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INFLUENCE OF FOLIAR APPLICATION OF MINERAL, CHELATED AND NANO ZINC ON GROWTH, SOME BIOCHEMICAL ASPECTS AND PRODUCTIVITY OF THYME IN BALOZA-NORTH SINAI AREA

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Zinc is vital for the growth of plants. Globally, zinc insufficiency is a widespread micronutrient shortfall in areas of agriculture, resulting in decreased yield and nutritional quality of agricultural plants. It is necessary for plants to grow normally and productively. The fieldwork was conducted in the 2021–2022 seasons at the Experimental Farm of Desert Research Center, Baloza Research Station, North Sinai Governorate. The experiments aimed to evaluate the effects of three different sources of zinc fertilizer as $ZnSO_4$, Zn-EDTA and ZnO NPs at 0, 50, 100 and 150 ppm on growth, yield, nutrients contents of thyme plants. As well as the elemental composition of thyme plants using qualitative energy-dispersive X-ray microanalysis (EDX) system attached with scanning electron microscopy (SEM) as EDX-SEM. Treatments were arranged in a split plot design with three replicates. The results showed that, the best treatment was Zn NPs at 100 mg l^{-1} that gave the maximum growth, components of yield, and nutrient concentrations of thyme plants. In this work, it was found that thyme plants contained a major concentration of C and O while N presented as moderate concentration, but Si, P, K, Ca, Cu and Zn presented trace concentration.

Keywords: Nanotechnology, green synthesis, zinc fertilizers, thyme, plant productivity

INTRODUCTION

Approximately two billion individuals worldwide are now known to be deficient in zinc due to dietary deficiencies (Prasad, 2008). The immune system, homeostasis, oxidative stress, apoptosis, and ageing all depend on zinc. Zinc deficiency is associated with significant ailments of broad public health interest. Zinc deficiency has been related to significant ailments of public health concern. According to the research published by Giacalone et al. (2021), taking zinc supplements orally has been suggested to treat and mitigate the effects of COVID-19 (coronavirus). Human zinc lack is mostly due to insufficient consumption of food containing a high percentage of zinc, due to the low zinc content in agricultural soils (Tabrez et al., 2022 and Hamdy et al., 2023). One of the major soil factors limiting agricultural growth is zinc deficiency, which can lower crop yields and nutritional quality (Lee et al., 2017). Due to the fact that plant-available zinc levels are low in about 50% of agricultural soils worldwide (Alloway et al., 2008), and hence, zinc insufficiency is a significant worldwide public health issue affecting humans (Tulchinsky, 2010). In fact, 31% of people worldwide are estimated to be zinc deficient, according to Caulfield and Black (2004). Raising the amount of zinc in the diet and increasing its bioavailability is therefore essential, mainly in undeveloped and developing countries (Welch and Graham, 2004 and Zhao and McGrath, 2009). Furthermore, zinc is one of the vital elements needed for plant growth, participating in numerous metabolic activities, such as the synthesis and degradation of proteins, lipids, carbohydrates, and nucleic acids (Tarafdar et al., 2014). This indicates that zinc oxide nanoparticles (ZnO NPs) actively regulates several mechanisms that help plants identify and respond to abiotic stresses. Based on Yan et al. (2016), it has been shown that zinc has a significant role in controlling reactive oxygen species (ROS) and preserving plant cells from oxidative damage.

Using nanotechnology to enhance crops worldwide has garnered a lot of interest lately (El-Ramady et al., 2021). Nanotechnology is one of the newest technologies to emerge recently, and new applications in plant biotechnology and agriculture are starting to become possible with the creation of nanomaterials and devices (Biswas, 2015). Agriculture is becoming more interested in nanotechnology because of the successful *in vitro* applications of several nanoplatforms. When compared to conventional methods, the use of nanomaterials can promote healthier plant growth, faster germination of seeds, increased tolerance to biotic and abiotic stresses, and efficient nutrient utilization.

Micronutrient NPs are thought to be an excellent option for plant absorption and biofortification because of their high surface-to-volume ratio and small size. Several studies have been conducted on the interactions between ZnO NPs and plants. Like for instance, the use of micronutrients as NPs to boost productivity in agricultural production has been shown to enhance yield, as illustrated by Reynolds (2002). Zhao et al. (2014) found that in an organic soil, cucumber (*Cucumis sativus*) growth was improved by 400 and 800 mg/kg ZnO NPs. According to Mahajan et al. (2011), mung (*Vigna radiata*) and gramme (*Cicer arietinum*) seedlings grew more quickly in plant agar medium when ZnO NPs were added at low quantities. Significantly higher growth and yield were noted in the study conducted by Prasad et al. (2012), on the effects of ZnO NPs on the germination and yield of peanuts. Additionally, Wang et al. (2018) stated that specific studies showed a significant reduction of tomato vegetative growth by ZnO NPs.

Thyme, or *Origanum syriacum* var. *synaicum* (*aegyptiacum*), which is related to the Lamiaceae family is an extensively used culinary and medicinal spice that is grown commercially in the North Sinai Governorate, locally referred to as zaatar in Arabic. According to Figueiredo et al. (2008), the plant is classified as an aromatic and medicinal plant with a broad chemical intraspecific. Thyme leaves, either fresh or dried, are used as a spice (Lee et al., 2004). Thyme contains between 0.32 and 4.9% of essential oil. Plant essential oils have numerous characteristics, including antioxidant, antifungal, and antibacterial properties. The essences have numerous of pharmacological and biological properties and are rich in phenolics (Bozin et al., 2006) antibacterial, carminative, antioxidative, and antiseptic according to Jackson and Hay (1995), Letchamo et al. (1995), Bozin et al. (2006), Nejad et al. (2008) and Carlen et al. (2010). The aim of study is to evaluate the effects of three different forms of zinc fertilizer as $ZnSO_4$, Zn-EDTA and ZnO NPs on growth, productivity, and biochemistry of thyme plants grown in sandy soils.

MATERIALS AND METHODS

1. Materials

Neem (*Azadirachta indica* A. Juss) leaves were the materials employed in the green synthesis of ZnO NPs. Fresh neem leaves were gathered from the garden of the Desert Research Center in Egypt, while $ZnSO_4$ with a high purity of ≥98% was obtained from Merck Chemicals Ltd. Fresh leaves of neem were washed with distilled water, drying in an oven at 60°C and grinding.

1.2. Preparation of ZnO NPs

The two main steps in the synthesis of ZnO NPs were the preparation of leaf extract from neem leaves and the synthesis of ZnO NPs according to Thi et al. (2020)

1.2.2. Preparation of neem extract

Neem leaves were cleaned from surface dust by repeatedly washing in distilled water. The leaves were then dried in an oven at 60°C for 48 hours. After being dried, the neem leaves were crushed, powdered, and sieved to the right size. Neem leaves were extracted at a concentration of 250 g L^{-1} at 25°C for 24 hours while being constantly shaked. After that, the extract was then filtered with filter paper.

1.2.2. Synthesis of ZnO NPs

The neem leaves that had previously been removed from the extraction solution were added to 100 ml aqueous solutions containing 20 g of $ZnSO_4$ and heated for one hour at 70°C to create ZnO NPs. After that, a white-colour solution was created by reducing the mixed solution. This shift in colour is thought to be an extraordinary sign that soluble components from the neem leaf extract was formed. The ZnO NPs mixture was given a pH adjustment of 5.39 by adding NH_4OH solution (0.1 M mol L^{-1}). After centrifugation, the precipitates were collected in a clay vessel, washed with deionized water several times, and heated at 600°C for three hours in a muffle to produce ZnO NPs, to get rid of organic materials and to obtain nanozinc with a high crystal form (Davar et al., 2015) as shown in Fig. (1). At last, ZnO NPs created a white precipitate, which was appropriately packaged for further analysis.

1.2.3. Characterization of ZnO NPs

1.2.3.1. FTIR spectroscopy was used to characterize ZnO NPs using a Nicolet Avatar 230 spectrometer.

1.2.3.2. SEM was used to determine the NPs morphology. A field emanation gun (FEG) type was used, with a quickening power of 30 kV and a magnification range of 250x to 20000x.

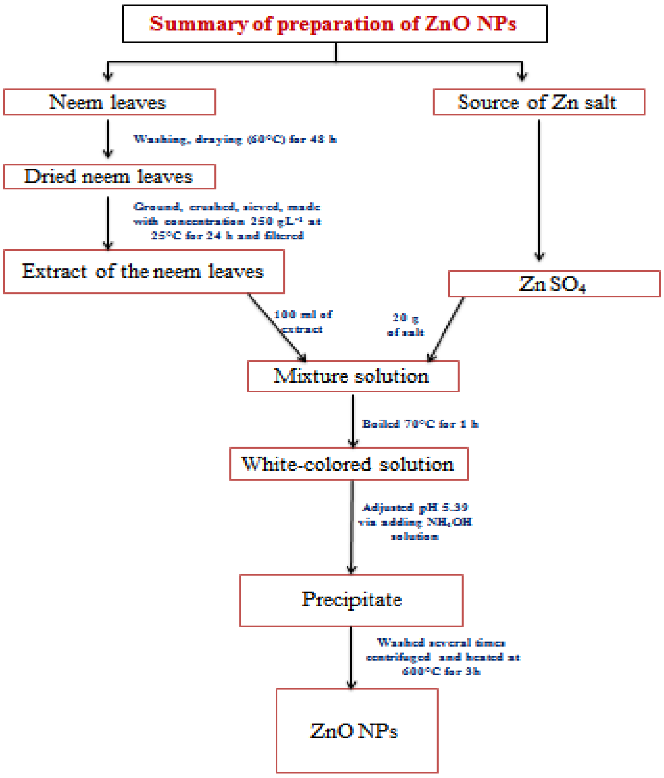

Fig. (1). A schematic view of the preparation of ZnO NPs.

