# Enhancing Salinity Tolerance in Fenugreek through Exogenous Stimulants: Physiological, Biochemical, and Yield Responses

*Esam A.A. Al-Azzony*, Mohamed A. Abdein*, Naglaa Khalafallah, Hadeer Darwish, Gehan F.A. Massoud, Yasser A.M. Hafez, Amani H.A.M. Gharib, Rabia R.A. El-Hawagry, Mossad K.M. Maamoun, Abd El-Fattah D. Badr, Fatma M.A. Elkady, Reda E. Essa, Ahmed M. Abd El-Kawy, Sonia A.S. Abdallah*

**Abstract**: *Salinity stress adversely affected seed yield and oil parameters, increased $Na^+$ and $Cl^-$ uptake, and decreased calcium and total chlorophyll contents. Under low, moderate, and severe salinity conditions, the application of different anti-salinity treatments significantly improved plant height, fresh and dry herb weights, pod number, seeds per pod, seed yield, and oil-related parameters. Among the tested treatments, glutathione and Pottassein-P showed the greatest effectiveness in alleviating the adverse effects of salinity. Twelve fatty acids were identified, with palmitic acid being the predominant saturated fatty acid. Fatty acid composition varied*

Esam A.A. Al-Azzony, Medicinal and Aromatic Plants Research Departments, Horticulture Research Institute, Agricultural Research Centre, Giza 12619, Egypt, Email: esam.karm221@gmail.com
Mohamed A. Abdein, Seeds Development Department, El-Nada Misr Scientific Research and Development Projects, Turrell, Mansoura, 35511, Egypt, Email: abdeingene@yahoo.com
Naglaa Khalafallah, Soil and Water Department, Faculty of Agriculture, Tanta University, 31527 Tanta, Egypt
Hadeer Darwish, Medicinal and Aromatic Plants Research Departments, Horticulture Research Institute, Agricultural Research Centre, Giza 12619, Egypt
Gehan F.A. Massoud, Medicinal and Aromatic Plants Research Departments, Horticulture Research Institute, Agricultural Research Centre, Giza 12619, Egypt
Yasser A.M. Hafez, Medicinal and Aromatic Plants Research Departments, Horticulture Research Institute, Agricultural Research Centre, Giza 12619, Egypt
Amani H.A.M. Gharib, Department of Breeding Vegetables, Medical and Aromatic Plants, Horticulture Research Institute, Agricultural Research Centre, Giza 12619, Egypt
Rabia R.A. El-Hawagry, Department of Breeding Vegetables, Medical and Aromatic Plants, Horticulture Research Institute, Agricultural Research Centre, Giza 12619, Egypt
Mossad K.M. Maamoun, Department of Breeding Vegetables, Medical and Aromatic Plants, Horticulture Research Institute, Agricultural Research Centre, Giza 12619, Egypt
Abd El-Fattah D. Badr, Self-Pollinated Vegetable Crops Department, Horticulture Research Institute, Agricultural Research Centre, Giza 12619, Egypt
Fatma M.A. Elkady, Botany Department, National Research Center, Dokki, Cairo, Egypt
Reda E. Essa, Field Crop Research Dept., National Research Centre, Dokki, Giza, Egypt
Ahmed M. Abd El-Kawy, Ministry of Agriculture and Land reclamation, 83523 Qena, Egypt
Sonia A.S. Abdallah, Department of Plant Production, Faculty of Environmental Agricultural Science, Arish University, North Sinai 45516, Egypt

*ISSN: 0262-8104 (print) 1756-3488 (online)*
*Waterlines Vol. 44 No. 2*

*significantly in response to salinity levels and anti-salinity treatments, with increased proportions of saturated and monounsaturated fatty acids under saline conditions. The highest oleic acid content was recorded with glutathione application under severe salinity. Furthermore, anti-salinity treatments enhanced plant performance and improved several growth and yield attributes under different salinity levels. Principal component analysis (PCA) revealed that tolerance indices contributed substantially to the variation among treatments, particularly under severe salinity. These findings highlight the importance of evaluating treatment responses under moderate and severe salinity conditions. Overall, foliar application of glutathione was the most effective treatment, followed by Pottassein-P application through irrigation water, in mitigating salinity-induced adverse effects in fenugreek.*

***Keywords:*** *Salinity stress tolerance, Seawater, Pottassein, Glutathione, Anti-salinity.*

## Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual dicotyledonous plant belonging to the family Fabaceae. It is widely used as an edible vegetable and valued as a medicinal plant and a source of bioactive compounds with potential health benefits. Its traditional medicinal applications are supported by experimental evidence demonstrating the antibacterial activity of fenugreek extracts against several pathogenic bacteria (Sethi et al., 2024; Nagulapalli Venkata et al., 2017), as well as their antifungal activity against important plant pathogenic fungi (Cristea et al., 2025). Fenugreek can consistently grow in dry or semi-arid environments under salt stress due to its adaptability to a variety of temperatures, ecological circumstances, and soil types (Narayana et al., 2022). Offering numerous nutritional, therapeutic, and agronomic advantages, fenugreek seeds are highly prized parts of this leguminous crop. These seeds are high in protein (20–30%), fat (6–7%), carbs (55–60%), and dietary fiber (Rao et al., 1996; Niknam et al., 2021; Faisal et al., 2024). They are nutritional powerhouses because they are high in vital amino acids like lysine and tryptophan, vitamins like niacin, folic acid, and vitamin C, and minerals like iron, calcium, magnesium, and potassium (Aljuhaimi et al., 2018). The detrimental effects of salt stress on fenugreek yield and quality were documented in earlier research. Medicinal plants may be subject to different environmental pressures than other plants, especially agricultural crops (Mohamadi Esboei et al., 2022). Even when abiotic stress lowers output in medicinal plants, the concentration of secondary metabolites, the most advantageous medicinal

chemicals, eventually rises (Yang et al., 2018; Li et al., 2020; Ogbe et al., 2020). According to recent research, the exogenous application of different elicitors, such as Osmo-protectants (Wang et al., 2017; Estaji et al., 2019), phytohormones (Sytar et al., 2019; Rhaman et al., 2021), and polyamines (Liu et al., 2016; El Amrani et al., 2019), may significantly reduce the negative effects of abiotic stresses on plants. Glutathione is an essential non-protein thiol component in plant cells (Foyer and Noctor 2005a) and a useful metabolite that performs several biochemical functions in the plant's response to stress (Hameed et al. 2014). By directly removing some ROS and preserving the activity of particular antioxidant components, glutathione improves resistance to oxidative stress (Hasanuzzaman et al. 2012; Foyer and Noctor, 2005b). Glutathione (GSH)enhances the synthesis of photosynthetic pigments, accelerates the preservation of chlorophyll, and integrates into primary metabolism (Khattab 2007). Under chromium stress, exogenous GSH increased endogenous GSH levels and decreased cysteine levels in leaf tissue while also improving rice seedling development and nutrient uptake (Qiu et al. 2013). Additionally, a number of studies have demonstrated that extracellular GSH increases nutritional levels to provide protective effects (Cai et al. 2011). Furthermore, GSH accelerates the uptake and utilization of nutrients and metals and inhibits the generation of ROS (Mostofa et al. 2014). Under both normal and stressed circumstances, GSH stimulates growth, development, gene expression, and protein activation. Potassium (K) is an essential nutrient that has a favorable effect on the metabolism of proteins, vitamins, nucleic acids, and growth factors (Bisson et al., 1994; Bednarz and Oosterhuis, 1999). Furthermore, the transfer of photosynthates from sources to sinks depends on K (Cakmak et al., 1994). In fenugreek, potassium fertilization has been reported to influence seed weight per plant and improve seed protein and potassium contents, particularly under water-deficit conditions (Żuk-Gołaszewska et al., 2015). Similarly, K application has been shown to significantly enhance crop yield and quality (Dotaniya et al., 2019). Therefore, the present study aimed to investigate whether the exogenous application of selected stimulants could enhance salinity tolerance in fenugreek plants by evaluating their effects on physiological and biochemical parameters, osmolyte accumulation, seed yield, and fixed oil yield. It also aimed to assess the effectiveness of these stimulants in improving fenugreek performance and seed quality under salinity stress.

## Material and Methods

This experiment was carried out in the El-Qassassin Horticultural Research Station, Ismailia Governorate, Horticulture Research Institute, Agricultural Research Center (A.R.C.), Egypt, during the two successive summer seasons (2022/2023 and 2023/2024); the planting date for both seasons was the 1st week of October. The variety Giza-3 which obtained from field crops institute, A.R.C., Egypt was used in this study. Table 1 shows the physicochemical analysis of the experimental soil according to the method of Blackemore et al. (1981) in addition to the anions and cations present in fresh water used in water salinity modification.

**Table 1. Analysis of the experimental soil and fresh water**

| | Anions | | | Cations | | | | EC | pH |
|---|---|---|---|---|---|---|---|---|---|
| | $HCO_3^-$ | $Cl^-$ | $SO_4^-$ | $Ca^{++}$ | $Mg^{++}$ | $Na^+$ | $k^+$ | dS/m | |
| Soil | 1.51 | 19.9 | 3.3 | 7.4 | 3.5 | 13.9 | 0.3 | 2.8 | 7.88 |
| Tab water | 2.7 | 1 | 0.14 | 1.8 | 1 | 0.86 | 0.17 | 0.387 | 8.3 |
| | Available nutrients in the soil (mg kg-1soil) | | | | | | | | |
| Properties | Clay | Silt | Sand | N | P | K | Fe | Zn | Mn | B |
| Value | 5.1 | 16.7 | 78.2 | 57 | 2.16 | 26.9 | 1.68 | 0.43 | 1.69 | 0.55 |

Salinity levels of 3.125, 6.25, and 9.375 dS/m were obtained by mixing Nile water (0.387 dS/m) with seawater of Suez Canal (51.6 dS/m) to produce saline irrigation water levels, *i.e.*, low, medium and sever of 2000, 4000, and 6000 ppm, respectively.

Three replicates of a split-plot, complete randomized block design were used. The main plot included 4 irrigation water treatments (normal, low, medium and high salinity. The subplots included 5 different salinity-tolerant stimulants. Therefore, the experiment included 20 treatments.

- The first subplot was sprayed with tap water (control, C).
- The second subplot was sprayed with 40 ppm vitamin E (T1).
- The third subplot was sprayed with glutathione (T2) at 2 mM ( $2\times10^{-3}$ mol/L).
- The fourth subplot (T3) had 2 L/feddan Pottassein P (32% Potassium Oxide

($K_2O$) + 5% Superphosphate ($P_2O_5$)), which was obtained from the Agriculture Research Center, Giza, and applied with irrigation water.

- The fifth sub-plot (T4) was sprayed with putrescine (Put) (tetramethylenediamine $C_4H_{12}N_2$, F.W. 88.15 g/mol) at 2.5 mM ($2.5\times10^{-3}$ mol/L), which was obtained from the local market (Techno-Gene).

All different application treatments were starting on day 45 after planting (DAP) and repeated three times, every 30 days. The plot area involved three rows spaced 50 cm apart, the seeds were planted in hills 25 centimeters apart. The seedlings were reduced to two plants per hill after three weeks of seeding. All of the experimental plot units received N, P, and K fertilizers in the form of potassium sulfate (50% $K_2O$) at 50 kg/feddan, ammonium sulphate (20.5% N) at 200 kg/feddan, and 200 kg/fed calcium superphosphate (15.5% $P_2O_5$). During soil arrangement, phosphorus fertilizer was applied. At 30-, 60-, and 90-days following sowing, three equal parts of nitrogen and potassium fertilizers were given to the soil. When necessary, all plants were treated according to standard agricultural procedures.

