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Research Article

Comparison of Growth Indices, Biomass, Anatomy, Phytochemical and Elemental Activity of Hydroponically Grown and Soil Grown *Amaranthus*

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ABSTRACT

Hydroponics has emerged as one of the most popular agricultural production methods today. However, whether hydroponically produce plants of comparable quality to soil grown plants is still unclear. Thus, in this study, morphology, biomass, growth indices, anatomy, phytochemical compounds, macro and microelements were determined and compared in hydroponically and soil grown *Amaranthus. Amaranthus hybridus* is naturally gluten-free and a good source of calcium, zinc, copper, vitamin B_6, folate, and an excellent source of fiber, iron, magnesium, phosphorus, and manganese. It not only provides healthy nutrition but also boosts our immune system. They have exceptional medicinal properties, making them in high demand in the society and various industries. Growth data revealed that it appears morphologically far better than soil. The roots were evident and long, leaves having large petioles with broad lamina and intense green color. So many branches were appeared as compared to soiled ones. In a hydroponic setup, dry weight was increased by seven times. It can be related to the presence of a more significant number of corticular vascular bundles in the cortex of hydroponically grown roots which help them to absorption of more nutrients and water. Phenolic compounds are found to be 15 times less than soiled plants because they are considered anti-nutrients and are only produced when plants are stressed, whereas flavonoid is found to be three times more abundant in hydroponic systems. Mineral elements are also found to be more abundant in hydroponic systems. To maximize profitability, plants should be grown in hydroponic systems in order to obtain healthy nutrition or therapeutic potential.

Keywords: Hydroponics; Soil grown plants; Nutrients

INTRODUCTION

Amaranthus hybridus L, sometimes known as "Amaranth or Pigweed," is an annual or short-lived herbaceous plant that grows 1 feet to 6 feet tall. The leaves are alternating petioled, 3 inches-6 inches long, dull green, rough, hairy, ovate or rhombic with wavy margins, and ovate or rhombic with wavy margins. The flowers are tiny and have greenish or crimson panicles at the end. The taproot is long and fleshy red or pink in color. The seeds are tiny and lenticellular in shape, averaging 1 mm-1.5 mm in diameter and weighing 0.6 g-1.2 g per 1000 seeds. It is one of the most common weeds in the tropics, subtropics, and warm temperate zones. This genus belongs to the family Amaranthaceae and the order Caryophyllales [1-3].

Amaranthus's popularity has surged nowadays, and it has garnered the attention of several food scientists since it is regarded as a functional food with various health-promoting properties. It is a fascinating crop since its vegetables and seeds are considered exceptionally nutritious and so are consumed by both humans and animals, as plants contain almost all of the vital nutrients that humans require. It is naturally gluten free and a good source of calcium, zinc, copper, selenium, vitamin B_6, and folate, as well as an excellent source of fiber, iron, magnesium, phosphorus, and manganese. Diabetes mellitus, cancer, malaria, hypercholesterolemia, atherosclerosis, helminthic and bacterial infections, inflammation, hepatic disorders, and cardiovascular problems have all been treated using A. *caudatus*. This plant is grown as a vegetable, ornamental

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plant, and other species as food, leaf vegetables, and cereals in India, Sri Lanka, and other tropical nations [4-7].

Amaranth has been identified as one of the most under valued and neglected plants with great promise for eradicating poverty and malnutrition. These are C4-type plants with excellent nutritional content in their seeds and leaves, and they are drought-tolerant species. Phytochemicals such as betalains and phenolic compounds are abundant in amaranth leaves. Furthermore, amaranths can accumulate significant oxalate levels in the form of calcium oxalate crystals, which is the major antinutrient.

