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Antioxidant activity of Superoxide Dismutase (SOD) and Catalase (CAT) in *Cyamopsis Tetragonoloba* under fluoride stress.

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ABSTRACT: *Fluoride stress is the individual for most significant abiotic stresses. Fluoride adversely affects plants by increasing the number of ROS species and negatively regulating plant growth. In the present study; Cyamopsis tetragonoloba was exposed to fluoride stress in medium strength Hoagland solution. In the current study, a concentration-dependent chemical analysis (0, 10, 20, 30, 40, 50 and 60 mM NaF) of the antioxidant enzyme activity against fluoride stress was performed. Growth inhibition, increased levels of MDA and decreased chlorophyll content in seedlings treated with NaF were observed. Antioxidant enzymes (SOD, CAT) showed highest activity with 30 mM NaF and showed a significant defense against fluoride stress in the culture. This study would maintain an understanding of the role of antioxidants in plant survival against fluoride stress.*

Keywords: *Antioxidant enzymes, cyamopsis tetragonoloba, Fluoride stress*

1. INTRODUCTION

Guar (*Cyamopsis tetragonoloba* L.) is one of the possible forage crops of spring-summer legumes. Guar is mainly grown in arid and semi-arid regions of Pakistan, India, the United States and South Africa [1].Guar plants have a degree of water stress tolerance due to their ability to extract water from the deep layers of soil through their deep root system[2]. It is an important vegetable forage crop for livestock. Guar seeds contain galactomannos that are used in a wide range of industries such as the pharmaceutical, textile, paint, cosmetic, detergent and food industries [3][4]. In view of these agrobotanical characteristics of this crop, it is receiving considerable attention to grow in areas affected by abiotic stress [5].Local guar germplasm has also been found to be very diverse in nature and possibly to adapt to different climatic conditions, such as the fresh Khyber Pakhtoon Kha (KPK) environment or the dry Bahawalpur environment. There are some reports available on the salt tolerance potential of this important forage crop [6].

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Cluster beam can be used for multiple uses (vegetable, beef / feed or green manure). It is a good source of nutrition, and soft green peels are also a cheap source of food. Poultry meal flour and seeds are also used as feed for high protein cattle [7]. Cluster bean gum is a naturally occurring hydrocolloid (also known as "guarania") found in the seed endosperm. It turned out to be the most biologically produced Toxic, ecological and safe agricultural chemicals. Gum is produced mainly from terrestrial endosperm after the dissolution of the seed [8].

2. MATERIALS AND METHODS:

2.1: Plant Materials: Fluoride (NaF) treatment and design

Guar (*Cyamopsis tetragonoloba L.*) Var. RGC-1038 was obtained from the Agricultural Research Station (Swami Keshwanand Agricultural University of Rajasthan SKRAU Bikaner), Rajasthan, India, for experimental purposes developed for the present studyThe seeds were cleaned with 0.5% sodium hypochlorite, used for 15 minutes, and then washed with distilled water. The sterilized seeds are germinated in a Petri dish with filter paper dipped in distilled water after 24 hours in the dark. 3 days after the seedling appeared, ten seedlings were placed in each plastic pot containing a medium-strength Hoagland nutrient solution, production tolerance maintained at 28 ± 2 ° C in a thermostatically prohibited indoor culture and 500 ° C photoperiod at 16 hours photoperiod at μmol - 2 h - 1. Seedlings (*Cyamopsis tetragonoloba L.*) acclimated to the 15-day climate were grown with different concentrations of NaF (0, 10, 20, 30, 40, 50 and 60 for 5 days mM). Control plant maintained in a strong Hoagland environment without fluoride treatment

2.2: Study of physiological parameters

The physiological parameter was calculated at the same time as the root elongation rate along with the rate of development by previously measuring root length (RL) and fresh weight with fluoride conductivity.

Root elongation rate (cm day-1): finally the longest mean RL - initially the longest RL / Δ (t2 - t1);

(RL: root length; t1: last day of treatment; t2: initial day of treatment).

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$$\text{Growth rate (FW g day 1)} \quad \frac{\ln W2 - \ln W1}{T2 - T1}$$

(W1: fresh weight record initially; W2: fresh weight of final record; T1: period before treatment; T2: period after treatment.

