Heliyon 9 (2023) e21646

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Combined application of SiO_2 and TiO_2 nanoparticles enhances growth characters, physiological attributes and essential oil production of *Coleus aromatics* Benth

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ARTICLE INFO

Keywords:
Coleus aromaticus
Chlorophyll fluorescence
essential oil
nanoparticles
Thymol

ABSTRACT

Nanoparticles (NPs) have gained considerable interest among researchers in the field of plant biology, particularly in the agricultural sector. Among the numerous NPs, the individual application of silicon (Si) or titanium (Ti), in their oxide forms, had a positive influence on growth, physiochemical and yield attributes of plants. However, the synergetic application of both these NPs has not been studied yet. Therefore, the current study was aimed to investigate the effect of combined application of silicon dioxide (SiO_2) and titanium dioxide (TiO_2) NPs on the growth characters, physiological parameters, and essential oil quality and production of *Coleus aromatics* Benth. Aqueous solutions of nanoparticles were applied to the foliage of the plants at varying combinations (Si50+Ti50, Si100+Ti50, Si100+Ti100, Si200+Ti100, Si100+Ti200 and Si200+Ti200 mg L^{-1}). Various morpho-physiological, biochemical and yield attributes were assessed at 120 days after planting. The results demonstrated that both Si and Ti NPs improved the growth and photosynthetic efficiency in a dose dependent manner. The best results were obtained by the combined application of Si100+Ti100 mg L^{-1}, and thereafter, the values declined progressively. The maximum improvement in fresh weight (39.5 %) and dry weight (40.8 %) of shoot, fresh weight (45.7 %) and dry weight (49.4 %) of root was observed as compared to respective controls. Moreover, the exogenous application of Si100+Ti100 mg L^{-1} increased photosynthetic attributes such as total content of chlorophyll (41.7 %), carotenoids (43.7 %), chlorophyll fluorescence (7.1 %), and carbonic anhydrase (23.8 %). All of these contributed to the highest accumulation in the content (129.0 %) and yield (215.5 %) of essential oil (EO), in comparison to the control. Thus, results encouraged the use of SiO_2 and TiO_2 NPs to be applied in combined form to boost the essential oil production of *Coleus aromaticus.* The findings of this study may serve agronomists to determine the optimal concentrations of NPs for enhanced production of bioactive compounds with a wide range of industrial applications.

1. Introduction

Coleus aromaticus Benth., is a perennial succulent herb, belongs to the family Lamiaceae [1]. This plant is distributed all over India

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https://doi.org/10.1016/j.heliyon.2023.e21646
Received 12 May 2023; Received in revised form 19 September 2023; Accepted 25 October 2023
Available online 9 November 2023

and attains a height of up to 90 cm. *C. aromaticus* has been extensively studied for its multiple potentials with regard to allelopathic, antibacterial, antimicrobial, insecticidal, free-radical scavenging and radio-protective properties [2]. Flavouring agents like carvacrol and thymol are found in this herb, whilst phenolic compounds like rosmarinic acid, chlorogenic acid, etc. contribute to its overall health. The herb's beneficial properties have made it useful not only in culinary but also in therapeutic contexts [2]. The potential effectiveness of C. aromaticus has been proven by earlier studies in treating severe illnesses such as helminthiasis, persistent bronchitis, epilepsy, malaria and asthma [3]. Phytochemical analysis of *C. aromaticus* conducted by El-hawary [1] has identified the existence of phenols, sterols, terpenoids, alkaloids, glycosides, flavonoids, and tannins, but no saponins or steroids. Essential oil, produced in diminutive glandular hairs, significantly contributes to the volatile compounds of *C. aromaticus*. Thymol has been identified as a prominent component of essential oil [3]. Considering its growing demand, the production of essential oil is very scanty and need some novel techniques to increase its production and quality. Therefore an attempt has been made to enhance the production of essential oil by applying different combinations of nanoparticles.

Nanotechnology has generated attention due to its revolutionary impact on food security and agricultural sustainability [4,5]. It is a growing field with enormous potential for the agriculture sector [6]. Nanotechnology innovation considerably stimulates investigation on the foliar spray of engineered NPs as biofertilizers, nanocapsules, and nanodevices in agricultural sector [7–11]. Scientists have used various NPs to reduce nutrient loss, prevent diseases, and boost up the crop yields, while promoting long-term crop production [12]. Comparatively larger surface area, smaller size and improved catalytic reactivity of NPs, allow them to interact and control several plant activities [7,13–15]. For instance, silicon is one of the most abundant elements on earth's surface and is regarded as a "quasi-essential" component [16]. It is a beneficial mineral-element, especially for members of the Poaceae family [17]. Silicon, primarily found in soil with silicic acid ($Si(OH)_4$) in between 0.1 and 0.6 mM concentrations, is considered crucial for the growth and development of several plants [18]. Application of silicon dioxide nanoparticles (SiO_2NPs) has proved as an important physiological tool for encouraging plant growth and reducing metal toxicity under heavy-metal stress. Moreover, the effect of SiO_2NPs depends on the plant concerned and the degree of heavy metal present in the rhizoshpere [19]. SiO_2NPs mediated enhancement in plant growth and development is attributed to improved photosynthetic parameters like effective photochemical efficiency, chlorophyll fluorescence, PS II activity, photochemical quenching and related enzymes [20]. Similarly, titanium dioxides (TiO_2) NPs are widely recognized for their significant role in modulating various plant processes [12]. The remarkable hydrophilic feature, and high conductivity of TiO_2NPs have led to their modern employment in widespread fields as diverse as medicine, nutrition, agriculture, and bioremediation [21,22]. TiO_2NPs have been found to enhance canola seed germination, radicle and plumule growth, promote wheat plant growth even in drought conditions, and regulate key enzyme activities like glutamate dehydrogenase, nitrate reductase, and glutamine synthase [23,24]). These effects contribute to improved nutrient assimilation, metabolite synthesis, and overall plant growth [25,26]. Furthermore, TiO_2NPs exhibit photocatalytic properties and instigate oxidation-reduction reactions [24]. Prior studies revealed that TiO_2 boosts the plant growth and development, improves chlorophyll levels, up-regulates photosynthetic activities and related enzymes, as well as synthesis of secondary metabolites [27]. In addition, TiO_2NPs have shown the specific function in various components of plant physiological-molecular biology, such as enhancing the light absorption, up-regulates the activity of Rubisco and antioxidative enzymes. Also, TiO_2NPs positively effects growth via alteration in thylakoid membranes and photosystem II [28].

