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Efficacy of TiO_2 nanoparticles in enhancing the photosynthesis, essential oil and khusimol biosynthesis in *Vetiveria zizanioides* L. Nash

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Abstract

Titanium dioxide nanoparticles (TiO_2NPs) have intrigued scientists due to their plant growth elicitor activity and potential to strengthen the photosynthetic machinery of plants. Therefore, we investigated if foliar application of TiO_2NPs could enhance the photosynthetic characteristics and the yield of essential oil-bearing multi-purpose crop, *Vetiveria zizanioides*. Of the various concentrations (30, 60, 90, 120, and 150 mg L^{-1}), 90 mg(TiO_2NPs) L^{-1} was found being significant enough in mproving most of the parameters studied. At 300 d after transplantation, foliar application of TiO_2NPs (90 mg L^{-1}) increased the total chlorophyll (Chl) content and maximum photochemical efficiency of PSII by 27.2 and 23.5%, respectively, compared with the control. Due to this treatment, the content and yield of essential oil (EO) increased by 23.6 and 55.1%, respectively. The khusimol (main active constituent of EO) content and yield were improved by 24.5 and 93.2%, respectively.

Additional key words: gas chromatography; photosynthetic parameter; secondary metabolite; surface characteristic; vetiver.

Introduction

Human interventions have intensely affected the edaphics of many of fertile lands, making most of them inhospitable for agricultural practices. However, exploitation of vetiver grass (*Vetiveria zizanioides* L. Nash) has emerged as a promising remedy for such repercussions. Vetiver, belonging to the family Poaceae, is a densely tufted plant. The miraculous nature of vetiver is realized on account of its multifarious utilities. Roots as well as aboveground parts of vetiver have greatly benefited the world. (Chomchalow and Chapman 2003). Intertwined network of vetiver roots sustain the soil environment in addition to producion of essential oil, which is highly valued for its aromatic and biological properties. Essential oil (EO) of vetiver has marked influence on perfumery industry and is extensively used commercially in food and cosmetic industries. Vetiver EO is also used in human healthcare as an aroma-therapeutic agent. It is highly beneficial to human health owing to its antioxidant (Kim *et al*. 2005) and anticancer activities (Chen *et al*. 2003). Moreover, the utilization of roots left after extraction of vetiver oil is also known to underpin country's economy to an extent. They are extensively used as refrigerant, handicrafts, brooms, paper, and straw boards, *etc*. Vetiver also suits to produce soft, durable fabric.

Application of nanomaterials in the field of agriculture aims at sustainable crop production, nutrient loss reduction, disease suppression, and increase of yield. Nanomaterials are known to influence vital plant life events ranging from seed germination and photosynthesis to flowering (Khan *et al.* 2017). Different types of nanomaterials have been applied to plants, amongst which TiO_2NPs are reported to regulate a variety of processes. Noteworthy among these are stimulation of carbohydrate production as well as enhanced growth and photosynthetic rate in plants (Owolade *et al*. 2008, Chen *et al*. 2014, Khodakovskaya *et al*. 2014). According to Chao and Choi (2005), application of TiO_2NPs augments the growth and yield of plants by approximately 30%. It has been documented that application of TiO_2NPs improves seed germination as well as radical and plumule growth in canola seedlings (Mahmoodzadeh *et al*. 2013). Additionally, application of TiO_2NPs has been reported to regulate important enzymatic activities (*e.g.*, nitrate reductase) that could be exploited to accumulate additional nutrients resulting in enhanced growth in plants (Yang *et al*. 2006, Mishra *et al*. 2014). However, previous studies showing the effect of TiO_2NPs

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Abbreviations: C_i – intercellular CO_2 concentration; CA – carbonic anhydrase; DAT – days after transplanting; DDW – double distilled water; DMRT – Duncan´s multiple range test; EO – essential oil; g_s – stomatal conductance; GC – gas chromatography; NPs – nanoparticles; NR – nitrate reductase; P_N – net photosynthetic rate; OD – optical density; TiO_2NPs – titanium dioxide nanoparticles.
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on photosynthetic machinery in the EO-bearing plants like vetiver are meagre and it has been analysed in the present study. In view of the positive effects of TiO_2NPs on growth, photosynthesis, enzymatic activities, and yield, an attempt was made to investigate whether the TiO_2NPs can augment growth and photosynthesis of vetiver and enrich the content and yield of its EO and its active constituent, khusimol. The results could provide a conceptual basis for enhancement of the leaf photosynthetic capacity in order to increase the essential oil yield and improve fertilization strategies in vetiver.

