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# Response of *Salvia officinalis* to zinc and silicon nanoparticles and pollen extract as alternates to traditional fertilizers

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*Salvia officinalis* is used in a variety of medicinal and aromatic products. The effects of various treatments on sage (*Salvia officinalis*) plants were investigated in an open-field experiment conducted between 2021 and 2022. During the experiment, ZnO nanoparticles (NPs) were used at concentrations of 1.0 and 1.5 g/L, SiO<sub>2</sub> NPs were used at concentrations of 0.1 and 0.2 g/L, and date palm pollen extracts (DPE) were used at concentrations of 15 and 25 g/L, in combination with NPK fertilizers at 75%, 50%, and 25%, respectively, with a control group of 100% NPK fertilizer. A treatment consisting of 75% NPK, 15 g/L DPE, 1.0 g/L ZnO NPs, and 0.1 g/L SiO<sub>2</sub> NPs significantly improved vegetative traits and essential oil yield. Compared to the control in the growing seasons of 2021 and 2022, this treatment resulted in increases in plant height, chlorophyll index, fresh and dry weights, and essential oil yield (EOY) per plant of 23.40% and 28.30%, 27.56% and 26.54%, 42.17% and 42.95%, 64.10% and 62.79%, and 93.38% and 91.08%, respectively. Combinations of 25% NPK + 25 g/L DPE + 1.5 g/L ZnO nanoparticles + 0.2 g/L SiO<sub>2</sub> NPs and 75% NPK + 0.1 g/L SiO<sub>2</sub> NPs produced the highest essential oil percentage (EO%). During the experimental seasons, these treatments increased EO% by 15.45% and 26.25%. In total, 58 substances were identified across the different treatments in the essential oil composition analysis. There were 11 compounds in the 25% NPK, 25 g/L DPE, 1.5 g/L ZnO NPs, and 0.2 g/L SiO<sub>2</sub> NPs treatments, and 32 in the 50% NPK, 25 g/L DPE, and 0.2 g/L SiO<sub>2</sub> NPs treatments. Oxygenated hydrocarbons, sesquiterpenes, and monoterpenes varied by application. Thujone, camphor, manool, and ledol were the major constituents of the EO. Leaf chemical composition, antioxidant activity, and total phenolic compounds were significantly influenced by the treatments. In

combination with DPE, ZnO and SiO<sub>2</sub> NPs reduced the need for higher amounts of mineral NPK fertilizers. These agents can therefore be useful for advancing sustainable agricultural practices in novel and advantageous ways.

#### KEYWORDS

date pollen, macroelements, sage plant, Si NPs, Zn NPs

## 1 Introduction

*Salvia officinalis* L. (commonly known as sage, kitchen sage, garden sage, culinary sage, or common sage) belongs to the family Lamiaceae. This Mediterranean plant is cultivated globally for its culinary and medicinal applications, making it one of the most prominent pharmaceutical herb (Khare et al., 2020). It is used as a native styptic, diuretic, antiseptic, tonic, anti-inflammatory, antifungal, menstruation promoter, and for spasmodic pain relief (Amer et al., 2019). The leaves are highly aromatic, and their essential oil contains more than 49 aromatic constituents, which can be useful as natural agents in cosmetics, food preservation, and pharmaceutical products (Prakash et al., 2015). Factors such as habitat, plant organ and age, genetic systems, stage of harvest (Maksimović et al., 2007), and soil conditions (Esetlili et al., 2016) strongly affect the sage essential oil percentage and chemical composition.

Nitrogen (N), phosphorus (P), and potassium (K) are essential macronutrients, frequently used in fertilization due to their critical roles in plant cell metabolism, enzymatic activity, and various physiological, chemical, and biochemical processes (Hawkesford et al., 2012). However, excessive use of chemical fertilizers can degrade soil quality, eutrophicate water bodies, and pollute air and groundwater (Congreves and Van Eerd, 2015). Furthermore, the overuse of chemical fertilizers can result in inefficient nutrient utilization and habitat disruption, posing significant challenges to sustainable agriculture. In several regions worldwide, soils have become non-responsive to NPK fertilizers, due to the decreased application of trace elements (Vanlauwe et al., 2010; Jones et al., 2013). The reduced nutrient use efficiency and environmental concerns associated with chemical fertilizer application remain significant challenges, hindering the achievement of sustainable agriculture. The use of nano-elements has been shown to enhance fertilizer efficiency (Lu et al., 2016). Zn in particular interacts with several soil elements, notably nitrogen (N) and phosphorus (P) (Loneragan and Webb, 1993). A deficiency of Zn in the soil is often linked to higher levels of available phosphorus (Takkar, 1993).

Furthermore, crops typically absorb less than half of the applied fertilizer (Chen and Wei, 2018), with the remaining amount either becoming fixed in the soil or contributing to water contamination (Liu and Lal, 2015). Specifically, N, P, and K added to the soil are lost at rates of 40-70%, 80-90%, and 50-90%, respectively (Solanki et al., 2015; Chen and Wei, 2018; Feregrino-Perez et al., 2018).

Therefore, a balanced application of macro and micronutrients is necessary. Consequently, new agricultural approaches advocate for the use of safe, ecologically acceptable products, with diverse applications. These techniques aim to enhance plant development, while mitigating environmental contamination caused by the excessive use of chemical fertilizers. The utilization of nanoparticles (NPs) derived from elements such as zinc and silicon, along with natural extracts like date palm pollen extract, represent a promising alternative to chemical fertilizers. These substitutes are both environmentally safe and friendly, offering a sustainable approach to improving agricultural productivity. Using nanotechnology to create new fertilizers is a promising approach to boost global horticultural production in a sustainable manner. This technology addresses the growing food demands of an increasing population, while promoting sustainability in the face of changing climatic conditions (Raliya et al., 2017; Feregrino-Perez et al., 2018). The application of nanoparticles (NPs) as fertilizers significantly enhances plant growth and development, while reducing the excessive use of chemical fertilizers (Acharya et al., 2020). However, numerous studies have shown that different plant species can respond variably to NPs, with effects that can be either beneficial or detrimental, depending on the nanoparticle size and dosage (El-Badri et al., 2021; Rai-Kalal and Jajoo, 2021). Nanoparticles, which range in size from 1–100 nm, offer multiple benefits, including improved plant growth, nutrition, and production (Cele, 2020). Moreover, nanotechnology has diverse applications across all stages of horticultural production, including storability, processing, packaging, and transportation. Therefore, the horticultural and food industries stand to benefit significantly from the application of nanotechnology (Swarnapriya and Vaibhao Ramesh, 2020). The interaction between nano-elements and traditional fertilizers is attributed to the high reactivity of nano-elements, which enhances the absorption of nutrients and essential materials by plants (Prasad et al., 2017). The efficiency of nanomaterials in nutrient uptake, distribution, and accumulation within plants is significantly influenced by intrinsic factors, such as particle size and surface coatings, as well as extrinsic factors, including organic matter content, soil pH, and texture. Additionally, the exposure route plays a critical role in this process (El-Ramady et al., 2018; Ma et al., 2018).

Recent reports have highlighted the effectiveness of zinc oxide nanoparticles in encouraging the development of plant species

(Adrees et al., 2021; Faizan et al., 2021). Factors like pH, soil physicochemical traits, and tolerance percentage of the plant species/variety can affect the soil's zinc availability and its impact on the crops (García-Gómez et al., 2018). Several researches indicate that foliar spray application with Zinc oxide nanoparticles (ZnONPs) is the most effective means of addressing defective microelements or the lack of them in plants (Rizwan et al., 2019; Adrees et al., 2021). During foliar spraying, plants readily and directly absorb NPs rather than depending on or utilizing the mineral fertilizers in the soil (Subramanian et al., 2015; Kah et al., 2018). Zn plays a crucial role in plants, serving as an essential component of various enzymes and acting as a key regulatory cofactor in processes such as protein synthesis, auxin production, cell division, photosynthesis, and sexual fertilization. Additionally, Zn is important for maintaining the structure and function of cell membranes (Marschner, 1995). Conversely, zinc deficiency can lead to a decline in cell growth and proliferation, negatively affecting photosynthesis, electron transport, photophosphorylation, and the leakage of electrolytes from the roots of plants (Kabata-Pendias, 2000; Auld, 2001; Genc et al., 2006). An understanding of the safety of crops and risk to the environment with application of nano zinc is still limited (Zhang et al., 2024), although, they added that there are no environmental risks from the application of ZnONPs in agriculture.

In recent times, silicon (Si) nanoparticles (NPs) have been utilized as an essential element in agricultural, mostly in arid environments, to bind other nutrients and hold onto water, thus boosting cell vigor (Sommer et al., 2006). It has been seen that utilizing Si enhances root system development, similar to the effect that a high dose of nitrogen has, resulting in improved plant chlorophyll content, photosynthesis, and quality of the product (Kah et al., 2018), thus, alleviating the adverse consequences of illnesses and abiotic pressures on plants (Farouk and Omar, 2020; Khator and Shekhawat, 2020). The electron transfer rate, stomatal conductance, and photochemical processes are all positively affected by silicon nanoparticles (SiNPs) (Ahanger et al., 2020). Silicon reduces the transpiration rate due to the thicker cuticle layer in silicon-treated plants (Rahmawati and Wulandari, 2024). Moreover, because safranal biosynthesis originates from zeaxan, the availability of Si influences the synthesis of carotenoids and the appropriate operation of chloroplasts. Silicon affects safranal biosynthesis due to its role as an intermediate in the carotenoid cycle (Pitsikas, 2016). Also, SiO<sub>2</sub> NPs have distinctive characters like bioactivity, stability, and customizable porosity, therefore, SiO<sub>2</sub> NPs are applied strongly in technological domains variety. However, there is no evidence to suggest that silica, the main component of Si NPs, is ecotoxic to microorganisms, fish, birds, invertebrates, and plants.

Pollen from male trees of date palms (*Phoenix dactylifera*; Palmaceae) is widely acknowledged as being among the most effective and is utilized more often throughout the Middle East, particularly in Egypt. The various constituents present in date palm pollen, including the enzymes that are estimated by electrophoresis (Al-Helal, 1992), proteins, saponins, sterols, triterpenes, vitamins A, C, and E, macro- and micro-nutrients like N, Zn, B, Mo, Se, Fe, Mn, as well as Cu, glycosides, and carbohydrates, have several amino

acids, and thirteen fatty acids (e.g., palmitic acid 34.45%), also phenylethane (8.75%), antioxidants, essential oils, flavonoids and phenols, in addition to several steroids, like, brassinosteroid (Mahran et al., 1985; Hassan, 2011; Basuny et al., 2013). Consequently, the utilization of date palm pollen significantly affects plant growth and secondary metabolism positively.

