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Original paper

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

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The lucrative approach of nanotechnology and it´s utilizing in the agricultural sector is prospective. Based on this fact a field trial was done through 2018 and 2019 seasons to scrutinize the consequence of nano N, P and K elements application individual or in combination and nano zeolite loaded nitrogen or not on a sage plant grown under water stress compared to the commercial NPK fertilisers at new reclaimed land. Nano NPK elements were prepared from their precursor as potassium persulfate ($K_2S_2O_8$), calcium phosphate ($Ca\ (H_2PO_4)_2{\cdot}H_2O$) and salt NH_4NO_3, urea ($CO\ (NH_2)_2$), while nano zeolite was hydrothermally synthesized. Water stress was applied via drip irrigation with 15 days intervals. The data revealed that, nano-fertilisers and nano-zeolites had a superior effects on the plant itself under stress conditions with concern on nano-zeolite loaded nitrogen and nano-NPK mixture as well which boosted vegetative growth (plant height, branches number, yield fresh weight, health index, herb fresh and dry weight, leaf area and oil yield), also improved photosynthetic rate, stomatal conductance, CO_2 concentration, water use efficiency and relative water content. The chemical composition (plant pigments, total carbohydrates, total phenolic, tannin, total flavonoids, oil constituents, macro and micro-elements) with indigenous hormones (gibberellic acid GA_3 and abscisic acid ABA) and antioxidant enzymes (peroxidase and superoxide dismutase) were also positively affected. The outcomes of current study emphasis global warning about chemical fertilisers pollution, particularly in new reclaimed areas and safety production of medicinal and aromatic plants.

Key words: sage plant, water stress, nano elements, nano-zeolite, NPK fertilisers

The global demand for medicinal and aromatic plants has been increased to match the global pharmaceutical and cosmetics industries expanding. Also, there is a need to mitigate the hazardous impacts on the environment induced by chemical fertilisation particularly NPK fertilisers. They are applied in huge amounts with low efficiency (N 30−35%, P 18−20% and K 35−40%) causing groundwater pollution or accumulate in soil body (Mir *et al*. 2018). Therefore, new agriculture practices must be applied for increasing the plant´s ability to absorb nutrients under harsh conditions.

The frequency and intensity of drought conditions have been increased in the Mediterranean and Africa regions with the projected global temperatures increasing (IPCC 2013). The tolerant plants to drought stress show different responses, including increment root/shoot ratio, growth reduction, leaf anatomy change, reduction of leaf size, total leaf area to limit water loss and guarantee photosynthe-

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sis (Toscano *et al.* 2019). The reduction of photosynthetic activity is related to the mechanisms of stomatal conductance. So, the first response of plants to water stress is stomatal closure for water potential adjustment resulting in a reduction in photosynthetic activity, which in turn leads to a decrease in plant growth and production (Shi *et al.* 2019). Natural zeolites overcome the drought effects in arid regions by acting as water distributors throughout the soil, that 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 infiltration and retention in the soil due to their porous and capillary properties which act as a slow-release source for water as well as macro and microelements. Hidayat *et al.* (2015) found that the zeolite is capable of retarding the release of urea and the potential to be developed as a controlled release of nitrogen from urea.

Therefore, in the last few years, there is an emphasis on nanoparticle fertilisers synthesis. Surface coatings of nano-materials on fertiliser particles make them more strongly due to higher surface tension than the conventional surfaces and thus help in gradual release (Manjunatha *et al.* 2016). Nano fertilisers cause an increase in nutrients use efficiency, reduces soil toxicity, minimizes the potential negative effects associated with over dosage and reduces the frequency of the application. Therefore, nanotechnology has a high potential for achieving sustainable agriculture, especially in developing countries (Tulasi *et al.* 2015). The nano fertiliser refers to a product that delivers nutrients to crops by one of three ways; the nutrients can be encapsulated inside nanomaterials such as nano-tubes or nano-porous materials, coated with a thin protective polymer film and/or delivered as particles or emulsions of nano-scale dimensions. Owing to a high surface area to volume ratio the effectiveness of nano-fertilisers may surpass the most innovative polymer-coated conventional fertilisers, which have seen little improvement in the past ten years (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

Table 1

Some physical and chemical properties of the experimental site

Physical properties		Chemical properties	
Particle size distribution [%]		Electrical conductivity (EC) [dS/m]	1.68
Coarse sand 2000 − 200 µm	80.20	pH (1:2.5) soil : water suspension	7.68
Fine sand 200 − 20 µm	12.50		
Silt 20 − 2 µm	4.25	Soluble cations [meq/l]:	
Clay < 2 µm	3.05	Ca^{2+}	5.20
Bulk density [g/cm³]	1.52	Mg^{2+}	4.18
		K^{+}	2.40
Total porosity [%]	52.80	Na^{+}	5.20
Pore size distribution as % of total porosity		Soluble anions [meq/l]:	
Macro (drainable) pores (> 28.8 µ)	82.98	CO_3^{2-}	0.00
Micro pores (< 28.8 µ)	17.02	HCO^{3-}	1.70
Water Holding Capacity (WHC)*	20.33	Cl^{-}	3.60
Field capacity (FC)*	8.55	SO_4^{2-}	11.50
Wilting percentage (WP)*	4.10	Total $CaCO_3$ [%]	0.20
Available moisture (FC-WP)*	4.45	Organic matter [%]	0.20
Hydraulic conductivity [cm/h]	6.25		

*on weight basis

Lamiaceae and its essential oil is used in industry as food preservation. Sage has been used as a gargle for sore throat, to fight gastroenteritis and other infections, to improve lipid status and liver function in general, to improve appetite and digestion, and to improve mental capacity (Jakovljevi *et al.* 2019). Sage essential oil is a complex mixture of compounds including monoterpenes, sesquiterpenes, and diterpenes. The chemical composition of *S. officinalis* would be varied depending on the environmental conditions such as climate, water availability (Ghorbani & Esmaeilizadeh 2017).

