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Original Article

Effect of Titanium Dioxide Nanoparticles on Essential Oil Quantity and Quality in *Thymus vulgaris* under Water Deficit

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Abstract

The aim of this study was to evaluate effect of foliar application of titanium dioxide nanoparticles on quantitative traits and essential oil, thymol and carvacrol percent of thyme under different levels of water stress. Factorial experiment was used based on a completely randomized design with four replications. Factors included water stress (50, 70 and 90% of field capacity) and titanium dioxide nanoparticles (0, 1 and 3 mg/l). Plant height, number of branches, fresh and dry weight of shoot, fresh and dry weight of root and also essential oils (thymol and carvacrol) content were measured using standard compounds by GC-Mass. Results showed that the water deficit affects significantly (P<0.01) on all studied traits. The greatest amount of essence (0.42% per dry matter), thymol (82.302%) and carvacrol (12.33%) at 70% field capacity and the lowest essence (0.30% per dry matter), thymol (77.53%) and carvacrol (11.62%) approached with 50% of field capacity. Analysis of variance showed significant effect (P<0.05) of titanium dioxide concentration on plant height and shoot fresh and dry weights and root dry weight (P<0.01). It was concluded that use of titanium dioxide nanoparticles as spraying under water deficit stress has incremental effect on plant growth characteristics of thyme but had no effect on essential oil components.

Keywords: *Thymus vulgaris*, Titanium dioxide, Water stress, Essential oil

Introduction

Medicinal herbs have been used since human creation. After providing his preliminary requirements, herbs were used to relief pain and cure simple to chronic diseases. They could also separate non-toxic from toxic plants [1].
Despites improvements in chemistry and pharmacy, modern societies have been tented to use traditional medicine and consumption of medicinal plants. As some drugs cannot be produced synthetically or artificial production of these drugs are not economically affordable. Furthermore, herbal medicines have fewer side effects on human health and they also have multiple effects (simultaneously positive effect on different body organs) as well as reducing concerns do to side effects of artificial drugs [1]**.**
Thymus vulgaris L. is a perennial plant the Lamiaceae family with shrub structure [2]. The ratio of essential oil components in this plant varies depending on genetic and agronomic conditions [3]. Essential oil is observed in all parts of the plant however the main part of its production is occurred in flowering branches. Thymus essential oil has antiseptic properties, anti-rheumatic, antioxidant, antifungal, natural food preservatives and delay aging in mammals which can be used in food engineering medicines and hygienic substances [4]. Thymol and Carvacrol are the most important components of the essential oil. More ever this plant contains Tannin, Flavonoids, Saponin and

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bitter substances. Regarding information based on traditional medicine, *Thymus vulgaris* is used to cure different diseases such as cough, sore throat, bronchitis and asthma, anti-inflammatory properties, antiseptic and antispasmodic with the attribute [5,6].

Medicinal plants need full vegetative and reproductive growth to produce active components and Limited water resources in arid and semi-arid regions like Iran. Drought stress which can lead to reduction in the quantity and quality of active components [7].

It was reported that height, stem diameter, leaf area, leaf area index, and yield and basil chlorophyll reduced in different levels of drought stress [8]. Dry matter content and essential oil concentration increased with higher levels of irrigation [9]. The effect of different levels of soil moisture on Basil showed that soil moisture reduction, decreased essential oil performance, but increased the percentage of essential oil [10]. Generally, water stress not only affects yield and performance of essential oil, but also affects the quality of essential oil. Plants respond to drought stress at the physiological, cellular and molecular level and this response depends on plant genotypes [11], duration and severity of water shortages [12], the age and developmental stage [13]. In dry conditions the content of phytochemicals such as proline, protein and chlorophyll can change significantly, which can be considered as the mechanisms of drought tolerance. Unlike agronomical plats, under water stress, medicinal plants produce more chemicals substances and consequently have higher economic efficiency agronomical plants [14].