Plants were chopped slightly above ground as they reached maturity. The height of the plant was measured from the ground to its highest point. Following harvesting, the fresh herbs were weighed and allowed to dry for a few days in order to eliminate any remaining moisture. After air-drying, each plant was weighed to determine its dry weight. Following their physical extraction from each plant and subsequent weighing, the quantity of pods (NP), seeds (NS), and seed yield (SW) were evaluated.

A Soxhlet device was used to remove fixed oil from the samples. In order to do this, 50 g of each sample were taken out and, after being roughly ground, placed in a Whatman thimble along with 250 ml of the solvent (hexane). The device was then filled for four hours.

The amount of fixed oil was then determined after the oil and solvent were separated using a rotary evaporator and weighed (A.O.A.C., 1990). A hand Spad-502 meters was used to measure the total chlorophyll content (mg/g F. Wt.) in leaves 80 days after planting, while the DuBois et al. (1956) method was used to calculate the total carbohydrate percentage.

Fatty acids analysis was determined by Gas Chromatography (GC) according to the procedure described by (Metcalf et al., 1966) and (AOAC, 1990). A dendrogram

was constructed based on “Euclidean distance" procedure. Fatty acids were clustered using unweighted pair group method (UPGMA) using arithmetic average as outlined by (Kovach, 1995).

***Stress Tolerance Indices:***

Principal component analysis (PCA) throughout the three stress levels was conducted separately for high, medium, and low salinity stress conditions to examine treatment performance based on six stress tolerance and sensitivity indices (Table 2)

***Statistical analysis***:

Analysis of variance (ANOVA) was used to evaluate the data in accordance with (Snedecor and Cochran, 1980). Statistical analysis was performed using Statistix 9 software (Analytical Software, 2008) was used to compare the means. At the 5% level, the least significant difference (L.S.D.) was used to determine significance.

**Table 2. List of the salinity stress tolerance indices and formula**

| **Index** | **Tolerance indices** | | | | **Susceptibility indices** | |
|---|---|---|---|---|---|---|
| | Geometric mean productivity | Yield Index | Modified Stress Tolerance Index 1 | Modified stress tolerance index 2 | Relative decrease in yield | Stress Susceptibility percentage index |
| **Abbr.** | GMP | YI | $MSTI_1$ | $MSTI_2$ | RDY | SSPI |
| **Formula** | $\sqrt{Yn} \times Ys$ | $Ys / \bar{Y}s$ | $[(Yn)^2/(\bar{Y}n)^2] \times STI$ | $[(Ys)^2/(\bar{Y}s)^2] \times STI$ | $100-(Ys/Yn \times 100)$ | $[(Yn-Ys) / (2 \times \bar{Y}n)] \times 100$ |
| References | Fernandez (1992) | Gavuzzi *et al.* (1997) | Farshadfar and Sutka (2002) | | Farshadfar *et al.* (2013) | Moosavi *et al.* (2008) |

Ys and Yn: Yield of each anti-salinity treatment under stress and non-stress conditions, respectively.

$\hat{Y}s$ and $\hat{Y}n$: Mean of yield overall treatments under stress and non-stress conditions, respectively

## Results

### *Morphological Responses:*

Plant height, herb fresh weight and dry fresh weight in both seasons, as well as the number of branches in the second season were all significantly impacted by salinity levels, anti-saline treatments, and their combination (Table 3).

### *Salinity effects:*

In proportion to the increase in salinity concentration, the growth parameters decreased. Salinity levels of 2000, 4000, and 6000 ppm reduced plant height by 17.65%, 36.70% and 51.34%; NB by 15.15%, 32.26% and 42.96%; HFW by 29.88%, 56.60% and 71.74%; and HDW by 29.94%, 56.71% and 71.86%, respectively, when compared to normal conditions (Fig.1).

**Table 3. Analysis of Variance for salinity effect, anti-salinity treatments and their interaction on growth parameters of Fenugreek plants in both seasons**

| S.O.V. | df | PH | NB | HFW | DFW |
|---|---|---|---|---|---|
| **1st season** | | | | | |
| **Salinity (S)** | 3 | 5549.8** | 186.4** | 6419.136** | 229.05** |
| **Error (S)** | 6 | 0.017 | 2.715 | 67.071 | 1.186 |
| **Treatments (T)** | 4 | 213.792** | 25.275** | 446.432** | 15.917** |
| **S×T** | 12 | 10.258** | 1.275ns | 42.034** | 1.504** |
| **Error (T)** | 32 | 0.017 | 0.895 | 1.141 | 0.018 |
| **2nd season** | | | | | |
| **Salinity (S)** | 3 | 5580.133** | 189.217** | 6845.111** | 200.126** |
| **Error (S)** | 6 | 0.017 | 0.017 | 51.597 | 2.83 |
| **Treatments (T)** | 4 | 207.442** | 24.267** | 441.03** | 12.91** |
| **S×T** | 12 | 10.175** | 1.3** | 38.429** | 1.122** |
| **Error (T)** | 32 | 0.017 | 0.017 | 1.269 | 0.065 |

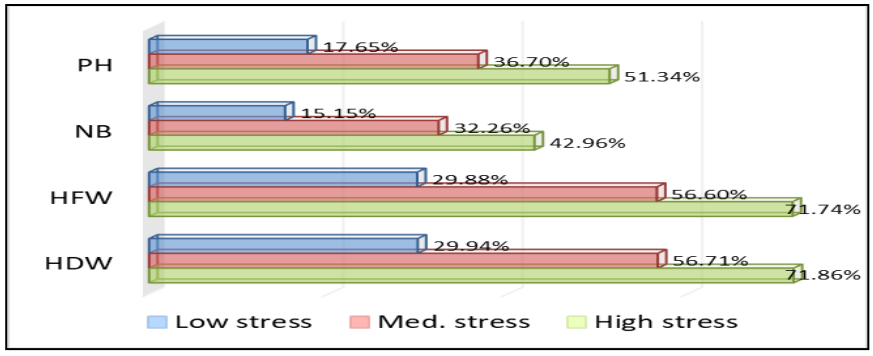



Fig. 1: Decreasing percentages of Plant height (PH), number of branches (NB), herbs fresh weight (HFW) and herbs dry weight (HDW) as affected by salinity stress

***Treatments effect:***

Under normal conditions, T1, T2, T3, and T4 of anti-salinity treatments, on average of both seasons (Fig.2), increased plant height by 5.03%, 8.81%, 15.09%, and 12.58%; HFW by 10.03%, 20.37%, 40.86%, and 31.16%; and HDW by 10.17%, 20.56%, 41.15%, and 31.38%, respectively, compared to the control.

Under low salinity stress (2000 ppm), T1, T2, T3, and T4 of anti-salinity treatments increased plant height by 9.45%, 20.47%, 15.75%, and 12.60%; HFW by 25.21%, 55.18%, 40.75%, and 28.79%; and HDW by 25.29%, 55.39%, 40.90%, and 28.87%, respectively, compared to the control.

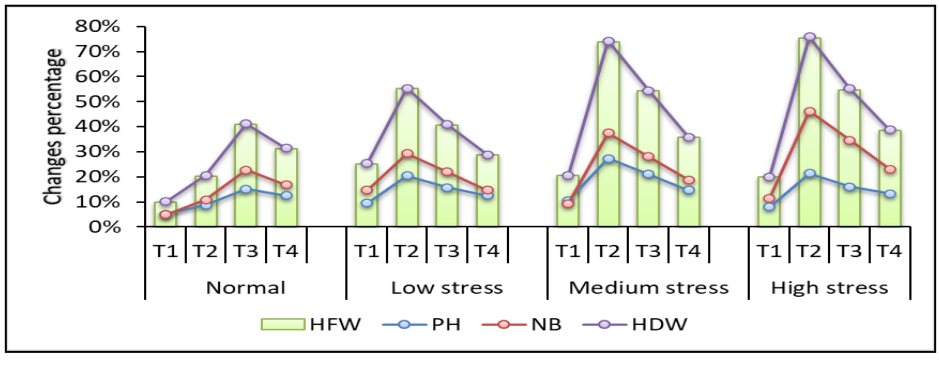



Fig. 2: Plant height (PH), number of branches (NB), herbs fresh weight (HFW) and herbs dry weight (HDW) as affected by stimulants treatments × salinity stress

Under moderate salinity stress (4000 ppm), T1, T2, T3, and T4 of anti-salinity treatments increased plant height by 10.53%, 27.37%, 21.05%, and 14.74%; HFW by 20.56%, 73.90%, 54.20%, and 35.66%; and HDW by 20.63%, 74.26%, 54.44%, and 35.82%, respectively, compared to the control. Under severe salinity stress (6000 ppm), T1, T2, T3, and T4 of anti-salinity treatments increased plant height by 8%, 21.33%, 16%, and 13.33%; number of branches by 11.54%, 6.15%, 34.62% and 23.08%; HFW by 19.95%, 75.38%, 54.73%, and 38.50%; and HDW by 20.05%, 75.87%, 55.1%, and 38.71%, respectively, compared to the control.

***Yield traits responses:***

Salinity levels, anti-saline treatments, and their interaction all had a highly significant/significant effect on number of pods per plant (NP), number of seeds per pod (NS), seed weight per plant (SW/P) and seed yield pr feddan (SW/Fed) in both seasons (Table 4).

**Table 4. Analysis of Variance for the effects of salt, anti-salinity treatments, and their interactions on fenugreek plant production metrics in both seasons**

| S.O.V. | df | NP | NS | SW/P | SW/Fed |
|---|---|---|---|---|---|
| 1st season | | | | | |
| Salinity (S) | 3 | 2138.8** | 270.4** | 474.504** | 535694** |
| Error (S) | 6 | 6.283 | 8.6 | 0.323 | 366.18 |
| Treatments (T) | 4 | 78.9** | 17.025** | 21.376** | 24130.9** |
| S x T | 12 | 3.8** | 2.525* | 2.49** | 2811.2** |
| Error (T) | 32 | 0.988 | 1 | 0.004 | 3.963 |
| 2nd season | | | | | |
| Salinity (S) | 3 | 2083.4** | 260.15** | 500.401** | 564933** |
| Error (S) | 6 | 0.717 | 2.183 | 0.331 | 731.002 |
| Treatments (T) | 4 | 86.775** | 19.725** | 27.007** | 30490** |
| SxT | 12 | 3.275** | 3.025** | 3.255** | 3674** |
| Error (T) | 32 | 0.7 | 1 | 0.2 | 9.714 |

***Salinity effects:***

The seed yield and associated parameters declined in direct proportion to the rise in saline concentration (Fig.3).

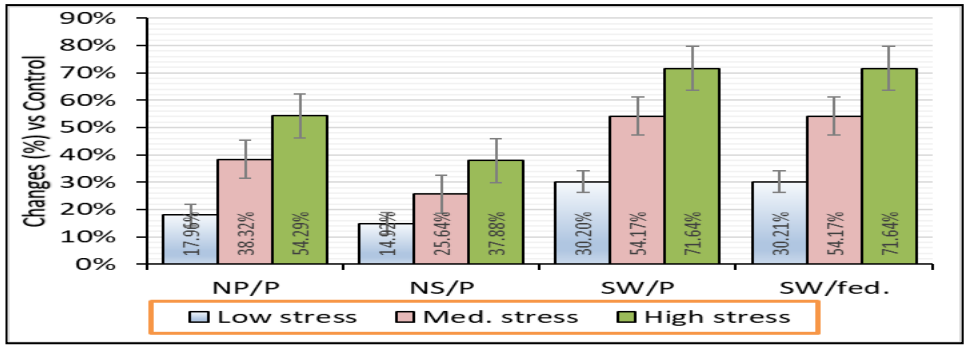



Fig. 3: Decreasing percentages of number of pods (NP), number of seeds NS, seeds weight per plant (SW/P) and seeds yield per feddan (SW/fed.) as affected by salinity stress

In comparison to normal conditions, salinity levels of 2000, 4000, and 6000 ppm decreased the number of pods per plant (NP) by 17.96%, 38.32% and 54.29%; the number of seeds per pod (NS) by 14.92%, 25.64% and 37.88%; the seed weight per plant (SW/P) by 30.20%, 54.17%, and 71.64%; and the seed yield per feddan (SW/fed.) by 30.21%, 54.17%, and 71.64%, respectively.

