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COMPARISON BETWEEN EFFECT OF COMMERCIAL AND NANO NPK IN PRESENCE OF NANO ZEOLITE ON SAGE PLANT YIELD AND COMPONENTS UNDER DROUGHT STRESS

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ABSTRACT: Lucrative approaching of nanotechnology and its application on plant is gradually growing recently. Based on this fact an investigation was done during two successive seasons 2018 and 2019 to scrutinize the consequence of nano macro elements application represented in NPK individually or in combination and nano zeolite with and without loaded nitrogen on a sage plant grown under drought stress in comparison with commercial NPK fertilizers at a newly reclaimed area of the desert. The outcome results revealed that nano-zeolite loaded nitrogen, as well as nano-NPK mixture, donated outstanding results with reference to vegetative growth (plant height, number of branches, herb fresh and dry weights, leaf area, yield fresh weight, health index, and oil yield) beside photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, water use efficiency, relative water content, and chemical composition symbolized in (plant pigments, total carbohydrates, total phenols, tannins, total flavonoids, oil constituents, macro, and micro-elements) alongside indigenous hormones characterized in (gibberellic acid GA3 and abscisic acid ABA), antioxidant enzymes (peroxidase and superoxide dismutase) in contrast to the commercial dose of chemical fertilizers NPK (as control) under the same conditions. Moreover, leaf anatomical structure supported the acquired vegetative growth parameters and chemical analyses results. The outcomes of the current study gave emphasis to global warning about pollution resulted from chemical fertilizers particularly in newly reclaimed areas and safety production of medicinal and aromatic plants.

Key words: *Salvia officinalis* L., drought stress, nano elements, nano-zeolite, npk fertilizers, leaf anatomy.

INTRODUCTION

The global demand for medicinal and aromatic plants increase to match the global pharmaceutical and cosmetics industries enlarge, and there is a need to reduce the negative environmental impacts of chemical fertilizers since efficiency of NPK fertilizers use is in the range of 30-35%, 18-20%, and 35-40%, respectively, leaving a large part of added fertilizer to accumulate in soil **(Mir *et al.*, 2018)**, that means a great part of chemical fertilizers are not absorbed and pollute the agriculture soil and underground water. In light of the climatic changes that affect the world, especially the high-temperature regions, the agriculture field also need solutions that increase the plants ability to absorb nutrients under the different stress conditions.

Increasing global warming may be caused gradually increase in the intensity of drought in the Mediterranean and Africa regions and increased risk of agricultural drought **(IPCC, 2013)**. It was mentioned that the plants tolerant to drought stress show different responses, including increment root/ shoot ratio, growth reduction, leaf anatomy change, minimize total leaf area to secure photosynthesis and reduce

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water loss **(Toscano *et al.*, 2019)**. The decrease of photosynthetic activity is linked to the system of stomatal conductance. So, plants close stomata as a first response to drought for water potential adjustment resulting in a reduction in the photosynthetic system, which in turn leads to an adverse effect on plant growth and production **(Shi *et al.*, 2019)**.

Natural zeolite overcomes the effects of drought in arid regions by acting as a water distributor over the soil, which in turn affecting water conduction in plants **(Ghazavi, 2015)**. Also, **Rastogi *et al.* (2019)** mentioned that nano zeolites may be effectively used in agriculture to facilitate water filtration and preservation in the soil due to their porous and capillary properties which act as a slow-release source for water. Slow-release of nutrients in the environments could be possible by application of natural materials as zeolites. Zeolite ensures a slow-release of nitrogen from urea **(Hidayat *et al.*, 2015)**.

Therefore, in the last few years, there is an emphasis on the synthesis of nanoparticles fertilizers. Surface coatings of nano-materials on fertilizer particles hold the material more strongly due to higher surface tension than the conventional surfaces and thus help in controlled release **(Manjunatha *et al.*, 2016)**. Recent researches stated that the use of nano fertilizers increases nutrients effectiveness and overcome soil perniciousness resulted from overdosage of chemical fertilizers. Hence, nanotechnology is a promising process for sustainable agriculture, especially in developing countries **(Tulasi Guru *et al.*, 2015)**. Moreover, it was mentioned that a nano fertilizer carries nutrients to plants in one of three ways. The nutrients can be encapsulated inside nanomaterial such as nanotubes or nano-porous materials, coated with a thin protective polymer film or delivered as particles or emulsions of nano-scale dimensions. Owing to a high surface area to volume ratio, the effectiveness of nano fertilizers may surpass the most polymer-coated traditional fertilizers **(De Rosa *et al.*, 2010)**.

Salvia officinalis L. (Sage) plant is an evergreen subshrub native of the Mediterranean region. Sage is one of the most important members of the family Lamiaceae. Its essential oil is used in industry as food preservation. Sage has multiple medicinal benefits; it had the ability to improve liver function, appetite, digestion, and mental capacity **(Jakovljevi *et al.*, 2019)**. Sage essential oil is a complex mixture of compounds including monoterpenes, sesquiterpenes, and diterpenes. Borneol, cineole, camphor, and thujone, are the most identified components from Sage plant. Caffeic acid and 3-Caffeoylquinic acid have been isolated from the sage extract which resulted in antioxidant effect. *S. officinalis* has a plentiful amount of rosmarinic acid and ellagic acid, followed by rutin, chlorogenic acid, and quercetin as flavonoids. The environmental conditions such as climate, water availability has a great effect on the chemical composition of the sage plant **(Ghorbani and Esmaeilizadeh, 2017)**.

The purpose of the present research was to examine the effect of nano macro elements represented in NPK individual or in combination beside nano zeolite with and without loaded nitrogen on a sage plant grown under drought stress in comparison with commercial NPK fertilizers at newly reclaimed area of the desert.

MATERIALS AND METHODS

The present investigation was carried out in the open nursery at a newly reclaimed area of desert located in Wadi El-Notron, Beheira Governorate (Longitude 28°54’ E, Latitude 28°20 N and Altitude 130 m) in Egypt, during two successive seasons (2018 and 2019). Physical and chemical analyses of the reclaimed soil were performed according to **Richards (1954) and Jackson (1973)** as shown in Table 1 at Soil, Water and Environment Research Institute, Agriculture Research Center (ARC).

Plant Material, Transplant and Harvest Dates

The seedlings of sage plant about 13 to 15 cm tall with intact roots were obtained from experimental farm of Faculty of Pharmacy, Cairo University, and planted on 21st February 2018 and 2019 in the experimental field as open area with a distance of 50 cm between rows, and 40 cm spacing between plants in plots with 3×5 m. Irrigation was done according to the plant needs for two months then drip irrigation with intervals 12 days and the flow rate was 4 l hr.$^{-1}$. Harvest was done on September 21st in both seasons.

Table 1. Physical and chemical properties of experimental site

Physical property		Chemical property	
Particle size distribution (%)		Electrical conductivity (EC) (dS/m)	1.59
Coarse sand 2000–200 μ	79.82	pH (1:2.5) soil : water suspension	7.57
Fine sand 200–20 μ	13.12	Soluble cations (meq/l):	
Silt 20–2 μ	4.05	Ca^{2+}	5.20
Clay < 2 μ	3.01	Mg^{2+}	4.18
Bulk density (g/cm^3)	1.50	K^+	2.38
Total porosity (%)	53.0	Na^+	5.18
Pore size distribution as (%) of total porosity		Soluble anions (meq/l):	
Macro (drainable) pores (> 28.8 μ)	82.88	CO_3^{2-}	0
Micro pores (< 28.8 μ)	17.02	HCO^{3-}	1.6
Water Holding Capacity (WHC)	19.83	Cl^-	3.5
Field capacity (FC)	8.51	SO_4^{2-}	11.70
Wilting percentage (WP)	4.09	Total $CaCO_3$ (%)	0.2
Available moisture (FC-WP)	4.54	Organic matter (%)	0.19
Hydraulic conductivity (cm/h)	6.30		

Land Preparation

Before planting, a half dose of organic matter (Table 2) was added to all plots (full dose as recommended 5 ton per faddan, the soil was first mechanically ploughed and planked twice till the soil surface has been settled, then plots established (fad. = 4200 m^2).

Fertilizers Added

The recommended doses of chemical fertilizers were added according to the Ministry of Agriculture and Land Reclamation. Calcium superphosphate (15.5% P_2O_5) at a rate of 150 kg/fad., was added during soil preparation. Ammonium sulphate (20.5% N) at a rate of 150 kg/fad., and potassium sulphate (48% K_2O) at a rate of 50 kg/fad., were divided into three doses at 45-days intervals between doses; the first was added after four weeks of transplanting.

