



Evaluation of the application of gibberellic acid and titanium dioxide nanoparticles under drought stress on some traits of basil (*Ocimum basilicum* L.)

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Abstract

This study is carried out to study the effect of *Gibberellic acid* (GA₃) and *Titanium Dioxide Nanoparticles* (Nano-TiO₂) on some characteristics of medicinal plant of *Ocimum basilicum Lamiaceae*) under drought stress. The experiment was conducted as a factorial arrangement in randomized complete block design with four replications in which A, B, C are the three factors and factor A is related to the irrigation content as fc 100%, fc 70% and fc 40% and factor B in three levels with GA₃ application with concentrations of 0 (control), 250 ppm, 500 ppm and factor C with three doses of titanium nanoparticles with concentrations of 0%, 0.01% and 0.03%. The results showed that the drought stress caused to decreasing of plant biomass and the foliar relative water content, and the increasing of catalase and the level of anthocyanin in the medicinal plant of basil, while, the application of gibberellin and titanium nanoparticles caused to improving of the negative effects of the stress. The results of the study indicated that the drought stress causes to decreasing of quantitative and qualitative characteristics of the plant. The best treatments were recognized as follows: in 100% irrigation regime, non-application of gibberellin and application of *Nano-TiO₂* with concentration of 0.01%; in 70% irrigation regime, the application of gibberellin with concentration of 250 ppm and *Nano-TiO₂* with concentration of 0.03%; and in 40% irrigation regime, the application of gibberellin with concentration of 500 ppm and *Nano-TiO₂* with concentration of 0.03%.

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Introduction

Drought is one of the commonest environmental stresses which confines about 25% of cultivating plants. In terms of the amplitude of the realm of drought stress, the priority is with dry and semi-dry areas. Drought stress belongs to the general stresses which hinders the growth of the plant and have undesirable effects on the plant performance. Water is the main growth limiting factor in these areas (Kaafi, 2006). Titanium nanoparticles increase the plant resistance against environmental stresses and decrease the free radicals. Doing this, in order to convert O_2 free radicals to O_2 , it changes from Ti^{4+} to Ti^{+3} and in order to retain its shape, it converts O_2 to H_2O_2 and then changes to Ti^{4+} (Hong *et al*, 2007). Titanium nanoparticles increases the performance of the plant through increasing the first photosystem light energy that is absorbed by chloroplast membrane and is transferred to the second photosystem and then by converting light energy to electrons energy, electrons transmission, and also by reinforcement and development of water photolysis and releasing oxygen (Mingo *et al*, 2007). Due to its smaller size, *Nano-TiO₂* can easily catalyze oxidation and regeneration reactions and accelerate the release of full energy electrons (Young *et al*, 2006). Since it causes to increasing of plants' meiosis and size, *TiO₂* is recommended as a growth regulator which acts like Gibberellin and Cytokinin (Albrecht *et al*, 2006). Using nanoparticles in plants leads to less chemical composts consumption which is in line with our objective to make use of modern agricultural technologies to boost performance and specifically, increasing essence and active ingredients for medicinal purposes. The application of nanoparticles on agricultural and medicinal plants affects the photosynthesis system, hence increases the photosynthesis and biomass of the plant. It is worth noting that in the present situation, increasing agricultural production to meet the needs of the growing population is very difficult. Also, having knowledge and research plans in physiologic and biologic grounds for stress resisting will help protect plants against the negative effects of stresses a lot. Therefore, attempts should be done to solve this

problem and provide better conditions for the plant regarding the application of anti-stress materials in the environment. It is possible to decrease the effects of drought stress on basil by the application of hormones and nanoparticles.

Materials and methods

Field conditions and treatments

This experiment was carried out in 2013 as factorial arrangement in randomized complete block design with four replications at the center for training and plant consultancy of Tehransar, region 21st of municipality of Tehran (49° E 46 .51' 15 35 42 11 29 " N). The alluvial sandy-loam type of soil was used in the experiment. Factor A was related to the irrigation amount as fc 100%, fc 70% and fc 40% and factor B in three levels with GA₃ application with concentrations of 0 (control), 250, 500 ppm and factor C with three doses of titanium nanoparticles with concentrations of 0%, 0.01% and 0.03%. 108 black pots with the height of 24 cm and opening diameter of 23 cm were filled with field soil up to 19 cm height. Pot drainages were built in order to prevent water logging. The first irrigation was done two days after cultivation and after that the irrigation was done every 2 to 4 days until the end of plant's growth depending on the growth stages and the existing water in the pot. It is worth noting that a heavy irrigation to soil saturation level was done 48 hours before cultivation of basil seeds. After two days, the pot soil was prepared for cultivation and the soil humidity was appropriate for blossoming, the cultivation of seeds was done on 7th May 2013. The soil was disinfected by Benomyl (2 to 1000) to avoid fungal and other pathogenic contaminations. 40 seeds were cultivated in each pot and were irrigated two days later. Basils blossomed 4 to 8 days after cultivation. After plants' growth and having them thinned, 20 plants remained in each pot. The low-irrigation stress was applied observing the appropriate time intervals after passage of different growth stages. The total application of titanium nanoparticles and gibberellin acid observing the standards mentioned earlier was done before sunrise or at the sunset. These treatments were applied during two phonological stages of growth with 50%

