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Photocatalytic Effect of TiO_2 Nanoparticles on Essential Oil of *Rosmarinus Officinalis*

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Abstract

In this study, the effect of TiO2 nanoparticles (NP) was evaluated on the essential oil of rosemary plant (*Rosmarinus officinalis*). The experimental treatment included spraying seven doses of TiO_2 NPs (0 (control), 20, 40, 60, 100, 200, and 400 ppm) on the rosemary leaf. The results indicated that the use of TiO_2 NPs significantly affected the quantity of rosemary essential oil. The peak area for most of the compounds increased with the application of TiO_2 NPs. However, at high concentrations of TiO_2 NPs (more than 200 ppm), the peak area decreased. The peak area of some compounds, such as myrecene, 2-butenal, 2-ethenyl-, 3-pinanone, isoborneol, and β-terpineol was observed in different conditions, and a significant increase in concentration was observed in the groups that received 60 or 100 ppm TiO_2 dose. This study was the first to evaluate the favorable effects of TiO_2 NPs on the essential oil of a highly valuable medicinal plant.

Keywords: Rosmarinus Officinalis, Titanium Dioxide, Nanoparticles, Essential Oil

Introduction

Agriculture plays an important role of the entire national economy (Said.et al., 2017), and fertilizers are natural or synthetic materials applied to soils or plant tissues to increase plant growth and provide one or more essential nutrients for the plant. It has been perceived that yields of many crops began to decline due to the excessive fertilization and the loss in soil organic matter (Hagab, et al., 2018). Therefore, exploitation of novel materials is required to increase the crop yield.

Nanotechnology is the accepted name for the production and utili zation of functional structures with at least one nanometer dimension characteristic (Jain and Jain, 2017).

Nanomaterials and nanotechnology can be employed for plant nutrition. Nanomaterials and nanotechnology can improve crop yield. The use of nanotechnology in agriculture has been rapidly gaining acceptance worldwide. For instance, nanometarials have been used to increase the efficiency of fertilizers' application in various plants.

Titanium dioxide (TiO_2) is mainly used as a pigment and bulk material. The global production of TiO_2 for all the uses reaches millions of tons per year, and nearly 70% of all TiO_2 produced is used as pigments in paints; TiO_2 is also used in pharmaceuticals, cosmetic industry medical applications, antimicrobial applications, energy storage, and catalysts for air and water purification (Weir et al., 2012).

Theoretical and technological assessment of the properties of nanoparticles (NPs) are required for their application in plants, and their physiological and molecular responses should also be investigated in every plant (Mutlu et al., 2018). The effect of NPs on plants has attracted considerable interest among the researchers in plant science (Mehmood and Murtaza, 2016).

Titanium dioxide NP is one of the most broadly used nano materials, but its interaction with plants has not been well investigated (Mutlu et al., 2018).

The effects of TiO_2 NPs on animals has been investigated to a greater extent than those on plants (Demir et al. , 2017). Some studies have shown that TiO_2 NPs exert positive and stimulating effects on plants (Ghosh et al., 2010), and particularly improved root and shoot growth increments, (Feizi et al., 2013; Asli and Neumann, 2009; Feizi et al., 2012; Azimi et al., 2013; Haghighi and da Silva, 2014). Nevertheless, the negative effects have also been reported, particularly genotoxicity (Pakrashi et al., 2014; Castiglione et al., 2011) and germination inhibition (Hong et al., 2005). Some reports have discussed the effect of nano TiO_2 on the photochemical reaction in some plants such as spinach (Gao et al., 2006; Hong et al., 2005; Yang et al., 2006), and Lemna minor (Song et al., 2012).

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Although, plant essential oils have been widely used as a medicinal and aromatic plant since the ancient times (Ojewumi,et al, 2018), there have not been any reports on the effects of NPs on the quality and quantity of essential oils. In this work, the effect of spraying different concentrations of TiO_2 NPs on the quantity and quality of essential oil of *Rosmarinus officinalis* was investigated.
Rosemary plant is cultivated for its aromatic oil, and "rosemary oil," is obtained by steam distillation of the fresh leaves and flowering tops of the plant. Its essential oil is colorless or pale yellow oil with the characteristic fragrance of rosemary, and a warm camphoraceous taste (Al-Sereiti et al., 1999).

