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Effect of salinization on health benefits of fenugreek seedlings

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Abstract: The current investigation was conducted to evaluate the potency of salinity to enhance the nutraceutical properties of fenugreek (*Trigonella foenum-graecum* L.) seedlings. Gradual doses of NaCl solution (0, 50, 100, 150 and 200 mM) were applied and the nutritional value of seedlings was determined 2 and 5 days' post- germination. Moreover, the antioxidant and antimicrobial activity along with the phenolic content of the considered seedlings were assessed. The obtained results manifested that salt application to different levels could increase the amount of protein, N, Zn and Cu in the 2- and 5- day old seedlings. Salinity could also raise the amount of vitamin C, B1, B2 and B9 but only in one stage. Conversely, the amount of reducing, non- reducing and total sugars as well as that of Fe declined under stress. Compared with their unsalted synonyms, water and methanolic extracts of salt- treated fenugreek seedlings had higher H_2O_2- scavenging activity in the two stages, while their reducing power and free radicles- scavenging activities were promoted in the second stage only. Regarding the antimicrobial activity, water and methanolic extracts of the stressed seedlings generally exhibited better antimicrobial activity against some pathogenic microbes especially *Erwinia carotovora*, *Bacillus subtilis* and *Candida albicans*. Only in the second stage, most of the employed salt concentrations increased the titer of tannins, saponins, alkaloids, flavonoids and total phenols in the aqueous and alcoholic extracts. Therefore, it is recommended to salinize young fenugreek seedlings to improve its health benefits in a simple, low-cost and low- risk approach.

Key words: fenugreek seedlings; salinity; nutritional value; antioxidant activity; antimicrobial potency; phenolics

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual herb belonging to family Fabaceae. As many as 260 species of genus *Trigonella* were identified with the species *foenum-graecum* most distributed throughout the world. It is cultivated as a semi- arid crop in Mediterranean countries, Europe and Asia [1]. According to Lust [2], fenugreek is one of the oldest known medicinal plants in the recorded history. Fenugreek leaves and seeds have been famed for their anti- diabetic, cholesterol-lowering, antitumor, antibacterial, antifungal, vermicidal and antioxidant activities [3]. Fenugreek seeds are also reputed for numerous medicinal virtues with tonic, demulcent, emollient, carminative, emmenagogue, diuretic, aphrodisiac and expectorant properties. Moreover, they have been widely used since millennia as a treatment for stomach irritation, mouth ulcers and chapped lips [4].

With the dramatic increase in population number all over the world along with the spread of diseases, there is an ever-increasing need to cultivate medicinal plants. Yet, different forms of environmental stresses could retard agricultural activities with eventual interference with the quantitative and qualitative productivity of medicinal plants [5]. Among such stresses, salinity is one of the most problematic issues threating the agricultural sector [6]. Studies on the negative effects of salt stress on growth, development and yield of various medicinal plants could be reported [7, 8]. However, and up to our knowledge, so little attention is paid to the utilization of environmental traumas in enhancing the medicinal value of certain plants.

In recent studies, Mickky *et al.*, [9, 10] could maximize the medicinal value of alfalfa plants by growing them with decreasing water supply either alone or combined with increasing sand amount in the cultivation soil. Such stressful conditions could significantly enforce the studied plants to over- produce some medically- active phytochemicals such as phenolics, vitamins, non-photosynthetic pigments and minerals. Furthermore, the extracts of alfalfa plants under such treatments possessed better antioxidant as well as antibacterial and antifungal activities.

Therefore, the present investigation was intended to assess the effect of gradual levels of salt stress on health benefits of fenugreek seedlings. The amount of some nutritive phytoconstituents was determined in addition to evaluating the antioxidant and antimicrobial potentialities of the extracts prepared from stressed and unstressed seedlings.

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Materials and Methods

Experimental Design

Seeds of fenugreek (*Trigonella foenum-graecum* L. variety Giza 3) were obtained from Sakha Agricultural Research Center, Kafr El-Sheikh Government, Egypt. Homogeneous lot of seeds was externally sterilized using 4% NaOCl. The seeds were hydro- primed for 8 hours then allowed to germinate on gauze in dark-painted plastic boxes at 25± 2°C. The germinated seeds were thereafter sprayed with NaCl solutions at 0, 50, 100, 150 or 200 mM when required till the sampling was performed after 2 and 5 days from the experiment beginning.

Evaluation of Nutritional Value of Fenugreek Seedlings

To assess the nutritional value of fenugreek seedlings under the studied conditions; moisture, protein, nitrogen and sugar contents were determined. Also, some vitamins and heavy metals were quantified.

Determination of chemical composition:

Moisture content of fenugreek seedlings was determined according to the method described by AOAC [11]. Also, crude protein amount in the studied samples was recorded by the official Kjeldahl method of AOAC [11]. The percentage of total nitrogen could be determined then protein content was calculated using 6.25 as a coversion factor. Total sugars content was determined according to phenol- sulfuric acid method cited from Masuko *et al.,* [12]. The amount of reducing sugars was determined by dinitrosalicylic acid method of Miller [13]. The titre of non- reducing sugars could be thus calculated by subtracting the value of reducing sugars from that of total sugars.

