FLUORIDE-INDUCED RESPONSES IN THE CHLOROPHYLL CONTENT AND THE ANTIOXIDANT SYSTEM IN TEA LEAVES (*CAMELLIA SINENSIS*)

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ABSTRACT: Experiments were performed to study the effects of different fluoride ion (F) concentrations (0, 1, 5, 10, 20, and 50 mg/L) on the chlorophyll content and the antioxidant system (enzymatic and non-enzymatic antioxidants) in tea leaves. The chlorophyll content in the tea leaves remained relatively stable when the concentrations of F were within 1–10 mg/L but reduced significantly when the F concentrations were ≥20 mg/L. Increased F-induced stress led to an increase in the release of superoxide anion ($^{\bullet}O_2^-$), indicating that the tea leaves were stressed by oxidation with a positive correlation between F concentration and oxidative stress. Lower concentrations of F (1–5 mg/L) increased the activities of superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), glutathione peroxidase (GPX) and dehydroascorbate reductase (DHAR) in the tea leaves, whereas higher concentrations inhibited their activities. Ascorbic acid (AsA) and reduced glutathione (GSH) remained relatively stable at lower F concentrations, but decreased at higher F concentrations. The results indicated that both the enzymatic and non-enzymatic antioxidant systems in the tea leaves would provide adequate protection against oxidative stress induced by lower F concentrations but that higher F concentrations led to an imbalance between the generation and elimination of reactive oxygen species (ROS).

Keywords: Antioxidant enzymes; Antioxidants; Chlorophyll; Fluoride; Oxidative stress; Tea leaves.

INTRODUCTION

Fluorine, accounting for 0.077% of the total earth crust, is one of the most widely distributed and abundant elements in the earth's crust. Human use of natural resources, such as in fertilizer manufacturing[1] and the smelting of bauxite,[2] increase the production and release of the fluoride ion (F), which causes environmental pollution.

Plants can absorb F from the soil, air, and water, and can also store it. The amount of F stored by the plants varies between the plant species. The baseline values of plant F content generally range from 0.5–25 mg/kg, and are usually less than 10 mg/kg. A F content of more than 50 mg/kg may lead to plant poisoning. Tea is a F-rich plant, with the vast majority of F concentrated in the leaves, especially the old leaves. The F content can be up to 2,000 mg/kg or more in the leaves.[3] Even though F is not one of the essential elements for tea trees,[4] the tree

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still grows well under high F content. However, the F-resistance mechanism in tea is unclear.

Numerous studies have shown that plants under F-induced stress exhibit reactive oxygen species (ROS) accumulation, decreased activities of antioxidant enzymes,[5] damage to DNA and protein,[6] and decreased membrane stability.[7] The plant uses the antioxidant system to tolerate stresses, that induce ROS by neutralizing the oxidative molecules. The antioxidant system comprises antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), glutathione peroxidase (GPX), and dehydroascorbate reductase (DHAR), and antioxidant compounds, such as ascorbic acid (AsA) and reduced glutathione (GSH) (Figure 1).

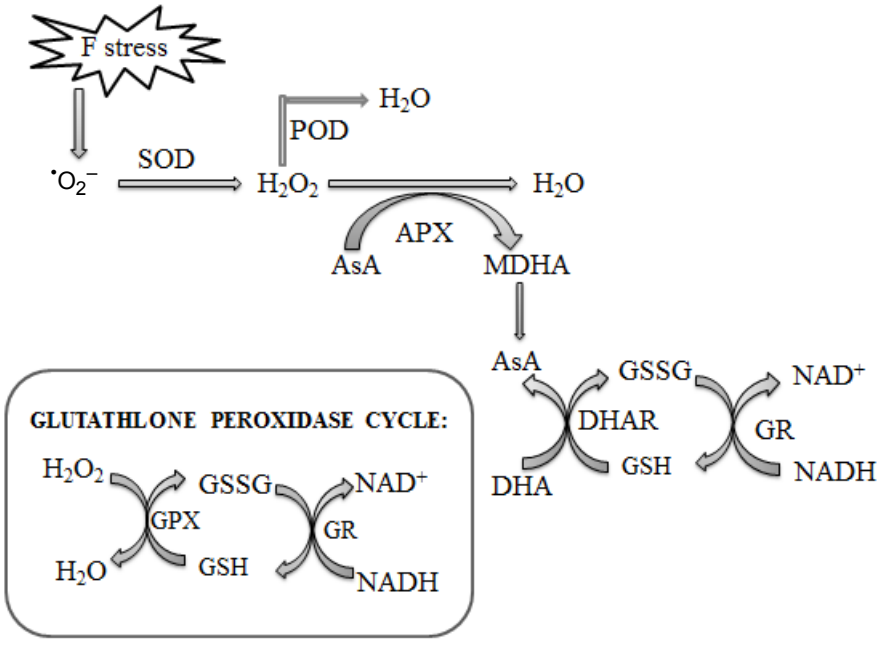

Figure 1. Reactive oxygen species (ROS) scavenging system in the tea plant with the antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), glutathione peroxidase (GPX) and dehydroascorbate reductase (DHAR), and the antioxidant compounds, ascorbic acid (AsA) and reduced glutathione (GSH). APX = ascorbate peroxidase, MDHA = monodehydroascorbate, DHA = dehydroascorbate, F = fluoride ion, H_2O_2 = hydrogen peroxide, H_2O = water, $^{\bullet}O_2^-$ = superoxide anion, GSSG = glutathione disulfide, NAD^+ = oxidised form of nicotinamide adenine dinucleotide (NAD), and NADH = reduced form of NAD.

The role of these antioxidants in F-induced stress has been studied in wheat,[8] strawberry,[9] St John's Wort (*Hypericum perforatum*),[10] mesquite,[11] and other plants. However, tea hyper-accumulates F and this enrichment of F in tea leaves compared with other plants indicates that tea is more tolerant to F. The high levels of F induces ROS accumulation as well as changes in the antioxidant system. While these are elements that must be key in the F tolerance mechanisms in tea plants, which accumulate high levels of F, there has been little investigation of the antioxidant system in F-enriched plants. Current reports on the effect of F on the

antioxidant system in tea plants looked at SOD, APX, and CAT but not at other components of the antioxidant system, such as ASA-GSH cycle or the non-enzymatic antioxidants. The present study sought to understand the effects of F stress on the antioxidant enzymes and the non-enzymatic antioxidants in a systematic manner. In the present study, F-enriched tea plants were used to study the response mechanism of tea plants to F-induced stress by examining (i) chlorophyll content (ii) lipid peroxidation of the membranes in the tea leaf by measuring malondialdehyde (MDA), (iii) ROS accumulation by measuring the superoxide anion ($^{\bullet}O_2^-$) levels, and (iv) the antioxidant system (SOD, POD, GR, DHAR, GPX, AsA, and GSH).

MATERIALS AND METHODS

Nutrient solution: One-year-old cuttings of *Camellia sinensis* (L.) O. Kuntze cv. *Shu Cha Zao*, were obtained from Shucheng County, Anhui province, China. Plants were hydroponically grown in a greenhouse with constant conditions (65% humidity; 25±2ºC during the day and 20±2ºC at night; irradiance at 250–280 μmol m^{-2} sec^{-1}) in plastic pots (with 8 seedlings each) filled with 1 dm^3 of nutrient solution (mg/L) containing 3.1 PO_4^{3-}, 30 NH_4^+, 10 NO_3^-, 40 K, 1.0 Mn, 25 Mg, 30 Ca, 0.35 Fe, 0.1 Zn, 10.8 Al, 0.025 Cu, 0.1 B, 0.05 Mo.[12] F (as NaF) was supplied at six treatment levels: 0, 1, 5, 10, 20, and 50 mg/L. Plant solutions were aerated continuously with an air bubbler and were replaced completely at 4 day intervals. After 30 days of treatment, the fully expanded leaves from the third to sixth position from the tip were frozen within liquid nitrogen and stored at –80ºC for further experiments.

Chlorophyll content: Chlorophyll levels were assayed according to Silva et al.[13] Approximately, 0.100 g leaf samples were ground in 100% (v/v) acetone and centrifuged at 5,000 g for 10 min at 4ºC, before reading the absorbance at 470, 663, and 645 nm.

