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BIOCHEMICAL AND FT-IR PROFILING OF *TRITIUM AESTIVUM* L SEEDLINGS IN RESPONSE TO SODIUM FLUORIDE TREATMENT

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ABSTRACT: Seed germination is widely used as a model to explore toxicity of various harmful chemicals in plants. High concentration of fluoride in water can be harmful for the plant kingdom at all stages of development. Seeds of *Tritium aestivum* L (wheat) were sown in Petri dishes and exposed to five different concentrations of sodium fluoride (20, 40, 60, 80, and 100 ppm) for two weeks. Seeds grown with distilled water were used as controls for comparison. Germination data for the seedlings was recorded and seedlings were harvested on 15th day after sowing. Freshly prepared extract of seedlings was examined for various biochemical assays (total protein, glutathione (GSH), glutathione S-transferase activity, and lipid peroxide) and by Fourier transform infrared spectroscopy (FT-IR) analysis. We found a gradual decrease in seed germination parameters as the concentration of sodium fluoride (NaF) increased. All measured biochemical parameters were also effected in dose dependent manner. The total protein level was slightly increased with 20, 40, and 60 ppm of NaF. However, with 80 and 100 ppm of NaF there was a slight decrease as compared to control. Both GSH and GST levels were decreased with increased concentration of NaF while lipid peroxide was increased with 20 ppm of NaF treatment but showed a slight decrease when treated with higher concentrations of NaF. Overall maximum effect was shown by highest NaF level (100 ppm) and moderate tolerance was observed up to 40 ppm. FT-IR profiles revealed some changes in functional groups of proteins, lipids, and carbohydrates with NaF treatments. Future studies on crop tolerance for plants irrigated with groundwater with high fluoride levels are recommended.

Keywords: Biochemical assay; FT-IR; Germination; Sodium fluoride; *Triticum aestivum* L.

INTRODUCTION

Nearly all plants, and animals contain fluoride in their bodies as they are exposed to it naturally, through water and air.[1-2] A high level fluoride can damage body parts and hence its concentration is very important for the health of plants and animals.[3-4.] Almost all natural waters, like freshwater, seawater, and spring water, contain a small amount of fluoride naturally which may not be at a toxic level but ground water and hot springs near volcanic and mountainous areas may contain much higher levels, such as more that 50 mg/L (50 ppm) of fluoride.[5-6] Some crops are dependent on groundwater for agricultural irrigation and are continuously being exposed to fluoride, which can be dangerous for some sensitive plants.[4]

In plants, fluoride is mainly absorbed by root tissue, and its toxic levels can effect primary plant metabolism, like photosynthetic process, retard the growth, and even reduce crop yields.[7-8] Toxic levels of fluoride can accumulate in plants which can lead to leaf injury and fruit damage. These plants can act as a risk factor for humans through the food chain.[9] A high level of fluoride can damage various body organs, such as the thyroid, and can cause dental, skeletal, and non-skeletal fluorosis in humans.[10-11] A toxic dose of fluoride is known to affect brain, liver, and kidney by

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inducing oxidative stress.[12-13] In plants, seedlings are usually more susceptible to fluoride toxicity as compared to older more mature plants.[14] Proper germination of seed to seedling is the most essential developmental part in the life cycle of plants.[15-16] Enzyme phytase releases inorganic phosphate and other nutrients from phytin in seeds during germination process. Fluorine can inhibit phytase enzyme present in the seeds which slows down the germination rate of seedling.[17] Plant antioxidant activity is influenced by fluoride toxicity due to stress which alters many cellular activities, like inhibition of lipid peroxidation, cell disruption, protein, and DNA damage.[18] Plants under any kind of stress can accumulate or lose various biochemical components, like carbohydrates, proteins, amines, or lipids, which can be investigated by using Fourier transform infrared spectroscopy (FT-IR), since it can detect functional groups of these molecules.[19] Plants can also overcome some sort of stress by their complex anti-oxidative defense mechanisms which mainly include GSH and some antioxidant enzymes.[20] All these biochemical parameters can be monitored in seeds during germination process.[21]

Sodium fluoride (NaF) is widely used in laboratory studies to explore fluoride toxicity at the various stages of growth. Wheat (*Tritium aestivum* L) is the most important edible crop all over the world. Hence, in the present study we monitored seed germination and biochemical change in wheat under different concentrations of sodium fluoride. Recently, FT-IR has being used to monitor changes in cellular activity at all plant developmental stages.[22] Thus, we also comparing the spectra of normal seedlings with fluoride-treated seedlings to investigate the changes if any.