The present research aims to examine the effect of nano NPK elements individually or in combination with nano zeolite loaded nitrogen or not on a sage plant grown under drought stress compared to commercial NPK fertilisers at the new reclaimed area.

MATERIAL AND METHODS

The current research was carried out in the open nursery at the new reclaimed area, Wadi El-Notron, Beheira Governorate (Longitude 28°54' E, Latitude 28°20 N and Altitude 130 m), Egypt, during two consecutive seasons (2018 and 2019). The soil physical and chemical analyses were performed according to Jackson (1973) as shown in Table 1 at Soil, Water and Environment Research Institute, Agriculture Research Centre (A.R.C).

Plant material, transplant and harvest dates

The seedlings of sage plants (≈ 13 to 15 cm tall with intact roots) were obtained from experimental farm of Faculty of Pharmacy, Cairo University, and planted on 21st of February, 2018 with 50 cm apart between rows, and 40 cm spacing between plants in plots (3×5 m^2). The plants were irrigated as the farmer does for 2 months using a drip irrigation system. Then the plants were exposed to water stress since they irrigated every 15 days. The first cut was taken on October 15th 2018 and the second one was taken on 20th June 2019.

Land preparation

Two weeks before planting, 2.5 ton/fed. (Fadden = 4,200 m^2) of organic matter (Table 2) was added to the soil; the soil was first mechanically ploughed and planked twice till the soil surface has been settled, then plots were established.

Fertilisers application

Chemical fertilisers in the recommended doses by the Ministry of Agriculture and Land Reclamation were added at the rate of 150 kg/fed. Ammonium sulphate (20.5% N), was divided into two doses; the first was added 2 weeks after planting, while the second was four weeks later. Both calcium superphosphate (15.5% P) at the rate of 150 kg/fed. and potassium sulphate (48% K) at the rate of 40 kg/fed. were added one day before planting.

Table 2

The chemical composition of used organic matter

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	2.5×10^7	2.5×10^4	20	0	1.82	1.25	1.06	18.1	784.12	96.31	31.05	251.23

Zeolite loaded nitrogen

Nano zeolite was synthesized (A.M.) according to (Hassan *et al.* 2015) then loaded nitrogen (Table 3) by soaking in 1M ammonium sulphate solution for 5 days (Junxi Li *et al.* 2013) then exposed to an open area (25°C) to be dried (Figure 1). The total N content was analyzed using the Kjeldahl digestion method (Helrich 1990). Both nano-zeolite and nano-zeolite loaded nitrogen were applied with drip irrigation (30 g/L/month) 4 times consistently from the planting date.

Synthesis of NPK nanoparticles

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 18 hours at room temperature in two-steps processes. In the first step, 0.23 g chitosan was dissolved in methacrylic acid aqueous solution (0.5%, v/v) for 5 h 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 h 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 were used separately. The loading of N fertilisers in Chitosan nanoparticles was obtained by dissolving of 2M N into 100 ml of chitosan nanoparticle solution under magnetic stirring for 8 h at 25°C, subsequently dried at 50°C for 72 h. The following concentrations of 1,000 ppm for each of N, P and K were finally obtained in each solution. The resulting solutions had a pH of 5.5.

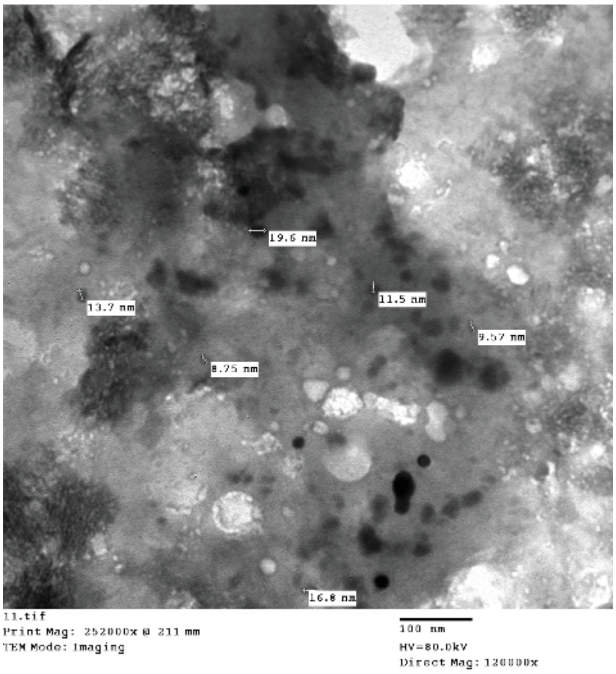

Figure 1. The image of nano-zeolite loaded nitrogen

The particles were uncontrolled in shape with a size range from 6.25 to 6.57 nm for nitrogen (Figure 2), from 5.30 to 12.3 nm for phosphorus (Figure 3), from 7.99 to 15.3 nm for potassium (Figure 4) and from 44.2 to 54.3 nm for mixed NPK (Figure 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

Table 3

The 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	–	–

nanoparticles were determined directly from the figure using an Image-Pro Plus 4.5 software. The value is an average size of three parallels.

