



## RESEARCH ARTICLE

# Morpho-physiological and biochemical alterations in fenugreek in response to salinity

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### Abstract

A pot experiment was conducted to study the growth and stress response of fenugreek plants at varying levels of salinity, and the observations were recorded at two stages. There was a significant decline in morpho-physiological parameters, viz., fresh and dry weight, root and shoot length, water status, and cell stability, along with degradation of photosynthetic pigments with increasing salinity. The salinity stress led to the accumulation of oxidative stress factors, viz., malondialdehyde. The compatible solutes like proline showed an increment of 80%; total soluble sugars showed an accumulation of 63 and 49% at 30 and 50 days after germination (DAG), respectively, in treatment T<sub>5</sub> (8 ds/m). Salinity stress led to an accumulation of Na<sup>+</sup> as reflected by the highest Na<sup>+</sup>-K<sup>+</sup> ratio at 50 DAG in treatment T<sub>5</sub>. Non-enzymatic antioxidants, viz. phenol content, resulted in an increment of 40% in treatment T<sub>5</sub>. The enzymatic antioxidants, viz., peroxidase and catalase, showed significant accumulation at the early stage followed by a decline at the later stage. Thus, we can conclude that the higher levels of salinity affect the growth and physiological performance of fenugreek, but the plants activate the defense response by the accumulation of compatible solutes and antioxidants, thereby tolerating the stress.

**Keywords:** Fenugreek, oxidative stress, compatible solutes, antioxidants

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### Introduction

Fenugreek (*Trigonella foenum-graecum* L.), belonging to the Fabaceae family, is one of the earliest medicinal herbs, which originated in Central Asia before 4000 BC. India, Pakistan, Afghanistan, Iran, Nepal, Egypt, France, Spain, Turkey, Morocco, North Africa, the Middle East, and Argentina are among the countries where it is grown commercially. In India, the annual production of fenugreek seed was 249,523 MT during the year 2023-24, which was cultivated over an area of 158,203 hectares (Spice Board of India, 2025). New crops that can withstand higher salt concentrations than traditional agricultural crops must be developed because fresh water resources for agriculture are limited and soil salinity is continuously rising globally (Glenn et al.1998). From seed germination to harvest, salinity is a significant abiotic factor that negatively impacts crop growth and development. Increasingly detrimental effects on agricultural output have been noted recently, particularly in arid and semiarid areas with high evapotranspiration and insufficient rainfall (Jha et al., 2019).

Within the next 25 years, almost 30% of the arable land may be lost due to the current steady increase in soil salinization. Global agricultural production needs to rise in order to feed the estimated 9.3 billion people by 2050. Thus,

extensive efforts are required to raise agricultural output. One of the main things preventing conventional agricultural production is soil salinity. Hundreds of farmed fertile areas have become saline due to ongoing farming and poor irrigation management. The utilization of saline resources for agricultural development has received more attention in recent years. Breeding hardy crops that can withstand salt is one practical solution to this problem. To improve plant breeding and lessen future food shortages, it would be beneficial to comprehend the mechanisms underlying plant salt tolerance (Honghong, 2018).

Depending on the severity and time-span of the stress, salinity stress alters a number of physiological and metabolic processes, which eventually decreases agricultural productivity. It is well known that soil salinity suppresses plant growth by osmotic stress, which is followed by ion toxicity. In the early stages of salinity stress, osmotic stress leads to a number of physiological changes, including membrane disruption, nutrient imbalance, impaired detoxification of reactive oxygen species (ROS), variations in antioxidant enzymes and reduced photosynthetic activity, and a decrease in stomatal aperture. Plant tissues under high concentrations of NaCl ions accumulate Na<sup>+</sup> and Cl<sup>-</sup> ions, which is one of the most harmful effects of salt stress. The uptake of K<sup>+</sup> ions is inhibited by high Na<sup>+</sup> concentrations, which reduces productivity and may even be fatal. Plants' essential biological processes can be disrupted by salinity-induced ROS production, which can cause oxidative damage to proteins, lipids, and DNA (Gupta & Huang, 2014). Fenugreek grows notably in arid and semi-arid environments where high salinity levels are one of the soil's most recognizable characteristics. In this regard, Habib *et al.* (1971) noted that fenugreek performs well in salty soils when no other legume is lucrative. The impact of salt stress on fenugreek has been the subject of numerous investigations. Fenugreek seedling survival is seriously threatened when soil salinity rises above 4 dS/m. The plant can, however, withstand higher saline levels of 4 to 12 dS/m as it grows toward maturity (Chowdhury *et al.*, 2014). In recent times, the escalating scarcity of fresh water for agricultural purposes has prompted researchers to investigate alternative irrigation strategies. Using saline water to irrigate crops is one such method that has drawn interest since it may help address water scarcity issues. In light of the aforementioned considerations, a study was conducted to examine the morpho-physiological and biochemical alterations in fenugreek crops in response to salinity stress.