1.2.3.3. Using DLS (Malvern Zeta size Nano-ZS nano series), the particle size dispersion of the various NPs dispersed in DI water was investigated.

1.2.3.4. Energy-dispersiveX-rayspectroscopy (EDX) spectroscopy was measured using an Oxford EDAX system-equipped SEM apparatus (a SEM Model Quanta field emission gun (FEG) with applying electrical power of 30 kV; JEOL Company, Tokyo, Japan).

2. Field Experiment

The current study was carried out in the recently reclaimed desert land at Baloza Research Station, North Sinai Governorate, Agricultural Experimental Station of the Desert Research Center, during the 2021–2022 seasons. The study was designed using three replicates using a split-plot design. Three forms of zinc fertilizer, $ZnSO_4$, Zn-EDTA and ZnO NPs were applied to the main plots. Zn at various concentrations (0, 50, 100, and 150 ppm) was included in the subplots. All plants were subjected to standard farming procedures.

Thyme seedlings were planted on the 15th of February, under drip irrigation, in rows 75 cm apart and 50 cm (11200 plants/faddan). The experimental site had sand-textured soil, so an irrigation system with four litres per hour of drippers was employed for thirty minutes a day. For each treatment, 200 kg N/faddan was added as ammonium sulphate (20.5% N) in three doses, 150 kg P_2O_5/faddan was added as calcium super phosphate (15% P_2O_5) and 75 kg K_2O/faddan was added as K sulphate (50% K_2O) as recommended by the Ministry of Agriculture in Egypt. During soil preparation, P and compost were added as organic manure at a rate of 20 m^3/faddan. The plant's vegetative portions were cut at a height of 10 cm above the soil. The first cut of aerial parts was carried out on the 15th of June and the second cut was on the 15th of October. For each cut in the two seasons, three plants were selected randomly from each treatment. Samples of the cultivated soil were taken before thyme planting to analyse the chemical and physical properties using standard methods as stated by Chapman and Pratt (1978) as shown in Table (1).

2.1. Characters of growth and yield

Height of plant (cm), fresh and dry weight (g/plant) and fresh and dry weight (ton/faddan) of herb.

2.2. Determination of macro and micro elements in the dry herb

Plant samples were dried in an electric oven at 70°C for 48 hours, before finely ground for chemical analysis according to A.O.A.C. (1990). In an acid-digested solution made in accordance with Cottenie et al. (1984), macro and micronutrients were determined.

2.3. Statistical analysis

Using the Statistix version 9 computer programmer (Analytical Software, 2008), the current work data was statistically examined. The differences between the means of the treatments were considered significant since they exceeded the least significant differences (L.S.D.) at the 5% level.

Table (1). Initial status of some chemical and physical properties of the experimental soil.

Chemical properties												
Depth (cm)	**pH***	**EC****	**Soluble ions in saturated soil extract (meq/L)**							**Available macronutrients (ppm)**		
		dS/m	**Na^{+}**	**K^{+}**	**Ca^{++}**	**Mg^{++}**	**Cl^{-}**	**HCO_3^{--}**	**SO_4^{--}**	**N**	**P**	**K**
0-30	8.19	1.365	5.11	0.50	3.61	4.40	3.35	3.80	6.50	38	3.66	48

Physical properties				
Depth (cm)	**Particle size distribution (%)**			**Texture class**
	Sand	**Silt**	**Clay**	
0-30	89.10	6.32	4.58	Sandy

*in 1:2.5 soil extraction ** in soil paste extraction

RESULTS

1. Characterization of NPs

The possible functional groups in the synthesised NPs and the neem leaf extract were examined using FTIR. The FTIR spectra of ZnO NPs and the extract from neem leaves are shown in Fig. (2). As shown in Fig. (3), DLS was used to measure the particle size distributions of ZnO NPs which have a particle size of 64.8 nm. Fig. (4) shows the surface morphology of ZnO NPs using scanning electron microscopy (SEM). Using neem extract, an energy dispersive X-ray spectrum (EDAX) from one of the densely populated areas of ZnO NPs was captured in the spot profile mode. With significant signals from the atoms of zinc and oxygen are shown in Fig. (5).

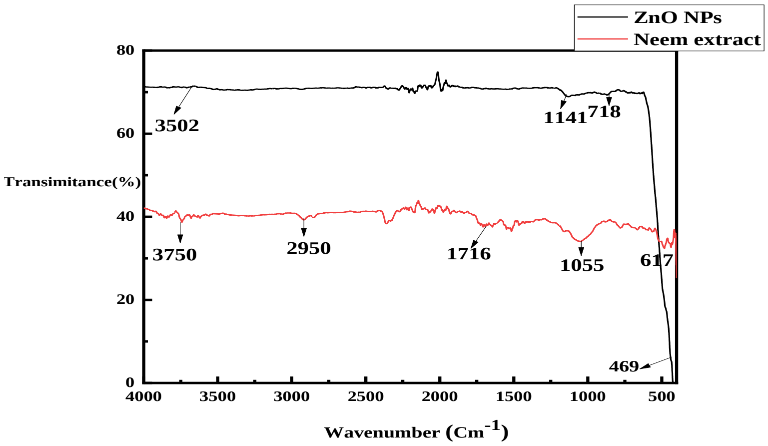

Fig. (2). FTIR spectra of neem extract and ZnO NPs.

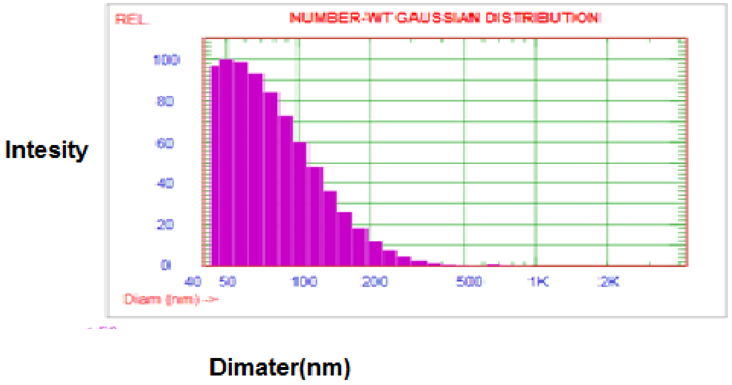

Fig. (3). Particle size distribution of ZnO NPs.

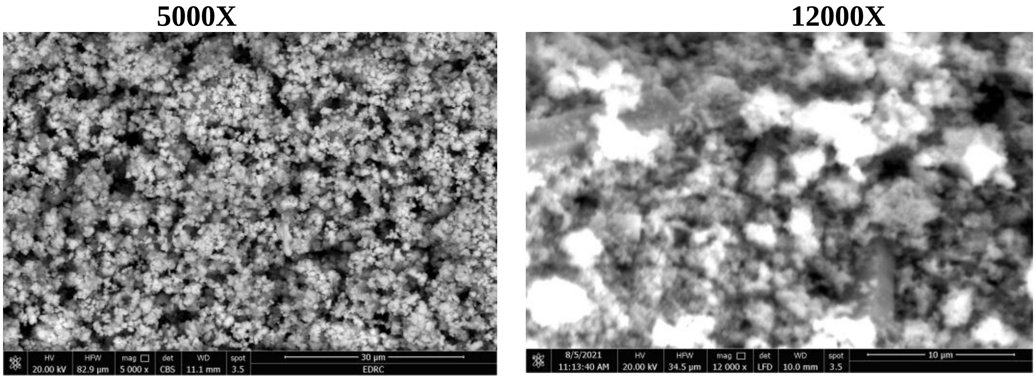
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Fig. (4). SEM of ZnO NPs.

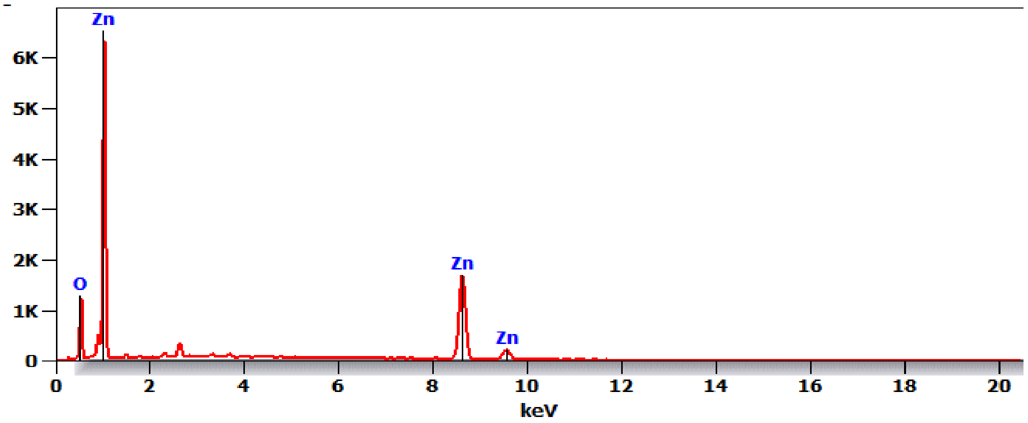


Fig. (5). EDX Spectrum images of ZnO NPs Synthesized using neem leaf extract.

