



Research Article

Effect of sodium fluoride on germination, seedling growth and biochemical attributes in linseed varieties (*Linum usitatissimum* L.)

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Abstract

Fluoride is a potential threat to crop plants due to its inherent toxicity and its ability to move within plant tissues. The goal of the current study was to determine how sodium fluoride affected physiological, morphological, and biochemical parameters during the germination and seedling development of the T-397 and Shekhar linseed cultivars. Seeds were exposed to various sodium fluoride (NaF) concentrations (0.1 mM, 0.5 mM, 1.0 mM, 2.5 mM, 5.0 mM, and 10.0 mM) during the experiment. The findings of this investigation unequivocally demonstrated the substantial detrimental effects of sodium fluoride on multiple aspects of linseed growth and development. As the NaF concentration increased, the adverse impacts were magnified, highlighting a concentration-dependent relationship. Notably, the levels of total soluble sugars and proline content exhibited a remarkable fourfold increase as the sodium fluoride concentration escalated. This observation suggests that sodium fluoride may disrupt the normal metabolic processes in linseed, leading to alterations in sugar and proline metabolism. Interestingly, even at the lowest tested concentration of 0.1 mM NaF, linseed displayed susceptibility to fluoride-induced growth inhibition. This suggests that even at very modest levels of contamination, linseed cultivars are susceptible to fluoride stress, which may have an impact on crop yield in fluoridated areas. It is important to remember that this study did not definitively show any differences in the examined cultivars' sensitivity to fluoride stress; therefore more investigation is required to identify which type of linseed is more susceptible to this stress. This study's conclusion emphasizes the substantial harm that sodium fluoride causes to a number of vital indicators of seedling growth and germination of linseed. These results highlight the significance of comprehending the mechanisms underlying plant toxicity to fluoride and the possible ramifications for crop agriculture.


Keywords proline, relative water content, seedling growth, sodium fluoride

Introduction


Fluoride (F), the 13th most frequent element and a member of the halogen family, is present in the earth's crust in amounts of about 0.3g per kg. Fluorides naturally occur in rocks, coal, clay, and soil as sodium fluoride or hydrogen fluoride, and they are released into the environment when minerals weather, as well as when volcanic ash and marine aerosols

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are discharged into the atmosphere Inorganic fluorides in water often persist in soil solution (as fluoride ions) under conditions of relatively low pH and hardness. Although F is non-essential for plants, it can sometimes affect how they operate structurally, biochemically, and physiologically even when there are no obvious symptoms of injury. The order of fluoride retention in onions, according to Jha et al., [1], is root > shoot > bulb.

It has been noted that the buildup of too much fluoride from the atmosphere can harm some plant species. Between 60 and 6,000 kilotons of hydrogen fluoride are released globally each year from volcanic sources through eruptions and passive venting. A little portion of this, about 10%, can be released directly into space. Additionally, fluoride may enter the soil from a number of anthropogenic sources, such as the production of phosphate fertilizers, pesticides (such as sulfuric fluoride), detergents, bricks, tiles, and ceramics, as well as air pollution from industrial activities (used in the production of aluminum and as a flux in the steel and fiberglass industries) and the burning of fossil fuels. Polytetrafluoroethylene (PTFE), one of the thermostable fluoropolymer plastics, is a widely available and useful fluoroplastic that is sold and used in domestic cooking utensils because of its heat resistance and non-stick qualities. In India as well as other countries, endemic fluorosis is a serious public health issue caused by high fluoride concentrations in groundwater. According to Ghosh et al., [2] there are several ecological factors, both natural and anthropogenic, that contribute to groundwater contamination. These factors include the release of hazardous wastes, an excess of fertilizers, liquid and soil waste from businesses, sewage disposal, surface impoundments, and more. Seed germination has been demonstrated to be decreased by sodium fluoride solutions at lower dosages. Variations within a species of plants as well as between species affect fluoride uptake, plant development, and yield. When seeds were exposed to solutions of sodium fluoride and hydrogen fluoride during germination, the inhibitory effects of the inorganic fluoride oxidizing agents were studied. However, even at very low quantities, ClF_3 or BrF_5 in the air can be quite dangerous. Interhalogens appear to react fast with the surfaces of plants or seeds, resulting in damage. Because fluoride acts as a metabolic inhibitor and germination is one sort of metabolism, the fresh weight of seedlings decreased monotonically as fluoride concentration rose. Fluoride is thought to have an impact on a number of physiological processes, including changes in biochemical levels, enzyme activity, pigments, photosynthesis, and biomass as observed by Baunthiyal et al., [3]. Grain crops have also been found to have cytogenetic alterations brought on to fluoride explained by Gritsan, et al., [4]. Elloumi et al., [5] analyzed the leaf tips and edges, necrotic lesions, chlorosis, and burning are the first observable signs of fluoride damage. The effects of chlorosis and necrosis on plants include decreased photosynthetic efficiency and reduced plant yields. Given the aforementioned information, the following study objectives will be investigated: the effects of different sodium fluoride concentrations on the germination of linseed as well as the morpho-physiological and biochemical changes that sodium fluoride causes in different linseed varieties during seedling growth.

