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Fluoride-induced physiological and biochemical responses in two species of fenugreek

Ramesh Kumar*, B R Gadi
Department of Botany, Stress Physiology and Molecular Biology Laboratory, Centre for Advanced Study (CAS), Jai Narain Vyas University, New Campus, Jodhpur, Rajasthan, India

Abstract
The aim of this study was to find out the effect of sodium fluoride (5, 10, 15 and 20 mM) on two species of fenugreek, *Trigonella corniculata* L. (Kasuri Methi) and *Trigonella foenum-graecum* L. variety RMt-305, at early seedling stage. The seeds were germinated on moist Whatman No.1.filter paper in petri plates and growth parameters were recorded at seven days assessed after seed sowing. (F^-) stress significantly decreased seed germination percentage, shoot and root length, shoot-root ratio and vigor index. NaF decreased the photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids), chlorophyll stability index (CSI), membrane stability index (MSI), with increasing concentrations of (F^-). Further, the reduction was more in RMt-305 than Kasuri methi. Results showed that increasing level of NaF enhanced level of non-enzymatic antioxidants (ascorbate and phenols) which may be associated with growth regulation and scavenging of reactive oxygen species. Overall, the present study suggests that fenugreek species Kasuri Methi is more tolerant than verity RMt-305 under fluoride stress at early seedling stage.

Keywords: Fenugreek, germination, phenols, pigments, vigor index

Introduction

Abiotic stresses like extreme temperatures, drought, flooding stress, salt stress, fluoride, heavy metal stress, and pollutants adversely affects number of developmental and metabolic processes of plants. Fluorine (F) a significant monovalent poisonous gaseous halogen and strongest oxidizing agent which occurs in air, soils, rocks, water, and plants (Singh *et al.* 2018) [45]. Accumulation of F^- in agricultural products, vegetable and cereal crops causes serious problem for food safety and human health (Sachdeva *et al.* 2023). In Rajasthan state of India all, 33 districts have high level of F^- in ground water. Organic and inorganic substances released into the atmosphere by brick kilns, fertilizers manufacturing factories, and other industrial industries i.e., cryolite, fluorspar, and apatite are main source of F^- pollution (Yadav *et al.* 2024) [48]. Oxidative stress triggered by fluoride as an accumulative poison in plants which reduces overall biomass (Banerjee and Roychoudhary 2019) [8]. F^- is not necessary for plant growth but excessive fluoride concentrations are phytotoxic, especially during the early seed germination seedling growth stages of plants (Fina *et al.* 2016) [20].
Different plants spices show different responses and adaptive strategies towards the fluoride toxicity (Boukhris *et al.* 2020) [11]. Higher level of fluoride causes fluorosis and neurological disorders in animals and humans, the world health organization (WHO) and bureau of Indian standards (BIS) declared 1.5 mg L^{-1} digestive safe limit of fluoride respectively (Choubisa 2013) [15]. Excessive F^- can cause physiological abnormalities in a variety of plants through interference with membrane integrity and enzyme activity, as well as through uptake and interaction with other mineral nutrients (Baunthiyal *et al.* 2014) [31]. It reduced photosynthesis by blocking the Hills reaction through reduction of chlorophyll formation and break down of chloroplasts (Kumar *et al.* 2017) [28]. Plants have F^- tolerance mechanisms like have homeostasis of F^- and gene activation associated with antioxidant, hormones, osmolytes, stress proteins, transporters and metabolites (Gadi *et al.*2021) [22].
The most significant effect of F^- on reduction of productivity globally particularly in arid region is by through low uptake and transport of water which changes enzyme activity, produces toxic reactive oxygen species (ROS) that can negatively affect cellular and metabolic activities of plant cells. As byproducts of numerous metabolic processes, ROS are also found in multiple cellular compartments including mitochondria, peroxisomes, and chloroplasts. Enzymatic and non-enzymatic antioxidants are involved in detoxification of ROS physiological, pathological and in processes of defence system (Kozlov *et al.* 2024) [27]. Exogenously applied calcium protects fenugreek by increasing antioxidant system and other functional characteristics (Aqeel *et al.*2022) [5].
Recent study of Ahmed *et al.* (2024) [3] state that primed seeds of fenugreek with SA under NaF stress showed improved growth and productivity. Osmolytes maintain osmotic potential and also protect the cellular machinery from oxidative stress-induced cellular damage. The most common osmolytes that are necessary for osmoregulation are proline, sugars and glycine-betaine, these osmolytes sustain cellular osmotic homeostasis under stress and protect the cells by increasing antioxidant capacity (Sharma *et al.*2019) [40]. Exposure to sodium fluoride triggers oxidative stress in mung bean seedlings, as induced by alterations in protein, MDA level, and secondary metabolites such as ascorbic acid and phenolic compounds (Bhat *et al.* 2025) [10].
ROS exerts wide range of physiological responses in plants along with changes in cellular structure and modification in enzymes, proteins, nucleic acid, antioxidants, pigments and lipids biomolecules resulting in enzyme inactivation and membrane damage. Phytohormones are involved in growth and detoxification of ROS (Sharma *et al.* 2019) [40]. Stress protectants i.e. salicylic acid (SA), polyamines (PAs), melatonin (Mel), glycine betaine, calcium (Ca^{2+}), and nanoparticles, when applied exogenously are differentially