Proper nutrition is the most important factor controlling plant growth [15]. In the present era, nanotechnology is considered as a prerequisite factor for opening new horizons in knowledge especially agricultural science. Nanotechnology is defined as the manipulation of single atoms, molecules, masses of molecules to produce new structures with different characteristics. Considerable attention has recently been focused on using Nano-titanium dioxide because of its growth stimulation effect [16, 17]. Therefore, the aim of this study was to evaluate the effect of water stress and titanium dioxide nanoparticles on growth and the amount of essential oil of *Thymus vulgaris*.

Material and Methods

This experiment, was conducted at Sareyn which is located at northwest of Iran at 38° 09′ 05″ N latitude 48° 04′ 15″ E longitude in summer 2015. Seeds of *Thymus vulgaris* were obtained from "Pakan Bazr" Company. Factorial experiment was used based on completely randomized design with four replications in the open air that Titanium dioxide nanoparticles with three levels (0, 1 and 3 mg per liter) and water stress at three levels (50, 70 and 90% of field capacity). Plant height, number of branches, fresh and dry weight and essential oil concentration were assessed. Water stress treatments were performed by weighing flower pots. Before performing the experiment, analyze soil samples were achieved in the laboratory of Soil Science, (Table 1). The contents of minerals were determined by Varian SpectrAA-400 plus atomic absorption spectrometer.

Flower pots were used with diameter 20 cm, height 30 cm with a capacity of 6 kg and had 3 holes in the bottom of the pot for drainage. Before starting the water stress treatment, all plants were watered regularly at field capacity. Weighing method was used for calculating the amount of water needed for each pot [18]. After preparing pots, 20 healthy seeds were cultured in each pot and after ensuring the emergence and seedling establishment, they were thinned in several stages and six plants were kept per pot. Until the bolting stage, the pots watered regularly and uniformly and then watering was done based on weights of pots. Nanoparticles of titanium were sprayed on each pot in three stages weekly for three weeks [9].

Plant Height, Fresh and Dry Weight of Plant, Root and Shoot

After completion of growth stage, 4 plants from each pot were randomly selected and disconnected from the crown. The height of plant was measure by a ruler. The weights of fresh and dry plants and roots were recorded with a digital scale.

Percentage and Essential Oil Yield

To keep the quality and the quantity of essential oil, twigs and leaves of *Thymus* were dried in the shade and at room temperature. Essential oil was extracted from dried leaves and branches by Clevenger [19]. To measured essence components was used GC-Mass device Where to identify the amount of thymol and carvacrol was calculated these compounds based on the chromatogram curve [20].

Table 1 Physical and chemical characteristics of the soil used

Soil Tissue	Sand %	Clay %	Lai %	Titanium (mg/Kg)	Na (mg/Kg)	K (mg/Kg)	P (mg/Kg)	The electrical conductivity(ds/m)
Sandy Loam	92	6	2	0.012	3	1.5	3.69	1.2

Table 2 Variance analysis of morphological Attributes and essential oil Percentage

Significance of effects	Items								
	Essential oil percentage	Thymol %	Carvacrol %	Root dry weight	Root fresh weight	Shoot dry weight	Shoot fresh weight	Number of lateral branches	Plant height
Drought	0.044^{**}	68.53^{**}	9.78^{**}	8.89^{**}	54.31^{**}	117.39^{**}	4110.27^{**}	234.77^{**}	43.51^{**}
Titanium Dioxide	0.001^{ns}	0.056^{ns}	0.044^{ns}	1.77^{**}	3.99^{ns}	10.87^{*}	73.78^{**}	13.36^{ns}	5.66^{*}
D * T	0.002^{ns}	0.047^{ns}	0.044^{ns}	0.53^{*}	2.51^{ns}	8.93^{*}	33.44^{*}	0.40^{ns}	1.01^{ns}
Error	.003	0.043	1.12	0.14	1.29	3.14	8.68	4.14	1.67
C.V.	15.73	7.83	8.63	9.52	14.5	8.16	4.24	9.72	7.75

ns, * and **: non-significant and significant at%5 and%1 level of probability respectively

Table 3 Mean comparison of different levels of drought stress for different characteristics in 5% level of Duncan's multiple earned probability

