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Original article

Effects of TiO_2 nanoparticles on morphological characteristics of chickpea (*Cicer arietinum* L.) under drought stress

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Extended abstract

Introduction

Drought stress is one of the most important environmental stresses affecting plant growth and yield. The use of nanoparticles can decrease the impact of drought stress. Therfore, the effect of spraying titanium dioxide nanoparticles (TiO_2-NPs) on chickpea was done under drought stress conditions, The experiments were conducted using a completely randomized design with three replications in greenhouse conditions. Different levels of drought stress consisted of 40, 60 and 90% as control of filed capacity and spraying titanium dioxide nanoparticles at five levels of 0, 5, 10, 20 and 40 mg/L. Increasing the concentration of TiO_2-NPs to 20 mg/L in the lowest of irrigation level increased the relative content of chlorophyll, stomatal conductivity, leaf number, leaf area, leaf dry weight, shoot dry weight, total root length, root diameter, root area and root volume in chickpea compared to control sample. With the increasing concentration of TiO2-NPs, these parameters showed a decreasing trend at all levels of drought stress. TiO_2-NPs increased the osmotic potential compared to control at all three levels of irrigation. Shoot dry weight at 90, 60 and 40% of field capacity was increased after treatment with 20 mg/L TiO_2-NPs 21, 28 and 42% , respectively. Furthermore, root dry weight was enhanced by 54, 49 and 23% at 90%, 60% and 40% of field capacity by using 20 mg/L TiO_2-NPs. Generally, based on the results obtained, the application of titanium dioxide nanoparticles at all three levels of field capacity at a concentration of 20 mg/L could moderate the negative effects of drought stress on the physiological and morphological characteristics of the plant.

Materials and methods

The experiment was carried out as a factorial experiment with three replications in a completely randomized design in a greenhouse. The morphological and physiological characteristics of the plant were assessed at different levels (40, 60 and 90 percentage) of field capacity (FC). FC was measured by calculating the amount of soil humidity. The titanium dioxide nanoparticles (TiO_2-NPs) are used in five concentration including 0, 5, 20, 10 and 40 mg/L. Firstly characteristics of nanoparticles were investigated by measuring zeta potential, XRD and TEM. Secondly, a 100 mg/l mother solution was prepared in deionized water. TiO_2-NPs were dispersed by ultrasonic bath for 40 min before sprayng the solution on the plants. The plants completely were soaked by sprayed solution 4 times each 14 days .Finally after the growth duration some morphological and physiological parameters were measured.

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The data were analyzed using ANOVA with Statistical Analysis System (Minitab .17) software and the significance of difference between means was determined by Tuky test.

Result and discussions

The results showed that the leaf area of chickpea was significantly affected by the test factors and their interactions. A 35%-increase in leaf area was observed at the lowest level of irrigation after exposure to 20 ppm of TiO_2-NPs. Chlorophyll index of chickpea was significantly affected by the test factors and their interactions. The interaction of two test factors showed that with an increase in the concentration of TiO_2-NPs to 20ppm, chlorophyll index of chickpea was increased in all levels of irrigation. At all levels of irrigation, using the concentration of 40 ppm of TiO_2-NPs, the chlorophyll index in chickpea leaves was reduced compared to the concentration of 20 ppm. The highest osmotic potential was observed in 40% capacity after treatment with 5 and 10 mg/L titanium dioxide nanoparticles. There was no significant differences between 40 and 20 mg/L at this level of irrigation. At all irrigation levels, the application of the nanoparticle produced the highest osmotic potential, and thus, the use of nanoparticles increases the osmotic potential compared to control plants. Osmotic regulation under the water shortage conditions decreases cellular inflammation by maintaining water and collecting material in the cell. Also, the percentage of dry weght of the whole chickpea plants were significantly affected by the test factors and their interactions. The interaction of two factors showed that with the increasing concentration of TiO_2-NPs to 20 ppm, the dry weight of chickpea in the lowest irrigation level was increased by10% compared to control plants. Stomatal conductivity in all irrigation levels had an upward trend by using TiO2-NPs.

