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Total Protein and Carbohydrate Determination in Leafy Vegetables Cultivated in Hydroponics and Soil

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ABSTRACT: In this study we are aiming to analyze the effect of change in plant cultivation technique associated with plant growth. Not only morphologically many anatomical and estimation of many phyto-chemicals can be considered to analyze the effect of plant cultivation technique. Seven leafy vegetable plant species were selected i.e. *Amaranthus viridis, Trigonella foenum graceum* L., *Chorchorus olitorius* L., *Coriandrum sativum, Mentha arvensis, Cicer arietinum, Spinacia oleracea* L. for cultivating under hydroponic system and in soil. Estimation of amount of protein, carbohydrate and moisture content were aimed to determine the effect on growth of the plant in hydroponics and soil cultivation technique. Hydroponically cultivated leafy plants was found rich in protein except *C. olitorius* (3.6 mg/g) and *T.F. graceum* L. (3.7 mg/g) as compared to soil cultivated plant *C. olitorius* (4.9 mg/g) and *T.F. graceum* L. (4.4 mg/g). Similarly, carbohydrate content was also consistently high in hydroponically cultivated leafy plants except one species of *S. oleracea* L. (3.9 mg/g) and it was found as 4.11 mg/g in soil cultivated leafy plant. Somehow growth rate was high in hydroponics under optimum condition. Some important factors in lifecycle of the plant like flowering and fruiting are still to be achieved.

Keywords: Plant cultivation, phyto-chemicals, hydroponics, soil cultivation, protein, carbohydrate, moisture, optimum.

INTRODUCTION

Food is consumed by all living organism to obtain nutritional support, necessary for their growth and survival. A big part of food resources are obtained from the plant by the humans like many other higher animals. Plant consumes energy from sunlight and prepares food for own benefits. Plant uses their stored food material for maintaining their own health and metabolic activities including giving rise to fruits and flower. Food is stored by the plants in form of protein and carbohydrates are also used by other higher organisms called consumers like human beings and animals belong to category of herbivores and omnivores too. Now the important thing is that the neutraceutical value of the food. If food is rich in various type of nutrients and minerals. It will directly reflect to the good health of the organisms who is consuming it as a source of nutrition. Leafy vegetables are one of an important part of nutrition for the people who live in Asia, especially in south Asian country like India. Varieties of leafy vegetables are found and listed in edible resources under Indian Territory. Some of them are cultivated by farmers and local cultivators. Some are also available seasonally and are of wild type. Some of the popular leafy vegetables in central India are *Oxalis corniculata*, *Cordia myxa* Roxb., *Cicer arietinum*, *Cassia tora*, *Amaranthus viridis* L., *Chorchorus olitorius*, *Leucas cephalotes*, *Amaranthus gangaticus* L., *Amaranthus tricolor* L., *Trigonella foenum graceum* L., *Spinacea oleracea* L., *Spinacea glabra* L., *Basella rubra* L., *Brassica compestris* L., *Coriandrum savtivum*, *Mentha arvensis*, *Allium cepa*, *Merremia emarginata* Burm f., *Moringa pterygosperma* Lam., *Ipomoea batatas* Lam., *Ipomoea aquatic* Frosk etc. These are only few examples as the climate and region of the Asiatic region changes, plant diversity also varies significantly (Chauhan *et al.*, 2014; Dhandore and Chandrakar 2021; Misra and Misra 2014; Sharma and Pandit 2022).

Almost all kind of leafy vegetables species are well known for their nutritional values obtained by analyzing various kinds of fibers, vitamins, minerals, protein content, carbohydrate, lipid, and total moisture (Kumar *et al.*, 2020). Hence, reason behind the

obtaining optimum growth by the plant would be maximum amount of stored nutrients. Rise in popularity and consumption of leafy vegetables causes limited productivity and high demand. This situation causes search of new technique and technology over traditional methods of obtaining nutritional resources. Modification and change is must in old plant cultivation methods. But changing plant cultivation technique may also possibly bring some negative result and decline in nutrient content of the cultivated plant. So, it is mandatory to check the effect of any novel methods of plant cultivation by scientific approaches before implementation. Here in this study hydroponic is a technique; in which controlled and optimum conditions for plant cultivation is provided artificially. On the other hand traditional soil cultivated plant under natural climate is cultivated with best possible facilities. Circulating hydroponic system setup has been established in the glass house facilitated with temperature control unit, humidity controller, Light sources, pH meter etc (Mukharjee and Dutta 2018; Seal, 2011).

MATERIAL AND METHOD

In earlier study the compatibility and acceptance of hydroponics for plant cultivation was analyzed successfully. Also the chlorophyll content was estimated and found consistently high in all plant species cultivated in hydroponic system as compared to soil cultivated plants. One plant species of *C. arietinum* was an exception among all other selected plant species after applying similar conditions/factors in both techniques (Prabhas and Ekka 2022).

