Phytochemical Research of *Methylobacterium* Species Seaweed Extraction Chilly and Tomato Plant Growth

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Abstract— Chemical fertilizers possess health risks and soil pollution, in addition to these very expensive and significantly higher production costs. Seaweed liquid fertilizer is proved to be the better alternative to nourish plants. Hence, the present study focused on the alternate from Sargassum Species seaweed. Extraction of sea weed with water, ethanol, chloroform, methanol, petroleum ether and ethyl acetate were tested for the efficiency with and without Microbial supplementation. Methanolic extract found to show higher protein components (670 µg/ml), carbohydrate (98µg/ml), total phenolic (0.256 mg/ml), chlorophyll (0.260 mg/ml) and lower levels of lipids (0.650gm/10mL), carotenoids (0.057mg/ml), and Total Flavonoid (1.087 mg/ml). Bacterial supplementation of seaweed with Methylobacterium species was found to influence plant growth positively.

Keywords :Liquid Fertilizer, organic, Sargassum Species, Sea weed

I. INTRODUCTION

Many viable options need to be researched to meet the increasing demand and one of these is the use of seaweed as a fertilizer. Seaweed nutrient composition varies with humidity, temperature, geographic place, weather and species [1, 2]. Biological properties of macro algae are more important for agriculture field. So in this study selected for liquid fertilizer from seaweed. Seaweed used primarily for liquid fertilizer, dung, agar, carrageenan and alginate production.In latest years, the use of seaweeds as biofertilizers has risen in horticulture and agriculture [3].*Methylobacterium* species, which are the primary genus in the microbial leaf group, have mutual interactions with some plants[4].A significant study issue for sustainable crop development in the 21st century is the use of plant growth supporting symbiotic and non-symbiotic free-living beneficial bacteria as an external cause of nitrogen [5]. In order to determine the impacts of inoculation with *Methylobacteriumsuomiense* CBMB120, an experiment was performed under regulated circumstances to decrease the fertilization on red pepper of external chemical nitrogen, plant growth promoting (PGP) root and stem colonizer [6].Hydroxyl radicals in the troposphere oxidize most of the methane produced into the atmosphere. The only biological source for methane is methanotrophs:they oxidize methane from terrestrial and aquatic environments as well as atmospheric methane generated before it enters the atmosphere [7].

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II. MATERIALS AND METHODS

A. Seaweed collection:

The brown Sargassumsp seaweeds were gathered from the coastal region, Palladam (10.9997° N, 77.2807° E), Rameshwaram (9.2876° N, 79.3129° E), Tamilnadu, India.The seaweed was cleaned with distilled water and then placed in a freezer (−40 ° C) instantly and was frozen for 24 hours in a freeze dryer.The dried sample was then ground to powder and stored for further analysis in a sealed bag at −40 ° C.

A. Extraction of Seaweed with various solvents:

The freeze-dried seaweed powder has been immersed in multiple solvents (1: 20, w / v), including ethyl acetate (80% v / v) methanol (80% v / v), chloroform (80% v / v) and alcohol (80% v / v), petroleum oxide and water at room temperature for 24 hours in shaking incubators to extract plant growth promoting component (Airanthi et al. 2011).The sample was then passed through the filter paper Whatman No.1, and supernatant was gathered in a container. The residues under the same circumstances were re-soaked again.The combined filtrate was evaporated to obtain the dried extract in a rotary evaporator under vacuum at 40 ° C.

B. Optimization of Seaweed:

Seaweed powder was weighed and soaked with the various volume of solvent to identify the solvent which produces more of extract. The ratio of seaweed solvent was optimized with the concentration ratio of seaweed and methanol solvent (w/v) of 1:250, 2:250, 3:250, 4:250 and 5:250. Extractions were repeated for 10 times and the values expressed as Standard Deviation.

Physicochemical Analysis of seaweed extract:

a) Total Phenolic Content (TPC):

Spectrophotometriccally defined the TPC of seaweed extracts (Matanjun et al. 2008).The diluted (5 mg mL^{-1})

seaweed extracts of 0.1 mL volume were introduced to the 1.0 mL of Folin – Ciocalteu reagent (diluted 10-fold)and the mixture was thoroughly mixed. The mixture was introduced to 0.8 mL of sodium carbonate (7.5 percent, w / v) after 3 min and held at room temperature in the dark for 30 min. The absorbance was evaluated against the blank solution at 765 nm after 30 minutes.