Zeolite Loaded Nitrogen

Nano zeolite was synthesized (AM) according to **Hassan and Mahmoud (2015)** then loaded nitrogen (Table 3) by soaking in 1M ammonium sulphate solution for 5 days **(Junxi *et al.*, 2013)** at 25°C and aeration condition (Fig. 1). The total N content was analyzed according to **Helrich (1990)**. Both zeolite only and zeolite loaded nitrogen were applied with dripping irrigation (30 gram per litter) monthly for 4 months continuously from the planting date.

Synthesis of Nitrogen, Phosphorus and Potassium Nanoparticles and Application

Chitosan (MW 71.3 kDa, degree of deacetylation (89%) was purchased from Aldrich (Germany). All reagents were of analytical grade from precursor potassium persulfate ($K_2S_2O_8$) and methacrylic acids were purchased from Aldrich (Germany). Calcium phosphate (Ca $(H_2PO_4)_2{\cdot}H_2O$), salt NH_4NO_3, urea (CO (NH_2)2) and potassium chloride KCl were purchased from Sigma Chemical Co. (St. Louis, USA).

Nanoparticles were obtained by (Top to bottom molecular chemical approach method under pressure 2 Mpa.) polymerizing methacrylic acid in chitosan solution as carrier coated in buffer solution for 5 hours at room temperature in two-steps processes. In the first step, 0.23 g chitosan was dissolved in methacrylic acid aqueous

Table 2. Chemical composition of compost

Chemical analysis																
pH (1:5)	EC (1: 5 extract) dS/m	Organic matter (%)	Organic-C (%)	Total content of bacteria	Phosphate dissolving bacteria	Humidity (%)	Weed seeds	Total-N (%)	Total-K (%)	Total-P (%)	C/N ratio	Fe ppm	Mn ppm	Cu ppm	Zn ppm
7.5	3.1	44.3	25.5	2.5 x 10^7	2.5 x 10^4	20	0	1.82	1.25	1.06	18.1	784.12	96.31	31.05	251.23

Table 3. Chemical composition of nano zeolite loaded by N

Chemical composition (%)	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	FeO	MnO	MgO	CaO	Na_2O	K_2O	SrO	P_2O_3	N
	45.50	2.81	13.30	5.40	8.31	0.51	6.30	9.52	2.83	0.87	0.22	0.67	2.70
Trace elements (ppm)	Ba	Co	Cr	Se	Cu	Zn	Zr	Nb	Ni	Rb	Y	-	-
	10	1.2	35	0.8	19	64	257	13	55	15	22	-	-

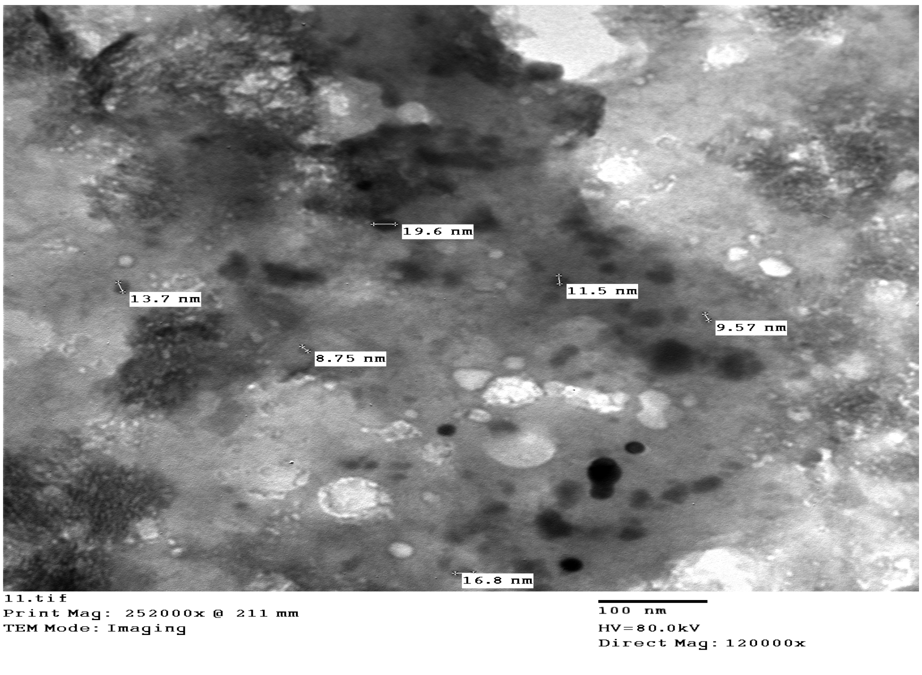

Fig. 1. Nano-zeolite loaded nitrogen

solution (0.5%, *V/V*) for 18 hr., under magnetic stirring. In the second step, with continued stirring, 0.2 mmol of $K_2S_2O_8$ was added to the solution, until the solution became clear. The polymerization was subsequently carried out at 75°C under magnetic stirring for 4 hr., which leads to the formation of nanoparticle solution, then centrifuged at 500 rpm for 30 minutes, which was thereafter cooled in an ice bath. The sources of N, P and K used were used separately. The loading of N fertilizers in Chitosan nanoparticles was obtained by dissolving of 2M N into 100 ml of chitosan nanoparticle solution under magnetic stirring for 8 hr., at 25°C, Subsequently dried at 50 C for 72 hr. The following concentrations: i) 1000 ppm of N; ii) 1000 ppm of P and iii) 1000 ppm of K were finally obtained in each solution. The resulting solutions had a pH of 5.5.

The particles were uncontrolled in shape with a size range of (6.25 to 6.57 nm) for nitrogen (Fig. 2), (5.30 to 12.3 nm) for Phosphorus (Fig. 3), (7.99 to 15.3 nm) for potassium (Fig. 4) and (44.2 to 54.3 nm) for mixed NPK, Fig. 5 with crystal structure and 98.5% purity.

The morphology and size of the nanoparticles were investigated using a JEOL 1010 transmission electron microscope at 80 kV (JEOL, Japan). One drop of the nanoparticle solution was spread onto a carbon-coated copper grid and was subsequently dried at room temperature for transmission electron microscopy (TEM) analysis. The sizes of the nanoparticles were determined directly from the figure using an Image-Pro Plus 4.5 software. The value is the average size of three parallels.

Nitrogen, phosphorus, and potassium (Nano NPK) were applied either individually or mixed as foliar monthly for 4 months continuously from the planting date.

During the two successive seasons the treatments were as follows:

- NPK fertilizers (recommended dose) as control (T1)
- Nano-nitrogen (T2)
- Nano-phosphorus (T3)
- Nano-potassium (T4)
- Nano-NPK (T5)
- Nano zeolite (T6)
- Nano zeolite loaded nitrogen (T7)

Data Recorded

The following data were recorded:

Vegetative growth parameters

- Plant height (cm)
- Number of branches per plant
- Herb fresh weight (g/plant)
- Herb dry weight (g/plant)
- Leaf area (cm^2)
- Leaf/stem ratio fresh (g)
- Yield fresh weight (ton/hectare)
- Health index
- Oil yield (litter/hectare)

Chemical Analyses

Photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and water use efficiency

Five different leaves per treatment were used to determine photosynthetic rate on an area basis (μmol CO_2 $m^{-2}s^{-1}$), leaf stomatal conductance (mol H_2O $m^{-2}s^{-1}$), intercellular CO_2 concentration and water use efficiency using a LICOR 6400 (Lincoln, Nebraska, USA).

Relative water content (RWC)

RWC were determined by the method of **Weatherly (1951).**

Nitrogen concentrations

In the dried leaves, the total nitrogen content was measured according to **Helrich (1990)**.

Phosphorus concentrations:

Phosphorus was determined calorimetrically in leaves according to **Jackson (1973)**.

Potassium and sodium concentrations

The flame photometer apparatus (CORNING M 410, Germany) was used to determine potassium and sodium concentrations in dried leaves.