2. Growth and Yield characters

Data of the effects of foliar spraying with Zn fertilizer forms on the yield components of thyme plants through two cuts during the studied two growth seasons are presented in Table (2). Plant height (cm) and fresh and dry weight of herbs (g/plant) significantly increased by using ZnO NPs form compared to other forms i.e. Zn-EDTA and $ZnSO_4$. The ZnO NPs treatment at rate of 100 ppm gave the greatest significant values of fresh weight (834.67 and 876.40 g), dry weight (243.48 and 248.35 g) and plant height (45.90 and 47.28 cm) of thyme herb in the 1st and 2nd cut, respectively), compared to control and all studied treatments. Also, Figs. (6 a and b) illustrate that foliar application of different Zn forms positively improved both vegetative growth and total yield of thyme (ton/faddan). Where, the maximum values of fresh and dry weight of vegetative were recorded in the ZnO NPs treatment at rate of 100 ppm. While control treatment recorded the lowest values and the second cutting had the greatest effect on vegetative growth as compared to the first.

3. Determination of Macronutrients Content

Table (3) shows that the treatments through two cuts had an influence on the nitrogen, phosphorus, and potassium content (%) in the thyme plant. ZnO NPs provided the most significant increase in N content when contrasted with Zn-EDTA and $ZnSO_4$. P and K did not show significant increases with the studied treatments. The ZnO NPs treatment at a rate of 100 ppm showed the greatest macronutrients content of 2.3273 and 2.5135% for nitrogen; 0.4990 and 0.4740% for phosphorus and 1.2662 and 1.3802% for potassium in the 1st and 2nd cuts, respectively, these values were followed by Zn-EDTA, and then $ZnSO_4$ treatments. The second cutting has better nutritional content than the first one.

4. Determination of Micronutrients Content

The data in Fig. (7) clearly show that the studied various treatments had significant effects on the micronutrients content of thyme plants. Generally, using ZnO NPs significantly resulted in rising the micronutrients content compared to using Zn-EDTA and $ZnSO_4$. The ZnO NPs treatment at rate of 100 ppm gave the highest values of micronutrients content in thyme herb i.e. 0.2846 and 0.2988% for Fe; 111.10 and 112.60 mg l^{-1} for Mn; 74.181 and 76.181mg l^{-1} for Cu and 92.344 and 96.961 mg l^{-1} for Zn in the 1st and 2nd cut, respectively.

Table (2). Effect of the studied treatments on plant height, fresh and dry weight of thyme plant in the two cuts as an average of both study seasons.

Treatments	**Plant height. (cm)**		**Fresh weight (g/plant)**		**Dry weight (g/plant)**	
	1st cut	**2nd cut**	**1st cut**	**2nd cut**	**1st cut**	**2nd cut**
Zn forms (A)						
Zn-EDTA	39.067B	40.239B	437.25B	445.99B	139.89B	146.88B
Zn-nano	41.020A	42.251A	620.12A	651.13A	199.86A	203.86A
$ZnSO_4$	35.893C	36.969C	271.43C	282.29C	114.39B	118.96B
LSD 0.05	0.6423	0.6615	29.225	30.595	28.146	29.602
Zn rates (B)						
Control	30.277D	31.185D	319.52C	331.12C	94.90C	98.19C
50 mg l^{-1}	40.043C	41.245C	429.87B	446.08B	150.93B	156.01B
100 mg l^{-1}	42.973A	44.263A	545.48A	566.78A	194.95A	201.78A
150 mg l^{-1}	41.346B	42.586B	476.87AB	495.23AB	164.73AB	170.28AB
LSD 0.05	0.6361	0.6552	86.474	89.893	37.307	38.691
A×B						
Zn-EDTA control	30.277GH	31.185GH	263.11EF	273.64FG	87.84DE	92.23CD
Zn-EDTA 50 mg l^{-1}	41.020D	42.251D	440.50D	449.31DE	127.43DE	133.80CD
Zn-EDTA 100 mg l^{-1}	43.950B	45.269B	495.33CD	505.24CD	197.21ABC	207.07AB
Zn-EDTA 150 mg l^{-1}	41.997CD	43.257CD	453.67CD	462.74D	147.06BCD	154.42BC
ZnO NPs control	31.253G	32.191G	283.78EF	295.13FG	117.31DE	120.99CD
ZnO NPs 50 mg l^{-1}	42.973BC	44.263BC	586.00BC	615.30BC	208.06AB	212.22AB
ZnO NPs 100 mg l^{-1}	45.903A	47.280A	834.67A	876.40A	243.48A	248.35A
ZnO-NPs 150 mgl^{-1}	43.950B	45.269B	693.17AB	727.83AB	229.29A	233.88A
$ZnSO_4$ control	29.300H	30.179H	232.39F	241.68G	117.84DE	81.36D
$ZnSO_4$ 50 mg l^{-1}	36.137F	37.221F	306.44EF	318.70EFG	144.16CD	122.00CD
$ZnSO_4$ 100 mg l^{-1}	39.067E	40.239E	366.67DE	385.00DEF	118.62DE	149.93BC
$ZnSO_4$ 150 mg l^{-1}	38.090E	39.233E	359.50DEF	366.69DEFG	78.23E	122.56CD
LSD 0.05	1.1018	1.1349	149.78	155.70	64.618	67.015

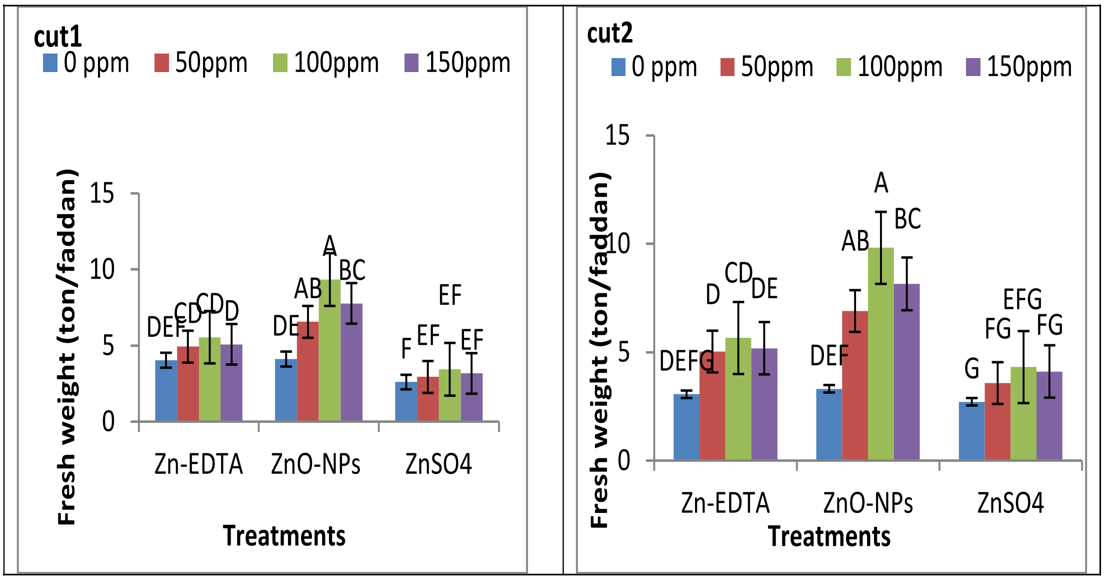


Fig. (6 a). Effect of the studied treatments on fresh weight (ton/faddan) during the two cuts as an average of both study seasons.

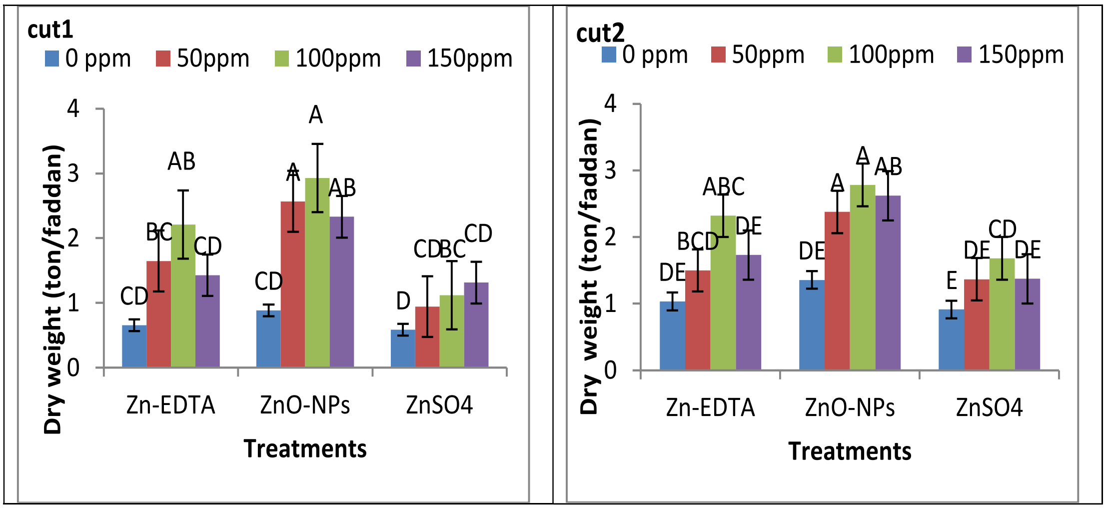


Fig. (6 b). Effect of the studied treatments on dry weight (ton/faddan) during the two cuts as an average of both study seasons.