Methodology

The current experiment was conducted in a laboratory and net house in the Department of Plant Physiology at Banaras Hindu University, Varanasi. The two kinds of bold, disease free (sterilized in HgCl_2 0.1%) seeds of linseed genotypes T-397 and Shekhar were chosen for this research study, procured from the Department of Agronomy, Institute of Agricultural Sciences, Banaras Hindu University. Seven treatments of sodium fluoride concentrations respectively T1 0.1mM, T2 0.5mM, T3 1.0mM, T4 2.5mM, T5 5.0mM and T6 10.0mM) with three replications were used. Each petri dish included 10 seeds, which were incubated for 3 days at 20°C in an incubator for germination. Similarly, each pot contained 10 seeds, which were duly planted and watered with irrigation water at intervals of 3-5 days. Pots were maintained in a polyhouse environment with regular maintenance. The experiment was statistically evaluated using a completely randomized design (CRD).



Germination percentage (%)

48 hours after treatment, the percentage of germination was recorded. The following formula was used to compute the percentage of germination:

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{total number of seeds sown for germination}} \times 100$$

Root length (cm)

Scale was used to measure the maximum root length at 7 days After Sowing (DAS) which is the distance between the shoot's base and the longest root tip.

Shoot length (cm)

The maximum shoot length was measured at 7 DAS using a scale, averaged, and represented in cm. The primary shoot's growing tip was the measurement point.

Relative water content (%)

Petri dishes holding solutions of a given concentration of Sodium fluoride concentrations are filled with seeds (10 seeds per Petri dish). Petri dish edges were impermeably covered with colorless parafilm in order to prevent water leaks. Petri dishes were placed in a germination chamber to achieve germination for 72 hours at $20 \pm 2^\circ\text{C}$ under a daylight photoperiod. Extracted roots and shoots were then placed in an oven (NSW-142) at 105°C for 5 minutes, then at 65°C until weight was constant. At 7 DAS, an electronic balance recorded the dry weight.

$$\text{RWC (\%)} = \frac{F_w - D_w}{T_w - D_w} \times 100$$

F_w = Fresh weight (mg), T_w = Turgid weight (mg), D_w = Dry weight (mg).

α -Amylase activity (mg g^{-1} fresh weight min^{-1})

Extraction of α -amylase

To assay the in vitro activity of α -amylase enzyme in linseed endosperm, the method of Bernfeld [6] was adopted. Three replications were taken into consideration for each treatment and each replication contained 100 mg of endosperm. The endosperm was taken from seeds kept for germination for 72 hours and before any radicle protrusion took place. The embryo portion was discarded.

Chlorophyll content (mg g^{-1} fresh weight)

By using the Arnon [7] approach, the chlorophyll content of the provided leaf sample was calculated. Using 20 ml of 80% acetone, 100 mg of leaf samples were used to extract the chlorophyll. Using the formula shown below, the amount of chlorophyll content was determined.

$$\text{Total chlorophyll content} = 20.2(A_{645}) + 8.02(A_{663}) \times V \div 1000 \times W \text{ (mg /g fresh Wt)}$$

Where V is the final extract volume, W is the fresh leaf weight, and A is the wavelength-specific absorbance. In terms of chlorophyll content, the values are represented as mg/g of sample

Soluble sugar content (mg g^{-1} Fresh Weight)

The method described by Dubois et al., [8] was used to determine the amount of soluble sugar in leaves at 20 DAS. The sugar content was given as mg glucose g^{-1} fresh leaf weight. 200 milligrams of Anthrone were dissolved in 100 mL of 98 percent H_2SO_4 to create an Anthrone reagent. This reagent was freshly made. 5 mL of 80% ethanol was used to homogenize the sample (100 mg fresh weight).

