Effect of Nitrobenzene granules and Seaweed extracts on biochemical contents of Arachis hypogaea callus culture.

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Abstract— *The present study is aimed to evaluate the effect of organic extracts (benzene, diethyl ether and water) of seaweeds (*Halimeda *gracilis, Ceramium rubrum and Cystophyllum muricatum) and nitrobenzene granuleson biochemical contents of Arachishypogea L. callusunder in vitro conditions. The callus of Arachishypogea L. was obtained from the leaf explants on MS medium containing 2, 4-D (1 mgL^{-1}) and BAP (0.5 mg L^{-1}). The mass multiplication of callus was achieved at 1mg L^{-1} of 2, 4-D and 0.5 mg L^{-1} of GA_3. The calli were then treated with different concentrations (0.5, 1.0 and 1.5 mg L^{-1}) of seaweed extracts and Nitrobenzene granules. Total carbohydrate, total protein and total chlorophyll contents were analyzed at 5, 10 and 15 days intervals. The total carbohydrate content was high (3.7mg/100mg) in callus treated with Benzene extract of Ceramium rubrumat 1.5 mg L^{-1} on 15^{th} day. The total protein content was increased (6.9mg/100 mg) in callus treated with Benzene extract of Cystophyllum muricatum at 0.5 mg L^{-1} on 5^{th} day and the total chlorophyll content was lower (0.36mg/100mg) in Nitrobenzene granules at 0.5mg L^{-1} in 5^{th} day when compare to control. The present study reveals the positive role of different extracts of seaweeds on increasing the biochemical contents of callus culture of A.hypogea. The extracts can be further evaluated for their role on enhanced regeneration of plants from callus culture.*

Keywords— Nitrobenzene, Seaweed extract, Arachis hypogaea, **Halimeda** ***gracilis, Ceramium rubrum and Cystophyllum muricatum.***

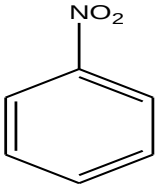
I. INTRODUCTION

Seaweed extracts are well-known biostimulants. The seaweed extract consist of trace elements and particularly plant growth regulators such as cytokinin, amino acids, antibiotics, and Vitamins. In modern trends the seaweeds were used along with the farm land as a soil conditioner in some European countries. There are different extraction methods can be used for seaweed extracts preparation i.e. water extraction under high pressure, alcohol extraction, alkaline extraction, microwave-assisted extraction (MAE) and supercritical CO_2 extraction. Cytokinins can be extracted using chilled 70% ethanol. Deuterium is used as co solvent in this process (Yokoya*et al.*,2010, Stirk*et al.,* 2009). Extraction in 85% methanol leads to obtainment of algae extract rich in gibberellins (Hytonen.*et al.,* 2009). Algal extracts improve plant resistance to frost and drought and increase crop yields. Plants sprayed with the use of seaweed extracts are also characterized by higher resistance to pests and pathogens and more efficient consumption of nutrients from soil (Matysiak K *et al*.,2010).

Groundnut (*Arachishypogaea* L.,) is one of the most important oil crops. The Groundnut provides major source of edible oil and vegetable protein. It contains 47-53% oil and 25-36% of protein. Groundnut is a self pollinated crop whereas flowers are produced above ground and after fertilization, pegs move towards the soil, and seed-contain pods are formed. The cultivated groundnut (*Arachishypogaea* L.,) originated in South America. China is the first country in cultivate groundnut and India is in second place. Peanut oil is often used in cooking, because it has a mild flavor and a relatively high smoke point. Due to its high monounsaturated content, it is considered healthier than saturated oils, and is resistant to rancidity. There are several types of peanut oil including: aromatic roasted peanut oil, refined peanut oil, extra virgin or cold pressed peanut oil and peanut extract. In the United States, refined peanut oil is exempt from allergen labeling laws. The top countries of peanut cultivars around the world in 2012.

Nitrobenzene is a greenish yellow crystal or yellow oily liquid with the odor of bitter. The nitrobenzene is soluble in water, acetone, benzene, diethyl ether, and ethanol. Nitrobenzene is applied with nitrogen for the enhancement of flowering and growth of agricultural crops. The nitrobenzene from seaweed extract has the capacity to increase flowering in plant (flower stimulant) and also prevent flower shedding.

1.1 Structure and Molecular formula:



$C_6H_5NO_2$

The science of tissue culture is historically linked to the discovery of the cell and subsequent propounding of the cell theory. Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. More than 234 years ago, Henri-Louis Duhamel du Monceau's(1756) pioneering experiments on wound healing in plants demonstrated spontaneous callus formation on the decorticated region of elm plants. The development of the multicellular or multiorganed body of a higher organism from a single-celled zygote supports the totipotent behaviour of a cell. . Haberlandt is regarded as the father of tissue culture. German botanist Gottlieb Haberlandt (1902) developed the concept of invitro cell culture. He was the first to culture isolated, fully differentiated cell in a nutrient medium containing glucose, peptone, and Knop's salt solution.

Callus, which shown stable characteristics under specific conditions after subculture through many successive passages, is a suitable material for cyto differentiation. The advantage of using such callus is that it is composed of fairly homogeneous mass cells and can be proliferated in large amounts under known culture conditions. Wetmore and Sorokin (1955) induced vascular strands in syringa callus derived from the cambial region of the stem or graft apices of shoots. Since then (1950) vascular or tracheary element differentiation has been induced in callus derived from tissues of many species. To Biostimulate the callus of *Arachishypogaea* L., using different seaweed extracts and it's biochemical assay. To collect seaweeds from Mandapam, Rameshwaram coastal Islands, Tamilnadu, India. To prepare seaweed extract by using three different organic solvents.To collect seed of *Arachis hypogaea* L. from Tamil Nadu Agriculture University Trichy,Tamilnadu,India.To prepare explants in half-strength MS Media.To induce callus in MS media with growth hormones.To Mass multiple the callus using growth hormones.To biostimulate callus by using crude seaweed extracts. To biostimulate callus by using nitrobenzene granules (Standard). To estimate the amount of total chlorophyll by Mackinney method. To estimate the amount of total carbohydrate by Anthrone method. To estimate the amount of total Protein by Lowery's method.

II. MATERIALS AND METHODS

2.1 Collection of Seaweeds:

The three different varieties of seaweeds such as *Halimeda gracilis*, *Ceramium rubrum* and *Cystophyllum muricatum*, were collected from Mandapam coastal Islands (Ramanathapuram District) Tamilnadu,India. Then the seaweeds were washed thoroughly with sea water to remove extraneous materials and brought to the laboratory in plastic bags containing water to prevent evaporation. Samples were then shade dried until constant weight obtained and ground in an electric mixer. The powder samples subsequently stored in refrigerator.

2.2 Preparation of seaweed extract:

The three seaweed were weighed 50 grams and extracted with 150 ml of diethyl ether, benzene and water each.

2.3 Collection of Seed:

The seed of *Arachis hypogaea* L., TMV-7 was collected from Tamilnadu Agriculture University Trichy, Tamilnadu,India.

2.4 Preparation of Explant:

The seed were surface sterilized by using 0.1% mercuric chloride and ethanol and it was inoculated in the half-strength MS medium. This was incubated in 12 hrs light and 12 hrs dark conditions at 25^0 C for 8 to 10 days.

2.5 Induction of Callus:

The explants were collected and that surface were damaged and inoculated in the MS medium and this was incubated in 12 hrs light and 12 hrs dark conditions at 25^0 C for 10 to 15 days.

2.6 Bio stimulation of Callus by using seaweed extract:

The callus was treated with three seaweed extracted with three different solvent in three different concentrations such as 0.5, 1.0 & 1.5 mg/l in three different incubation time such as (5, 10 & 15 days) and was incubated in 12 hrs light and 12 hrs dark conditions at 25°C under aseptic condition.

2.7 Biostimulation of Callus by using Nitrobenzene:

The callus was treated with nitrobenzene purchased from Greenland Bio-science Vadodara, Gujarat,India in three different concentration 0.5,1 & 1.5 mg/l.

2.8 Estimation of Chlorophyll by Mackinney 1941:

2.8.1 Procedure:

20 mg of each callus was taken and ground separately using 80% acetone (the volume make up to 2 ml).The extract was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and absorbance was read at 645nm and 663nm against the blank (80% acetone).

2.8.2 Calculation:

Use Arnon's equation (below) to convert absorbance measurements to mg Chl g-1 leaf tissue.

Chl a (mg g-1) = [(12.7 × A663) - (2.6 × A645)] × ml acetone / mg leaf tissue/1000.

Chl b (mg g-1) = [(22.9 × A645) - (4.68 × A663)] × ml acetone / mg leaf tissue/1000.

Total Chl = Chl a + Chl b.

2.9 Estimation of Carbohydrate by Anthrone Method (Hansen J, Møller IB, 1975) :

Dissolve 0.2g of anthrone in 5 ml of ethanol Add slowly 75%of sulphuric acid till the mark reaches 100 ml in standard measuring flask.

2.9.1 Procedure:

20 mg of callus wash taken and that was ground by using 1ml of distilled water this was took as test sample. Keep the test tube in an ice bath and slowly add 5 ml of the cold Anthrone reagent and mix properly. Close the test tubes with aluminium foil and place it in a boiling water bath for 10 min. Cool the tubes and measure OD at 620 nm. Blank should be prepared as per previous steps without adding test solution.

2.9.2 Observation:

Green color formation is noted.

2.10 Estimation of Protein Lowery's Method et al 1940:

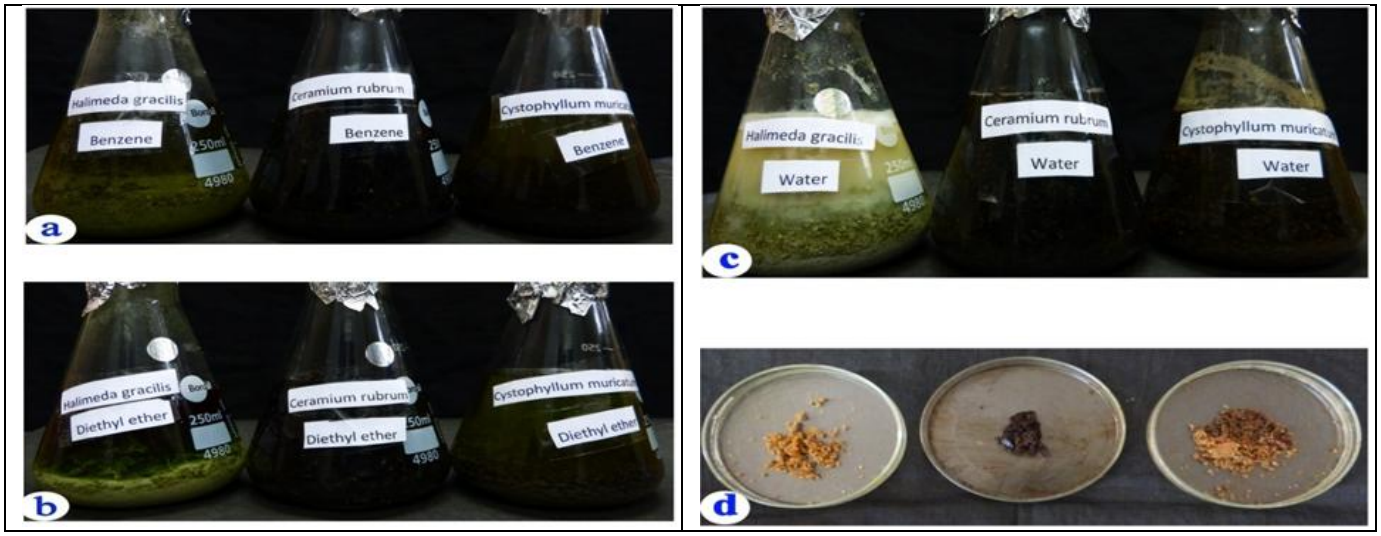
2.10.1 Procedure:

20 mg of callus wash taken and that was ground by using 1ml of distilled water from this 0.2ml was took as test sample. To each test tube add 1 mL of the mixed reagent and mix thoroughly and allow to stand at room temperature for 10 min or longer. Add 0.3 mL of diluted Folin-Ciocolteau reagent rapidly and mix properly. Incubate all tubes for 60 minutes. Measure OD of the standard and test solution at 660nm and plot the standard graph. Run the blank.

