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TRIGONELLA FOENUM-GRAECUM L. SEED GERMINATION UNDER SODIUM HALIDE SALTS EXPOSURE

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ABSTRACT: Germination of seed to seedling is a physiological process that triggers a cascade of biochemical reactions. The quality of irrigation water can directly affect the early growth stages of a plant. Seeds of *Trigonella foenum-graecum* L. (fenugreek) were exposed to three sodium halides, i.e., NaCl (50 & 100 ppm), NaI (50 & 100 ppm), and NaF (50 & 100 ppm) for two weeks. Seeds grown without the addition of sodium halides were taken as a control for comparison. Seedlings were harvested on the 15th day of sowing and the root and shoot length were recorded. Leaves, shoot, and root was separated, encapsulated in potassium bromide, and then loaded for Fourier transform infrared spectroscopy (FTIR) analysis. A slight increase (16.66%↑) in shoot length was observed with 50 ppm NaCl but 100 ppm NaCl did not show any change in seedling length as compared to the control. 50ppm NaI did not show any change in root length but shoot length was slightly decreased (16.66%↓), and 100ppm NaI showed reduced root (12.5%↓) and shoot length (25%↓) as compared to control. The seeds treated with NaF 100 ppm had the lowest shoot (50%↓) and root length (37.5%↓) among all treated and untreated groups. Unique FT-IR spectral patterns in control and treated seedlings indicate some conformational changes in macromolecules with sodium halide treatment. The overall absorption peaks of treated roots and stems were slightly different from the leaves. The effect of treatment on seedlings was found in the following order; NaCl < NaI< NaF.

Keywords - FT-IR; Sodium fluoride; Sodium chloride; Sodium iodide; *Trigonella foenum- graecum L*

INTRODUCTION

The halogens are universally present in nature and act as micro-nutrients for humans, animals, and plants[1-3]. Among them, fluorine and chlorine have the highest crustal abundance while iodine stands at the lowest[4]. Fluorine and chlorine are found somewhat in high amounts in crops due to being there in agricultural fields from waste-materials[5-6] and iodine levels are increased in the soil through iodinated irrigation water[7]. Total fluoride content of soil ranges from 150–400 mg/kg which is mainly because of phosphorous fertilizers containing 1 – 1.5% fluorine[8]. Also, fluoride naturally occurs in form of metal fluoride salt with sodium in clay and soil. Sodium fluoride (NaF) present in water or soil can alter plant physiology, biochemistry, and structural activities[9,10]. Likewise, chlorine exists in combination with some inorganic substances like sodium. Sodium chloride (NaCl) mainly contributes to salinity stress in agricultural land which is composed of a range of dissolved salts [11-13]. Chlorine is able to accumulate in leaf tissue, resulting in leaves with a scorched or burned appearance. Chlorine is present in a small amount and acts as a micronutrient and participates in several physiological processes in plants [14]. Iodine is considered a micro-nutrient not only for humans but also for plants. Sodium iodide (NaI) is added to table salt to treat iodine deficiency. The biofortification of crops with iodine is growing these days in order to increase iodine content in plants to

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fulfill the daily intake requirements for humans [15]. Iodine is not an essential nutrient for plants and its high levels in irrigation waters can be toxic[16].

The seed germination phase is considered a vital part of the plant's life cycle as the yield of the crops depends fully on the germination process. Elements that are present in water or soils can directly affect the germination process. Recently, Fourier-transform infrared spectroscopy (FTIR) has been used to observe the biochemical changes in plants' response to abiotic stresses in all stages of plant development[17]. An acquisition time of FTIR is very short and suitable to examine the initial response of seedlings to stress[18]. Fenugreek *(Trigonella foenum-graecum* L.*)* is known for its medicinal value all over the world. Fenugreek is a fast-growing plant and needs only 5–10 days for germination [19]. In the present study, we explored the effect of different sodium halides on seedling growth and biochemical changes during the germination process in fenugreek (*Trigonella foenum-graecum* L) seeds.

