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NUTRITIONAL SUPERIORITY OF SEAWEED BASED ORGANIC GREEN LEAFY VEGETABLE, *AMARANTHUS RETROFLEXUS* L.

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ABSTRACT

One of the most important challenges for agriculture, besides enhancing food production, is to provide almost all the essential minerals and organic nutrients to humans for maintenance of health and proper organ function through organic farming. In this study seaweed liquid fertilizer (SLF) of *Hypnea musciformis* was used as an organic fertilizer and studied its influence on nutritional status of the leaves of *Amaranthus retroflexus* L. There were eighteen amino acids found in treated and control plants. Essential amino acids viz., leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine and histidine were found in both control and treated plants. Moreover these amino acids except leucine, lysine and threonine were found plenty in treated plants. Phenylalanine, methionine and tryptophan were respectively 236%, 107% and 107% higher than that of control. Nonessential amino acids were slightly higher than control except tyrosine and proline. Water soluble vitamins (B1, B2, B3, B6, B9, B12 and vitamin C) and fat soluble (A, D, E, K) vitamins were found to be increased in treated plants than control. The results clearly indicated that SLF of *Hypnea musciformis* (Wulfen) J.V. Lamouroux, an eco-friendly cost effective biostimulant, could be used as an organic supplement for improving nutritional status of vegetable crops.

KEY WORDS: **Essential Amino Acid, Non-Essential Amino Acid, water soluble vitamins, fat soluble vitamins and *Hypnea musciformis.***

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INTRODUCTION

Green leafy vegetables (GLV) are used since ancient periods as a source of food as they contain many nutrients and minerals which are helpful in maintaining human health. The health and nutrition of expanding world populations are major upcoming challenges especially in developing countries. India is endowed with an array of leafy vegetables suited for tropical, sub-tropical and temperate climates to be grown all the year round. Leafy vegetables are appreciated because they not only supply the protective nutrients and add variety to a monotonous diet, but also have an alternative taste, pleasing appearance and aroma[1]. WHO recommended a minimum daily intake of 400g fruits and vegetables[2]. Among all vegetables, green leafy vegetables have occupied a unique place because of their colour, flavor and health benefits. They are rich in source of β-carotene, ascorbic acid, iron, zinc, folate and dietary fiber[3]. Green leafy vegetables are the cheapest of all the vegetables within the reach of poor man, being richest in their nutritional value and also the medicinal values. Greens are good for health, increase blood level, clear vision, cure sore in stomach and mouth, prevent jaundice, cure heart disease, release knees pain, sugar disorder and also the greens are good food for children and give essential nutrient for pregnant women[4,5,6]. The leaves of amaranth constitute an inexpensive and rich source of protein, carotenoids, vitamin C and dietary fiber[7], minerals like calcium, iron, zinc, magnesium[8]. Amaranth protein has a high concentration of essential amino acids, especially lysine which is a limited amino acid in other crops[9,10]. This study aims to bring out the role of soil application of seaweed liquid fertilizer (SLF) as a biostimulant, for producing organic leafy green vegetables.

MATERIALS AND METHODS

(i) Collection of seaweeds

Hypnea musciformis (Wulfen) J.V. Lamouroux was collected during low tide, at Hare Island, Thoothukudi from November 2013 to February 2014. The samples were washed thoroughly with seawater followed by fresh water to remove the sand particles and macroscopic epiphytes. After draining, the seaweeds were shade-dried, powered, sieved and used for the preparation of seaweed concentrate.

(ii) Preparation of seaweed liquid fertilizer for soil application

About 20g dried seaweed powder with 200ml distilled water was added. It was heated to 60^{o}C and maintained at the same temperature for 24hr using a hot air oven. The extract was filtered and then centrifuged at 10000 rpm to remove most of the suspended impurities. The filtrate was stored in air tight bottles at 4^{o}C (100% seaweed concentrate)[11] suitable dilution (1%) was used for soil application on leafy green vegetable crop, *Amaranthus retroflexus* L.

(iii)Experimental design

A pot culture experiment was conducted during February to April 2014 at Plant Research Centre, St. Mary's College Campus, Thoothukudi. The pots were filled with 3kg of garden soil. 50 seeds were sown in each pot. After the emergence of seedlings, they were thinned to ten plants per pot and allowed to grow for a period of 30days. Weeding and watering were done at regular intervals throughout the experimental period. SLF was applied in soil (along with 100ml of distilled water in the ratio of 1:100) after the expansion of first leaf and was continued for twenty days.

