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STUDIES ON THE GROWTH, PHOTOSYNTHETIC FUNCTIONING AND PHYSIOLOGICAL CHARACTERIZATION OF *WITHANIA somnifera* L., TAINTED WITH FLUORIDE CONTAMINATED WATER

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ABSTRACT

Purpose
Fluoride (F^-) pollution is one of the most serious issue facing the soil, water and agroclimatic variables as it does not occur naturally in its state but have always been found as the reactive substance in the earth's crust. It is always present in organic and inorganic forms, i.e., cryolite, fluorspar, and apatite, which is released into the atmosphere by brick kilns, fertilizers manufacturing factories, and other industrial industries.

Methods
The present study assessed the impact of F^- on growth, photosynthetic performance, and physiology of *Withania somnifera* L. plants.

Results
Results presented that high dose of F^- (200 ppm) significantly affects the plant growth in terms of root, shoot length, and dry weight and exhibited approx. two-fold reduction in root length and shoot (22.65%) as evident by shortened internode and leaf number of *W. somnifera* L. The physiological characteristics viz., vapor pressure deficit (VPD), water-use efficiency (WUE), transpiration rate (E), and stomatal conductance (gs) also showed alterations in F^- treated *W. somnifera* L. plants. Further, decreased soil plant analysis development (SPAD) values and photosynthetic pigments such as chlorophyll a, b, and a+b (12-43%) expressed toxicity response of F^-.

Conclusions
The study demonstrates a negative impact of F^- on growth, physiology, and medicinal value (metabolite content). Therefore, ecophysiological studies are prerequisites to the large-scale cultivation of *W. somnifera* L. plants for large-scale production.

Keywords: *Chlorophyll; SPAD value; Photosynthetic response; Growth; Harvest index; Fluoride; Withania somnifera* L.

INTRODUCTION

Fluoride concentration in the environment is a global issue.[1,2] Fluorine, a superreactive element, occurs in almost all waters and soil as inorganic or organic fluoride compounds (e.g., Freon). The natural and anthropogenic source of F^- is weathering of fluoride mineral, volcanic eruption, marine aerosol, fluoridated municipal discharge, aluminum smelters, glass and fluoride chemical, consumer goods, and the development of fluorochemical industries.[3,4,5] The concentration of F^- in soil and water depends on various factors such as temperature, pH, the solubility of fluorine-bearing minerals, anion exchange capacity of aquifer materials (OH^- for F^-), and the nature of geological formation drained by water.[6] Fluoride is substantially absorbed by the plants' roots as F^- ions from the soil rich in an acidic environment. The acute and chronic toxicity of F^- depends on the concentration, duration, and frequency of its exposure. Fluoride exerts toxicity in plants by altering the F-sensitive biochemical processes, including growth, physiology, and nucleic acid metabolism.[7] High F^- level inside the plant cells severely affects the physiological responses, nutrient imbalance, chlorophyll degradation, low seedling establishment, growth rate, and photosynthetic pigments. In addition, enormous exposure to F^- in recent years has caused toxicity by synthesizing reactive oxygen species (ROS) in plants, leading to membrane damage, DNA damage, and altered growth[7,8,9] while in human fluoridation causes neurotoxicity, endocrine disruption, dental fluorosis, and long term deposition in bones.[5,10]

Fluoride in water is the major source of contamination. However, plants and animals using soft water are more prone and adversely affected by F^- toxicity because the availability of F^- reduces with the increasing water hardness. Fluoride acts as enzymatic poison inhibiting enzyme activity, photosynthesis, glycolysis, protein synthesis, and other important enzyme-mediated processes of plants and animals.[11,12,13] High F^- concentrations (>1.5 mg F^-/L) have been reported in groundwater of over 20 developed and developing countries, including India, China, South Africa, Bangladesh, etc., suffer from endemic fluorosis.[14,15] Kumar et al.[16] reported that about 200 million people across the globe are at high risk from crippling fluorosis. Besides, F^- also influences the metabolites in aromatic and medicinally important plants, which leads to diminishing the therapeutic values of the plants.[13]