Although some studies have examined the effects of SiO_2NPs or TiO_2NPs on the growth of various plants, there is a lack of research specifically investigating their influence on medicinal plants. Moreover, no studies have explored the cumulative effect of SiO_2 and TiO_2 NPs on the production and quality of essential oil (EO) in plants. Given the significant pharmaceutical importance of *C. aromaticus* and the known benefits of SiO_2 and TiO_2NPs in plant systems, this study aimed to investigate the effects of the NPs on photosynthetic parameters, peltate glandular trichomes (PGTs), EO production, and the quality and composition of EO in *C. aromaticus* plants. This effort was made to meet the rapidly expanding demands for pharmaceutically important secondary metabolites of *C. aromaticus*.

2. Materials and methods

2.1. Growth conditions and plant material

The experiment was conducted in a net house situated at Aligarh Muslim University's Botany Department, Uttar Pradesh, India. The experiment was carried out in earthen pots with dimensions of 40 cm in diameter and 45 cm in height. *Coleus aromaticus* Benth plantlets of similar size were obtained from a local nursery of Aligarh, Uttar Pradesh. Pots were filled up with soil and organic manure in a ratio of 6:1, with each pot containing approximately 8.5 kg of soil. Before transplanting the plantlets, randomly collected soil samples were tested for soil physico-chemical parameters at Central Laboratory for Soil and Plant Analysis (Indian Agricultural Research Institute, New Delhi). A basal dose of nitrogen (N, 24.78 mg kg^{-1}), phosphorous (P. 13.80 mg kg^{-1}), and potassium (K, 28.0 mg kg^{-1}) were applied in the form of urea, single superphosphate and muraite of potash, respectively.

2.2. Nanoparticles

The Department of Chemical Engineering, King Saud University, Riyadh, Kingdom of Saudi Arabia, provided the SiO_2NPs as Aerosol - 200, which is hydrophilic fumed-silica with a specific surface area of 200 m^2 g^{-1}. Whereas, TiO_2NPs with specific surface area of 90 m^2 g^{-1} were procured from Applied Physics Department, Aligarh Muslim University, Aligarh. Both the NPs were diluted with double distilled water (DDW) in order to prepare aqueous concentrations of the NPs viz. Si50+Ti50, Si100+Ti50, Si100+Ti100, Si200+Ti100, Si100+Ti200 and Si200+Ti200 mg L^{-1} (Si and Ti correspond to SiO_2 and TiO_2, respectively, while numbers 50, 100 and 200 correspond to mg L^{-1} of the respective NPs).

2.3. Characterization of NPs using electron microscopy

The structure and surface morphology of SiO_2 and TiO_2 NPs was identified using SEM (JEOL JSM–6510, Japan) operated at 20 kV. The SEM images were captured at multiple magnifications and secondary imaging detector was used for imaging at μm scale. The elemental composition was determined through EDX analysis by Thermo EDX, Oxford equipment connected with SEM. The qualitative and quantitative morphology of NPs was characterized using a transmission electron microscopy (TEM, JEOL JEM-1230, Japan) operating at a voltage of 120 kV. Prior to TEM observations, a carbon-coated copper grid was loaded with a solution containing NPs.

2.4. Experimental plan

The pot experiment was carried out using simple randomized design. Aerosols of SiO_2NPs and TiO_2NPs were sprayed in required amount. The control plants were sprayed with DDW. Using a hand sprayer, foliar-sprays with regard to each treatment were applied at 5 days interval, starting from 90th day after planting (DAP). The foliar-spray treatments were applied for 5 times. As and when required, weeding and watering were performed. The growth attributes, physiological and biochemical parameters, and EO-related parameters of *Coleus aromaticus* were assessed at 120 days after planting (DAP) to determine the crop response.

2.5. Estimation of growth parameters

At 120 DAP, randomly-selected plants from each treatment were ripped out of ground cautiously, and washed to remove adherent soil particles, followed by surface-drying with a blotting sheet. Afterwards, the fresh weight of shoot and root was assessed. The obtained plants were dried in an oven at 60 °C for about 48 h to measure their dry weights.

2.6. Determination of physiological and biochemical attributes

2.6.1. Total contents of chlorophyll and carotenoids

The Lichtenthaler and Buschmann [29] method was used to measure the total chlorophyll and carotenoid content in leaves. Fresh leaf tissue (1 g) was homogenized with 100 % acetone and collection of supernatant was made after centrifugation at 10000 rpm for 10 min. Afterwards, the content of chlorophyll *a*, chlorophyll *b* and carotenoids, was measured with a spectrophotometer at 662, 645, and 480 nm, respectively (Shimadzu UV-1700, Japan). The total chlorophyll content was calculated by adding the amounts of chlorophyll *a* and chlorophyll *b*. The total chlorophyll and carotenoid contents were measured and expressed as mg g^{-1} leaf fresh-weight.

2.6.2. Chlorophyll fluorescence (fv/fm)

Saturation-Pulse Fluorometer (PAM-2000, Germany) was used to measure chlorophyll fluorescence (Fv/Fm) on the upper side of fully expanded leaves between 11:00 a.m. and 12:00 p.m. The accurate readings were recorded by keeping the leaves in the dark for 30 min before measurement. This helped to stabilize the reaction centre and produce more reliable results. Low measuring beams with a light intensity of 125 mol m^2 s^{-1} were used to determine the minimum (Fo) and maximum (Fm) fluorescence of dark-adapted leaves. The variable fluorescence (Fv) was calculated using the values of Fm-Fo and maximal efficiency of PSII (chlorophyll fluorescence) by using Fv/Fm.