Currently, the available data on the combined effects of NPK, DPE, and NPs of ZnO and SiO<sub>2</sub>, evaluated in terms of vegetative development, EO productivity, and chemical composition, are limited. Thus, the goal of this field experiment is to assess how NPK fertilizers, ZnO and SiO<sub>2</sub> NPs, and date palm pollen extract, affect the growth, development, essential oil productivity, and chemical and biological composition of *Salvia officinalis*. The study also seeks to determine a safe and affordable substitute fertilizer source that can partially substitute chemical fertilizers (NPK) to reduce pollution in the environment and guarantee the production of safe agricultural products.

## 2 Materials and methods

The study was conducted in an open field at the Experimental Farm of the Horticulture Department, Faculty of Agriculture, (Saba Basha), Alexandria University. The Experimental Farm was at Abees village, which is located in West Alexandria Governorate, Egypt, with an elevation of 2 m above mean sea level, temperature through the experimental period of each season ranged from 25°C–37°C, and relative humidity was 55%–70%. This experiment took place during the 2021 and 2022 seasons.

### 2.1 Analysis of the soil at the experimental site

Before planting, 200 g of soil was collected at the experimental site, at various locations, from a depth of 0 to 30 cm. The samples of soil were accurately mixed into a single sample in order to evaluate their physical and chemical properties (Table 1).

Following an air-drying method, the soil samples were crushed using a mortar and pestle. The samples were collected and divided into fractions of less than 2 mm, using a stainless steel test sieve (Cools and De Vos, 2011). The hydrometer (US 21 CFR 1040.10 AND 1040.11, USA) method was used to assess the distribution of the particle size (Gee and Bauder, 1986). Twenty grams of dried soil and 100 mL of deionized water were mixed in the ratio of 1:5 to obtain the soil chemical character measurements. Before the extract was filtered, the mixture was left to sit for a full day. The following measurements were made and the extract filtered: The soil EC was measured using an EC meter (MI 170, SZ egged, Hungary, Italy) (Jackson, 1973). Additionally, methods described by (Jackson, 1973) were used to estimate the concentrations of magnesium (Mg<sup>++</sup>), calcium (Ca<sup>++</sup>), and chloride (Cl<sup>-</sup>)\_ENREF\_40. The method of quantifying total carbonate and organic matter was utilized (Nelson and Sommers, 1996). The amount of accessible N was estimated by the micro Kjeldahl (DNB.1500 NP S.N. 33848 made in Spain RAYPA) method (Bremner and Mulvaney, 1983).

TABLE 1 The physical and chemical parameters of the experimental site soil.

Physical parameters		Chemical parameters			Available macronutrients (mg/kg)					
Clay	60.33%	pH (1:2.5)	Organic matter %	EC (ds/m)	N	P	K			
Sand	30.40%									
Silt	09.27%									
Soil texture clayey sand		8.1	2.36	1.81	799		28.3	51.6		
		Anions (meq/l)				Cations (meq/l)				
		CO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>-2</sup>	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>++</sup>	Ca <sup>2++</sup>	Zn <sup>++</sup>
		0.0	5.9	2.9	13.3	10.2	0.81	4.11	10.2	0.71

+, cation monovalent; ++, cation bivalent.

The method of [Olsen and Sommers \(1982\)](#) was used to estimate the available P. Zn<sup>++</sup> availability was estimated using an atomic absorption spectrophotometer (AAS) ([Page et al., 1982](#)); even as Na<sup>+</sup> and K<sup>+</sup> were measured using a PSC 7 flame photometer (JENEWAY, Staffordshire, UK) ([Black et al., 1965](#)). After 30 minutes, a pH-meter (JENEWAY3510, Staffordshire, UK) was used to estimate the soil pH in the suspension (1:2.5, soil: distilled water) ([Black et al., 1965](#)).

## 2.2 Sowing of seeds

Sage seeds were obtained from Egypt's Department of Medicinal and Aromatic Plants, Horticulture Research Institute, Agricultural Research Center, Ministry of Agriculture. After soaking the seeds in a 5% commercial Na hypochloride solution for five minutes, they were cleaned using deionized water. The seeds were sown in the experimental units (2 x 2 m of each unit) on the hills (3 seeds/hill) 30 cm apart, on March 15<sup>th</sup>, in the 2021 and 2022 seasons. There were four rows, 60 cm apart in each unit, and seven hills in every row. Thus, every experimental unit contained 28 hills. As soon as the seeds were sown, the experimental area was watered. A surface system of irrigation in the Experimental Farm was utilized using water from the Nile, which had a pH of 7.33 and an EC of 0.35 dsm<sup>-1</sup>. After full germination the seedlings (12 cm in height) were thinned (one seedling/hill) on first May, in the two seasons. The agricultural practices before seed sowing and after the germination of seeds (edging and leveling the soil in the used area, weeding, and insecticide and pesticide control) were done when needed.

## 2.3 The fertilizers used

### 2.3.1 Nitrogen, phosphorus, and potassium fertilizers

The N, P, and K fertilizers at levels of 300, 200, and 100 kg/ha — ammonium sulfate (20.5% N), calcium super phosphate (15.5% P<sub>2</sub>O<sub>5</sub>), and potassium sulfate (48% K<sub>2</sub>O), were utilized in succession as a recommended dose (as per Ministry of Agriculture, Egypt). Calcium super phosphate was applied as one application during soil preparation. The amounts of nitrogen and potassium fertilizers were applied in four equal doses, the first dose was on

second May after the thinning, the second dose was on first June, the 3<sup>rd</sup> addition was 15 days after the first cut, and the 4<sup>th</sup> dose was done 30 days after the 3<sup>rd</sup> addition, during the experimental seasons.

### 2.3.2 Nanoparticles

Nanoparticles of zinc oxide (ZnO NPs) at 1 and 1.5 g/L, according to [Prasad et al. \(2012\)](#) and [Pirzad and Barin \(2018\)](#). The nanoparticles of silicon (SiO<sub>2</sub> NPs) at 0.1 and 0.2 g/L, as per [Adhikari et al. \(2013\)](#). The used nanoparticles were used as foliar spraying. Zinc oxide nanoparticles and silicon oxide nanoparticles were applied thrice. The first and second applications were sprayed 3 and 33 days after thinning of the sage seedlings and the 3<sup>rd</sup> spray 20 days after the first cut. A powder of the NPs was dissolved in deionized water.

#### 2.3.2.1 Synthesis of NPs of ZnO and SiO<sub>2</sub>

The ZnO nanoparticles were produced according to [Ali et al. \(2017\)](#). In brief, 50 mL of 2 M NaOH and 100 mL of 1 mM Zn (CH<sub>3</sub>COO)<sub>2</sub> · 2H<sub>2</sub>O were combined dropwise and stirred continuously for two hours. The white precipitate was collected using centrifugation (model 58 10r, Eppendorf Corporation, Hamburg, Germany) at 9508 rpm, for 5 minutes, at room temperature (25 ± 2°C). To eliminate the impurities, it was rinsed thrice with distilled water. The ZnO NPs were dried at 60°C for the entire night in a drying incubator (Thomas Scientific, Swedesboro, NJ, USA). The sol-gel procedure described by [Hench and West \(1990\)](#) was employed to prepare SiO<sub>2</sub> NPs. Thirty-five milliliters of water and 65 milliliters of 100% alcohol were combined and stirred mechanically for five minutes. The preceding ethanol/water solution was then mixed with 25 mL of tetraethyl orthosilicate (TEOS) dropwise and allowed to sit at room temperature for 60 minutes, with mechanical stirring. In order to achieve this, an ammonia solution was gradually added until the gel's full formation was determined, for which the sol-gel method was used to record that the solution had transformed into a gel. The gel that had formed was subjected to two hours of ultra-centrifugation at 7000 rpm. Ultimately, the precipitated wet gel was gathered and subjected to three rounds of distilled water washings to eliminate any unwanted chemical or any chemical that had not reacted (TEOS). The moist gel was left for calcination at 700°C for 5–7 hours in an Empyrean PAN analytical X-ray diffractometer with Bragg-Brentano geometry and