Nitrogen, phosphorus and potassium were applied either individually or mixed as foliar monthly for 4 months continuously from planting date.
The treatments were as follows:

- NPK fertilisers (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

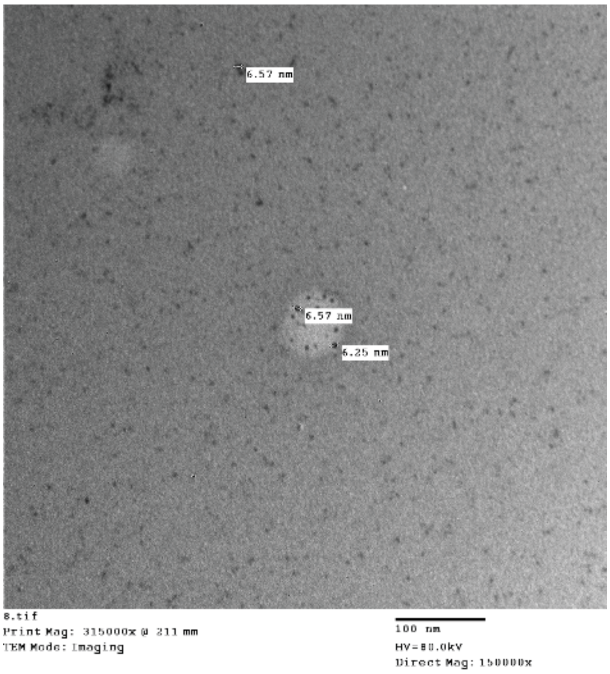

Figure 2. The image of nano-nitrogen

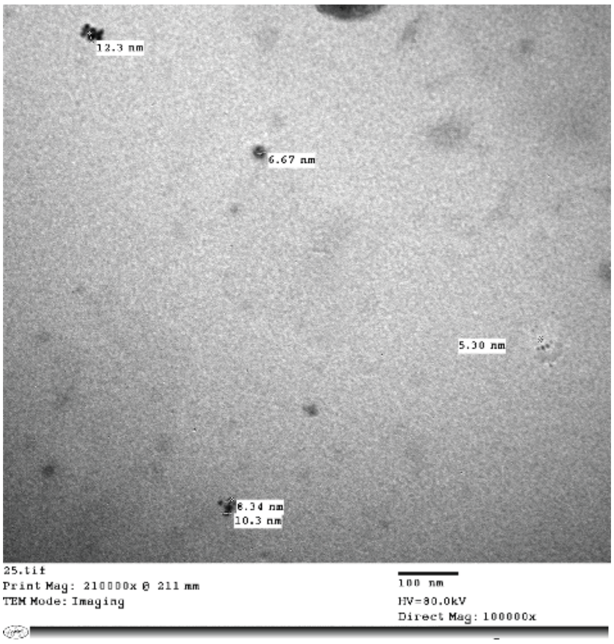

Figure 3. The image of nano-phosphorus

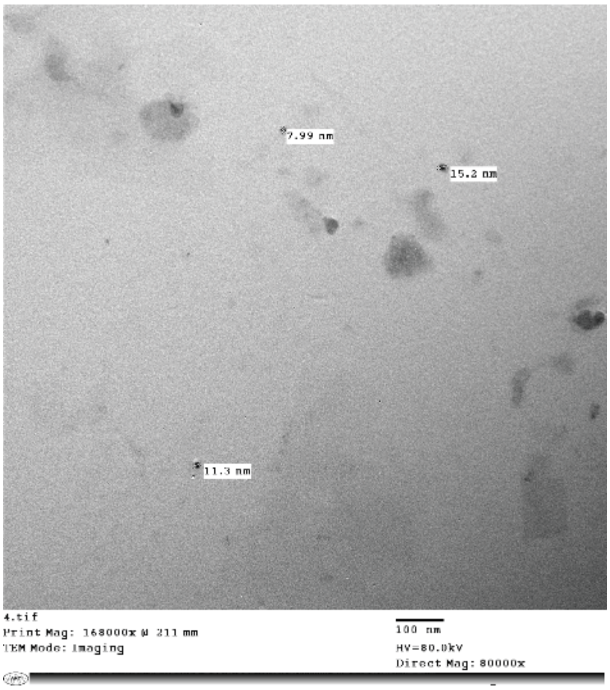

Figure 4. The image of nano-potassium

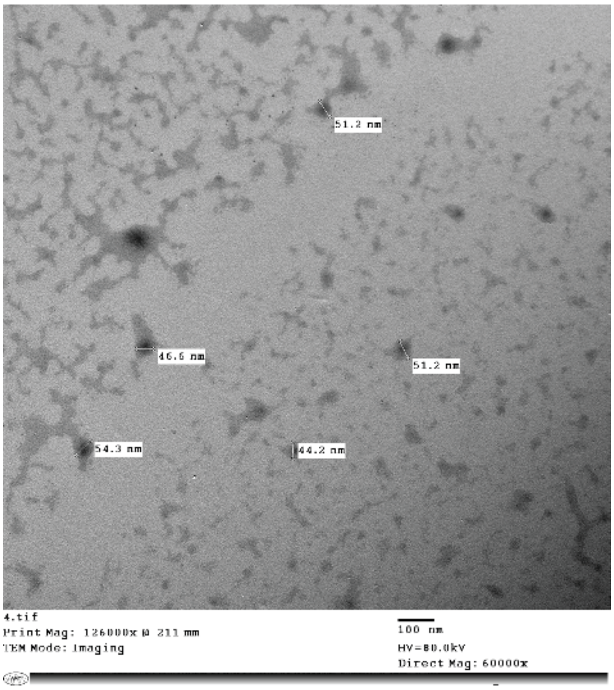

Figure 5. The image of nano-NPK

Data recorded

A. Vegetative growth parameters: plant height [cm], branches number per plant, herb fresh weight [g/plant], herb dry weight [g/plant], leaf area [cm^2], leaf/stem ratio, yield fresh weight [t/ha], health index, oil yield [L/ha]

B. Chemical analysis

Total chlorophylls concentrations [%] were measured using the spectrophotometer and calculated according to Moran (1982).