Determination of heavy metals:

To extract heavy metals, dry plant ashes were digested in concentrated nitric acid coupled with sulfuric acid in presence of perchloric acid. After being filtered, clear plant extracts were analyzed for Fe, Zn and Cu using atomic absorption spectrophotometer (Buck Scientific Series model 214; USA) as described by Allen *et al.,* [14].

Determination of vitamins:

For determination of vitamin C, the titration routine of Ogunlesi *et al.,* [15] was followed using 2, 6-dichlorophenol indophenol dye solution. Meanwhile, the amounts of vitamin B1, B2 and B9 were colorimetrically determined as described by Poornima and Ravishankar [16], Uraku *et al.,* [17] and AOAC [11]; respectively.

Evaluation of the Medicinal Efficacy of Fenugreek Seedlings

To study the medicinal efficiency of the seedlings, water and methanolic extracts were prepared to determine their antioxidant and antimicrobial activities. In addition, the amount of some medically-active phenolics was recorded in those extracts.

Preparation of water and methanolic plant extracts:

To obtain water extracts, known weight of the tissues dried at 50°C was incubated in distilled water at 70°C for 20 minutes then filtered. Meanwhile, the dry plant tissues were shaken in methanol at 37°C for 3 hours followed by filtration to obtain the methanolic extracts.

Determination of antioxidant activity:

The procedure given by Ebrahimzadeh *et al.,* [18] was adopted to demonstrate 2, 2'-diphenyl-1-picrylhydrazyl (DPPH)- scavenging capacity of the plant extracts. The plant tissue (in mg) required to reduce initial DPPH concentration by 50% (IC_{50}) was computed graphically. Moreover, the antiradical power (ARP) of the extracts was calculated as the reciprocal of IC_{50}. To explore 2, 2'-azino-bis 3-ethyl benzothiazoline-6-sulfonic acid (ABTS)- scavenging capability of the extracts, the method of Re *et al.,* [19] was followed. The extracts were also assessed for their H_2O_2-scavenging ability following the spectrophotometric protocol of Keser *et al.,* [20]. In addition, the reducing power was investigated following Yildirim *et al.,* [21].

Determination of antimicrobial activity:

Antimicrobial assay was performed *via* filter paper disc method described by Murray *et al.,* [22]. Stock cultures of *Klebsiella pneumonia*, *Escherichia coli*, *Erwinia carotovora*, *Bacillus subtilis*, *Proteus vulgaris*, *Streptococcus pyrogenes*, *Staphylococcus epidermidis*, *Enterobacter cloacae*, *Pseudomonas aerugenosa* and *Candida albicans* were obtained from Laboratory of Microbiology, Faculty of Medicine, Mansoura University, Egypt. Nutrient agar medium for bacteria as well as Czapek dox agar for the fungus was used. Ampicillin as an antibacterial agent and clotrimazole as an antifungal drug were employed as positive controls. The microbial susceptibility was evaluated by measuring the inhibition zone diameter.

Determination of phenolic compounds:

Following the spectrophotometric analyses cited from Lin and Tang [23] and Sadasivam and Manickam [24], Folin-Ciocalteu reagent and vanillin hydrochloride reagent were used to record the amount of total phenols and tannins; respectively. In addition, gravimetric methods were adopted to determine the amount of saponins [25], flavonoids [26] and alkaloids [27].

Results

Changes in Nutritional Value of Fenugreek Seedlings

In comparison with the unsalted fenugreek seedlings, application of NaCl at 50 and 200 ppm increased moisture content of the 2- day old seedlings; while 100, 150 and 200 ppm NaCl increased their moisture content when they became 5- day old. Otherwise, a drop-in moisture content of the studied seedlings was recorded. During the two stages, all salt concentrations could raise protein and nitrogen content of the seedlings with corresponding reduction in the amount of reducing, non- reducing and total sugars (Figure 1).