## Materials and Methods

A greenhouse experiment was conducted during *Rabi* - 2023 at the Department of Biochemistry, Junagadh Agricultural University, Junagadh. Fenugreek seeds of variety Gujarat

Methi-2 were used, and about 35–40 seeds were grown per pot (30 × 25 cm) in four replications for further experiments. After germination the seedlings were thinned to maintain 20 plants per pot. Different sets were maintained for observations to be taken at 30 and 50 days after germination (DAG). Saline water obtained from coast of Mangrol, Dist. Junagadh was used after appropriate dilution for treatment. The sea water had the following composition: EC (dSm<sup>-1</sup>) - 52.67; pH- 8.4; Na<sup>+</sup> (mEq/L) - 526.43 and K<sup>+</sup> (mEq/L) - 4.72. The treatment details are as follows: T<sub>1</sub>- Control (Ground water), T<sub>2</sub>- 2 dS/m EC Saline water, T<sub>3</sub>- 4 dS/m EC Saline water, T<sub>4</sub>- 6 dS/m EC Saline water and T<sub>5</sub>- 8 dS/m EC Saline water. The soil was well-drained with pH range of 7.6 to 7.9, slightly alkaline in nature and calcareous in texture having normal electrical conductivity. Initial sample of soil was analyzed before seed sowing and final sample analyzed after the completing experiment in the last stage (Table 1). Germination of seed was observed after three days of sowing in all the pots. Initially treatment of ground water was given up to five leaf stage and after that salinity treatment was implemented in 3 to 4 days of interval. Sampling for various parameters was performed at 30 and 50 days after germination. The methods used for the estimation of different parameters are mentioned below.

### Morphological parameters

Fresh weight of plant was directly measured on weighing balance. The dry weight of plants was measured by exposing them to a constant temperature in hot air oven at 100 °C for 2 hours. and measured on weighing balance. Root and shoot length of the plant were measured using measuring scale.

### Physiological parameters

The relative water content (RWC) and membrane stability index (MSI) and total chlorophyll content of leaf tissue were estimated according to the method of Sairam *et al.* (2002). These parameters were calculated using the following formulas:

$$\text{Relative Water Content (\%)} = \frac{\text{Fresh weight (g)} - \text{Dry weight (g)}}{\text{Turgid weight (g)} - \text{Dry weight (g)}} \times 100$$

$$\text{Membrane Stability Index (\%)} = \left[ 1 - \frac{C_1}{C_2} \right] \times 100$$

where, C<sub>1</sub> = Conductivity of sample kept at 40°C, C<sub>2</sub> = Conductivity of sample kept at 95 °C

$$\text{Total Chlorophyll (mg/g FW)} = 20.2 (A_{645}) + 8.02 (A_{663}) \times \frac{V}{\text{wt} \times 1000}$$

where, A<sub>663</sub> = Absorbance at 663 nm, A<sub>645</sub> Absorbance at 645 nm

V= Final volume of extract in DMSO (ml), W= Fresh weight of the sample (g)

### Biochemical parameters

Fresh leaves were extracted in 80% methanol and centrifuged at 5,000 rpm for 10 minutes. Suitable aliquot was taken and the total soluble sugar content was estimated by phenol sulphuric acid method (Sadasivam & Manickam, 1992). Suitable aliquots from sugar extract were taken and the free amino acid content was determined using ninhydrin reagent (Lee & Takahashi, 1966). Proline content in leaf tissues was extracted in 3% sulphosalicylic acid and measured by reaction with acid ninhydrin (Sadasivam & Manickam, 1992). For malondialdehyde (MDA) content determination, the TBA reaction, which measures the quantity of thiobarbituric acid reactive compounds (TBARS), was used to quantify lipid peroxidation (Abdelhameed *et al.*, 2021). For total phenol estimation suitable aliquot was taken from methanol extract prepared for total soluble sugar analysis and evaporated to dryness in water bath. The reaction was preceded by addition of Folin Ciocalteu's phenol reagent and 20% sodium carbonate and the absorbance was read at 650 nm on spectrophotometer (Sadasivam & Manickam, 1992).

