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Effect of *Grateloupia lithophila* (sea weed) extract as biofertilizer on edible *plant Amaranthus tricolor var.tristis*

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Abstract

Seaweeds are large group of marine algae belonging to Chlorophyceae, Pheophyceae and rhodophyceae with more than 20,000 species in there in the world. *Grateloupia lithophila* was collected from kovalam and used as bio fertilizer for vegetable amaranthus, *Amaranthus tricolor vartristis*. Four different concentration of seaweed extract were prepared, such as1:200, 1:400, 1:600, and 1:800. Prepared bio fertilizer was treated with the edible plant *Amaranthus tricolor vartristis* and subjected to protein and carbohydrate analysis. Among the various concentrations tested 1:400 concentration of *Grateloupia lithophila* extract shows higher amount of protein and carbohydrates. The results shows that there is increase in the nutritive value of the vegetable Amaranthus compared to control plants. This increase due to this seaweed bio fertilizer. Primary pigment chlorophyll, carotenoid and secondary pigment phycocyanin were analysed for three samples (control, 1:400 seaweed treated plant and seaweed samples). The pigments were found to be higher in the seaweed treated sample. It is evident that pigments like phycocyanin shows hepatoprotective and neuroprotective activities. The antioxidant property of the plant sample was tested. The result shows that there is increased amount of reductone in the treated sample compared to other samples. This may be due to presence of secondary metabolites in samples. Recent studies on seaweed shows that it not only used as a bio fertilizer it will be also health promoting properties and pharmaceutical applications. So we can use these sea weed as a bio fertilizer.

Key words: *Phycocyanin, Biofertilizer, Hepatoprotective, Chlorophyll, Carotenoid*

1. Introduction

Population is growing at a fast rate, the agro based product also should need to increase Disadvantages of using chemical fertilizer is also clearly visible. So now a days farmers are surely turns towards organic fertilizer. According to Chhaya ND 1997, one of such option is the use of seaweed as the bio fertilizer. The effect of seaweed extract is due to the microelements and plant growth regulators such as cytokinin present in it. Seaweed extract is used as a foliar spray. Application to soil and soaking of seeds before sowing. It enhances the germination of seeds and protect the seeds from frost and fungal diseases. Seaweed extract is increasing the quality of product and serves as an excellent agents that maintain soil conditions.

Many chronic diseases will get resolved by adding seaweeds to the diet. Eating sea vegetables regularly can facilitate the excretion of heavy metals, radioactive elements, dioxins and PCBs from our bodies, promote a healthy immune system, prevent thyroid disease, obesity, cancers and metastases, cardiovascular disease, type 2 diabetes, nervous system disorders, osteoporosis, reduce chronic inflammation, inhibit viruses (including herpes and human papilloma virus), and help regulate menses. In fact, the Japanese people's remarkable longevity and extremely low incidence of cardiovascular disease, thyroid disease, breast cancer and prostate cancer may largely be due to the fact that they have the consumed more seaweed.

Vegetable Amaranthus includes *Amaranthus tricolor var. tricolor*, *Amaranthus tricolor var. tristis*; Vegetable Amaranthus can be easily distinguished by inflorescence features like mostly or

exclusively axillary glomerules or short spikes, Origin of flower bud from leaf axil, 3 tepal lobes, 3 stamens, brownish black seed, indeterminate growth habit. Vegetable Amaranthus can be easily distinguished by inflorescence features like mostly or exclusively axillary glomerules or short spikes, the seed character is also very useful in demarcating vegetable, grain and weed Amaranthus (Das 2012). Vegetable group shares striking similarity with weed group in having brownish-black or black seeds with undifferentiated folded Inflorescences of selected Amaranthus species Cooked amaranth leaves are an excellent source of vitamin A, vitamin C, calcium, manganese, and folate. Grain amaranth has been used for food by humans in a number of ways. The ground grain is used in breads, noodles, pancakes, cereals, granola, cookies and other flour-based products. The grain can be popped like popcorn or flaked like oatmeal. More than 40 products containing amaranth are currently on the market. Nutritional value of these plant is high, so use of seaweed will also increase the nutritive value of this plant.it is easily available to all kind of people.