Release rate of $^{\bullet}O_2^-$: The release rate of $^{\bullet}O_2^-$ was determined following the protocol of Wang et al.[14] A 1 mL aliquot of crude enzyme extract was mixed with 1 mL sodium phosphate buffer (50 mM, pH 7.8) and 1 mL hydroxylammonium chloride (10 mM). The mixture was kept for 20 min at 25ºC and then centrifuged for 10 min at 10,000 g. The supernatant was mixed with 17 mM sulphanilic acid and 7 mmol L^{-1} 1-naphthylamine. Ethyl ether (3 mL) was added after incubation at 25ºC for 20 min. The mixture was centrifuged at 10,000 g for 5 min. Absorbance of the water phase at 530 nm was recorded.

MDA content: Leaf samples (0.500 g) were homogenized in an aquatic solution of trichloroaceticacid (TCA, 5% w/v, 5 mL) and then centrifuged at 10,000 g at 4ºC for 15 min. The supernatant was used for determination of malondialdehyde (MDA) content by thiobarbituric acid (TBA) method.[15]

Determination of activities of antioxidative enzymes: For determination of antioxidative enzymes activities, leaf samples (0.500 g) were ground with a pre-cooled mortar and pestle in 5 mL ice-cold extraction buffer containing 50 mM K_2PO_4-KOH (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 5%

(w/v) insoluble polyvinylpyrrolidone (PVPP). Homogenate was centrifuged at 12,000 rpm for 20 min at 4ºC. The supernatant was used to measure the activities of POD, DHAR, SOD, GPX, and GR. The activity of DHAR was measured in a reaction solution containing 50 mM PBS (pH 7.0), 0.5 mM DHA, 2.5 mM GSH.[16] Activity of POD in tea leaves was measured according to Kochba et al.[17] The activities of SOD, GPX, and GR were determined by using the assay kit (Nanjing Jiancheng Bioengineering Institute, China).

Determination of AsA and GSH: Leaf issues were homogenized at 4ºC in ice-cold 5% trichloroacetic acid (w/v). After centrifugation at 10,000 g for 15 min, the supernatant was brought up to 10 mL, which was used to determine the content of AsA, dehydroascorbic acid (DHA), GSH, and oxidized glutathione (GSSG). Determination of AsA and DHA was according to Kampfenkel et al.[18] The analysis of GSH and GSSG in the samples was according to Nazar et al.[19]

Statistical analysis: Statistical analysis was performed using the SPSS statistical package (version 17.0). Analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test were carried out to determine the significance of difference ($p<0.05$). All values reported are means of at least three independent measurements.

RESULTS

Chlorophyll content: Chlorophyll content is an important indicator of the photosynthetic capacity of a plant. Differences in chlorophyll a and b content in the leaves of plants treated with 0–50 mg/L F are shown in Table 1.

Table 1. Effect of fluoride (F) on the chlorophyll content of tea plant leaves. (Values are mean±SE, n=3, FW=fresh weight)

F concentration (mg/L)	Chlorophyll a+b (mg/g leaves FW)	Chlorophyll a (mg/g leaves FW)	Chlorophyll b (mg/g leaves FW)	Ratio of chlorophyll a/b
0	2.728±0.285^{a}	2.010±0.208^{a}	0.717±0.077^{a}	2.804±0.033^{c}
1	2.672±0.146^{abc}	1.998±0.104^{a}	0.674±0.043^{ab}	2.966±0.045^{b}
5	2.786±0.153^{a}	2.082±0.123^{a}	0.703±0.031^{a}	2.958±0.062^{b}
10	2.418±0.298^{abcd}	1.837±0.232^{ab}	0.580±0.066^{bc}	3.163±0.068^{a}
20	2.385±0.178^{bcd}	1.809±0.137^{ab}	0.576±0.041^{c}	3.142±0.052^{a}
50	2.300±0.062^{d}	1.727±0.049^{b}	0.573±0.016^{c}	3.015±0.063^{b}

^{abcd}Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

The chlorophyll content in the tea leaves decreased with an increase in the F concentration. The chlorophyll a+b content in the 20 mg/L and 50 mg/L F treatment groups decreased by 12.6% and 15.7%, respectively, compared with the

control group (0 mg/L F). The chlorophyll a and b levels in the tea leaves responded differentially to F-induced stress. Chlorophyll a was not sensitive to F-induced stress and only the leaves in the 50 mg/L F treatment group showed differences compared with the control. However, the chlorophyll b content reduced significantly when the F concentration was ≥10 mg/L. This was reflected in changes in the ratio of chlorophyll a/b.

Levels of ROS: Fluoride treatment resulted in an increased release rate of $^{\bullet}O_2^-$ for all F treatment groups, except for the 1 mg/L group, compared with the control group. The superoxide anion release rate increased by 84.53% and 94.28% (Figure 2), respectively, in the plants treated with 20 mg/L and 50 mg/L F, compared to the control group. MDA concentrations in the leaves of tea plants treated with 0–5 mg/L F remained stable. However, when the F concentration was increased to 10 mg/L, the MDA concentration increased significantly, with a positive correlation to the F concentration (data not shown).

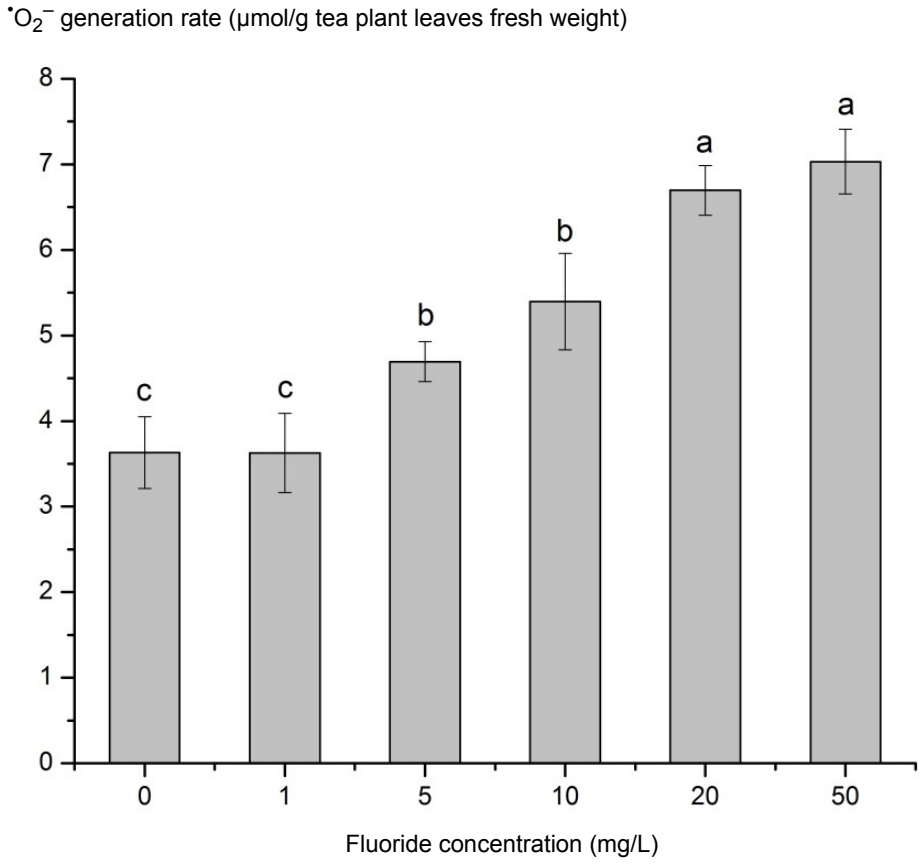

Figure 2. Effect of fluoride on the superoxide anion, $^{\bullet}O_2^-$, generation rate in tea plant leaves (μmol/g tea plant leaves fresh weight).
Values are mean±SE, n=3.
[abc]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

Activities of antioxidant enzymes: SOD, GR, POD, DHAR, and GPX are enzymatic antioxidants that play important roles in the antioxidant system. SOD activity increased significantly when the tea leaves were treated with 1 mg/L F, reaching a peak value 45.86% greater than the control (Figure 3). F concentrations higher than 5 mg/L inhibited SOD activity, which decreased gradually in accordance with the F concentration to a final value 71.25% lower than in the control group.

