Print ISSN: 0375-9237
Online ISSN: 2357-0350

EGYPTIAN JOURNAL OF BOTANY (EJBO)

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PUBLISHED BY

Studying the behavior of Titanium (bulk and nanoparticles) in the soil and its effect on the growth and productivity of *Salvia fruticosa* L. plant

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The present experiment was undertaken to study the behavior of titanium in soil and the effect of application methods (foliar spraying or soil injection) and TiO_2 concentrations (0, 5, 7.5, and 10 ppm as nanoparticles or 25, 50, and 75 ppm as bulk particles) as well as their interaction treatments on *Salvia fruticosa* (three-lobed sage) plant. In most cases, the tallest plants, the maximum yield of dry herb/feddan, yield of volatile oil/feddan, values of chlorophyll, and nitrogen, phosphorous, and potassium (NPK) values of *Salvia fruticosa* were achieved from the interaction treatment between foliar spraying and 10 ppm TiO_2 nanoparticles while the main components of three-lobed sage essential oil were 1,8-cineole, camphor, α-pinene, and camphene, respectively. Also, from the sequential extraction method of Ti in soil, the highest fraction of Ti was found in residual forms at each of the treatments. This confirmed that using TiO_2 (bulk or nanoparticles) in either soil or foliar application is harmless and improved chlorophyll production and consequently improved photosynthesis which leads to increased productivity of the plants under this study.

Keywords: Laser, TiO_2, application methods, three-loped plant

ARTICLE HISTORY
Submitted: December 05, 2024
Accepted: May 02, 2024

CORRESPONDANCE TO
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Email: saskhater@hotmail.com
DOI: 10.21608/ejbo.2024.253273.2594

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INTRODUCTION

Three-lobed sage (*Salvia fruticosa* L.), also known as Greek sage or Turkish sage, is a fascinating aromatic and medicinal shrub that is among the family of Lamiaceae. It may be well known about *Salvia officinalis* by its trifoliate leaves. The three-lobed sage plant is unique to the Eastern Mediterranean Basin, and the entirety of its native stretches from West Syria through Cyrenaica, Sicily, and Southern Italy. Three-lobed sage is mostly imported into the world market from Greece, Turkey, Albania, Cyprus, and Crete, where most of the production is gathered from natural populations (Putievsky *et al.*, 1986; Rivera *et al.*, 1994; Kintzios, 2003; and International Trade Center Report, 2015).

In traditional medicine, the plant known as maramia in the Middle East is frequently used as a medicinal tea to treat liver diseases, regulate menstruation, treat mouth sores, accelerate wound healing, and lessen the symptoms of colds and memory loss. The volatile oil that was extracted from the herb has antifungal and antibacterial properties, especially the one that includes 1,8-cineole as a main component. Given the significance of this wild plant, various Middle Eastern nations, like Lebanon, have passed legislation limiting the wild harvest and transmission of *Salvia fruticosa*. Ministerial Decision 179/1 was issued in 2012 (Undp and Lari, 2013; Yaniv and Dudai, 2014; and European Medicines Agency, 2015).

The recent advancements in nanobiotechnology have expanded the applications of nanomaterials in the field of agriculture to enhance seed germination and growth of plants. Nanomaterials' distinctive properties, such as their size and higher surface area when compared to their respective bulk forms, are what cause them to have positive effects (Scrinis and Lyons, 2007). Plant biotechnology and agricultural research are being attached to developing new methods in nanotechnology. In the past ten years, sufficient research has been done to utilize the growth-promoting effects of nanoparticles, and novel approaches to nano-agriculture have been developed (Mehrnaz and Mansour, 2014; Elkhateeb et al., 2024; Soliman et al., 2024). The size range of nanoparticles (NPs) is 1-100 nm. There are several uses for nanotechnology in numerous fields of study, including agriculture and medicine, etc. (Mohanraj and Chen, 2006).

The secondum is the ninth most abundant element and the second most common transition metal in the earth's crust. The most significant effects of Ti compounds on plants are improvement of the growth of several plants. It raises the levels of some essential components and increases the activities of peroxidase, catalase, and nitrate reductase in plant tissues. TiO_2 nanoparticles promote germination of seed and growth of spinach (Zheng *et al.*, 2005). At an appropriate concentration, TiO_2 NPs can encourage plant photosynthesis and nitrogen uptake, which so significantly improve spinach yield (Zheng *et al.*, 2005; Hong *et al.*, 2005; and Yang *et al.*, 2006). When there is UV radiation present, TiO_2 nanoparticles have a variety of impacts on redox systems of reactive oxygen species (ROS) (Kim *et al.*, 2010) and they have an increasing effect on fennel seed germination (Feizi *et al.*, 2013).

Ti behavior in soil solution is dependent on the chemical and physical conditions. The bioavailability, toxicity, and mobility of Ti are related to its species. The various chemical forms or mechanisms of metals which bond to soil constituents are ascertained through sequential extraction operations (Jena *et al*., 2013). Therefore, the current investigation is carried out to study the methods of application (soil injection or foliar spraying) and behavior of titanium dioxide (TiO_2) concentrations (bulk or nanoparticles) in the soil and its effect on the growth and productivity of *Salvia fruticosa* (three-lobed sage) plant and the partitioning of Ti in Baloza soil to confirm its safety or toxicity for use as fertilizers.

MATERIALS AND METHODS

This experiment was conducted during the two sequential seasons of 2020 and 2021 in Baloza Station which belongs to Desert Research Center (30^o 07$^\backslash$ N and 31^o 20$^\backslash$ E), North Sinai, Egypt, to study the impact of the application methods (soil injection or foliar spraying) and behavior of titanium dioxide (TiO_2) concentrations as bulk particles (B.) or nanoparticles (N.) in the soil and its effect on the growth and productivity of *Salvia fruticosa* (three-lobed sage) plant under North Sinai conditions. *Salvia fruticosa* seedlings were graciously provided by the Baloza Experimental Station. The seedlings were cultivated in the open field in March 2020/2021 for the two seasons, respectively. According to Chapman and Pratt (1971), the mechanical and chemical characteristics of the utilized soil are given in Table 1.

Plants were placed in rows 75 cm apart and with 50 cm between their rows using a drip irrigation system. Table 2 revealed the chemical analysis conducted on the used water. During the soil preparation process, 10 m^3/feddan of compost fertilizer was applied. Chemical fertilization was applied as the recommended dose for sage plants in newly reclaimed soil (Abd El-Azim, 2003). All agricultural processes were done in accordance with the references of the Egyptian Ministry of Agriculture.

This work consists of 14 treatments, which were the combination between two application methods (soil injection or foliar spraying) and seven concentrations from titanium dioxide as follows: 0, 5, 7.5, and 10 ppm as nanoparticles, 25, 50, and 75 ppm as bulk particles. Titanium dioxide was added to plants three times, the first addition being carried out 21 days after transplanting date. Meanwhile, the second and third times were conducted at 30-day intervals after the first and second one and were conducted again after 15 days from the first cut. Moreover, the second and third ones were conducted at 30-day intervals after the first and the second ones.

Titanium dioxide (TiO_2) in a powder form with a reagent grade of 99.9% was obtained from Sigma Aldrich company.

Preparation of TiO_2 Nanoparticles

The following process was used to create the initial TiO_2 nanopowder. A colloidal solution was created using pulsed laser ablation in titania target liquid (99.9%). Pulsed laser ablation in a liquid was performed using a Nd: YAG laser. For 3 hours, the ablation was performed in a 100 mL cylindrical reactor. The solution was then dried in air at 60°C. The initial sample was annealed at temperatures ranging from 200 to 1000°C. Kanitz *et al*. (2019) and Gavrilenko *et al*. (2019) discuss in detail the equipment and the experiment for preparing nanopowders.

Characterization of TiO_2 Nanoparticles

Transmission Electron Microscopy (TEM) was used to measure the images of the particles. A little drop of the produced solutions was placed onto a copper microgrid and allowed to dry to create TEM samples. The photos of the TiO_2 nanoparticles show that they have a spherical shape and an average particle size of 5 nm to 100 nm (Figure 1). The plants were cut twice in the season, *i.e.*, in July and November. Harvesting was done by cutting the vegetative parts of plants 15 cm above the soil surface leaving two branches for regrowth.

Recorded Data Were as Follows
Growth and Productivity Characteristics

Plant height (cm), herb fresh yield/plant (g), herb fresh yield/feddan (kg), herb dry yield/plant (g), and herb dry yield/feddan (kg).

Table 1. Some chemical and physical characteristics of the experimental soil.