Materials and methods

Growth conditions and plant material: The experiment was conducted using plastic pots (40-cm diameter × 45-cm height) in the natural conditions of the net house at the Department of Botany, Aligarh Muslim University, Aligarh (27°52'N, 78°51'E, and 187.45 m a. s. l.). The slips of vetiver were procured from Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. Prior to transplanting, each pot was filled with 8.5 kg of homogenous mixture of soil and organic manure. The soil samples were randomly collected from different pots and subsequently analyzed for the soil characteristics before transplantation of plant material. Soil samples were analyzed in the Central Laboratory for Soil and Plant Analysis, Indian Agricultural Research Institute, New Delhi. Physico-chemical characteristics of the soil were: texture of sandy loam, pH 8.07, available N, P, and K of 167.4, 94.6, and 286.0 kg ha^{-1}(soil), respectively. A uniform recommended basal dose of N (as urea), P (as diammonium phosphate), and K (as muriate of potash) was applied before transplantation at 85.5, 102.3, and 45.0 mg kg^{-1}(soil), respectively.

TiO_2 nanoparticles were supplied by Department of Applied Physics, Aligarh Muslim University, Aligarh, India. They were dissolved in double distilled water (DDW) and thereafter different aqueous concentrations (0, 30, 60, 90, 120, and 150 mg L^{-1}) were prepared for foliar spray.

Physico-chemical data of TiO_2 nanoparticles.

Parameter	Test method	Unit	TiO_2 nanoparticles
Specific surface area	BET	$m^2 g^{-1}$	90 ± 20
pH	in 4% dispersion	-	3.2 – 4.2
Tamped density	acc. to DIN EN ISO 787	g L^{-1}	approx. 120
Moisture	2 h at 105°C	%	< 2.1
Ignition loss	2 h at 1,000°C based on material dried for 2 h at 105°C	%	< 2.1
TiO_2 content	based on ignited material	%	>99.8
Average particle size	SEM	nm	14

Scanning electron microscopy (SEM): With the help of SEM (*JSM-6510 LV*, *JEOL*, Japan), morphological structure of TiO_2NPs was examined. Prior to analysis, samples were coated by gold. The same procedure was carried out (Fig. 1) at Ultra Sophisticated Instrumentation Facility Centre of Aligarh Muslim University, Aligarh, India. Surface characteristics of the samples (Fig. 1) were evaluated at accelerating voltage of 10 kV, magnification of 1,500× with spot size of 30. A secondary electron imaging detector was used for imaging at 10-μm scale.

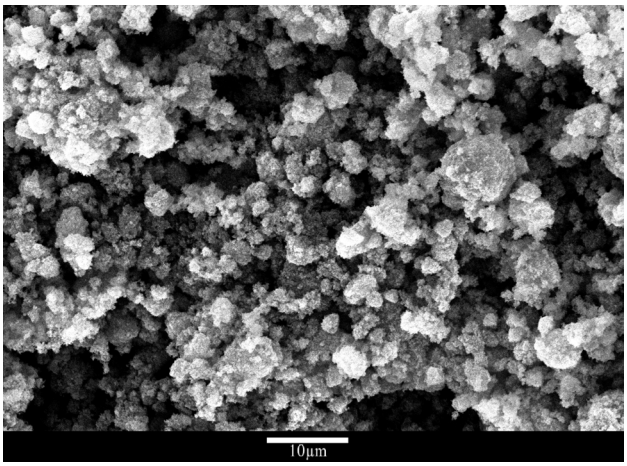

Fig. 1. Scanning electron microscopic (SEM) image of titanium dioxide nanoparticles (TiO_2NPs) taken at 10 μm scale with a magnification of 1,500 ×. The average primary particle size of TiO_2NPs was found to be 14 nm.

Experimental setup: TiO_2NPs were dissolved using DDW at different concentrations, *i.e.*, 0 (control), 30, 60, 90, 120, and 150 mg L^{-1}. Different aqueous concentrations were sonicated before spraying on the plants. De-ionized water was used as a control. Spray treatments were applied five times at an interval of 7 d with a hand sprayer. Each treatment was replicated five times. Crop performance was assessed in terms of growth attributes, physiological and biochemical parameters, and content as well as the yield of EO including kushimol in *Vetiveria zizanioides* L. Nash. Sampling (plant analysis) was carried out at 300 DAT.

Growth attributes, namely fresh mass (FM) and dry mass (DM) of shoots and roots were evaluated at 300 DAT. Five plants of each treatment were uprooted and washed

carefully with tap water in order to remove all adhering foreign particles; then surface was dried using blotting paper. FM of shoots and roots was recorded thereafter. Plants were dried at 80°C for 48 h using a hot-air oven; DM of plants was recorded subsequently.