MATERIAL AND METHODS

Plant material: Seeds of fenugreek were purchased from the local market in Riyadh, Saudi Arabia, and taxonomic identification was confirmed by Dr. Mona. S. Alwhibi in Botany and Microbiology Department, College of Science, King Saud University. An equal number of seeds were placed in different Petri dishes containing sterile cotton, dispersed in 10 mL of aqueous solutions of different halogen elements. Seeds were germinated for 15 days with different concentrations of aqueous solutions of NaCl, NaI, and NaF as mentioned below

Treatment: The experimental design with the different treatments was arranged below

1. *Control*: Fenugreek seeds germinated in distilled water;

2. *NaCl treated*: Fenugreek seeds germinated in 50 & 100 ppm NaCl aqueous solution

3. *NaI treated:* Fenugreek seeds germinated in 50 & 100 ppm NaI aqueous solution.

4. *NaF treated:* Fenugreek seeds germinated in 50 & 100 ppm NaF aqueous solution.

All Petri dishes were placed in a growth chamber for two weeks. 1 mL of the respective solutions was added to each Petri dish every day in order to maintain adequate moisture.

Growth analysis:

After two weeks of treatment, seedlings were harvested carefully. The root and shoot length was recorded with the help of a ruler. After harvesting, tiny leaves, shoots, and roots were collected separately for Fourier-transform infrared spectroscopy.

Fourier-transform infrared spectroscopy (FTIR): Freshly collected leaves, shoots, and roots were homogenized with distilled water and left to dry. The dried samples were encapsulated in potassium bromide and then loaded onto an FTIR spectroscope and results were recorded on Thermo Scientific-Nicolet -6700 FTIR spectrometer at a scan range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

