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Mitigation of Salinity Stress in Fenugreek Plants Using Zinc Oxide Nanoparticles and Zinc Sulfate

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ABSTRACT

Salinization is increasing on a global scale, and improving the tolerance of plants to salinity is urgent to study, so this study was conducted at the Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Giza, Egypt during two successive seasons 2019 and 2020 to evaluate the effect of zinc sulfate and ZnO nanoparticles treatments as a foliar application at the level of 0, 20, and 40 ppm on some morphological and chemical features of *Trigonella foenum-graecum* plants grown under three salinity levels (0,1500, and 3000 ppm) aiming to reduce the negative effect of salinity on plant growth. The present study revealed that salinity stress caused a decrease in morphological traits (shoot length, number of branches/ plant, number of leaves/ plant, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight) and harvest traits (number of pods/plant, number of seeds /pod, and 100 seed weight), as well as total phenols, and K^+ /Na^+ ratio. While, using zinc sulfate and ZnO nanoparticles gave high results with all studied morphological traits, and also improved the harvest characteristics of the fenugreek plants. The highest 100-seed weight and total saponins were obtained by the interaction between the treatments of ZnO nanoparticles at 40 ppm and salinity at 1500 ppm. According to the obtained results, it could be concluded that ZnO nanoparticles and zinc sulfate mitigated the negative effects at each level of salinity under this study.

Keywords: *Trigonella foenum-graecum*, salinity stress, nano-zinc, zinc sulfate, saponins, flavonoids.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual plant belonging to the Fabaceae family, (Yunus and Gulen, 2021). Its leaves consist of three small ovate to oblong leaflets. It is grown all over the world as a semi-dry crop. Its seeds and leaves are common ingredients in dishes from the Indian subcontinent. It is an important medicinal crop cultivated throughout the world. Fenugreek seeds are used locally as a yellow pigment, for beauty and pharmaceutical purposes. Fenugreek is a good soil renovator and has widely been used as green manure (Abdelgani *et al.*, 1999). Fenugreek seeds and leaves are rich in carbohydrates, proteins, and minerals, yet low in oil (Gad *et al.*, 1982) and (Khaled *et al.*, 2021). Fenugreek seeds (per 100 grams) are a rich source of dietary fiber, B vitamins, protein (46% DV), and dietary minerals, especially manganese (59% DV) and iron (262% DV). Fenugreek seeds are rich in polyphenolic flavonoids, such as trigonelline, saponin (4%-8%), quercetin, and phytic acid. Fenugreek seeds also contain proteins rich in lysine, tryptophan, mucous fibers, and other rare chemical components, such as coumarin, folic acid, nicotinic acid, phytic acid, scopoletin, saponin, and trigonelline which may be responsible for inhibited cholesterol absorption, and decrease blood sugar concentration (Singh *et al.*, 2022).

Salinity is one of the major problems for increasing production in crop-growing areas around the world. Every 100 g of fenugreek seeds contains 9% water, 58% carbohydrates, 23% protein and 6% fat, with calcium at 40% of the Daily Value (DV) and 1,350 kilojoules (323 kcal) of food energy.

Salinity decreased the micronutrients solubility (such as Mn, Mo, Cu, Fe, and Zn) and so the plants have an elements deficiency appearance. Manganese (Mn) and Zinc (Zn) deficiency have been observed in barley (*Hordeum vulgare* L.) with increasing sodicity. Foliar application of sodium antagonistic minerals could eliminate the ion disequilibrium and osmotic stress resulting from the excess of sodium and chloride ions in the rhizosphere. Foliar application of minerals such as Mn, Fe, B, and Cu may be more practical than application to the soil as they are absorbed into soil particles and are less available for rooting medium. (Pandya *et al.*, 2005).

Diffusion of different chemical fractions of zinc is supported by several factors such as soil management practices, the inherent ability of the soil to maintain the supply of the element. When Zn is put to the soil from outer resources to correct a deficiency, the nature, it undergoes transformation to different chemical ponds and sheer of which, may however, differ across soils, based on Physico-chemical characteristics and the associate environmental requirements. Its shortage generally occurs in soil having coarse texture, high pH and $CaCO_3$, and low organic carbon content (Sakal, 2001). Continuously high use of (residual sodium carbonate) water increases the pH and (displaceable sodium) of the soil which reduces the presence of zinc. It has been shown that the presence of zinc is an indicator of the solubility of zinc metal. The concentration of zinc in the soil

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increases as the pH of the soil solution decreases. (Lindsay, 1972). The knowledge regarding the distribution of Zn fractions in soil helps in understanding the input of odd Zn fraction to plant availability. Zinc applied to the soil reacts with soil constituents, forming a number of sparingly soluble compounds, which together determine the concentrations of Zn in the soil solution and its availability to plants. Zinc ions also inhibited enzymes that create free radicals and led salt-stressed plants to survive (Weisany *et al*., 2012).

