codium decorticatum* (green algae). Nitrogen contents surveyed in seaweed species from Hiroshima Bay, Inland Sea ranged from 0.71-3.5% (Yoshida, 2001).

ii. Phosphorus

In the present study a high phosphorus content (5.5%) was noticed in *Gracillaria corticata* (red algae) as compared to that in brown and green algae (Table 4).

Phosphorus content in seaweeds of *Undaria pinnatifida* and *Aminaria digitata* has been reported by Kolb *et al,* (2004). According to Fuller and Roger (1952) plants respond well to phosphorus applied as algal fertilizer than to inorganic phosphates. Vicente *et al.* (1980) reported values for phosphorus in *Thallassia testudinum* that were similar to the levels of these minerals in *Hypnea decipiens* and *Hypnea hawaiiana*.

Phosphorus is an integral component of important compounds of plant cells, including DNA, RNA, sugar–phosphate intermediates of respiration and photosynthesis, and the phospholipids that make up plant membranes.

iii. Potassium

Potassium content was maximum in *Sargassum lilicifolium* (4.6%) as compared to *Gracillaria corticata and Ulva fasciata* (2.8 to 3.1% respectively) (Table 4). The observed concentrations were higher than in other seaweeds (1.5 to 3.9%) such as *Caulerpa lantillifra, Gracillaria parpispora, Monostroma oxysperum, Enteromorpha flexuosa* and *P. vietnamensis* (Subba Rao *et al.*, 2007). Several seasonal, environmental and physiological factors including the seaweed species, oceanic residence time, geographical place of harvest, wave exposure and types of processing, method of mineralization etc. affect the concentration of K (Honya *et al.*, 1993).

Vicente *et al.* (1980) reported values for potassium in *Thallasia testudinum* that were similar to the levels of these minerals in *Hypnea decipiens* and *Hypnea hawaiiana*. Joko *et al,* 2006 found a high K concentration in brown algae *Kappaphycus alvarezii, Turbinaria conoides,* and *Sargassum polycystum.* A large variation (32-115mg/g) was found in K- content of edible brown and red algae in Japan and Spain (Ruperez, 2002).

Among different mineral nutrients the indispensability of potassium for growth and metabolism is indisputable. K is the major plant nutrient which plays biophysical role in cellular water relation and biochemical role in a variety of metabolic processes such as protein synthesis (Hsiao and Lauchli, 1986), enzyme activities (Evans and Sorger, 1966), photoreduction and photophosphorylation (Pfiuger and Mengle, 1972), starch formation (Hawker *et* *al*., 1974). potassium plays a key role in the process of stomatal behaviour and assimilate translocation.

iv. Calcium

Calcium content in *Gracillaria corticata* was marginally higher (4.34%) than in the other two species of seaweeds.

According to Ruperez, (2002) Ca values in different edible algae ranged from 0.4-10%. Calcium is of fundamental importance for membrane functioning and the maintance of cell integrity. It is important for synthesis of pectin in the middle lamella of the cell wall. It plays an important role in the regulation of membrane permeability to various ions, in particular to inorganic cations (Van Steveninck, 1965). According to Clarkson and Hanson (1980) a major role of calcium appears to be its binding with proteins, nucleic acids, lipids and enzyme.

v. Magnesium

Magnesium content in *Gracillaria corticata* (3.5%) and *Ulva fasciata* (3.4%) was slightly more than that in *Sargassum ilicifolium* (3.3%).

In plant cells, magnesium ions (Mg2+) have a specific role in the activation of enzymes involved in respiration, photosynthesis, and the synthesis of DNA and RNA. The most well known role of Mg is its contribution to the center of the chlorophyll molecule. Magnesium stabilizes the ribosomal particles in the configuration necessary for protein synthesis and it is belived to have a similar stabilizing effect in the matrix of the nucleus (Menel and Kirkby, 1982). Mg is highly essential in many enzyme reactions (Clark, 1984).

vi. Iron

Iron content was found to be highest in *Sargassum* as compared to *Ulva fasciata* and *Gracillaria corticata*, in the present study.

Iron composition of edible seaweeds *Porphyra vietnamensis, Undaria pinnatifida* and *Aminaria digitata* varied from 1.54 to 13.7 mg/ 100g. (Kolb *et al.,* 2004 and Rao, 2006). It was also reported that Fe content in *Caulerpa racemosa*, was higher. Compared with the Japanese seaweeds *Hizikia fusiformis*, *Porphyra yezoensis* and *Enteromorpha intestinalis* the Indonesian seaweed samples had slightly lower Fe content (Yoshie *et al.* 1999).

Furthermore, Fe contents of brown algae (*Fucus vesiculosus, Laminaria digitata*, and *Undaria pinnatifida*) and red algae (*Chondrus cryspus* and *Porphyra tennera*) grown in Spain were almost the same, with ranges of 0.33 – 10.3 mg/100g dry weight, respectively (Ruperez, 2002).

According to El-Baroty (2007) the alga *Asparagopsis taxiformis* was found to be rich in iron (1.500 mg/100g). Similarly it was reported to be highest (9.6-11 mg/100g) in *Gracillaria* (Joko *et al*, 2006).

Among the various microelements iron has perhaps the greatest biological significance. This is due to its association with proteins. According to Sandmann and Boger (1983), there are two groups of well defined iron-containing proteins: hemoprotiens and iron sulfur proteins. The role of iron in the biosynthesis of chlorophyll is well established. Iron is required for the formation of protochlorophyllide from Mg-protoporphyrin (Machold and Stephan, 1969).

vii. Manganese

In the present study we noticed that manganese content (9.62 mg/100g dry weight) in *S. lilicifolium* was higher than in red and green algae.

Manganese composition of edible seaweed *Porphyra vietnamensis* is 9mg/100g dry wt. as reported by Rao (2006). According to Kolb *et al,* (2004) manganese content in of *Undaria pinnatifida* and *Aminaria digitata* varied from 0.332 to 29.4mg/100g dry weight. El-Baroty (2007) reported 1.4mg/100g manganese in *Asparagopsis taxiformis..*

Manganese ions (Mn^{2+}) activate several enzymes in plant cells. In particular, decarboxylases and dehydrogenases involved in the tricarboxylic acid (Krebs) cycle are specifically activated by manganese. The best defined function of manganese is in the photosynthetic reaction through which oxygen is produced from water (Marschner 1995).

Manganese is essential in both lower and higher plants for the Hill reaction. In chloroplast larger fraction of Mn is held in a less tightly combined state and seems to be most closely involved in oxygen evolution, whereas the smaller fraction may be more directly involved in thylakoid structure of stability (Takahashi and Asada, 1977). It is directly involved as component of biotin enzyme in the biosynthesis of fatty acids (Marschner, 1986).

b. Microelements (trace metals)

i. Copper

The content of copper was found to be very less in different seaweeds under investigation and ranged from 0.9 – 1.2 mg/100g dry weight (Table 4)

According to Yoshie *et al.* (1999), The Cu content of Indonesian seaweeds varied from 0.25 to 2.51mg/g dry weight. According to El-Baroty (2007), the alga *Asparagopsis taxiformis* was found to be rich in copper.

Kolb *et al.,* (2004) analyzed and reported copper contents in seaweeds *U. pinnatifida* and *A. digitata* (0.185 to 0.247 mg/ 100g dry weight respectively). Copper composition of edible seaweed *P. vietnamensis* is 0.83mg/100g dry wt (Rao 2006).

Copper acts as a cofactor in multiple enzymes, energy conversion etc. It is taken up by the plants in very small quantities. Copper is a component of several metalloenzymes and it appears to act as an intermediate electron acceptor in the direct oxidation of substrate by molecular oxygen (Gupta, 1979). Cu also plays an important role in maintaing membrane structure and thylakoids (Henriques, 1989). Copper is associated with enzymes involved in redox reactions being reversibly oxidized from Cu^{+} to Cu^{2+}.

ii. Zinc

Zinc content was 6.4, 4.52 and 5.96 mg/100g dry weight respectively in *Sargassum*, *Ulva* and *Gracillaria* (Table 4).

Zinc plays very important role as an enzyme catalyst. It enhances the catalytic, structural and regulatory function, stabilizes membranes, hormones and nucleic acids (Norziah and Ching, 2000). Zn is required for the activity of various types of enzymes, including aldolases, isomerases, transphosphorylases and RNA and DNA polymerases (Marschner, 1986).

Compared with the Japanese seaweeds *Hizikia fusiformis, Porphyra yezoensis* and *Enteromorpha intestinalis* the Indonesian seaweed samples had a slightly lower Zn content which ranged from 0.82-5.24 mg/100g dry weight (Yoshie *et al.* 1999).

In brown and red algae (*Fucus vesiculosus, Laminaria digitata, Undaria pinnatifida* and *Chondrus cryspus, Porphyra tennera)* Zn content ranged from 1.7- 07.1 mg/100g dry weight (Ruperez, 2002). According to El-Baroty (2007), the alga *Asparagopsis taxiformis* was rich in zinc (01.20 mg/100g).

iii. Nickel

Nickel metal content varied from 0.74–1.2 mg/100g in *Sargassum*, *Ulva* and *Gracillaria* (Table 4). Nickel contents in *Undaria pinnatifida* and *Aminaria digitata* was 0.265 and 3.25mg/ 100g dry weight, as recorded by Nada Kolb *et al,* 2004. In *Porphyra* it was 0.29mg/100g (Rao, 2006). Ni is a relevant element and is of immediate concern due to their potential toxicity for living organisms.

Urease is the only known nickel-containing enzyme in plants, although nitrogen-fixing micro-organisms require nickel for the enzyme that reprocesses some of the hydrogen gas generated during fixation. Nickel- deficient plants accumulate urea in their leaves and, consequently, show leaf tip necrosis.

In the present study low levels of Ni content is beneficial as far as theses extract are sprayed on vegetables which may reduce the chances of Ni toxicity.

C. Seaweed Application to Trigonella foenum-graecum

1. Germination

Effect of fresh extract, of the three seaweeds viz. *Ulva fasciata, Sargassum lilicifolium* and *Gracillaria corticata* at various concentrations (5 - 100%) on the germination of fenugreek was analysed in a petri plate experiment. It was noticed that the fresh extract up to 50 - 60% promoted germination in fenugreek. At higher concentration slowly the rate of germination declined and was completely inhibited at 100% concentration of all the seaweeds. A cent percent germination was recorded on the third/ fourth day after treatment for the concentrations ranging from 5 to 50% which was not seen in control. This trend was similar to that occurred for the Hoagland treated seeds which also exhibited 100% germination after the third day of treatment (Table 5, 6 and 7).

When extract was prepared by boiling dried seaweed in d.w. and applied to fenugreek seeds a similar influence on germination was observed in the present study. The concentrations which caused a beneficial effect on germination were from 10 to 50% in all the seaweeds. Even at the lower concentration of 5% (*Ulva* and *Sargassum*) the germination percentage was greater than the control seeds.

For *Sargassum* treatment 100% germination was recorded for all the concentration upto 50% whereas in case of other two extracts it was slightly below 100% for lower concentrations.

Table 5: Effect of SWC of *U. fasciata* on seed germination of *T. foenum-graecum*.

