

Nano-Fe (Magnetic-Fe) and Se Foliar Application Tranquilize the Salinity Adverse-Effects on *Satureja Mutica* *Fisch* and *Satureia Spicigera* (C. Koch) Boiss

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Abstract

Background: The secondary metabolites from savory species are widely used in food and pharmaceutical industries. Salt accumulation in the growing medium adversely affects the growth and yield of plants. The hyper-availability of Na^+ and Cl^- triggers nutrient imbalances, leading to secondary ionic stress. Under salinity exposure, the reactive oxygen species (ROS) over-generation drives oxidative stress in cells. Moreover, when facing environmental stress factors; the availability of essential nutrients and especially micro-elements strongly declines. Foliar application of micro-nutrients principally as nano-form is a promising strategy in meeting the nutritional demands of plants under stress environments with progressive nutrient shortages. Nano-materials and the supply of nutrients as foliar treatments meliorate the growth, biochemical reactions, and nutrient use efficiency of plants under salinity. The idea with the present experiment was to assay the effects of nano-Fe (magnetized-Fe) and selenium foliar application on the growth and some physiological responses of two *Satureja* species under saline-sodic conditions.

Results: When studying the foliar application of Se and nano-Fe (0 and 3 mg L⁻¹) on *Satureja mutica* and *Satureia spicigera* via two separate experiments, under normal no-saline conditions; the highest catalase activity was recorded in magnetized-Fe treated plants in both species. Independent effects of foliar application and plant species influenced total phenolics and Mg content of leaves. Foliar sprays reduced MDA content in plant tissue. In the second experiment, foliar applications were evaluated under salinity conditions. No-saline × Se and magnetized-Fe treated plants attained the highest data for aerial parts biomass in *S. spicigera*.

Conclusion: The results demonstrated that salinity adversely influenced the growth and physiological responses, nevertheless, foliar spray with Se and magnetized-Fe partially ameliorated the salinity depression on *Satureja* species.

Background

Satureja species are annual/perennial plants from the Lamiaceae family [1], rich in minerals and vitamins. The essential oil is enriched with thymol, carvacrol, camphene, linalool, and some other terpenoids [1]. Moreover, they have antioxidant, anti-cancer, appetizer, diuretic, and purifying properties. The oil and other compounds from savory species are widely used in food, perfume, and pharmaceutical industries [2, 3]. *Satureja spicigera* and *Satureja mutica* are herbaceous plants reaching the height of about 60 cm. They have fine stems, dense small leaves, and numerous branching growth patterns. Both species are in flower during autumn. The species have diverse distribution patterns in many localities and habitats of Iran and, there is a great interest in the mass agricultural production of both species in the various locations and especially in saline-prone lands.

Environmental (abiotic) factors are the predominant constraints of plants growth, development, and productivity. Salt accumulation in the rhizosphere adversely affects the growth and yield of plants. Salinity retains seed germination, reduces crop yield, and causes remarkable morpho-physiological modification in plants [4]. The over-accumulation of Na^+ and Cl^- induces ionic imbalances, eventually leading to secondary ionic stress. Under salinity, ROS over-generation triggers oxidative stress in cells. In addition to that, salinity by causing ionic stress, oxidative damage, and osmotic stress drastically retards the plant's normal metabolism [5]. Salt stress reduced the yield, essential oil, and proline content of *Pelargonium graveolens* [6]. Increased salt concentration inhibits plant growth through osmotic potential, unbalanced nutrient ratios, modulation of plant growth regulators, reduction of photosynthetic pigments, and drives cell wall deterioration [5, 7, 8]. Under abiotic stress, the availability of essential nutrients and especially micro-elements strongly decline [9]. Foliar application of micro-nutrients principally as nano-form vs common forms is a promising strategy in reducing the chemical fertilizers input. They activate the antioxidants system and enhance plants' tolerance against ROS molecules [10]. Nano-elements are flexible in their physical, chemical, and catalytic properties and are more suitable for plant nutrition [10]. Due to the huge specific area of nano-elements and their high catalytic action,

those compounds promisingly interact with plants in favor of growth improvement. Several reports revealed that nano-materials meliorate the growth, biochemical reactions, and nutrient use efficiency of plants under salinity and even normal conditions. The compounds have immense roles in photosynthesis, stomatal conductance, and lipids metabolism under normal and particularly stressful environments [11, 12]. Nano-elements could link to the active bio-molecules like proteins, nucleic acids, and sub-cellular structures capable of protecting them from oxidative damage [11, 12]. Foliar treatment of *Momordica charantia* with chitosan–selenium nanoparticles resulted in the increased K⁺ content, proline content, and antioxidant enzymes activity under salinity conditions [13].

Se improved the activity of antioxidant enzymes, photosynthetic pigments content, photosynthesis potential, free radicals scavenging potential, reduced electrolytic leakage percentage, improved cell membrane integrity, delayed senescence, reduced Na⁺ uptake as well as stimulated K⁺ absorption [14, 15, 16].

Iron is an essential macro-nutrient that holds un-substitutable roles in the chloroplast structure and is considered as a major electrolyte in all plants with dominant actions in respiration, photosynthesis, enzyme function, hormonal regulation, oxidation, and reduction processes, and chlorophyll formation [17].

In *Mentha piperita*, foliar application of nano-Fe significantly improved the yield and plant growth-related traits [18]. Nowadays, researchers are trying to employ several efficient compounds to overcome salt stress on plants in the hope to reduce the over-application of chemical fertilizers which may cause environmental pollution. In the present study, we tried to assay the effects of nano-Fe (magnetized-Fe) and selenium foliar application on the growth and some physiological traits of two *Satureja* species under saline-sodic conditions.

Results

In the first experiment, ANOVA results revealed that the treatments did not affect TSS, chlorophyll a, proline, Na, Zn, Ca, K, P, and N content of plants. Total phenolics and Mg content were influenced by the individual effects of species and foliar treatments. Catalase activity was responded to the interaction effects of species × foliar application. Chlorophyll b, flavonoids, and MDA content as well as SOD activity were responsive to the sole effects of foliar applications. Fe and Mn content was affected by species (Table 1 and 2).

Table 1 ANOVA for the effects of foliar applications on the physiological traits of *Satureja* species (First experiment).