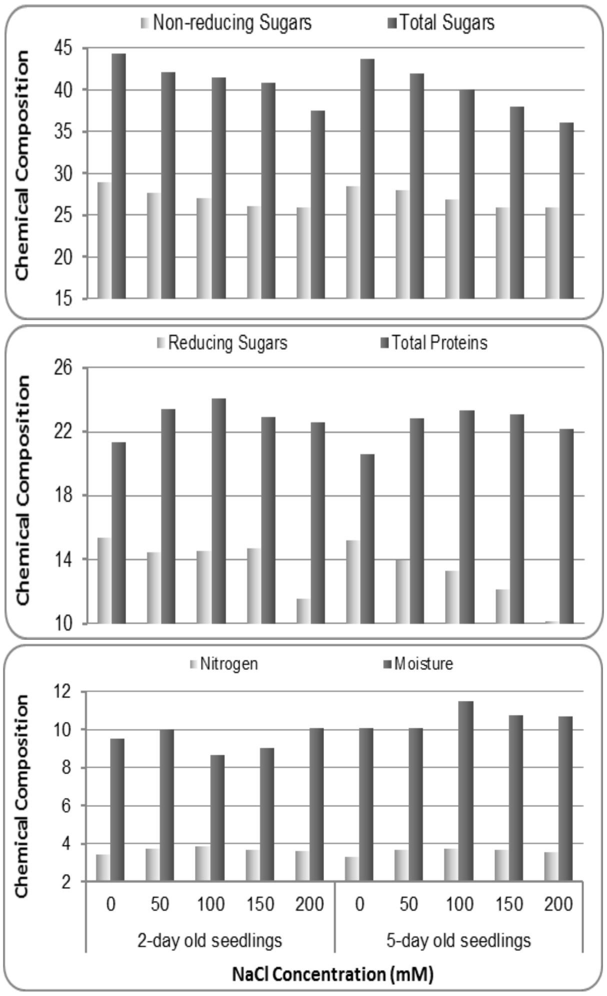

Figure 1: Effect of different salt concentrations on chemical composition (%) of fenugreek seedlings at two different germination stages.

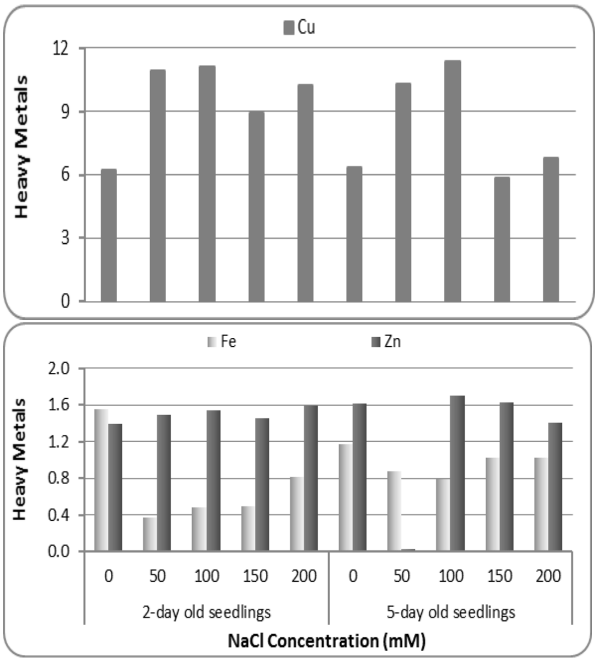

Figure 2: Effect of different salt concentrations on heavy metals contents (mg 100 g^{-1}) of fenugreek seedlings at two different germination stages.

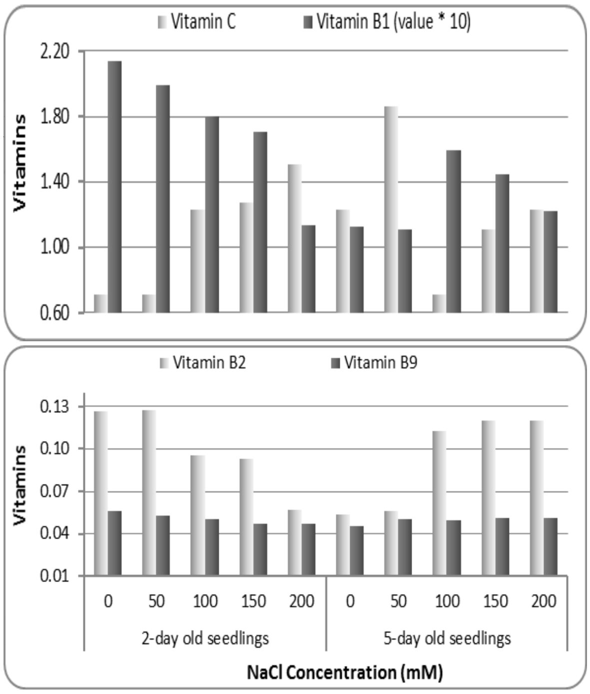

Figure 3: Effect of different salt concentrations on vitamins contents (mg g^{-1}) of fenugreek seedlings at two different germination stages.

With respect to heavy metals, the amount of Fe generally decreased by salt application at the two addressed stages. In the 2- day old seedlings, Zn and Cu amounts increased by almost all salt levels; while these metals increased by some salt levels when the seedlings were 5- day old (Zn by 100 and 150 ppm and Cu by 50, 100 and 200 ppm). Except under these levels, a decline in the two metals in the 5- day old seedlings was noticed (Figure 2).

Regarding the assayed vitamins, vitamin C increased by most the applied salt concentrations (100, 150 and 200 ppm) in the first stage and the converse was observed in the second stage. For vitamin B1, B2 and B9; they were increased by almost all salt concentrations in the second stage but decreased in the first one because of salt (Figure 3).