2.3: Estimation of lipid peroxidation

Lipid peroxidation was examined in the degree of oxidation of polyunsaturated membrane fatty acids (130) to form malondialdehyde (MDA), as described by [9].The plant tissue of the plants (0.5 g) is extracted in 5 ml of 2-thiobarbituric acid with 0.25% (w / v) in 5% trichloroacetic acid. The mixture is incubated at 95 ° C in a water bath, shaking for 30 minutes blocking the reaction, cooling the tube in an ice water bath. The sample is then centrifuged at 3000 rpm for 10 minutes during supernatant absorption and examined for 532 nm and 600 nm (extinction coefficient: 155 mM^{-1} cm^{-1}).

2.4: Chlorophyll content

Leaf samples (0.1 g) were selected guar *(Cyamopsis tetragonoloba L.)* at random from the seedling. The sample was homogenized in an 80% acetone mortar. The extract was centrifuged at 10,000 rpm for 10 minutes at 4 ° C. After cooling, the sample was heated in an oven at 65 ° C shortly before room temperature. Supernatant uptake was recorded spectrophotometrically at 663, 645 and 450 nm. Chlorophyll content was calculated using the method provided by [10].

Chl a: (12.7 × A663–2.69 × A545).

Chl b: (22.9 × A645–4.68 × A663).

Total chlorophyll=20.2 × A645 + 8.02 A663 × V/W × 1000

2.5: Estimation of proline content.

Root and leaves exposed according to [11]. Sample of plants with 0.3% freshly prepared sulfosalicylic acid (0.3 g). The sample was centrifuged at 3000 rpm for 20 minutes. Then 1 ml of nitric acid is mixed with 1 ml of glacial acetic acid with the proline content in the upper

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phase. The mixture was incubated in a shaking water bath at 100 ° C for 1 hour. The effect stopped when the tube cooled in an ice and water bath. The mixture was added to 2 ml toluene and stirred for 1 minute. Absorbance of proline content is supernatant explained at 520 nm.

2.6: Prepare for the antioxidant test:

2.6. 1: Extract of the enzyme preparation

For antioxidative enzyme tests**,** fresh plant samples (0.5 g) in 50 mM cooled potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, Triton-x 100, 2% PVP and 1 mM ascorbate acid were homogenized at 4 ° C. The homogenate was pressed through four layers of cold cheese and the extract so obtained was centrifuged at 14,000 g for 20 minutes, the supernatant being used for another enzyme test when stored at -20 ° C.

2.6.2: Superoxide dismutase assay (SOD) (EC 1.15.1.1)

The SOD was assayed through determining its capability to diminish the photochemical reduction of nitrobluetetrazolium (NBT) according to the method of [12]. 0.1 ml enzyme extract with 2.5ml of reaction mixture (0.1mM EDTA, 13mM methionine and 70 µM NBT) and 0.4 ml of riboflavin (16.7mM) in 50mM phosphate buffer (pH 7.8) was incubated in light for 30 minutes. The absorbance was recorded at 560 nm condition.

2.6.3: Catalase test (CAT) (EC 1.11.1.6)

CAT activity was determined by [13].who controlled the disappearance of H_2O_2. Crude enzyme extract (0.1 ml) was added to 3 ml reaction mixture containing 50 mM phosphate buffer (pH 7), 30 mM H_2O_2. H_2O_2 in addition to the reduce into absorbance be measured for 5 min at 240 nm.

2.7: Statistical Analysis

The every part of experiments was performed here in triplicates (n = 3). The value in the table, text, and figures signifies mean value ± standard deviation (SD). The difference between control and treatment was examined statistically in means of the t-test, with the level of significance be $P < 0.05$.

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3. RESULTS

3.1: Physiological Parameters of Effect under the Fluoride Stress

During this study, the root elongation rate of (*Cyamopsis tetragonoloba L.*) increased significantly with reduced fluoride concentration (P <0.05) [Table 1]. The growth rate was significantly reduced by increasing the fluoride concentration (P <0.05).

Table 1: Change in growth parameters in (*Cyamopsis tetragonoloba L.*at different NaF concentrations.