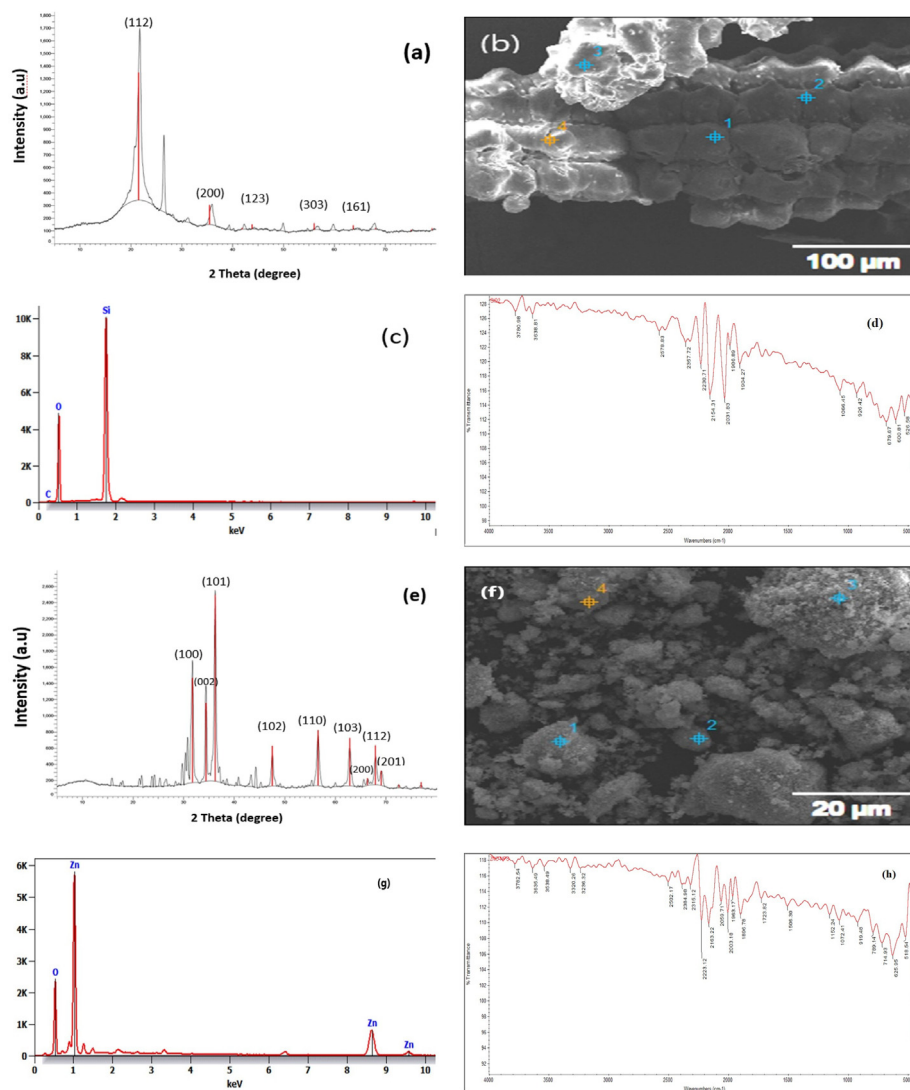


FIGURE 1

(A) XRD of SiO<sub>2</sub> NPs; (B) SEM of SiO<sub>2</sub> NPs; (C) EDX of SiO<sub>2</sub> NPs; (D) FTIR of SiO<sub>2</sub> NPs; (E) XRD of ZnO NPs; (F) SEM of ZnO NPs; (G) EDX of ZnO NPs; and (H) FTIR of ZnO NPs.

Cu K $\alpha$  radiation ( $R = 1.54\text{\AA}$ ). The powder patterns of the silicon oxide and zinc oxide nanoparticles were registered. The step scan spanned the angular range of 20 to 80 at a step of 0.02. The size of the crystallite was calculated using the Scherrer equation ( $D = Kh/B \cos B$ ). The crystallite size ( $D$ ), the X-ray radiation wave length ( $h$ ), ( $K$ ) the constant (0.94), the line width at half the peak's greatest intensity ( $B$ ), and the diffraction angle ( $QB$ ), are all represented in this equation. (Figures 1–D) show SEM, FTIR, XRD, EDX of the SiO<sub>2</sub> NPs and (Figures 1E–H) show SEM, FTIR, XRD, EDX of ZnO NPs.

The chemical bonding nature of the nanoparticles was assessed using the Fourier-transform infrared (FTIR) absorption spectroscopy (THERMO NICLOT, 50.UK). The powder's shape and elemental content were investigated using scanning electron microscopy (SEM; JEOL, JSM-6360LA, Tokyo, Japan) during the removal process (Naddaf et al., 2020). One method for describing crystalline materials was X-ray diffraction XRD device (Panalytical Empirian, Istanbul, Turkey). It offered details on phases, preferred

crystal orientations (texture), and additional structural characteristics, such as, average grain size, strain, crystallinity, and crystal defects (Pandey et al., 2021). Energy-dispersive X-ray spectroscopy (EDX; JEOL JSM-5300, USA) was a method for elemental analysis related to electron microscopy that used the production of distinctive X-rays, to identify the elements that were present in the samples (Scimeca et al., 2018).

The specification, physical and chemical properties, and acute toxicity of the used ZnO NPs and SiO<sub>2</sub> NPs are presented in (Table 2).

### 2.3.3 Date pollen extract

In Damanhur city, Elbehyra Governorate, Egypt, pollen from Egyptian date palms (*Phoenix dactylifera* L.) was collected in the last week of March, in the seasons of 2021 and 2022, during the male inflorescence cover opening. The pollen extract was prepared according to Nagai et al. (2002) method, with some changes: For

one hour, 0.1g of pollen was added to 10.0 mL of deionized water. Next, the mixture was centrifuged (Sigma 3-18KS, SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany) at 5000 rpm for 10 minutes at a temperature of 20°C. The ultrasonic probe, VCX 750 (SONICS & MATERIALS, INC., Newtown, CT, USA), was used to sonicate the mixture for 30 seconds at a frequency of 6 KHZ. In every experiment, the resultant supernatant was used as a water pollen extract. Distilled water was then added to complete the extract, to obtain the used levels (15 and 25g/L), as per [Abou-Sreeda and Yassen \(2016\)](#). Date pollen extract was sprayed thrice. The first and second applications were carried out 2 and 31 days after thinning of the sage seedlings, and the 3rd application was performed 22 days after the first cut.

TABLE 2 The specification, physical and chemical properties, and acute toxicity of the used ZnO NPs and SiO<sub>2</sub> NPs.

	Silica oxide nanoparticles	Zinc oxide nanoparticles
	Specification	
Appearance	white powder	white powder
Average Particle size	15 ± 10 nm	20nm
Morphology	Spherical.	Spherical.
Surface area	109.356 m <sup>2</sup> /g	2.7534 m <sup>2</sup> /g
Average pore radius	3.53198e+01Å	40.5965 nm
Total pore volume	1.931e-02 cc/g	0.042062 cm <sup>3</sup> /g
Chemical Composition	silicon = 46.83% Oxygen 53.33%	Zn= 80.34% O= 19.6
<b>Physical and chemical properties</b>		
Odor	odorless	odorless
Density	at 20 °C (1.973 g/cm <sup>3</sup> )	at 20 °C (5.6 g/cm <sup>3</sup> )
Melting Point	1600-1728 °C	1957°C
Boiling Point	2230°C	2360 °C
Solubility:	insoluble in water	insoluble
Acute Toxicity	Inhalation human LD50 = 3000mg/kg Intravenous rat LD50 = 90mg/kg Intravenous mouse LD50 = 40mg/kg Oral rat LD50>3000mg/kg Dermal rabbit LD50>5000 mg/kg	The lethal dose 50 (LD50) of intravenously administered =0.3 mg/kg in mice TheLD50of intratracheal instillation= 493.85 µg/kg in mice.

## 2.4 The used fertilizer types and fertilization treatments

In this experiment, ten fertilizer applications were performed ([Table 3](#)).

## 2.5 Experimental layout

In this investigation, a randomized complete block design (RCPD) was used. Three replicates of the experiment were done with ten treatments in each replicate ([Snedecor and Cochran, 1989](#)). Each treatment contained three experimental units in one way ANOVA.

## 2.6 Data recorded

### 2.6.1 Growth parameters

The first and second cuts of sage were done on July first and November first of the 2021 and 2022 seasons, with five plants randomly harvested from each experimental unit. The vegetative characteristics were measured, as the average of the two cuts of each season: Plant height (cm) was estimated from the soil surface to the plant's top; fresh weight and constant air dry weights of aerial organs/plant (g) and chlorophyll index of the fifth leaf from the top of the branch was estimated using SPAD units, measured with a Minolta SPAD chlorophyll meter model-502 ([Yadava, 1986](#)).

### 2.6.2 Essential oil percentage and yield

Air-dried sage herb samples at constant weight (50 g/sample) were used for hydro distillation with sterile water, 1 L for 3 hours, in a Clevenger-type apparatus. The collected essential oil (EO) was dried over anhydrous sodium sulfate and kept for later use at 4°C ([Elmsellem et al., 2019](#)). Where,

TABLE 3 The used fertilization treatments.

Treatments No.	The fertilization treatments
T1	100% NPK (recommended dose) as a control
T2	75% NPK + 15 g/L date pollen extract (DPE)
T3	50% NPK + 25 g/L DPE
T4	75% NPK + 0.1 g/L SiO <sub>2</sub> NPs
T5	50% NPK + 0.2 g/L SiO <sub>2</sub> NPs
T6	75% NPK + 1 g/L ZnO NPs
T7	50% NPK + 1.5 g/L ZnO NPs
T8	50% NPK + 25 g/L DPE + 0.2 g/L SiO <sub>2</sub> NPS
T9	50% NPK + 15 g/L DPE + 1.0 g/L ZnO NPs + 0.1 g/L SiO <sub>2</sub> NPs
T10	25% NPK + 25 g/L DPE + 1.5 g/L ZnO NPs + 0.2 g/L SiO <sub>2</sub> NPs.

$$EO\% = \frac{\text{oil volume in graduated tub}}{\text{plant sample weight}} \times 100$$

$$\text{Plant essential oil (mL)} = EO\% \times \text{plant dry weight}$$

Where the percentage and yield of the essential oil/plant were determined as the average of the two cuts of each season.

### 2.6.3 Gas chromatography/mass spectrometry analysis of oil

For identifying the EO compounds during the first cut of the second season, gas chromatography/mass spectrometry (GC–MS) analysis was utilized. The sample was obtained and filtered, to ensure that it wouldn't impact the column, and then one microliter of the sample was inserted into the GC. An Agilent 6890 N gas chromatograph with a capillary column DB-5 MS (30 m × 250 μm × 0.25 μm) from Agilent Technologies, USA, and a 5975 B mass selective detector spectrometer from the same firm were attached to the gas chromatograph. A split mode maintained the front inlet at 250°C. This was the temperature program: 60°C was the starting point and was held for two minutes; after that, it was programmed to reach 120°C at a pace of 6°C per minute, and was kept for two minutes; finally, it was designed to reach 230°C at a rate of 4°C per minute, and was maintained for five minutes. The split injection flow rate was one milliliter per minute. As a carrier gas, 1.0 mL of helium per minute was employed. An ionization voltage of 80 eV was employed when using the MS detector in the EI mode. The temperature of the ion source was 230°C. It reached 280°C in the transfer line. The mass range (m/z) 30–1000 was covered by the spectrum collection. The retention indices were computed using the retention periods of n-alkanes, C6–C26, that were injected under identical chromatographic conditions. They were identified by using the Nits 08.L library of essential oil compounds, by comparing the mass spectra and relative retention indices of the volatile components.