stem elongation (vegetative stage) and 50% flowering (natal stage) in 13th June, 2013 and 14th August, 2013, respectively in a way that the whole stems along with upper and lower leaves of all bushes except for control treatment bushes (0% concentration) were impregnated with this solution and the required notes were taken. The sampling was done after the application of the required spraying observing the appropriate time interval. The second phase of spraying was done in which to prepare gibberellin concentrations, the required amount of hormone powder was weighed in and bulked with distilled water and dissolved completely with electric mixer. After the solution was made in the laboratory, the spraying was done on all the considered bushes at the sunset as mentioned before and the sampling was done one month later to study the biochemical characteristics such as carotenoids, anthocyanin, and catalase.

Catalase Calculation

The Dhinsa and Motowe method (1981) was used to calculate the amount of catalase. The reactive compound which comprised 50 mM potassium phosphate (PH=7), 15 mM hydrogen peroxide, and 100 microliter enzyme distillate was used to evaluate this enzyme. The catalysis of hydrogen peroxide was followed by absorption decrease in 240 nanometer wavelength using spectrophotometer and expressed as per one mg protein in enzyme distillate.

Anthocyanin Calculation

The amount of anthocyanin was calculated by Wanger method (1979). For this purpose, 0.1 gr of leaf tissue was mixed by 10 mL acidic methanol (pure methanol and pure chloridric acid with volume factor to be at 1:99). The distillate of each treatment was put at 25°C for 24 hours and then was centrifuged at 4000 rpm for 10 minutes. The solution was poured in cell to be evaluated by spectrophotometer in 550 nanometer wavelength. The following formula has been used to calculate the amount of anthocyanin: $A_{Abs\ 550} = \epsilon bc$, $\epsilon=33000$ where "A" stands for the read absorption equivalence, "B" stands for Cout width (1 cm), and "C" for

Anthocyanin concentration in terms of mM/g fresh weight.

Biomass Calculation

In order to calculate the biomass, before the shoot is dried, it is removed from the crown and it was dried in an oven at 75°C for 48 hours, then it was weighed by a digital scale.

Relative water content

The fresh weight of two leaves sliced from each treatment was measured, then they were put into distilled water for 4 hours in low-light intensity to determine the Turgescence weight. After that, the slices were dried for 48 hours at 80°C and the RWC was determined through the following equation:

$$RWC = \frac{\text{fresh weight} - \text{dry weight}}{\text{Turgescence weight} - \text{dry weight}} \times 100\%$$

Statistical analysis

In this experiment, the statistical analysis was carried out by SAS 9 software. It is worth noting that the calculations were done after performing normal data test.

Results

Biomass

The main effects of drought stress, and the application of *Nano-TiO₂* and the reciprocated drought and Gibberellin stress on biomass trait on the statistical level of 1% and the main effects of gibberellin and the reciprocated effects of drought stress and *Nano-TiO₂* and the reciprocated effects of gibberellin and *Nano-TiO₂* on the statistical level of 5% was significant on this trait. However, the reciprocated triple effects of drought stress, Gibberellin, and *Nano-TiO₂* on this trait were not statistically significant (Table 1).

The of application of 250 ppm of gibberellin and control treatment had the highest amounts of basil biomass in the 100% irrigation regime with 15.09 g, and 16.24 g, respectively. However and regarding these amounts, the control treatment obtained the lowest biomass with 10.98 g and 5.35 g in drought

stress of 70% and 40%, respectively, Khazeh *et al* (2015). The spraying treatment of 250 ppm and 500 ppm of gibberellin in 70% and 40% drought stress

obtained higher amount of biomass as compared to other treatments in the same irrigation regime (Table 1).

Table 1. The variance results of Basil characteristics in evaluating the effects of drought stress of Gibberellin and Titanium nanoparticles.