Particle size distribution (%)			Texture class	EC^{*} (dSm^{-1})	pH^{**}	Soluble ions (meq/L)								Available nutrients (ppm)		
						Cations				Anions						
Sand	Silt	Clay				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	SO_4^{--}	Cl^{-}	N	P	K
87.9	7.37	4.73	Sand	1.19	8.03	3.92	2.04	3.91	2.03	-	3.43	5.02	3.45	15.1	5.9	70.3

Some total trace elements (mg kg^{-1})							
Ti	V	Cr	Sr	Fe	Mn	Zn	Cu
0.036	46.30	45.97	28.62	14004	186.94	97.90	28.67
Available trace elements (mg kg^{-1})							
Ti	V	Cr	Sr	Fe	Mn	Zn	Cu
0.006	0.20	0.15	0.19	7.93	5.04	1.99	0.09

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

Table 2. Chemical composition of irrigation water.

Samples	pH	EC (ppm)	SAR	Soluble cations (mM/L)				Soluble anions (mM/L)			
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	SO_4^{--}	Cl^{-}
1st season	7.35	1456	4.92	2.89	3.18	8.55	0.58	0.10	5.58	2.05	7.47
2nd season	7.05	1512	5.35	3.24	3.03	9.45	0.40	0.50	3.76	3.67	8.19

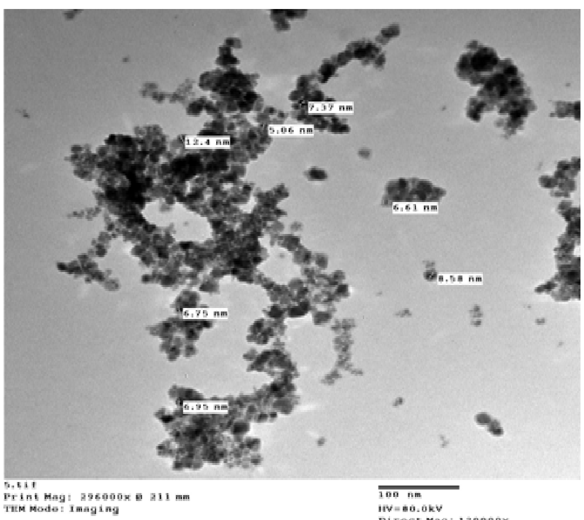

Figure 1. TEM images and size distribution of titanium dioxide nanoparticle.

Determination of some Chemical Constituents

Volatile Oil Percentage. This assay was performed according to British Pharmacopoeia (1963), volatile oil yield/plant (mL), yield of volatile oil/feddan (L), and essential oil chemical components. The GC-MS analysis of volatile oils was carried out in the first season by Gas Chromatography-Mass Spectrometry (GC-MS) instrument stands at the Laboratory of Medicinal and Aromatic Plants, National Research Center, Egypt.

Total Chlorophyll Content in Leaves. Using a Minolta chlorophyll meter (model SPAD 502), the total chlorophyll in plant leaves was measured in SPAD units. Chlorophyll measurements were made using the recently fully expanded leaf and 10 readings were averaged per experimental unit in accordance with Markwell *et al*. (1995).

Measurement of Nitrogen, Phosphorus, and Potassium in Plant׳s Dry Herb. N, P, and K percentages were measured in an acid-digested solution that was made in accordance with Cottenie *et al.* (1984).

Soil Properties at the End of the Experiment

Determination of Available Nitrogen, Phosphorus, and Potassium in Soil

Available nitrogen in soil samples was extracted by 2M potassium chloride solution and determined according to Dhank and Johnson (1990). Available potassium and phosphorous were extracted by DTPA+ammonium carbonate solution, and the measurements were made according to the method described by Soltanpour (1985).

Evaluation of Ecological Risk Assessment of Titanium

The principles of the Risk Assessment Code (RAC) were applied to assess the reactivity of sediments. Based on the proportion of exchangeable and carbonate-bound titanium in the sediment, the RAC scale is used to evaluate potential mobility and risk (Karak *et al*., 2011). Sequential extraction techniques were used to calculate the Ti fractions (Tessier *et al*., 1979). Ti is categorized into the following five fractions operationally:

F1 (Soluble and Exchangeable). Through mechanical shaking for one hour at 20°C, 20 milliliters of 1.0 M

$MgCl_2$ solution adjusted to pH 7 was used to extract the Ti soluble and exchangeable from the soil.

F2 (Bound to Carbonates). Through mechanical shaking for four hours at 25 °C, the residual from the previous step was extracted with 20 ml of 1.0 M sodium acetate solution adjusted to pH 5 with acetic acid.

F3 (Bound to Fe-Mn Oxides). 50 ml of 0.04 M $NH_4OH.HCl$ in 25% acetic acid at 96°C for 5.5 hours was used to shake the residue of the second extraction to extract Ti bound to Fe and Mn oxides.

F4 (Bound to OM). The Ti bound to organic matter (OM) was extracted by adding 7.5 ml of pH 2 adjusted solution (0.02 M HNO_3 and 12.5 ml of 30% H_2O_2) to the F3 residual. This was followed by two hours of constant agitation at 85°C, followed by 3 hours of adding 7.5 ml of 30% H_2O_2 (pH 2) at 85 °C, then cooling to room temperature, and shaking for 30 minutes.

F5 (Residual Fraction or Bound to Soil Matrix). Ti bound to soil matrix was extracted by transferring the residue from F4 into a digestion container and adding aqua regia.

Determination of Bioconcentration Factors (BCFs)

Titanium and some heavy metals contents were determined in tissues of *Salvia fruticosa* plant and the soil using Inductivity Coupled Argon Plasma (ICAP) after digestion. The biological absorption coefficient was then used to quantify the degree of element uptake by plants from soil. Nagaraju and Karimulla (2002) defined it as the ratio of element concentration in plant ash to total metal concentration in soils. Mountouris *et al*. (2002) defined the bioconcentration factor (BCF) or translocation factor as the ratio of metal concentration in vegetable edible parts such as leaves, seeds, and roots to the total metal concentration in soil.

The bioconcentration factors (BCFs) were calculated as ratio of titanium and some heavy metal in tissue to that in the soil:

BCF = plant tissue trace element concentration / soil trace element concentration.

The application method (soil injection or foliar spraying) and the concentrations of TiO_2 (bulk or nanoparticles) were the two factors that were tested in a factorial fashion using a split blot design. The application method was represented by the main plots, while the concentrations of TiO_2 were included in the subplots with three replicates. Using the Statistix computer program, the differences in means were evaluated using the least significant difference (LSD) test at 5% (Analytical Software, 2008).

RESULTS

Growth and Yield Characteristics

From the data presented in Figure 2 and Tables 3 and 4, results show that there are significant differences between the two methods of application (soil injection or foliar spraying) in plant height, herb fresh weight, and herb dry weight per plant and per feddan. The foliar spraying was better than soil injection, which recorded a significant increase in this regard in the second cut of the first season and both of cuts in the second season.

All concentrations of titanium dioxide as nanoparticles (N. Ti) or bulk particles (B. Ti) gave significant differences in this respect compared to the control treatment. The concentration 10 ppm N. Ti was the superior treatment, which gave significant increase in plant height, herb fresh weight, and herb dry weight per plant and per feddan followed by 50 ppm B. Ti compared to other concentrations and control treatment.

The results presented in the same figure and tables reveal that the interaction treatments between application method (soil injection or foliar spraying) and concentrations of TiO_2 (as nano- or bulk particles) recorded a significant increase in plant height and herb fresh and dry weights of three-lobed sage plants in comparison with control treatment. Also, increasing titanium dioxide concentrations under each method of application increased plant height as well as herb fresh and dry weight per plant and per feddan. Moreover, the interaction treatment between foliar spraying by 10 ppm N. Ti gave significant increase in this regard compared to other interaction treatments and control treatment. These results were found in the first and second cuts of the two seasons.