In last years, a greet betterment in salinity tolerance was induced in some types of crops by nanotechnology (Chen and Yada, 2011). Nanoparticles (NPs) with mini size and major surface area are anticipated to be the preferable material for use as a Zinc fertilizer in plants. Nanomaterial is an effective application for releasing micro elements gradually (Naderi *et al.,* 2011).The soil content of zinc decreased with the increase of the soil salinity (Gunes *et al.* 1996; Jamalomidi *et al*. 2006; Khoshgoftarmanesh *et al.* 2006). Cakmak (2008) prognosis that stress caused by zinc deficiency may dampen the activities of several antioxidant enzymes. Spraying the vegetative parts of crops with enough micronutrients during different growth stages may be necessary to solve the immediate agronomic problems, meet human nutrition needs and achieve economic goals (Johnson *et al*., 2005).

A number of researchers described the key role of ZnO nanomaterial in crop growth (Wang *et al.,* 2004), including nitrogen uptake, respiration, photosynthesis, and the activation of other physiological and biochemical processes, hence its importance in obtaining higher yields (Zozi *et al*., 2012). Nano-particles with the tiniest particle size and big surface area are anticipated to be the perfect material for use as Zn fertilizer in plants. Application of micronutrients in the form of nanoparticles is an important route to release required nutrients gradually and in a controlled way, which is essential to mitigate the problems of soil contamination triggered by the extra use of chemical fertilizers.

This research aims to evaluate the effect of foliar application with zinc sulfate and zinc nanoparticles on some morphological and chemical properties of fenugreek plants grown under three levels of salinity in order to reduce the negative effect of salinity on crop growth.

MATERIALS AND METHODS

Pot Experiment

An investigation was conducted at the Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Giza, Egypt during two successive seasons 2019 and 2020 to evaluate the effect of zinc treatments as a foliar application on some morphological and chemical features of *Trigonella foenum-graecum* plants grown under salt stress.

This experiment was designed using randomized complete block design (RCBD) with two factors; the first was salt stress and the second was zinc treatments. The experiment included 15 treatments (Each treatment included 21 pots with three replicates) that were a mixture of three salinity levels (0, 1500, and 3000 ppm) and four zinc treatments at the rates of 20 and 40 ppm from two different sources (ZnO nanoparticles and zinc sulfate), in addition control treatment (without foliar application) and were applied 45 and 75 days after sowing.

Seeds were obtained from Agricultural Research Center -Legumes Department. On the 1st of November, Fenugreek seeds were sown in pots (40 cm diameter) filled with light loamy soil, in both seasons. Plants were irrigated weekly with gradually increasing salinity concentration until reaching the desired salinity levels. Plants were irrigated with three levels of saline water. Artificial seawater was prepared by mixing sodium chloride, calcium chloride, and magnesium chloride at the ratio of 1:1:0.5, respectively, according to methods described by Kester *et al*. (1967). Calcium superphosphate (15.5% P_2O_2) was added during preparation of soil mixture at the rate of 7 g per pot, while potassium sulphate (48% K_2O) at the rate of 3 g per pot was added 45 days after planting.

Zinc Oxide nanoparticles preparation

Zinc acetate ($ZnAc_2$, $2H_2O$, public purpose Reagent, minimum 98.5%) was used. The necessary amounts were firstly dissolved in 100 ml of ultrapure water, and then 20 ml of aqueous 1M NaOH solution was added under streaming control and slow magnetic stirring at a temperature of 50°C. The mixture was kept at this temperature for one hour and then cooled to room temperature. It was immediately filtered by centrifugation, then washed with ultrapure water and later dried by lyophilization. The size and shape of Zinc Oxide nanoparticles were described in Fig. 1 using electron microscopy, after preparation of Negative Electron Microscopy Stains Protocol, according to Desoukey *et al*. (2019).

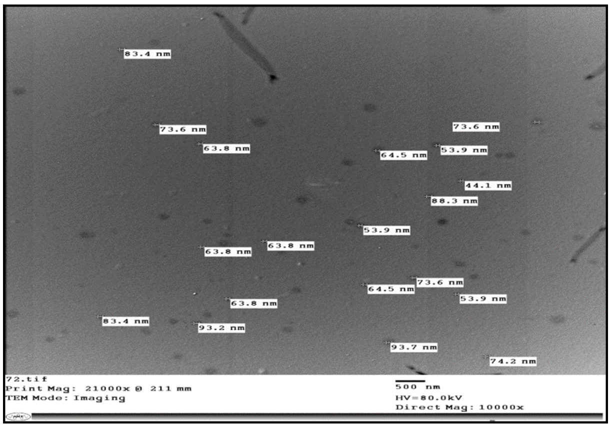

Fig.1.TEM characterization for Zinc Oxide nanoparticles in sizes ranged between 44.1-93.0 nm.