MATERIAL AND METHODS

Growth and seed treatment: Seeds of *Tritium aestivum* L (accession-no. 188) were collected from seed center of Ministry of Agriculture, Saudi Arabia. Healthy seeds were selected and placed on wet filter paper in a Petri dish and treated with 0, 20, 40, 60, 80, and 100 ppm of NaF for two weeks. The seedlings were analyzed for germination parameters, biochemical changes, and scanned by FT-IR spectroscopy.

Germination parameters: Different germination parameters, which included germination percentage, germination index, vigor index, relative injury rate, root length, and shoot length, were assessed in all the treated groups and compared with control.[23]

Extraction and biochemical assays: The fresh seedlings from control and all treated groups were homogenized in phosphate buffer pH 7 (1:10 W/V) separately with the help of mortar and pestle. The extract was centrifuged at 4ºC at 3,000 g for 10 min and the supernatant was used for the following biochemical assays:

(i) Quantitative estimation of crude protein, which was done by the method described by Bradford.[24] (ii) Glutathione (GSH) assay, which was carried out according to the method of Beutler et al.[25] (iii) Glutathione S-transferase activity (GST) activity, which was assessed based upon the GST-catalyzed reaction between GSH, GST substrate, and CDNB (1-chloro-2, 4-dinitrobenzene)[26] and (iv) Lipid peroxide level, which was estimated according to the TBA test of Ruiz-Larrea et al.[27]

Fourier-transform infrared spectroscopy (FT-IR): FT-IR (functional group) analysis of all groups was measured by FT-IR spectrometer at a scan range of 400–4000 cm^{-1} (Thermo Scientific-Nicolet-6700, USA).

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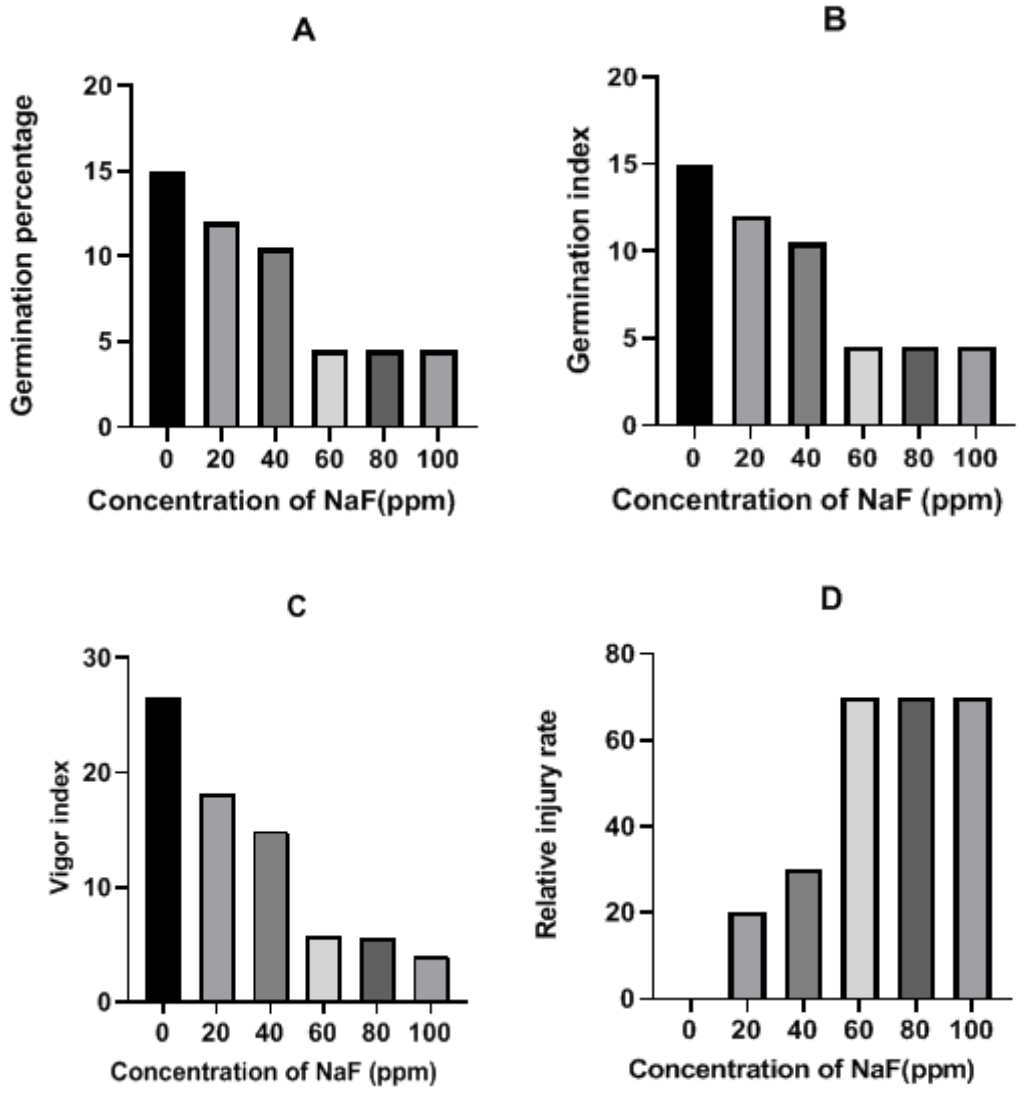
RESULTS

