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Stress-Induced Variations in Total Flavonoid and Phenolic Content of *Trigonella foenum-graecum*, *Coriandrum sativum*, and *Spinacia oleracea* Microgreens

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Abstract*— *Background* –** *Microgreens because of their high concentrations of vital nutrients and bioactive substances like flavonoids, phenolic compounds, minerals, vitamins and others additional secondary metabolites is often referred as functional food. The effect of different stress condition on phytoconstituent composition is unaddressed.* ***Objective* –** *This study is focus on the impact of various stress conditions on the total flavonoid content (TFC) and as well as total phenolic content (TPC) in three different distinct edible baby green species like: Trigonella foenum - graecum (Fenugreek), Coriandrum sativum (Coriander), and Spinacia oleracea (Spinach) in controlled stress factors, including environmental impact like- Temperature, Relative humidity etc.* ***Methodology* –** *Absorbance values were measured and calculated for Total phenolic content (TPC) and Total flavonoid Contents (TFC) contents for different stress conditions of microgreen growth like proper water supplying, Inadequate water supplying, sand as growing medium etc.* ***Results- *Results indicated the stress conditions significantly increase the total flavonoid as well as phenolic content across all species, shows stress dependent variation with highest content in freshly grown microgreen and optimum water supply. Coriander and spinach both are greater value on sand media conditions rather than inadequate media and proper watering supply.* ***Conclusion-*** *This research contributes to correlate plant resilience mechanisms and offers insights into improving the health benefits of microgreens on applying the various stressors. This work also focusses on elucidating the presence of the secondary metabolites variety like polyphenol which involved a potent response and exploring the long-term effects of stress on microgreen quality.*

Keywords– *Microgreen, Stress Condition, TFC, TPC, Water supply.*

I. Introduction

Microgreens called 'Baby green' is vegetables which are consider as a first true pair of leaf. It's had a greatly sources of food & nutritional value rather than the mature medicinal as well as others general vegetable (1). Generally, it had 4-40 times more nutritional value as well as beneficence of pharmacological and biological activity. From seedling to developed cotyledons are harvested time is as general 7-21 days (2). Microgreens are prone on various stress conditions like – Growing media, Water supply, not only that also involved Environmental factors like Temperature, Light condition as well as Relative humidity also modify the presence of bio-functional and the quality of the microgreens nature that are different to each other's. Microgreens species key preharvest factors affects the edible quality of genetic modification, climatic changes. Here this study mainly focusing on three different microgreen families' species, their different growing conditions that can affects the variety of biochemical changes (3). The selected microgreen species are- Fabaceae (Medhi), Apiaceae (Coriander), Amranthacae (Spinach). Under various stress condition like-Changing different media are applied to grow the microgreen species which was further analysis for the different analytical way (4,5).

Fig 1. Spinach, Fenugreek and Coriander Microgreen from left to right

Variety of water condition like inadequate & proper water supplying how affected the physical as well as chemical changes on the development of the species. On the other hand, Challenging variety of media like sand & soil where applied the equal amount of the seed for harvesting & further observe the changing of the growing criteria in different media which also must be affected the different bioactive characteristics (2). As per different growing it must be depended on the nutritional component that are analysis by various methods like Phenolic content, flavonoid content estimation (6,7). These tiny greens exhibit strong antioxidant properties that contribute to human health by combating oxidative stress and reducing the risk of chronic diseases (8,9). As per general criteria Baby green have greater nutritional value as well as much greater bioactive compound & secondary metabolite content like- Alkaloid, Flavonoid, Tannin, saponin, Glycoside etc. which must have different types of the activity(10). The background of the study

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is the scarcity of the nutritional elements is extensively spread in worldwide so it's can induce the tendency of the malnutrition and also increase the inadequate supply of the Nutritional candidates. To overcome the issue Microgreen's popularity can overcome that with wide variety of advances like early growing stages, enhance nutritional quality & quantity, Space efficiency, easy to grow etc. (11,12). The variation of different stress condition affects the TFC and TPC of microgreens (13). The objective of the study is Exploring Stress Introduce Changes in Total Flavonoid Content (TFC) and Total Phenolic Content (TPC) of Three Different Microgreen Species investigates how various stressors affect the levels of flavonoids and phenolics in microgreens, highlighting their nutritional and health benefits.