effective in reducing F^- accumulation and toxicity by regulating various pathways in plants (Gadi *et al.*2021) [22].Salicylic acid is a phenolic signalling molecules that mitigates the toxicity of stresses and protect the plants in several stress including heat, cold, drought, metals and pollutants (Liu *et al.*2024; Fang *et al.*2025) [19, 30]

Fenugreek (Trigonella) is economic important annual crop plants species of family Fabaceae. *Trigonella foenum-graecum* L and *Trigonella corniculata* (commonly known as pan methi or Kasuri methi) are cultivated spices in tropical as well as temperate regions of India (Meena *et al.*2021). The leaves and seeds are used in vegetable, spices and various indigenous medicines. The basic chemical constitutes of the *Trigonella* are sapogenins, steroid, and therapeutic alkaloids (Pal and Mukherjee 2020) [34]. The present research work was carried out to investigate the effects of NaF on early seedlings growth, metabolites and activities of antioxidants enzymes of *Trigonella foenum-graecum* L. variety RMt-305 and *Trigonella corniculata* L. The main objective of present work was to find out the stress tolerance mechanism of these medicinal and economically important crops under fluoride stress.

Materials and Methods

Seed germination and growth parameters

The seeds of fenugreek, (*Trigonella foenum-graecum* L.) variety RMt-305 and Kasuri Methi (*Trigonella corniculata* L.) were surface sterilized in 0.1 % sodium hypochlorite solution and were kept for germination on moist Whatman No. 1 filter paper in petri plates. The seeds were exposed to different concentrations (5, 10, 15 and 20 mM) of sodium fluoride (NaF) and incubated at 18 °C ± 2 °C. Seed germination percentage was assessed on third day after sowing. Seedling growth was evaluated in seven-day-old seedlings by measuring seedling growth (sum of root length and shoot length). According to Abdul-Baki and Anderson, (1973) [2] using the following formula:

Vigor index = Germination percentage X Total seedling length.

Relative water content (RWC): RWC was determined according to Barrs and Weatherley (1962) [9]. Fresh weight (FW) of ten seedlings per treatment was recorded and then, immediately soaked in distilled water for hydration. Turgid weight (TW) was measured after 4 h of hydration. The dry weight (DW) was recorded after drying at 80 °C for 24 h. For measuring RWC using the was formula:

$$RWC = (FW - DW) / (TW - DW) \times 100$$

Pigments: The quantitative estimation of the photosynthetic pigments (Chl a, Chl b, total Chl and carotenoids) were calculated by Arnon's(1949) [6] method. 100 mg fresh cotyledons were homogenized in 10 mL of acetone (80%) and centrifuged at 10,000 rpm for 15 min. The absorbance of the Supernatant was noted at 450, 645 and 663 nm by spectrophotometer.

Chlorophyll stability index (CSI): Fresh leaf samples (100 mg) of each treatment were divided into two sets i.e; normal and heated leaves and samples were homogenized in 80% acetone and absorbance of the extract was measured at 652 nm. (Koleyoreas's, 1958) [26]. CSI was assessed by the formula: CSI = [absorbance of heated leaves/ absorbance of normal leaves] × 100.

