THE EFFECT OF CALCIUM ON GROWTH AND DEVELOPMENT OF SELECTED MICROGREENS

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ABSTRACT
Microgreens, the edible seedling of vegetables and herbs, received increasing attention nowadays. Microgreens are considered functional food or superfood due to their health-promoting properties. Mustard (Brassica nigra L.) and Radish (Raphanus sativus L.) were selected species for treatment. Five sets of these microgreens were prepared for treatment, two sets of both greens were treated with calcium chloride and the other two with calcium sulphate. Both solutions had different concentrations and one set was under control without any treatment. Calcium solutions were applied for 5 days. After the treatment studied plant growth and development, harvested microgreens were analyzed for biochemical metabolites. As a result, most of the treated microgreens had more physiological growth and increased metabolites than control.

Keywords: *Microgreens, Plant metabolites, Calcium Chloride, Calcium Sulphate*

INTRODUCTION
In recent times, people around the world tend to have good immunity in the context of the COVID-19 pandemic threat. Normally, this generation suffers from a low level of immunity due to an unhealthy diet, stress, pollution, sedentary lifestyle, and lack of exercise. Nowadays, society is being health-conscious more than ever before. This subject has to be taken seriously to improve human health with nutritious food (Patras *et al.*, 2021). Microgreens are considered a functional food because its health-promoting food, it contains high antioxidants and bioactive compounds. Microgreens are advanced edible with lots of nutrition. Microgreens have more bioactive components than their seeds and mature plant that is why it is known as a "superfood". The initial stage of plant development is considered as a microgreen, the first rapid stage of plant growth. After the appearance of two green culinary leaves, this stage is known as the "cotyledon growth stage" (Renna *et al.*, 2020). The most preferable substrate to grow in soil, is peat moss, coco coir or a similar substance. The harvesting period is within 5 to 21 days depending on lifecycle, cut the upper portion of soil which is edible consists of a stem and two cotyledon leaves. Microgreens have increased attention in the past decade because of their culinary especially. They are defined by various tastes, colors, and textures, these soft greens are found in different species and families. Microgreens are the type of distinction crop that presents the soft juvenile greens from seeds of vegetables, grains, herbs as well as wild type (Marchioni *et al.*, 2021).
Microgreens can be derived from different kinds of seeds. The well-known species are harvested using from these families. Brassicaceae, Asteraceae, Apiaceae, Amaryllidaceae, Amaranthaceae, Cucurbitaceae. Radish and Mustard microgreens are included in the Brassicaceae family. As regards minerals, mustard microgreens include calcium, iron, manganese, phosphorus, zinc. Mustard seeds are a good source of selenium, which suggests that improves the cancer risk in many different modes (Marchioni *et al.*, 2021).

ROLE OF CALCIUM
The important role of calcium in plant growth and development is diverse and has good documentation. Calcium is involved in various cell functions and contributes to the structure of the cell wall and membrane integrity. Binds to pectic acid in the lamella in the center of

the cell wall provide structural support and strength and prevent the building of connections that strengthen cells' demolition of the wall. It acts as an intracellular signaling mechanism activation of physiological stress responses and developmental signals such as germination and rooting lengthen hair.

There are three different physiological types in which plants are classified according to their characteristics Specific Ca requirements. Calcitrophs belong to a family of plants containing more soluble Ca Organization. Calcium accumulation in the offspring of calcitrophs may be directly related to the quantity Of Ca available in soil solutions or growth media. Plants belonging to Brassicaceae A physiological form of calcitrophs, a popular botanical family used to produce microgreens (Kou *et al.*,2015).

MATERIALS AND METHOD

Two plants *Brassica nigra* common name mustard, *Raphanus sativus* common name radish both belong to the Brassicaceae family. This family has the most grown microgreens. Collection of authentic seeds of Radish and Mustard microgreens done from the urban platter. To investigate of Ca effect for the growth and development of plants, five sets of both microgreens with three replicas were prepared. Two sets of them were treated with calcium chloride ($CaCl_2$) and the other two were treated with calcium Sulphate ($CaSO_4$). One set was under control without any treatment. $CaCl_2$ and $CaSO_4$ both were prepared with two different concentrations 0.01M and 0.1M. 1.1098g in 100ml distilled water made 0.1M solution and 0.11098g in 100ml distilled water is made up with 0.01M solution of $CaCl_2$. 1.3614g in 100ml distill water made 0.01M solution and 0.13614g in 100ml distill water made 0.01M solution of $CaSO_4$. Each concentrated solution was applied for 5 days. Harvested after 10 days of development and washed with distilled water. Harvested plants were used for growth experiments and estimation of metabolites.

