Original Research Article

Qualitative analysis of curcumin content and total phenolic compounds in distinct turmeric (*Curcuma longa L.*) germplasms along with statistical evaluation.

Abstract

Aim: Nowadays, turmeric value has increased with the presence of curcumin content and total phenols, as they are highly advantageous and useful in different areas of study. Salinity stress is also a major problem in certain areas under which many turmeric cultivars show reduced phenolic compounds and curcumin content. Therefore, the present study is aimed at identifying the different varieties that are diverse with high yields and maximum production so that the salt stress effects can be studied.

Methodology: This study is carried out using Factorial Completely Randomized Design (FCRD). Statistical analysis is done by applying the Duncan Multiple Range Test (DMRT) in relation to the concentrations of total phenolic compounds and curcumin content.

Results: We observed, a gradual rise in the total phenol content and curcumin content in all the cultivars. In particular, treatments including maximum salinity stress like 100 mM concentration gave better results compared to the control and 50 mM concentration. These observations can be considered as stress-responsive mechanisms developed by the turmeric plants under salt stress conditions. Compared to all the selected cultivars, Vallabh Sharad and New Selection 1 gave maximum output for total phenol content. 2.12 (leaf) and 0.28 (rhizome) values, which are maximum compared to controls observed in the case of 100 mM concentration. Also, New Selection 1 gave 0.58 (rhizome) curcumin content, which is the maximum compared to the control.

Conclusions: Therefore, this increase in the phytochemicals in plants, helps in the increase of the medicinal properties in the turmeric plants also, internally protecting them from salt stress.

Key words: Total phenolic compounds, Curcumin content, Salt stress, Plant resistance.

Introduction:

Turmeric is an important medicinal herb which is commonly used as a spice and has several advantages in curing many important diseases like cancers, kidney ailments, and heart attacks. This spice has gained much importance in the ancient culture as it is used in treating many wounds, so it is commonly called as a wound dresser [1]. Also, in many places like China and Myanmar, turmeric powder is used as a paste for applying to inflammation and to other allergies. Also, in India, turmeric paste is taken early in the morning to avoid many health issues and cleanse the gut. Not only this, turmeric is well known for its flavour, colour, and is also considered a super spice. Almost every Indian dish calls for a pinch of turmeric to add flavour, colour, and aroma. Also, turmeric is considered a natural colour for dyeing clothes and has many other advantages [2,3].

This importance of turmeric is obtained due to the presence of very unique compounds in the rhizomes of this crop called phytochemicals [4]. These compounds promote several health benefits in humans, making this crop famous world-wide. This importance of the turmeric crop is achieved due to the availability of total phenolic compounds and curcumin (phytochemicals) in the young and mature rhizomes of turmeric [5].

The levels of these phytochemicals increase as the rhizomes mature. These compounds are known to play a unique role in protecting human health from severe cancers. These compounds are well-known constituents of plants, promoting anti-oxidant activity. Along with this, these compounds facilitate the elimination of the free radicals in the human body, which are highly responsible for causing cancers in humans. Such cancerous cells, that are formed will be eliminated with the presence of the total phenolic compounds in the turmeric rhizomes [6,7].

With respect to the many health benefits and medicinal properties in turmeric, which are internally achieved by the presence of the curcumin content in the rhizomes, this curcumin is the most abundant curcuminoid present in the growing rhizomes of turmeric plants [8]. Apart from curcumin alone which occupies almost 85%, there are other curcuminoids like dimethoxy curcumin, at around 15%, and bis-dimethoxy curcumin, at around 5%, indicating that out of total curcuminoids present in the rhizomes, curcumin stands out in its concentration in the young and maturing rhizomes of turmeric [9].

It is known that curcumin alone aids in curing many diseases like Cancers, Alzheimer's, Gall bladder stones and many others. Therefore, the presence and the content of the curcumin per rhizome also depends upon the variety of the turmeric plant selected [10]. The maximum rhizomes production with curcuminoids production indicates the high-quality turmeric which is useful in different industries like medicines, ayurvedic, homeopathy, drug preparations and nutritional experiments. So, proper understanding of the curcumin production and presence of total phenolic compounds in the turmeric rhizomes is an important factor in keeping many diseases at bay [11].