Total carbohydrates concentrations [%] in leaves was determined as reported by Helrich (1990).

Net photosynthesis, stomatal conductance and water use efficiency

Measurements of net photosynthesis in leaves on an area basis [$\mu mol/CO_2/m^2/s$], leaf stomatal conductance [$mol/H_2O/m^2/s$], and water use efficiency of five different leaves per treatment was monitored using a LICOR 6400 (Lincoln, Nebraska, USA) infrared gas analyzer (IRGA). Light intensity (Photosynthetically active radiation, PAR) within the sampling chamber was set at 1,500 $\mu mol/m^2/s$, using a Li-6400-02B LED light source (LI-COR). The CO_2 flow into the chamber was maintained at a concentration of 400 µmol/mol using an LI-6400-01 CO_2 mixer (LI-COR).

Relative water content (RWC) was determined by the method outlined by Weatherly (1951).

The total nitrogen content of the dried leaves was determined as described by Helrich (1990).

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

Potassium and sodium concentrations were determined in dried leaves using the flame photometer apparatus (CORNING M 410, Germany).

Calcium, magnesium, iron, manganese, boron, and zinc concentrations were determined using Inductively Coupled Plasma Emission Spectrometer "ICP" The Agilent 720/730 series US).

Total phenolics contents of the leaves extracts were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton & Rossi 1965).

Tannin contents were determined using Folin-Ciocalteu reagent method as described by Chahardehi *et al.* (2009).

Total flavonoids concentrations were determined in leaves using the method of Meda *et al.* (2005).

Endogenous phytohormones

Freeze-dried plant leaves (equivalent 5 g FW) were ground to a fine powder within a mortar and pestle the analysis was performed according to Fales *et al.* (1973) for the determination of indole-acetic acid (GA_3) and abscisic acid (ABA). 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). Quantification of phytohormones were performed by using external authentic hormones and a Microsoft program to calculate the concentrations of the identified peaks.

Peroxidase following the method of Macheix and Quessada (1984).

Superoxide dismutases was measured as described by Dhindsa *et al.* (1981).

The essential oil in leaves

The leaves of *Salvia officinalis* were hydro-distilled for 3 hours with a Likens–Nickerson-type apparatus, using diethyl ether to get yellowish oil yield.

Gas chromatography

GC–MS was carried out with a Hewlett-Packard with a flame ionization detector (FID) on a capillary column (TC-WAX FFS fused silica 60 m ´ 0.25 mm i.d). The column temperature was programmed from 60°C to 240°C at a rate of 3°C/min and held at 240°C. The injector and detector temperatures were 250°C and 280°C, respectively.

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. The difference between treatments then analysed using DMRT (Duncan Multiple Range Test) at a 5%.

RESULTS AND DISCUSSION

A. Growth parameters

The acquired data from sage plant growth characters are represented in Table 4. It divulged that both treatments nano zeolite loaded nitrogen (T7) and nano NPK (T5) significantly enhanced the growth parameters compared to all other treatments. The increments in each of plant height were 72 and 78%, branches number 162 and 239%, herb fresh weight 156 and 178%, and herb dry weight 133 and 184% in the 1st and 2nd season, respectively as a result of T7 implementation compared to control treatment (T1).

The same trend was obtained with the other parameters as shown in Table 5. The T7 application gave significantly an increment in each of leaf area 49 and 76%, leaf/stem ratio 122 and 138%, yield fresh weight 138 and 179% and health index 114 and 244% in the 1st and 2nd season, respectively judged against control treatment (T1). Concerning oil yield, T7 donated significantly higher oil yield than all other treatments during both seasons. The augmentation values were 220 and 255% in the 1st and 2nd season, respectively compared to control treatment (T1).

It is worth to mention that, there were insignificant differences in response to most plant growth characters between T7 and T5 treatments applica-

Table 4

Effect of different treatments on growth characters of *Salvia officinalis* during season 2018 and 2019

Treatments		Plant height [cm]		Branches number/plant		Herb fresh weight [g/plant]		Herb dry weight [g/plant]	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control (NPK)	T1	24.6^{d}	22.7^{d}	5.87^{d}	4.21^{c}	23.68^{d}	20.61^{e}	6.92^{c}	4.96^{c}
Nano-N	T2	33.5^{b}	29.3^{b}	10.50^{b}	9.31^{b}	42.11^{b}	39.73^{c}	9.78^{b}	8.05^{b}
Nano-P	T3	28.6^{c}	25.8^{c}	8.41^{c}	8.10^{b}	33.79^{c}	30.18^{d}	8.29^{b}	6.64^{b}
Nano-K	T4	27.5^{c}	23.7^{c}	9.30^{c}	8.77^{b}	31.53^{c}	28.44^{d}	7.06^{c}	5.10^{c}
Nano-NPK	T5	43.5^{a}	38.2^{a}	14.66^{a}	12.50^{a}	58.42^{a}	50.36^{b}	16.05^{a}	13.88^{a}
Nano-Zeolite	T6	35.4^{b}	31.5^{b}	11.37^{b}	9.42^{b}	40.98^{b}	40.22^{c}	9.55^{b}	8.77^{b}
Nano-Zeolite + N	T7	42.3^{a}	40.5^{a}	15.42^{a}	14.30^{a}	60.61^{a}	57.34^{a}	16.11^{a}	14.09^{a}

Means with the same letter in a column are not significantly different by DMRT 5%
1st – first season; 2nd – second season