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RESULTS

Effect of different halogen elements on the growth of the seedling

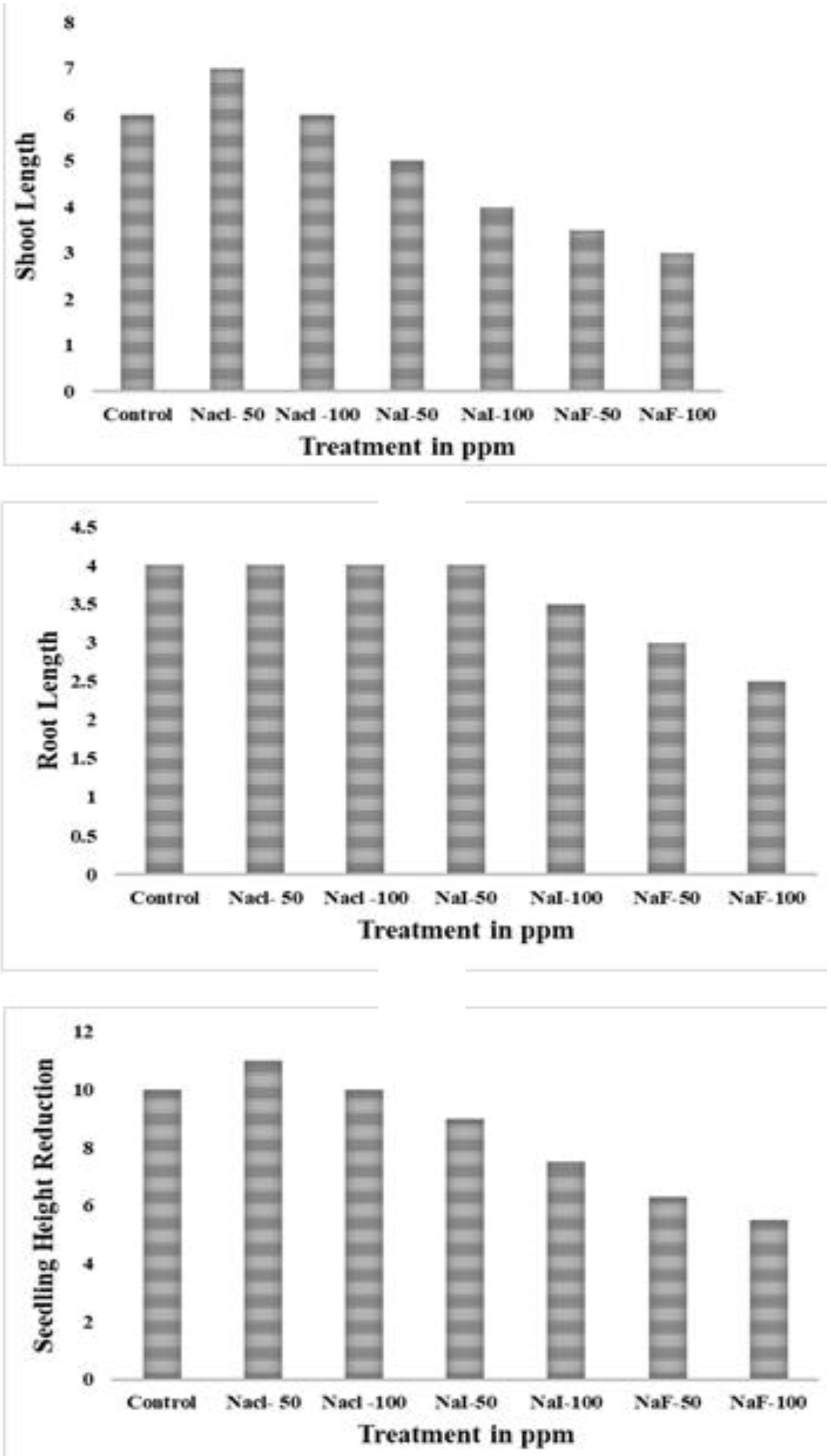
The Table and Figure 1 show the effect of different concentrations of sodium halides on seedling height. We found a slight increase in shoot length with no change in root length in seeds treated with 50 ppm of NaCl while seeds treated with 100 ppm NaCl did not show any change in seedling height when compared with the control. 50 ppm NaI did not show any change in root length but shoot length was slightly decreased, and 100 ppm NaI showed reduced root and shoot length as compared to the control. The seeds treated with NaF (50 &100 ppm) had the lowest shoot and root length among all treated and untreated groups. As a result, the effect of halogen elements on seedling growth was found in the following order; NaCl < NaI< NaF (Figure 1).

Table. Percentage change of shoot length; root length, and seedling height reduction (SHR) in all groups compared to control

Group	Percent change		
	Shoot length	Root length	SHR
Control	100.00	100.00	100.00
NaCl-50	116.66	100.00	110.00
NaCl-100	100.00	100.00	100.00
NaI-50	83.84	100.00	90.00
NaI-100	75.00	87.50	75.00
NaF-50	66.66	75.00	63.00
NaF-100	50.00	62.50	55.00

Effect of different halogen elements on FTIR analysis

FTIR analysis of roots, shoots, and leaves samples collected from seeding treated with different concentrations of sodium halides, are shown in Figure 2, Figure 3, and, Figure 4, respectively. The recorded spectral result shows some unique spectral patterns in control and treated seedling at certain wavenumbers which indicates some conformational changes in macromolecules such as lipids (3000–2800 cm^{-1}), proteins and lignin (1700–1500 cm^{-1}), lipids, and pectin (1790–1720 cm^{-1}), cellulose and hemicellulose (1300–1180 cm^{-1}) and other carbohydrates (fingerprint region 1200–900 cm^{-1}). In the root sample, the differences in band intensities between control and halogen treated plants were prominent at wavenumbers 3980, 3059, 3025, 2921, and 2852 cm^{-1} indicating conformational changes in lipids and 1635, 1601, 1600, 1492, 1449, 1152, 1069, 1027, and 905 cm^{-1} denoted cell wall region (Figure 2). In shoot samples, the same pattern of band shifts was found with few additional bands at 2366 and 2340 cm^{-1} mentioning more changes in lipids. We observed changes in band intensities in leaf tissue at 3389, 3060, 2922, 2853, and 2368 cm^{-1} indicating lipids 1944, 1636, 1605, 1493, 1449, and 1428 cm^{-1}indicating proteins, 1069, 906, 839, 754, 696, 536 cm^{-1} indicating carbohydrates (Figure 3). The average absorbance of lipids, protein, carbohydrates, and cell wall components of sodium halide-treated root, stem, and leaf is shown in Figure 5.