Table (3). Effect of the studied treatments on NPK content (%) of thyme plant in two cuts as an average of both two study seasons.

Treatments	**N%**		**P%**		**K%**	
	1st cut	**2nd cut**	**1st cut**	**2nd cut**	**1st cut**	**2nd cut**
Effect of Zn fertilizers						
Zn- EDTA	1.986 B	2.085B	0.379A	0.386A	1.055AB	1.097B
ZnO NPs	2.108 A	2.277A	0.415A	0.394A	1.154A	1.257A
$ZnSO_4$	1.889 C	1.946 C	0.3773A	0.3886A	0.968B	0.987B
LSD 0.05	0.0745	0.0801	0.0522	0.0516	0.1358	0.0516
Effect of rate						
Control	1.693C	1.784C	0.295C	0.295C	0.923C	0.295 C
50 mg l^{-1}	1.985B	2.093B	0.401B	0.400B	1.039B	0.400B
100 mg l^{-1}	2.231A	2.352A	0.462A	0.461A	1.179A	0.461A
150 mg l^{-1}	2.068B	2.180B	0.402B	0.402B	1.094AB	0.402B
LSD 0.05	0.1386	0.1478	0.0244	0.0238	0.0899	0.0238
Effect of interaction						
Zn-EDTA control	1.676 GH	1.760FG	0.296D	0.302D	0.912DE	0.302D
Zn-EDTA 50 mg l^{-1}	1.901DEFG	1.996CDEF	0.392C	0.399C	1.001BCDE	0.399C
Zn-EDTA 100 mg l^{-1}	2.264AB	2.378AB	0.429 BC	0.437ABC	1.178A	0.437ABC
Zn-EDTA 150 mg l^{-1}	2.102BCD	2.207BC	0.398BC	0.406C	1.127ABC	0.406 C
ZnO NPs control	1.776FGH	1.9188EF	0.305D	0.292D	0.940CDE	0.292D
ZnO NPs 50 mg l^{-1}	2.177AB	2.3513AB	0.436BC	0.414BC	1.176AB	0.414BC
ZnO NPs 100 mg l^{-1}	2.327A	2.513A	0.499A	0.474A	1.266A	0.474A
ZnO NPs 150 mg l^{-1}	2.152ABC	2.324AB	0.420 BC	0.399C	1.205A	0.399 C
$ZnSO_4$ control	1.6267 H	1.6755 G	0.284D	0.290D	0.890E	0.290D
$ZnSO_4$ 50 mg l^{-1}	1.8769EFG	1.9332DEF	0.377C	0.388C	0.948CDE	0.3883C
$ZnSO_4$ 100 mg l^{-1}	2.102BCDE	2.1652BCD	0.4597AB	0.473AB	1.092ABCD	0.473AB
$ZnSO_4$ 150 mg l^{-1}	1.952CDEF	2.010CDE	0.388C	0.400C	0.966CDE	0.400C
LSD 0.05	0.2401	0.2350	0.0631	0.0621	0.1557	

5. Elemental Composition Analysis for Thyme Plants Using SEM-EDX

This study showed that elemental composition estimation in atomic percentage for powder samples may be quickly and easily determined using scanning electron microscopy with an energy dispersive X-ray analytical equipment (SEM-EDX). The results of the elemental composition of thyme using EDX analysis of powder from plant tissues treated with Zn fertilizer are shown in Fig. (8) and Table (4). In all these elements, C and O were presented as high concentrations while N was presented as moderate concentrations, but Si, P, K, Ca, Cu and Zn were presented in trace concentations. The variation in percentage of elemental concentration is mainly attributed to the addition of Zn fertilizer, the Zn NPs treatmenta at rate of 100 ppm gave the maximum value of the chemical composition (atomic %) in plant tissue, these values were followed by Zn-EDTA, and then Zn and $ZnSO_4$ treatments.

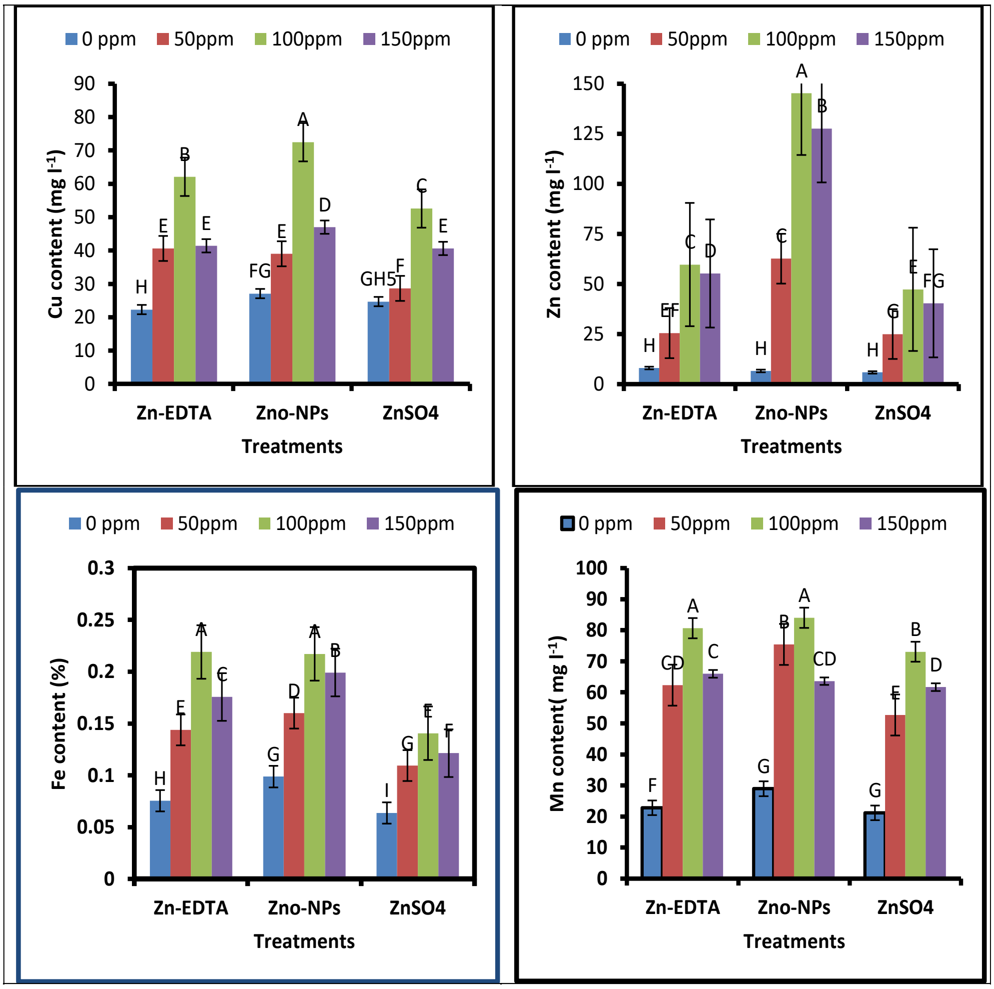


Fig. (7a). Effect of the studied treatments on micronutrients content in herbs of thyme during cut 1 as an average of both study seasons.