BIOCHEMICAL TEST: It includes the estimation of the following metabolites.

ESTIMATION OF PROTEIN:

This estimation was followed for the standard protocol of total protein by Bradford in 1976. This procedure is based on the quantitative capacity of a dye. Phosphate buffer (0.1M, pH 7.2), Bradford Reagent and Bovine Serum Albumin (BSA) are the main reagents for this estimation. Take optical density at 595nm and plot it versus the sample. The result is expressed as (mg/ml) plant material.

ESTIMATION OF PHENOL:

Total phenol was estimated by Bray *et al.*, 1954. This process contains folin-ciocalteau's reagent, ethanol is used as a solvent and 20% Sodium carbonate. Gallic acid or Tannic acid are used as standard. Optical density at 660nm and result performed in mg/ml.

ESTIMATION OF STARCH:

For starch estimation standard methods given by Hodge, J E Hofreiter, B T, 1962. In this process sample is treated with 80% alcohol to remove sugars and after that starch is extracted from perchloric acid. For standards in a hot acidic medium, starch is hydrolyzed to glucose and dehydrated to hydroxymethylfurfural. This reagent forms a green color product with anthrone.

ESTIMATION OF SUGAR:

The standard protocol of total protein by Somogyi, M 1952. This estimation contains Alkaline copper tartrate and arsenomolybdate reagent. The reducing sugar got heated with alkaline copper tartrate decreasing the copper from the cupric to cuprous state and thus cuprous oxide is formed. After cuprous oxide is treated with arsenomolybdic acid, the lessening of molybdic acid to molybdenum blue takes place. The blue color developed is equated with a set of standards at 620nm.

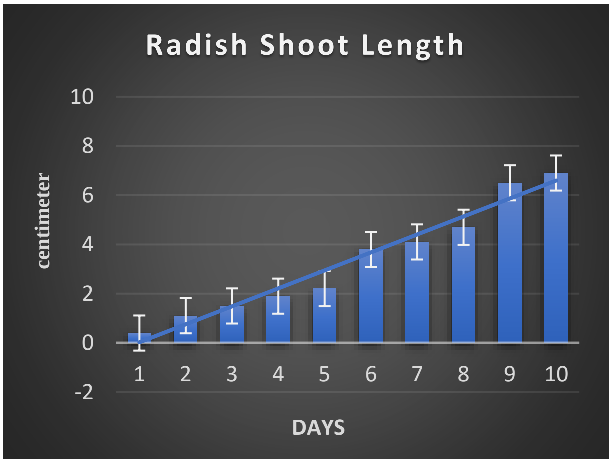
ESTIMATION OF CHLOROPHYLL:

The standard protocol of chlorophyll a, chlorophyll b, and total chlorophyll did by D.L. Arnon, 1949. Chlorophyll is found in the green pigments of plants. Chlorophyll a and Chlorophyll b occur in higher plants. Chlorophyll b is readily extracted in organic solvents such as acetone and ether. Take the absorbance of the solution at 663nm, 652nm, and 645nm.

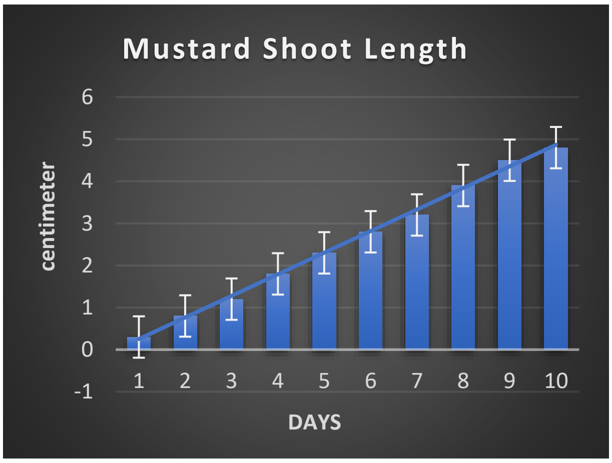
RESULTS AND DISCUSSION

Calcium is a very essential macronutrient for plants. After treatment of Ca on plants, it showed very effective change morphologically and biochemically.