Concentration / Days	Fresh extract				Boiled extract				Soaked Extract			
	1	2	3	4	1	2	3	4	1	2	3	4
Control	00	43	67	78	00	43	67	78	00	52	88	100
5%	--	07	70	10	00	57	66	80	00	08	68	90
10%	--	68	76	100	00	77	82	90	19	67	99	100
20%	01	77	96	100	38	69	79	98	31	92	99	100
30%	12	54	100	100	47	70	90	100	29	80	89	100
40%	23	62	100	100	48	70	98	100	30	69	89	100
50%	22	59	100	100	52	67	99	100	29	88	98	100
60%	02	08	38	100	00	00	89	90	10	55	85	89
70%	--	01	03	03	00	00	38	39	00	22	33	51
100%	--	--	01	01	00	00	00	00	00	00	00	00
Hoagland*	48	87	100	100	48	87	100	100	48	87	100	100

Table 6: Effect of SWC of *S. ilicifolium* on seed germination of *T. foenum-graecum*.

Concentration / Days	Fresh extract				Boiled extract				Soaked Extract			
	1	2	3	4	1	2	3	4	1	2	3	4
Control	00	43	67	78	00	43	67	78	00	52	88	100
5%	00	69	94	100	00	67	89	100	11	68	70	99
10%	19	78	100	100	19	68	99	100	22	66	69	100
20%	29	72	100	100	29	70	90	100	18	36	69	100
30%	27	89	100	100	27	79	92	100	30	68	90	100
40%	38	88	100	100	38	78	98	100	40	88	98	100
50%	29	80	100	100	29	78	100	100	39	66	69	100
60%	00	19	28	42	00	21	39	42	30	50	90	90
70%	00	00	22	32	00	00	32	32	20	20	20	20
100%	00	00	00	00	00	00	00	00	00	00	00	00
Hoagland*	48	87	100	100	48	87	100	100	48	87	100	100

Values represent percent germination
* Full strength

When extract obtained after soaking the seaweed, in d.w. was used for treatment, more or less a similar trend was observed. Germination was influenced upto 50% of concentration and later on a reduction in germination percentage occurred in all the seaweeds used.

A proper establishment of crop stand mainly depends on the successful seed germination. Seed germination thus, represents the most important phase in the life cycle of crop plants and it is regulated by several environmental factors. Various types of chemicals and biofertilizers are used for enhancing germination, growth and yield of crop plants. Hoagland is one of the nutrient media used for growing plants under laboratory condition for studying several physiological aspects.

Wilczek *et al.* (1982) reported that seaweed extracts improved seed germination in table beet. Improved seed germination in faba bean due to seaweed extracts has been reported by El-Sheekh and El-Saled (2000). Hong (2007) demonstrated that the seeds soaked in aqueous extract of *S. wightii* performed better than the water-soaked controls. According to Moller and Smith (1999) under optimal conditions, seaweed extract shows a limitation in seed germination, but under stress condition seed germination is more prominent. A wide range of beneficial effects of seaweed extract applications on plants, including seed germination and establishment have been reported, (Beckett and van Staden 1989; Blunden 1991)

Table 7: Effect of SWC of *Gracillaria corticata* on seed germination of *T. foenum-graecum*.

Concentration / Days	Fresh extract				Boiled extract				Soaked Extract			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Control	00	43	67	78	00	43	67	78	00	52	88	100
5%	01	48	86	100	00	45	66	69	00	71	89	90
10%	02	58	93	100	00	65	70	90	18	68	69	90
20%	13	66	99	100	07	60	78	100	31	79	99	99
30%	16	89	100	100	08	68	90	100	30	96	99	100
40%	26	94	100	100	08	70	79	100	29	84	99	100
50%	20	97	100	100	24	94	99	100	33	73	92	100
60%	18	46	46	46	10	10	20	20	28	60	70	70
70%	07	11	11	11	00	00	00	00	12	22	23	24
100%	00	00	00	01	00	00	00	00	00	00	00	00
Hoagland*	48	87	100	100	48	87	100	100	48	87	100	100

Values represent percent germination
* Full strength

Demir *et al*., (2006) studied the effect of seaweed suspension of three alage namely *Codium tomentosum*, *Gracillaria gracilis* and *Cystoseria barbata* on seed germination of tomato, pepper and aubergine. They found that pepper seeds treated with green seaweed had higher germination than those of others and the percentage recorded was 52% and 68% at 15°C and 25 °C respectively. Further the germination percentage of aubergine and tomato seeds treated with green seaweeds was much higher at low temperature (91% and 99%, respectively).

According to El-Sheekh and El-Saled (2000) green algae have a higher cytokinin content than red seaweeds while Jennings (1968) reported that green algae and brown algae contain gibberlic acid which play important role in seed germination.

Effect of different seaweed concentrates on the germination and growth was analysed by growing the plants in a mixture of coco-peat and soil (1:1) in plastic trays. A few selected concentrations of seaweed liquid fertilizers were used for this study. Results of germination studies are presented in Tables 8,9 and 10.

In control seeds of fenugreek no germination was observed on the first day. But fresh extracts of *Sargassum* and *Gracillaria* (10 and 25%) and *Ulva*, (25%) gave upto 20% germination on the first day. On the third day a cent percent germination was recorded in all the treated seeds when control seeds had 90% germination.

Table 8: Effect of selected concentrations of *U. fasciata* on seed germination of *T. foenum-graecum.*

Sr. No.	Concentration	Germination % after day											
		Fresh extract				Boiled extract				Soaked Extract			
		1	2	3	4	1	2	3	4	1	2	3	4
1	Control	00	70	90	100	00	40	70	100	00	50	70	100
2	10%	00	80	100	100	10	50	80	100	00	50	80	100
3	25%	10	70	100	100	20	70	90	100	40	60	80	100
4	50%	00	80	100	100	30	60	100	100	30	90	100	100
5	Hoagland	20	09	100	100	40	80	100	100	40	90	100	100

Table 9: Effect of selected concentrations of *S. ilicifolium* SWC on seed germination of *T. foenum-graecum.*

Sr. No.	Concentration	Germination % after day											
		Fresh extract				Boiled extract				Soaked Extract			
		1	2	3	4	1	2	3	4	1	2	3	4
1	Control	00	70	90	100	00	40	70	100	00	50	70	100
2	10%	10	80	90	100	10	80	90	100	00	60	90	100
3	25%	10	80	100	100	30	70	90	100	10	60	100	100
4	50%	00	70	100	100	40	80	100	100	20	80	100	100
5	Hoagland	20	90	100	100	40	80	100	100	40	90	100	100

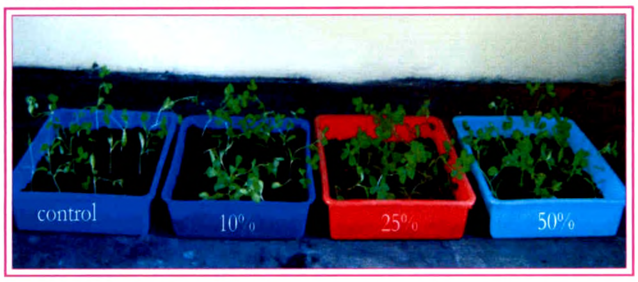
Table 10: Effect of selected concentrations of *G. corticata* SWC on seed germination *of T. foenum-graecum.*

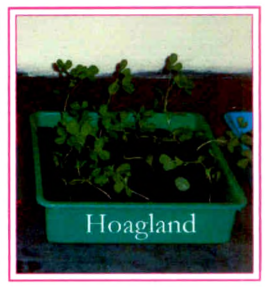
Sr. No.	Concentration	Germination % after day											
		Fresh extract				Boiled extract				Soaked Extract			
		1	2	3	4	1	2	3	4	1	2	3	4
1	Control	00	80	90	100	00	40	70	100	00	50	70	100
2	10%	10	70	100	100	00	30	70	100	00	70	90	100
3	25%	20	50	100	100	20	60	90	100	10	70	100	100
4	50%	00	60	100	100	30	70	100	100	30	90	100	100
5	Hoagland	20	90	100	100	40	80	100	100	40	90	100	100

Seeds were sown in coco peat and soil mixture in plastic tray.