Figures 1A–1C. Effects of different treatment on 1A: shoot length; 1B: Root length; and 1C seedling height reduction.

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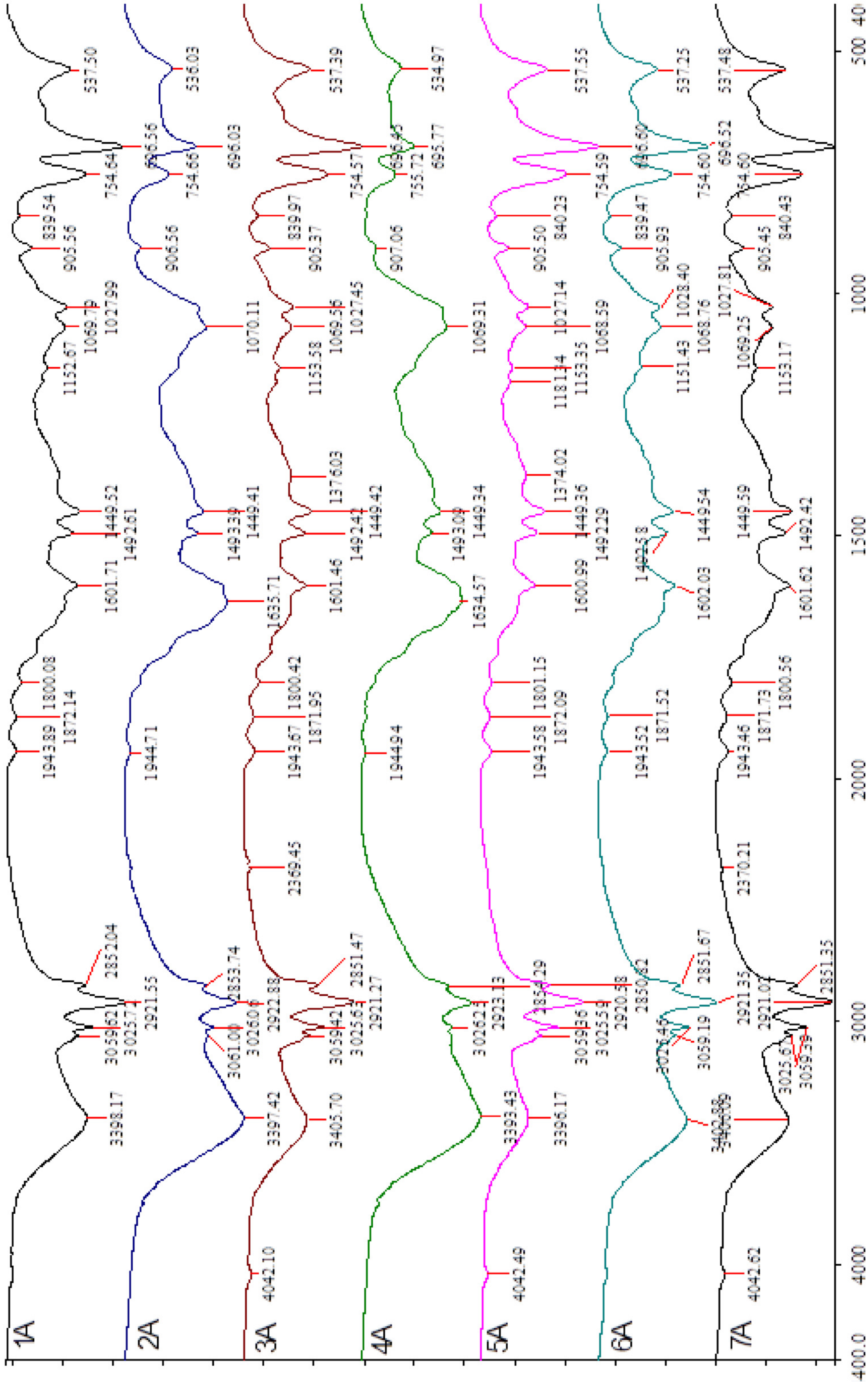