Changes in Medicinal Efficacy of Fenugreek Seedlings

Away from DPPH- scavenging activity, the methanolic extracts of fenugreek seedlings generally possessed higher antioxidant capacity than water extracts. Both of water and methanolic extracts of the 2- and 5- day old seedlings had enhanced H_2O_2- scavenging activity when treated with salt at different concentrations. For the reducing power as well as ABTS- and DPPH- scavenging activities of the extracts, these were decreased in the 2- day old seedlings but increased in the 5- day old ones grown under stress (Figure 4).

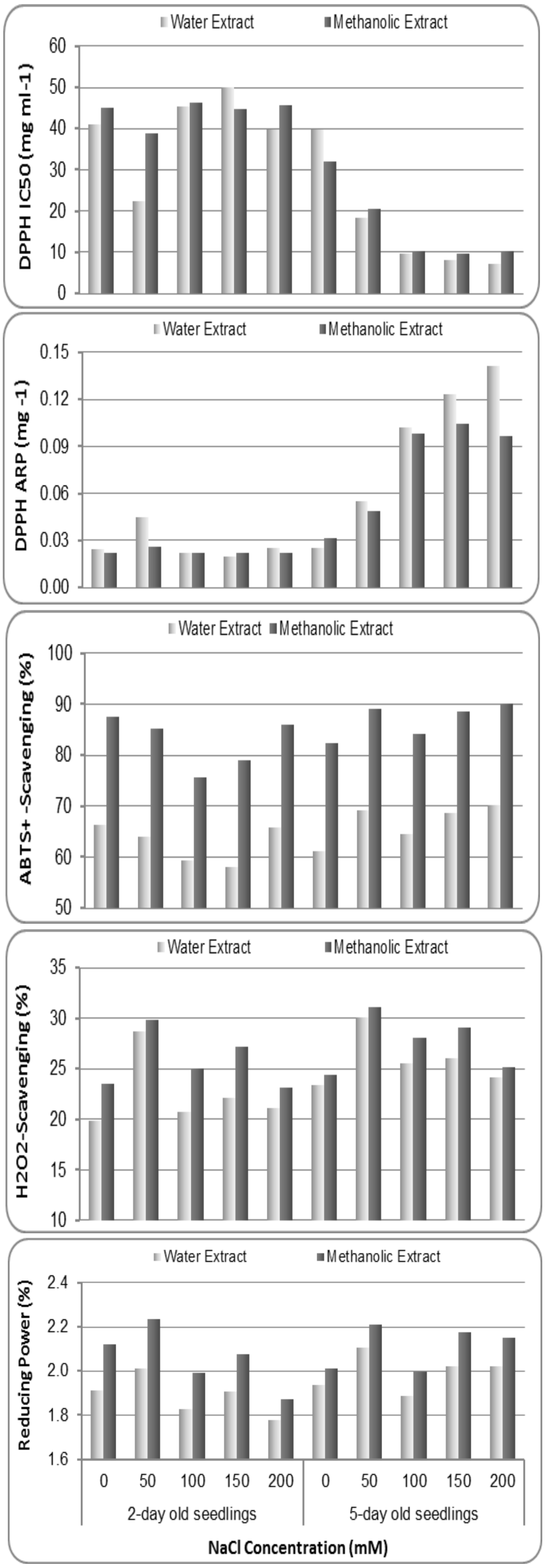

Figure 4: Effect of different salt concentrations on antioxidant activities of fenugreek seedlings at two different germination stages.