The objective of the study is to make a comparison of the *Amaranthus* was grown in the field as well as in hydroponic systems. The question that arises here is why it is necessary because if we go to our statistics, then it is FAO who indicates in their report that 11% (1.5 billion ha) of the globe's land surface (13.4 billion ha) is used in crop production. According to World Bank 2018 report, 60.43% of the land is used for agricultural practice. Agriculture is the primary source of livelihood for about 58% of the Indian population [8,9]. Global human population growth amounts to around 83 million annually or 1.1% per year (world population prospectus, 2017). As per the United Nations report 2019, the world population is expected to increase by 2 billion people in the next 30 years, from 7.7 billion currently to 9.7 billion in 2050. The more people there are, the more land we will need. More buildings must be built, more resources must be discovered, more food must be grown, and more waste must be generated. When land is cleared for human activities such as building, mining, farming, and garbage disposal, there will be no land left for agricultural practice. Therefore, we need to improve our agricultural practice; hence hydroponics is the best solution [10].

Hydroponics is an emerging concept to grow plants in nutrient media with the mechanical support of sand, gravel, vermiculite, perlite, cocopeat, rockwool, peat moss, coco coir, etc. It can be of open as well as closed types. In the earlier one, water cannot be reused once it is uptake by plant roots, and in the latter, water can be reused and recycled [11]. The nutrients needed for the growth and development of plants are C, O, and H, which are obtained through air and water, and N, P, Si, B, K, Ca, Mg, Cl, Mn, Na, Fe, Zn, Cu, Ni) and Mo obtained from the soil. The most common nutrient solutions used are those proposed by Hoagland and Arnon, Hewitt, Cooper, and Steine, which are modified depending on climatic conditions and the type of water.

This method could plant up anywhere. It controls various environments easily as nutrients, pH, temperature, oxygen, etc. Crop yield and quality were more stable and consistent than growing in soil. Water and fertilizer were used decreased by about ten times and 40% of the cropping in the ground, respectively. Plants grow faster and yield more than those planted in the soil, and hydroponic methods could provide proportion and good nutrient content to balance the plant's needs. It saved time because it could reduce harvest age shorter than planting in the ground, cost savings of labor in planting and maintenance, and transportation costs due to select crop production areas closer to the market [12-15]. It could control the disease, and pest problems were more manageable because the soils mainly caused the main pest problem. The growing site was used most effectively. That was the same plant in the exact location throughout the year and had continued to grow. This method could have crops density than plants grown in soil because it did not have to compete for food and water. Products were clean and safe for both the consumer and the environment. These are some of the benefits listed by Keeratiurai and Prayong in 2013.

Plants produce a variety of metabolites in response to their surroundings. Even though numerous writers have conducted various types of studies on this species, no one has compared plants grown in soil to plants grown in the absence of soil. As a result, the goal of the research was to look into the morphological, anatomical, phytochemical, and mineral profiles of soil and soilless-grown plants. The study's findings are valuable to people from all walks of life, such as those working in the technological business, an industry of pharmaceuticals, chemical industry, food industry, and farming industry. This research is especially beneficial to landless farmers regarding their economic growth and environmental protection [16].

MATERIALS AND METHODS

Chemicals and reagents

Folin and Ciocalteu's reagent was purchased from Thomas Baker (Chemicals) limited (Maharashtra, India). Gallic acid, rutin, and solvents like acetone and methanol were purchased from Sisco Research Laboratories Pvt. Ltd. (New Mumbai, India). Potassium acetate and aluminium chloride were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Sodium carbonate and hydrochloric acid were purchased from Merck Life Science Pvt. Ltd. (Mumbai, India).

Site description

A hydroponic system was built, and *Amaranthus* plants were cultivated in an open environment at the Netaji Subhas University of Technology in New Delhi, India, from September 6 to November 8, 2021. The average monthly temperature are between 780 F and 950 F, humidity 55.4% to 98.2% and sunlight hours 2600 hours to 3200 hours were recorded during the testing period.