Plate VII : Effect of fresh SWC on *Trigonella foenum-graecum*

A. *Ulva* SWC treatment





B. *Sargassum* SWC treatment

C. *Gracillaria* SWC treatment

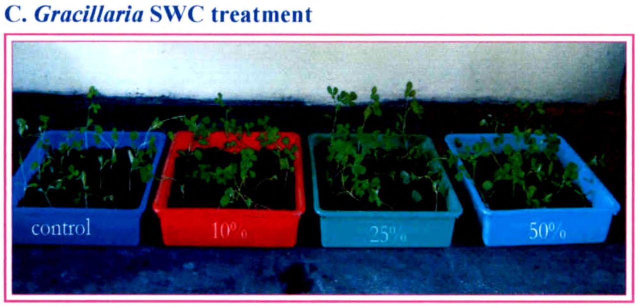


Plate VIII : Effect of boiled SWC on *Trigonella foenum-graecum*

A. *Ulva* SWC treatment

B. *Sargassum* SWC treatment

C. *Gracillaria* SWC treatment

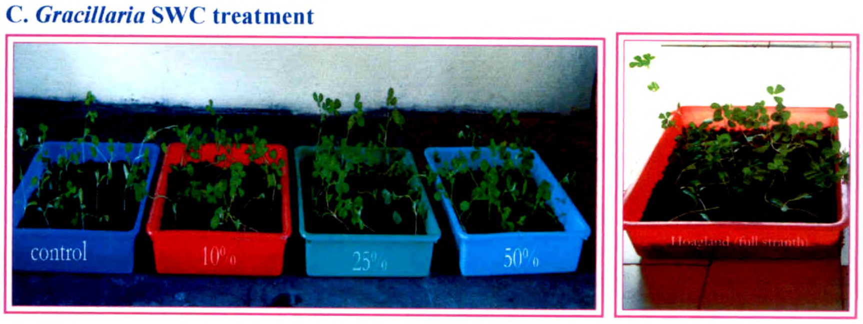


Plate IX : Effect of water soaked SWC on *Trigonella foenum-graecum*

A. *Sargassum* SWC treatment

B . *Ulva* SWC treatment

C. *Gracillaria* SWC treatment

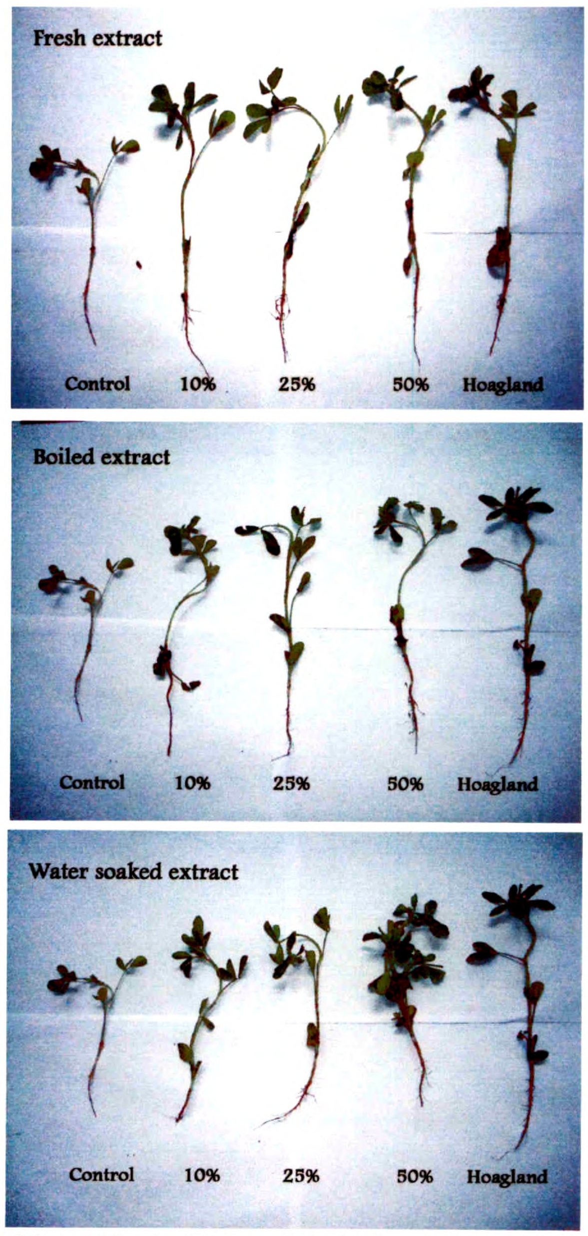


Plate X : Effect of *Ulva fasciata* on growth of *Trigonella foenum-graecum*

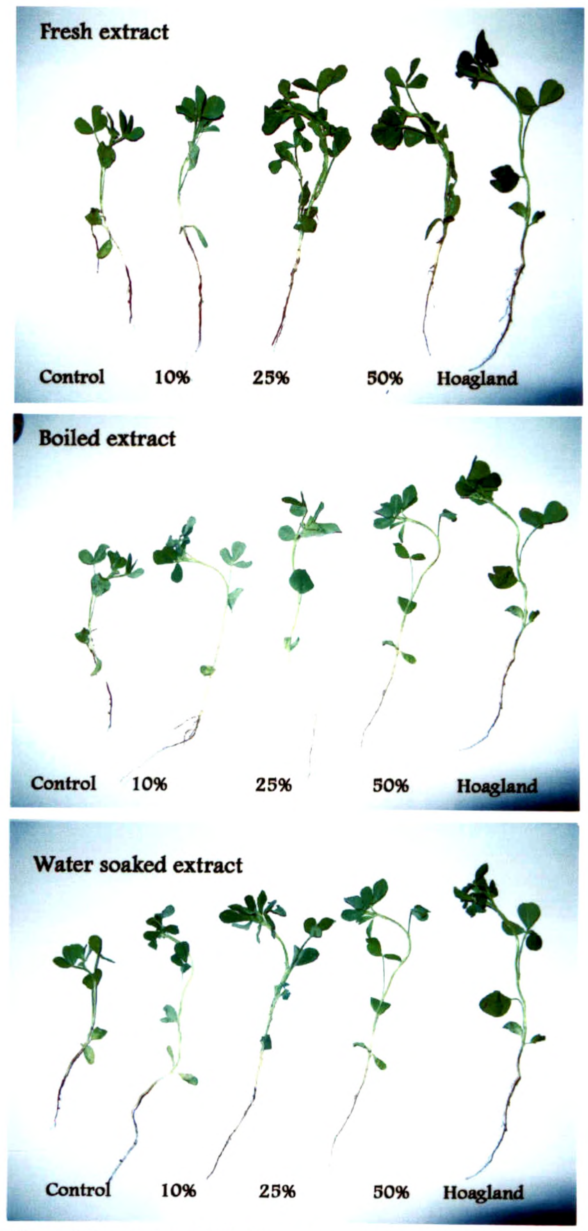


Plate XI :Effect of *Sargassum ilicifolium* on growth of *Trigonella foenum-graecum*

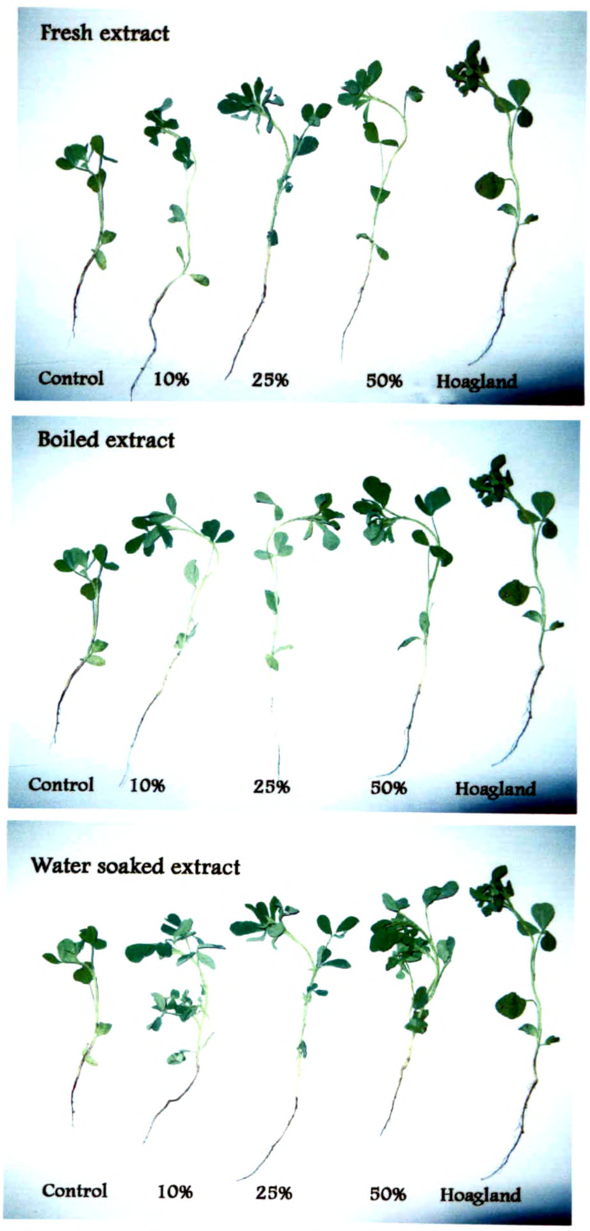


Plate XII :Effect of *Gracillaria corticata* on growth of *Trigonella foenum-graecum*

When boiled extract was used for treatment, the number of seed germinated on the first day was increased upto 40% and this was similar to the Hoagland treated seeds. At 50% boiled extract of all the seaweeds 100% germination occurred on the third day which was more than that in control but simillar to Hoagland.

Soaked extract of seaweeds also promoted germination giving cent percent values for 25 and 50% extract of *Sargassum* and *Gracillaria* on the third day of treatment. The lowest concentration used for treatment (10%) also influenced germination in fenugreek but the effect was visible slowly and 100% germination took place on the fourth day.

Thus all the types of exacts, fresh, boiled and soaked positively influenced seed germination in fenugreek.

2. Growth

a. Shoot Growth

Effect of fresh, boiled and soaked extracts of seaweeds on the growth of fenugreek was investigated using a few selected concentrations viz. 10, 25 and 50%. As the concentration of treatment increased, growth also was found promoted. Maximum height of the plants was recorded for 50% concentration of all the fresh seaweed extracts and compared to control a significant increase was evident (Table 11). The values observed at 25% concentration were only slightly at the lower level than those at 50% extract in all the seaweeds. The rise in the height of plants was more or less similar for all the respective treatments of different seaweeds. The growth of seaweed treated plants was

Table 11: Effect of fresh SWC on the shoot length of *T. foenum-graecum*.

Days	*Ulva*			*Sargassum*			*Gracillaria*			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10 %	25%	50%		
5	2.4	2.8	2.8	2.2	2.8	2.7	2.3	2.6	2.8	1.69	1.74
10	3.7	3.8	3.9	3.8	3.9	4.1	3.7	4.1	4.1	3.31	4.54
15	7.6	7.9	8.2	7.8	7.9	8.5	7.7	8.4	8.3	4.23	8.75
20	10.1	10.4	10.4	10.2	10.5	10.5	10.1	10.2	10.5	4.62	10.82
25	12.4	12.6	12.7	12.5	12.4	12.8	12.1	12.4	12.9	9.25	13.60

(Height of the plant in c.m.)

Table 12: Effect of boiled SWC on the shoot length of *T. foenum-graecum*.

Days	*Ulva*			*Sargassum*			*Gracillaria*			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10 %	25%	50%		
5	1.59	1.68	1.83	1.68	1.94	2.70	1.7	2.2	2.8	1.69	1.74
10	3.39	3.47	3.68	3.39	3.57	3.80	3.9	3.5	3.9	3.31	4.54
15	5.53	5.73	6.65	4.19	4.70	4.90	4.5	7.2	7.8	4.23	8.75
20	6.41	7.39	8.02	5.18	5.99	8.30	5.1	9.9	8.8	4.62	10.82
25	10.92	10.92	13.10	9.54	9.75	10.25	10.7	11.8	12.1	9.25	13.60

(Height of the plant in c.m.)

Table 13: Effect of water soaked SWC on the shoot length of *T. foenum-graecum*.

Days	*Ulva*			*Sargassum*			*Gracillaria*			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10 %	25%	50%		
5	1.55	2.1	2.7	1.9	2.2	2.1	1.4	2.2	1.22	1.69	1.74
10	3.22	3.4	3.9	3.51	3.7	4.1	3.7	4.0	2.87	3.31	4.54
15	5.00	6.9	7.7	4.8	4.7	7.5	4.7	5.3	4.03	4.23	8.75
20	5.73	8.2	8.5	5.8	6.1	9.8	5.5	7.9	7.74	4.62	10.82
25	10.28	11.9	12.7	10.12	10.2	12.6	11.2	12.1	12.40	9.25	13.60

(Height of the plant in c.m.)