II. MATERIALS AND METHOD

1.1. *Chemicals & Standard* - Gallic acid (Merck Specialities P.L), Quercetin, Folin- coicaltue reagent (Merck Specialities P.L), Sodium carbonate (Merck Specialities P.L), Sodium nitrate (Merck Specialities P.L), Aluminium chloride (Merck Specialities P.L), Sodium hydroxide (Merck Specialities P.L), Methanol (CS chemical) and Distilled water etc.

1.2. *Collection of Seed and of Microgreen Production* - All seeds & uses are study was purchase from the local nearest market of Hooghly, West Bengal, India. After collection of the fresh seed washed it properly with distilled water then soaked it for the overnight for proper germination(14). Thereafter the overnight soaking seed are harvested on equal weight over the growing tray with equal space maintaining then cover it properly with the specific growing media. The selected microgreens species and their common name and also family are mentioned bellow the Table:1. These selected three species are Grow in Different stress conditions like- Proper water supplying, Inadequate water supplying and proving sand media for growing. Each and every Conditions should take for individual timing for proper optimum development. Others factor like sunlight, Temperature, Relative humidity are also more all less impactful for the growing of each and every microgreen species(15). After growing of the microgreen species each and every condition microgreen sample collected for the drying purpose. Thereafter with proper shade drying it was further move to extraction.

TABLE 1. Different microgreen species and their common name and also their family

Sl No.	Microgreen Species	Common Name	Family	References
1.	*Trigonella foenum - graecum*	Fenugreek (Methi)	Fabaceae	(16)
2.	*Coriandrum sativum*	Coriander	Apiaceae	(16)
3.	*Spinacia oleracea*	Spinach	Amaranthaceae	(16)

TABLE 2. Stress conditions applied to microgreens

SL NO.	Different stress condition for cultivation of microgreens
1	Sufficient water supplying (PW)
2.	Inadequate water supplying (IW)
3.	Sand media condition (S)

In the research study various stress conditions could be applied to understand how these environmental or physiological stress factors influence the phytochemical composition, specifically the flavonoid and phenolic content, in microgreens. Specifically, some specific media like Proper water suppling where water is supplied in accurate sufficient optimum level and providing sand & soil media which impact a variety change in the phytochemical as well as the nutritional content. In this study are also focused on the Inadequate quantity of the water suppling media also can observed in the variety changes in the Nutritional value.

1.3. *Microgreen Morphology & Yield Calculation*- On the Morphology of the Microgreen's varies by the species to species which also impacted on the different media, as well as various watering condition also. Overall growing cycle, length of the species, structural features like- leaf area also noticeable depending on the microgreen's species. After completing the growing of the microgreen to calculate the percentage yield the following formula is following-

$$\% \text{ of Yield} = (\text{Harvested weight} / \text{Initial seed weight}) \times 100 \ldots\ldots [1]$$

1.4. *Extraction* - From the collection of the drying sample it was triturated with the mixer grinder. Then each of the Crude powdered samples are collected 2 gm which was used for the maceration purpose. This Powdered sample are prepared on 10 ml methanolic extract on Iodine flask with tight binding. Placed it for 7 days then filtered it properly & weighing the sample which was further analyze(17).