Total Flavonoids Content (TFC)

According to Chang et al. (2002), the TFC of seaweed extract obtained from multiple solvents and water was calculated by the colorimetric technique.The 0.5 mL of seaweed extract blended with 0.1 mL of 1 M potassium acetate, 0.1 mL of aluminum chloride (10%), 1.5 mL of methanol and 2.8 mL of distilled liquid. The solution was held for 30 minutes in the dark.The solution's absorbance was evaluated at 415 nm.

Total Carotenoids Content

The complete carotenoid concentration of solvents and water samples from the seaweed*Sargassumsp*was defined by Kirk and Allen (1965) procedure.With 20 ml of 80% Acetone, 1 gm of seaweed extract was taken and added. The samples were then centrifuged for 30 minutes at 3000 rpm. The residue was re-extracted with 10 ml of 80% acetone and evaluated for the content of carotenoids. Absorbance was read at 645nm in UV-Spectrophotometer. The formulae used to calculated the complete carotenoid content in the sample: Carotenoid (mg/g fresh weight) = ΔA 480 + (0.114× ΔA 663) – (0.638 × ΔA 645).

b) Total Chlorophyll Content (TCPC)

The TCPC of *Sargassum*sp of solvents and water extracts were determined by the technique of Kivk and Allen (1965). The method used for Chlorophyll estimation is the same as for carotenoid content analysis rather the final extract was analyzed spectrophotometer at 480 nm.

c) Estimation of Protein

Lowry's Method (Raymont et. al. 1964) measured the protein contentProtein reacts with FCR to give 660 nm of a blue-colored mixture. About 0.5 gm of sample was mixed with water in mortar and pestle and centrifuged at 5000 rpm for 10 minutes. The supernatant has been used to estimate protein. A standard graph with concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard solution was plotted using Bovine serum albumin (BSA).The 0.4 mL sample was taken in distinct tubes with a water volume of up to 1 mL.Added 5ml of Solution C (1 vol. Folin-Ciocalteau reagent-diluted reagent with 15 vol.) and then mix well and incubate at room temperature for 10 minutes, then add 0.5 mL FCR and 30 minutes incubation.

d) Estimation of Carbohydrate

Anthrone technique (Roe, 1955) measured the carbohydrate content. Samples of Seaweed were immersed in 80% ethanol and centrifuged at 4000 rpm. In 0.5 ml of supernatant, 5 ml of anthrone reagent was introduced. The tubes were held for 15 minutes in a boiling water bath and held for 10 minutes in a dark room.The color developed was read at 650 nm in a spectrophotometer.

e) Estimation of Lipid

According to Folch et al. (1957), the lipid quantity was measured using mixture of chloroform-methanol. 5 ml of Chloroform-methanol (2:1) mixture was added to 400 mg of sample. At room temperature, the mixture was incubated for 24 hours.The mixture was filtered after incubation and collected in a pre-weighted beaker of 10 ml. The mixture of chloroform-methanol has been evaporated on a hot plate leaving a residue at the beaker's bottom. The beaker was calculated with the residue and weight of the empty beaker to understand the weight of the lipid in the sample.

f) Estimation of Amino acids

Ninhydrin technique (Moore and Stein, 1948) predicted the complete free amino acid quantity of newly gathered algae frozen tissues. To obtain the total quantity at 4.0 ml, water was added to the appropriate algal extract aliquots.Then add 1,0 ml of ninhydrin reagent and mixed and stored for 15 minutes in a boiling water bath. The tubes were then separated, cooled and added 1.0 mL of 50% ethanol. The pink colour developed in Spectrophotometer was evaluated at 550 nm.

III. RESULTS AND DISCUSSION

a) Starting material (Sargassumspecies)

Seaweed which was collected from coastal region Palladam, Rameshwaram, Tamilnadu, India was brown algae *Sargassum* species. *Sargassum, one of the Phaeophyceae class of marine macroalgal genera, is commonly found in temperate and tropical oceans.*It refers to the Sargassaceae family and the Fucales order. It is an enormous brown algae that is economically essential and ecologically dominant in manytropics.It is commonly allocated on Tamilnadu, India a nd many areas of Asia's southern shores.

Extraction of liquid fertilizer from seaweed using various solvents

The dry weight of seaweed extracts was analyzed using different solvents like methanol, ethanol, chloroform, ethyl acetate and petroleum ether. The methanol solvent showed the highest amount of dry weight in seaweed extract (1.370 grams).

b)Optimization of Seaweed extract with methanol

In this study the *Sargassum* seaweed gave a higher yield of methanol extract of about 5.97±0.2%.