Recorded data

On the 1st of February, data such as shoot length (cm), number of leaves/plant, number of branches/ plant, shoot fresh weight (g), shoot dry weight (g), root length (cm), root fresh weight (g), root dry weight (g), total chlorophylls as well as total carotenoids (mg/g FW) using spectrophotometer according to Saric *et al.* (1967), proline content (mg/g D.W) according to Bates *et al*. (1973), and K^+: Na^+ ratio according to Piper (1947) were recorded in both seasons. On the first week of April in both seasons, the plants were harvested and data were recorded including, number of pods/plant, number of seeds/pod, and 100-seed weight (g), while seeds content of total saponins, total phenols, and total flavonoids (mg/100 g D.W) were recorded only in the second season, according to methods described by Uematsu *et al.* (2000), Singleton and Rossi (1965), and Ranganna (1986). The chemical analyses were conducted in Food Safety & Quality Control Laboratory, Faculty of Agriculture, Cairo University, Giza, Egypt.

Statistical analysis

Data were statistically analyzed, and separation of means was performed using the Least Significant Difference (L.S.D.) test at the 5% level of probability (Little and Hills, 1978).

RESULTS AND DISCUSSION

The data presented in Tables 1, 2, and 3 illustrated the effect of ZnO nanoparticles and zinc sulfate on the vegetative growth of *Trigonella foenum-graecum* plants grown under three levels of salinity (0, 1500, and 3000 ppm).

It is obvious from Table 1 that the mean shoot length, number of leaves/plant and number of branches/plant decreased with salinity compared with control. Shoot length, number of leaves/plant, and number of branches/plant of control plants were the highest (29.87 and 29.40 cm), (33.60 and 32.73), and (3.27 and 2.93), compared to the salinity treatments in the two seasons, respectively. The results were in okayt with Ibrahim and Faryal (2014) and Saberali and Moradi (2019) that attributed the reason for this deficiency to soil salinity which reduced growth by decreasing assimilates provide and hydrostatic pressure. Salinity may produce toxic effects on the embryo and the seedlings and therefore, it affects the morphological traits (Ratnakar *et al*., 2013).

The effect of zinc treatments on the vegetative growth of fenugreek plants was clear with nano-zinc at 40 ppm which recorded the highest results on mean shoot length (31.33 and 29.11cm) in the first and second seasons, respectively. While the highest mean numbers of leaves/plant (35.33 and 32.44) and branches/plant (3.67 and 3.00) were recorded with the treatment of zinc sulfate at 20 ppm in both seasons.

Regarding the interaction between salinity and zinc treatments, it was observed that, zinc sulfate at 40 ppm gave the highest shoot length (34.00 and 35.33 cm) with plants irrigated with tap water in the two seasons as well as the highest number of leaves/plant (41.33) in the first season, while in the second season, the highest number of leaves/plant (42.33) was recorded with the treatment of nano zinc at 40 ppm, these results are in harmony with the results of Ibrahim and Faryal (2014). Salinity at 1500 ppm without Zinc gave the highest number of branches/ plant in the first season (5.33), whereas at the same level of salinity (1500 ppm) using zinc sulfate at the rate of 20 ppm produced also the highest number of branches/plant in the second season.

Table 1. Effect of salinity, ZnO nanoparticles, and zinc sulfate treatments on shoot length (cm), number of leaves/plant, and number of branches/plant of *Trigonella foenum-graecum* plants in the 1st and 2nd seasons.

Salinity	Treatments	Shoot length (cm)		Number of leaves/plant		Number of branches/plant	
		1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
0		29.87	29.40	33.60	32.73	3.27	2.93
1500		25.60	23.97	31.93	28.13	3.20	2.27
3000		26.47	25.00	26.13	23.13	1.60	1.00
$LSD_{0.05}$		1.19	1.27	4.79	3.55	0.660	0.645
	Control	22.33	21.00	28.11	27.11	2.89	2.11
	Nano20	27.94	26.67	31.22	27.67	2.78	2.33
	Nano40	31.33	29.11	32.00	29.56	2.44	1.67
	Zn20	28.50	27.11	35.33	32.44	3.67	3.00
	Zn40	26.44	26.72	26.11	23.22	1.67	1.22
$LSD_{0.05}$		1.54	1.64	6.19	4.59	0.852	0.832
Zero	Control	23.33	24.00	26.67	30.33	3.33	3.33
	Nano20	28.33	28.00	24.33	21.67	1.00	0.67
	Nano40	33.67	30.33	39.67	42.33	3.67	3.67
	Zn20	30.00	29.33	36.00	33.67	4.00	3.33
	Zn40	34.00	35.33	41.33	35.67	4.33	3.67
1500	Control	23.00	20.00	40.33	34.00	5.33	3.00
	Nano20	27.00	24.33	34.67	32.00	4.00	3.33
	Nano40	31.00	28.67	31.33	24.00	2.67	1.00
	Zn20	25.67	25.33	34.67	34.00	3.33	4.00
	Zn40	21.33	21.50	18.67	16.67	0.67	0.00
3000	Control	20.67	19.00	17.33	17.00	0.00	0.00
	Nano20	28.50	27.67	34.67	29.33	3.33	3.00
	Nano40	29.33	28.33	25.00	22.33	1.00	0.33
	Zn20	29.83	26.67	35.33	29.67	3.67	1.67
	Zn40	24.00	23.33	18.33	17.33	0.00	0.00
$LSD_{0.05}$		2.67	2.84	10.72	7.94	1.84	1.44