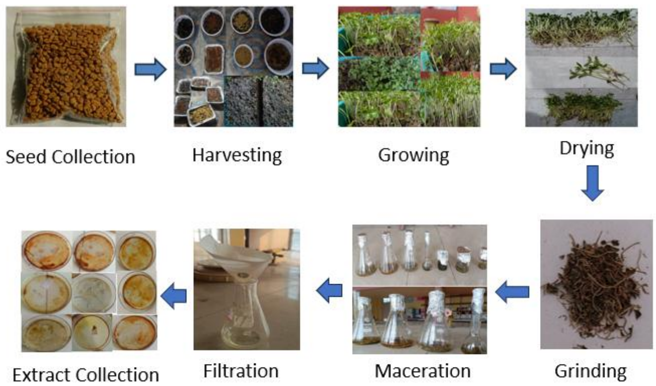

Fig. 2: Extraction process of crude microgreen Sample.

1.5. *Determination of Total Flavonoid Content*-

1.5.1. Preparation of Standard quercetin for Calibration curve- By using Colorimetric Aluminium chloride test Total flavonoid content (TFC) was performed(18,19). A stock solution of Quercetin was made with methanol (1 mg/ml) then from the stock solution prepared serially diluted solution which concentration was – 20,40,60,80 mcg/ml. 1ml of this each and every serial dilution added 4ml of distilled water and 0.3 ml of the 5% sodium nitrate ($NaNO_2$) solution and kept it for 5 minutes. The mixture was then treated with 0.3 ml of the 10% aluminum chloride ($AlCl_3$) solution and maintained for six minutes. After proper mixing of the solution then at last added 1M sodium hydroxide ($NaOH$) solution 2 ml. After few minutes later a

UV spectrophotometer was used for measuring at 510 nm absorbance(20).

1.5.2. Preparation of sample for the Total Flavonoid content- The Microgreen extract prepared 0.5 mg/ml concentration with the methanol where we are using sample 1.25 mg & 2.5 ml methanol. Then the methanolic extract (0.5 mg/ml) were further proceed to the similar procedure of preparation of standard quercetin calibration curve and lastly measure the absorbance at 510 nm with the help of UV spectrophotometer. The entire flavonoid content was determined using the absorption value. Using the linear equation based on the calibration curve, The concentration of flavonoid was represented as Quercetin equivalent (mg QE/100g) (21).

1.6. Determination of Total phenolic content-

1.6.1. Preparation of Standard Galic acid for Calibration curve- To measure the Total phenolic content by using Folin- Ciocalteu reagent. Here Firstly prepared a standard Galic acid solution with methanol (1mg/ml)(22). From that stock solution another prepared 20,40,60,80 mcg/ml concentrated serial diluted sample. Each and every diluted sample taking 1 ml of the solution and added 5m 10% Folin-Ciocalteu (F-C) reagent, allow it for 6 minutes. After that add 1ml of 7% sodium carbonate (Na2Co3) solution and incubate for 30 minutes on the water bath at 40° C. After that absorbance measure with the help of UV spectrophotometer at 760 nm (23).

1.6.2. Preparation of sample for the Total Flavonoid content- Prepared a methanolic extract of 0.1 mg/ml. Then as per standard calibration curve preparation followed by others everything was performed and at last measure the absorbance on the 760 nm via UV spectrophotometer. The Total phenolic content of each & every microgreen species was calculated as per Galic acid equivalent (mg GAE/g). All the experiment were triplicate (24).

III. Results

A. *Morphological Characteristics* **–** In this Microgreen species morphology focus on the actual growing time and calculate the leaf area (length × width) & also the measurement of the average Hight of the microgreen species(cm) that were calculated finally at the overall development of the individual microgreen species separately. Below (Table 3) this table no those data are mentioned.

B. *Yield Calculation***-** This are mentioned here between the difference of how much amount of the crude extract was taken (Theoretical value) to perform on the extraction & how much amount of the obtaining sample (practical value) taken from the extraction (maceration)(25). From these two values we determined the percentage yield that was mentioned below this Table 4.

TABLE 3. Different microgreen species conditions with their growing cycle & Hight & also leaf area.

