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Impact of Titanium on Growth and Forage Production of Maize (*Zea mays* L.) under Different Growth Stage

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ABSTRACT

This study was conducted to evaluate the effect of titanium dioxide and titanium oxide foliar application during two growing stage on growth and forage production of maize during 2012 growing season in the research farm of Islamic Azad University of Varamin-Pishva Branch. The experimental design was randomized complete blocks arranged in factorial with four replications. The factors were included two growing stages i.e. four leafy stage and stem elongation for titanium application and five titanium concentration and sources including control (water) titanium oxide (Bulk) and three concentrations of 0.01, 0.02 and 0.03% of titanium dioxide nanoparticles. The results showed that titanium dioxide nanoparticle especially at stem elongation stage increased plant growth and dry matter accumulation in maize plant. Although, titanium oxide (bulk) had positive effect on biological yield, titanium dioxide nanoparticle produced the highest forage yield. In addition, among different concentrations of titanium dioxide nanoparticle, application 0.02% significantly improved plant growth; dry matter and final forage yield so that it can be recommended to earning more forage.

Keywords*:* *Corn, Foliar application, Nanoparticle, Yield.*

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INTRODUCTION

Maize is one of the most important silage plants in the world because of its high yield, high energy forage produced with lower labour and machinery requirements than other forage crops (Roth *et al.*, 1995). It is a crop that is planted and consumed more and more the last years (Kara, 2001). The maize is used mainly to make silage to feed the cattle and sheep because it produces abundant amount of green herbage and its silage has high nutriment value and palatable (Acikgoz, 1991). For the 2002-2004 periods, world average yields of maize and winter wheat were estimated for 4.57 and 2.77 $t.ha^{-1}$, respectively (FAO, 2005). Trace element, also called micronutrient, in biology, any chemical element required by living organisms in minute amounts, usually as part of a vital enzyme, a cell-produced catalytic protein. Exact needs vary among species, but commonly required plant micronutrients include copper, boron, zinc, manganese, and molybdenum. Lack of a necessary plant micronutrient in the soil causes plant deficiency diseases. Some elements are not necessary for plants but when these elements are provided for plants, there are some positive responses to these elements. Among these elements titanium has been identified as promoting elements for plant growth. A few studies relate TiO_2 nanoparticles uptake by plant roots (Lin and Xing 2008), and root to shoot transfer (Zhu *et al.*, 2008). Concerning TiO_2, TiO_2-based nanocomposites have recently been shown to be accumulated and translocated to the shoots of *Arabidopsis thaliana* (Kurepa *et al.*, 2010). It has been reported that titanium had positive effects on physiological traits of plants. Titanium has been suggested to play a role in the fixation of nitrogen by legumes and increasing the yields of crop plants (Nand *et al.*, 1983). Titanium is non-toxic even in large doses and does not play any natural role inside the human body. An estimated quantity of 0.8 milligrams of titanium is ingested by humans each day, but most passes through without being absorbed. It does, however, have a tendency to bio-accumulate in tissues that contain silica. An unknown mechanism in plants may use titanium to stimulate production of carbohydrates and encourage growth. This may explain why most plants contain about 1 part per million (ppm) of titanium, food plants have about 2 ppm, and horsetail and nettle contain up to 80 ppm. Application of titanium dioxide on food crops has been reported to promote plant growth, increase the photosynthetic rate, reduce disease severity and enhance yield by 30% (Chao *et al.*, 2005). This work investigates the effects of titanium dioxide nanoparticle and titanium oxide (bulk) applied in a foliar spray at different stages of development (four leafy and stem elongation stage) on the growth and forage yield of maize plants.

MATERIAL AND METHODS

Study site and climate

Field experiment was conducted at the agricultural research farm in Islamic Azad University, Varamin Pishva Branch, Varamin, Iran during the 2012 growing season. The highest and lowest temperature in study site was registered -20 and 38 ^{o}C, respectively. Also mean annual precipitation was recorded 235 mm.

Experimental design and treatments

The experimental design was randomized complete blocks arranged factorial with four replications. The first factor was growth stages at two levels (four leafy stage and stem elongation stage) in addition, different dose of titanium (Control, 0.01%, 0.02%, 0.03% titanium nanoparticle and bulk titanium) were allocated to second factor.

Filed preparation and seed sowing

Prior to experiment soil samples were collected to determine the soil characteristics. The soil had clay loam texture containing 28.6% sand, 25.2% silt and 46.2% clay. The soil pH and EC was measured as 7.7 and 3.4 mmhos.cm^{-1}, restively. When plots were prepared there were 40 plots with 3 m width and 4 m length. There was 2 m alley between plots and blocks to avoid lateral water movement. Before seed sowing 150 kg.ha^{-1} super phosphate triple was mixed into the soil. Corn seeds (*Zea mays* L. Single Cross 704) were sown by hand on 15th June at depth of 3 cm. The plant density was 10 plants per square meter. Irrigation was performed as furrow irrigation, immediately. Second irrigation was done three days after first one to get the best seed germination and seedling establishment. Weeds were controlled manually during growing season. It is worth mentioning that nitrogen fertilizer (300 kg.ha^{-1} Urea) was applied at eight leafy stage and tasseling.