Volatile Oil Production: Data recorded in Figure 3 and Table 5 indicate that there were no significant differences between method of addition (foliar spraying and soil injection) in volatile oil percentage but showed significant differences in volatile oil yield per plant and per feddan of three-lobed sage plant. At the same time, the foliar spraying method recorded a significant increase in volatile oil yield per plant and per feddan of three-lobed sage plant compared to the soil injection method. The same figure and table pointed out that volatile oil percentage, oil yield/plant

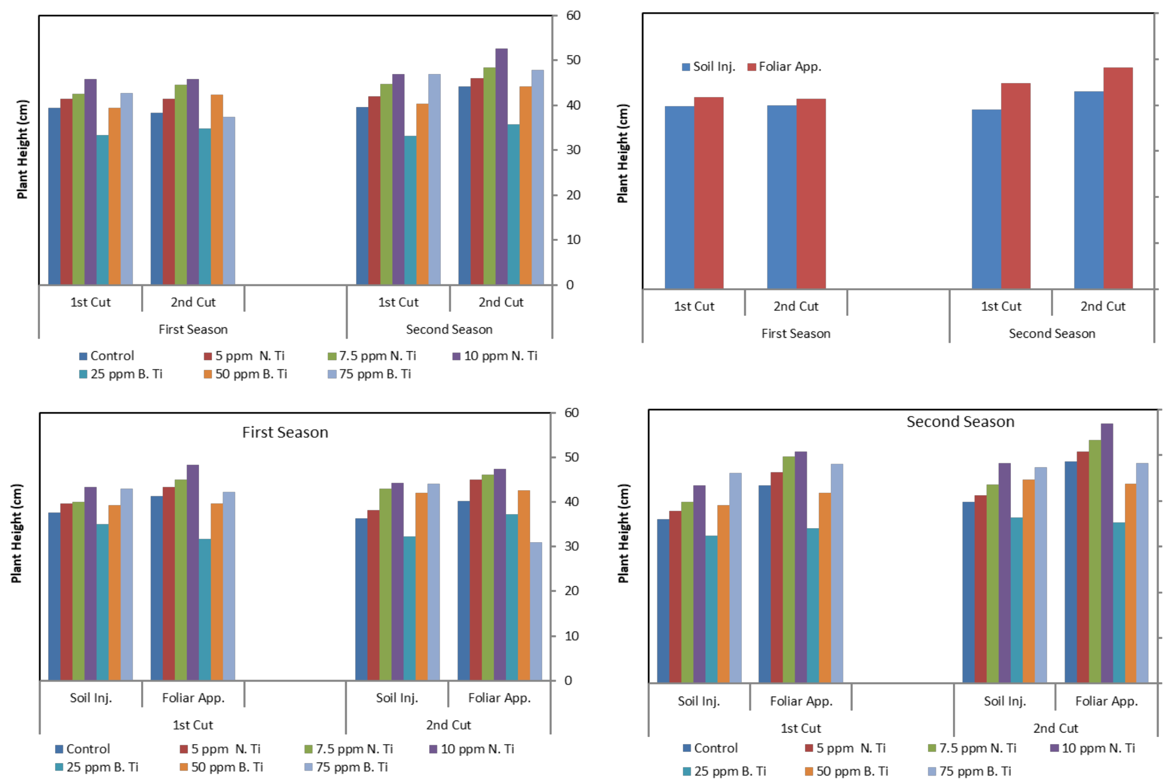

Figure 2. Effect of application method and titanium concentrations as well as their interaction treatments on plant height (cm) of *Salvia fruticosa* (three-lobed sage) during two cuts of the two seasons (2020/2021).

Table 3. Effect of application method and titanium concentrations as well as their interaction treatments on herb fresh weight of Salvia fruticosa (three-lobed sage) plant during two cuts of the two seasons (2020/2021)

Application Method (A) Ti form (T)	Herb fresh weight/plant (g)						Herb fresh weight/feddan (kg)					
	First season											
	1st cut			2nd cut			1st cut			2nd cut		
	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)
Control	67.39	65.93	66.66	96.99	119.22	108.11	1132.20	1107.60	1119.9	1629.40	2003.00	1816.20
5 ppm N. Ti*	83.30	79.25	81.28	125.28	171.25	148.26	1399.5	1331.30	1365.40	2104.60	2877.00	2490.80
7.5 ppm N. Ti	85.96	99.72	92.84	148.39	222.58	185.49	1444.10	1675.20	1559.70	2492.90	3739.40	3116.10
10 ppm N. Ti	93.51	143.60	118.55	157.69	310.00	233.85	1570.90	2412.40	1991.70	2649.20	5208.00	3928.60
25 ppm B. Ti**	76.02	101.53	87.86	161.17	145.00	153.08	1246.40	1705.70	1476.00	2707.60	2436.00	2571.80
50 ppm B. Ti	74.19	112.61	94.32	168.75	170.00	169.38	1277.10	1891.80	1584.50	2835.00	2856.00	2845.50
75 ppm B. Ti	81.56	50.00	65.78	222.00	70.00	146.00	1370.30	840.0	1105.10	3729.00	1176.00	2452.80
Means (A)	80.27	93.23		154.32	172.58		1348.60	1566.30		2592.60	2899.30	
LSD at 5% for	A=3.5	T=2.7	A*T=4.6	A=4.5	T=6.0	A*T =8.8	A=58.2	T=45.1	A*T =78.3	A=75.3	T=101.5	A*T =148.1
Second season												
Control	83.51	79.76	81.64	144.41	215.00	179.71	1403.00	1340.00	1371.50	2426.10	3612.00	3019.10
5 ppm N. Ti	122.92	104.72	113.82	228.33	246.50	237.42	2065.00	1759.30	1912.10	3836.00	4141.20	3988.60
7.5 ppm N. Ti	146.60	140.75	143.67	247.92	258.15	253.03	2462.80	2364.60	2413.70	4165.00	4336.90	4251.00
10 ppm N. Ti	160.99	194.87	177.91	269.68	337.50	303.59	2704.60	3273.30	2988.90	4530.70	5670.00	5100.30
25 ppm B. Ti	92.18	129.33	110.76	162.50	203.93	183.22	1548.60	2172.70	1860.70	2730.00	3426.00	3078.00
50 ppm B. Ti	107.85	136.22	122.03	175.17	291.25	233.21	1811.80	2288.40	2050.10	2942.80	4893.00	3917.90
75 ppm B. Ti	124.12	50.00	87.06	242.72	50.00	146.36	2085.20	840.00	1462.60	4077.60	840.00	2458.80
Means (A)	119.74	119.37		210.10	228.90		2011.60	2005.50		3529.80	3845.60	
LSD at 5% for	A=N.S.	T=3.6	A*T =5.9	A=3.2	T=7.8	A*T =10.5	A=N.S.	T=61.2	A*T =100.1	A=54.3	T=130.7	A*T =177.4

*N. Ti = titanium dioxide as nanoparticles; **B. Ti = titanium dioxide as bulk particles; N.S. = not significant

Table 4. Effect of application method and titanium concentrations as well as their interaction treatments on herb dry weight of Salvia fruticosa (three-lobed sage) plant during two cuts of the two seasons (2020/2021)

Application method(A) Ti form (T)	Herb dry weight/plant (g)						Herb dry weight/feddan (kg)					
	First season											
	1st cut			2nd cut			1st cut			2nd cut		
	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)
Control	23.80	23.44	23.62	32.92	34.58	33.75	399.84	393.91	396.87	553.10	580.90	567.00
5 ppm N. Ti*	34.55	34.66	34.61	40.16	46.51	43.34	580.55	582.50	581.48	674.70	781.50	728.10
7.5 ppm N. Ti	37.88	37.55	37.72	43.86	61.83	52.84	636.50	630.95	633.73	736.80	1038.80	887.80
10 ppm N. Ti	40.55	56.67	48.61	46.22	76.66	61.44	681.35	952.06	816.71	776.60	1287.90	1032.20
25 ppm B. Ti**	25.99	37.67	31.83	46.88	46.20	46.54	436.69	632.86	534.77	787.60	776.20	781.90
50 ppm B. Ti	36.16	44.50	40.33	47.44	43.33	45.39	607.60	747.60	677.60	797.10	728.00	762.60
75 ppm B. Ti	36.50	10.00	23.25	58.39	20.00	39.19	613.20	168.00	390.60	981.10	336.00	658.50
Means (A)	33.63	34.93		45.12	47.02		565.10	586.83		758.14	789.91	
LSD at 5% for	A= 0.07	T= 1.31	A*T= 1.72	A= 2.42	T=2.11	A*T=3.48	A= 1.19	Ti= 22.11	A*Ti=28.97	A= N.S.	Ti= 35.48	A*Ti=58.62
Second season												
Control	33.83	27.00	30.41	42.59	48.33	45.46	453.60	568.50	511.00	715.60	812.10	763.80
5 ppm N. Ti	44.00	45.00	44.50	60.05	54.25	57.15	739.20	756.00	747.60	1009.00	911.40	960.20
7.5 ppm N. Ti	54.66	56.83	55.75	63.46	62.18	62.82	918.30	954.90	936.60	1066.20	1044.70	1055.50
10 ppm N. Ti	62.16	67.83	64.99	65.55	75.55	70.55	1044.40	1139.50	1092.00	1101.40	1269.20	1185.30
25 ppm B. Ti	35.48	34.50	34.99	51.66	55.06	53.36	596.10	579.60	587.80	867.90	925.10	896.50
50 ppm B. Ti	39.44	43.16	41.30	54.48	68.17	61.33	662.60	725.20	693.90	915.40	1145.40	1030.40
75 ppm B. Ti	42.05	9.00	25.52	62.67	9.00	35.83	706.60	151.20	428.90	1053.00	151.20	602.10
Means (A)	43.54	41.45		57.21	53.22		731.54	696.41		961.21	894.16	
LSD at 5% for	A=1.43	T=1.26	A*T=2.07	A=1.71	T=1.51	A*T=2.48	A=23.98	T=21.16	A*T= 34.79	A=28.79	T=25.42	A*T= 41.77

*N. Ti= titanium dioxide as nanoparticles; **B. Ti= titanium dioxide as bulk particles.