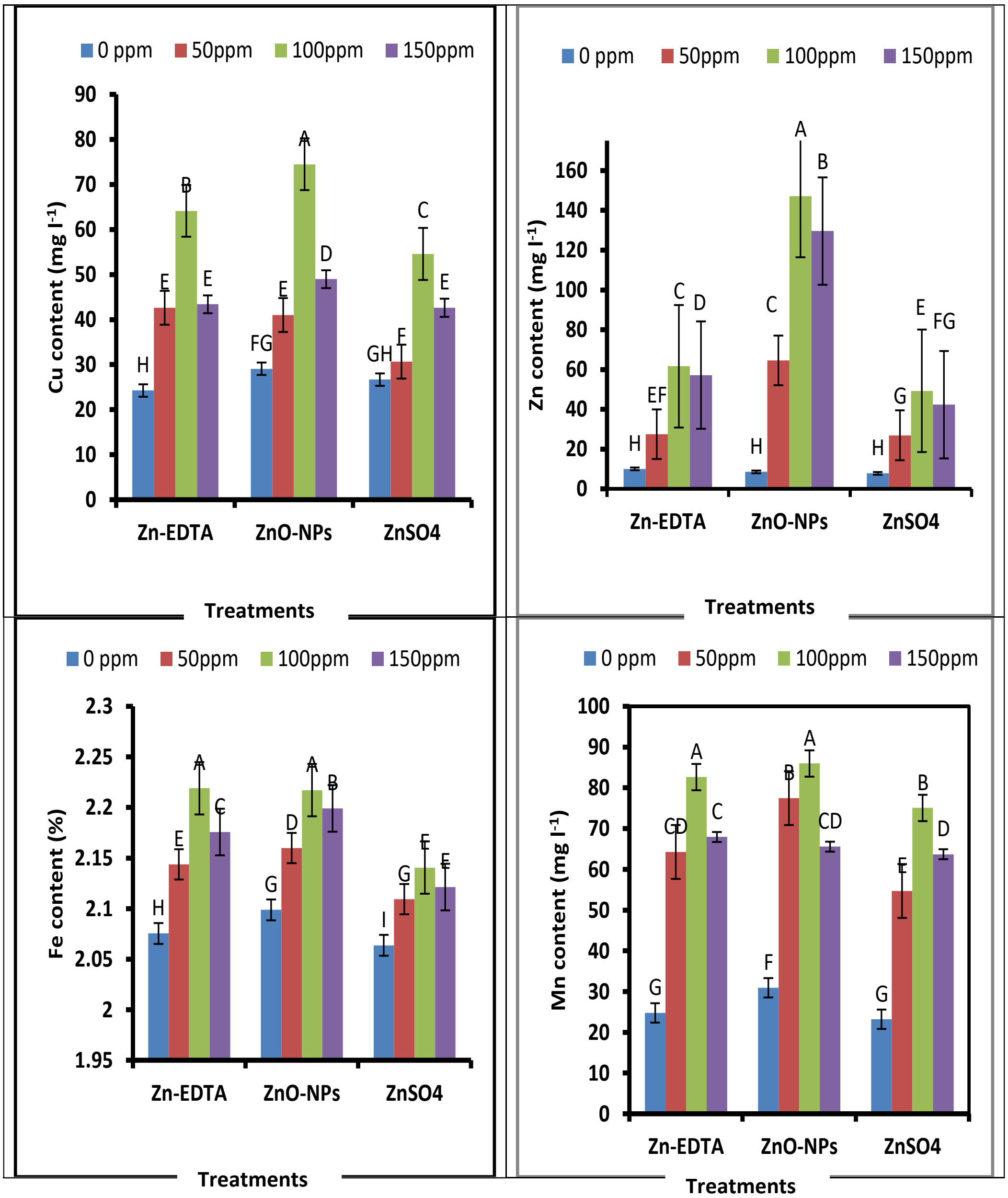
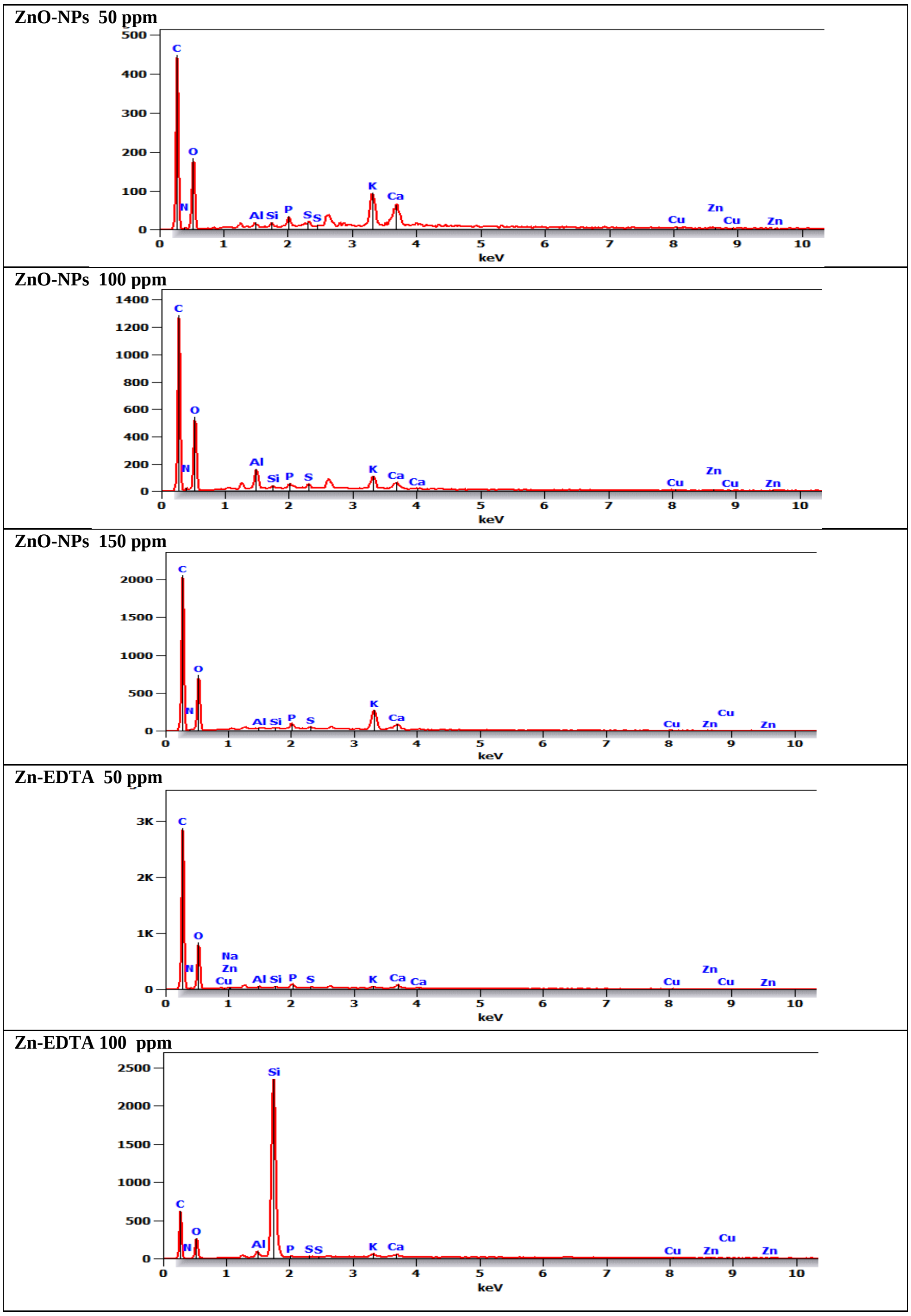


Fig. (7 b). Effect of the studied treatments on micronutrients content in herbs of thyme during cut 2 as an average of both study seasons.


ZnO-NPs 50 ppm
C
O
N
Al Si
P
S S
K
Ca
Cu
Zn
Cu
Zn
keV
ZnO-NPs 100 ppm
C
O
N
Al
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ZnO-NPs 150 ppm
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Zn-EDTA 100 ppm
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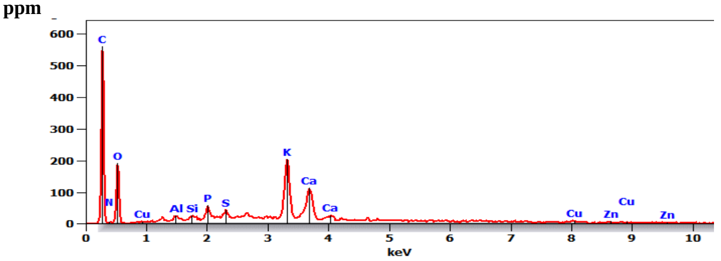
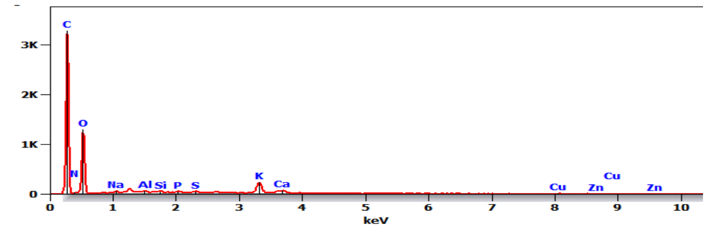
Continued

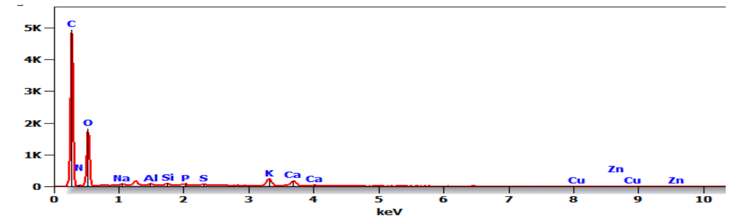
Zn-EDTA 150 ppm



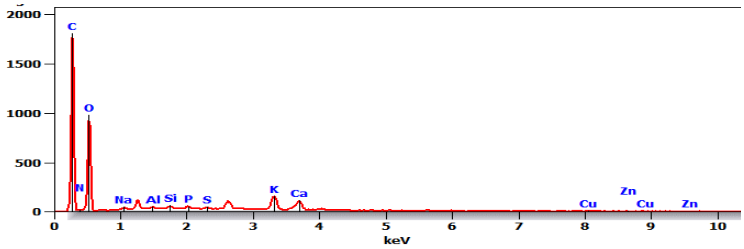
ZnSO$_4$ 50 ppm



ZnSO$_4$ 100 ppm



ZnSO$_4$ 150 ppm



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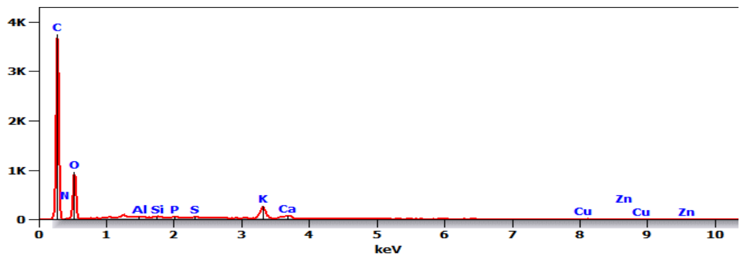

Fig. (8). EDX analysis of thyme plants.