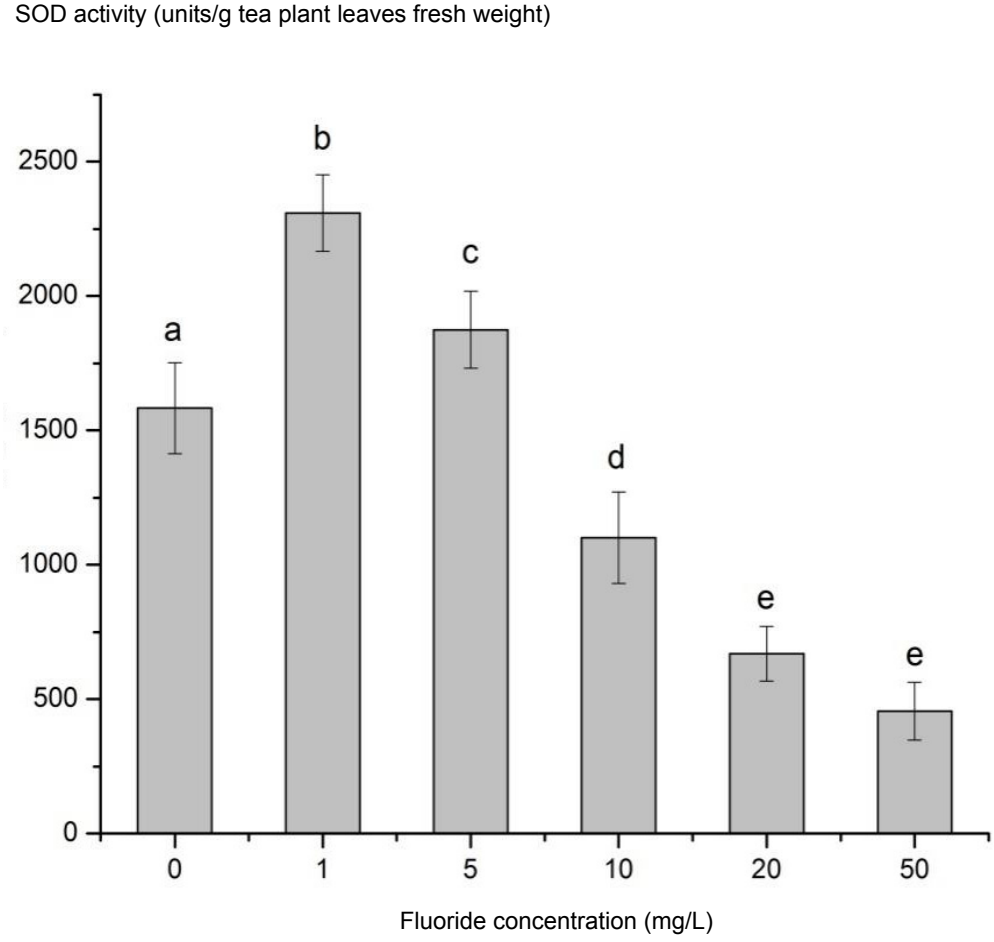

Figure 3. Effect of fluoride on activity of the antioxidant enzyme superoxide dismutase, SOD, in tea plant leaves (units/g tea plant leaves fresh weight).
Values are mean±SE, n=3.
[abcde]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

The activity of POD increased significantly by 19.20% with treatment of 5 mg/L compared with the control group. POD activity decreased significantly when the F concentration was greater than 10 mg/L and at 50 mg/L the activity decreased by 41.17% (Figure 4).

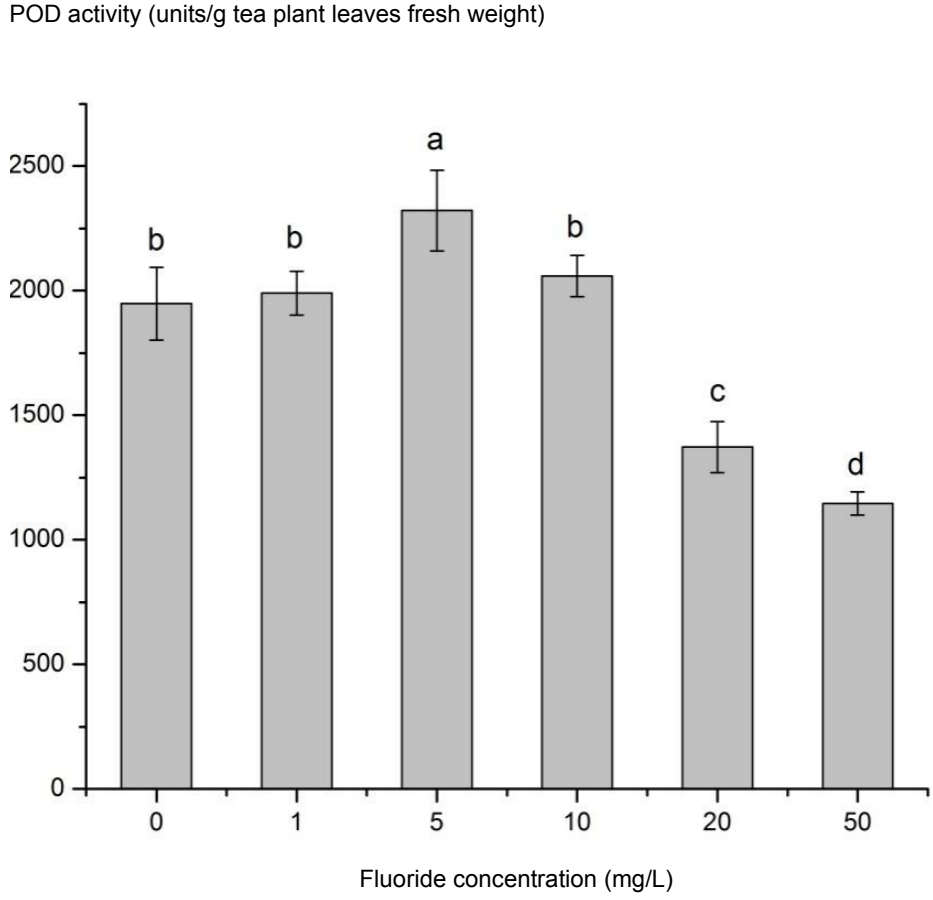

Figure 4. Effect of fluoride on activity of the antioxidant enzyme peroxidase, POD, in tea plant leaves (units/g tea plant leaves fresh weight).
Values are mean±SE, n=3.
[abcd]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

The activity of GPX showed a decreasing trend with increasing F concentrations (Figure 5). The activity of tea leaves decreased by about 11.34% when the concentrations of F were within 1–5 mg/L. When the F concentration was greater than 5 mg/L, the activity of GPX significantly declined with increasing concentration of F, decreasing by 52.96% at the highest F treatment compared with the control group.

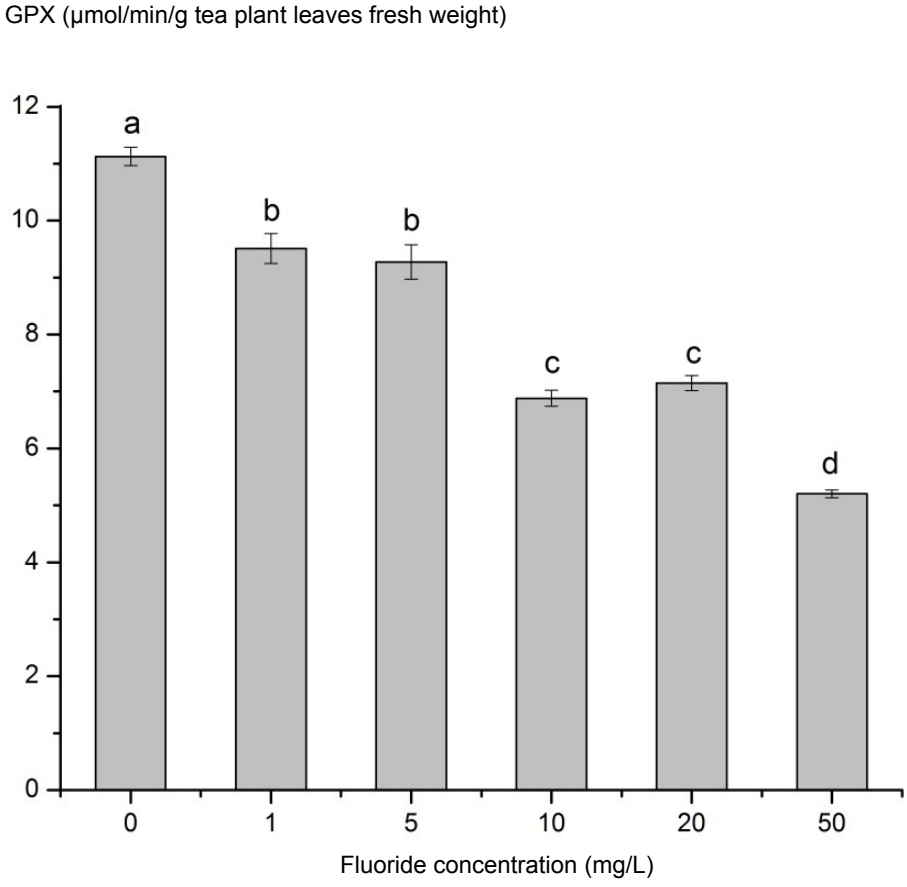

Figure 5. Effect of fluoride on activity of the antioxidant enzyme glutathione peroxidase, GPX, in tea plant leaves (μmol/min/g tea plant leaves fresh weight).
Values are mean±SE, n=3.
[abcd]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

GR enzyme activity increased slightly in leaves treated with 5 mg/L F, and reached a peak value at 10 mg/L F with an increase of 61.54% compared to the control group. Increased concentration of F over that suppressed the GR activity, in a declining trend (Figure 6).