Nano 20= ZnO nanoparticles at the rate of 20 ppm - Nano40= ZnO nanoparticles at the rate of 40 ppm
Zn20= zinc sulfate at the rate of 20 ppm - Zn40= zinc sulfate at the rate of 20 ppm

Data in Table 2 showed shoot fresh and dry weight results in the two seasons. A significant decrease was observed in the results of salinity treatments compared with control. The control treatment gave the highest results (7.20 and 5.60, 0.89 and 0.72 g), respectively. The reduction in shoot dry weight could also be accompanied by a reduced rate of leaf production, which leads to reduced photochemical reaction and dry matter aggregation (Puvanitha and Mahendran, 2017).

Table 2. Effect of salinity, ZnO nanoparticles, and zinc sulfate treatments on shoot fresh and dry weights (g) of *Trigonella foenum-graecum* plants in the 1st and 2nd seasons.

Salinity	Treatments	Shoot fresh weight (g)		Shoot dry weight (g)	
		1st Season	2nd Season	1st Season	2nd Season
0		7.20	5.60	0.89	0.72
1500		4.73	3.67	0.70	0.55
3000		4.09	3.07	0.65	0.48
$LSD_{0.05}$		1.37	0.83	0.12	0.09
	Control	4.27	3.67	0.62	0.45
	Nano20	4.99	4.00	0.73	0.57
	Nano40	6.11	4.78	0.87	0.69
	Zn20	7.22	4.78	0.93	0.71
	Zn40	4.11	3.39	0.58	0.49
$LSD_{0.05}$		1.77	1.07	0.15	0.12
Zero	Control	6.00	5.00	0.67	0.52
	Nano20	4.33	4.33	0.57	0.56
	Nano40	8.33	7.00	1.05	0.91
	Zn20	10.33	6.00	1.23	0.87
	Zn40	7.00	5.67	0.91	0.75
1500	Control	4.30	3.67	0.76	0.44
	Nano20	5.33	4.00	0.80	0.62
	Nano40	5. 67	4.00	0.85	0.67
	Zn20	5.33	4.67	0.70	0.67
	Zn40	3.00	2.00	0.41	0.34
3000	Control	2.50	2.33	0.43	0.40
	Nano20	5.33	3.67	0.83	0.52
	Nano40	4.33	3.33	0.72	0.49
	Zn20	6.00	3.67	0.87	0.59
	Zn40	2.33	2.33	0.42	0.38
$LSD_{0.05}$		3.06	1.85	0.26	0.21

Zinc sulfate 20 ppm treatment recorded high results (7.22 and 4.78, 0.93 and 0.71g) in the two seasons, respectively, followed by the treatment of nano-zinc 40 ppm without significant differences.

Interaction between zinc treatments and salinity concentrations gave the highest shoot fresh and dry weights (10.33 and 1.23g), with the treatment of zinc sulfate 20 ppm in plants irrigated with tap water in the first season. While in the second season, nano-zinc 40 ppm gave the highest shoot fresh and dry weights (7.00 and 0.91 g) in plants irrigated with tap water.

The results in Table 3 explain the mean root length and root fresh and dry weights. It was found that the best treatment for root length and root fresh weights was tap water with mean values (10.17 and 10.47 cm) and (0.148 and 0.133 g) in the two seasons, respectively. The results are in agreement with Ibrahim and Faryal (2014) and Puvanitha and Mahendran (2017), the poisonous rate of sodium chloride may be the reason for the shortfall in root length and unequal elements uptake by the seedlings (Ratnakar *et al*., 2013), while the treatment of 1500 ppm salinity in the first season and 3000 ppm in the second season recorded the highest mean root dry weight results with values of 0.099 and 0.096 g, respectively, with no significant differences between all salinity treatments.

In respect of the effect of zinc treatments on root length, root fresh weight, and root dry weight, it was observed that nano-zinc at 40 ppm recorded the highest results with them (10.83 and11.28 cm), (0.172 and 0.164 g) and (0.127 and 0.117 g), respectively, in the two seasons, this could be due to micronutrients that improved root growth thus increasing elements uptake to overcome the salinity (Ibrahim and Faryal, 2014).