SL NO	MICROGREEN SPECIES		GROWTH CYCLE (DAYS)	MICROGREEN HIGHT (Cm)	LEAF AREA	
					LENGTH (cm)	WIDTH (cm)
1	Spinach	Sand	9	6.5	0.9	0.5
2		Proper water	10	6.8	0.9	0.4
3		Inadequate water	11	6.2	0.8	0.4
4	Methi	Sand	9	5.9	0.7	0.4
5		Proper water	9	5.9	0.6	0.3
6		Inadequate water	12	5.8	0.7	0.4
7	Coriander	Sand	10	6.5	0.8	0.4
8		Proper water	10	6.3	0.8	0.3
9		Inadequate water	12	6.1	0.8	0.4

TABLE 4. Different microgreen species conditions with their obtaining theoretical, practical value as well as Yield calculation data.

SL NO	MICROGREEN SPECIES		THEORETICAL VALUE (gm)	PRCATICAL VALUE (gm)	% OF YIELD
1	Spinach	Sand	2	1.6	80
2		Proper water	2	1.6	80
3		Inadequate water	2	1.2	60
4	Methi	Sand	2	1.6	80
5		Proper water	2	1.5	75
6		Inadequate water	2	1.1	55
7	Coriander	Sand	2	1.6	80
8		Proper water	2	1.5	75
9		Inadequate water	2	1.1	55

C. *Total flavonoid content determination***-** The total flavonoid content of these edible microgreen species was determined using a UV-Visible spectroscopy technique that employed aluminum chloride. In this study focused on the equivalent weight of the Quercetin present in a particular amount of the dry extract of the particular species using the Calibration curve. The result was shown below on the (Fig. 3) on the base of the equation of $y = 0.0024x + 0.1651$, $R^2 = 0.9976$ on Figure no:1. As compare to the Mean ± Standard deviation value the quantity of the quercetin determines of the specific sample. On the quantitative study the amount of the quercetin present in Spinach species better in proper water supplying media then sand media and at last in the Inadequate water supply medium. In case of Fenugreek (Methi) species Proper water supplying & inadequate water supplying both are better result than the sand media. At last, on the species of the coriander species Sand the ideal media for the suitable then inadequate and at last proper water supplying media are resulted medium as shown in Table 5.

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ANOVA studies (two-way) were conducted for the results of TPC values as shown in Fig 4.