Irrigation Level (%FC)	Essential oil percentage	Carvacrol%	Thymol%	Root fresh weight	Number of lateral branches	Plant height
50	0.30 b	11.61 b	77.52 c	6.37 b	16.66 c	14.79 c
70	0.42 a	13.32 a	82.30 a	6.86 b	20.66 b	16.65 b
90	0.33 b	11.98 b	79.69 b	10.27 a	25.5 a	18.6 a

Similar letters in each column shows non-significant difference according to Duncan’s Multiple Range Test in 5% level of probability

Table 4 Mean comparison of different levels of foliar application of the titanium dioxide Nano-particles on different characteristics in 5% level of Duncan's multiple earned probability

Titanium Dioxide	Essential oil percentage	Carvacrol%	Thymol%	Root fresh weight	Number of lateral branches	Plant height
0	0.34 a	12.32 a	79.88 a	7.33 b	20 b	16.24 b
1	0.37 a	12.24 a	79.76 a	7.70 ab	20.75 ab	16.33 b
3	0.35 a	12.36 a	79.88 a	8.46 a	22.08 a	17.47 a

Similar letters in each column shows non-significant difference according to Duncan’s Multiple Range Test in 5% level of probability

Table 5 Mean comparison of double interaction effects drought stress and titanium on experimented traits

Irrigation Level (FC%)	Titanium Dioxide (mg/l)	Root dry weight	Shoot dry weight	Shoot fresh weight
50	0	3.28 d	17.65 c	52.25 d
	1	3.58 d	18.61 c	46.85 e
	3	3.55 d	18.81 c	55.25 d
70	0	3.34 d	22.38 b	65.10 c
	1	3.78 cd	22.21 b	67.95 bc
	3	3.72 d	22.08 b	71.85 b
90	0	4.32 c	22.94 b	85.7 a
	1	5.88 a	27.55 a	89.95 a
	3	4.88 b	23.15 b	89.6 a

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test in 5% level of probability

Statistical Analysis

All results were analyzed using the general linear model (GLM) procedure of SAS institute Inc., 2003. Least square means (LSMEANS) was used to detect the difference between treatments when $p < 0.05$ was detected

Results

Height of Plant

The results showed that water stress significantly decreased plant height and the highest and lowest height was observed in control and 50% water restriction, respectively (18.6 vs. 14.79 cm). The height of plant increased by increasing the level of titanium. The highest height of plant was detected in Titanium at 3 mg/l (17.47), however the lowest height was observed in control treatment (16.24)(Table 2 and 3).

Essential Oil Content

As shown in table 2, be concentration of essential oil was significantly affected by water stress, regardless of titanium level. 70% water restriction lead to the highest amount of essential oil content (47%) (Tables 2 and 3). The effect of irrigation levels were significant ($P<0.01$) on essential oil and the component of them as the highest value; essences (0.42% per dry matter), thymol (82.302% per essence) and carvacrol (12.333% per essence) in the irrigation treatment based on 70% field capacity and the lowest; essence (0.30% per dry matter), thymol (77.529% per essence) and carvacrol (11.619% per essence) in a water deficit stress based on 50% of field capacity (tables 2 and 3). But the effect of nanoparticles of titanium and interaction of titanium nanoparticles and water deficit was not significant on the essence and the component (thymol and carvacrol) of them (Table 2 and 4).

Fresh and Dry Weight of Shoots

The greatest weight of fresh and dry shoots were detected in 90% water restriction with 1mg/l titanium dioxide 89.95 and 27.55 respectively (table 3).

Fresh and Dry Weight of Root

Water stress had significant (P≤% 1) effect on the fresh and dry weight of root (Table 2), so that the reduction in water, reduced fresh and dry weight root (Table 2 & 3). Generally addition of titanium dioxide increased dry matter content and the best response was detected at the level of 1 milligram per liter.

Number of Lateral Branches

According to the results, the effect of water stress had significant effect on the number of side branches (P≤% 1) (Table 2). Severe stress (50% FC) reduced the number of branches (Table 3). The nanoparticles had no significant effect on the number of branches.

