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Research Article

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Evaluation of the effects of titanium dioxide Nano particles on some morphological traits and oil content of Lemon balm (*Melissa officinalis l.*) under lead stress

Mahdis Sayyadizadeh, Amir Behzad Bazrgar*, Saeed Bakhtiyari

Department of Agronomy, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran

Abstract Effects of Nano-particles of unknown and their effect on living organisms and ecosystems, causes a lot of concern. In order to investigate the effect of dioxide titanium along with lead stress on Melissa, an experiment was conducted at experimental greenhouse of Ferdowsi University of Mashhad, in 2014. Experimental design was factorial with six lead levels (0, 100, 300, 600, 900 and 1200 PPM) and six titanium dioxide (control, 2, 4, 6 and 8 PPM) based on completely randomized design with 3 replications. Low concentration of dioxide titanium increased some characteristics as wet and dry root weight, aerial dry weight, leaf area, chlorophyll content and the percentage of essential oils. Mean comparison showed that lead stress decrease the percentage of essential oils while had no significant effects on other traits. The results of analysis of variance showed that taking titanium dioxide in low concentrations may have a positive effect on lead stress.

Keywords chlorophyll content, leaf area, dry weight, essential oil percent

Introduction

Contamination of soils by heavy metals is a big concern caused by human, agricultural and industrial activities. Lead is a potential pollutant among heavy metals that easily accumulates in soils. Although lead is not an essential element for plants, it absorbed easily and accumulated in different plant parts [1]. It exerts adverse effect on morphology, growth and photosynthetic processes of plants. A high lead level in soil induces abnormal morphology in many plant species [2]. Heavy metal accumulator plants can be used as alternative solution for solving the problem. Researches on certain medicinal and aromatic plants (mint, lavender, thyme, pot marigold, hollyhock, garden sorrel, *black nightshade sp.* etc) showed that they can be more resistant to some heavy metals and other pollutants than other crops [3]. Studies of different experiments showed that total chlorophyll content declined progressively with increasing concentrations of heavy metals. The amount of chlorophyll was reduced in *Triticum aestivum* cv. Vergina grown on Cu-enriched soil and in *Brassica oleracea* var. *Botrytis* cv. exposed to Cu^{2+}, Co^{2+} and Cr^{2+} (chlorophyll). Lead inhibits growth, reduces photosynthesis (by inhibiting enzymes unique to photosynthesis), interferes with cell division and respiration, reduces water absorption and transpiration, accelerates abscission ordefoliation and pigmentation, and reduces chlorophyll and adenosine triphosphate (ATP) synthesis [4]. It is reported that the Pb concentration in garden soils in London can be as high as 20000 µg $g^{-1,}$ whereas Pb occurs naturally in the earth's crust at average levels of 5 to 50 µg g^{-1} [5]. Some reports demonstrated that, with planting variety of medicinal plants such as Basli, Peppermint and Anet (Dill), instead of crops in area contaminated by heavy metals like lead, cadmium and copper, although, plants growth decreased, but there is no change in the oils [6]. TiO_2 is odorless, low-solubility crystal which has thermal stability and combustibility, excellent physical properties, corrosion resistance, biocompatibility and excellent electrical and optical performance [7]. Titanium has biological significant effects on plants, being beneficial at low and toxic at higher concentration. Although Ti is beneficial, it is not essential, Ti deficiency does not exist [8]. Lei *et al.*, reported that nanoTiO_2 could promote photosynthesis and greatly improve spinach growth. It was proved that nanoanatase TiO_2 could greatly improve whole chain electron transport, photoreduction activity of photosystem II, O_2-evolving and photophosphorylation activity of spinach Chl not only under visible light, but also energy-enriched electron from nanoanatase TiO^2, which entered Chl under ultraviolet light and was transferred in photosynthetic electron transport chain and made $NADP^+$ be reduced into NADPH, and coupled to photophosphorylation and made electron energy be transformed to ATP [9].

According to Raskar S and LawareSL experiment (2013), TiO_2 NPs at lower concentration enhances seed germination, promptness index and seedling growth. The goal of this study was to assess the effects of titanium dioxide on some morphological traits of Lemon balm *(Melissa officinalis l.*) under lead stress [10].