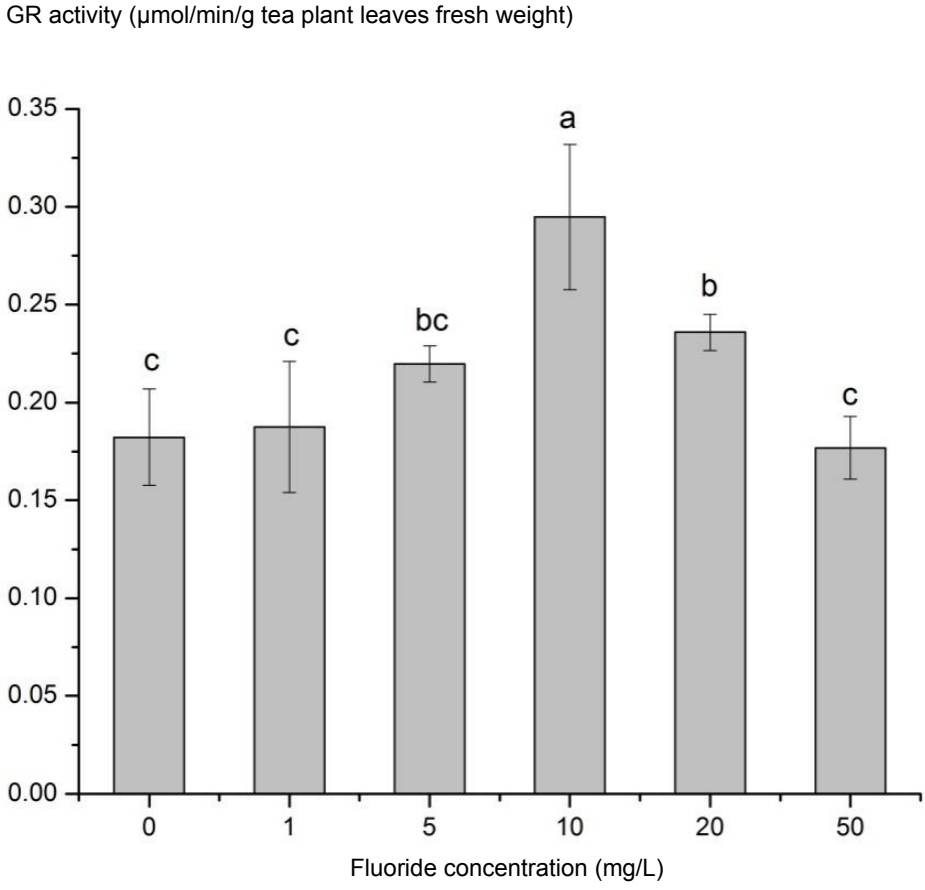

Figure 6. Effect of fluoride on activity of the antioxidant enzyme glutathione reductase, GR, in tea plant leaves (μmol/min/g tea plant leaves fresh weight).
Values are mean±SE, n=3.
[abc]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

DHAR activity increased slightly with 1 mg/L F treatment, but no significant differences were found between 5 mg/L F treatment and control group (Figure 7). However, DHAR activity declined sharply in plants treated with ≥10 mg/L F, with decreases of 32.69%, 35.58%, and 42.27% in plants treated with 10 mg/L, 20 mg/L, and 50 mg/L F, respectively, compared to the control group.

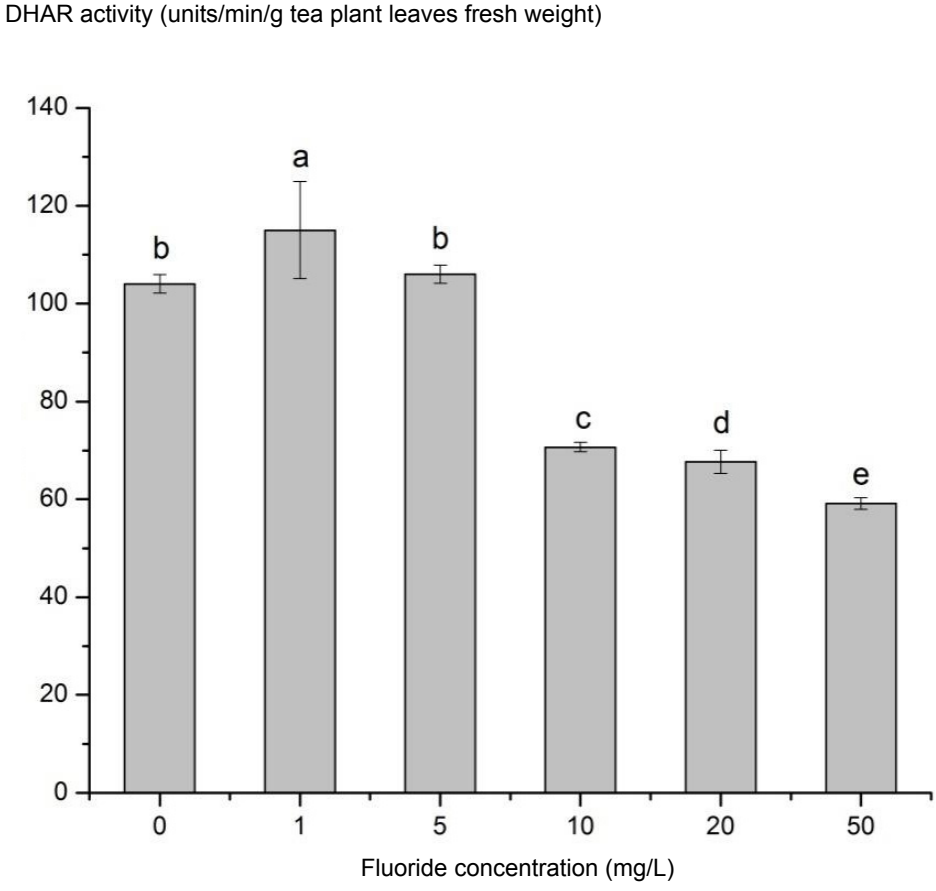

Figure 7. Effect of fluoride on activity of the antioxidant enzyme dehydroascorbate reductase, DHAR, in tea plant leaves (units/min/g tea plant leaves fresh weight).
Values are mean±SE, n=3.
[abcde]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

Content of non-enzymatic antioxidants: AsA and GSH are important non-enzymatic antioxidants in plants. Treatment of tea leaves with F impacts the contents of both AsA and GSH. At 1–20 mg/L F treatment, AsA showed no differences with the control group but the level significantly decreased by 57.14% at 50 mg/L F treatment (Figure 8).