Protein content (mg g^{-1} fresh weight)



Using a cold pestle and mortar, 100 mg of fresh leaves were dissolved in 5.0 mL of 1.5% NaCl. 20 minutes were spent centrifuging the extract at 10,000 g. The 2 mL extraction media was used twice during the extraction process. The total volume of all supernatants was pooled, and it was set at 10 mL. Bradford [9] used the Coomassie Brilliant Blue G-250 dye binding method to quantify the amount of protein in the crude extract.

Proline content (mg g⁻¹ fresh weight)

The procedures outlined by Bates et al., [10] were used to determine the proline content in the Leaf sample (100 mg).

Statistical analysis

Three replications of each observation were made, and mean values were computed for all of the data. The chemicals were of the reagent-grade variety for analysis. According to Panse and Sukhatme [11], the data were analyzed using a factorial randomized design. Critical difference (C.D.) values at the 1% level were computed.

Results and Discussion

Germination percentage (%)

At 48 hours, the impact of varied Sodium fluoride concentrations on the germination % was noted (Table 1).

Table 1. Effect of sodium fluoride on germination percent of linseed varieties

Variety/ Treatments	Germination percent (%)		
	T-397	Shekhar	Mean
Control	96.67	96.67	96.67
T ₁ (0.1mM)	90.00	86.67	88.33
T ₂ (0.5mM)	86.67	83.33	85.00
T ₃ (1.0mM)	83.33	76.67	80.00
T ₄ (2.5mM)	80.00	66.67	73.33
T ₅ (5.0mM)	76.67	56.67	66.67
T ₆ (10.0mM)	50.00	20.00	35.00
Mean	80.48	69.52	75.00
SEm±	Variety (V)	2.01	
	Treatment (T)	2.85	
	Interaction (V×T)	4.03	
CD at 1%	Variety (V)	7.92	
	Treatment (T)	11.20	
	Interaction (V×T)	15.84	

According to the findings, the percentage of germination for both kinds fell from T1 to T6 as the concentration of Sodium fluoride was raised. The minimum germination percentage (20%) was recorded in T₆ (10.0mM) for Shekhar variety. The maximum germination percentage was recorded in Control, for both varieties. The data for germination percentage under variety and treatment were found to differ significantly. The data for variety and treatment interaction with respect to percent germination did not differ significantly. The results of the current study showed that increasing fluoride levels in the root zone typically resulted in a decrease in germination percentage. Bhargav et al., [12] also found that sodium fluoride had a similar effect on seed germination and seedling growth of *Triticum aestivum* Var. Raj, 4083. According to Song et al., [13], lower seed germination with an increase in salinity in their study may have been caused by a decrease in water intake. Al-Karaki [14] asserts that internal osmotic stress or restricted ingestion may have a more negative impact on seed



germination in barley than ion toxicity effects. Al Mansouri et al., [15] noted that α -amylase activities were, in contrast, slightly stimulated in all seeds of cultivars of durum wheat (*Triticum durum* D esf.). This resulted from the possible cause of the germination percent decreased by salt stress, all osmotica reduced endosperm starch and soluble sugars content as well as α -amylase activities recorded after 48 h of treatment.

Root length (cm)

Table 2 represents data of seedling root length at the 7th day of observation. A significant difference was observed in root length under Sodium fluoride induced stress for variety. Treatment and interaction of variety and treatment did not differ significantly. The maximum root length (2.90 cm) was observed in T-397 variety T₃ (1.0mM) of treatment, whereas, the minimum root length (0.97cm) was recorded at T₆ (10.0mM) treatment of same variety.