Table 5

Effect of different treatments on growth characters of *Salvia officinalis* during season 2018 and 2019

Treatments		Leaf area/cm^2		Leaf/stem ratio		Yield fresh weight [t/ha]		Health index		Oil yield [L/ha]	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control (NPK)	T1	9.88^{c}	8.12^{c}	2.37^{d}	2.12^{c}	1.51^{d}	1.22^{d}	1.14^{c}	0.89^{d}	5.42^{e}	5.08^{e}
Nano-N	T2	10.57^{b}	10.40^{b}	3.62^{c}	3.02^{b}	2.69^{b}	2.53^{b}	1.54^{b}	1.45^{c}	10.12^{b}	8.79^{c}
Nano-P	T3	8.91^{d}	8.48^{c}	3.45^{c}	2.94^{c}	2.27^{b}	1.84^{c}	1.24^{b}	1.10^{c}	7.21^{c}	6.92^{d}
Nano-K	T4	8.43^{d}	8.30^{c}	2.86^{d}	2.30^{c}	2.05^{c}	1.63^{c}	1.05^{c}	0.88^{d}	6.83^{d}	5.81^{e}
Nano-NPK	T5	13.39^{a}	12.51^{a}	5.31^{a}	4.25^{a}	3.79^{a}	3.33^{a}	2.34^{a}	2.24^{b}	17.33^{a}	15.48^{b}
Nano-Zeolite	T6	12.65^{a}	12.77^{a}	4.87^{b}	4.53^{a}	2.67^{b}	2.62^{b}	1.45^{b}	1.50^{c}	10.26^{b}	9.15^{c}
Nano-Zeolite + N	T7	14.69^{a}	14.36^{a}	5.28^{a}	5.06^{a}	3.60^{a}	3.41^{a}	2.44^{a}	3.06^{a}	17.36^{a}	18.08^{a}

Means with the same letter in a column are not significantly different by DMRT 5%
1st – first season; 2nd – second season

tion 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 shown that when plants are subjected to drought, they exhibit reductions in growth traits correlated with the degree of stress severity (Scarascia-Mugnozza *et al.* 1996; Nayyar & Gupta 2006).

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

Hence increment of volatile oil productivity with T7 treatment could be explained on the basis of available elements, vitamins, gibberellins, cytokines, hormone-like substances, amino acids and sugars that lead to an increase in biochemical processes within the plant (the luxury of metabolism) consequently an increase in volatile oil content.

Photosynthetic rate, stomatal conductance, 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 (Tables 6 and 7) undeniably revealed that plants under T7 treatment significantly donated higher values for each of net photosynthesis rate 25 and 33%, stomatal conductance 8 and 8.5%, intercellular CO_2 concentration 7 and 9%, water use efficiency 90 and 83% and relative water content 9 and 12% in the 1st and 2nd season, respectively.

Despite of treatments, photosynthesis rate values were the highest in the 1,200 hours and could be referred to the significant availability of photosynthetic active radiation throughout the examined period. There is a positive relationship between photosynthesis rate and stomatal conductance where the higher stomatal conductance increased photosynthesis rate.

Subject to confirmation drought stress-induced stomatal closure, hence the uptake of CO_2 notably decreased intercellular (Cronic 2000), as a result of the consumption of NADPH+H for the CO_2 fixation via Calvin-cycle turn downs remarkably. But the 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 could be diagnostic to the straight suppression of biochemical processes through ionic, osmotic or other conditions were induced by the diminishing of cellular water. A few other reasons that contributed to this diminish might be the limited CO_2 diffusion into the intercellular spaces of the leaf as the end result of reduced stomatal conductance (Lawlor 2002).

Commonly water use efficiency for crops is a principally vital consideration as long as irrigation water resources are inadequate or diminishing and rainfall is a restricting factor as the condition of arid and semi-arid areas including Egypt. Moreover, the main constituent of a management system 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 plants are in harmony with those obtained by Melaine *et al.* (2010) and Filipa *et al.* (2016) on sage plants, Mahmoud and Soliman (2017) on evening primrose plant and Mahmoud *et al.* (2017) on *Carum carvi.*

B. Chemical analysis

I. *Macro and micro-elements*

As declared of growth parameters data, the results of chemical analysis (Tables 8 & 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 were significantly increased as a result of nano zeolite loaded nitrogen application (T7). The augmentations were 78 and 54% for nitrogen, 21 and 26% for phosphorus, 8 and 18% for potassium, 13 and 14% for calcium and 64 and 61% for magnesium in the 1st and 2nd season, respectively compared to control treatment T1. On the other hand, there was the discrepancy between sodium concentration and earlier macro-elements, where control treatment T1 gave a significant increase in sodium concentration compared to all other treatments particularly T7 treatment.

Come into the sight of micro-elements, it was observed, that the augmentations were 29 and 40% for zinc, 95 and 88% for iron, 59 and 83% for manganese and 19 and 22% for boron in the 1st and 2nd season, respectively in the favour of T7 compared to the control T1.