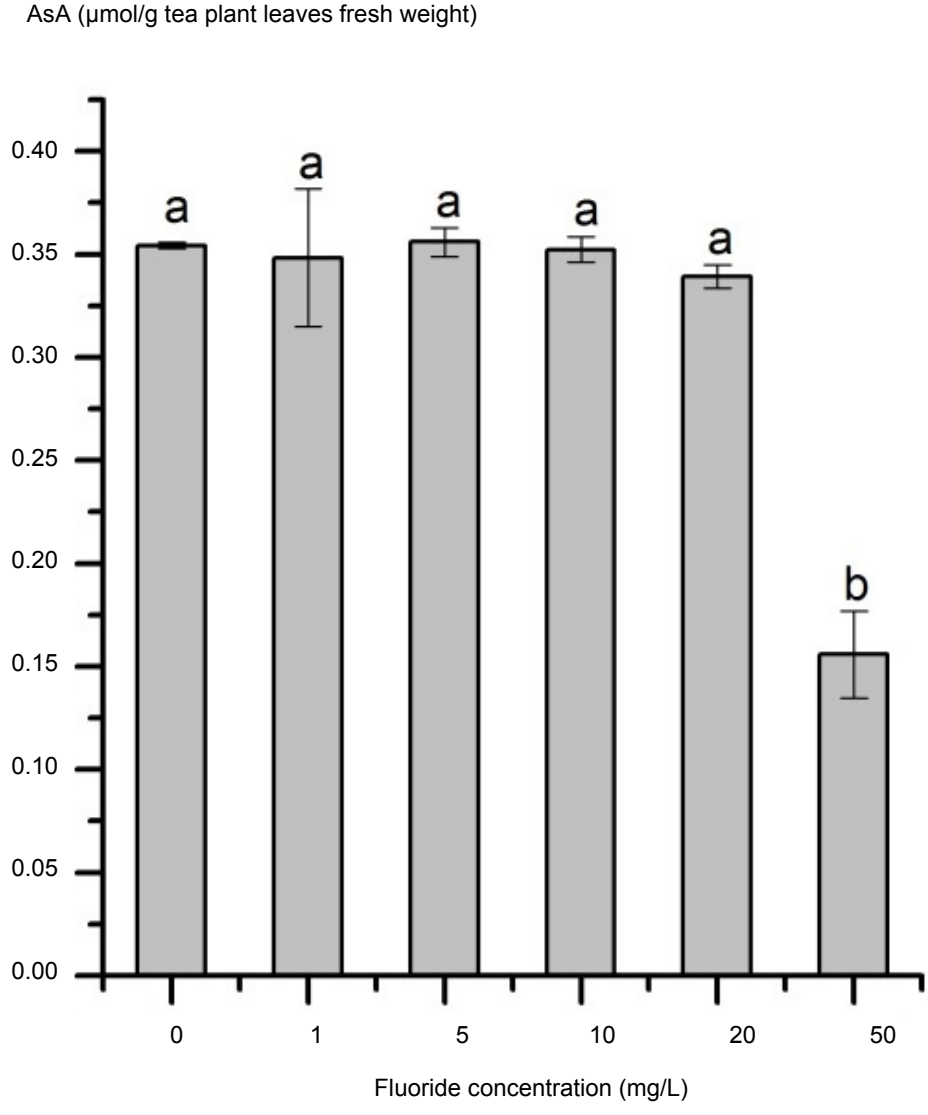

Figure 8. Effect of fluoride on content of the antioxidant compound, ascorbic acid, AsA, in tea plant leaves (µmol/g tea plant leaves fresh weight).
Values are mean±SE, n=3.
[ab]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

DHA contents in the tea leaves increased with an increase in the F concentration (Figure 9).

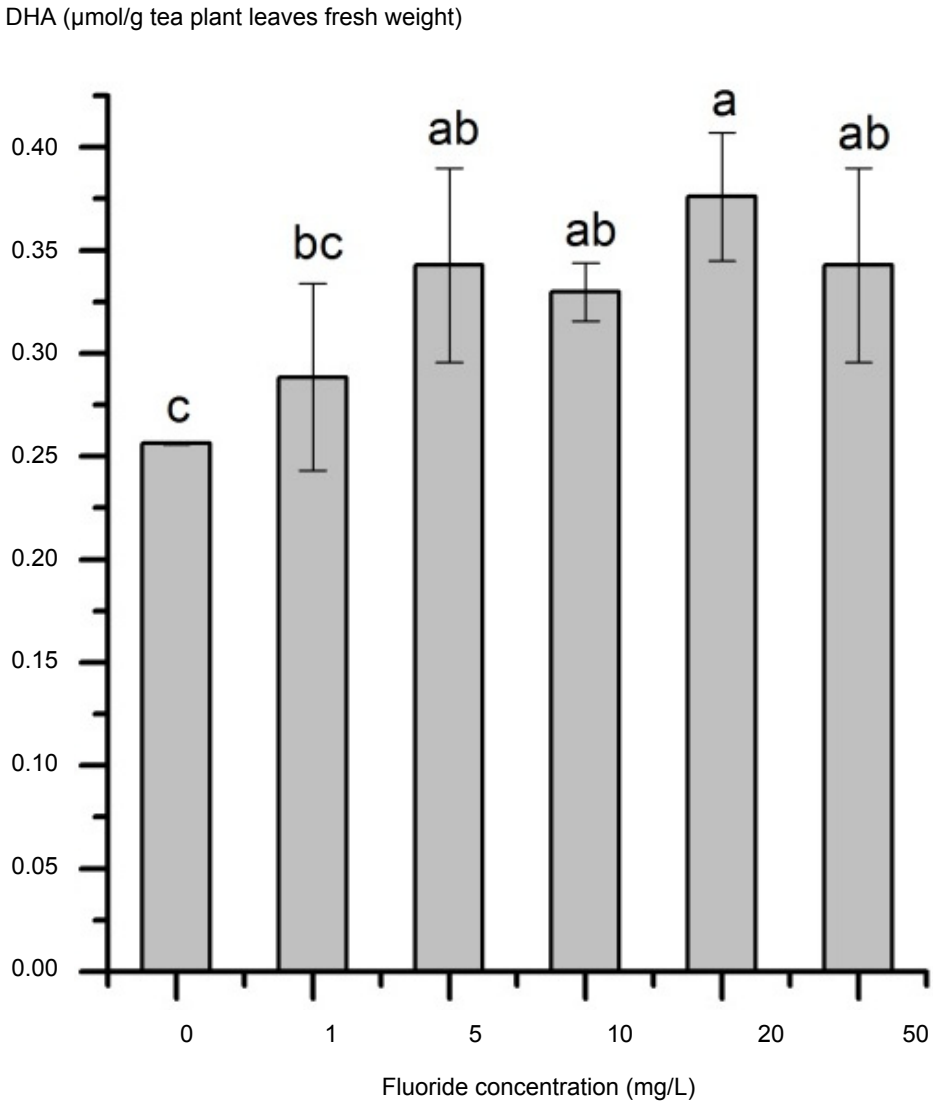

Figure 9. Effect of fluoride on content of dehydroascorbate, DHA, in tea plant leaves (μmol/g tea plant leaves fresh weight).
Values are mean±SE, n=3.
[abc]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

The GSSG level was not significantly changed at 1 mg/L F concentration compared to the control group, but rapidly declined at and above 5 mg/L F concentration (Figure 10).