Discussion

Plant Height

Water restriction can lower the number of nodes and internode length. In fact water stress can reduce growth period [18]. Our result was accordance with the result of Pourmousavi *et al.*, [21] who reported that titanium dioxide and water stress decrease plant height. They stated that the decrease in stem height as a result of water stress can be attributed to the number and length of nodes and internodes. Water stress had great effect on growth and retards development of process, reducing stem elongation and leaf growth which may be due to the negative effect of water stress on the photosynthesis phenomenon, nutrition, and water-hormonal relationship in plant [22]. Plant growth not only depends on the accumulation of raw materials through photosynthesis and nutrient uptake, but also depends on high water potential to elongation of the cells. Potassium affects water retention and water absorption of cells [23]. Spraying titanium dioxide nanoparticles was significant on height, dry and fresh weights of plant and dry weight of root (Table 2). The highest (17.47 cm) and the lowest (16.24 cm) plant height were obtained in 3 and zero milligrams per liter, respectively (Table 4). Moaveni *et al.*, [22] reported that plant height increased in response to different treatments of titanium dioxide nanoparticles.

Essential Oil

Dry matter content and essential oil yield increased with higher levels of irrigation in peppermint [24]. In addition to the negative impacts of water scarcity and drought on growth rate and production of all plants, such as medicinal plants, there are many reports about the positive effects of water stress and deficit irrigation on the chemical constituents of plants. For example, high irrigation (60 mm per irrigation) caused a sharp decline in Silybin active ingredient of milk thistle seeds and the highest concentration of this substance was achieved at 20 mm irrigation [10]. Although the most content of essential oil was obtained by watering at 70% of field capacity, water stress (55% field capacity irrigation) resulted in maximizing the components of essential oil [1]. Our results showed that titanium dioxide nanoparticles had no effect on essential oil content.

In peppermint, the essential oil yield was increased to 60% of field capacity [25] But in another study [26] in Badrashbu the essential oil yield was reduced based decreasing of field capacity but there was no effect on essential oil component meanwhile the highest percentage of essence (0.35 mm per 100g of dry matter) was obtained in 70% field capacity. Meanwhile, in addition to the negative effects of water stress on growth and production of plants, but most reports suggest a positive effect on the chemical constituents of the plant's For example, the study results show that excess water (60 mm per each time irrigation) led to a sharp decline in the silybin Milk thistle seeds and maximum of this material was achieved based on 20 mm of irrigation [10]. In another study in Basil results showed that, although most of the oil is achieved by watering up to 70% of field capacity, but high peak of the components of essence was conducted with water stress (55% field capacity irrigation) [1]. It has been reported [27] that drought stress had significant effects on growth parameters, yield, vegetative organs, proline and thymol thyme and also with increasing drought stress some traits were decreased like; plant height, number of lateral shoot, dry weight and fresh weight, root volume, root dry weight and root length but thymol and proline increased as in 55% field capacity (vigor stress) proline and thymol increased. Salinity and drought stress are main factor on the quantity and quality of essence in plants [28] But drought stress is the most factor on increasing of the amount of thymol thyme [27, 28], and for example, water stress was changed essence yield of Rosemary (*Rosmarinus officinalis* L) and anise (*Pimpinella anisum* L) but had no impact on essential components in Rosemary [14]. In addition, drought stress had significant effect on growth and yield essence of lavender Hindi (cymbopogon winteriness juwit) per acre [29]. Therefore it can be sure that secondary metabolites and their components are affected by environmental factors and water shortages [30]. In the present study, in addition to water stress increased essence, increased essence component (thymol and carvacrol) but has no significant on essence component

Number of Lateral Branches

Our results were in agreement with the results of Haghighi and Daneshmand, [31] who reported that Nano-titanium dioxide had no significant effect on stem diameter. However, it was reported that high concentrations of titanium dioxide nanoparticles had a negative effect on shoot dry weight and length of canola stem [32].

