

EFFECT OF TiO₂ NANOPARTICLES IN THYME UNDER REDUCED IRRIGATION CONDITIONS

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ABSTRACT

The nanotechnology is a relatively new technology that has recently entered the field of agriculture. Nanotechnology covers the integration or manipulation of individual atoms, molecules or molecular masses to a diverse array of structures allowing the production of new characteristics and traits of interest. The aim of this study was to evaluate the effects of foliar application of TiO₂ nanoparticles on quantitative traits (plant height, number of branches, dry weight of shoots and roots) and the essential oil content of thyme under different levels of field capacity. Our results showed that the application of TiO₂ nanoparticles had significant effects on thyme growth, while the essential oil concentrations not affected. These results imply that the application of TiO₂ nanoparticles in plants increase agronomic value under reduced irrigation conditions but has not different significant on essential oil.

Keywords: *Thymus vulgaris*; essence; nano dioxide titanium; nanotechnology

INTRODUCTION

Health concerns have been one of the main drivers increasing the knowledge and use of medicinal plants worldwide in folk medicine since prehistorically times (Trebichalský et al., 2015; Fazeli-Nasab and Mirzaei, 2018). Currently, economic, social, and ethical demands have shaped the use of herbal medicines. Some drugs can hardly be synthesized in the lab, or their artificial production is costly. Furthermore, the social perceptions that herbal medicines have fewer negative collateral effects on human health and reduce more concerns towards their use than artificial drugs have been installed in the human minds of modern societies (Alizadeh-Salteh et al., 2010; Omidbeigi, 2002).

The increasing consumption of medicinal plants in the last decades is a challenge as, inevitably, agronomic practices should be applied to optimize the production rates of several species under culture to fulfill the current demands on a world-wide scale (Fazeli-Nasab et al., 2017).

Medicinal plants usually need full vegetative and reproductive growth to produce enough yields of active compounds (Eftimova et al., 2018). These requirements may be critical in arid and semi-arid regions, where limited water resources for agriculture cause drought stress which can lead to reduction in the quantity and quality of the desired plant components (Khodadadi Dehkordi, 2016a; Khodadadi Dehkordi, 2016b).

Plants respond to drought stress at the physiological, cellular and molecular level. This response depends on the

genotype of the plant (Distelfeld et al., 2014), the duration and severity of water shortages (Kunrath et al., 2018) and the age and developmental stage at which drought stress was imposed (Sheoran et al., 2014).

Nanotechnology (the application of single atoms, molecules, molecular aggregates to produce new structures with different characteristics) is considered as a promising field in agricultural practices. Specifically, considerable attention has recently been devoted to the use of Nano-titanium dioxide particles in plants because of their reported growth stimulation effects (Wang et al., 2016).

In this study we evaluated the effects of titanium dioxide nanoparticles (TiO₂ nanoparticles) on growth features and the amount of essential oil in the medicinal plant thyme (*Thymus vulgaris* L.) under drought stress conditions.

Thyme (*Thymus vulgaris* L.) belong to Lamiaceae and also is a small shrub native to the Western Mediterranean basin. Its use is reputed to alleviate different diseases such as cough, sore throat, bronchitis and asthma, and it shows anti-inflammatory, antiseptic, and antispasmodic properties (Adwan et al., 2007; Lixandru et al., 2010). Essential oil is produced in all parts of the plant; however, the highest yields occurred in the flowering branches at anthesis. The essential oil shows anti-rheumatic and anti-aseptic properties, as well as antioxidant and antifungal effects (Shokri and Sharifzadeh, 2017). The essential oil components in this plant varies depending on genetic and agronomic conditions (Kouchaki, 2009; Kouchaki et al., 2008).

Scientific hypothesis

The purposes of this study were to assess whether the foliar application of TiO₂ nanoparticles stimulates plant growth, and whether it increases essential oil yield in thyme plants subjected to different levels of field capacity.

MATERIAL AND METHODS

Material

Seeds of *Thymus vulgaris* were obtained from Pakan Bazr Ishafan (Ishafan, Iran). Twenty healthy seeds were sown in 20 cm (diameter) x 30 cm (height) filled with commercial substrate. The soil samples were analyzed (Laboratory of

Soil Science, Faculty of Agriculture, and University of Zabol) to assess several physical and chemical characters (Supplementary Table 1). The contents of minerals were determined by a Varian SpectrAA-400 plus atomic absorption spectrometer. After radicle emergence and seedling establishment (plants growth at fresh air) six plants were kept per pot. Before starting the experimental work all plants were watered regularly at field capacity according to **Haghighi and Daneshmand. (2012)**. The experimental work was conducted during the 2015 summer in Sareyn (Iran; 38° 09' 05" N, 48° 04' 15" E).

