Plant Archives Vol. 19 No. 1, 2019 pp. 221-227 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF FOLIAR SPRAY WITH NANO TITANIUM, ZINC AND BULK OXIDES IN SOME BIOCHEMICAL AND ACTIVE SUBSTANCES OF *MORINGA OLEIFERA* LAM

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Abstract

The experiment was carried out during the season of 2017-2018 in plastic house at the research station of the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Diyala to study the effect of foliar spray with nano particles and bulk of titanium dioxide (TiO_2) and zinc oxide (ZnO) at concentration of 50 $mg.l^{-1}$ for each one of them, in addition to spray with distilled water as control treatment in the biochemical qualities and active substances content of moringa. The results can be summarized as follows:

Foliar spray with nano particles and bulk titanium dioxide and zinc oxide lead to enhancing all biochemical qualities and active substances content of moringa. Treatment with concentration of 50 $mg.l^{-1}$ of zinc oxide nano particles gave the best results in terms of plant Ca, Fe content, protein percentage, percentage of total carbohydrate, â-carotene, niaziridin, chlorogenic acid, gallic acid, vitamin C content in leaves and values of these qualities reached 2.20, 29.62, 21.98, 13.47, 34.12, 28.69, 30.67, 900.25, 29.29 respectively, while treatment with concentration of 50 $mg.l^{-1}$ of titanium dioxide nano particles gave the best results in terms of Kaempferol content in leaves reaching 26.21 mg.100 g^{-1}.

Key words : *Moringa oleifera*, nano titanium, nano zinc, active substances.

Introduction

Moringa (*Moringa oleifera* Lam.) belongs to the moringacea family and it is one of ornamental trees and its native habitat is northwest India and cultivated in Africa, Central and South America (Abdul Karim *et al*., 2016; Pachauri *et al*., 2013). Moringa family contains only one genus moringa, which has thirteen species. The most important species are *Moringa oleifera* (Katayon *et al*., 2006). It's called the miracle tree for its nutritional, medical and industrial importance as well as environmental importance (Saini *et al*., 2016). The leaves contain high levels of minerals, carbohydrates, proteins, carotenoids, polyphenols, alkaloids, tannins, and saponins, so the leaves are the most frequently used part (Oladeji *et al*., 2017) as well as glycosides, flavonoids and phytosterols (Yadav *et al*., 2016). Ali *et al*. (2013) and Singh (2017) reported that moringa contains many active compounds such as carotene, vitamin C, A and E, which are antioxidants substances.

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Nanotechnology is a new and fascinating field of science and discoveries in nanotechnology can open up innovative applications in biotechnology and agriculture. In the fields of electronics, energy, medicine and life sciences, nanotechnology has provided extensive research in the science of reproduction, technology, the conversion of agricultural residues and food into energy, and other by-products through nano scale biological processes, chemical sensors, water cleaning and disease prevention and treatment of plants using various nano pesticides (Carmen *et al*., 2003; Nair *et al*., 2010). Nano particles are particles ranging in size from 1 to 100 nanometers. In nanotechnology, particles are defined as a small object acting as a whole unit in relation to its transition and properties (Giraldo *et al*., 2014).

Titanium is found in the soil at a relatively high concentration from several tenths of a percentage point to several percentage points. The vast majority of titanium is not available to the plant, because it is mostly found in minerals that are insoluble in water. Titanium is found in plants with relatively low concentrations (0.1-10 ppm) (Wait, 1986; Dumon and Ernst, 1988). Titanium nano particles have photovoltaic properties and improve light absorption and conversion from photovoltaic to electrical and chemical energy, as well as pushing carbon dioxide to protect the green plastid from aging when exposed to long-lasting illumination (Hong *et al*., 2005a; Yang *et al*., 2006). In addition, titanium nano particles increase cell growth by improving metabolism and nitrogen metabolism and thus increasing plant weight (Hong *et al*., 2005; Mingyu *et al*., 2007). Several studies have shown the positive effects of nano particles dioxide in the content of some biochemical and active ingredients, Morteza *et al*. (2013) on maize, Haag-Whitted *et al*. (2014) on Chrysanthemum and Khater (2015) on coriander.