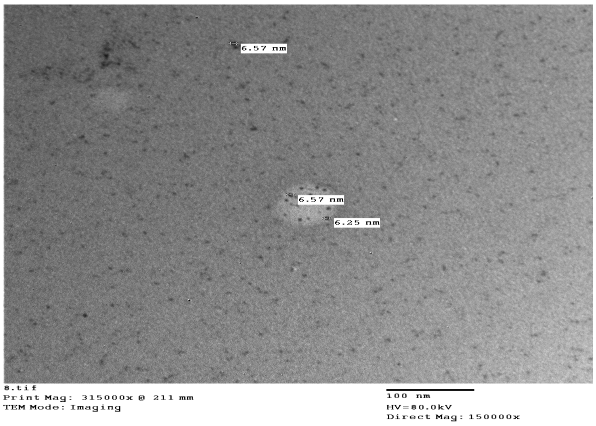

Fig. 2. Nano-nitrogen

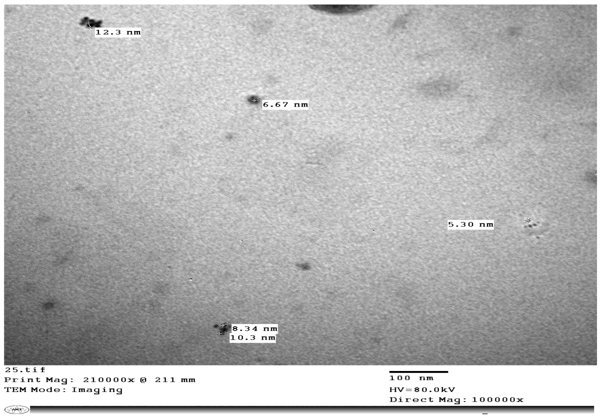

Fig. 3. Nano-phosphorus

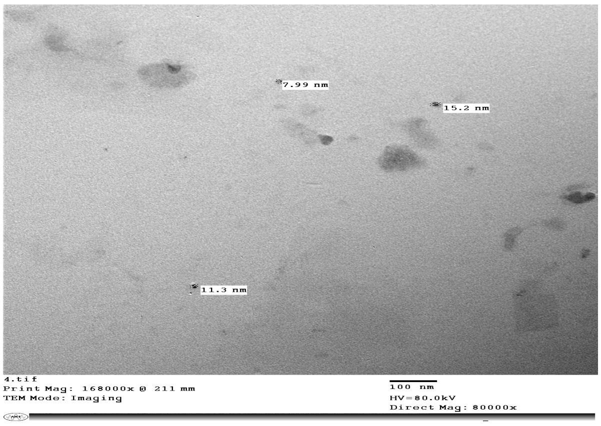

Fig. 4. Nano-potassium

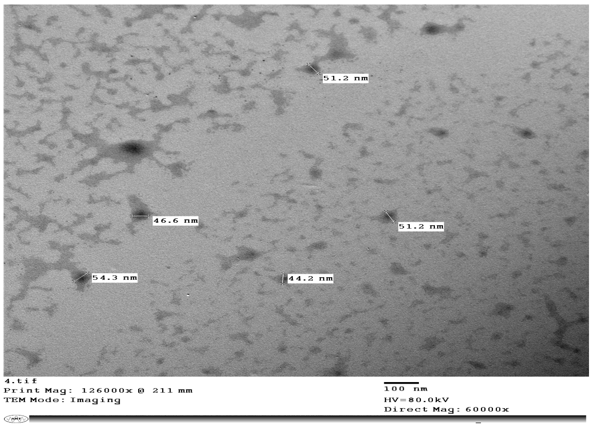

Fig. 5. Nano-NPK

Calcium, magnesium, zinc, iron, manganese and boron concentrations

Ca, Mg, Zn, Fe, Mn and B were determined using Inductively Coupled Plasma Emission Spectrometer "ICP" The Agilent 720/730 series US).

Total chlorophylls contents

Total chlorophylls contents were analyzed as reported by **Moran (1982)**.

Total phenols

Total phenolic contents of the leaves were spectrophotometrically determined according to **Singleton and Rossi (1965)**.

Total carbohydrates content

Total carbohydrates concentrations (%) in leaves were determined according to **Helrich (1990)**.

Tannins content

Tannins content was determined using Folin-Ciocalteu reagent method as described by **Chahardehi *et al.* (2009)**.

Total flavonoids concentrations

Total flavonoids were determined in leaves according to **Meda *et al.* (2005)**.

Endogenous phytohormones

Freeze-dried plant leaves (equivalent 5 g FW) were ground to a fine powder. The determination of gibberellic acid (GA3) and abscisic acid (ABA)were performed according to **Fales *et al.* (1973)**.

The quantification of the endogenous phytohormones was carried out with Ati-Unicumgas-liquid chromatography, 610 Series, equipped with flame ionization detector according to the method described by **Vogel**

(1975). Enzyme activity was determined using the method described by **Macheix and Quessada (1984)** for Peroxidase and **Dhindsa *et al.* (1981)** for Superoxide dismutases.

Volatile oil in leaves

The fresh herb of *Salvia officinalis* was hydro-distilled for 3 hours to get yellowish volatile oil using Clevenger apparatus according to **Guenther (1961)**.

Gas Chromatography

The oil was analysed using DsChrom 6200 Gas Chromatography with a flame ionization detector (FID) for separation of volatile oil constituents. The apparatus was fitted with capillary column (BPX-5, 5%phenyl (equiv.) polysillphenylene-siloxane 30 m × 0.25 mm ID × 0.25 µm film). The column temperature was programmed from 70°C to 200°C at a rate of 10°C/min. The temperature of the detector and injector were 300°C and 250°C, respectively. The quantitative determination was performed using the methods described by **Tatjana *et al.* (2009).**

Anatomical Study

Specimens, 1 cm long were taken from the middle portion of Sage leaf lamina and petiole on the 4th node from the top of the main stem including midrib after 5 months of planting through the second season (2019). All specimens were subjected to microtechnique procedures according to **Willey (1971),** sectioned to a thickness of 20 microns, double stained with crystal violet-erythrosin combination, cleared in xylene and mounted in Canada balsam. Sections were microscopically examined and photomicrographed. The averages of ten counts and measurements of different tissues were recorded.

Data Analysis

The experimental design was randomized with a block design using 5 replicates. Data were subjected to statistical analysis using ANOVA at 5% significance level. Duncan Multiple Range Test (DMRT) at 5% was used to detect the significant differences between treatments according to **Duncan (1955)**.

RESULTS AND DISCUSSION

Vegetative Growth Parameters

The acquired results from Sage plant growth characters represented in Table 4 revealed that both treatments nano zeolite loaded nitrogen (T7) and nano nitrogen, phosphorus, potassium (T5) significantly had the upper hand compared to all other treatments including control (commercial NPK) treatment in both seasons. The increments in each of plant height were (72, 78%), number of branches (162, 239%), herb fresh weight (156, 178 %) and herb dry weight (133, 184%) respectively were the result of the implementation of (T7) treatment in comparison to control treatment.

Same trend was obtained with other parameters in Table 5 as a consequence of (T7) treatment application significantly gave an increment in both seasons represented in (49, 76%) for leaf area, (122, 138%) for leaf/stem ratio, (138, 179%) for yield fresh weight and (114, 244 %) for health index, respectively judged against control treatment. Concerning oil yield, appliance of (T7) significantly donate oil yield higher than all other treatments during both seasons under drought stress, the augmentations were (220, 255 %) respectively compared to control plants.

It worth mentioning that, there were insignificant differences in response to most plant growth characters between (T7) and (T5) treatments application albeit (T7) treatment was more efficient.

Previous morphological characters were found to be accompanied by a reduction growth, particularly in control plants. On the other side, earlier reductions were compensated mainly due to (T7) treatment application then (T5) treatment. Many investigations have found that when plants are exposed to water stress, they had limited growth traits correlated with the degree of stress severity **(Scarascia-Mugnozza *et al.*, 1996; Nayyar and Gupta, 2006).**

It could be concluded that, nano-zeolite loaded nitrogen (T7) and nano NPK (T5) led to increase plant parameters over control due to their favorable effects on plant growth represented in available of nutrients, retention of water by zeolite **(Abdel Wahab *et al.*, 2017)** and improved soil physical and chemical prosperities **(Hassan and Mahmoud, 2015)**.

Table 4. Effect of different treatments on growth characters of *Salvia officinalis* L. during 2018 and 2019 seasons

Treatment	Plant height (cm)		Number of branches/plant		Herb fresh weight (g/plant)		Herb dry weight (g/plant)	
	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S
Control (NPK)	24.6d	22.7d	5.87d	4.21c	23.68d	20.61e	6.92c	4.96c
Nano-N	33.5b	29.3b	10.5b	9.31b	42.11b	39.73c	9.78b	8.05b
Nano- P	28.6c	25.8c	8.41c	8.1b	33.79c	30.18d	8.29b	6.64b
Nano- K	27.5c	23.7c	9.3c	8.77b	31.53c	28.44d	7.06c	5.10c
Nano -N PK	43.5a	38.2a	14.66a	12.5a	58.42a	50.36b	16.05a	13.88a
Nano- Zeolite	35.4b	31.5b	11.37b	9.42b	40.98b	40.22c	9.55b	8.77b
Nano-Zeolite + N	42.3a	40.5a	15.42a	14.3a	60.61a	57.34a	16.11a	14.09a

Means with the same letter in a column are not significantly different by DMRT 5%.