***Treatments effect:***

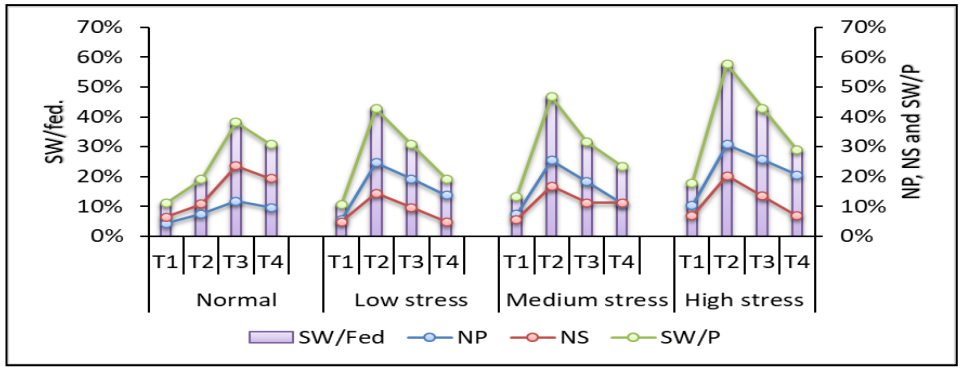
Table 5 and Fig. 4 show the yield and associated characteristics in the seawater-stressed and anti-salinity-treated fenugreek plants. The yield attributes of NP/P, NS/P, SW/P, and SW/fed significantly increased when diluted seawater was mixed with the various stimulants. Anti-salinity treatments boosted the plant resistance to stress via boosting the examined yield attributes among the studied groups (low, medium & high salinities).

Application of 2 mM glutathione (T2) showed the highest stimulation in yield and related traits of fenugreek plants, followed by Pottassein P (T3) under the three salinity levels compared to their corresponding control, with no significant differences between them in most yield traits. Moreover, the three stimulant treatments gave higher stimulations in the four yield traits under the normal conditions (non-saline water irrigation without any stimulant treatments), in which Pottassein P treatment (T3) exhibited the highest stimulations, followed by Putrescine (T4) at 2.5 mM., with no significant differences between them in NP and NS.

As shown in Fig. 4, Under low salinity stress (2000 ppm), T1, T2, T3, and T4 of anti-salinity treatments increased the number of pods by 5.48%, 24.66%, 19.18%, and 13.70%; the number of seeds/pods by 4.80%, 14.40%, 9.60%, and 4.80%; SW/P by 10.57%, 42.72%, 30.70%, and 19.21%; and seed yield per feddan (SW-fed.) by 10.57%, 42.73%, 30.70%, and 19.21%, respectively, compared to the corresponding control.

Table 5. Number of pods per plant (NP), number of seeds per pod (NS), seeds weight per plant (SW/P) and seed yield per feddan (SW/fed.) as impacted by the interaction of stress level× stimulants treatments on average of two seasons

| **Treatments** | | **NP** | **NS** | **SW/P** | **SW/fed.** |
|---|---|---|---|---|---|
| Non-stress (Normal) | Control | 47 | 23.333 | 15.3185 | 514.696 |
| | T1 | 49 | 24.833 | 17.003 | 571.301 |
| | T2 | 50.5 | 25.833 | 18.235 | 612.696 |
| | T3 | 52.5 | 28.833 | 21.164 | 711.088 |
| | T4 | 51.5 | 27.833 | 20.038 | 673.299 |
| Low stress | Control | 36.5 | 20.833 | 10.617 | 356.72 |
| | T1 | 38.5 | 21.833 | 11.739 | 394.430 |
| | T2 | 45.5 | 23.833 | 15.153 | 509.13 |
| | T3 | 43.5 | 22.833 | 13.876 | 466.245 |
| | T4 | 41.5 | 21.833 | 12.656 | 425.242 |
| Medium stress | Control | 27.5 | 17.833 | 6.837 | 229.712 |
| | T1 | 29.5 | 18.833 | 7.749 | 260.367 |
| | T2 | 34.5 | 20.833 | 10.033 | 337.12 |
| | T3 | 32.5 | 19.833 | 8.995 | 302.232 |
| | T4 | 30.5 | 19.833 | 8.439 | 283.573 |
| High stress | Control | 19.5 | 14.833 | 4.02 | 135.083 |
| | T1 | 21.5 | 15.833 | 4.737 | 159.152 |
| | T2 | 25.5 | 17.833 | 6.337 | 212.934 |
| | T3 | 24.5 | 16.833 | 5.745 | 193.021 |
| | T4 | 23.5 | 15.833 | 5.18 | 174.048 |
| LSD 0.05 | | 1.88 | 2.193 | 0.572 | 13.257 |




Increment (%) of number of pods (NP), number of seeds NS, seeds weight per plant (SW/P) and seeds yield per feddan (SW/fed.) as affected by stimulants treatments × salinity stress compared to corresponding control

Under moderate salinity stress (4000 ppm), T1, T2, T3, and T4 of anti-salinity treatments increased the number of pods by 7.27%, 25.45%, 18.18%, and 10.91%; the number of seeds/pod by 5.61%, 16.82%, 11.22%, and 11.22%; SW/P by 13.34%, 46.75%, 31.56%, and 23.44%; and seed yield per feddan (SW-fed.) by 13.34%, 46.76%, 31.57%, and 23.45%, respectively, compared to the corresponding control. Under severe salinity stress (6000 ppm), T1, T2, T3, and T4 of anti-salinity treatments increased the number of pods by 10.26%, 30.77%, 25.64%, and 20.51%; the number of seeds/pod by 6.74%, 20.23%, 13.48%, and 6.74%; SW/P by 17.82%, 57.64%, 42.90%, and 28.86%; and seed yield per feddan (SW-fed.) by 17.82%, 57.63%, 42.89%, and 28.85%, respectively, compared to the corresponding control.

***Osmolyte, Ion Accumulation, Total chlorophyll, and Carbohydrate Contents:***

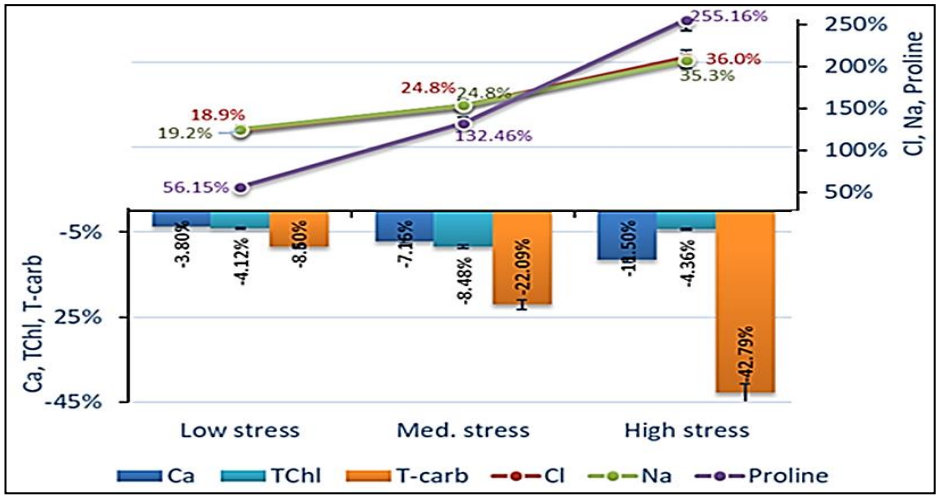
Important factors affecting plant stress responses and adaptation mechanisms include osmolytes, ion accumulation, photosynthetic pigments, and carbohydrate contents. These compounds (e.g., proline, $Ca^{2+}$, $Cl^{-}$, $Na^{+}$, chlorophyll, and carbohydrates) are essential for cellular stability, osmoregulation, and photosynthetic efficiency, all of which support the health and productivity of plants. Salinity levels, anti-saline treatments, and their interaction all had a significant effect on ion accumulations ($Ca^{2+}$, $Cl^{-}$, $Na^{+}$; mg/100g), proline (µmol/g), Total chlorophyll (mg/g), and Carbohydrate content (%) in both seasons (Table 6).

Table 6. Analysis of Variance for salinity effect, anti-salinity treatments and their interaction on an ion accumulations (mg/100g), proline (µmol/g), Total chlorophyll (mg/g), and Carbohydrate content (%)

| S.O.V. | df | $Ca^{2+}$ | $Cl^{-}$ | $Na^{+}$ | Proline | TChl | T-carb |
|---|---|---|---|---|---|---|---|
| 1st season | | | | | | | |
| Salinity (S) | 3 | 1463** | 2674** | 1811.4** | 160.4** | 0.039** | 1030** |
| Error (S) | 6 | 28.817 | 12.417 | 17.133 | 0.006 | 0.00001 | 0.002 |
| Treatments (T) | 4 | 1165.50** | 441.92** | 296.10** | 0.707** | 0.051** | 12.56** |
| S x T | 12 | 33.500** | 11.450** | 9.900** | 0.055** | 0.049** | 1.408** |
| Error (T) | 32 | 3.150 | 2.129 | 1.225 | 0.002 | 0.00001 | 0.0001 |
| 2nd season | | | | | | | |
| Salinity (S) | 3 | 1485** | 2720** | 1840** | 155.9** | 0.038** | 1030** |
| Error (S) | 6 | 2.450 | 4.417 | 6.050 | 0.002 | 0.0001 | 0.020 |
| Treatments (T) | 4 | 1145.40** | 427.35** | 287.025** | 1.077** | 0.051** | 12.516** |
| S x T | 12 | 31.000** | 11.950** | 10.325** | 0.072** | 0.049** | 1.416** |
| Error (T) | 32 | 0.450 | 2.550 | 0.425 | 0.001 | 0.00003 | 0.007 |

***Salinity effects:***

Proline content significantly increased with the rise of salt concentrations. This was confirmed by the linear analysis, which showed a highly significant relation for treatment, salinity level, and their interaction on proline contents of the three tested treatments ($R^2$=0.969). In comparison to normal conditions, salinity levels of 2000, 4000, and 6000 ppm increased the proline content by 56.15%, 132.46%, and 255.16%, respectively (Fig.5).