It has been known that existing N in soil is strongly related to the capacity of plant roots to absorb water from the soil. Nevertheless few types of soils are

Table 6

Effect of different treatments on photosynthetic rate, stomatal conductance and CO$_2$ concentration of *Salvia officinalis* during season 2018 and 2019

Treatments		Photosynthetic rate [µmol/m^2/s]		Stomatalconductance [mmol/m^2/s]		Intercellular CO$_2$ concentration [ppm]	
		1st	2nd	1st	2nd	1st	2nd
Control (NPK)	T1	17.24±1.03^c	17.08±1.06^b	258.3±2.13^c	250.6±1.05^c	153.3±1.05^b	150.8±1.22^b
Nano-N	T2	18.64±1.29^b	18.22±1.90^b	263.8±3.07^c	255.1±1.12^b	150.4±2.11^b	147.6±2.04^b
Nano-P	T3	18.18±1.79^b	17.88±1.52^b	274.2±2.11^a	267.8±1.15^a	146.7±2.09^c	141.9±1.34^c
Nano-K	T4	17.28±1.12^c	16.89±1.64^c	270.3±2.30^b	258.2±1.26^b	144.8±3.01^c	144.2±1.78^c
Nano-NPK	T5	20.85±1.62^a	21.85±1.27^a	273.6±1.23^a	269.5±1.23^a	160.8±2.91^a	159.5±2.11^a
Nano-Zeolite	T6	18.67±1.57^b	17.96±1.78^b	275.6±1.40^a	262.3±1.34^b	152.1±2.13^b	150.7±2.41^b
Nano-Zeolite + N	T7	21.53±1.25^a	22.76±1.33^a	278.5±3.25^a	271.8±1.22^a	164.5±3.02^a	165.2±2.37^a

Means with the same letter in a column are not significantly different by DMRT 5%
1st – first season; 2nd – second season

Table 7

Effect of different treatments on transpiration rate, water use efficiency and relative water content (RWC) of *Salvia officinalis* during season 2018 and 2019

Treatments		Transpiration rate [mmol m^2/s]		Water-use efficiency [µmol/mmol]		RWC [%]	
		1st	2nd	1st	2nd	1st	2nd
Control (NPK)	T1	3.81±1.03^a	3.86±1.11^a	4.52^b	4.42^b	83.06^d	80.89^b
Nano-N	T2	3.52±1.02^a	3.60±1.09^a	5.29^b	5.06^b	87.42^b	85.47^a
Nano-P	T3	3.32±1.11^a	3.41±1.16^a	5.47^b	5.24^b	85.59^c	83.71^b
Nano-K	T4	2.91±1.07^b	3.12±1.09^a	5.93^b	5.41^b	83.89^d	81.55^b
Nano-NPK	T5	3.54±1.05^a	3.62±1.25^a	5.88^b	6.03^a	90.06^a	87.91^a
Nano- Zeolite	T6	3.15±1.12^a	3.22±1.20^a	5.91^b	5.57^b	88.31^b	86.35^a
Nano-Zeolite + N	T7	2.50±1.17^b	2.81±1.22^b	8.61^a	8.09^a	91.03^a	90.62^a

Means with the same letter in a column are not significantly different by DMRT 5%
1st – first season; 2nd – second season

poor in N content especially in arid or semi-arid regions and the new reclaimed area (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 (Heckathorn *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, it was noticed an increase of micronutrients uptake such as Zn, Cu, Mn, and Fe (Bagayoko *et al.* 2000). If truth be told, soil conditions and particularly fer-

Table 8

Effect of different treatments on N, P, K, Ca and Na of *Salvia officinalis* during season 2018 and 2019

Treatments		N [%]		P [%]		K [%]		Ca [%]		Na [%]	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control (NPK)	T1	1.35^{d}	1.72^{c}	0.78^{b}	0.82^{c}	2.89^{b}	2.85^{b}	1.55^{b}	1.58^{b}	3.02^{a}	2.61^{a}
Nano-N	T2	1.55^{b}	1.80^{b}	0.77^{c}	0.76^{d}	2.75^{b}	2.79^{b}	1.48^{c}	1.50^{c}	2.89^{b}	2.37^{b}
Nano-P	T3	1.44^{c}	1.38^{d}	0.80^{b}	0.80^{c}	2.68^{b}	2.70^{b}	1.40^{d}	1.48^{c}	2.79^{b}	2.40^{b}
Nano-K	T4	1.33^{d}	1.40^{c}	0.75^{c}	0.77^{d}	3.04^{a}	3.09^{a}	1.46^{c}	1.53^{c}	3.00^{a}	2.55^{a}
Nano-NPK	T5	2.05^{a}	1.83^{b}	0.81^{b}	0.86^{b}	3.00^{a}	3.12^{a}	1.77^{a}	1.78^{a}	2.12^{c}	2.03^{c}
Nano-Zeolite	T6	1.33^{d}	1.48^{d}	0.76^{c}	0.81^{c}	2.61^{b}	2.90^{b}	1.50^{b}	1.55^{b}	2.09^{c}	1.69^{d}
Nano-Zeolite + N	T7	2.41^{a}	2.65^{a}	0.94^{a}	1.03^{a}	3.11^{a}	3.36^{a}	1.76^{a}	1.80^{a}	2.07^{c}	1.44^{d}

Means with the same letter in a column are not significantly different by DMRT 5%
1st – first season; 2nd – second season

Table 9

Effect of different treatments on Mg, Zn, Fe, Mn and B of *Salvia officinalis* during season 2018 and 2019