1st S: First season 2nd S: Second season.

T_1: Control (NPK); T_2: Nano-N, T_3: Nato-P, T_4 : Nano- k, T_5: Nano – NPK, T_6: Nano-Zealite and T_7: Nano – Zeolite + N

Table 5. Effect of different treatments on growth characters of *Salvia officinalis* L. during 2018, and 2019 seasons

Treatment	Leaf area (cm^2)		Leaf/stem ratio fresh weight (g)		Yield fresh weight (ton/ha)		Health index		Oil yield (l/ha)	
	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S
Control (NPK)	9.88c	8.12c	2.37d	2.12c	1.51d	1.22d	1.14c	0.89d	5.42e	5.08e
Nano-N	10.57b	10.40b	3.62c	3.02b	2.69b	2.53b	1.54b	1.45c	10.12b	8.79c
Nano- P	8.91d	8.48c	3.45c	2.94c	2.27b	1.84c	1.24b	1.10c	7.21c	6.92d
Nano- K	8.43d	8.30c	2.86d	2.30c	2.05c	1.63c	1.05c	0.88d	6.83d	5.81e
Nano -N PK	13.39a	12.51a	5.31a	4.25a	3.79a	3.33a	2.34a	2.24b	17.33a	15.48b
Nano- Zeolite	12.65a	12.77a	4.87b	4.53a	2.67b	2.62b	1.45b	1.50c	10.26b	9.15c
Nano-Zeolite + N	14.69a	14.36a	5.28a	5.06a	3.60a	3.41a	2.44a	3.06a	17.36a	18.08a

Means with the same letter in a column are not significantly different by DMRT 5%.

1st S: First season 2nd S: Second season.

T_1: Control (NPK); T_2: Nano-N, T_3: Nato-P, T_4 : Nano- k, T_5: Nano – NPK, T_6: Nano-Zealite and T_7: Nano – Zeolite + N

Hence increment of volatile oil productivity with (T7) treatment could be explain on the basis of available elements, vitamins, hormones, hormone like substances, amino acids and sugars that lead to richness of metabolism consequently an increase in volatile oil content.

Photosynthetic Rate, Stomatal Conductance, Intercellular CO_2 Concentration, Water-Use Efficiency and Relative Water Content

The diurnal mean leaves photosynthesis rate of *Salvia officinalis* L. under different treatments as influenced by drought stress publicized in Tables 6 and 7 undeniably revealed that, plants under (T7) treatment significantly donated higher values of net photosynthesis rate (25, 33%), stomatal conductance (8, 8.5%), intercellular CO_2 concentration (7, 9%), water use efficiency (90, 83%) and relative water content (9, 12%) respectively in both seasons compared to control.

Despite of treatments, photosynthesis rate values were the highest in the 1200 hours and could be referred to the significant availability of photosynthetic active radiation throughout the examined period. There is positive relationship between photosynthesis rate and stomatal conductance where the higher stomatal conductance increased photosynthesis rate.

Subject to confirmation drought stress induced stomata closure, hence the uptake of CO_2 notably decreased intercellular**,** as a result the consumption of NADPH+H for the CO_2 fixation *via* Calvin-cycle turn downs remarkably **(Cronic, 2000)**. But application of (T7) treatment may be formulated and returns standard conditions and this increased intercellular CO_2 concentration, moreover, excess of reductive power was successfully dispersed either by photochemical extinguishing **(Muller *et al.*, 2001)** or by an effective re-oxidation of NADPH+H. The decreased of either photosynthesis rate or stomatal conductance in other treatments can be diagnostic to the straight suppression of biochemical pathway through ionic, osmotic or other factors that could be stimulated by restricting cellular water **(Lawlor, 2002)**.

Water use efficiency for crops is a crucial consideration as long as water resources are inadequate and rainfall is a restricting factor as the condition of arid and semi-arid areas including Egypt. Moreover, the main factor that affects water use efficiency is soil conditions and fertility; therefore almost entire fertility was given symbolized in nano zeolite loaded nitrogen treatment (T7)_which provides plants with roots that extant around and deep in soil volume for water and nutrients uptake, these consequences in healthier plants that can more easily withstand seasonal drought stresses **(Stewart, 2001)**.

Preceding results related to morphological characters of Sage plant are in harmony with those obtained by **Melanie *et al.* (2010) and Filipa *et al.* (2016)** on sage plant, **Abdel Wahab and Soliman (2017)** on evening primrose plant and **Abdel Wahab *et al.* (2017)** on *Carum carvi.*

Chemical Analyses

Macro and microelements

As declared of growth parameters results, the results of chemical analysis (Tables 8 and 9) had the same way due to the information that, concentrations of macro and micronutrients in the shoot of *Salvia officinalis* L. through both seasons significantly increased as a result of nano zeolite loaded nitrogen (T7) treatment application, the augmentations were (78, 54%) for nitrogen, (33, 40%) for phosphorus, (8, 18%) for potassium, (13, 14%) for calcium and (64, 61%) for magnesium, respectively in comparison with control treatment (T1). On the other hand, there was discrepancy between results of sodium concentration and earlier macro-elements, where control treatment (T1) gave significant increase in sodium concentration compared to all other treatments particularly (T7) treatment (31, 45%) respectively in favor of control.

Come into the sight of micro-elements, it was observed that, the augmentations during two seasons were (29, 40%) for zinc, (95, 88%) for iron, (59, 83%) for manganese and (19, 22%) for boron respectively in the favor of (T7) compared to control (T1).

Table 6. Effect of different treatments on photosynthetic rate, stomatal conductance, intercellular CO_2 concentration of *Salvia officinalis* L. during 2018 and 2019 seasons

Treatment	Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$)		Stomatal conductance (mol H_2O $m^{-2}s^{-1}$)		Intercellular CO_2 concentration (ppm)	
	1st S	2nd S	1st S	2nd S	1st S	2nd S
Control (NPK)	17.24c	17.08b	258.3c	250.6c	153.3b	150.8b
Nano-N	18.64b	18.22b	263.8c	255.1b	150.4b	147.6b
Nano- P	18.18b	17.88b	274.2a	267.8a	146.7c	141.9c
Nano- K	17.28c	16.89c	270.3b	258.2b	144.8c	144.2c
Nano -N PK	20.85a	21.85a	273.6a	269.5a	160.8a	159.5a
Nano- Zeolite	18.67b	17.96b	275.6a	262.3b	152.1b	150.7b
Nano-Zeolite + N	21.53a	22.76a	278.5a	271.8a	164.5a	165.2a

Means with the same letter in a column are not significantly different by DMRT 5%.

1st S: First season 2nd S: Second season.

T_1: Control (NPK); T_2: Nano-N, T_3: Nato-P, T_4 : Nano- k, T_5: Nano – NPK, T_6: Nano-Zealite and T_7: Nano – Zeolite + N

Table 7. Effect of different treatments on transpiration rate, water use efficiency and relative water content (RWC) of *Salvia officinalis* L. during 2018 and 2019 seasons

Treatment	Transpiration rate (mmol $m^{-2}s^{-1}$)		Water-use efficiency (µmol $mmol^{-1}$)		RWC (%)	
	1st S	2nd S	1st S	2nd S	1st S	2nd S
Control (NPK)	3.81a	3.86a	4.52b	4.42b	83.06d	80.89b
Nano-N	3.52a	3.60a	5.29b	5.06b	87.42b	85.47a
Nano- P	3.32a	3.41a	5.47b	5.24b	85.59c	83.71b
Nano- K	2.91b	3.12a	5.93b	5.41b	83.89d	81.55b
Nano -N PK	3.54a	3.62a	5.88b	6.03a	90.06a	87.91a
Nano- Zeolite	3.15a	3.22a	5.91b	5.57b	88.31b	86.35a
Nano-Zeolite + N	2.50b	2.81b	8.61a	8.09a	91.03a	90.62a

Means with the same letter in a column are not significantly different by DMRT 5%.

1st S: First season 2nd S: Second season.