2.6.3. Carbonic anhydrase activity

Carbonic anhydrase (CA) activity in fresh leaves was measured using the protocol developed by Dwivedi and Randhawa [30]. Fresh leaf sample (0.2 g) devoid of veins were sliced into small rectangular pieces and submerged in 10 mL of 0.2 M aqueous cysteine hydrochloride solution. The reaction mixture was maintained at 4°C for 20 min. After incubation, the samples were transferred to a test tube containing 4 mL of a phosphate buffer (pH 6.8), 4 mL of a 0.2 M sodium bicarbonate ($NaHCO_3$) solution, 0.2 mL of a 0.02 M sodium hydroxide (NaOH) solution, and 0.2 mL of 0.002 % bromothymol-blue indicator. The reaction mixture was shaken for 20 s before being incubated in an ice box for 2 h. Afterwards, the sample was titrated against 0.01 N HC1 using methyl-red as an indicator. The volume of HCl used to develop light purple color was recorded. The CA activity was measured and expressed as mol CO_2 kg^{-1} leaf FW s^{-1}.

2.6.4. Nitrate reductase activity

Nitrate reductase (NR) activity was measured by the method pioneered by Jaworski [31], 0.2 g of freshly diced leaves were transferred to a plastic vial with 0.5 mL of 0.2 M potassium nitrate solution, 2.5 mL of phosphate buffer (pH 7.5), and 2.5 mL of 5 % isopropanol. After that, the reaction mixture was incubated for 2 h using a BOD incubator maintained at 30 °C in dark. Post incubation, 0.4 mL of the reaction mixture was transferred to a test tube, followed by adding 0.3 mL each of 1 % sulphanilamide and 0.02 % N-(1-naphthyl) ethylenediamine dihydrochloride solution. After incubation at room temperature for 20 min to attain maximum colour intensity, the content was diluted with DDW to a final volume of 5 mL. Optical density of the final solution was measured at 540 nm using the spectrophotometer. NR activity was expressed as nmol NO_2^- g^{-1} FW h^{-1}.

2.7. Essential oil parameters

Fresh leaves (200 g) were collected from each treatment and chopped leaf-tissue was used to obtain essential oil (EO) using the hydro-distillation method. Leaf EO content was quantified gravimetrically in accordance with method of Guenther [32]. Clevenger's apparatus was used to distil the chopped leaves for 3 h, and the resulting EO was dehydrated with anhydrous sodium sulphate before being stored in sealed glass vials at 4 °C until the GC analysis was carried out.

The US-made Agilent 7890B gas chromatography instrument was used for GC analysis. The instrument was equipped with a

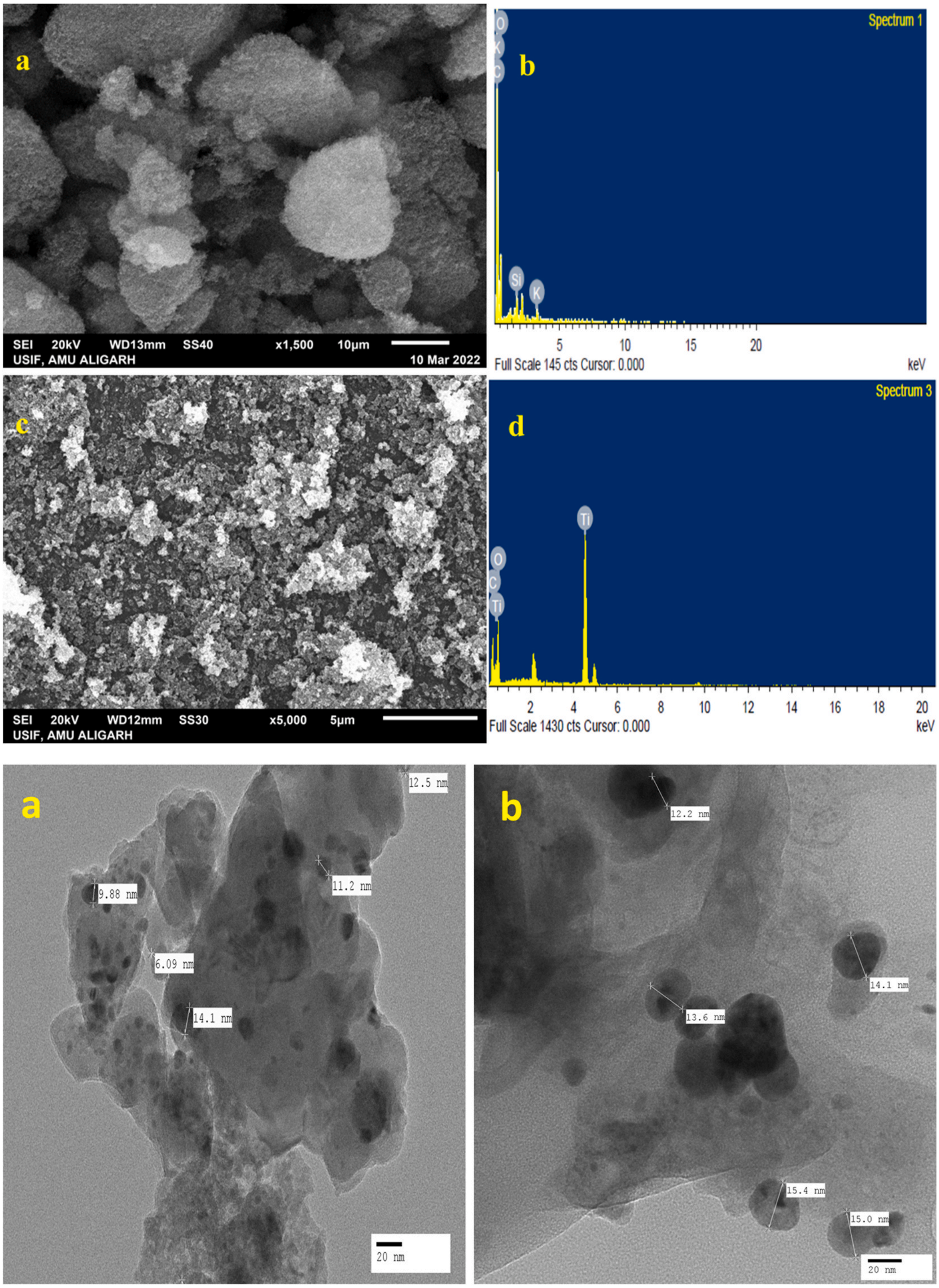

Fig. 1. A: SEM image of SiO_2NPs (**a**); SEM-EDX of SiO_2NPs (**b**), and SEM image of TiO_2NPs (**c**), SEM-EDX of TiO_2NPs (**d**)B: TEM image of SiO_2NPs **(a)** and TiO_2NPs (**b**).

capillary column coated with fused silica carrying 30 m length and 0.32 mm inner diameter in addition to an injector and flame ionisation detector. Nitrogen was employed as the carrier gas. Detector temperature 270 °C, oven temperature 260 °C, and injector temperature 250 °C constituted the GC temperature profile. A sample size of 0.2 μL was used constantly. The first temperature was 40 °C, maintained for 2 min, and the second and final temperature were 260 °C, maintained for 10 min. Active components of EO, such as thymol and *trans*-caryophyllene, were identified using retention time. Content of EO and the active constituents was determined by comparing the chromatogram peaks of the sample to the peaks obtained from the reference standard according to Adams [33].