Determination of physiological activities

Total Chl content was estimated in fresh leaves according to the method of Lichtenthaler and Buschmann (2001). Fresh tissue taken from interveinal area of leaf was ground with 100% acetone using mortar and pestle. The optical density (OD) of the pigment solution was recorded at 662 and 645 nm to determine the contents of Chl *a* and Chl *b*, respectively, by a spectrophotometer (*Shimadzu UV-1700*, Tokyo, Japan). Total Chl content was estimated by adding the contents of Chl *a* and *b*. The content of each photosynthetic pigment was finally expressed as mg g^{-1}(FM).

Carbonic anhydrase (CA, EC 4.2.1.1) activity was measured in fresh leaves, using the method described by Dwivedi and Randhawa (1974). Chopped leaf pieces (0.2 g) were transferred to Petri plate. Then, the leaf pieces were dipped in 10 mL of 0.2 M cystein hydrochloride solution for 20 min at 4°C. To each test tube, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.002% bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. The enzyme activity was expressed as $\mu M(CO_2)\ kg^{-1}(FM)\ s^{-1}$.

Nitrate reductase (NR, E.C. 1.7.1.1) activity was estimated by the intact tissue assay method developed by Jaworski (1971). Fresh chopped leaves, weighing 0.2 g, were transferred to a plastic vial. Each vial contained 2.5 mL of phosphate buffer (pH 7.5), 0.5 mL of potassium nitrate solution, and 2.5 mL of 5% isopropanol. After incubation for 2 h at 30°C, 0.4 mL of the vial content was transferred to a test tube. Then, 0.3 mL of both 1% sulphanilamide and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride solution were added. Finally, the vials were kept at room temperature for 20 min for maximum color development; the vial content was diluted to a volume of 5 mL with distilled water. The OD of the content was recorded at 540 nm using the spectrophotometer. NR activity was expressed as $nmol(NO_2)^{-1}\ g^{-1}(FM)\ h^{-1}$.

Chl fluorescence (F_v/F_m) was measured in diurnal time using a saturation-pulse fluorometer *PAM-2000* (*Walz*, Effeltrich, Germany). All measurements were carried out on first pair of unifoliate, fully expanded leaves. The upper surface of leaf was clipped to measure the maximum photochemical efficiency of PSII. Light intensity, temperature, CO_2 concentration, and relative humidity were maintained at 1,000 µmol(photon) $m^{-2}\ s^{-1}$, 25°C, 580 µmol mol^{-1}, and 80%, respectively.

Net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s) were measured on sunny days at 11:00 h using the youngest fully expanded randomly selected leaves from five replicates of vetiver by infrared gas analyzer (IRGA) portable photosynthetic system (*LI-COR 6400*, *LI-COR* Lincoln, Nebraska, USA) at 300 DAT. Before recording the measurements, IRGA was calibrated and zero was adjusted approximately every 30 min during the measurement period. All the above mentioned parameters were recorded three times for each treatment and air temperature, relative humidity, CO_2 concentration, and PPFD were maintained at 25°C, 85%, 600 µmol mol^{-1}, and 800 µmol $mol^{-2}\ s^{-1}$, respectively.

Yield and quality parameters

Essential oil (EO) content: Vetiver EO was extracted from roots through hydro-distillation method using Clevenger's apparatus (*Borosil*, India) and then quantified gravimetrically according to Guenther (1972). Fresh roots (200 g) were chopped into small pieces. EO was extracted by distillation of roots for 10 h. The extracted oil was dried using anhydrous sodium sulphate and subsequently preserved in sealed glass vials at 4°C for the gas chromatography analysis of the EO. The amount of EO obtained from the plant material (roots) was calculated as:

EO content (%, v/w) = (observed volume of oil [mL]/ mass of sample [g]) × 100

Gas chromatography (GC) analysis: The active constituent (khusimol content) of EO was determined using gas chromatography apparatus [*GC System 7890B*, *Agilent*, USA] equipped with a capillary column *HP5* (coated with polyimide and fused silica) of the size 30 m × 0.320 mm, flame ionization detector, and an injector. Nitrogen was used as the carrier gas. GC temperature schedule was as follows: detector temperature, 300°C; oven temperature, 250°C; injector temperature, 250°C. The sample size was 0.2 µL invariably. Initial temperature was 100°C with a hold time of 20 min; it was increased to 270°C at the rate of 4°C per min. Identification of the khusimol was based on retention time. It was quantified as the percent content comparing the experimental peaks with the peaks obtained from the reference standard reported in the literature (Adams 2007).