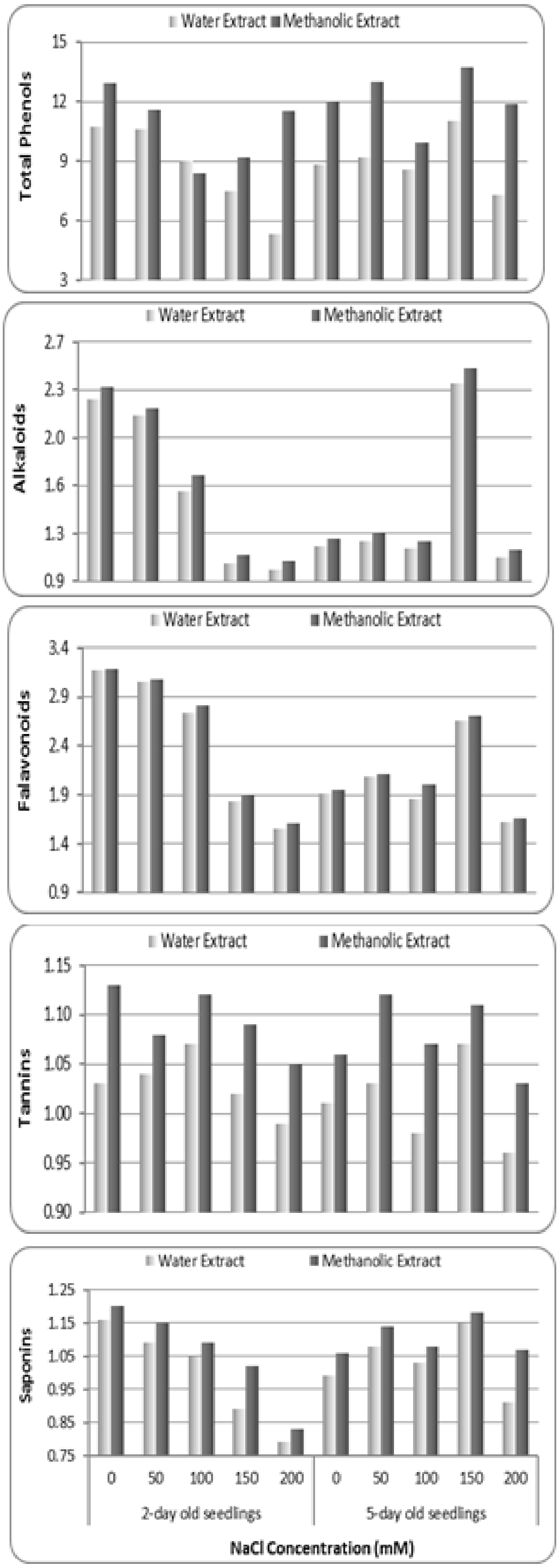

Figure 5: Effect of different salt concentrations on phenolics contents (%) of fenugreek seedlings at two different germination stages.

Concerning the antimicrobial activity represented in table 1, the suppressing effect of water extracts was enhanced by some salt concentrations particularly against *Klebsiella pneumonia* (by 50, 100 and 150 ppm NaCl in the second stage), *Erwinia carotovora* (by 50, 100 and 150 ppm in the first stage), *Bacillus subtilis* (by 100 ppm in the first stage), *Candida albicans* (by 50 ppm in the second stage) as well as *Streptococcus pyrogenes* and *Staphylococcus epidermidis* (both by 50, 100 and 200 ppm in the first stage in addition to 100 and 200 ppm in the second stage). For methanolic extracts, some salt concentrations could enhance the antimicrobial potentiality against *Erwinia carotovora* (200 ppm in the first stage), *Bacillus subtilis* (50 ppm in the first stage) and *Candida albicans* (150 ppm in the second stage).

Escherichia coli, *Proteus vulgaris*, *Enterobacter cloacae* and *Pseudomonas aerugenosa* all seemed to be resistant to the extracts of both stressed and unstressed fenugreek seedlings so these were not represented in table 1.

As observed for the antioxidant activity, methanolic extracts of fenugreek seedlings generally contained higher amount of phenolic compounds than water extracts. In the first stage, tannins in water extracts were the only class of phenolics that increased when applying NaCl particularly at 50 and 100 ppm. In the second stage and in both water and methanolic extracts; tannins, alkaloids, flavonoids and total phenols were all increased by 50 and 150 ppm NaCl solution while saponins increased by 50, 100 and 150 ppm (Figure 5).

Table 1: Effect of different salt concentrations on the antimicrobial activity of fenugreek seedlings at two different germination stages. WE = water extract; ME = methanolic extract; Nt = not tested.

Germination Stage	Salt Concentration (mM)	Inhibition Zone Diameter (mm) *Klebsiella pneumonia*		*Erwinia carotovora*		*Bacillus subtilis*		*Streptococcus pyrogenes*		*Staphylococcus epidermidis*		*Candida albicans*	
		WE	ME	WE	ME	WE	ME	WE	ME	WE	ME	WE	ME
2- Day Old Seedlings	0	0	0	0	0	**9**	0	**7**	0	**7**	0	**7**	**7**
	50	0	0	**7**	0	**7**	**8**	**10**	0	**8**	0	0	0
	100	0	0	**7**	0	**10**	0	**8**	0	**9**	0	0	0
	150	0	0	**7**	0	0	0	0	0	**7**	0	0	**6**
	200	0	0	0	**7**	**7**	0	**9**	0	**8**	0	0	0
5- Day Old Seedlings	0	**7**	0	0	0	**8**	0	**7**	0	0	0	**7**	0
	50	**8**	0	0	0	**7**	0	**8**	0	**8**	0	**8**	0
	100	**8**	0	0	0	**7**	0	**8**	0	**8**	0	0	0
	150	**8**	0	0	0	0	0	**8**	0	**8**	0	**7**	**8**
	200	0	0	0	0	0	0	**8**	0	**8**	0	**7**	0
Antibiotic Drugs		**27**		Nt		Nt		**25**		Nt		**15**	

Discussion

Plants can synthesize vast array of chemicals in the form of primary or secondary metabolites; and these phytochemicals can be utilized by human as valuable nutrients and to cure or prevent various ailments. *Via* the present study, the impact of salt stress on phytonutrients of fenugreek seedlings was addressed. The moisture content of the studied 2- and 5- day old seedlings increased under most of the applied NaCl concentrations. A similar increase in moisture content of fenugreek plants at successive growth stages under salt treatment was lately recorded by Kapoor and Pande [28] who ascribed that pattern of change in moisture content to the deteriorative status of the plant cells under stress.