Withania somnifera L., commonly known as Ashwagandha, is very revered, sacred, and one of the most important herbs of Ayurveda with diverse medicinal properties including antitumor, anti-diabetic, antioxidants, anti-inflammatory, anti-stress, and improve vigor and fatigue.[17,18,19]

The present study hypothesizes that the large-scale cultivation of Ashwgandha in soil tainted with F^- pollution may influence the growth, physiology, and medicinal value of the plant, which directly or indirectly hampered the perspective of the plant being used as super feedstock in pharmaceutical, and nutraceutical industries, and socio-economy and thus lives of human being of the world. In the present study, Ashwagandha plants were treated with different concentrations of F^- (as NaF) to evaluate the impact of F^- on growth, photosynthetic performance, and metabolite content.

MATERIAL AND METHODS

Collection and growth of plants

The certified saplings of *W. somnifera* L. Dunal var. Poshita was collected from the polyhouse of the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, Uttar Pradesh, India (26.8948° N, 80.9824° E). The collected plant saplings were further grown in a glasshouse. After 60 days of growth in the polyhouse, plants were transferred to earthen pots (9×12 inch) filled with fertile soil and left for environmental acclimatization. The acclimatized plants shifted to the natural environment for experimental analysis.

Experimental design

The different concentrations of sodium fluoride (NaF), such as 10, 20, 50, 100, and 200 ppm, were prepared by directly mixing the required amount of the test substance in pre-weighed (wt) sterilized soil. The plant of *W. sominfera* L. was grown in different concentrations of NaF and irrigated with tap water at regular intervals for proper growth and development. All the experiments were carried out in five replicates. Plants grown in normal soil without F^- application served as control. The harvesting was done after 60 days of growth, and the samples were stored at 4 ^{0}C and carried to the laboratory for physiological and biochemical analysis.

Physico-chemical analysis of soil

The physicochemical parameters of soil, viz., pH, electrical conductivity (EC), temperature, and Na content were estimated by APHA.[20] The pH content of the soil sample was measured by dissolving soil in distilled water (DW, 5:1). After proper dissolution, pH and EC were measured by pH meter (Orion ion meter, USA). The Na content was measured according to Kumar and Rao.[21]

Phenotypic parameters

The shoot length of the plant *W. somnifera* L. was measured with the help of a metric scale at 15 day of interval after treatment. To ensure the accuracy of the length, at least five values were measured from different treatments. The fresh weight of the plants was measured by uprooting, followed by washing to remove the soil debris. After washing, the plant was used to soak in bolting paper to remove excess water and then measured by a weighing machine. All plants' physiologically active leaves were manually counted every 15 days of treatment until the flowering stage.

Leaf chlorophyll index (SPAD)

Leaf chlorophyll index observed by Chlorophyll Content Meter, Apogee Instrument Inc. CCM-200 (Opti-Sciences). The sensor was placed over the middle part of the leaf, avoiding the midrib of the leaf, to estimate the leaf chlorophyll SPAD values.

Harvest Index

Plant performance indicates how efficiently dry mass is translocated into usable below-ground components. All plant components, viz., shoot (stem + leaves + crown with berries) and roots, were kept to dry at room temperature until constant weight. These parts are weighed separately with the help of a single-pan digital balance. The HI was calculated according to Singh and Verma[22] as stated below:

$$\text{Harvest Index } (gg^{-1}) = \frac{\text{Total root dry weight}}{\text{Shoot (stem+leaves+crown with berries) dry weight}}$$