Membrane stability index (MSI): Sairam *et al.* (1997) [39] method was used for determination of membrane stability index. For MSI, 0.1 g of leaf sample in 10 mL of distilled water was heated at 40º C for 30 min (C1). Similarly, 0.1 g of leaf sample was heated for 10 min at 100 ºC (C2). Electrical conductance was measured using conductivity meter. It was assessed with the following formula: MSI % = (1-C1/C2) X 100

Non-enzymatic antioxidants: Ascorbate content was measured by 2,6-dichlorophenoleindophenol dye-based titration method (Ranganna,1977) [37]. Standard ascorbic acid solution was prepared by dissolving ascorbate (0.1g) in 100 mL oxalic acid (4%). Plant material (0.1g) was homogenized with 10 mL of 4% oxalic acid and centrifuged at 10,000 rpm for 15 min to separate the supernatant. The 5 mL supernatant was added to 10 mL of oxalic acid and titrated with dye till appearance of pink color as end point.

Total phenolic content: Total phenolic content was assessed by Folin-Ciocalteu reagent-based method of Singleton *et al.* (1999) [47]. 0.1 g fresh seedlings were crushed in 80 % acetone and centrifuged (10,000 rpm for 20 min) to separate the supernatant. 0.2 mL supernatant was mixed with distilled water to make up 2 mL final volume. The reaction mixture was consisting of 1 mL of the Folin-Ciocalteu reagent and 2 mL of 20% Na_2CO_3 solution. These test tubes containing reaction mixture were incubated at 37 °C for 30 min. and absorbance was measured at 760 nm with blank (without plant extract).

Statistical analysis: All biochemical assay were performed in five replicates. Data are presented as mean ± standard error (SE). Mean comparisons were performed using a multivariate general linear model followed by Tukey's b test. All statistical analysis was carried out by IBM SPSS v.16.0 software at $p \geq 0.05$ significance level.

Results

Seed germination and growth parameters

Results revealed that increasing concentration of NaF differentially regulated the seed germination and vigor index of both *T. corniculata* (Kasuri) and *T. feanum-gracecum* L *var*. RMt-305.Seed germination, vigor index of the seedlings are crucial phases in life cycle of crop plants. Present results indicate that different levels of NaF (5, 10, 15 and 20 mM) decreased the seed germination, seedling growth and vigor index of the Kasuri (Table1). Seed germination was reduced by 23.18 %, 29.05 %, 30.18 % and 39.62 % with 5, 10, 15 mM and 20 mM NaF, respectively. Seedling length was also affected by 2.58 %, 4.26 %, 12.03 %, 30.53 % at 5,10,15 and 20 mM NaF respectively. NaF decreased maximum vigor index by 58.05% at 20 mM NaF and minimum at 5 mM NaF 25.37 %. Similarly, different concentrations of NaF (5, 10, 15, and 20 mM) decreased the vigor index, seed germination, and seedling growth of RMt-305 (Table 2). Seed germination percentage reduced by 20.76 %, 39.62 %,49.00 % and 62.12 %, at 5,10,15 and 20 mM NaF, respectively. Additionally, there was an impact on seedling length; the treatments with 20 mM NaF showed the maximum reduction in seedling length by 46.15% and 1.53 %, 35.38 %,18.46 % by 5, 10, 15 mM NaF respectively. The effect of NaF is also seen in the low vigor index of seedlings, which decreased by 79.36 % at 20 mM NaF and by 22.43 % at 5 mM NaF, as well as by 58.58 % at 15 mM NaF and 61.02 % at 10 mM NaF. In the present study, reduction in RWC (4.26 %) by 5 mM NaF,5.46 % by 10 mM NaF, 10.99 % and 20.23 % by 15 mm NaF and 20 mM NaF noted in Kasuri (Fig.1c). Similarly decreased in RMt-305 (Fig. 1c) by 4.83%, 13.29%, 16.80%, and 22.45% at 5,10,15 and 20 mM NaF, respectively.

