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Fluoride Uptake in Edible Parts of Plants and Its Impact on Plant Defence Mechanism

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Abstract: **Radish ((*Raphunus sativa*), Coriander (*Coriandrum sativum*), Mustard (*Brassica juncea*) and Spinach (*Spinacea oleracea*) plants were grown in earthen pots watered with aqueous solutions of 0.31(tap water), 5, and 10mgL^{-1} fluoride (NaF). Inorganic F uptake and its influence on pigments like total chlorophyll (mg/g), carotenoids (µg/g) and antioxidants like catalase, peroxidase (unit/min/g) activity, free sugar, ascorbic acid and free amino acid content (mg/g) were estimated from the edible plant parts which were harvested after 60 days. The results show that the peroxidase acitivity, free amino acid content and F uptake was enhanced with increasing F exposure whereas catalase activity, total chlorophyll, carotenoids, ascorbic acid and free sugar content decreases with similar exposure. Peroxidase activity was maximum increased in mustard where overall F toxicity effect was found to be minimum. Overall observation of all the entities indicates that F induced stress influences the test plants species in order radish > coriander > spinach > mustard. The results indicate that plant species maximum tolerant to F toxicity shows maximum peroxidase activity. Maximum increase in free amino acid content was observed in radish plant may be an adaptive reaction in plant cells to neutralize the damaging effects of reactive oxygen species (ROS) generated during F induced stress.**

Keywords: **Fluoride contaminated water; Fluoride uptake; Radish, Coriander, Spinach and Mustard; catalase, peroxidase, Free sugar, Free amino acid, ascorbic acid, chlorophyll, Carotenoids.**

1. INTRODUCTION

Application of fluoride contaminated groundwater for irrigation is prevalent in many fluoride endemic areas, which can affect the crops considerably. Fluoride is absorbed by plant roots and then transported via xylematic flow to different parts [1, 2] where it can get accumulated. Earlier studies revealed that exposure to elevated fluoride can cause retarded plant growth [3], chlorosis [4] and leaf necrosis [5]. Fluorine is not considered as an essential element for plants. However, in fluorosis endemic areas, the fluoride content in plant parts have shown higher concentrations [6-8]. Fluoride can interfere with the metabolism of proteins, lipids, and carbohydrates [9-12], inhibits soluble sugar composition in germinated mung bean [13].The effect of fluoride on plant growth may be complex, varying from positive to negative effects [14]. Previous studies have shown that the growth and productivity of many crops are adversely affected by fluoride [15-18]. The chlorophyll content of the *Triticum aestivum* leaves reduces with increasing concentration of NaF exposure. Ascorbic acid content initially decreased and then increased with increasing concentration of fluoride i.e 20mgL^{-1} [19], as it is an antioxidant that plays an important role in protection against physiological stress [20].

The effects of fluoride on the activities of enzymes, Peroxidase [21] and chloroplast ATP-ase [22] have been discussed by many workers. The effects of fluoride on photosynthesis rates, and chlorophyll destruction have been reported [23]. Fluoride exerts its effects on carbohydrate metabolism principally on Photosynthetic carbon fixation and on starch degradation in *Abies alba* leaves [24]. Along with accumulating in chloroplasts and reducing the chlorophyll concentration, ultra structural have shown that fluoride disrupts chloroplasts membrane [25]. Due to sodium fluoride exposure marshy halophyte like *Salicornia brachiata* shows decreased Photosynthetic pigments (chlorophylls and carotenoids) content and Peroxidase (POX), superoxide dismutase (SOD), activities were negatively regulated [26]. A

significant alleviation in activities of peroxidase, catalase and ascorbic acid oxidase was recorded on Mullberry (*Morus alba*) on exposure to fluoride, leading to production of reactive oxygen species with simultaneous increase in tissue levels of peroxidases [27].

Although most F accumulation in the human body occurs through F contaminated drinking water, substantial amounts of F can also be ingested through crops and vegetables irrigated with F contaminated water [28, 29]. The high level of fluoride in edible parts of plant which is consumed by human health is of great concern.

The objective of the study is to understand the accumulation and translocation of fluoride in the plants and its impact on plant pigments and antioxidants like carotenoids, chlorophyll, catalase, peroxidase, free sugar, free amino acid and ascorbic acid content in radish, coriander, spinach and mustard in a controlled condition.