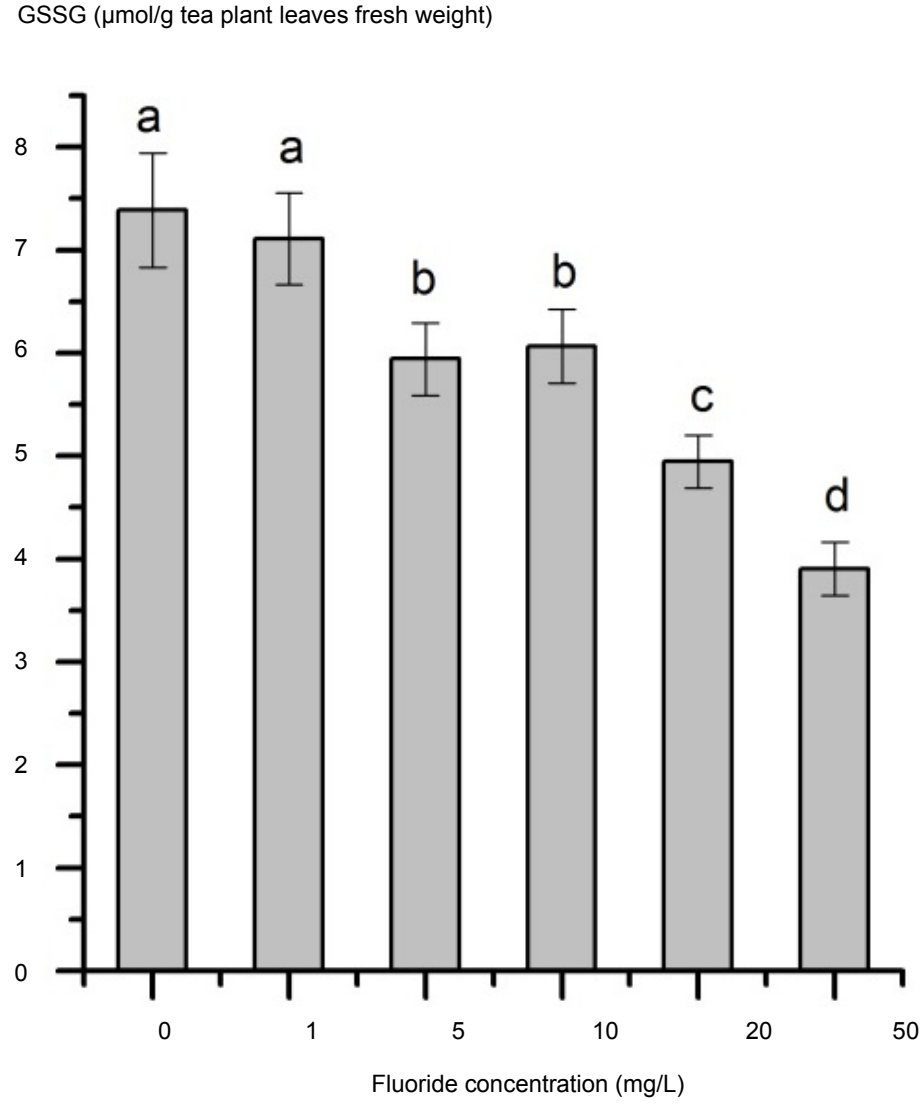

Figure 10. Effect of fluoride on content of glutathione disulfide, GSSG, in tea plant leaves (μmol/g tea plant leaves fresh weight).
Values are mean±SE, n=3.
[abcd]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

The GSH level was not significantly changed at 1 mg/L F concentration compared to the control group, but rapidly declined at and above 5 mg/L F concentration (Figure 11).

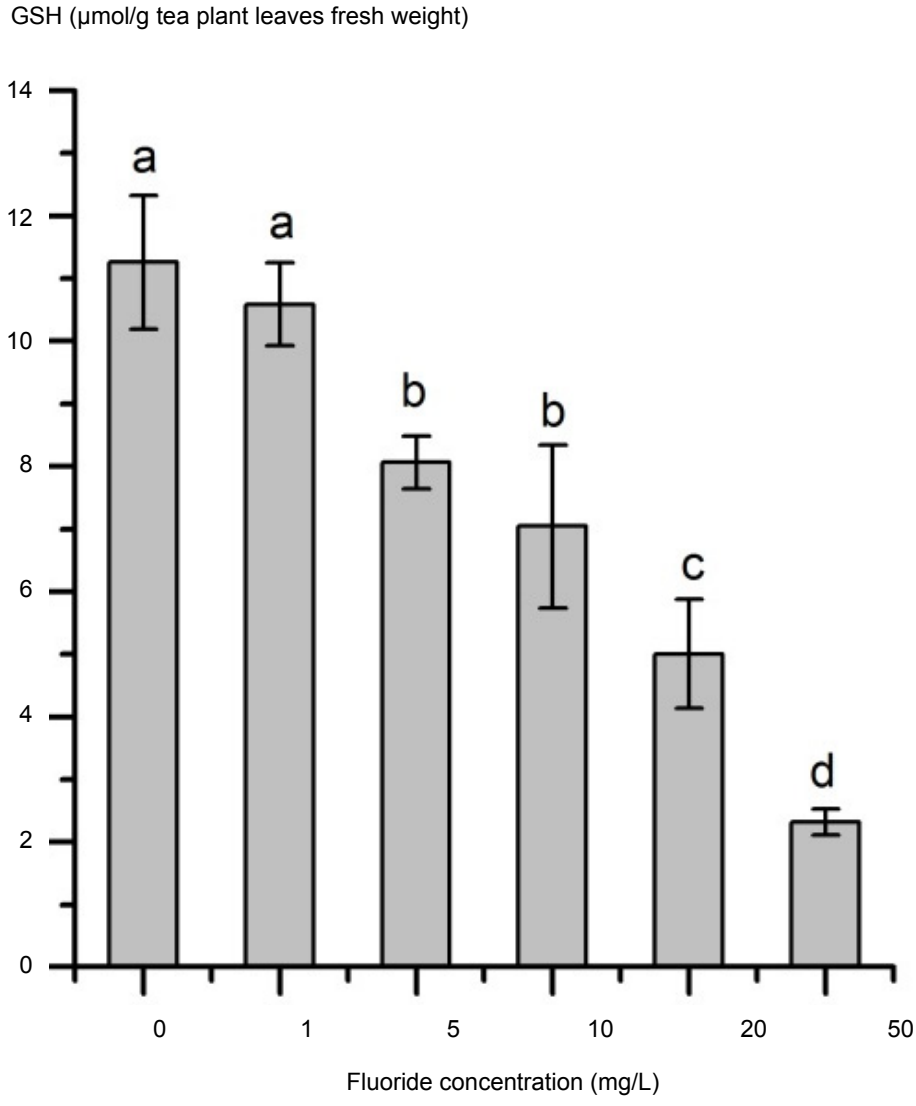

Figure 11. Effect of fluoride on content of the antioxidant compound reduced glutathione, GSH, in tea plant leaves (µmol/g tea plant leaves fresh weight).
Values are mean±SE, n=3.
[abcd]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

The redox pairs GSH/GSSG and AsA/DHA are important reduction/ oxidation pairs in plants relevant to stress tolerance. The GSH/GSSG ratios were similar to the control in plants treated with low F concentrations (1–5 mg/L), but declined significantly when the F concentration was greater than 10 mg/L (Figure 12).

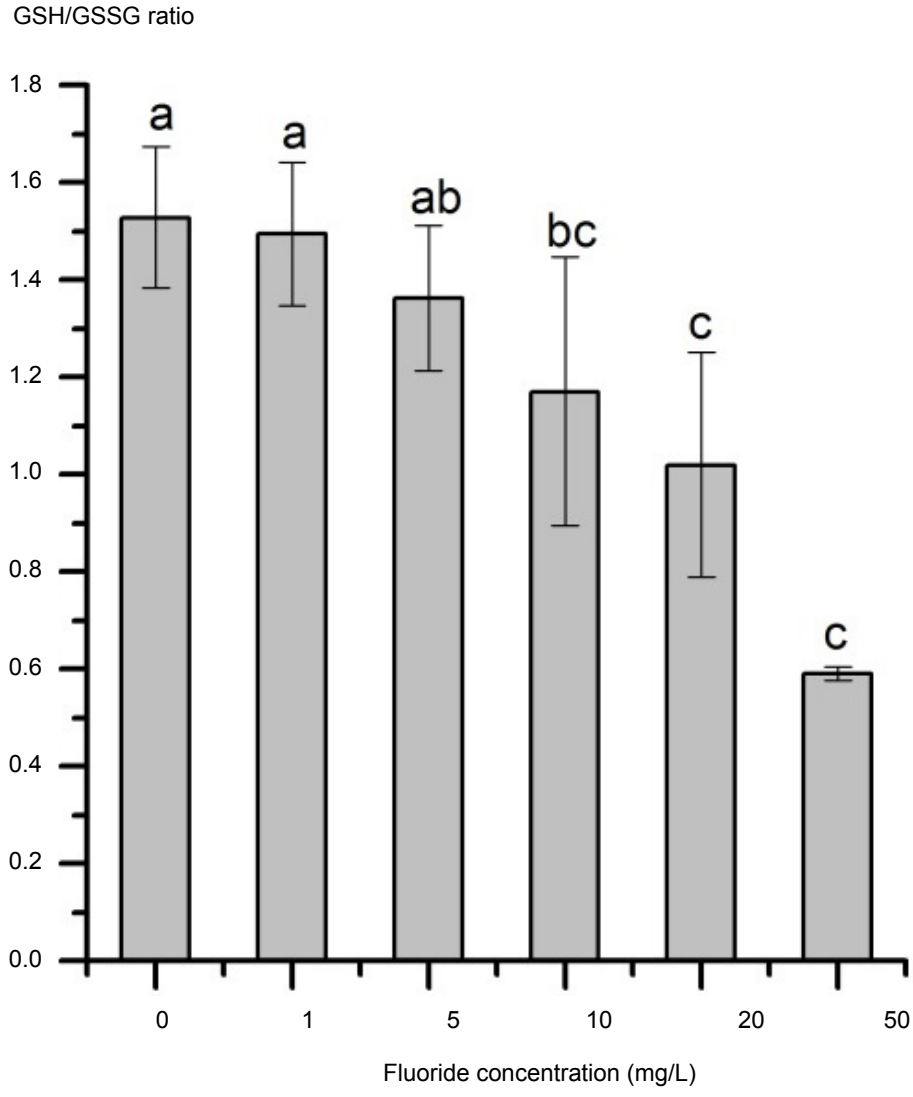

Figure 12. Effect of fluoride on the reduced glutathione/glutathione disulfide ratio (GSH/GSSG) in tea plant leaves.
Values are mean±SE, n=3.
[abc]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

The AsA/DHA ratios decreased significantly with increasing F concentrations. Together, this data indicated that F-induced stress first disrupted the AsA/DHA balance, whereas the GSH/GSSG balance was altered only under high F-induced stress (Figure 13).