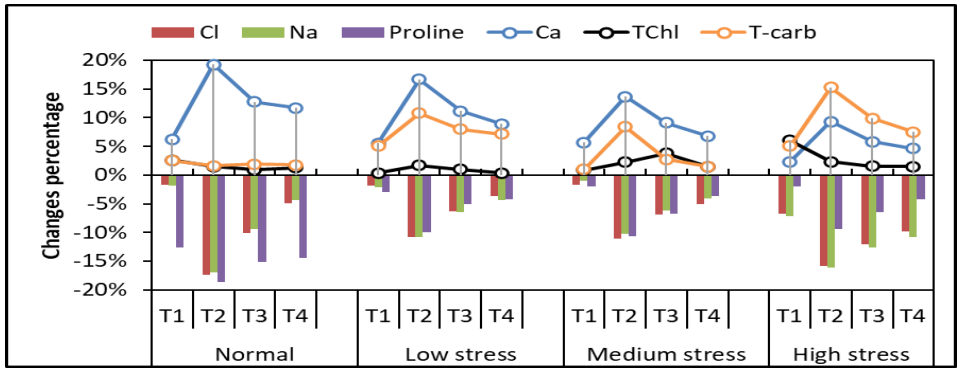

Changes percentages of Ca, Na, Cl, proline, chlorophyll (TChl) and carbohydrates (T-carb) as affected by salinity stress

Results showed that the three salinity levels exported high quantities of Na+ and Cl into the plant organs, which confirms the "inclusive" character of this variety. Based on the Na+ and Cl uptake and proline as well as $Ca^{2+}$, total chlorophyll and soluble carbohydrate contents, it appears that salt stress increased the accumulation of $Na^{+}$ and $Cl^{-}$ However, low, medium and high salinity levels increased the $Na^{+}$ uptake by 19.20%, 24.84% and 35.32% (Fig.) and $Cl^{-}$ by 18.92%, 24.76% and 35.98%, respectively compared to the control of normal water irrigation (Fig.). However, accumulation of such amount of salts can affect the hormonal balance or osmolyte accumulation which may initiate salt adaptive responses (Albacete et al. 2008; Munns and Tester 2008). On opposite, Results showed that the three salinity levels decreased the Ca content by 3.80%, 7.16% and 11.50%; Consequently, $Ca^{2+}/Na^{+}$ ratios decreased significantly in the organs of fenugreek irrigated with the three saline water levels (Table 2). Similar results have been reported by Ashraf et al. (2004). Also, the three salinity levels decreased the total chlorophyll by 4.12%, 8.48% and 4.36%; and soluble carbohydrate content by 8.50%, 22.09% and 42.79%, respectively compared to the control of normal water irrigation (Fig.5).

***Treatments effect:***

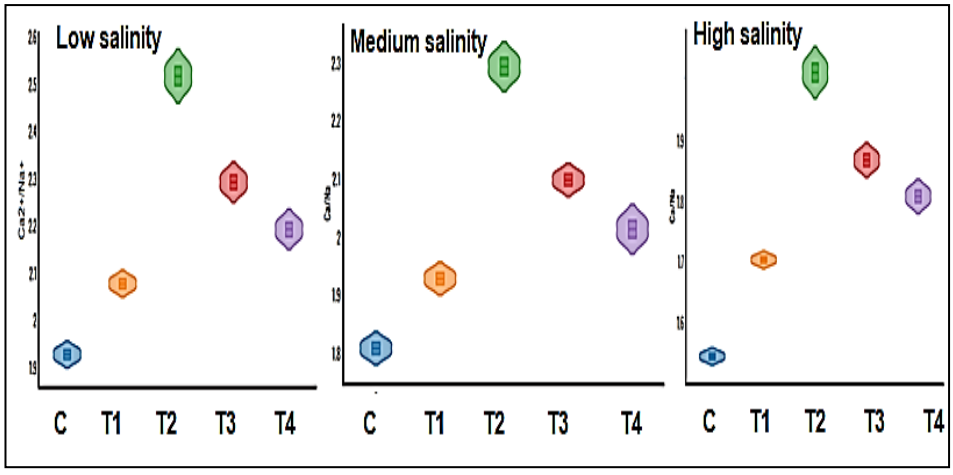
As shown in Fig. 6, Under low salinity stress (2000 ppm), T1, T2, T3, and T4 of anti-salinity treatments decreased $Cl^{-}$ by 1.8%, 10.81%, 6.31% and 3.6%; Na+ by 2.15%, 10.73%, 6.44% and 4.29% and Proline by 2.91%, 9.99%, 5% and 4.16%. In meantime, these treatments increased the $Ca^{2+}$ by 5.57%, 16.70%, 11.13% and 8.91%, respectively causing increased of Ca/Na ratio by 7.88% (T1), 30.73% (T2),

18.78% (T3) and 13.79% (T4) compared to the corresponding control (Fig.6). On the other hand, under low salinity stress, T1, T2, T3, and T4 of anti-salinity treatments increased the chlorophyll content by 0.39%, 1.72%, 1% and 0.39%, and 10.91% and total carbohydrate by 5.05%, 10.82%, 7.97% and 7.17%, respectively, compared to the corresponding control.

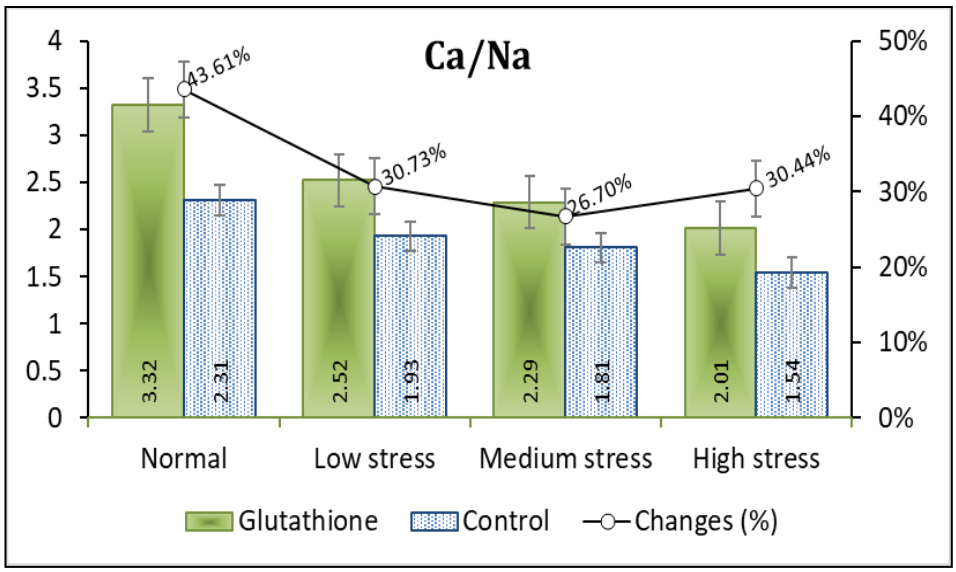



Ca, Na, Cl, proline, chlorophyll and carbohydrates as affected by stimulants treatments × salinity stress

Under both moderate and high salinity stress, T1, T2, T3, and T4 of anti-salinity treatments decreased $Cl^-$, $Na^+$ and Proline and increased $Ca^{2+}$, chlorophyll content and total carbohydrate, however, Glutathione (T2) applying exhibited the highest desirable effects with changes by 10.81% ($Cl^-$), 10.73% ($Na^+$), 9.99% (Proline), 16.70% ($Ca^{2+}$), 1.72% (Chl) and 10.82% (Carb) under low salinity; 11.11% ($Cl^-$), 10.29% ($Na^+$), 10.61% (Proline), 13.66% ($Ca^{2+}$), 2.27% (Chl) and 8.40% (Carb) under medium salinity and also, by 15.79% ($Cl^-$), 16.19% ($Na^+$), 9.34% (Proline), 9.32% ($Ca^{2+}$), 2.36% (Chl) and 15.24% (Carb) under high salinity. Effects of Glutathione on both Ca and Na (Figs.7&8) caused increasing in the Ca/Na ratio under all salinity levels and normal conditions with increment by 43.61% (non-stress), 30.73% (low stress), 26.70% (medium stress) and 30.44% (high stress) compared to the corresponding control.




Violin plot of $Ca^{2+}/Na^{+}$ ratio determined in plant tissues as affected by four different salinity stimulants (T1, T2, T3, T4) under low, medium and high salinity levels.




$Ca^{2+}/Na^{+}$ ratio determined in plant tissues as affected by Glutathione (T2) under low, medium and high salinity levels as well as non-stressed (control) treatments.

***Oil related traits:***

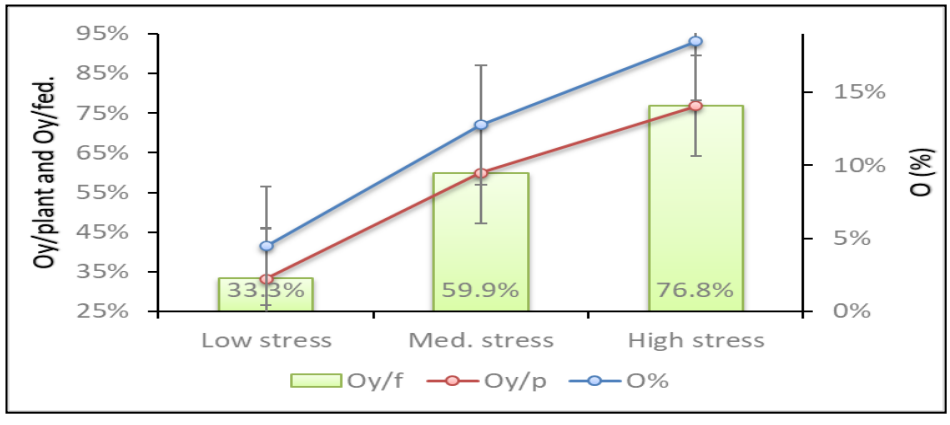
Salinity levels, anti-saline treatments, and their interaction all had a significant effect on oil yield per plant (g/plant) and per feddan (kg /fed.) in both seasons, as well as fixed oil percentage (%) in the 2nd season (Table 7).

Table 7. Analysis of Variance for salinity effect, anti-salinity treatments and their interaction on fixed oil percentage (%) as well as oil yield per plant (g/plant) and per feddan (kg /fed.)

| S.O.V. | df | O% | Oy/p | Oy/f |
|---|---|---|---|---|
| **1st season** | | | | |
| **Salinity (S)** | 3 | 5.559** | 2.951** | 3330** |
| **Error (S)** | 6 | 0.048 | 0.007 | 8.187 |
| **Treatments (T)** | 4 | 0.078** | 0.116** | 130.762** |
| **S × T** | 12 | 0.011ns | 0.017** | 19.335** |
| **Error (T)** | 32 | 0.008 | 0.0001 | 0.080 |
| **2nd season** | | | | |
| **Salinity (S)** | 3 | 5.559** | 3.219** | 3636** |
| **Error (S)** | 6 | 0.032 | 0.009 | 8.345 |
| **Treatments (T)** | 4 | 0.078** | 0.150** | 169.326** |
| **S × T** | 12 | 0.011** | 0.022** | 25.576** |
| **Error (T)** | 32 | 0.002 | 0.001 | 0.607 |

***Salinity effects:***

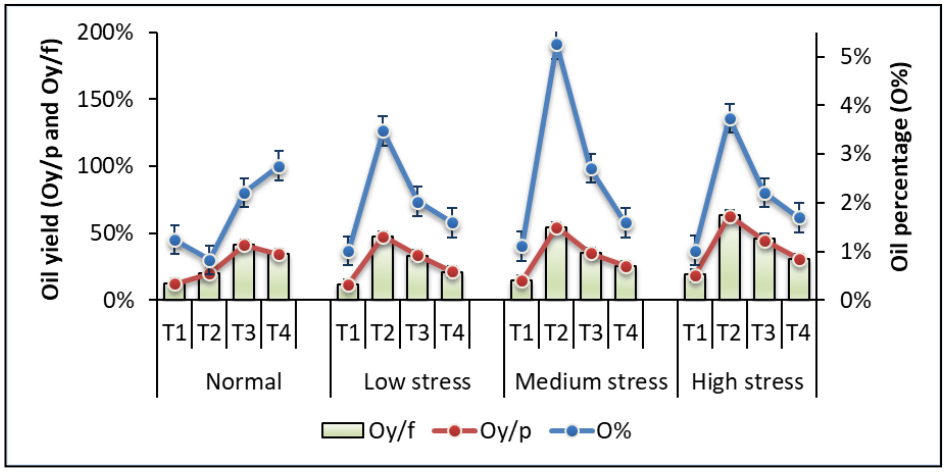
In proportion to the increase in salinity concentration, the oil parameters decreased. Salinity levels of 2000, 4000, and 6000 ppm reduced fixed oil percentage by 4.49%, 12.76% and 18.50%; oil yield per plant by 33.19%, 59.90% and 76.84% as well as oil yield per feddan by 33.29%, 59.94% and 76.84%, respectively, when compared to normal conditions (Fig.9).