Table (4). Elemental analysis of thyme plant.

Treatments		**Chemical composition (atomic %)**									
		C	**N**	**O**	**Si**	**P**	**S**	**K**	**Ca**	**Cu**	**Zn**
Control	**0**	46.0	12.0	38.8	0.1	0.1	0.1	2.1	0.5	0.0	0.2
Zn-EDTA	**50**	47.0	11.8	38.8	0.2	0.5	0.3	0.4	0.8	0.1	0.3
	100	50.2	23.7	18.7	4.7	0.2	0.1	0.7	0.7	0.1	0.8
	150	36.1	12.8	37.9	0.3	0.9	0.6	5.9	4.2	0.5	0.8
ZnO NPs	**50**	38.4	9.9	42.5	0.3	0.8	0.4	3.6	2.9	0.2	1.1
	100	42.7	9.6	40.0	0.7	0.2	0.2	2.6	2.0	0.3	1.8
	150	41.3	11.5	41.6	0.2	0.5	0.6	1.3	0.9	0.2	1.7
Zn SO_4	**50**	41.9	11.0	41.6	0.1	0.6	0.4	2.9	1.0	0.0	0.3
	100	31.9	9.4	46.5	0.4	0.6	0.4	6.3	3.4	0.4	0.5
	150	38.6	8.9	47.8	0.4	0.3	0.5	1.7	1.3	0.0	0.6

DISCUSSION

From Fig. (2 a), the wide peak in the 3000–3500 cm^{-1} range in the spectra of the neem leaf extract could be attributed to the stretching vibration of the phenol hydroxyl group, which also merged with the amines' N–H due to of various bioactive substances (like proteins or phytochemicals) found in neem (Alzohairy, 2016). At about 2950 cm^{-1}, there were two minor intensity peaks that matched the symmetric and asymmetric C–H members of the aliphatic group. The bending vibration of the OH group is responsible for a big broad peak of about 1716 cm^{-1}. This peak may be caused by moisture that has been chemisorbed or physiosorbed on the surface of the NPs, according to Zheng et al. (2007). The presence of the alcohol's C–O stretching vibration and the ethylene system's C–H vibration is indicated by the bands at 1055 and 617 cm^{-1}, respectively. A comparison between these peaks and the ZnO NPs spectrum indicated a decrease in ZnO NPs peak broadening. In ZnO NPs, distinctive ZnO stretching vibration are caused by the prominent peak at around 450 cm^{-1} (Pirhashemi and Habibi-Yangjeh, 2016).

$ZnSO_4$ was changed into $Zn(OH)_2$ by the bio molecules before the intermediate product was calcined to produce ZnO NPs. The FTIR study have verified that the phenolic chemicals found in flavonoids have a greater similarity for metals. This shows that a phenolic group could produce metal NPs to stabilize a medium and prevent aggregation. This suggests that biological molecules may play two distinct functions in the aqueous solution-mediated synthesis and stabilisation of ZnO NPs. ZnO NPs in this study may have been stabilised and capped in large part by phytochemicals found in neem leaf extract, including phenols, terpenoids, flavonoids, and alkaloids (Azam et al., 2007). As illustrated in Fig. (3), the particle size distributions of ZnO NPs were 64.8 nm.

SEM–EDX ZnO NPs (Figs. 4 and 5) showed that uniformly distributed ZnO NPs were in the agglomerated form and formed larger clusters, the EDX spectrum of produced ZnO NPs reveals their chemical composition and verifies their purity (Moharram et al., 2014). The atomic percentage values found were 27.4% for O and 72.6% for Zn. These results agree with the expected chemical composition of the green synthesis ZnO NPs.

The current study examined the effects of foliar application of zinc fertilizer at three doses (50, 100, and 150 mg l^{-1}) as ZnO NPs, Zn-EDTA, and Zn SO_4 on various growth and biochemical parameters in thyme plants. The most significant values of thyme's total production and vegetative growth were due to application of ZnO-NPs treatment at a rate of 100 ppm.

Plant growth and development require zinc as a vital metal (Pathak et al., 2012). Zinc NPs have different impacts on plants depending on some factors, such as size, shape, concentration of the NPs, exposure time, the plant species, and environmental conditions. According to Kataria et al. (2019), NPs enter plants through their roots or leaves. The cuticle on leaves acts as the first line of barrier of NPs, allowing only particles smaller than 5 nm to pass through stomata. As noted by Achari and Kowshik (2018), most of NPs enter through stomata, which are always larger than 20 nm, and then go through the ground tissues to phloem, which can be transported to other parts and collect in things like stems, leaves, fruit, and grains. In root, NPs pass through several barriers like endodermis, cortex, epidermis, and casparid strip. Apoplastic and symplastic pathways help in the internal transportation of ZnO NPs taken up by the plant roots (Lin and Xing, 2008). NPs can absorb particles 15–20 times more efficiently than their bulk particles (Srivastav et al., 2016). NPs can enter cells through channels, pores, or the process of endocytosis, they can also be transported to other organelles within cells, including vacuoles, plastids, and the nucleus. NPs move across cells via plasmdesmata.

The schematic diagram of the uptake and distribution of ZnO NPs in plant tissues are presented in Fig. (9). The effects of zinc nano fertilizer on photosynthesis, chlorophyll production, mitochondrial respiration, and hormone biosynthesis, including ethylene, gibberellic acid, and jasmonic acid, may be responsible for thyme improved vegetative growth (Hänsch and Mendel, 2009).

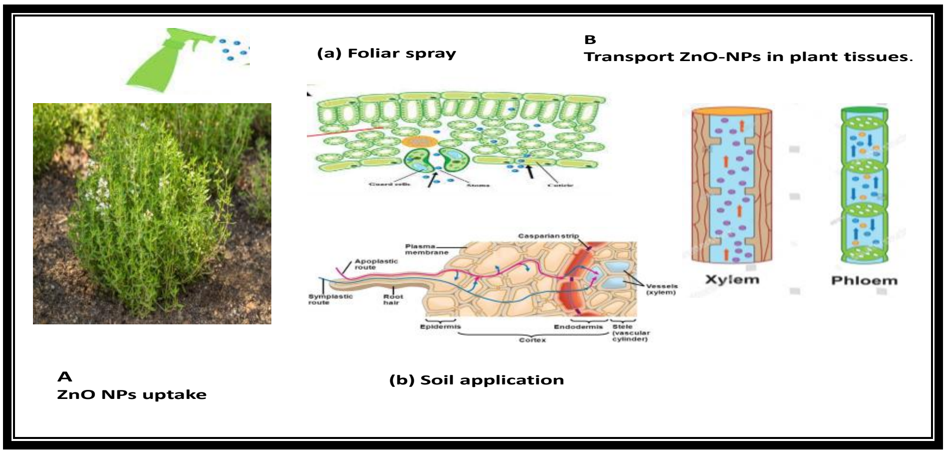

Fig. (9). Schematic of uptake and transport of ZnO NPs in thyme plant.

Higher solubility of metal ions in water may limit their capacity to pass through the lipophilic cuticle, but as these compounds become more mobile and soluble in the cuticle's transport-limiting barrier, so does the permeability of lipophilic organic molecules through the cuticle as a result, ZnO NPs that are less hydrophilic and have a greater capacity to disperse in lipophilic substances may have a higher chance of penetrating the leaf surface and releasing ions through the cuticle than ions that dissolve in water (Da Silva et al., 2006). According to Prasad et al. (2012), the nano size and decreased solubility in water of ZnO NPs in peanuts contribute to their increased bioavailability, these characteristics also explain why ZnO NPs yield more in peanuts than chelated $ZnSO_4$. Gonzalez-Melendi et al. (2008) also reported the presence of NPs in *Cucurbita pepo* extracellular spaces and inside its cells. Along with findings from studies on pearl millet by Tarafdar et al. (2014) and snap bean plants by Nahla et al. (2017), foliar spraying zinc nano-fertilizer has been shown to have favourable impacts on vegetative development indices.