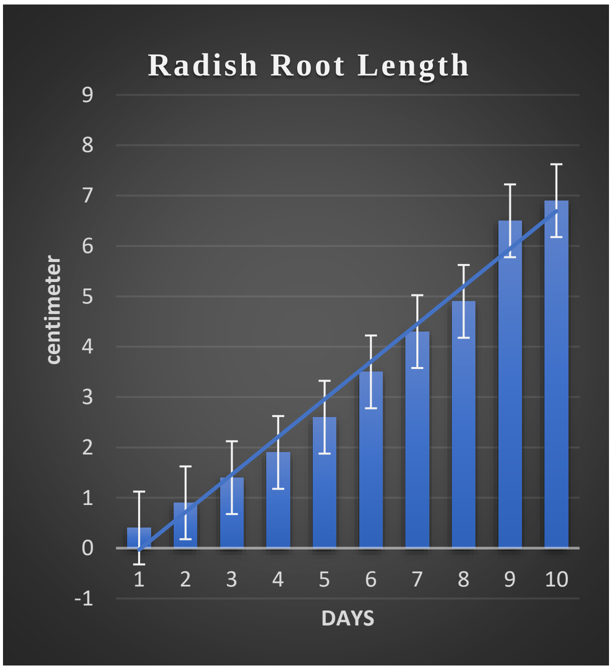
Effect of Ca on Plant Growth



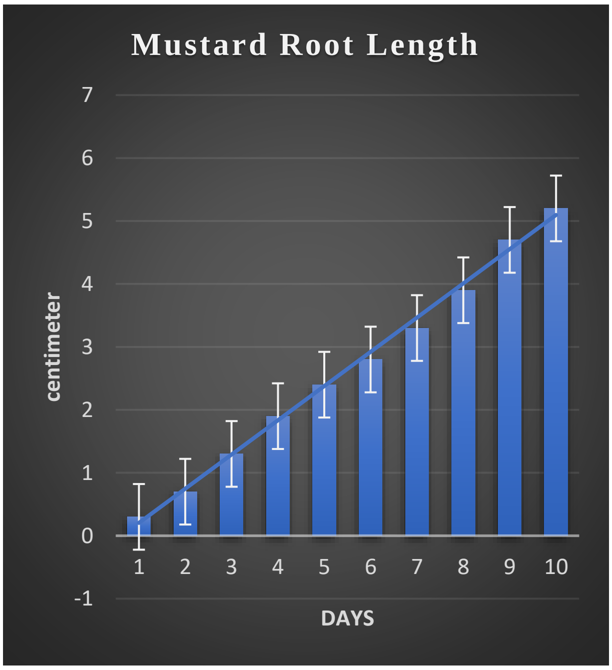
Graph 1: effect of $CaCl_2$ on plant height. Values represent mean ± S.E. (n=3)



Graph 2: Effect of $CaCl_2$ on plant height. Values represent mean ± S.E. (n=3)



Graph 3: Effect of $CaCl_2$ on root growth of plants. Values represent mean ± S.E. (n=3)



Graph 4: Effect of $CaCl_2$ on the root growth of plants. Values represent mean ± S.E. (n=3)

When $CaCl_2$ was applied to the plant, growth of root and shoot both increased than that of control.