Decreasing percentages of oil percentage (O%), oil yield per plant (Oy/p) and oil yield per feddan (Oy/fed.) as affected by salinity stress

***Treatments effect:***

As shown in Fig.10, Under low salinity stress (2000 ppm), T1, T2, T3, and T4 of anti-salinity treatments increased oil percentage by 1.01%, 3.47%, 2.03% and 1.59%; oil yield per plant by 11.77%, 47.62%, 33.54% and 21.36% and oil yield per feddan by 11.69%, 47.66%, 33.34% and 21.10%, respectively, compared to the corresponding control.




: Oil percentage (O%), oil yield per plant (Oy/p) and oil yield per feddan (Oy/fed.) as affected by stimulants treatments × salinity stress

Under moderate salinity stress (4000 ppm), T1, T2, T3, and T4 of anti-salinity treatments increased oil percentage by 1.11%, 5.26%, 2.71% and 1.59%; oil yield per plant by 14.77%, 54.65%, 35.23% and 25.58% and oil yield per feddan by 14.60%, 54.41%, 35.09% and 25.38%, respectively, compared to the corresponding control.

Under severe salinity stress (6000 ppm), T1, T2, T3, and T4 of anti-salinity treatments increased oil percentage by 1.02%, 3.74%, 2.21% and 1.70%; oil yield per plant by 18.24%, 62.89%, 44.65% and 30.82% and oil yield per feddan by 19.00%, 63.45%, 46.00% and 31.02%, respectively, compared to the corresponding control.

***Fatty acids:***

In all, 12 distinct fatty acids were identified in this investigation (Table 8 and Fig.11). The predominant saturated fatty acid found in this investigation was palmitic acid (C16:0). The primary polyunsaturated fatty acids (PUFA) found in

fenugreek seeds were linoleic acid (C18:2n6) and linolenic acid (C20:3n6), whereas oleic acid (C18:1n9) was the primary monounsaturated fatty acid.

**Table 8. Fatty acids of fenugreek seeds as affected by Salinity levels × glutathione treatments**

| Fatty acids | Molecular | | Salinity levels × glutathione treatments | | | |
|---|---|---|---|---|---|---|
| | Format | Weight (g/mol) | Control | Low | Medium | High |
| **Myristic acid (C14:0)** | $C_{14}H_{28}O_2$ | 228.37 | 0.18 | 0.19 | 0.2 | 0.21 |
| **Palmitic acid (C16:0)** | $C_{16}H_{32}O_2$ | 256.42 | 9.6 | 10.31 | 11.28 | 11.41 |
| **Heptadecanoic acid (C17:0)** | $C_{17}H_{34}O_2$ | 270.5 | 0.26 | 0.31 | 0.43 | 0.52 |
| **Stearic acid (C18:0)** | $C_{18}H_{36}O_2$ | 284.5 | 4.03 | 4.21 | 4.29 | 4.31 |
| **Arachidic acid (C20:0)** | $C_{20}H_{40}O_2$ | 312.5 | 1.12 | 1.42 | 1.35 | 1.27 |
| **Behenic acid (C22:0)** | $C_{22}H_{44}O_2$ | 340.6 | 0.61 | 0.52 | 0.45 | 0.36 |
| **Palmitoleic Acid (C16:1n7)** | $C_{16}H_{30}O_2$ | 254.41 | 0.59 | 0.49 | 0.29 | 0.19 |
| **Vaccenic acid (*trans* C18:1n7)** | $C_{18}H_{34}O_2$ | 282.5 | 0.22 | 0.34 | 0.41 | 0.52 |
| **Oleic acid (*cis* C18:1n9)** | $C_{18}H_{34}O_2$ | 282.5 | 15.23 | 15.22 | 15.66 | 15.73 |
| **Linoleic acid (C18:2n6)** | $C_{18}H_{32}O_2$ | 280.4 | 36 | 33.42 | 31.2 | 29.52 |
| **γ-Linolenic acid (C18:3n6)** | $C_{18}H_{30}O_2$ | 278.4 | 32 | 33.36 | 34.2 | 35.69 |
| **Gondoic acid (C20:1)** | $C_{20}H_{38}O_2$ | 310.5 | 0.16 | 0.21 | 0.24 | 0.27 |

As shown in (Table 8 and Fig.12), the fatty acid profile in the fenugreek seeds differs considerably between the different salinity levels. The amounts of fatty acids in the control treatment were in the order of UFAs > PUFAs >MUFAs > SFAs. Salinity × glutathione interaction treatments, on the other hand, showed that SFAs were more than MUFAs, although the percentage proportion of each specific fatty acid category varied Coban et al. (2024).

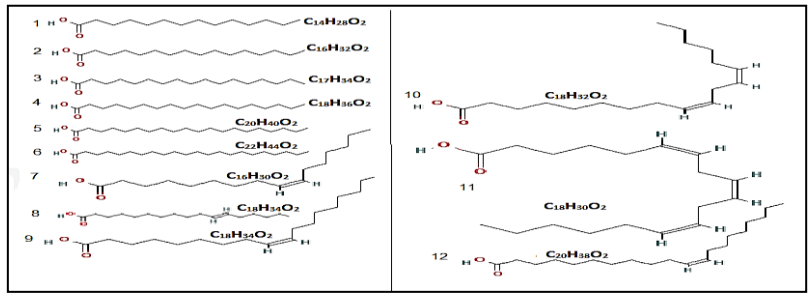
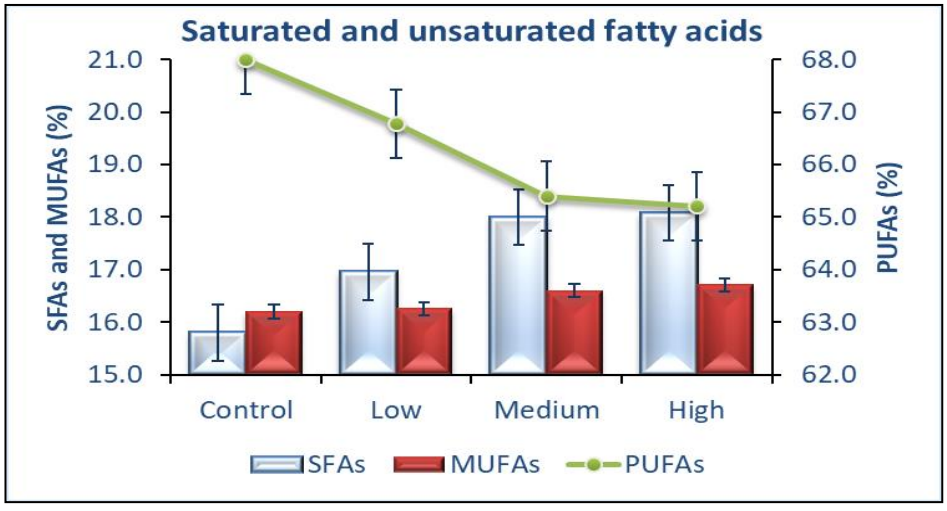



Fig. 11: Fatty acids

1. Myristic acid, 2. Palmitic acid, 3. Heptadecanoic acid, 4. Stearic acid, 5. Arachidic acid, 6. Behenic acid, 7. Palmitoleic Acid, 8. Vaccenic acid, 9. oleic acid, 10. Linoleic acid, 11. γ-Linolenic acid, 12. gondoic acid

The lowest amounts of SFAs and MUFAs were found in the control treatment, compared with the content of these fatty acids in the seeds under salinity (P = 0.005) where both SFAs and MUFAs increased *versus* PUFAs decreased, with increasing salinity levels up to medium level (4000 ppm) with no significant differences between medium and high salinity levels.




2: Relative distribution of saturated and unsaturated fatty acids as affected by salinity × glutathione foliar spray treatments

Findings showed that seeds contained from 15.8-18.08% SFAs and 81.92-84.2% UFAs in which 16.2-16.71% MUFAs and 65.21-68% PUFAs of total fatty acids. The contribution of oleic acid varied significantly across the monounsaturated fatty acids. The control had the lowest content ($P<0.05$), while the interaction treatment of glutathione x high salinity level (15.73%).

Monounsaturated fatty acids can contribute to dietary energy. Furthermore, they may have beneficial effects on blood lipid profiles and help reduce plasma cholesterol levels (Grela et al., 2017), particularly oleic acid. Dietary fats are also important sources of energy and provide essential fatty acids that must be obtained from the diet (Institute of Medicine, 2002).

***Principal components analysis (PCA) for stress indices:***

Principal component analysis (PCA) throughout the three stress levels was conducted separately for high, medium, and low salinity stress conditions to

examine treatment performance based on stress tolerance and sensitivity indices (Fig. 13).

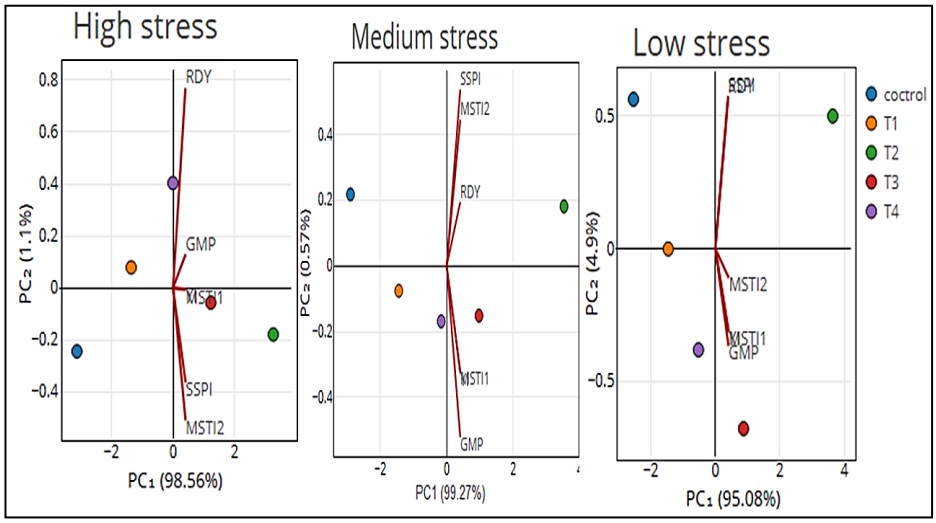



Fig. 13: Summarizes PCA results for high, medium, and low stress conditions; differences in biplot structure reflect stress-dependent variance among indices. Control: Without anti-salinity; T1: V.E; T2: Glutathione; T3: Pottassein-P; T4: Putrescine treatments

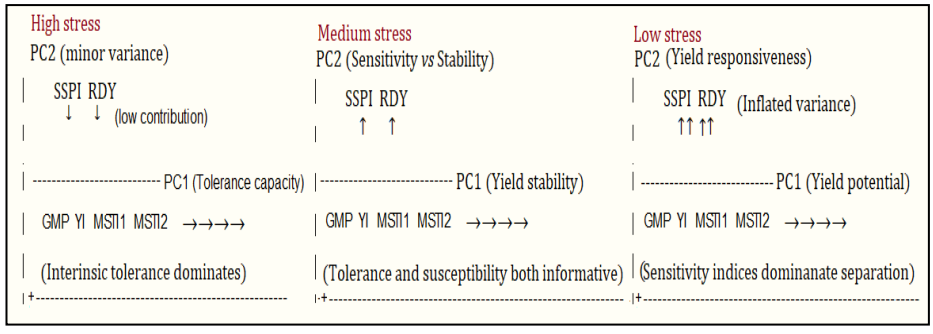
The multidimensional architecture of the data - and hence the PCA results - varied significantly with stress magnitude, according to the joint assessment of stress tolerance and sensitivity indicators under high, medium, and low salinity stress. This demonstrates that tolerance indexes' physiological significance and discriminating ability depend on stress levels. The tolerance-related parameters (GMP, YI, MSTI1, and MSTI2), which loaded positively (Table 9) and consistently on the first principal component (PC1), were among the primary factors of PCA under high stress settings.