2. MATERIALS AND METHODS

2.1 Plant Material

The fresh and healthy seeds of four winter crops like, Radish, Spinach, Coriander and Mustard were selected for the pot cultivation experiment. The seeds were collected from the local farmers and was allowed to germinate in the labelled pots using only tap water (0.31mgL^{-1} F). 4kg of soil was given to each pot. For each three desired sodium fluoride concentration i.e, control (tap water containing 0.31mgL^{-1} F), 5mgL^{-1} and 10mgL^{-1}, triplicate set up was made in three separate pots. Three healthy seedlings were allowed to grow in each pot. The experimental setup for each each variety of crop consists of 12 pots. The pots were kept in open field conditions and were irrigated regularly with labelled concentration of NaF solutions. Representative edible parts of plant samples were collected after harvesting for estimation of fluoride content and estimation of pigments like chlorophyll, carotenoids, enzymes and antioxidants like catalase, peroxidase, ascorbic acid free amino acid and free sugar.

2.2 Estimation of Available Fluoride

The 0.5g of sampled edible plant parts from each pot were air dried and blended separately. Then extracts were prepared in 25ml of 0.1M perchloric acid. The fluoride contents in the extracts were measured by ion selective electrode (Orion Thermo scientific, Model 1119000, USA) [30]. The mean of the three values at each treatment were calculated and used for comparison.

2.3 Estimation of Total Chlorophyll

For estimation of total chlorophyll, in each of the crop variety extraction was made in 80% acetone (v/v) and was measured in spectrophotometer i.e. by Arnon's (1949) method [31]. The mean of the three values at each treatment were calculated and used for comparison.

2.4 Estimation of Ascorbic Acid

The ascorbic acid content in the plant materials was estimated by titrimetric method after extraction in 4% (w/v) oxalic acid following Sadashivam and Manikam (1996) [32].

2.5 Estimation of Carotenoids

For estimation of carotenoids the plant materials were extracted in 80% (v/v) acetone and then was measured spectrohotometrically following Davis (1976) method [33].

2.6 Estimation of Free sugar

1 gm of Plant material was crushed in 5ml of hot 80% ethanol and centrifuged at 5000 g for 10 mins and the content of total soluble sugar (water soluble monosaccharides and disaccharides) was determined by Anthrone method [34], an unspecific colorimetric method.

2.7 Estimation of Catalase activity

catalase activity of the plant materials exposed to different NaF concentration was determined by Colorimetric method [35]. 500mg of fresh healthy leaves (without the midrib portion) were taken and Crushed in 5ml of cold phosphate citrate buffer (extraction buffer – 960mg citric acid and 1.42g Na_2HPO_4 dissolved in 100 ml distilled water). Then $Ti(SO_4)_2$ was added to the reaction mixture.

2.8 Estimation of peroxidase activity

peroxidase activity of the plant materials exposed to different NaF concentration was determined by Colorimetric method [36]. 500mg of fresh healthy leaves (without the midrib portion) were taken and Crushed in 5ml of cold phosphate citrate buffer (extraction buffer: 960mg citric acid and 1.42g Na_2HPO_4 dissolved in 100 ml distilled water). The diluted enzyme extract was reacted with 1ml of 300µM phosphate buffer and the reaction was terminated by adding 1ml of 5% H_2SO_4.

2.9 Estimation of free amino acid

The content of α amino nitrogen was estimated in a 80% ethanolic extract of 0.5g plant material using ninhydrin reagent [37]. The plant tissues were homogenised in 5ml of 80% ethanol and centrifuged at 5000g for 10mins. Pellets were again extracted with 80% ethanol and supernatants were pooled together. The supernatant was diluted 20 times with distilled water. 1ml of this extract was mixed with 1ml of 0.2 M ninhydrin reagent and kept in boiling water bath for 20 mins. The ninhydrin reagent was prepared by dissolving separately 800mg of hydrated stannous chloride in 500ml of citrate buffer (pH=5) and 20g of ninhydrin in 500ml of ethylene glycol monoethyl ether and mixing the two solutions. After completion of the reactions, the tubes were cooled down to room temperature and the volume was made upto 5ml with a diluents solution made up of equal proportions of n-propanol and distilled water. The absorbance of purple colour developed measured at 580nm in spectrophotometer. A standard curve was prepared from L-leucine.

2.10 Estimation of superoxide dismutase

The specific activity of superoxide dismutase in the plant materials exposed to different NaF concentrations was determined by the method described by Paoletti et al. (1986) [38] . Plant material 0.2gm was extracted in 0.6ml of 100mM Tea-dea buffer. In 1ml cuvette 0.8ml buffer solution, 0.04ml NADH solution, 0.025ml EDTA solution and 0.1ml of sample extract was added. The absorbance was studied in spectrophotometer at 340nm at 2mins interval upto 10 mins. The SOD specific activity was determined in terms of unit/mg protein/min. The protein content in plant materials was determined by Lowry method. Enzyme unit= % of inhibition / 50 × dilution factor.