NaF(fluoride) concentration (mM)	**Root elongation rate (cm/day)**	**Plant growth rate (g fresh weight/day**)
Control	0.0376 ±0.121	0.242±0.009
10Mm	0.004±0.100*	0.039±0.007*
20Mm	0.066±0.011*	0.061±0.005*
30Mm	0.082±0.019*	0.076±0.014*
40Mm	0.524±0.014*	0.045±0.011*
50Mm	0.066±0.007*	0.058±0.0144*
60Mm	0.0666±0.0078*	0.060±0.007*

Data presented are mean±standard deviation (n=3); *significant mean difference from control at P<0.05 according to t-test

3.2. Chlorophyll Content, Lipid Peroxidation, and Proline Estimation of Effect under the Fluoride Stress.

In the current chlorophyll estimation experiment, a significant decreased of Chla was observed with all treated leaf samples except 20 mM (P <0.05). On the other hand, Chlb was

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also significantly decreased in all treated leaf samples except 10and 20 mM ($P < 0.05$). Total chlorophyll was also significantly decreased .The greatest decreased in total chlorophyll with Chla and Chlb was recorded in 60 mM NaF compared to approximately 1.8-fold in control [fig 1(a)]

In root sample, significant increase in MDA content was observed in 20 mM and 30 mM NaF treated sample in comparison to control. In leaf sample, all NaF treated leaf sample showed significant increase in comparison to control [Fig. 1b].

Under fluoride stress, proline content was significantly increased in the leaf and root sample of *Cyamopsis tetragonoloba L.* ($P < 0.05$). An extremely high proline level is observed in the root in comparison to control [Fig. 1c].

3.3 Response of Antioxidants (SOD and CAT) to Fluoride Stress

In leaf tissues, the level of SOD is significantly ($P < 0.05$) higher than that of the control at all treatment (Fig 2a) SOD activity was highest with the roots and leaves with 30 mM NaF treatment.

In the leaf and root sample, CAT activity increased significantly toward 30mM NaF ($P < 0.05$), followed by increase in Root sample (Fig. 2b) in the case of the Leaf sample, CAT activity was highest at 30 mM in comparison to control.

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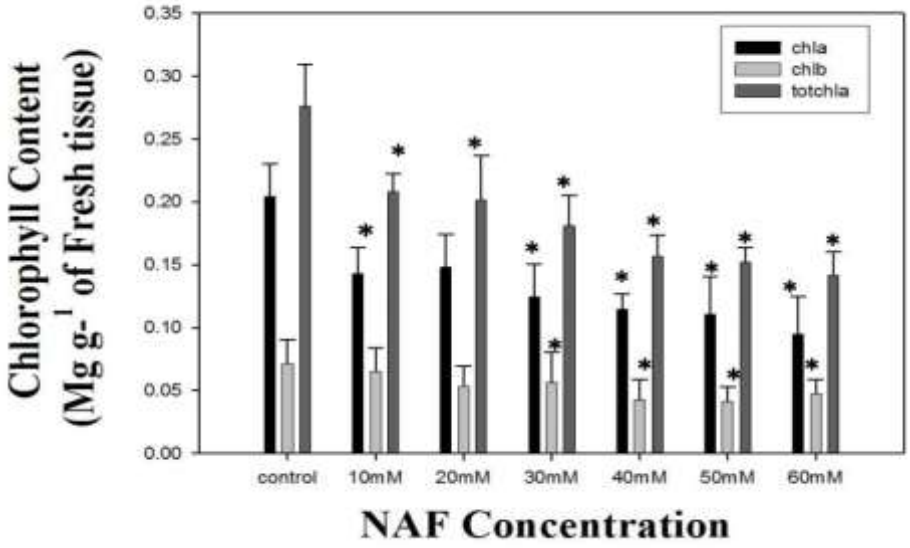

Figure 1: (a) Chlorophyll contented seedling treat by 0–60 mM fluoride (NaF) concentration used for 5 days. Bar shows standard deviation (SD), also statistics point marked by asterisks indicate to mean value be significantly dissimilar among treatment with control (*P < 0.05).

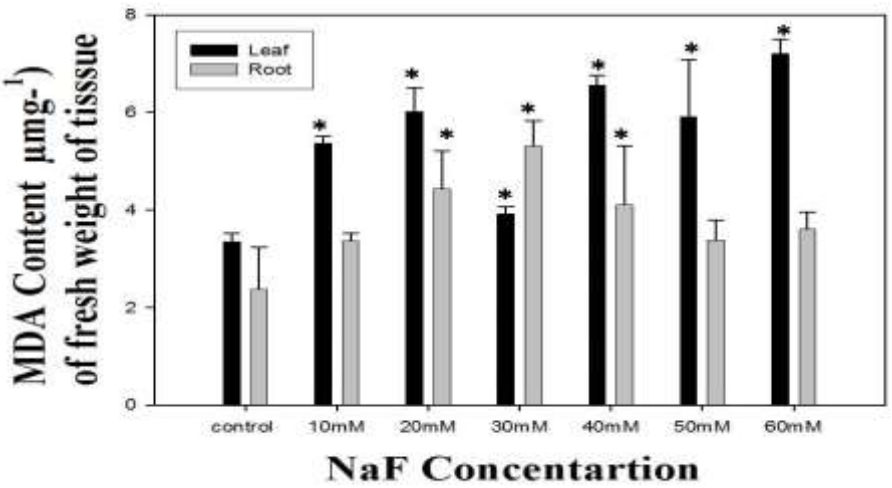

Figure 1: (b) malondialdehyde (MDA) contented level seedling treat by 0–60 mM fluoride (NaF) concentration used for 5 days. Bar shows SD, also statistics point marked