**Table 9. Eigenvalues, eigenvectors and variances percentage of Principal Component Analysis**

| | **High stress** | | | **Medium stress** | | | **Low stress** | | |
|---|---|---|---|---|---|---|---|---|---|
| | PC1 | PC2 | PC 3 | PC1 | PC2 | PC 3 | PC1 | PC2 | PC 3 |
| **GMP** | **0.410** | 0.129 | **-0.489** | **0.408** | **-0.520** | -0.122 | **0.410** | **-0.368** | -0.412 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **YI** | **0.411** | -0.005 | **-0.347** | **0.409** | **-0.326** | -0.088 | **0.413** | -0.315 | -0.148 |
| **MSTI1** | **0.411** | -0.008 | **-0.348** | **0.409** | **-0.323** | -0.095 | **0.413** | -0.312 | -0.105 |
| **MSTI2** | **0.407** | **-0.510** | 0.234 | **0.408** | **0.443** | -0.106 | **0.418** | -0.111 | 0.877 |
| **SSPI** | **0.408** | **-0.365** | **0.496** | **0.407** | **0.534** | **-0.455** | **0.398** | **0.576** | -0.096 |
| **RDY** | **0.402** | **0.768** | **0.467** | **0.408** | 0.193 | **0.866** | **0.398** | **0.569** | -0.138 |
| **Eigenvalue** | 5.913 | 0.066 | 0.021 | 5.956 | 0.034 | 0.009 | 5.705 | 0.294 | 0.001 |
| **Variance (%)** | 98.557 | 1.100 | 0.343 | 99.273 | 0.569 | 0.158 | 95.077 | 4.898 | 0.025 |
| **Cumulative (%)** | 98.557 | 99.657 | 100 | 99.273 | 99.842 | 100 | 95.077 | 99.975 | 100 |

As a result of their restricted variability at high stress levels, SSPI and RDY, on the other hand, showed minimal or opposing impacts. Susceptibility index values decreased at high salinity because yield decrease was consistently noticeable across treatments. As a result, PC1 successfully illustrates intrinsic tolerance potential under extreme stress, highlighting the effectiveness of treatments, especially T2 and T3, to preserve relative yield stability rather than reduce actual yield loss. The graphic (Fig. 14) illustrates how treatment perspectives shift under different stressful situations.




: Schematic conceptualization of PCA axis orientation in relation to varying stress levels.

tress: PC1 shows true physiological tolerance; Medium stress: PC axes indicate both tolerance and susceptibility; Low stress: PC2 increasingly illustrates yield response, not stress resistance.

The PCA structure became more balanced at medium stress. Sensitivity indices (SSPI, RDY) and tolerance indices (GMP, YI, MSTI1, MSTI2) both made significant contributions to PC1 and PC2, suggesting more phenotypic diversity between treatments. This stress level is an example of an intermediate state where stress response and yield potential are expressed together. In this case, PCA is distinguished between treatments based on both the degree of yield decline and yield maintenance, leading to a more distinct division between T2, T3, and T4.

SSPI and RDY's greater contribution under medium stress emphasizes their increased significance when stress is high enough to cause varied damage but not high enough to reduce yield in all treatments. PCA results under low stress conditions were significantly different from those under high stress. SSPI and RDY displayed excessive variance, especially for T2 and T3, but tolerance indices remained to load positively. Small absolute yield variations between stressed and non-stressed conditions cause overestimation in susceptibility indices under moderate stress, increasing SSPI and RDY values. This modified PCA structure represents a statistical rather than biological reality. Because of this, PCA at low stress tended to highlight yield sensitivity measurements, occasionally moving otherwise tolerant treatments away from the tolerance axis.

This clarifies why applying parameters from high-stress environments to interpret PCA under low stress may seem inconsistent. Crucially, driven by the greatest GMP, YI, MSTI1, and MSTI2 values, Glutathione (T2) treatment continuously

occupied the advantageous region of PCA space at all stress levels. However, inflated SSPI and RDY values caused their relative position to move under low stress, confirming that high sensitivity indicators under moderate stress do not always signal poor tolerance but rather reflect increased yield potential under non-stress conditions. When taken as a whole, these results show that PCA is context-sensitive and that its biological interpretation needs to be grounded in the degree of stress.

PCA reflects both tolerance and susceptibility at medium stress, real tolerance mechanisms at high stress, and yield responsiveness instead of stress resistance under low stress. Therefore, rather than depending solely on one stress state, the integrated interpretation across stress levels offers a more reliable and physiologically pertinent evaluation of treatment efficiency.

Taken together, these findings indicate that treatment evaluation for salinity tolerance should prioritize PCA outcomes under high and medium stress conditions, where multivariate patterns are biologically meaningful. Based on consistent dominance across tolerance indices and PCA positioning, T2 (Glutathione) is recommended as the most promising treatment for cultivation and breeding programs targeting saline environments, followed by T3 (Pottassein P) as a secondary option.

**Discussion**

According to Tani et al. (2018), Farsaraei et al. (2021) and Malik et al. (2022), the first organ adversely affected by salinity stress is vegetative growth. Additionally, the general trend in salinity-stressed seedlings is a loss of fresh and dry weight (Farsaraei et al., 2021; Malik et al., 2022). The toxicity of sodium and chlorine ions, as well as an imbalance in nutrient absorption and a decrease in water absorption, may be the cause of the growth decline under salinity stress (Çirka et al., 2021; Bahrasemani et al., 2024). The positive effects of glutathione treatments may have arisen from improving osmotic tolerance and/or regulating the biochemical processes (Sadak et al., 2017) and, by decreasing the passive Na influx, glutathione maintains plasma membrane stability, lowers lipid peroxidation, and improves plant salt tolerance (Kesawat et al., 2023). The positive role of GSH may also be due to its effect on increasing membrane permeability (Khattab, 2007). Plants treated with glutathione accumulate proline acid; the growth of these osmolytes increases the primary enzymes involved in proline production, which results in osmotic control

under salinity stress (Sadak et al., 2017). By raising nitrogen, phosphorus, and potassium levels, glutathione foliar spray may mitigate the negative effects of salt stress on ion content (Semida et al., 2021; Rajabi Dehnavi et al., 2022). Therefore, improving osmotic tolerance, preserving integrity of membranes, and halting the loss of photosynthetic pigments could have all contributed to glutathione's positive effects. Under salinity stress, these reactions enhanced plant development (Kattab, 2007; Hasanuzzaman et al., 2017; Gul et al., 2022).

The activation of amylase and protease during germination may be the reason for the growth increase brought on by putrescine under normal settings and stress conditions brought on by sodium chloride (Zeid, 2004). According to the reports, polyamines help plants protect themselves from environmental stressors. Putrescine has been shown to boost grain output and lessen plant growth inhibition in drought-stressed plants (Aydin et al., 2015). In order to reduce the negative effects of salt stress on tomato seedling plant growth, Rama Rao et al. (2025) evaluated how exogenous putrescine (PUT) administration influences and facilitates the initiation of defense mechanisms. They also showed the potential advantages of PUT applications for improving crop growth and salt stress tolerance.

Our findings are in line with other research showing that the use of putrescine in medicinal pumpkin seeds (Farsaraei et al., 2021) and wheat (Aydin et al., 2015) increases plant growth by raising the fresh weight of roots, shoots, and seedling dry matter percentage under stress. As demonstrated in a salt-sensitive rice variety, plant growth induced by external putrescine in salty conditions can decrease the buildup of sodium ions in the root, boost putrescine and grafted polyamine biosynthesis, and hence promote plant development (Quinet et al., 2010). On the other hand, as Ca rises, Na and Cl ion accumulation falls. According to Yasar and Uzal (2020), there is some effectiveness in mitigating the adverse effects of salt by boosting calcium levels during salt stress. A high Ca content may lessen the Na toxicity under salinity, according to Cachorro et al. (1994). According to Yaşar et al. (2006, 2013) and Üzal and Yıldız (2014), plant tolerance to salinity stress is strongly influenced by potassium (K) and calcium (Ca) uptake and accumulation. The findings further imply that the Giza 3 variety's capacity to preserve photosynthetic activity and maintain larger levels of proline and carbohydrates in leaves is linked to its relative resistance to salt stress. Osmotic correction is facilitated by these osmolytes. Additionally, the study highlighted the importance of glutathione treatment as a salt-tolerant medication in the present research circumstances.

Glutathione application (2 mM) under increasing salinity levels was associated with distinct changes in the relative fatty acid composition of fenugreek seed fixed oil compared with the control (Table 8). Among saturated fatty acids, myristic, palmitic, heptadecanoic, and stearic acids increased progressively with increasing salinity. Palmitic acid exhibited the most pronounced increase, suggesting a substantial shift in its relative contribution to the total fatty acid composition. In contrast, behenic acid decreased progressively, whereas arachidic acid increased at lower salinity levels before declining slightly under higher salinity, indicating differential responses among individual saturated fatty acids. Such variations may reflect changes in the fatty acid profile of seed fixed oil under saline conditions (Dutta et al., 2025; Guo et al., 2022). Monounsaturated fatty acids exhibited variable responses across salinity levels in the presence of glutathione. Oleic acid remained relatively stable, with a slight increase, whereas palmitoleic acid decreased markedly. In contrast, vaccenic and gondoic acids increased progressively, indicating changes in the relative proportions of individual monounsaturated fatty acids. These variations suggest differential responses of fatty acid components to the combined conditions of glutathione application and salinity stress (Dutta et al., 2025; Guo et al., 2022). Polyunsaturated fatty acids also showed contrasting responses to increasing salinity.

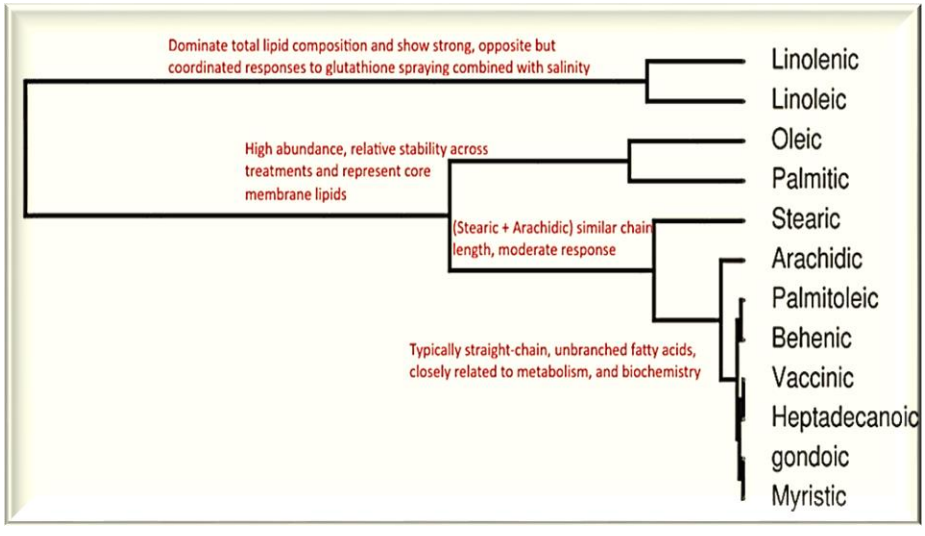



Fig.15: Dendrogram showing the classification of fatty acids based on their responses to the interaction between foliar glutathione application and salinity levels.