(iv) Analyses

High Performance Liquid Chromatographic system (Shimadzu-UFLC Prominence), equipped with an auto sampler (Model-SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) was used for analyzing water soluble vitamins (B-complex and C) and fat soluble vitamins (A, D, E and K). The data were recorded using LC-solution software. The quantitative amino acid analysis was carried out on Biochrome automatic amino acid analyser by Cation Exchange Chromatography process using Sparkman method[12].

(v) Statistical Analysis

Data collected in this study was analysed by using Microsoft Excel 2007[13], One way ANOVA was used to compare difference in the means of amino acid and vitamins profile of control and treated plants. A significant difference was considered at the level of $p \leq 0.05$[14].

RESULTS AND DISCUSSION

Amino acids are the building blocks of proteins. The 22 known amino acids, essential and non-essential, affect a broad range of physical and mental process. Recent studies witnessed the findings that amino acids are cell signaling molecules as well as being regulators of gene expression and the protein phosporylation cascade, and key precursors for syntheses of hormones and low-molecular-weight nitrogenous substances with enormous biological importance[15]. Physiological concentration of amino acids and their metabolites are required for the normal biological functions of our body. Thus an optimal balance among amino acids in the diet and circulation is crucial for whole body homeostasis. Some amino acids regulate key metabolic pathways that are necessary for maintenance, growth, reproduction and immunity. They are called functional amino acids which include arginine, cysteine, glutamine, leucine, proline and tryptophan. In the present study, *Amaranthus retroflexu*s grown with soil application of *Hypnea musciformis* (Table-1) possessed greater amount of these amino acids especially cysteine (44%), arginine (19%), tryptophan (107%) in comparison with control. Dietary supplementation of these amino acids rich *Amaranthus retroflexus* may be beneficial for ameliorating health problems at various stages of life cycle. The nine essential amino acids (EAA) viz., isoleucine, valine, lysine, threonine, tryptophan,

methionine, phenylalanine and histidine were found in both control and treated plants. Moreover, three amino acids except leucine, lysine and threonine were found in plenty in the *Hypnea musciformis* treated plants. Phenylalanine, methionine and tryptophan were respectively 236%, 107% and 107% higher than that of control. Cysteine is one of the effective free radical destroyers and works suitably when taken along with selenium and vitamin E. Cysteine concentration was 44% higher than the control evidencing the role of seaweeds in organic fortification of consumable products. Tryptophan, a precursor of serotonin and melatonin and its deficiency may underlie many types of brain diseases such quality of sleep and disturbance in sleep and was found in significant higher level ($p \leq 0.05$) in SLF treated *Amaranthus retroflexus* leaves. All the other amino acids especially non-essential amino acids were seemed to be enhanced in SLF treated plants. However the increment was 20.5%, 13.8% and 19.6% in glycine, aspartic acid and arginine respectively (Table-1). Green leafy vegetables are a good source of water soluble (Vitamin B1, B2, B3, B9, B12 and C) and fat soluble (Vitamin A, D, E and K) vitamins. The known B-complex vitamins are B1 (Thiamine), B2 (Riboflavin), B3 (Niacin), B6 (Pyridoxine, pyridoxal, pyridoxamine), B12 (Cyanocobalamin), biotin and folic acid (folate, folacin, pteroylglutamin). The B-complex vitamins provide the body with energy by aiding in the conversion of carbohydrate to glucose, which the body "burns" to produce energy. They are also vital in the metabolism of fat and protein. In addition, the B vitamins are necessary for normal functioning of the nervous system[16]. The status of water soluble vitamins namely thiamine, riboflavin, niacin, pyridoxine, folic acid and cyanocobalamin under SLF of *Hypnea musciformis* treatment are shown in Table (2). Results indicated that except pyridoxine all the B vitamins were notably increased in leaves of *Hypnea musciformis* SLF treated plants. Riboflavin was boosted to a greater extent and was 627% more than the control. Folic acid was also found to be 219% higher. Thiamine, niacin and cyanocobalamin were increased by 18%, 80% and 22% respectively. Vitamin C prevents scurvy disease and also aids in the formation of folic acid derivatives, which are essential for DNA synthesis[17]. Also vitamin C is a non-enzymatic antioxidant, free radical scavenger, recycles vitamin E and maintaining enzymes in their reduced state[18]. Vitamin C was found in considerable amount in SLF treated plants than control (Table -2). Vitamin E is one of the most important fat-soluble vitamins with a strong antioxidant activity. Its function is protection of lipids from peroxidation[19]. Vitamin E level did not vary much between SLF treated and the control. Vitamin A is lipophilic, free radical scavenger and $^1O_2$ quencher. It is fundamentally needed for reproductive and proliferative processes in human[20, 21]. As a result, dietary source of vitamin A is a major concern. The study established that vitamin A was found comparatively low in control, however it was increased due to SLF treatment (Table-2). The findings of the present study indicated that SLF may be substituted as organic fertilizer for enhancing not only the quantity but also the quality of consumable produced to alleviate malnutrition in a cost effective manner.