Figure 2. IF-IR spectra of roots of seedlings treated with different halogen elements with concentration in ppm. 1A: control; 2A: NaCl 50; 3A: NaCl 100; 4A: NaI 50; 5A: NaI 100; 6A: NaF 50; and 7A: NaF100.

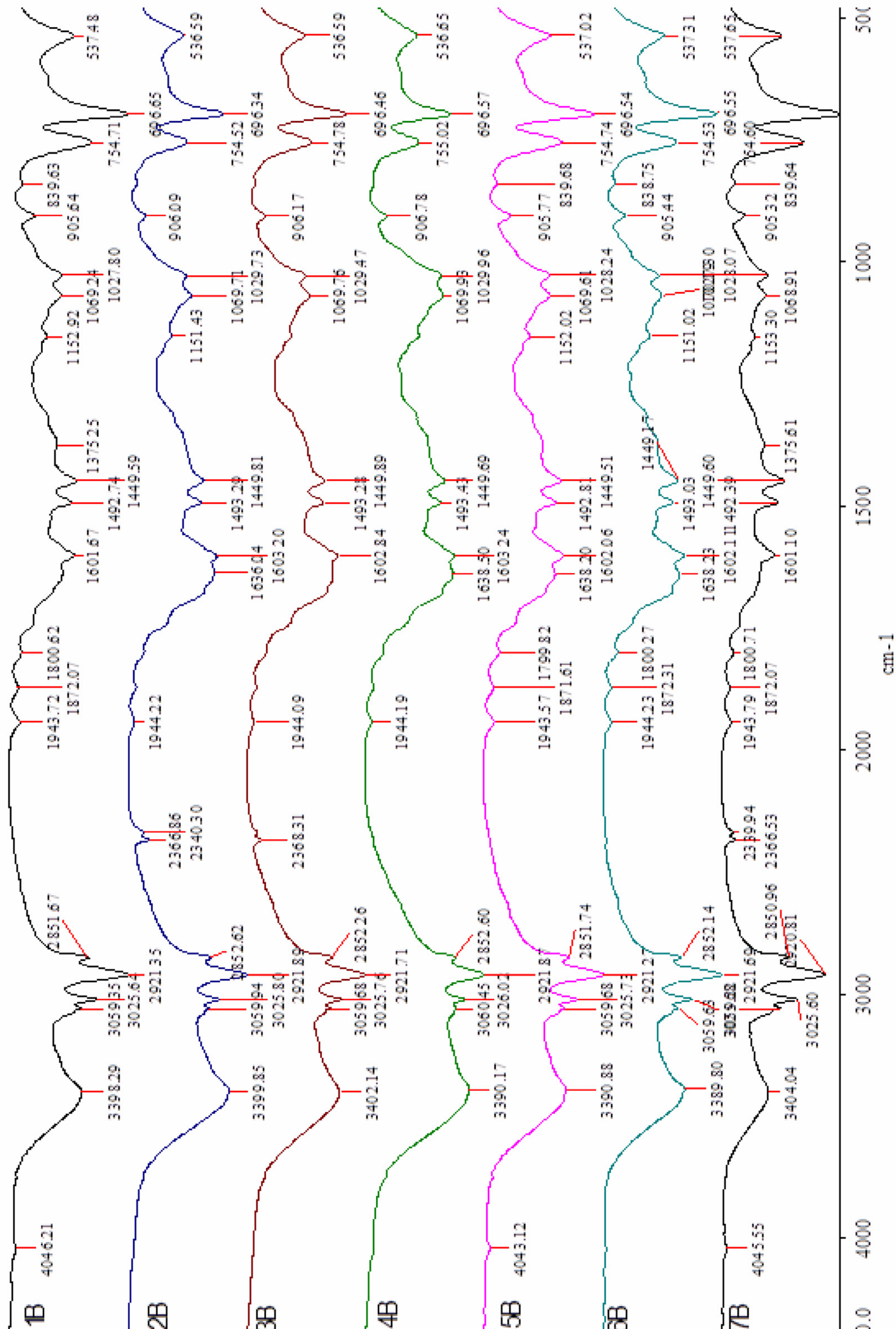