Variables	Degree of Freedom	Biomass	Foliar relative water content	Catalase	Anthocyanin
Replications	3	47/1	**263/51	8067/62 ns	88/67 ns
Drought Stress (A)	2	39/570**	**5627/82	659176/05**	**4149/29
Gibberellin (B)	2	14/97*	162/81*	17771/97*	1039/21**
Nano-TiO ₂ (C)	2	46/28**	138/16*	25045/84**	1096/57**
× Ba	4	26/33**	** 156/13	28297/42**	229/33**
× Ca	4	93/11*	**189/79	8852/62 ns	212/38**
× Cb	4	01/15*	ns1/56	24206/95**	108/26 ns
B×Ca×	8	44/8 ns	ns0/14	19009/92**	284/57**
Mistake		50/4	33/87	87/87	47/87
Changes Index		16/58	18/16	19/40	19/99

Table 2. The mean comparison of the reciprocated effects of drought stress and different concentrations of TiO₂ on the traits.

Biomass (gr)	Foliar relative water content (%)	Anthocyanin weight)	(mM/g fresh TiO ₂ (%)	Drought stress
15/18A	90/23AB	20/16E	(control) 0	FC %100
16/30A	93/31A	23/96E	0/01	FC %100
14/67AB	86/32B	23/26E	0/03	FC %100
10/28D	71/05D	30/10D	(control) 0	FC %70
12/46 C	72/62D	40/66 BC	0/01	FC %70
13/14BC	78/69C	44/72B	0/03	FC %70
6/26 F	61/53F	36/64 C	(control) 0	FC %40
7/44EF	65/14EF	39/87 BC	0/01	FC %40
8/64 E	69/17 ED	52/02A	0/03	FC %40

** significant in statistical level of 1%; * significant in statistical level of 5%; ns: not significant.

The application of 0.01% nanoparticle in 100% irrigation regime with control and 0.03% treatments showed no difference in terms of biomass creation as compared with other treatments in this irrigation regime. However, it had the maximum amount of biomass with 16.30 g while the same control treatment in the 40% irrigation regime obtained the minimum amount of biomass with 6.26 g that showed no great statistical difference with 0.01% titanium nanoparticles with 7.44 g of biomass in the same irrigation regime. Having only 8.64 g of biomass, they

showed the minimum amount as compared with the application of 0.03% titanium nanoparticles (Table 2) (Figure 1).

Navaro *et al* (2008) also showed that Nano-TiO₂ can store nutrient elements on their surface and act as the nutrition source for the plant. Table 3 shows that the highest amount of biomass (13.73 g) is obtained by the application of compound treatment of 500 ppm gibberellin and 0.03% titanium nanoparticle and lowest amount (10.10 g) comes from the compound

treatment of 500 ppm gibberellin and non-application of titanium nanoparticles. The latter showed no great statistical difference (10.10 g) with the non-application of gibberellin and 0.03% titanium nanoparticles. Based on the studies on Spearmint by Nadjafi (2006), decreasing irrigation cycle from 21 days to 7 days caused the increasing of the biomass. Studies carried out by Ghaffari *et al*, (2012) indicated that the drought tension led to decreasing the dry weight of Canola, but the application of gibberellin caused to increasing this trait under drought stress. Akbari *et al*, (2008)

reported that under gibberellin stress, the biomass of Vetch increased. Pandi *et al* (2010) showed that the consumption of *Nano-ZnO* through increasing in the surface of *Indole acetic acid* in the root of pea caused to increase the plant growth. Ashkani *et al*, (2007), has also stated that the biomass trait was affected under the water stress significantly, and therefore in this study, the highest biomass is observed when the water is supplied 100% for the plant. Mahajan *et al*. (2011) determined that the seedling's root and stem of vetch and pea that were under *Nano-ZnO* treatment, had higher growth rate compared with control plant.

Table 3. The mean comparison of the reciprocated effects of drought stress and Gibberellin on the traits.

Biomass (gr)	Foliar relative water content (%)	Catalase (mg/mmol)	(Protein Anthocyanin (mM/g fresh weight)	Gibberellin (ppm)	Drought stress
A16/24	A92/30	ED 259/28	D21/50	0	100 %FC
AB15/09	A89/39	EF230/00	D24/42	250	100 %FC
AB14/82	A88/18	F199/58	D21/45	500	100 %FC
DC10/98	C70/72	D297	C31/79	0	%70FC
B13/37	B78/92	D310/40	42/24B	250	%70FC
C11/53	BC74/72	C416/05	B41/44	500	%70FC
F5/35	E61/20	BC 465/37	C32/01	0	%40FC
E7/34	DE65/24	AB501/26	A48/15	250	%40FC
D9/66	DC 69/40	A530/17	A48/37	500	%40FC

Table 4. The mean comparison of the reciprocated effects of gibberellin and *TiO2* on the trait

Gibberellin (ppm)	<i>TiO2</i> (%)	Catalase (protein mg/mmol)
0	0 (control)	330/99dc
0	0/01	355/85 bc
0	0/03	334/82 dc
250	0 (control)	352/75bc
250	0/01	285/05d
250	0/03	403/88ab
500	0 (control)	327/11dc
500	0/01	397/74ab
500	0/03	420/96a

(The similar alphabets in each column do not have statistically significant difference).