Regarding the interaction between salinity and zinc treatments, results showed that zinc sulfate at 40 ppm with plants irrigated with tap water gave the highest results of root length (12.67 cm), root fresh weight (0.276 g), and root dry weight (0.148 g) in the first season, while the highest root length, root fresh weight, and root dry weight in the second season were recorded by using nano-zinc at 40 ppm with values of 12.33 cm, 0.219 g, and 0.141g.

Table 3. Effect of salinity, ZnO nanoparticles, and zinc sulfate treatments on root length (cm), root fresh weight (g), and root dry weight (g) of *Trigonella foenum-graecum* plants in the 1st and 2nd seasons.Salinity

Salinity	Treatments	Root length (cm)		Root fresh weight (g)		Root dry weight (g)	
		1stSeason	2nd Season	1st Season	2nd Season	1st Season	2nd Season
0		10.17	10.47	0.148	0.133	0.094	0.089
1500		8.93	8.73	0.132	0.111	0.099	0.091
3000		9.27	10.10	0.116	0.114	0.095	0.096
$LSD_{0.05}$		0.803	0.856	0.027	0.025	0.013	0.014
	Control	8.67	9.67	0.106	0.098	0.079	0.075
	Nano20	8.22	8.22	0.109	0.099	0.083	0.085
	Nano40	10.83	11.28	0.172	0.164	0.127	0.117
	Zn20	10.28	11.06	0.122	0.124	0.098	0.101
	Zn40	9.28	8.61	0.151	0.111	0.093	0.084
$LSD_{0.05}$		1.03	1.105	0.035	0.032	0.016	0.018
Zero	Control	8.83	9.33	0.065	0.071	0.036	0.039
	Nano20	8.67	8.83	0.094	0.088	0.070	0.073
	Nano40	11.17	12.33	0.194	0.219	0.137	0.141
	Zn20	9.50	10.50	0.111	0.124	0.080	0.091
	Zn40	12.67	11.83	0.276	0.164	0.148	0.102
1500	Control	9.50	10.00	0.155	0.125	0.116	0.102
	Nano20	6.83	6.17	0.115	0.099	0.078	0.085
	Nano40	10.33	10.67	0.166	0.142	0.127	0.111
	Zn20	11.00	10.83	0.135	0.114	0.103	0.099
	Zn40	7.00	6.00	0.088	0.076	0.071	0.060
3000	Control	7.67	9.67	0.098	0.097	0.084	0.083
	Nano20	9.17	9.67	0.117	0.111	0.101	0.097
	Nano40	11.00	10.83	0.155	0.133	0.119	0.098
	Zn20	10.33	11.33	0.119	0.133	0.111	0.114
	Zn40	8.17	8.50	0.090	0.095	0.061	0.089
$LSD_{0.05}$		1.79	1.91	0.060	0.055	0.028	0.031

Table 4 appears effect of ZnO nanoparticles and zinc sulfate on the harvest characteristics of fenugreek plant (number of pods/plant, number of seeds per pod, and 100 seed weight) under three levels of salinity (0, 1500, and 3000 ppm).

The results showed a decrease in the harvest characteristics with salinity compared with the control. Control plants gave the highest results for number of pods/plants (8.91 and 8.85), number of seeds per pod (13.85 and 13.77), and100-seed weight (4.76 and 4.73g), in the two seasons, respectively. Salinity can affect plant metabolism, resulting in decreased growth and yield (Ritu, 2016).

Zinc treatments improved the harvest characteristics of fenugreek plant, and the highest results were found with the number of pods/plant and number of seeds per pod (9.07 and 8.99) and (13.45 and 13.68) in the two seasons, respectively, using zinc sulfate at 20 ppm treatment. This increase may be explained by the action of zinc in the production of dole acetic acid (IAA), and activation enzymes of photosynthesis (Priyanka *et al*., 2019). Nano-zinc treatment (20 ppm) gave the highest mean weight of 100-seeds (4.36 g) without significant difference among other zinc treatments, but with a significant difference with control plants in both seasons.

Plants irrigated with tap water as well as treated with zinc sulfate 20 ppm gave the highest number of pods/plant and number of seeds per pod (11.27 and11.37) and (15.73 and 15.63) in the two seasons, respectively. There was also an expected reduction in the harvesting characteristics as a response to salinity treatment, but the interaction with zinc treatment led this reduction to be insignificant in most cases. Zinc treatments had a positive effect when interacted with salinity, especially with the treatment of nano-zinc 40 ppm which produced the highest 100-seed weight (4.86 and 4.87 g) in the two successive seasons, respectively, with saline-treated plants at 1500 ppm.

Table 4. Effect of salinity, ZnO nanoparticles, and zinc sulfate treatments on number of pods/plant, number of seeds per pod, and 100-seed weight of *Trigonella foenum-graecum* plants in the 1st and 2nd seasons.