Figure 3. IF-IR spectra of stem of seedlings treated with different halogen elements with concentration in ppm. 1B: control; 2B: NaCl 50; 3B: NaCl 100; 4B: NaI 50; 5B: NaI 100; 6B: NaF 50; and 7B: NaF100.

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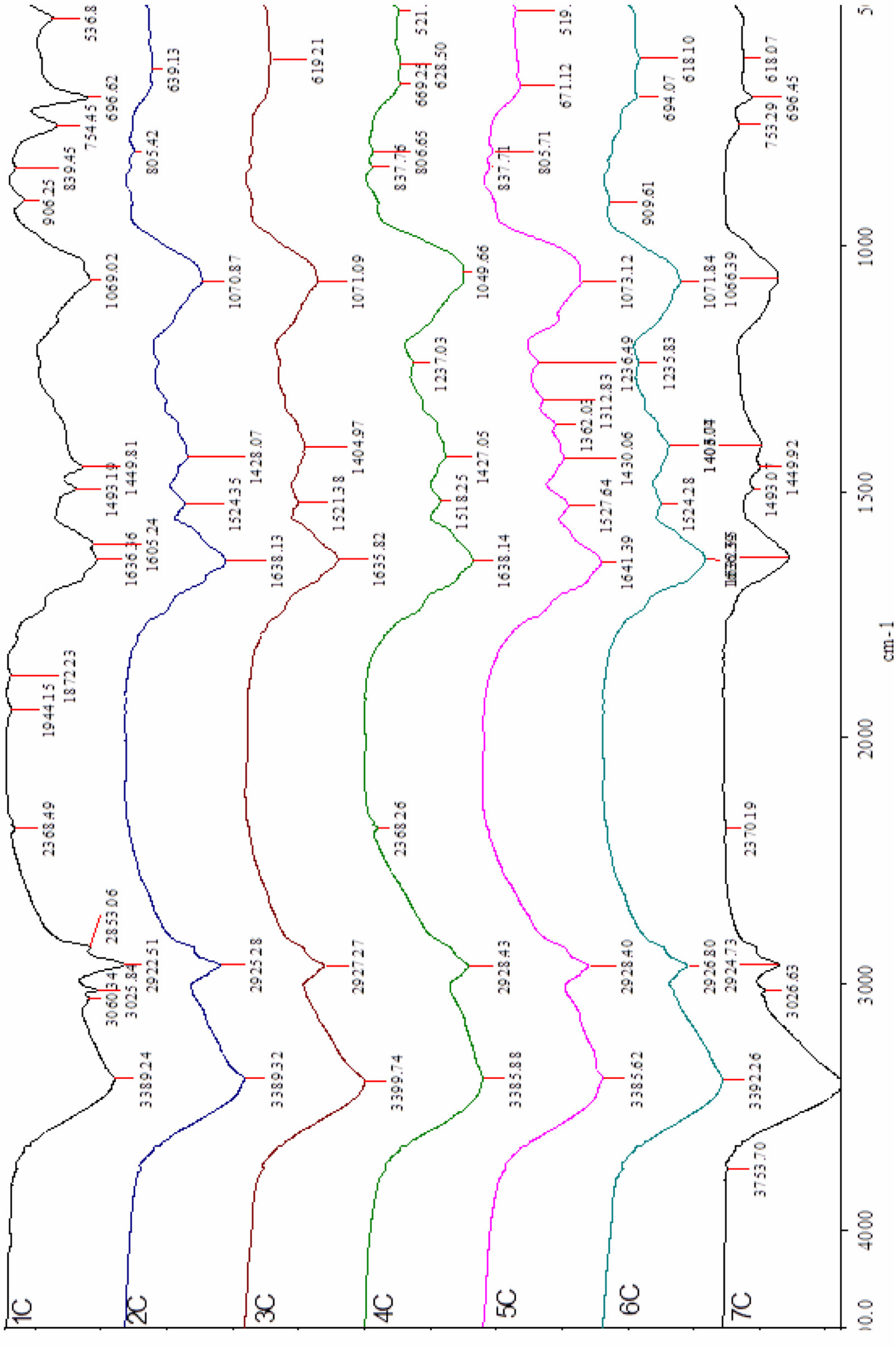