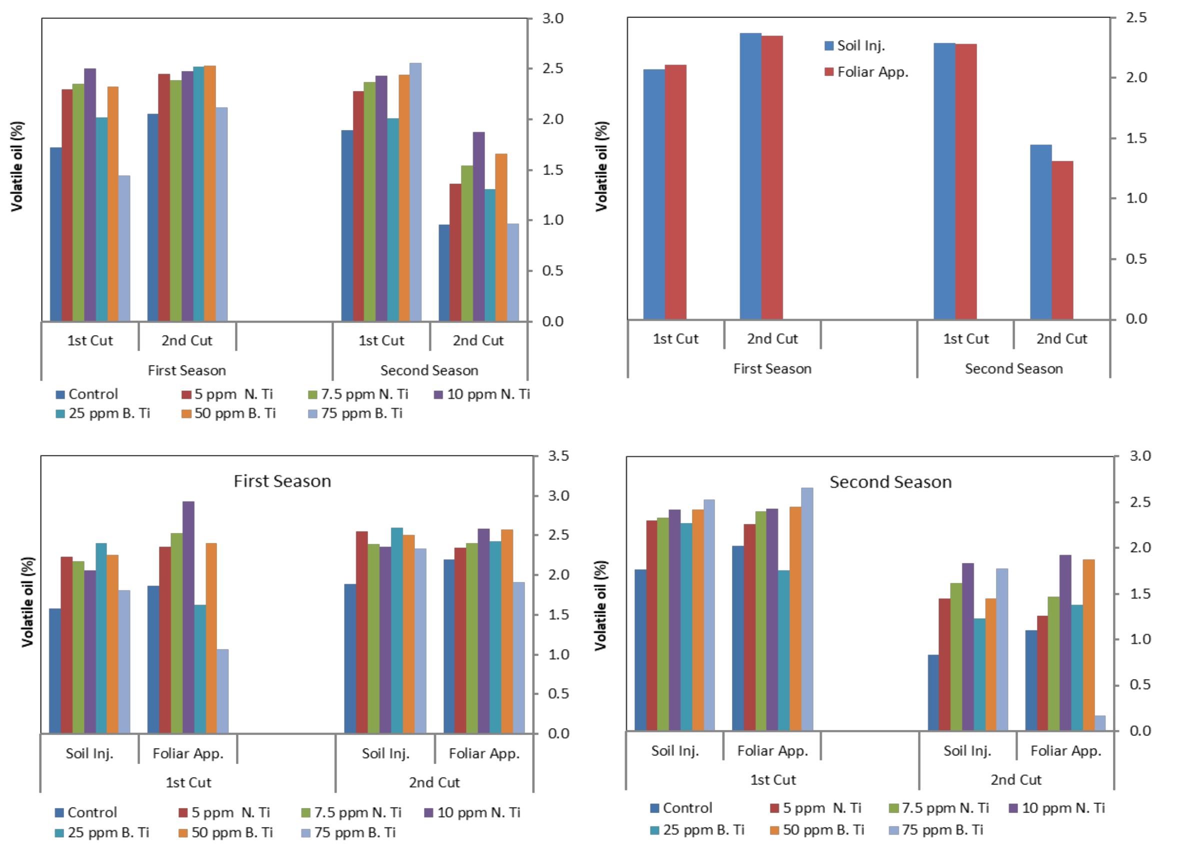

Figure 3. Effect of application method and titanium concentrations as well as their interaction treatments on volatile oil percentage (%) of *Salvia fruticosa* (three-lobed sage) during two cuts of the two seasons (2020/2021).

Table 5. Effect of application method and titanium concentrations as well as their interaction treatments on volatile oil of *Salvia fruticosa* (three-lobed sage) plant during two cuts of the two seasons (2020/2021)

Application Method (A) Ti form (T)	Volatile oil/plant (mL)						Volatile oil/feddan (L)					
	First season											
	1st cut			2nd cut			1st cut			2nd cut		
	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)
Control	0.37	0.43	0.40	0.62	0.76	0.69	6.31	7.36	6.84	10.44	12.78	11.61
5 ppm N. Ti*	0.77	0.82	0.79	1.02	1.08	1.05	12.93	13.76	13.34	17.21	18.29	17.75
7.5 ppm N. Ti	0.82	0.95	0.88	1.05	1.48	1.26	13.81	16.00	14.91	17.58	24.89	21.23
10 ppm N. Ti	0.83	1.66	1.25	1.09	1.98	1.53	14.05	27.99	21.02	18.32	33.40	25.86
25 ppm B. Ti**	0.62	0.61	0.62	1.22	1.12	1.17	10.48	10.34	10.41	20.48	18.87	19.67
50 ppm B. Ti	0.81	1.07	0.94	1.18	1.11	1.15	13.67	18.06	15.87	19.90	18.73	19.31
75 ppm B. Ti	0.66	0.10	0.38	1.35	0.38	0.87	11.10	1.80	6.45	22.82	6.41	14.62
Means (A)	0.70	0.81		1.07	1.13		11.76B	13.62A		18.11	19.05	
LSD at 5% for	A= 0.10	T=0.08	A*T=0.14	A=0.06	T= 0.06	A*T=0.10	A=1.77	T=1.46	A*T=2.47	A=1.04	T=1.14	A*T=1.75
Second season												
Control	0.47	0.68	0.58	0.82	1.10	0.96	7.97	11.49	9.73	13.88	18.49	16.18
5 ppm N. Ti	1.01	1.016	1.01	1.44	1.26	1.35	17.00	17.08	17.04	24.25	21.17	22.71
7.5 ppm N. Ti	1.27	1.36	1.31	1.61	1.47	1.54	21.43	22.92	22.17	27.09	24.69	25.89
10 ppm N. Ti	1.50	1.65	1.57	1.83	1.91	1.87	25.27	27.73	26.50	30.76	32.16	31.46
25 ppm B. Ti	0.80	0.60	0.70	1.23	1.38	1.30	13.53	10.20	11.86	20.69	23.27	21.98
50 ppm B. Ti	0.95	1.05	1.00	1.45	1.86	1.65	16.03	17.79	16.91	24.38	31.38	27.88
75 ppm B. Ti	1.06	0.24	0.65	1.77	0.16	0.97	17.88	4.02	10.95	29.80	2.81	16.30
Means (A)	1.01	0.94		1.45	1.30		17.02	15.89		24.41	21.99	
LSD at 5% for	A=0.08	T=0.03	A*T=0.09	A=0.062	T=0.063	A*T=0.09	A=1.50	T=0.61	A*T=1.59	A=1.03	T=1.05	A*T=1.64

*N. Ti= titanium dioxide as nanoparticles; **B. Ti= titanium dioxide as bulk particles.

and oil yield/feddan were increased as TiO_2 concentrations increased (as nanoparticles or bulk particles). In addition, TiO_2 concentration at 5, 7.5, or 10 ppm as N. Ti or 25 and 50 ppm as B. Ti showed a significant increase in this respect compared to control treatments. Also, the concentration at 10 ppm of N. Ti was the superior treatment in this regard, which gave the maximum values in volatile oil percentage, volatile oil yield per plant and per feddan compared to the other concentrations.

The data given in Figure 3 and Table 5 show that the interaction treatments between application methods and TiO_2 concentrations recorded a significant increase in volatile oil percentage, volatile oil yield per plant and per feddan compared to control treatments (as foliar spraying or soil injection). In addition, increasing the concentration of titanium dioxide under each method of application increased volatile oil percentage and volatile oil yield per plant and per feddan. Moreover, the interaction treatment between foliar spraying method and N. Ti at 10 ppm was the best treatment in this regard and gave the maximum values in volatile oil percentage and volatile oil yield per plant and per feddan compared to those of the other interaction ones. These results were similar in the two cuts of the two seasons.