### 2.6.4 Total phenol compounds and antioxidant activity

The dried leaf samples from the two cuts in the second season were the only ones used to assess the total phenol compounds (TPC) and antioxidant activity (AOA). The air-dried leaves were ground and soaked in methanol. The mixture was filtered after 24 hours, and the amount of total phenols was measured using the filtrate. With external calibration using gallic acid and the Folin–Ciocalteu reagent, the amount of TPC in the crude extracts was ascertained. To summarize, 0.2 milliliters of extract solution and 0.2 milliliters of the Folin–Ciocalteu reagent were added, and their contents were properly mixed (Singleton et al., 1999). One milliliter of 15% Na<sub>2</sub>CO<sub>3</sub> was added after four minutes, and the mixture was then left to stand at room temperature for two hours. A Spectro (Thermo Fisher Scientific, Waltham, MA, USA model 4001/4) spectrophotometer was used to detect the absorbance at 760 nm. Using an equation derived from the gallic acid calibration curve, the concentration of TPC was determined as a milligram of gallic acid equivalent/g dry weight (D.W). The results were an average of the three separate measurements that were made of the TPC, in each of the fractions (Martínez-Esplá et al., 2014).

The 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay was used to evaluate the antioxidative capacity of dry leaves (Binsan et al., 2008), with a tiny alteration. To a 0.1% protein solution (in 5 mM poly (1,4-butylene succinate) (PBS) buffer, pH 7.2), 0.15 mM of 2,2-diphenyl-1-picryl hydra Zyl (DPPH), in 95% ethanol, was added, in a ratio of 1:1 (v/v). The blend was combined and allowed to sit at room temperature for half an hour, in the dark. A spectrophotometer (Helios Gamma; Thermo Fisher Scientific) was used to measure the absorbance of the resultant solution at 517 nm. The preparation of the blank was identical to that of the sample, with the exception that the 5 mM PBS buffer (pH 7.2) was utilized. Trolox was used to produce the calibration curve in the 12.5–100 μM range. The Trolox equivalent (TE)/mg of dry leaves was the unit of expression for the activity. The data of TPC and AOA have been calculated as an average of the two cuts in the 2022 season.

## 2.7 Leaf chemical composition

Leaves samples of the two cuts in 2<sup>nd</sup> season were dried in the oven at 72°C for 36 h and ground to obtain a homogenous powder in a metal-free mill (Ika-Werke, M 20 Germany). Concentrated sulfuric acid (95%, 5 mL) was added to the 0.2 g sample, then a sand hotplate was used to heat the mixture for 10 min. After that, 0.5 mL of perchloric acid was dropwise, and heating was continued to obtain a clear solution. The solution was left to cool, and filtered, then it was diluted to 50mL (Evenhuis and de Waard, 1980). The measurements of N, P, and K percentages were estimated by a modified micro- Kjeldal method (Chemists, 1990), spectrophotometer (GT 80+, UK) (Murphy and Riley, 1962), and an atomic absorption spectrophotometer (Avanta E; GBC, Victoria, Australia) (Cottenie et al., 1982), respectively. Silicon, Zn and total carbohydrates percentages were measured using the techniques of Hogendorp et al. (2012); Jackson (1973), and Herbert et al. (1971), consecutively. Data on leaf chemical composition were calculated as the average of the two cuts in 2022 seasons.

## 2.8 Statistical analysis

The data underwent analysis of variance using the SAS software (Version 6.12; SAS Institute Inc., Cary, NC, USA). The mean separations ( ± SE) were computed by a one-way ANOVA, with significance established at  $p \leq 0.05$ , by using the Duncan's multiple range test (DMRT).

## 3 Results

### 3.1 Vegetative growth characters

During the experimental seasons, treatments combining 75%, 50%, and 25% NPK RD with ZnO NPs, SiO<sub>2</sub> NPs, or DPE at various concentrations notably increased the height of sage plants compared to the control (NPK RD) with some exceptions

(Figure 2A). Additionally, the tallest plants were those that received T9 in the two seasons. This treatment resulted in a height of 96.66 and 90.66 cm against 78.33 and 70.66 cm for the control in both seasons, respectively. It is obvious that the variations between the used fertilization treatments were unable to arrive at the significant level ( $p \leq 0.05$ ) in some cases.

With regard to the chlorophyll index, the majority of fertilization applications exhibited positive effects on the SPAD values over the respective control (Figure 2B). The highest significant SPAD values ( $p \leq 0.05$ ) were recorded for plants that received T9 of 41.33 and 41.00 SPAD units against 32.40 and 32.40 SPAD units for the control in the two seasons, respectively.

The findings of the aerial part fresh weight (APFW) and aerial part dry weight (APDW) of the sage plant clearly showed that fertilization treatments had differently affected the APFW and APDW (Figures 2C, D). Whereas, in the two seasons the treatment of T9 resulted in a higher significant APFW of 783.83 and 777.66 g/plant and APDW of 262.00 and 252.33 g/plant, consecutively. Furthermore, the fertilization treatments significantly increased the APFW and APDW compared to NPK RD in the two seasons, with some exceptions; even as the control gave an APFW of 551.00 and 544.00 g/plant and APDW of 159.66 and 155.00 g/plant in the two seasons, in succession. The differences among the used applications reached the significant level in a majority of cases during the experimental seasons. The application of ZnO and SiO<sub>2</sub> nanoparticles, along with DPE, has shown positive effects on vegetative growth parameters, particularly when reducing NPK by 100%, with T9 showing the most notable improvement.

## 3.2 Essential oil productivity

Fertilization treatments influenced EO% and EO yield (EOY) (based on herb dry weight) (Figures 3A, B). All treatments caused significant increases in EO% and EOY in relation to 100% NPK (NPK RD) throughout the experimental duration, except for T3 in the first season and T6 and T7 during the two seasons in case of EO%. The maximum significant EO% reached 2.96% for the T10 treatment in the first season. Although, in the second season the EO% reached 3.03% for the T4 treatment. At the same time, the T1 plants had 2.33% and 2.40% EO in both the seasons, consecutively. Moreover, the T9-treated plants produced a maximum significant EOY of 7.60 and 7.07 mL/plant in both the seasons, respectively. The EOY in the control plants reached 3.93 and 3.70 mL/plant in both the seasons, successively. Also, the impact of the used fertilization treatments on EO% and EOY reached the significant level ( $p \leq 0.05$ ) among most of the applications used, in the both seasons. It is obvious from data of EO% and EOY that the treatments containing ZnO NPs, SiO<sub>2</sub> NPs and DPE had positive effect on such traits or decreasing NPK RD.

GC-MS analysis of sage EO (Table 4) showed the identification of 58 compounds distributed among the 10 used treatments. T8 resulted in the maximum EO compounds of 32, while T10 showed the least EO compounds of 11 compounds. The other treatments produced intermediate numbers of compounds. Specifically, T3, T7,

and T8 exhibited compound numbers in EO that were higher than those observed with 100% NPK. In contrast, the remaining treatments resulted in compound numbers in EO that were lower than those with 100% NPK. The total compounds represented 98.60% for the control to 99.99% for T4, for the EO of sage. Oxygenated hydrocarbons ranged from 79.05% in T9 to 88.94% in T6 of the total EO chemical structure. These were followed by sesquiterpene hydrocarbons reaching 9.38% for T6 to 16.29% for T9. Then, monoterpene hydrocarbons had the lowest content of sage EO ranged from 0.00% for T8 to 3.61% for T2. The analysis exhibited that the dominant constituents (> 3%) were thujone (9.96% in T8 to 29.86% in T6), (+)-2-bornanone/(15)-(-)-1-camphor (4.61% in T8 to 15.77% in T6), manool (6.84% in T7 to 14.23% in T10), ledol (8.45% in T8 to 13.78% in T4 and T9), cineol (6.34% in T8 to 12.77% in T1), Estragole (0.87% in T7 to 11.86% in T8), Endo-Borneol (4.40% in T5 to 8.96% in T3), caryophyllene (3.03% in T6 to 8.76% in T8), Humulene (4.76% in T8 to 7.43% in T1) and caryophyllene oxide (3.06% in T8 to 5.88% in T4). The EO analysis revealed that nine compounds were observed in all treatments, while some constituents were noticed in one or two treatments only.

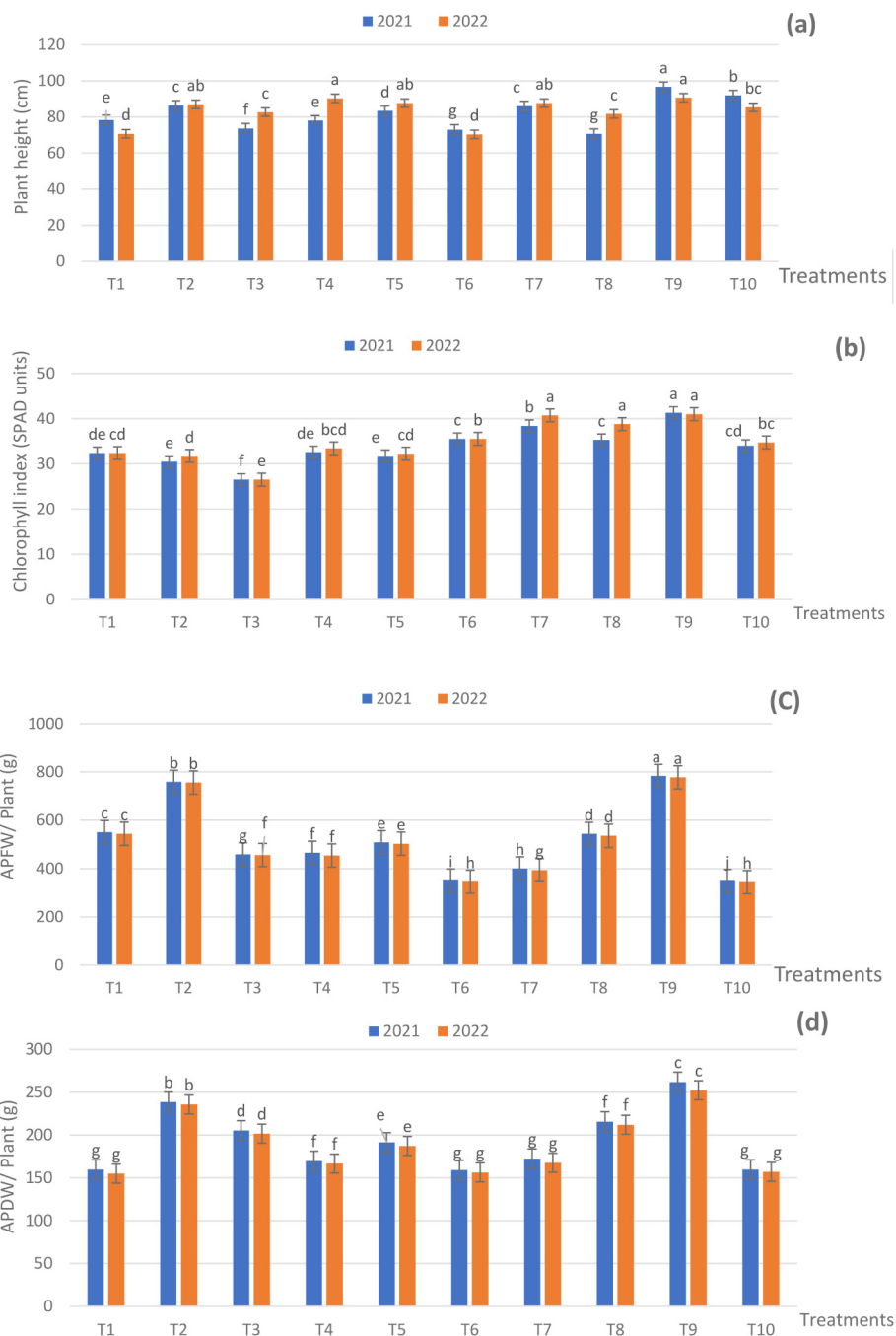
## 3.3 Total phenol compounds and antioxidant activity