Significance	TSS content	Chl a content	Chl b content	Flavonoids content	Total phenolics content	Proline content	SOD activity	CAT activity	MDA content
Species (A)	ns	ns	ns	ns	**	ns	ns	ns	ns
Foliar application (B)	ns	ns	**	*	**	ns	*	*	**
A × B	ns	ns	ns	ns	ns	ns	ns	*	ns

ns, *, and ** show non-significant and significance at P≤0.05 and P≤0.01, respectively.

Table 2 ANOVA for the effects of foliar applications on *Satureja mutica* and *Satureja spicigera* elemental content (First experiment).

Significance	N	P	K	Na	Fe	Ca	Mg	Mn	Zn
Species (A)	ns	ns	ns	ns	**	ns	**	**	ns
Foliar application (B)	ns	ns	ns	ns	ns	ns	**	ns	ns
A × B	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns, *, and ** show non-significant and significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Chlorophyll b content

Foliar application of nano-Fe and Se influenced chlorophyll b content of plants; 62 and 50% more than control, respectively. Nano-Fe foliar application increased chlorophyll b content 12% more than Se treatment (Table 3).

Total phenolics and flavonoids

Foliar treatment with Fe and Se improved phenolic and flavonoids content compared to control ones (Table 3). Foliar treatments of nano-Fe and Se improved flavonoids content up to 53 and 35% compared to control, respectively. Even though, both foliar treatments increased phenolics content compared to control, nano-Fe raised phenolics 4.3% more than Se treatment (Table 3). Species type was prominent on total phenolics content. Figure 1 A, shows that *S. mutica* contained 19% more phenolics than *S. spicigera*.

Table 3 Mean comparisons for the effects of foliar application on physiological traits and Mg content of *Satureja* species.

Foliar application (mg L ⁻¹)	Chl b content (mg g ⁻¹ FWt)	Flavonoids content (mg g ⁻¹ FWt)	Total phenolics content (mg g ⁻¹ FWt)	SOD activity (μmg ⁻¹ protein)	MDA content (nmol ⁻¹ g FWt)	Mg content (g kg ⁻¹)
0	0.371 ^b	9.51 ^b	72.5 ^b	5.88 ^b	13.83 ^a	2.56 ^a
nano-Fe	1.00 ^a	14.7 ^{ab}	96.4 ^a	9.93 ^a	7.13 ^b	1.91 ^c
Se	0.88 ^a	20.1 ^a	100.8 ^a	7.03 ^b	6.18 ^b	2.15 ^b

Similar letters in the columns are non-significant based on Duncan's multiple range test.

MDA content

Both foliar treatments reduced MDA accumulation compared to control plants; depicting the melioration role of treatments in maintaining cell membranes integrity. Moreover, Se treatment was more efficient in retarding MDA production compared to nano-Fe application (Table 3).

Mn, Fe, and Mg content

The species and foliar treatments influenced Mg content of plant samples. The greatest Mg content was recorded for *S. spicigera* (Fig. 1 C). Foliar treatments reduced Mg content (28% with nano-Fe and 8% with Se spray) of plants and the top Mg content belonged to the control treatment (Table 3). *S. mutica* had more Fe and Mn²⁺ content compared to *S. spicigera* (Fig. 1 B).

Similar letters on the columns are non-significant based on Duncan's multiple range test.

SOD and CAT activity

SOD activity was reacted to the foliar treatments. Nano-Fe spray increased (41%) SOD activity compared to no-foliar treatment and, Se treated ones had 16.3 % more SOD activity compared to control (Table 3). Interaction effects of species × foliar treatments influenced CAT activity. Foliar use of nano-Fe × *S. mutica* increased CAT activity up to 33% compared to no-foliar treatment in the same species. In *S. spicigera*, Se foliar treatment improved CAT activity up to 22% more than the control treatment. Under the control of no-saline conditions, CAT activity in *S. spicigera* was 8% more than *S. mutica* (Fig. 2).

The Second Experiment

Dry weight and plant height

Aerial parts and roots dry weight were impacted by the species type, salinity, and foliar treatments (Table 4). Foliar applications of Se and nano-Fe × no-saline conditions in *S. spicigera* increased aerial parts dry weight up to 14 and 27% compared to the no-saline × no-foliar treatment (Table 5). There was a difference between the foliar treatments on the dry weight of *S. spicigera* as well. So that, nano-Fe application × NaCl₀ led to 13% more aerial parts dry weight compared to Se × NaCl₀. Under the salinities of 50 and 100 mM, aerial parts' dry weight in both cultivars was decreased even with foliar treatments, indicating the low efficiency of foliar sprays in keeping the normal growth and aerial parts biomass of plants. Under no-saline conditions, foliar Se and nano-Fe treatments improved aerial parts' dry weight of *S. mutica* up to 54 and 53%, respectively. With the same conditions (NaCl₀ × no-foliar), *S. spicigera* attained 55% more yield than *S. mutica* (Table 5).

Salinities of 50 and 100 mM in both species and even with foliar treatments reduced root dry weight. The root dry weight under no-saline × selenium and nano-Fe treatments were 45 and 51% more than NaCl₀ × no-foliar treatments in *S. spicigera* (Table 5).

Plant height was influenced by foliar treatment (Table 4). No-saline × Fe and Se treatments and, NaCl_{50 mM} × Fe and Se treatment added up the plant height. NaCl₀ × Fe increased the height of plants up to 14% compared to NaCl_{50mM} × Fe. Similarly, NaCl₀ × Se attained 6% more height than NaCl_{50mM} × Se. Salinities of 50 and 100 mM under no-foliar treatments reduced plant height compared to their foliar sprayed ones (Table 6).

Total soluble solids content

TSS was influenced by the individual effects of species and, salinity × foliar spray (Table 4). The highest TSS content belonged to *S. spicigera* (12% more than *S. mutica*) (Table 7). Under NaCl_{50 and 100 mM}, foliar application of Fe and Se increased TSS content compared to control (no saline, no foliar treatment), (Table 6). The lowest TSS content was obtained with no saline × no-foliar treatment (Table 6).