To determine the  $\text{Na}^+ - \text{K}^+$  ratio, the dry leaf powder was digested in di-acid (3:1 ratio of nitric acid and perchloric acid) on a hot plate. The direct reading on flame photometer was measured against standard.

To determine the antioxidant enzyme activity, the fresh leaf tissue was homogenized in a pre-chilled mortar and pestle with 50 mM sodium phosphate extraction buffer (pH 7.0). The homogenates were centrifuged at 10,000 rpm for 15 minutes and the supernatant was used for the assay of antioxidant enzymes *viz.* peroxidase (POD) and catalase (CAT) immediately. The POD activity was determined using the reaction mixture comprising of 0.003%  $\text{H}_2\text{O}_2$ , 0.01% o-dianisidine and enzyme extract. The colour change of the oxidized dye was monitored at 460 nm (Sadasivam & Manickam, 1992). CAT activity was assayed following  $\text{H}_2\text{O}_2$  decomposition measuring the decrease in the absorbance at 240 nm (Sadasivam & Manickam, 1992).

### Statistical analysis

A completely randomized design was used for the statistical data analysis (Snedecor & Cochran, 1963). The treatment means were compared using Duncan's New Multiple Range Test (DMRT) at significance level of  $p < 0.05$ .

## Results and Discussion

### Morphological parameters

Salinity stress significantly reduced both fresh and dry weight of fenugreek plants at 30 and 50 DAG (Table 2), with the reduction becoming more pronounced as salt concentration increased. At 30 DAG, fresh weight declined from 0.72 g in the control ( $T_1$ ) to 0.53 g in  $T_5$ , while dry weight decreased from 0.18 g to 0.15 g. By 50 DAG, fresh weight further declined from 1.38 g in the control to 0.93

g in  $T_5$ , and dry weight showed the greatest reduction (51.40%) under  $T_5$  compared with the control. The decline in dry weight under salinity is largely due to osmotic stress, which limits water uptake and causes cellular dehydration, along with ion toxicity and nutrient imbalance that disrupt photosynthesis, cell expansion, and dry matter accumulation. As a result, nutrient uptake and biomass production are reduced, leading to poor plant growth. The greater reduction observed at 50 DAG suggests that prolonged exposure to salinity intensifies its adverse effects on biomass accumulation. Similar findings have been reported in fenugreek by Ratnakar & Rai (2013). Dadkhah & Griffiths (2006) also reported that restricted water uptake and impaired nutrient utilization are major factors contributing to biomass reduction under saline conditions.

Salinity stress significantly reduced both root and shoot length of fenugreek plants at 30 and 50 DAG (Table 2), with the reduction becoming more pronounced as salinity increased. At 30 DAG, the shortest root length was recorded in  $T_5$  (3.31 cm), which was statistically at par with  $T_4$  (3.67 cm), while the control ( $T_1$ ) resulted in the longest shoot (16.42 cm) and  $T_5$  the shortest (10.69 cm). At 50 DAG, root length decreased by 8.00 % in  $T_2$  and up to 63.26 % in  $T_5$  compared with the control, whereas shoot length declined from 32.24 cm in the control to 16.97 cm in  $T_5$ . The reduction in root and shoot growth under salinity is mainly associated with osmotic stress,  $\text{Na}^+$  and  $\text{Cl}^-$  toxicity, and nutrient imbalance, which collectively restrict cell division, cell elongation, and the uptake of water and essential nutrients. As a result, overall plant growth is adversely affected, with the greater reduction at 50 DAG indicating that the inhibitory effects of salinity become more severe with prolonged exposure. Similar responses have been reported by Pandey *et al.* (2015), Kapoor & Pande (2015) and Ghosh *et al.* (2015).

### Physiological parameters

Salinity stress markedly affected the physiological performance of fenugreek plants, resulting in progressive declines in RWC, MSI, and total chlorophyll content as salinity increased (Table 3). At both growth stages, RWC decreased steadily, with the highest reduction (approximately 19%) observed in  $T_5$  compared with the control, indicating poor plant water status under saline conditions. Membrane stability was also adversely affected, as MSI declined from 50.73 to 43.01% at 30 DAG and from 60.43 to 48.57% at 50 DAG, suggesting greater membrane damage under salt stress. Likewise, total chlorophyll content decreased significantly, with nearly a 26% reduction in  $T_5$  relative to the control, reflecting impaired photosynthetic capacity. The deterioration of these physiological traits can be largely attributed to osmotic stress,  $\text{Na}^+$  and  $\text{Cl}^-$  toxicity, and oxidative damage. These factors reduce water uptake, disrupt membrane integrity, and promote chlorophyll degradation, thereby lowering photosynthetic efficiency