The fresh and dried leaves of rosemary are both used as ingredients of various fool products, or consumed in small amounts as herbal tea because of the plant's characteristic aroma. And rosemary extracts are frequently used as natural antioxidants because they improve the shelf lives of perishable foods.

Rosemary has significant pharmacological effects, such as antibacterial (Bozin et al., 2007), anticancer (Cheung and Tai, 2007; Inatani et al., 1983), and antidiabetic (Bakırel et al., 2008) effects.

The other major use of rosemary is in the perfumery industry, where the essential oils are used as the natural ingredients of perfumes.

Owing to the high importance of this plant, the photocatalytic effect of TiO_2 NPs on the essential oil of rosemary was investigated under controlled conditions in this study.

Materials and Methods

This study was conducted in the Laboratory of Islamic Azad University of Damghan, Iran in 2017 using a completely randomized design with three replications.

Rosemary plant samples were prepared and transferred to perlite and pit moss (50 to 50) pots (21 pots, each containing three rosemary plants) and placed in a growth chamber at 25 °C under photoperiod, namely, 18 h light and six h darkness for 10 days to grow and take roots. During the deployment, light watering was done on the first day and every two days, thereafter, the nutrition was fed combined with the ratio of 50 to 50 with Hoagland and water.

Titanium dioxide nanoparticles with an average size of 30 nm and high chemical purity of~99.99% were purchased from US Nano Company. It was applied as suspension. The experimental treatment included 7 levels of TiO_2 nanoparticles (0, 20, 40, 60, 100, 200, and 400 ppm).

After the deployment, the treatment was performed on the eleventh day, and TiO_2 nanoparticles were sprayed (at the determined concentrations) on the leaves of all the plants. The next step of treatment was done one week later, whereas the last step was done two weeks later (Fig. 2).

At the end of the experiment, fresh aerial parts were dried in the shade and at room temperature before the extraction of the essential oils by hydro distillation.

Hydrodistillation

Conventional hydrodistillation was carried out using a commercially available circulatory Clevenger apparatus. Accordingly, 50-g portions of the dried rosemary leaves were exactly weighed and immersed in water in the ratio of 1:10, followed by collecting the volatile oils at sequential times. The distillation period was 30 minutes.

Gas chromatography and gas chromatography–mass spectrometry

Quantitative and qualitative evaluations of the oils were performed by means of GC and GC-MS instruments. The GC analyses were done on a Varian (CP 3800) gas chromatograph which had a split/splitless (10:1) injector (280 °C), and a flame ionization detector (250 °C) N_2 was applied as the carrier gas (0.8 mL/min). CP-Sil 5 CB (30 m × 0.25 mm × 0.25 µm film thickness) was used as a the capillary column. The oven temperature was kept at 60 °C for 3 min, then heated to 220 °C at a rate of 6 °C min^{-1}, and finally kept for 3 min. Quantitative data were obtained through system area percentage.

GC-MS determinations were performed on an HP-6890 GC system coupled with a 5973 network mass selective detector, and equipped with an HP-5MS capillary fused silica column (30 m × 0.25 mm I.D. × 0.32 µm film thickness). The operating conditions were the same as described above, but the carrier gas was He with a flow rate of 0.8 mL/min. The mass spectra were taken at 70 eV, and were recorded over the m/z range 20-500 amu. All the chromatographic measurements were carried out in triplicate, and the mean of the retention times and percentage compositions of each component were taken into consideration. Duplicate times were discarded if they differed by more than 1 s, and the experiments were repeated again in duplicate.

Surface morphology

The surface of the sprayed samples with TiO_2 nanoparticles was analyzed with a scanning electron microscopy (SEM) system (model VEGA, TESCAN, Brno, Czech Republic). This microscopy system was equipped with a field-emission gun through a previously described protocol. The nanoparticle distribution on pistachio hulls and the surface morphologies were investigated (Vaezi et al., 2008).

Statistical Analysis

All the statistical operations were done using SPSS 24th version. For the analysis of variance (ANOVA), $p < 0.05$ was considered significant.

Results

Effect of different concentrations of TiO_2 NPs on the extraction yield of R. officinal essential oil

The effects of different concentration of TiO_2 NPs on the extraction yield were investigated. Different concentrations of TiO_2 NPs were sprayed (0, 20, 40, 60, 100, 200, and 400 ppm) on the leaves of all the plants.