3. Statistical analysis

Foliar-spray treatments were carried out five times, with each experimental pot serving as a replicate. Statistical analysis was accomplished according to simple randomized design, employing SPSS-22 statistical software (SPSS Inc., Chicago, IL, USA). To compare the means of data set, Duncan's multiple range test (DMRT) was used at the $p \leq 0.05$ significance level. In addition, standard error (±SE) values were used to compare the data means. The LSD values were calculated using OPSTAT.

4. Results

Various parameters of *C. aromaticus* were examined to measure the impact of varying combined-concentrations of SiO_2 and TiO_2 NPs.

4.1. Characterization of NPs using electron microscopy

The surface characteristics, morphology and size of SiO_2 and TiO_2 NPs were examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques. The characterization was further validated via SEM-EDX to observe their elemental composition. The SEM images revealed the presence of spherical-shaped SiO_2NPs, whereas knitted ball-like crystalline structure was observed in TiO_2 NPs (Fig. 1). Furthermore, the size of both SiO_2 and TiO_2 NPs were observed using TEM. As clear from the figure, the average size of SiO_2NPs ranges between 6.09 and 14.1 nm and the average diameter of TiO_2 NPs was about 12.2–15.4 nm (Fig. 1).

4.2. Growth parameters

The values of all growth attributes were improved by the combined application of SiO_2 and TiO_2 NPs, in a dose dependent manner. The utmost increase was observed by spray-treatment of Si100+Ti100 mg L^{-1} concentration compared to the control. Contrastingly, at Si200+Ti100, Si100+Ti200, and Si200+Ti200 mg L^{-1}, the values of all growth parameters were progressively decreased. The optimum treatment (Si100+Ti100 mg L^{-1}) boosted the shoot and root length by 38.5 % and 44.6 %, respectively. This treatment also increased the shoot fresh weight (39.5 %), shoot dry weight (40.8 %), root fresh weight (45.7 %) and root dry weight (49.4 %) in comparison to the control (Table 1).

4.3. The activities of CA and NR enzymes

The activities of CA and NR were up regulated by the exogenous application of SiO_2 and TiO_2 NPs (Fig. 3), in a dose dependent manner. The maximum activities were noted in the plants treated with Si100+Ti100 mg L^{-1}; however, the values were gradually decreased with increasing the concentration of NPs. The optimum treatment (Si100+Ti100 mg L^{-1}) boosted the, levels of CA and NR activities by 23.8 % and 19.6 %, respectively as compared to control plants (Fig. 3).

Table 1
Effect of foliar application of various concentrations of SiO_2 and TiO_2 nanoparticles on growth attributes of *Coleus aromaticus.*

Nanoparticle treatments (mg L^{-1})	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
Control*	17 ± 0.32^{e}	38.7 ± 0.73^{e}	43.3 ± 0.8^{d}	19.2 ± 0.03^{e}	92.3 ± 1.7^{d}	39.2 ± 0.7^{d}
Si50+Ti50	18.2 ± 0.35^{d}	40.02 ± 0.76^{de}	47.6 ± 0.9^{cd}	25.8 ± 0.04^{d}	98.1 ± 1.8^{cd}	46.8 ± 0.8^{cd}
Si100+Ti50	20.4 ± 0.38^{c}	46.75 ± 0.89^{c}	53.8 ± 1.0^{b}	26.2 ± 0.04^{bc}	106.2 ± 2.0^{b}	48.6 ± 0.9^{b}
Si100+Ti100	24.5 ± 0.46^{a}	53.6 ± 1.02^{a}	63.1 ± 1.2^{a}	28.7 ± 0.05^{a}	128.7 ± 2.4^{a}	55.2 ± 1.0^{a}
Si200+Ti100	23.1 ± 0.44^{b}	50.03 ± 0.95^{b}	57.4 ± 1.0^{b}	27.3 ± 0.05^{b}	119.1 ± 2.2^{b}	50.3 ± 0.9^{b}
Si100+Ti200	21.05 ± 0.40^{c}	42.35 ± 0.80^{d}	49.6 ± 0.9^{c}	23.4 ± 0.04^{c}	95.4 ± 1.8^{c}	42.1 ± 0.8^{c}
Si200+Ti200	17.24 ± 0.32^{e}	40 ± 0.76d^{e}	36.2 ± 0.6^{d}	15.6 ± 0.2^{d}	88.9 ± 1.6^{d}	35.5 ± 0.6^{d}
LSD	0.12	0.24	0.38	0.20	0.63	0.29

Each value represents the mean of 5 replicates with ±SE. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). * Spray of double distilled water.

4.4. Peltate glandular-trichome (PGT) analysis

In the current study, combined foliar-application of NPs resulted in a considerable rise in the area of PGTs (Fig. 2a,b). The maximum enhancement was recorded in plants supplemented with Si100+Ti100 mg L^{-1}. In comparison to control, an increase of 3756.8 cm^2 was witnessed in the area of PGTs due to the optimum foliar-application of Si100+Ti100 mg L^{-1} (Fig. 2b).