**Table 1:** Properties of soil

| Parameters              | Initial sample | Final sample |        |        |        |        |
|-------------------------|----------------|--------------|--------|--------|--------|--------|
|                         |                | $T_1$        | $T_2$  | $T_3$  | $T_4$  | $T_5$  |
| EC (dSm <sup>-1</sup> ) | 1.00           | 1.00         | 1.12   | 1.72   | 2.21   | 2.78   |
| pH                      | 7.78           | 7.71         | 7.70   | 7.73   | 7.62   | 7.89   |
| Na <sup>+</sup> (ppm)   | 179.87         | 154.50       | 169.20 | 209.20 | 211.50 | 218.80 |
| K <sup>+</sup> (ppm)    | 65.00          | 44.47        | 36.50  | 44.60  | 44.20  | 30.94  |

**Table 2:** Effect of different salinity treatments on morphological parameters in fenugreek

| Parameters      | Fresh weight (g)  |                    | Dry weight (g)    |                   | Root length (cm)   |                   | Shoot length (cm)  |                    |
|-----------------|-------------------|--------------------|-------------------|-------------------|--------------------|-------------------|--------------------|--------------------|
|                 | 30 DAG            | 50 DAG             | 30 DAG            | 50 DAG            | 30 DAG             | 50 DAG            | 30 DAG             | 50 DAG             |
| $T_1$ (control) | 0.72 <sup>a</sup> | 1.38 <sup>a</sup>  | 0.18 <sup>a</sup> | 0.27 <sup>a</sup> | 4.47 <sup>a</sup>  | 8.71 <sup>a</sup> | 16.42 <sup>a</sup> | 32.24 <sup>a</sup> |
| $T_2$ (2 dS/m)  | 0.63 <sup>c</sup> | 1.28 <sup>ab</sup> | 0.16 <sup>b</sup> | 0.25 <sup>b</sup> | 4.35 <sup>a</sup>  | 8.07 <sup>b</sup> | 15.09 <sup>b</sup> | 29.78 <sup>b</sup> |
| $T_3$ (4 dS/m)  | 0.67 <sup>b</sup> | 1.20 <sup>bc</sup> | 0.17 <sup>a</sup> | 0.23 <sup>b</sup> | 4.13 <sup>a</sup>  | 7.41 <sup>c</sup> | 13.91 <sup>c</sup> | 25.64 <sup>c</sup> |
| $T_4$ (6 dS/m)  | 0.60 <sup>d</sup> | 1.15 <sup>c</sup>  | 0.16 <sup>b</sup> | 0.22 <sup>b</sup> | 3.67 <sup>ab</sup> | 6.50 <sup>d</sup> | 11.52 <sup>d</sup> | 19.70 <sup>d</sup> |
| $T_5$ (8 dS/m)  | 0.53 <sup>e</sup> | 0.93 <sup>d</sup>  | 0.15 <sup>c</sup> | 0.18 <sup>c</sup> | 3.31 <sup>b</sup>  | 5.34 <sup>e</sup> | 10.69 <sup>e</sup> | 16.97 <sup>e</sup> |
| S.Em. $\pm$     | 0.006             | 0.040              | 0.002             | 0.008             | 0.249              | 0.127             | 0.194              | 0.347              |
| CD @ 5%         | 0.019             | 0.118              | 0.005             | 0.024             | 0.740              | 0.378             | 0.575              | 1.030              |

**Note:** The different letter(s) in each column shows values are significantly different ( $p < 0.05$ ) as evaluated by the DMRT

and overall plant growth. The more pronounced reductions at 50 DAG further indicate that the negative effects of salinity intensify with prolonged exposure. Similar decreases in RWC, MSI, and chlorophyll content under saline conditions have been reported in fenugreek by Kapoor & Pande (2015) and Meena *et al.* (2016). Comparable findings were also documented by Belmecheri-Cherifi *et al.* (2019), Banakar *et al.* (2022), and Sarker & Oba (2020), who related these physiological changes to reduced water availability, membrane deterioration, and loss of photosynthetic pigments under salt stress.