Fig 1. Effect of *Ulva fasciata* SWC on growth of *Trigonella foenum-graecum*

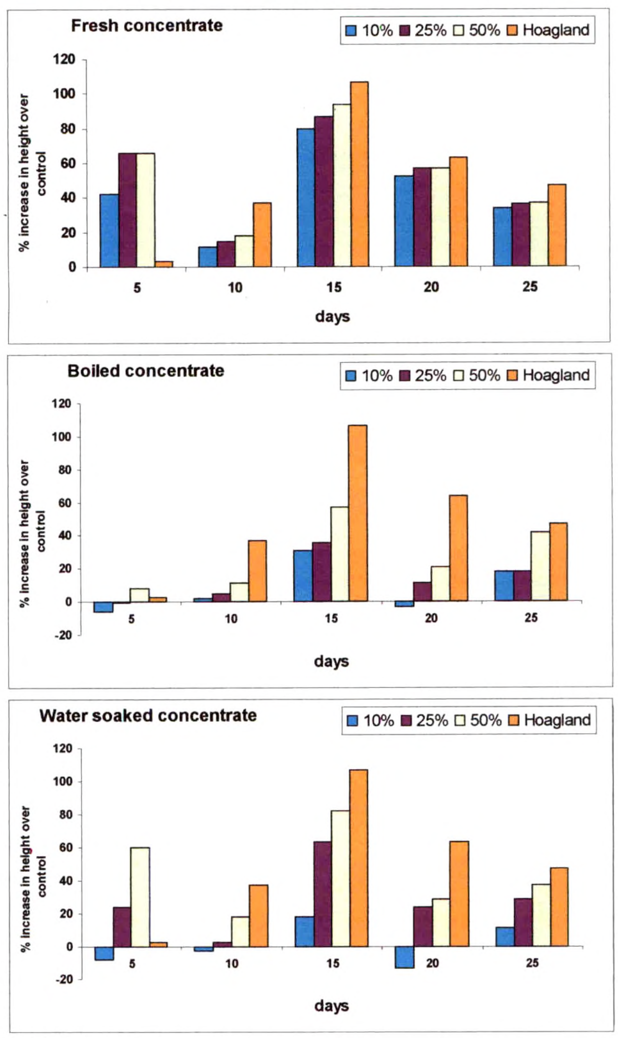


Fig 2. Effect of *Sargassum ilicifolium* SWC on growth of *Trigonella foenum-graecum*

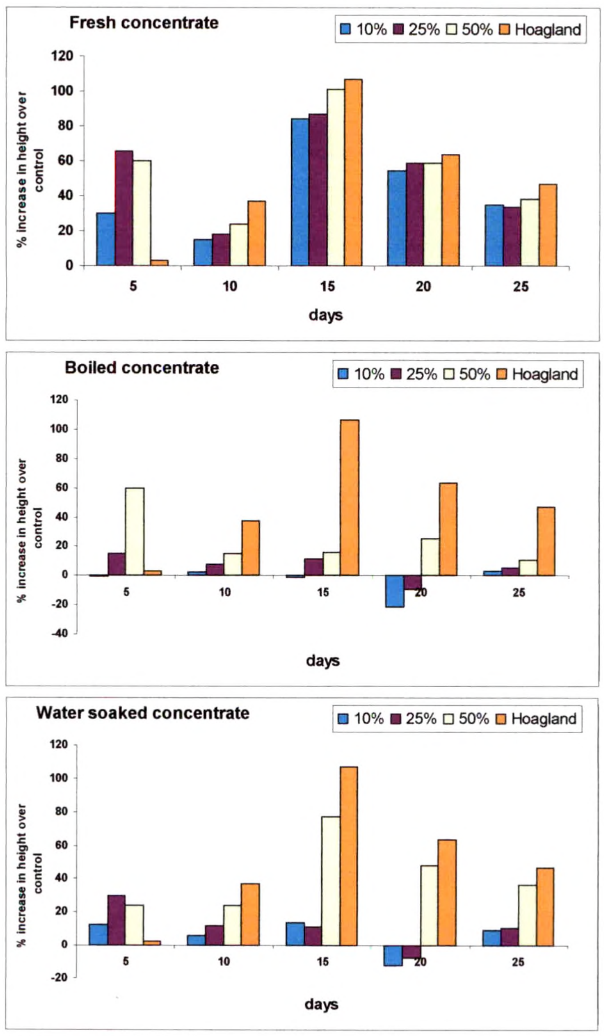
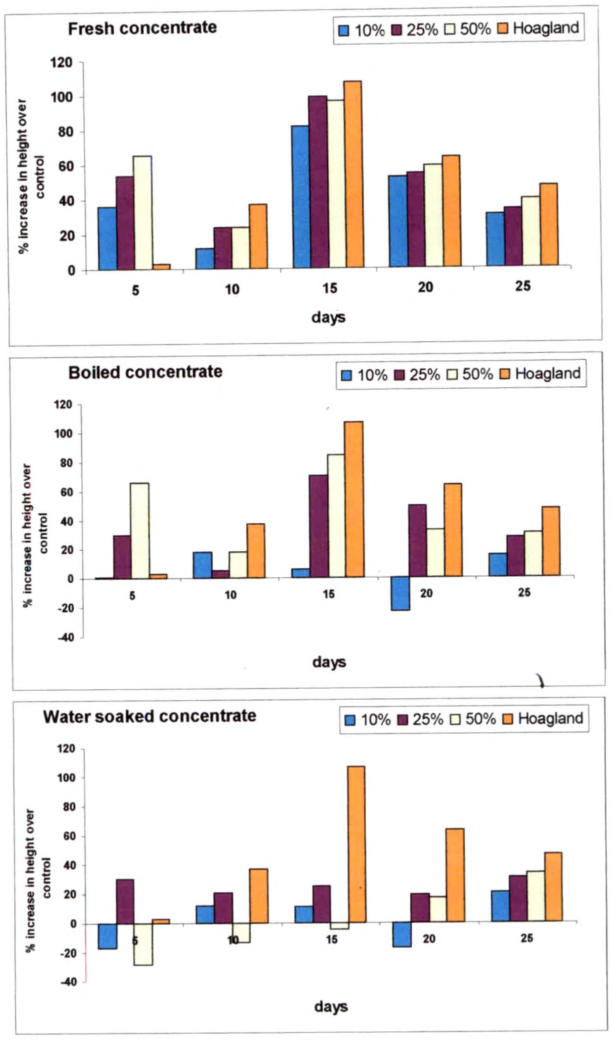


Fig 3. Effect of *Gracillaria corticata* SWC on growth of *Trigonella foenum-graecum*



comparable to those of Hoagland treated ones and only a marginal difference was present in growth values recorded. This indicated a positive influence of seaweed extract on the growth of fenugreek.

Boiled seaweed extract was found less effective than the fresh one however the length of shoot in treated plants was more than the control plants and this was true for all the three extracts prepared after boiling the seaweeds (Table 12). The concentration of 50% was more effective than 10 and 25% and when compared to the Hoagland the growth values were at the lower level. The best response was obtained for *Ulva* extract followed by *Gracillaria* and then *Sargassum* (Plates 7,8 & 9). Thus after boiling nutrient loss might be more in *Sargassum* and *Gracillaria* as compared to *Ulva.*

Results of soaked seaweed extract on the height of fenugreek are presented in Table 13. All the three concentrations promoted shoot growth in fenugreek over that of control and the values were comparable to the Hoagland treatment applied at full strength. *Gracillaria* extract exhibited better response than the other two soaked SWC. However no much variation was evident in the growth readings.

Zodape *et al.* (2009) studied the effect of liquid seaweed fertilizer of brown seaweed *Kappaphychus alvarezii* on *Abelmoschus esculantum* and wheat var. GW322 and found that growth, yield of grain and quality was greatly influenced. The brown algae, like *Ascophyllum nodosum* and *Laminaria hyperborean* are generally used for preparation of commercial seaweed concentrates. However, in experimental studies it was shown that growth of seedlings was stimulated by the crude extracts of green (*Cladophora* *dalmatica, Enteromorpha intestinalis, Ulva lactuca, Caulerpa chemnitzia*), brown (*Sargassum wightii*) and red algae (*Corallina mediterranea, Jania rubens, Pterocladia pinnate*) (El-Skeekh and El- Saled, 2000 and Sivasankaria *et al.*, 2006)

Seaweed products exhibit growth stimulating activities, and the use of seaweed formulations as biostimulants in crop production is well established. Biostimulants are defined as materials other than fertilizers that promote plant growth when applied in small quantities and are also referred to as metabolic enhancers (Zhang and Schmidt, 1997). Seaweed components such as macro- and microelements, amino acids, vitamins, cytokinins, auxins, and abscisic acid like growth substances affect cellular metabolism in treated plants leading to an enhanced growth and crop yield (Crouch and van Staden, 1992, 1993; Reitz and Trumble, 1996; Durand *et al.,* 2002 and Stirk *et al.,* 2002). Although many of the various chemical components of seaweed extracts and their modes of action remain unknown, it is possible that these components exhibit synergistic activity (Fornes *et al.*, 2002 and Vernieri *et al.,* 2005). The effect of SWC on plant growth often depends on the concentration used (Van Satnden *et al.*, 1994) and on the mode of application (Crouch and Van Satnden, 1992, 1993 and Atzmon & Van Satnden, 1994).

Influence of SWC on seedling establishment, rooting, flowering, fruit production and yield has been reviewed extensively (Crouch & Van staden, 1994). In tomato not only seedling growth, yield and fruit ripening were improved, but also resistance to infection with *Meloidogyne incognita* (Crouch

& Van Satnden, 1993). SWC also enhanced the growth of potato plantlets *in vitro* (Crouch & Van Satnden, 1991).

According to Taylor and Wilkinson, (1977), increased seedling growth may be due to the presence of phenyl acetic acid (PAA) and other closely related compounds (P-CH-PAA) in the SWC as well as the presence of some growth promoting substances. In *Cajanus cajan* and *Vigna radiata* the higher concentrations of S.L.F. showed a decreased growth and maximum seedling growth was observed at lower concentrations (Mohan *et. al.,* 1994 and Kumar *et al.*, 1993). Dhargalkar and Untawale (1983) also reported similar findings with *Hypnea musciformis, Spatoglossum asperum, Stoechospermum marginatum* and *Sargassum* on the growth of crops such as green chillies, turnips and pineapple.

Seaweed fertilizers contained sufficient amount of trace elements in available form, as there were no symptoms of deficiency found under the conditions of the experiment. The growth was maintained well at all the concentration of seaweed fertilizer in the present study. Fresh seaweed extracts at 50% concentration can be applied to the leafy vegetables for increasing shoot growth.

Percent increase in shoot height of fenugreek after treatment was analyzed. The effect of fresh extract of all the seaweeds showed maximum promotion in height in fenugreek. The rate of increase was maximum 15th day of growth and later on a decline was reported in all the seaweed extracts (Fig. 1,2&3). The rate of shoot growth at 50% concentration was maximum in all the types of extracts and seaweeds and also comparable to that of Hoagland treated plants. The growth of individual fenugreek plants as affected by various seaweed treatments is displayed in Plates 10,11 and 12.

b. Fresh weight:

Effect of seaweed fertilizers on fresh weight of fenugreek is recorded in the Tables 14, 15 and 16. All the three types, i.e. fresh, boiled and soaked seaweed extracts enhanced fresh weight of treated plants as compared to control. As the concentration of seaweed concentrate increased the fresh weight per plant also increased significantly, in case of all the seaweeds. The values recorded were greater than 1 g per plant for 50% concentrate of all the seaweeds whereas in control plants, they were 0.711g per plant. Lower concentrations of SWC also enhanced the fresh weight of fenugreek plants.

Zodape *et al*., (2008) reported a decline in fresh weight at higher concentrations of *K. alvarezii* seaweed liquid fertilizers (10%) in *Abelmoscus esculantum*. A positive correlation between the concentration of seaweed fertilizer of *Codium liyengarill* and fresh weight per plant was observed by Zahid (1999) in gram and spinach.

c. Dry weight:

Effect of seaweed fertilizers on dry weight of fenugreek is depicted in Tables 1, 15 and 16. As was noticed in case of fresh weight the concentration of 50% of water soaked and boiled extract of *Gracillaria* greatly influenced the dry weight of fenugreek, which was even greater than that recorded for Hoagland. Among the three seaweeds, soaked and boiled extract of *Ulva* and *Gracillaria* were quite effective than the *Sargassum*. It was also found that the dry weight was directly proportional to the concentration of seaweed extract

Fig 4. Effect of *Ulva fasciata* SWC on fresh and dry weight of *T. foenum-graecum* in percent variation.