All fertilization treatments of 75, 50, and 25% NPK RD added to ZnO NPs, SiO<sub>2</sub> NPs, and DPE at various levels, significantly promoted TPCs, as compared to T1 (Figure 4A). Also, the differences among the mean values of all treatments were significant ( $p \leq 0.05$ ). Among all the treatments, the significant maximum value of TPCs was found in plants that received T8, resulting in 14.91 mg GAE/g D.W compared to 11.04 mg GAE/g D.W for T1. The other treatments resulted in intermediate values of TPCs. Even as the mean values of AOA (Figure 4B) ranged from 0.0260 micromole Trolox equivalent ( $\mu$ MTE)/10g D.W for T1 to 0.0324  $\mu$ M TE/10g for T7. It was noticed that the AOA values of the used applications were close. Thus, AOA was positively improved by applying ZnO nanoparticles, SiO<sub>2</sub> nanoparticles, and DPE in combination with 75%, 50%, and 25% of the NPK, compared to the 100% NPK treatment.

## 3.4 Leaf chemical composition

To estimate the impact of different fertilization treatments on leaf chemical composition, the levels of nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), silicon (Si), and total carbohydrates (%) in the leaves were measured (Table 5). The data indicate that the applied treatments had significantly different effects on leaf chemical composition. Specifically, the differences in the mean values of N, P, K, Zn, Si, and total carbohydrates among the various fertilization treatments were significant in most cases. The highest significant values were recorded as follows: N at 2.06% for both T1 and T2, P at 0.86% for T1, K at 3.03% for T9, Zn at 0.16% for T7, Si at 0.02336% for T10, and total carbohydrates at 1.73% for





**FIGURE 2** Effect of fertilization treatments during 2021 and 2022 seasons on, (A) plant height; (B) chlorophyll index; (C) APFW; and (D) APDW. The means have similar letters within the figures that denote non-significance ( $p \leq 0.05$ ), according to Duncan’s multiple range test. T1 - 100% NPK (recommended dose) as a control, T2 - 75% NPK + 15g/L date pollen extract (DPE), T3 - 50% NPK + 25 g/L DPE, T4 - 75% NPK + 0.1 g/L SiO<sub>2</sub> NPs, T5 - 50%NPK + 0.2 g/L SiO<sub>2</sub> NPs, T6 - 75% NPK + 1 g/L ZnO NPs, T7 - 50% NPK + 1.5 g/L ZnO NPs, T8 - 50% NPK + 25 g/L DPE + 0.2 g/L SiO<sub>2</sub> NPs, T9 - 50% NPK + 15 g/L DPE + 1.0 g/L ZnO NPs + 0.1 g/L SiO<sub>2</sub> NPs, and T10 - 25% NPK + 25g/L DPE + 1.5 g/L ZnO NPs + 0.2 g/L SiO<sub>2</sub> NPs.

T7. Conversely, the lowest significant values were N at 1.11% for T8, P at 0.35% for T5, K at 1.61% for both T5 and T8, Zn at 0.05% for T9 and T10, Si at 0.00012% for T1, and total carbohydrates at 0.83% for T2 and T4.

The most effective fertilization treatment improved leaf chemical composition compared to the 100% NPK treatment, with the exception of nitrogen (N%) and phosphorus (P) content.

## 4 Discussion

Climatic conditions (such as temperature, relative humidity, photoperiod, and light intensity) and soil conditions (including pH and physical and chemical properties) influence the physico-chemical properties of nanoparticles. Consequently, the study was conducted in an open field to account for these variables.

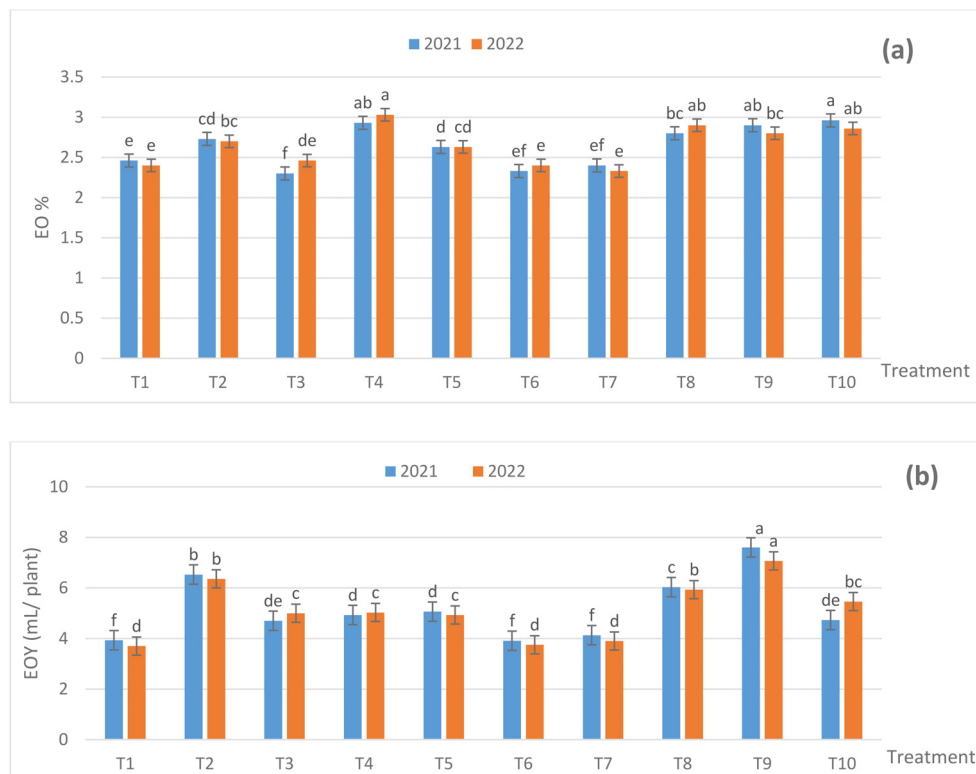


FIGURE 3

Effect of fertilization treatments during 2021 and 2022 seasons on (A) EO% and (B) EOY (ml/plant). The Duncan’s multiple range test indicates that there was no significant difference ( $p \leq 0.05$ ) among the means, as seen in the figures that are followed by the same letters. T1- 100% NPK (recommended dose) as a control, T2 - 75% NPK + 15 g/L date pollen extract (DPE), T3 - 50% NPK + 25 g/L DPE, T4 - 75% NPK + 0.1 g/L SiO<sub>2</sub> NPs, T5 - 50% NPK + 0.2 g/L SiO<sub>2</sub> NPs, T6 - 75% NPK + 1 g/L ZnO NPs, T7 - 50% NPK + 1.5 g/L ZnO NPs, T8 - 50% NPK + 25 g/L DPE + 0.2 g/L SiO<sub>2</sub> NPs, T9 - 50% NPK + 15 g/L DPE + 1.0 g/L ZnO NPs + 0.1 g/L SiO<sub>2</sub> NPs, and T10 - 25% NPK + 25g/L DPE + 1.5 g/L ZnO NPs + 0.2 g/L SiO<sub>2</sub> NPs.

TABLE 4 EO composition of *Salvia officinalis* as affected by the utilizations used at first cut in the second season.

Compound name (%)		Treatments									
		Control (T1)	T2	T3	T4	T5	T6	T7	T8	T9	T10
1	Trilinolein	0.21	-	-	-	-	-	-	-	-	-
2	Beta-pinene	1.09	0.52	-	-	0.43	-	1.51	-	0.53	-
3	Butyl	0.28	-	-	-	-	-	-	-	-	-
4	P-Cymene	0.46	0.80	0.63	0.65	0.70	-	0.67	-	0.78	-
5	Cineole	12.77	12.7	12.22	11.12	9.91	10.8	10.41	6.34	12.71	10.82
6	Thujone	24.96	29.21	21.14	25.00	26.17	29.86	21.35	9.96	22.53	26.6
7	(+)-2-bornanone/ (1S)-(-)-Camphor	8.45	11.28	12.04	8.74	12.66	15.77	10.35	4.61	10.65	7.02
8	Endo-Borneol	5.49	5.54	8.96	4.97	4.40	4.53	6.39	5.46	5.25	6.09
9	Estragole	1.55	-	1.84	2.15	0.87	-	5.30	11.5	-	-
10	Á-copaene	0.23	-	0.28	0.24	0.22	-	0.57	0.46	0.35	-
11	Caryophyllene	5.22	4.79	5.37	3.80	5.13	3.03	5.53	8.76	7.03	7.79
12	Humulene	7.43	7.24	6.36	6.19	6.32	6.35	6.09	4.76	7.01	6.69
13	À-ylangene	0.31	-	-	0.26	-	-	0.36	-	0.20	-

(Continued)