The results showed that the extraction yield after adding TiO_2 NPs was increased. Nonetheless, the percentage of the extraction yield showed no significant increase at more than 200 ppm of TiO_2 NPs. The results are tabulated in Table 1.

Table 1- Effect of TiO_2 nanoparticles on the percent of the extraction yield of *Rosmarinus officinalis* essential oil using HD technique

TiO_2 nanoparticles	20	40	60	100	200	400
Yield (%)	1.7 [a]	1.9[b]	2,2[c]	2.7[d]	2.76[d]	2.6[d]

Different superscript letters in a row indicate significant difference (p=0.05)

Identification and quantification of essential oil compounds

In this study, the determination and characterization of the oil constituents were performed through the following steps:

i. The comparison of their mass spectral fragmentation patterns regarding authentic samples and linear retention indices relative to C_9-C_{21} *n*-alkanes with those provided in previously published papers was done ;
ii. The data were stored in Wiley 275 MS library; and
iii. The fragmentation pattern was matched with that of the National Institute of Standards

and Technology Mass Spectra Library package with a resemblance percentage above 85%. The relative percentage amounts of the components were directly assessed from the peak area using an HP-6890 GC system on the HP-5MS column, considering the sum of all eluted peaks as 100% without using the correction factor. Similar volumes of each essence were separately analyzed by GC-MS, and the respective results are shown in Table 2, where the constituents are grouped based on the order of their elution from an HP-5MS column.

Through the HD method (Table 2), 49 components were identified in the oil from the *Rosmarinus officinalis.*

Table 2- The components of the essential oils from *rosemary*

				0	20	40	60	100	200	400
1	Tricyclene	15.5517	932	0.082		0.106	0.083		0.087	
2	α pinene	16.0217	936	11.163	2.409	14.076	11.085	10.693	12.252	11.732
3	Camphene	16.6537	947	2.797	0.123	5.096	3.816	3.281	4.535	2.963
4	Cyclobutene, 2-propenylidene	16.7456	954	0.103	0.466		3.816			0.144
5	β-Pinene	17.6315	973	0.578	0.904	0.548		0.294		0.319
6	3-Octanone	17.8098	964	1.326	1.487	6.177	0.435	2.025	4.781	1.847
7	Myrecene	17.9016	982	2.029	0.134		0.127	0.123	0.153	1.293
8	1-Octen-3-ol	17.9772	985	0.165	0.167	0.152	3.471			
9	3-Octanol	18.485	989	0.163	0.509	0.266		0.097	0.234	0.111
10	a-Thujene	18.6363	930	0.099	3.579	0.17	0.155	0.174	0.186	0.215
11	Limonene	19.5222	1023	5.499	0.329	13.958	0.186	8.587	11.054	7.861
12	1,8-Cineole	19.614	1022	9.286	0.341	5.969	9.963	4.37	3.677	6.758
13	g-Terpinene	20.4027	1246	0.434	1.038	0.26	5.509	0.3	0.321	0.365
14	Phenol, 4-(1-methylpropyl)-	20.6728	1050	0.097		0.095	0.328	0.077	0.0889	0.42
15	Terpinolene	21.3265	1052	0.491	3.7		0.083	0.375	0.332	
16	FILIFOLONE	21.8559	1083	0.876	0.163	0.864	0.31	0.611	0.734	0.797
17	Linalool	22.0611	1091	3.794	0.129	2.165	0.675	2.757	3.085	3.087
18	2-Butenal, 2-ethenyl-	22.261	1120	0.119		0.072	0.202			0.18
19	Alloocimene	22.6553	1125	0.19	12.787	0.192	1.966	0.159	0.177	0.107
20	Fenchone	22.9795	1135	0.091	1.169	0.071	0.159	0.08		14.909
21	2-Bornanone	23.7087	1145	12.375	0.172	13.763	12.882	14.848	13.66	1.025
22	3-Pinanone	24.0274	1172	1.121	2.015	1.033	0.927	0.869	0.828	0.269
23	2,3-Hexadiene, 2-methyl-	24.4218	1179	0.147	20.674	0.15	0.086	0.088	0.13	1.293
24	3-Pinanone , (1.alpha.,2.beta.,5.alpha.)-	24.5568	1183	2.028	0.235	1.656	1.621	1.437	14.266	18.705
25	endo-Borneol	24.8269	1186	15.962	0.471	12.041	11.14	12.368		
26	β-Terpineol	25.3725	1193			0.126		0.246		2.294
27	2-Pinen-4-one,	25.3671	1201	1.651	2.522	1.573	3.485	3.426	2.532	1.359
28	a-Terpineol	25.4481	1209		0.122	2.27	3.729	4.099	3.674	3.499
29	Verbenone	25.6264	1202	3.282	0.234	4.806	3.628	4.514	3.375	
30	trans-3-Caren-2-ol	26.3125	1210	0.121	1.206	0.153	0.162	0.17	0.157	0.218
31	Carvone	26.6096	1218	0.223	1.24	0.153	0.181	0.203	0.168	