Essential Oil Constituents: The results obtained in Tables 6 and 7 show that the volatile oil constituents in the first season were affected by the interaction treatments between method of application and titanium dioxide concentrations (as nanoparticles or bulk particles). Twenty-five components were identified in volatile oil of three-lobed sage under different treatments that represented 98.54, 89.91, 98.82, 99.64, 98.46, 92.15, and 96.32%, respectively, in seven interaction treatments between soil injection and TiO_2 concentrations. Moreover, the results in Table 6 shows that 1,8-cineole was the major component (peroxides, 21.98, 24.38, 41.10, 26.01, 39.46, 20.11, and 30.03%), followed by camphor compound (ketone, 17.07, 14.73, 17.00, 13.71, 15.94, 16.23, and 13.28%), α-pinene (monoterpene hydrocarbon, 6.89, 6.62, 6.22, 6.28, 6.93, 6.08, and 6.18%), camphene (9.25, 7.24, 6.15, 6.57, 6.60, 7.62, and 5.55%) and β–pinene (6.49, 6.85, 6.48, 7.12, 7.09, 5.89, and 7.33%), respectively, in the seven interaction treatments under this study. On the other hand, based on the interaction treatments between the foliar spraying method and TiO_2 concentrations (0, 5, 7.5, and 10 ppm as nanoparticles or 25, 50, and 75 ppm as bulk particles), it was observed that 1,8-cineole was the major component followed by camphor compound, β–pinene, α–pinene, and camphene, respectively, in the seven interaction treatments under this study. Investigation of interaction treatments on volatile oil constituents of three-lobed sage plants showed that 1,8-cineole reached the highest percentage (41.10%) because of the interaction treatment between the foliar spraying method and N. TiO_2 at 7.5 ppm and the lowest percentage (20.11) at soil injection method with 50 ppm TiO_2 as bulk particles. Camphor recorded the greatest value (17.07%) at the interaction treatment between the soil injection method and 0 ppm TiO_2 concentration and the lowest value (9.90%) at the interaction treatment between the foliar spraying method and 25 ppm TiO_2 as bulk particles. Also, β–pinene recorded the highest value (8.35%) at the interaction treatment between foliar spraying type and 50 ppm TiO_2 as bulk particles and lowest value (4.05) at soil injection method with TiO_2 at 25 ppm concentration as bulk particles.

Total Chlorophyll, Nitrogen, Phosphorus, and Potassium Percentages in Plants

The data recorded in Tables 8 and 9 indicate that there was insignificant difference among application method treatments on total chlorophyll in the first cut of two seasons. However, there were significant differences in this regard in the second cut of both seasons. On the other hand, application method treatments recorded significant differences in nitrogen, phosphorus, and potassium percentages. In addition, the highest values in this respect were obtained from the foliar spraying treatment compared to the soil injection treatment.

On the other hand, TiO_2 at all concentrations of 5, 7.5, and 10 ppm as nanoparticles or 25 and 50 ppm as bulk particles gave a significant increase in total chlorophyll, N, P, and K percentages in plants compared to control or 75 ppm as bulk particles. Moreover, total chlorophyll, N, P, and K percentages in plants were increased as TiO_2 concentrations increased to reach the maximum values by using that of 10 ppm as nanoparticles and 50 ppm as bulk particles.

From the data presented in the same Tables 8 and 9 it is shown that the interaction treatment between foliar spraying and TiO_2 at 75 ppm as bulk particles increased total chlorophyll in plants compared to other interaction treatments under this study.

Table 6. Effect of soil injection by titanium dioxide on volatile oil constituents of *Salvia fruticosa* (three-lobed sage) plant during the first season (2020)

No.	RT	Components	Control	5 ppm N. Ti*	7.5 ppm N. Ti	10 ppm N. Ti	25 ppm B. Ti**	50 ppm B. Ti	75 ppm B. Ti
1	3.76	Tricyclene	0.95	0.74	0.41	0.64	-	0.87	0.59
2	3.91	α-Pinene	6.89	6.62	6.22	6.28	6.93	6.08	6.18
3	4.22	Camphene	9.25	7.24	6.15	6.57	6.60	7.62	5.55
4	4.70	β-Pinene	6.49	6.85	6.48	7.12	7.09	5.89	7.33
5	4.83	α-Myrcene	3.69	3.39	2.03	3.98	3.12	3.22	3.24
6	5.40	α-Terpinene	0.44	0.47	0.25	0.45	-	1.07	0.40
7	5.64	DL-Limonene	4.21	3.71	2.52	4.38	4.32	3.72	3.17
8	5.76	1,8-Cineole	21.98	24.38	41.10	26.01	39.46	20.11	30.03
9	6.22	Terpinene	0.59	0.62	0.33	0.57	-	2.21	0.63
10	6.77	α-Terpinolene	0.28	0.25	0.15	0.26	-	0.53	-
11	7.16	L-Linalool	0.22	-	-	0.30	-	-	-
12	7.36	β-Thujone	1.94	1.71	1.62	2.17	1.26	1.86	3.20
13	7.61	α-Thujone	0.64	1.64	0.44	0.88	-	0.53	1.57
14	8.32	Camphor	17.07	14.73	17.00	13.71	15.94	16.23	13.28
15	8.56	Trans-3-pinanone	1.52	0.53	0.12	1.07	-	-	0.71
16	8.76	Linalyl propionate	-	1.33	-	1.18	-	-	1.22
17	8.84	Endo-borneol	4.88	4.22	3.25	2.90	2.46	5.01	2.04
18	8.94	4-Terpineol	1.81	1.93	0.94	1.30	0.64	3.31	1.13
19	9.31	α-Terpineol	2.86	3.77	1.31	4.81	1.50	3.24	3.88
20	11.00	Bornyl acetate	3.08	2.73	2.69	3.58	2.59	3.56	1.72
21	12.26	α-Terpinyl acetate	2.59	4.77	1.79	3.95	2.31	2.96	4.64
22	13.63	Trans-Caryophyllene	3.07	3.23	2.19	3.37	2.41	2.76	3.08
23	14.36	α-Humulene	1.06	1.27	0.86	0.97	0.48	0.42	0.93
24	16.86	Caryophyllene oxide	1.39	1.23	0.77	2.42	0.64	0.52	1.19
25	17.10	Viridiflorol	1.64	1.55	0.20	0.77	0.71	0.43	0.61
TOTAL			98.54	98.91	98.82	99.64	98.46	92.15	96.32

*N. Ti= titanium dioxide as nanoparticles; **B. Ti= titanium dioxide as bulk particles

Table 7. Effect of foliar spraying by titanium dioxide on volatile oil constituents of *Salvia fruticosa* (three-lobed sage) plant during the first season (2020)

No.	RT	Components	Control	5 ppm N. Ti*	7.5 ppm N. Ti	10 ppm N. Ti	25 ppm B. Ti**	50 ppm B. Ti	75 ppm B. Ti
1	3.76	Tricyclene	0.44	0.89	0.84	0.86	0.67	0.55	0.56
2	3.91	α-Pinene	5.32	7.11	7.85	6.30	6.03	5.74	5.73
3	4.22	Camphene	2.92	8.17	7.89	7.90	4.44	4.05	4.90
4	4.70	β -Pinene	7.43	7.82	8.12	7.40	8.11	8.35	8.01
5	4.83	α-Myrcene	3.40	3.23	3.73	3.58	3.46	4.29	4.02
6	5.40	α-Terpinene	0.38	0.49	0.49	0.54	0.57	0.59	0.39
7	5.64	DL-Limonene	2.44	3.37	3.85	4.11	3.07	3.97	3.76
8	5.76	1,8-Cineole	38.05	26.52	26.27	25.82	31.29	27.84	33.13
9	6.22	Terpinene	0.55	0.72	0.70	0.78	0.97	0.92	0.59
10	6.77	α-Terpinolene	-	0.28	0.24	0.31	0.31	0.39	0.25
11	7.16	L-Linalool	-	0.31	0.16	0.31	-	-	-
12	7.36	β-Thujone	2.58	1.22	1.91	2.32	2.93	2.52	2.23
13	7.61	α-Thujone	1.28	1.21	0.66	0.85	0.75	1.68	1.13
14	8.32	Camphor	9.34	15.80	12.87	16.46	9.90	13.87	16.74
15	8.56	*trans*-3-Pinanone	1.82	0.69	0.71	1.39	0.84	1.30	1.06
16	8.76	Linalyl propionate	1.08	1.03	-	1.00	1.59	1.37	0.96
17	8.84	Endo-Borneol	1.52	3.78	4.92	3.76	1.65	1.09	1.98
18	8.94	4-Terpineol	1.87	2.09	1.35	2.18	1.35	1.87	1.89
19	9.31	α-Terpineol	3.59	3.31	2.23	4.04	5.18	4.88	3.54
20	11.00	Bornyl acetate	0.89	2.42	3.65	2.82	1.59	3.04	1.49
21	12.26	α-Terpinenyl acetate	4.70	4.26	3.18	2.73	5.45	2.67	1.95
22	13.63	*trans*-Caryophyllene	3.32	1.82	3.51	1.72	4.20	3.21	2.19
23	14.36	α-Humulene	1.07	0.49	1.44	0.59	1.62	1.37	0.65
24	16.86	Caryophyllene oxide	1.82	0.90	1.33	0.82	2.30	0.59	0.53
25	17.10	Viridiflorol	1.65	1.47	0.77	0.81	0.35	1.37	0.49
		TOTAL	97.46	99.40	98.67	99.40	98.62	97.52	98.17

*N. Ti= titanium dioxide as nanoparticles; **B. Ti= titanium dioxide as bulk particles.