2. Materials and Methods:

2.1 Sample Collection and Preparation

The seaweed sample was collected from the kovalam beach. The seaweeds after collection washed properly in water to remove unwanted materials and were brought to the laboratory. The collected seaweed sample was shade dried for three days. Then the dried sample was mechanically blended with 300ml of water to get homogenized sample. Then this sample was making up to 1ml with clean water. It was stored in dark to avoid direct sunlight. Seeds of *Amaranthes tricolour vartristis* were collected from Besent nagar shop

Table.1 Preliminary qualitative phytochemical analysis of extracts of control, treated plant and seaweed

S.no.	Test	Control PLANT	Treated plant	Seaweed
1 Alkaloids	Hanger's reagent Test	+ve	+ve	+ve
	Wagner's reagent Test	+ve	+ve	+ve
	Mayer's reagent Test	+ve	+ve	+ve
	Dragendroff Test	+ve	+ve	+ve
2.Phenol	Ferric chloride Test	+ve	+ve	+ve
	Lead acetate test	+ve	+ve	+ve
3.Flavonoids	Tannins test	+ve	+ve	+ve
	Lead acetate test	+ve	+ve	+ve
4.Steroids	Libermann-Burchardit	+ve	+ve	+ve

2.2 Cultivation of *Amaranthes tricolour vartristis*

Then these seed was cultivated in Hindustan College of arts & science garden. For cultivation the 10 x 10 meter land was used and it was separated into five different lanes. Lanes was marked as control, Group A (1:200), Group B (1:400), Group C (1:600) and Group D (1:800). The bio-fertilizer was treated in different concentration in different lane Group A, Group B, Group C and Group D from the germination stage of plant at 5 days interval. After 5 days it is again treated with the bio-fertilizer. No fertilizer is added to control plants. Then observe the growth parameter of control and different concentrated treated plants.

2.3 Preparation of plant extract:

Treated and control plant leaf was collected in five polytene bags. The collected plant leafs are washed with distilled water and dried. Then it was grinded in motor and pestle with acetone. This extract was used in all the tests except protein and carbohydrate.

2.4 Analytical methods

Preliminary phytochemical analysis for alkaloids, flavonoids, phenols, Tannins and steroids were made by standard procedures. Total proteins are estimated by Folin phenol reagent (Lowry *et al.*, 1951) and carbohydrates was estimated by Anthrone method (Hedge, J E and Hofreiter, B T 1962). In vitro antioxidant properties were evaluated by assessing DPPH (Blois, 1958). Analysis of pigment as carried out by Thin layer chromatography.

3. Result and Discussion:

3.1Phytochemical Analysis:

Present study revealed that control treated plant and seaweed extract contain alkaloids, phenol, flavonoids and steroids (Table 1).These secondary metabolites have therapeutical properties. (Vishnu R *et al.*, 2013, Benedec D *et al.*, 2013, Charalampos P, *et al.*, 2013, Narender PD, 2012). Result shows that plant and seaweed have many medicinal properties

3.2 Estimation of protein:

The amount of protein in the sample was estimated in control, treated and seaweed. The results shows more amount of protein is present in treated (1:400) sample, compared to the other two sample (Table-2). The total protein package is more important for both plant and animal protein sources contribute valuable beneficial nutrients and bioactive compounds to the diet. Diet focusing on plant-based protein sources can be beneficial for helping to prevent diabetes, cardiovascular disease and mortality. Proteins are the most important organic constituents of animal tissues including insects and

play an important role in energy production (Taşkın and Aksoylar, 2011).

Table .2 : Estimation of Total Protein

S.NO	Experimental Groups	A $_{660}$nm	Concentration, µg/ml
1	Control plant	0.98	0.65 ± 0.023
2	Seaweed	0.24	0.16 ± 0.349
3	Group A	0.73	0.49 ± 0.025
4	Group B	1.26	0.84 ±0.021
5	Group C	1.01	0.67 ± 0.026
6	Group D	1.15	0.73 ± 0.021

Mean ± SE of 3 individual observations of each group

3.3 Estimation of carbohydrate

The amount of carbohydrate was estimated in control, treated and seaweed. The results shows more amount of carbohydrate is present in treated (1:400) sample and seaweed compared to the control (Table-3). In plants, they have two main purposes. First, they provide building blocks for plant structural components, such as cellulose (important in building cell walls). Secondly, carbohydrates are molecules that deliver energy for plant growth. This result indicates that seaweed increases amount of carbohydrate in plants that indirectly help the plant to grow.