Micronutrients significantly affect plant growth and development. Among the micronutrients, zinc is involved in the metabolism of RNA and ribosomal content in plant cells that stimulate carbohydrate, protein and DNA synthesis. Zinc is also required for the manufacture of amino acid tryptophan, the initial initiator of IAA, which acts as a catalyst for growth (Amberger, 1982). Zinc also plays a key role in plant physiology, activating certain enzymes and associated with the metabolism of carbohydrates, auxin and ribosomes (Memon *et al*., 2013). Zinc has a role in the management of reactive oxygen species and the protection of the plant cell from oxidative stress, as well as regulating the function of stomata by regulating potassium content in plant cells (Samreen *et al*., 2013; Amiri *et al*., 2016; Venkatachalam *et al*., 2017). Its functions to form nucleic acids, proteins, pollen formation, fertilization and germination (Hegazy *et al*., 2016).

Nano-zinc oxide is a semiconductor material that constantly shrinks to a nanometer or even smaller size (Wang *et al*., 2004) and has anti-staphylococcal, antifungal, antimicrobial, anticonvulsant, antimicrobial and bacterial properties. Nanozinc used as sterilizers for food industry equipment and confectionery against bacterial contamination that causes food borne diseases (Molina *et al*., 2006). Zinc nano particles are widely used in cosmetics, foodstuffs and many biological and pharmaceutical applications. They act as an antibacterial agent, regulate immunity and promote growth, several human and animal studies have shown the use of zinc nano particles as mineral supplements with better results than bulk zinc sources and indirectly inhibit environmental pollution (Swain *et al*., 2016). Several studies have shown the positive effects of nano particles oxide in the content of some biochemical and active ingredients Najafivafa *et al*. (2015) on *Satureja hortensis* and Davarpanah *et al*. (2016) on pomegranate, Mohsenzadeh and Moosavian (2017) on rosemary.

The main objective of the study is to investigate the effects of foliar spray with nano particles and bulk titanium and zinc oxides in the content of some biochemical components and active substances of moringa.

Materials and Methods

The experiment was carried out during the season of 2017-2018 in plastic house at the research station of horticulture department and landscape gardening, College of Agriculture, University of Diyala to study the effect of foliar spray with nano particles and bulk of titanium (TiO_2) and zinc (ZnO) oxides at concentration of 50 $mg.l^{-1}$ for each one of them, in addition to spray with distilled water as control treatment, in the biochemical qualities and active substances content of moringa. The experiment was carried out according to Randomized Complete Block Design (RCBD) with three replicates.

The study was conducted for the period from 1/10/2017 to 20/6/2018. Seeds were planted in plastic pots of 25 cmin diameter, two seeds per pot, containing loamy sand soil, the physical and chemical properties shown in table 1. After germination and emergence, seedlings thinning to one in each pot. The experiment included foliar spray with nano titanium dioxide($NTiO_2$) and nano zinc oxide (NZnO) (size 10-30 nm)(produced by Sky Spring Nano materials Company, Inc.USA) at concentration of 50 $mg.l^{-1}$ and symbolizing them as NTi_{50} and NZn_{50} respectively and spraying with bulk (TiO_2) and zinc (ZnO) with a concentration of 50 $mg.l^{-1}$ for each and symbolized as Ti_{50} and Zn_{50} respectively, as well as spraying with

Table 1 : Some physical and chemical properties of cultivation soil.

Property		Value	Unit
pH(1:1)			7.11
E.C.		4.66	dS/m
Soil separates	Clay	96	$gm.kg^{-1}$
	Silt	32	$gm.kg^{-1}$
	Sand	872	$gm.kg^{-1}$
Soil Texture		**Loamy sand**	
$CaCO_3$		28.07	$gm.kg^{-1}$
Organic Matter		0.91	%
Available Nitrogen		43.60	$mg.kg^{-1}$
Available Phosphorus		12.98	$mg.kg^{-1}$
Available Potassium		255.2	$mg.kg^{-1}$

distilled water as a comparative treatment and symbolized as MO. Plants were sprayed twice, 60 and 75 days after transplanting.