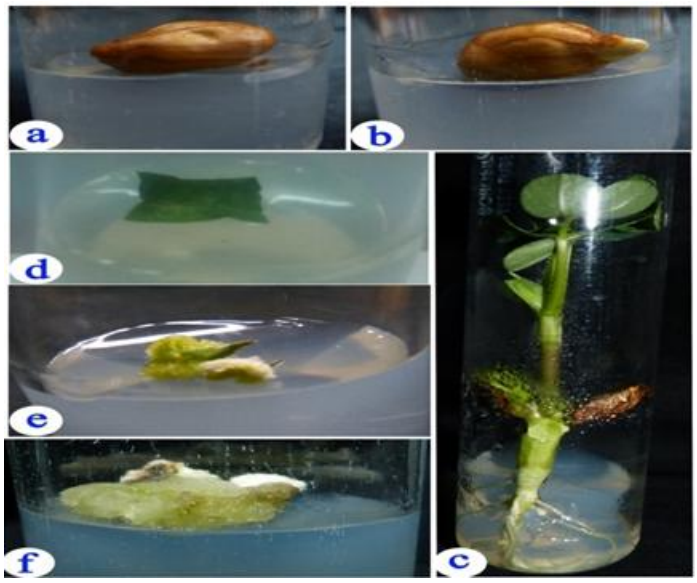
2.10.2 Observation:

Blue colour is noted and read using spectrophotometer.

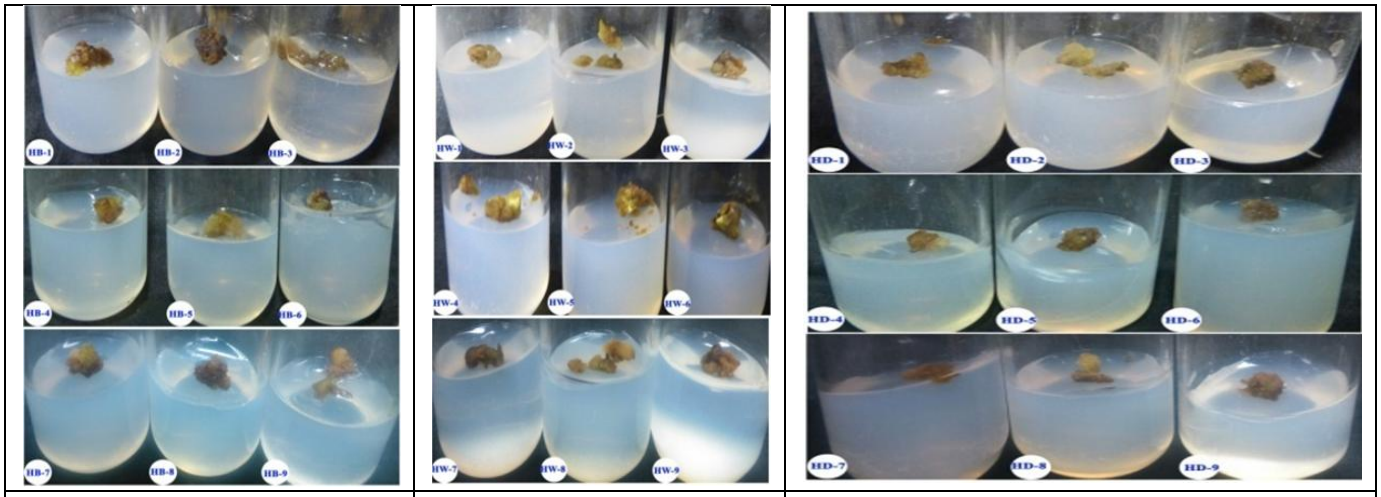
2.11 Extraction of Seaweed in Solvents



2.11.1 CALLUS INDUCTION OF *Arachis hypogiea L.*

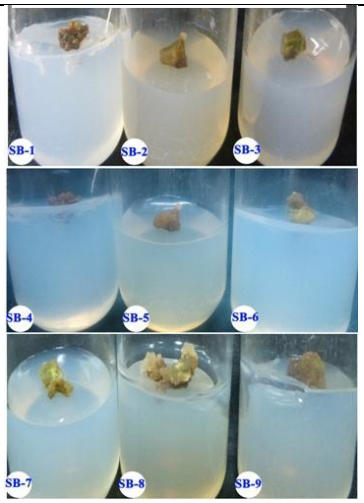
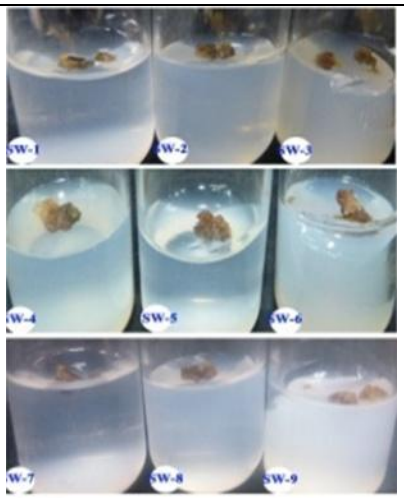
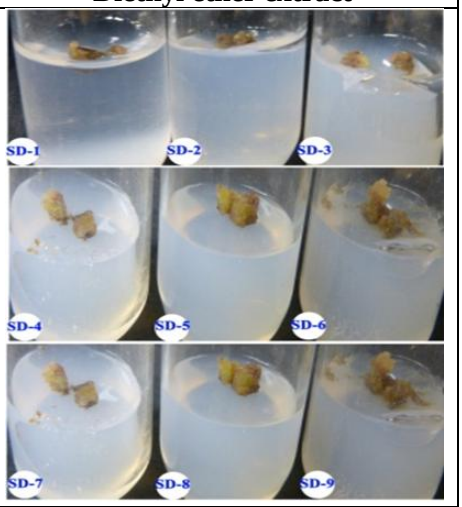


2.11.2 Callus treatments of Halimeda gracillis at different concentrations:

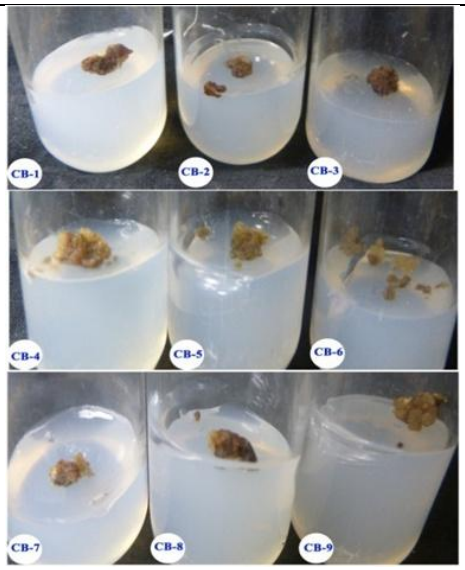
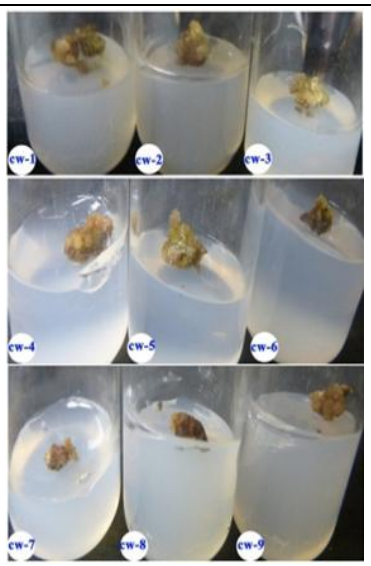
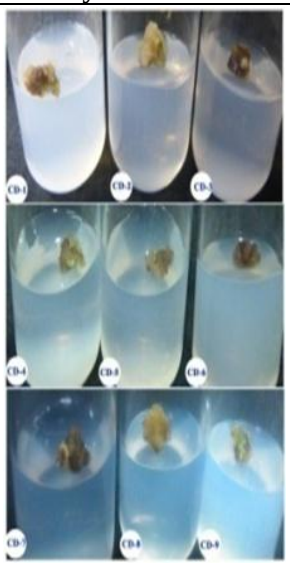


Benzene extract	Water extracxt	Diethyl ether extract

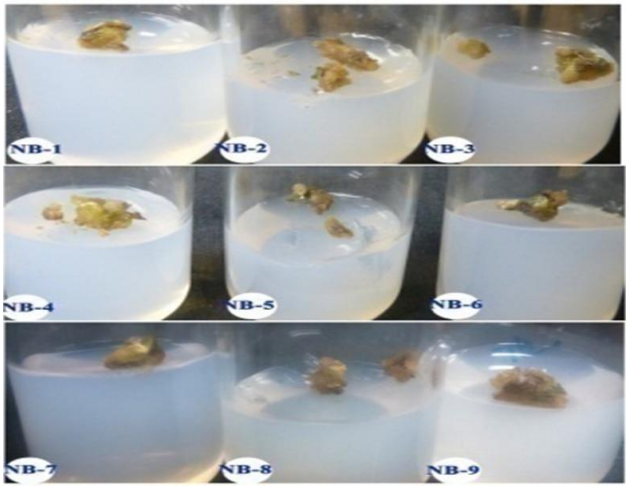
2.11.3 Callus treatments of *Halimeda gracillis* at different concentrations:

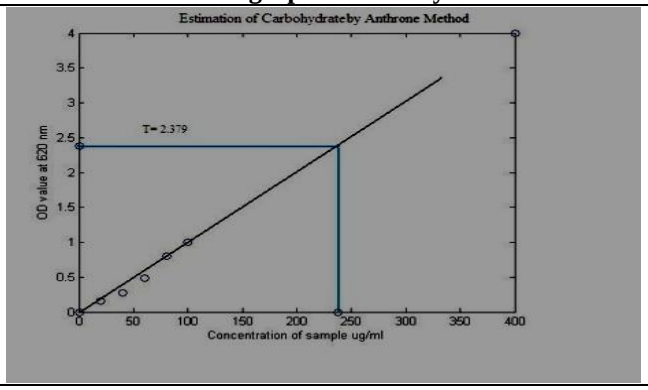
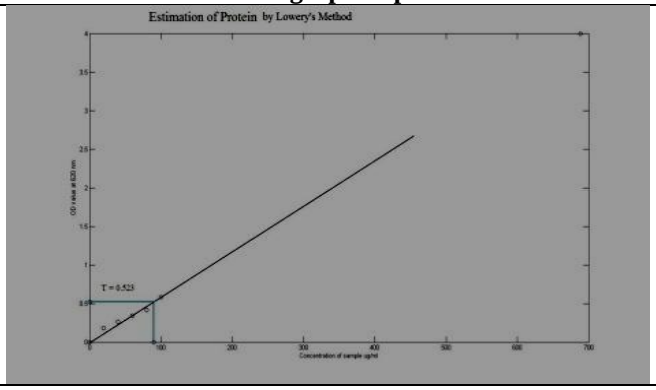
Benzene extract	Water extracxt	Diethyl ether extract
		

2.11.4 Callus treatments of *Cystopyllum muricatum* at different concentrations:

Benzene extract	Water extracxt	Diethyl ether extract
		

2.11.5 Callus treated with Nitrobenzene granules



Standard graph of carbohydrate	Standard graph of protein
	

III. RESULT AND DISCUSSION

3.1 Extraction of seaweeds in solvents:

A total of three seaweeds were extracted with three different organic solvents and incubated for 25 days at room temperature. The solvent extracts were air evaporated slowly and the aqueous extract was dried in hot air oven. After evaporation, the sample was weighed and used as standard stock. Extracts of three seaweeds with different organic solvents are shown in Fig; 5.1a-d. All the extracted samples were weighed after evaporation and their list are given in **Table 4**. There are lot of methods used for seaweed extraction *Saragassum wightii, Gelidella aerosa* and *Ulva lactuca* extracted with 5% acetone (Immanuel and Subramanian, 1999). *Saragassum plgiophyllum* was extracted by soaked and boiled (Anantharaj and venkatasalu, 2001 and Ashok et al., 2004). *Saragassum polycystum, Ulva lactuca* and *Tubinaria conoides* were extracted with 50% ethyl alcohol (Ramamoorthy and Sujatha 2007). In present study, seaweeds were extracted with 100% Benzene and Diethyl ether and Water. Solvents were selected on the basis of polarity. Liquid extract of *Saragassum wightii* increased height and number of branches in *Arachis hypogaea* under field condition when compared to chemical treatment (Sridhar and Rengasamy 2010). Seaweed extract not only increased the vegetative growth of the plant but it also triggers the early flowering and fruiting in crops. Another experiment conducted by Zodape et al., 2008 showed that treatment of seaweed extract increased shoot length (31.7%), diameter (18.2%) and yield (37.4%) of *Abelmoschus esculentus* than the control. There is no previous study on treatment of callus with seaweed extracts.

3.2 Callus induction in Arachis hypogaea L.

The surface sterilized peanut seeds were inoculated in half strength MSB_5 medium (**Fig: 4 a**). The seeds germinated after 3-4 days (**Fig: 4 b**), the seedling grown after 10-12 days (**Fig: 4 c**). From this the leaf was chosen as explants and inoculated in MSB_5 medium containing 2,4, D and BAP at various concentration was shown in **(Fig: 4 d).** The maximal level of callus was obtained on MSB_5 medium supplemented with 2,4, D(1 mg L^{-1}) and BAP(0.5 mg L^{-1}) at 10-12 days(**Fig 4 e**). The callus induced on MSB_5 media was green in colour. Mass multiplication of the induced callus was achieved on MSB_5 medium containing 2,4, D (1 mg L^{-1}) and GA_3 (0.5 mg L^{-1}) (**Fig: 4 f**). Callus of *A.hypogea* was induced by using different concentration of 2, 4, D. The 2 mg / L 2,4-D gives maximum level of callus (Alam and Khaleque 2010). The callus from hypocotyls explants were obtained in 0.5 mg/ L of 2, 4-D along with BAP (Muthusamy et al.,2007). Maximum level of callus from mature tissues was obtained from 3.0 mg /L of IAA and 1.0 mg /L of BAP (Plalanivel et al., 2000). In our studies the explants was treated with 2,4-D alone, BAP alone and combination of 2,4-D with BAP at various concentration the maximal level of callus was obtained in (1 mg L^{-1}) of 2,4-D and (0.5 mg L^{-1}) of BAP. The mass multiplication was achieved in (1 mg L^{-1}) of 2,4-D and (0.5 mg L^{-1}) of GA_3.

3.3 Treatment of callus with Benzene extract of *Halimeda gracillus* and its biochemical assay

MS medium supplemented with Benzene extract of *Halimeda gracillus* as bio stimulant in place of plant growth regulators at various concentrations (0.5, 1.0 &1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5,10 & 15 days) (**Fig : 5**). Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1,** total chlorophyll estimation values is given in **Table 2**, The bar diagram representation of total chlorophyll estimation is shown in (**Fig : 16 a**). The total carbohydrate and total protein values is in **Table 3 ,** The total carbohydrate bar diagram representation in (**Fig :17 a**) and the total protein bar diagram representation in (**Fig :18 a**). Among

them, HB-3 showed 0.33 mg/ 100 mg and the minimum level is HB-1 showed 0.07 mg/100 mg. This result indicates that total chlorophyll content is lowering treated callus tissues when compared to control HB-7 showed 3.8 mg/100 mg and the minimum level in HB-5 showed 2.2 mg/ 100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. HB-8 showed 3.7 mg/ mg and the minimum level is HB-6 showed 0.8 mg / 100 mg. This result indicates that the protein content is higher in treated callus tissues when compared to control.

3.4 Treatment of callus with aqueous extract of *Halimeda gracilus* and its bio chemical assay

MS medium supplemented with aqueous extract of *Halimeda gracilus* as bio stimulant in place of plant growth regulators at various concentrations (0.5, 1.0 & 1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5,10 &15 days) **(Fig:6).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig: 16 b)**. The total carbohydrate and total protein value is given in **Table 3**, the total carbohydrate bar diagram representation in **(Fig: 17 b)** and the total protein bar diagram representation in **(Fig: 18 b).** Among them, HW-3 showed 0.34 mg/100mg and the minimum level is HW-8 showed 0.29 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissues when compared to control. HW-7 showed 3.5 mg/100 mg and the minimum level is HW-6 showed 2.1 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. HW-7 showed 6.3 mg/100 mg and the minimum level is HW-5 showed 0.5 mg/ 100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.5 Treatment of callus with Diethyl ether extract of *Halimeda gracilus* and its biochemical assay

MS medium preparation with Diethyl ether extract of *Halimeda gracilus* as bio stimulant in place of plant growth regulators at various concentrations (0.5, 1.0 & 1.5) mg L^{-1} used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 7).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table: 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 17 f)** and the total protein bar diagram representation in **(Fig: 18 f)**. Among them, SD-7 showed 0.33mg/100 mg and the minimum level is SD-3 showed 0.020 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. SD-9 showed 3.4 mg/ 100 mg and the minimum level is SD-6 showed 2.2mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. SD-8 showed 3.8 mg/100 mg and the minimum level is SD-5 showed 0.5 mg/ 100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.6 Treatment of callus with benzene extract of *Ceramium rubrum* and its biochemical assay

MS medium supplemented with benzene extract of *Ceramium rubrum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 8).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 d).** The carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 d)** and the total protein bar diagram representation in **(Fig: 18 d)**. Among them, SB-3 showed 0.35mg/100 mg and the minimum level is SD-7 showed 0.30 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. SB-9 showed 3.7 mg/100 mg and the minimum level is SB-5 showed 2.1 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. SB-7 showed 4.2 mg/ 100 mg and the minimum level is SB-5 showed 0.6 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.7 Treatment of callus with aqueous extract of *Ceramium rubrum* and its biochemical assay

MS medium supplemented with aqueous extract of *Ceramium rubrum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 9).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of

total chlorophyll estimation is shown in **(Fig 16 e).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 e)** and the total protein bar diagram representation in **(Fig: 18 e)**. Among them, SW-6 showed 0.38mg/100 mg and the minimum level is SW-8 showed 0.27 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. SB-8 showed 3.1 mg/100 mg and the minimum level is SW-6 showed 2.3 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. SB-8 showed 6.8 mg/ 100 mg and the minimum level is SW-5 showed 0.6 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.8 Treatment of callus with diethyl ether extract of *Ceramium rubrum* and its biochemical assay

MS medium supplemented with diethyl ethern extract of *Ceramium rubrum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 10).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 f).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 f)** and the total protein bar diagram representation in **(Fig: 18 f)**. Among them, SD-7 showed 0.33mg/100 mg and the minimum level is SD-3 showed 0.20 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. SD-9 showed 3.4 mg/100 mg and the minimum level is SD-6 showed 2.2 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. SD-8 showed 3.8 mg/ 100 mg and the minimum level is SD-5 showed 0.5 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.9 Treatment of callus with benzene extract of *Cystophyllum muricatum* and its biochemical assay

MS medium supplemented with benzene extract of *Cystophyllum muricatum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 11).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 g).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 g)** and the total protein bar diagram representation in **(Fig: 18 g)**. Among them, CB-6 showed 0.35mg/100 mg and the minimum level is CB-3 showed 0.07 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. CB-7 showed 3.7mg/100 mg and the minimum level is CD-6 showed 2.6 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. CB-7 showed 6.9 mg/ 100 mg and the minimum level is CB-4 showed 1.4 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.10 Treatment of callus with aqueous extract of *Cystophyllum muricatum* and its biochemical assay

MS medium supplemented with aqueous extract of *Cystophyllum muricatum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 12).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 h).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 h)** and the total protein bar diagram representation in **(Fig: 18 h)**. Among them, CW-2 showed 0.35mg/100 mg and the minimum level is CB-7 showed 0.27 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. CW-9 showed 3.1mg/100 mg and the minimum level is CW-4 showed 2.2 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. CB-8 showed 4.2 mg/ 100 mg and the minimum level is CB-5 showed 0.4 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.11 Treatment of callus with diethyl ether extract of Cystophyllum muricatum and its biochemical assay

MS medium supplemented with diethyl ether extract of*Cystophyllum muricatum* as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 13).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 i).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 i)** and the total protein bar diagram representation in **(Fig: 18 i)**. Among them, CD-2 showed 0.34mg/100 mg and the minimum level is CD-8 showed 0.25 mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. CD-7 showed 3.1mg/100 mg and the minimum level is CD-6 showed 2.1 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. CD-9 showed 4.2 mg/ 100 mg and the minimum level is CD-6 showed 1.0 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

3.12 Treatment of callus with Nitrobenzene granules and its biochemical assay

MS medium supplemented with Nitrobenzene granules as biostimulant in place of plant growth regulators at various concentration (0.5, 1.0&1.5) mg L^{-1} was used. Approximately 100 mg of callus was weighed and inoculated in MS medium. Triplicates were maintained for each concentration and used for three incubation periods (5, 10 &15 days) **(Fig: 14).** Estimation of total chlorophyll, total carbohydrate and total protein was done after each incubation period. The weight of each sample is given in **Table 1**, the total chlorophyll estimation value is given in **Table 2**, the bar diagram representation of total chlorophyll estimation is shown in **(Fig 16 j).** The total carbohydrate and total protein value is given in **Table 3,** the total carbohydrate bar diagram representation in **(Fig: 17 j)** and the total protein bar diagram representation in **(Fig: 18 j)**. Among them, NB-3 showed 0.29mg/100 mg. This result indicates that the total chlorophyll content is lower in treated callus tissue when compared to control. NB-9 showed 3.6mg/100 mg and the minimum level is NB-4 showed 2.0 mg/100 mg. This result indicates that the total carbohydrate content is higher in treated callus tissues when compared to control. NB-8 showed 3.7 mg/ 100 mg and the minimum level is NB-4 showed 0.6 mg/100 mg. This result indicates that the total protein content is higher in treated callus tissues when compared to control.