Total phenolics content

Species type and treatment were independently influenced the phenolics content (Table 4). *S. mutica* had 10% more phenolics than *S. spicigera* (Table 7). NaCl_{50 mM} × Fe foliar spray, and NaCl_{100 mM} × Fe and Se treatment increased phenolics content in plants as well (Table 6). Nano-Fe foliar application × NaCl_{100mM} increased phenolics content up to 45% compared to control. NaCl_{50 mM} × nano-Fe raised up phenolics by 4% compared to Se treatment. Furthermore,

under 100 mM salinity, nano-Fe treatment attained 6% more phenolics content compared to Se treatment, showing the high efficiency of Fe compared to Se in phenolics biosynthesis and accumulation (Table 6).

Table 4 ANOVA for the effects of salinity and foliar applications on physiological traits of *Satureja* species.

Significance	Shoot dry Weight	Root dry Weight	Plant height	Chlorophyll a content	Chlorophyll b content	Carotenoids content
Species (A)	**	**	ns	ns	ns	ns
Treatment (B)	**	**	**	ns	ns	ns
A × B	**	**	ns	ns	ns	ns

Table 4 continued

Significance	Total soluble solids content	Proline content	Total phenolics content	Flavonoids content	MDA content	CAT activity	SOD activity
Species (A)	**	**	**	ns	**	**	ns
Treatment (B)	**	**	**	ns	**	**	**
A × B	ns	ns	ns	ns	ns	ns	ns

ns, *, and ** show non-significant and significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Treatment (B): foliar application (0, 3 mg L⁻¹ Se and nano-Fe) + salinity (0, 50 and 100 mM)

Proline content

The results from table 7 show an 11% increase in proline content of *S. spicigera* compared to *S. mutica* (Table 7). NaCl_{100 mM} × no foliar and Fe sprayed treatment increased proline content up to 80% compared to no-saline control. Under the salinity of 50 and 100mM; Se treatment compared to nano-Fe had less impact on the proline content of plants. Foliar treatments with Se (34.7 µg⁻¹g FWt) and nano-Fe (41.4 µg⁻¹g FWt), under the control of no-saline conditions, increased proline content of plants (Table 6).

MDA content

The independent effects of species and the treatments influenced MDA content of plants (Table 4). *S. spicigera* had 10% more MDA content than *S. mutica* (Table 7). Under NaCl_{100 mM} × no foliar treatment, MDA content was comparably higher than other treatment combinations. Foliar treatment of Se and nano-Fe reduced MDA content under no saline and salinity of 50 and 100 mM. An increase of 19% in MDA content was recorded in NaCl_{50mM} × no-foliar compared to control treatments (Table 6).

SOD and CAT activity

Under NaCl_{100 mM} × foliar Fe and Se treatment, SOD activity was superior. SOD activity showed 41% increase in NaCl_{100mM} × Fe and Se combination compared to control (Table 6).

CAT activity of *S. spicigera* was higher than *S. mutica* (Table 7). $\text{NaCl}_{100\text{mM}} \times \text{Se}$ treatment attained 33% more CAT activity compared to control. Under a no-saline environment $\times \text{Se}$ foliar application, CAT activity was 7% more than $\text{NaCl}_0 \times \text{Fe}$ treatment. Furthermore, with the salinity of 50 and 100 mM; Se treatment improved CAT activity 9 and 10% compound to the same conditions but foliar sprayed with nano-Fe (Table 6).

Table 5 Mean comparisons for the interaction effects of salinity and foliar applications on elemental content and plant dry weight of *Satureja* species.

Species	Salinity (mM)	Foliar application (3 mg L ⁻¹)	Aerial parts dry weight (g m ⁻²)	Root dry weight (g m ⁻²)	Ca content (g kg ⁻¹)	K/Na ratio
<i>S. mutica</i>	0	0	55.76 ^e	0.14 ^f	21.00 ^{b-e}	30.77 ^{b-d}
<i>S. mutica</i>	0	Fe	119.1 ^{bc}	0.67 ^b	19.07 ^{c-h}	34.70 ^{ab}
<i>S. mutica</i>	0	Se	123.3 ^{bc}	0.42 ^c	25.37 ^a	38.73 ^a
<i>S. mutica</i>	50	0	42.38 ^e	0.19 ^f	18.67 ^{c-h}	1.733 ^e
<i>S. mutica</i>	50	Fe	56.23 ^e	0.34 ^e	18.53 ^{c-h}	1.633 ^e
<i>S. mutica</i>	50	Se	56.33 ^e	0.28 ^e	19.47 ^{c-h}	2.267 ^e
<i>S. mutica</i>	100	0	30.66 ^e	0.33 ^e	16.33 ^{gh}	0.933 ^e
<i>S. mutica</i>	100	Fe	43.6 ^e	0.41 ^d	16.63 ^{eh}	1.067 ^e
<i>S. mutica</i>	100	Se	35.81 ^e	0.60 ^b	20.53 ^{b-e}	1.167 ^e
<i>S. spicigera</i>	0	0	125.4 ^{bc}	0.44 ^d	19.37 ^{b-g}	29.30 ^{cd}
<i>S. spicigera</i>	0	Fe	172.5 ^a	0.97 ^a	20.57 ^{b-f}	26.27 ^d
<i>S. spicigera</i>	0	Se	150.0 ^{ab}	0.86 ^a	25.43 ^a	32.07 ^{bc}
<i>S. spicigera</i>	50	0	36.33 ^e	0.66 ^b	20.83 ^{b-d}	1.300 ^e
<i>S. spicigera</i>	50	Fe	83.0 ^{cd}	0.68 ^b	21.03 ^{b-e}	1.400 ^e
<i>S. spicigera</i>	50	Se	65.90 ^{de}	0.56 ^c	16.77 ^{eh}	1.867 ^e
<i>S. spicigera</i>	100	0	51.00 ^e	0.56 ^c	16.00 ^h	0.866 ^e
<i>S. spicigera</i>	100	Fe	94.75 ^{cd}	0.67 ^b	17.50 ^{d-h}	0.900 ^e
<i>S. spicigera</i>	100	Se	51.95 ^e	0.41 ^d	20.10 ^{b-e}	1.233 ^e

Similar letters in the columns are non-significant based on Duncan's test.

Table 6 Mean comparisons for the effects of salinity and foliar applications on plant height and some physiological traits of *Satureja* species.