Recently, salinity stress in the turmeric plants growing, near water bodies or under improper field management practices is observed to show reduction in yields as well as important compounds like curcuminoids and phenolic contents in both the leaves and rhizomes [12]. This problem of salt stress with respect to curcumin content and other important compounds, effects the quality of turmeric. Different plants exposed to different salt concentrations are known to develop varied symptoms like dried leaves, stunted growth, yellow patches on leaves etc., leading to the yield losses and reduction in phytochemicals. Therefore, our study is aimed at identifying the suitable cultivar that gives maximum phytochemicals under salt stress conditions so that it can be adopted and cultivated in the areas facing the problem of salinity. Along with this, the significance of these treatments is checked using FCRD (Factorial CRD) and the observations are analysed using DMRT (Duncan Multiple Range Test) [13].

Materials and Methods:

A total of five germplasms were selected for the work, which were known to have diverse backgrounds and beneficial characteristics like high yields and varied climatic adaptability. These germplasms were given salinity treatment in two concentrations, like 50 mM and 100mM. Following the salinity treatment, the germplasms was separated into control (untreated) and treatments (50 mM and 100 mM) groups. The NaCl solution mixed in required proportions with water was given to the plants and allowed to absorb the salt solution for two days without watering them [14]. The characteristics, growth and other parameters of the plants were recorded and observed at each and every month up to three months after the salt treatment to understand the effect of salinity on the plant's growth and phytochemicals. Three months after the salinity treatment, the rhizomes of the plants were collected for observing and the estimation of total phenolic and curcumin contents [14].

Phytochemical portray of different turmeric germplasms under salt stress conditions:

Figure 1: Stage (3 months after salinity treatment) of germplasms selected for phytochemical analysis:

All the five selected germplasms were estimated for their total phenolic content and curcumin content. All these germplasms were selected in the final stage of the treatment, i.e., three months after salinity treatment. These germplasms contain control, 50 mM concentration, and 100 mM salt concentrations of each cultivar. Their appearance, size, and growth varied with each other within the same cultivar due to different concentrations of salt. Also, some plants developed stress symptoms like leaf rolling, yellowing, light green patches on leaves, and reduced chlorophyll content, etc. These characters were seen only in treated cultivars of all the varieties, but the control plants were normal and showed normal growth conditions (Figure 1.). Therefore, two plants of each cultivar, including both 50mM and 100mM, were taken for phytochemical analysis to understand the medicinal properties of turmeric cultivars even under salt stress conditions.

Extraction and Estimation of total phenolic compounds in turmeric germplasms under salt stress:

The total phenolic compounds in the turmeric plants that were salt treated, were estimated by using the protocol followed by [15]. Here, the total phenolic compounds were estimated from the developing leaves and rhizomes of all the control and salt-treated plants. For this estimation, 0.5 gms of developing leaf and rhizome samples were taken and properly amalgamated with 10 ml of 80% ethanol using a mortar and pestle.

Spectrometric determination of total phenolics in turmeric germplasm: Leaf and rhizome samples of 0.5g of all the plants were mixed with 8ml of double distilled water followed by adding Folin-Cio-Calteau's reagent (diluted in a 1:2 ratio with double distilled water). This reaction was incubated for 3 min at room temperature and immediately 1 ml of Na2CO3 (25% mixed in water) was added to halt the chemical reaction. This reaction mixture prepared was incubated for 60 min at room temperature, after which the absorbance was recorded at 725 nm. The blank value was recorded prior to the control and treatment values. These values differ for all the cultivars depending upon the control and the treatment concentrations.

Estimation of curcumin content:

In our study, young rhizomes were selected for the rhizome sample needed for curcumin content estimation. Therefore, 1 gram of each rhizome was collected from the salt-treated and control plants from all the selected germplasms and were properly amalgamated using a few drops of methanol to which methanol was added further and the volume was made up to 100 ml and was kept overnight to infuse the curcumin present in the samples into the methanol. From the previously prepared solutions, a volume of 5 ml was pipetted out from them into fresh tubes, and the absorbance was recorded at 425 nm using a spectrophotometer. For the estimation of curcumin, the following formula was used, which was given by [15].

Curcumin content gm /100 gm =

$$\frac{0.0025 \times A425 \times \text{volume made up} \times \text{dilution factor} \times 100}{0.42 \times \text{weight of the sample (gm)} \times 1000}$$

The total mean values of both the phenols and curcumin contents were statistically represented using the Factorial CRD method, done using R-software [16] to check the significance of the data [17]. Also, with the help of Duncan's new Multiple Range Test, it helps in providing the variations in the mean values of the cultivars taken and the treatments given that were significant to each other [18].