Seaweed machinery such as macro- and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid like growth substance have an effect on cellular metabolism in treated plants leading to enhanced growth and crop yield(Crouch and others 1992; Crouch and van Staden 1993; Reitz and Trumble 1996; Durand and others 2003; Stirk and others 2003; Ordog and others 2004). Seaweed and seaweed-derived products has commonly used as amendments in crop production systems due to the attendance of a quantity of plant growth-stimulating compound (Wajahatullah Khan *et al*., 2009). 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) The biostimulant present in seaweed extract increase the vegetative growth (10%), the leaf chlorophyll content (11%), the stomata density (6.5%), photosynthetic rate and the fruit production (27%) of the plant (Spinelli *et al*., 2010). Seaweed extractsimplies the presence of more than one group of plant growth-promoting substances/hormones (Tay and others 1985; Crouch and van Staden 1993). Cytokinins have been detected in fresh seaweeds (Hussein and Boney 1969) and seaweed extracts (Brain and others 1973).Marine algae are also hypothetically rich in auxins and auxin-like compounds (Crouch and van Staden 1993). Chemical analysis of the aqueous extract of (*Padina pavonica*) showed the attendance of macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) required for maturity and growth of plants (Asma Chbani 2013). A chain of poly pheonolic compounds, flavonoids, flavonol glycosides have been identified from methanol extract of red and brown algae (Santoso *et al*., 2002). The HPLC analysis of *Ulva lactuca.* L in acetone extract shown high amount of phenolic compound (Vanillin, p-coumaric acid are major compounds) (Hassan and Ghareib 2009). The cytokinine such as Trans-zeatin, dihydrozeatin and iso-pentenyladenosine has been identified from *Fucus serratus* L. by GC-MS analysis.(Stirk and Van Staden 1997). *Kappaphycus alvarezii*, seaweed has been reported to be various organic extracts has confirmed the presence of the plant growth regulators (PGRs) Indole 3-acetic acid, Gibberellin GA_3, Kinetin, and Zeatin (Kamalesh prasad *et al*., 2010). Recent research suggests that application of seaweed extract as seed treatment and/or foliar spray helps significant growth of plants. The extract contains micro-nutrients, auxins and cytokinins and other growth promoting substances (Spinelli *et al*., 2010).

TABLE 1
DETERMINATION OF CALLUS WEIGHT IN THREE SEAWEEDS AT THREE DIFFERENT SOLVENT EXTRACTIONS, IN THREE CONCENTRATIONS UPTO 15TH DAY.

	Control mg	5 days			10 days			15 days		
Halimedagracilis		0.5 mg-L	1 mg-L	1.5 mg-L	0.5 mg-L	1 mg-L	1.5 mg-L	0.5 mg-L	1 mg-L	1.5 mg-L
Benzene	20	HB-1 95	HB-2 92	HB-3 69	HB-4 61	HB-5 56	HB-6 65	HB-7 135	**HB-8 158**	HB-9 98
H_2O	20	HW-1 82	HW-2 92	HW-3 67	HW-4 61	HW-5 56	HW-6 62	HW-7 63	**HW-8 84**	HW-9 60
Di ethyl ether	20	HD-1 41	HD-2 63	HD-3 62	HD-4 56	HD-5 63	HD-6 63	HD-7 42	**HD-8 66**	HD-9 44
Ceramiumrubrum										
Benzene	20	SB-1 78	SB-2 78	SB-3 72	SB-4 52	SB-5 98	SB-6 102	SB-7 80	SB-8 63	**SB-9 86**
H_2O	20	SW-1 50	SW-2 47	SW-3 60	SW-4 75	SW-5 87	SW-6 59	**SW-7 95**	SW-8 85	SW-9 71
Di ethyl ether	20	SD-1 110	SD-2 92	SD-3 85	SD-4 120	SD-5 110	**SD-6 130**	SD-7 51	SD-8 88	SD-9 98
Cytophyllummuricatum										
Benzene	20	CB-1 55	CB-2 60	CB-3 77	**CB-4 97**	CB-5 48	CB-6 79	CB-7 83	CB-8 58	CB-9 48
H_2O	20	CW-1 102	CW-2 109	CW-3 113	CW-4 43	CW-5 77	**CW-6 120**	CW-7 57	CW-8 60	CW-9 106
Di ethyl ether	20	CD-1 92	**CD-2 98**	CD-3 84	CD-4 95	CD-5 77	CD-6 84	CD-7 92	CD-8 76	CD-9 92
Nitrobenzene	**20**	NB-1 73	NB-2 83	NB-3 75	NB-4 112	NB-5 62	NB-6 78	NB-7 96	**NB-8 113**	NB-9 108

TABLE 2
CHLOROPHYLL A AND B OD VALUES AND CONCENTRATION

Samples	OD at 663	OD at 645	Chl a ug/ ml	Chl b ug/ml	Total Chl mg/100mg
HB1	0.073	0.047	0.040245	0.036733	0.076978
HB2	0.266	0.223	0.13992	0.193091	0.333011
HB3	0.254	0.23	0.13139	0.203914	0.335304
HB4	0.221	0.206	0.113555	0.184156	0.297711
HB5	0.238	0.218	0.12279	0.193918	0.316708
HB6	0.206	0.196	0.10533	0.176216	0.281546
HB7	0.243	0.217	0.126095	0.191603	0.317698
HB8	0.204	0.201	0.10341	0.182409	0.285819
HB9	0.246	0.216	0.12813	0.189756	0.317886
HW1	0.251	0.227	0.129875	0.201181	0.331056
HW2	0.228	0.22	0.11618	0.198548	0.314728
HW3	0.285	0.224	0.151855	0.18979	0.341645
HW4	0.241	0.217	0.124825	0.192071	0.316896
HW5	0.226	0.207	0.1166	0.184131	0.300731
HW6	0.235	0.208	0.122185	0.18317	0.305355
HW7	0.228	0.21	0.11748	0.187098	0.304578
HW8	0.222	0.205	0.11432	0.182777	0.297097
HW9	0.264	0.223	0.13865	0.193559	0.332209
HD1	0.267	0.223	0.140555	0.192857	0.333412

HD2	0.232	0.209	0.12015	0.185017	0.305167
HD3	0.195	0.185	0.099775	0.166195	0.26597
HD4	0.255	0.225	0.132675	0.197955	0.33063
HD5	0.262	0.228	0.13673	0.199752	0.336482
HD6	0.24	0.217	0.12419	0.192305	0.316495
HD7	0.223	0.21	0.114305	0.188268	0.302573
HD8	0.219	0.202	0.112805	0.180044	0.292849
HD9	0.189	0.181	0.096485	0.163019	0.259504
SB1	0.236	0.211	0.12243	0.186371	0.308801
SB2	0.268	0.224	0.14106	0.193768	0.334828
SB3	0.288	0.24	0.15168	0.207408	0.359088
SB4	0.238	0.217	0.12292	0.192773	0.315693
SB5	0.275	0.232	0.144465	0.20129	0.345755
SB6	0.252	0.224	0.1309	0.197512	0.328412
SB7	0.245	0.217	0.127365	0.191135	0.3185
SB8	0.254	0.219	0.13282	0.191319	0.324139
SB9	0.263	0.213	0.139315	0.182343	0.321658
SW1	0.244	0.209	0.12777	0.182209	0.309979
SW2	0.223	0.203	0.115215	0.180253	0.295468
SW3	0.249	0.216	0.130035	0.189054	0.319089
SW4	0.228	0.211	0.11735	0.188243	0.305593
SW5	0.242	0.216	0.12559	0.190692	0.316282
SW6	0.329	0.253	0.176025	0.212699	0.388724
SW7	0.234	0.211	0.12116	0.186839	0.307999
SW8	0.197	0.194	0.099875	0.176032	0.275907
SW9	0.293	0.24	0.154855	0.206238	0.361093
SD1	0.226	0.211	0.11608	0.188711	0.304791
SD2	0.204	0.199	0.10367	0.180119	0.283789
SD3	0.161	0.14	0.084035	0.122626	0.206661
SD4	0.22	0.203	0.11331	0.180955	0.294265
SD5	0.227	0.212	0.116585	0.189622	0.306207
SD6	0.242	0.224	0.12455	0.199852	0.324402
SD7	0.254	0.231	0.13126	0.205059	0.336319
SD8	0.192	0.179	0.09865	0.160027	0.258677
SD9	0.239	0.215	0.123815	0.190249	0.314064
CB1	0.221	0.205	0.113685	0.183011	0.296696
CB2	0.25	0.218	0.13041	0.19111	0.32152
CB3	0.03	0.066	0.01047	0.06855	0.07902
CB4	0.24	0.217	0.12419	0.192305	0.316495
CB5	0.203	0.222	0.100045	0.206688	0.306733
CB6	0.286	0.238	0.15067	0.205586	0.356256
CB7	0.23	0.211	0.11862	0.187775	0.306395
CB8	0.255	0.221	0.133195	0.193375	0.32657
CB9	0.199	0.217	0.098155	0.201899	0.300054
CW1	0.261	0.226	0.136355	0.197696	0.334051
CW2	0.262	0.251	0.13374	0.226087	0.359827
CW3	0.255	0.222	0.133065	0.19452	0.327585
CW4	0.214	0.203	0.1095	0.182359	0.291859
CW5	0.204	0.197	0.10393	0.177829	0.281759
CW6	0.233	0.213	0.120265	0.189363	0.309628
CW7	0.211	0.192	0.109025	0.170466	0.279491
CW8	0.216	0.191	0.11233	0.168151	0.280481
CW9	0.203	0.198	0.103165	0.179208	0.282373
CD1	0.216	0.2	0.11116	0.178456	0.289616
CD2	0.259	0.239	0.133395	0.213049	0.346444
CD3	0.244	0.209	0.12777	0.182209	0.309979
CD4	0.226	0.2	0.11751	0.176116	0.293626
CD5	0.26	0.226	0.13572	0.19793	0.33365