Table 1 Variance analysis of quantitative traits and essential oil yield under different treatments.

Significance of effects	Items						
	Essential oil percentage	Root dry weight	Root fresh weight	Shoot dry weight	Shoot fresh weight	Number of lateral branches	Plant height
Reduced Irrigation (RI)	0.069**	9.17**	56.75*	122.88**	3185.67**	350.96**	48.29*
Titanium dioxide (T)	0.026 ^{ns}	1.9**	6.43*	16.36**	84.95**	18.22 ^{ns}	8.42**
RI * T	0.024 ^{ns}	0.78*	4.95 ^{ns}	14.42*	48.59*	0.36 ^{ns}	2.03 ^{ns}

Note: ns – non-significant, * – significant at $p < 0.5$, and ** – significant at $p < 0.01$.

Experimental design

TiO₂ nanoparticles (30nm; were obtained from US Research Nanomaterial's (US-Nano) of course with collaboration of Iranian nano-Pishgaman Company) at three concentrations (0, 0.25 and 0.5 mg per liter) were applied to plants subjected to three reduced irrigation levels (50, 70 and 90% of field capacity) using a completely randomized design with a three-factorial arrangement. Titanium nanoparticles were sprayed weekly for three weeks (started from Four leaves stage) (**Nezami et al., 2012**). For each treatment four replicates were performed.

Quantitative character assessment

After completion of the growth stage (about 60 days), four plants from each pot were randomly selected and the plant height, number of branches, fresh and dry weight of the aerial part and roots, and the essential oil concentration were assessed. Essential oil concentration was assessed from dried leaves and stems according to hydro distillation method in a Clevenger-type apparatus (**Arazmjo et al., 2010**) In this way, 30 gr of shoots and dried leaves of thyme harvested and then poured into a 500 mm balloon, then added to 300 ml of sterilized water and placed on a stove for 2 hours and after extraction of essential oil, Its value was measured.

Statistical analysis

Results were analyzed using the general linear model (GLM) procedure implemented in the SAS/STAT package (SAS Institute, 2003). Least square means (LSMEANS)

was used to detect difference between treatments at $p < 0.05$ level.

RESULTS

Experimental results are summarized in Table 1, Table 2, Figure 1 and Figure 2. Overall, reduced irrigation significantly decreased plant height, number of branches, fresh and dry weight of the aerial part and roots in thyme plants and the lowest of them were observed in plants under a 50% field capacity (water restriction). However, a positive effect on plant height and number of branches, fresh and dry weight of roots was noted as the level of titanium applications increased. Increases in fresh and dry weight of shoots were detected at 90% FC when concentrations of 0.5 mg. L⁻¹ titanium dioxide were added to plants. In contrast, reduction in water supply significantly decreases root biomass of plants. However, the addition of titanium dioxide at high concentration (0.5 mg. L⁻¹) increased root fresh and dry weight.

The concentration of essential oils was significantly changed under reduced irrigation, and the highest amounts were recorded at 70% water restriction levels. No reversal of this trend was observed when titanium nanoparticles were added to thyme plants.

In Figure 1, similar letters within each column are non-significant values according to Duncan's Multiple Range Test at $p < 5\%$.