Results and Discussions:

The estimation of total phenolic compounds and curcumin content in turmeric germplasms under salt stress conditions was important to understand the effect of salinity on the growth and especially other parameters like curcumin content and total phenolic content [19,20]. Turmeric has gained much importance due to the presence of these two compounds called curcumin and total phenolics. It was well-known that total phenolics play a major role in treating many diseases in humas. Also, these total phenolics were known to act as very important anti-oxidants as they have the highest anti-oxidant properties [21].

Nevertheless, curcumin content was known for its famous role in protecting the human body against several cancers, heart failures, and kidney-liver dysfunctions. Apart from this, curcumin was known for its medicinal and therapeutic properties for wound healing and in the beauty industry [22,23]. The proper estimation of these important compounds of turmeric was useful in understanding the effect of salt stress on these varieties and thereby, cultivating these resistant varieties in the areas that were frequently affected by salinity [24,25].

This method of estimation and quantitative analysis of total phenolics and curcumin was possible by following certain methods which were previously designed [26,27]. For the estimation of these phytochemical compounds, not many chemicals were needed. Also, spectrophotometric evaluation and chromatographic techniques were known to be accurate in identifying and quantifying different phytochemical compounds [28,29].

Table 1. Phytochemical parameters under salt stress.

		TOTAL PHENOLIC CONTENT (LEAF)			TPC (RHIZOME)		CURCUMIN CONTENT (RHIZOME)			
S. No	**Turmeric germplasms**	**Control**	**50mM**	**100 mM**	**Control**	**50mM**	**100 mM**	**Control**	**50mM**	**100mM**
1	**VALLABH SHARAD**	1.74	1.85	2.12	0.17	0.18	0.28	0.5	0.51	0.55
2	**NEW SELECTION 1**	1.32	1.53	2.09	0.17	0.15	0.19	0.48	0.54	0.58
3	**VALLABH PRIYA**	1.06	1.46	1.87	0.16	0.15	0.19	0.47	0.51	0.51
4	**AZAD**	0.98	1.42	1.77	0.17	0.15	0.18	0.43	0.5	0.51
5	**NEW SELECTION 2**	0.56	1.21	1.36	0.15	0.12	0.16	0.41	0.42	0.42

The total phenolic content in both the leaves and rhizomes along with curcumin content were observed in the selected turmeric germplasms treated with salt stress (Table 1). Positive results were observed in all the treatments under salinity stress. It was observed that the 100mM concentration of all the cultivars gave good results compared to 50mM and controls for total phenols estimation and curcumin content as a stress adaptive mechanism (Table1).

The total phenolic compounds present in the leaves and rhizomes, as well as the curcumin content in the rhizomes, were found to be normal in control conditions, slightly increased in 50 mM concentrations, and maximum in 100 mM concentrations. Based on this experiment, out of all the five selected cultivars, Vallabh Sharad and New Selection 1 gave good results compared to other varieties for the total phenolic content both in leaves and rhizomes.

In the case of total phenolic content in leaves, Vallabh Sharad gave normal results in control conditions and there was a slight increase in 50 mM concentration. But the value of 100 mM was maximum in a way that this cultivar was responsive to salt stress and was able to cope with the stress conditions. The values observed in leaf samples were 1.74, 1.85, and 2.12 for control, 50 mM, and 100 mM concentrations, respectively. Following Vallabh Sharad, New Selection 1 responded similarly with respect to the salt stress in which 1.32, 1.53 and 2.09 were observed for control, 50 mM and 100 mm concentrations. Similarly, total phenolic compounds in rhizomes were highest in Vallabh Sharad, with 0.17, 0.18, and 0.28 for control, 50 mM, and 100 mM concentrations, respectively. Also, New Selection1 showed similar patterns, i.e., 0.17, 0.15, and 0.19 for control, 50 mM, and 100 mM concentrations, respectively (Table 1). Here, in New Selection 1 for rhizome samples, there was a slight reduction in Total Phenolic Content in 50 mM concentrations and it was greatly increased in 100 mM concentrations.

As a result, total phenol concentrations in control plants were normal in all varieties, slightly increased in 50 mM concentrations, and maximum in 100 mM salt concentration. It was understood that under normal conditions the plants showed normal concentrations of total phenolic compounds, but in the case of 50 mM concentrations, the plants were trying to undergo stress tolerance mechanisms and were able to produce maximum phenolic compounds. But in the case of 100 mM, it was clear that the plants were trying to cope with the stress of producing maximum phenolic compounds. This indicates that the increased salt concentrations in the samples improved the phytochemical accumulation in plants, i.e., increasing the total phenolic concentrations in a plant, which was one way of promoting improvement in growth of the plants through the increase in their phytochemical concentrations, which provide many useful effects in plant development and also health benefits in humans.