Table 8. Effect of application method and titanium concentrations as well as their interaction treatments on total chlorophyll and N% of *Salvia fruticosa* (three-lobed sage) plant during two cuts of the two seasons (2020/2021)

Application method (A) Ti form (T)	Total chlorophyll (SPAD)						N (%)					
	First season											
	1st cut			2nd cut			1st cut			2nd cut		
	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)
Control	33.53	30.73	32.13	34.33	34.60	34.46	0.611	0.755	0.683	0.601	0.745	0.673
5 ppm N. Ti*	34.80	34.76	34.78	36.23	37.43	36.83	0.690	0.771	0.731	0.680	0.815	0.748
7.5 ppm N. Ti	35.10	35.56	35.33	36.86	39.53	38.20	0.706	0.830	0.768	0.694	0.855	0.774
10 ppm N. Ti	36.23	36.66	36.44	38.30	41.90	40.10	0.754	0.865	0.809	0.735	0.883	0.809
25 ppm B. Ti**	34.00	34.43	34.21	35.36	39.46	37.41	0.646	0.789	0.717	0.646	0.751	0.698
50 ppm B. Ti	35.33	36.23	35.78	37.40	42.10	39.75	0.669	0.888	0.777	0.641	0.774	0.707
75 ppm B. Ti	38.26	41.2a	39.74	39.33	43.96	41.65	0.602	0.729	0.665	0.592	0.724	0.658
Means (A)	35.66	35.32		36.83	39.85		0.668	0.804		0.656	0.792	
LSD at 5% for	A= N.S.	T=0.66	A*T=1.42	A=0.78	T= 0.55	A*T=1.00	A=0.006	T=0.011	A*T=0.016	A=0.008	T=0.012	A*T=0.017
Second season												
Control	32.33	28.63	30.48	35.86	36.66	36.26	0.654	0.840	0.747	0.644	0.830	0.737
5 ppm N. Ti	34.00	34.03	34.01	36.93	40.76	38.85	0.779	0.894	0.836	0.770	0.879	0.824
7.5 ppm N. Ti	34.53	36.13	35.33	37.36	42.53	39.95	0.790	0.924	0.857	0.777	0.914	0.845
10 ppm N. Ti	35.06	38.43	36.75	39.40	45.03	42.21	0.833	0.964	0.899	0.817	0.959	0.888
25 ppm B. Ti	35.03	35.03	35.03	35.83	35.33	35.58	0.722	0.853	0.788	0.707	0.833	0.770
50 ppm B. Ti	35.80	37.00	36.40	38.06	41.73	39.90	0.747	0.878	0.812	0.737	0.863	0.800
75 ppm B. Ti	40.63	39.40	40.01	39.76	44.80	42.28	0.534	0.814	0.674	0.529	0.809	0.669
Means (A)	35.34	35.52		37.60	40.98		0.723	0.881		0.711	0.870	
LSD at 5% for	A=N.S.	T=0.86	A*T=1.59	A=1.25	T=0.86	A*T=1.58	A=0.037	T=0.018	A*T=0.041	A=0.037	T=0.019	A*T=0.042

*N. Ti= titanium dioxide as nanoparticles; **B. Ti= titanium dioxide as bulk particles; N.S.: not significant

Table 9. Effect of application method and titanium concentrations as well as their interaction treatments on P% and K% of *Salvia fruticosa* (three-lobed sage) plant during the two cuts of the two seasons (2020 and 2021)

Application method (A) Ti form (T)	P (%)						K (%)					
	First season											
	1st cut			2nd cut			1st cut			2nd cut		
	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)
Control	0.083	0.140	0.111	0.079	0.135	0.107	0.912	1.217	1.064	0.897	1.227	1.062
5 ppm N. Ti*	0.129	0.150	0.140	0.125	0.143	0.134	1.143	1.362	1.252	1.129	1.342	1.235
7.5 ppm N. Ti	0.131	0.214	0.173	0.125	0.209	0.167	1.183	1.490	1.336	1.164	1.475	1.320
10 ppm N. Ti	0.142	0.290	0.216	0.133	0.287	0.210	1.239	1.528	1.385	1.215	1.518	1.367
25 ppm B. Ti**	0.107	0.146	0.126	0.092	0.136	0.114	1.059	1.262	1.160	1.039	1.237	1.138
50 ppm B. Ti	0.114	0.149	0.131	0.116	0.141	0.128	1.062	1.295	1.178	1.047	1.275	1.161
75 ppm B. Ti	0.049	0.136	0.092	0.042	0.133	0.088	0.743	1.240	0.992	0.733	1.205	0.969
Means (A)	0.108	0.175		0.102	0.169		1.049	1.342		1.032	1.326	
L.SD at 5% for	A= 0.014	T=0.006	A*T=0.015	A=0.011	T= 0.004	A*T=0.012	A=0.048	T=0.034	A*T=0.061	A=0.051	T=0.036	A*T=0.065
Second season												
Control	0.079	0.148	0.114	0.074	0.143	0.109	0.701	1.019	0.860	0.687	1.005	0.846
5 ppm N. Ti	0.128	0.194	0.161	0.123	0.187	0.155	0.910	1.222	1.066	0.896	1.202	1.049
7.5 ppm N. Ti	0.132	0.203	0.167	0.126	0.198	0.162	0.948	1.355	1.151	0.929	1.340	1.134
10 ppm N. Ti	0.154	0.220	0.187	0.144	0.217	0.181	1.028	1.773	1.401	1.005	1.763	1.384
25 ppm B. Ti	0.111	0.181	0.146	0.105	0.174	0.140	0.772	1.092	0.932	0.750	1.072	0.911
50 ppm B. Ti	0.113	0.162	0.138	0.105	0.152	0.129	0.807	1.045	0.926	0.794	1.020	0.907
75 ppm B. Ti	0.057	0.137	0.097	0.054	0.134	0.094	0.652	0.999	0.825	0.640	0.988	0.814
Means (A)	0.110	0.178		0.105	0.172		0.831	1.215		0.815	1.198	
LSD at 5% for	A=0.007	T=0.008	A*T=0.012	A=0.007	T=0.008	A*T=0.013	A=0.099	T=0.101	A*T=0.159	A=0.099	T=0.101	A*T=0.158

*N. Ti= titanium dioxide as nanoparticles; **B. Ti= titanium dioxide as bulk particles.

Moreover, each of nitrogen, phosphorus, and potassium percentages recorded the highest values at the interaction treatment between foliar spraying and TiO_2 at 10 ppm as nanoparticles, which gave a significant increase in this respect in comparison to other interaction treatments and control during the two cuts of the two seasons.

Nutrient Availability in Soil after the Experiment

The data recorded in Table 10 indicates that there were significant differences among application method treatments for NPK availability in soil after the experiment. Furthermore, the soil injection gave a higher value compared to the foliar application method. The results reveal that TiO_2 at all concentrations of 5, 7.5, and 10 ppm as nanoparticles or 25 and 50 ppm as bulk particles gave an increase in N, P, and K availability (mgkg^{-1}) in soil compared to control or 75 ppm as bulk particles. Also, the concentration at 10 ppm N. TiO_2 recorded the highest values in this regard. Furthermore, the most effective treatment was the interaction treatment between soil injection and TiO_2 at 10 ppm as nanoparticles that recorded higher concentrations of nitrogen, phosphorus, and potassium availability (mgkg^{-1}) in soil than other interaction treatments and control (104.97, 11.375, and 104.56 mg kg^{-1}), respectively, at 0-30 cm soil depth.