The experiment included five levels as follows :

1) MO (spray with distilled water only).
2) NTi_{50} (50 $mg.l^{-1}$ nano-titanium dioxide).
3) Ti_{50} (50 $mg.l^{-1}$ bulk titanium dioxide)
4) NZn_{50} (50 $mg.l^{-1}$ nano-zinc oxide).
5) Zn_{50} (50 $mg.l^{-1}$ bulk zinc oxide).

The data were analyzed according to the statistical program (SAS, 2003). Means were compared using Duncan's Multiple Range Test (DMRT) (P>0.05). Experimental measurements included some biochemical components such as: Ca percentage in leaves, Fe content in leaves ($mg.100g^{-1}$ dry weight), percentage of protein in leaves (%), percentage of total carbohydrate in leaves (%), β-carotene content in leaves (mg.100 g^{-1} dry weight) and some active substances such as: niaziridin content in leaves ($mg.100g^{-1}$ dry weight), chlorogenic acid content in leaves ($mg.100g^{-1}$ dry weight), gallic acid content in leaves ($mg.100g^{-1}$ dry weight),vitamin C content in leaves ($mg.100g^{-1}$ dry weight) and kaempferol content in leaves ($mg.100g^{-1}$ dry weight).

The percentage of Ca in the leaves was estimated according to Leggett and Westermann (1973). Percentage of total carbohydrate in the leaves was estimated, according to Pearson *et al* (1976). Percentage of protein in leaves was estimated, according to USDA (2016). β-carotene and Fe content in leaves was estimated, according to A.O.A.C. (1970). Niaziridin, chlorogenic acid, gallic acid, vitamin C and kaempferol in leaves were estimated according to Harborne (1984).

Results

I- Biochemical properties

I-1- Ca percentage in leaves (%)

Results of table 2 showed that the percentage of calcium in the leaves increased significantly when spraying plants with concentration of 50 $mg.l^{-1}$ of nano-zinc oxide as it reached 2.20% compared with the control treatment which gave a percentage of 1.94%.

I-2- Fe content in leaves (mg.100 g^{-1} dry weight)

Foliar spraying with titanium and zinc nano particles and bulk oxides affect in Fe content in leaves. Results of table 2 showed a significant increase in the content of Fe in leaves when spraying with 50 $mg.l^{-1}$ of nano zinc oxide, and reached 29.62 mg.100 g^{-1} dry weight compared with control treatment, which Fe content in leaves was 25.59 mg.100 g^{-1} dry weight.

I-3- Percentage of protein in leaves (%)

Results of table 2 showed significant differences in percentage of protein in leaves when spraying with titanium and zinc nano particles and bulk oxides. Treatment at concentration of 50 $mg.l^{-1}$ of nanozinc oxide gave highest percentage of protein in leaves reached 21.98% in comparison with control treatment, which recorded lowest percentage of protein in leaves reached 18.32%.

I-4- Percentage of total carbohydrate in leaves (%)

Results of table 2 showed that the highest percentage of total carbohydrates in leaves was achieved when spraying with a concentration of 50 $mg.l^{-1}$ of nano zinc oxide, reached 13.7%, compared to control treatment (10.67%).

I-5- β-carotene content in leaves (mg.100 g^{-1} dry weight)

Results of table 2 showed that the highest content of β-carotene in leaves was achieved when spraying with a concentration of 50 $mg.l^{-1}$ of nano zinc-oxide, reached 34.12 mg.100 g^{-1} dry weight compared to control treatment, which recorded the lowest content of β-carotene in leaves reached 23.96 mg.100 g^{-1} dry weight.

II- Content of some active substances

II-1- Niaziridin content in leaves (mg.100 g^{-1} dry weight)

Results of table 3 showed a significant increase in content of niaziridin in leaves when spraying with a concentration of 50 $mg.l^{-1}$ of the zinc oxide nano particles reached 28.69 mg.100 g^{-1} dry weight compared to control treatment (18.22 mg.100 g^{-1} dry weight).