Preparation of titanium dioxide nanoparticles and bulk titanium dioxide

In order to preparation of titanium dioxide nanoparticles 20 g titanium dioxide was dissolved in to the water and then 0.01 ml of solution was filled up to 1000 ml. Thus different concentrations of titanium dioxide (0.01, 0.02 and 0.03%) were prepared. Ultrasound instrument was used to homogeneity of the solution. Bulk titanium dioxide was purchased from Advanced Material Company (United State). To make bulk solution, 6 g titanium was dissolved in to 100 ml distilled water then 1 ml of solution was filled up to 1000 ml.

Titanium foliar application

Titanium dioxide nanoparticles and titanium oxide (bulk) were sprayed on plants by a calibrated pressurized back-pack sprayer (20-l capacity) at four leafy stage and stem elongation. Plants were treated by 500 ml titanium solution per square meter. Control plants were treated by distilled water.

Sampling and date collection

At kernel milk stage, 2 m^2 from middle of each plot was harvested and weighted. Leaves and ears were detached and then leaf, ear and stem fresh weight was registered separately. Stem and ear diameter and stem length were measured. Samples were dried at 50 ^oC for 72 h and then dry weigh was recorded.

Statistical analysis

Analysis of variance and mean comparisons were done via SAS (Ver.9) software and Duncan multiple range test at 5% probability level.

RESULT

Analysis of variance (Table 1) demonstrated that interaction between maize growing stages and titanium foliar application on all traits was significant except for leaf dry weight. Comparison of main effects showed that titanium foliar application at stem elongation increased leaf fresh weight, ear fresh weight, ear diameter, leaf dry weight, stem dry weight, ear dry weight and biological yield while had not significant effect on stem fresh weigh, stem diameter and stem length (Table 2). Ti dioxide nanoparticle (0.02%) application significantly increased leaf fresh weight compared to control (Table 2).

Although, titanium oxide (bulk) application increased leaf fresh weight, there was o significant difference between titanium oxide and titanium dioxide at 0.01 and 0.03% treatments (Table 2). In addition, stem fresh weight increased considerably on account of titanium foliar application whether titanium dioxide or titanium oxide (Table 2). There was obvious increment in ear fresh weight because of titanium foliar application; however, titanium dioxide nanoparticle (0.02%) had more effect on ear fresh weight (Table 2). It is interest to remark that ear diameter significantly increased due to application of both titanium sources. Similar results were observed when titanium was applied on plants so that leaf dry weight increased dramatically. Stem dry weight increased due to titanium dioxide (0.02%) as well. The results showed that ear dry weight increased by titanium foliar application compared to control. Among the titanium treatments titanium dioxide nanoparticle (0.02 and 0.03%) produced the heaviest dried ears (Table 2). Stem diameter affected by titanium application so that titanium dioxide and titanium oxide increased stem diameter compared with control (Table 2). The highest plants were observed from those plots which were treated by 0.02 and 0.03% titanium dioxide nanoparticle or titanium oxide (bulk) (Table 2). Biological yield as the most important trait, increased on account of 0.01 or 0.02% titanium dioxide nanoparticle application. However titanium oxide (bulk) had significance difference with control treatment as well (Table 2). As can be seen form table 3, the highest leaf fresh weight was observed when maize plants were treated titanium dioxide nanoparticle (0.02%). On the contrary the lowest leaf fresh weight was related to control treatment (Table 3).

Table 1. ANOVA result of measured traits

S.O.V	df	Leaf fresh weight	Stem fresh weigh	Ear fresh weight	Ear diameter	Leaf dry weight
Replication	3	27546.42^{ns}	20838.53^{ns}	62303.29^{ns}	19.82^{ns}	3752.10^{ns}
Growth stage (G)	1	170172.02**	3312.40^{ns}	552015.02**	87.025**	5428.90*
Titanium (T)	4	124308.28**	75565.10^{ns}	302328.90**	48.02**	2243.40^{ns}
G × T	4	105066.83**	133316.40*	214752.65*	22.15*	2480.40^{ns}
Error	27	5272.18	42862.90	65579.10	7.13	1121.28
C.V	-	8.61	9.19	24.79	7.16	12.27

*, ** and ns: significant at 0.05, 0.01 probability level and no significant, respectively.