In the present study, the foliar application of zinc fertilizer enhanced the NPK content of thyme plants, The ZnO NPs treatment at a rate of 100 mg l^{-1} produced the highest percentage of thyme's NPK content (Table 3). These findings are consistent with earlier research that discovered that the Kalmi plant's levels of K and P increased because of the nano fertilizer treatments (Adhikari, 2011). In this regard, Yang et al. (2021) found that ZnO NPs at 25 and 100 mg kg^{-1} considerably increased rice aboveground N uptake. This was mostly because rice plants had a higher sink capacity (aboveground dry weight). According to their later research, ZnO NPs at 10 mg l^{-1} increased P-solubilizing enzyme activity, which in turn raised the mung beans uptake of P (Raliya and Tarafdar, 2013). Eissa et al. (2022)

observed that adding NPs to the soil system improved the marjoram plant's total NPK concentration. Foliar spray of nano phosphor zinc fertilizer and inoculation with phosphate solubilizing bacteria treatment gave the maximum significant growth and N, P, K, and Zn content of the barley crop (Ibrahim and Hegab, 2022).

The foliar application of zinc nano fertilizer significantly increased Fe, Mn, Cu and Zn content in thyme plant (Fig. 7 a and b). According to Kouhi et al. (2015), NPs promote the production of organic acids that the root exudates, especially fumaric, lactic, citric, and oxalic acids, which aid in plants absorption of nutrients. This explains the increase in the concentration of these elements. However, in contrast to the low treatment, microelement absorption was reduced by high concentrations of ZnO NPs (150 mg l^{-1}). After being soaked in 100 mg l^{-1} of ZnO NPs, the leaves of *Coriandrum sativum* and *Spinacia oleracea* similarly had higher concentrations of Fe (Alvarez-Parrilla et al., 2011). ZnO NPs at 400 mg kg^{-1} were found to improve Mn uptake in cucumber plants by Zhao et al. (2014). The contents of Fe, Mn, Zn and Cu in the thyme plant were found to be lower than the maximum permissible level reported by Reeves and Baker (2000) and Kabata-Pendias and Pendias (1992).

Considering Fig. (8), it shows that the most common element in all herbs are C, O, followed by N, Si, P, K, Ca, Cu and Zn. Considering that, like all other living things, plants consist of carbon (C), this result is consistent with other studies. One of the primary components of photosynthesis, carbon, is present in the environment as carbon dioxide (CO_2). This enables the plant to absorb CO_2 for healthy growth. Carbon appeared to be the most common element in this investigation (Rusli et al., 2022). Also, the findings indicated that Zn was present in levels that were distributed with Cu. Zhao et al. (2014) discovered that because Zn^{+2} and Cu^{+2} were competing for the same transporter, ZnO NPs at 400–800 mg kg^{-1} reduced Cu absorption.

CONCLUSIONS

In the current work, zinc NPs were green synthesised sustainably to determine whether they impact thyme productivity more than zinc sources. The experiments' findings investigated that application of zinc nano source plays a significant position in increasing productivity, improved the nutritional status by thyme plant. In this study, the most effective treatment Zn NPs at 100 mg l^{-1} gave the significantly maximum values of yield components by 42.97, 9.348 and 2.928 for plant height (cm), fresh and dry weight (ton/faddan), respectively in the first cut. However, compared to the first cut, the second cut had far higher yield component increases. the nutritional content followed the same pattern of the nutritional content. Also, the resultes of the elemental composition of thyme using SEM-EDX analysis attributed to the application of zinc fertilizer. The zinc NPs treatment at rate of 100 ppm gave the maximum value of the chemical composition (atomic %) in plant tissue. Also, it can recommend that, thyme plants should be treated with nano ZnO at rate of 100 ppm to give the highest productivity of thyme herb under sandy soil and drip irrigation system of Baloza region conditions.

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REFERENCES

A.O.A.C. (1990). In: ‘Official Methods of Analysis’. Association of Officinal Analytical Chemists. 16th Ed., Washington. D.C., USA, 17 p.

Achari , A.G. and M. Kowshik (2018). Recent developments hon nanotechnology in agriculture: plant mineral nutrition, health, and interactions with soil microflora. Journal of Agricultural and Food Chemistry, 66 (33): 8647–8661.

Adhikari, T. (2011). Nano-particle Research in Soil Science: Micronutrients. In: ‘National Symposium on Applications of Clay Science: Agriculture, Environment and Industry’. NBSS & LUP, Nagpur, pp. 74-75.

Alloway, B.; Graham, R., and S. Stacey (2008). In: ‘Micronutrient Deficiencies in Global Crop Production’. Alloway B. (Ed.), Springer, Netherlands, Chapter 3, pp. 63–92.

Alzohairy, M.A. (2016). Therapeutics role of *Azadirachta indica* (Neem) and their active constituents in diseases prevention and treatment. Evidence-Based Complementary and Alternative Medicine, Article ID 7382506. DOI: 10.1155/2016/7382506

Analytical Software (2008). Statistix Version 9, Analytical Software, Tallahassee, Florida, USA.

Alvarez-Parrilla, E., L.A. de La Rosa, R. Amarowicz and F. Shahidi (2011). Antioxidant activity of fresh and processed jalapeno and serrano peppers. Journal of Agricultural and Food Chemistry, 59: 163-173.

Azam, A., A.S. Ahmed, M. Oves, M.S. Khan, S.S. Habib and A. Memic (2007). Antimicrobial activity of metal oxide nanoparticles against gram-positive and gram-negative bacteria: A comparative study. International Journal of Nanomedicine, 2012 (7): 6003–6009.

Biswas, A.K. (2015). Characterization of zinc oxide nano particles and its effect on growth of maize (*Zea mays* L.) Plant. Journal of Plant Nutrition, 38: 1505-1515.

Bozin, B., N. Mimica–Dukic, N. Simin and G. Anackov (2006). Characterization of the volatile composition of essential oils of some Lamiaceae species on the antimicrobial and antioxidant activities of the entire oils. Journal of Agricultural and Food Chemistry, 54: 1822-1828.

Carlen, C., M. Schaller, C.A. Carron, J.F. Vouillamoz and C.A. Baroffio (2010). The new *Thymus vulgaris* L. hybrid cultivar (Varico 3) compared to five established cultivars from Germany, France, and Switzerland. Acta Horticulturae, 860: 161-166.

Caulfield, L.E. and R.E. Black (2004). In: 'Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attribution to Selected Major Risk Factors'. World Health Organization.

Chapman, H.D. and P.E. Pratt (1978). In: 'Methods of Analysis for Soil and Waters'. University of California. Dept. Agric. Sci., USA, pp. 1-309.

Cottenie, A., M. Verlso, L. Kilkens, G. Velghe and R. Camerlynck (1984). In: 'Chemical Analysis of Plants and Soils'. Lab. Agroch. State Univ. Gent, Belgium.

Da Silva, L.C., M.A. Oliva, A.A. Azevedo and M.J. De Araujo (2006). Response of resting plant species to pollution from an iron palettization factory. Water, Air and Soil Pollution, 175: 241-256.

Davar, F., A. Majedi and A. Mirzaei (2015). Green synthesis of ZnO nanoparticles and its application in the degradation of some dyes. Journal of the American Ceramic Society, 98 (6): 1739-1746.

Eissa, D., H.R. Hegab, A. AbouShady and H.Y. Kotp (2022). Green synthesis of ZnO NPs, MgO and SiO_2 nanoparticles and its effect on irrigation water, soil properties, and *Origanum majorana* productivity. Scientific Reports, 12: 5780.

El-Ramady, H., N. Abdalla, H. Elbasiouny, F. Elbehiry, T. Elsakhawy et al. (2021). Nano-biofortification of different crops to immune against COVID-19: A review. Ecotoxicology and Environmental Safety, 222: 112500.

Figueiredo, A.C., J.G. Barroso, L.G. Pedro and J.J.C. Scheffer (2008). Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour and Fragrance Journal, 23: 213-226.

Giacalone, A., L. Marin, M. Febbi and M.R. Tovani-Palone (2021). Current evidence on vitamin C, D, and zinc supplementation for COVID-19 prevention and/or treatment. Electronic Journal of General Medicine, 18 (5): em311.

Gonzalez-Melendi, P., R. Fernandez Pacheco, M.J. Coronado, E. Corredor, P.S. Testillano et al. (2008). Nanoparticles as smart treatment delivery systems in plants: Assessment of different techniques of microscopy for their visualization in plant tissues. Annals of Botany, 101: 187- 195.

Hamdy, M.N., Z.E. El Sayed, V.X. Allam, V. Bashir, V.X. Taha et al. (2023). Nanotechnology and its prospective role in using bioactive compounds to fight covid 19 infection in this era. Egyptian Journal of Desert Research, 73 (2): 513-549.

Hänsch, R. and R. Mendel (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Current Opinion in Plant Biology, 12 (3): 259–266.

Ibrahim, G.A.Z. and R.H. Hegab (2022). Improing yield of barley using bio and nano fertilizers under saline conditions. Egypt. J. Soil Sci., 62 (1): 41-53.

Jackson, S.A.L. and R.K.M. Hay (1995). Characteristics of varieties of thyme (*Thymus vulgaris* L.) for use in the UK: oil content, composition, and related characters. Annals of Horticulture, 69: 275-281.

Kabata-Pendias, A. and H. Pendias (1992). Trace elements in soils and plants. 2nd Ed. USA: CRC Press, Inc. Boca Raton, Florida.

Kataria, S., M. Jain, A. Rastogi, M. Zivcak, M. Brestic, S. Liu and D.K. Tripathi (2019). Role of Nanoparticles on Hotosynthesis: Avenues and Applications. In: ‘Nanomaterials in Plants, Algae and Microorganisms’. Academic Press, pp. 103–127.