Statistical analysis: Each pot was treated as one replicate and all the treatments were repeated five times. The data were analyzed statistically using *SPSS-22* statistical software (*SPSS Inc*., Chicago, IL, USA). Data means were compared using *Duncan*'s multiple range test (DMRT) at $p<0.05$. Standard error (± SE) was also employed to separate the means in the tables and figures.

Results

TiO_2NPs are nontoxic, white colored with specific high surface area. TiO_2NPs are popular commercially as the suitable catalyst for photocatalytic degradations (Gupta and Tripathi 2011). The text table enlists all the important physico-chemical data of TiO_2NPs including average primary particle size which is estimated to be 14 nm (Fig. 1).

Among various TiO_2NPs concentrations, 90 mg L^{-1}

proved to be the optimum that maximally increased the values of most of the parameters in comparison with the control.

Growth attributes: Foliar application of varying doses of TiO_2NPs resulted in significant improvement in the growth attributes as compared with control at 300 DAT. Compared with the control (water spray), foliar application of TiO_2NPs at 90 mg L^{-1} enhanced shoot FM and DM by 25.8 and 27.9%, respectively, and root FM and DM by 24.8 and 28.1% respectively (Table 1).

Physiological and biochemical parameters: Foliar application of TiO_2NPs showed a positive effect on total leaf Chl content of vetiver. Out of various TiO_2NPs concentrations, 90 mg L^{-1} increased total Chl content by 27.2% at 300 DAT, compared with that of control (Table 2). Activities of CA and NR also increased significantly with increasing TiO_2NPs concentrations. TiO_2NPs applied at 90 mg L^{-1} increased activities of CA and NR by 23.9 and 25.2%, respectively, over the control (Table 2). It also increased the F_v/F_m by 23.5% over the control (Table 2). Foliar application of TiO_2NPs also resulted in significant increment in P_N, C_i, and g_s of the plants. TiO_2NPs applied at 90 mg L^{-1} exceeded the control with regard to P_N, C_i and g_s by 27.5, 24.8, and 26.2%, respectively (Table 2).

Yield and quality parameters: A significant enhancement of the EO and khusimol content due to TiO_2NPs application, as compared with control, was observed in the present study. TiO_2NPs applied at 90 mg L^{-1} showed an increase of 23.6 and 24.5% in EO and khusimol content, respectively (Fig. 2). Likewise, EO and khusimol yield of vetiver, recorded at 300 DAT, was noticeably affected by the foliar application of various concentrations of TiO_2NPs. As compared with the control, TiO_2NPs application at 90 mg L^{-1} significantly enhanced the yield of EO and khusimol by 55.1 and 93.1% at 300 DAT, respectively (Fig. 2).

Discussion

The growth of plants is critically determined by different exogenous and endogenous factors (plant growth promoters, elicitors) through establishment of strong source–sink relationship leading to better supply of nutrients and hence, better cell metabolism (Patel and Golakia 1988). At lower concentrations, titanium is known to increase plant biomass (Pais 1993), nutrient content (Giménez *et al*. 1990), content of photosynthetic pigments (Carvajal *et al*. 1994), *etc*. In order to exploit the beneficial effects of this element, it is added to various micronutrient fertilizer complexes. However, the application of elements as nanoparticles (NPs) may be a suitable and promising fertilization method because of their peculiar physicochemical characteristics compared to other chemical forms. The efficacy of NPs depends on their concentration and varies from plant to plant (Singh *et al.* 2015) and is determined by their chemical composition, size, surface

Table 1. Effect of foliar spray of titanium dioxide nanoparticles (TiO_2NPs) on growth attributes of vetiver recorded at 300 DAT. Values represent the mean of five replicates ± standard error. Values of means within row followed by *the same letter(s)* are not significantly different according to DMRT ($p \leq 0.05$). FM – fresh mass, DM – dry mass.