Linoleic acid decreased substantially, whereas γ-linolenic acid increased progressively, indicating a shift in the relative distribution of the major polyunsaturated fatty acids in fenugreek seed fixed oil. Changes in fatty acid unsaturation are recognized as an important aspect of plant responses to abiotic stress; however, the present findings specifically demonstrate changes in seed oil fatty acid composition rather than direct alterations in membrane lipid composition (Dutta et al., 2025; Guo et al., 2022). Cluster analysis (Fig. 15) revealed distinct relationships among the identified fatty acids. Linoleic and γ-linolenic acids formed a closely related pair, while oleic and palmitic acids also clustered together. The remaining fatty acids were distributed among other branches of the dendrogram, reflecting differences in their response patterns across the treatments. Overall, these findings indicate that increasing salinity in the presence of foliar glutathione (2 mM) was associated with selective changes in the relative fatty acid composition of fenugreek seed fixed oil. These observations may be discussed in the context of the reported role of glutathione in enhancing antioxidant defense and improving plant adaptation to salinity-induced oxidative stress (Rehman et al., 2021).

## Conclusion

The findings of this study clearly demonstrate that salinity stress adversely affected the growth parameters, yield attributes, photosynthetic pigments, and seed and oil yields of fenugreek plants, while increasing the accumulation of $Na^+$ and $Cl^-$ ions and decreasing $Ca^{2+}$ content and the $Ca^{2+}/Na^+$ ratio. However, the exogenous application of anti-salinity stimulants significantly alleviated these detrimental effects by reducing $Na^+$ and $Cl^-$ accumulation, increasing $Ca^{2+}$ content and the $Ca^{2+}/Na^+$ ratio, and improving photosynthetic pigments and total carbohydrate content. Among the tested treatments, glutathione (T2), applied as a foliar spray at 2 mM, exhibited the most pronounced beneficial effects, improving plant salt tolerance, regulating proline accumulation, and enhancing plant growth, yield attributes, and seed and oil productivity across the tested salinity levels. Pottassein-P (T3), applied through irrigation water, was the second most effective treatment. Furthermore, principal component analysis (PCA) highlighted the context-dependent nature of stress tolerance indices, emphasizing the importance of evaluating treatment performance under both moderate and high salinity conditions. Overall, foliar application of glutathione at 2 mM is recommended as a promising approach for improving fenugreek productivity and salinity tolerance under saline irrigation conditions.

**References**

AOAC (1990) Official methods of analysis of the Association of Official Analytical Chemists. 15th edn. Edited by K. Helrich. Arlington. VA: Association of Official Analytical Chemists

Albacete, A., Ghanem, M.E., Martínez-Andújar, C., Acosta, M., Sánchez-Bravo, J., Martínez, V., Lutts, S., Dodd, I.C., & Pérez-Alfocea, F. (2008). Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. Journal of Experimental Botany, 59, pp. 4119–4131. https://doi.org/10.1093/jxb/ern251

Aljuhaimi, F., Şimşek, Ş., Özcan, M.M., Ghafoor, K., & Babiker, E.E. (2018). Effect of location on chemical properties, amino acid and fatty acid compositions of fenugreek (*Trigonella foenum-graecum* L.) seed and oils. Journal of Food Processing and Preservation, 42, article e13569

Analytical Software (2008). Statistix 9: User's Manual. Tallahassee, FL: Analytical Software

Ashraf, M., Mukhtar, N., Rehman, S., & Rha, E.S. (2004). Salt-induced changes in photosynthetic activity and growth in a potential medicinal plant Bishop's weed (*Ammi majus* L.). Photosynthetica, 42(4), pp. 543–550

Aydin, M., Hossein Pour, A., Haliloğlu, K., & Tosun, M. (2015). Effect of putrescine application and drought stress on germination of wheat (*Triticum aestivum* L.). Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 46(1), pp. 43–55

Bahrasemani, S., Seyedi, A., Fathi, S., & and Jowkar, M. (2024). The seed priming using putrescine improves germination indices and seedling morphobiochemical responses of indigo (*Indigofera tinctoria*) under salinity stress. Journal of Medicinal Plants and By-products, 13(1), p. 179. https://doi.org/10.22034/jmpb.2023.128870

Bednarz, C.W., & Oosterhuis, D.M. (1999). Physiological changes associated with potassium deficiency in cotton. Journal of Plant Nutrition, 22, pp. 303–313.

Blakemore, L.C., Searle, P.L., & Daly, B.K. (1981). Methods for chemical analysis of soils. New Zealand Soil Bureau Scientific Report 10A. Lower Hutt, New Zealand: Department of Scientific and Industrial Research. https://doi.org/10.7931/DL1-SBSR-10A

Bisson, P., Cretenet, M., & Jallas, E. (1994). Nitrogen, phosphorus and potassium availability in the soil: physiology of the assimilation and use of these nutrients by the plant in Constable, G.A. and Forrester, N.W. (eds.) Challenging the future:

Proceedings of the World Cotton Research Conference-1, Brisbane, Australia, 14–17 February. Melbourne: CSIRO, pp. 115–124

Cachorro, P., Ortiz, A., & Cerdá, A. (1994). Implications of calcium nutrition on the response of *Phaseolus vulgaris* L. to salinity. Plant and Soil, 159(2), pp. 205–212. https://doi.org/10.1007/BF00009282

Cai, Y., Cao, F., Wei, K., Zhang, G., & Wu, F. (2011). Genotypic dependent effect of exogenous glutathione on Cd-induced changes in proteins, ultrastructure and antioxidant defense enzymes in rice seedlings. Journal of Hazardous Materials, 192(3), pp. 1056–1066. https://doi.org/10.1016/j.jhazmat.2011.06.011

Cakmak, I., Hengeler, C., & Marschner, H. (1994). Partitioning of shoot and root dry matter and carbohydrates in plants suffering from phosphorus, potassium and magnesium deficiency. Journal of Experimental Botany, 45, pp. 1245–1250

Çirka, M., Kaya, A.R., & Eryiğit, T. (2021). Influence of temperature and salinity stress on seed germination and seedling growth of soybean (*Glycine max* L.). Legume Research, 44(9), pp. 1053–1059. https://doi.org/10.18805/LR-628

Coban, F., Özer, H., & Lan, Y. (2024). Genetic and environmental influences on fatty acid composition in different fenugreek genotypes. Industrial Crops and Products, 222(Part 3), article 119774. Available at: https://doi.org/10.1016/j.indcrop.2024.119774

Cristea, S., Perisoara, A., Tihauan, B.M., Ene, M.D., Constantin, M., Florea, A.M., Ivan, E.Ş., Zala, R.C., Purcăreanu, B., Mihaiescu, D.E., & Pirvu, L. (2025). In vitro evaluation of the antifungal activity of Trigonella foenum-graecum seed extract and its potential application in plant protection. Plants, 14(21), article 3320. https://doi.org/10.3390/plants14213320

Dotaniya, C.K., Niranjan, R.K., Kumar, U., Lata, M., Regar, K.L., Doutaniya, R.K., Mohbe, S., & Jadon, P. (2019). Quality, yield and nutrient uptake of fenugreek as influenced by integrated nutrient management. International Journal of Plant & Soil Science, 29(3), pp. 1–7. https://doi.org/10.9734/ijpss/2019/v29i330142

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28(3), pp. 350–356. https://doi.org/10.1021/ac60111a017

Dutta, S., Islam, Z., Das, S., Barman, A., Chowdhury, M., Mondal, B.P., Ajnabi, J., & Manna, D. (2025). Harmonizing plant resilience: unveiling the symphony of membrane lipid dynamics in response to abiotic stresses: a review. Discover Plants, 2, article 61. https://doi.org/10.1007/s44372-025-00152-0

El Amrani, A., Couée, I., Berthomé, R., Ramel, F., Gouesbet, G., & Sulmon, C. (2019). Involvement of polyamines in sucrose-induced tolerance to atrazine-mediated chemical stress in Arabidopsis thaliana. Journal of Plant Physiology, 238, pp. 1–11. https://doi.org/10.1016/j.jplph.2019.04.012

Mohamadi Esboei, M., Ebrahimi, A., Amerian, M.R., & Alipour, H. (2022). Melatonin confers fenugreek tolerance to salinity stress by stimulating the biosynthesis processes of enzymatic, non-enzymatic antioxidants, and diosgenin content. Frontiers in Plant Science, 13, article 890613. https://doi.org/10.3389/fpls.2022.890613

Estaji, A., Kalaji, H.M., Karimi, H.R., Roosta, H.R., & Moosavi-Nezhad, S.M. (2019). How glycine betaine induces tolerance of cucumber plants to salinity stress. Photosynthetica, 57(3), pp. 753–761. https://doi.org/10.32615/ps.2019.053

Faisal, Z., Irfan, R., Akram, N., Manzoor, H.M.I., Aabdi, M.A., Anwar, M.J., Khawar, S., Saif, A., Shah, Y.A., Afzaal, M., & Desta, D.T. (2024). The multifaceted potential of fenugreek seeds: from health benefits to food and nanotechnology applications. Food Science & Nutrition, 12(4), pp. 2294–2310. https://doi.org/10.1002/fsn3.3959

Farsaraei, S., Mehdizadeh, L., & Moghaddam, M. (2021). Seed priming with putrescine alleviated salinity stress during germination and seedling growth of medicinal pumpkin. Journal of Soil Science and Plant Nutrition, 21(3), pp. 1782–1792. https://doi.org/10.1007/s42729-021-00479-z

Farshadfar, E., & Sutka, J. (2002). Screening drought tolerance criteria in maize. Acta Agronomica Hungarica, 50(4), pp. 411–416. https://doi.org/10.1556/AAgr.50.2002.4.3

Farshadfar, E., Poursiahbidi, M.M., & Safavi, S.M. (2013). Assessment of drought tolerance in land races of bread wheat based on resistance/tolerance indices. International Journal of Advanced Biological and Biomedical Research, 1(2), pp. 143–158

Fernandez, G.C.J. (1992). Effective selection criteria for assessing plant stress tolerance', in Kuo, C.G. (ed.) Adaptation of food crops to temperature and water stress: Proceedings of the International Symposium on Adaptation of Food Crops to Temperature and Water Stress, Shanhua, Taiwan, 13–18 August. Shanhua, Taiwan: Asian Vegetable Research and Development Center (AVRDC), pp. 257–270. https://doi.org/10.22001/wvc.72511

Foyer, C.H., & Noctor, G. (2005a). Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant,

Cell & Environment, 28(8), pp. 1056–1071. https://doi.org/10.1111/j.1365-3040.2005.01327.x

Foyer, C.H., & Noctor, G. (2005b). Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. The Plant Cell, 17(7), pp. 1866–1875. https://doi.org/10.1105/tpc.105.033589

Gavuzzi, P., Rizza, F., Palumbo, M., Campanile, R.G., Ricciardi, G.L., & Borghi, B. (1997). Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. Canadian Journal of Plant Science, 77(4), pp. 523–531. https://doi.org/10.4141/P96-130

Grela, E.R., Samolińska, W., Kiczorowska, B., Klebaniuk, R., & Kiczorowski, P. (2017). Content of minerals and fatty acids and their correlation with phytochemical compounds and antioxidant activity of leguminous seeds. Biological Trace Element Research, 180(2), pp. 338–348. https://doi.org/10.1007/s12011-017-1005-3

Gul, N., Ahmad, P., Wani, T.A., Tyagi, A., & Aslam, S. (2022). Glutathione improves low temperature stress tolerance in Pusa Sheetal cultivar of *Solanum lycopersicum*. Scientific Reports, 12, article 12548. https://doi.org/10.1038/s41598-022-16440-8

Guo, Q., Liu, L., Rupasinghe, T.W.T., Roessner, U., & Barkla, B.J. (2022). Salt stress alters membrane lipid content and lipid biosynthesis pathways in the plasma membrane and tonoplast. Plant Physiology, 189(2), pp. 805–826. https://doi.org/10.1093/plphys/kiac123

Hameed, A., Sharma, I., Kumar, A., Azooz, M.M., Lone, H.A., & Ahmad, P. (2014). Glutathione metabolism in plants under environmental stress in Ahmad, P. (ed.) Oxidative Damage to Plants: Antioxidant Networks and Signaling. San Diego, CA: Academic Press, pp. 183–200. https://doi.org/10.1016/B978-0-12-799963-0.00006-X

Hasanuzzaman, M., Nahar, K., Alam, M.M., & Fujita, M. (2012). Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by modulating the antioxidant defense and glyoxalase system. Australian Journal of Crop Science, 6(8), pp. 1314–1323.