Statistical analysis

Three replicates were considered at each observation for every plant species and the mean ± standard deviation values were calculated. The comparison of the treatment means for each parameter were done by ANOVA using SPSS version 17.0.and the level of significance were determined at p = 0.05

3. RESULTS AND DISCUSSION

3.1 Uptake of available fluoride

Significant accumulation of fluoride in the edible parts of mature radish, coriander, spinach and mustard plants (Table 1) were observed in the present study. The uptake in all the varieties of plant increased many fold with increasing F-concentrations. Among all the varieties of crops, only radish roots and leaves show a little available F accumulation at control exposure. Obtained results show that radish and coriander have greater tendency for accumulation of fluoride followed by spinach leaves, mustard leaves and fruits. Under $10mgL^{-1}$ fluoride exposure the uptake of fluoride increased 90.33% in mustard fruit, 98% in mustard leaf, 111.38% in spinach leaf, 123.47% in radish leaf, 125% in coriander leaf and 128.2% in radish root in comparison to their uptake under $5mgL^{-1}$ F exposure. This may be an indicator of high translocation factor (ratio of fluoride concentration in plant's shoot parts to that of plant's root parts). The soluble fluoride content for all soils is biologically much important to plants and animals [39]. All the vegetables do not accumulate fluoride to the same extent and variations among vegetables are high [40]. A high concentration of fluoride in plant shoots may be due to the fact that when a high concentration of fluoride is added to the soil or soil solution the pH becomes more alkaline, fluoride could increase in the soil solution and more fluoride would be potentially available for uptake by the plant root [41]. The radish root retained more fluoride than shoot this may be partly due to dilution of fluoride in shoot due to increased shoot biomass and partly due to restricted translocation of fluoride from the root to shoot [42]. Higher concentration of fluoride in root is probably due to low permeability through the endodermis. Leafy and roots vegetables accumulate more fluoride [43].

In the present study all the four selected crops, mainly radish and coriander can efficiently accumulate available fluoride in the edible parts. It enhances the risk of increased dietary intake of fluoride in fluorosis endemic areas. Radish and coriander tends to accumulate more fluoride than spinach and mustard indicating their more susceptibility to F- pollution.

3.2 Chlorophyll

The total chlorophyll content gradually decreases with increasing NaF concentration (Table 2). This reduction is observed to be maximum in radish leaves (43.76%) at $10mgL^{-1}$ F exposure in comparison to control. Followed by coriander (40.97%), spinach and mustard leaves. This reduction is found to be lesser (30.79%) in mustard leaves from control to $10mgL^{-1}$ F exposure and the reduction is chlorophyll content was found to be least (21.4%) in spinach. The decrease in chlorophyll content may be due to the breakdown of chlorophyll due to fluoride induced stress or inhibition of chlorophyll biosynthesis as a consequence of inhibition of Y-aminolevulinic incorporation due to increasing F exposure. Fluoride accumulates in chloroplasts reducing the chlorophyll concentration and disrupting chloroplasts membrane [25].

3.3 Carotenoids

The carotenoids concentration gradually decreases (Table 2) with increasing F- concentrations and this is found to be highest in coriander leaf, where at 10 mgL^{-1} F exposure the carotenoid content reduces 53.71% than carotenoids content in control. This reduction is followed by radish, mustard and spinach leaves. In radish at $10mgL^{-1}$ exposure the carotenoids reduction is 46.74%, in mustard it was 44.82% and in spinach the reduction was about 43.44% in comparison to control. Carotenoids are important antioxidants in plants which helps to defense against abiotic stress [44]. Thus reduction in carotenoids content may be due to inhibition under fluoride induced stress. The high electronegativity of F destroys the chlorophyll molecule accelerates the disintegration of chloroplasts [45].