### Biochemical parameters

#### Compatible solutes

Salinity stress caused significant changes in the biochemical composition of fenugreek plants, leading to increased accumulation of TSS and proline, while reducing FAA content (Table 4). TSS increased steadily with increasing salinity, reaching its highest value in  $T_5$  (4.43 mg/g) at 30 DAG, which was 63.50% higher than the control, and remained elevated at 50 DAG across all salinity treatments. Proline content followed a similar trend, increasing significantly with salinity and attaining the highest levels in  $T_5$  at both 30 DAG (35.55  $\mu$ mol/g) and 50 DAG (27.23  $\mu$ mol/g) amounting to 81% increase over control, highlighting its important role in osmotic adjustment under salt stress. In contrast, FAA content declined progressively as salinity increased, decreasing from 7.56 to 4.27 mg/g at 30 DAG and from 6.69 to 2.84 mg/g at 50 DAG. The increased accumulation of TSS

and proline represents an adaptive mechanism to maintain osmotic balance, protect cellular structures, and stabilize proteins and membranes under salt stress. The decline in FAA at higher salinity may be attributed to impaired amino acid synthesis and enhanced protein degradation caused by ionic and osmotic stress. These findings are consistent with the reports of Banakar *et al.* (2022), Athar *et al.* (2022), and Shahi & Srivastava (2024).

#### Malondialdehyde

MDA, a widely recognized indicator of lipid peroxidation and oxidative membrane damage, increased significantly with increasing salinity in fenugreek plants (Table 4). At 30 DAG, MDA content rose progressively with salinity, with  $T_3$  (0.30 nmol/mL),  $T_4$  (0.33 nmol/mL), and  $T_5$  (0.40 nmol/mL) recording significantly higher values than the control. A similar trend was observed at 50 DAG, where  $T_5$  exhibited the highest MDA content, representing a 34.36% increase over the control. This pattern indicates that prolonged exposure to salinity intensifies oxidative damage. The increase in MDA under saline conditions is primarily associated with excessive production of ROS, which trigger lipid peroxidation and compromise membrane integrity. The progressive rise in MDA with increasing salinity therefore reflects enhanced oxidative stress and greater membrane damage, making it a reliable biochemical marker of salinity-induced oxidative injury. Similar increases in MDA under salt stress have also been reported by Kapoor *et al.* (2013) and Abdelhameed *et al.* (2021).

**Table 3:** Effect of different salinity treatments on physiological parameters in fenugreek

| Parameters               | RWC (%)            |                    | MSI (%)            |                    | Total chlorophyll (mg/g) |                   |
|--------------------------|--------------------|--------------------|--------------------|--------------------|--------------------------|-------------------|
| Treatment/Stage          | 30 DAG             | 50 DAG             | 30 DAG             | 50 DAG             | 30 DAG                   | 50 DAG            |
| T <sub>1</sub> (control) | 87.57 <sup>a</sup> | 80.43 <sup>a</sup> | 50.73 <sup>a</sup> | 60.43 <sup>a</sup> | 3.19 <sup>a</sup>        | 3.54 <sup>a</sup> |
| T <sub>2</sub> (2 dS/m)  | 76.53 <sup>b</sup> | 74.81 <sup>b</sup> | 46.58 <sup>b</sup> | 54.77 <sup>b</sup> | 3.00 <sup>b</sup>        | 3.33 <sup>b</sup> |
| T <sub>3</sub> (4 dS/m)  | 76.34 <sup>b</sup> | 71.29 <sup>c</sup> | 47.52 <sup>b</sup> | 52.44 <sup>c</sup> | 2.81 <sup>c</sup>        | 3.19 <sup>c</sup> |
| T <sub>4</sub> (6 dS/m)  | 74.88 <sup>b</sup> | 68.74 <sup>d</sup> | 43.01 <sup>c</sup> | 48.57 <sup>d</sup> | 2.67 <sup>d</sup>        | 2.98 <sup>d</sup> |
| T <sub>5</sub> (8 dS/m)  | 73.38 <sup>c</sup> | 67.73 <sup>d</sup> | 44.58 <sup>c</sup> | 49.62 <sup>d</sup> | 2.54 <sup>e</sup>        | 2.80 <sup>e</sup> |
| S.Em. $\pm$              | 0.950              | 0.634              | 0.555              | 0.514              | 0.011                    | 0.009             |
| CD @ 5 %                 | 2.822              | 1.883              | 1.650              | 1.526              | 0.033                    | 0.027             |

**Note:** The different letter(s) in each column shows values are significantly different ( $P < 0.05$ ) as evaluated by the DMRT