CD6	0.271	0.228	0.142445	0.197646	0.340091
CD7	0.22	0.208	0.11266	0.18668	0.29934
CD8	0.195	0.179	0.100555	0.159325	0.25988
CD9	0.199	0.206	0.099585	0.189304	0.288889
NB1	0.316	0.234	0.17024	0.193986	0.364226
NB2	0.276	0.235	0.14471	0.204491	0.349201
NB3	0.231	0.202	0.120425	0.177236	0.297661
NB4	0.241	0.221	0.124305	0.196651	0.320956
NB5	0.249	0.227	0.128605	0.201649	0.330254
NB6	0.243	0.228	0.124665	0.204198	0.328863
NB7	0.244	0.219	0.12647	0.193659	0.320129
NB8	0.24	0.222	0.12354	0.19803	0.32157
NB9	0.228	0.208	0.11774	0.184808	0.302548

TABLE 3
CARBOHYDRATE AND PROTEIN OD VALUE AND CONCENTRATION

	CARBOHYDRATE		PROTIEN	
	OD at 620nm	100mg/mG	OD at 660nm	100mg/g
HB-1	2.635	2.635	0.371	3.2
HB-2	2.352	2.352	0.429	3.7
HB-3	2.469	2.469	0.184	1.6
HB-4	2.529	2.529	0.084	0.7
HB-5	2.271	2.271	0.099	0.8
HB-6	2.215	2.215	0.077	0.6
HB-7	3.496	3.496	0.214	1.8
HB-8	3.082	3.082	0.170	1.4
HB-9	2.492	2.492	0.085	0.7
HW-1	2.811	2.811	0.729	6.3
HW-2	2.670	2.670	0.315	2.7
HW-3	2.809	2.809	0.330	2.8
HW-4	2.135	2.135	0.152	1.3
HW-5	2.800	2.800	0.066	0.5
HW-6	2.158	2.158	0.163	1.4
HW-7	3.557	3.557	0.347	3.0
HW-8	3.533	3.533	0.326	2.8
HW-9	2.538	2.538	0.230	2.0
HD-1	2.717	2.717	0.319	2.7
HD-2	2.352	2.352	0.557	4.8
HD-3	2.469	2.469	0.357	3.1
HD-4	2.529	2.529	0.064	0.5
HD-5	2.271	2.271	0.135	1.1
HD-6	2.215	2.215	0.105	0.9
HD-7	3.496	3.496	0.194	1.7
HD-8	3.082	3.082	0.421	3.6
HD-9	2.492	2.492	0.264	2.3
SB-1	2.773	2.773	0.485	4.2
SB-2	2.728	2.728	0.292	2.5
SB-3	2.785	2.785	0.342	2.9
SB-4	2.516	2.516	0.109	0.9
SB-5	2.119	2.119	0.070	0.6
SB-6	2.270	2.270	0.135	1.1
SB-7	3.617	3.617	0.306	2.6
SB-8	3.134	3.134	0.147	1.2

SB-9	3.708	3.708	0.401	3.4
SW-1	2.693	2.693	0.520	4.5
SW-2	2.805	2.805	0.791	6.8
SW-3	2.784	2.784	0.595	5.1
SW-4	2.883	2.883	0.103	0.9
SW-5	2.489	2.489	0.073	0.6
SW-6	2.359	2.359	0.108	0.9
SW-7	2.973	2.973	0.221	1.9
SW-8	3.134	3.134	0.442	3.8
SW-9	2.566	2.566	0.209	1.8
SD-1	2.770	2.770	0.384	3.3
SD-2	2.614	2.614	0.440	3.8
SD-3	2.745	2.745	0.417	3.6
SD-4	2.565	2.565	0.101	0.8
SD-5	2.378	2.378	0.061	0.5
SD-6	2.238	2.238	0.168	1.4
SD-7	2.874	2.874	0.248	2.1
SD-8	2.932	2.932	0.094	0.8
SD-9	3.450	3.450	0.230	2.0
CB-1	2.792	2.792	0.806	6.9
CB-2	2.615	2.615	0.487	4.2
CB-3	2.748	2.748	0.539	4.6
CB-4	2.956	2.956	0.163	1.4
CB-5	2.936	2.936	0.165	1.4
CB-6	2.533	2.533	0.214	1.8
CB-7	3.708	3.708	0.601	5.1
CB-8	2.069	2.069	0.281	2.4
CB-9	3.516	3.516	0.614	5.3
CW-1	2.770	2.770	0.320	2.7
CW-2	2.796	2.796	0.488	4.2
CW-3	2.770	2.770	0.384	3.2
CW-4	2.232	2.232	0.105	0.9
CW-5	2.977	2.977	0.045	0.4
CW-6	2.327	2.327	0.052	0.4
CW-7	2.990	2.990	0.116	1.0
CW-8	2.695	2.695	0.221	1.9
CW-9	3.186	3.186	0.265	2.4
CD-1	2.551	2.551	0.493	4.2
CD-2	2.740	2.740	0.240	2.0
CD-3	2.777	2.777	0.498	4.3
CD-4	2.549	2.549	0.161	1.3
CD-5	2.729	2.729	0.119	1.0
CD-6	2.160	2.160	0.119	1.0
CD-7	3.129	3.129	0.258	2.2
CD-8	2.223	2.223	0.323	2.8
CD-9	2.950	2.950	0.314	2.7
NB-1	2.614	2.614	0.340	2.9
NB-2	2.766	2.766	0.425	3.7
NB-3	2.717	2.717	0.375	3.2
NB-4	2.037	2.037	0.072	0.6

NB-5	2.272	2.272	0.103	0.9
NB-6	2.958	2.958	0.122	1.0
NB-7	3.342	3.342	0.198	1.7
NB-8	3.510	3.510	0.245	2.1
NB-9	3.640	3.640	0.267	2.3

FIG: 1-10. TOTAL CHLOROPHYLL ESTIMATION CHARTS:

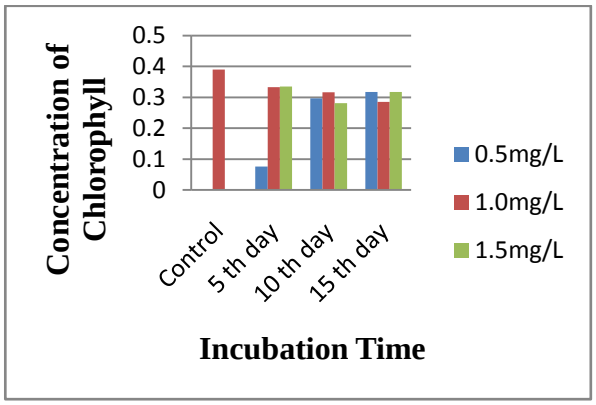


FIG:1. TOTAL CHLOROPHYLL FOR *HALIMEDA GRACILIS* IN BENZENE

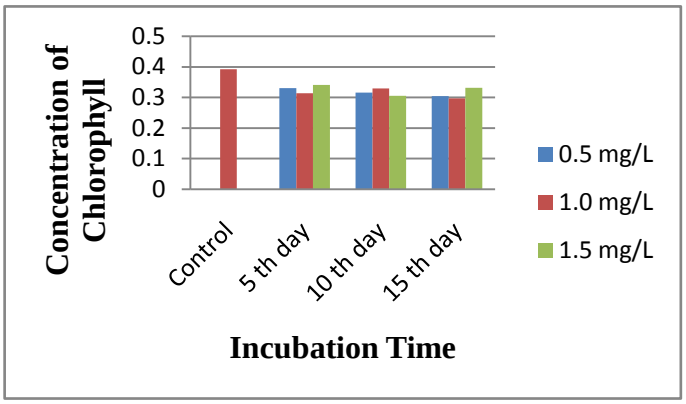


FIG: 2. TOTAL CHLOROPHYLL FOR *HALIMEDA GRACILIS* IN WATER

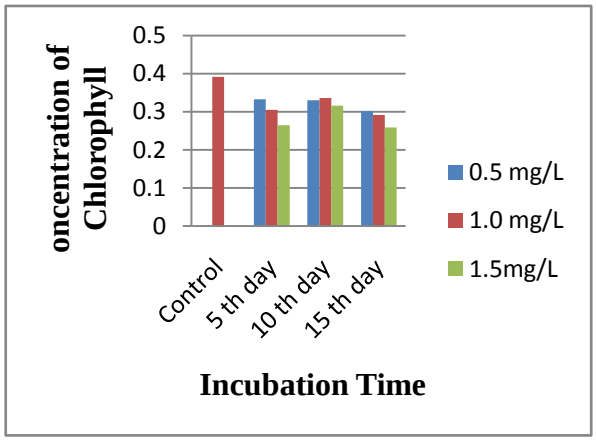


FIG: 3. TOTAL CHLOROPHYLL FOR *HALIMEDA GRACILIS* IN DIETHYL ETHER

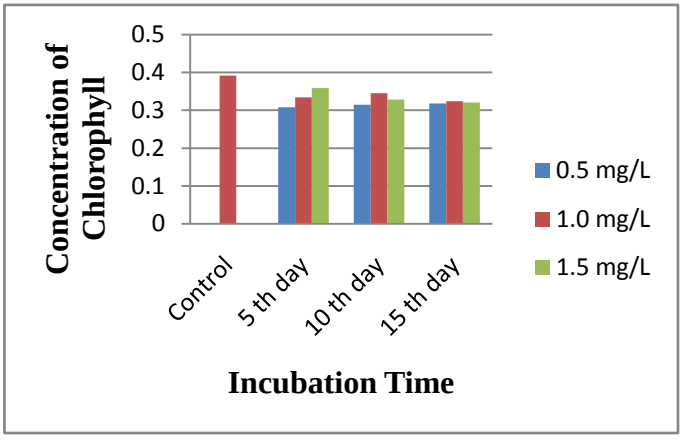


FIG: 4.TOTAL CHLOROPHYLL FOR *CERAMIUM RUBRUM* IN BENZENE

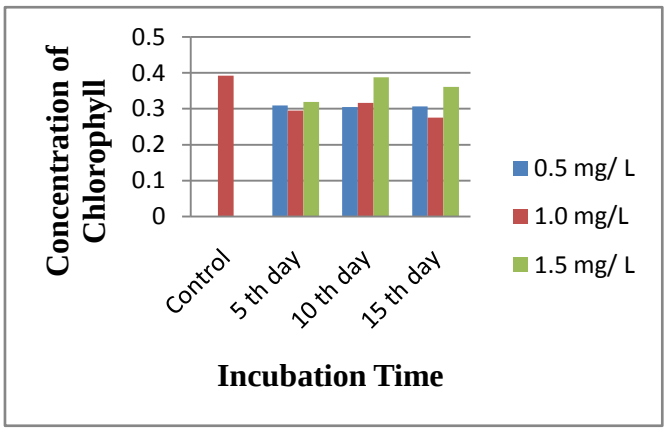


FIG: 5.TOTAL CHLOROPHYLL FOR *CERAMIUM RUBRUM* IN WATER

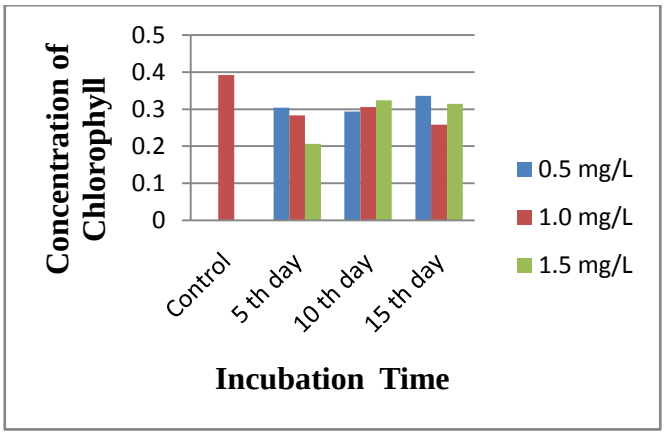


FIG: 6. TOTAL CHLOROPHYLL FOR *CERAMIUM RUBRUM* IN DIETHYL ETHER

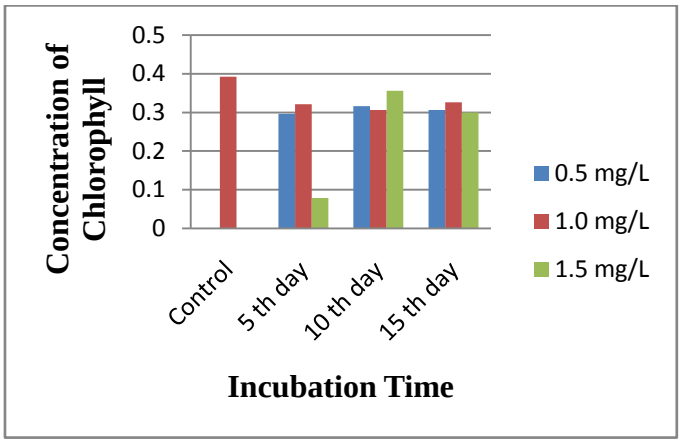


FIG: 7. TOTAL CHLOROPHYLL FOR *CYSTOPHYLLUM MURICATUM* IN BENZENE

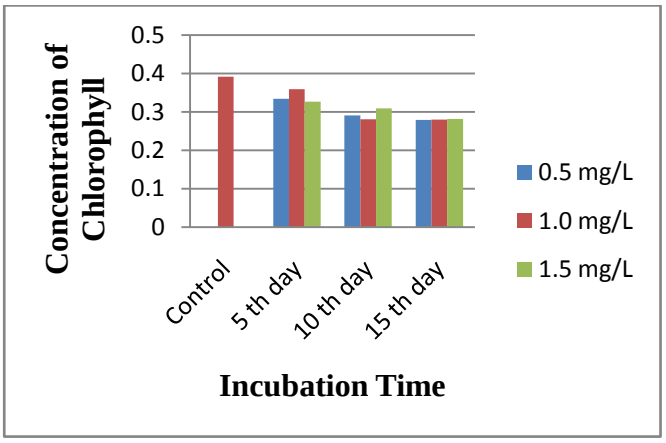


FIG: 8.TOTAL CHLOROPHYLL FOR *CYSTOPHYLLUM MURICATUM* IN WATER

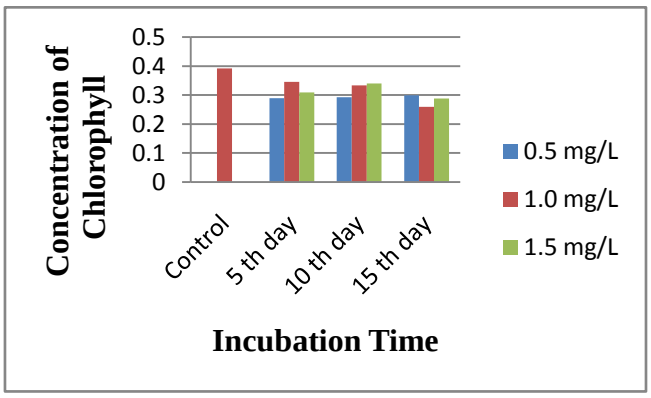


FIG: 9. TOTAL CHLOROPHYLL FOR *CYSTOPHYLLUM MURICATUM* IN DIETHYLETHER

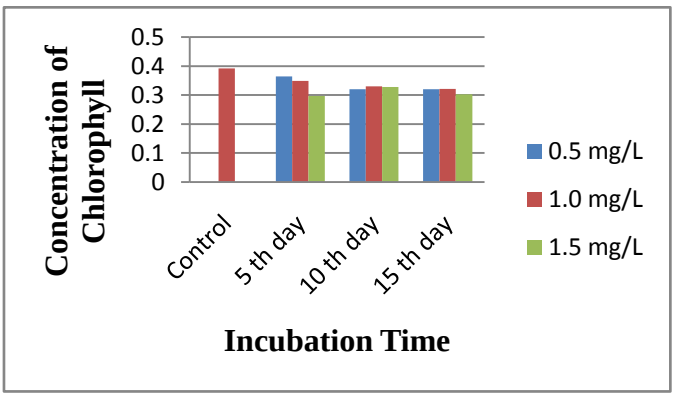


FIG: 10.TOTAL CHLOROPHYLL FOR *NITROBENZEN*

FIG: 11. TOTAL CARBOHYDRATE ESTIMATION CHARTS (FIG. 12-21):