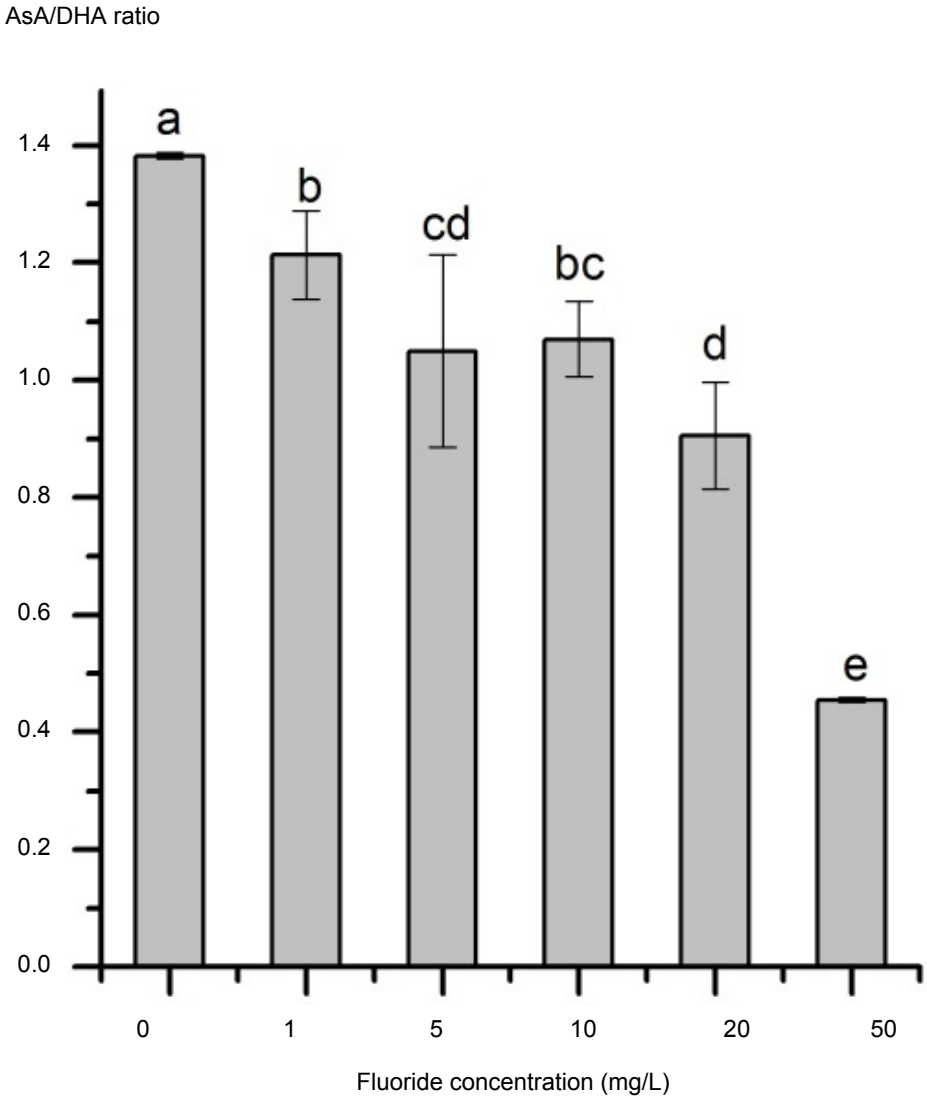

Figure 13. Effect of fluoride on the ascorbic acid/dehydroascorbate ratio, AsA/DHA, in tea plant leaves.
Values are mean±SE, n=3.
[abcde]Different lower case letters indicate the presence of a significant difference, $p<0.05$, whereas the same lower case letters denote no significant difference.

DISCUSSION

Chlorophyll content and its stability play an important role in the maintenance of normal photosynthesis in plants under stress, thereby enhancing the abiotic stress tolerance of the plants. In this study, we found that chlorophyll a + b content decreased by 15.7% when tea leaves were treated with a high concentration of F (50 mg/L). Joshi et al.[20] also found that F contamination in wheat (2.26–13.0 mg/L) reduced the content of wheat chlorophyll to varying degrees. Similarly, Chakrabarti et al.[21] demonstrated that 30 mg/L of NaF reduced the chlorophyll content of chickpea seedlings by 72.34%. Eyini et al. found that F treatment reduced the content of chlorophyll in plants.[22] All these studies have shown that chlorophyll content under F-induced stress maintained high stability in plants other than tea trees. High F may reduce chlorophyll by entering the chloroplasts in the form of F ions, which can bind to the central complexed Mg^{2+} in the porphyrin ring, thereby undermining the chlorophyll molecules and resulting in decreased chlorophyll content.[23] Tea chlorophyll a/b ratio increased with an increase in the F concentration (Table 1), indicating that chlorophyll b may be more sensitive to F in response to stress.

Active oxygen metabolism plays a very important role in plant defense and tolerance to abiotic stress.[24] Reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$), are produced in plants experiencing abiotic stresses such as drought, high salinity, extreme temperatures, high irradiance, UV light, nutrient deficiency, and air pollution.[25] ROS levels within a certain range do not damage the plants but excessive ROS accumulation results in oxidative damage, thereby affecting the normal metabolism of tea leaves. Plant cells generate ROS in a variety of ways, resulting in oxidative damage and the accumulation of malondialdehyde (MDA), a marker for oxidative stress, and this damage is strengthened under environmental stress.[11] In the present study, we showed that the $^{\bullet}O_2^-$ levels increased significantly when the F concentration was ≥5 mg/L compared to the control group. That is, F stress resulted in an increased level of ROS in tea leaves. Moreover, when the F concentration was ≥10 mg/L, the accumulation of MDA in the tea leaves caused oxidative stress. Wilde et al.[5] reported that F caused an accumulation of $^{\bullet}O_2^-$ in green beans. $^{\bullet}O_2^-$ in plants can be transformed into toxic $^{\bullet}OH$, which causes oxidation of fatty acids, promotes the formation of harmful peroxides, and leads to increased accumulation of MDA.

Under adverse stress, ROS levels in plants are regulated by both enzymatic and non-enzymatic antioxidants. SOD is the first line of defense against ROS. SOD converts $^{\bullet}O_2^-$ into H_2O_2. The resulting H_2O_2 is catalyzed by POD and other enzymes to form harmless H_2O and O_2. POD thus has a dual role, both in conversion of $^{\bullet}O_2^-$ into H_2O_2 and in direct removal of H_2O_2.[26] GPX uses GSH to reduce H_2O_2 and organic and lipid hydroperoxides and therefore helps plant cells avoid oxidative stress.[27] DHAR and GR are also key enzymes in the antioxidant system that eliminate ROS. Thus, the antioxidant capacity of plants is a result of the synergy between various antioxidant enzymes. In this study, F treatment

resulted in an initial increase in SOD, POD, DHAR, and GR activities in the tea leaves, activity levels which later declined with increasing F concentrations. Wilde and Yu[5] and Saini et al.[11] observed the same trend when they studied SOD in almond seedlings and mimosa leaves, respectively, under F-induced stress. Reddy and Kaur[28] found that under F-induced stress, POD activity decreased significantly in the salt-tolerant dicot *Salicornia*. Different antioxidant enzyme activity responses and trends were also found in different plants at different levels under varied F concentrations, which may be due to species differences in the genus. These results demonstrated that tea leaves showed enhanced activity of antioxidant enzymes that were able to eliminate excessive ROS in response to low F concentrations. However, at higher F concentrations, these enzyme activities were eventually inhibited.