**Table 4:** Effect of different salinity treatments on biochemical parameters in fenugreek

| Parameters               | TSS (mg/g)        |                   | FAA (mg/g)        |                   | Proline ( $\mu$ mol/g) |                    | MDA (nmol/mL)     |                   | Total phenol (mg/100 g) |                     |
|--------------------------|-------------------|-------------------|-------------------|-------------------|------------------------|--------------------|-------------------|-------------------|-------------------------|---------------------|
| Treatment/Stage          | 30 DAG            | 50 DAG            | 30 DAG            | 50 DAG            | 30 DAG                 | 50 DAG             | 30 DAG            | 50 DAG            | 30 DAG                  | 50 DAG              |
| T <sub>1</sub> (control) | 1.62 <sup>d</sup> | 3.50 <sup>e</sup> | 7.56 <sup>a</sup> | 6.69 <sup>a</sup> | 6.46 <sup>e</sup>      | 5.22 <sup>e</sup>  | 0.21 <sup>d</sup> | 0.27 <sup>e</sup> | 139.64 <sup>e</sup>     | 116.20 <sup>e</sup> |
| T <sub>2</sub> (2 dS/m)  | 2.55 <sup>c</sup> | 4.14 <sup>d</sup> | 6.97 <sup>b</sup> | 6.30 <sup>b</sup> | 14.82 <sup>d</sup>     | 12.01 <sup>d</sup> | 0.23 <sup>d</sup> | 0.30 <sup>d</sup> | 161.61 <sup>d</sup>     | 131.66 <sup>d</sup> |
| T <sub>3</sub> (4 dS/m)  | 3.10 <sup>b</sup> | 4.61 <sup>c</sup> | 6.21 <sup>c</sup> | 5.16 <sup>c</sup> | 21.75 <sup>c</sup>     | 18.68 <sup>c</sup> | 0.30 <sup>c</sup> | 0.33 <sup>c</sup> | 191.49 <sup>c</sup>     | 155.33 <sup>c</sup> |
| T <sub>4</sub> (6 dS/m)  | 3.41 <sup>b</sup> | 5.44 <sup>b</sup> | 5.39 <sup>d</sup> | 4.53 <sup>d</sup> | 29.90 <sup>b</sup>     | 23.77 <sup>b</sup> | 0.33 <sup>b</sup> | 0.38 <sup>b</sup> | 208.65 <sup>b</sup>     | 181.58 <sup>b</sup> |
| T <sub>5</sub> (8 dS/m)  | 4.43 <sup>a</sup> | 6.27 <sup>a</sup> | 4.27 <sup>e</sup> | 2.84 <sup>e</sup> | 35.55 <sup>a</sup>     | 27.23 <sup>a</sup> | 0.40 <sup>a</sup> | 0.41 <sup>a</sup> | 234.99 <sup>a</sup>     | 204.44 <sup>a</sup> |
| S.Em. $\pm$              | 0.106             | 0.047             | 0.066             | 0.079             | 0.252                  | 0.236              | 0.005             | 0.006             | 2.069                   | 1.292               |
| CD @ 5 %                 | 0.316             | 0.140             | 0.196             | 0.233             | 0.750                  | 0.702              | 0.015             | 0.016             | 6.148                   | 3.837               |

**Note:** The different letter(s) in each column shows values are significantly different ( $p < 0.05$ ) as evaluated by the DMRT

#### Total phenol

Salinity stress significantly increased the accumulation of total phenols in fenugreek plants at both growth stages (Table 4). At 30 DAG, total phenol content increased steadily with salinity, reaching its highest value in T<sub>5</sub> (234.99 mg/100 g), while the control recorded the lowest value (139.64 mg/100 g). A similar pattern was observed at 50 DAG, where T<sub>5</sub> showed the highest phenol content (204.44 mg/100 g), followed by T<sub>4</sub>, T<sub>3</sub>, T<sub>2</sub>, and the control. Overall, T<sub>5</sub> exhibited an approximately 42% increase in total phenol content compared with the control across both growth stages. The enhanced accumulation of phenolic compounds under salinity represents an important protective response against salt-induced oxidative stress. Phenolics act as non-enzymatic antioxidants that scavenge ROS, thereby reducing oxidative damage and helping to maintain membrane stability. Salinity also stimulates the phenylpropanoid pathway, leading to increased phenolic biosynthesis and improved stress tolerance. The higher phenol content observed with increasing salinity therefore reflects activation of the plant's antioxidant defense system. Similar responses have been reported by Nair *et al.* (2017) and Abdelhameed *et al.* (2021).