Figure 4. IF-IR spectra of leaves of seedlings treated with different halogen elements with concentration in ppm. 1C: control; 2C: NaCl 50; 3C: NaCl 100; 4C: NaI 50; 5C: NaI 100; 6C: NaF 50; and 7C: NaF100.

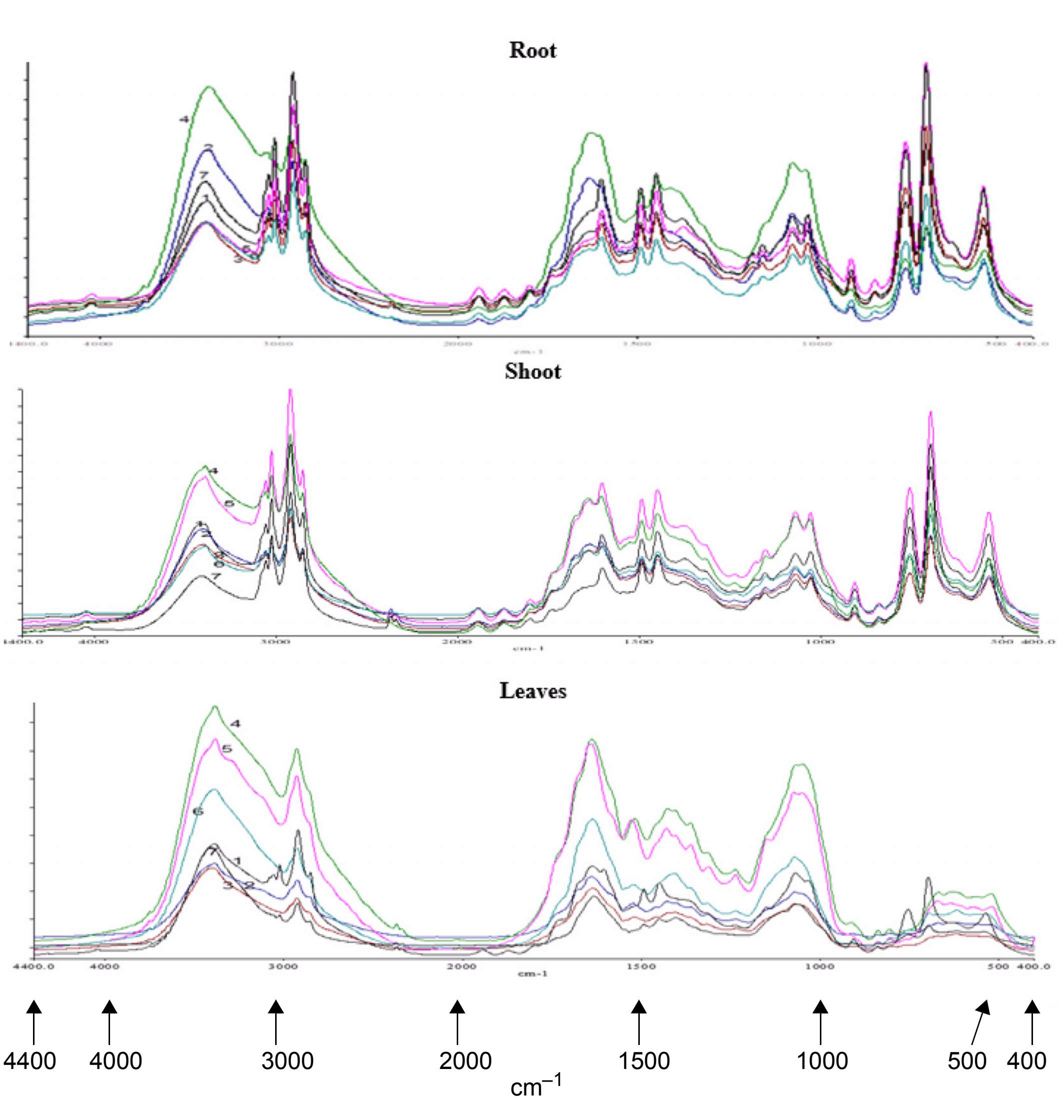

Figure 5. Average lipid (3000–2000 cm^{-1}) absorbance; average proteins (1800–1500?cm^{-1}) absorbance; average carbohydrates (1500–1200 cm^{-1}) and average cell wall components (1000–600 cm^{-1}) of seedlings parts, (root, shoot, and leaves), treated with different halogen elements.with concentration in ppm. 1: control; 2: NaCl 50; 3: NaCl 100; 4: NaI 50; 5: NaI 100; 6: NaF 50; and 7: NaF100.

DISCUSSION

Water is very essential for seed germination. The quality of water used for irrigation can directly affect seedling growth. Fenugreek seed germinated fine with NaCl solutions as compared to NaI and NaF. 100ppm NaCl did not show any change in the root or shoot length but, surprisingly, 50ppm NaCl stimulated the growth of seedlings (Figure 1). NaCl is known to be toxic for plants but not at lower concentrations. NaCl can provide a better ion balance, without causing any change to

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cellular metabolism and the root-to-shoot distribution of other essential nutrients [20]. Improvement in growth at the low level of NaCl in plants has also been reported earlier[21-23]. The same concentration (50 and 100ppm) of NaI and NaF reduced both root and shoot length (Figure 1). Some earlier reports concluded that toxic levels of fluoride and iodine can cause reduce root and shoots length due to imbalanced nutrient uptake by seedling [23-25].