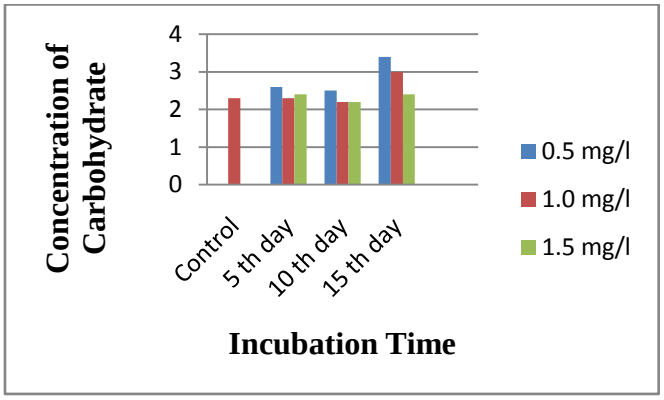


FIG: 12. TOTAL CARBOHYDRATE FOR *HALIMEDA GRACILIS* IN BENZENE

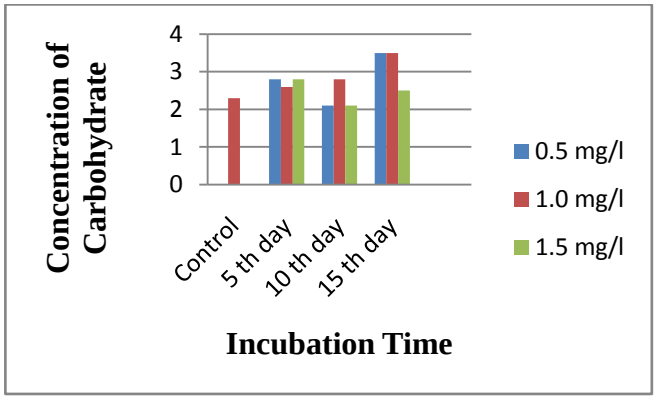


FIG: 13.TOTAL CARBOHYDRATE FOR *HALIMEDA GRACILIS* IN WATER

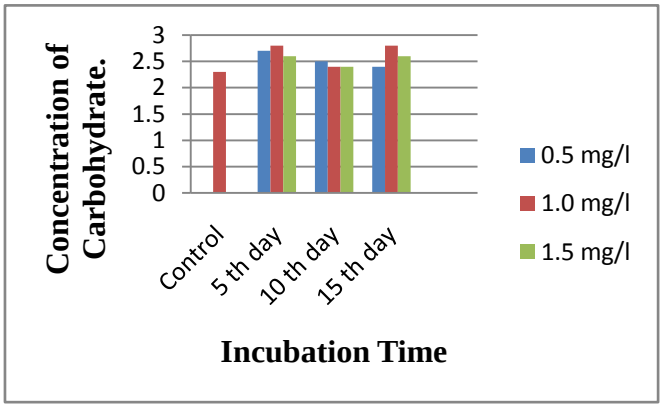


FIG: 14. TOTAL CARBOHYDRATE FOR *HALIMEDA GRACILIS* IN DIETHYEL ETHER

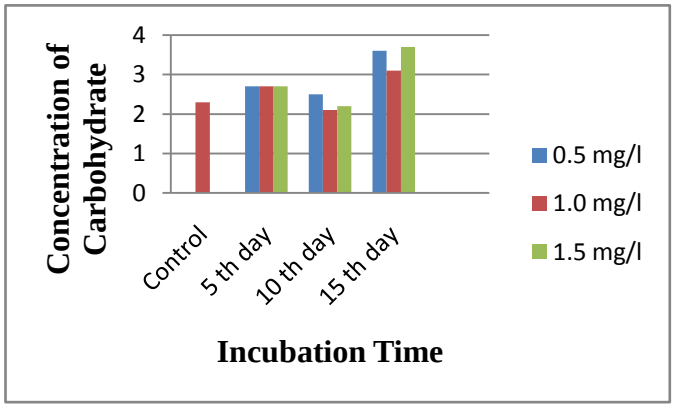


FIG: 15.TOTAL CARBOHYDRATE FOR *CERAMIUM RUBRUM* IN BENZENE

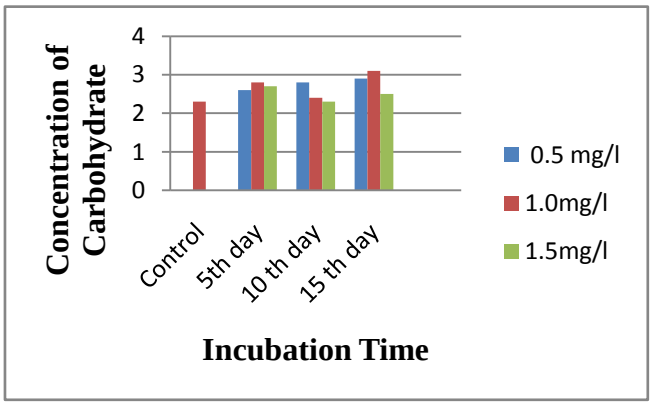


FIG: 16.TOTAL CARBOHYDRATE FOR *CERAMIUM RUBRUM* IN WATER

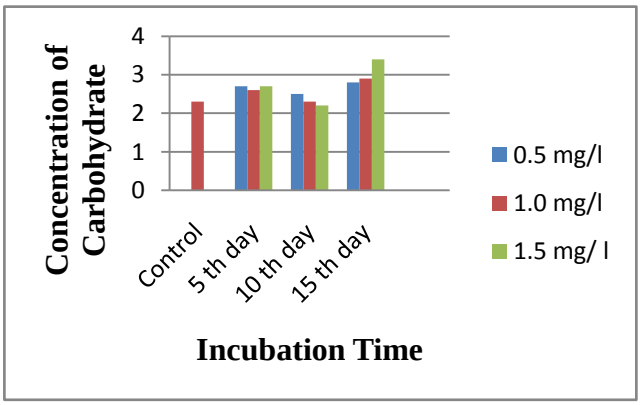


FIG: 17.TOTAL CARBOHYDRATE FOR *CERAMIUM RUBRUM* IN DIETHYEL ETHER

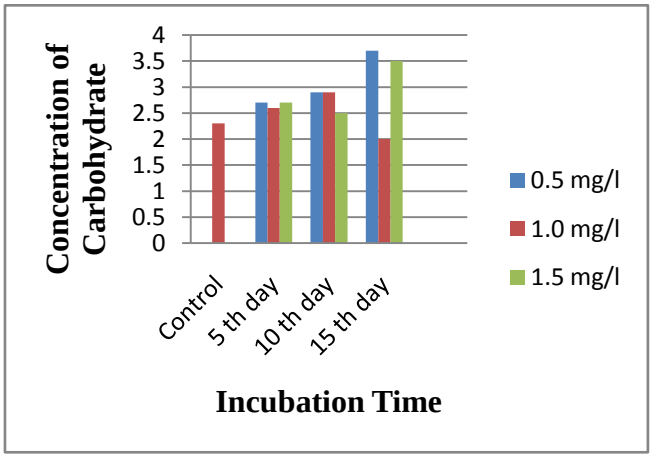


FIG : 18.TOTAL CARBOHYDRATE FOR *CYSTOPHYLLUM MURICATUM* IN BENZENE

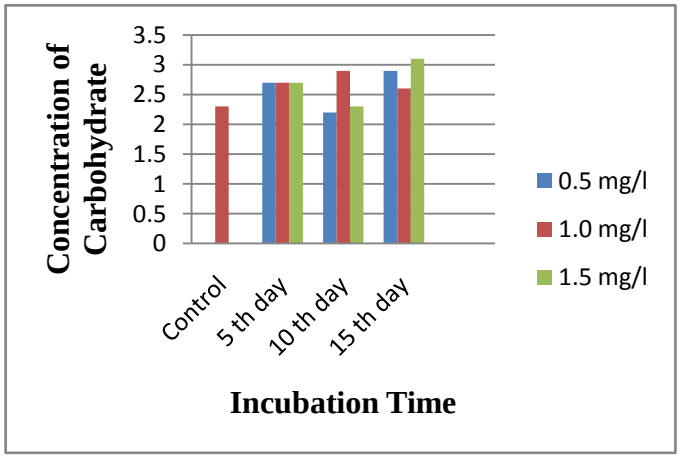


FIG: 19.TOTAL CARBOHYDRATE FOR *CYSTOPHYLLUM MURICATUM* IN WATER

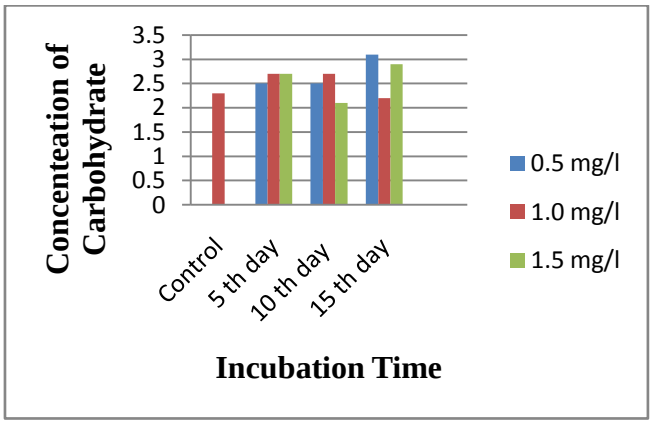


FIG: 20. TOTAL CARBOHYDRATE FOR *CYSTOPHYLLUM MURICATUM* IN DIETHYL ETHER

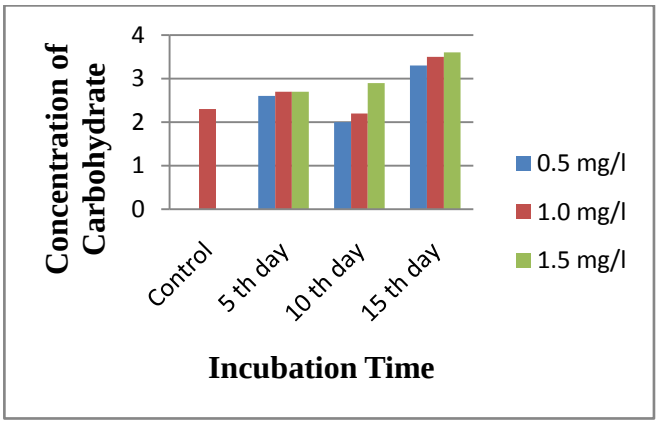


FIG: 21.TOTAL CARBOHYDRATE OF NITROBENZENE

FIG: 22. TOTAL PROTEIN ESTIMATION CHARTS (FIG. 23- 32):