Treatment (Salinity + foliar spray)	Plant height (cm)	TSS (°Brix)	Total phenolics content (mg g ⁻¹ FWt)	Proline content (µg ⁻¹ g FWt)	MDA content (nmol ⁻¹ g FWt)	CAT activity (Units ⁻¹ mg protein)	SOD activity (µmg ⁻¹ protein)
NaCl ₀ + no spray	14.5 ^c	0.80 ^e	58.4 ^d	18.6 ^e	1.46 ^{de}	14.0 ^e	6.77 ^e
NaCl ₀ + Fe	32.8 ^a	0.98 ^{cd}	83.8 ^c	41.4 ^c	1.21 ^{ef}	14.2 ^{de}	8.44 ^{cd}
NaCl ₀ + Se	32.1 ^a	1.03 ^{b-d}	78.4 ^c	34.17 ^d	1.15 ^f	15.3 ^{cd}	7.45 ^{de}
NaCl ₅₀ + no spray	10.3 ^c	0.95 ^b	79.5 ^c	33.17 ^d	1.81 ^b	14.8 ^{cd}	8.32 ^{cd}
NaCl ₅₀ + Fe	28.3 ^a	1.17 ^{ab}	92.3 ^{a-c}	44.2 ^c	1.41 ^e	16.4 ^c	9.18 ^{bc}
NaCl ₅₀ + Se	30.1 ^a	1.18 ^{ab}	89.0 ^{bc}	29.5 ^d	1.24 ^{ef}	18.1 ^b	9.68 ^{bc}
NaCl ₁₀₀ + no spray	14.1 ^c	1.03 ^{b-d}	87.1 ^{bc}	91.6 ^a	2.35 ^a	16.1 ^c	9.53 ^{bc}
NaCl ₁₀₀ + Fe	23.0 ^b	1.12 ^{a-c}	106 ^a	95.1 ^a	1.52 ^d	18.9 ^b	11.47 ^a
NaCl ₁₀₀ + Se	22.1 ^b	1.23 ^a	100 ^{ab}	77.1 ^b	1.67 ^c	21.1 ^a	11.62 ^a

Similar letters in the columns are non-significant based on Duncan's test.

Table 7 Mean comparison for K and Zn content and some physiological traits of two *Satureja* species.

Species	TSS content (°Brix)	Total phenolics content (mg g ⁻¹ FWt)	Proline content (µg ⁻¹ gFWt)	MDA content (nmol ⁻¹ g FWt)	CAT activity (Units ⁻¹ mg protein)	K content (g kg ⁻¹)	Zn content (mg kg ⁻¹)
<i>Satureja mutica</i>	0.99 ^b	90.63 ^a	48.67 ^b	1.66 ^b	16.16 ^b	37.21 ^a	24.26 ^a
<i>Satureja spicigera</i>	1.12 ^a	81.71 ^b	54.66 ^a	1.84 ^a	16.96 ^a	33.86 ^b	21.88 ^b

Similar letters in the columns are non-significant based on Duncan's test.

Elemental content

N, P, Na, K, Fe, Mn, Zn, and Mg contents were responsive to the salinity and foliar applications (Table 8). The highest N and P content belonged to no saline × Fe and Se foliar sprayed plants and, no-salinity × no foliar treatment (Fig. 3 A). The salinity of 50 and 100 mM with no foliar treatment, reduced N and P content of plants compared to their foliar sprayed ones (Fig. 3 A). The highest K content belonged to NaCl₀ × Se (Fig. 3 B). Under the salinity of 50 mM with no-foliar treatment, K content was declined. Se foliar use under 50 mM salinity, improved K content up to 15% compared to

NaCl_{50mM} × no-foliar treated plants. With the salinity of 100mM, selenium foliar spray increased K content (18%) (Fig. 3 B). Potassium content was influenced by the species type. The more K content belonged to *S. mutica*, which was 9.1% more than *S. spicigera* (Table 7).

NaCl_{100 mM} × no-foliar application increased Na content in plants (Fig. 3 B). The least Na⁺ content was belonged to no-saline × Fe and Se foliar treatment and even with no foliar sprays. With a salinity of 50mM and no-foliar treatment, Na⁺ content of plants increased. However, foliar spray of Fe and Se under 50 mM salinity, declined Na⁺ content of plants. The same results in reducing Na⁺ content of plants was traced with 100 mM salinity levels foliar treated with Fe and Se (Fig. 3 B).

K/Na ratio and Ca²⁺ content were influenced by the interactions of species × salinity × foliar sprays (Table 8). Se and nano-Fe treatment under control (non-saline conditions) in *S. mutica* increased K/Na ratio by about 21 and 12% compared to NaCl₀ × no-foliar conditions (Table 5). With salinity increase to 50 and 100 mM, there was no difference between foliar treatments of *S. mutica* considering K/Na ratio. In *S. spicigera*, the same trend in K/Na ratio was recorded to emphasize the low tolerance of both species against salinity depression (Table 5). The highest Ca²⁺ content for both species was attained by Se foliar treatment under no-saline conditions (Table 5). Ca²⁺ content in NaCl₀ × Se treated plants demonstrated 17% increase in *S. mutica* and 24% more in *S. spicigera* compared to NaCl₀ × non-foliar treatment. Salinity levels in both species even with foliar treatments had low Ca content (Table 5).

Fe content was influenced by salinity × foliar treatments. The highest Fe content was recorded for NaCl₀ × Fe and NaCl_{50mM} × nano-Fe. There was no difference in Fe content between NaCl₀ × no foliar and NaCl_{100mM} × Fe foliar spray (Fig. 3 C).

Zn content was responsive to species type. *S. mutica* attained 10% more Zn content than *S. spicigera* (Table 7). The highest Zn (10% more than control) and Mn content were recorded in NaCl₀ × Se. With a salinity of 50 and 100 mM and foliar spray of nano-Fe and Se; Zn content was not affected and the least Zn content belonged to NaCl_{50mM} × Se (Fig. 3 D).

The top Mg content (12.5% more than control) was attained by NaCl₀ × Se spray. There was no meaningful difference in Mg content of control, NaCl₀ × nano-Fe, and NaCl_{50mM} × Se. The least Mg content (36% lower than control) was traced for NaCl_{100mM} × nano-Fe (Fig. 3 E).

Table 8 ANOVA for the effects of salinity and foliar applications on elemental content of *Satureja* species.