Treatments		Mg [%]		Zn [ppm]		Fe [ppm]		Mn [ppm]		B [ppm]	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control (NPK)	T1	0.39^{b}	0.44^{b}	97.6^{b}	95.2^{d}	166.8^{c}	160.2^{e}	82.5^{b}	70.6^{c}	30.7^{b}	25.8^{b}
Nano-N	T2	0.38^{b}	0.41^{c}	91.8^{c}	90.5^{d}	189.7^{b}	181.3^{d}	85.1^{b}	78.6^{b}	22.5^{c}	17.7^{c}
Nano-P	T3	0.37^{b}	0.40^{c}	90.4^{c}	89.2^{d}	190.7^{b}	178.8^{d}	81.4^{b}	75.4^{b}	23.4^{c}	19.8^{c}
Nano-K	T4	0.38^{b}	0.43^{c}	93.5^{c}	85.4^{d}	193.5^{b}	180.5^{d}	80.3^{b}	77.2^{b}	24.7^{c}	20.4^{c}
Nano-NPK	T5	0.60^{a}	0.65^{a}	122.5^{a}	100.7^{c}	201.6^{b}	193.2^{c}	89.5^{b}	81.3^{b}	25.1^{c}	22.8^{c}
Nano-Zeolite	T6	0.40^{b}	0.49^{b}	100.3^{b}	119.8^{b}	307.2^{a}	256.8^{b}	135.2^{a}	125.3^{a}	31.8^{b}	27.7^{b}
Nano-Zeolite + N	T7	0.64^{a}	0.71^{a}	125.7^{a}	133.5^{a}	325.2^{a}	301.4^{a}	131.5^{a}	129.5^{a}	36.6^{a}	31.5^{a}

Means with the same letter in a column are not significantly different by DMRT 5%
1st – first season; 2nd – second season

tility 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 the T7 application may be owing to the extent in the root surface per unit of soil volume which in turn contribute greatly to the enhance of nutrient uptake (Ghallab & 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 fertilisers to be more effective by preventing leaching and holding important elements (NH4, K, Mg and Ca) as well as trace nutrients as slow-release upon required (Kallo *et al.* 1986). Supportive evidence for these results was reported by Safaei *et al.* (2014) who mentioned that application of nano-fertilisers and humic acid improve N content, sativa performance and diminished environmental pollution. So, it could be used as a natural material to increase and stabilize field crop production.

II. *Chlorophyll content, total phenolic, total carbohydrates, tannins and total flavonoids*

Data represented in Table 10 indicated that chlorophyll content, total phenolic, total carbohydrates and total flavonoids were significantly recorded the highest amount 49 and 46%, 25 and 36%, 39 and 45% and 23 and 20% in the 1st and 2nd season, respectively as a result of T7 treatment application compared to control treatment (T1). Contrarily, in the case of tannins, the application of nano-NPK (T5) significantly surpassed all other treatments where the increment was 6 and 6.2% in the 1st and 2nd season, respectively compared to (T1)

The elevated amount in total chlorophyll content might be due to the beneficial effects of nano-zeolite loaded nitrogen T7 on plant pigments. Since it liberates more nutrients from the unavailable reserves as correcting iron and zinc deficiency in sandy soil caused efficient photosynthesis process and available macro and micronutrients. Moreover, their role in increasing root surface per unit of soil volume as well as the high capacity of the plants building metabolites, which in turn contribute much to the increase of nutrient uptake (Tisdale *et al.* 1985). While increment in total carbohydrate, total phenolic and total flavonoids might be due to the increase of photosynthesis as a result of the increase in photosynthetic pigments content in leaves. Also could be refer to the fact that zeolite with cages structure together with a depiction of the straight and zigzag channel appears to be a source of water retention and number of essential elements that might play an im-

Table 10

Effect of different treatments on chlorophyll, total phenolic, total carbohydrates, tannins and total flavonoids of *Salvia officinalis* during season 2018 and 2019

Treatments	Chlorophyll content [mg/g FW]		Total phenolic [mg GAE/g DW]		Total carbohydrates [%]		Total tannin [g/kg]		Total flavonoids [mg/g]	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control (NPK) T1	4.32^{b}	4.45^{b}	33.41^{b}	32.45^{c}	13.5^{b}	12.8^{c}	139.7^{b}	142.3^{b}	57.8^{b}	62.7^{b}
Nano-N T2	5.12^{a}	5.32^{a}	34.12^{b}	35.61^{b}	15.6^{b}	13.5^{b}	130.9^{c}	129.5^{d}	60.2^{b}	63.6^{b}
Nano-P T3	4.35^{b}	4.66^{b}	32.09^{b}	37.20^{b}	12.8^{c}	13.2^{b}	133.6^{c}	130.7^{d}	55.6^{c}	59.2^{c}
Nano-K T4	4.28^{b}	4.59^{b}	35.71^{b}	40.25^{a}	14.7^{b}	15.6^{b}	136.5^{b}	134.4^{c}	61.1^{b}	63.8^{b}
Nano-NPK T5	6.05^{a}	6.26^{a}	38.55^{a}	37.41^{b}	15.2^{b}	17.3^{a}	148.2^{a}	151.3^{a}	68.5^{a}	72.3^{a}
Nano- Zeolite T6	5.87^{a}	5.80^{a}	32.11^{b}	30.84^{c}	13.1^{b}	12.8^{c}	135^{b}	131.6^{d}	58.3^{b}	60.1^{b}
Nano-Zeolite+ N T7	6.43^{a}	6.51^{a}	41.89^{a}	44.09^{a}	18.8^{a}	18.6^{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 – first season; 2nd – second season

portant role in plant metabolism, conspicuously the most significant function would appear to involve carbohydrate metabolism and photosynthesis and drought resistance as aftereffect (Tisdale & Nelson 1975). On the ground of previous data, all the secondary metabolites might 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, a medium-intensity of drought stress increases the accumulation of flavonoids and tannins, as reported by (Yang & Li 2011).

Drought stress has a strong influence on secondary metabolic pathways accountable for the accumulation of secondary products. Nevertheless, it was mentioned that the corresponding consequences are not definite and a thorough review might 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 shown that plants which are exposed to water stress produce higher amounts of secondary metabolites. Unemotional, in comparison to nano-fertilisers as an external source of nutrients in the form of chemical fertilisers donate more than 50% of crop productivity enhancement. But the nutrient use efficiency by crops is near to the ground due to the loss of nutrients through fixation, leaching, volatilization and microbial mineralization (Samra & Sharma 2009). Similar results were obtained by Mahmoud *et al.* (2017) on *Carum carvi*, Ramesh *et al.* (2016) on watermelon and Mahmoud *et al.* (2019) on radish plants.