Ecological Risk Assessment of Titanium

The presented data in Table 11 indicate that the mean ratio of titanium content of F1 soluble, exchangeable fractions ranged between 0.143 and 0.981, its ratio of F2 carbonate fraction ranged between 0.100 and 0.582, its ratio of F3 bound to Fe-Mn oxyhydroxides (Fe-Mn oxides) ranged between 0.149 and 2.370, its ratio of F4 bound to organic matter (OM) ranged between 0.049 and 0.991, and its content ratio of F5 residual (Res.) ranged between 95.006 and 99.513. This is because the foliar spraying treatment targets plants more than soils. The values of RAC were increased with increasing the rate of addition for all treatments. Values of RAC for bulk particle concentrations are more effective than those for nanoparticle concentrations. Because the nanoparticles are more mobile, they had been absorbed by plants and part of them had been lost in drainage water during the irrigation process. Therefore, it is preferable to add nanoparticles in capsules at soil application treatments. So, they are of slow release and can be used during the growth period of plants. So, TiO_2 bulk particles are more effective for RAC values after harvesting *Salvia fruticosa* plants. The concentration 75 ppm B. Ti gave the highest values in RAC (0.981) at soil injection, while the concentration 5 ppm N. Ti gave the least values of RAC (0.198) at foliar spraying but still more than those of the control treatment.

Table 10. Effect of application method and titanium concentrations as well as their interaction treatments on N, P, and K availability (mg/kg) in soil after the experiment.

Treatments	N (mg/kg)			P (mg/kg)			K (mg/kg)		
	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)	Soil injection	Foliar app.	Means (T)
Control	17.35	15.68	16.51	6.16	6.04	6.74	73.30	73.56	73.86
5 ppm N. Ti*	70.71	33.68	52.19	9.22	8.54	8.88	86.23	77.99	82.11
7.5 ppm N. Ti	75.99	68.55	72.26	11.00	8.58	9.79	89.99	84.30	87.14
10 ppm N. Ti	104.97	69.68	87.32	11.37	10.66	11.02	104.56	99.70	102.13
25 ppm B. Ti**	69.21	34.68	51.94	8.28	7.83	8.05	74.43	77.99	75.99
50 ppm B. Ti	54.99	33.41	44.20	8.00	7.31	7.02	74.43	73.30	73.86
75 ppm B. Ti	13.99	13.99	13.98	5.73	4.41	5.07	65.82	59.76	62.79
Means (A)	58.17	38.52		8.70	7.46		80.98	78.08	
LSD at 5% for	A=2.45	B=1.99	A*B=2.81	A=0.50	B=0.42	A*B=0.59	A=3.72	B=3.72	A*B=5.27

*N. Ti= titanium dioxide as nanoparticles; **B. Ti= titanium dioxide as bulk particles

Table 11. Average ratio of Ti in different fractions and RAC of two seasons

Treatments		F1%	F2%	F3%	F4%	F5%	RAC
Foliar spraying	Control	0.018	0.125	0.161	0.183	99.513	0.143
	5 ppm N. Ti*	0.058	0.140	0.280	0.362	99.160	0.198
	7.5 ppm N. Ti	0.184	0.245	0.438	0.657	98.476	0.429
	10 ppm N. Ti	0.336	0.100	0.297	0.345	98.922	0.436
	25 ppm B. Ti**	0.140	0.302	0.149	0.049	99.360	0.443
	50 ppm B. Ti	0.157	0.309	0.170	0.436	98.928	0.466
	75 ppm B. Ti	0.354	0.113	0.671	0.467	98.396	0.467
Soil injection	Control	0.018	0.125	0.161	0.183	99.513	0.143
	5 ppm N. Ti	0.243	0.241	0.210	0.239	99.067	0.484
	7.5 ppm N. Ti	0.155	0.372	0.919	0.661	97.894	0.527
	10 ppm N. Ti	0.374	0.391	2.370	0.710	96.006	0.765
	25 ppm B. Ti	0.120	0.708	1.005	0.332	97.836	0.828
	50 ppm B. Ti	0.634	0.324	0.441	0.404	98.198	0.958
	75 ppm B. Ti	0.399	0.582	0.978	0.991	96.998	0.981

*N. Ti= titanium dioxide as nanoparticles; **B. Ti= titanium dioxide as bulk particles. Titanium content in F1 soluble, exchangeable fraction, F2 carbonate fraction, F3 bound to Fe-Mn oxyhydroxides (Fe-Mn), F4-bound to organic matter (OM), and F5 residual (Res.)

The Bioconcentration Factors (BCFs)

The results in Table 12 reveal that there were differences between the two methods of application (soil injection and foliar spraying) in all BCFs of Ti values. The foliar spraying was more effective than soil injection. Also, the values of BCF for N. Ti treatments were more effective than B. Ti treatments. BCF of Ti values increased with increasing Ti concentrations. The concentration of 10 ppm N. Ti gave the highest values in BCF of Ti value (0.270) at foliar spraying, while the concentrations of 5 ppm N. Ti and 25 ppm B. Ti gave the lowest values in BCF of Ti values (0.067 and 0.067, respectively), at soil injection but were still more than those of control treatment. Regarding BCF of Fe, results obtained that there were differences between the two application methods (soil injection and foliar spraying) in all BCF of Fe values. Foliar spraying was more effective than soil injection. BCF of Fe values increases with increasing the rate of addition for all treatments except at 75 ppm B. Ti, and this agrees with El-Ghamry *et al.* (2018). Moreover, the values of BCF of Cu for nanoparticle treatments increased with increasing the rate of addition for all treatments for two methods of application except at 10 ppm N. Ti. Furthermore, the results of BCF of V, showed that there were no differences between the two methods of application (soil injection or foliar spraying). However, the values of BCF of V for nanoparticle treatments are more effective than bulk particles for treatments. All values of BCF of V values were more than its control treatment. Also, for BCF of Sr, Mn, Zn, and Cr, results indicated that there were no differences between the two methods of application (soil injection or foliar spraying). Also, there were no differences between Ti nano- and bulk particles.

Table 12. Average of bioaccumulation factor (BCF) of titanium and some heavy metals in *Salvia fruticosa* plant in the two seasons.

Treatments		BCF-Ti	BCF-Cr	BCF-V	BCF-Sr	BCF-Fe	BCF-Mn	BCF-Zn	BCF-Cu
Foliar spraying	Control	0.065	0.498	0.174	0.470	0.070	0.089	0.214	0.544
	5 ppm N. Ti*	0.112	0.692	0.362	0.328	0.138	0.107	0.102	0.650
	7.5 ppm N. Ti	0.219	0.954	0.480	0.216	0.233	0.082	0.074	0.594
	10 ppm N. Ti	0.270	0.215	0.839	0.615	0.347	0.106	0.069	0.527
	25 ppm B. Ti**	0.073	0.356	0.565	0.434	0.159	0.083	0.050	0.405
	50 ppm B. Ti	0.090	0.308	0.202	0.038	0.230	0.066	0.136	0.409
	75 ppm B. Ti	0.112	0.515	0.227	0.360	0.089	0.070	0.168	0.507
Soil injection	Control	0.065	0.498	0.174	0.470	0.070	0.089	0.214	0.544
	5 ppm N. Ti	0.067	0.565	0.671	0.543	0.077	0.110	0.080	0.625
	7.5 ppm N. Ti	0.073	0.438	0.505	0.556	0.087	0.076	0.029	0.640
	10 ppm N. Ti	0.084	0.435	0.671	0.641	0.109	0.138	0.021	0.637
	25 ppm B. Ti	0.067	0.450	0.271	0.427	0.107	0.094	0.145	0.401
	50 ppm B. Ti	0.077	0.420	0.245	0.625	0.114	0.109	0.226	0.423
	75 ppm B. Ti	0.080	0.451	0.214	0.438	0.080	0.074	0.149	0.397

*N. Ti= titanium dioxide as nanoparticles; **B. Ti= titanium dioxide as bulk particles

DISCUSSION

From the above-mentioned results, it is indicated that the foliar spraying of titanium dioxide (TiO_2) as nanoparticles or bulk particles increased each of plant height, herb fresh yield, herb dry yield, and volatile oil yield as well as some of the chemical components (total chlorophyll, nitrogen, phosphorus, and potassium percentages) in three-lobed sage plants compared to the soil addition method. These results agreed with those obtained by El-Sagan and Shokry (2019) and Elsherpiny *et al*. (2022). So, foliar spraying may be advantageous because Ti delivery through soil is ineffective since this element has limited root uptake and is known for its low mobility in soil. In terms of its negative effects, neither Ti plant damage when taken at higher levels than advised (Frazer, 2001) nor adverse effects have been related to eating Ti-sprayed agricultural products to date (Nano-Plant Technology, 2002; and Fernando *et al.*, 2017). On the other side, it was found that the most successful way to deliver Ti was through foliar treatment, which was followed by soil injection. This could be because of the high efficiency of the foliar spraying method. To put it in another way, the foliar spraying method could decrease the time between the addition of TiO_2 and its absorption by the three-lobed sage plant. In addition, the soil application method may be encouraged N-fixation in soil (Wang *et al.*, 2012).