Photosynthetic pigments

To estimate chlorophylls and carotenoids, the mature leaf of all plants was subjected to different fluoride treatments viz., 0, 10, 20, 50, 100, and 200 ppm separately. 100-mg fresh leaf tissues immersed in 10 ml Dimethyl sulfoxide (DMSO) in the dark overnight[23] for pigment extraction. The extract was centrifuged at 5000 rpm for 5 min, and the absorbance was observed at different intensities such as 480, 510, 645, and 663 nm using a spectrophotometer (Synergy HTX 1901231C Reader Software version 3.05.11). The pigment value of chl a, chl b, total chl (a+b), and carotenoids were estimated according to Arnon[24] and Maclachlan and Zalik.[25]]

Measurement of leaf gas exchange

To measure the exchange of CO_2 and H_2O gases, a portable photosynthesis system (PPS) with a Multiphase Flash TM Fluorometer (LI-6800, LI-COR, Lincoln, NE, Nebraska, USA) was used. To examine the differences in CO_2 and water vapor gas concentrations before and after the leaf cuvette, PPS contains two infrared gas analyzers. On the third or fourth completely developed leaves from the top of the plant, the gas exchange parameters, such as net photosynthesis (P_N), stomatal conductance (gs), and transpiration efficiency (E), were observed between 7:00 to 9:00 h. The measurements were performed with a light intensity of 600 mol (photons) $m^{-2}s^{-1}$, leaf temperature of 34± 2 °C, CO_2 level maintained at 400 mol (CO_2) mol^{-1}, and leaf-to-air vapor pressure deficit (VPD) of <3 kPa. The instantaneous water-use efficiency (WUE) is calculated as the ratio between P_N and E.[22]

Statistical analysis

Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were applied to determine the significance level of the difference between treatments by SPSS 16.0 software.

RESULTS

Physicochemical analysis of soil

The physico-chemical characteristics of the F^- treated and normal soil were collected from the experimental field of the Department of Botany, University of Lucknow (New Campus), Lucknow, India. The soil was initially treated with different concentrations of NaF, i.e., 0, 10, 20, 50, 100, and 200 ppm, and presented changes in pH ranges from 5.99 to 7.53. However, variations in the soil pH were found to be nonsignificant, with a concentration of up to 50 ppm compared to the control. The maximum alteration (20.35%) was found in the soil treated with 200 ppm of NaF compared to control. The soil applied with 20 ppm NaF exhibited no significant change compared to normal soil (Table 1).

Electrical conductivity (EC) of the soil treated with different concentrations of NaF was maximum observed at 200 ppm of NaF, and the lowest content was observed at 20 ppm NaF compared to control. The Na content in the soil was also analyzed and found that maximum Na content was present in the soil treated with 20 ppm NaF compared to the control. In the higher concentration of NaF, Na content was found to be significantly decreased by 15 and 34% in 100 and 200 ppm relative to control plants, respectively (Table 1).

Table 1. Physico-chemical parameters of soil samples.

F^- Concentration (ppm)	pH	EC	Na
0	7.52±0.71^{b}	0.21±0.03^{a}	68.7±6.50^{bc}
10	7.41±0.87^{ab}	0.27±0.03^{a}	74.7±7.81^{cd}
20	7.53±0.88^{b}	0.23±0.02^{a}	82.3±9.61^{d}
50	7.18±0.90^{ab}	0.25±0.03^{a}	60.9±5.76^{b}
100	6.91±0.65^{ab}	0.36±0.04^{b}	58.6±6.84^{b}
200	5.99±0.63^{a}	0.34±0.04^{b}	45.4±5.71^{a}

All the values are means of three replicates (n = 3) ± S.D. Different letters within the same column indicate significantly different values between treatments (DMRT, $p<0.05$).

Effect of fluoride on growth and harvest index

The effect of growth on *W. somnifera* L. plants grown under different concentrations of F^--tainted soil has been shown in Figure 1 and Table 2. Results showed that the *W. somnifera* L. plants treated with different concentrations represented the root-shoot length and dry mass alterations. Growth response in terms of root-shoot length was found to be decreased with the increasing level of F^- application. The maximum loss in root-shoot length was observed in 200 ppm NaF, which was 17.58 and 49.5cm, respectively, compared to control (i.e., 33 and 64 cm). However, the minimum reduction in root-shoot length was observed in 10 ppm F^- compared to control plants.