32	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	26.8041	1224	0.98	11.382					1.087
33	2-Butenal, 2-ethenyl-	27.0417	1252	0.963	0.233	0.65	1.152	1.212	0.28	1.312
34	Bicyclo[5.1.0]oct-3-ene	27.1173	1266			0.902	1.446	1.624	1.321	8.949
35	Bornyl acetate	27.5224	1283	7.885	0.363	6.87	7.791	10.634	10.022	0.236
36	Cyclohexene, 1-methyl-4-(1-methylethylidene)	28.8244	1293	0.161	0.272	0.134	2.685	0.245	0.184	0.238
37	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-	29.4348	1300	0.193	0.449	0.292	0.221	0.278	0.274	0.262
38	Myrcenylacetat	29.7967	1326	0.217	0.292	0.135	0.28	0.319	0.233	0.383
39	2,2-dimethyl-3-methylene-bicycloheptane	30.0074	1330	0.378	4.216	0.185	0.415	0.453	0.378	0.271
40	Valerenol	30.7745	1401	0.292	0.507	0.294	0.304	0.293	0.28	2.379
41	Caryophyllene	31.5956	1405	3.536	0.563	0.963	1.876	2.247	1.965	0.204
42	Geranylacetone	31.9035	1453	0.443	1.413	0.073	0.202	0.235	0.167	0.267
43	.alpha.-Humulene	32.5463	1460	3.536	0.276	0.095	0.201	0.259	0.19	0.573
44	Caryophyllene oxide	35.9118	1580	0.443	0.249	0.275	0.561	0.472	0.386	
45	β-copaene	36.4412	1585	0.434	0.127		0.105	0.099		0.112
46	1-Cyclooctene-1-carboxaldehyde	36.5978	1594	0.772	0.753		0.087	0.081		0.104
47	Caryophylladienol	37.3757	1624	0.172	0.192	0.203	0.254	0.335	0.244	0.316
48	Caryophyllene oxide	37.7863	1657	0.16	0.621	0.592	0.08	0.946	0.69	1.015
49	.alpha.-Bisabolol	38.0185	1668	0.641	0.103	0.139	0.069	0.239	0.169	0.299

The major components in the volatile oil of the *R. officinalis* were α pinene, camphene, 1,8-cineole, linalool, endo-borneol, verbenone, bornyl acetate, and caryophyllene.

No change in the chemical profiles and percentages of ingredients in the *R. officinalis* essential oil was obtained in rosemary plants that received TiO_2 NPs. The peak areas of chemical compounds of the extracted essential oils revealed that the peak areas for most compounds increased with the application of TiO_2 nano particles. However, at high concentrations of TiO_2 NPs (more than 200 ppm), a decrease in the peak area was observed, and TiO_2 had the opposite effect on the quantity of chemical compounds in *R. officinalis* essential oil.

However, some compounds, such as myrecene, 2-butenal, 2-ethenyl-, 3-pinanone, isoborneol, and β-terpineol had different conditions, and significant increases in their concentrations were observed in plants sprayed with 60 or 100 ppm TiO_2 NPs. The comparative diagrams of some compounds are shown in Figures 1-3.

Figs. 1–3 show the peak area of the α pinene, caryophyllene, 3-pinanone in different concentrations of TiO_2 NPs.