Anthocyanin

The main effects of drought stress, and the application of *Nano-TiO2* and the reciprocated drought and gibberellin stress on biomass trait on the statistical level of 1% and the main effects of gibberellin and the reciprocated effects of drought stress and *Nano-TiO2* and the reciprocated effects of

gibberellin and *Nano-TiO2* on the statistical level of 5% was significant on this trait. However, the reciprocated triple effects of drought stress, gibberellin, and *Nano-TiO2* on this trait were not statistically significant (Table 1).

Table 5. The mean comparison of the reciprocated effects of drought stress, gibberellin, and TiO_2 on traits.

Catalase mg/mmol)	(Protein Anthocyanin (mM/g fresh weight)	Nano-TiO ₂ (%)	Gibberellin (ppm)	Drought stress
ji236/85	i10/47	0	0	100
fghi 308/92	fgh28/69	0/01	0	100
ji232/09	gh25/33	0/03	0	100
k119/73	fgh29/39	0	250	100
fghij301/95	h22/18	0/01	250	100
jhi268/33	h21/70	0/03	250	100
jki196/16	h20/62	0	500	100
jk191/21	h21/01	0/01	500	100
jki212/37	h22/73	0/03	500	100
eggh 360/86	efg34/58	0	0	70
ji242/71	h22/77	0/01	0	70
ghij 287/44	def38/02	0/03	0	70
jhi274/75	fgh28/47	0	250	70
jhi249/03	abcd49/11	0/01	250	70
sdef407/43	abcd49/13	0/03	250	70
ghij289/78	fgh27/24	0	500	70
abdc496/18	abc50/09	0/01	500	70
bcd462/20	abcd46/99	0/03	500	70
defg395/25	gh25/97	0	0	40
abc515/90	gh23/40	0/01	0	40
abcd484/94	abcd46/65	0/03	0	40
abcd481/53	cde41/24	0	250	40
abcd486/38	abcd49/13	0/01	250	40
ab535/86	ab54/08	0/03	250	40
abcd496/38	bcd42/72	0	500	40
abcd505/82	abcd47/08	0/01	500	40
a588/32	a55/33	0/03	500	40

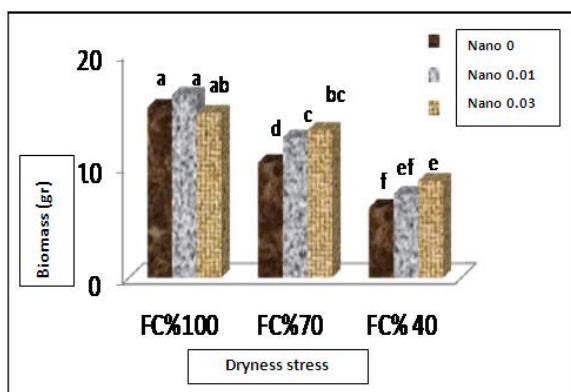


Fig. 1. The reciprocated effects of dryness stress and Titanium dioxide on Biomass.

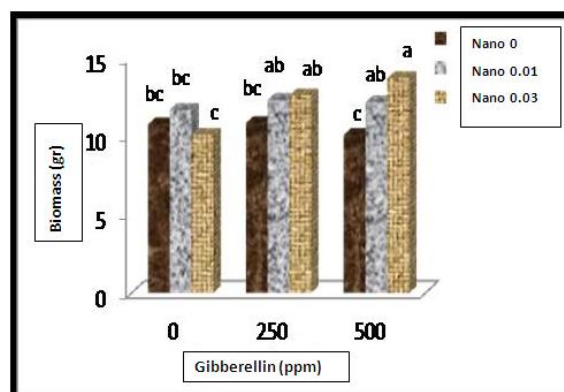


Fig. 2. The reciprocated effects of Gibberellin and Titanium dioxide onnanoparticles on Biomass.

The main effects of drought stress, gibberellin, and $Nano-TiO_2$ and the reciprocated effects of drought stress and gibberellin, the reciprocated effects of drought stress, and the application of $Nano-TiO_2$ the reciprocated triple effects of drought stress, gibberellin, and $Nano-TiO_2$ on anthocyanin trait were significant in statistical level of one percent (Table 1).