Figure 1. Effect of fluoride on plant growth and development of *W. somnifera* L. plants after 60 days of F^- contaminated irrigation water.

The root and shoot dry mass of *W. somnifera* L. plants exhibited a similar reduction pattern; the maximum reduction was observed in 200 ppm NaF, which was 33.05% and 19.84%, respectively, compared to control. Reduction in root dry weight in plants treated with different concentrations of NaF, such as 10 ppm (12.30%), 20 ppm (22.82%), 50 ppm (29.32%), and 100 ppm (29.59%), while in shoot it was 10 ppm (4.21%), 20 ppm (8.17%), 50 ppm (10.39%) and 100 ppm (15.01%), respectively in comparison to control (Table 2).

The harvest index measures the reproductive efficiency of the plants. *Withania somnifera* L. treated with different concentrations of F^- exhibited reduction with the increasing concentration of F^-. The maximum loss in HI was observed at 200 ppm, while the minimum was at 10 ppm of NaF compared to control plants (Table 2).

Table 2. Effect of fluoride on the shoot-root length, dry weight, and harvest index of *W. somnifera* L. Plants after 60 days of treatment.

F^- Concentration (ppm)	Length (cm)		Dry Weight (g)		Harvest Index
	Shoot	Root	Shoot	Root	
0	64.00 ± 3.22^{d}	33.01 ± 1.66^{e}	28.27 ± 2.68^{b}	14.46 ± 1.82^{c}	0.516 ± 0.05^{c}
10	61.52 ± 3.81^{cd}	29.03 ± 1.80^{d}	27.08 ± 3.16^{ab}	12.68 ± 1.33^{bc}	0.472 ± 0.06^{c}
20	60.50 ± 4.04^{cd}	27.10 ± 1.36^{d}	25.96 ± 2.46^{ab}	11.16 ± 1.06^{ab}	0.448 ± 0.04^{bc}
50	56.00 ± 3.11^{bc}	23.88 ± 1.48^{c}	25.33 ± 2.96^{ab}	10.22 ± 1.19^{a}	0.426 ± 0.04^{abc}
100	53.50 ± 2.69^{ab}	20.43 ± 1.36^{b}	24.00 ± 3.02^{ab}	10.18 ± 0.96^{a}	0.382 ± 0.05^{ab}
200	49.50 ± 3.07^{a}	17.58 ± 0.98^{a}	22.66 ± 2.65^{a}	9.68 ± 1.01^{a}	0.355 ± 0.04^{a}

All the values are means of five replicates (n=5) ±S.D. Different letters within the same column indicate significantly different values between treatments (DMRT, $p<0.05$).

Effect of fluoride on the number of leaves and internodal length

Withania somnifera L. plants treated with different concentrations of NaF indicated loss in the number of leaves and thus affected the phyllotaxy of the plants. The number of leaves in plants treated with 200 ppm of NaF concentration decreased compared to the control plants, i.e., 22. However, the number of leaves remained unchanged in the 10 ppm NaF-treated plants. The number of leaves in the *W. somnifera* L. plants was found to be 21, 19, and 17 with respect to 20, 50, and 100 ppm of NaF compared to control plants.

Table 3. Effect of fluoride on the number of leaves and position on *W. somnifera* L. plants after 60 days of treatment.