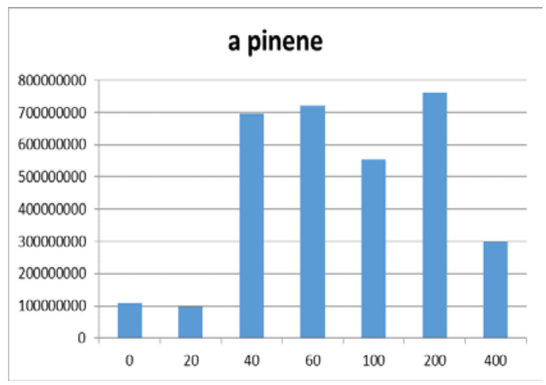

Fig. 1: Peak area of the α pinene in different concentrations of TiO_2 NPs.

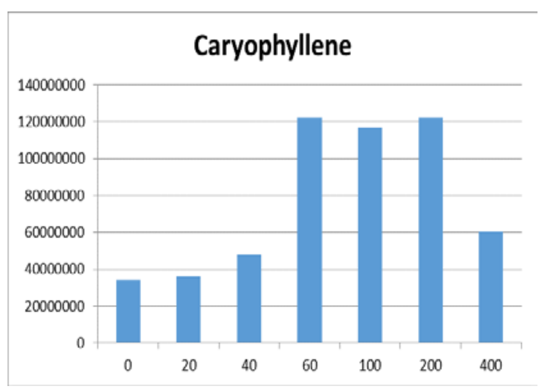

Fig. 2: Peak area of the caryophyllene in different concentrations of TiO_2 NPs.

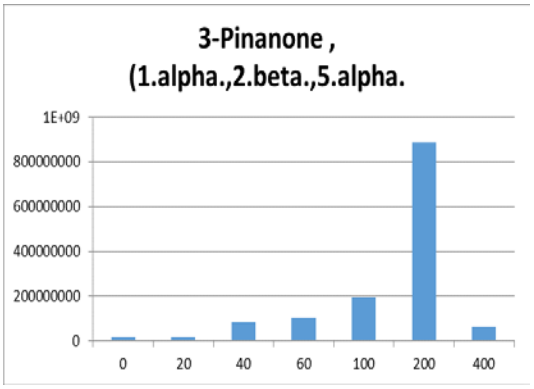

Fig. 3: Peak area of the 3-pinanone in different concentrations of TiO_2 NPs.

After comparing the peak areas of α pinene, caryophyllene, and other compounds in the essential oil, it was found that the amount of the compounds increased when the concentrations of TiO_2 NPs were increased and reached maximum values at 200 ppm TiO_2 NPs before decreasing significantly, as shown in Figs.1–2. This condition was also observed in the most chemical compounds in the essential oils extracted from plants sprayed with TiO_2 NPs.

The high peak areas for myrecene, 2-butenal, 2-ethenyl-, 3-pinanone, isoborneol, and β-terpineol, were observed in the medium concentration of TiO_2, which could be caused by the photocatalytic properties of nano TiO_2.

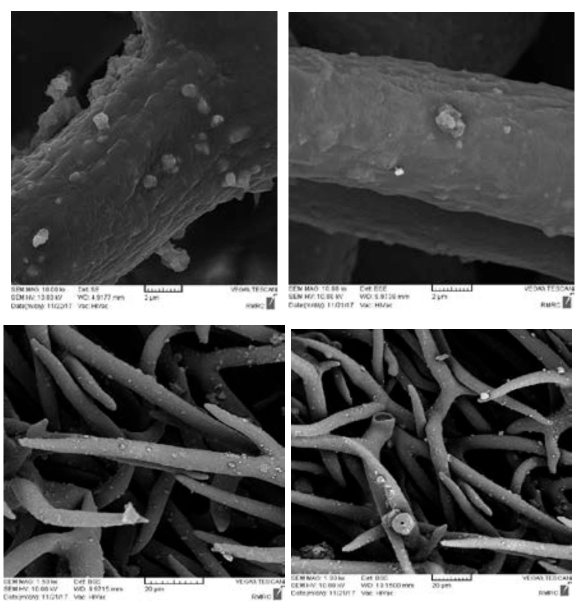

Fig. 4. SEM image of rosemary sprayed with TiO_2 nanoparticles (400 ppm)

Table 3- Surface analysis of rosemary sprayed with TiO_2 nanoparticles (400 ppm)

Element	**Percent**
Carbon	58.02
Oxygene	29.24
Silicone	1.01
Clorine	1.49
Calsium	1.02
Titaniume	9.21

Discussion and Conclusion

The results indicated that the use of TiO_2 NPs significantly affected the quantity of the essential oils of the rosemary plant. In particular, up to TiO_2 NP concentration of 200 ppm, the amount of the chemical compounds in all the essential oil samples increased.