Parameter	TiO_2 [mg L^{-1}]					
	0	30	60	90	120	150
Shoot FM [g]	348.7 ± 5.20^{e}	373.6 ± 5.21^{d}	393.5 ± 4.80^{c}	438.8 ± 5.34^{a}	421.2 ± 3.87^{b}	405.9 ± 4.50^{c}
Shoot DM [g]	102.7 ± 1.24^{e}	110.7 ± 1.91^{d}	117.6 ± 1.28^{c}	131.4 ± 1.59^{a}	124.6 ± 1.33^{b}	121.3 ± 1.25^{bc}
Root FM [g]	210.9 ± 3.30^{e}	224.5 ± 3.23^{d}	234.7 ± 2.63^{c}	263.2 ± 3.25^{a}	252.4 ± 2.70^{b}	243.6 ± 2.55^{bc}
Root DM [g]	61.2 ± 0.85^{f}	65.6 ± 0.53^{e}	68.9 ± 0.56^{d}	78.4 ± 0.51^{a}	74.7 ± 1.01^{b}	71.8 ± 0.58^{c}

Table 2. Effect of foliar spray of titanium dioxide nanoparticles (TiO_2NPs) on physiological and biochemical parameters of vetiver recorded at 300 DAT. Values represent the mean of five replicates ± standard error. Values of means within a row followed by *the same letter(s)* are not significantly different according to DMRT ($p \leq 0.05$).

Parameter	TiO_2NPs [mg L^{-1}]					
	0	30	60	90	120	150
Total Chl [mg g^{-1}(FM)]	1.84 ± 0.014^{f}	1.98 ± 0.023^{e}	2.08 ± 0.017^{d}	2.34 ± 0.024^{a}	2.26 ± 0.012^{b}	2.18 ± 0.020^{c}
NR activity [nM(NO_2) g^{-1}(FM) h^{-1}]	321.4 ± 4.69^{e}	342.6 ± 3.20^{d}	351.7 ± 2.80^{d}	398.3 ± 4.56^{a}	376.8 ±3.72^{b}	364.5 ± 2.29^{c}
CA activity [µM(CO_2) kg^{-1}(leaf FM) s^{-1}]	233.4 ± 2.65^{f}	251.5 ± 2.71^{e}	264.9 ± 2.51^{d}	292.3 ± 2.71^{a}	281.8 ± 2.34^{b}	273.6 ± 2.10^{c}
F_v/F_m	0.667 ± 0.008^{f}	0.705 ± 0.006^{e}	0.743 ± 0.007^{d}	0.824 ± 0.009^{a}	0.793 ±0.005^{b}	0.768 ± 0.007^{c}
P_N [µmol(CO_2) m^{-2} s^{-1}]	13.90 ± 0.2^{f}	14.79 ± 0.17^{e}	15.48 ± 0.23^{d}	17.72 ± 0.25^{a}	16.86 ± 0.30^{b}	16.21 ± 0.36^{c}
C_i [ppm]	271.20 ± 3.75^{f}	289.74 ± 3.18^{e}	303.38 ± 3.39^{d}	338.50 ± 3.83^{a}	326.60 ± 2.62^{b}	315.60 ± 2.87^{c}
g_s [µmol(CO_2) m^{-2} s^{-1}]	0.221± 0.006^{d}	0.235 ± 0.005^{c}	0.246 ± 0.006^{c}	0.279 ± 0.006^{a}	0.269 ± 0.005^{ab}	0.258 ± 0.005^{b}