T_1: Control (NPK); T_2: Nano-N, T_3: Nato-P, T_4 : Nano- k, T_5: Nano – NPK, T_6: Nano-Zealite and T_7: Nano – Zeolite + N

Table 8. Effect of different treatments on N, P, K, Ca and Na of *Salvia officinalis* L. during 2018 and 2019 seasons

Treatment	N (%)		P (%)		K (%)		Ca (%)		Na (%)	
	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S
Control (NPK)	1.35d	1.72c	0.48b	0.52c	2.89b	2.85b	1.55b	1.58b	3.02a	2.61a
Nano-N	1.55b	1.80b	0.47c	0.46d	2.75b	2.79b	1.48c	1.50c	2.89b	2.37b
Nano- P	1.44c	1.38d	0.50b	0.50c	2.68b	2.70b	1.40d	1.48c	2.79b	2.40b
Nano- K	1.33d	1.40c	0.45c	0.47d	3.04a	3.09a	1.46c	1.53c	3.00a	2.55a
Nano -N PK	2.05a	1.83b	0.51b	0.56b	3.00a	3.12a	1.77a	1.78a	2.12c	2.03c
Nano- Zeolite	1.33d	1.48d	0.46c	0.51c	2.61b	2.90b	1.50b	1.55b	2.09c	1.69d
Nano-Zeolite + N	2.41a	2.65a	0.64a	0.73a	3.11a	3.36a	1.76a	1.80a	2.07c	1.44d

Means with the same letter in a column are not significantly different by DMRT 5%.
1st S: First season 2nd S: Second season.
T_1: Control (NPK); T_2: Nano-N, T_3: Nato-P, T_4 : Nano- k, T_5: Nano – NPK, T_6: Nano-Zealite and T_7: Nano – Zeolite + N

Table 9. Effect of different treatments on Mg, Zn, Fe, Mn and B of *Salvia officinalis L.* during 2018 and 2019 seasons

Treatment	Mg (%)		Zn (ppm)		Fe (ppm)		Mn (ppm)		B (ppm)	
	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S
Control (NPK)	0.39b	0.44b	97.6b	95.2d	166.8c	160.2e	82.5b	70.6c	30.7b	25.8b
Nano-N	0.38b	0.41c	91.8c	90.5d	189.7b	181.3d	85.1b	78.6b	22.5c	17.7c
Nano- P	0.37b	0.40c	90.4c	89.2d	190.7b	178.8d	81.4b	75.4b	23.4c	19.8c
Nano- K	0.38b	0.43c	93.5c	85.4d	193.5b	180.5d	80.3b	77.2b	24.7c	20.4c
Nano -NPK	0.60a	0.65a	122.5a	100.7c	201.6b	193.2c	89.5b	81.3b	25.1c	22.8c
Nano- Zeolite	0.40b	0.49b	100.3b	119.8b	307.2a	256.8b	135.2a	125.3a	31.8b	27.7b
Nano-Zeolite + N	0.64a	0.71a	125.7a	133.5a	325.2a	301.4a	131.5a	129.5a	36.6a	31.5a

Means with the same letter in a column are not significantly different by DMRT 5%.
1st S: First season 2nd S: Second season.
T_1: Control (NPK); T_2: Nano-N, T_3: Nato-P, T_4 : Nano- k, T_5: Nano – NPK, T_6: Nano-Zealite and T_7: Nano – Zeolite + N

It has been known that, existing N in soil is strongly related to the capacity of plant roots to absorb water from soil. Nevertheless, a few types of soils are poor in N content especially in arid and semi-arid regions (**Hernández *et al*., 1997**) that may make the plants more vulnerable to drought stress taking place in these regions.

Under water stress, stomata turn to be close causes a decrease in transpiration hence reduction in water transport through plant vessels; which, in turn, affects the capability of roots to absorb water and nutrients from the soil (**Waraich *et al*., 2011**) as the case in control plants (T1). Moreover, drought provoked N deficiency mainly hinders plant growth under water deficit (**Heckathor *et al*., 1997**) represented in decreasing leaf size due to decreased cell number and size beside the whole plant shoot (**MacAdam *et al*., 1989**). Meanwhile, the ameliorative effects of N, P and K together on plant growth under drought have been ascribed to an enhancement in stomatal conductance (**Brück *et al.,* 2000**), photosynthesis (**Ackerson, 1985**), higher cell-membrane constancy, enhanced plant water positive relation and raised drought tolerance (**Sawwan *et al.,* 2000**) also increased of micronutrients uptake such as Zn, Cu, Mn, and Fe (**Bagayoko *et al.,* 2000**). If truth be told soil conditions and particularly fertility improved the ability of plants to sustain relatively normal growth, stomatal conductance, and photosynthesis under drought conditions (**Kleiner *et al.,* 1992**).

Escalating macro and micronutrient concentrations in *Salvia officinalis* L leaves as a result of (T7) application may be owing to the extent in root surface per unit of soil volume which in turn contribute greatly to the enhance of nutrient uptake **(Ghallab and El-Gahadban, 2004)**, along with the essential role of zeolite which containing macro and micronutrients, and its channels that grant large surface areas on which chemical reactions can take place and turn fertilizers to be more beneficial by limiting nutrients leaching and holding important and trace nutrients as slow release as required **(Kallo *et al.,* 1986).** Supportive evidence for this result was reported by **Safaei *et al.* (2014)** who mentioned that application of nano-fertilizer and humic acid as natural material having nutritional elements and physiological effects, had enhanced *Nigella sativa* growth parameters and could be used to sustainable agriculture system.

Chlorophyll Content, Total Phenols, Total Carbohydrates, Tannins and Total Flavonoids

Results represented in Table 10 indicate that, chlorophyll content, total phenolic, total carbohydrates and total flavonoids significantly recorded the highest amount (49, 46%), (25, 36%), (29, 33%) and (23, 20 %) respectively in both seasons as a result of (T7) treatment application compared to control plants (T1). Contrarily, in the case of tannins, appliance of nano-NPK (T5) significantly surpassed all other treatments including both (T7) and (T1) treatments where the increment was (6, 6.2 %) respectively during both seasons in comparison with (T1). The increment in total chlorophyll content may be due to the positive effects of nano-zeolite loaded nitrogen (T7) on plant pigments since liberate more nutrients that modify iron and zinc deficiency in sandy soil which positively reflected on efficiency of photosynthesis process besides their role in increasing plant metabolites and root surface per unit of soil volume, which in turn increase nutrient uptake (**Tisdale *et al.,* 1985).** While increment in total carbohydrate, total phenols, and total flavonoids may be due to the elevation of photosynthesis as a result of increase in chlorophyll content, also could be refer to the fact that zeolite with cages structure together with a depiction of the straight and zigzag channel which is considered a source of water retention and some nutrients that may play a vital role in plant metabolism, conspicuously the most significant function would be the drought resistance as aftereffect (**Tisdale and Nelson, 1975).** On the ground of previous data, all the secondary metabolites may not enhance in the same ratio in reaction to water stress since plants show uneven or changeable response to drought stress for different secondary products For example, medium-intensity drought stress increases the accumulation of flavonoids and tannins, as reported by **Yang and Li (2011)**.

Drought stress has a strong influence on secondary metabolic pathways accountable for accumulation of secondary products. Nevertheless, it was mentioned in many researches, the corresponding consequences are not definite and a thorough review of the literature may assist to reach the influential conclusions concerning the effects of water stress on the increase of natural products within plant tissue. In a wide array of both in vivo and in vitro experiments, it could be concluded that plants grown under water stress has higher quantity of secondary components. In comparison to nano-fertilizers as external sources of nutrients in the form of chemical fertilizers donate more than 50% of crop productivity enhancement, but the nutrient use efficiency by crops is poor due to fixation, leaching, volatilization and microbial mineralization (**Samra and Sharma, 2009).**

Similar results were found by **Abdel Wahab *et al.* (2017)** on *Carum carvi* and **Ramesh *et al.* (2016)** on watermelon and **Abdel Wahab *et al.* (2019)** on radish plant.

Table 10. Effect of different treatments on chlorophyll, total phenolic, total carbohydrates, tannins and total flavonoids of *Salvia officinalis* L. during 2018 and 2019 seasons

Treatment	Chlorophyll content (mg/g FW)		Total phenols (mg $GAEg^{-1}DW$)		Total carbohydrates (%)		Total tannins (g/kg)		Total flavonoids (mg/g)	
	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S
Control (NPK)	4.32^{b}	4.45^{b}	33.41^{b}	32.45^{c}	18.5^{b}	17.8^{c}	139.7^{b}	142.3^{b}	57.8^{b}	62.7^{b}
Nano-N	5.12^{a}	5.32^{a}	34.12^{b}	35.61^{b}	20.6^{b}	18.5^{b}	130.9^{c}	129.5^{d}	60.2^{b}	63.6^{b}
Nano- P	4.35^{b}	4.66^{b}	32.09^{b}	37.20^{b}	17.8^{c}	18.2^{b}	133.6^{c}	130.7^{d}	55.6^{c}	59.2^{c}
Nano- K	4.28^{b}	4.59^{b}	35.71^{b}	40.25^{a}	19.7^{b}	20.6^{b}	136.5^{b}	134.4^{c}	61.1^{b}	63.8^{b}
Nano -N PK	6.05^{a}	6.26^{a}	38.55^{a}	37.41^{b}	20.2^{b}	22.3^{a}	148.2^{a}	151.3^{a}	68.5^{a}	72.3^{a}
Nano- Zeolite	5.87^{a}	5.80^{a}	32.11^{b}	30.84^{c}	18.1^{b}	17.8^{c}	135^{b}	131.6^{d}	58.3^{b}	60.1^{b}
Nano-Zeolite + N	6.43^{a}	6.51^{a}	41.89^{a}	44.09^{a}	23.8^{a}	23.6^{a}	132.4^{c}	136.8^{c}	71.3^{a}	75.4^{a}