The present research study has introduced a new approach for studying the fate of NPs in medicinal plants in terms of uptake, translocation, and alteration of metabolic pathways in a concentration-dependent manner.

TiO_2 NPs can increase the fresh and dry weights of plants by improving light absorption, and the activities of Rubisco enzymes (Mingyu, Hong, et al., 2007 a & b), thereby increasing nitrate uptake (Yang et al., 2006) and accelerating the conversion of inorganic into organic materials (Nair et al., 2010).

The positive effects of TiO_2 NPs on photosystem II and tilacoid membranes (Hong et al., 2005) have been reported as well. TiO_2 NPs stimulated cell division, increased the cell size, and stimulated the callus induction in dark conditions and may affect plant hormones (cytokinin and gibberellin) (Mandeh et al., 2012). TiO_2 NPs also increased the light absorption, accelerated the transmission and conversion of light energy, prevented decay of chloroplasts, and increased the length of the photosynthetic period of chloroplasts (Hong et al., 2005).

Furthermore, TiO_2 NPs transmitted the light energy to electrons, converted them into chemical energy, and ultimately resulted in CO_2 stabilization (Gao et al., 2006). An important property of TiO_2 NPs that made them extremely important and effective was their photocatalytic properties.

TiO_2 photocatalysts have been the mostly used in the photon decomposition of organic compounds. TiO_2 has been used as a photocatalyst in the disinfection (sterilization) of various environmental contaminants, such as organic matters, viruses, bacteria, fungi, algae, and cancer cells (Duffy et al., 2004).

After interacting with CO_2, TiO_2, was converted into harmless water and inorganic anions (Frazer, 2001). This process was attributed to the high oxidation activities of cavities and hydroxyl radicals (OH^0), which are well-known oxidizing agents. The potential oxidation of this radical was 2.8 eV.

TiO_2 NPs could increase Reactive Oxygen Species (ROS) and antioxidant anzymes in plants (Castiglione et al., 2016) and increased the activities of enzymes related to nitrogen metabolism, such as glutamate dehydrogenase, glutaminsentase and glutamate, and pyroxin transaminase. It has been also a stimulant for absorption nitrate and other organic and inorganic nitrogenous compound and finally increased the total protein and chlorophyll in plants (Ma et al., 2010). TiO_2 NPs with increased photon absorption stimulated redox reactions in plants and consequently promoted photosynthesis and prevented chloroplast aging (Ma et al., 2010; Hatami et al., 2016).

These NPs increased the activity of carboxylation of the enzyme rabisovu and promoted the growth of plants (Hong et al., 2005). Nano-antase-induced marker genes in Rubisco activase (rca) mRNA, and the enhanced protein levels and activities of Rubisco activase improved Rubisco carboxylation and the high rate of photosynthetic carbon reaction. The exogenous application of TiO_2 NPs improved the net photosynthetic rate, the conductance of water, and the transpiration rate in plants (Qi, Liu, and Li, 2013), and nano-anatase strongly promoted the entire chain electron transport, photoreduction activity of photosystem п, and O_2-evolving and photophosphorylation activity of chlorophyll under visible and ultraviolet light (Mingyu, Fashui, et al., 2007). This NP has increased photosynthesis activity, and promoted the production of intermediate sugar. Erythrose-4-phosphate was one of the intermediate sugars, thus was used as precursor in the synthesis of phenyl amine and tyrosine amino acid (Schimidt path) (Turhan and Eris, 2005). Then, phenyl alanie was converted to cinnamic acid, and phenyl alanaze catalyzed this reaction. It had an important role in flavonoid compound synthesis (Castiglione et al., 2011). TiO_2 NPs could promote the activity of Phenyl ammonilase, and finally increased the production of sugary compounds (Castiglione et al., 2011). Glyceraldehyde 3-phosphate, the main sugar in the Kelvine cycle, was used as a substrate in methyl erythritol phosphate route, which produced terpenoid compounds. Terpenoid and flavonoid compounds in rosemary were affected by TiO_2 concentration. The amount of primary metabolites increased in rosemary that received a specific amount of NP concentration.

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