Effect of Ca on Soil pH

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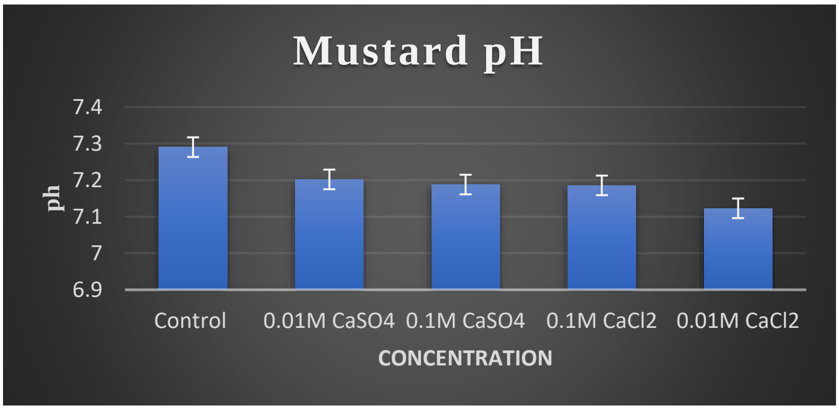
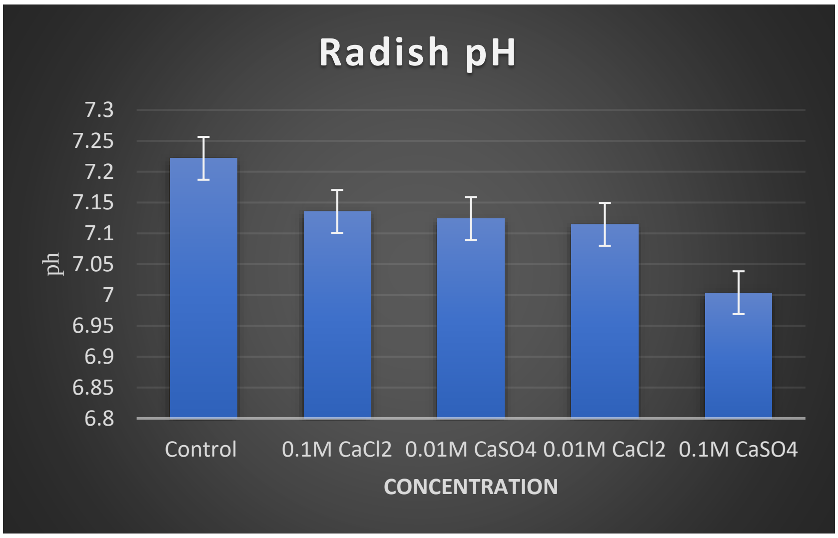


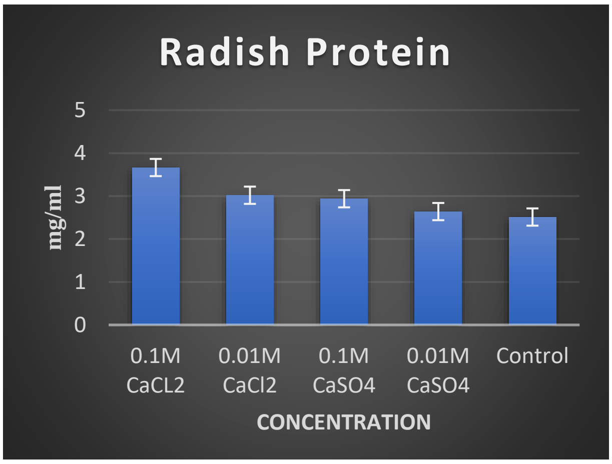
Graph 5: Effect of Ca on soil pH
Values represent mean ± S.E. (n=3)

After the application of Calcium Chloride ($CaCl_2$) and Calcium Sulphate ($CaSO_4$), soil pH decreased more than in the control. In comparison concentrated soil was found with less pH.

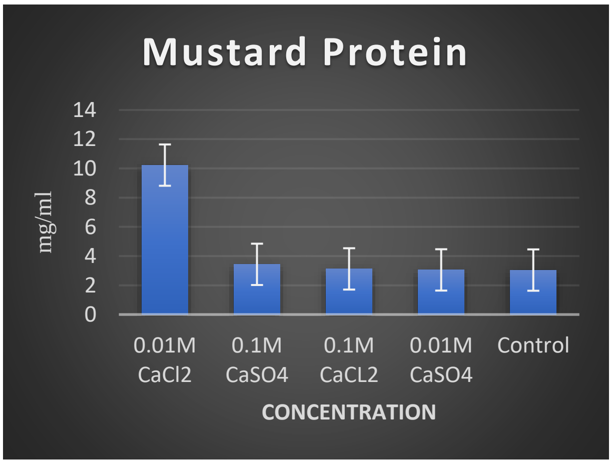


Graph 6: Effect of Ca on Soil pH
Values represent mean ± S.E. (n=3)

Effect of Calcium on Metabolites
When this treatment is done with plants, it shows a very decent effect. It increases the number of metabolites in plants.