Means with the same letter in a column are not significantly different by DMRT 5%.
1st S: First season 2nd S: Second season.
T_1: Control (NPK); T_2: Nano-N, T_3: Nato-P, T_4 : Nano- k, T_5: Nano – NPK, T_6: Nano-Zealite and T_7: Nano – Zeolite + N

Endogenous Phytohormones and Enzymes

Focusing on hormonal and enzyme analysis as shown in Figs. 6 and 7, the obtained results in this concern clearly revealed that, application of (T7) treatment significantly recorded the highest amount of gibberellic acid (GA_3) Fig. 6 in plant tissues (43, 58%) respectively for the period of both seasons compared to control plants (T1). On the other side, both treatments (T4) and (T3) significantly resulted in the highest amount of abscisic acid (ABA) compared to all other treatments including control (T1). While the lowest amount of (ABA) significantly resulted from (T7) application (25, 47%) during the two seasons compared to control plants (T1).

In this connection, enzymes results represented in Fig. 7 discerned that, the highest amount of both enzymes peroxidase and superoxide dismutase emerged within plant tissues as a consequence of (T7) treatment implementation since significantly recorded (86, 104%) respectively for both seasons.

The aforementioned results may explain as; the task of plant growth regulators under water deficiency stress is essential in changing physiological reactions that finally lead to adaptation to an adverse environment. Hormonal balance, steadiness, concentrations, biosynthesis, and distribution play a crucial role in fast adaptation to abiotic stress (**Mitchell *et al.*, 2013).** These negative impacts of drought were significantly overcome by the application of (T7) treatment represented in low amount of ABA within plant tissues and high amounts of antioxidant system activation symbolized in peroxidase and superoxide dismutase enzymes which increase under water stress (**Li *et al.*, 2006)** and has vital task in the defense against damage by oxygen radicals (**Kardish *et al.*, 1994)** accompanied with high level of GA3. Moreover, it has been proven that the decline in gibberellic acid (GA_3) level and raise in abscisic acid (ABA) level under drought conditions suggested that the decrease of growth may possibly as a result of the drought-induced changes in permeability of cells membrane and water uptake due to change hormonal balance (**Saeidi-Sar *et al.*, 2007).** Hence high concentration of GA3 with (T7) treatment may have indication in alleviating drought-imposed adverse effects on Sage plant. *Vice versa*; drought-induced reduction in dry matter production, chemical constituents and yield was more well-defined with (T1) treatment application and this reaction may be because synergic interaction between ABA in response to drought stress appeared.

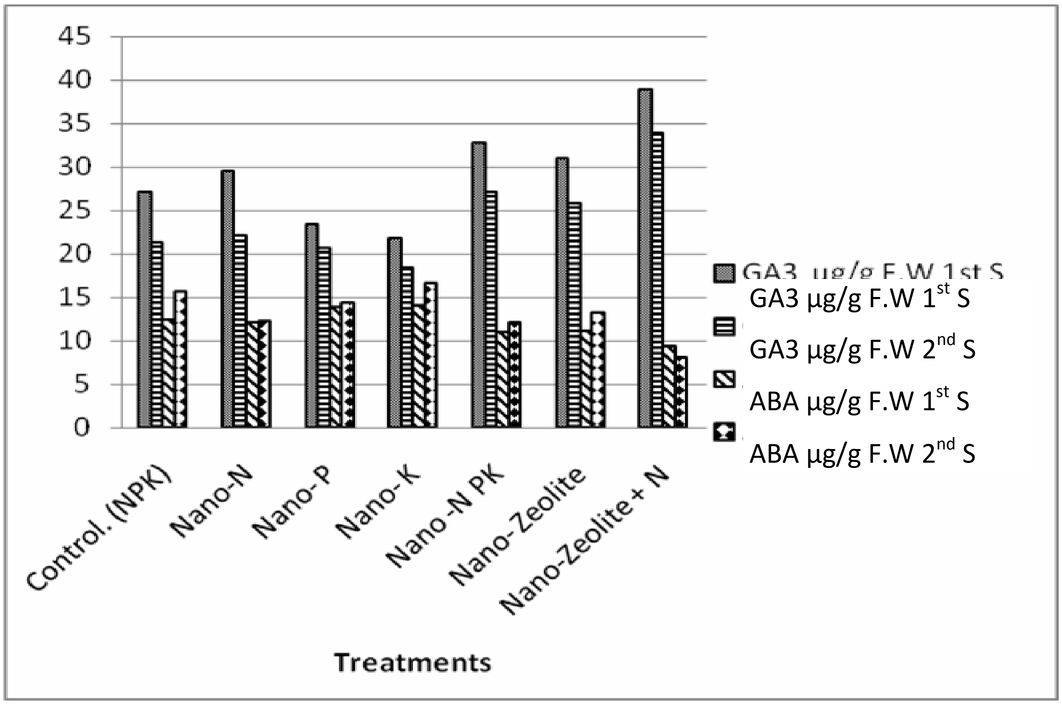

Fig. 6. Effect of different treatments on GA3 and ABA hormones

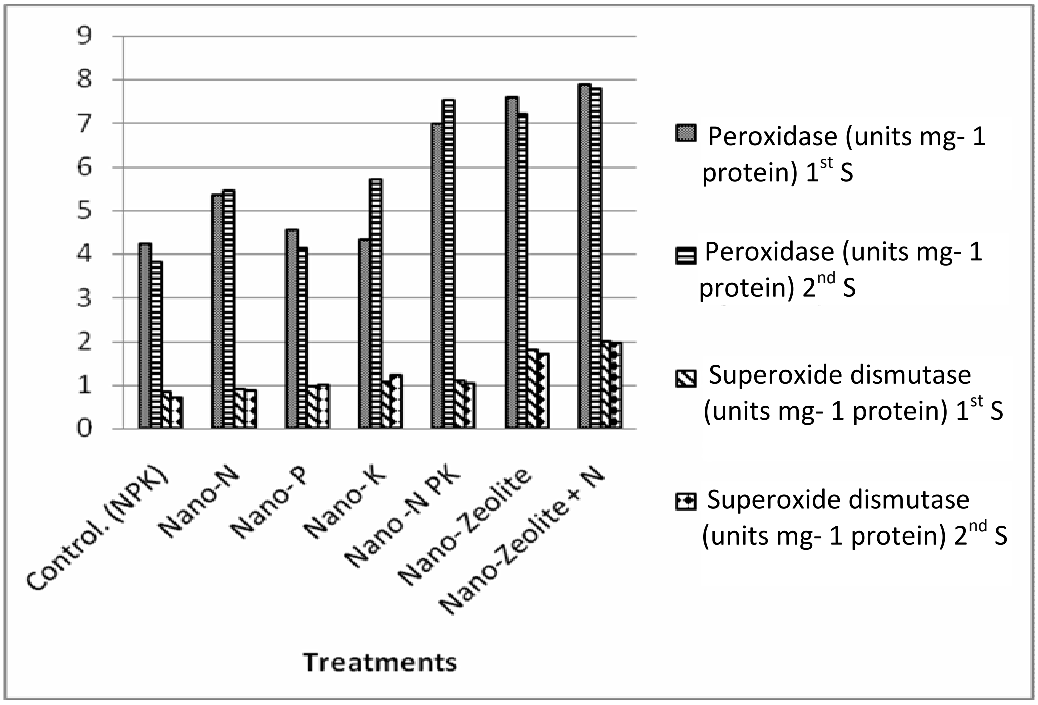

Fig. 7. Effect of different treatments on peroxidase and superoxide dismutase enzymes

For these reasons applied of (T7) treatment during two seasons might have enhanced endogenous phytohormones accumulation represented in GA3 as a result of initialized normal conditions, mitigation drought stress and stimulate protected enzymes which also help growth and survival of the plant to tolerate water stress (**Hoque *et al.*, 2007**).

The abovementioned results are in consonance with **Gill *et al.* (2011), Meijón *et al.* (2011)** and **Abdel Wahab and Soliman (2017)**.