With respect to the curcumin content, which was estimated from the rhizomes of the plants that were three months old after the salinity treatment, normal results were obtained in control, a slight increase in 50 mM concentrations, and better results were obtained in 100 mM concentrations. In Vallabh Sharad, where 0.50, 0.51 and 0.55 were observed for control, 50 mM concentration and 100 mM concentrations, respectively. Along with Vallabh Sharad, better results were observed in New Selection1 where 0.48, 0.54, and 0.58 concentrations were observed in control, 50 mM, and 100 mM concentrations, respectively. Therefore, it was observed that the curcumin content in untreated plants was normal, in treated plants like 50 mM concentration, the curcumin content increased a little, and in 100 mM concentration, a maximum increase in curcumin content was observed. This was considered as a common phenomenon in which plants tend to develop certain characteristics against stress conditions and try to impose resistance. The increase in the curcumin content in 100 mM salt concentration increases anti-oxidant properties in plants, which helps humans in many ways.

Therefore, in terms of total phenolic content, Vallabh Sharad gave good results compared to New Selection1 in both the leaf and rhizome samples. Whereas, New Selection 1 gave the maximum results in curcumin content compared to Vallabh Sharad. In both the cases, there was a gradual increase in their phytochemical concentrations from control to 50 mM and 100 mM, which indicates that this increase in the total phenolic content and the curcumin content increases the ability of the plants to cope with the salt stress conditions, which in turn protects the crops from low yields and improves the quality of rhizomes.

Statistical Analysis:

With the help of Factorial CRD, the mean values for the cultivars along with the treatments were recorded in each phytochemical test. Here, DMRT helps in identifying those cultivars and treatments that were on par with each other and also certain cultivars and treatments showed significant variations among them [30].

Total phenolic content in leaf and rhizome:

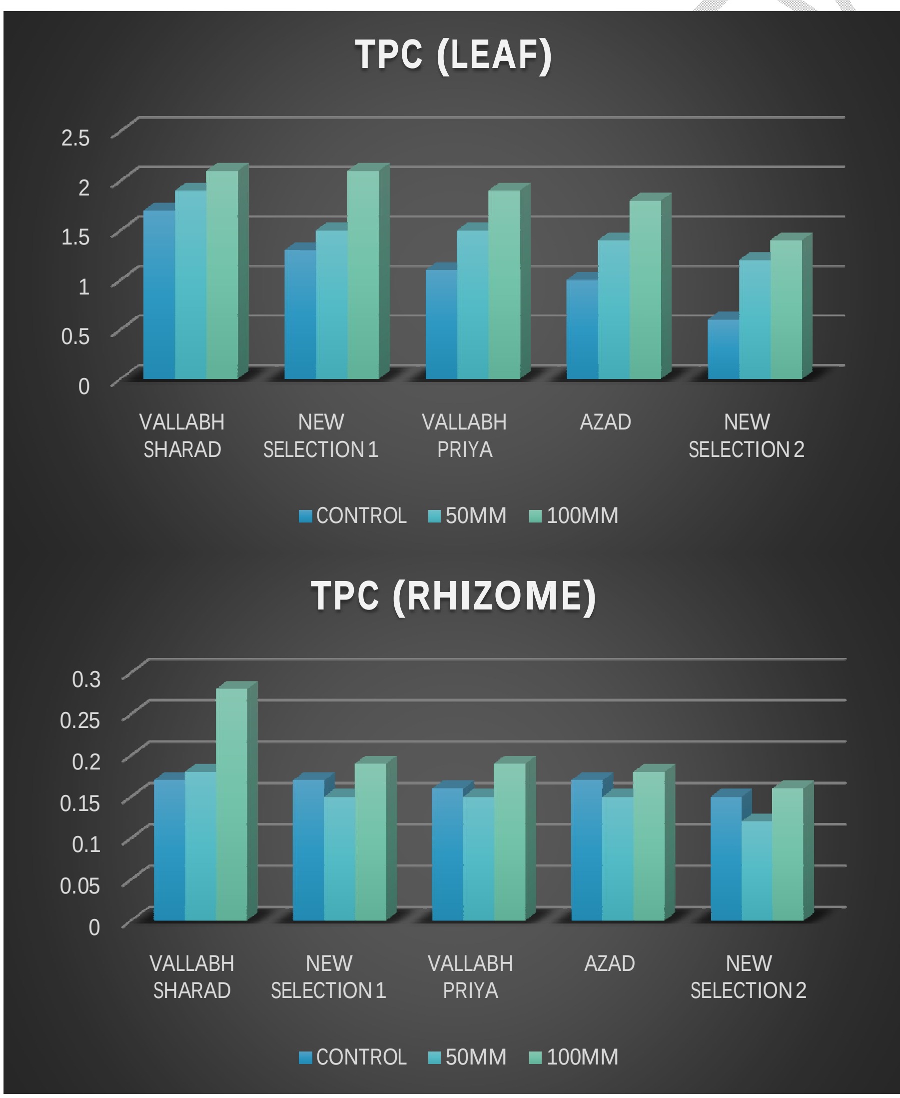
The total phenolic compounds were considered as the most important phytochemicals in plants for their specific role in promoting growth under unfavorable or salt stress conditions.