Materials and Methods

The experiment was conducted at experimental greenhouse, Faculty of agriculture, Ferdowsi University of Mashhad, in 2014. Soil samples were collected from different fallow lands of university field, and analysed prior to experiment. The soil texture was sandy loam. Based on laboratory experiments, requirement elements were added to this soil. The experiment was conducted based on RCBD, in factorial design in three replication with six lead levels (0, 100, 300, 600, 900 and 1200 PPM) and six titanium dioxide (control, 2, 4, 6 and 8 PPM). Seeds of *Melissa officinalis l.* were collected from Botanical garden (Section of medicinal plants), in Masshhad. Seeds were sterilized, planted in 5kg pots and irrigated with tap water until required field capacity. Plants were allowed to become established for 1 month before TiO_2 application. Different concentration of Titanium dioxide (control, 2, 4, 6 and 8 PPM) was supplemented to pots in three stages of 30, 45 and 60 days after planting, using 2.5L sprayer on leaves in constant pressure of 2 atm. After plant establishment, plants treated with different concentration of lead (0, 100, 300, 600, 900 and 1200 PPM) in two stages of 30 and 45 days after planting.

Table 1: Physical and Chemical Characteristics of the Soil

Soil texture	**Loamy sandy**
%Clay	**8**
%silt	**18**
%Sand	**74**
Soil acidity (pH)	**7.4**
EC (dSm^{-1})	**1.901**
CEC	**17.2**
Organic Carbon (%)	**0.30**
Total Nitrogen (%)	**0.02**
Phosphorous (mg/kg)	**8**
Potassium (mg/kg)	**140**
Calcium (mg/kg)	**14.83**

After five month, at the beginning of flowering, plants were carefully removed from the pot and washed twice with distilled water. The plants were blotted dry with tissue papers and then the various morphological parameters such as wet and dry root weight, wet and dry root weight, leaf area, chlorophyll content and the percentage of essential oils per plant were recorded. The collected data were analysed using SAS software. Mean comparison was done by LSD method, Significant F-values were tested at the $p \leq 0.05$ significance level.

Results and Discussion

Root fresh weight

Based on Analysis of variance (Table 2), the effect of effect of titanium dioxide nano on root fresh weight was statistically significant ($p \leq 0.05$), while the effect of lead and interaction effect of lead and titanium dioxide nano on this trait was insignificant. Mean comparison showed that root fresh weight generally increased with increasing levels of titanium dioxide nano and the highest weight was obtain at concentration of 8 ppm titanium and that was 1.22 times higher compare to the control (0 ppm) (Figure 1).

Table 2: Analysis of variance for effect of titanium dioxide nano and lead application on some studied traits of Lemon balm

Source of variation	df	Root fresh weight	Root dry weight	Aerial fresh weight	Aerial dry weight	Leaf area index	Essential oil percent	Chlorophyll content
Block	2	79.82^{ns}	0.02^{ns}	500.73^{ns}	0.57^{ns}	0.56^{ns}	2.70**	608.23^{ns}
Lead (A)	5	64.59^{ns}	0.09^{ns}	555.49^{ns}	0.38^{ns}	0.13^{ns}	12.51**	1488.70^{ns}
Titanium dioxide nano (B)	4	125.79*	1.10**	481.02^{ns}	1.68**	2.48*	16.87**	1923.9*
A×B	20	39.38^{ns}	0.27**	346.02^{ns}	0.11^{ns}	0.13^{ns}	1.01**	611.27^{ns}
Error	58	44.48	0.11	384.68	0.34	0.95	0.06	762.52

ns: Not significant, * Significant at p≤0.05, * * Significant at p≤0.01

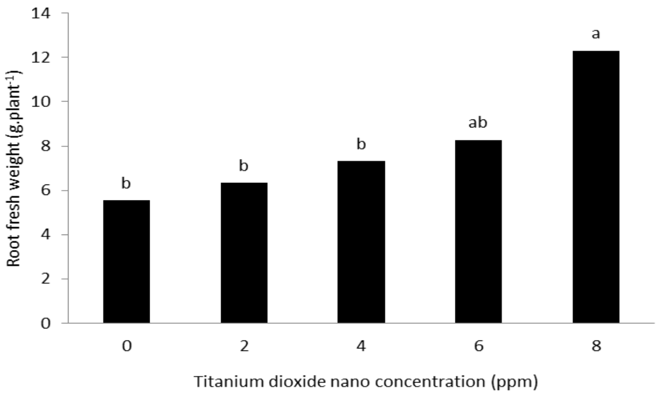

Figure 1: The effect of titanium dioxide nano concentration on root fresh weight

Root dry weight

The results indicated that the interaction effect of titanium dioxide nano and lead on root dry weight was significant (p≤0.01) (Table 2). Based on mean comparison, the highest root dry weight (2.33 g) was obtained at treatment with concentration of 100 ppm lead supplemented with titanium dioxide nano at 8 ppm level. The lowest dry weight was obtained (0.12 g) with application of 100 ppm lead and no titanium dioxide nano. In all lead levels, the root dry weight increased with increasing concentration of titanium dioxide. There was no significant difference between titanium dioxide nano treatments on root dry weight in all lead levels except 100 ppm lead.