Significance	N	K	P	Ca	Mg	Fe	Zn	Mn	Na	K/Na ratio
Species (A)	ns	**	ns	ns	ns	ns	**	ns	ns	**
Treatment (B)	**	**	**	**	**	**	**	**	**	**
A × B	ns	ns	ns	*	ns	ns	ns	ns	ns	*

ns, *, and ** show non-significant and significance at P≤0.05 and P≤0.01, respectively.

Treatment (B): foliar application (0, 3 mg L⁻¹ Se and nano-Fe) + salinity (0, 50 and 100 mM)

T1: NaCl₀ + no-spray; T2: NaCl₀ + nano-Fe spray; T3: NaCl₀ + Se spray; T4: NaCl₅₀ + no-spray; T5: NaCl₅₀ + nano-Fe spray; T6: NaCl₅₀ + Se spray; T7: NaCl₁₀₀ + no-spray; T8: NaCl₁₀₀ + nano-Fe spray; T9: NaCl₁₀₀ + Se spray.

Discussion

Overall results demonstrate the adverse effects of salinity stress on the growth potential and physiological responses of both *Satureja* species. Salinity reduces plants growth, photosynthesis potential, and productivity [5, 7, 19]. In *Satureja* species and under salinity; electrolytes leakage and MDA content were increased [7]. Similar results have been reported in *Satureja hortensis* [20]. The results are clearly showing that MDA content in both species was meaningfully increased in response to salinity. Saline conditions initiate chaos in the overall metabolism of plants and even go to huge morpho-physiological variations. The elevated Na and Cl absorption under salinity impedes the sorption of K and Ca and subsequently; the ionic imbalances trigger strong oxidative stress and a drastic disturbance in plant growth and productivity [8, 21].

Na⁺ over-availability under saline-sodic conditions deteriorates the cell membranes and leads to very high electrolytes leakage [22]. In rice, Se application reduced MDA and H₂O₂ generation under salinity [23]. In our experiment, Se foliar application declined MDA accumulation and simultaneously increased the activity of SOD and TSS content in *Satureja* species. With *Zea mays* plants [24], lemon [25], and tomato [26]; Se foliar treatment improved SOD activity, photosynthetic potential, carotenoid, and TSS content of plants. Se is a preferential essential nutrient that has prominent roles versus stressors effects and, improves the growth potential and yield and retards the senescence of plants [23]. Se fortifies the antioxidant pool and activity under stressful environments. Furthermore, Se is an integral part of the enzyme glutathione peroxidase and hence holds pivotal protective roles in cells against oxidative damages [27] in the main part by diminishing of H₂O₂ and MDA contents [15] In the presence of Se; H₂O₂ is initially scavenged by the action of GSH and PX followed by the catalase activity. In the present study, CAT activity was improved by Se foliar treatment under salinity.

A reasonable increase in CAT activity by the Se foliar treatment has been reported by Hernandez-Hernandez et al. [26] More possibly, the increase in plant biomass with Se application could be ascribed by the reduced ROS generation, the protected photosynthetic apparatus, and the stimulated absorption of other essential minerals under saline stressful environments. Furthermore, with the studies on wheat [28], *Moringa peregrine* [29], and *Pimpinella anisum* [30]; foliar spray with nano-Fe under salinity, increased the height, dry biomass, chlorophylls content as well as sugars content of plants. Fe, especially as nano-form, played pivotal roles in the activity of SOD and CAT under salinity in grape [19] and *Linum usitatissimum* [5] Similar results have previously been reported demonstrating the positive effects of nano-Fe foliar application on chlorophyll content, SOD activity, K/Na ratio as well as on proline and flavonoids content of plants.

CAT and SOD are fundamental enzymes in scavenging the oxidative damage caused by diverse ROS radicles. The activity and the proportional ratio of these enzymes are largely important in battling ROS radicles to protect cells against stressors' side-effects. SOD is in the front-line of plant defense versus stress factors; acts by scavenging H₂O₂ into water and molecular O₂ and declines the reactive radicles adverse effects [31].

Under salinity, the biosynthesis of non-enzymatic antioxidants like phenolics, flavonoids, and proline plays chief roles in the demolition of free radicles and the regulation of osmotic potential and so fortifies plants survival under stressful environments [32, 33]. Proline accumulation under salinity imposition greatly inhibits cells acidification and prevents high respiration rates and hence maintains the cell energy reserves which give the plant high withstand potential under stress conditions [33]. Se treatment of plants under salinity stimulates proline accumulation and in contrast, declines Na⁺ absorption and translocation [23].

The intensified phenolics and flavonoids biosynthesis under salinity reduces ROS molecules' genesis and further negative actions [32]. Phenolics are a major category of plant secondary metabolites with an important function in protecting plants versus stress factors. In rosemary, Fe and Zn foliar treatment increased phenolics and flavonoids content of plant [34].