Hydroponic set up

Amaranthus was grown using the ebb and flow method in a hydroponic system. The technique made use of PVC pipes with 3-inch diameter pores. A steady flow of nutritional solutions was maintained in the growth channels. The plants were planted in net pots with coco-peat and lacca balls as the support medium, and the roots were hung in a nutritious solution running through the channels. The reservoir's feeding solution was pumped out. The flow was directed into the other channel, which was equipped with an end cap and spout at the decreasing end of the increasing channel. Before returning to the reservoir, the nutrient solution passed through all of the system's channels (closed system). To prevent contamination of the nutrient solution, a reservoir with a surface area of 0.053 m^2 was placed beneath the growing system and covered [17-20].

The *Amarantus* plant was chosen as the source material for this objective. This experiment was divided into two groups one was carried out in the greenhouse of NSUT DELHI, and the other was in the laboratory of NSUT DELHI. *Amarantus* seeds were purchased from a local nursery and verified by NSUT BSE DEPT. Three replicates of 20 seeds were placed in a tray filled with cocopeat and moistened with water. Similarly, seeds were grown in a greenhouse in the soil as well. At 3, 4 leaves stage three replicates of 10 good plants were transferred in a hydroponic setup and exposed to an external environment. In the hydroponic setup, we have used modified Hoagland solution as a nutrient media with pH 6.9. 10 L nutrient media were added every week and maintained the EC with nutrient media and pH with orthophosphoric acid. Observed plants morphologically and recorded their growth parameters such as plant height, number of leaves, and number of branches every week. Plant height was measured from the soil level to the terminal leaf using a meter rule and in the case of hydroponic set up just above the system to the terminal leaf. After ten weeks of transfer, when plants attained full height and entered into harvesting period, we collected the sample and calculated their biomass (fresh weight and dry weight); dry weight was determined by oven drying at 800°C overnight. Observe their root anatomy under a compound microscope, and lastly, phytochemical and mineral analysis was done.

Anatomical studies

A mature and fresh portion of the plant's roots was taken, then cleaned and sectioned with a sharp blade. The sections were stained with safranine and mounted in glycerin before being examined using a compound light microscope.

Phytochemical analysis

Preparation of plant extract: The crude plant extract was prepared using the Soxhlet extraction technique. About 20 g of powdered plant material was evenly packed into a thimble and extracted with 250 ml of solvents. As a solvent, acetone was used. The extraction procedure is repeated for another 24 hours or until the extractor's syphon tubes solvent becomes colorless. The extract was then placed in a beaker and cooked on a hot plate at 30°C-400°C until the solvent had evaporated completely. The dried extract was kept at 400°C in the fridge for future phytochemical study.

Quantification of total phenolic compounds: The Folin-Ciocalteu reagent technique was slightly modified to detect the quantity of phenol in the aqueous extract. 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of 2% Na_2CO_3 solution were added to 1 ml of plant extract. The resultant mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765 nm. Gallic acid (1 mg/ml) was utilized as a control. All of the tests were performed three times. The findings were computed and represented as gallic acid equivalent (mg/g of extracted substance) using the standard curve.

Quantification of flavonoid: To determine flavonoid content, the aluminium chloride colorimetric method was used with some modifications. 1 ml of the sample plant extract was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 M potassium acetate, and 5.6 ml of distilled water for 30 minutes at room temperature. At 420 nm, the absorbance was measured. As a control, 1 mg/ml of quercetin was used. All of the tests were carried out in triplicate. The flavonoid content was calculated using the standard curve and expressed as quercetin equivalent mg/g of extracted compound.

Mineral analysis

Mineral such as sodium, magnesium, phosphorus, potassium, calcium, manganese, iron and zinc were determined by swift ED3000 EDX manufactured by oxford instruments.