Concerning total protein and nitrogen contents of the concerned fenugreek seedlings, these were increased in response to the application of all the used salt concentrations. Matching our findings, Kumar *et al.,* [29] recorded an increase in protein content of some rice cultivars as a result of increasing salinity level up to 100 mM NaCl. Also, Abdul Qados [30] reported a comparable increase in protein content of bean plants with raising NaCl concentrations till 240 mM. Such an alteration in the amount of protein and nitrogen may be an adaptive response to salt stress since some plants can resist osmotic stress by accumulating certain osmolytes such as soluble proteins and other nitrogenous compounds that enable their cells to absorb more water. Coinciding with this assumption, Kosová *et al.,* [31] pointed out that an active salinity acclimation is conferred by stimulated biosynthesis of certain novel proteins with osmoprotective functions.

With respect to sugar content, a reverse trend was observed herein where salt stress decreased the amount of reducing, non- reducing and total sugars in fenugreek seedlings. Similarly, and at 12 dS m^{-1} salinity level, the amount of reducing, non- reducing and total sugars in the tissues of five Malaysian rice varieties decreased in comparison with their unstressed equivalents [32]. In the current investigation, the decline noticed in seedling sugar content under salt stress may result from lower ability of the stressed seedlings to synthesize sugars and/ or higher rate of sugar degradation to compensate the disturbing conditions [33].

Regarding the amount of heavy metals in the explored fenugreek seedlings, the amount of Fe generally decreased by salt application at the two addressed stages, while Zn and Cu amounts fluctuated among the various treatments. Almost all the studies conducted to assess heavy metal composition of certain plants under the effect of salt stress have highlighted the impact of salinity on the plant ability to uptake heavy metal from the growing medium [34, 35]. However, the case in the present study is completely different since the growing medium of fenugreek seedlings herein is simply NaCl solution at different concentrations (0, 50, 100, 150 or 200 ppm). So, the fluctuation in heavy metals concentrations in the addressed seedlings in response to raising salt concentration can be explained only on the basis of the metabolism of metals already present in the seeds before applying NaCl stress.

In the current investigation, the reduction in Fe content of salinized fenugreek seedlings may indicate higher consumption of Fe reserve under stress conditions in a trial to cope with salinity. Fe is an essential micronutrient for plant cells particularly at the juvenile stage when it is involved in various biochemical and physiological pathways. Fe plays an important role in protein and DNA biosynthesis [36]. Furthermore, Fe acts as a component of some vital enzymes such as cytochromes of the respiratory electron transport chain [37]. Fe is also an active cofactor for many enzymes required for synthesis of the plant hormones ethylene and abscisic acid which are active in many plant development pathways and their adaptive responses to the fluctuating environmental conditions [38].

Unlike the case for Fe, the fenugreek seedlings addressed herein contained higher amounts of Zn and Cu when treated by almost all salt levels in the first stage and some salt levels in the second stage (higher Zn by 100 and 150 ppm; and Cu by 50, 100 and 200 ppm). The higher content of such metals in the salt- stressed seedlings may refer to less ability of the seedlings to benefit from the reserve of these elements when stressed. The two elements are essential for plant cells and are involved in various metabolic processes.

For plants, Zn is an essential micronutrient involved in a wide range of physiological processes [39]. Generally, Zn is involved in protein synthesis [40], auxin anabolism [41] and stabilization of cellular membranes [42]. More interestingly and according to Tavallali *et al.,* [43], Zn can reduce the harmful impact of salt stress where Zn can affect the plant capacity for both water uptake and transport. In cellular membranes, some ligands such as cysteine and histidine can bind to Zn minimizing the production of toxic hydroxyl radicals [41]. Furthermore, there are some evidences that Zn is involved in oxidative stress- induced expression of genes encoding antioxidant defense enzymes such as ascorbate peroxidase and glutathione reductase [44].

With respect to Cu, it is also one of the essential micronutrients required for growth and development of plants. *In vivo*, Cu can exist in different oxidation forms; mainly Cu^{2+} and Cu^{+}. According to Raven *et al.,* [45], Cu is a redox- active metal that serves as a structural element in various regulatory proteins. In addition, Cu participates in cell wall metabolism, hormonal signaling as well as respiratory reactions. Furthermore, Cu plays an important role in oxidative stress responses. Cu ions also act as cofactors for many essential enzymes such as amino oxidase, cytochrome c oxidase, superoxide dismutase and polyphenol oxidase. Moreover, Cu is vital for iron mobilization [46].

In addition to moisture content as well as the amount of protein, nitrogen, sugars and heavy metals investigated in fenugreek tissues in the present study, some vitamins were also quantified under the experimental conditions. The amount of vitamin c in the seedlings generally increased by salt application in first stage but decreased in the second stage. The reverse was recorded for vitamin B1, B2 and B9 that generally increased in the second stage because of salt treatments but decreased in the first stage. A wide range of observations was reported concerning the alteration in vitamins content in plants exposed to salinity. However, it is well documented that some plants suffering different forms of stressful conditions tend to over- synthesize certain water soluble vitamins (vitamin C and some vitamins of B group) with potent antioxidant activity [47].