Fe is the cofactor of several enzymes, has prominent roles in chlorophylls and chloroplasts development [35], improves photosynthesis, and also has inevitable functions in oxidation/reduction reaction in plants [35]. Salinity stress goes to the chlorophyll's breakdown and reduces photosynthesis potential which retards the growth and productivity of plants [5]. Under the situation explained; it seems that the utilization of nano-elements plays crucial actions in subtracting salinity side-effects in the main part via their intensified absorption rate from the leaf surfaces as well as their feasible translocation and further metabolism. Moreover, nano-fertilizers accelerated uptake and metabolism greatly reduce the chemical fertilizers' input and hence, drastically decline the soils and water resources pollutions [36]. Singh et al. [5] reported that with salinity, Na, and Cl content of *Linum usitatissimum* was increased while Ca and K content reduced. Saline-sodic salinity interferes with the other nutrients absorption mainly via antagonistic competition. The ionic balance instability induced by the hyper-accumulation of Cl^- and Na^+ excessively hinder the uptake of Mg^{2+} , Ca^{2+} , and K^+ leading to reduced growth and yield [37]. In rice [23] and lettuce [38], Se application under salinity improved plant growth, Mg^{2+} availability and K/Na ratio and so, protected the plants against toxic Na impacts [38]. Se has a substantial role in Ca^{2+} homeostasis inside cells [39] and the regulation of growth and development and also stabilizes cell membranes integrity [40, 41]. ROS over-production under salinity perturbs signaling cascade inside cells. Variations in the cells Ca^{2+} content under saline-sodic environments, stimulate the movement of Ca^{2+} from the endoplasmic reticulum, Golgi apparatus, and vacuoles and/or depletes the Ca^{2+} specific sites in cell walls that damage the structure and function of the cell and threaten the cells viability and plant survival. Se foliar treatment slakes the free radicals (ROS) side-effects and, the regulation of Ca^{2+} homeostasis secures plant normal growth and productivity [39, 42]. The research conducted on *Pimpinella anisum* [30], grapevine [19], and linseed [5] showed that Fe foliar treatment increased N content, K/Na ratio and P content. Very small diameter of nano-Fe molecules hastens their absorption and assimilation even under stressful environments and thereby, diminishes the salinity depression [43]. When salinity stress is imposed on plants, the toxic ions are received at the membrane and/or cell wall levels. Therefore, a cascade of signal transduction events initiates the expression of salt-tolerance-related genes; their expression rate is species-dependent hence, several transcription factors and tolerance-related end-products are produced to reduce the ions toxicity and to induce ion equilibrium. Stress-responsive genes occurrence, expression, transcription, and translation rate are the tolerance determinants under saline-prone environments. The tolerance is quite dependent upon the compatible osmolytes such as proline and sugars accumulation. Furthermore, with ion equilibrium in the tolerant species, cell turgor and tissue intactness are secured and specifically, the photosynthetic tissues continue their normal activities for the assimilates genesis and partitioning. The immediate salinity effect apart from osmotic stress is the harmful ionic toxicity (chemical stress) resulting from the accumulation of harmful ions and, especially, Na^+ influx is a matter of great concern. Na^+ over-absorption triggers stress signaling pathways in the tolerant species leading to Na^+ excretion and compartmentalization in vacuoles to cope with the Na^+ over-accumulation. Furthermore, long-lasting signaling spends more energy despite the limited stomatal aperture. Those stress sensing and signaling events in the main part stimulate oxidative burst and ABA-dependent signaling processes which hugely impart plant growth indices and quality attributes. In the tolerant species, the re-sequestration of Na^+ into vacuoles assuages the salinity depression. Furthermore, the anatomical and intercellular localization of toxic ions mainly Na^+ relieves the pressure on metabolizing functional tissues to give the plant a reliable resilience under stressful environments. So, in-plant reclamation strategies and particularly sodium sequestration and osmo-protection have been defined as the chief tolerance mechanisms against salinity lesion. Hopefully, the integrated phenomics and genomics along with hormone-guided tolerance studies would be the hallmarks of salinity stress tolerance/avoidance

research themes in the major agricultural crops to combat the sudden damages and to ensure the prolonged adaptation behavior for the extended cultivation of agricultural produce under saline-sodic conditions and, even with water shortage saline-prone environments. Agricultural practices have inevitable functions to reach the reasonable tolerance and the guaranteed yield and quality attribute under harsh stressful situations as well. Meeting the appropriate nutritional demands of plants under formidable saline-sodic conditions is possibly the more convenient procedure to combat the stress factors and to reach the desirable productivity.

Conclusions

Foliar application of plants positively influenced physiological traits (phenolics, flavonoids, chlorophyll b content, CAT, and SOD activity) of both species. Under salinity, Ca^{2+} content, plant dry weight, and K/Na ratio were affected by the independent effects of treatments and species type. The highest Mn, Zn, K, and Mg were recorded with no saline \times Se treatment. SOD activity was influenced by salinity \times Fe and Se foliar use. Eventually, the idea is that salinity stress adversely affected the growth-related traits and physiological responses of plants. However, foliar selenium and magnetic-Fe treatments were able to partially smoothen the adverse side-effects of salinity on plants. The results with more detailed complementary studies would be advisable to the extension sections and pioneer farmers. The recommendation is frequently emphasized since these elements i.e Se and nano-Fe are easily available at low costs.

Methods

Foliar applications of Fe and Se (0 and 3 mg L⁻¹) were assayed on the growth and physiological responses of two *Satureja* species (*mutica* and *spicigera*) under salinity (0, 50, and 100 mM). Two separate experiments were conducted as factorial based on the completely randomized design with three replications. The pots were filled with medium-sized perlite provided by a local supplier. The temperature regime of the greenhouse was 27 \pm 1 and 18 \pm 1°C at day and night, respectively and, the relative humidity was 65 \pm 5%. The plant material (seeds) of two above-mentioned *Satureja* species were provided by Pakan Bazr Seed Company, Esfahan, Iran. Experimental research on the plants was comply with the institutional, national, or international guidelines. The seeds were initially planted in trays and ten plantlets were transferred to each 5-liter pot when the real leaves emerged. The plantlets were daily nourished with the Hoagland's nutrient solution (electrical conductivity of 2.2 mS cm⁻¹) (34). The optimal pH of the nutrient solution was 5.7 and was recorded every other day and adjusted accordingly by using H₂SO₄ (5% v/v).

In the first experiment, foliar applications including dH₂O, nano-Fe [34], and Se [6] were applied on plants having four real leaves. Ten days later, 1/3 of plants were sampled to determine elemental content, enzymes activity, and some physiological traits.

Afterward, in the second successive experiment, the remaining plants were subjected to salinity. The salinity levels began with 25 mM and, gradually increased to reach the final level within 10 days by adding an adequate amount of NaCl to the Hoagland's nutrient solution. To avoid the salinity shock, the pots were washed with tap water once a week. Following the salinity application, the EC of nutrient solution was 2.2 mS cm⁻¹ (0 mM NaCl), 4 mS cm⁻¹ (50 mM NaCl) and 8.4 mS cm⁻¹ (100 mM NaCl). One week after the salinity initiation, the second foliar treatments were applied and one month later, samplings were done to record the growth and physiological responses of plants.

Reagents and materials for the synthesis of Fe₃O₄ Magnetic Nano-Particles (Fe₃O₄ MNPs)

All chemicals (iron (III) chloride hexahydrate and iron (II) sulfate heptahydrate) were obtained from Merck (Darmstadt, Germany) except ethanol which was purchased from Hamon Teb Markazi (Zarandieh, Iran) and deionized water from

Instrumentation

FT-IR spectrum of Fe_3O_4 nanoparticles was recorded on a Vector 22 (Bruker, Ettlingen, Germany) instrument using KBr as the mulling agent. X-ray diffraction analysis (XRD) of Fe_3O_4 nanoparticles were done on Bruker D8 Advance (Bruker AXS, Karlsruhe, Germany) instrument with Cu-K_α radiation source (1.54 Å) between 8 and 80° generated at 35 mA and 40 kV at room temperature. Furthermore, a heater (IKA, model RHB2) and an ultrasonic bath (DSA100-SK₂-4.0L Fuzhou Desen Precision Instruments Co., Ltd, China) were used in the different steps of the synthesis process.