#### Na<sup>+</sup>-K<sup>+</sup> ratio

Salinity stress significantly increased the Na<sup>+</sup>-K<sup>+</sup> ratio in fenugreek plants at both growth stages, with T<sub>5</sub> recording an approximately 19 % higher ratio than the control (Table 5). The Na<sup>+</sup>-K<sup>+</sup> ratio increased progressively with increasing salinity, indicating greater Na<sup>+</sup> accumulation and reduced K<sup>+</sup> uptake, which reflects a disruption in ionic balance within the plant. The rise in the Na<sup>+</sup>-K<sup>+</sup> ratio under saline conditions is primarily due to the preferential uptake of Na<sup>+</sup> over K<sup>+</sup> by the roots. Excessive Na<sup>+</sup> competes with K<sup>+</sup> for uptake and transport, leading to K<sup>+</sup> deficiency and ionic imbalance that adversely affects enzyme activity, osmotic regulation, and several metabolic processes. Consequently, the Na<sup>+</sup>-K<sup>+</sup> ratio is widely recognized as a reliable indicator of salinity-induced ionic stress. Similar responses have been reported by Belmecheri-Cherifi *et al.* (2019) and Banakar *et al.* (2022), who also attributed the increased Na<sup>+</sup>-K<sup>+</sup> ratio to the antagonistic interaction between Na<sup>+</sup> and K<sup>+</sup> during ion uptake.

#### Antioxidant enzymes activity

Salinity stress significantly affected the activities of the antioxidant enzymes peroxidase (POD) and catalase (CAT)

**Table 5:** Effect of different salinity treatments on biochemical parameters in fenugreek

| Parameters               | Na <sup>+</sup> -K <sup>+</sup> ratio |                   | Peroxidase ( $\Delta$ OD/min/g FW) |                    | Catalase ( $\Delta$ OD/min/g FW) |                    |
|--------------------------|---------------------------------------|-------------------|------------------------------------|--------------------|----------------------------------|--------------------|
| Treatment/Stage          | 30 DAG                                | 50 DAG            | 30 DAG                             | 50 DAG             | 30 DAG                           | 50 DAG             |
| T <sub>1</sub> (control) | 0.44 <sup>d</sup>                     | 0.57 <sup>c</sup> | 4.60 <sup>c</sup>                  | 5.70 <sup>a</sup>  | 4.11 <sup>b</sup>                | 4.49 <sup>b</sup>  |
| T <sub>2</sub> (2 dS/m)  | 0.49 <sup>c</sup>                     | 0.58 <sup>c</sup> | 5.08 <sup>bc</sup>                 | 5.55 <sup>a</sup>  | 4.29 <sup>b</sup>                | 5.40 <sup>a</sup>  |
| T <sub>3</sub> (4 dS/m)  | 0.51 <sup>bc</sup>                    | 0.61 <sup>b</sup> | 5.36 <sup>b</sup>                  | 5.25 <sup>ab</sup> | 4.69 <sup>b</sup>                | 5.78 <sup>a</sup>  |
| T <sub>4</sub> (6 dS/m)  | 0.53 <sup>ab</sup>                    | 0.62 <sup>b</sup> | 5.53 <sup>ab</sup>                 | 4.75 <sup>b</sup>  | 5.45 <sup>a</sup>                | 5.16 <sup>ab</sup> |
| T <sub>5</sub> (8 dS/m)  | 0.55 <sup>a</sup>                     | 0.69 <sup>a</sup> | 6.16 <sup>a</sup>                  | 5.40 <sup>a</sup>  | 5.89 <sup>a</sup>                | 5.02 <sup>ab</sup> |
| S.E.m. $\pm$             | 0.008                                 | 0.005             | 0.210                              | 0.172              | 0.227                            | 0.253              |
| CD @ 5 %                 | 0.024                                 | 0.014             | 0.623                              | 0.510              | 0.676                            | 0.751              |