Salinity	Treatments	Number of pods/plant		Number of seeds/pod		100-seed weight (g)	
		1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
0		8.91	8.85	13.85	13.77	4.76	4.73
1500		7.12	7.28	11.66	11.87	4.62	4.58
3000		6.91	6.61	11.58	11.58	3.53	3.52
$LSD_{0.05}$		0.97	0.85	1.53	0.95	0.13	0.11
	Control	7.07	7.16	11.99	12.37	4.18	4.20
	Nano20	7.39	7.19	12.70	12.58	4.36	4.36
	Nano40	7.57	7.32	11.91	11.79	4.34	4.36
	Zn20	9.07	8.99	13.45	13.68	4.33	4.26
	Zn40	7.14	7.24	11.77	11.62	4.30	4.22
$LSD_{0.05}$		1.25	1.09	1.98	1.23	0.17	0.14
Zero	Control	8.27	8.27	13.00	13.00	4.81	4.79
	Nano20	8.50	8.27	14.10	13.97	4.84	4.86
	Nano40	8.53	8.33	13.33	13.30	4.75	4.78
	Zn20	11.27	11.37	15.73	15.63	4.68	4.57
	Zn40	8.00	8.00	13.10	12.97	4.71	4.65
1500	Control	6.27	6.70	10.00	11.33	4.33	4.41
	Nano20	5.97	6.17	10.83	10.50	4.63	4.57
	Nano40	7.77	7.47	12.50	12.33	4.86	4.87
	Zn20	8.87	9.17	13.53	13.73	4.60	4.51
	Zn40	6.73	6.90	11.43	11.43	4.69	4.52
3000	Control	6.67	6.50	12.97	12.77	3.40	3.41
	Nano20	7.70	7.13	13.17	13.27	3.62	3.64
	Nano40	6.40	6.17	9.90	9.73	3.42	3.41
	Zn20	7.13	6.43	11.08	11.68	3.70	3.69
	Zn40	6.63	6.83	10.77	10.47	3.49	3.49
$LSD_{0.05}$		2.17	1.89	3.42	2.13	0.29	0.24

The data presented in Table 5 illustrated the effect of ZnO nanoparticles and zinc sulfate on the chemical compounds (total chlorophylls and total carotenoids in the two seasons) of *Trigonella foenum-graecum* plants grown under three levels of salinity (0, 1500, and 3000 ppm).

Fenugreek plant treated with 1500 ppm salinity gave the highest result for mean total chlorophylls (1.54 and 1.53 mg / g) and mean total carotenoids (0.70 and 0.67 mg/g) compared to other salinity treatments in the two seasons.

The results obtained on the effect of zinc treatments showed that, nano-zinc at 40 ppm recorded the highest mean of total chlorophylls (1.53 and 1.66 mg/g) in the two seasons compared to all treatments. It also gave the highest result (0.73 and 0.77 mg/g) in the mean of total carotenoids. The results are in agreement with those found by Khadijeh *et al.* (2017).

Regarding the interaction between salinity and zinc treatments, it was observed that nano- zinc at 40 ppm gave the best total chlorophylls (1.79 and 1.94 mg/g) in the two seasons, respectively. It was found that plants stressed by 1500 ppm salinity with zinc treatments contained total chlorophylls and carotenoids close to those obtained from unstressed plants in most cases in both seasons. Khadijeh *et al.* (2017) reported that nano treatments could enhance chlorophyll content and improve the growth of the stressed plant. Also, the interaction between salinity at 1500 ppm and nano-zinc at 40 ppm resulted in the highest total carotenoids

(0.87 and 0.94 mg/g) in the two seasons, respectively compared to the other treatments.

Table 5. Effect of salinity, ZnO nanoparticles, and zinc sulfate treatments on total chlorophylls and total carotenoids (mg/g F.W) of *Trigonella foenum-graecum* plants in the 1st and 2nd seasons.

Salinity	Treatments	Total chlorophylls (mg/g F.W)		Total carotenoids (mg/g F.W)	
		1st Season	2nd Season	1st Season	2nd Season
0		1.52	1.52	0.66	0.67
1500		1.54	1.53	0.70	0.67
3000		1.15	1.25	0.45	0.51
$LSD_{0.05}$		0.123	0.11	0.037	0.031
	Control	1.22	1.35	0.56	0.53
	Nano20	1.45	1.51	0.61	0.68
	Nano40	1.53	1.66	0.73	0.77
	Zn20	1.51	1.56	0.64	0.72
	Zn40	1.28	1.07	0.48	0.40
$LSD_{0.05}$		0.16	0.136	0.048	0.039
Zero	Control	1.04	1.69	0.65	0.60
	Nano20	1.56	1.43	0.67	0.76
	Nano40	1.79	1.94	0.81	0.82
	Zn20	1.76	1.36	0.69	0.78
	Zn40	1.44	1.16	0.47	0.40
1500	Control	1.49	1.37	0.62	0.58
	Nano20	1.54	1.70	0.68	0.58
	Nano40	1.68	1.71	0.87	0.94
	Zn20	1.55	1.71	0.76	0.79
	Zn40	1.42	1.16	0.59	0.46
3000	Control	1.14	0.99	0.41	0.41
	Nano20	1.26	1.40	0.49	0.70
	Nano40	1.13	1.34	0.49	0.54
	Zn20	1.24	1.60	0.47	0.58
	Zn40	0.98	0.90	0.39	0.34
$LSD_{0.05}$		0.275	0.235	0.082	0.069