Effects of NaF on final germination of seedlings: All germination indices of seedlings treated with various concentrations of NaF (0, 20, 40, 60, 80, and 100 ppm) are presented in Figures 1A–1F. Seeds treated with various concentrations of NaF showed low germination as compared to control (seeds watered with distilled water) and the effect was concentration dependent. Remarkable changes in all the germination parameters including germination percentage, germination index, vigor index, relative injury rate, root length, and shoot length were observed above 40 ppm NaF while seeds treated with 20 and 40 ppm NaF showed a less than 50% effect.



Figures 1A–1D: Effects of different concentration of NaF on 1A: germination percentage, 1B: germination index, 1C: vigor index, and 1D: relative injury rate.

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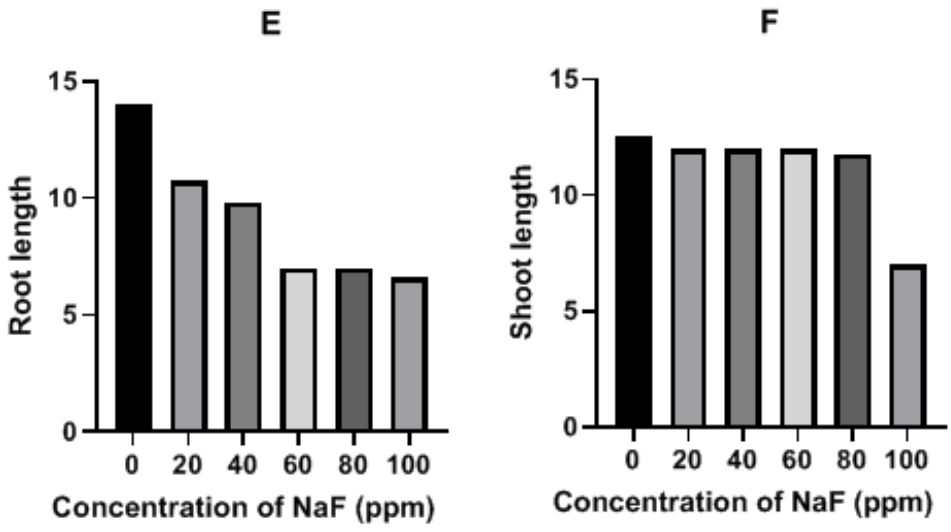
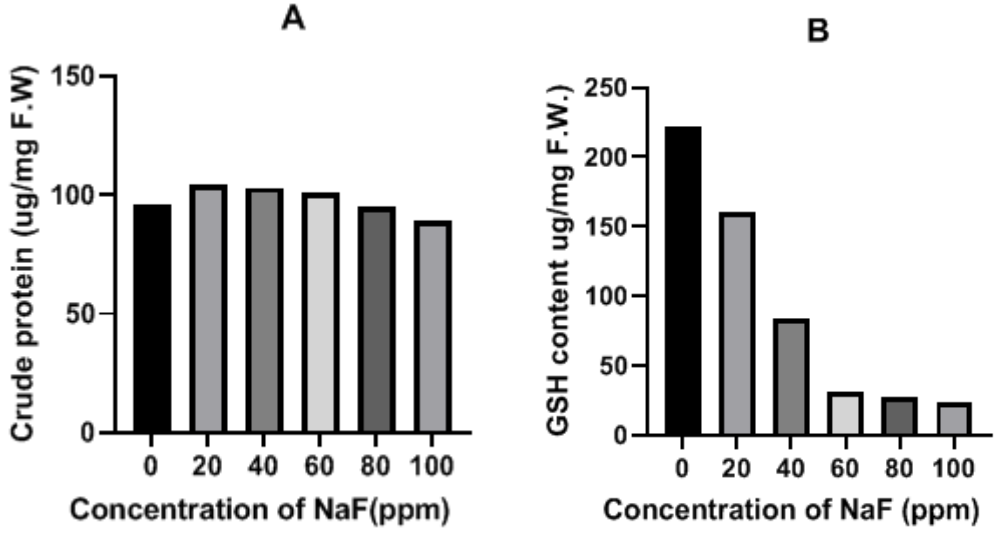