Synthesis of Fe_3O_4 MNPs

For the preparation of Fe_3O_4 MNPs; 50 mL deionized water was degassed into an ultrasonic bath for 10 min and next, 4.86 g iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and 3.34 g iron (II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) were added and the solution was heated at 100°C and vigorously stirred to dissolve the iron salts. Then, 12 mL concentrated ammonia solution was rapidly added under vigorous stirring and allowed to complete the reaction (2 h) and to form black iron oxide MNP_s. The formed Fe_3O_4 MNPs were cooled at room temperature and collected using a magnet. Later, Fe_3O_4 MNPs were first washed with a mixture of ethanol: water (50:50, v/v) and then with pure ethanol. Finally, Fe_3O_4 MNPs were dried at 80°C for 5 h in an oven.

Characterization of Fe_3O_4 MNPs

The FT-IR spectrum of Fe_3O_4 MNPs (Fig. 4-B) shows a strong peak at around 582 cm^{-1} more possibly related to the Fe–O bond in Fe_3O_4 . This peak was shifted to a high wave number compared to the Fe–O bond peak of bulk magnetite at 570 cm^{-1} due to NPs size [44]. Therefore, this verifies that Fe_3O_4 MNPs have been successfully synthesized. Moreover, figure 4-A shows the X-ray diffraction pattern (XRD) of Fe_3O_4 MNPs. The diffraction peaks in 2θ region of 5–80° (30.007, 35.601, 43.239, 53.782, 57.372, and 63.058°) which marked by their indices (220, 311, 400, 422, 511, and 440) confirmed the formation of Fe_3O_4 MNPs [44].

The fresh and dry weight of plants (biomass)

The fresh and dry weight of plants was determined by oven drying at 48°C for 3 days.

Pigments content

Chlorophyll a and b and carotenoids content were calculated spectrophotometrically following the methods of Prochazkova et al. [45].

Mineral elements

Na and K were determined in the dried grounded leaves by flame-photometer according to the methods described by Chrysargyris et al. [46]. The content of Zn, Ca, Mg, and Fe were measured by atomic absorption spectroscopy (Shimadzu, AA6300, Japan) as previously described by Honarjoo et al. [47]. Nitrogen and P contents were quantified by Kjeldahl and vanadate molybdate methods, respectively.

Total Soluble Solid (TSS) content

TSS was quantified by a hand refractometer (Erma, Tokyo, Japan) and the data were reported as °Brix.

Proline content

Proline content was assayed according to the method of acid-ninhydrin with toluene as standard reagent at 520 nm as described by Fedina et al. [48].

Malondialdehyde content

Malondialdehyde (MDA) content (nmol g^{-1} FWt) was recorded according to Nareshkumar et al. [49].

Determination of catalase enzyme activity

Catalase enzyme activity was determined according to the methods of Sairam et al. [50]. Leaf samples (0.5 g) were homogenized in ice-cold 0.1M phosphate buffer (pH 7.5) containing 0.5 mM ethylenediamine tetra-acetic acid (EDTA) with pre-chilled pestle and mortar. The homogenate was centrifuged at 4°C in T80+ refrigerated centrifuge for 15 min at 15000g. The supernatant was transferred to a 30 ml tube for enzyme extraction [50].

Total phenolics and flavonoids content

Total flavonoids content was determined according to the method of Quettier-Deleu et al. [51]

Phenolics content was assessed using Folin-Ciocalteu reagent according to the procedure described by Kim et al. [52].

SOD activity

SOD activity was traced via the method described by Rios-Gonzalez et al. [53]

Data analysis

The experiment was conducted as factorial based on the completely randomized design with three replications. The data were analyzed by SPSS (ver.15). Figures were drawn by Excel (2016) and the means were compared by Duncan's multiple range test at $p \leq 0.05$ and $p \leq 0.01$.

Abbreviations

ANOVA: Analysis of variance

CAT: Catalase

MDA: Malondialdehyde

MNPs: Magnetic nanoparticles

SOD: Superoxide dismutase

ROS: Reactive oxygen species

TSS: Total soluble solids

Declarations

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Author's Contribution

Conceptualization: MBH and LVM; **data curation,** LVM, MBH, ZM and AS; **Methodology,** MBH, LVM, ZM, AS and DE; **Visualization,** MBH and LVM; **Writing & Editing,** MBH, LVM and DE; All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publish in BMC Plant Biology.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon request.