II-2- Chlorogenic acid content in leaves (mg.100 g^{-1} dry weight)

Results of table 3 indicated that chlorogenic acid in the leaves increased significantly when spraying with50 $mg.l^{-1}$ of zinc oxide nano particles reached 30.67 mg.100 g^{-1}dry weight compared with control treatment, which recorded lowest content reached 16.36 mg100 g^{-1} dry weight.

II-3- Gallic acid content in leaves (mg.100 g^{-1} dry weight)

Results of table 3 revealed that foliar spray with titanium and zinc nano particles and bulk oxides resulted in a significant increase in gallic acid content in leaves.Treatment of 50 $mg.l^{-1}$ of nano zinc oxide gave the highest content reached 900.25 mg.100 g^{-1} dry weight, which on par with treatment of 50 $mg.l^{-1}$ of nano titanium dioxide, which recorded 853.50 mg100 g^{-1} dry weight,

Table 2 : Effect of foliar spray with nano titanium, zinc and bulk oxides in some biochemical properties of moringa.

Treatments	Ca (%)	Fe (mg.100g^{-1})	Protein (%)	Total carbohydrate(%)	β-carotene (mg.100g^{-1})
M0	1.94e	25.59e	18.32d	10.67d	23.96d
NTi50	2.19b	29.21b	21.37b	12.41b	31.19b
Ti50	2.02c	27.36d	18.01e	11.40c	27.17c
NZn50	2.20a	29.62a	21.98a	13.47a	34.12a
Zn50	1.99d	27.64c	18.54c	12.10b	31.43b

Means in each column followed by similar letters are not significantly different (P>0.05) according to Duncan's Multiple Range Test (DMRT).

Table 3 : Effect of foliar spray with nano titanium, zinc and bulk oxides in content of some active substances of moringa.

Treatments	Niaziridin content (mg.100g^{-1})	Chlorogenic acid content (mg.100g^{-1})	Gallic acid content (mg.100g^{-1})	Vitamin C content(mg.100g^{-1})	Kaempferol content(mg.100g^{-1})
M0	18.22d	16.36d	638.33c	18.45e	16.31e
NTi50	26.98b	29.74b	853.50a	24.08b	26.96a
Ti50	19.17c	15.56e	754.00b	19.85d	21.53d
NZn50	28.69a	30.67a	900.25a	29.29a	26.21b
Zn50	19.17c	18.90c	718.33b	21.34c	22.37c

Means in each column followed by similar letters are not significantly different (P>0.05) according to Duncan's Multiple Range Test (DMRT).

compared to control treatment, which gave lowest content reached 638.33 mg.100 g^{-1} dry weight.

II-4- Vitamin C content in leaves (mg.100 g^{-1}dry weight)

Results in table 3 showed that all foliar spray treatments with titanium and zinc nano particles and bulk oxides had a significant effect on vitamin C content in leaves. Treatment of 50 mg.l^{-1} of nano zinc oxide resulted significant increase in vitamin C content in leaves reached 29.29 mg.100 g^{-1} dry weight compared to control treatment, which gave the lowest vitamin C content in leaves reached 18.45 mg.100 g^{-1} dry weight.

II-5- Kaempferol content in leaves (mg. 1 g^{-1} dry weight)

Results of table 3 showed that all foliar spray treatments with titanium and zinc nano particles and bulk oxides had a significant effect on kaempferol content in leaves. Treatment of 50 mg.l^{-1} of nano titanium dioxide recorded highest kaempferol content in leaves reached 26.96 mg.100 g^{-1} dry weight compared to control treatment, which gave lowest content reached 16.31 mg.100 g^{-1} dry weight.

Discussion

The results showed that foliar spraying of moringa with titanium and zinc nano particles and bulk had a positive effect on biochemical components and some active substances in leaves of the moringa and spray treatment at concentration of 50 mg.l^{-1} of nano zinc oxide gave the best results in terms of percentage of Ca, Fe content, percentage of protein, percentage of total carbohydrate, β-carotene content, niaziridin, chlorogenic acid, gallic acid and vitamin C content in leaves.