Table: 1 Effect of solvent and seaweed ratio on seaweed liquid fertilizer (SLF) extraction

Seaweed : Methanol Ratio	**Quantity of Extracts Obtained(g)**
10:250	2.563± 0.4*
20:250	3.278± 0.2*
30:250	4.789± 0.3*
40:250	**5.972± 0.2***

*****SD (Standard Deviation) value of the extract obtained with different ratio of the Seaweed and methanol

Physicochemical Analysis of Sargassum Species

i. Total Phenolic Content (TPC):

The total phenolic content found to be high of about 0.456mg/mL with methanol than other solvents like ethanol of about 0.149 mg/mL and lower in petroleum ether as it is a non- polar solvent (Fig 1).

ii. Total Flavonoids Content [TFC]

The total Flavonoids content found to be high of about 1.587mg/ mL with methanol than other solvents like ethanol of about 0.227 mg/mL and lower in petroleum ether as it is a non- polar solvent (Fig 1).

iii. Total Chlorophyll Content [TCPC]

The TCPC of the seaweed extracts with the Methanol is 0.78 mg/g which exhibited significantly less when compared to the chlorophyll content in seaweed extracted with ethanol is 0.07 mg/g (Fig 1).

iv. Total Carotenoids Content [TCC]

Additionally, the TCC of the seaweed extract was significantly high in methanol (5.27 mg/g of extract) when compared to ethanol (2.8 mg/ml) (Fig 1).

v. Total Protein content

The amount of protein present in 1 ml of seaweed sample. The protein content was the maximum in methanol sample 0.63 mg/mL when compared to ethanol sample 0.27 mg/mL (Fig 1).

vi. Total Carbohydrate content

The carbohydrate content of seaweed with methanol is about 0.98 mg/mL when compared to seaweed with ethanol which is about 0.65 mg/mL (Fig 1).

vii. Amount of Lipid present

The total lipid contents present in the seaweed was 2.850gm/10mL DW of methanol when compared to ethanol of about 0.521gm/10mL (Fig 1).

viii. Estimation of Amino acids

The amount of amino acids present in the seaweed extract with methanol is about 0.546 µg/mL and it shows the highest amount of amino acids present when compared to seaweed extract with ethanol 0.043 µg/mL (Fig 1).

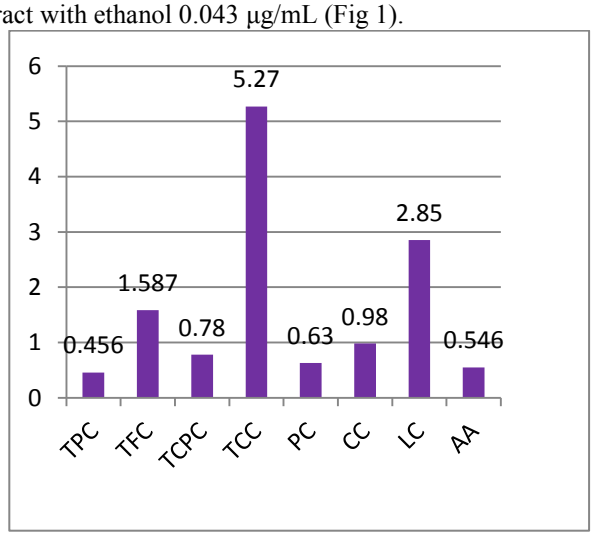

Fig 1: Physicochemical analysis of seaweedextract

TPC – Total phenolic content; TFC – Total flavonoids content; TCPC – Total chlorophyll content; TCC – Total Carotenoid content; PC- Protein Content; CC – Carbohydrate content; LC- Lipid Content; AA- Aminoacids

Compared to the consequence, production enhanced with improved solvent polarity (the methanol and diethyl alcohol polarity index is 5.1 and 2.8 respectively), implying that more polar compounds were observed in seaweed extracts. Variations in the yields of distinct products are ascribed to the polarities of multiple compounds existing in the crops.Because methanol extracts produced considerably greater yields for all seaweeds than diethyl ether, methanol extracts (dry weight base) were used.The peak growth parameter (fresh weight and wet weight, root length, shoot length) was reported in crops that were fed with *Methylobacterium* species by 40&% SLF of Sargassum.

IV. CONCLUSION

Seaweed liquid fertilizers will be helpful for greater plant development, as the extract includes growth promoting amino acids, antibiotics, micronutrients, vitamins, trace elements, hormones, gibberellins and cytokinins. The quantity of the extract was high when it was compared with *Avrainvilleaerecta*which have yielded only 681.2 mg with methanol extraction. It was discovered that the carotenoid content was two times greater than that of methanolic extracts of C. calcitrans[(2.33 ± 0.14) mg / g DW] as indicated by Goiris et al 2012.This may be owing to variations in growth stage or culture circumstances that influence the quantity of carotenoids generated by the microalgae cells immediately.

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