4.5. Essential oil parameters

The essential oil (EO) content (Fig. 4A) and yield (Fig. 4B) were assessed in both control and treated plants, revealing a significant enrichment in the content (129.0 %) and yield (215.5 %) of EO with the application of Si100+Ti100 mg L^{-1} NPs (Fig. 4). Further increases in concentration led to a gradual decline in the values; nevertheless, they remained higher compared to the control. Moreover, both these nanoparticles specifically the application Si100+Ti100 mg L^{-1} proved equally significant in enhancing the contents of active constituents of EO as compared to untreated plants. The maximum value reported in active constituents, viz. Thymol and *trans*-β-caryophyllene were found to be 29.4 % (Figs. 4C) and 27.3 % (Fig. 4E) respectively, compared to their respective controls (Fig. 4). Also, the significant increase in the yield of thymol (Fig. 4D) and *trans*-β-caryophyllene (Fig. 4F) were observed in treated plants as compared to control. The results were further validated by analyzing the bioactive compounds using GC-MS analysis (Fig. 5 A, B).

5. Discussion

Plant growth is influenced by a combination of external and internal factors, including plant growth promoters and elicitors, which establish a strong source-sink relationship and enhance nutrient supply for improved cellular metabolism [34]. Among the various techniques, the use of nanoparticles has proven to be one of the most effective and sustainable way to improve plant growth and development. Several studies have documented the positive effects of exogenously applied NPs on plant growth, physiology, and the production of secondary metabolites [12,26,35,36]. Due to their widespread applicability, nanoparticles (NPs) are considered as a promising new class of elicitors for increasing the yield of high-value bioactive compounds (secondary plant products) of plants [37–39]. The unique physicochemical properties of NPs and their ability to increase plant metabolism have led to their recent use in agriculture [40,41]. Researchers have recommended the applications of NPs in agriculture through several ways. In corroboration with this, the current study shows a positive effect of combined application of SiO_2 and TiO_2 NPs on various growth, quality, physiological parameters and essential oil content of Coleus *aromaticus*.

In the present study, the foliar application of $SiO_2 + TiO_2$ NPs enhanced the growth attributes (viz., root and shoot length, fresh and dry weights of root and shoot) in a dose dependent manner (Table 1). The utmost increase was reported by the combined application of Si100+Ti100 mg L^{-1}. The enhancement in plant growth may be attributed to the improved photosynthetic parameters (Fig. 3A), activities of carbonic anhydrase and nitrate reductase (Fig. 3B) and total chlorophyll content (Fig. 3C). In corroboration with our findings, a similar increase in growth attributes were also reported in *Foeniculum vulgare* [42], *Phaseolus vulgaris* [43] and *Ocimum basilicum* [44]. Besides, Chahardoli et al. [45] revealed that foliar application of TiO_2NPs amplified the shoot and root length as well as total biomass of *Nigella arvensis* L. In another study, Ahmed et al. [46] demonstrated the positive impact of both SiO_2 and TiO_2 NPs on fresh and dry weight of *Vetiveria zizanioides*. SiO_2NPs mediated enhancement in plant growth and development might be due to its modulation in plant hormone and sugar metabolism [47]. Similar results were also obtained in various plant species [12,26,48].

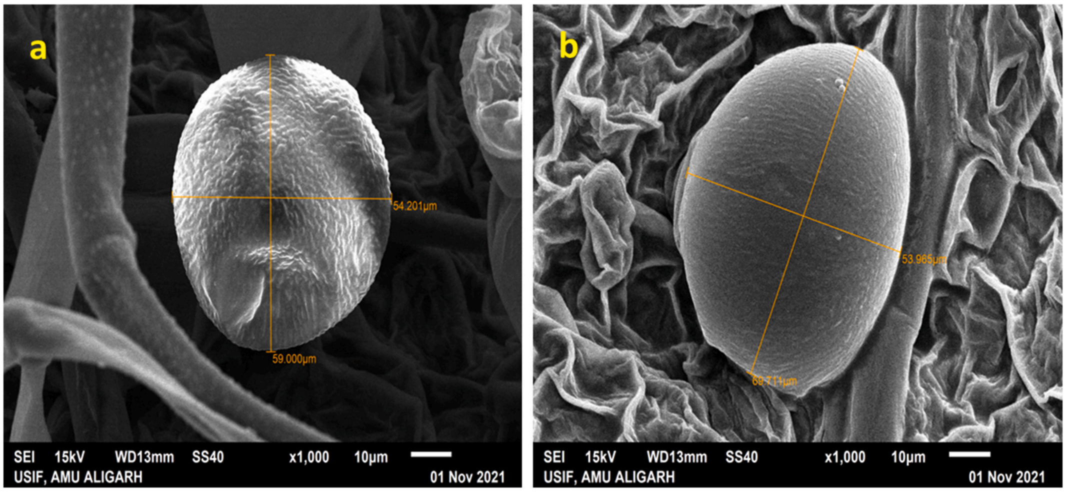

Fig. 2. Effect of combined application of SiO_2 and TiO_2 nanoparticles on peltate glandular trichomes of Coleus aromaticus leaves as observed under scanning electron microscope at 120 DATregarding **(a)** water-spray control and **(b)** optimum treatment (Si100+Ti100 mg L^{-1}).