Increment in biochemical components and active substances may be due to the fact that some nano particle can be absorbed, transported and accumulated at specific sites in the cell such as the gaps, nuclei and the plasma network that bind cells. These nano particles can therefore alter plant physiological processes and influence the plant growth and development (Wang *et al*., 2016). According to Govorov and Garmeli (2007), nano particles can stimulate the efficiency of chemical energy production in photosynthesis systems. Chlorophyll acts at the center of the optical reaction to bind the crystals and thus forms a new hybrid system that produces electrons up to ten times higher, this can help in design of an artificial light harvesting system. Increment in dry weight and protein content in leaves, when treated with zinc oxide nano particles is due to the role of zinc in plant growth and its effective contribution to the major biological processes of the cervical cells leading to the formation of plant biomass (Venkatachalam *et al*., 2017). The results showed that treatment of zinc oxide nano particles led to a significant increase in percentage of Ca and Fe content, percentage of total carbohydrates, β-carotene content in leaves corresponds to what Khalifa *et al*. (2011) reported on Iristhat all vegetable traits and biochemical components such as N, P, K, Fe, Mn, Zn, and B, percentage of carbohydrates and pigments were increased when treated with zinc.

Zinc has been shown to affect photosynthetic pigments and this is due to its role in increasing photochemical stimulation, structure of green plastids, transmission of photonics, as well as photosynthesis (Kumar *et al*., 1988; Romheld and Marschner, 1991). Increment in total carbohydrates and protein may be due to the role of zinc in increasing vegetative growth and its involvement in process of photosynthesis and ribosome content in plant cell, which stimulates formation of carbohydrates, proteins and nucleic acids, and helps synthesis of tryptophan, which acts as an improvement growth substance (Jat *et al*., 2007). As mentioned by Abu Dhahi and Al-Younis (1988) that zinc is necessary for the process of phosphorus and glucose formation and therefore the lack stops the process of representation of starch, and helps in process of chlorophyll formation because of its direct impact in processes of formation of amino acids, carbohydrates and energy compounds, and increases formation of vitamin C as well as vitamin B complex, and zinc has a role in transformation of nitrogen, where prove its importance in formation of DNA, which necessary in process of protein synthesis.

The results showed an increase in content of active substances in leaves such as niaziridin, chlorogenic acid and gallic acid and vitamin C in leaves, this may be due to the role of zinc in increasing some properties of plant and its role in increasing efficiency of photosynthesis in leaves and activation of carbonic anhydrase enzyme and then increase carbohydrates and proteins synthesis by stimulating the effectiveness and activity of Ribonuclease enzyme, which reflected on photosynthesis and production of metabolic materials. The results showed that treatment of 50 $mg.l^{-1}$ of nano particle titanium was superiority in giving highest content of kaempferol in leaves, this increase is due to the optical stimulation properties of titanium dioxide and the optical system, thus increasing the activity of photosynthesis. It is expected that increase in all plant production characteristics including morphological, biochemical, active substances and productivity will be associated with increased photosynthesis (Lin and Xing, 2007). Titanium treatment also encourages absorb nitrates and promote transformation of inorganic nitrogen into organic nitrogen such as protein and chlorophyll and improve plant growth and development. Increment in kaempferol content in leaves is attributed to the fact that use of nano particles of titanium dioxide stimulates photosynthesis and increases dry matter in plants and improves biomass production by stimulating plants metabolic activities (Ma *et al*., 2008; Raliya *et al*., 2015).

Conclusion

From the results of the present study, it can be concluded that foliar spray with titanium and zinc nano particles and bulkpositively affected all biochemical components and active substances in leaves of moringa. Foliar spray with nano zinc oxide at concentration of 50 $mg.l^{-1}$ was surpassed in giving the best results in terms of percentage of Ca, Fe content, percentage of protein, percentage of total carbohydrate, β-carotene content, niaziridin, chlorogenic acid, gallic acid and vitamin C content in leaves as compared to control treatment, while foliar spray with nano titanium dioxide at concentration of 50 $mg.l^{-1}$ gave the best results in terms of kaempferol in leaves.

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