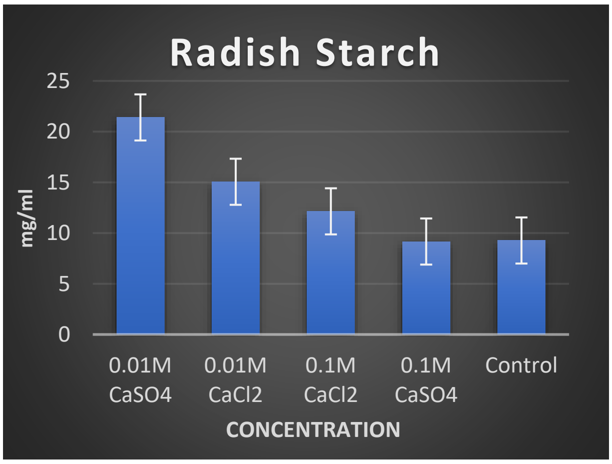


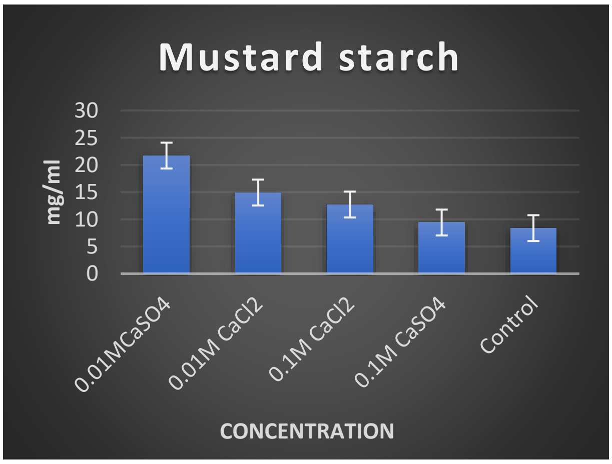
Graph 7: Effect of Ca on Total Protein of Plants. Values represent mean ± S.E. (n=3)



Graph 8: Effect of Ca on total protein of plants. Values represent mean ± S.E. (n=3)

This bar graph illustrates that total protein content is more in the treated plant with Calcium Chloride and Calcium Sulphate compared to control.

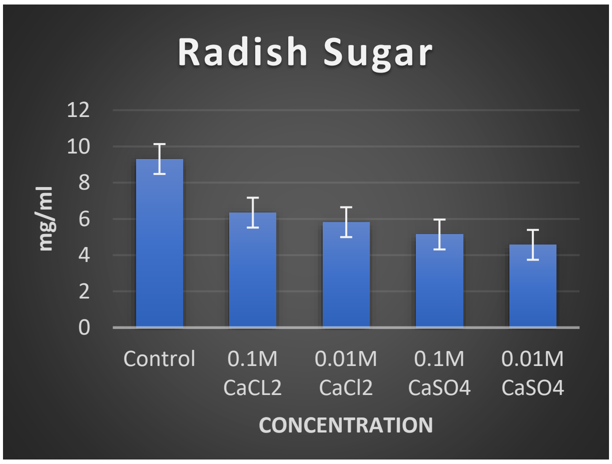




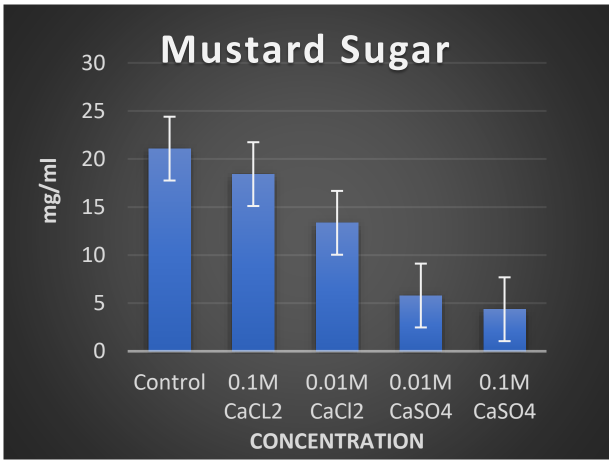
Graph 9: Effect of Ca on Starch. Values represent mean ± S.E. (n=3)

Graph 10: Effect of Ca on Starch. Values represent mean ± S.E. (n=3)

After the application of Calcium Chloride and Calcium Sulphate, Starch content in both microgreens increased more than control.