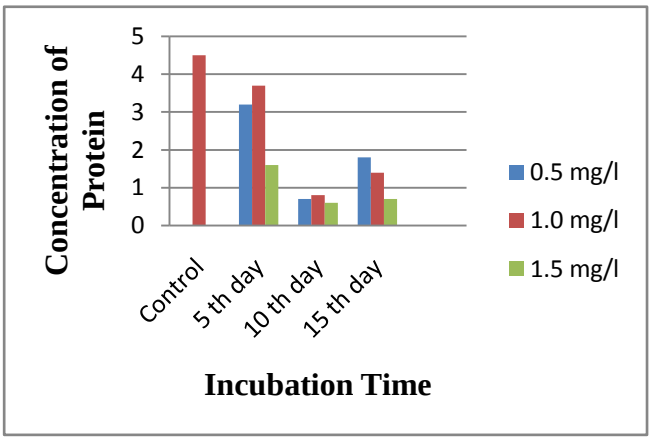


FIG: 23. TOTAL PROTEIN FOR *HALIMIDA GRACILIS* IN BENZENE

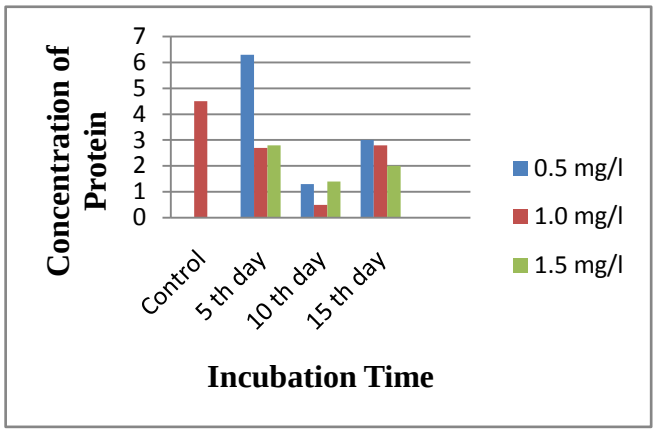


FIG: 24.TOTAL PROTEIN FOR *HALIMIDA GRACILIS* IN WATER

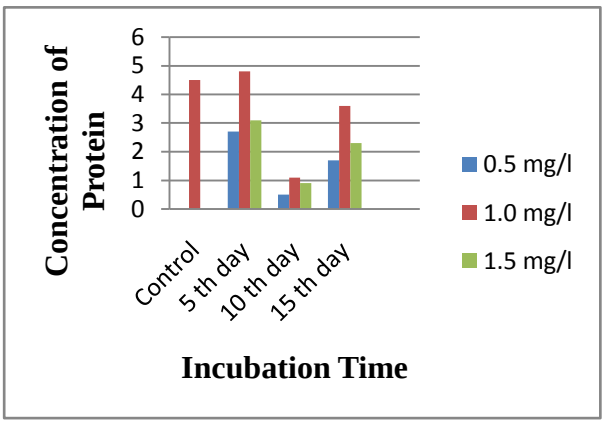


FIG: 25.TOTAL PROTEIN FOR *HALIMIDA GRACILIS* IN DIETHYL ETHER

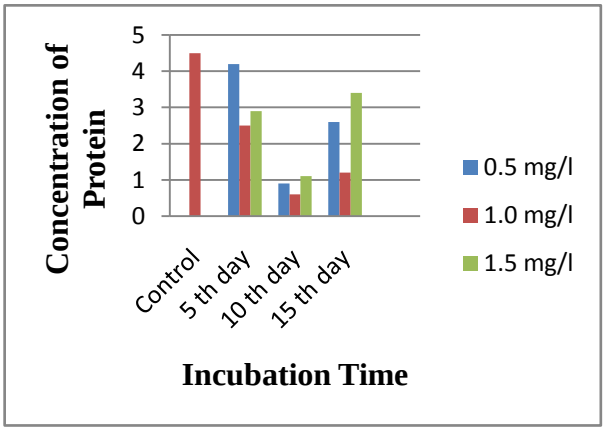


FIG: 26. TOTAL PROTEIN FOR *CERAMIUM RUBRUM* IN BENZENE

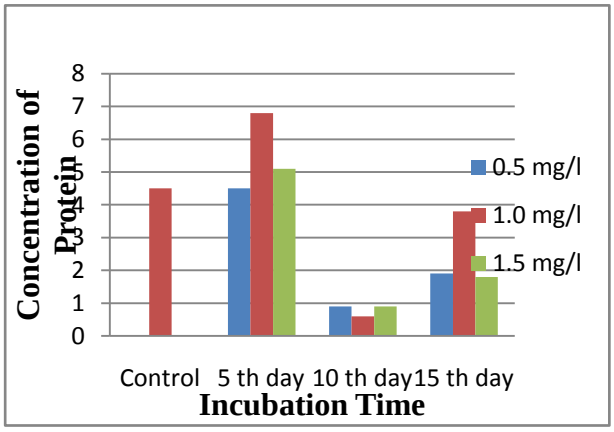


FIG: 27. TOTAL PROTEIN FOR *CERAMIUM RUBRUM* IN WATER

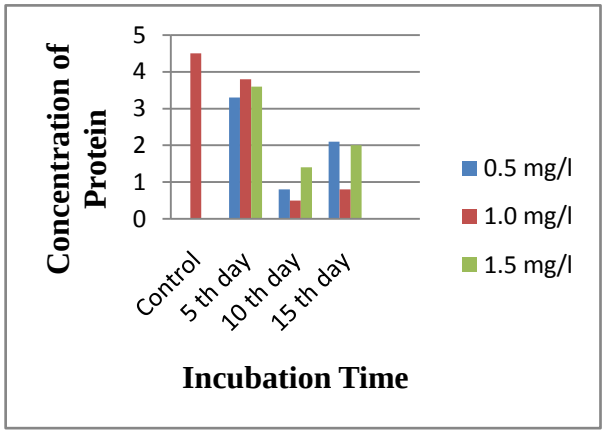


FIG: 28. TOTAL PROTEIN FOR *CERAMIUM RUBRUM* IN DIETHYL ETHER

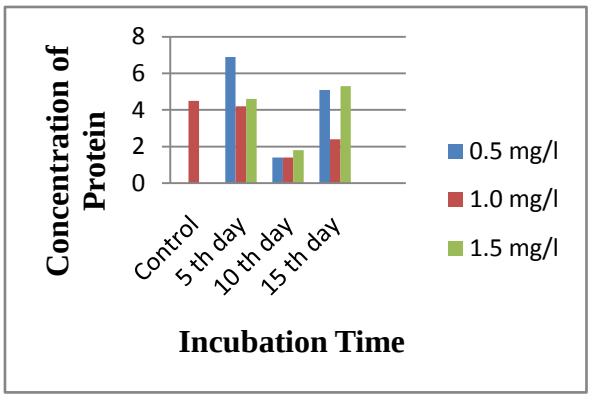


FIG: 29. TOTAL PROTEIN FOR *CYSTOPHYLLUM MURICATUM* IN BENZENE

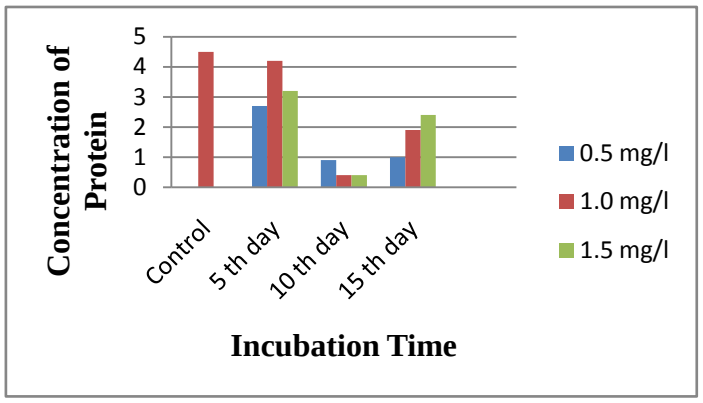


FIG: 30.TOTAL PROTEIN FOR *CYSTOPHYLLUM MURICATUM* IN WATER

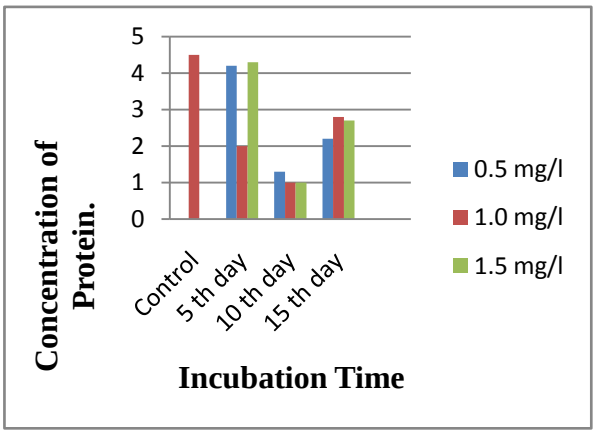


FIG: 31.TOTAL PROTEIN FOR *CYSTOPHYLLUM MURICATUM* IN DIETHYLETHER

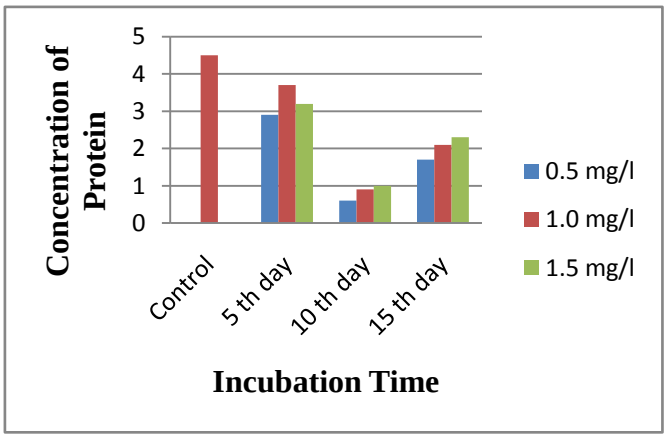


FIG: 32.TOTAL PROTEIN FOR NITROBENZENE

Different extraction methods can be used for seaweed extracts preparation i.e. water extraction under high pressure, alcohol extraction, alkaline extraction, microwave-assisted extraction (MAE) and supercritical CO2 extraction. Conditions of the process depend on the active substances of interest. Extracts rich in auxins can be produced by alkaline extraction. The process is carried out under low pressure. Previously dried probes are extracted with the use of sodium hydroxide (Booth 1996). Extraction in 85% methanol leads to obtainment of algae extract rich in gibberelins. Biomass should be previously homogenized. The temperature of the process is 4°C (Hytonen *et al*., 2009).

Seaweed liquid fertilizer was used for coconut plantation in Tamilnadu and Kerala (Kalimuthu *et al*., 1987). An experimental field trail report of CMFRI (Central Marine Fisheries Research Institute), Mandapam reported that by using 3 months old Hypnea and cowdung compost on Bhendi crop gave 73% higher yield than that of control. Seaweeds are not only used as compost but can also be used as a liquid fertilizer. Liquid seaweed extract when applied to seed, soil or sprayed on crops it increased seed germination percentage, nutrient uptake, growth (Immanuel and Subramanian, 1999).SWC treatment enhanced both root shoot ratios and biomass accumulation in tomato seedlings by stimulating root growth (Crouch and van Staden 1992). Seaweeds and seaweed products enhance plant chlorophyll content (Blunden 1997). Seaweed concentrate trigger early flowering and fruit stein a number of crop plants (Abetz and Young 1983). Tomato seedlings treated with SWC set more flowers earlier than the control plants and this was not considered to be a stress response (Crouch and van Staden 1992). Seaweed extracts have been shown to improve plant resistance against insect and diseases (Allen and others 2001). Our study has helped to extend the period of growth and improved the quality of the plants. Ultimately, the fabricated support presented fertilizer properties, water retention and biodegradability and could serve in horticulture as an alternative to plastic pots and chemical fertilizer (Asma Chbani 2013). The extract of *Enteromorpha intestinalis* enlarged seed germination, root, shoot length and chlorophyll content of *Sesamum indicum* (Gandhiyappan and Perumal 2001). The effect of the liquid extract from *Sargassum wightii* on *Arachis hypogaea* which showed boost in height and quantity of branches of the plant in comparison to chemical treatment (Sridhar and Rengasamy., 2010). The callus from the *Withania somnifera* treated with seaweed extract in vitro in various concentration, the good results were obtain in medium with 40% of seaweed extract, it gives 8.6 shoots / callus. While in higher concentration of seaweed in medium ie: 80% produce 4.3 shoots / callus (Sathees kannan.,2014).The *Arachis hypogea* L. seeds were treated with seaweed extract in various concentration and the fantastic result were obtain in 2%. The fresh weight, dry weight, root, shoot length, number of branches, leaf, protein, lipid, chlorophyll, carbohydrate were obtain high while compare to others. The leaf tissue of 2% SLF treated groundnut sample and control plant leaf tissue were analysis in scanning electron microscopic with energy Dispersive spectroscopic analysis the elements varies (Ganapathy selvam and Sivakumar 2014).

Seaweed *Caulerpa racemosa* extract 3% spray at vegetative stage and flowering stage increase in chlorophyll, crop growth rate, Seed weights, while compare to control (Sujatha and Vijayalakshmi 2013). The GC-MS analysis of *Caulerpa racemosa* extract shows much of plant nutrients, trace elements and antioxidant. The foliar spray of *Kappaphycus alvarezii* 5% enhances the fruit and the foliar spray plant leaves shown antibacterial activity and resistance to fruit borers compare to control (Zodape *et al.,* 2011).

According to Jeyakumar, Department of Crop biology Tamilnadu Agricultural University, Coimbatore, Nitrobenzene is a Combination of nitrogen and plant growth regulators, extracted from seaweeds. Nitrobenzene produces best results in combination of plant growth regulators, which have capacity to increase flowering in plant and also prevent flower shedding. Yield contributing characters like plant height increase by 8-10% and number of branches per plant increase by 15-20%. Four sprays of Nitrobenzene during 40,55,80 and 105 DAS improve the yield up to 40%.

Groundnut is a legume of economic importance, its improvement could greatly benefit from the integration of both classical and modern techniques. Although biotechnology techniques such as tissue culture and gene transformation have been reported in peanut. The different concentration of 2,4-D induced callus. The 2 mg/L 2,4-D gives maximum level of Callus (Alamand Khaleque 2010). The callus from hypocotyls explants were obtained in 0.5 mg/ L of 2,4-D long with BAP (Muthusamy *et al*., 2007). Maximum level of callus from mature tissues were obtained from 3.0 mg/ L of IAA and 1.0 mg/L of BAP (Palanivel *et al*., 2002).

IV. CONCLUSION

In present study totally three seaweeds namely *Halimeda gracilus, Ceramium rubrum* and *Cystophyllum muricatum* were used. The seaweeds were extracted with three different organic solvents such as benzene, diethyl ether and water. Totally nine extracts were obtained from three seaweeds. Among the nine extracts, *Cystophyllum muricatum* aqueous extract gave

high amount of crude extract 3.150grams, Callus Bioassay was done at different concentration (0.5,1.0,1.5 mg L^{-1}) at different incubation (5, 10, 15 days). *Ceramium rubrum* benzene extract showed increased level of total carbohydrate content, while the total protein level increased in *Cystophyllum muricatum* benzene extract and there was no increase in total chlorophyll content with all the extracts tested. This is the first report on application of seaweed extract and nitrobenzene granule, a flower enhancing hormone in *A.hypogaea* callus culture. GC – MS and LC-MS have to be done for further characterization of the bio molecules present in the three seaweed different solvent, water extracts and TLC for confirmation of Nitrobenzene compound.

ACKNOWLEDGEMENTS

We would be thankful to M.B.Viswanathan, Head, Department of Botany, Bharathidasan University, Trichy-24 for providing the Lab facilities to carry out the research.

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