Statistical analysis

Statistical analysis was performed using GraphPad Prism Software (version 9.0, GraphPad software, La Jolla California, USA). All experiments were carried out as mean ± standard deviation of three replicates. Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test was used to determine the statistical significance at $p<0.05$

RESULTS AND DISCUSSION

Growth indices

During the entire *Amaranthus* cycle, there was an increase in growth parameters. When the plants were moved to the hydroponic system, they sought to adapt to their new habitat. As a result, development during the initial vegetative stage was moderate, and the increase in plant height, number of leaves, and number of branches from week 1 week to 4 were not substantial. From week 6 (43.78 cm ± 15.27 cm) to week 8 (57.66 cm ± 12.72 cm), the plant height increased at a breakneck speed (Figure 1 and Table 1). The number of branches was also growing at its own pace. When the plants reached maturity, the average number of leaves increased to 95-100, the average plant height increased to 60 cm, and the average number of branches increased to 20. The increase in plant development characteristics each week compared hydroponically cultivated plants to soil cultivated plants (Figure 2 and Table 2) was insignificant. In the case of hydroponically cultivated plants, 71.4 percent more height was documented. If we look at the branches of soil-cultivated plants, we can find that just 3% of them have grown. In the case of leaves, hydroponically grown plants grow at an average of 86.3 percent, while soil-cultivated plants grow at an average of 36.36 percent. Morphologically in the case of hydroponic cultivated *Amaranthus* sp. tap roots were evident and long (Figure 3), with adventitious roots; the stem was robust, solid, and thickened; and the leaves were green, with a long petiole and broad, flattened lamina with wavy margins (Figure 4). Above all, the data demonstrates that hydroponically maintained plants have attained better morphological growth than conventional ones (Figures 5-12).

Figure 1: Morphology of *Amaranthus* plant growing in hydroponic system *vs.* soil.

Figure 2: Root morphology of hydroponic and soil grown *Amaranthus*.

Table 1: Biometric attributes (Plant height, number of leaves and number of branches) recorded every week of hydroponically cultivated *Amaranthus* plant. The values are expressed at mean ± standard deviation (n=10).

Date	Height	Branches	Leaves
Week 1	11.81 ± 2.3	2.29 ± 0.46	6.65 ± 1.21
Week 2	18.29 ± 2.95	4.55 ± 0.57	18.74 ± 4.57
Week 3	21.37 ± 6.69	9.59 ± 4.08	19.22 ± 11.93
Week 4	22.29 ± 7.72	10.22 ± 3.76	25.14 ± 12.24
Week 5	28.56 ± 12.18	10.44 ± 2.6	22.96 ± 9.51
Week 6	43.78 ± 15.27	10.55 ± 2.81	56.29 ± 13.98
Week 7	46.18 ± 13.61	11.48 ± 2.08	73.14 ± 15.76
Week 8	57.66 ± 12.72	13.07 ± 1.83	76.73 ± 15.38
Week 9	58.38 ± 12.39	17.57 ± 2.35	81.76 ± 10.1
Week 10	58.42 ± 13.26	18.57 ± 2.38	95.38 ± 11.32

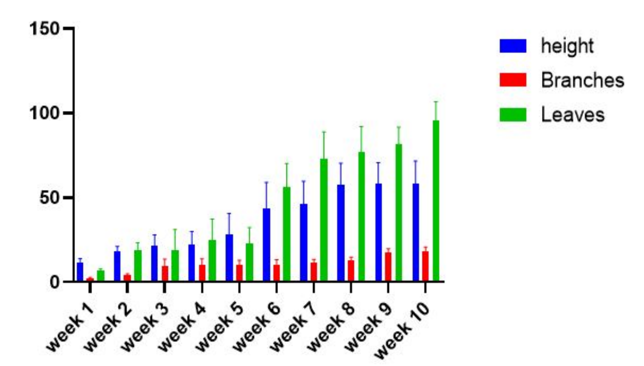

Figure 3: Growth indices of hydroponically grown plants. Vertical bars represent standard deviation.

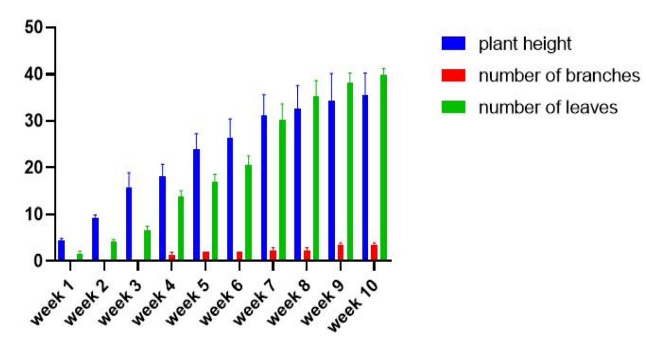

Figure 4: Graph showing growth indices of soil cultivated plants. Vertical bars represent standard deviation.