Pigments content and CSI

In the present study photosynthetic pigments such as Chl a, Chl b, Chl a+b, carotenoids were negatively affected by the increasing level of NaF in the both Kasuri and RMt-305 (Fig.1a,1b). NaF treated seedlings showed the reduction of total Chl by 11.85 %, 16.81 %, 26.06% and 31.74% with the 5, 10, 15 and 20 mM NaF, respectively. Chl b tends to be reduced by 10.88% to 41.88 % with 5 to 20 mM NaF treatments. Whereas, Chl a decreased by 12.51 %, 13.74 %, 24.16% and 24.98 % by given treatments of 5,10,15 and 20 mM NaF, respectively. When compared the control, carotenoids content reduced by 11.08 %, 13.02 %, 17.14 % and 18.89 % at 5,10,15,20 mM NaF, respectively.

CSI percentage also reduced with increasing of concentration 5-20 mM NaF by 11.54% - 44.70 % by in Kasuri (Fig.2a). It is revealed from the results that the elevated concentration of NaF have detrimental effects on photosynthetic pigments, including carotenoids and Chl a, Chl b, and Chl a+b (Fig.1b). Application of 5,10,15 and 20 mM NaF to RMt-305 reduced total Chl by 16.53 %, 21.74 %, 27.33 % and 33.31 % respectively in. Similarly, reduction was observed from 20.81%, 31.48, 39.05 %, and 52.19 % by 5,10,15,20 mM NaF respectively in Chl b. Whereas Chl a decreased by 13.49 %, 14.82 %, 19.01 % and 19.88 % with treatments of 5,10,15 and 20 mM NaF. Carotenoids reduced by 11.52 %, 13.54 %, 17.82 % and 19.64 % at 5,10,15,20 mM NaF. CSI percentage also reduced with increasing NaF concentration by 17.69 %, 34.65 %, 44.94 % and maximum 59.89 % by 5, 10, 15 and 20 mM NaF respectively in var. RMt-305(Fig.2a).

MSI

Under stress lipid peroxidation-based electrolyte leakage is a common phenomenon which reduces the membrane stability. In this study, low MSI was associated with loss of membrane integrity in NaF treated seedlings of Kasuri and RMt-305(Fig.2b). The present study concedes more destructive effect of higher 20 mM NaF concentration on MSI than lower concentration of 5 mM NaF in Kasuri. It was evident from the data that 15 mM and 20 mM NaF decreased MSI by 50.37% and 59.88% as compared to control. The present study recognizes that in Kasuri and RMt-305, high concentration of NaF (20 mM) is more toxic to MSI (Fig.2b) than lower concentration of 5 mM NaF. The results suggests that at 5 mM and 20 mM NaF decreased MSI by 10.97 % and 33.69%, respectively. Results of present study clear that the NaF caused decrease in MSI.

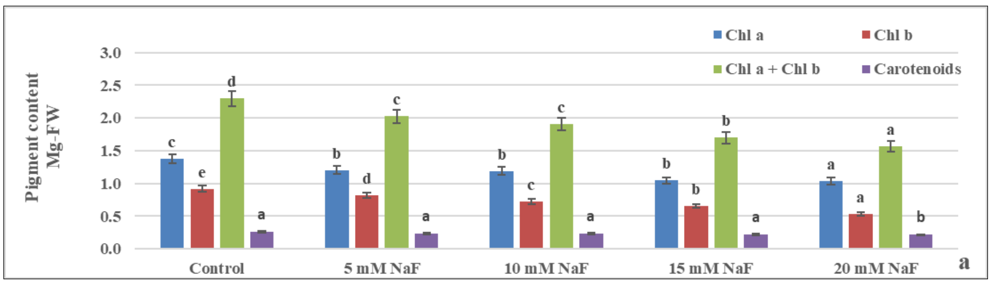
Non-enzymatic antioxidant: The influence of NaF on the non-enzymatic antioxidant (AsA and phenols) content was estimated in Kasuri and RMt-305 seedlings. The AsA (Fig.3b) content increased by 25.00 %,41.08 %,52.21% and 60.16 % with 5,10,15 and 20 mM NaF treatment, respectively. The phenols (Fig.3a) content was also increased by 9.13 %, 12.02 %, 27.37% and 40.03% with 5, 10, 15 and 20 mM NaF treatment, respectively. The effect of NaF on the non-enzymatic antioxidants (AsA and phenols) was computed in RMt-305 seedlings. There were 32.87 %, 43.43 %, 48.28% and 52.41% increase in the AsA content by 5.10, 15 and 20 mM NaF (Fig.3b) were employed, respectively. Accumulation of phenols in RMt-305 seedlings 14.80 %, 16.75 %, 24.51 % and 36.38 % increase in phenols by 5, 10, 15 and 20 mM of NaF (Fig.3a), respectively.