Table 3: Estimation of Carbohydrate

S.NO	Experimental Groups	A_{530} nm	Concentration, µg /ml
1	Control plant	0.15	0.12± 0.43
2	Seaweed	0.90	0.72± 0.041
3	Group A	0.19	0.15± 0.047
4	Group B	0.86	0.68± 0.042
5	Group C	o.28	0.22± 0.043
6	Group D	0.38	0.03± 0.040

Mean ± SE of 3 individual observations of each group

3.4 Pigment Analysis

Chlorophyll & Carotenoid

The amount of chlorophyll a and chlorophyll b and total chlorophyll and carotenoid pigment was estimated in the sample control and seaweed treated samples. The results shows more amount of Carotenoid was present in control sample compared to the treated (1:400) sample(Table- 4). Chlorophylls, which are green, and carotenoids, which are yellow, orange or red, play pivotal roles in photosynthesis (Bauernfeind, 1981; Dailey, 1990; Young and Britton, 1993) Carotenoids protect the chlorophylls from photo-oxidation and are accessory, light-harvesting pigments and photoreceptors. These pigment play an important role in human health. Plants containing carotenoids, anthocyanins, and other flavonoids are believed to function as "chemopreventers". It protect us from certain cancer and reduce cardiovascular diseases.

Table. 4. Estimation of Total Chlorophyll A and carotenoid

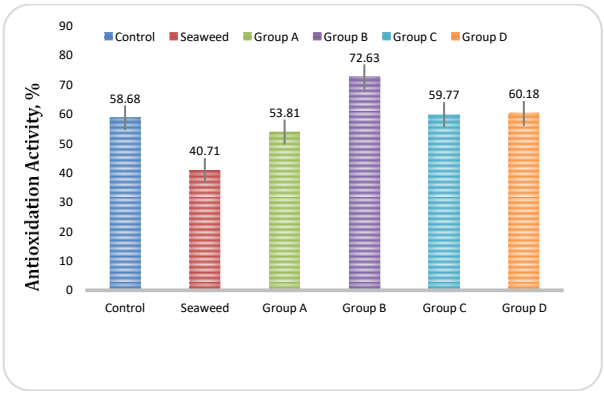
S.No.	Experimental Group	Chlorophyll A, µg/ml	Chlorophyll B, µg/ml	Total Chlorophyll, µg/ml	Carotenid
1	Control sample	37.457± 0.003	41.078± 0.003	78.510± 0.004	2.043± 0.034
2	Treated sample	40.209±0.008	48.783± 0.001	88.964± 0.005	1.679± 0.031

Mean ± SE of 3 individual observations of each group

3.5 Antioxidant Property

The amount of antioxidant property was estimated in the sample control, treated and seaweed. The results shows more amount of reductones was present in test plant extract (1:400) compared to other samples. Free radical may be defined as any atom possessing unpaired electrons. The reactive oxygen species are oxygen derived free radicals such as superoxide anion (O_2), hydroxyl ($OH^{\bullet}$), hydroperoxyl ($OOH^{\bullet}$), peroxyl ($ROO^{\bullet}$) and alkoxyl ($RO^{\bullet}$) radicals and non-free radicals such as hydrogen peroxide (H_2O_2), hypochorous acid (HOCl), ozone (O_3) and singlet oxygen (O_2)[Halliwell B, Gutteridge J, 1999]. These will be formed by living organisms endogenously and exogenously [Irshad M, Chaudhuri PS, 2002]. These free radicals are produced by our body and to stabilize the body's natural function, but the excess amount could cause the cell and tissue damage [Sen S, *et al.*, 2010]. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule [Yamagishi S and Matsui T, 2011]. The synthetic antioxidants are now replaced because the natural antioxidants could be considered as safer without any side effects [Meenakshi S, *et al.*,2011]. The result shows that Seaweed extract in (1:400) concentration will be the good in increasing antioxidant property and safer compared to synthetic one(Graph- 1).

Graph.1: Estimation of Antioxidant Activity



4. Conclusion:

Vegetable Amaranthus includes *Amaranthus tricolor var. tritis. Amaranthus tricolor* is recognized as an easy-to-grow, productive, tasty and nutritious vegetable. In recent years, seaweeds are abundantly consumed by the people for its rich source of nutrients. In this study sea weed is used as a bio fertilizer and the result shows that this increase the nutrient profile of Amaranthes as well as it increases antioxidant property of the plant. The result shows that this sea weed can be used as effective bio fertilizer.

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