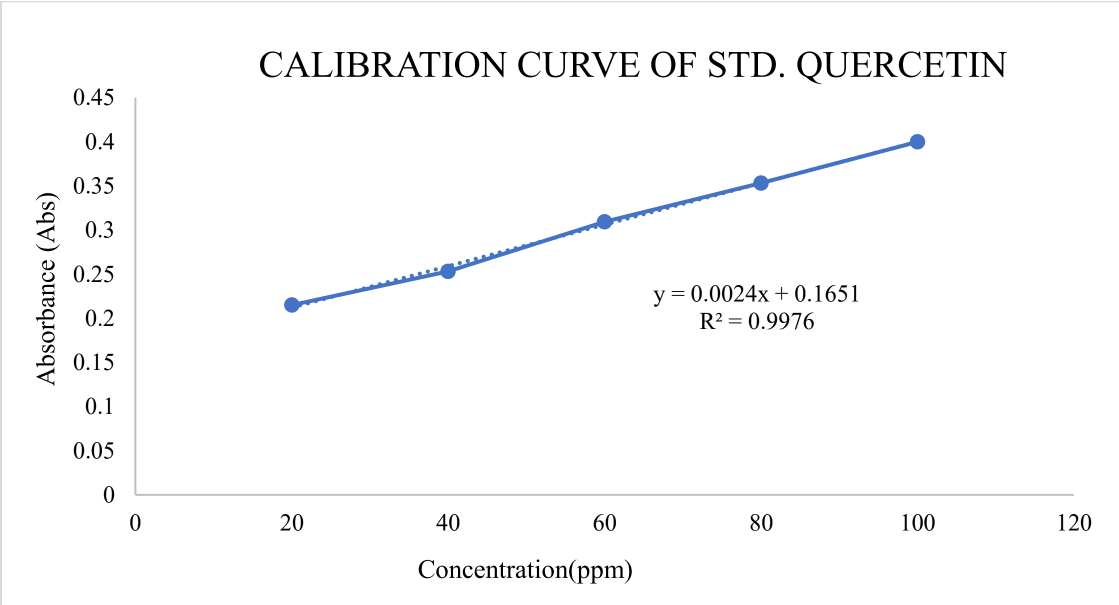

Fig. 3: Calibration curve of Standard quercetin.

TABLE 5. TFC content of different microgreens at stress conditions

SL NO	MICROGREEN SPECIES		Quercetin Content (Mean ± SD mg/ 100 g dry weight)
1	Spinach	Sand	49.0616 ± 0.0704
		Proper water	47.4828 ± 0.4093
		Inadequate Water	35.5051 ± 1.488
		Seed	24.6740±0.3176
2	Methi	Sand	43.6534 ± 0.0905
		Proper water	56.0602±0.0993
		Inadequate water	44.1732 ± 0.5105
		Seed	29.092±3.4453
3	Coriander	Sand	46.3593 ± 0.6729
		Proper water	47.4948± 0.6066
		Inadequate water	47.8428±0.04097
		Seed	43.0543±2.5881

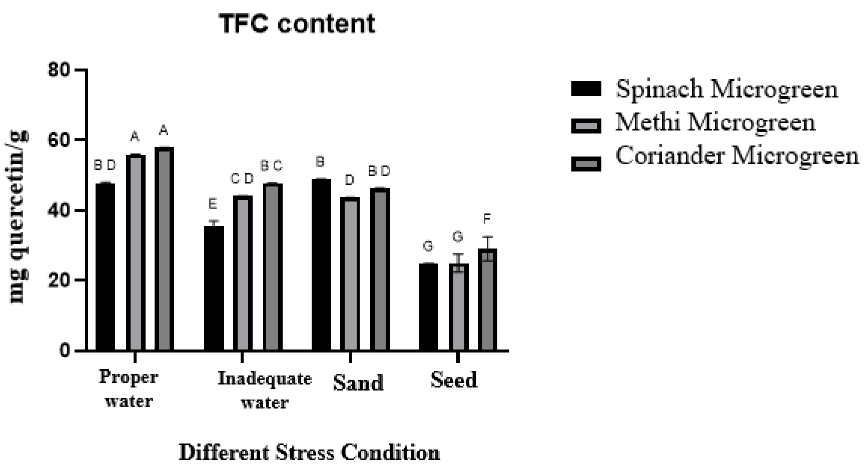

Fig. 4: Two-way ANOVA studies for TFC content of microgreens at different stress conditions. Different values indicate that the readings are significantly different (P<0.05).

D. *Determination of Total phenolic Content*- With the help of Folin - Ciocalteu (F-C) reagent we determine the Total phenolic quantity. In this quantitative measurement of Phenolic assay performed through Galic acid as a standard. Through this calibration curve of the gallic acid the phenolic quantity of the dry extract of these three different types of the microgreen are obtained. From the Equation y = 0.0227x - 0.2209, R^2 = 0.9803 Shown on Fig 4 denoted the amount of the phenols present in specific types of stress condition microgreen species. Here on the spinach species Sand and proper water supplying condition were contains greater amount of the Phenolic content compare to the Inadequate water supplying media. On the other hand, Fenugreek and the coriander species both were shown much greater phenolic level in the proper water supplying media rather than the sand and the inadequate water providing media as depicted in Table 6. Two-way ANOVA studies were conducted to determine the statistical significance of the values and is shown in Fig 6:

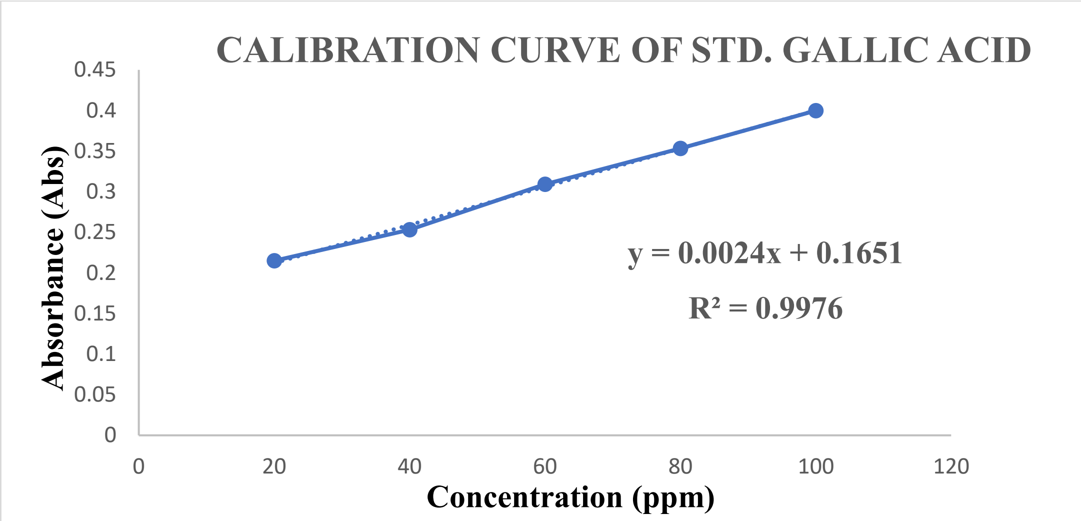

Fig. 5. Calibration curve of Standard gallic acid.

TABLE 6. Different microgreen species condition with various gallic acid content (Mean ± SD mg/ 100 g dry weight).

SL NO	MICROGREEN SPECIES		Gallic acid Content (Mean ± SD mg / 100 g dry weight)
1	Spinach	Sand	79.5417 ± 1.3825
		Proper water	71.6805 ± 14.7921
		Inadequate water	60.2361±4.7931
		Seed	32.5833±4.1971
2	Methi	Sand	52.1527 ± 2.1990
		Proper water	92.6527 ± 1.1105
		Inadequate water	57.9444 ± 2.1050
		Seed	38.1111±6.8605
3	Coriander	Sand	92.1527± 2.1990
		Proper water	89.7916 ± 0.6009
		Inadequate water	63.6527 ± 2.6582
		Seed	51.5694±0.4935

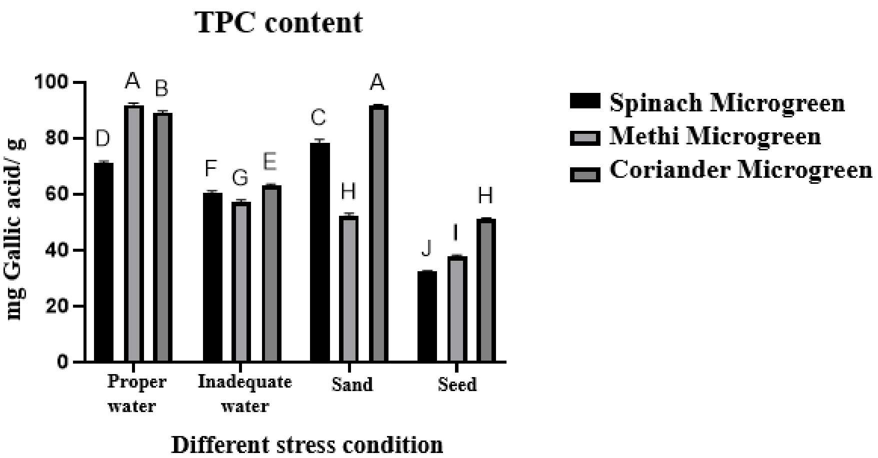

Fig. 6. Two-way ANOVA studies for TPC content of microgreens at different stress conditions. Different values indicate that the readings are significantly different (P<0.05)