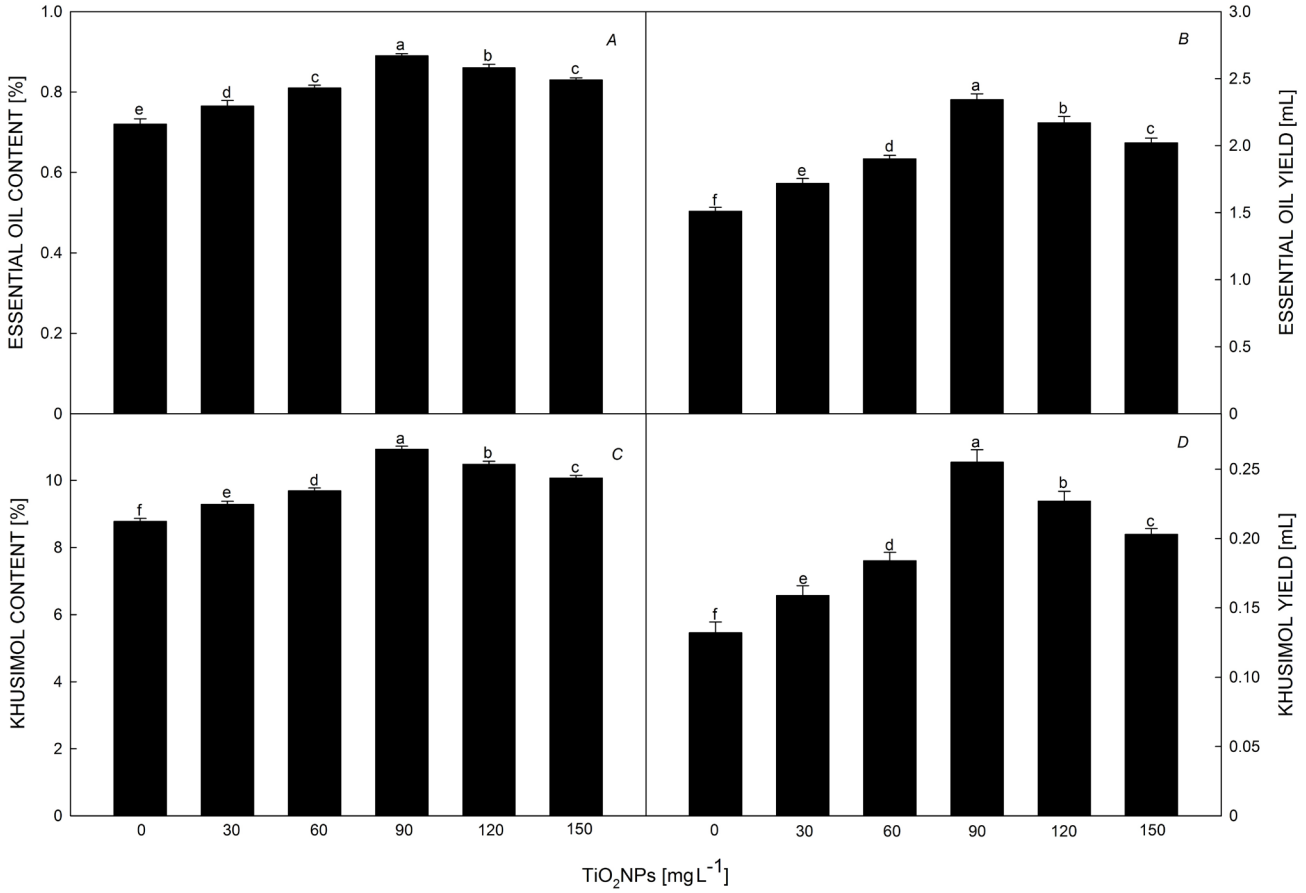

Fig. 2. Effect of six concentrations of foliar sprays of TiO_2NPs [0 (control), 30, 60, 90, 120, and 150 mg(TiO_2NPs) L^{-1}] on essential oil content (*A*), essential oil yield per plant (*B*), khusimol content (*C*), khusimol yield per plant (*D*) of vetiver (*Vetiveria zizanioides* L. Nash) at 300 DAT. Means within a column followed by *the same letter(s)* are not significantly different ($p \leq 0.05$). Error bars (┬) show SE.

area, reactivity (Khodakovskaya *et al.* 2009). NPs have been observed to act as elicitors (Morteza *et al.* 2015). In this regard, the efficiency of NPs, being concentration dependent, depends on the ligand (NPs) concentration required to evoke an effective response the plant cells for the stimulation of a particular signaling pathway. The agrochemical sprays of different fertilizers, nanoparticles, elicitors, *etc*., to aerial parts of the plants has proven to be a successful agricultural practice worldwide (Fernández and Brown 2013, Morteza *et al*. 2015). Moreover, absorption and translocation of metal oxide nanoparticles in different parts of the plants depend on their bioavailability, concentration, solubility, and exposure time (Siddiqi and Husen 2017). Foliar-sprayed beneficial elements may be absorbed by the foliage *via* the cuticle, cuticular irregularities, stomata, and other epidermal structures such as trichomes (Fernández and Brown 2013). Though, the mechanism of penetration of aqueous solution of NPs is not completely understood, the entry into the plant cells is well reported. Singh *et al.* (2015) suggested that NPs accumulated in the plant cells are transported by apoplast or symplast through plasmodesmata. However, further study needs to be conducted to explore the exact mechanisms by which plants absorb NPs. Our results showed exemplary effect of lower concentrations of TiO_2NPs on growth characteristics, physiological attributes, and EO content and yield in vetiver. Values of most of the parameters increased at 90 mg L^{-1}, however, at increasing concentrations of TiO_2NPs beyond 90 mg L^{-1}, a progressive decrease in the values was observed; nevertheless, the values were still greater in comparison with the control. A significant increase in the values of growth parameters was observed over control by the application of 90 mg L^{-1} TiO_2NPs (Table 1). Moaveni *et al.* (2011) reported an improvement in growth characteristics in response to exogenous application of TiO_2NPs in *Calendula officinalis* L., while Morteza *et al.* (2013) observed similar results in maize, Khater and Osman (2015) in fennel, and Jiang *et al.* (2017) in wheat. Physiological and biochemical parameters also responded effectively to application of TiO_2NPs (Table 2). TiO_2NPs of 90 mg L^{-1} proved to be optimum for total Chl content and NR and CA activity (Table 2). Significant increase in the photosynthetic pigments (Chl *a*, Chl *b*, total Chl content, carotenoids, and anthocyanin contents) of maize has been reported after the foliar application of TiO_2NPs by Morteza *et al*. (2013). Nitrogen metabolism is known to be regulated by TiO_2NPs directly by regulating the synthesis and activation of the key enzymes, such as glutamine synthase, glutamate dehydrogenase, glutamate-pyruvate transaminase, and more importantly nitrate reductase itself, facilitating the absorption of nitrate to plants (Yang *et al.* 2006). These enzymes are important as