Fresh and Dry Weight of Shoot

The results obtained from the effect of water stress and titanium addition on fresh and dry weight was accordance with the results of Janssen *et al.,* [33] who stated that fresh and dry weight decreased by increasing water stress in Spanish Thyme. Feizi *et al.,* [34] reported that the addition of 60 milligrams per liter titanium dioxide nanoparticles to fennel could increase shoot dry weight by 76%compared to other treatments (non-carrier particles) Martinez-Sanchez *et al*., [35] showed that spraying titanium dioxide Nano-particles on pepper leaves positively affect the growth rate. In contrast to the result of Kuzel *et al.,* [36], using titanium dioxide Nano-particles as a nutrient solution had no effect on growth rate of tomato [31]. Many studies suggested better growth arte by spraying titanium dioxide Nano-particles rather than nutrient solution. However, further researches are needed to identify whether spraying or using spraying titanium dioxide Nano-particles as nutrient solution can resulted in better performance.

Use of Nanoparticles of Titanium

The results in soybean showed that titanium movement is very slow but is more effective in the organ that treatment with spraying [37]. Meanwhile in this experiment, treatment of titanium by spraying boost growth and was similar based on some reports that is related the positive effect of titanium on the growth of plants such as rice [38], cowpea [39] and Apple [40]. The nanoparticles in this study had no positive effect on essence and fresh weight roots that the reason of them was reported by Wojcicki and Klamkvvsky [41]. That they observed when titanium was applied in the optimum diet on potato had not effect but titanium in non-optimal conditions have been effective for the growth and where in this research the nanoparticles of titanium was used in optimum conditions then it justify lack significant amount of essence component and root fresh weight. The present study showed that the use spraying form of nanoparticles of titanium has positive impact on the growth of thyme that had confirmed by Kazl *et al.*, [36] and Haghighi and Daneshmand [31].

Fresh and Dry Weight of Roots

In a result [42] related Increases in fresh and dry weight of shoots at 90% FC when concentrations of 0.5 mg/L titanium dioxide were added to plants and also reduction in water supply significantly decreases root biomass of plants and then related that the addition of titanium dioxide at high concentration (0.5 mg/L) increased root dry weight but not significant on root fresh weight. Bagheri *et al*., [43] noted that all evaluated parameters (fresh and dry root, stem and shoot) significantly decreased with increasing drought stress, and at high levels of drought, growth was completely stopped. Nano-titanium and titanium had a significant effect on root fresh and dry weight and addition of titanium and Nano-titanium, increased fresh and dry weight of root and the highest fresh and dry weight was achieved in the treatment of a 1 milligram per liter Nano Titanium by 91.40 and 92.27 percent respectively compared to control [31]. However, higher concentrations lead to a reduction in fresh and dry weight. Shafiei and Tadvin, [32] reported that all concentrations of titanium dioxide nanoparticles had negative effect on all parameters studied including root and shoot dry weight, germination percentage, germination rate, mean duration of germination, vigor index, root length and stem length It was reported that that high concentrations of titanium dioxide nanoparticles had a negative impact on canola seed germination and all parameters were dose-dependent. Yang and Watts [44] showed that concentrations of 2 To 200 mg of aluminum had no effect on seed germination and root length of four cultivated species. However, at a concentration of 2000 mg per liter, germination and root length decreased. However, it was also shown that seed germination and root length response was related to the concentration of nanoparticles and different species respond to different threshold concentrations.

Research results have shown that the titanium soybean plant is very slow and is treated in the organ that is most effective [37]. Generally, it has been established that titanium nanoparticles have different effects depending on the type of plant. If nanoparticles used as a foliar application (spraying) because it increases the photosynthesis and produce organic juice and pour the juice into the ultimate root, dry weight can be increased. However, if nanoparticles added to nutrient solution, it will cause more soil water absorption by roots and consequently root fresh weight will increase. As reported by Karamdkelath-Melo, [45] the use of 6 mg per kg Nano-titanium dioxide in soil with 30 tons of municipal solid waste compost coated with sulfur and 30 kg per ha humid acid enhanced organic carbon soil saturated hydraulic conductivity, mean weight diameter, the index increased stability of soil structure and porosity as well as increasing minerals such as N, P, K, Fe, Mn, Cu and Zn compared to the control. Various factors such as pathogens, low temperature, salinity, drought, flooding stress, heat and heavy metals can negatively affect plant performance. Satellite images showed that damage drought in 2000, along with heat stress was more than $ 4.2 billion [46]. The effect of drought stress on plants is influenced by genotype, length of drought period, climate conditions and growth stage of the drought. Moreover, the time of drought is more important than the severity drought as drought stress not only occurred suddenly but also increases gradually. Therefore, time is an important factor for survival in drought conditions [46] as Osborn *et al*., [47] reported that the impact of drought stress on crop performance depends on duration and severity of stress and plant growth stage, i.e. and before, during flowering and after flowering, corn yield decreased by 25, 50 and 21% compared to control plants.