Table 2. Effect of sodium fluoride on root length (cm) of linseed varieties

Variety/ Treatments	Germination percent (%)		
	T-397	Shekhar	Mean
Control	2.63	2.55	2.59
T ₁ (0.1mM)	2.52	2.57	2.54
T ₂ (0.5mM)	2.57	2.15	2.36
T ₃ (1.0mM)	2.90	2.35	2.63
T ₄ (2.5mM)	2.25	2.13	2.19
T ₅ (5.0mM)	2.42	2.27	2.35
T ₆ (10.0mM)	0.97	1.21	1.09
Mean	2.32	2.18	2.25
SEm±	Variety (V)	0.06	
	Treatment (T)	0.08	
	Interaction (V×T)	0.12	
CD at 1%	Variety (V)	0.23	
	Treatment (T)	0.33	
	Interaction (V×T)	0.47	

The data depicted that the root length of variety Shekhar was increased up to T₁ (0.5mM) and showed maximum root length but after that, a sudden fall was observed at T₂ (1.0mM). It again increased from T₄ (2.5mM) to T₅ (5.0mM). Due to a lack of water, the osmotic effect of salt causes a decline in early plant growth. Because salt and plant nutrients interact negatively, salt stress also causes a nutritional imbalance in plants. This reduced the uptake of nutrients by plants and the growth of plants as prescribed by Feigin et al., [16]. Similar to the present study which was conducted on *Vicia faba* by Davies et al., [17] confirmed the same effect on root.

Shoot length (cm)

Table 3 shows the impact of various concentrations of Sodium fluoride on seedling shoot length of linseed varieties, T-397 and Shekhar. The observation was taken at the 7th day after germination. In general, when a plant grew older, the shoot length grew as well. As the concentration of Sodium fluoride increased, the length of the shoots dropped. The T-397 variety's maximum shoot length (10.27 cm) was recorded under Control conditions with no NaF stress, whereas the Shekhar variety's minimum shoot length (2.37) was noted with T₆ (10.0 mM) treatment. The data for variety and treatments and interaction of variety and treatments with respect to shoot length was reported to differ significantly. When compared to the control, the drop in shoot length per plant in T-397 was

Table 3. Effect of sodium fluoride on shoot length (cm) of linseed varieties

Variety/ Treatments	Germination percent (%)		
	T-397	Shekhar	Mean
Control	10.27	9.13	9.70
T ₁ (0.1mM)	9.42	8.42	8.92
T ₂ (0.5mM)	8.03	7.93	7.98
T ₃ (1.0mM)	7.47	7.07	7.27
T ₄ (2.5mM)	7.13	6.25	6.69
T ₅ (5.0mM)	6.42	4.97	5.69
T ₆ (10.0mM)	3.47	2.37	2.92
Mean	7.46	6.59	7.02
SEm±	Variety (V)	0.05	
	Treatment (T)	0.07	
	Interaction (V×T)	0.10	
CD at 1%	Variety (V)	0.19	
	Treatment (T)	0.27	
	Interaction (V×T)	0.39	

11.10% at both fluoride levels, while it was only 7% in Shekhar (Table 3).A similar observation was found in the previous study done for wheat, mustard, and cluster beans by Sharma, [18] and Sabal et al., [19].

Relative water content

A significant difference was observed in relative water content (%) under Sodium fluoride-induced stress for treatments (Figure 1). The varieties and interaction of variety and treatments did not differ significantly. The data demonstrated that when the concentration of Sodium fluoride increased, the relative water content (%) decreased. The maximum relative water content (70.50%) was reported in the T-397 variety at Control under no Sodium fluoride stress, whereas, the minimum (45.33%) was recorded in the Shekhar variety at T₆ (10.0mM). According to reports, a high fluoride environment reduces plant dry weight, root and shoot length, and leaf size, Kumar and Rao [20]. It is therefore conceivable that the relative water content (%) dropped as well. Singh [21] have noted comparable effects on the morphological parameters in wheat and barley.

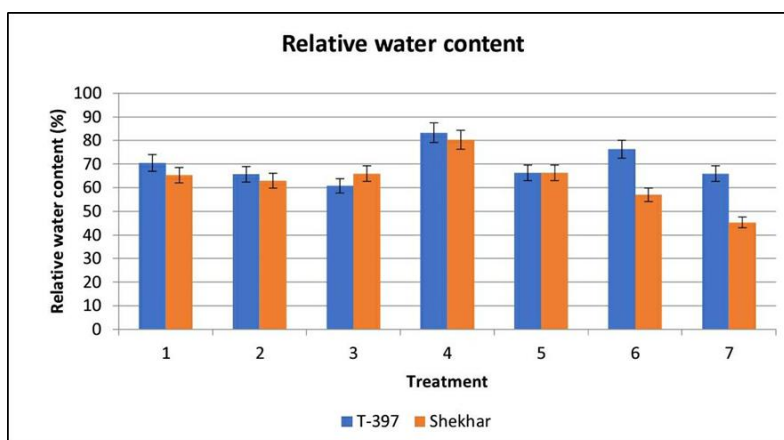


Figure 1. Effect of sodium fluoride on relative water content (%) of linseed varieties