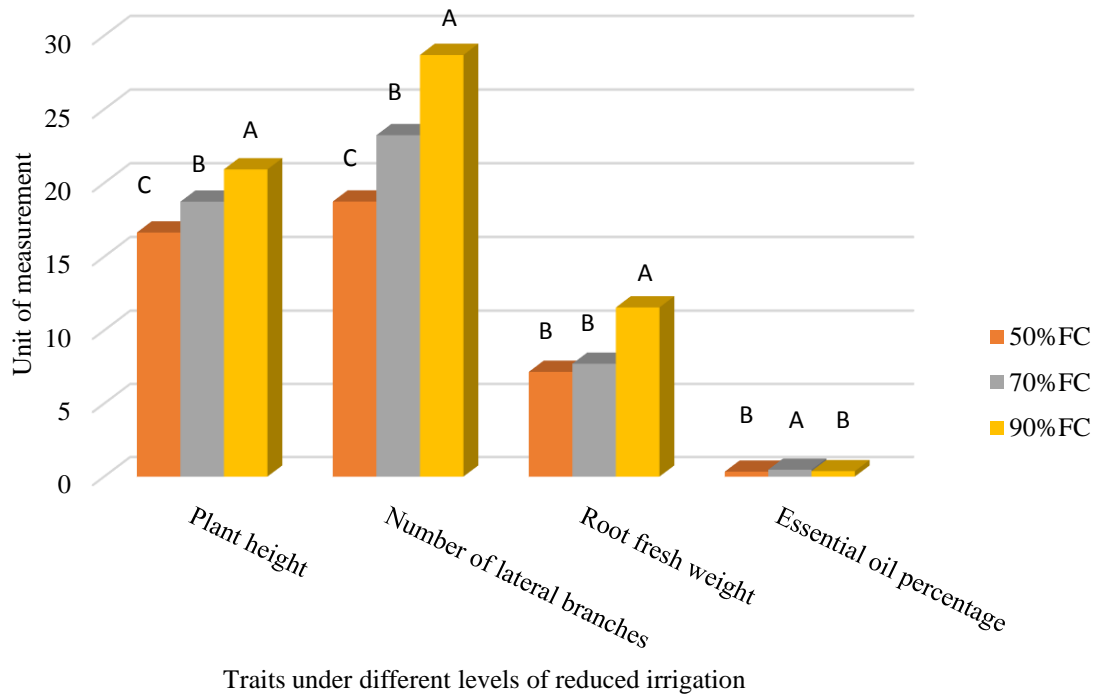


Figure 1 Mean comparison of four measured traits under different levels of reduced irrigation.

Note: similar letters within each column are non-significant values according to Duncan’s Multiple Range Test at $p < 5\%$.

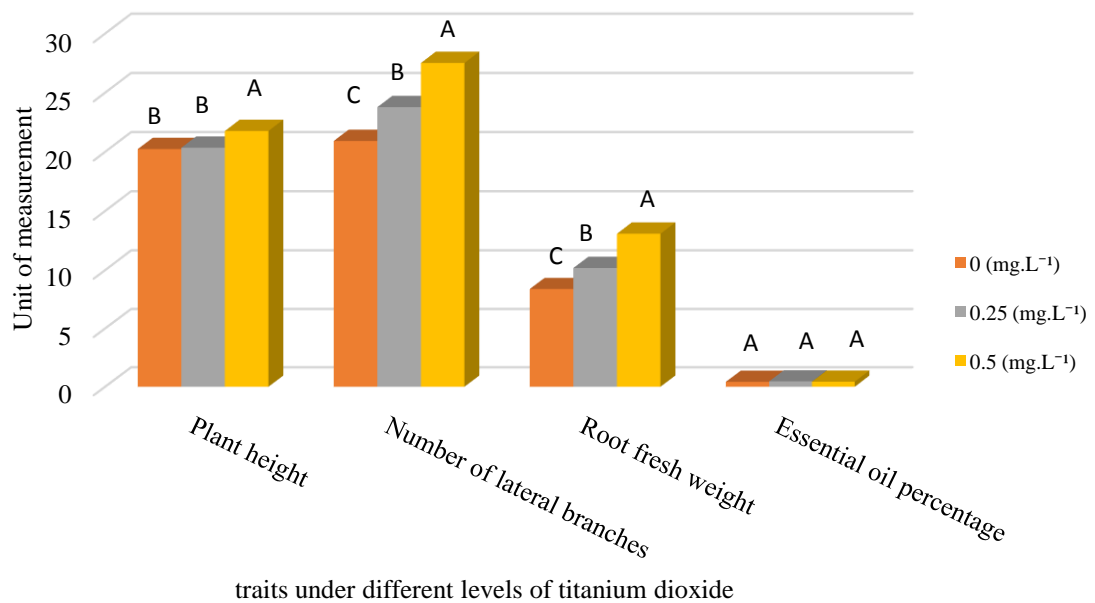


Figure 2 Mean comparison of four measured traits under different levels of foliar application of titanium dioxide.

Table 2 Mean comparison of double interaction effects, reduced irrigation and titanium, on four measured traits.

Irrigation Level (% FC)	Titanium dioxide (mg. L ⁻¹)	Root dry weight	Shoot dry weight	Shoot fresh weight
50	0	3.84 ^d	20.06 ^c	44.19 ^e
	0.25	4.09 ^d	21.15 ^c	49.29 ^d
	0.50	4.13 ^d	21.38 ^c	52.12 ^d
70	0	3.91 ^d	25.24 ^b	58.47 ^{cd}
	0.25	4.18 ^d	25.21 ^b	64.1 ^c
	0.50	4.42 ^{cd}	25.32 ^b	67.78 ^b
90	0	4.86 ^c	26.08 ^{ab}	75.82 ^{ab}
	0.25	5.42 ^b	26.29 ^{ab}	84.85 ^a
	0.50	6.42 ^a	31.31 ^a	84.52 ^a

Note: Similar letters within each column are non-significant values according to Duncan's Multiple Range Test at $p < 5\%$.

DISCUSSION

The application of titanium nanoparticles in *Thymus vulgaris* individuals under reduced irrigation conditions revealed a diversity of effects which were not fully concordant neither between the scored morphological characters nor with the level of reduced irrigation.