In drought conditions, dry matter loss may be due to cell swelling pressure, reduction in leaf area, reduction in photosynthetic rate as a result of biochemical constraints such as a decrease in photosynthetic pigments, especially chlorophyll [48]. Potassium also plays an important role in maintaining osmotic potential and water absorption by preventing water losses which will lead to improve in plant growth and yield [49]. Furthermore, one of the most important factors that affect potassium uptake is moisture content of the soil. In arid areas, plant response to potassium fertilizers is not considerable, because decrease in solubility of nutrients causes the lack of plant response to potassium fertilizers [49].

Buttar *et al*., [50] reported that no irrigation significantly reduced canola yield by 45 to 58 percent compared to irrigation. Kazi *et al*., [51] reported a significant positive correlation between the levels of irrigation and grain yield, thus 54% of the variation in yield was related to changes in water levels. Water stress decreased stem weight and seed yield by 23 and 55%, respectively, while the mycorrhizal symbiosis decreased those parameters by 12 and 31%, respectively [52]. Paygozar *et al*., [53] also reported that drought stress reduced dry matter yield of the millet. Other researchers also noted that under water stress the dry matter content and plant biomass of corn, sunflower and wheat were decreased [54, 55] which was consistent with our results.

The main reasons for the decrease in the weight of the plants during the period of stress can be attributed to its adverse effects on growth and plant physiology including growth, photosynthetic systems, nutrient uptake and nitrogen metabolism. The growth of a plant is dependent to cell division, growth and differentiation of cells. Cellular growth is one of the most sensitive responses of plants to drought stress. Therefore, reduction in cell size regarding to plant growth pattern depends on the time of water shortage in terms of phenology. If water stress occurs at the beginning of the growth cycle of the plant, leaf area will reduce and thus carbon fixation will diminish in the growing season. Other secondary effects of leaf area reduction involve changes in the pattern of water and nitrogen usage [56]. Mirza *et al*., [57] stated that flowering occurs earlier in Amelanchier which was under water stress than other plants and water stress also reduced fresh and dry weight of root, stem and stem height.

Previous results showed that the transportation of titanium across the cell of soybean plants was very slow and was more effective on the part of the plant which was subjected to titanium treatment [37]. In our trial, spraying titanium dioxide on thymus resulted in higher growth rate which was consistent with other studied conducted on rice [38], cowpea [39] and Apple [40]. Treating thymus with nanoparticles of titanium dioxide had no positive effect on essential oil concentration and fresh weight of root which maybe do to the optimal nutritional status as Wojcik *et al*., [41] stated that inclusion of titanium dioxide increased growth rate of the apple when it suffered from good nutrition. However, its effect on growth rate is still controversial. Kuzel *et al*., [36] noted that inclusion of titanium as nutrient solution was more effective on growth rate than using as spraying, but Martinez-Sanchez *et al*., [35] reported that addition of titanium dioxide as spraying was more effective on growth rate than using as nutrient solution.

Conclusion

Growth and height of *Thymus vulgaris* were significantly affected by titanium dioxide nanoparticle and water stress which these parameters decreased and increased by water stress and titanium dioxide respectively. However the interaction of titanium dioxide and water stress was not significant in terms of growth and plant height. Concentration of essential oil was not affected by titanium dioxide nanoparticle but affected by water stress. Therefor it is suggested using titanium dioxide to consider vegetative tissue while the other substances than titanium dioxide maybe used to increase essential oil concentration.

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