III. *Phytohormones and enzymes*

Focus on hormonal and enzymes analysis of sage plants as shown in Figures 6 and 7. The obtained data clearly revealed that the application of T7 treatment significantly recorded the highest amount of gibberellic acid (GA_3) in plant tissues since it was 43 and 58% in the 1st and 2nd season, respectively (Figure 6) 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. While the lowest amount of ABA resulted from the T7 application since it was 25 and 47% in the 1st and 2nd season, respectively compared to control treatment (T1).

In this connection, enzymes data represented in Figure 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 their values were 86 and 104%, respectively for both seasons.

The aforementioned data might explain that 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 adverse effects of drought were significantly alleviated by the application of T7 treatment represented in the low amount of ABA within plant tissues and high amounts of antioxidant system activation symbolized in peroxidase and superoxide dismutase enzymes which increase under

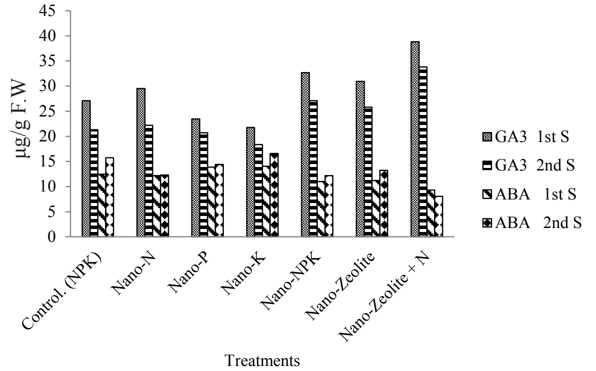

Figure 6. Effect of different treatments on GA_3 and ABA hormones through both seasons

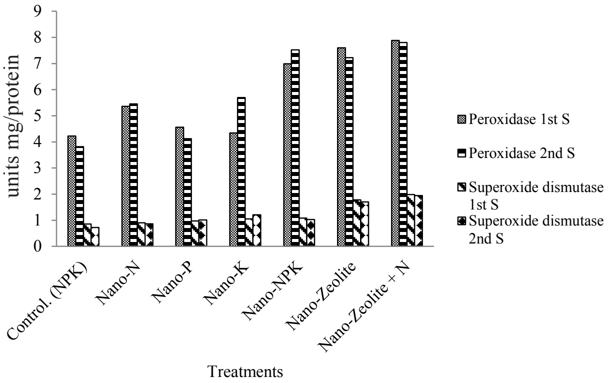

Figure 7. Effect of different treatments on peroxidase and superoxide dismutase enzymes through both seasons

water stress (Li *et al.* 2006). It has a vital task in the defense against damage by oxygen radicals (Kardish *et al.* 1994) accompanied with a high level of GA_3. 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 the high concentration of GA_3 with T7 treatment might have an 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 might be attributed to synergic interaction between ABA in response to drought stress appeared.

For these reasons applied of T7 treatment during two seasons might have enhanced endogenous phytohormones accumulation represented in GA_3 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 above-mentioned results are in consonance with those obtained by Meijón *et al.* (2011), Gill *et al.* (2011) and Mahmoud and Soliman (2017).

IV. *Volatile oil components*

The data of *Salvia officinalis* L. essential oil (yellowish liquid with a warm camphoraceous) components resulted from G.C. mass analysis is represented in Table 11 and can be discussed as follows – among identified components, it was found that the utmost amount of *α-Pinene* content (3.87 and 3.71%) was resulted from the application of T7 treatment while the lowest amount (0.10 and 0.29%) in the 1st and 2nd season, respectively outputted from T6 treatment. Regarding *β-Pinene*, it was recorded the highest amount with T5 treatment application (4.05 in the 1st and 4.16% in the 2nd season,) whereas the lowest amount presented in the first season

Table 11

Effect of different treatments on essential oil components of *Salvia officinalis* during season 2018 and 2019

Component [%]	Control (NPK) T1		Nano-N T2		Nano-P T3		Nano-K T4		Nano-NPK T5		Nano-Zeolite T6		Nano-Zeolite + N T7	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
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
ɣ-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

(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 and 17.81%) respectively for both seasons from T6 treatment. As for *Camphor*, it was found that T4 treatment application donated the highest amount (33.01 and 33.52% in the 1stand 2nd season, respectively).

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

Variation in the oil constituents' amounts of extracted essential oil might be due to one or combined different factors mentioned 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. The essential oil terpene compounds concentration in stressed leaves possibly would 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 data presented in this research support the hypothesis that the substances of secondary plant products could 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 fertilisers), which consider the reason of raised biomass production also, might be accompanied by reduced concentrations of relevant essential oil compounds. These results concurred with those of Mahmoud *et al.* (2017) on *Carum carvi* and Mahmoud and Taha (2018) on *Eruca sativa*.

CONCLUSIONS

The application of nano-zeolite-loaded nitrogen (T7) and mixture of nano nitrogen, phosphorus and potassium (T5) resulted in positive outcomes on both plant under study sage (*Salvia officinalis*) and environment, represented in; high growth characteristics, chemical composition of the plant and reduce water stress effects as well in contrast to results derived from commercial dose of chemical fertilisers NPK (T1). Therefore taking into account both quality and quantity parameters with both nano-fertilisers and nano soil amendments application are very important factors particularly with medicinal and aromatic plants regardless economic costs 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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