Table 2: Biometric attributes (Plant height, number of leaves and number of branches) recorded every week of soil cultivated *Amaranthus* plant. The values are expressed at mean ± standard deviation (n=10).

Date	Height	Branches	Leaves
Week 1	4.4 ± 0.54	0	1.6 ± 0.54
Week 2	9.4 ± 0.54	0	4.2 ± 0.44
Week 3	15.8 ± 3.19	0	6.6 ± 0.89
Week 4	18.2 ± 2.58	1.4 ± 0.54	13.8 ± 1.3
Week 5	24 ± 3.31	2	17 ± 1.58
Week 6	26.4 ± 4.07	2	20.6 ± 1.98
Week 7	31.2 ± 4.44	2.4 ± 0.55	30.2 ± 3.49
Week 8	32.6 ± 4.98	2.4 ± 0.54	35.4 ± 3.29
Week 9	34.4 ± 5.77	3.4 ± 0.55	38.2 ± 2.05
Week 10	35.6 ± 4.72	3.4 ± 0.55	39.8 ± 1.48

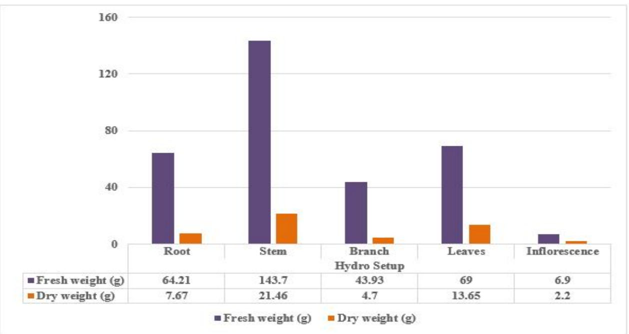

Figure 5: Variation in fresh weight and dry weight of different plant organs of hydroponically cultivated plants.

Biomass contribution

Data has revealed that hydroponic plants' fresh and dry weights were significantly higher than those of soil-cultivated plants (Figure 4). Fresh weight and dry weight of roots were 1346% and 1044% (Figure 5) higher than soil-grown plants, respectively. Stem fresh weight and dry weights were 409.5% and 523.8%

higher, respectively. Leaves outweighed soil-cultivated plants (Figure 6) by 360%, 394%, respectively. Similarly, fresh and dry inflorescence weights are 301 and 746 percent higher than soil. If we calculate the total dry biomass, hydroponically grown plants have achieved 49.68 g, which is seven times higher, and soil-grown plants have achieved 7.13 g (Figure 7). If we use the hydroponic method, we can get a higher yield.

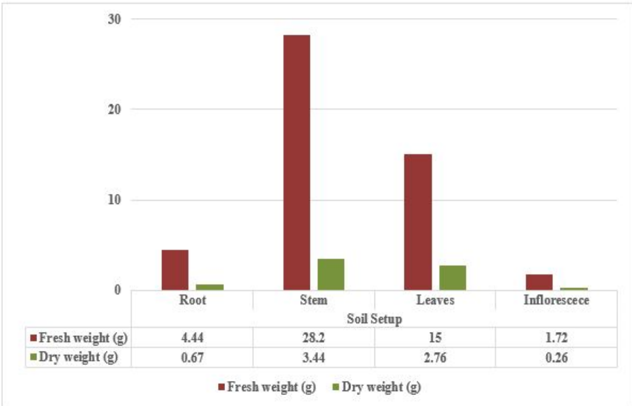

Figure 6: Variation in fresh weight and dry weight of different plant organs of soil cultivated plants.