TABLE 4 Continued

	Compound name (%)	Treatments									
		Control (T1)	T2	T3	T4	T5	T6	T7	T8	T9	T10
14	Caryophyllene oxide	3.36	3.32	4.55	5.88	3.90	4.42	5.28	3.06	3.89	5.52
15	Ledol/Viridiflorol	13.69	9.61	11.71	13.73	12.17	11.08	13.52	8.45	13.73	11.96
16	Bornyl acetate	1.09	1.76	1.84	1.56	1.15		1.50		1.09	1.77
17	Caryophylla-4 (12),8 (13)-dien-5 $\alpha$ -ol	0.65	0.25	0.21	0.44	0.25	-	-	0.95	0.76	-
18	Labda-8 (20),14-dien-13-ol, (13R)-; Manool	10.41	7.55	8.77	12.33	12.52	10.17	6.84	9.50	8.93	14.23
19	Aromadendrene	0.44	1.12	0.32	0.50	0.56	-	0.27	0.77	0.81	-
20	$\Delta$ -Pinene	-	2.12	1.77	1.41	1.46	-	0.39	-	2.05	-
21	Cis-ocimene	-	0.17	-	-	-	-	-	-	0.20	-
22	Guaia-1 (10),11-diene	-	0.51	-	-	-	-	-	-	-	-
23	$\Delta$ -cadinene	0.51	0.30	0.43	0.41	0.35	-	-	-	-	-
24	Linalool	-	-	0.34	0.21	-	-	0.33	-	-	-
25	Cis- $\alpha$ -Bergamotene	-	-	0.27	-	-	-	-	-	-	-
26	LEDEN	-	0.51	0.30	-	-	-	-	-	0.34	-
27	Cis- $\alpha$ -Bisabolene	-	-	0.20	-	-	-	-	-	-	-
28	Methyleugenol	-	-	-	-	-	-	0.83	-	-	-
29	Retinal	-	-	-	-	-	0.60	-	-	-	-
30	Isobornyl acetate	-	-	-	-	-	1.24	-	-	-	-
31	$\Delta$ -acorenil	-	-	-	-	-	-	0.70	-	-	-
32	Eugenol	-	-	-	-	-	-	0.56	-	-	-
33	$\Delta$ -Guaiene	-	-	-	-	-	-	0.19	-	-	-
34	(-)-Spathulenol	-	0.38	-	-	-	-	0.27	0.97	-	-
35	Diethyl Phthalate	-	-	-	-	-	-	0.29	-	-	-
36	9-OCTADECENOIC ACID (Z)-	-	-	-	-	-	-	-	2.72	-	-
37	TETRADECANE, 1-CHLORO	-	-	-	-	-	-	-	0.74	-	-
38	1-Hexadecanol, 2-methyl-	-	-	-	-	-	-	-	3.10	-	-
39	3-OCTADECYNE	-	-	-	-	-	-	-	2.60	-	-
40	Isobornyl thiocynoacetate	-	-	-	-	-	-	-	2.24	-	-
41	Trans-Sesquisabinene hydrate	-	-	-	-	-	-	-	0.82	-	-
42	10-Heptadecen-8-ynoic acid, methylEster, (E)-	-	-	-	-	-	-	-	0.79	-	-
43	1,3-benzodioxole, 5-[[2- (2-butoxyethoxy)ethoxy]methyl]-6-propyl-	-	-	-	-	-	-	-	0.50	-	-
44	1-Heptatriacotanol	-	-	-	-	-	-	-	1.35	-	-
45	9-octadecenoic acid (z)-	-	-	-	-	-	-	-	1.65	-	-
46	Thunbergol	-	-	-	-	-	-	-	2.01	-	-
47	Patchouli alcohol	-	-	-	-	-	-	-	1.67	-	-
48	EPISTEPHAMIERSINE	-	-	-	-	-	-	-	0.50	-	-

(Continued)

TABLE 4 Continued

Compound name (%)		Treatments									
		Control (T1)	T2	T3	T4	T5	T6	T7	T8	T9	T10
49	7-Hydroxy-6,9a-dimethyl-3-methylene-decahydro-azuleno [4,5-b]furan-2,9-dione	-	-	-	-	-	-	-	0.54	-	-
50	(2-Aminocyclohexyl)-phenyl-methanol	-	-	-	-	-	-	-	0.48	-	-
51	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	-	-	-	-	-	-	-	1.08	-	-
52	Galactopyranose, 5TMS derivative	-	-	-	-	-	-	-	0.81	-	-
53	3-Buten-2-ol, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-, (3E)-	-	-	-	-	-	1.91	-	-	-	-
54	3-METHYL-5-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-YL)-1-PENTYN-3-OL	-	-	-	-	0.77	-	-	-	-	-
55	CYCLOHEXENE, 1,5,5-TRIMETHYL-6-METHYLENE	-	-	0.14	-	-	-	-	-	-	-
56	TETRADECANE, 1-CHLORO-	-	-	-	-	-	-	-	0.74	-	-
57	Doconexent	-	-	-	0.40	-	-	-	-	-	-
58	Trans-p-mentha-1 (7),8-dien-2-ol	-	-	-	-	-	-	-	-	-	0.49
Total Compounds (%)		98.60	99.68	99.69	99.99	99.94	99.76	99.50	99.89	98.84	98.98
Monoterpene Hydrocarbons (%)		1.09	3.61	0.63	2.06	2.59	1.44	2.66	0.00	3.50	1.33
Sesquiterpene Hydrocarbons (%)		14.5	13.71	14.29	11.44	12.58	9.38	13.81	15.57	16.29	14.48
Oxygenated Hydrocarbons (%)		83.01	82.36	84.77	86.49	84.77	88.94	83.03	84.32	79.05	83.17
Number of Compounds (%)		20	20	22	20	19	12	24	32	19	11

T1- 100% NPK (recommended dose) as a control, T2- 75% NPK +15g/L date pollen extract (DPE), T3- 50% NPK +25 g/L DPE, T4- 75% NPK + 0.1 g/L SiO<sub>2</sub> NPs, T5-50%NPK + 0.2 g/L SiO<sub>2</sub> NPs, T6- 75% NPK + 1 g/L ZnO NPs, T7-50% NPK + 1.5 g/L ZnO NPs, T8- 50% NPK + 25 g/L DPE + 0.2 g/L SiO<sub>2</sub> NPs, T9- 50% NPK + 15 g/L DPE + 1.0 g/L ZnO NPs + 0.1 g/L SiO<sub>2</sub> NPs, and T10- 25% NPK +25g/L DPE+ 1.5 g/L ZnO NPs + 0.2 g/L SiO<sub>2</sub> NPs.

Our investigation tested the potential of combining NPs of ZnO and SiO<sub>2</sub>, and DPE with 75%, 50%, and 25% NPK RD, as a means of reducing the risk associated with traditional fertilizers. The study revealed a positive effect on the reduction of NPK application through the use of ZnO NPs, SiO<sub>2</sub> NPs, and DPE. This observation suggested that a balance could be achieved between 75%, 50%, and 25% NPK RD and either DPE or NPs of the used elements, leading to an increase in vegetative traits. Noreen et al. (2018) revealed that Zn has an important function in plant growth, being a principal component or a principal co-factor of several proteins, as well as enzymes. Nano zinc has a vital role in various processes in cells, for example, in physiological, chemical, as well as biochemical activities (Todeschini et al., 2011; Marschner, 2012). Micronutrients such as Zn and Si have been found to provide several benefits under various crop conditions, including enhancing the N uptake and using efficiency and increasing production of crop biomass over the NPK levels (Angle et al., 2017). Zn NPs play a crucial role in various anatomical and physiological processes in plants (Agarwal et al., 2017). ZnO is essential for the activity of many enzymes, including dehydrogenases and superoxide

dismutase (Narendhran et al., 2016). ZnO NPs are utilized as chemical absorbents, antibacterial agents, catalysts, and polymer additives due to their low toxicity, large specific surface area, high pore volume, long lifespan, and photodegradability (Abbasi Khalaki et al., 2021). Thus, zinc is vital for chlorophyll and biomass production, pollen function, RNA metabolism, and DNA formation (Pandey et al., 2006; Cakmak, 2008).

The used levels of ZnO NPs and SiO<sub>2</sub> NPs did not exhibit any evidence of toxicity on the sage plant. Previous researches on other plant species including *Alyssum desertorum*, *Borago officinalis*, *Calendula officinalis*, and *Thymus vulgaris* (Yadegari, 2017), documented that Zn and Fe had improving impacts on the growth parameters of such plants. ZnO NPs increased chlorophyll pigments in *Borago officinalis* (Mohammadi et al., 2018). Likewise, on *Origanum majorana* El-Khateeb et al. (2020), on rosemary Hassanpouraghdam et al. (2020), and on *Mentha piperita* Lafmejani et al. (2021). They found that spraying of nano zinc singly or together with other elements enhanced the vegetative characteristics of such plants. They added that the effect of nano zinc was related to the level, kinds of plants, and utilization time