TiO₂ nanoparticles increased plant height, but not lateral branch growth under different drought regimes.

Reduced irrigation can reduce the active plant growth period retarding development processes involving stem elongation and leaf growth, the number of nodes and internode length (Ribas-Carbo et al., 2005). Pourmousavi et al., (2007) reported that both titanium dioxide and reduced irrigation decrease plant height in soybean. These observations are in contrast with those obtained in wheat by Moaveni et al., (2011) who reported that plant height increased in response to different treatments of TiO₂ nanoparticles.

Our results in thyme agree with the later report and suggest that the application of titanium nanoparticles may partly counteract the decrease in stem height as a result of water drought. However, the behavior of other aerial growth parameters, like stem diameter and lateral branch number, remains unchanged when titanium nanoparticles were added. Our observations agree with the results of Haghghi and Daneshmand, (2012) who reported that Nano-titanium dioxide had no significant effects on the stem diameter on tomato plants.

Since fresh and dry weight of the aerial parts are partially related to plant height it is reasonable to assume that congruent effects on the effects of titanium nanoparticles would be found. Our results showed that this is the case, in agreement with other works reporting that spraying TiO₂ nanoparticles on leaves positively affects the overall growth rate as measured by the increase in fresh and dry weights of plants from several species (Martínez-Sánchez et al., 1991; Murbach Teles Andrade et al., 2014). However, negative effects on shoot dry weight and length of stem in canola were reported when high concentrations of TiO₂ nanoparticles were supplied (Shafiei and Tadayon, 2014).

Moreover, it should be stated that the method of application of the TiO₂ nanoparticles to plants could have influence on the results obtained. Thus, it has been reported that the application of titanium as a nutrient solution showed no effects on growth rate in tomato (Haghghi and Daneshmand, 2012). It has been suggested that better performances on growth rates are obtained when spraying TiO₂ nanoparticles rather than adding them to conventional nutrient solutions.

The growth rate of fresh roots weight was not affected by the addition of TiO₂ nanoparticles.

Previous results on soybean plants showed that the transportation of titanium across cells was very slow and was more effective on the organs of the plant which were subjected to titanium treatment (Lu et al., 2001). Since in our experimental design we sprayed titanium dioxide on the aerial parts of thyme it might be expected a higher growth rate in the aerial parts than in the root system. Our results agree with this prediction and they were consistent with other experimental work conducted on canola (Mahmoodzadeh et al., 2013), cowpea (Owolade et al., 2008) and apple (Grzyb et al., 2014; Wojcik and Wojcik, 2001).

However, opposite data were obtained by several authors and it is still controversial whether a consistent pattern in the growth rate of roots may be predicted. Even, it has been reported that the inclusion of titanium dioxide only increased the growth rate in apple when the plants showed an optimal nutritional state (Wojcik and Klankowski, 2005). Also, the threshold concentrations of titanium dioxide used may show contrasting results in different species (Bagheri et al., 2010; Shafiei and Tadvin, 2014).

TiO₂ nanoparticles show no effects on the essential oil content of thyme under reduced irrigation.

Several studies have reported a positive association between the essential oil yield and the levels of irrigation in medicinal species from Lamiaceae (Charles et al., 1990; Taarit et al., 2010). However, these observations appears to be genotype specific, since the highest content of essential oils and the most diversity of the individual components of the essential oil have been obtained in some

species at moderate reduced irrigation conditions (e.g., 55% field capacity irrigation; (Omidbeigi, 2007)). Our results obtained in thyme agree with this view and suggest that reduced irrigation may increase the overall concentration of essential oils of course only up to 70% FC.

Apparently, the application of titanium nanoparticles resulted in no significant quantifiable changes in oil content. However, our study was not addressed to assess the metabolic turnover of the individual components of the essential oil. Therefore, it should be further assessed whether several chemical compounds of the essential oil may change their relative concentration when TiO₂ nanoparticles are supplied to plants.

CONCLUSION

Our experimental results in thyme has shown that the addition of titanium nanoparticles in cultivated thyme plants may be an interesting choice for the agricultural practices of this species in arid areas. Nevertheless, the effects of spraying of titanium nanoparticles in this species are varied. On the one hand, growth and height of the aerial parts were significantly affected by TiO₂ nanoparticles. On the other, changes in the growth rate of roots were not observed. In addition, titanium dioxide did not increase the yield of the essential oils. Our data suggest that while the use of titanium dioxide may improve the agronomic features linked to the vegetative aerial parts of thyme, other substances should be screened to increase the essential oil concentration.

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Conflict of interest

The authors declare that they have no conflict of interests.

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