**Note:** The different letter(s) in each column shows values are significantly different (P<0.05) as evaluated by the DMRT

in fenugreek plants (Table 5). POD activity increased during the early growth stage, with T<sub>5</sub> and T<sub>4</sub> showing 25.32% and 16.87% higher activity, respectively, than the control at 30 DAG. However, POD activity declined slightly by 50 DAG, suggesting a weakening of the antioxidant response with prolonged exposure to salinity. In contrast, CAT activity increased across all salinity treatments at 30 DAG, with the highest activity recorded in T<sub>5</sub> (5.89 unit/min/g FW), which was statistically at par with T<sub>4</sub> (5.45 unit/min/g FW). At 50 DAG, CAT activity increased up to moderate salinity, with the greatest enhancement (22.31%) observed in T<sub>3</sub> compared with the control. The initial increase in POD and CAT activities reflects the activation of the plant's antioxidant defense system to detoxify ROS produced under salt stress. However, the decline in POD activity and the lower CAT activity under higher salinity at the later growth stage suggest that prolonged or severe stress can overwhelm the antioxidant system, reducing its efficiency because of oxidative damage. These changes highlight the dynamic response of antioxidant enzymes in protecting plants against salinity-induced oxidative stress. Similar observations have been reported by Nair *et al.* (2017), Abdelhameed *et al.* (2021) and Banakar *et al.* (2022).

## Conclusion

Salinity severely impairs the growth and development of fenugreek by affecting key morphological, physiological, and biochemical processes. Salt-induced osmotic stress limits water uptake, while excessive Na<sup>+</sup> accumulation disrupts ionic balance and promotes oxidative stress, resulting in increased lipid peroxidation, as reflected by higher MDA content. To cope with these adverse effects, fenugreek enhances the accumulation of compatible osmolytes such as proline and total soluble sugars and strengthens its antioxidant defense through increased phenolic compounds and the activities of CAT and POD enzymes. These responses help maintain cellular homeostasis and reduce oxidative damage under saline conditions. The present findings demonstrate that traits such as growth parameters, relative water content, membrane

stability index, chlorophyll content, Na<sup>+</sup>-K<sup>+</sup> ratio, osmolyte accumulation, antioxidant enzyme activity, and MDA content provide reliable measures of salinity tolerance in fenugreek. Together, these morpho-physiological and biochemical attributes can be effectively used to identify salt-tolerant genotypes and support breeding programs focused on developing climate-resilient fenugreek cultivars.

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## सारांश

लवणता के विभिन्न स्तरों पर मेथी (*Trigonella foenum-graecum* L.) के पौधों की वृद्धि एवं तनाव प्रतिक्रिया का अध्ययन करने के लिए एक गमला प्रयोग किया गया तथा प्रेक्षण दो अवस्थाओं पर दर्ज किए गए। लवणता की वृद्धि के साथ आकृतिक एवं शारीरिक मानकों, जैसे ताज़ा एवं शुष्क भार, जड़ एवं तना लंबाई, जल स्थिति तथा कोशिका स्थिरता में उल्लेखनीय कमी देखी गई। साथ ही, प्रकाश-संश्लेषी वर्णकों का भी ह्रास हुआ। लवणता तनाव के कारण ऑक्सीडेटिव तनाव सूचक मालोनडायाल्डिहाइड (MDA) का संचय बढ़ गया। अनुकूल घुलनशील पदार्थों (Compatible solutes) में प्रोलीन की मात्रा उपचार T<sub>s</sub> (8 dS/m) में क्रमशः 30 तथा 50 दिन अंकुरण के बाद (DAG) पर लगभग 80% तक बढ़ी, जबकि कुल घुलनशील शर्करा का संचय क्रमशः 63% तथा 49% दर्ज किया गया। लवणता तनाव के परिणामस्वरूप Na<sup>+</sup> का संचय बढ़ा, जिसके कारण उपचार T<sub>s</sub> में 50 DAG पर Na<sup>+</sup>/K<sup>+</sup> अनुपात सर्वाधिक पाया गया। गैर-एंजाइमिक प्रतिऑक्सीकारकों में फिनॉल की मात्रा उपचार T<sub>s</sub> में लगभग 40% तक बढ़ी। एंजाइमिक प्रतिऑक्सीकारकों, जैसे पेरोक्सीडेज तथा कैटालेज़, की सक्रियता प्रारम्भिक अवस्था में उल्लेखनीय रूप से बढ़ी, परंतु बाद की अवस्था में इसमें कमी देखी गई। अध्ययन से निष्कर्ष निकला कि लवणता के उच्च स्तर मेथी की वृद्धि एवं शारीरिक क्रियाओं पर प्रतिकूल प्रभाव डालते हैं, किन्तु पौधे अनुकूल घुलनशील पदार्थों तथा प्रतिऑक्सीकारकों का संचय करके अपनी प्रतिरक्षा प्रणाली को सक्रिय करते हैं, जिससे वे लवणता तनाव को सहन करने में सक्षम होते हैं।