3.4 Catalase

The catalase activity gradually decreases with increasing concentration of F exposure. Maximum decrease in catalase enzyme activity was observed in radish leaves (Table 3). This reduction in enzyme activity is found to be followed by coriander, mustard and spinach. In radish leaves under 5 and $10mgL^{-1}$ F exposure catalase activity reduces 43.15% and 66.5% respectively than catalase activity under control. In coriander at $10mgL^{-1}$ and $5mgL^{-1}$ fluoride exposure the catalase activity reduces 52.5% and 25.25% respectively in comparison to control exposure. In mustard leaf, under 5 and $10mgL^{-1}$ F exposure catalase activity reduces 16.44% and 43.15% respectively. In spinach under similar F exposure catalase activity reduced 21.78% and 43.15% than control. The changes in catalase activity may vary with the intensity of abiotic stress. The reduction in catalase activity concludes that hydroxyl ions attached to iron atoms in catalase may be replaced by low molecular weight anions in sufficient concentration leading to breakdown and inhibition of catalase activity [27].

3.5 Peroxidase

The peroxidase enzyme activity was found to be increased with increasing F concentration exposure in all variety of test plant. The increase of peroxidase activity was found to be highest in mustard leaf followed by spinach, coriander and radish leaves (Table 3). In comparison to the enzyme activity under control the peroxidase activity was found to be increased by 226% in mustard leaf, 144.63% in spinach leaf, 121.4% in coriander leaf and 107.3% in radish leaf under $10mgL^{-1}$ F exposure. The peroxidase activity increases under fluoride stress condition. Maximum increase in mustard and spinach indicates that peroxidase fights to combat F stress and the increase in peroxidase activity is found to be lesser in radish and coriander. Peroxiredoxins are ubiquitous antioxidant enzymes that participate in cellular redox homoestasis and have been shown to increase under several abiotic stresses [46]. The oxidative stress induced by fluoride in plant leaves appears to be an indirect effect of fluoride toxicity leading to production of reactive oxygen species with simultaneous increase in tissue levels of peroxidases [27].

3.6 Free amino acid

In all the test plant parts of radish, coriander, spinach and mustard, the free amino acid content gradually increases with increasing concentration of F exposure (Table 4). In comparison to the free amino acid content in control the increase in free amino acid was found to be highest in radish, followed by coriander, spinach and mustard. In radish, maximum enhancement was observed in root. Under 5 and $10mgl^{-1}$ F exposure the free amino acid content increased 28% and 63.37% respectively in comparison to control. In radish leaf under same exposure the increase was by 20.5% and 45% respectively. In coriander leaf the increase was by 18.8% and 42.7% respectively compared to control. In spinach leaf under same exposure the free amino acid content enhanced by 16% and 36.5% respectively. In mustard the enhancement was found to be higher in leaf than fruit. In leaf the increase was 13% and 32% respectively. In mustard fruit the enhancement was 11% and 26.6% respectively. The free amino acid pool in tissues depends on the relative contributions of degradation of storage protein, amino acid synthesis and amino acid utilizations for protein synthesis and for respiration thus the enhancement in free amino acid content may be due to fluoride stress induced increased respiratory rate [47]. This enhancement may also be attributed to degradation of protein due F induced stress.

3.7 Superoxide dismutase specific activity

In all the test plant parts of radish, coriander, spinach and mustard the specific activity of SOD/mg protein/min, gradually increases with increasing concentration of F exposure (Table 4). In comparison to the specific activity of SOD in control the increase was found to be highest in radish, followed by coriander, spinach and mustard. In radish, maximum increase in SOD activity was observed in root, followed by leaf. Under 5 and 10mgL^{-1} F exposure the SOD specific activity was increased by 13% and 62% respectively in comparison to control. In radish leaf under 5 and 10mgL^{-1} F exposure the SOD specific activity was increased by 12% and 59% respectively. In coriander leaf the enzyme activity increased by 10.5% and 52% respectively compared to control. In spinach leaf under 5 and 10mg/l F exposure the SA of SOD was enhanced by 8% and 45% respectively. In mustard the enhancement was found to be higher in leaf than fruit. In leaf the increase was 6.8% and 38% respectively. In mustard fruit the SA of SOD increased by 6.2% and 34% respectively. This increase in specific activity of superoxide dismutase (unit/mg protein/min) may be due to the F- induced oxidative stress (superoxide radicals). Superoxide dismutase is an antioxidant enzyme which helps to combat oxidative stress [48] and increase in SOD activity may be partly attributed to an increased metabolic activity or fluoride induced increase in SOD biosynthesis [49]. In order to avoid the oxidative damage, higher plants raise the level of endogenous antioxidant defense [50].