they promote the biosynthesis of Chl, amino acids, proteins (enzyme), nucleic acid, *etc*. (Buchanan *et al.* 2002, Wu 2003). Moreover, the quality and yield of the crops are affected by the activities of these enzymes (Ji *et al.* 2001, Buchanan *et al.* 2002, Yang and Gao 2002, Wu 2003). As observed in present study, TiO_2NPs increased the activity of important enzymes such as NR and CA. TiO_2NPs-mediated increase in the NR activity has been reported by Yang *et al.* (2006) in spinach and by Lu *et al.* (2002) in soybean. Castiglione and Cremonini (2009) have observed increased growth, dry mass, photosynthetic pigments, and photosynthetic rate by the application of TiO_2NPs. The increased Chl content is believed to have a positive effect on photosynthesis resulting in increased FM and DM of the plant (Hong *et al.* 2005a, Yang *et al*. 2006, Mishra *et al*. 2014). This favorable effect on photosynthesis can also be a result of increased activity of enzymes involved in the process. Previously, enhanced activity of key carbon fixation enzyme, Rubisco, in spinach was also well-documented (Zheng *et al*. 2005, Yang *et al*. 2006, Gao *et al*. 2006). TiO_2NPs are also known to influence positively the Hill reaction and stimulate chloroplast activity which in turn improve ferrodoxin-cytochrome reduction and facilitate increased cyclic photophosphorylation (Hong *et al*. 2005a). As suggested by Hong *et al*. (2005b) and Zheng *et al*. (2007), interactions of nanoparticles with the chloroplasts speeds up electron transport and oxygen evolution. Giraldo *et al.* (2014) reported that single-walled carbon nano-tubes were passively transported within the lipid envelope of extracted plant chloroplasts and were irreversibly localized in it, increased the photosynthetic activity up to three times than that of control and maximally enhanced electron transport rates. Moreover, the process of photosynthesis is inhibited in N-deficient environment and application of TiO_2NPs have been observed to aid in the process by increasing the nutrient availability to the plant as observed by Yang *et al*. (2006) in case of spinach. Our finding can be corroborated with those of Hong *et al*. (2005a,b), Yang *et al*. (2006), Castiglione and Cremonini (2009), Morteza *et al*. (2013), who have reported increase in growth attributes (dry mass), enzyme activities (NR, Rubisco), activity of chloroplasts (efficient light absorption, photosynthetic rate), and decrease in Chl degradation resulting in an improved assimilation of fixed CO_2 and in turn an enhanced yield of the plants.

EOs are directly as well as indirectly linked to the process of photosynthesis as they are synthesized from carbohydrate precursors. Increased growth, enzyme activities, nutritional status, and photosynthesis, as also evident from our study, is a key reason for proficient assimilation, efficient translocation, and partitioning of photosynthates, resulting in better growth of the plant and increased biosynthesis of carbohydrates. Increased carbohydrate contents and their potential diversion to