Figures 1E and 1F: Effects of different concentration of NaF on 1E: root length and 1F: shoot length.

Effects of NaF on biochemical parameters of seedlings: Biochemical responses of seedlings treated with NaF were determined by analyzing anti-oxidative defense mechanisms by measuring total protein, GSH content, GST, and lipid peroxide levels as shown in Figures 2A–2D. Total protein was increased initially with 20, 40, and 60 ppm NaF concentration but then decreased when NaF reached 80 and 100 ppm as compared to control (Figure 2A). GSH and GST showed same trend and decreased with an increased concentration of NaF, as clearly seen in Figures 2B and 2C. Lipid peroxide was initially increased with 20 ppm of NaF and then with increasing concentrations it slightly decreased as compared to control (Figure 2D)



Figures 2A and 2B: Effects of different concentration of NaF on 2A: crude protein and 2B: GSH content. (F.W.= fresh weight).

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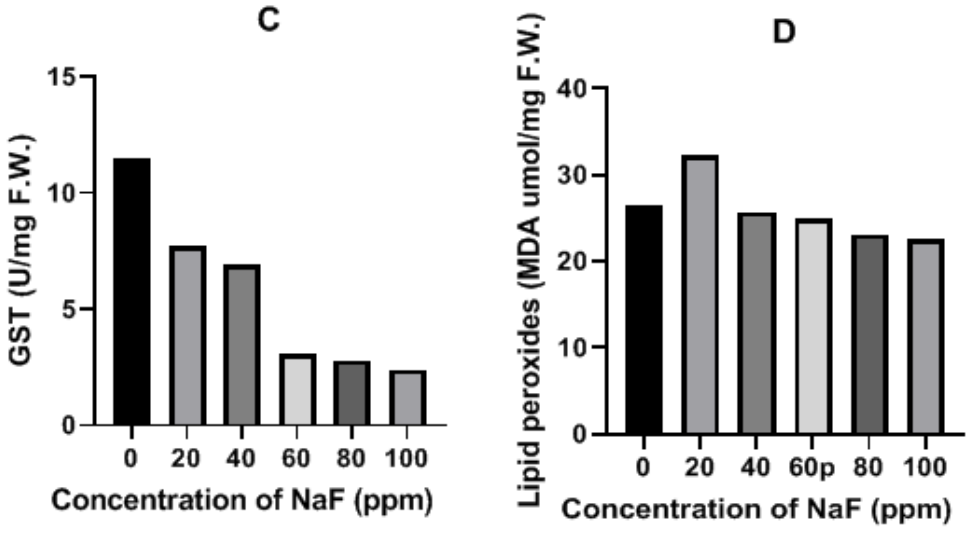

Figures 2C and 2D: Effects of different concentration of NaF on 2C: GST level and 2D: lipid peroxides. (F.W.= fresh weight).