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by asterisks indicate to mean value be significantly dissimilar among treatment with control (*P < 0.05).

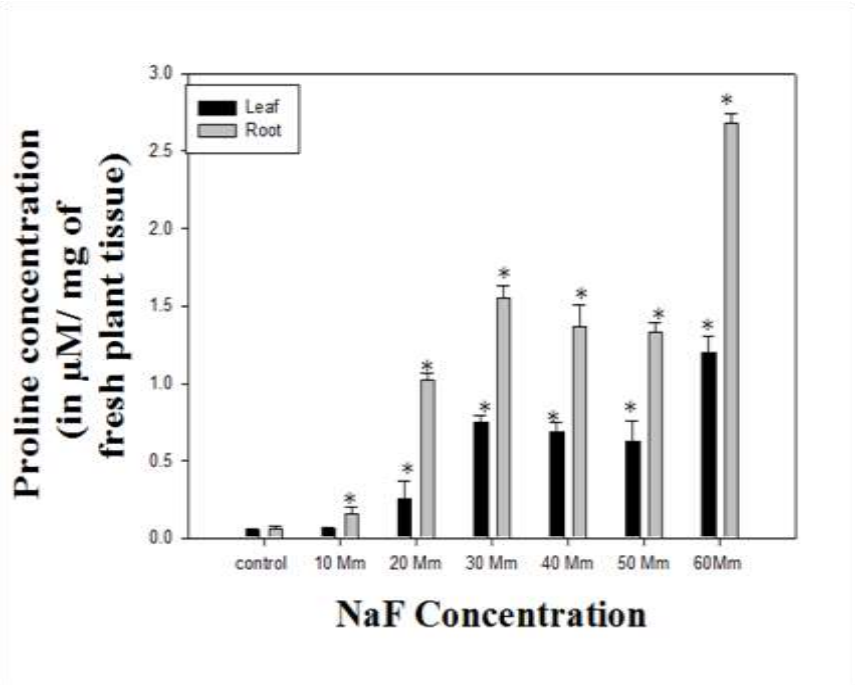

Figure 1: (c) Proline content seedling treats by 0–60 mM fluoride (NaF) concentration used for 5 days. Bar shows SD, also statistics point marked by asterisks indicates to mean value be significantly dissimilar among treatment with control (*P < 0.05)

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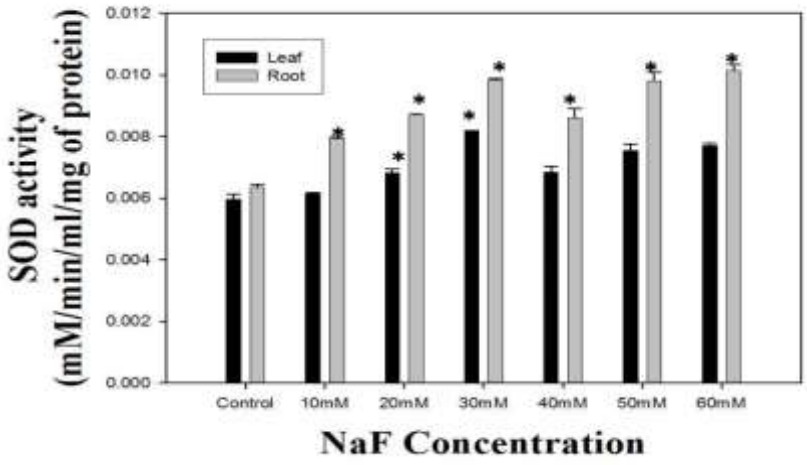

Figure 2: (a) change inside Superoxide dismutase activity within (Cyamopsis tetragonoloba L.) underneath NaF (0–60 mM) concentration value be means of three replicates, in addition to bar explain SD. Statistics point marked by asterisks signify to mean value be significantly dissimilar among treatment with control (*P < 0.05)