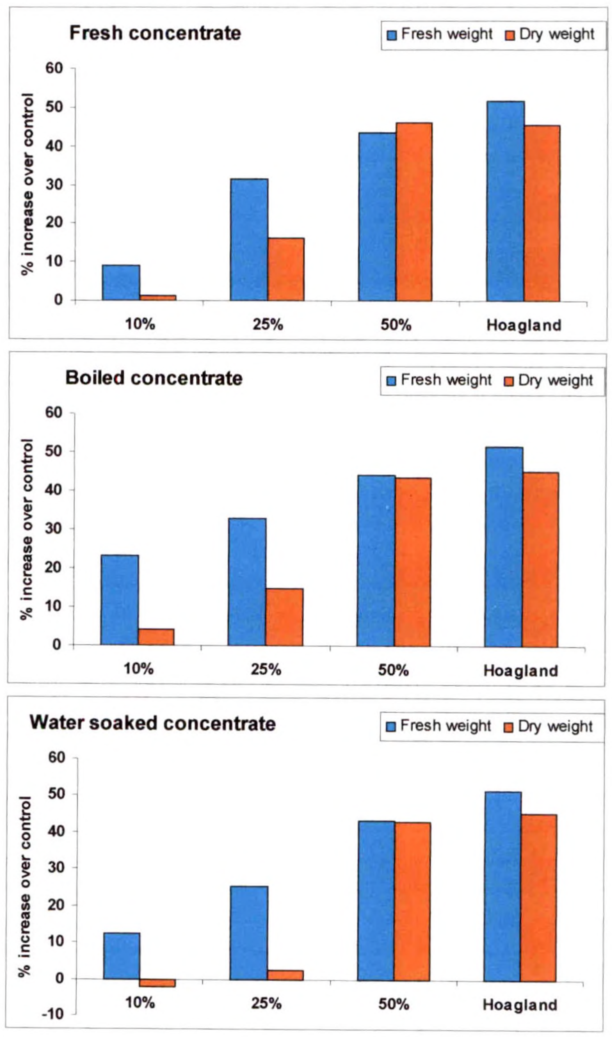


Fig 5. Effect of *Sargassum ilicifolium* SWC on fresh and dry weight of *T. foenum-graecum* in percent variation.

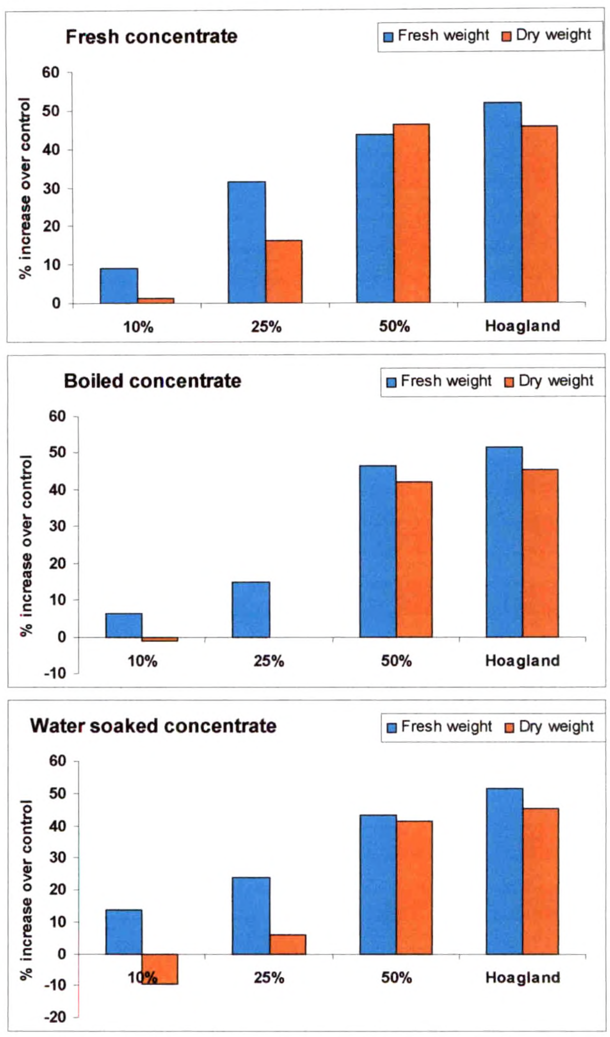
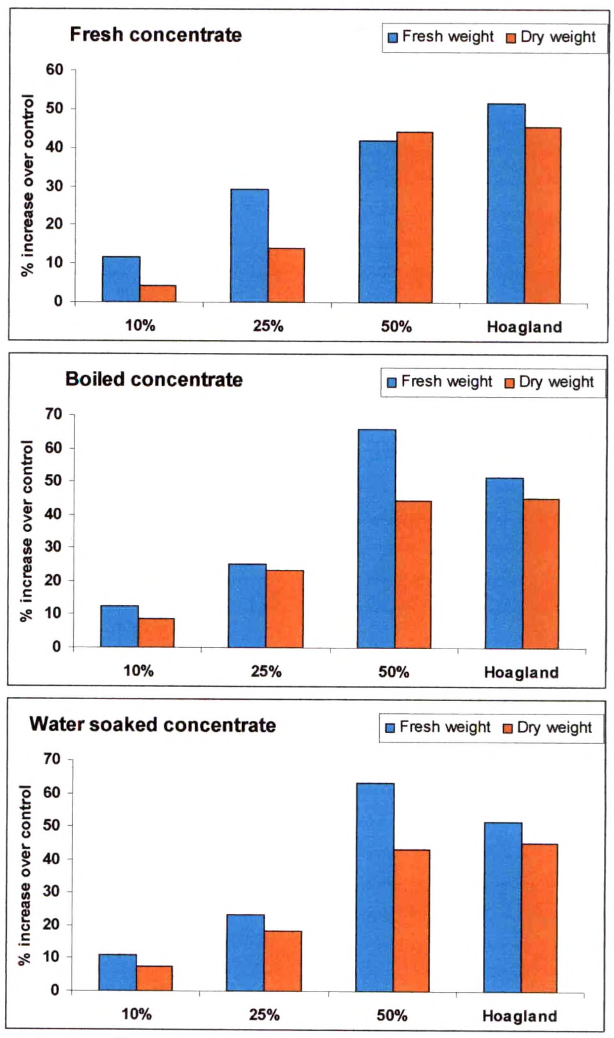


Fig 6. Effect of *Gracillaria corticata* SWC on fresh and dry weight of *T. foenum-graecum* in percent variation.



and A loss in dry weight by treatment of (10%) of *Kappaphycus alvarezii* liquid fertilizers to *Abelmoscus* has been recorded by Zodape *et al.,* (2008). Zahid (1999) also reported a decrease in dry weight per plant with increase in seaweed concentration of *Codium lyengarli* in gram and sunflower. In our studies, a rise in dry matter is reported for all the SWC treatments.

d. Moisture percentage

Content of moisture declined as the concentration of treatment increased. A minimum moisture percentage was found in the fenugreek plants treated with 50% of all SWC. However it was greater than that in control plants in most of the treatments. Due to increased amount of cellular ingradients, i.e. organic materials, a reduction in the moisture percentage was quite obvious in the present study.

Effect of individual seaweeds on fresh and dry weight of fenugreek is figured graphically. Fresh, boiled and soked concentrate of *Ulva fasciata* caused about 45-50% increase in the fresh biomass and dry matter content of fenugreek plants when applied at 50% rate and the results were well comparable to those of Hoagland. (Fig 4). Quite similar observations were reported for 50% fresh and boiled concentrate of *Sargassum ilicifolium* (Fig.5). Soaked extract of *Sargassum* showed a slightly less stimulation at 50% rate. Water soaked and boiled extracts of *Gracillaria corticata* at 50% significantly enhanced the fresh weight of fenugreek and a rise of 70%, giving higher values even than the Hoagland treated plants was recorded (Fig 6). However stimulation in dry matter content was less than 50% for all the types of concentrates of *Gracillaria*. as the concentration increased the dry weight per plant also increased.

Table 14. Effect of *Ulva* SWC on biomass of *T. foenum-graecum.*

Sr. No.	Parameter	fresh extract			boiled extract			soaked extract			control	Hoagland
		10%	25%	50%	10%	25%	50%	10%	25%	50%		
1	Fresh weight (g/plant)	0.775	0.935	**1.020**	0.875	0.944	**1.025**	0.799	0.890	**1.018**	0.711	1.078
2	Dry weight (g/plant)	0.237	0.272	**0.342**	0.244	0.269	**0.336**	0.229	0.240	**0.335**	0.234	0.340
3	Moisture %	69.03	70.90	**66.47**	72.11	71.50	**67.21**	71.33	73.03	**67.09**	65.82	68.46

Table 15. Effect of *Sargassum* SWC on biomass of *T. foenum-graecum.*

Sr. No.	Parameter	fresh extract			boiled extract			soaked extract			control	Hoagland
		10%	25%	50%	10%	25%	50%	10%	25%	50%		
1	Fresh weight (g/plant)	0.775	0.935	**1.015**	0.757	0.816	**1.040**	0.810	0.880	**1.019**	0.711	1.078
2	Dry weight (g/plant)	0.240	0.272	**0.334**	0.231	0.234	**0.332**	0.212	0.248	**0.331**	0.234	0.340
3	Moisture %	69.03	70.90	**67.70**	69.48	70.58	**68.07**	73.82	71.81	**67.51**	65.82	68.46

Table 16. Effect of *Gracillaria* SWC on biomass of *T. foenum-graecum.*

Sr. No.	Parameter	fresh extract			boiled extract			soaked extract			control	Hoagland
		10%	25%	50%	10%	25%	50%	10%	25%	50%		
1	Fresh weight (g/plant)	0.794	0.918	1.009	0.799	0.889	**1.027**	0.789	0.877	**1.020**	0.711	1.078
2	Dry weight (g/plant)	0.244	0.267	0.337	0.254	0.289	**0.388**	0.252	0.277	**0.382**	0.234	0.340
3	Moisture %	69.26	70.91	66.50	68.21	67.49	**62.22**	68.06	68.41	**62.54**	65.82	68.46

Values are averge of three detreminations.

3. Biochemical analysis of T. foenum-graecum

a. Photosynthetic pigments

Effect of seaweed concentrates on photosynthetic pigments of *Trigonella foenum-graecum* is depicted in Tables. 17, 18 and 19.

All the seaweed concentrates had a positive effect on the photosynthetic pigments at all the concentrations used. But comparatively, the fresh seaweed concentrate had shown a better effect than the boiled and water soaked concentrates. The amount of chl.a, chl.b and total chlorophyll levels enhanced by about 5–10% over control in different treatments. The treatment of 50% seaweed concentrate has shown significant results and a maximum increase in total chlorophyll content was recorded at this concentration with *Sargassum ilicifolium* application. Other two fresh SWC (*Ulva* and *Gracillaria*) also caused a similar influence at 50% concentration and these values were very near to those reported in Hoagland treated plants (Fig. 7).