Table 1: Effect of NaF on seed germination, shoot and root length, seedling length and vigor index of *Trigonella corniculata* L. Data represent the mean of five replicates (n=5) ± SE. Different letters indicate significant differences by Tukey's test ($P \leq 0.05$)

Treatment	Germination%	Shoot length (cm)	Root length(cm)	Seedling length(cm)	Vigor index
Control	88.33 ± 3.28^{a}	5.27 ±0.41^{a}	2.47 ±0.07^{a}	7.73 ± 0.47^{a}	682.79 ±64.97^{a}
5 mM NaF	67.67 ± 3.93^{b}	5.73 ± 0.27^{a}	1.80 ±0.31^{b}	7.53 ± 0.52^{a}	509.55 ±29.08^{ab}
10 mM NaF	62.67 ± 7.26^{b}	5.60 ± 0.12^{a}	1.80 ±0.12^{b}	7.40 ± 0.00^{a}	426.15 ±39.66^{bc}
15 mM NaF	61.67 ± 7.26^{b}	5.47 ±0.18^{a}	1.33 ±0.07^{b}	6.80 ± 0.20^{a}	419.35 ±28.48^{b}
20 mM NaF	53.33 ± 7.26^{b}	4.07 ± 0.35^{b}	1.30 ±0.15^{b}	5.37 ± 0.23^{b}	286.38 ±29.90^{c}

Table 2: Effect of NaF on seed germination percentage, shoot and root length and vigor index in *Trigonella foenum-gracecum* L.*var.*RMt-305. Data represent the mean of five replicates (n=5) ± SE. Different letters indicate significant differences by Tukey's test ($P \leq 0.05$)

Treatment	Germination %	Shoot length (cm)	Root length(cm)	Seedling length(cm)	vigor index
Control	88.89±2.00^{a}	3.3±0.07^{a}	3.2±0.12^{b}	6.5±0.06^{a}	577±15.24^{a}
5 mM NaF	70.43±10.41^{ab}	3.3±0.27^{a}	3.1±0.35^{a}	6.4±0.44^{a}	450±45.73^{b}
10 mM NaF	53.67±7.54^{bc}	2.5±0.17^{b}	2.8±0.03bc	5.3±0.18^{b}	284±45.32^{b}
15 mM NaF	45.33±7.86^{c}	1.7±0.35^{c}	2.5±0.18b^{c}	4.2±0.20^{c}	190±32.99^{b}
20 mM NaF	33.67±3.18^{c}	1.5±0.13^{c}	2.1±0.37^{c}	3.5±0.44^{c}	119±28.89^{b}



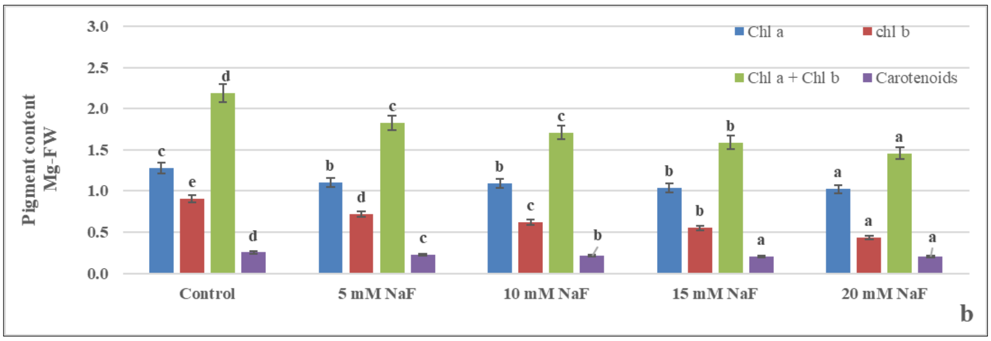


Fig 1: Effect of NaF on pigments content in seedlings of *T. corniculata L. (a)* and *T. foenum-gracecum L. var.* RMt-305 *(b)Values represent* the mean of five replicates *(n=5)* ± SE. Different lower-case letters indicate significant differences among treatments at P ≤ 0.0