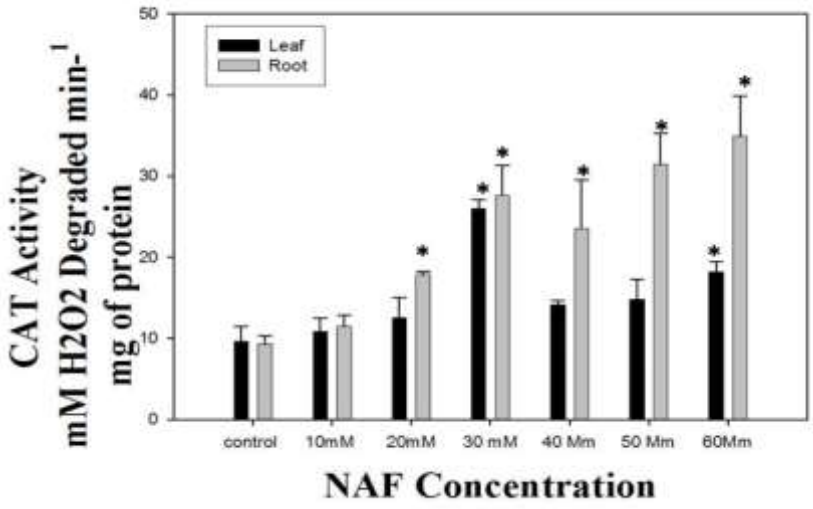

Figure 2: (b) Change inside activity CAT within (Cyamopsis tetragonoloba L.) Underneath NaF (0–60mM) concentration value is means of three replicates, in addition to bar explain standard deviation (SD). Statistics point marked by asterisks signify to mean value be significantly dissimilar among treatment with control (*P < 0.05).

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4. DISCUSSION

Similar results have been reported in *Cyamopsis tetragonoloba* L. [14]. This is combined with reduced metabolic activity in the presence of fluoride because the latter acts as a metabolic inhibitor. Increased NaF concentration showed phytotoxic effects on physiology and biochemical parameters of seedling growth. Fluoride may affect some developmental processes in grain germination. [15]. suggested that NaF can inhibit carbohydrate metabolism in sprouted seedlings. In addition, at 50 mg the moisture content of the leaves is 71.4%, which indicates that increased NaF levels cause root elongation and root length, and hence reduced length.

It is clear that by increasing the sodium fluoride treatments in,chlorophyll a, b and chlorophyll decrease because in our result in decrease In the root population, tremuloids exposed to sodium fluoride for a longer period do not affect the chlorophyll content of the level, but [16].observed that clear photosynthesis was reduced. The chloroplast membrane, stabilized by high CSI polyunsaturated lipids, indicates a more harmonious response to stress tolerance. In some studies, the inhibitory effect of fluoride on chlorophyll accumulation in sunflower (*Helianthus annuus*)[17]. [18]. *Triticum aestivum* [19].*Pisum sativum* [20]. Therefore, fluoride its effect on chlorophyll content is uncertain, but the tendency of chlorophyll accumulation under fluoride stress indicates that photosynthetic activity is adequately maintained.

One of the first signs of fluoride damage in plants is the loss of chlorophyll, which seems to be related to the damage of chloroplasts. The total chlorophyll content of the leaves was significantly reduced with increasing nutrient concentration Fluoride. The results obtained in this study, [21].The decrease in chlorophyll concentrations is due to the decomposition of chlorophyll during stress or inhibition of the participation of aminolevulinic acid in the synthetic route of chlorophyll [22] [23].The mechanisms by which visible fluoride-depressed photosynthesis are not clear are uncertain, but recommended fluoride mechanisms include enzyme inhibition, subcellular loss of organization, and chloroplast granulation [24].

In the current study, MDA content increaded with the increasing concentration of NaF. Similar results were found in previous studies. Lipid peroxidation levels increased with increasing NaF concentration.When treated with 200 mM NaF, Elite showed higher lipid peroxidation rates than those.Our results also showed an increase in H_2O_2 levels with

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increased salt treatment. However, H_2O_2 accumulation was relatively lower in *Brassica napus (*SLM046) with 200 mM NaCl treatment, Elite showed more accumulation compared to *Brassica napus* (SLM046) [25]

Plasma membrane permeability is reflected by electrolyte leakage and increases with excessive lipid peroxidation. In addition, the fluoride strain in *S. polyrhiza* resulted in increased lipid peroxidation as reported by [26].[27] observed an increase in similar electrolyte leakage rate due to fluoride toxicity in tea plants. Electrolyte leakage has been observed to increase due to increased fluoride exposure of populus deltoids[28] .