F^- Concentration (ppm)	Number of leaves	Leaf positions between internode									
		(1-2)	(2-3)	(3-4)	(4-5)	(5-6)	(6-7)	(7-8)	(8-9)	(9-10)	(10-11)
0	22±2.08^{c}	2±0.20	2±0.19	2.2±0.26	2.8±0.27	2±0.23	2.5±0.24	2±0.25	2.2±0.21	1.8±0.17	2±0.23
10	22±2.57^{c}	2±0.21	2±0.21	2±0.19	2±0.25	3±0.31	2±0.25	2.5±0.29	1.5±0.18	2±0.23	2±0.25
20	21±1.99^{c}	1.5±0.14	2±0.19	1.5±0.19	2±0.21	2±0.19	2±0.21	2.5±0.31	1.8±0.23	2.5±0.26	2.3±0.27
50	19±1.99^{bc}	1.5±0.18	2±0.23	2±0.21	1.5±0.14	2. ±0.29	2.3±0.22	1.5±0.16	1.5±0.18	2.5±0.24	2.5±0.31
100	17±2.14^{bc}	1.8±0.23	1.6±0.15	1.5±0.14	1.6±0.19	1.6±0.15	1.6±0.19	1.5±0.14	1.5±0.16	1.5±0.18	2±0.19
200	14±1.63a	1.5±0.18	1.2±0.15	1.5±0.18	1.3±0.14	1.7±0.18	1.8±0.23	1.2±0.14	1.3±0.12	1.5±0.16	2±0.21

All the values are means of five-replicate (n=5) ±S.D. Different letters within the same column indicate significantly different values between treatments (DMRT, $p<0.05$).

Effect of fluoride on photosynthetic pigments and soil plant analysis development

Photosynthetic pigments in *W. somnifera* L. Plants treated with different concentrations of NaF have been depicted in Figure 2. Results revealed that the concentration of photosynthetic pigments (Chl a, b, and total Chl) decreased with the increasing concentration of NaF. The maximum decrease in photosynthetic pigments was observed in 200 ppm, i.e., 0.573±0.07, 0.326±0.04, and 0.922±0.11 mg/g FW compared to control. The percentage loss of total chlorophyll (a+b) content in *W. somnifera* L. plants was found to be 12, 28, 39, and 41%, respectively, at 10, 20, 50, and 100 ppm NaF. Similarly, the concentration of Chl a was found to be reduced in the order of nearly 14, 32, 43, and 45%, while in Chl b was about 13, 21, 29 and 31 in plants treated with different fluoride-contaminated water (10, 20, 50 and 100 ppm NaF), respectively.

Carotenoid content in *W. somnifera* L. plants treated with different concentrations was found to be upregulated with increasing concentrations of NaF. The carotenoid level in W. somnifera L. plants was found to be maximum increased by 27% while minimum as 7% compared to control plants.

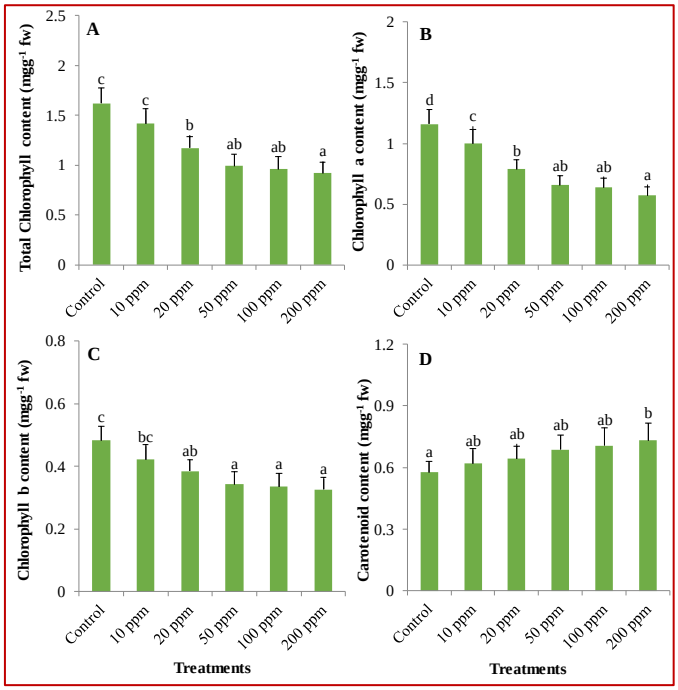

Figure 2. Changes in the level of total chlorophyll (A), Chl a (B), Chl b (C), and carotenoids (D) of the *W. somnifera* L. plants under F^- stress conditions. All the values are means of five replicates (n = 5) ±S.D. Different letters indicate significantly different values between treatments (DMRT, $p<0.05$).