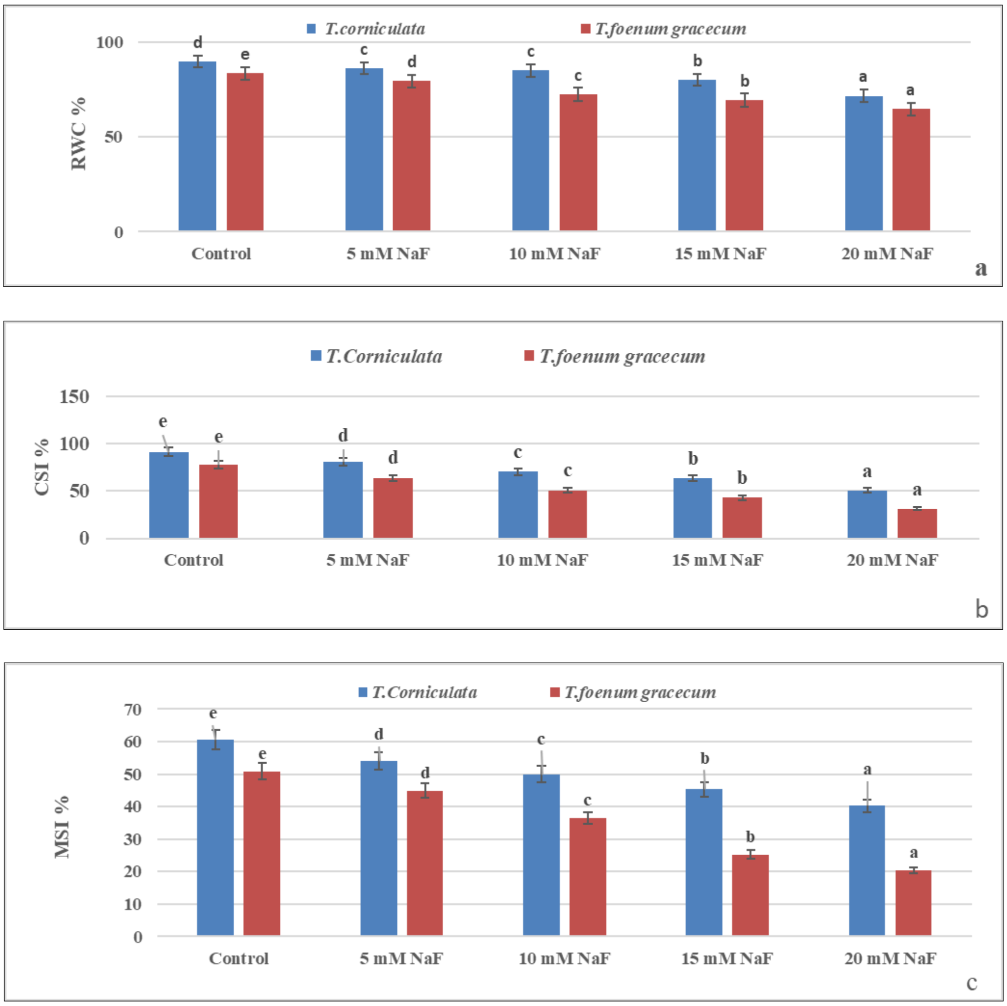
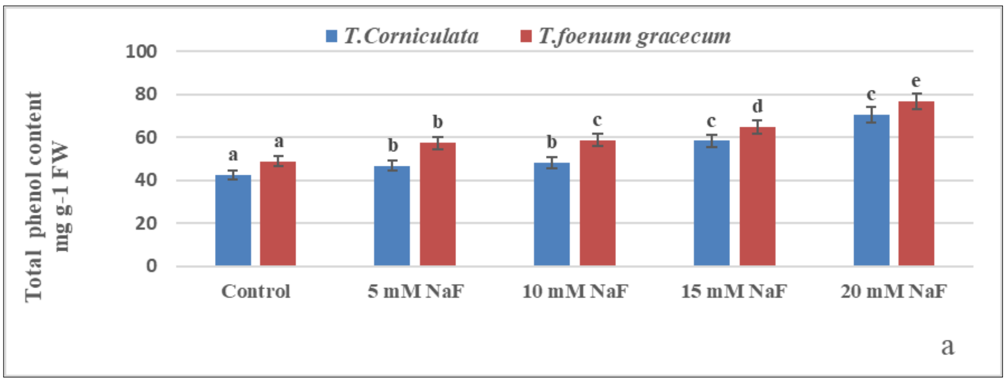