Table 2. Total phenolic content along with DMRT in leaf and rhizome of selected turmeric cultivars under salt stress.

	TPC (LEAF)				TPC (RHIZOME)			
	CONTROL	**50MM**	**100MM**	**MEAN**	**CONTROL**	**50MM**	**100MM**	**MEAN**
VALLABH SHARAD	1.7	1.9	2.1	1.9 a	0.17	0.18	0.28	0.21 a
NEW SELECTION 1	1.3	1.5	2.1	1.6 b	0.17	0.15	0.19	0.17 b

VALLABH PRIYA	1.1	1.5	1.9	1.5 c	0.16	0.15	0.19	0.17 b
AZAD	1	1.4	1.8	1.4 d	0.17	0.15	0.18	0.16 b
NEW SELECTION 2	0.6	1.2	1.4	1.0 e	0.15	0.12	0.16	0.14 c
MEAN	1.1 c	1.5 b	1.8 a		0.16 b	0.15 c	0.20 a	
CV				**4.79**				**5.42**

Figure 2: Graphical representation of total phenolic contents in leaf and rhizome samples



It was clear that the total phenolic content in the leaves was maximum in the case of Vallabh Sharad, i.e., 1.7, 1.9 and 2.1 in the case of control, 50 mM and 100 mM concentrations, followed by New Selection 1, i.e., 1.3, 1.5 and 2.3 for control, 50 mM and 100 mM concentrations (Table 2). It was also observed that compared to control, plants in the 100mM treatment gave the maximum results as a mechanism of salinity stress tolerance, which was beneficial and advisable to cultivate these two varieties under stress conditions (Figure 2). Also, for DMRT, it was observed that all the three treatments and all the five cultivars showed significant differences among themselves; where the treatments were recorded as 1.1, 1.5 and 1.8 for control, 50 mM and 100 mM concentrations, followed by cultivars with their mean values of 1.9, 1.6, 1.5, 1.4, and 1.0 respectively. Finally, the CV value of 4.79 indicates the significance of this salinity treatment.

The total phenols present in the rhizomes of the selected turmeric cultivars were maximum in Vallabh Sharad with the highest in 100mM concentration i.e., 0.17, 0.18, and 0.28 followed by New Selection1 with the highest in 100mM concentration i.e., 0.17, 0.15, and 0.19 for control 50mM and 100mM concentrations respectively (Table 2). In the case of DMRT, all the three treatments showed significant variation with each other, i.e., 0.16, 0.15, and 0.20 for control, 50mM and 100mM concentrations, and the cultivars New Selection 1, Vallabh Priya, and Azad were observed to be on-par with each other with their mean values of 0.17, 0.17, and 0.16 respectively, leaving Vallabh Sharad and New Selection 2 as they showed significant variation, i.e., 0.21 and 0.14 compared to others (Figure 2). Finally, a CV value of 5.42 indicates the maximum significance of the treatment.

Curcumin Content:

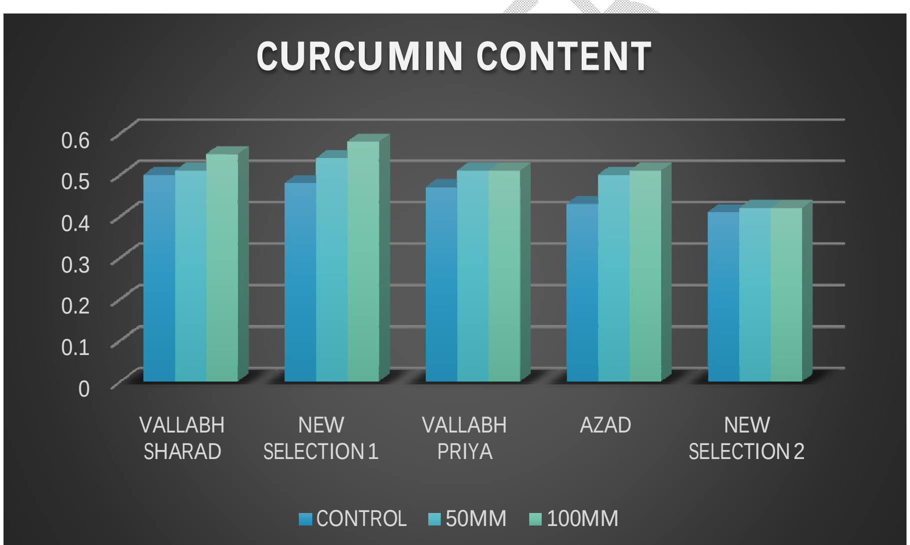
Curcumin content was known to promote anti-cancerous and anti-diabetic properties along with many other health benefits. This curcumin helps in maintaining the human immune system due to the fact that its production was needed in maximum concentration to determine the quality of the plants.

Table 3: Mean values along with DMRT of curcumin content

	CONTROL	**50MM**	**100MM**	

VALLABH SHARAD	0.5	0.51	0.55	0.52 a
NEW SELECTION 1	0.48	0.54	0.58	0.54 a
VALLABH PRIYA	0.47	0.51	0.51	0.50 b
AZAD	0.43	0.5	0.51	0.48 b
NEW SELECTION 2	0.41	0.42	0.42	0.42 c
MEAN	0.46 c	0.50 b	0.52 a	
CV				**5.21**

Figure 3: Graphical representation of curcumin content:



Curcumin content was typically highest during rhizome developmental stages, as observed in our experiment, with New Selction1 having 0.48, 0.54, and 0.58 for control, 50 mM, and 100 mM concentrations, respectively, followed by Vallabh Sharad having 0.50, 0.5, and 0.55 for control, 50 mM, and 100 mM concentrations, respectively (Table 3). Also, the DMRT observed for curcumin content showed significant variation in all the treatments with their mean values of 0.746, 0.50, and 0.52 for control, 50 mM, and 100 mM treatments, respectively. In the case of cultivars, Vallabh Sharad and New Selection1 were on-par with each other with mean values of 0.52 and 0.54, respectively, followed by Vallabh Priya and Azad, i.e., 0.50 and 0.48, respectively, where New Selection2 was observed to be showing significant variation compared to other cultivars (Figure 3). Also, the CV of curcumin content, which was 5.21, shows maximum significance in the treatment.

Finally, these two varieties, Vallabh Sharad and New Selction1, gave maximum output for both the phytochemical parameters, i.e., total phenolic content and curcumin content, where it was very clear that Vallabh Sharad has the maximum total phenolic content in both the leaf and rhizome samples. Whereas, New Selection 1 gave good results compared to Vallabh Sharad for curcumin content. Therefore, in the light of this information, it can be concluded and recommended for the use of these two cultivars, Vallabh Sharad and New Selection 1, for cultivation in salt-affected soils to obtain maximum curcumin content in the growing rhizomes so that they will be beneficial to the farmers, industries, and consumers as well.

Conclusion:

Turmeric importance was primarily due to the presence of important and useful compounds known as total phenols and curcuminoids, which were referred collectively as phytochemicals. Out of all the curcuminoids, curcumin occupies 85% of them, and so has many advantages for humans as well as for the plant itself. Total phenols were significant due to their anti-oxidant, anti-bacterial, and cardioprotective properties, among other benefits. These compounds protect human health and promote immunity. Curcumin has many advantages and was called an active compound useful in treating cancers, heart diseases, kidney failures, Alzheimer’s and many more. Both the compounds were highly significant for their anti-oxidant properties, which were useful in promoting health and immunity in humans. Not only this, curcumin under stress conditions protects the plants from death with its anti-oxidant properties, thus being beneficial to the plant itself.

In our study, we have observed that the total phenolic compounds and curcumin content vary in turmeric plants under salinity stress. Out of all the selected turmeric germplasms, only two gave good results compared to the other three with respect to the presence of these compounds under salt stress conditions. The samples were tested three months after the salinity treatment, at the time when the rhizomes were young or just emerged. Under such circumstances, it was understood that the concentrations of the total phenolic compounds were more in 50 mM treatment, followed by 100 mM, in both leaf and rhizome samples. Whereas, curcumin content was observed to be increasing gradually from control to 100 mM concentration and maximum content was observed at 100 mM concentration, which was a method of plant protection against stress conditions. Therefore, our studies reveal that some plants were able to tolerate salt stress conditions up to a maximum of 100 mM by scavenging the free radicals produced due to the stress and to increase the production of the phytochemicals as they provide many health benefits in humans due to their rich anti-oxidant nature.