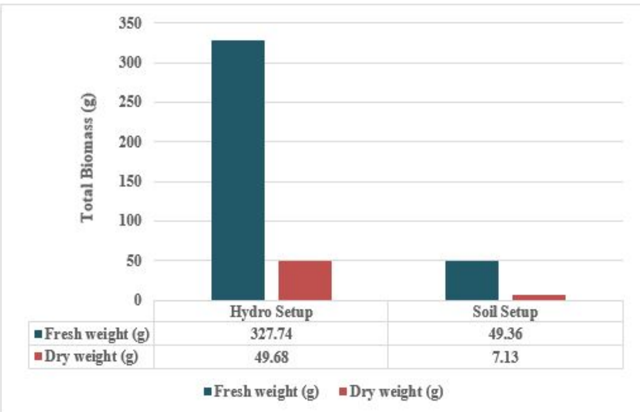

Figure 7: A comparison of the fresh and dry weights of plants grown in soil and in the hydroponic set up.

Anatomical study

The anatomical section of roots (Figure 8) clearly shows that the number of corticular vascular bundles is numerous and scattered in hydroponically cultivated plants. In contrast, significantly few vascular bundles were present in soil-grown plants, implying that nutrient absorption is more remarkable in hydroponic as xylem and phloem help in water and nutrient conduction. The medullary rays are a few layers of radially elongated cells that lie between the vascular bundles. All of the roots' pith was very clear and extended from the vascular bundles to the center (Figure 9).

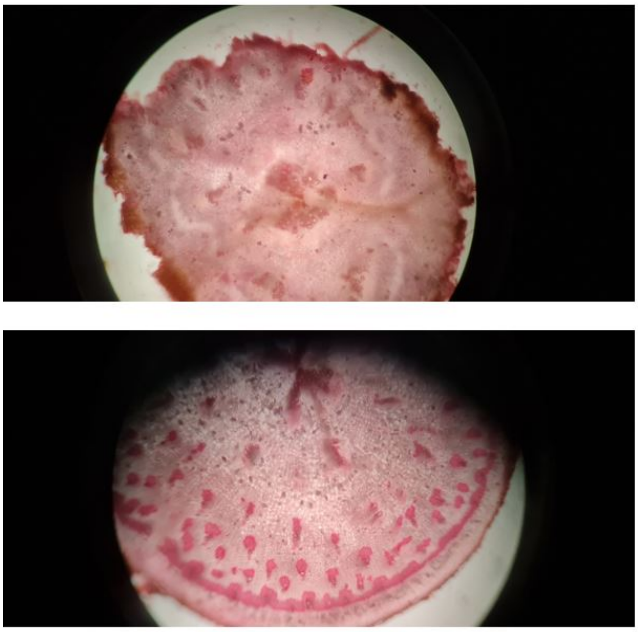

Figure 8: Cross section of roots of hydroponic grown *vs.* soil grown *Amaranthu* upper-soil cultivated, lower-hydroponic cultivated.

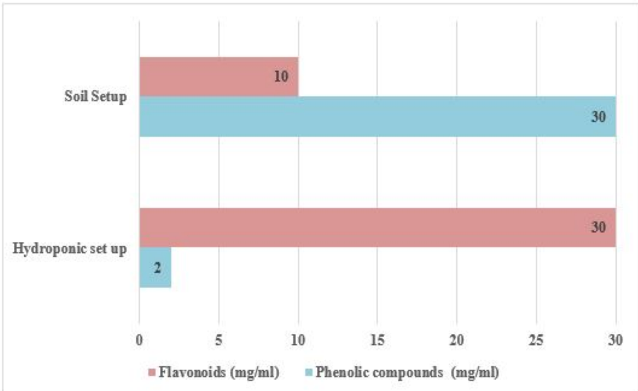

Figure 9: Comparison of flavonoids and phenolic compounds in *Amaranthus* grown in soil and hydroponic systems.