Fig 2: Effect of NaF on Relative water content (RWC)(a) and chlorophyll stability index (CSI) (b) and membrane stability index MSI (c) in seedlings of *T. corniculata* L. and *T. foenum-gracecum* L.*var*.RMt-305. Values represent the mean of five replicates (n=5) ± SE. Different lower-case letters indicate significant differences among treatments at $P \leq 0.05$



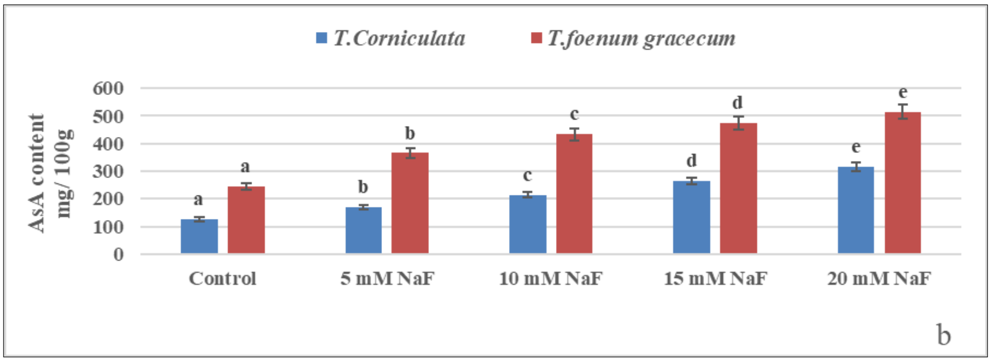


Fig 3: Effect of NaF on total phenols content (a) ascorbate (AsA) content (b) in seedlings of *T. corniculata* L. and *T.foenum-gracecum* L.*var*.RMt-305.Values represent the mean of five replicates (n=5) ± SE. Different lower case letters indicate significant differences among treatments at $P \leq 0.05$

Discussion

Trigonella is diverse crop spices of dry and semi-arid areas of Rajasthan where high concentration of fluoride in water and soil affects several crops. F^- is extreme electronegative, highly reactive and occurs in nature almost exclusively as fluoride salts (Han *et al*. 2021) [33]. Fluoride causes toxicity at seed germination and early seedling growth stages of plants. Seed vigor and performance are influenced by genetic as well as fluoride like environmental stresses. Plants are sensitive to fluoride stress mainly due to production of ROS and other cytotoxic byproducts but the response severity depend on the species and concentration of F^- (Li *et al*. 2013; Singh and Roychoudhury 2023) [44]. In the present study, 5, 10, 15 and 20 mM NaF differentially reduced the seed germination, early seedling growth and vigor index of both species *Trigonella corniculata* (Kasuri) and *Trigonella foenum-gracecum* L variety RMt-305.

It was found that fluoride reduces the seed germination and germination of both the fenugreek species but Kasuri is more tolerant then RMt-305 due to the better growth performance (Table.1 and 2). The reduction of seed germination increased with increasing fluoride levels significant differences in reduction of seed germination with respect to fluoride have been observed in both *Trigonella* sps. Seed germination, seedling length and vigor index were reduced maximum by 20 mM NaF (Table.1 and 2). Similarly, earlier works showed that F^- significantly decreased the seed germination in agricultural crops like *Triticum astivum* (Pelc *et al*. 2020) [35], *T. foenum-graecum* (Burgohain and Chowardhara 2022) [12] and *Sphenostylis stenocarpa* variety Jesca Elevated (F^-) lowered root and shoot biomass, growth and vigor index (Chahine *et al*. 2024) [13]. F^- resistant bacteria in *Triticum aestivum* L. cultivars Chakwal-50 and Galaxy-13 show remeditive effect on seed germination, growth and productivity which was adversely affect by NaF 350 ppm in these plants, F^- toxicity inhibits metabolic activity of plants which causes overall poor plant growth (Mushtaq *et al*. 2021) [33]. In present study, high concentration of NaF (20 mM) showed maximum reduction in RWC in both Sps. but more RWC reduction in RMt-305 compare to Kasuri (Fig.1c) which indicating more tolerance nature of the Kasuri than RMt-305. Similar to other stresses, F^- also reduces RWC in *T.foenum- gracecum* (Rasafi *et al*. 2021) [18] *Triticum aestivum* (Sodani *et al.*2024) and *T. foenum-graecum* (Bhati *et al*. 2024). Compare to RMt-305, Kasuri showed maximum germination,vigor index, and RWC tolerance to (F^-) stress.