Therefore, the importance of total phenols and curcumin along with its derivatives was needed for plant protection under severe salt stress conditions, and total phenolic compounds exclusively were important for plants and humans in promoting many health benefits, which were observed even under 100 mM salt concentration. Considering the above factors, Vallabh Sharad and New Selection 1 germplasms gave maximum total phenolic compounds and curcumin content under salt stress conditions compared to their controls. Therefore, these two varieties can be used and were highly recommendable in salt-stressed areas as they gave good phenolic and curcumin content even under severe salt stress conditions (100 mM) and were able to perform better than other selected turmeric cultivars under salinity stress influence.

References:

1. Akter J, Hossain MA, Takara K, Islam MZ, Hou D-X. Antioxidant activity of different species and varieties of turmeric (Curcuma spp): isolation of active compounds. *Comp Biochem Physiol Part C: Toxicol Pharmacol.* 2019; 215:9–17.
2. Maghimaa M, Alharbi SA. Green synthesis of silver nanoparticles from Curcuma longa L. and coating on the cotton fabrics for antimicrobial applications and wound healing activity. *J Photochem Photobiol B: Biol*. 2020; 204:111806.
3. Priyadarsini KI, Gandhi VV, Kunwar A. Important chemical structural features of curcumin and its derivatives: How do they influence their anticancer activity? Indian *Journal of Biochemistry & Biophysics* Vol. 57, April 2020, pp. 228-235.
4. Chaaban A, Richardi VS, Carrer AR, Brum JS, Cipriano RR, Martins CEN. Insecticide activity of Curcuma longa (leaves) essential oil and its major compound α-

phellandrene against Lucilia cuprina larvae (Diptera: Calliphoridae): histological and ultrastructural biomarkers assessment. *Pesticide Biochem Physiol.* 2019; 153:17–27.

5. Chen Z, Quan L, Zhou H, Zhao Y, Chen P, Hu L. Screening of active fractions from Curcuma longa radix isolated by HPLC and GC-MS for promotion of blood circulation and relief of pain. *J Ethnopharmacol.* 2019; 234:68–75.
6. Pintatum A, Maneerat W, Logie E, Tuenter E, Sakavitsi M E, Pieters L. In vitro anti-inflammatory, anti-oxidant, and cytotoxic activities of four curcuma species and the isolation of compounds from Curcuma aromatica rhizome. *Biomolecules.* 2020; 10(5):799.
7. Mahesh S, Kavi Priya S, Prasanth RM, Anantharaman P. In silico analysis on macro-algae metabolites against skin cancer protein, phylogenetic and statistical analysis. *Indian Journal of Biochemistry & Biophysics* Vol. 58, 2021; June pp. 219-228.
8. Nazanin Mirmohseni Namini, Alireza Abdollahi, Monireh Movahedi, Amirnader Emami Razavi & Reza Saghiri. Association of SERPIND1 expression with grade, stage and presence of metastasis in breast cancer. *Indian Journal of Biochemistry & Biophysics* Vol. 58, 2021; February pp. 71-82.
9. Tirumala, M.G.; Anchi, P.; Raja, S. Rachamalla, M.; Godugu, C. Novel Methods and Approaches for Safety Evaluation of Nanoparticle Formulations: A Focus towards in Vitro Models and Adverse Outcome Pathways (AOP). *Front. Pharmacol.*, 2021;12, 612659.
10. Sabir S, Zeb A, Mahmood M, Abbas S, Ahmad Z, Iqbal N. Phytochemical analysis and biological activities of ethanolic extract of Curcuma longa rhizome. *Braz J Biol*. 2020; 81:737–40.
11. Lee S-Y, Cho S-S, Li Y, Bae C-S, Park KM, Park D-H. Anti-inflammatory effect of Curcuma longa and allium hookeri Co-treatment via NF-κB and COX-2 Pathways. *Sci Rep*. 2020; 10(1):5718.
12. GLOBOCAN. New Global Cancer Data|UICC. Available online: https://www.uicc.org/news/globocan-2020-new-globalcancer-data 2020; (accessed on 27 September 2021)
13. Samriddhi Mehta, Shrishty Bakshi, Sangeeta Choudhury, Sudeep Bose & Ranu Nayak. Hierarchical gold nanostructures-based sensor for sensitive and fast detection of cancer biomarker. *Indian Journal of Biochemistry & Biophysics* Vol. 58, 2021; April pp. 135-140.