Flavonoids and the other phenolic compounds extracted from medicinal plants are natural antioxidants, and these phytochemicals are used as alternative sources for pharmaceutical and medicinal applications due to their antibacterial, anti-inflammatory, anti-cancer, and cardio protective effects, as well as boosting the immune system (Tungmunnithum *et al.*, 2018).

Fenugreek is wealthy in saponins, polyphenolics, diosgenin, soluble fiber, and 4-hydroxy isoleucine. Dietary fiber, flavonoids, and saponins may be responsible for the hypoglycemic and hypolipidemic actions, and the beneficial effects may be due to decreased infection. Seeds of fenugreek quite reduce insulin concentrations and postprandial glucosby decreasing oxidative stress (Singh *et al.*, 2022). Fenugreek seeds have anti-diabetic actions and may be due to the presence of steroidal, fiber content, alkaloids, and saponins (Singh *et al.*, 2020).

Due to the previously mentioned importance of the bioactive compounds of *Trigonella foenum-graecum* seeds, the data presented in Table 6 illustrated the effect of ZnO nanoparticles and zinc sulfate on the seed content of total saponins, total phenols, and total flavonoids of fenugreek plants grown under three levels of salinity (0, 1500, 3000 ppm) in the second season.

Fenugreek plants treated with 1500 ppm salinity gave the highest mean total saponins (453.1mg/100g), and mean total flavonoids (429.5 mg/100g), while the control plants had the highest mean total phenols (283.6 mg/100g).

The treatment of nano-zinc at 40 ppm had a superior effect on the total saponin content resulting in the highest mean total saponins (399.2 mg/100 g). On the other hand, zinc treatment did not have a positive effect on total flavonoids and total phenols and led to a significant decrease. Plants without zinc treatments gave the highest mean total phenols (305.0 mg/100g) and mean total flavonoids (455.5 mg/100g).

Regarding the interaction between salinity and zinc treatments, it was observed that the treatment of 1500 ppm salinity with nano-zinc at 40 ppm gave the highest total saponins (552.8mg/100 g), while the control plants recorded the highest total phenols (335.6 mg/100 g) and total flavonoids (477.5 mg/100 g) compared to all other treatments.

Table 6. Effect of salinity, ZnO nanoparticles, and zinc sulfate treatments on total saponins, total phenols, and total flavonoids (mg/100 g) of *Trigonella foenum-graecum* plants in the 2nd season only.

Salinity	Treatments	Total saponins (mg/100 g)	Total phenols (mg/100 g)	Total flavonoids (mg/100 g)
		2nd season	2nd season	2nd season
0		243.6	283.6	427.7
1500		453.1	276.4	429.5
3000		333.9	271.0	415.1
$LSD_{0.05}$		10.51	5.20	6.34
	Control	338.3	305.0	455.5
	Nano20	334.3	284.4	438.9
	Nano40	399.2	265.0	419.5
	Zn20	249.3	270.7	413.4
	Zn40	396.6	259.9	393.4
$LSD_{0.05}$		13.58	6.72	8.19
Zero	Control	124.1	335.6	477.5
	Nano20	164.8	291.1	437.0
	Nano40	281.7	267.4	430.9
	Zn20	201.1	268.6	413.9
	Zn40	446.3	255.2	380.0
1500	Control	534.2	264.6	447.7
	Nano20	479.7	291.0	440.5
	Nano40	552.8	264.8	410.8
	Zn20	290.4	287.3	434.2
	Zn40	408.4	274.4	414.1
3000	Control	356.6	314.9	441.3
	Nano20	358.3	271.1	439.1
	Nano40	363.2	262.9	416.9
	Zn20	256.5	256.3	392.1
	Zn40	335.1	250.0	386.1
$LSD_{0.05}$		23.51	11.64	14.19

Table 7 showed the effect of ZnO nanoparticles and zinc sulfate on proline content and K^+/Na^+ ratio of *Trigonella foenum-graecum* plants grown under three levels of salinity (0, 1500, and 3000 ppm). High salinity level (3000 ppm) gave high proline content (4.96 and 5.05 mg/g) in the two seasons. Similar results with Pereira *et al.* (2000) and Ritu (2016); who reported that there was an increment in proline content with increasing salinity concentration which is consistent with the results of this study, but the control recorded a high K^+/Na^+ ratio (1.52 and 1.62) in the two seasons. Zinc sulfate at 40 ppm gave the highest mean proline content with values of 5.60 and 5.58 mg/g, while control treatment resulted in the highest K^+/Na^+ ratio of 1.14 and 1.22), respectively, in the two seasons.