The SPAD value in *W. somnifera* L. plants treated with different concentrations of NaF was found to be downregulated. The SPAD value was decreased by 65% at 200 ppm NaF while with 10 ppm at 6% as compared to control. In the case of plants applied with 20, 50, and 100 ppm NaF, the reduction in SPAD was 12, 20, and 45%, respectively, compared to control (Figure 3A).

Impact of vapor pressure deficit, stomatal conductance, water-use efficiency, and transpiration rate on *W. Somnifera* L. Plants during F^- stress condition

The vapor pressure deficit in *W. somnifera* L. plants has been presented in Figure 3B. Results revealed that the VPD value was maximally increased by 56% at 20 ppm compared to the control and started to decrease with the increasing concentration of F^- contaminated water irrigation. The reduction in VPD value was found to be 32% as compared to the control. Results concluded that the VPD value increased to some extent up to the concentration of 50 ppm NaF; afterward, sharp decline trends were observed.

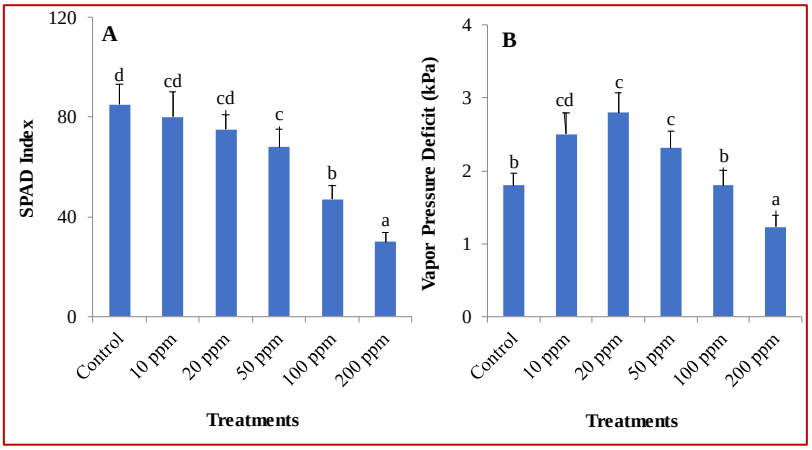

Figure 3. Changes in the value of soil plant analysis development (A) and Vapor Pressure Deficit (B) on *W. somnifera* L. plants under F^- stress condition. All the values are means of five replicates (n = 5) ± S.D. Different letters indicate significantly different values between treatments (DMRT, $p<0.05$).

Fluoride toxicity highly influences the gs. Results showed that F^--treated plants exerted diminished gs ranges from 30-38%. Maximum reduction in gs was observed in 200 ppm NaF while minimum in 10 ppm NaF compared to control plants (Figure 4A).

The water-use efficiency of *W. somnifera* L. plants treated with different concentrations of NaF was found to be upregulated with the increasing concentration. However, water-use efficiency was not more affected at lower concentrations, i.e., 10 and 20 ppm, and showed increased levels at 100 and 200 ppm NaF treatment. In comparison, 11% increase in WUE was observed in 200 ppm NaF compared to control plants (Figure 4B).

The transpiration rate in *W. somnifera* L. plants treated with different concentrations of NaF revealed a decreasing trend ranging from 11 - 22%. The maximum loss in E (22%) was observed in 200 ppm NaF relative to control plants. However, the least reduction (11%) was observed in 10 ppm compared to control (Figure 4C).