Table 1
Effect of soil application of SLF of *Hypnea musciformis* on amino acids profile of *Amaranthus retroflexus* L

S.No.	Amino Acids (mg g^{-1} DW)	Control	Treated
Essential Amino Acid (EAA)			
1	Isoleucine	4.03±0.143	4.61±0.125(14)*
2	Leucine	4.13±0.168	4.29±0.216(3.8)*
3	Lysine	3.82±0.140	3.94±0.175(3)*
4	Methionine	1.05±0.021	2.18±0.057(107.6)*
5	Threonine	2.85±0.084	2.95±0.049(3.5)*
6	Phenylalanine	0.49±0.005	1.65±0.078(236.7)*
7	Tryptophan	0.13±0.001	0.27±0.001(107.6)*
8	Histidine	0.74±0.001	0.96±0.001(29.7)*
9	Valine	2.35±0.102	3.42±0.175(45.5)*
Non-Essential Amino Acid(Non EAA)			
10	Tyrosine	0.96±0.002	0.68 ± 0.001^{NS}
11	Cysteine	0.68±0.002	0.98±0.002(44)*
12	Arginine	2.09±0.827	2.5±0.0749(19.6)*
13	Alanine	2.46±0.425	2.63±0.542(6.9)*
14	Serine	3.05±0.117	3.16±0.203(3.6)*
15	Glycine	4.03±0.610	4.86±0.569(20.5)*
16	Glutamic acid	10.85±0.154	11.19±0.237(3.1)*
17	Asparic acid	4.69±0.215	5.34±0.176(13.8)*
18	Proline	0.83±0.013	0.42 ± 0.010^{NS}

Note : *indicates mean values significant at p≤0.05 level. NS = Non-significant Values are the mean of three replicates ± standard deviation. Values within parentheses indicate percentage increase over control. Dry samples were used for analysis. Plants were grown in potted condition. All the variables were recorded on 30 days old plant. Control= Control plants were irrigated with water. SLF = Soil application of 1% seaweed liquid fertilizer.

Table 2
Effect of Soil application of SLF of *Hypnea musciformis* on vitamins profile of *Amaranthus retroflexus* L.

Vitamins (mg g^{-1} DW)	Control	Treated
Vitamin E	17.89±0.571	18.16±0.430 (1.5)*
Vitamin A	0.0120±0.000	0.0290±0.001 (141.6)*
Vitamin C	3.8±0.174	5.8±0.215 (52.6)*
Vitamin B1 (Thiamine)	0.00123±0.000	0.00146±0.000 (18.6)*
Vitamin B2 (Riboflavin)	0.0110±0.000	0.0800±0.000 (627)*
Vitamin B3 (Niacin)	0.0405±0.001	0.0731±0.001 (80)*
Vitamin B6 (Pyridoxine)	24.91±0.493	25.48±0.607 (2.28)*
Vitamin B9 (Folic acid)	0.0125±0.001	0.0399±0.001 (219)*
VitaminB12(Cyanocobalamin)	7.49±0.211	9.17±0.125 (22)*

Note: *indicates mean values significant at p≤0.05 level.
Values are the mean of three replicates ± standard deviation.
Values within parentheses indicate percentage increase over control.
Plants were grown in potted condition.
All the variables were recorded on 30 days old plant.
Control= Control plants were irrigated with water.
SLF = Soil application of 1% seaweed liquid fertilizer.

CONCLUSION

The upward impact of SLF application on amino acids and vitamins in *Amaranthus retroflexus* suggests that SLF-based organic foods have nutritional superiority than conventional ones. The study also evidenced that organic vegetables deliver more nutrients per average serving, including all important protective phytonutrients.

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