Conclusion

Generally, TiO_2-NPs showed a positive effect on the total dry and fresh weight of the whole chickpea plants. The application of 20 ppm anatase TiO_2-NPs at all levels of irrigation reduced drought stress and prevented further plant losses. The application of low concentration of nanoparticles promoteed plant growth and at high levels showed inhibitory effects on growth. Taken all together, due to the increasing use of chemical fertilizers in agriculture, nano compounds can be used as an appropriate alternative that increases product quality.

***Keywords**:* Chlorophyll Index, Field Capacity, Leaf Area, Root, Stoma conductivity.

Table 1. Physochemical properties of soils culture.

C	Electrical conductivity	$CaCo_3$	P	K	N	Soil texure
(%)	($dS.m^{-1}$)	(%)	($mg.kg^{-1}$)	($mg.kg^{-1}$)	(%)	Loamy sand
3.5	2.12	13.00	7.00	151	0.05	

Table 2. Characteristics of Titanium dioxide nanoparticles (TiO_2-NPs).

Crystalline mean size	Crystalline phase	Surface	True density	Bulk density	Molecular weight
(nm)		($m^{-2}.g$)	($g.cm^{-3}$)	($g.cm^{-3}$)	(g.mol)
13	Tetragonal	200-240	3.91	3.91	79.9

Table 3. Mean squars, source of variation and degree freedom of morphologic chracteristics of chickpea under different concentration of TiO_2-NPs and drought stress.

	Source of varation				
	Field capacity(FC)	Nanoparticles TiO_2(TiO_2-NPs)	FC×TiO_2 (TiO_2-NPs)	Erorr	CV%
df	2	4	8	30	
Spad	262^{**}	26.0^{**}	2.68^{**}	0.441	*2.62*
Conductive stoma	222^{**}	50.7^{**}	4.04^{*}	1.53	*8.8*
Potential osmotic	1.049^{**}	0.046^{**}	0.012^{**}	0.002	*5.02*
Plant highet	148^{**}	4.78^{ns}	1.17^{ns}	4.93	*7.05*
Number of leaves	49.9^{**}	8.39^{**}	1.49^{*}	0.531	*4.86*
Leaves area	27722^{**}	15355^{**}	2353^{**}	213	*3.3*
Dry Leaf weight	0.118^{**}	0.036^{**}	0.003^{**}	0.0009	*8.43*
Dry stem weight	0.070^{**}	0.021^{**}	0.009^{**}	0.0003	*7.07*
Leaf/shoot	0.040^{**}	0.059^{**}	0.100^{**}	0.004	*5.045*
Dry shoot weight	0.037^{**}	0.011^{**}	0.019^{**}	0.0015	*6.41*

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

Table 4. Effect of different concentrations of TiO_2-NPs on relative chlorophyll content, stoma conductivity, osmotic potential, high stem, leaf number and leaf area under different field capacity.

Field capacit	Nanoparticles Titanium dioxide	Spad	Stomatal conductance	Osmotic potential	Plant highet	Number of leaves	Leaf area
(%)	(mg)		$Mmol.m^{-2}s$	Mpa	cm		$cm.plant^{-1}$
	0	28.0^{bc}	16.8^{bc}	0.50^{h}	33.4^{a}	16.5^{bc}	561^{ab}
	5	27.7^{b-d}	16.5^{bc}	0.66^{fg}	34.5^{a}	16.1^{b-d}	572^{ab}
90	**10**	28.7^{b}	16.6^{bc}	0.78^{ef}	36.4^{a}	16.3^{b-d}	555^{bc}
	20	33.2^{a}	23.5^{a}	0.65^{fg}	35.8^{a}	19.1^{a}	602^{a}
	40	27.4^{b-e}	15.4^{b-d}	0.56^{gh}	34.5^{a}	15.0^{b-f}	497^{d}
	0	26.0^{c-f}	12.7^{d-f}	0.90^{de}	29.6^{a}	15.0^{b-f}	506^{d}
	5	25.7^{d-g}	13.7^{c-e}	0.97^{cd}	31^{a}	15.5^{b-e}	513^{cd}
60	**10**	25.6^{e-g}	15.0^{b-d}	1.01^{b-d}	31^{a}	14.3^{c-g}	480^{d}
	20	27.6^{b-e}	18.3^{b}	0.82^{e}	31.8^{a}	16.7^{b}	472^{d}
	40	25.4^{fg}	11.3^{e-g}	1.01^{b-d}	30.6^{a}	15.1^{b-f}	414^{e}
	0	18.8^{i}	9.5^{fg}	1.05^{bc}	28.4^{a}	13.0^{f-h}	279^{f}
	5	20.3^{hi}	9.3^{fg}	1.25^{a}	29.1^{a}	12.0^{h}	288^{f}
40	**10**	21.4^{h}	10.7^{eg}	1.20^{a}	30^{a}	13.3^{f-h}	310^{f}
	20	23.9^{g}	12.2^{dg}	1.13^{ab}	28.1^{a}	14.1^{d-h}	377^{e}
	40	19.3^{i}	8.6^{g}	1.13^{ab}	28.3^{a}	12.6^{gh}	207^{g}