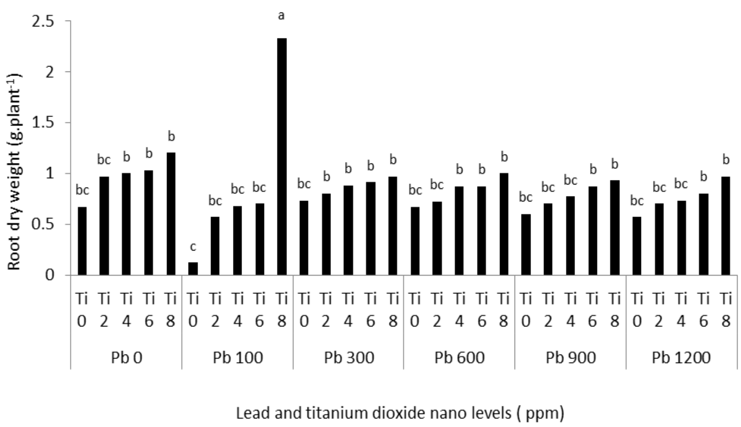

Figure 2: The interaction effect of different lead concentration and titanium dioxide nano on root dry weight

Shoot fresh/dry weight

Based on Analysis variance table 2, the effect of titanium dioxide nano and lead levels was not significant on shoot fresh weight. The interaction effect of effect of different lead concentration and titanium dioxide was not significant on this trait as well. The effect of titanium dioxide nano on shoot dry weight was significant ($p \leq 0.01$). Shoot dry weight progressively increased with increasing concentration of titanium dioxide nano (Figure 3). Among the titanium dioxide nano treatments, 8 ppm concentration of titanium exhibited higher shoot dry weight (3.30 g) and there was no significant difference among 4, 6 and 8 ppm treatments statically. The lowest shoot dry weight (2.55 g) was observed in control.

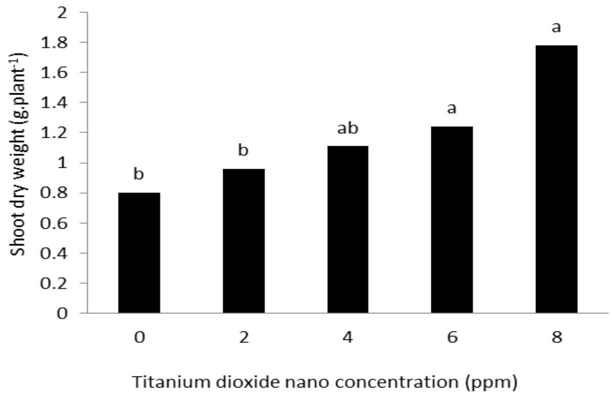

Figure 3: The effect of titanium dioxide nano on shoot dry weight

Leaf area index

Results showed that only the simple effect of titanium dioxide nano on leaf area index was significant ($p \leq 0.05$). Results of mean comparison demonstrated that. Leaf area index increased with increasing concentration of titanium dioxide nano. Plant treated with 8 ppm of titanium dioxide nano showed higher leaf area index (1.78), while the lowest leaf area index (0.80) was found in control (Figure 4).

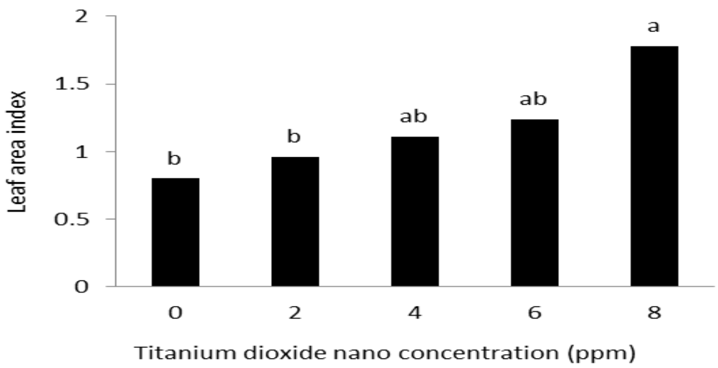

Figure 4: The effect of titanium dioxide nano on leaf area index

Chlorophyll content

Our results demonstrated that titanium dioxide nano had significant effect on chlorophyll content ($p \leq 0.05$). Data pertaining the effect of titanium dioxide nano on chlorophyll content indicated that chlorophyll content progressively increased with increasing concentration of titanium dioxde nano. The highest chlorophyll content was obtained at concentration of 8ppm which was 87.54% higher compare to the control.