A. Selection of plant

Seven leafy vegetable were selected i.e. *Amaranthus viridis,* (Chaulai), *Trigonella foenum graceum* L., (Methi), *Chorchorus olitorius* L., (Chech), *Coriandrum sativum* (Coriander), *Mentha arvensis* (Mint), *Cicer arietinum* (Gram), *Spinacia oleracea* L. (Palak). All selected leafy vegetables are selected on the basis availability and popularity for consumption among the people of south Asiatic region in Chhattisgarh – India (Mahamane *et al*., 2003).

B. Qualitative Test of Protein

At first Biuret test has been performed to confirm the presence of protein inside tissue's of selected leafy vegetable plant's species. Principle behind Biuret test is based on reaction between copper (II) ion and nitrogen atoms of protein peptides resulting into disarticulation of peptide hydrogens. Qualitative analysis is based on presence of amino acids. Amino acids are basic structural units of proteins. Usually, all twenty amino acids are found in plant cell (Sharma *et al*., 2016).

C. Quantitative estimation of Protein

Fresh leaves of each leafy vegetable species were collected and placed in tap water immediately for surface cleaning and removal of dust particles. After that all samples were transferred to beakers containing distilled water. 1gm leaf of all each plant samples were measured and macerated using mortar pestle and phosphate buffer saline (PBS) was added to make up final volume of 20ml. Then extracted solution was centrifuged at 10000 rpm for 10 minutes. After centrifugation supernatant is transferred to conical flask of 25ml. Standard curve is obtained by obtaining and plotting graph for absorbance value of Bovine serum albumin (BSA) as standard reagent. 1 ml of extracted supernatant of sample was added to reagent A containing 48 ml of 2% sodium carbonate prepared in 0.1N sodium hydroxide, 1ml of 0.5 % copper sulphate and 1ml of 1% sodium potassium tartarate. After incubation for 15 mintues 0.5 ml of reagent B containing Folin Ciocltеau reagent and water in ratio of 1:1. After incubation for 30 minutes absorbance was measured in 660 nm (Ghaly and Alkoiak 2010; Sarkar *et al*., 2020; Nomura *et al*., 2019).

D. Qualitative estimation of carbohydrates (sugar)

Qualitative test was completed first. Fehling's test is performed to confirm the presence of total sugar (reducing and non-reducing) in all samples of leafy vegetables. This test is based on principle of reducing aldehyde (R-CHO) or ketone R-CO) groups. A simple but popular Iodine test was also performed to confirm the presence of starch.

E. Quantitative estimation of carbohydrates

Principle of the reaction is based on the reaction occurs between carbohydrates of the plant sample react with sulphuric acid (H_2SO_4) to form bluish-green compound known as furfural. Absorption maximum of furfural can be measured at 620 nm with the help of spectrophotometer. 2 grams of each macerated sample was extracted using 10 ml distilled water and transferred to 25ml conical flask. 200 mg of anthrone reagent dissolved in 100 ml of concentrated H_2SO_4 (Sulphuric acid) in conical flask or glass beaker separately. Prepared reagent could be transferred and stored in reagent bottle.1ml of each sample was taken in separate conical flask of 25ml and 2 ml of anthrone's reagent was added. Final volume make up to 25 ml using distilled water. Blank solution was prepared dissolving 2ml of anthrone's reagent in distilled water and make up the final volume of 25 ml. Reading for absorbance was measured in UV-visible spectrophotometer at wavelength of 750 nm (Mustapha and Babura, 2009; Mukhaerjee *et al.,* 2019; Mannem *et al*., 2012).

F. Estimation of moisture content (percentage)

Mean value of replicates in thrice was estimated for each model plant species. 1000 mg (1.0) of each sample was dried at 110°C and incubated for cooling at room temperature. Porcelain crucibles were used for the entire process. Weight of porcelain crucibles were determined separately before. Calculations were made according to below mentioned formula -

Total moisture content (%) = [(wB-wA) × 100]/weight of sample

w = weight of sample

wA = weight of crucible + leaf sample before drying

wB = weight of crucible + leaf sample after drying (Jayasinghe *et al*., 2019).

G. Statistical Analysis

Values are shown as mean ± SE (standard error of the mean) in the table 1. Mean was obtained by three replicates of each sample. A confidence level of 95% (or significance of 5%) is considered for data management.

RESULT AND DISCUSSION

Data obtained after statistical analysis can be described for two different variables 1. Plant cultivated in hydroponic 2. Plant cultivated in soil in terms of total protein, total carbohydrate and total moisture content found in the leaf per gram weight. Hydroponically cultivated leafy plants was found rich in protein except *C. olitorius* (3.6 mg/g) and *T. F. graceum* L. (3.7 mg/g) as compared to soil cultivated plant *C. olitorius* (4.9 mg/g) and *T.F. graceum* L. (4.4 mg/g). Similarly, carbohydrate content was also consistently high in hydroponically cultivated leafy plants except one species of *S. oleracea L*. (3.9 mg/g) and it was found as 4.11 mg/g in soil cultivated leafy plant. Total moisture content was also measured constantly high in case of hydroponics. Reason behind this was availability of optimum amount of water to the plant root in hydroponic condition while in case of soil it depends upon texture and physiology of the soil particles. Amount of the salts, mineral and ions may also affect the water transport from soil to plant cell (inside).