As shown in table 2, the highest amount of anthocyanin is obtained by the irrigation regimes of 40%, 70% and 100% and the compound treatment of 500 and 250 ppm gibberellin (48.37 and 48.15 mM/g fresh weight, respectively) and lowest amount (21.45 mM/g fresh weight) comes from the treatment of 500 ppm gibberellin in 100% irrigation regime. The latter

showed no great statistical difference (21.50 mM/g fresh weight) with the non-application of gibberellin and the application of 250 ppm gibberellin (24.42 mM/g fresh weight) in the same irrigation regime. Likewise, in 40% and 70% irrigation regimes, the application of 250 and 500 ppm gibberellin obtained high amounts of anthocyanin and were categorized in one group (Table 1).

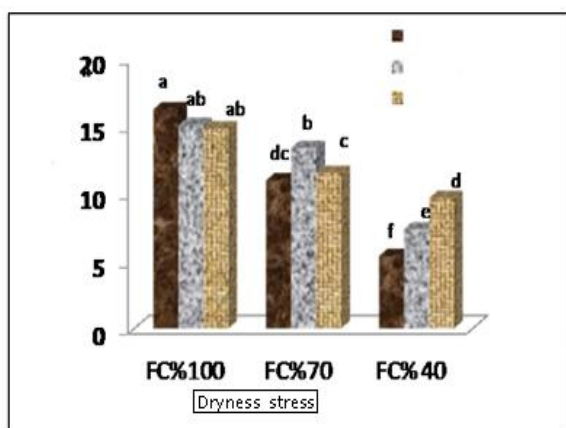


Fig. 3. The reciprocated effects of drought stress and gibberellin on The Biomass (gr).

The study conducted by Jaafar *et al*, (2012) on the effect of drought stress (normal irrigation; moderate,

high, and intense drought stress) on biological characteristics of *Labisiapumila* Benth showed that the high and intense drought stress, the highest contents of anthocyanin was obtained. In line with this study, Hassanpour Asil *et al* (2012) observed in their study that gibberellin causes to increasing of anthocyanin in Iris. The highest amount (52.02 mM/g fresh weight) was achieved by the application of 0.03% *Nano-TiO₂* and the lowest amount (20.16 mM/g fresh weight) was attained by non-application of *Nano-TiO₂* in 100% irrigation regime which showed no significant statistical difference with 0.01% and 0.03% *Nano-TiO₂* (23.96 and 23.26 mM/g fresh weight, respectively). The application of 0.03% titanium nanoparticles in 70% irrigation regime showed higher amount of anthocyanin as compared with control treatment (non-application of *Nano-TiO₂*), while in 40% irrigation, the application of 0.01% titanium nanoparticles did not have statistically significant difference with non-application approach but showed higher amount of anthocyanin compared with the application of 0.03% treatment (Table 2).

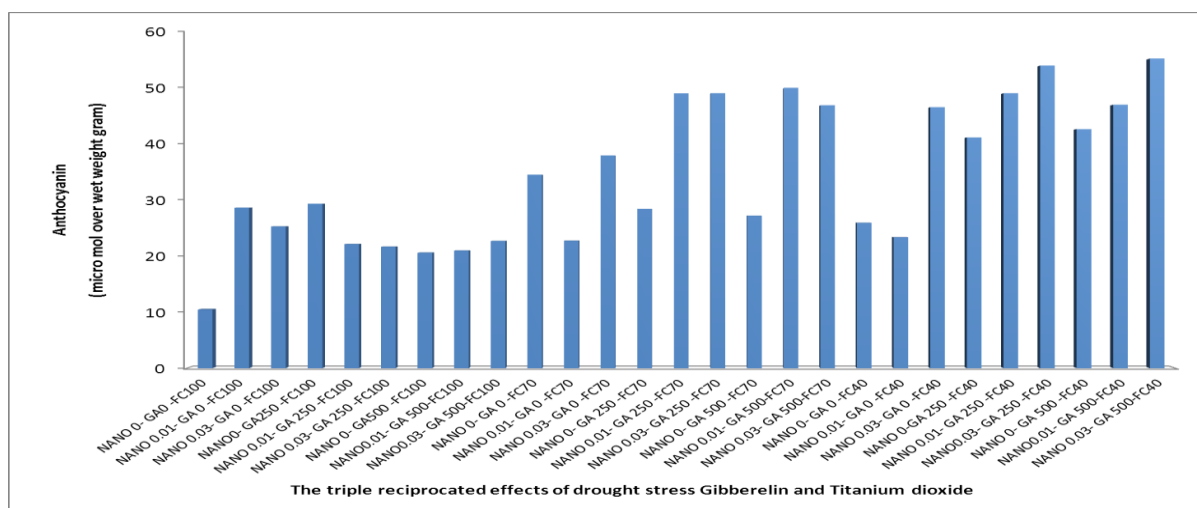


Fig. 4. The triple reciprocated effects of drought stress, Gibberellin and Titanium on Anthocyanin.