From obtained results, it was found that the interaction between high level of salinity (3000 ppm) and zinc sulfate at 40 ppm produced the highest content of proline (6.25 and 6.24 mg/g) in the two seasons, respectively. Similar results reported by Khadijeh *et al.* (2017). While control treatment recorded the highest K^+/Na^+ ratio (2.03 and 2.38), respectively, in the two seasons. These results are in agreement with Himabindu *et al.* (2016) who reported that, since salt stress often induces disturbance in the cellular K^+ homeostatic balance, which coincides with changes in all physiological processes, maintaining a high cytosolic K^+/Na^+ ratio would constitute a stress tolerance strategy.

Table 7. Effect of salinity, ZnO nanoparticles, and zinc sulfate treatments on proline content (mg/g D.W.) and K^+/Na^+ ratio of *Trigonella foenum-graecum* plants in the 1st and 2nd seasons.

Salinity	Treatments	Proline content (mg/g D.W.)		K^+/Na^+ ratio	
		1st Season	2nd Season	1st Season	2nd Season
0		3.32	3.23	1.52	1.62
1500		3.82	3.82	0.91	0.86
3000		4.96	5.05	0.73	0.75
$LSD_{0.05}$		0.103	0.085	0.133	0.083
	Control	2.11	2.17	1.14	1.22
	Nano20	3.09	3.01	0.993	1.06
	Nano40	4.22	4.32	1.07	1.15
	Zn20	5.16	5.08	1.06	0.98
	Zn40	5.60	5.58	1.01	0.96
$LSD_{0.05}$		0.133	0.111	0.172	0.107
Zero	Control	1.60	1.81	2.03	2.38
	Nano20	2.23	2.15	1.42	1.52
	Nano40	3.91	3.85	1.45	1.68
	Zn20	4.34	4.02	1.45	1.31
	Zn40	4.53	4.33	1.25	1.20
1500	Control	2.11	1.95	0.75	0.68
	Nano20	2.26	2.70	0.85	0.87
	Nano40	3.59	3.16	0.96	1.00
	Zn20	5.14	5.11	0.98	0.85
	Zn40	6.02	6.18	1.01	0.88
3000	Control	2.64	2.75	0.65	0.60
	Nano20	4.78	4.18	0.71	0.79
	Nano40	5.16	5.96	0.79	0.77
	Zn20	5.99	6.11	0.75	0.77
	Zn40	6.25	6.24	0.76	0.80
$LSD_{0.05}$		0.231	0.192	0.297	0.185

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تخفيف إجهاد الملوحة في نباتات الحلبة باستخدام جزيئات أكسيد الزنك النانوية وكبريتات الزنك

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تتزايد الملوحة على نطاق عالمي ، وتحسين تحمل النباتات للملوحة أمر ضروري للدراسة ، لذلك أجريت هذه الدراسة في قسم بساتين الزينة ، كلية الزراعة ، جامعة القاهرة ، الجيزة ، مصر خلال موسمين متتاليين ٢٠١٩ و ٢٠٢٠ لتقييم تأثير رش كبريتات الزنك وجزيئات أكسيد الزنك النانونية بمستويات (٠ و ٢٠ و ٤٠ جزء في المليون) على الصفات المورفولوجية والكيميائية لنبات الحلبة النامي تحت تأثير مستويات الملوحة (٠ و١٥٠٠و٣٠٠٠ جزء في المليون) بهدف تقليل التأثير السلبي للملوحة على نمو النبات. وكشفت هذه الدراسة أن الملوحة تسببت في انخفاض في الصفات المورفولوجية بما في ذلك (طول المجموع الخضري وعدد الفروع/النبات وعدد الأوراق/النبات والوزن الطازج والجاف للمجموع الخضري وطول الجذر والوزن الطازج والجاف للجذر) وعلى الصفات المحصولية (عدد القرون/النبات وعدد البذور/قرن ووزن ١٠٠ بذرة)، وأيضا الفينولات الكلية ونسبة أيوني الصوديوم والبوتاسيوم.بينما أعطى إستخدام كبريتات الزنك وجزيئات أكسيد الزنك النانونية أفضل النتائج لجميع الصفات المورفولوجية وأيضا حسن من الصفات المحصولية لنبات الحلبة. تم ملاحظة زيادة في وزن ال ١٠٠ بذرة والمجموع الكلي للفينولات عند التركيز ٤٠ جزء في المليون من جزيئات أكسيد الزنك النانونية مع التركيز ١٥٠٠ جزء في المليون من الملوحة ، ووفقا للنتائج التي تم الحصول عليها ، يمكن إستنتاج أن جزيئات أكسيد الزنك النانونية وكبريتات الزنك خففت من الآثار السلبية لكل مستوى من مستويات الملوحة.