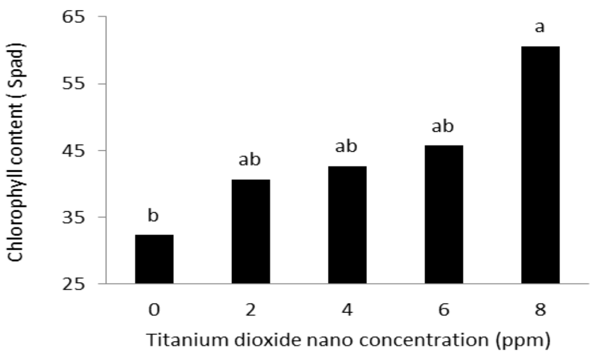

Figure 5: The effect of titanium dioxide nano on chlorophyll content

Essential oil percentage

It was observed that the interaction effect of titanium dioxide nano and lead concentrations on essential oil was significant (($p \leq 0.01$). Maximum increase in essential oil percentage (6%) was recorded at 8ppm titanium dioxide nano treatment with no lead. In all lead levels essential oil percentage increased with increasing concentration of titanium dioxide nano (Figure 6).

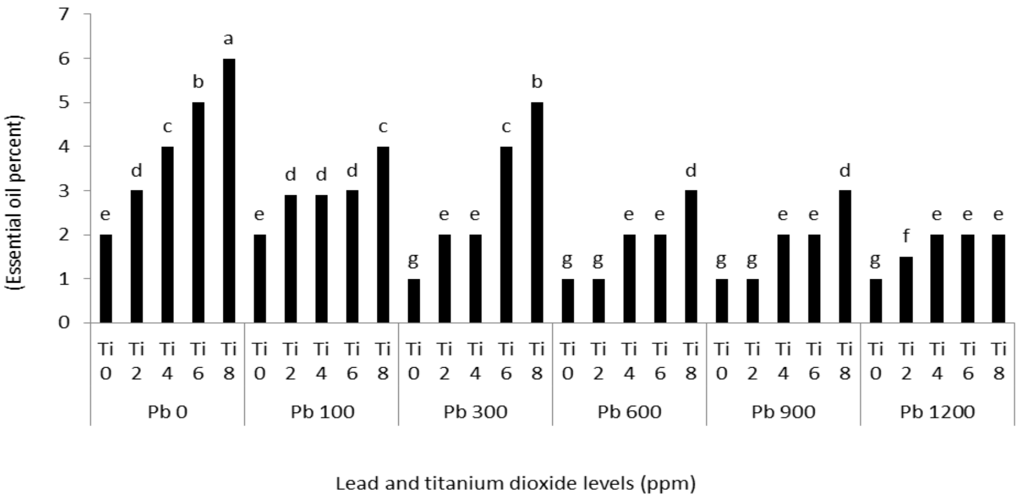

Figure 6: The effect of different lead concentrations on oil percentage

Discussion

Melissa officinalis L. is a medicinal plant and is used for food purposes. In order to understand the possible positive effect of applying nanoparticle in nutrient and medicinal industry, it is important to investigate the effect of nanoparticle such as titanium dioxide nano (NP TiO_2) on some morphological and physiological characteristics on plants. Results of our study indicated that the applied NP TiO_2 had increased effect on morphological traits in high concentration. Some studies have reported no negative effects of titanium dioxide nano on plant growth, especially at low concentration. Titanium dioxide nano (NP TiO_2) can encourage and accelerate seed germination by improving water absorption in spinach seeds and inhibit photosynthesis in *Chlamydomonas reinhardtii* [11]. NP TiO_2 improves solar photosynthetic system and plant nutrient uptake that are effective in chlorophyll production such as Fe, Mg and Nitrogen by enhancing electron transfer [12-15]. Results of this study showed that lead stress had negative effect on all plant growth stages and reduced plant yield and plant growth stages, while titanium application can improve plant growth and reduce negative effect caused by lead stress in high and appropriate concentration.

Phytoremediation of heavy metal-contaminated soil is an emerging method that aims to extract or inactivate metals in soil [16]. Heavy metals inhibit root and shoot growth and yield production, affect nutrient uptake, induced reduction in the photosynthesis and accelerate plant senescence [17]. Stresses caused by Heavy metals are also known to reduce development of plant cells and stem length [18]. Plants have ability to absorb lead through their roots, while lead transformation toward aerial plant parts is highly limited [19]. Root and shoot biomass reduction can be mentioned as negative effect of lead pollution. Lead decreases photosynthetic rate by distorting chloroplast ultrastructure, inhibiting activities of Calvine cycle, reducing chlorophyll synthesis and obstructing electron transport [1]. Pb contamination caused disorder in stomata operation; cells water balance and respiration process in physiological level [5].

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