Continue of Table 1. ANOVA result of measured traits

S.O.V	df	Stem dry weight	Ear dry weight	Stem diameter	Plant height	Biological yield
Replication	3	4966.50^{ns}	310.53^{ns}	6.62^{ns}	207.62^{ns}	20980.63^{ns}
Growth stage (G)	1	103428.90**	35742.46**	24.02^{ns}	305.80^{ns}	264712.90**
Titanium (T)	4	77346.90**	12116.84**	58.97**	865.96**	198342.03**
G × T	4	43559.90**	22954.64**	38.77*	475.45**	61181.21**
Error	27	4408.57	1090.69	10.62	93.59	7241.93
C.V	-	8.19	14.94	14.53	4.66	6.49

^{ns}, * and **: non significant, significant at 5% and 1% of probability level, respectively.

Similar results were obtained regarding stem fresh weight, the highest stem fresh weight was produced when 0.01 and 0.02% titanium dioxide nanoparticle were applied (Table 3). Ear fresh weight significantly increased when titanium dioxide nanoparticle was applied at both growing stages. On the other hand, the highest ear diameter was observed when 0.02 titanium dioxide nanoparticle was used at stem elongation stage (Table 3). In addition, we found that leaf dry weight was affected by titanium foliar application. Stem dry weight increased due to 0.01 and 0.02% titanium dioxide nanoparticle; however there was no significant difference between 0.01 and 0.02% titanium concentrations (Table 3). The highest ear dry weight was achieved from treated plants with titanium dioxide nanoparticle (0.02%) at stem elongation stage (Table 3). Stem diameter increased on account of titanium application so that the thickest stems were related to bulk titanium application at four leafy stage (Table 3). Furthermore, the highest plants were related to those plots which were treated by titanium oxide (bulk) at both growing stages (Table 3). Finally the highest biological yield was produced when titanium dioxide nanoparticle (0.02%) was applied at stem elongation stage (Table 3).

Table 2. Mean comparison effect of growth stages and titanium on measured traits

Treatments	Leaf fresh weight	Stem fresh weigh	Ear fresh weight	Ear diameter	Leaf dry weight
Growth stages					
Four leafy	777.45[b]	2241.60[a]	915.30[b]	35.80[b]	261.20[b]
Stem elongation	907.90[a]	2259.80[a]	1150.25[a]	38.75[a]	284.50[a]
Titanium					
Titanium dioxide 0.01	885.00 [b]	2360.80 [a]	897.01[bc]	38.37 [a]	270.50 [ab]
Titanium dioxide 0.02	1000.50 [a]	2334.80 [ab]	1207.80 [a]	38.50 [a]	296.75 [a]
Titanium dioxide 0.03	839.63 [b]	2218.30[ab]	1158.02 [ab]	37.50 [a]	278.00 [ab]
Bulk titanium	833.63 [b]	2217.30[ab]	1141.50[ab]	39.00 [a]	268.50 [ab]
Control	654.63 [c]	2122.50[b]	759.61[c]	33.00 [b]	250.50 [b]

*Similar letters in each column show non-significant difference at 5% level in Duncan's test.

Continue of Table 2. Mean comparison effect of growth stages and titanium on measured traits

Treatments	Stem dry weight	Ear dry weight	Stem diameter	Plant height	Biological yield
Growth stages					
Four leafy	759.00[b]	191.07[b]	23.20[a]	209.98[a]	1228.70[b]
Stem elongation	860.70[a]	250.85[a]	21.65[a]	204.45[a]	1391.40[a]
Titanium					
Titanium dioxide 0.01	837.00 [b]	223.50 [bc]	21.62 [b]	204.75 [b]	1424.38 [ab]
Titanium dioxide 0.02	934.50 [a]	264.66 [a]	25.25 [a]	216.62 [a]	1465.25 [a]
Titanium dioxide 0.03	857.00 [b]	249.25 [ab]	24.25 [ab]	215.28 [a]	1363.00 [b]
Bulk titanium	724.25 [c]	208.75 [c]	22.75 [ab]	208.66 [ab]	1208.88 [c]
Control	696.50 [c]	162.63 [d]	18.25 [c]	190.75 [c]	1088.75 [d]

*Similar letters in each column show non-significant difference at 5% level in Duncan's test.

DISSCUSSION

In general we found that titanium foliar application increases maize growth and forage production. Previously, Nand *et al*. (1983) indicated that foliar spray of water soluble Ti led to significantly higher production of fresh and dry matter yields of the whole plant and leaf and stem dry yields. It is worth mentioning that growth, biomass, productivity and fruit quality of many plant species are improved by titanium, as well as plant concentrations of some essential elements such as nitrogen, phosphorus, calcium and magnesium (Pais, 1983; Carvajal *et al*., 1998). Beneficial effects of titanium are due to intensification of the Fe activity in leaf chloroplasts and fruit chromoplasts and consequently increased metabolic activity and nutrient absorption (Carvajal *et al*., 1998; Carvajal and Alcaraz, 1995). From the plant nutrition point of view, titanium has been suggested to play a role in the fixation of nitrogen by legumes and increasing the yields of crop plants (Pais *et al*., 1977). In addition, beneficial effects of titanium on various physiological parameters, biomass yield (Pais *et al*., 1977), essential element contents (Alcaraz *et al*., 1990), chlorophyll contents (Simon *et al*., 1988; Carvajal *et al*., 1994) has been reported. Also it has been reported that titanium dioxide nanoparticles improve spinach (*Spinacia oleracea*) seed germination and plant growth (Zheng 2005).