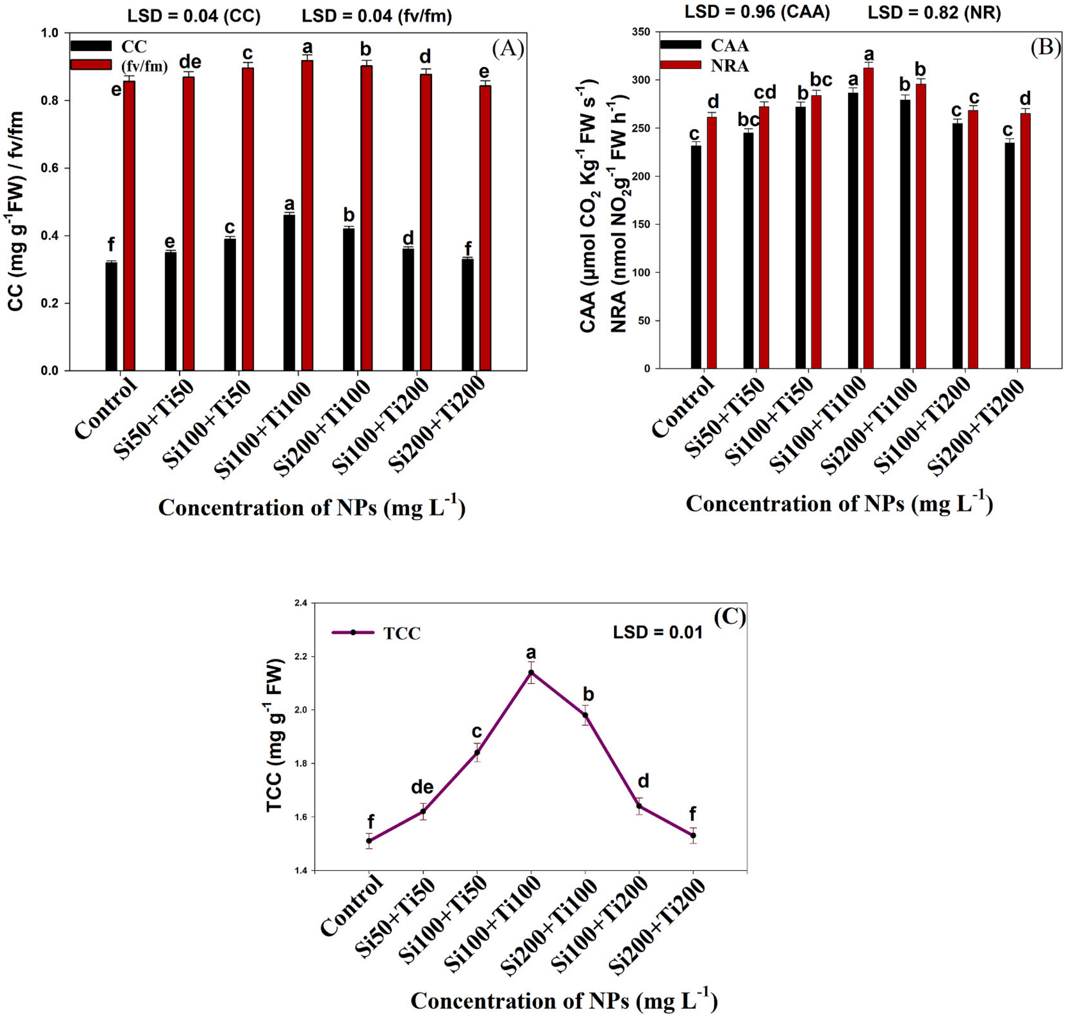

Fig. 3. Effect of combined application of SiO_2 and TiO_2 nanoparticles on (**A**) carotenoid content (CC) and chlorophyll fluorescence (fv/fm), (**B**) carbonic anhydrase activity (CAA) and nitrate reductase activity (NRA), and (**C**) total chlorophyll-content (TCC) recorded in the leaves of Coleus aromaticus at 120 DAT. The values shown are the average of five replicates.

Our studies with regard to Coleus *aromaticus* revealed that the combined application of SiO_2 and TiO_2 NPs not only improved the growth traits but also upgraded the photosynthetic parameters significantly (Fig. 3). The optimum combined-dose of the two NPs (Si100+Ti100 mg L^{-1}) maximally improved the PS II activity and carotenoids (Fig. 3A), CAA activity (Fig. 3B) and total chlorophyll content (Fig. 3C). The increased leaf chlorophyll-content consequently improved the photosynthesis, which in turn boosts the plant's fresh and dry weights, and secondary metabolite production in plants [25,48]. The observed increase in chlorophyll content mediated by SiO_2NPs may be attributed to changes in intrinsic nutrient levels such as magnesium and sulfur [49]. Similarly, the role of TiO_2NPs in enhancing chlorophyll content aligns with previous findings by Ebrahimi et al. [43] conducted on bean plants. The improvement in chlorophyll content can be attributed to the positive impact of SiO_2NPs and TiO_2NPs on enzymatic activities related to nitrogen metabolism, facilitating nitrate uptake by plants and promoting the production of organic nitrogen (such as proteins and chlorophyll) from inorganic nitrogen sources [25,48,50]. In agreement with the present findings, Sharifi-Rad et al. [51] reported that application of SiO_2NPs considerably boosted the photosynthesis in several agricultural and medicinal crops. Similar results were observed in *Mentha piperita* [12], *Vetiveria zizanioides* [34] and *Cymbopogon flexuosus* [52]. Furthermore, Ahmed et al. [46]. demonstrated a positive impact of SiO_2 and TiO_2 NPs on photosynthetic pigments as well PSII activity of vetiver.

The present study also demonstrated that both these NPs influenced the efficiency of PS II as compared to untreated plants. Noji et al. [53] observed that silicon NPs interacted with PS II and enhanced the photolysis of water, a critical process involved in photosynthetic oxygen evolution. This interaction resulted in the accelerated flow of electrons from water to quinone molecules, thereby improving electron transport. Similarly, the role of TiO_2NPs in enhancing PSII activity in our current study aligns with the findings of Gohari et al. [24] in Moldavian balm. The application of TiO_2NPs promotes photosynthesis due to their photo-catalytic nature, facilitating the capture and conversion of light energy into chemical energy, ultimately leading to increased CO_2 fixation