In accordance with our finding in the first stage, increased ascorbic acid (vitamin c) content in response to salt treatment was previously recorded in *Hordeum vulgare* [48], *Lycopersicon esculentum* [49] and *Cicer arietinum* [50]. Accumulation of ascorbic acid in stressed plants may be an adaptive strategy to cope with the unsuitable conditions. According to Asensi-Fabado and Munné-Bosch [51], ascorbic acid can scavenge reactive oxygen species (ROS), particularly singlet oxygen, preventing the oxidative damage of essential macromolecules (nucleic acids, lipids and proteins) in cytosol, chloroplasts, mitochondria and peroxisomes. However, lowered ascorbate content in the elder fenugreek seedling (5 days old) reported herein in response to salinity may reveal lower capability of seedlings to synthesize such vitamin or higher degrading rates.

Ratnakar and Rai [52] reported that thiamine (vitamin B1) content of *Atriplex hortensis* plants decreased under NaCl treatment at 60 mM compared to the unstressed plants. On the other hand, Tunc-Ozdemir *et al.,* [53] found that salt- stressed *Arabidopsis thaliana* seedlings had more thiamine content than their unstressed relatives. Also, Rapala-Kozik *et al.,* [54] observed upgraded levels of thiamine content in NaCl- treated *Zea mays* plants. According to Tunc-Ozdemir *et al.,* [53], stress- modulated increase in thiamine amount may at least in part result from the enhanced expression of genes coding thiamine synthesis. They also documented that thiamine over- production in stressed plants could stimulate their tolerance to salt- induced oxidative stress. In this context, thiamine was proven to have the ability to quench certain types of ROS especially superoxide anions and hydroxyl radicals [53]. Moreover, thiamine was suggested to have an indirect role in the antioxidative defense system through the provision of more NAD(P)H under stressful conditions [47].

In the leaves of 20 mM NaCl- treated *Atriplex hortensis* plants, riboflavin (vitamin B2) content remained as the same as that in control plants but it was significantly decreased at 40 and 60 mM NaCl concentrations [52]. On contrary, Mickky *et al.,* [9] found that riboflavin amount in *Medicago sativa* plants was significantly increased in response to water stress. Although riboflavin was not recognized as a direct antioxidant, some studies have indicated that this vitamin has the ability to induce the accumulation of potent antioxidants more obviously in plants grown under stress [55]. In addition, some riboflavin derivatives, especially FAD, were found to be needed for the activity of many antioxidant enzymes involved in H_2O_2 scavenging [56]. Moreover, riboflavin was recently recognized as an important regulator of the cellular redox status and is hence involved in the complex network forming the antioxidant defense system [47].

To the best of our knowledge, studies on the stress-induced changes in folic acid (vitamin B9) content of higher plants are to somewhat scarce. However, the common trend in the past few years is directed to exogenous application of folate [57] or folate biofabrication through metabolic engineering [58] to induce potent effects on plant response to stress. Generally, folate and its derivatives have various metabolic functions the most critical of which is the biosynthesis of amino acids, nucleic acids and pantothenate. Moreover, this vitamin is essential for lignin formation and is involved also in photorespiration [59]. Like riboflavin, folate was also recognized as a regulator of the cellular redox state [47].

The antioxidant activity of fenugreek seeds, germinated seeds as well as the vegetative parts is well documented using various antioxidant assays. A study by Dixit *et al.,* [60] has revealed potent antioxidant activity of germinated fenugreek seeds indicated by ferric reducing power, scavenging of DPPH and ABTS radicals, oxygen radical absorbance capacity, pulse radiolysis and inhibition of lipid peroxidation. In that study, the authors ascribed fenugreek antioxidative activity at least partly to the presence of flavonoids and polyphenols. In addition, Kaviarasan *et al.,* [61] recorded that fenugreek seed extract could scavenge DPPH, ABTS as well as hydroxyl radicals and inhibit H_2O_2- induced lipid peroxidation. They similarly assigned the potency of fenugreek extracts to protect cellular structures from oxidative damage to their high phenolic contents. More recently, methanolic extract of fenugreek seeds were assessed for their antioxidant properties by Pathak *et al.,* [62] who proved that such extract exhibited significant potentiality for iron reduction as well as the scavenging of hydroxyl and DPPH radicals. They also attributed these activities to fenugreek phenolic contents.

Nevertheless and to our best knowledge, almost no attention is paid to study the antioxidant activity of fenugreek grown or germinated under stressful conditions. In the current research, both aqueous and methanolic extracts of fenugreek seedling were evaluated for their *in vitro* antioxidative activities. In most cases, H_2O_2- scavenging activity of water and methanolic extracts of 2- and 5- day old seedlings were enhanced under the treatment of almost all the tested salt doses. However, the reducing power as well as ABTS- and DPPH- scavenging activities of the extracts were enhanced by salt application only in the 5- day old seedlings; and these were suppressed by salt when the seedlings were younger (2 days old).