IV. Discussion

This research study is much more crucial to ignore the underlying molecular mechanisms governing these changes when examining how stress affects the total flavonoid content (TFC) and total phenolic content (TPC) of three distinct edible microgreen species because of their high nutritional content and bioactive components, microgreens also referred to as baby greens respond dynamically to a range of stressors and growing medium, including consistent temperature and relative humidity. This discussion synthesizes findings from recent studies to elucidate how these stressors influence the variety of the TFC and TPC in various edible microgreens species. According to this research investigation, stressors can greatly increase the manufacturing of flavonoids and phenolic compounds in microgreens (26). For example, it has been demonstrated that drought stress activates the phenylpropanoid pathway, which increases the synthesis of flavonoids like kaempferol and quercetin. This is accomplished by upregulating important biosynthetic genes involved in the production of flavonoids, which are frequently triggered by stress-related heat shock factors. The response of microgreens to stress is not uniform; it varies significantly among species.

The accumulation of TPC and TFC can differ based on genetic factors and specific growth conditions. In one study, kohlrabi microgreens exhibited a marked increase in TPC when subjected to nutritional stress, highlighting the potential for optimizing cultivation practices to enhance phytochemical content like Flavonoid, Alkaloid, Tannin etc. The increase in flavonoids and phenolics under stress conditions is often linked to enhanced antioxidant activity(27). These compounds play a critical role in plant defense mechanisms against oxidative stress induced by environmental challenges. For instance, thermal stress has been associated with increased antioxidant capacities in various microgreen species, correlating with elevated levels of TPC and TFC. At last Understanding how different stressors influence TFC and TPC can inform agricultural practices aimed at improving the nutritional quality of microgreens. By manipulating environmental conditions-such as Temperature, Lighting availability, Presence of moisture availability that can optimize the production of these valuable compounds. Furthermore, applying moderate stress could serve as a strategy for enhancing the health benefits of microgreens without compromising yield. Here for this three-microgreen species preliminary observation study, it is observed that the optimum condition of the growing time of the specified species and also noticed that the morphology of the species like Hight, leaf area (length × width) etc. and also measure the percentage yield of the specific condition of the microgreens. Total Phenolic and Flavonoid content estimation study shows that generally all microgreen species have to fulfilled more Phenolic as well as flavonoid content rather than the Seed of the specific species. The ANOVA studies shown that the values are significantly different indicating that there is a wide variation in TPC and TFC content of different microgreens in different stress conditions.

Then as per comparative study in case of flavonoid content estimation study in spinach species on sand media conditions, Fenugreek (Methi) on proper water supplying condition & Coriander in both watering condition have shown greater optimum result than other stress condition. On the Phenolic content estimation study Spinach on Proper water supplying conditions, Fenugreek (Methi) & Coriander on the sand media condition shown a greater value than other stress conditions.

V. Conclusion

From the research work, it is evident that effect of growing conditions of microgreens of three different species, especially the stress induction has profound impact on the phytoconstituent content in them. This leads to significant differences in the TPC and TFC content of the microgreens when grown in stress conditions. This stress induced change in flavonoid and phenolic content implies that microgreens have an extraordinary capacity to alter their metabolic pathways in reaction to stress, which in turn improves their nutritional content. These substances are known for their antioxidant qualities and function in preventing disease. The molecular processes behind these stress responses should be clarified in future studies, and the possibility of breeding or engineering microgreens with higher phytochemical content should be investigated. Furthermore, knowing how various effect of different stress condition on the content of individual phytoconstituent can help the agriculturalist to focus on cultivation of specific compound rich phytoconstituent which can be incorporated to daily diet by dietician.

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