3.8 Free Sugar

The free sugar content in all test crop variety gradually reduces with increasing F concentration. The obtained results (Table 5) show that the reduction in free sugar content was found to be maximum in radish root, followed by radish leaf, coriander leaf, spinach leaf, mustard leaf and mustard fruit. In radish root under 5 and 10mgL^{-1} F exposure the reduction in free sugar content was found to be 11.86%, 18.67% and 25.67% respectively in comparison to control. In radish leaf under similar F exposure the reduction was found to be 9.27% and 17.95% respectively. In coriander leaf reduction was found to be 9.02% and 16.19% respectively. In spinach leaf the reduction was found to be 7.98% and 15.3% in comparison to control. In mustard leaf the reduction was 6.9% and 13.81%. In mustard fruit in comparison to control the reduction was found to be 6.2% and 11.76% respectively. Soluble sugars can have important role in reactive oxygen species scavenging mechanisms as increased glucose levels can increase the production of NADPH. Under metal stress photosynthetic metabolism and photosynthetic electron transport decreases and these alterations in photosynthetic metabolism lead to overproduction of ROS such as O2•−, •OH, and H_2O_2 [51]. Thus reduction in free sugar content may be due to the inhibition of photosynthesis or due to fluoride induced oxidative stress [52]

3.9 Ascorbic acid

The ascorbic acid content in edible parts of different plants gradually decreases with increases fluoride concentration which indicates that ascorbic acid is an important antioxidant which breakdown due to fluoride stress. The decrease in ascorbic acid content is maximum in mustard leaf (Table 5) under 10mgL^{-1} F exposure 44.76%, which is followed by mustard fruit (38%), radish root (28.77%), coriander leaf (26.24%), radish leaf (22.76%) and spinach leaf (22.7%) compared to control at 10mgL^{-1} F exposure. However at 5mgL^{-1} F exposure maximum reduction in ascorbic acid content was observed in radish root (16.9%), followed by mustard leaf (15.57%) followed by mustard fruit (11.31%), radish leaf (11%), spinach leaf (10.43%) and coriander leaf (10.4%) compared to control. The decrease in ascorbic acid content with increasing concentration of fluoride indicates its breakdown under F stress which is supported by earlier workers [53]. However lesser reduction in more F susceptible plants indicates that it helps to combat F stress.

3.10 Plant Morphology

During the experiment, it was also observed that with increasing fluoride exposure, the plants showed retarded growth and delayed flowering and maturing.

4. CONCLUSION

Generally antioxidant enzymes and non enzymatic antioxidants have synergistic effects on free radical scavenging, thus the generation and removal of free radical is balanced [54]. However, the reduced activity of antioxidant enzymes under stressed condition can lead to lipid peroxidation and membrane damage [55]. Our findings are supported by some earlier workers. Under salinity induced stress the activity of plant enzymes like catalase, peroxidase, superoxide dismutase increases and ascorbic acid concentration declines in *Cicer arietinum* [56]. *Kandelia candel*, a mangrove species, grown under multiple heavy metal stress like Pb^{2+}, Cd^{2+}, and Hg^{2+} showed fluctuation in superoxide dismutase (SOD) and

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peroxidase (POD) activities, while catalase (CAT) activity increased [57]. Activities of ascorbate peroxidase (APX) and superoxide dismutase (SOD) were increased whereas catalase (CAT) was decreased in two varieties of *Zygophyllum* species under heavy metal exposure [58], which is quit comparable to our findings

In the present study all the four selected crops, mainly radish and coriander can efficiently accumulate available fluoride in the edible parts. It enhances the risk of increased dietary intake of fluoride in fluorosis endemic areas. Radish and coriander tends to accumulate more fluoride than spinach and mustard indicating their more susceptibility to F- pollution. The major biochemical parameters like chlorophyll, carotenoids, free sugar content and catalase activity also found to be more decreased in radish roots, leaves and coriander leaves. The SOD activity and free amino acid content was found to be increased more in radish and coriander. As all the four varieties of winter crop plants showed retarded growth and delayed flowering and maturing, it can be concluded that all the four varieties are susceptible to F- stress. The decrease in chlorophyll, carotenoid, free sugar, content and changes in catalase, peroxidase, SOD activity, ascorbic acid, free amino acid content suggests that antioxidant defense system did not sufficiently protect the plant tissue under elevated fluoride stress. Fluoride is not only gets accumulated to its edible parts but also inhibits its metabolic processes.Thus care should be taken to avoid use of fluoride contaminated water during cultivation of these crops.