Influence observed by the treatment of boiled SWC was less but the values were maximum at 50% SWC of *Sargassum ilicifolium* and much greater than the control plants (Fig. 5). Water soaked SWC of *Sargassum ilicifolium* and *Ulva fasciata* stimulated the chlorophyll content in fenugreek at 50% SWC and values though were less than those in Hoagland treated plants, were higher than in control (Fig. 6).

Table 17: Effect of fresh SWC on photosynthetic pigments of *T. foenum-graecum*.

Concentrations		Photosynthetic Pigments			
		Chl.a	**Chl.b**	**Total Chl.**	**Carotenoid**
Ulva	10%	56.4	77.1	133.0	12.69
	25%	56.8	77.2	133.8	12.96
	50%	**57.2**	**81.6**	**138.9**	**13.12**
Sargassum	10%	53.0	70.4	128.3	12.88
	25%	56.8	82.5	139.1	13.08
	50%	**56.7**	**86.0**	**142.6**	**13.16**
Gracillaria	10%	56.6	79.7	136.2	12.60
	25%	56.6	80.3	136.9	12.72
	50%	**57.1**	**83.7**	**140.6**	**12.80**
Control		53.1	72.2	125.2	11.88
Hoagland		**57.6**	**86.5**	**144.0**	**13.16**

Table 18: Effect of boiled SWC on photosynthetic pigments of *T. foenum-graecum*.

Concentrations		Photosynthetic Pigments			
		Chl.a	**Chl.b**	**Total Chl.**	**Carotenoid**
Ulva	10%	47.3	76.8	123.9	12.84
	25%	48.8	81.6	130.4	13.22
	50%	**50.5**	**85.0**	**135.5**	**13.36**
Sargassum	10%	47.0	77.1	123.9	12.64
	25%	48.9	83.1	132.0	12.76
	50%	**55.2**	**87.4**	**142.5**	**13.28**
Gracillaria	10%	46.9	75.2	122.0	12.76
	25%	48.7	80.8	129.5	12.80
	50%	**50.5**	**84.4**	**139.6**	**13.40**
Control		47.4	69.9	117.3	12.44
Hoagland		**57.7**	**90.2**	**147.8**	**13.60**

Tables 19: Effect of water soaked SWC on photosynthetic pigments of *T.foenum-graecum*.

Concentrations		Photosynthetic Pigments			
		Chl.a	Chl.b	Total Chl.	Carotenoid
Ulva	10%	58.6	69.4	127.8	12.6
	25%	55.5	81.0	134.9	12.6
	50%	**56.1**	**86.2**	**142.2**	**13.12**
Sargassum	10%	58.2	54.1	128.1	12.80
	25%	55.8	80.0	135.7	12.76
	50%	**56.3**	**86.7**	**142.7**	**13.20**
Gracillaria	10%	57.0	68.6	126.5	12.28
	25%	57.8	77.3	135.0	12.76
	50%	**58.3**	**81.6**	**139.9**	**13.12**
Control		47.4	69.9	117.3	12.44
Hoagland		**57.7**	**90.2**	**147.8**	**13.60**

Values in mg 100^{-1}g fresh weight

Fig 7. Effect of fresh SWC on photosynthetic pigments of *T. foenum-graecum*

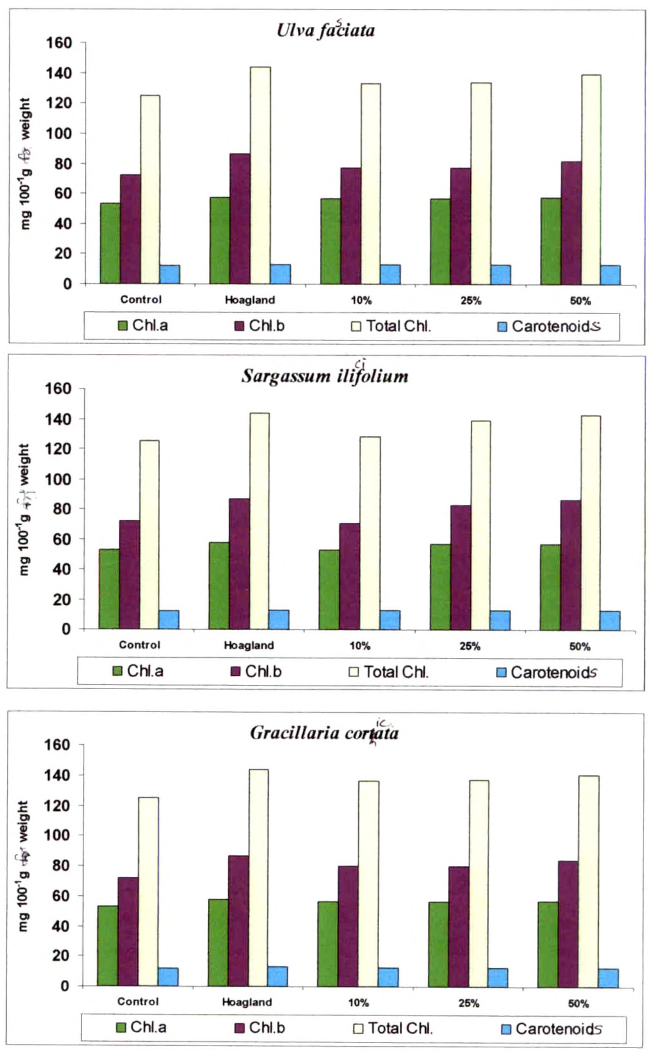


Fig 8. Effect of boiled SWC on photosynthetic pigments of *T. foenum-graecum*

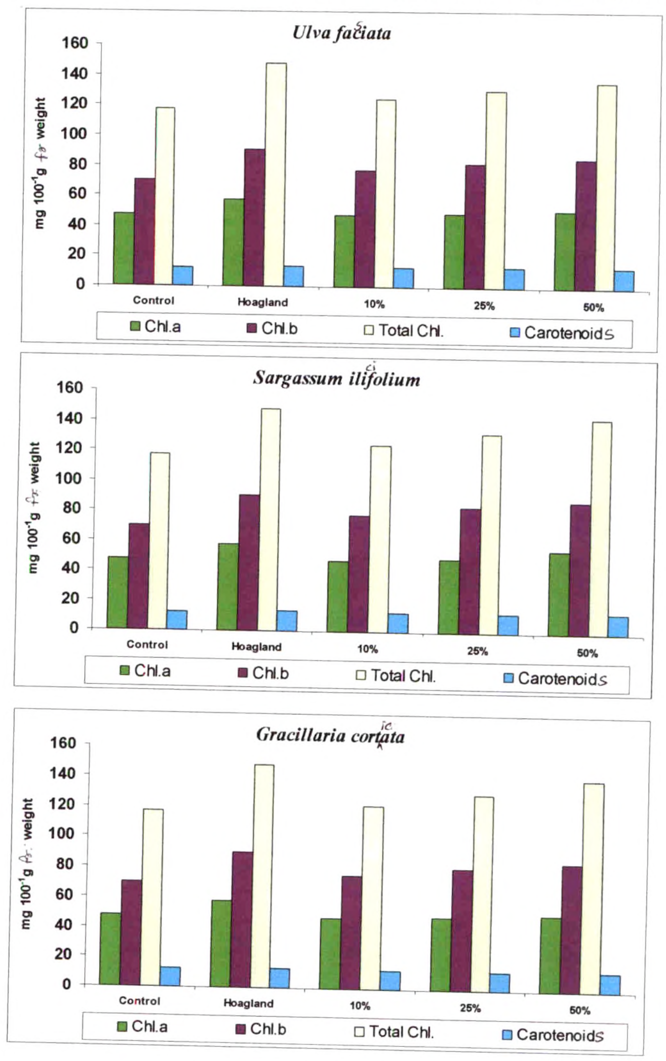
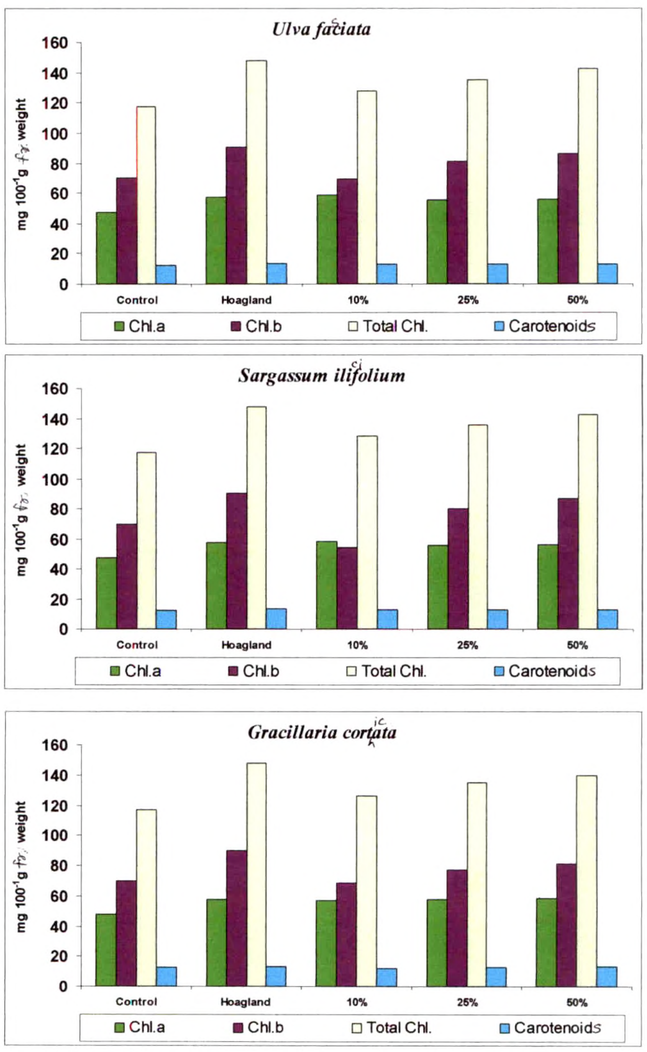


Fig 9. Effect of water soaked SWC on photosynthetic pigments of *T. foenum-graecum*



Among various plant pigments, chlorophylls occupy a unique place in the life of green plants as these are primarily involved in harvesting the solar energy and converting it into chemical energy in green plants. Photosynthetic pigments in seaweed treated plants have been studied by some workers. Blunden *et al.* (1997) demonstrated that seaweeds and seaweed products enhance plant chlorophyll content. Application of a low concentration of *Ascophyllum nodosum* extract to soil or on foliage of tomatoes produced leaves with higher chlorophyll content than those of untreated control. The increase in chlorophyll content was a result of reduction in chlorophyll degradation, which might be caused in part by betaines in the seaweed extract (Whapham *et al.*, 1993). Genard *et al.*, (1991) had the opinion that glycine betaine present in seaweeds delays the loss of photosynthetic activity by inhibiting chlorophyll degradation during storage conditions in isolated chloroplasts.