14. AlBasher, G.; Abdel-Daim, M.M.; Almeer, R.; Ibrahim, K.A.; Hamza, R.Z.; Bungau, S.; Aleya, L. Synergistic Antioxidant Effects of Resveratrol and Curcumin against Fipronil-Triggered Oxidative Damage in Male Albino Rats. *Environ. Sci. Pollut. Res.,* 2020; 27, 6505–6514.
15. Rizk, M.A.; El-Sayed, S.A.E.-S.; Igarashi, I. Effects of Methanolic Extract from Turmeric (Curcuma longa) against the In Vitro Multiplication of Several Babesia Species and Theileria Equi. *Parasitologia,* 2021; 1, 20.
16. Warasi, M.S., Hungerford, L.L. & Lahmers, K. Optimizing Pooled Testing for Estimating the Prevalence of Multiple Diseases. *JABES. 2022.*
17. Grover, M.; Behl, T.; Sachdeva, M.; Bungao, S.; Aleya, L. Setia, D. Focus on Multi-Targeted Role of Curcumin: A Boon in Therapeutic Paradigm. *Environ. Sci. Pollut. Res.* 2021; 28, 18893–18907.
18. Mostajeran, A., Gholaminejad, A., & Asghari, G. Salinity alters curcumin, essential oil and chlorophyll of turmeric (Curcuma longa L.). *Research in pharmaceutical sciences*, 2014; *9*(1), 49–57.
19. Bray, H. G. and Thorpe, W. V. Estimation of phenols. *Methods of Biochemical Analysis*. 1954; 1: 27-52.
20. Sadasivam, S. and Manickam. Biochemical Methods, Third edition, *New Age International Publishers,* 2018; New Delhi, 202
21. Dafaallah, Awadallah. 12 Duncan's multiple range test (DMRT). 2018; 10.13140/RG.2.2.16262.93764.
22. Dafaallah, Awadallah. 13 Design and Analysis of Factorial Experiments using Completely Randomized Design (CRD). 2019; 10.13140/RG.2.2.29684.71045.
23. Tomeh, M.A.; Hadianamrei, R.; Zhao, X. A Review of Curcumin and Its Derivatives as Anticancer Agents. *Int. J. Mol. Sci.,* 2019; 20, 1033.
24. Braga MC, Vieira ECS, de Oliveira TF. Curcuma longa L. leaves: characterization (bioactive and antinutritional compounds) for use in human food in Brazil. *Food Chem.* 2018;265:308–15.
25. Ahatotu E.O, Lawal. Determination of proximate and minerals content of Turmeric (Curcuma longa linn) leaves and Rhizomes. *Journal of Food, Nutrition and Packaging* Vol 2019; 6:01- 04.
26. Gezici, S. A study on turmeric (Curcuma longa L.): Multifunctional agents for the management of oxidative damage, neurodegeneration and cancer. *Current Research In Pharmaceutical Sciences*. 2019; 09(03): 46-53

27. Vishakha Burman, Vaishali, Khyati Lehari, Naresh Pratap Singh, Bijendra Singh, Pooran Chand, Jitender Singh and Sengar, R. S. Quantification of Total Phenolics and Curcumin Content in Different Turmeric Germplasm. *Int.J.Curr.Microbiol.App.Sci.* 2020; 9(11): 1753-1758.
28. Vishakha, B., K. Himanchal, L. Khyati, Aastha, P.S. Naresh and Vaishali. "Characterization of phenolic compounds of turmeric using TLC" published in *Journal of Pharmacognosy and Phytochemistr*y. 2019; SP2: 994-99.
29. Pınar Kılıçaslan Sönmez, Ali Turhan, Mustafa Öztatlıcı & Kemal Özbilgin, Effects ofverteporfin-mediated photodynamic therapy in breast cancer cells. Indian *Journal of Biochemistry & Biophysics* Vol. 57, 2020; October pp. 560-566.
30. Annie, S. L., Singh, D., & Umesha, C. Performance of Turmeric (Curcuma longa L.) Genotypes on Growth and Yield under Prayagraj Agro-Climatic Zone. *International Journal of Environment and Climate Change*, 2022; *12*(11), 976-982. https://doi.org/10.9734/ijecc/2022/v12i1131073