Volatile Oil Components

The results of *Salvia officinalis* L essential oil (yellowish liquid with a warm camphoraceous) components resulted from G.C. analysis represented in Table 11 can be discussed as follows:

Among identified components, it was found that the utmost amount of α-Pinene content (3.87, 3.71%) resulted from application of (T7) treatment while the lowest amount (0.10, 0.29%) for both seasons was outputted from (T6) treatment. Regarding β-Pinene, it was recorded the highest amount with (T5) treatment application (4.05, 4.16%) whereas the lowest amount presented in first season (0.1%) from (T6) treatment and (1.25%) in the second season from (T2) treatment.

The maximum amount of 1.8 Cineol achieved from (T5) treatment in the first season (61.35%) but (T7) treatment in the second season produced the highest amount (58.62%), In this field, the highest amount of α–Thujone (17.73, 17.81%) respectively for both seasons was obtained from (T6) treatment. As for Camphor, it was found that (T4) treatment application donated the highest amount (33.01, 33.52%), respectively for two seasons.

With reference to Caryophyllene compound, application of (T6) treatment resulted in the highest amount (8.47, 6.51%) during the two seasons, respectively.

Variation in the oil constituents' amounts of extracted essential oil may be due to one or combined different factors represented in effect of applied treatments, drought stress, environmental conditions under which sage plant has been grown, as well as the variation in conditions of analysis. On the ground of previous researches, the essential oil terpene compounds concentration in stressed leaves possibly will be improved when the rate of biological synthesis remains unchanged, but the whole biomass production of the plant turns down due to the stress conditions applied. The results presented in this research support the hypothesis that the substances of secondary plant products can be improved on purpose by applying moderate drought stress with respect to quality and concentrations of the active ingredients. In other words, when the drug should be extracted, it is not the matter of concentration other than the overall amount of the substance which becomes pertinent. Consequently, a scrupulous and comprehensive evaluation of the supposed effects of drought stress is required, *i.e.,* the positive influence on the boost of secondary plant products on one hand, and its negative effects on biomass production on the other hand. Additionally, it should be considered that the application of certain growth conditions (such as nano-particles or nano fertilizers), which consider reason of raised biomass production also, might be accompanied by reduced concentrations of relevant essential oil compounds.

These results concurred with those of **Abdel Wahab *et al.* (2017)** on *Carum carvi* and **Abdel Wahab and Sahar (2018)** on *Eruca sativa*.

Effect of Nano Zeolite Loaded Nitrogen on Leaf Anatomical Structure of Sage (*Salvia officinalis* L.) Plant

Leaf lamina

The microscopic parameters of the transverse sections of the leaf lamina of Sage plants treated with nano zeolite loaded nitrogen (T7) compared with commercial recommended dose of NPK (T1) as control are presented in Table 12 and Fig. 8 A and B. Counts and measurements proved considerable differences between both leaf laminas.

These differences involved 20% increase in lamina thickness of plants treated with (T7) due to the increase in thickness of upper epidermis (33.3%), palisade tissue (16.7%), and spongy tissue (17.8%). There was also an increase in the

Table 11. Effect of different treatments on essential oil components of *Salvia officinalis* L. during 2018 and 2019 seasons

Component	Control (NPK)		Nano-N		Nano- P		Nano- K		Nano-NPK		Nano-Zeolite		Nano-Zeolite+ N	
	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S
α-Pinene	1.27	1.14	1.39	1.36	0.78	0.84	1.02	1.22	3.87	3.71	0.10	0.29	1.69	1.55
Camphene	1.63	1.55	1.80	1.82	0.78	0.80	1.37	1.40	1.54	1.60	0.64	1.42	1.88	1.76
β-Pinene	1.54	1.57	1.28	1.25	2.39	2.25	3.34	3.50	4.05	4.16	0.10	2.38	3.71	3.68
1.8 Cineol	37.45	35.28	38.14	36.51	38.49	35.81	35.85	36.11	61.53	55.39	19.44	22.06	47.72	58.62
□-Terpinene	10.25	11.03	13.58	14.20	11.48	10.63	8.68	8.90	6.09	6.13	6.07	7.11	5.21	3.31
P-Cymene	3.72	2.81	2.30	1.77	1.85	3.42	1.54	1.62	1.72	1.05	5.74	5.88	4.10	3.19
α - Thujone	12.12	11.61	4.60	7.43	7.68	8.07	5.67	5.81	13.08	12.11	17.73	17.81	12.19	8.34
Camphor	19.65	18.05	21.89	19.05	25.65	22.11	33.01	33.52	2.70	2.85	19.27	17.35	20.12	14.78
Caryophyllene	3.19	1.42	1.51	1.03	0.63	0.66	0.33	0.38	4.35	4.62	8.47	6.51	2.27	1.80
Total terpenic components	21.60	20.47	21.86	20.43	17.91	17.68	16.28	18.37	21.62	24.47	21.12	23.45	21.88	25.08
Total oxygenated	69.22	63.99	64.63	63.95	71.82	66.91	74.53	74.09	77.31	67.15	56.44	57.36	77.01	71.95
Total identified	90.82	84.46	86.49	84.42	89.73	84.59	90.81	92.46	98.93	91.62	77.56	80.81	98.89	97.03
Un identified	9.18	15.54	13.51	15.58	10.27	15.41	9.19	7.54	1.07	8.38	22.44	19.19	1.11	2.97
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100

T_1: Control (NPK); T_2: Nano-N, T_3: Nato-P, T_4 : Nano- k, T_5: Nano – NPK, T_6: Nano-Zealite and T_7: Nano – Zeolite + N

Table 12. Averages of counts and measurements (in microns) of certain anatomical characters in transverse sections through the middle portion of leaf lamina of Sage (*Salvia officinalis* L.) plant as affected by different treatments (Averages of 10 readings)

Character	NPK (Control)	Nano zeolite + N	± % to control
Leaf lamina thickness	210	264	20.0
Upper epidermis thickness	15	20	33.3
Lower epidermis thickness	10	10	0.0
Palisade tissue thickness	150	175	16.7
Spongy tissue thickness	45	53	17.8
No. of palisade tissue layers	2	2	0.0
Midrib dimensions	490x408	700x764	42.9x87.3
Midrib bundle dimensions	160x140	240x420	50.0x200.0
Xylem tissue thickness	70	120	71.4
Phloem tissue thickness	50	90	80
No. of xylem rows	5	16	220
Mean No. of xylem vessels/row	4	5	25
Mean vessel diameter	6-9	6-16	0.0-77.8
Thickness of collenchyma above midrib bundle	50	70	40
Thickness of collenchyma below midrib bundle	63	84	33.3
Thickness of parenchyma above midrib bundle	44	91	106.8
Thickness of parenchyma below midrib bundle	101	154	52.5

T_1: Control (NPK); T_2: Nano-N, T_3: Nato-P, T_4 : Nano- k, T_5: Nano – NPK, T_6: Nano-Zealite and T_7: Nano – Zeolite + N