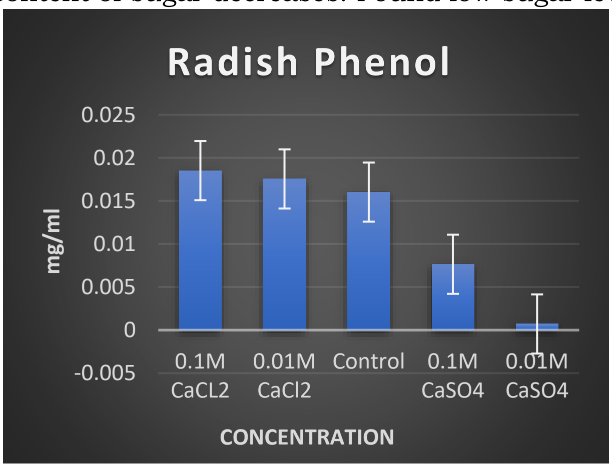


Graph 11: Effect of Ca on Total Sugar. Values represent mean ± S.E. (n=3)

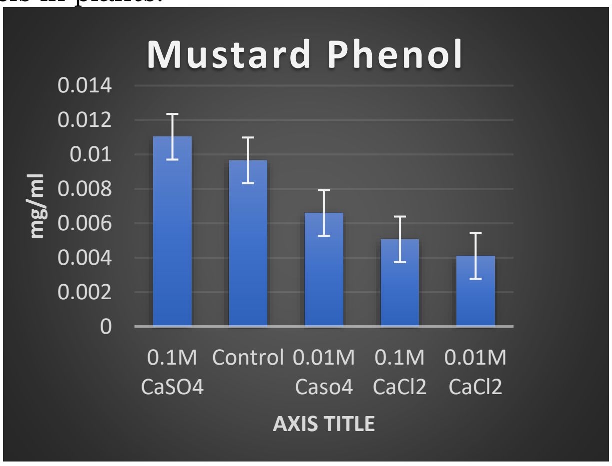


Graph 12: Effect of Ca on a Total Sugar. Values represent mean ± S.E. (n=3)

When Calcium Chloride ($CaCl_2$) and Calcium Sulphate ($CaSO_4$) are applied in soil sugar content of sugar decreases. Found low sugar levels in plants.

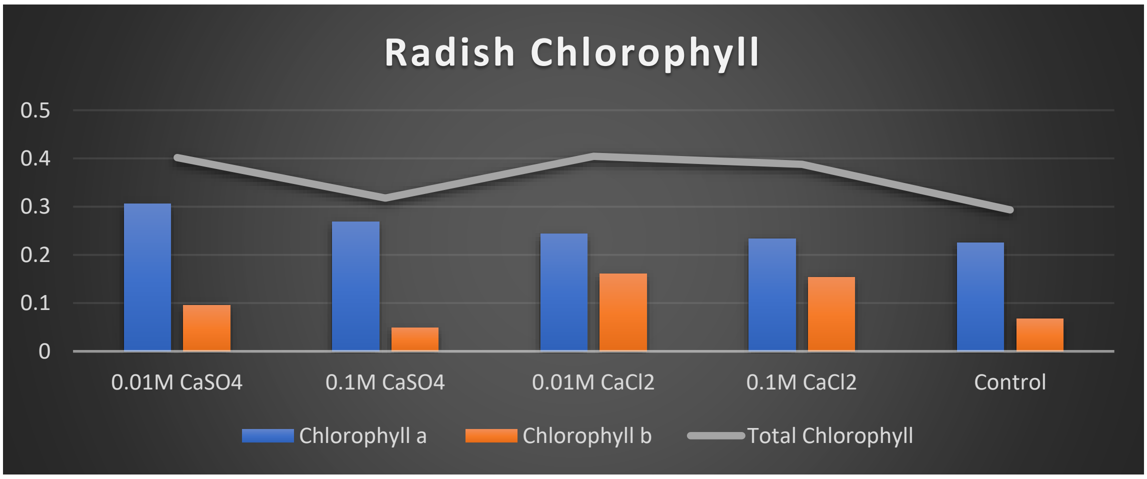


Graph 13: Effect of Ca on Phenols. Values represent mean ± S.E. (n=3)