The results taken from the triple effect mean comparison table (Table 5) showed that the highest amount of anthocyanin (55.33 mM/g fresh weight) was obtained by the application of 40% 500 ppm gibberellin and 0.03% titanium nanoparticles and the lowest amount (10.47 mM/g fresh weight) was achieved by the 100% irrigation treatment and non-Kiapour *et al*.

application of gibberellin and nanoparticles. The studies carried out by Ghorbani (2012) on basil; Gholampour (2011) on amaranth; and Rezaei Adl (2013) indicated that the anthocyanin level of these plants was affected by the different concentrations of titanium nanoparticles.

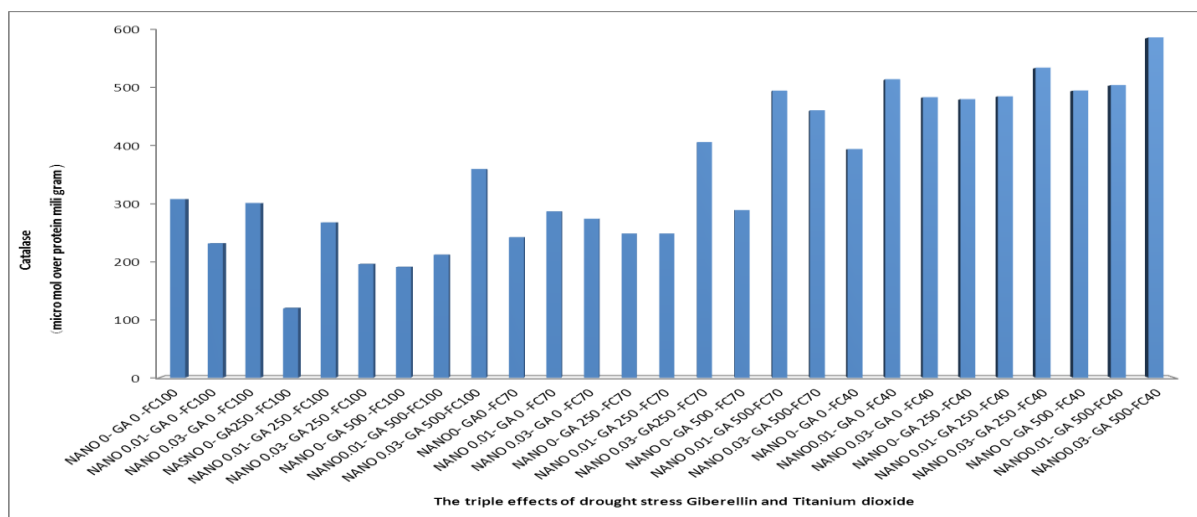


Fig. 5. The triple effects of drought stress, gibberellin, and *Nano-TiO₂* on Catalase

Catalase

The main effects of drought stress and *TiO₂* nanoparticle, the reciprocal twofold effects of drought stress and gibberellin, the reciprocal twofold effects of gibberellin and *Nano-TiO₂* and the triple reciprocated effects of drought stress, gibberellin and *Nano-TiO₂* were significant on 1% statistical level on catalase characteristics, however the twofold reciprocated effects of gibberellin and *Nano-TiO₂* were not statistically significant (Table 1). Regarding the results taken from the mean comparison, the highest amount of catalase (530.17 protein mg/mmol) was obtained from 40% irrigation regime and application of 500 ppm gibberellin and the lowest (199.58 protein mg/mmol) was achieved by the 100% irrigation regime and application of 500 ppm gibberellin. Increasing drought stress will increase catalase amount. 100% irrigation regime, non-application of gibberellin, application of gibberellin 250 ppm and 500 ppm were categorized the same statistical level and were not statistically different and more amount of catalase was obtained compared with control treatment. The highest and the lowest amount of catalase was achieved in condition of 40% irrigation, the applications of 500 ppm and non-application of gibberellin, respectively (Table 3). In the studies carried out by Ali *et al* (2012), it was indicated that the application of gibberellin on sour tea will increase the amount of catalase under drought stress. Regarding the results taken from the mean comparison of the reciprocated effects of