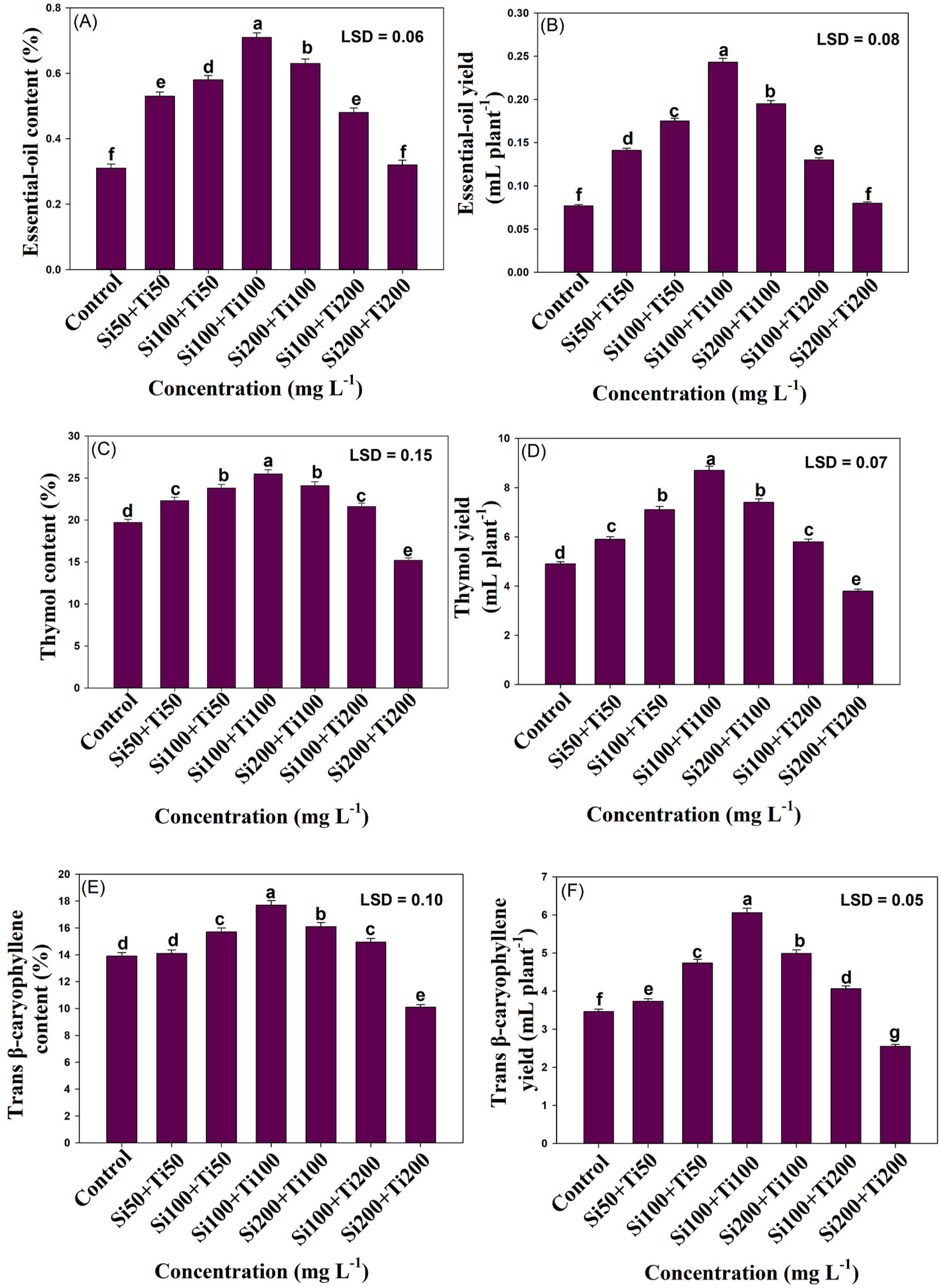

Fig. 4. Effect of combined application of SiO_2 and TiO_2 nanoparticles on essential-oil content (**A**), essential oil yield (**B**), thymol content (**C**), thymol yield (**D**), *trans*-β-caryophyllene content (**E**) and *trans*-β-caryophyllene yield (**F**) recorded in the leaves of Coleus aromaticus at 120 DAT. The values shown are the average of five replicates.

[54]. Moreover, the ability of TiO_2NPs to penetrate chloroplasts and participate in redox reactions may contribute to the improvement of electron transport rate (ETR) and the release of oxygen, thereby enhancing chloroplast activity.

Activities of CA and NR are immensely associated with nitrogen and carbon metabolism, respectively, both of which directly