α -Amylase activity (mg fresh weight min⁻¹)

The activity of α -Amylase was seen in the endosperm of linseed varieties at 72 hours after germination (Figure 2). Significant difference was observed in α -Amylase activity under Sodium fluoride stress for treatment, whereas, varieties and interaction of variety and treatments were not significantly differed. The maximum α -Amylase activity (3.92 mg glucose per gram fresh weight min⁻¹) was observed in the T-397 variety at Control and the minimum (2.10 mg glucose per gram fresh weight min⁻¹) was recorded in the Shekhar variety in Sodium fluoride induced stress at T₆ (10.0mM) treatment. Under controlled circumstances, the impact of salt stress on α -amylase activity in three cotton cultivars (NIAB-Karishma, NIAB-86, and K-115) was investigated during germination and the early stages of seedling growth, by Ashraf et al., [22]. All cultivars' starch was broken down into reducing and non-reducing sugars and α -amylase activity decreased as the concentration of NaCl rose. Figure 2 showed a similar trend of declining α -amylase activity.

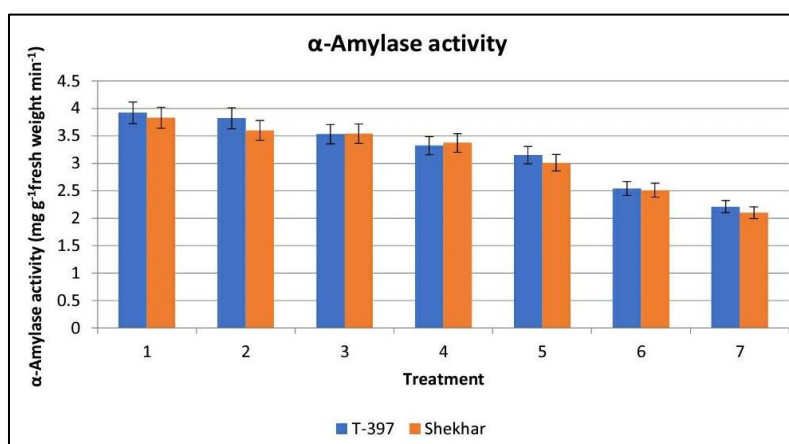


Figure 2. Effect of sodium fluoride on α -Amylase activity (mg g⁻¹ fresh weight min⁻¹) of linseed varieties

Total chlorophyll content (mg g⁻¹ fresh weight)

Perusal of data presented in Figure 3 revealed that the varieties and treatments have a significant effect on total chlorophyll content 15 days after germination. The interaction between varieties and treatments were not differs significantly. The total chlorophyll content of the Shekhar variety without treatment (Control) gained maximum total chlorophyll content (4.38 mg g⁻¹ fresh weight). The minimum total chlorophyll content (1.58 mg g⁻¹ fresh weight) was observed in the T-397 variety at T₆ (10.0mM). According to reports of Yamazoe [23], fluoride toxicity caused a decrease in the concentration of certain photosynthetic pigments. Nearly the same observations were made in the current experiment. When the degree of damage was assessed relative to control using various treatments, it was shown that T-397 had a larger percentage reduction in these parameters than Shekhar. While Shekhar showed the opposite pattern, T-397 experienced less alteration in total chlorophyll content as a result of fluoride treatment. While there was only a slight increase in total chlorophyll in Shekhar following this treatment, T-397 experienced a greater percent loss in the amounts of these pigments from 5.0mM to 10.0 mM fluoride. According to Gupta et al., [24], chlorophyll breakdown or the inhibition of chlorophyll production, which is a main symptom of fluoride-induced chlorosis, may be the cause of the decrease in chlorophyll concentration during Sodium fluoride stress. It was found that Shekhar is more resilient to fluoride toxicity on chlorophyll content than T-397.