Carotenoids act as the accessory light harvesting pigments and as triplet quencher to provide protection against the photooxidative damage (Bacon, 2001). In all the treatments of SWC, carotenoid level increased gradually with higher concentration in present study. At 50% concentration, the carotenoid content was almost equal to that of Hoagland plants. *Sargassum* treated plants with 50% concentration showed slightly higher carotenoid level than the other two species of seaweeds.

The positive effect on the photosynthetic pigments can be directly correlated with the increase in overall growth of the treated plants. The increased photosynthesis can lead to increase in yield and quality of fenugreek.

b. Organic constituents

i. Total carbohydrates

Effect of fresh, boiled and soaked extract of three seaweeds on the carbohydrate content in fenugreek is depicted in Table 20. Fresh extract of all seaweeds caused an increase in the content of carbohydrates and amongst the concentrations employed for the treatment, 25 and 50% of *Sargassum* and *Gracillaria* caused a remarkable changes in the sugar content. Fresh extract of *Ulva* enhanced carbohydrate content only at the 50% concentration. The amount of carbohydrates found in the treated plants (50%) was near to the values recorded for Hoagland treated plants (Fig 10).

Water soaked extracts of all the three seaweeds had nearly the similar effect on carbohydrate content as that of control and only a marginal increase was recorded in the treated plants. *Sargassum* and *Gracillaria* boiled extract were found to enhance the content of carbohydrates. Boiled extract of *Ulva* also did not have much positive effect, as compared to control.

There are very few reports regarding the effect of SWC on carbohydrates. Compared to control, plants sprayed with one percent *K. alvarezii* extract showed an increase in nutritional quality of wheat and sugar beet including, carbohydrate (Blunden *et al.*, 1979, Zodape, 2009).

Fig 10. Effect of SWC on total carbohydrates of *Trigonella foenum-graecum*

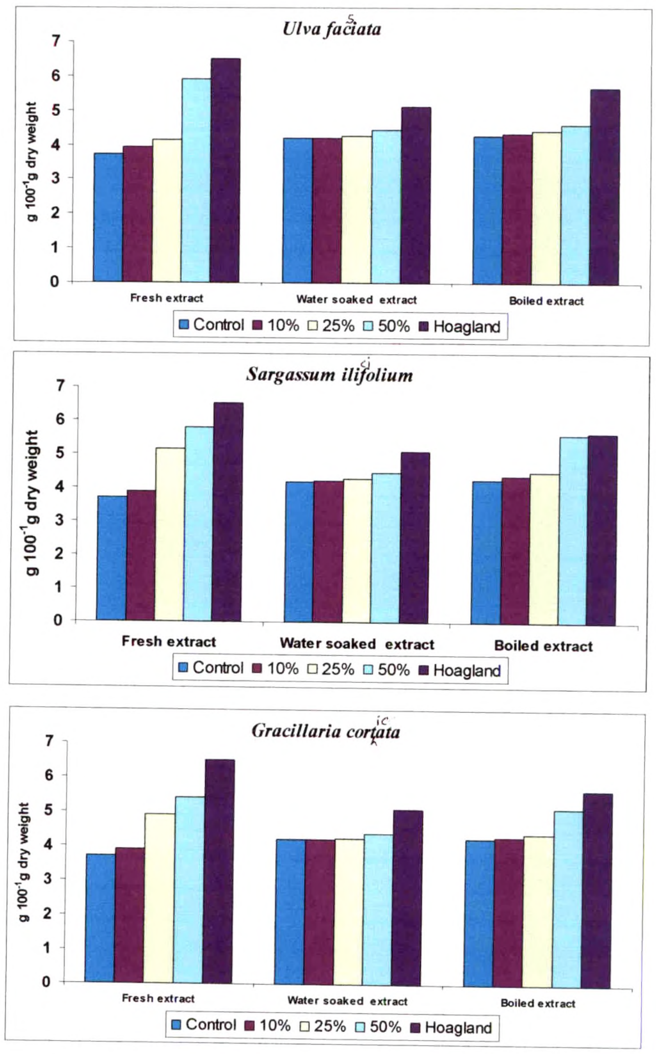


Table 20: Effect of SWC on total carbohydrates of *T. foenum-graecum*

SWC	*Ulva fasciata*			*Sargassum ilicifolium*			*Gracillaria corticata*			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10%	25%	50%		
Fresh extract	3.93	4.13	5.91	3.87	5.16	5.79	3.91	4.92	5.41	3.71	6.51
Water soaked extract	4.21	4.26	4.42	4.20	4.27	4.46	4.22	4.25	4.37	4.20	5.10
Boiled extract	4.3	4.39	4.57	4.35	4.48	5.59	4.3	4.39	5.13	4.25	5.64

Values are expressed in g $100g^{-1}$ dry weight

ii. Total soluble proteins

Effect of seaweed concentrate on total soluble proteins of *Trigonella foenum-graecum* is depicted in Table 21. Protein content in fenugreek was enhanced by all the seaweed extracts applied at 25% and 50% concentrations. The fresh extract of all the seaweeds caused a maximum enhancement in the protein content which was near about to Hoagland treated plants. At the lowest concentration (10%) the protein content was similar to that observed in the control plants in case of all the extracts.

Sivasankari *et al.*, (2006) reported highest protein content in *Vigna sinensis* treated *with* extract of *Sargassum ilicifolium* at 20% concentration. Zahid (1999) recorded maximum amount of protein in gram plant at 10% concentration, while in sunflower and spinach the highest values were observed at 20% concentration of the extract of *Codium lyengaril.* In the present study 50% concentrate of all the seaweeds, exhibited a stimulation in protein concentration in fenugreek plants. The response to *Sargassum ilicifolium* SWC was better in all, followed by *Ulva fasciata* and then *Gracillaria corticata* (Fig. 11)

iv. Total free amino acids

Effect of different liquid extract, of three seaweeds on the free amino acid content in fenugreek is presented in Table 22 .

A remarkable increase in free amino acids was observed in fenugreek plants treated with fresh extract of seaweed and all the three fresh

Fig 11. Effect of SWC on total soluble proteins of *Trigonella foenum-graecum*

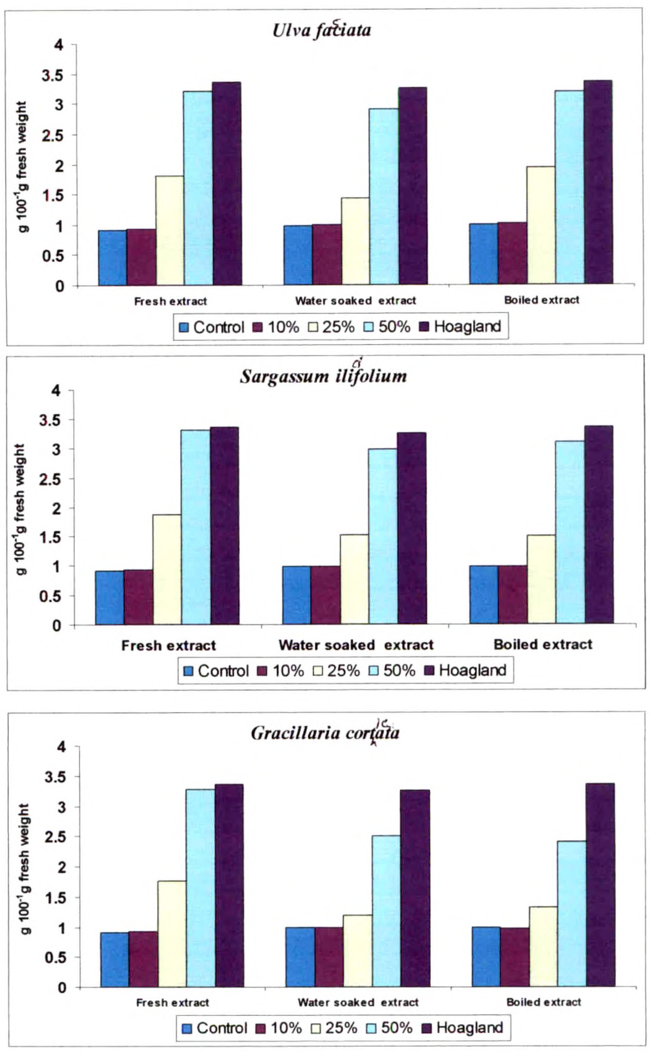


Fig 12. Effect of SWC on total amino acids of *Trigonella foenum-graecum*

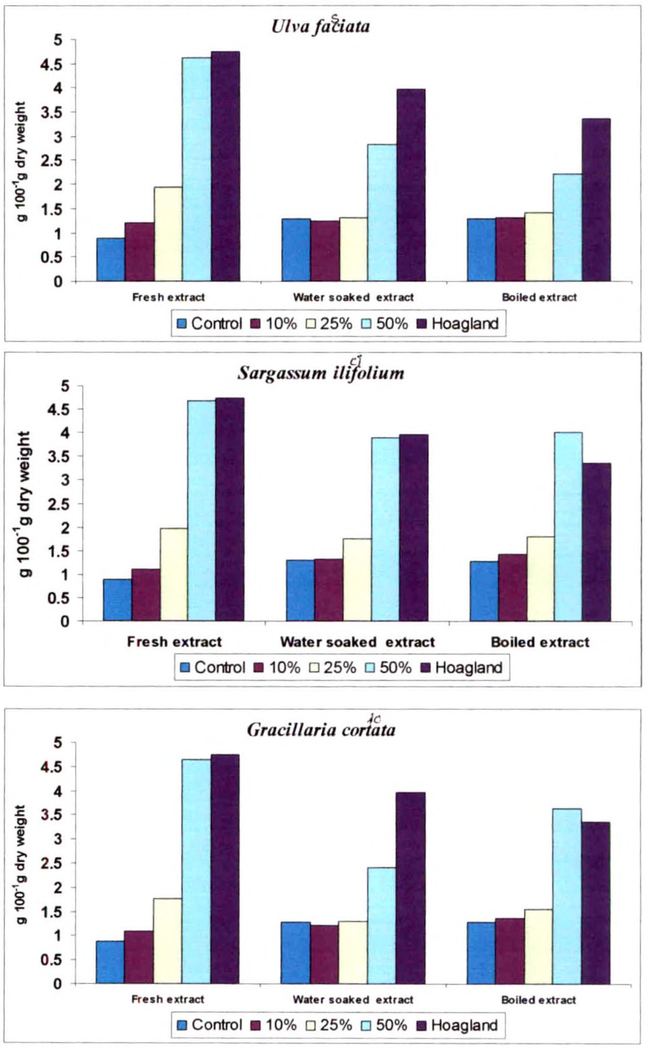


Table 21: Effect of SWC on total soluble proteins of *T. foenum-graecum*

SWC	*Ulva fasciata*			*Sargassum ilicifolium*			*Gracillaria corticata*			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10%	25%	50%		
Fresh extract	0.92	1.8	3.21	0.94	1.87	3.31	0.92	1.77	3.28	0.91	3.36
Water soaked extract	1.00	1.43	2.91	0.99	1.52	2.99	0.99	1.20	2.51	0.99	3.26
Boiled extract	1.008	1.94	3.183	0.991	1.512	3.091	0.982	1.327	2.41	0.991	3.36

Values are expressed in g $100g^{-1}$ fresh weight

Table 22: Effect of SWC on total amino acids of *T. foenum-graecum*

SWC	*Ulva fasciata*			*Sargassum ilicifolium*			*Gracillaria corticata*			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10%	25%	50%		
Fresh extract	1.21	1.94	4.63	1.1	1.98	4.68	1.09	1.77	4.65	0.89	4.75
Water soaked extract	1.25	1.32	2.84	1.32	1.75	3.89	1.22	1.31	2.41	1.29	3.97
Boiled extract	1.31	1.41	2.21	1.42	1.79	4.01	1.36	1.56	3.64	1.28	3.36

Values are expressed in g $100g^{-1}$ dry weight

extracts exhibited a marked increase in amino acids content at 50% concentration.