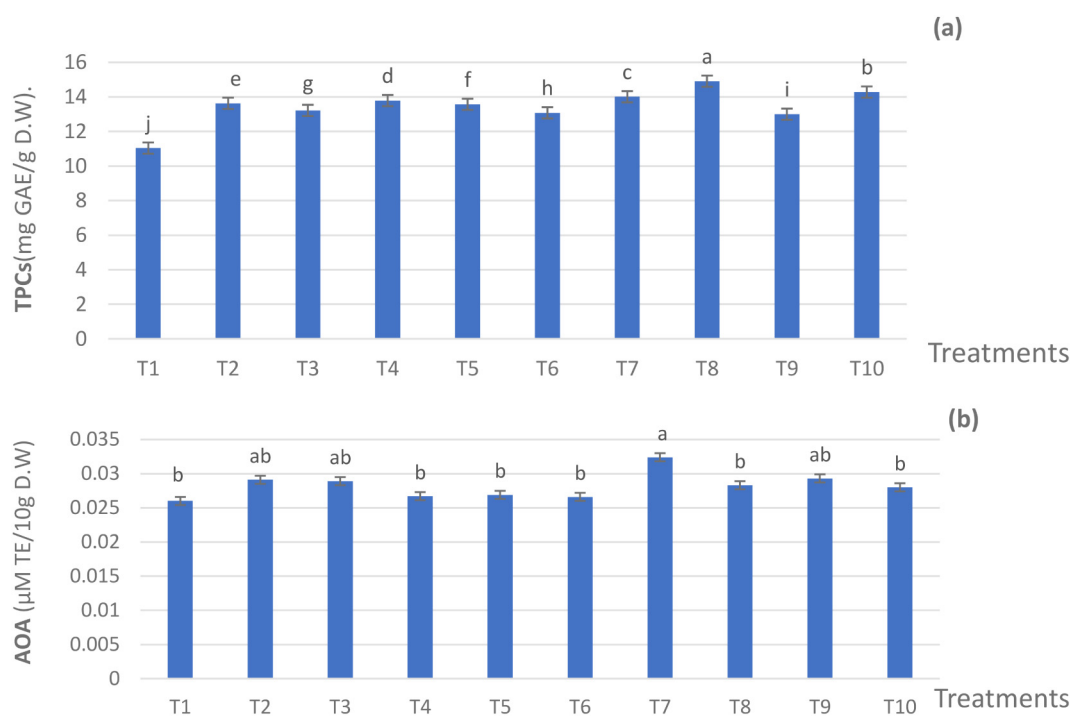


FIGURE 4

Effect of the fertilization treatments during the 2022 season on (A) TPC and (B) AOA. The Duncan's multiple range test indicates that there is no significant difference ( $p \leq 0.05$ ) among the means in the figures that are followed by the same letters. T1 - 100% NPK (recommended dose) as a control, T2 - 75% NPK + 15 g/L date pollen extract (DPE), T3 - 50% NPK + 25 g/L DPE, T4 - 75% NPK + 0.1 g/L SiO<sub>2</sub> NPs, T5 - 50%NPK + 0.2 g/L SiO<sub>2</sub> NPs, T6 - 75% NPK + 1 g/L ZnO NPs, T7 - 50% NPK + 1.5 g/L ZnO NPs, T8 - 50% NPK + 25 g/L DPE + 0.2 g/L SiO<sub>2</sub> NPs, T9 - 50% NPK + 15 g/L DPE + 1.0 g/L ZnO NPs + 0.1 g/L SiO<sub>2</sub> NPs, and T10 - 25% NPK + 25 g/L DPE + 1.5 g/L ZnO NPs + 0.2 g/L SiO<sub>2</sub> NPs.

(plant age). It has been reported that 6 g/L Zn NPs gave the maximum fresh and dry weights of sweet basil shoots in relation to 0, 2, and 4 mg/L Zn NPs or 2, 4, and 6 mg/L of either iron NPs or potassium NPs (Danaee and Abdossi, 2021). According to Khan et al. (2023), ZnO at 10 mg/L induced more biomass and chlorophyll content of *Echinops macrochaetus* than the untreated plants. Additionally, El-Mahrouk et al. (2024) found that 2g/L nano ZnO +20g/L date pollen extract, combined with half dose of the recommended NPK fertilizer was the most positive and effective treatment for the vegetative traits of sweet basil. Nano zinc at 10–800 mg/kg soil improved the quality of potato tubers (Zhang et al., 2024). The beneficial impact of nano SiO<sub>2</sub> on plant metabolism leads to improved sage growth efficiency. Silicon nanoparticles possess physiological characteristics that enable them to affect a plant's metabolic processes (Rastogi et al., 2019). SiO<sub>2</sub> NPs are capable of transferring elements and DNA into plant tissues (Torney et al., 2007). Additionally, nano-silica enhances germination rates, radicle height, and plant dry weight (Azimi et al., 2014). SiO<sub>2</sub> NPs also improve nutrient availability in maize (Suriyaprabha et al., 2012).

Additionally, silicon spraying affected the relative chlorophyll content of orchids (Mantovani et al., 2018). The development of *Arabidopsis thaliana* seedlings was stimulated with use of 10 mg/L SiO<sub>2</sub> NPs and the chlorophyll level was increased to the maximum rate of 500 mg/L (Azhar et al., 2021). The spraying of silicon or nitric oxide, especially a combination of them, boosted leaf chlorophyll, which led to an improvement in photosynthesis, and

in turn accounted for the weight of shoots of the sage plant under Cu-stress (Pirooz et al., 2021). Fenugreek plants treated with 200 kg/ha NPK (20:20:20) + NPK NPs at 2 g/L (20:20:20) + 2 g/L of nano elements (B, Mo, Cu, at 0.5%, Zn and Mg at 1.5%, and Fe at 8%) + nano seaweed extract at 2 g/L resulted in maximum values of the growth characteristics, when compared to untreated plants (Al-Saidi et al., 2022). Also, SiO<sub>2</sub> NPs at 250, 500, and 1000 mg/L could prove the significant efficiency in seed germination and growth seedling length of *Calendula officinalis* and *Mellisa officinalis* (Bovand et al., 2023). Silicon NPs at 500 mg/kg soil improved root, shoot, length, as also the chlorophyll content of *Eruca sativa* (Mathur and Goswami, 2024). SiO<sub>2</sub> NPs at 6, 12, and 18 g/L significantly increased the number and fresh and dry weights of shallot tubers (Rahmawati and Wulandari, 2024). Notably, NPs can have varying impacts on plant growth and development, depending on factors such as plant species and the characteristics of the nanoparticles, including their nature, composition, reactivity, and dosage (Khodakovskaya et al., 2012).

Enhancing impacts of DPE foliar sprays on sage growth and development were demonstrated in our results, which align with the previously mentioned constituents of DPE. In general, many authors have documented the improving impact of DPE on the development of plant species, attributing it to the presence of auxins and cytokinins in pollen (Alferez et al., 2000; Merwad et al., 2015). Hassan (2011) found that number, length, and weights (fresh and dry) of banana shoots were increased as a result of adding 200 mg/L of water pollen extract to a tissue culture medium. Similarly, Abou-

TABLE 5 Impact of the fertilization treatment on *Salvia officinalis* leaf N, P, K Zn, Si and total carbohydrate % in 2022 season.

Fertilization treatments	N%	P%	K%
T1	2.06 ± 0.01a	0.86 ± 0.03a	2.09 ± 0.001e
T2	2.06± 0.01a	0.60 ± 0.01e	2.10± 0.00 d
T3	1.76± 0.01 b	0.70 ± 0.00c	2.21± 0.02 c
T4	1.30± 0.00d	0.73± 0.03 b	1.89± 0.001 e
T5	1.23± 0.01 d	0.53± 0.01 f	1.61± 0.02 f
T6	1.50± 0.01 c	0.55± 0.01 f	2.50± 0.01 b
T7	1.50 ± 0.01c	0.59± 0.01 e	2.50± 0.01 b
T8	1.11 ± 0.00e	0.63 ± 0.02d	1.61± 0.02 f
T9	1.30 ± 0.00d	0.75 ± 0.00 b	3.03± 0.06 a
T10	1.53 ± 0.01c	0.73 ± 0.01 b	2.52± 0.03 b
	Zn%	Si%	Total carbohydrate %
T1	0.07± 0.00 g	0.00010 g	1.13 ± 0.05 d
T2	0.08± 0.00ef	0.00020 g	0.86 ± 0.11 e
T3	0.07 ± 0.01 fg	0.00443 e	1.13± 0.02 d
T4	0.10± 0.00 c	0.00143 f	0.83 ± 0.05 e
T5	0.09± 0.01 cd	0.00196 f	0.93 ± 0.05 e
T6	0.12± 0.00 b	0.00183 f	1.23± 0.06 cd
T7	0.16± 0.00 a	0.00873 c	1.73 ± 0.10 a
T8	0.08± 0.01 de	0.01406 b	1.53± 0.02 b
T9	0.05 ± 0.01 h	0.00623 d	0.93 ± 0.05 e
T10	0.05± 0.01 h	0.02336 a	1.33± 0.06 c

Means in each column followed by the such letters are not significantly different ( $p \leq 0.05$ ) by Duncan's Multiple Range Testing.

T1- 100% NPK (recommended dose) as a control, T2- 75% NPK +15g/L date pollen extract (DPE), T3- 50% NPK +25 g/L DPE, T4- 75% NPK + 0.1 g/L SiO<sub>2</sub> NPs, T5- 50% NPK + 0.2 g/L SiO<sub>2</sub> NPs, T6- 75% NPK + 1 g/L ZnO NPs, T7-50% NPK + 1.5 g/L ZnO NPs, T8- 50% NPK + 25 g/L DPE + 0.2 g/L SiO<sub>2</sub> NPs, T9- 50% NPK + 15 g/L DPE + 1.0 g/L ZnO NPs+ 0.1 g/L SiO<sub>2</sub> NPs, and T10- 25% NPK +25g/L DPE+ 1.5 g/L ZnO NPs + 0.2 g/L SiO<sub>2</sub> NPs.

Sreea and Yassen (2016) revealed that 20 g/L DPE had the greatest impact on improving the vegetative parameters of *Strelitzia reginae*, compared to the control with 5-15 g/L of DPE.

Based on the previous data, there are several factors, such as, climatic conditions and environmental factors, which can affect the EO% (Russo et al., 2013). The agricultural practices such as fertilization, controlling of weeds, insects, etc., as well as temperature and flowering stage development at harvesting time can positively regulate EO content (Hassiotis et al., 2014). Seemingly a balance had occurred among macro- and micro-nutrients as a consequence of adding NPK, NPs of ZnO and SiO<sub>2</sub>, or DPE (that contains several constituents). This balance may enhance various physiological, chemical, and biochemical processes or enzymatic activities in plant cells, including secondary metabolism, leading to increased EO synthesis. The concentrations of Ca<sup>++</sup> and K<sup>+</sup> strongly affect the EO yield

(Yavari et al., 2010). According to Alhasan (2020), there is a non-linear relationship between the NPK rate and basil EO (% v/w). Zinc plays a role in carbohydrate synthesis, protein metabolism, and auxin regulation, which in turn affects the EO content (Broadley et al., 2007). Furthermore, the positive effect of nano Zn on EO productivity, individually or in combination with another element, has been documented in studies on *Pimpinella anisum* (Pirzad and Barin, 2018), *Origanum majorana* (El-Khateeb et al., 2020), *Brassica nigra* (Zafar et al., 2020), and *Mentha piperita* (Lafmejani et al., 2021). Si stimulated secondary metabolism content of many species of plants (Ahanger et al., 2020; Santisree et al., 2020). Furthermore, Si is involved in EO synthesis, as demonstrated by numerous studies on basil (Farouk and Omar, 2020) and rose (Farahani et al., 2021) under drought stress and sage under Cu stress (Al-Saidi et al., 2022). Currently, there is very limited literature available on the use of DPE on medicinal and aromatic plants. However, it can be inferred that the improvement in sage EO synthesis may have been caused by the stimulative influences of various chemical and biochemical constituents of DPE on the different metabolic activities of plant cells, leading to enhanced essential oil synthesis.