Phytochemical analysis

Our data have clearly shown that flavonoids are more in hydroponically grown plants (Figure 9), whereas in soil cultivated plants, it is less. The phenolic compounds are among the most numerous and widespread groups of plant metabolites. They have biological properties such as anti-apoptosis, antiaging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, endothelial function improvement, and inhibition of angiogenesis and cell proliferation. Natural antioxidants are primarily found in plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols, and so on. Flavonoids are hydroxylated phenolic substances that plants produce in response to microbial infection and have been shown to be antimicrobial *in vitro* against a wide range of microorganisms. In the case of phenolic compounds, soil cultivated plants possess more. Polyphenols, saponins, tannins, and oxalates are phytochemicals found in *amaranth* grain that are not considered nutrients but may be antinutrient factors. Cooking reduces these compounds' content and anti-nutrient effect.

Mineral analysis

Macronutrients and micronutrients are essential components for proper plant growth and development because they play a vital role in disease control and management. Understanding the dynamics of nutrient uptake, transport, assimilation, and biological interactions is critical for improving crop plant yield. We used an EDX instrument to analyze both macro and microelements. The results (Figure 10) revealed that hydroponic *Amaranthus* has a higher concentration of minerals, including sodium 1.715 wt%, magnesium 8.814 wt%, phosphorus 13.502 wt%, calcium 2.521 wt%, manganese 0.33 wt%, iron 5.3 wt%, and zinc 2.032 wt%. In contrast, soil cultivated plants have sodium 0.214 wt%, magnesium 9.984 wt %, phosphorus 9.51 wt%, potassium 78.5 wt%, manganese 0.285 wt%, iron 0.075 wt%, and zinc 1.432 wt%.

Phosphorus is known to be an essential constituent of plant cell membranes, and it also plays a vital role in the formation of adenosine triphosphate, ribonucleic acid, and deoxyribose nucleic acid. Photosynthesis, glucose metabolism, energy production, redox-homeostasis, and signaling are all cellular functions that require it. In plants, P acts as an activator for over 60 enzymes, regulates water content, and decreases the adverse effects of salts. It helps in nutrient movement within the plant and the transfer of genetic characteristics from one generation to another. Potassium is a mobile and essential plant macronutrient abundant in all young plant parts required to ensure optimal plant growth. Figure depicts a graphical representation of mineral analysis in which potassium is very high, 71.086% in hydroponic setup and 78.5% in soil setup. K is an activator of essential enzymes such as protein synthesis, sugar transport, nitrogen, carbon metabolism, and photosynthesis. It also regulates osmotic potential. Calcium is another necessary element of living beings that is required as a calcium ion and participates in various cellular functions. Calcium is a crucial component for cell wall synthesis, plant growth and development, enzyme activation, salt balance, and water transport in plant cells, which activates potassium to govern the opening and closing of stomata.

Similarly, magnesium is the primary metal atom of chlorophyll, which plays an important role in photosynthesis in plants. Magnesium deficiency causes chlorophyll degradation and yellowing of the leaves, known as chlorosis. However, appropriate magnesium availability maintains plants healthier. Manganese divalent ions are transformed into trivalent and tetravalent ions and play an important part in various oxidation-reduction processes, including the electron transport chain in photosynthesis. Manganese also can activate several enzymes involved in the citric acid cycle, carbohydrates metabolism, carboxylation processes, and oxidation reactions. In the case of calcareous soil with higher pH values, the zinc is taken up by the soil in the form of a divalent cation. Zinc is either linked with an organic acid or transferred to divalent cations *via* chemical changes in the xylem, whereas zinc forms organic complexes with low molecular weight and larger quantities in the phloem sap. Zn is also necessary for optimal plant growth since it affects various biological processes such as cell proliferation, glucose metabolism, and P-Zn interactions (Figure 11). For all six kinds of enzymes (hydrolases, oxidoreductases, lyases, transferases, ligases, and isomerases), Zn is the only metal required. Although it has a structural role in some regulatory proteins, it is toxic to the cell at larger concentrations. Deformed chlorotic leaves, interveinal necrosis, decreased photosynthesis, and reduced biomass production are all symptoms of Zn deficiency in plants, resulting in diminished plant development, lower yield, and poor nutritional quality of the produce. Fe plays critical roles in crop plant physiological activities, yet they are only required in trace levels (Figure 12). Chlorophyll production and the maintenance of chloroplast structure and functions both require Fe. Although it is found at greater levels in soil, its bioavailability is restricted in aerobic and neutral pH settings. Fe is primarily contained in the Fe^{+3} form in aerobic soils, with very poor solubility, and does not meet the plant's iron needs. As a result, Fe deficiency has become a prevalent nutritional problem in many crop plants, causing interveinal chlorosis in young leaves, delayed root growth, low yield, and poor nutritional quality.