gibberellin and nanoparticle (Table 4), the highest amount of catalase (420.96 protein mg/mmol) was obtained by the application of 500 ppm gibberellin and 0.03% nanoparticles, while the lowest amount (285.05) was achieved by the application of 500 ppm gibberellin and 0.01% nanoparticles concentration which did not have significant difference with the compound treatment of non-application of gibberellin and nanoparticles and the compound treatment of 500 ppm gibberellin and non-application of nanoparticles. Based on results taken from tables of triple reciprocated treatments (Table 5), it is observed that in 40% irrigation regime, the application of 500 ppm gibberellin and 0.03% nanoparticles (588.32 protein mg/mmol), the highest catalase amount was obtained and the lowest amount (119.73 protein mg/mmol) was taken out of the application of 250 ppm gibberellin and non-application of nanoparticles in 100% irrigation regime. Approving these results, in the study of Feizi *et al* (2012) on wheat it was shown that the appropriate concentrations of *Nano-TiO₂* causes to increasing of catalase in wheat seedlings while the higher concentrations showed less impacts. Moaveni *et al* (2011) reported that the effect of titanium nanoparticles on the trait of seed performance, biologic performance, and harvest index in wheat was significant in 5% statistical level in which the highest amounts reported to be obtained for nanoparticles concentrations of 0.01% and 0.03%, respectively. Hruby and Kuzel (2012) also stated that the application of *Nano-TiO₂* had positive effects on

the plant traits of green alga and pepper which showed increase in reductase nitrate, peroxidase and catalase. Moaveni and Kheiri (2011) in the study of the application of *Nano-TiO₂* on corn in the two stages of stem elongation and flowering concluded that the foliar number and the dry weight and the seed performance were significant in reaction to nanoparticles treatment in 5% statistical level. It had the highest impact on the seed characteristics, dry weight, and seed performance of corn and caused to increasing of these factors. Also, the findings of Lu *et al* (2002) indicated that nanoparticles treatment of soya increases catalase in burgeoning seeds and increased water and compost consumption (Table 5).

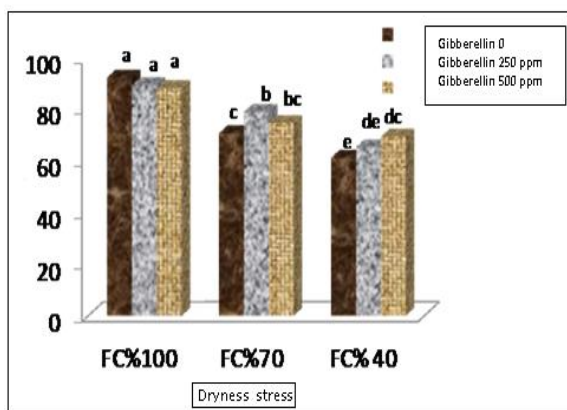


Fig. 6. The reciprocated effects of drought stress and gibberellin on The Foliar Relative Water content.

The Relative Water Content

The main effects of drought stress, and the reciprocated drought and gibberellin stress the reciprocated drought and nanoparticles stress was significant on the foliar relative water content on the statistical level of 1%. The main effect of gibberellin and nanoparticles on this trait was significant in 5% level. However, the twofold reciprocated effects of gibberellin and titanium and the triple reciprocated effects of drought stress, gibberellin and titanium was not significant on this trait (Table 1). Increasing the intensity of drought stress, will decrease the foliar relative water content on basil. The highest amount of the foliar relative water content (92.30%) was obtained in 100% irrigation regime and non-application of gibberellin in which no significant statistical difference was observed compared with 250 ppm gibberellin (89.39%) and 500 ppm (88.18%).

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The lowest amount (61.20 %) was obtained from 40% irrigation regime and non-application of gibberellin. In 70% irrigation, the highest relative foliar water content was achieved by the application of 250 ppm gibberellin and the non-application of gibberellin gave the lowest amount. Also, in 40% irrigation, the application of 500 ppm gibberellin achieved the highest relative foliar water content and the 40% irrigation treatment and non-application of gibberellin gave the lowest amount for the trait (Table 3). This is in line with the results reported from Pazoki *et al*, (2012) in which the drought stress leads to less relative foliar water content while the application of gibberellin increased that amount in contrast with non-application approach (Table 6). The highest amount of relative foliar water content (93.39%) was achieved by the application of 0.01% titanium nanoparticles in 100 irrigation regime in which no significant statistical difference was observed compared with control treatment (90.23%) with the same irrigation regime, while the lowest amount (61.535) was obtained by control treatment in 40% irrigation regime in which no significant difference was observed compared with 0.01% non-application of nanoparticle treatment (65.14%). In 70% irrigation regime, the non-application of nanoparticle treatment and 0.01% treatment had no significant differences and showed the lowest relative foliar water content, while the highest record of this trait was achieved by the application of 0.03% nanoparticles in 70% irrigation regime. In 40% irrigation regime, the application of 0.03% and 0.01% control treatment showed the lowest relative foliar water content, respectively (Table 2) (Figure 7).