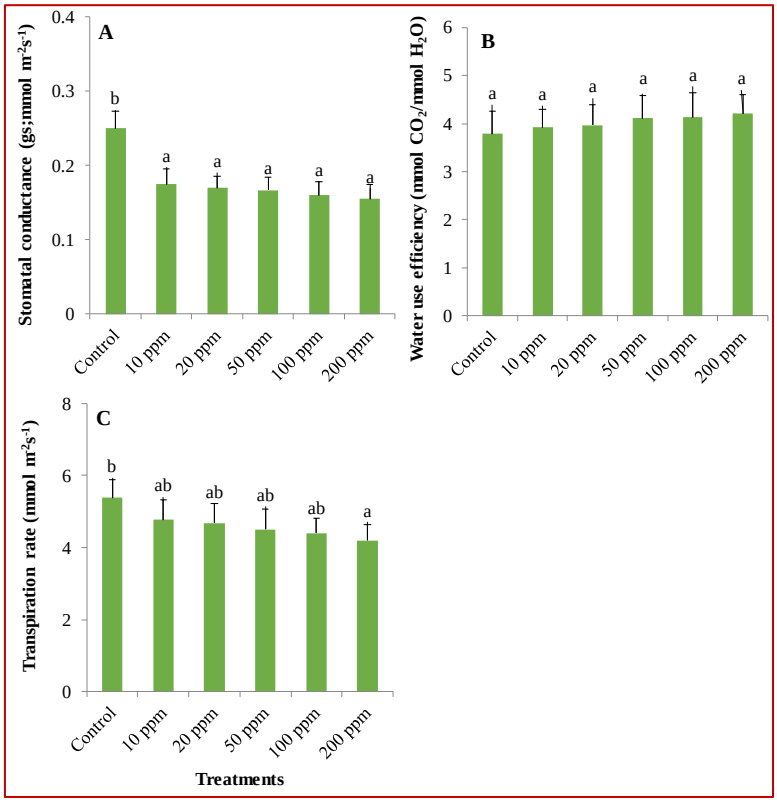

Figure 4. Responses of stomatal conductance (A), water-use efficiency (B), and transpiration rate (C) in *W. somnifera* L. plants under different fluoride concentrations. All the values are means of five replicates (n=5) ± S.D. Different letters indicate significantly different values between treatments (DMRT, $p<0.05$).

DISCUSSION

As reported by various authors, fluoride contamination in soil affects the health of the plants and soil owing to its insoluble nature.[5,13,14,26] However, high concentration alters the soil properties, as reported in the present study. High fluoride concentration reduces the pH of the soil, which may be ascribed to the occurrence of fluorine in protonated form (HF). After demineralization, it releases a large amount of hydrogen in the environment, which reduces the pH of the environment towards more acidic.[1,27] The electrical conductivity is related to the release of the mineral into the soil. The positive correlation of dose-dependent increase in EC in the present study reflects the influence on soil salinity and release of cations present in the soil in the form of the complex which following this study of increased Na level upon high exposure of NaF. Various authors have also reported increased EC and metal content under high NaF.[14,28]

The loss in the growth of *W. somnifera* L. plants due to fluoride toxicity is associated with alteration in phytohormones synthesis, leading to diminished cell wall extensibility and photosynthetic efficiency and thus growth of root and shoot as reported by various authors.[29] Ram et al.[30] reported that fluoride reduces cell division, cell expansion, and seeding development by degenerating Gibberellic acid in the aleuron layer, affecting the nutrient uptake and growth of the root and shoot. Similar results of reduced growth under fluoride stress have been reported by Gadi et al.[31] Reduction in root length may be ascribed to diminished availability of important minerals required for growth due to NaF toxicity. The loss in the total biomass of plants exposed to increasing concentrations of F^- was observed in various crops, i.e., barley, rice, wheat, lady's finger, onion, soybean, pea, radish, spinach, triticale, etc. Further, overall reduced biomass of the plants showed alteration in the metabolic pathways responsible for the growth of the plants. Yadu et al.[7] have also reported a similar study of dry weight reduction.