Kouhi, S.M.M., M. Lahouti, A. Ganjeali, M.H. Entezari (2015). Comparative effects of ZnO nanoparticles, ZnO bulk particles, and Zn^{2+} on *Brassica napus* after long-term exposure: changes in growth, biochemical compounds, antioxidant enzyme activities, and Zn bioaccumulation. Water, Air and Soil Pollution, 226: 1–11.

Lee, S.J., K. Umano, T. Shibamoto and K.G. Lee (2004). Identification of volatile components in basil *(Ocinum basilicum* L.) and thyme leaves *(Thymus vulgaris* L.) and their antioxidant properties. Food Chemistry, 91: 131–137.

Lee, J., M. Wissuwa and O.B. Zamora (2017). Biochemical indicators of root damage in rice (*Oryza Sativa* L.) genotypes under zinc deficiency stress. Journal of Plant Research,130: 1071-1077.

Letchamo, W., H.L. Xu and A. Gosselin (1995). Variations in photosynthesis and essential oil in thyme. Journal of Plant Physiology, 147: 29-37.

Lin, D. and B. Xing (2008). Root uptake and phytotoxicity of ZnO nanoparticles. Environmental Science and Technology, 42: 5580–5585.

Mahajan, P., S.K. Dhoke and A.S. Khanna (2011). Effect of nano-zno particle suspension on growth of mung (*Vigna radiata)* and gram

(*Cicer arietinum*) seedlings using plant agar method. Journal of Nanotechnology, Article ID 696535. DOI:10.1155/2011/696535

Moharram, A.H., S.A. Mansour, M.A. Hussein and M. Rashad (2014). Direct precipitation and characterization of zno nanoparticles. Journal of Nanomaterials, 2014 (7): 1–5.

Nahla, M., M.S. Abdelhakeem and A. Mohamed (2017). Zinc foliar spray on snap beans using nano-Zn with N-soil application using mineral, organic and biofertilizer. Middle East Journal of Agriculture Research, 6 (4): 1301–1312.

Nejad, E.S., J. Haddian, M.H. Mirjalilli, A. Sonboli and M. Yousefzadi (2008). Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phenological stages. Food Chemistry, 110: 927–931.

Pathak, G.C., B. Gupta and N. Pandey (2012). Improving reproductive efficiency of chickpea by foliar application of zinc. Brazilian Journal of Plant Physiology, 24: 173–180.

Pirhashemi, M. and A. Habibi-Yangjeh (2016). Ultrasonic-assisted preparation of novel ternary ZnO NPs/Ag_3VO_4/Ag_2CrO_4 nanocomposites and their enhanced visible-light activities in degradation of different pollutants. Solid State Sciences, 55: 58–68.

Prasad, A.S. (2008). Zinc in human health: Effect of zinc on immune cells. Molecular Medicine, 14 (5-6): 353-357.

Prasad, T.N.V.K.V., P.Y. Sudhakar, P. Sreenivasulu, V. Latha, K. Munaswamy et al. (2012). Effect of nanoscale zinc oxide particles on the germination, growth, and yield of peanut. Journal of Plant Nutrition, 35: 905- 927.

Raliya, R. and J.C. Tarafdar (2013). ZnO NPs nanoparticle biosynthesis and its effect on phosphorous-mobilizing enzyme secretion and gum contents in cluster bean (*Cyamopsis tetragonoloba* L.). Agricultural Research, 2: 48–57.

Reeves, R.D. and A.J.M. Baker (2000). Metal Accumulating. In: ‘Raskin, I.B. and D. Ensley (Eds.), Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment’. Wiley, New York, pp: 193 - 229.

Reynolds, G.H. (2002). Forward to the future nanotechnology and regulatory policy. Pacific Research institute, 24: 1-23.

Rusli, N.S., Z. Embong, N.Z.N. Hashim, N. Muhammad, M.N. Sunar et al. (2022). Assessment of trace element (Mg, Al, K and Mo) in 14 types of raw herbs and spices using SEM-EDX analysis. ASM Science Journal, 17. DOI: 10.32802/asmscj.2022.1039

Srivastav, A.K., M. Kumar, N.G. Ansari, A.K. Jain, J. Shankar et al. (2016) A comprehensive toxicity study of zinc oxide nanoparticles versus their bulk in wistar rats: toxicity study of zinc oxide nanoparticles. Human and Experimental Toxicology, 35: 1286–1304.

Tabrez, S., A.U. Khan, M. Hoque, M. Suhail, M.I. Khan and T.A. Zughaibi (2022). Biosynthesis of ZnO NPs from pumpkin seeds' extract and elucidation of its anticancer potential against breast cancer Nanotechnology Reviews, 11 (1): 2714–2725.

Tarafdar, J., R. Ramesh, M. Himanshu and R. Indira (2014). Development of zinc nano fertilizer to enhance crop production in pearl millet (*Pennisetum americanum*). Agricultural Research, 3(3). DOI: 10.1007/s40003-014-0113-y.

Thi, T.U.D., T.T. Nguyen, Y.D. Thi, K.H.T. Thi, B.T. Phan and K.N. Pham (2020). Green synthesis of ZnO nanoparticles using orange fruit peel extract for antibacterial activities. RSC Advances, 10 (40): 23899-23907.

Tulchinsky, T.H. (2010). Micronutrient deficiency conditions: global health issues, Public Health Reviews., 32: 243–255.

Wang, X.P., Q.Q. Li and Z.M. Pei (2018). Effects of zinc oxide nanoparticles on the growth, photosynthetic traits, and antioxidative enzymes in tomato plants. Biological Plantarum, 2018: 1-8

Welch, R.M. and R.D. Graham (2004). Breeding for micronutrients in staple. Journal of Experimental Botany, 55 (396): 353–364.

Yan, S., H. Zhang and Y. Huang (2016). Single-wall and multi-wall carbon nanotubes promote rice root growth by eliciting the similar molecular pathways and epigenetic regulation. IET Nanobiotechnology, 10: 222-229.

Yang, G.Y., H.Y. Yuan, H.T. Ji, H.J. Liu and Z. Guo (2021). Effect of ZnO NPs nanoparticles on the productivity, Zn biofortification, and nutritional quality of rice in a life cycle study. Plant Physiology and Biochemistry, 163: 87-94.

Zhao, F.J. and S.P. McGrath (2009) Biofortification and phytoremediation. Current Opinion in Plant Biology, 12 (3): 373-80.

Zhao, L., Y. Sun, J.A. Hernandez-Viezcas, A.D. Servin, J. Hong, J. et al. (2014). Influence of CeO_2 and ZnO Nnanoparticles on cucumber physiological markers and bioaccumulation of Ce and Zn: A life cycle study. Journal of Agricultural and Food Chemistry, 61 (49): 11945-11951.

Zheng, Y., C. Chen, Y. Zhan, X. Lin, Q. Zheng et al. (2007). Luminescence and photocatalytic activity of ZnO NPs nanocrystals: Correlation between structure and property. Inorganic Chemistry, 46: 6675–6682.

تأثير الرش الورقي بالزنك المعدني والمخلبي والنانوي على نمو والمحتوى البيوكيميائي وانتاجية نبات الزعتر بمنطقة بالوظة - شمال سيناء

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الزنك عنصر حيوي وضروري لنمو النباتات. ويعتبر نقصه مشكلة على مستوى العالم، وخاصة في المناطق الزراعية، مما يؤدي إلى انخفاض المحصول وتناقص الجودة الغذائية في المنتجات الزراعية. تم إجراء هذا البحث في محطة بحوث بالوظة بمركز بحوث الصحراء، شمال سيناء، مصر. خلال موسم ٢٠٢١-٢٠٢٢، تهدف التجربة إلى تقييم تأثير ثلاثة مصادر مختلفة لسماد الزنك وهي $ZnSO_4$ وZn-EDTA وZnO NPs عند تركيزات (صفر، ٥٠، ١٠٠، ١٥٠ جزء في المليون) على النمو والمحصول والمحتوى المعدني من العناصر لنبات الزعتر. وكذلك التركيب العنصري للزعتر باستخدام التحليل النوعي للأشعة السينية المشتتة للطاقة (EDX). صممت التجربة بتصميم القطع المنشقة مرة واحدة بثلاث مكررات. أظهرت النتائج أن أفضل المعاملات كانت Zn NPs بتركيز ١٠٠ مجم لكل لتر التي أعطت أعلى نمو وانتاجية لنبات الزعتر ومحتوى النبات من العناصر الكبرى والصغرى. أيضًا، تم تحديد التركيب العنصري في أنسجة النبات للعناصر الكبرى والصغرى باستخدام نظام تحليل الأشعة السينية من dispersive الطاقة (EDX) المرفق بالمجهر الإلكتروني الماسح (SEM-EDX) وأظهرت النتائج أن العينة تحتوي على تركيز كبير من الكربون (C) والأكسجين (O)، بينما وجدالنتروجين (N) بتركيز متوسط. أما عناصر السليكون (Si)، الفوسفور(P)، بوتاسيوم (K)، الكالسيوم (Ca)، النحاس (Cu) والزنك (Zn) فقد وجدت بتركيزات ضئيلة.