Hasanuzzaman, M., Nahar, K., Anee, T.I., & Fujita, M. (2017). Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. Physiology and Molecular Biology of Plants, 23(2), pp. 249–268. https://doi.org/10.1007/s12298-017-0422-2.

Institute of Medicine (IOM) (2002). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, DC: The National Academies Press. https://doi.org/10.17226/10490

Kesawat, M.S., Satheesh, N., Kherawat, B.S., Kumar, A., Kim, H.U., Chung, S.M., & Kumar, M. (2023). Regulation of reactive oxygen species during salt stress in plants and their crosstalk with other signaling molecules—Current perspectives and future directions Plants, 12(4), article 864. https://doi.org/10.3390/plants12040864

Khattab, H. (2007). Role of glutathione and polyadenylic acid on the oxidative defense systems of two different cultivars of canola seedlings grown under saline conditions. Australian Journal of Basic and Applied Sciences, 1(3), 323–334

Kovach, W.L. (1995). MVSP – A multivariate statistical package for IBM PC and compatibles. Kovach Computing Services, Pentraeth, Anglesey, Wales, U.K.

Li, Y., Kong, D., Fu, Y., Sussman, M.R., & Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiology and Biochemistry, 148, 80–89. https://doi.org/10.1016/j.plaphy.2020.01.006

Liu, Y., Liang, H., Lv, X., Liu, D., Wen, X., & Liao, Y. (2016). Effect of polyamines on the grain filling of wheat under drought stress. Plant Physiology and Biochemistry, 100, 113–129. https://doi.org/10.1016/j.plaphy.2016.01.003

Malik, J.A., AlQarawi, A.A., AlZain, M.N., Dar, B.A., Habib, M.M., & Ibrahim, S.N.S. (2022). Effect of salinity and temperature on the seed germination and seedling growth of desert forage grass *Lasiurus scindicus* Henr. *Sustainability*, 14(14), Article 8387. https://doi.org/10.3390/su14148387

Metcalfe, L.D., Schmitz, A.A., & Pelka, J.R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Analytical Chemistry, 38(3), 514–515. https://doi.org/10.1021/ac60235a044

Moosavi, S.S., Yazdi Samadi, B., Naghavi, M.R., Zali, A.A., Dashti, H., & Pourshahbazi, A. (2008). Introduction of new indices to identify relative drought tolerance and resistance in wheat genotypes. Desert (BIABAN), 12, 165–178

Mostofa, M.G., Seraj, Z.I., & Fujita, M. (2014). Exogenous sodium nitroprusside and glutathione alleviate copper toxicity by reducing copper uptake and oxidative damage in rice (*Oryza sativa* L.) seedlings. Protoplasma, 251(6), 1373–1386 https://doi.org/10.1007/s00709-014-0639-7

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Nagulapalli Venkata, K.C., Swaroop, A., Bagchi, D., & Bishayee, A. (2017). A small plant with big benefits: Fenugreek (*Trigonella foenum-graecum* Linn.) for disease prevention and health promotion. Molecular Nutrition & Food Research, 61(6), Article 1600950. https://doi.org/10.1002/mnfr.201600950

Narayana, P.K., Bueno, E., Baur, A., Ahmed, S., & von Wettberg, E.J. (2022). Fenugreek, a legume spice and multiuse crop adapted to a changing climate. In: Jha, U.C. et al. (eds.), Developing Climate Resilient Grain and Forage Legumes. Singapore: Springer Nature Singapore, pp. 105–123. https://doi.org/10.1007/978-981-16-9848-4_5

Niknam, R., Kiani, H., Mousavi, Z.E., & Mousavi, M. (2021). Extraction, detection, and characterization of various chemical components of *Trigonella foenum-graecum* L. (fenugreek) known as a valuable seed in agriculture. In: Naeem, M., Aftab, T. and Khan, M.M.A. (eds.), Fenugreek: Biology and Applications. Singapore: Springer, pp. 189–217. https://doi.org/10.1007/978-981-16-1197-1_9

Ogbe, A.A., Finnie, J.F., & Van Staden, J. (2020). The role of endophytes in secondary metabolites accumulation in medicinal plants under abiotic stress. South African Journal of Botany, 134, 126–134. https://doi.org/10.1016/j.sajb.2020.06.023

Qiu, B., Zeng, F., Cai, S., Wu, X., Haider, S.I., Wu, F., & Zhang, G. (2013). Alleviation of chromium toxicity in rice seedlings by applying exogenous glutathione. Journal of Plant Physiology, 170(8), 772–779. https://doi.org/10.1016/j.jplph.2013.01.016

Quinet, M., Ndayiragije, A., Lefèvre, I., Lambillotte, B., Dupont-Gillain, C.C., & Lutts, S. (2010). Putrescine differently influences the effect of salt stress on polyamine metabolism and ethylene synthesis in rice cultivars differing in salt resistance. Journal of Experimental Botany, 61(10), 2719–2733. https://doi.org/10.1093/jxb/erq118

Rajabi Dehnavi, A., Zahedi, M., Ludwiczak, A., & Piernik, A. (2022). Foliar application of salicylic acid improves salt tolerance of sorghum (*Sorghum bicolor* (L.) Moench). Plants, 11(3), 368. https://doi.org/10.3390/plants11030368.

Rama Rao, Y., Yadav, P., Rani, V., Muley, D., Sahoo, R.K., Brajendra, Bhushan Kumar, S., Gill, R., Gill, S.S., Ansari, M.W., & Tuteja, N. (2025). Putrescine mitigates NaCl-induced stress by modulating gene expression, antioxidants, and

ethylene level in tomato. Plant Signaling & Behavior, 20(1), Article 2515431. https://doi.org/10.1080/15592324.2025.2515431

Rao, P.U., Sesikeran, B., Rao, P.S., Naidu, A.N., Rao, V.V., & Ramachandran, E.P. (1996). Short term nutritional and safety evaluation of fenugreek. *Nutrition Research*, 16(9), 1495–1505. https://doi.org/10.1016/0271-5317(96)00163-7

Rehman, H.U., Alharby, H.F., Bamagoos, A.A., Abdelhamid, M.T., & Rady, M.M. (2021). Sequenced application of glutathione as an antioxidant with an organic biostimulant improves physiological and metabolic adaptation to salinity in wheat. Plant Physiology and Biochemistry, 158, 43–52. https://doi.org/10.1016/j.plaphy.2020.11.041

Rhaman, M.S., Imran, S., Rauf, F., Khatun, M., Baskin, C.C., Murata, Y., & Hasanuzzaman, M. (2021). Seed priming with phytohormones: An effective approach for the mitigation of abiotic stress. Plants, 10(1), Article 37. https://doi.org/10.3390/plants10010037

Sadak, M.S., Abd Elhamid, E.M.A., & Ahmed, M.M.R. (2017). Glutathione induced antioxidant protection against salinity stress in chickpea (*Cicer arietinum* L.) plant. Egyptian Journal of Botany, 57(2), 293–302. https://doi.org/10.21608/ejbo.2017.636.1029

Semida, W.M., Abd El-Mageed, T.A., Abdalla, R.M., Hemida, K.A., Howladar, S.M., Leilah, A.A.A., & Rady, M.O.A. (2021). Sequential antioxidants foliar application can alleviate negative consequences of salinity stress in *Vicia faba* L. Plants, 10(5), 914. https://doi.org/10.3390/plants10050914

Sethi, G., Sood, S., Bhardwaj, S.B., & Jain, A. (2024). In vitro evaluation of anti-microbial efficacy of *Trigonella foenum-graecum* and its constituents on oral biofilms. Journal of Indian Society of Periodontology, 28(3), pp. 304–311. https://doi.org/10.4103/jisp.jisp_540_23

Snedecor, G.W., & Cochran, W.G. (1980). Statistical Methods. 7th ed. Ames, Iowa, USA: Iowa State University Press

Sytar, O., Kumari, P., Yadav, S., Brestic, M., & Rastogi, A. (2019). Phytohormone priming: Regulator for heavy metal stress in plants. Journal of Plant Growth Regulation, 38, 739–752. https://doi.org/10.1007/s00344-018-9886-8

Tani, E., Sarri, E., Goufa, M., Asimakopoulou, G., Psychogiou, M., Bingham, E., Skaracis, G.N., & Abraham, E.M. (2018). Seedling growth and transcriptional responses to salt shock and stress in *Medicago sativa* L., *Medicago arborea* L., and

their hybrid (Alborea). Agronomy, 8(10), 231. https://doi.org/10.3390/agronomy8100231

Üzal, Ö., & Yıldız, K. (2014). Reactions to salt stress of some strawberry (*Fragaria × ananassa* L.) cultivars. *Yüzüncü Yıl* University Journal of Agricultural Sciences, 24(2), 159–167

Wang, Y., Gu, W., Meng, Y., Xie, T., Li, L., & Li, J. (2017). γ-Aminobutyric acid imparts partial protection from salt stress injury to maize seedlings by improving photosynthesis and upregulating osmoprotectants and antioxidants. Scientific Reports, 7, Article 43609. https://doi.org/10.1038/srep43609

Yang, L., Wen, K.S., Ruan, X., Zhao, Y.X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. Molecules, 23(4), Article 762. https://doi.org/10.3390/molecules23040762

Yasar, F., Uzal, O., & Yaşar, O. (2013). Identification of ion accumulation and distribution mechanisms in watermelon seedlings (*Citrullus lanatus* (Thunb.) Mansf.) grown under salt stress. Yüzüncü Yıl University Journal of Agricultural Sciences, 23(3), 209–214

Yasar, F., Uzal, O., Tufenkci, S., & Yildiz, K. (2006). Ion accumulation in different organs of green bean genotypes grown under salt stress. Plant, Soil and Environment, 52(10), 476–480. https://doi.org/10.17221/3469-PSE

Yasar, F., & Uzal, O. (2020). Effect of calcium applications on ion accumulation in different organs of pepper plant under salt stress. BIO Web of Conferences, 17, Article 00231. https://doi.org/10.1051/bioconf/20201700231

Zeid, I.M. (2004). Response of bean (*Phaseolus vulgaris* L.) to exogenous putrescine treatment under salinity stress. Pakistan Journal of Biological Sciences, 7(2), 219–225. https://doi.org/10.3923/pjbs.2004.219.225

Żuk-Gołaszewska, K., Wierzbowska, J., & Bieńkowski, T. (2015). Effect of potassium fertilization, Rhizobium inoculation and water deficit on the yield and quality of fenugreek seeds. Journal of Elementology, 20(2), 513–524. https://doi.org/10.5601/jelem.2014.19.4.775