Non-enzymatic antioxidants (AsA and GSH) are also involved in active oxygen scavenging in plants. AsA is involved in the clearance of H_2O_2 and the regeneration of the lipophilic antioxidant α-tocopherol.[29] GSH can be involved in glutathione-S-transferase-mediated peroxide scavenging reactions, as well as in glutathione peroxidase-mediated reduction of lipid peroxides and organic peroxide.[30] High F concentrations decreased AsA and GSH levels significantly, which may be due to reduction of AsA and GSH regeneration and decreased DHAR, GR, and GPX activities.[31] Adaptation of plants to environmental stress is not associated with an increase or reduction of a single component of the various oxidized or reduced forms of GSH or AsA, but is associated with the balance formed in the entire redox system.[32,33] Therefore, the protective effects of AsA and GSH in plants rely more on regulation of the entire oxidation and reduction potentials. The GSH/GSSG and AsA/DHA ratios decreased with increasing F concentrations and when the F concentration was 50 mg/L, the ratios decreased by 61.18% and 67.39%, respectively, indicating that high F concentrations produced excessive ROS, thereby leading to oxidative stress.

CONCLUSIONS

Our data demonstrated that the tea plant is able to cope with low levels of F stress (1–10 mg/L). The chlorophyll content in tea leaves remained relatively stable, which helps maintain normal photosynthesis and improves the ability to resist F-induced stress. With increasing F concentrations, the levels of ROS and MDA were also increased. The whole antioxidant system, both enzymatic and non-enzymatic systems, cooperates to regulate the balance of the redox reactions in tea plant, thereby tolerating the F stress. At lower F concentrations (1–5 mg/L), tea leaves can regulate their own antioxidant system to prevent the production of excess ROS, which may be one of the possible mechanisms for F-tolerance. When the F-induced stress exceeds the limit that the tea leaves can withstand, the antioxidant enzyme activity was inhibited, allowing changes in the GSH/GSSG and AsA/DHA ratios and resulting in ROS accumulation and, ultimately, oxidative stress.

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REFERENCES

1 Loganathan P, Hedley M, Wallace G, Roberts A. Fluoride accumulation in pasture forages and soils following long-term applications of phosphorus fertilisers. Environ Pollut 2001;115(2):275-82.
2 Mackowiak C, Grossl P, Bugbee B. Biogeochemistry of fluoride in a plant-solution system. J Environ Qual 2003;32(6):2230-7.
3 Cai HM, Peng CY, Chen J, Hou RY, Gao HJ, Wan XC. X-ray photoelectron spectroscopy surface analysis of fluoride stress in tea (*Camellia sinensis* (L.) O. Kuntze) leaves. J Fluorine Chem 2014;158:11-5.
4 Chan L, Mehra A, Saikat S, Lynch P. Human exposure assessment of fluoride from tea (Camellia sinensis L.): a UK based issue? Food Res Int 2013;51(2):564-70.
5 Wilde LG, Yu M. Effect of fluoride on superoxide dismutase (SOD) activity in germinating mung bean seedlings. Fluoride 1998;31:81-8.
6 Dey U, Mondal NK, Das K, Datta JK. Dual effects of fluoride and calcium on the uptake of fluoride, growth physiology, pigmentation, and biochemistry of bengal gram seedlings (*Cicer arietinum* L.). Fluoride 2012;45(4):389-93.
7 Ram A, Verma P, Gadi BR. Effect of fluoride and salicylic acid on seeding growth and biochemical parameters of watermelon (*Citrullus lanatus*). Fluoride 2014;47(1):49-55.
8 Bhargava D, Bhardwaj N. Effect of sodium fluoride on seed germination and seedling growth of *Triticum aestivum* var. Raj. 4083. J Phytol 2010;2(4):41-3.
9 Kumar KA, Varaprasad P, Rao AVB. Effect of fluoride on catalase, guiacol peroxidase and ascorbate oxidase activities in two varieties of mulberry leaves (*Morus alba L.*). Res J Earth Sci 2009;1:69-73.
10 Fornasiero RB. Phytotoxic effects of fluorides. Plant Sci 2001;161(5):979-85.
11 Saini P, Khan S, Baunthiyal M, Sharma V. Effects of fluoride on germination, early growth and antioxidant enzyme activities of legume plant species *Prosopis juliflora*. J Environ Biol 2013; 34(2):205-9
12 Konishi S. Promotive effects of aluminium on tea plant growth. Jarq-Jpn Agr Res Q 1992;26:26.
13 Silva S, Pinto G, Dias MC, Correia CM, Moutinho-Pereira J, Pinto-Carnide O, et al. Aluminium long-term stress differently affects photosynthesis in rye genotypes. Plant Physiol Bioch 2012;54:105-12.
14 Wang R, Chen S, Zhou X, Shen X, Deng L, Zhu H, et al. Ionic homeostasis and reactive oxygen species control in leaves and xylem sap of two poplars subjected to NaCl stress. Tree Physiol 2008;28(6):947.
15 Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot 1981;32(1):93-101.
16 Hossain MA, Asada K. Inactivation of ascorbate peroxidase in spinach chloroplasts on dark addition of hydrogen peroxide: its protection by ascorbate. Plant Cell Physiol 1984;25(7):1285-95.
17 Kochba J, Lavee S, Spiegel-Roy P. Differences in peroxidase activity and isoenzymes in embryogenic and non-embryogenic 'Shamouti' orange ovular callus lines. Plant Cell Physiol 1977;18(2):463-7.
18 Kampfenkel K, Vanmontagu M, Inze D. Extraction and determination of ascorbate and dehydroascorbate from plant tissue. Anal Biochem 1995;225(1):165-7.

19 Nazar R, Iqbal N, Syeed S, Khan NA. Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mung bean cultivars. J Plant Physiol 2011;168(8):807-15.
20 Joshi M, Bhardwaj N. Effect of fluoride on growth parameters and its accumulation in *Triticum aestivum* var. Raj 3675. Fluoride 2012;45(3 Pt 2):297-301.
21 Chakrabarti S, Patra PK, Mandal B, Mahato D. Effect of sodium fluoride on germination, seeding growth, and biochemistry of Bengal gram (*Cicer arieninum*). Fluoride 2012;45(3 Pt 2):257-62.
22 Eyini M, Sujanandini K, Pothiraj C, Jayakumar M, Kil BS. Differential response of *Azolla microphylla* Kaulf. and *Azolla filiculoides* Lam. to sodium fluoride. J Plant Biol 1999;42(4):299-301.
23 Weinstein LH, Davison A. Fluorides in the environment: effects on plants and animals. Wallingford, Oxon, OX, UK: CABI Publishing, a division of CAB International; 2004.
24 Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 2002;7(9):405-10.
25 Lin CC, Kao CH. Effect of NaCl stress on H_2O_2 metabolism in rice leaves. Plant Growth Regul 2000;30(2):151-5.
26 Šimonovičová M, Huttová J, Mistrík I, Široká B, Tamás L. Root growth inhibition by aluminum is probably caused by cell death due to peroxidase-mediated hydrogen peroxide production. Protoplasma 2004;224(1):91-8.
27 Noctor G, Gomez L, Vanacker H, Foyer CH. Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signalling. J Exp Bot 2002;53(372):1283-304.
28 Reddy MP, Kaur M. Sodium fluoride induced growth and metabolic changes in *Salicornia brachiata* Roxb. Water Air Soil Poll 2008;188(1-4):171-9.
29 Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Bioch 2010;48(12):909-30.
30 Noctor G, Foyer CH. Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Biol 1998;49(1):249-79.
31 Chaparzadeh N, D'Amico ML, Khavari-Nejad R-A, Izzo R, Navari-Izzo F. Antioxidative responses of *Calendula officinalis* under salinity conditions. Plant Physiol Bioch 2004;42(9):695-701.
32 Amor NB, Jiménez A, Megdiche W, Lundqvist M, Sevilla F, Abdelly C. Response of antioxidant systems to NaCl stress in the halophyte *Cakile maritima*. Physiol Plantarum 2006;126(3):446-57.
33 Asada K. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu Review Plant Biol 1999;50(1):601-39.