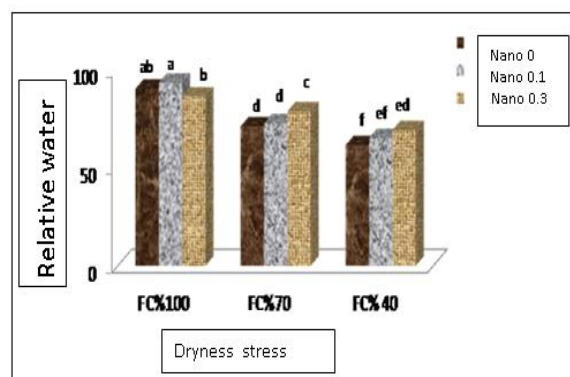


Fig.7 . The reciprocated effects of drought stress and Nano-TiO₂ on The Foliar Relative Water content.

Discussion

As shown in the results of this study, the drought stress caused to increasing the foliar relative water content and catalase and biomass of basil and the application of gibberellin and titanium nanoparticles improved the negative effects of the stress. Due to producing free radicals and decreasing inner cells carbon dioxide concentration, the drought stress condition causes to increasing peroxidation of membrane's lipids and malondialdehydes in which enhances antioxidant enzymes such as catalase and non-enzyme systems like anthocyanin to fight against free radicals (Sharma and Dubey, 2005). The external application of plant growth regulators are used to overcome disorders under stress. Different types of phytohormones such as gibberellin, kinetin, oxine and etc. are used to decrease the negative effects of environmental stresses (Ashraf *et al*, 2002). Having the great role of increasing meiosis and cell elongation, gibberellin increases plant height, number of knots, biomass, the performance of flowered branches, number of flowered branches, the dry and fresh weight of flowered branches, carotenoids, total chlorophyll, "a" and "b" chlorophyll, seeds weight, seed performance and basil essence especially under drought stress. In this experiment, catalase increased more with the application of nanoparticles and that has made the plant stronger. The reason is that plants possess effective defense-mechanism composed of superoxide dismutase, catalase, and peroxidase ascorbate, and glutathione reductase (Blokhin *et al*, 2003) against oxidative stresses that can eliminate or neutralize free radicals. The number and frequency of defense-mechanisms is because of the different types free radicals produced in the cells which show different reactions facing with different biologic molecules. The study indicated that the highest level of enzymes' activities was at 0.03% concentration and in 40% and 70% irrigation regimes which shows the effect of 0.03% titanium nanoparticles in decreasing the oxidative damages. The peroxidation of unsaturated fat and lipids increases when the oxidative stress occurs. Different aldehydes are created by the attack of free radicals to the lipids such as malondialdehyde. The free oxygen radicals attack

proteins and create partial changes in special places of amino acids by the catalysis of peptides chain. The sensitivity of amino acids of a peptide is different to an oxidative attack and there are various forms of activated oxygen in terms of reactivity potential. In this experiment of titanium nanoparticles, less malondialdehyde is created, thus the strength of the membranes is enhanced, and the antioxidant defense-mechanism is strengthened by this nanoparticle and is less likely to be attacked and destroyed by free radicals. In line with Buon *et al* (1992), due to a strong defense-mechanism and the accumulation of smolites under drought stress, the plant will have more relative foliar water content and due to this performance, it produces more flower, seed, and essence. Increasing photosynthesis increases the production of plant's dry material and morphological characteristics of the plant and ultimately it enhances the performance of the plant. Regarding biomass it should be noted that the application of this nanoparticle causes to increasing of the accumulation of photosynthetic in outer limbs due to increasing of solar radiance absorption, plant's growth speed, leaf surface index, plant's height, the number of knots, dry weight of leaf and stem which results in increasing biomass and the performance of the plant.

Conclusion

In this study, the drought stress caused to decreasing of the qualitative and quantitative characteristics of basil. Regarding the fact that basil is cultivated in seed and essence in order to reach the utmost biological performance, to reach these objectives, in 100% irrigation regime, the non-application of gibberellin and 0.01% application of *Nano-TiO₂* and in 70% irrigation regime, the application of 250 ppm gibberellin and 0.03% *Nano-TiO₂* and in 40% irrigation regime, the application of 500 ppm gibberellin and 0.03% *Nano-TiO₂* were identified as the best possible treatments.

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