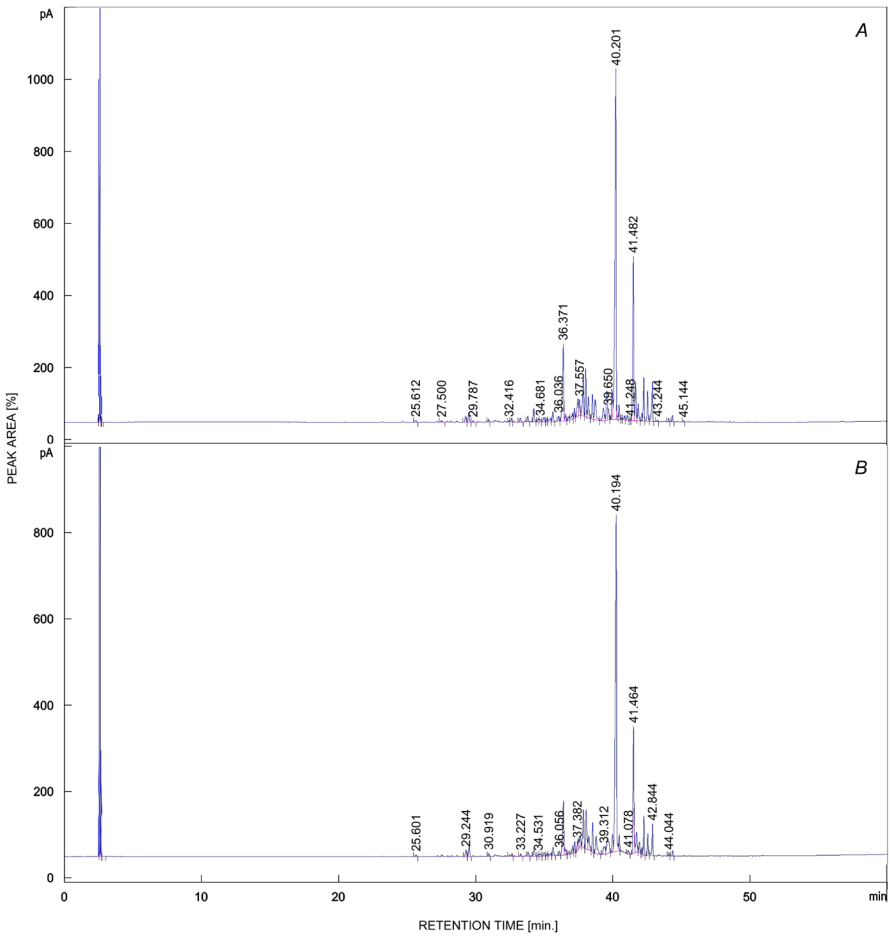

Fig. 3. (*A*) GC chromatogram of EO obtained from vetiver treated with TiO_2NPs at 90 mg L^{-1}. The khusimol content in the EO was 10.9%. Therefore, TiO_2NPs treatment increased the khusimol content by 24.5% as compared with the control. (*B*) GC chromatogram of EO obtained from vetiver control plants (deionised-water spray treatment). The khusimol content in the EO was 8.8%.

secondary metabolism might contribute to the elevated contents of EO in plants (Swamy and Rao 2009). The increase of 23.6% was found in the EO content after the application of 90 mg(TiO_2NPs) L^{-1} in comparison with the control (Fig. 2*A*). Maximum increase of 55.1% in EO yield of vetiver was reported at 90 mg L^{-1} followed by 120 mg(TiO_2NPs) L^{-1}, which exhibited an increase of 43.7% in comparison with control (Fig. 2*B*). Improved quality and yield of crop by foliar application of TiO_2NPs has been previously reported by Khader and Osman (2015) and Morteza *et al.* (2015). Further, TiO_2NPs are believed to have a positive effect on the expression of some important enzymes involved in terpene biosynthetic pathway. Additionally, elicitor exposure may lead to signaling of jasmonic acid and its methyl ester in plants (Doares *et al*. 1995); the latter has been found to be associated with the increased secondary metabolites (Walker *et al*. 2002). Thus, increased EO production may also be attributed to the elicitor effect of TiO_2NPs through jasmonic acid and its methyl-ester signaling, in addition to the improved carbohydrate biosynthesis as discussed above. As per GC reports, significant increase over water-sprayed control was reported in the active constituent (khusimol) of vetiver by application of TiO_2NPs (Fig. 3). This increase (Fig. 2*C*,*D* and 3) is perhaps because of increased expression of the enzyme involved in the biosynthesis of this sesquiterpene. According to Schalk and Deguerry (2013), khusimol is the oxidation product of zizaene which is apparently synthesized from farnesyl pyrophosphate (FPP) by the activity of zizaene synthase. As per the results of yield attributes shown in the GC chromatograms (Fig. 3*A,B*), we may suppose that TiO_2NPs may have some stimulatory role in the biosynthetic pathway mainly at two steps: one at conversion of zizaene from FPP by zizaene synthase and another at oxidation of zizaene into khusimol by cytochrome P_{450} reductase.

Conclusion: The present study indicated elicitor effect of TiO_2NPs which led to significant improvement in some of the important photosynthetic parameters, enzyme activities, and yield attributes of vetiver. The content of EO as well as its quality were positively influenced as percentage of the key active constituent (khusimol) was significantly enhanced. Furthermore, the nanoparticles were observed to be effective in a very small quantity and employing them in the form of foliar sprays can be used as a suitable alternative strategy to raise the production of high-value agricultural crops bearing EOs.

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