Table 1: Showing values obtained by quantitative estimation of protein and carbohydrates.

Scientific name of the selected plant species.	Total Protein (mg/1g)	Total Carbohydrate (mg/1g)	Total Protein (mg/1g)	Total Carbohydrate (mg/1g)	Total moisture content (%)	Total moisture content (%)
	Hydroponics		Soil Cultivation		Hydroponics	Soil
A. viridis	4.1 ± 0.554	9.3 ± 1.063	3.5 ± 0.924	8.10 ± 1.853	90.28	87.71
C. arietinum	19.7 ± 3.75	14.8 ± 1.571	10.2 ± 0.589	14.7 ± 1.572	80.79	75.10
C. olitorius L.	3.6 ± 0.739	9.2 ± 0.739	4.9 ± 1.848	7.8 ± 0.924	83.14	83.16
C. sativum	5.5 ± 1.294	4.6 ± 2.08	3.2 ± 1.201	3.9 ± 0.277	92.26	90.55
M. arvensis	1.1 ± 0.924	0.90 ± 0.833	0.2 ± 0.046	0.41 ± 0.185	96.42	96.92
S. oleracea L.	3.7 ± 1.478	3.9 ± 0.739	2.9 ± 0.739	4.11 ± 22.93	93.33	87.98
T.F. graceum L.	3.7 ± 1.663	8.1 ± 1.525	4.4 ± 1.294	7.7 ± 1.571	80.16	74.13

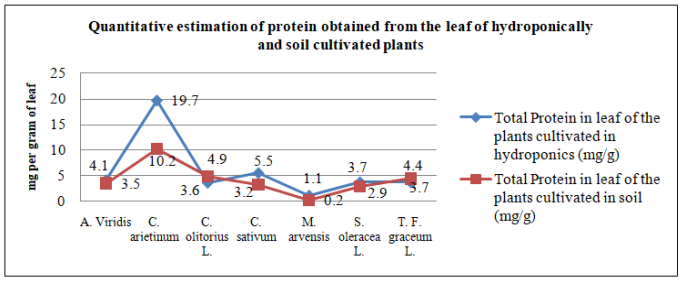

Fig. 1. Estimation of protein in the leaf of plants cultivated in hydroponics and soil.

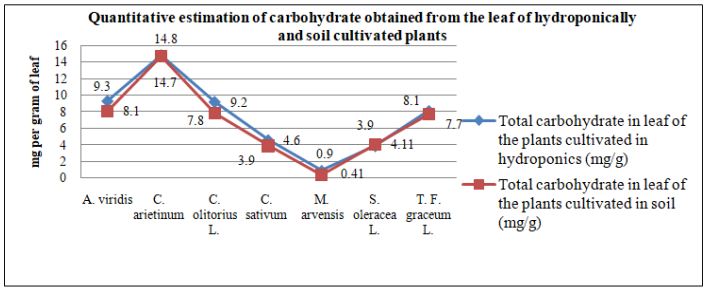

Fig. 2. Estimation of carbohydrate in the leaf of plants cultivated in hydroponics and soil.

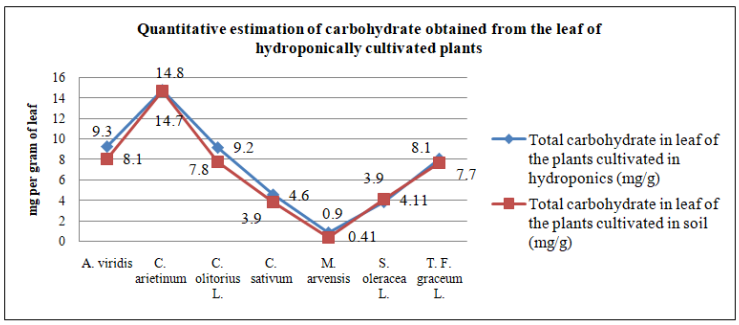

Fig. 3. Estimation of total moisture in the leaf of plants cultivated in hydroponics and soil.

CONCLUSION AND FUTURE SCOPE

Growth of all leafy vegetable plants was high in hydroponics as compared to soil cultivation. Important factors like growth rate of the plant, chlorophyll content of the leafy vegetables (Prabhas and Ekka 2022), total protein estimation, total carbohydrate estimation, total moisture content in the leaf are helpful to describe the overall effect on growth of the selected plant species when we switch plant cultivation technique.

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Conflict of Interest. None.

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