The use of titanium dioxide (TiO_2) improved crop productivity efforts. This was achieved by Ti, which is classified as beneficial for the plant, improving their growth and development. Plants treated with titanium are characterized by a higher chlorophyll content and more intensive photosynthesis. Also, Ti affects the uptake of nutrients and enzymatic activity (Malinowska and Kalembasa, 2012; Kleiber and Markiewicz, 2013; and Radkowski, 2013). As the above-mentioned results which indicated that using of titanium dioxide (TiO_2) at concentrations of 5, 7.5, and 10 ppm as nanoparticles or 25 and 50 ppm as bulk particles increased each of plant height, herb fresh yield, herb dry yield, volatile oil yield, and some of the chemical components *i.e.*, total chlorophyll, nitrogen, phosphorus, and potassium percentages as well as the bioconcentration factor (BCF) in three-lobed sage plants compared to the control treatment.

Moreover, all the characteristics under this study increased by increasing Ti concentrations from 5 to 10 ppm as nanoparticles or 25 to 50 ppm as bulk particles then decreased by using Ti at 75 ppm as bulk particles in comparison with the control treatment. These results were, respectively, in agreement with those recorded by Bieleski and Ferguson, 1983; Zheng *et al.*, 2005; Yang *et al.*, 2006; Lei *et al.*, 2008, and El-Ghamry *et al.,* 2018), who revealed that plants treated by Ti contained higher concentrations of P, Fe, Cu, Mn, and Zn in leaves in comparison to the control. This is in addition to the improvement of growth, biomass, and productivity quality of many plant species, which were treated with Ti, and increasing the contents of some essential elements such as nitrogen and phosphorus (Pais, 1983; Carvajal *et al.*, 1998; Khater and Osman, 2015; Khater, 2015; Fernando *et al*., 2017; El-Ghamry *et al.*, 2018; and Elsherpiny *et al*., 2022).

In view of the chemical properties of N. TiO_2 which are more stable, they could be retained in the surface region of soil for a relatively long time. However, Ti has been shown to be mobile in soils. Also, it could positively interact with one or more essential elements; thus, NPKs are essential elements for plants, while Ti plays a beneficial role. Notice that synergism interaction between Ti and NPK). The previous results agreed with those obtained by Lyu *et al*. (2017), El-Ghamry *et al*. (2018), and Eissa *et al*. (2022) who found that the values of N, P, K, and Fe (mg kg^{-1}) in the soil after harvesting of lettuce plants significantly decreased with increasing titanium level. Meanwhile, for Ti (mg kg^{-1}), the values of residual titanium significantly increased with increasing Ti addition rates. It can be mentioned that the plants treated by Ti as a foliar spraying possessed better performance than that of the soil injection method. Consequently, it can be demonstrated that plants benefited from applying Ti at low concentrations (5, 7.5, and 10 ppm N. Ti or 25 and 50 ppm B. Ti), which may be because of its essential function in nonbiological nitrogen fixation. Additionally, the emergence of Ti toxicity on plants may be the cause of the progressive decline in performance linked to increasing Ti levels above 50 ppm as mentioned by Al-Taani (2008) and El-Ghamry et al. (2018) who attested to the occurrence of toxicity brought on by the addition of Ti at high concentrations.

On the other hand, it can be noted that the decrease in all the traits under this study at 75 ppm TiO_2 as bulk particles may be due to the decrease in the bioconcentration factor (BCF) for Fe, Mn, Zn, and Cu in plant, as shown in Table 12. These elements are considered among the microelements that have an important role in all biological processes within the plant, thus decreasing all the characteristics under this study. These results agree with that recorded by Radkowski (2013) and El-Ghamry (2018).

Because of its possible toxicity and mobility, Ti fractionation is a crucial problem (Maiz *et al.*, 2000). The exchangeable and carbonate-bound fractions are examples of bioavailable species, which are the fractions most affected by human activities. Average of ecological risk assessment of titanium (RAC) was <1 % of the total. Titanium is soluble and exchangeable, and Ti bound to carbonate fractions is considered safe, in no-risk category for all treatments, as shown in Table 11. These results agreed with those noticed by Singh *et al.* (2005) who reported that soluble and exchangeable Ti and Ti coupled to carbonate are easily mobilized, which increases their bioavailability.

Also, the criteria of risk assessment code (RAC) as given in the same table indicate that the soil that can release <1 % of the total titanium in soluble, exchangeable form and carbonate fractions is considered safe, i.e., in no-risk category while the soil that releases >50% of the total titanium in the same fraction is under very high-risk category. A release is considered low risk if it is between 1 and 10%, medium risk if it is between 11 and 30%, high risk if it is between 31 and 50%, and very high risk if it is beyond 50% (Perin *et al.*, 1985).

Bioconcentration factor (BCF) is an excellent indicator of titanium and elements accumulation capacity because it considers the ratio of titanium and elements concentration in the plant. Meanwhile, if the BCF ≤1.0, it indicates that *Salvia fruticosa* plant can only absorb but not accumulate the metal. However, the plant may have potential to accumulate metal if the BCF > 1.0 (Liu *et al*., 2009; Sulaiman and Hamzah, 2018). Generally, the mean values of BCF for titanium and some heavy elements in different plant tissues of *Salvia fruticosa* plant were less than 1. It may be noted that there is no noticeable difference in BCF values between nanoparticles or bulk particles of TiO_2 and the method of application (foliar spraying or soil injection) does not affect the value of BCF. Liu *et al*. (2009) found that the bioconcentration factor (BCF) is lower than 1. So, the *Salvia fruticosa* plant cannot accumulate titanium and some heavy metals. Thus, from that, *Salvia fruticosa* plant is considered safe when growing in high Ti concentration soil under this study. Table 13 shows the comparison of the effect of Ti applications on some plants for some previous and current study.

CONCLUSION

The achieved results show that adding Ti at a low concentration, i.e., 10 ppm as nanoparticles or 50 ppm as bulk particles either as foliar spraying or as soil injection to *Salvia fruticosa* plants is suitable, but its toxicity started to appear at the high concentration of 75 ppm Ti as bulk particles. Also, it can be concluded that the interaction treatment between foliar spraying and 10 ppm N. Ti was the most effective on all traits compared to other interaction treatments in this study. In general, a greater comprehension of titanium toxicity in plant tissues may encourage risk analysis and safe use of it. This study also emphasizes the requirement for carefully adjusting the titanium working rates in accordance with the plant species, application technique, and stage of plant development. As a result, the findings can provide an

Table 13. Comparison of the effect of Ti applications on some plants for some previous and current study.

Plant name	Concentration (mg L^{-1})	Effects on plants	References
Fennel	5, 20, 40, 60, and 80	Decreased shoot biomass at 40 mg L^{-1} and enhanced germination rate and vigor index at low concertation.	Feizi *et al*., 2013
Parsley	10, 20, 30, and 40	Increased germination rate, root and shoot length, fresh weight, vigor index and chlorophyll content	Dehkourdi and Mosavi, 2013
Coriander	2, 4, and 6	Significant increase in plant height, fruit yield, and number of branches.	Khater, 2015
Fennel	2, 4, and 6	Increased plant height, number of branches, fruit yield, values of pigments, carbohydrates, sugars, nitrogen, phosphorus, and potassium.	Khater and Osman, 2015
Lettuce, Basil	5, 10, 50, and 100	Basil was more sensitive to cytostatic effects. Lettuce was more sensitive to genotoxic effects.	Silva *et al*., 2017
Basil	125, 250, 500, and 750	Homeostasis of essential elements was affected, and NPs reduced reducing sugar	Tan *et al*., 2017
Lettuce	25, 50, and 75	Many positive beneficial influences.	El-Ghamry *et al*., 2018
Turnip	0, 1, and 2	The highest values of growth, yield parameters, and chemical contents were obtained with TiO_2 application at the rate 2 mg/L, followed by the rate of 1 mg/L.	El-Sagan and Shokry, 2019
Three-lobed sage	5, 7.5, and 10 as nanoparticles or 25 and 50, as bulk particles	Increased Each of plant height, herb fresh yield, herb dry yield, and volatile oil yield as well as some of the chemical components (total chlorophyll, nitrogen, phosphorus, and potassium percentages) in three-lobed sage plants compared to soil addition method.	Current study

excellent foundation for the creation of titanium fertilizers. In the coming years, titanium might be regarded as one of the important nutrients for plants with additional research using cutting-edge methods from high-tech scientific instruments.

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