Fourier-transform infrared spectroscopy: FT-IR profiling was done to explore the physiological response of seedlings to varying concentrations of NaF treatment and results are presented in Figure 3 and the Table.

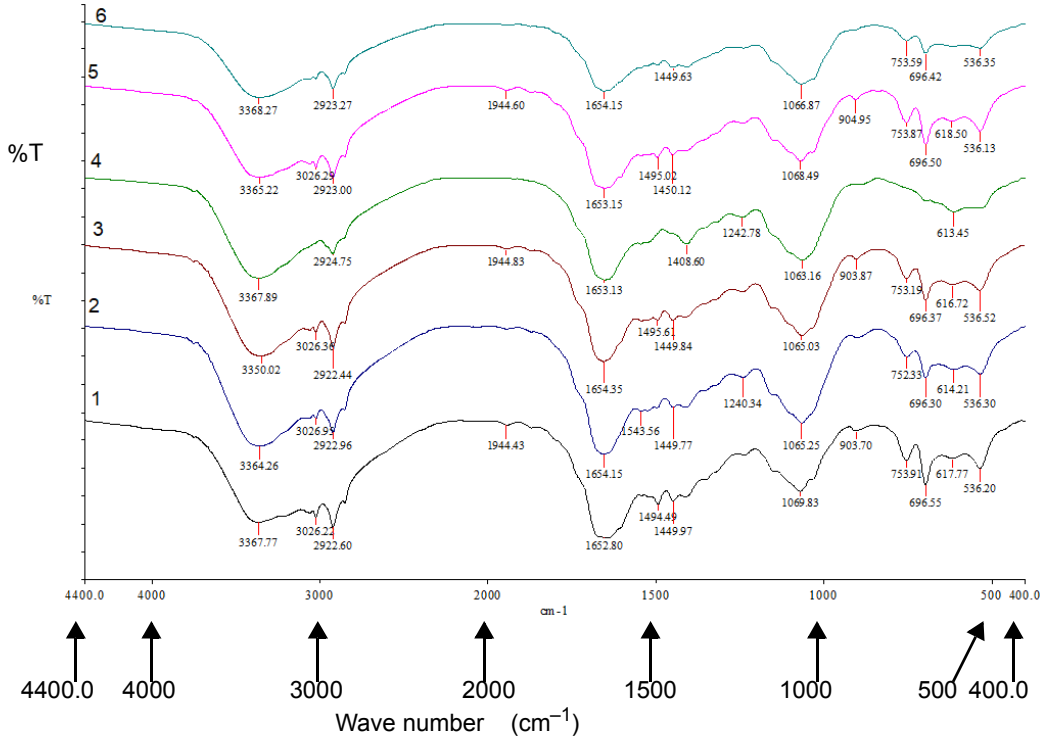

Figure 3. FT-IR spectra of the seedlings treated with different concentrations of NaF: 1=0 ppm, 2=20 ppm, 3=40 ppm, 5=80 ppm, and 6=100 ppm. (%T=% transmittance).

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Table. FT-IR spectra showing observed peaks and probable functional groups in the seedlings treated with various concentrations of NaF (0, 20, 60, 80, and 100 ppm)

Wave number (cm^{-1}) and fluoride concentration (ppm)						Functional group
0 ppm	20 ppm	40 ppm	60 ppm	80 ppm	100 ppm	
Lipids						
3367.77	3364.36		3367.98	3365.22	3368.27	1
		3350.02				2
3026.22	3026.95	3036.36		3036.29		3
2922.60	2922.94	2922.44	2924.75	2923.00	2923.23	4
Proteins						
1944.43		1944.83		1944.60		5
1652.80	1654.15	1654.15	1653.13	1653.15	1654.15.	6
	1543.46					7
Carbohydrates						
1494.49		1492.61		1495.02		8
1449.97	1449.77	1449.84		1450.12	1449.63	9
			1408.60			10
	1240.34		1242.78			11
Cell wall components						
1069.83	1065.25	1065.03	1063.16	1068.49	1066.87	12
903.70		903.87		904.95		13
735.91	752.33	753.19		753.87	753.59	14
696.55	696.30	696.37		696.50	696.42	15
617.77	614.21	616.72	613.45	618.50		16
536.20	536.30	536.52		536.13	536.35	17