FTIR analysis is involved in the identification and quantification of the plant substances like cell wall components, proteins, and lipids in order to understand the depth of biotic and abiotic stresses in plants[26,27]. Different trends of peaks positions from the spectra in different regions of lipids, proteins, carbohydrates, and cell walls were slightly different in control plants as compared to treated seedlings (Figures 2–5). Biochemical changes in samples can be achieved by analyzing the peak width, position, and intensity of absorption [28-30]. The band height changes indicate some modifications of chemical groups [31] in lipids, carbohydrates, proteins, and cell wall regions. The absorption peaks of treated roots and stems were slightly different from those of leaves. Lipids, carbohydrate, and protein regions of treated leaves showed different peaks when compared to treated roots and stems. The lipids and cell wall region of roots and shoots showed the almost same pattern. The leaf is very sensitive to water stress whereas the root is more resistant[31]. Quality water is vital for the growth and development of a plant and can bring physical or chemical changes in which many biological molecules such as nucleic acids, proteins, carbohydrates, and lipids are involved [32]. Higher or lower absorption levels for most wave numbers in NaI-treated and NaF-treated seedlings than for the control indicate an accumulation or reduction of various biochemical components in order to cope with stress [33-34].

CONCLUSIONS

Fenugreek seedlings were tolerant to NaCl salinity up to 100 ppm but the same amount of NaI and NaF caused reduced growth and biochemical changes. Changes in leaves were slightly different than root and shoot. FTIR spectroscopy can be used to detect conformational changes of biological molecular components in young seedlings as it only requires small sample sizes.

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