For water soaked and boiled seaweed extract, similar results were obtained as that of fresh seaweed extract. Lower concentration did not promote amino acid content in any of the seaweed concentrate but it was equal to or slightly grater than the control plants. All the three seaweeds were effective in influencing the content of amino acids as depicted by Fig. 12.

v. Total Polyphenols

Total polyphenol content in *Trigonella foenum-graecum* under the influence of SWC is are depicted in Table 23.

Fresh and boiled extract of all the seaweeds used at 25 and 50% concentration caused a significant increase in the polyphenol content in fenugreek. With *Sargassum* and *Gracillaria* boiled extracts, the content was even higher than in the Hoagland treated plants. Water soaked extracts were able to change the polyphenol content only slightly, in all the three seaweed extracts employed (Fig. 13)

Polyphenols are the secondary metabolites with aromatic ring structures and are abundant in the plant kingdom. These substances have more than one hydroxyl group in the nucleus. This group comprises a large variety of aromatic substance such as tannins and betalins, anthocynins, leucoanthocyanins and anthoxanthins, hydroxyl benzoic acids, glycosides, flavonoids, sugar esters of quinine, shikkimic acid esters and coumarine derivatives.

Fig 13. Effect of SWC on total polyphenols of *Trigonella foenum-graecum*

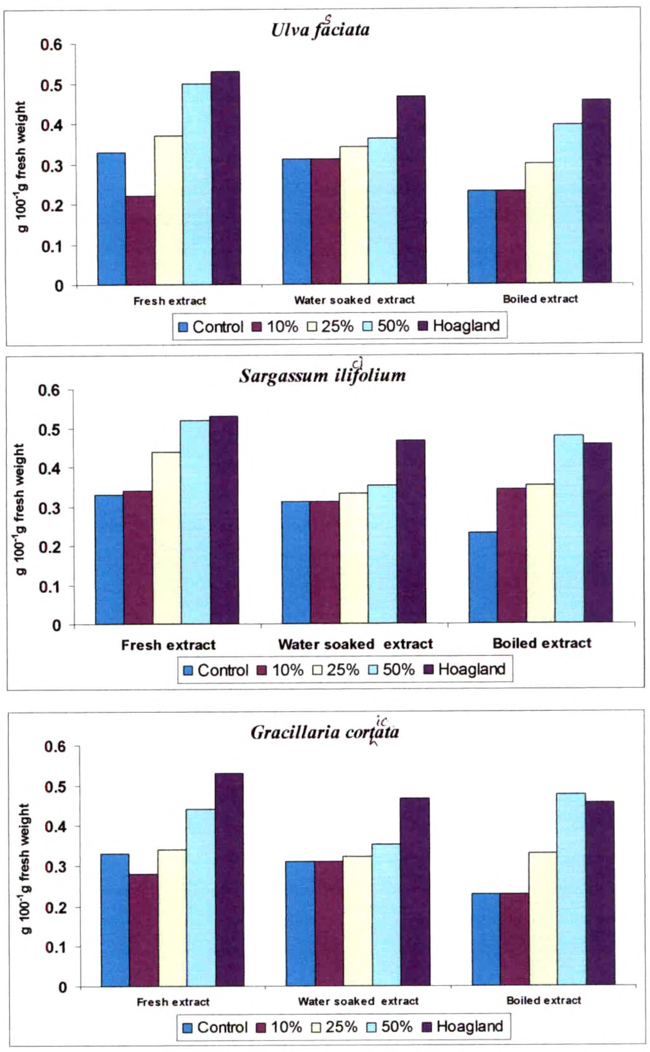


Table 23: Effect of SWC on total polyphenols of *T. foenum-graecum*

SWC	*Ulva fasciata*			*Sargassum ilicifolium*			*Gracillaria corticata*			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10%	25%	50%		
Fresh extract	0.22	0.37	0.5	0.34	0.44	0.52	0.28	0.34	0.44	0.33	0.53
Water soaked extract	0.31	0.34	0.36	0.31	0.33	0.35	0.31	0.32	0.33	0.31	0.47
Boiled extract	0.23	0.3	0.39	0.34	0.35	0.48	0.23	0.33	0.48	0.23	0.46

Values are expressed in g $100g^{-1}$ fresh weight

A number of processes which are essential for normal growth and development of plant may be disturbed due to small changes in phenol metabolism. The phenolic compounds , in some instances, affect fundamental plant processes such as photosynthesis, chlorophyll production, plant water relations (Rice,1979), protein synthesis (Dank *et al.*, 1975), respiration (Demos *et al* .,1975) and membrane permeability (Glass and Dunlap, 1974).

In the present study, only certain SWC were found to affect the polyphenol content in fenugreek. The accumulation of polyphenols may suggest increased resistance of plants after application of seaweed extract.

vi. Total nitrogen content

Total nitrogen content in fenugreek after treatment with macroalgae (Ulva, *Sargassum* and *Gracillaria*) as organic fertilizer is shown in Table 24.

Seaweeds are rich in nitrogen and their addition improves physical condition of the soil (Lean and Nalewajko, 1976). Nitrogen content was not much affected by the treatment of seaweeds and only a marginal elevation was noticed at 25 and 50% concentration of all the three types of extracts. The values of nitrogen were slightly higher when fresh extract of algae was used for treatment. The response obtained for different species of seaweed was more or less similar (Fig. 14). The amount of nitrogen in Hoagland treated fenugreek plants was negligibly higher than in the treated plants.

Fig 14. Effect of SWC on total nitrogen of *Trigonella foenum-graecum*

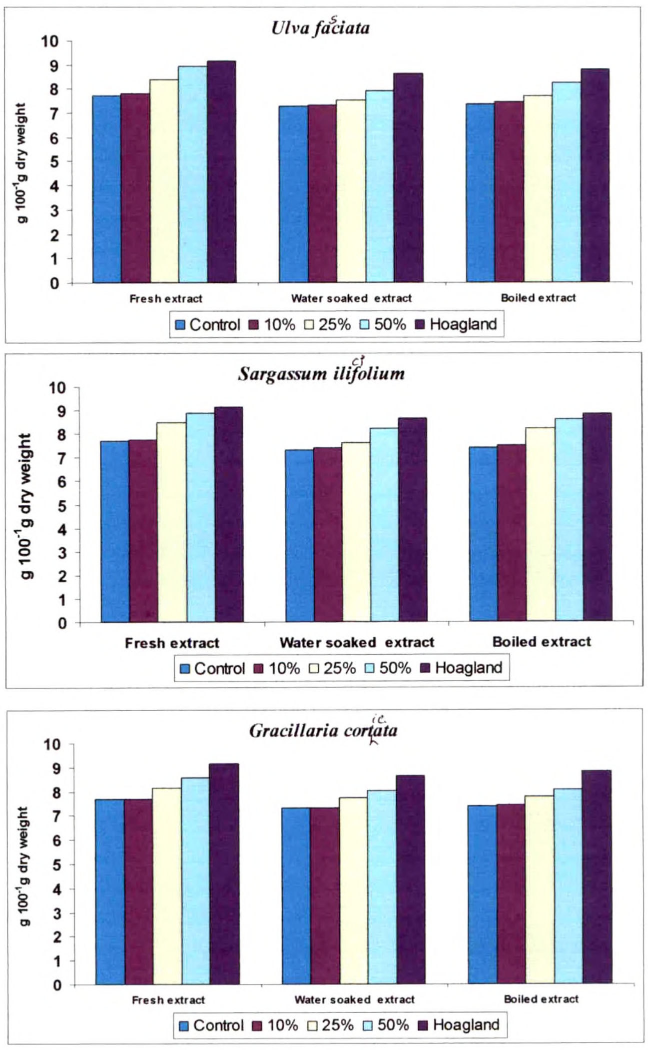


Table 24: Effect of SWC on total nitrogen of *T. foenum-graecum*

SWC	*Ulva fasciata*			*Sargassum ilicifolium*			*Gracillaria corticata*			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10%	25%	50%		
Fresh extract	7.82	8.39	8.96	7.74	8.49	8.89	7.71	8.14	8.57	7.71	9.14
Water soaked extract	7.33	7.53	7.93	7.40	7.63	8.23	7.29	7.73	8.03	7.29	8.66
Boiled extract	7.45	7.72	8.28	7.48	8.24	8.62	7.45	7.76	8.07	7.38	8.83

Values are expressed in g $100g^{-1}$ dry weight

Table 25: Effect of SWC on total ash content of *T. foenum-graecum*

SWC	*Ulva fasciata*			*Sargassum ilicifolium*			*Gracillaria corticata*			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10%	25%	50%		
Fresh extract	7.1	8	10.2	7.3	8.4	10.2	6.9	7.8	9.8	7.2	10.8
Water soaked extract	6.5	6.6	7.8	6.6	6.8	7.9	6.2	6.5	7.1	6.2	8.9
Boiled extract	7.3	7.8	8.8	7	7	8.9	7.1	7.3	8.1	6.9	9.6

Values are expressed in g $100g^{-1}$ dry weight

C. Total ash content

Effect of seaweed fertilizers on the total ash content in fenugreek is presented in Table 25.

With the fresh extract of all the seaweeds a rise in the ash content was found in fenugreek and the concentrations which gave maximum values of ash were 25 and 50%. When water soaked extract was used for treatment a marginal increase occurred in ash content only at the highest concentration (50%). Similar was true for the boiled extract of all the seaweeds. The values recorded at 50% concentration of all the extract of seaweeds were near to those reported for Hoagland treatment. No much variation occur in the mineral content with respect to different species of seaweeds used (Fig.15).

From the observations reported so far, it is obvious that 50% seaweed concentrate was effective in introducing significant changes in the experimental plants. Further fresh extract of seaweed are more promising than the boiled and soaked ones.

Biochemical analysis revealed that the application of seaweeds positively affected the cellular metabolism and promoted biosynthesis of macromolecules such as carbohydrates, proteins in fenugreek. They improve uptake of minerals as indicated by the elevated ash content in the treated plants. Increased content of photosynthetic pigments suggested enhanced rate of photosynthesis in the fenugreek, indicating better growth and development.

Treatment of fresh extracts of *Sargassum* was found beneficial to the crop plant under investigation.

Fig 15. Effect of SWC on total ash content of *Trigonella foenum-graecum*