1: N__H stretch (amines)
2: O__H stretch (alcohols), N__H stretch (amines, amides)
3: N__H stretch (amides), O__H stretch (alcohols), S, O__H stretch (carboxylic acids)
4: S, O__H stretch (carboxylic acids), C__H stretch (alkenes)
5: C=O stretch (ketone), C=C (benzene)
6: N__H bend (nitro compounds, amides), C__C stretch (amides), C¼O stretch (carboxylic acid, ketone), C¼C (benzene, alkenes)
7: N__H bend (nitro compounds), C__O stretch (amides, ketone), C¼C (benzene)
8: N_H bend (nitro compounds), C_O stretch (amides), C=C (benzenes), C=O (ketones)
9: N_H bend (nitro compounds), C__O stretch (amides), C=C (benzenes), C=O (ketones)
10: S(=O)2 stretch (sulfones), N¼O stretch (nitro compounds), O__H bend (carboxylic acids, alcohols)
11: C__N stretch (amines), C__O stretch (esters), C__O stretch (ethers, alcohols), O__H band (carboxylic acids)
12: S=O stretch (sulfoxides), C__N stretch (amines), C__O stretch (esters, ether, alcohol), =C__H bend (alkenes)
13: C=C__H bend (alkenes) (pectin)
14: C__N stretch (amines), =C__H bend (benzene), C__C stretch (chlorides)
15: C__N stretch (amines), =C__H bend (benzene), C__C stretch (chlorides)
16: C__N stretch (amines), =C__H bend (benzene), C__C stretch (chlorides)
17: Alkyne C-H bend

DISCUSSION

Fluorine is considered to be a ubiquitously distributed toxic element for almost all living organisms, including plants, animals, and bacteria.[28-29] Most crops in the world are irrigated with ground water which often contains higher concentrations of fluoride.[30] Almost all solutions of NaF used in the study were found to delay or inhibit seed germination (Figures 1A and 1B). Germination process is initiated by breaking seed dormancy to restore metabolic activity which mainly depends on some internal and environmental conditions. Many studies have reported fluoride as a metabolic inhibitor in plants, especially in breaking seed dormancy.[14] Fluoride can inhibit phytase enzymes, and reduce carbohydrate metabolism and amylase activity during the germination process.[30-31] It is well known that fluoride toxicity can lead to the accumulation of reactive oxygen species (ROS) and can thus change the enzymatic and non-enzymatic antioxidant systems in a living cell. Many studies have reported oxidative damage as a major effect of F toxicity in plants.[32-3.] Increase of total protein content in seedlings exposed to 20, 40, and 60 ppm of NaF solution may be due to the induction of stress proteins (Figure 2A).[34] Decrease in total protein content in seedlings treated with 80 and 100 ppm of NaF solutions can be due to protein degradation and accelerated proteolysis due to a high level of toxicity.[35] Glutathione and glutathione-related enzymes are widely used to mark oxidative stress in plants in response to any kind of environmental stress. Decreased level of GST and GSH in NaF-treated seedlings can be directly related to the oxidative stress response (Figures 2B and 2C).[36] Oxidative stress in plants is also well assessed by measuring peroxidation of unsaturated fatty acids. Seedlings treated with NaF may produce an imbalance in ROS which can trigger oxidation of polyunsaturated lipids. FT-IR profiling of seedlings was done for detection of chemical and conformational changes due to NaF stress (Figure 3 and the Table). Slight changes in chemical composition and functional groups of proteins, lipids, and carbohydrates due to NaF treatments indicate stress-induced metabolic changes [22].

CONCLUSIONS

Irrigation water contaminated with fluoride can induce biochemical changes and delay growth in plants. Hence, it is inferred that crops irrigated with fluoride containing groundwater should be tested for fluoride tolerance.

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