Table 1: F_{H2O} accumulation (mg/kg) in edible parts of different plant parts at increasing levels of F exposure

Plant parts	control	$5mgl^{-1}$ F	$10mgl^{-1}$ F
Mustard Leaf	0.00	4.15±0.1	8.21±0.08
Mustard Fruit	0.00	2.69±0.05	5.12±0.1
Radish Root	0.021 ±0.003	26.2±0.03	59.78±0.05
Radish Leaf	0.016±0.001	21.09±0.09	47.13±0.04
Coriander Leaf	0.00	22.3±0.03	50.17±0.05
Spinach Leaf	0.00	8.61±0.03	18.2±0.04

Table 2: Impact of different concentration of fluoride on leaf pigment content of different crops

	Chlorophyll (mg/g)			**caroteinoids(µg/gm)**		
plant Leaf	Control	$5mgl^{-1}$	$10mgl^{-1}$	Control	$5mgl^{-1}$	$10mgl^{-1}$
Mustard	3.02±0.02	2.52±0.015	2.09±0.012	47.65±0.4	34.08±0.2	26.24±0.08
Radish	3.236±0.03	2.56±0.02	1.82±0.02	45.79±0.08	33.01±0.12	24.34±0.11
Coriander	4.032±0.008	3.29±0.02	2.38±0.013	47.72±0.2	35.16±0.2	22.08±0.16
Spinach	6.54±0.04	5.86±0.03	5.14±0.03	52.51±0.2	41.06±0.12	29.69±0.18

Table 3: Impact of different fluoride concentration on defence enzymes of selected crops

	catalase(unit/min/gm)			**peroxidase (unit/min/gm)**		
plant Leaf	Control	$5mgl^{-1}$	$10mgl^{-1}$	Control	$5mgl^{-1}$	$10mgl^{-1}$
Mustard	2.92±0.05	2.44±0.03	1.66±0.02	25.61±0.4	56.72±0.3	83.5±0.51
Radish	1.97±0.02	1.12±0.01	0.66±0.01	28.25±0.2	37.96±0.15	58.56±0.3
Coriander	3.01±0.015	2.25±0.02	1.43±0.008	25.65±0.23	49.25±0.2	56.79±0.16
Spinach	2.02±0.012	1.58±0.01	1.32±0.01	26.75±0.2	38.39±0.3	65.44±0.12

Table 4: Impact of fluoride exposure on free amino acid and SOD content of different plant parts

	free aminoacid content (mg/gm)			**superoxide dismutase activity (unit/mgprotein/min)**		
plant parts	Control	$5mgl^{-1}$ F	$10mgl^{-1}$ F	Control	$5mgl^{-1}$ F	$10mgl^{-1}$ F
Mustard Leaf	1.89±0.02	2.136±0.03	2.495±0.03	1.42±0.02	1.516±0.03	1.96±0.02
Mustard Fruit	2.13±0.03	2.36±0.02	2.7±0.02	1.507±0.02	1.6±0.02	2.02±0.03
Radish Root	2.02±0.03	2.585±0.04	3.3±0.04	1.47 ±0.04	1.66±0.04	2.38±0.02
Radish Leaf	2.04±0.01	2.46±0.02	2.958±0.02	1.327±0.03	1.48±0.03	2.11±0.04
Coriander Leaf	4.29±0.04	5.096±0.03	6.12±0.02	1.37±0.02	1.514±0.02	2.08±0.02
Spinach Leaf	2.34±0.02	2.714±0.03	3.194±0.03	1.855±0.03	2.003±0.01	2.69±0.02

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Table 5: Impact of fluoride on ascorbic acid and free sugar content in different plant parts

	ascorbic acid (mg/gm)			free sugar (mg/gm)		
plant parts	Control	$5mgl^{-1}$	$10mgl^{-1}$	Control	$5mgl^{-1}$	$10mgl^{-1}$
Mustard Leaf	0.411±0.02	0.347±0.03	0.229±0.012	16.07±0.1	14.96±0.08	13.81±0.1
Mustard Fruit	0.389±0.012	0.345±0.01	0.241±0.01	6.12±0.04	5.74±0.06	5.43±0.04
Radish Root	0.278±0.004	0.231±0.006	0.198±0.003	41.56±0.1	36.63±0.12	30.89±0.2
Radish Leaf	0.391±0.02	0.348±0.04	0.302±0.02	15.32±0.1	13.9±0.06	12.57±0.04
Coriander Leaf	0.423±0.013	0.379±0.01	0.312±0.02	10.31±0.06	9.38±0.05	8.64±0.02
Spinach Leaf	0.489±0.006	0.438±0.01	0.378±0.012	18.04±0.06	16.6±0.12	15.28±0.2

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