In accordance with our results, Chunthaburee *et al.,* [63] found that salt stress could promote the antioxidant activities, evaluated by ferric reducing power as well as DPPH- and ABTS- scavenging activity, in the grains of four *Oryza sativa* cultivars. In a similar trend, the antioxidant potential of three plants, *Salsola baryosma*, *Trianthema triquetra* and *Zygophyllum simplex*, was found to be elevated under salt treatment as indicated by DPPH-scavenging activity and the total reducing power [64]. Furthermore, Abbas *et al.,* [65] reported an enhancement in DPPH- and H_2O_2- scavenging activities as well as total antioxidant capacity and reducing power of *Medicago sativa* plants suffering water deficit. In 2-month old rosemary plants subjected to three salt concentrations (50, 100, and 150 mM NaCl), DPPH-scavenging ability of the plant methanolic extracts was enhanced as compared with the unstressed plants [66].

Concerning the antimicrobial activity of fenugreek seedlings studied herein, the suppressing effect of their water extracts were enhanced by some salt concentrations particularly against *Klebsiella pneumonia*, *Erwinia carotovora*, *Bacillus subtilis*, *Candida albicans*, *Streptococcus pyrogenes* and *Staphylococcus epidermidis*. For methanolic extracts, some salt concentrations could enhance their antimicrobial potentiality against *Erwinia carotovora*, *Bacillus subtilis* and *Candida albicans*.

The antibacterial and antifungal activity of different parts of fenugreek plant, especially seeds and germinated seeds, was previously established in many reports. Walli *et al.,* [67] proved potent antimicrobial activity of boiling water extract of unpowdered fenugreek against three *Staphylococcus* genera namely *S. aureus*, *S. epidermis* and *S. saprophyticus*. El Nour *et al.,* [68] also recorded that petroleum ether extract of fenugreek seeds showed marked antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*. Moreover, the methanolic extracts of fenugreek calli derived from hypocotyls and cotyledons showed antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli* compared to methanolic extracts of seeds that showed antifungal activity against *Candida albicans*.

According to Barnes *et al.,* [69], fenugreek seeds are rich with the polysaccharide galactomannan in addition to disogenin, yamogenin, gitogenin, tigogenin, neotigogens, choline and trigonelline; all of which are bioactive constituents that could explain the antimicrobial potency of fenugreek. More importantly, the phenolic skeleton of fenugreek may contribute to their antimicrobial and antioxidant efficiency [70]. Therefore, it was of special significance in the current investigation to study the pattern of change in the amount of total phenols and the main phenolic classes as well in response to the applied NaCl stress.

With respect to phenolics estimated in the present study, tannins in the water extracts of fenugreek seedlings were the only class of phenolics that increased when applying NaCl (50 and 100 ppm) in the first stage. In the second stage and in both water and methanolic extracts; tannins, alkaloids, flavonoids and total phenols were increased when applying 50 and 150 ppm salt solutions while saponins increased by 50, 100 and 150 ppm NaCl in the concerned fenugreek seedlings. Coinciding with the results obtained herein and working also on fenugreek, Hussein and Aqlan [71] found that 0.1% NaCl solution could increase the total amount of phenolics, flavonoids and tannins as well. Meanwhile, higher NaCl concentrations (0.3%) decreased the amount of such metabolites in comparison with the control plants. In a more or less similar manner, Abd Elhamid *et al.,* [72] as well as Sadak [73] recently recorded that water stress caused marked increase in the total phenolic contents as well as total flavonoid and tannin contents in fenugreek plants as compared with their unstressed correspondings. In other plants, various studies have revealed marked overproduction of phenolic compounds in response to stressful conditions. Among those, data recorded by Mickky *et al.,* [9] revealed that application of water stress caused marked increase in the amount of total phenols, saponins, tannins, flavonoids and alkaloids of alfalfa plants; and the increase in the amount of those phenolics was directly proportional to the level of water stress.

Within the plant tissues, phenolics are well documented as stress induced metabolites over- produced in response to various stress stimuli. The accumulation of phenolics under unfavorable growth conditions may be a mechanism exerted by the stressed plants to withstand stress by participating in ROS scavenging mainly through the antioxidative enzymes utilizing polyphenols as co-substrates [74]. In a series of experimental observations, the increase in the titer of total phenols, saponins, tannins, flavonoids and alkaloids in plants growing in a stressing environment was attributed to the enhanced activity of the enzymes involved in phenols biosynthesis and/ or the inhibited activity of the enzymes responsible for their degradation [75, 76].

Conclusion

Based on the results obtained herein, it can be inferred that salinizing fenugreek seedlings can be simply employed to enhance their nutritional value by increasing the amount of some vital phytonutrients such as proteins and certain vitamins. In addition, such strategy can also improve the medicinal value of fenugreek seedlings by upgrading their antimicrobial and antioxidant activities as well as increasing the amount of some medically- active phenolics.

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