Mean with common letter(s) in each column are not statictically significant at 5% probability levels.

Table 5. Effect of different concentrations of TiO_2-NPs on leaf dry weight, stem dry weight, leaf/shoot and shoot dry weight of chickpea under different field capacity.

Field capacit	Nanoparticles Titanium dioxide	Leaf dry weight	Stem dry weight	Leaf/Shoot	Shoot dry weight
(%)	(mg)	(g)	(g)		(g)
	0	0.38^{cd}	0.29^{bc}	1.32^{c-e}	0.67^{c-e}
	5	0.42^{bc}	0.28^{bc}	1.49^{ab}	0.70^{b-d}
90	**10**	0.49^{ab}	0.32^{b}	1.62^{a}	0.81^{b}
	20	0.58^{a}	0.54^{a}	1.07^{g}	1.13^{a}
	40	0.38^{cd}	0.28^{bc}	1.24^{c-f}	0.66^{c-e}
	0	0.30^{d-g}	0.27^{bc}	1.14^{e-g}	0.58^{ef}
	5	0.32^{d-f}	0.26^{b-d}	1.20^{d-g}	0.59^{d-f}
60	**10**	0.38^{cd}	0.26^{b-d}	1.38^{b-d}	0.65^{c-e}
	20	0.44^{bc}	0.29^{bc}	1.46^{a-c}	0.74^{bc}
	40	0.28^{e-g}	0.26^{b-d}	1.10^{fg}	0.56^{e-g}
	0	0.24^{fg}	0.19^{ef}	1.21^{d-g}	0.43^{h}
	5	0.23^{g}	0.17^{f}	1.30^{c-e}	0.40^{h}
40	**10**	0.25^{fg}	0.20^{ef}	1.22^{d-g}	0.45^{gh}
	20	0.36^{c-e}	0.24^{c-e}	1.55^{ab}	0.61^{d-f}
	40	0.30^{d-g}	0.21^{d-f}	1.32^{c-e}	0.51^{f-h}

Mean with common letter(s) in each column are not statictically significant at 5% probability levels.

Table 6. Mean squars, source of variation and and degree freedom of root chracteristics of chickpea under different concentration of TiO_2-NPs and drought stress.

Source of variation	df	Total length root	Root diameter	Root area	Root volume	Root dry weight
Field capacity(FC)	2	1909935^{**}	0.0009^{**}	16309^{**}	25.9^{**}	0.074^{**}
Nanoparticles TiO_2(TiO_2-NPs) FC×TiO_2	4	6391298^{**}	0.0002^{**}	11055^{**}	0.53^{**}	0.032^{**}
FC×TiO_2(TiO_2-NPs)	8	1075740^{**}	3.80^{**}	2489^{**}	0.175^{**}	0.006^{*}
Erorr	30	6.32	8.83	56.25	0.0125	0.002
C.V%		*0.094*	*3.73*	*5.12*	*4.47*	*12.43*

** Significant at 1% level of probability

Table 7. Effect of different concentrations of TiO_2-NPs on total root length, average root dimeter, root volume, and root dry weight of chickpea under different field capacity.