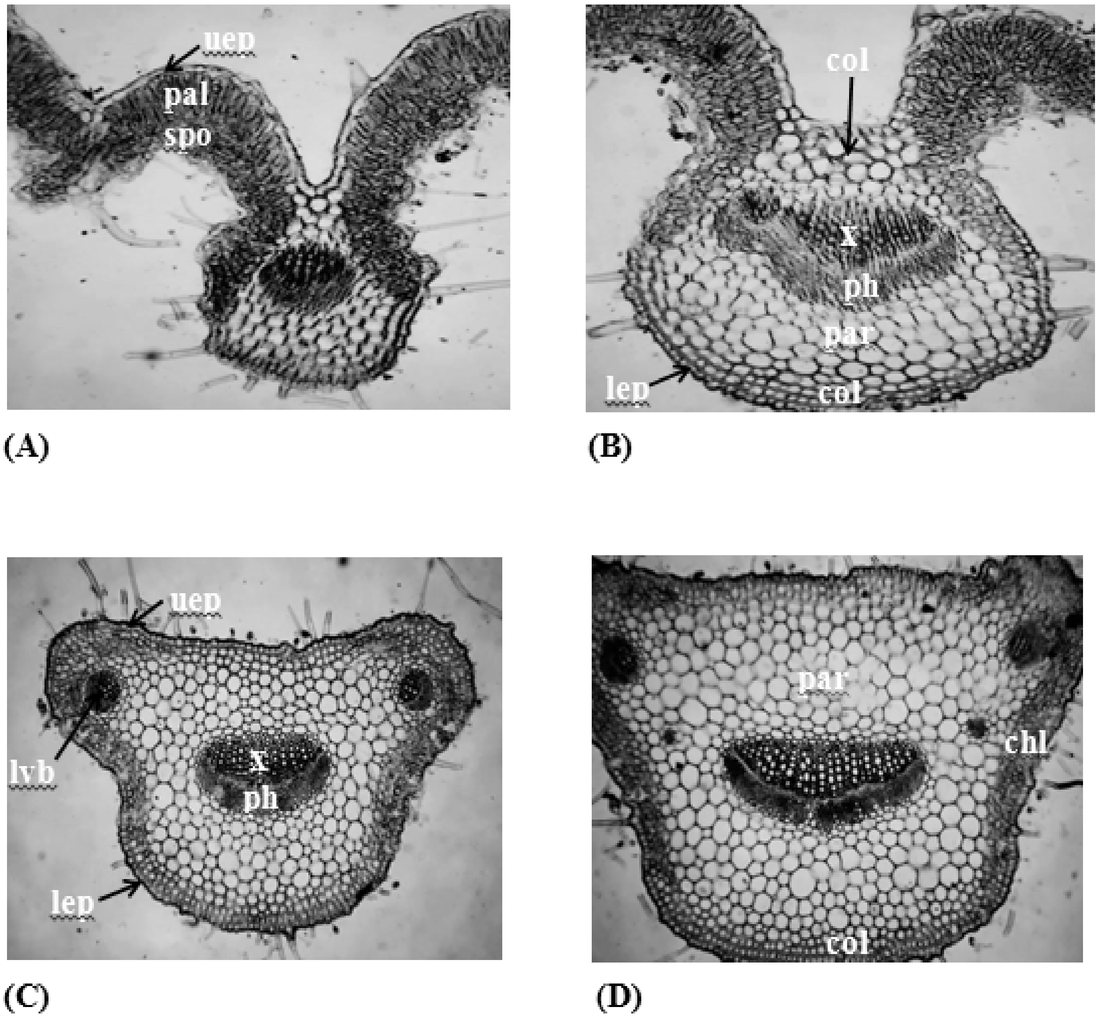

Fig. 8. Transverse sections through the leaf lamina and petiole on the 4th node of Sage (*Salvia officinalis* L.) plant. (X 40) (A and C): Control plant; (B and D): Plant treated with nano zeolite loaded nitrogen

chl, chlorenchyma; **col**, collenchyma; **lep**, lower epidermis; **lvb**, lateral vascular bundle; **pal**, palisade tissue; **par,** parenchyma; **ph**, phloem**; spo**, spongy tissue; **uep**, upper epidermis; **x**, xylem.

dimensions of the midrib as the increase percentages were 42.9% in length and 87.3% in width, which refers to the increase in the dimensions of the midrib vascular bundle, as it reached 50% in length and 200% in width, along with the thickness of the collenchyma above the vascular bundle (40%) and below the vascular bundle (33.3%) as well as the thickness of the parenchyma above the vascular bundle (106.8%) and below the vascular bundle (52.5%).The increase in the dimensions of the midrib vascular bundle is due to the increase in the thickness of the phloem tissue by 80% and xylem tissue by 71.4%. The latter is due to the increase in the No. of xylem rows by 220%, mean No. of xylem vessels per row by 25%, and mean diameter of vessel by 77.8%, compared with control plants.

Leaf petiole

The anatomical manifestations of the leaf petioles of Sage plants treated with (T7) compared to control (T1) are displayed in Table 13 and Fig. 8 C and D. The results showed that the (T7) treatment led to leaf petiole larger than control (T1), as the percentages of increase were

Table 13. Averages of counts and measurements (in microns) of certain anatomical characters in transverse sections through the middle portion of leaf petiole of Sage (*Salvia officinalis* L.) plant as affected by different treatments (Averages of 10 readings)

Character	NPK (Control)	Nano zeolite + N	± % to control
Leaf petiole dimensions	750x1225	1050x1575	40.0x28.6
Upper epidermis thickness	19	19	0.0
Lower epidermis thickness	15	25	66.7
Main vascular bundle dimensions	225x400	250x625	11.1x56.3
Xylem tissue thickness	120	135	12.5
Phloem tissue thickness	63	81	28.6
No. of xylem rows	16	28	75.0
Mean No. of xylem vessels / row	5	5	0.0
Mean vessel diameter	25-50	38-75	52.0-50.0
No. of lateral (subsidiary) bundles	2	2	0.0
Thickness of collenchyma	68-75	75-150	10.3-100.0
No. of collenchyma layers	3-4	3-4	0.0
Thickness of chlorenchyma	55	75	36.4
No. of chlorenchyma layers	3	4	33.3
Thickness of parenchyma above mainvascular bundle	158	283	79.1
Thickness of parenchyma below mainvascular bundle	180	260	44.4

40% in length and 28.6% in width of leaf petiole. This increase is attributed to the increase in the thickness of the lower epidermis (66.7%), collenchyma thickness (10.3-100%), the chlorenchyma thickness (36.4%), the number of chlorenchyma layers (33.3%), the parenchyma thickness above the main vascular bundle (79.1%), the parenchyma thickness below the main vascular bundle (44.4%), and the dimensions of the main vascular bundle (11.1% in length and 56.3% in width). The increase in the main vascular bundle dimensions is attributed to the increase in the thickness of the xylem tissue by 12.5%, the thickness of phloem tissue by 28.6%, No. of xylem rows by 75%, and the mean vessel diameter by 50-52% compared to the control.

This confirmed the advantageous effects of nano zeolite loaded nitrogen (T7) on the anatomical structure of Sage plant and in the region of earlier results relevant to morphological and chemical analysis. The present results were similar to those obtained by **El-Feky *et al.* (2013)** on basil who found that application of nano Fe_3O_4 improved anatomical structure of leaves, and **Abdel Wahab *et al.* (2017)** on *Carum carvi* who declared that, application of nano-zeolite enhanced anatomical structure compared to control (NPK).

Conclusions

On the ground of previously mentioned results, which lead to the conclusion that application of both nano-zeolite-loaded nitrogen and nano nitrogen, phosphorus and potassium mixture gave eminent outcomes on either plant under study sage (*Salvia officinalis*) and environment represented in higher growth characteristics and chemical composition in contrast to results derived from_commercial recommended dose of chemical fertilizers NPK taking into account both quality and quantity parameters particularly with medicinal and aromatic plants, unthinking or regardless economic factor since such plants are considered infrastructure of pharmaceutical, cosmetics industries and a source of national income, in addition to decreasing environmental pollution mainly in newly reclaimed areas.

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مقارنة بين تأثير الأسمدة التجارية وأسمدة النانو نيتروجين وفوسفور وبوتاسيوم في وجود النانوزيوليت على محصول ومكونات نبات المريمية تحت إجهاد الجفاف

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يزداد التقدم نحو تقنيات النانو وتطبيقاتها على النبات تدريجيا في الآونة الأخيرة، بناءً على هذه الحقيقة، تم إجراء بحث خلال موسمين متتاليين ٢٠١٨ و ٢٠١٩ لدراسة نتائج تطبيق العناصر النانوية الكبرى الممثلة في NPK منفردة أو مجتمعة والنانو زيوليت المحمل بالنيتروجين أو غير المحمل بالنيتروجين بالمقارنة مع الأسمدة NPK التجارية على نبات المريمية المزروع تحت إجهاد الجفاف في منطقة مستصلحة حديثا في الصحراء، كشفت النتائج أن الزيوليت المحمل بالنيتروجين، وكذلك خليط النانو- NPK، أعطى نتائج بارزة في النمو الخضري (ارتفاع النبات، عدد الفروع، الوزن الطازج والجاف للعشب، مساحة الورقة، المحصول الطازج، مؤشر الصحة ومحصول الزيت) بجانب معدل التمثيل الضوئي والتوصيل الثغري وتركيز ثاني أكسيد الكربون بين الخلايا وكفاءة استخدام المياه والمحتوى النسبي للماء والتركيب الكيميائي ممثلا في (الصبغات النباتية والكربوهيدرات الكلية والفينولات الكلية والتانينات والفلافونويدات الكلية ومكونات الزيت والعناصر الكبرى والصغرى) جنبًا إلى جنب مع الهرمونات الداخلية ممثلة في (حمض الجبريليك GA3 وحمض الأبسيسيك ABA)، والأنزيمات المضادة للأكسدة (بيروكسيديز وسوبراكسيد ديسموتيز) في مقابل الجرعة التجارية للأسمدة الكيماوية NPK (كنترول) في ظل نفس الظروف، علاوة على ذلك، دعم التركيب التشريحي للأوراق نتائج قياسات النمو الخضري والتحاليل الكيميائية المتحصل عليها، ركزت نتائج الدراسة الحالية على التحذير العالمي من التلوث الناجم عن الأسمدة الكيماوية وخاصة في المناطق المستصلحة حديثًا والإنتاج الآمن للنباتات الطبية والعطرية.

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