Soluble sugar content (mg g^{-1} fresh weight)

The data on soluble sugar content with respect to Sodium fluoride treatments is presented in Figure 4. The maximum soluble sugar content was observed in the Shekhar variety at T₆ (10.0mM) treatment (11.95 mg fresh weight), whereas, the minimum soluble sugar content was recorded also in the Shekhar variety at control. The data depicted for varieties and interaction of variety and treatments were not significantly different. The treatment has a significant effect on soluble sugar content. Changes in soluble sugar, protein, and proline contents under the influence of fluoride toxicity have been reported earlier by Kumar et al., [25]. In the current study, T-397 and Shekhar plants had higher soluble sugar content than control plants when treatment concentrations were increased. Sugar content in both genotypes increased to almost similar magnitude. The trend of total soluble sugar was found to increase sugar levels with increasing Sodium fluoride concentration, as described in Figure 4. Similar effect of sugar was observed by Gupta et al., [26] in rice. They stated that the sugar content of plants is closely correlated with the stress factor and that the lowering sugar content initially fell and subsequently grew with increasing Sodium fluoride concentration. The sugar concentration rose 118 times from 10 mg NaF/L to 30 mg NaF/L. It's possible that reducing sugar is present in tissues at higher levels because it isn't being converted to non-reducing sugar. This could be a strategy used by paddy (rice) in particular to lessen the effects of fluoride stress.

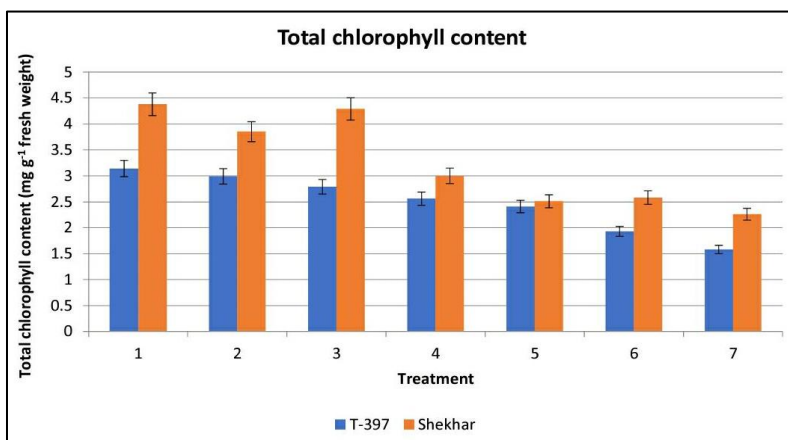


Figure 3. Effect of sodium fluoride on total chlorophyll content (mg g^{-1} fresh weight) of linseed varieties

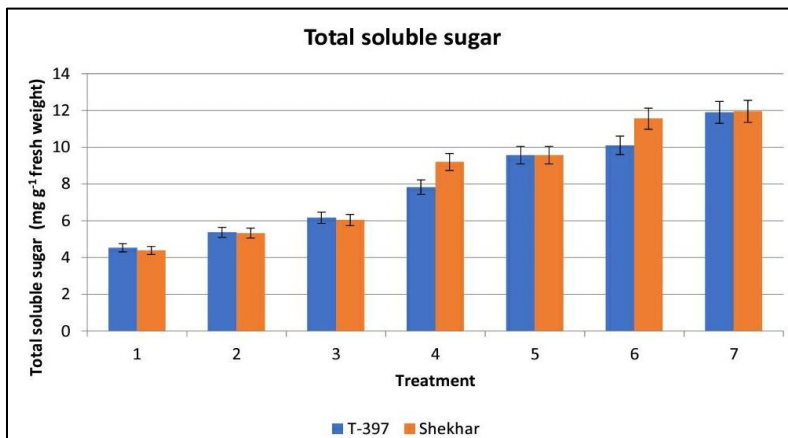


Figure 4. Effect of sodium fluoride on total soluble sugar (mg g^{-1} fresh weight) of linseed varieties

Protein content (mg fresh weight)

Table 4 represents the data of protein content recorded at 20 days after sowing. The data depicted that maximum protein content (13.32 mg g⁻¹fresh weight) was recorded at (Control) in the T-397 variety, whereas minimum protein content (7.12 mg g⁻¹fresh weight) was observed in Shekhar variety at the T₆ (10.0mM) level. The data shown in the table depicted that variety was not observed as significant. However, significant differences were found due to varietal and treatment interaction at 20 DAS. Protein content decreased as NaF concentration increased (Table 4). A similar effect was seen by Parida et al., [27], they studied salt-induced (NaCl at 100, 200, and 400mM) biochemical changes in *Bruguiera parviflora* (mangrove) on total soluble proteins. They discovered that the amount of protein in leaves dropped as salinity rose, which may point to a breakdown in the protein process or, more likely, to an increase in proteolytic activity.