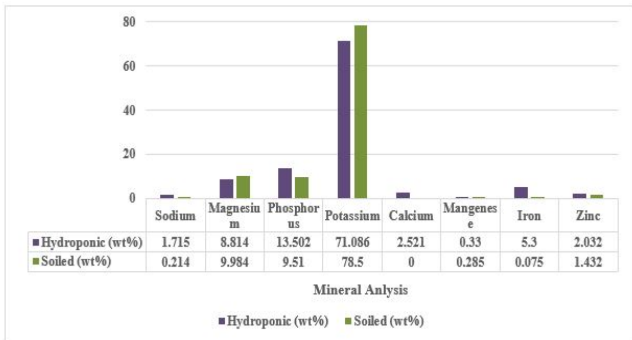

Figure 10: Mineral profiling comparison and variation in hydroponic cultivated plants and soil plants.

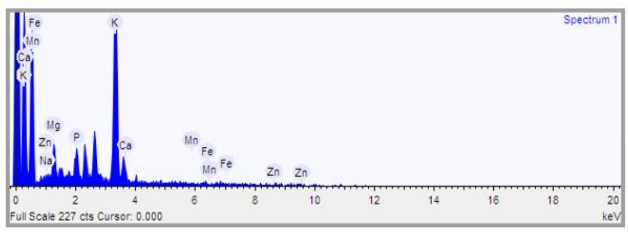

Figure 11: Spectrum of mineral analysis of soil cultivated plants.

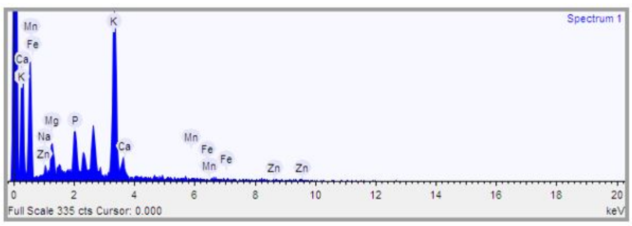

Figure 12: Spectrum of mineral analysis of hydroponically cultivated plants.

CONCLUSION

A growing and the increasingly wealthy global population is putting unprecedented strain on the planet's limited land and water resources, raising concerns about global food security. To meet the nutritional needs and food demands of a rapidly rising population, agricultural techniques must advance beyond the past, focusing on increased productivity and a healthy environment, social well-being, and public health. It's also critical to look into different options for controlling plant diseases caused by soil that are otherwise harmless to the environment and have been shown to improve product quality and per capita yield. The comparative study in every domain like morphology, anatomy, a phytochemical and elemental analysis in hydroponic as well as in soil reveals that hydroponics technology is better for harvesting of *Amaranthus* plant not only because of improved quality, but also because of higher yield, system ease of operation, and water efficiency. Small-scale farmers should use an open field hydroponic system instead of a controlled environment hydroponic system because it requires less capital. To our best knowledge, this is the first comprehensive growth research of *Amaranthus* grown hydroponically and in soil that examined and compared from various aspects. Further research into hydroponic production and optimization of its ability to assure acceptable product quality and the selection of suitable types to deliver better hydroponic products is also worthwhile.

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CONFLICT OF INTEREST

There is no conflict of interest.

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