Regarding yields, when soils were contaminated with F^-, dose-dependent downregulation in yield of variable magnitude was observed in various crops. Decreased yield in the form of HI reflected the deleterious effect of F^- causing growth retardation, the process of glycolysis (by binding with the enolase enzyme), and the diminished production of secondary metabolites with high therapeutic values.

The primary cause of total chlorophyll reduction is the inhibition of chlorophyll synthesis, stomatal conductance, rate of CO_2 entering into the plants, and NaF-induced chlorosis. Reduced chlorophyll biosynthesis in the present study might be due to the binding of F^- with Fe^{+2}/Fe^{+3} and Mg ion, an important constituent of chlorophyll, resulting in lowering its translocation to the leaves and thus chlorophyll degradation and photosynthesis inhibition.[32] Total chlorophyll reduction is primarily caused by the Chl collapse or inhibition of chlorophyll synthesis due to F^- stress. In addition, decreased gs reduces the intracellular CO_2 fixation and assimilation, which imbalance the PSII activity and electron movement, damaging the photosynthetic machinery.[22,33] Carotenoid acts as an accessory pigment and important parameter for the toxicity indicator. The result showed the increased carotenoid level with increased toxicity. This may be ascribed to photoprotective and tolerance response as well as antioxidant nature, which protects the plants against oxidative damage due to stress.[15] Increased carotenoid level in plants due to stress has been reported by various authors.[34] The soil-plant analysis development acts as an index for chlorophyll synthesis and also exhibited decreasing trends with increasing concentrations of F^- revealing the toxicity in plants leading to chlorosis and photosynthetic inhibition.[15]

Stomatal conductance measures the rate of CO_2 entering, degree of stomatal opening, water use efficiency, and potential indicator of stress of the plants. Decreased gs and E with increased NaF exposure is a common response to fluorosis.[35] Leaf number of *W. somnifera* L. plants treated with different concentrations of NaF showed no significant change at lower concentrations compared to the control. However, the number of leaves decreases with the increasing concentration of F^-. The internodal length between the two leaves was examined, and NaF significantly affects the shoot length and thus intermodal length between the two leaves at the highest concentration of NaF compared to the control. Decreased leaves number in the plants exhibited toxicity exerted by NaF, which may be ascribed to a reduction in turgor pressure, stomata closure, and low intake of CO_2 resulted into, reduced synthesis of food, ATP generation, and growth, especially in the aerial part of the plants.[36] Similar results were reported by various authors.[37,38,39]

Stomata are tiny openings that regulate gaseous exchange to optimize WUE and are controlled by various environmental factors, including VPD and the water status of the plants.[40] Vapor pressure deficit, a microclimatic factor, represents the morpho-physiological behavior of plants, including transpiration, plant growth, and productivity.[41] In addition, VPD, E, gs, and WUE are correlated to each other and plant-expressed change by altering one of the factors. In the present study, enhanced VPD at lower doses of NaF indicates the high transpiration rate and water loss from the cells resulted in diminished growth and productivity of the plant.[42] However, low VPD at higher concentration support the plant's growth; thus, NaF plays a supportive role by enhancing the WUE of the plant under NaF stress. Similarly, low transpiration under NaF treatment in the present study might be due to lower VPD, which in turn enhances WUE, gs, photosynthetic efficiency, and yield of the plants.[8,22,43]

CONCLUSIONS

The results of this study indicated that high concentration of F^- (200 ppm) could induce severe damage to plant growth and development of *W. somnifera* L. plants. Cell membrane stability was injured, photosynthetic responses were inhibited, and nutrient uptake was interrupted in *W. somnifera* L. plants after applying F^- contaminated water. The guiding significance of the major result of this work for safe crop cultivation is to prohibit the use of groundwater or contaminated high amounts of F^- (200 ppm) for the cultivation of *W. somnifera* L. plants. Also, it would be interesting to study more adaptive mechanisms and mechanisms of F^- uptake by the plant at molecular and genetic levels to determine its exact potential for phytoremediation.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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