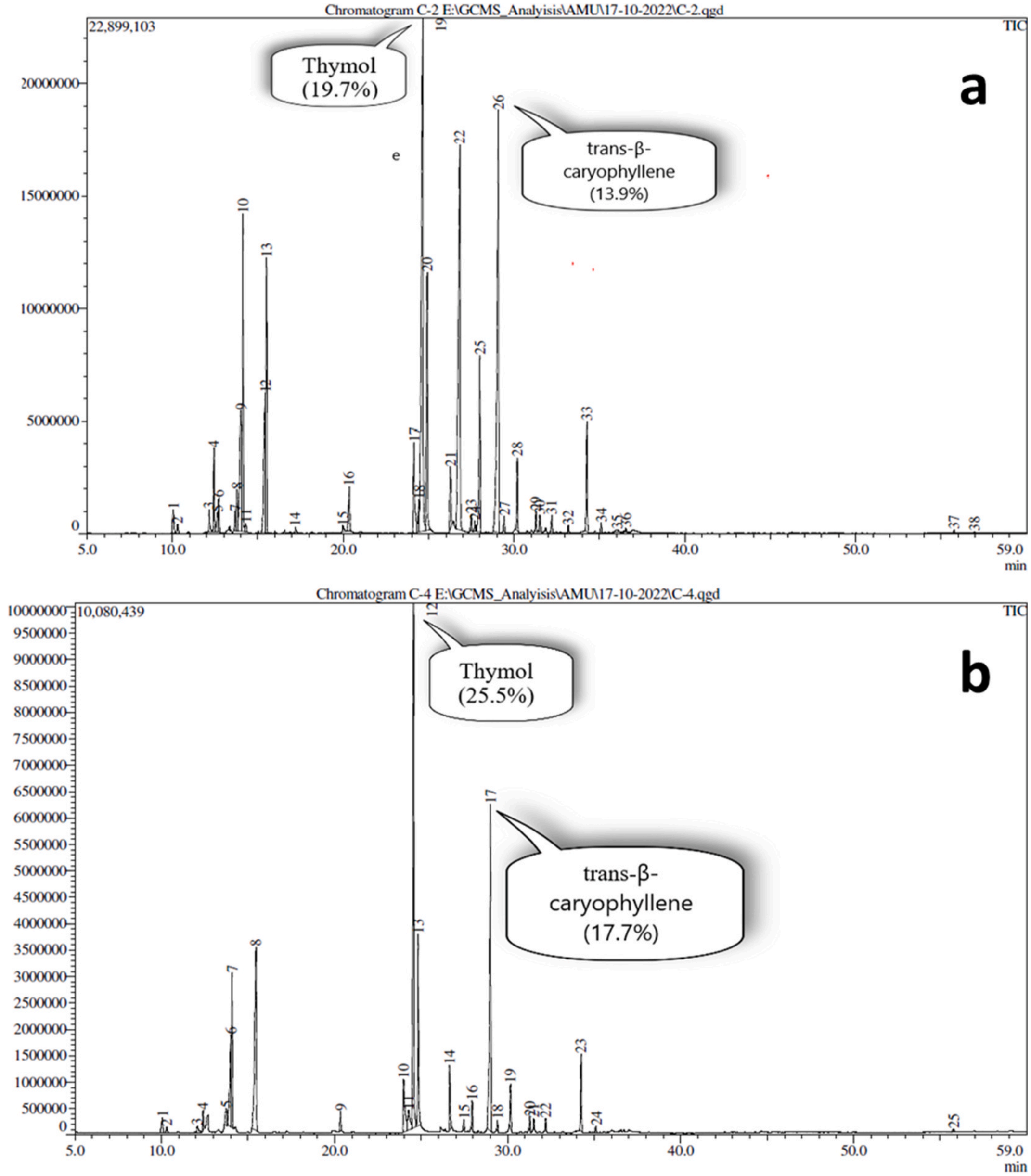

Fig. 5. GC chromatogram of leaf-EO of *Coleus aromaticus* treated with (**a**) water-spray (control) and (**b**) optimum treatment comprising the SiO_2 and TiO_2 nanoparticles (Si100+Ti100 mg L^{-1}) recorded *at* 120 DAT.

contribute to enhance photosynthesis, growth and biomass production of the crop plants [55]. It is evidently demonstrated by our results, a significant increase in growth characteristics, and photosynthetic parameter due to foliar application of SiO_2 and TiO_2 NPs (Table 1; Fig. 2). The application of SiO_2 and TiO_2 NPs (Si100+Ti100 mg L^{-1}) resulted in the utmost enhancement in the activities of CA (23.8 %) and NR (19.6 %) compared to the control (Fig. 3). In agreement with our findings Lu et al. [56], also reported the improved activity of NR in soybean due to application of various nanomaterials. Yang et al. [50], noted a similar increase in NR activity of spinach as a result of TiO_2NPs, suggesting that TiO_2NPs might directly affect nitrogen metabolism by modulating the activity of nitrate reductase (in addition to other enzymes), promoting the nitrate assimilation by plants. Ahmad et al. [12] also reported a significant increase in the activities of NR (17.7 %) and CA (19.1 %) in TiO_2-treated *Mentha piperita*. Besides, Shabbir et al. [34] observed a substantial increment in the activities of NR and CA in TiO_2NPs treated *Vetiveria zizanioides*. Similar enhancement in the activities of CA and NR was observed by the application of SiO_2 NPs [12,50].

In the current study, foliar-sprays of SiO_2 and TiO_2 NPs boosted the contents and yield of essential oil (EO), with the highest values being obtained by the combined dose of Si100+Ti100 mg L^{-1} (Fig. 4). Our findings are in line with the findings of Ahmad et al. [12] and Golami et al. [57], who reported the improvement in the content and yield of EO in *Mentha piperita*'s and *Rosmarinus officinalis* respectively, by the foliar application of TiO_2NPs. Similar findings were recorded by Ahmed et al. [46], where the application of SiO_2, TiO_2, or ZnO NPs, resulted in significant increase in the content and yield of EO in vetiver. Also, Gohari et al. [24] observed an increase in the EO (1.19 %) in TiO_2NPs treated *Dracocephalum moldavica* plants. Furthermore, Mukarram et al. [41] demonstrated that foliar application of SiO_2NPs assisted in the biosynthesis of EO in *Cymbopogon flexuosus*, resulted an increase in EO content (22 %) and yield (44 %). Similarly, TiO_2NPs boost the activity of many key enzymes involved in the terpene biosynthesis-pathway, increasing the overall yield of EO in vetiver [34]. In corroboration with these findings, our results demonstrated an increase in the content (29.4 %) and yield (27.3 %) of EO in *Coleus aromaticus* by the foliar application of SiO_2 and TiO_2 NPs (particularly Si100+Ti100 mg L^{-1}).

The current study revealed that combined application of SiO_2 and TiO_2 NPs increased not only the content and yield of EO but also increased the bioactive components of EO. The maximum increase in thymol (29.4 %) and β-caryophyllene (27.3 %) was observed in plants treated with Si100+Ti100 mg L^{-1} (Fig. 4). As per GC-MS reports, combined application of SiO_2 and TiO_2-NPs demonstrated a significant increase in the concentration of thymol and β-caryophyllene, principal constituents of *C. aromaticus* essential oil, in comparison to control plants (Fig. 5 A, B). The present investigation also revealed that the optimum treatment (Si100+Ti100 mg L^{-1}) of NPs had a substantial effect on the area of peltate glandular trichomes (an increase of 3756.8 cm^2), in the leaves, which evidently might have facilitated the achievement of high EO content of treated plants compared with those of the control. As per the above discussion, it may be concluded that foliar-spray treatment of SiO_2 and TiO_2 NPs may significantly increase the content as well as yield EO in Coleus aromaticus.

6. Conclusion

The unique physicochemical properties of nanoparticles enable them to induce diverse responses in plants. In the present study the application of SiO_2 and TiO_2 NPs promoted growth by improving the photosynthetic efficiency and pigment composition in plants. Moreover, the applied concentration (particularly Si100+Ti100 mg L^{-1}) of NPs improved the peltate glandular trichome area as compared to untreated plants. All of these contributed to the enhanced accumulation of essential oil in C. aromaticus. *Furthermore, the foliar application of NPs resulted in an increase in both the content and yield of the pharmaceutically important active constituent, thymol, thereby enhancing the quality of the essential oil. The NPs used in the present study have demonstrated effectiveness in improving plant growth and EO content.* These findings offer valuable insights into maximizing the potential benefits for its use in industrial applications.

Author contribution statement

Moinuddin and M. Masroor A. Khan: Conceived and designed the experiment.
Urooj Hassan Bhat: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Sarika Singh, Aman Sobia Chishti and Sangram Singh: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

There are no financial or personal relationships that could be interpreted as having influenced the work presented here.

Acknowledgment

The essential facilities provided by the Chairperson of the Department of Botany Aligarh Muslim University are highly appreciated. The authors would also like to thank the University Sophisticated Instruments Facility of A.M.U. (Aligarh) for the assistance extended for electron microscopy analysis.

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