Field capacity	Nanoparticles titanium dioxide	Total root length	Root diameter	Root area	Root volume	Root dry weight
(%)	(mg)	($mm.plant^{-1}$)	(mm)	($cm.plant^{-1}$)	($cm^3.plant^{-1}$)	($g.plant^{-1}$)
	0	2281^{h}	0.029^{c}	107^{f-h}	3.75^{b}	0.39^{b-e}
	5	2055^{j}	0.029^{c}	109^{f-h}	3.70^{b}	0.39^{b-e}
90	**10**	2807^{f}	0.035^{b}	125^{d-f}	4.12^{a}	0.45^{b-e}
	20	3181^{e}	0.045^{a}	143^{d}	4.20^{a}	0.60^{a}
	40	2084^{i}	0.024^{de}	98^{gh}	3.18^{c}	0.53^{ab}
	0	1761^{m}	0.019^{f}	103^{gh}	2.43^{ef}	0.35^{de}
	5	1987^{i}	0.022^{e}	116^{e-g}	2.15^{f}	0.38^{c-e}
60	**10**	2527^{g}	0.024^{de}	126^{d-f}	2.68^{de}	0.41^{b-e}
	20	4006^{c}	0.034^{b}	185^{c}	2.81^{d}	0.52^{a-c}
	40	1767^{m}	0.025^{d}	137^{de}	2.72^{de}	0.47^{a-d}
	0	1383^{n}	0.012^{h}	91.6^{h}	1.08^{h}	0.30^{e}
	5	2044^{k}	0.017^{fg}	142^{d}	1.06^{h}	0.34^{de}
40	**10**	4232^{b}	0.017^{fg}	219^{b}	1.12^{h}	0.35^{de}
	20	4302^{a}	0.022^{e}	250^{a}	1.56^{g}	0.37^{c-e}
	40	3341^{d}	0.015^{gh}	199^{bc}	1.00^{h}	0.31^{e}

Mean with common letter (s) in each column are not statictically significant at 5% probability levels.

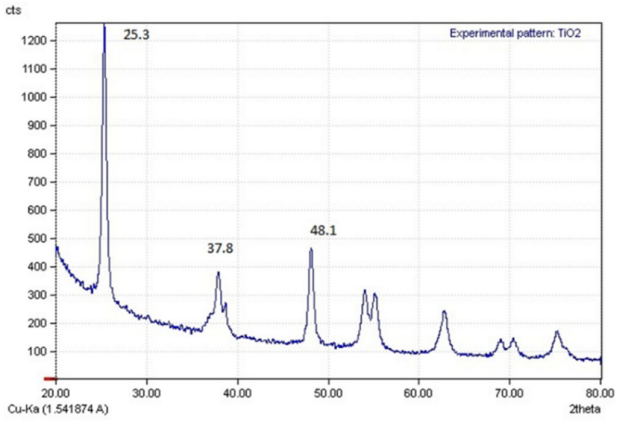

Fig 1. Image of XRD Patrren of Titanium dioxide (TiO_2-NPs) by using a X-ray diffractometer

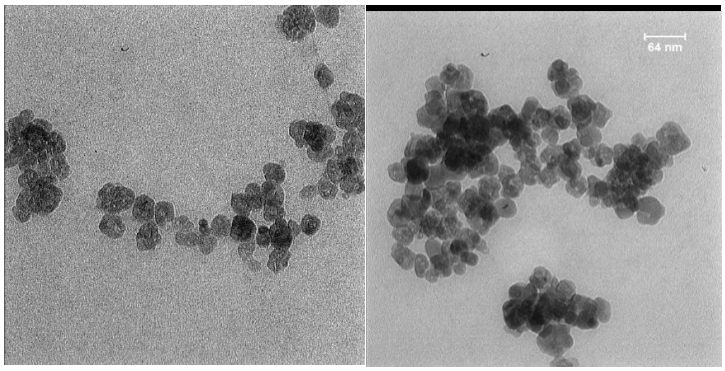

Fig. 2. Image of Titanium dioxide nanoparticles (TiO_2-NPs) prepared by transmission electron microscope (TEM).

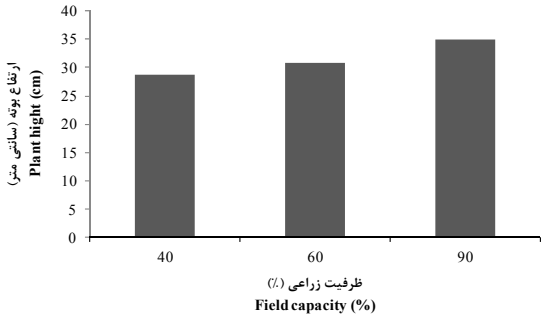

Fig. 3. Effect of diffrent moisture regimes on plant hight of chickpea.