Disclosure statement

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

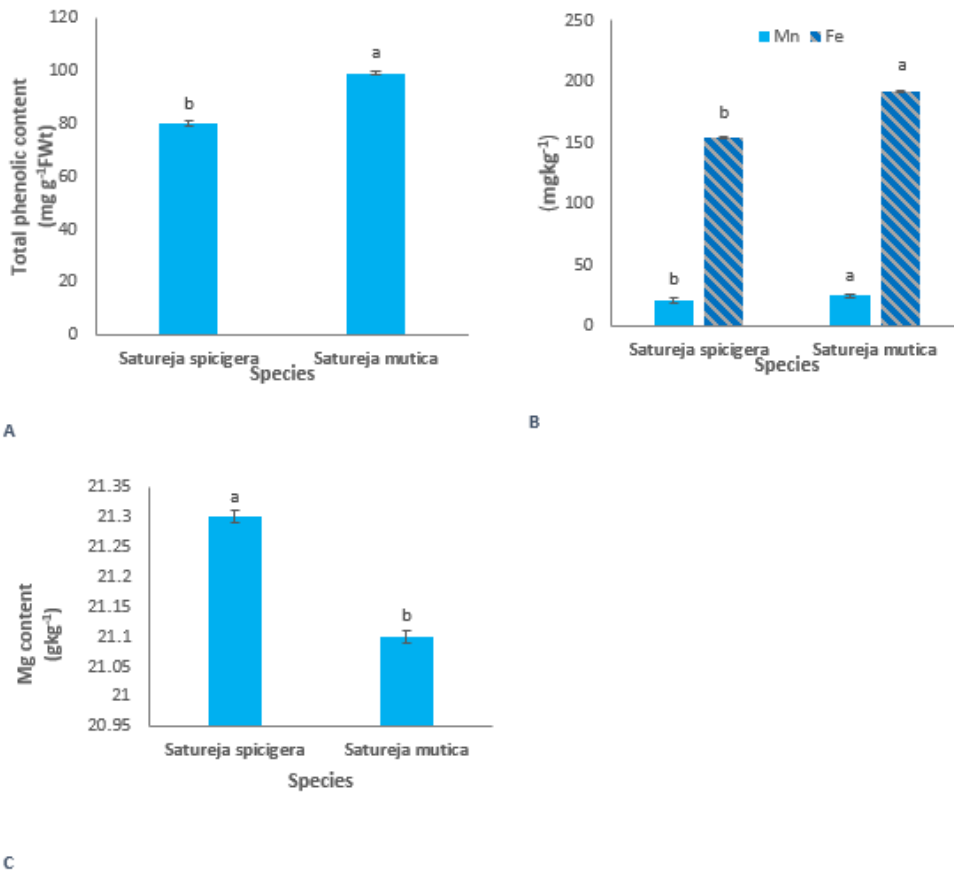


Figure 1

Mean comparison for Mn and Fe as well as total phenolics content in Satureja species.

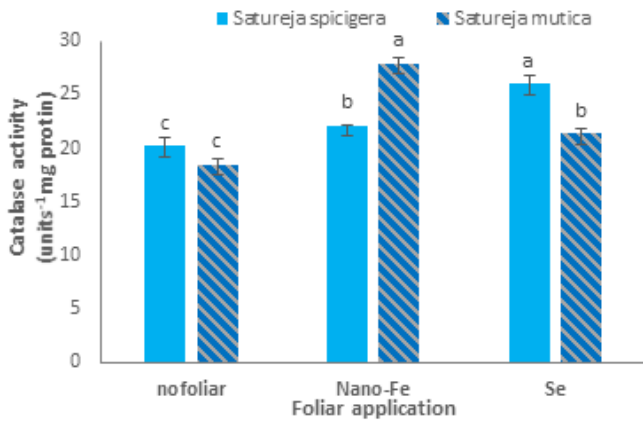


Figure 2

Mean comparison for the interaction effects of foliar application on catalase activity of Satureja species. Similar letters on the columns are non-significant based on Duncan's multiple range test.

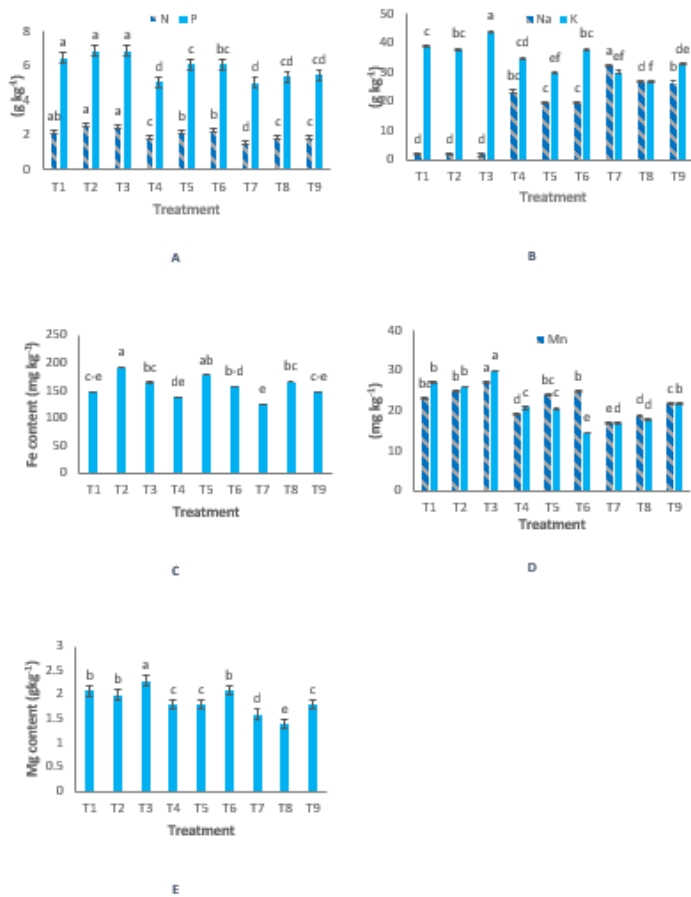


Figure 3

Mean comparisons for the interaction effects of salinity and foliar applications on elemental content of Satureja species. Similar letters in the columns are non-significant based on Duncan's test.

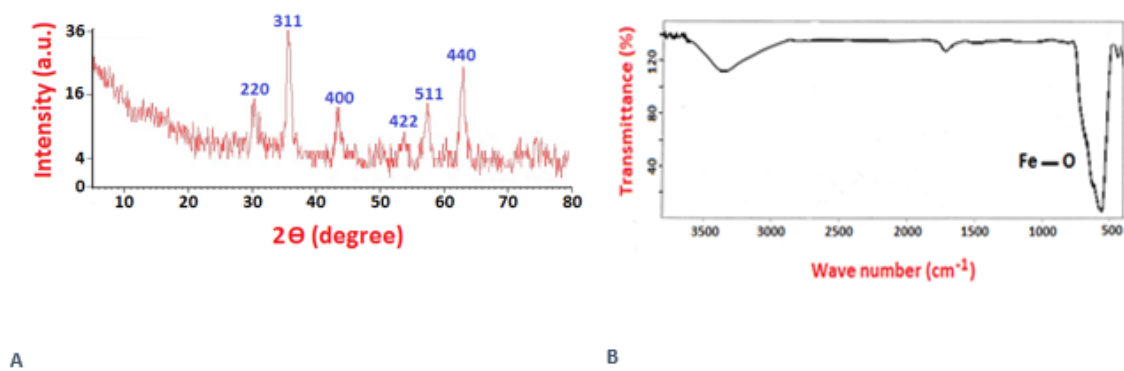


Figure 4

XRD pattern (A) and FT-IR spectrum (B) of Fe₃O₄ MNPs, respectively.