Table 3. Mean comparison interaction effect of growth stages and titanium on measured traits

Growth stages	Titanium	Leaf fresh weight	Stem fresh weigh	Ear fresh weight	Ear diameter	Leaf dry weight
Four leafy	Titanium dioxide 0.01	826.00 cd	24569.5 ab	910.0 cd	38.125 abc	264.00 bc
	Titanium dioxide 0.02	768.00 de	2295.5 ab	1406.5 ab	34.750 cde	271.00 abc
	Titanium dioxide 0.03	890.00 bc	2221.0 bc	1139.0 abcd	37.250 cd	295.00 ab
	Bulk titanium	720.00 def	2108.5 c	1009.0 bcd	36.250 cde	272.00 ab
	Control	683.25 ef	2123.5 bc	766.0 d	32.500 e	229.00 c
Stem elongation	Titanium dioxide 0.01	944.00 b	2262.0 abc	1406.5 ab	38.500 abc	277.00 abc
	Titanium dioxide 0.02	1233.00 a	2561.0 a	1510.5 a	42.250 a	322.50 a
	Titanium dioxide 0.03	789.25 cde	2215.5 bc	1177.0 abc	37.750 bcd	261.00 bc
	Bulk titanium	947.25 b	2139.0 bc	884.0 cd	41.750 ab	290.00 ab
	Control	626.00 f	2121.5 bc	773.3 cd	33.500 de	247.00 bc

*Similar letters in each column show non-significant difference at 5% level in Duncan's test.

Continue of Table 3. Mean comparison interaction effect of growth stages and titanium on measured traits

Growth stages	Titanium	Stem dry weight	Ear dry weight	Stem diameter	Plant height	Biological yield
Four leafy	Titanium dioxide 0.01	920.00 a	266.50 bc	22.000 ab	200.000 cd	1441.50 bc
	Titanium dioxide 0.02	885.50 ab	159.50 e	24.500 ab	209.325 bcd	1193.25 ef
	Titanium dioxide 0.03	651.00 ed	180.50 e	22.000 ab	205.250 bcd	1126.50 fg
	Bulk titanium	695.00 cde	200.50 ed	27.000 a	230.325 a	1344.00 cd
	Control	643.50 e	148.50 e	20.500 b	184.750 e	1038.25 g
Stem elongation	Titanium dioxide 0.01	983.50 a	180.50 e	25.000 ab	213.000 bc	1481.00 b
	Titanium dioxide 0.02	979.00 a	370.00 a	24.000 ab	208.000 bcd	1655.50 a
	Titanium dioxide 0.03	795.50 bc	237.00 cd	23.500 ab	204.250 cd	1291.25 ed
	Bulk titanium	794.00 bc	290.00 b	21.250 b	220.250 ab	1382.00 bcd
	Control	794.50 cd	176.75 e	18.500 c	196.750 de	1139.25 fg

*Similar letters in each column show non-significant difference at 5% level in Duncan's test.

In this study we observed that titanium dioxide nanoparticle was more effective on plant growth and dry matter production rather than titanium oxide (bulk). Ruffini and Cermonini (2009) suggested that nanoparticles can explain their actions depending on both the chemical compound and on the size and/or shape of the particles. Zheng *et al*. (2005) reported that the significant effect of nano-sized titanium on spinach germination is probably attributed to the small particle size, which allows its penetration into the seed during the treatment period, exerting its enhancing functions during growth. It seems that bulk titanium could not penetrate into the seeds of wheat in the same way which Zheng *et al*. (2005) reported. In conclusion our results indicated that foliar application of titanium dioxide nanoparticle can improve maize growth and forage production. It is worth to remark that titanium dioxide nanoparticle at 0.02% concentration was identified as the best treatment to improve forage yield and it would be recommend attaining high forage yield.

CONCLUSION

Titanium dioxide nanoparticle especially at stem elongation stage increased plant growth and dry matter accumulation in maize plant. Although, titanium oxide (bulk) had positive effect on biological yield, titanium dioxide nanoparticle produced the highest forage yield. In addition, among different concentrations of titanium dioxide nanoparticle, application 0.02% significantly improved plant growth; dry matter and final forage yield so that it can be recommended to earning more forage.

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