In present study,there was a gradual increase in proline concentration with an increase in fluoride. In a previous study, Significantly higher proline accumulation with 200 mM NaF was observed *Brassica napus* (SLM046) compared to other cultivars. Our results also showed significant changes in glycine betaine concentrate. The effects of different concentrations of NaCl and $CaCl_2$ on germination of mung grains. The absorbance concentrations indicates an increase in the concentration of NaCl and with a decrease in the percentage of $CaCl_2$ germination and an increase in catalase activity in terms of H_2O_2 degradation with increased salinity strain [29][30],[31].

Our data also revealed that proline accumulation and electrolyte leakage increased during stress. It has a proline osmolite can be suggest as non-enzymatic antioxidants in the fight against ROS effects produced [32].Increase the level of proline under ororide stress, maintain the osmotic potential of plants by eliminating -Oline by accumulating proline, and maintain plant damage [33] to maintain homeostasis [34].

In our current study, stimulation of increased SOD activity during all concentration periods confirms the important role of detoxification of ROS levels produced during fluoride induction. SOD in the chloroplast disrupts $O2^-$ and H_2O_2, froms H_20_2 thus preventing harmful effects [35]. The authors observed that increased SOD activity could be due to de novo synthesis of enzyme proteins. [36]. reported a similar increase in SOD activity under high fluoride stress in rice (*Oryza sativa*). The likely cause of increased SOD activity may be due to the increased rate of SOD biosynthesis at the facility during long-term fluoride exposure [37].Meanwhile, CAT, APX, POD, and GR activities were increased simultaneously during fluoride stress conditions, suggesting that *S.polyrhizac* tolerates stress by activating antioxidant defense mechanisms. Catalase directly decomposes H_2O_2 to form water and

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oxygen. Catalase activity increased steadily with increasing Fluoride concentration, 50 mg increase was 3.2-fold. a significant correlation between increased CAT activity and NaF concentration ($r = 0.969$, $p < 0.01$). Was observed Similar trends were found to increase CAT activity with increased salinity levels in *Triticum aestivum* [38]. Higher CAT activity indicates that Fluoride-tolerant lines are better able to dig H_2O_2. From the results, it is clear that *Prosopis juliflora* treated with different Fluoride concentrations shows increased CAT activities.

The increase in CAT is strongly related to the concentration of Fluoride ions, which can be compared with the previous report on the effect of cadmium in the Colomassia esculentum on enzymatic activity [39].It is known that antioxidants has an important task role in defending against free radicals in plants. Fluoride toxicity causes oxidative stress in plant leaves, and antioxidant enzymes play important roles in plants to counteract oxidative stress. Unlike iron, fluoride is not an oxidized metal, so oxidative stress in plants and tissue levels of fluoride-induced antioxidant enzymes [40].

CAT activity also increased with increasing salinity. In a greater elevation increase in CAT activity was observed compared to control variants, while Elite showed a double increase in 200 mM salt concentration. Salt-induced changes in GR activities in canola varieties are shown in. In addition, the ectofluoride stimulant of CAT activity has revealed that plants find high oxidative damage. Our results are well matched with previous reports showing that oxidative stress causes CAT activity to increase or inhibition depending on duration, type and intensity of stress [41]. [42] also observed increased catalase activity in three water macrophytes, eg Pistia stratiotes, Eichhornia crassipes, *Spirodela polyrhiza* under a concentration of 20 ppm fluoride for 10 days. Increased CAT activity includes an effective shift from H_2O_2 to H_2O and O_2, and the tolerance planting capacity has been confirmed to be confirmed to stress capacity [43].

5. CONCLUSION

This study showed that the genetic sources of SOD and CAT played an important role in protecting the antioxidants of various abiotic strains. Reduction in Growth, enhanced levels of MDA and decreased chlorophyll level in seedlings treated with NaF were recorded. Antioxidant enzymes (SOD, CAT) showed their highest elevated activity at 30 mM NaF

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treated root and leaf samples and showed a significant defense against fluoride stress in the culture. The results of this study explain that the basic plant defense response to fluoride stress occurs through SOD and CAT antioxidant enzymes. This study allows us to better understand the complexity of the defense system, including SOD and CAT against stress of NaF, which will determine its use in future research to improve fluoride-resistant strains.

6. ACKNOWLEDGMENT

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