Environmental stresses caused reduction in pigments through different ways, these pigments play key role in photosynthesis process. In our study, fluoride treatment reduces total chlorophyll, Chl a, Chl b, and carotenoids content and CSI in Kasuri and Rmt-305(Fig.2a).Goswami *et al*. (2016; 2020) [21, 23] also studied the effect of drought and salinity on seedlings of *Lasiurus scindicus* and suggested that both the stress reduced chlorophyll stability index (CSI) and pigments (chlorophyll a,b and carotenoids) content. F^- Toxicity affects activity of chlorophyll metabolism and damages chlorophyll a in *Lemna minor* L. (Kaminski *et al.*2024) [24]. *Withania somnifera* L. plants grow under fluoride (200 ppm) contaminated water show toxicity of F^- in plant growth and development, reduction in photosynthetic pigments (Chl a, b and total Chl), inhibited photosynthetic response due to damage cell membrane stability and disturb nutrient uptake (Yadav *et al*. 2024). Bhati *et al*. (2024) [48] reported phytotoxic effects of (F^-) on fenugreek germinating seeds in which (F^-) decreases pigments like chlorophyll a, chlorophyll b, and total chlorophyll.

A frequent occurrence that lowers the membrane stability of stressed seedlings is electrolyte leakage in reaction to lipid peroxidation. Yadu *et al*. (2018) also reported that seeds of *Cajanus cajan* L. showed inhibition in growth, MSI and protein content, due to high level of ROS under (F^-) treatment. It was evident from the data that higher concentration of NaF (20Mm) decreased MSI both in Kasuri and Rmt - 305 seedlings (Fig.2 b). An increase in electrolyte leakage under low level of F^- (100 mg/kg) in wheat (*Triticum aestivum* L.) seedlings showed F^- toxicity while decrease membrane damage under high level of F^- (7600 mg/kg) may be due to F^- adaptability of the plants (Shitova *et al.*2023) [41]. In the present study, increasing concentrations of fluoride causes the degradation of membrane through stress ROS or direct degradation of polyunsaturated fatty acids (Darvizheh *et al.*2019) [17]. Current results are similar with the earlier works of (Methenni *et al.*2018) in *Olea europeae* and (Katiyar *et al.*2025) [25] in *Oryza sativa.*

In order to scavenge ROS and support the preservation of redox equilibrium non-enzymatic antioxidants such phenols and ascorbate (AsA) are essential (Taibi *et al.*2016). In the present study fluoride treated seedlings showed high level phenols and (AsA). The influence of NaF on the AsA content was estimated which was increased maximum by 20 mM NaF in Kasuri and Rmt-305 (Fig.4b). The effect of NaF on phenols (Fig.3a) content was also increased maximum by

20 mM NaF in Kasuri and Rmt-305. F^- increased the phenols and AsA contents in *Citrullus lanatus* (Ram *et al.*2014) [36] and in *Oryza sativa* seedlings (Singh *et al.* 2025) [43].

Conclusion

Present study revealed that fluoride have negative effect on seed germination, growth and vigor index of Kasuri and RMt-305. Fluoride induced reduction in pigments, MSI while enhancement in non-enzymatic antioxidants (ascorbate, phenols) might for regulation of growth, osmotic potential and reactive oxygen species. Comparative study of all growth parameters, clearly indicating that Kasuri showed to be more tolerate against fluoride than RMt-305.

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