Table 4. Effect of sodium fluoride on Protein content (mg g⁻¹fresh weight) of linseed varieties

Variety/ Treatments	Germination percent (%)		
	T-397	Shekhar	Mean
Control	13.32	13.23	13.28
T ₁ (0.1mM)	12.69	12.66	12.68
T ₂ (0.5mM)	11.34	12.00	11.67
T ₃ (1.0mM)	10.62	10.82	10.72
T ₄ (2.5mM)	9.71	10.74	10.22
T ₅ (5.0mM)	9.38	9.02	9.20
T ₆ (10.0mM)	7.57	7.12	7.34
Mean	10.66	10.80	10.73
SEm±	Variety (V)	0.08	
	Treatment (T)	0.11	
	Interaction (V×T)	0.16	
CD at 1%	Variety (V)	0.32	
	Treatment (T)	0.45	
	Interaction (V×T)	0.63	

Proline content (mg fresh weight)

The data of proline content was recorded 20 days after sowing in plant leaves (Figure 5). The data depicted that maximum proline content (2.95 mg g⁻¹ FW) was recorded at T₆ (10.0mM) in the

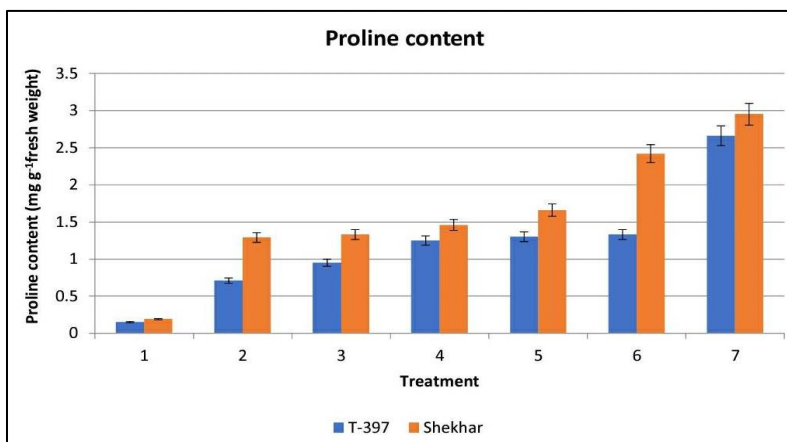


Figure 5. Effect of sodium fluoride on proline content (mg g⁻¹fresh weight) of linseed varieties



Shekhar variety of linseed, whereas, minimum proline content (0.15 mg g⁻¹ fresh weight) was observed in the T-397 variety of linseed at Control. The data was found statistically significant. According to Hong et al., [28], transgenic tobacco (*Nicotiana tabacum*) is affected by salt stress. He came to the conclusion that the enzyme 1-pyrroline-5-carboxylate synthetase (P5CS), which is subject to feedback inhibition by proline, is the rate-limiting enzyme in proline production in plants. So as the sodium fluoride concentration increased, Proline content increased up to T₂ to T₆ for both varieties but in Shekhar, significant increment was observed under each treatment. Datta et al., [29] observed similar findings in 2012 as well. On the basis of present observations, it is inferred that crop genotype response differed to increased sodium fluoride levels, and their differential behavior is due to differential responses of their morpho-physiological and biochemical parameters.

Conclusion

As a result of the aforementioned experiment, it is clear that Sodium fluoride toxicity had a significant impact on all morphological and biochemical parameters. With increasing Sodium fluoride toxicity, the morphological parameters, such as germination percentage, shoot length, root length, relative water content (%), and biochemical parameters, such as amylase activity, chlorophyll content, and protein content, significantly decreased, whereas total soluble sugar and proline content increased many folds. According to the results of the current investigation, even at lower concentrations of Sodium fluoride (0.1 mM), linseed is sensitive to growing under hazardous conditions.

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