It was noticed that higher percentages of monoterpene hydrocarbons, total compounds, and oxygenated hydrocarbons had resulted from usage of 75% NPK, combined with lower levels of DPE, SiO<sub>2</sub> NPs, and ZnO NPs, respectively; even as a higher percentage of sesquiterpene hydrocarbons were observed in the plants EO that received 50% NPK plus high levels of DPE and ZnO NPs. The synthesis of these compounds might be correlated with the concentrations of elements in the fertilization treatments used and the balance between the NPK level and DPE or concentrations of the NPs used. Although N is a necessary component of secondary metabolism, P has the necessary roles for the transport of carbohydrates and energy in leaf cells (Hawkesford et al., 2012). K has a role in a number of essential plant metabolic activities that enhance phloem transport, osmotic equilibrium, and photosynthesis (Hamzei et al., 2014). Additionally, NPs stimulate secondary metabolism such as terpenoid compounds (Mei et al., 2020) and promoted gene expression of secondary metabolism (Wang et al., 2021). DPE constituents have an impact on the metabolic processes, leading to elevated secondary metabolism (El-Mahrouk et al., 2024) in sweet basil. In the sage plant (Amer et al., 2019), in which NPK at 100%, 75%, and 50% alone or in combination with a biofertilizer (N-fixing bacteria and phosphate solubilizing bacteria) differently affect the total EO-identified compounds ranged from 9 to 22, comprising 83.6% to 99.9% of EO, including monoterpenes (0% to 13.4%), oxygenated monoterpenes (83.4% to 96.8%), sesquiterpenoids (0% to 4.1%), oxygenated sesquiterpenes (0% to 0.9%), and oxygenated diterpenes (0% to 0.8%), and the highest  $\alpha$ -thujone of 56.2% was observed with 100% NPK combined with biofertilizer, while 75% NPK with biofertilizer showed the highest percentage of B-thujone (55.8%) and camphor (29.2%). Also, Sharma et al. (2019) mentioned that 49 aromatic compounds were observed in sage EO and the principal constituents were 1,8- cineole, camphor,  $\alpha$ -thujone,  $\alpha$ -humulene, rosmarinic acid, and quercetin. Basil EO components (percentage and amount) were impacted by nano-chelate fertilizers (K, F, and Zn) at 2–6 mg/L (Danaee and Abdossi, 2021).

Total phenolic compounds and antioxidant activity essentially have beneficial roles in plant protection. TPCs have significant roles in plants because of their ability to scavenge free radicals, which is attributed to their hydroxyl groups. Consequently, the presence of plant TPCs can be immediately linked to their AOA (Tosun et al., 2009). Moreover, it has been established that phenols possess antioxidant characteristics and are employed in the enzyme activity system and principle metabolic production (Jokar and Ronaghi, 2015). Additionally, studies on sunflower (Kiarostami et al., 2010), rosemary (Rady et al., 2011), lavender (Chrysargyris et al., 2018), and sweet basil (Ahmed et al., 2019) have demonstrated an association between TPCs and antioxidant activity that is good for plants.

According to Lingyun et al. (2016), the beneficial impacts on AOA may be attributed to important zinc fractions in phenolic synthesis, maintaining membrane integrity, enhancing the scavenging molecule levels, protecting fundamental molecules, preserving groups of sulfhydryl, and preventing unnecessary interactions between iron and groups of other chemicals. Thus, zinc has an important function of maintaining cell membranes under harmful conditions. Application of Zn or Zn NPs at various levels increased TPCs in various plant species like basil (Fallahi et al., 2016; Danaee and Abdossi, 2021), *Chrusanthemum balsamita* (Derakhshani et al., 2011), and rosemary (Hassanpouraghdam et al., 2020). Also, Zn improved the antioxidant system in wheat (Adrees et al., 2021). Similarly, ZnO NPs alone or in combination with Si NPs, positively influenced antioxidant enzyme activity in two *Brassica napus* species (El-Badri et al., 2021).

Furthermore, Nourozi et al. (2019) documented that SiO<sub>2</sub> NPs can enhance TPC and flavonoid content, rosmarinic acid, and xantomicro, and raise AOA in the hairy roots of *Dracocephalum kotschy* Boiss via upregulated rosmarinic acid synthase and phenylalanine ammonia lyase (PAL) expression genes. Over and above all this, the role of Si in improving TPC biosynthesis in cells of plants by enhancing the enzymatic activities conjunct in the pathway of phenylpropanoid, such as PAL, has been recognized (Ahangar et al., 2020). Moreover, treating *Salvia officinalis* plants with 1 μM Si or 200 μM Si NPs has been shown to enhance TPCs and the DPPH scavenging activity (Al-Saidi et al., 2022). The application of 75% NPK + biofertilizer led to improvements in total phenol content and antioxidant activity compared to NPK at 50% and 100% (Amer et al., 2019). Additionally, K NPs have been found to have a positive effect on total phenol contents in basil (Danaee and Abdossi, 2021). Total phenols and antioxidant activity biosynthesis have been significantly increased in leaves of sweet basil, by applying different combinations of three-fourth or one-half of an NPK dose, with ZnO NPs, SiO<sub>2</sub>NPs, and date pollen extract, in comparison to a full dose of NPK (El-Mahrouk et al., 2024). Silicon oxide NPs at 100–1000 mg/kg soil raised the total protein, phenolic and flavonoid contents, and antioxidants in comparison to the control plants of *Eruca sativa* (Mathur and Goswami, 2024).

Our study focused on the chemical composition of sage leaves to evaluate the role of various applications on the percentages of nitrogen (N), phosphorus (P), potassium (K), zinc (Zn), silicon (Si), and total carbohydrates. The concentrations of these parameters in

leaves under different treatments may depend on their levels and the balance between them in the applications. Zinc can be stored in plant leaves through ZnO nanoparticle (NP) vegetative applications, and these nanoparticles may act as an active zinc source in plant metabolism (Li et al., 2018, 2019). Zinc plays a crucial role in the synthesis of carbohydrates and proteins (Soliman et al., 2015). Additionally, Sadak and Bakry (2020) demonstrated that ZnO NPs at 20, 40, and 60 mg/L gradually and significantly increased total carbohydrates in *Linum usitatissimum* compared to the control. Similarly, Hassanpouraghdam et al. (2020) found that foliar spraying of Zn NPs at 3 mg/L increased rosemary Zn content, while the highest K content was observed in the control. Notably, foliar sprays of Zn and K nano-chelates at 2, 4, and 6 mg/L increased basil Zn and K contents, respectively, compared to the control (Danaee and Abdossi, 2021). A study on *Pimpinella anisum* by Pirzad and Barin (2018) found a significant increase in leaf Zn with 6 g/L of Fe+Zn foliar spraying, with maximum leaf N at 2 g/L Fe + 6 g/L Zn, and the highest leaf P and K contents at 4 g/L Zn. Nano Zn foliar spray at 50 and 100 mg/L increased total carbohydrates in marjoram more than in untreated plants (El-Khateeb et al., 2020). Furthermore, Zafar et al. (2020) reported a steady increase in Zn content in all parts of *Brassica nigra* after applying ZnO NPs at 200–600 mg/kg soil. Zinc content also increased in various tissues of potato grown in soil containing 10–800 mg ZnO NPs/kg soil (Zhang et al., 2024). Additionally, Si foliar spraying improved uptake and increased Si content in *Phalaenopsis* and *Dendrobium orchids* (Mantovani et al., 2018).

There is great interest in using DPE because it contains several compounds, including macro and micro-elements, which enhance the elements in plant tissues. This was supported by Abou-Sreya and Yassen (2016) showed that 10g/L DPE foliar application stimulated the uptake and concentrations of N, P, K and total carbohydrates in *Strelitzia reginae* relative to control. Amer et al. (2019) treated sage with a 75% NPK dose plus *Azotobacter chroococcum*, *Bacillus megaterium* var. phosphaticum, and *B. cereus* resulting in the maximum carbohydrate value compared to the control, and 50 and 100% NPK alone or together with biofertilizer. Thus, the utilization of macronutrients such as N, P, K, Mg, S, and Ca, in combination with NPs, allows for precise nutrient delivery to plants while reducing the bulk requirements and associated costs (Ditta and Arshad, 2016; Chhipa, 2017).

## 5 Conclusions

The current research suggests the positive benefits of spraying NPs of ZnO and SiO<sub>2</sub> and natural extracts, such as DPE. These can be used as partial substitutes of traditional NPK fertilizers. Whereas, ZnO NPs, SiO<sub>2</sub> NPs, and DPE at various levels, combined singly or together with 75%, 50%, and 25% NPK RD have proved to be effective in enhancing the growth traits (plant height, chlorophyll index, and aerial fresh and dry weights/plant), EO productivity (EO % and yield, and chemical composition of EO), biochemical composition (TPCs and AOA) and leaf chemical composition (N, P, K, Zn, Si, and total carbohydrates percentages) of the sage (*Salvia officinalis*) plant, in relation to 100% NPK RD, with some

exceptions. The used treatments have varied impacts on the studied traits, with significant differences among themselves in most cases. Fifty-eight compounds which were distributed among the fertilization treatments were identified and ranged from 11 to 32. Also, the fertilization treatments had different effects on the EO composition (monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated hydrocarbons, and their constituents). Consequently, the application of nanoparticles of various elements and DPE could be considered, to reduce the excessive utilization of conventional fertilizers, with the aim of producing safe medicinal and aromatic products. A future study on sage will be regarding the effects of NPs of some nutrients and natural extracts on sage production, under different environmental conditions.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

EME: Supervision, Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. EA: Formal analysis, Methodology, Writing – original draft. MG: Investigation, Methodology, Writing – original draft. MAA: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. AM: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. EE: Formal analysis, Validation, Writing –

original draft, Writing – review & editing. MG: Formal analysis, Validation, Writing – original draft, Writing – review & editing.

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## Conflict of interest

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