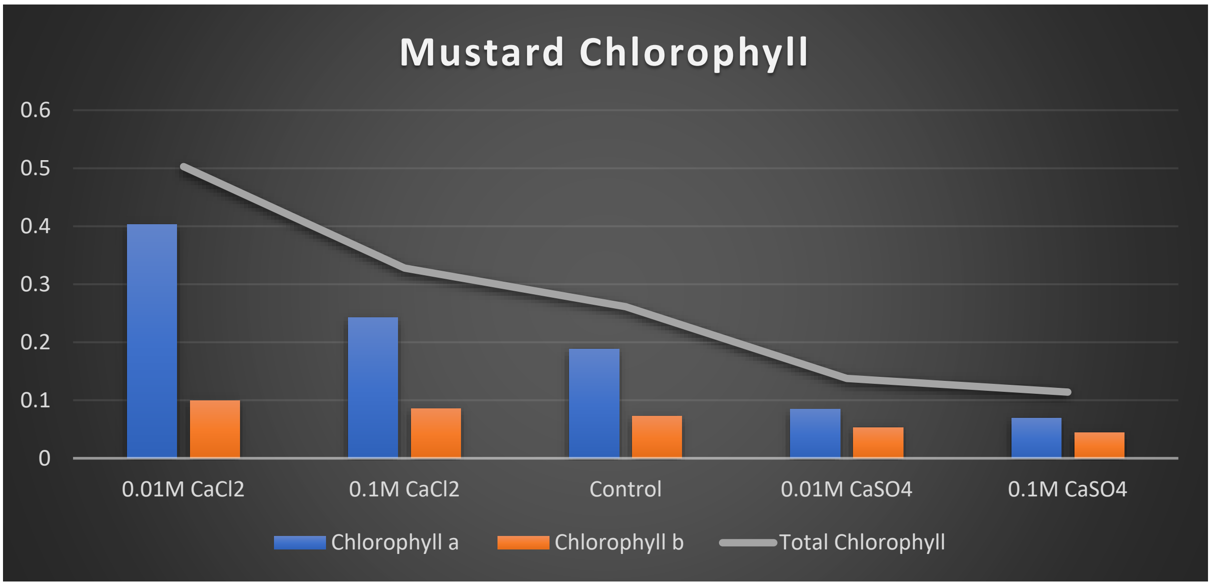
Graph 14: Effect of Ca on Phenols. Values represent mean ± S.E. (n=3)

In phenol, the results were found different in both microgreens. In radish microgreens, Calcium Chloride has more phenolic content than control and Calcium Sulphate treated plants have less phenolic content than control.



Graph 15: Effect of Ca in Chlorophyll Content.
Values represent mean ± S.E. (n=3)

In chlorophyll examination, particular differences were found in both microgreens. Radish after treatment plant has increased chlorophyll content than control. While in mustard, Calcium chloride has more chlorophyll content than control and Calcium Sulphate is found with less chlorophyll content than control.



Graph 16: Effect of Ca in Chlorophyll Content.
Values represent mean ± S.E. (n=3)

CONCLUSION

Microgreens are an emerging specialty crop that has attracted increasing attention over the past decade because of both nutritional and sensory characteristics. When calcium was applied to the plants, it showed a very positive result. Plant growth was quicker after the treatment than control. A great effect was found in metabolites, most of them were increased due to this effect with different concentrations. Ca is a macronutrient, very essential for plant growth and recently this calcium treatment is useful in Horticulture, especially in hydroponics. This treatment is very useful for growth and biochemical components. It must be used in nurseries for faster and healthy germination.

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