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Effect of Paclobutrazol on Some Morphological and Physiological Characteristics of Petunia under Drought Stress

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Abstract

This experiment was conducted to investigate the effect of paclobutrazol as an anti-gibberellin on some morpho-physiological characteristics of *Petunia* × *grandiflora* 'Bravo Blue', and determine the optimum concentration of paclobutrazol for drought resistance in this plant. The experiment was carried out as factorial based on completely randomized design with three replications in greenhouse. The studied factors were included paclobutrazol in four levels (0, 25, 50 and 75 mg a.i. l^{-1}) and drought stress in three levels (irrigation when soil moisture reached 90%, 75% and 50% of field capacity). Results showed that the application of paclobutrazol had increased the number of flowers, aerial part dry weight and number of lateral branches, and the application of this substance decreased the plant height, root dry weight and amount of carotenoids. While, paclobutrazol did not have significant effect on the electrolyte leakage, main branch number and leaf area of petunia. Also, the results showed that drought stress increased the number of flowers per plant, and reduced the content of chlorophyll, carotenoids, flower diameter, shoot and root dry weight and leaf area of petunia. Application of 75 mg a.i. l^{-1} paclobutrazol had the greatest effect on height controlling of petunia plant under drought stress. Carotenoids and chlorophyll content was increased by increasing the paclobutrazol concentration under 50% of field capacity. Generally, the results indicated that the application of paclobutrazol under severe drought stress could prevent chlorophyll and carotenoids decline, and had partially been effective in overcoming adverse effects of drought stress.

Keywords: Anti gibberellin, Carotenoids, Chlorophyll, Dry weight, Electrolyte leakage.

INTRODUCTION

As the most prevalent environmental stress, drought affects the growth of many plants. When plants do not receive sufficient water they are subjected to a stress called water deficit. Under drought conditions, soil water content drops, which does not favor water movement into the root cells. As a defense against water loss, transpiration decreases (Bray, 1997). Some plant growth retardant compounds such as triazole, pyrimidine and onium compounds prevent stem elongation by inhibiting sterols and gibberellin (GA) biosynthesis (Arteca, 1996). Plants treated with triazole are dwarf and more compact and have darker and thicker green leaves. These plants have higher amounts of chlorophyll, carotenoids, xanthophyll and nucleic acid too (Fletcher, 1985). Paclobutrazol is chemically a very active triazole and is effective on the most plant species (Barrett, 2001). Paclobutrazol (PBZ) is a triazole type plant growth retardant which blocks gibberellin biosynthesis (Hua *et al*., 2014; Watson and Jacobs, 2012). Paclobutrazol (S-enantiomer) keeps certain structural similarities with ent-kaurene and ent-kaurenol which are key compounds in the gibberellins (GAs) biosynthesis path-way and therefore may inhibit cytochrome P450 monooxygenases, impairing the oxidation of entkaurene to ent-kaurenoic acid (Ribeiro, 2011; Magnitskiy *et al.*, 2006; Denek and Keever, 1992). It has been reported that paclobutrazol has beneficial effects on physiological and morphological traits of different plants. Application of paclobutrazol reduces vegetative growth rate by improving water use efficiency, and consequently increases drought tolerance. Blocking gibberellin synthesis leads to decreasing in internode length, leaf area and number of leaves, and consequently to reducing growth (Magnitskiy *et al*., 2006; Hua *et al.,* 2014).

Its most notable feature is the reduction of vegetative growth followed by a change in the distribution pattern of photosynthetic products, thereby, diverting them towards reproductive growth and the formation of flower buds (Lever, 1986; Werner, 1993). Generally, triazols reduces vegetative growth and decreases competition for photosynthetic products between vegetative and reproductive organs.

Petunia belongs to the Solanaceae family with fast-growing, semi-woody and hairy stems. This plant is one of the most popular warm-season plants in the landscape owing to its versatility and variety. Like other ornamental plants, the petunia has become a challenge for the growers who seek to increase the quality of their production with lower and more compact plants (Francescangeli and Zagabria, 2008). The present study was designed to investigate the effect of paclobutrazol as an anti-gibberellin on some morphological and physiological characteristics of *Petunia* × *grandiflora* ‘Bravo F1 Blue’ and to determine the optimum concentration of paclobutrazol for drought resistance in this plant.

MATERIALS AND METHODS

The experiment was conducted in a factorial based on completely randomized design (CRD) with three replications at the greenhouse of Islamic Azad University, Shiraz branch. The studied factors comprised paclobutrazol in four levels (0, 25, 50 and 75 mg a.i. l^{-1}) and drought stress in three levels (irrigation at field capacity of 90 % (control), 75 % and 50 %).

Petunia seeds (*Petunia* × *grandiflora* ‘Bravo F1 Blue’) were planted in the seed tray, and seedlings were transplanted in pots (containing a mixture of soil, sand and leaf composts in a volume ratio of 1:1:1) after reaching to the 6-8 leave stage. After the establishment of plants in pot the paclobutrazol was applied as foliar application, and drought stress treatments were applied after a week. To determine field capacity (FC) [Steadman *et al*., 2004], two pots were filled with soil. The weight of the pots containing the dry soil was measured. Pots were watered, allowed to drain for 24 hours, and reweighed to determine the field (pot) capacity.

The parameters such as electrolyte leakage, content of chlorophyll and carotenoids, number of flowers, leaf area, flower diameter, plant height, main and lateral branch numbers and, shoot and root dry weight were measured at the end of the experiment (90 days after transplanting).

Chlorophyll and carotenoids contents were measured according to Arnon (1949),s method.

Fresh young leaves (1 g) were selected from plants within each treatment, washed with deionized water and grinded by acetone 80 % in a porcelain mortar. Remained leftovers in the mortar were completely washed and final volume was reached to 25 ml by acetone 80 %. After centrifugation at 6000 rpm, absorption of samples was read at wavelengths of 663, 645, 510 and 480 nm. The device was first calibrated by control sample of acetone 80%. The following formulas were used for estimation of total chlorophyll and carotenoids contents:

Total Chl. (mg g^{-1} FW) = [20.2 (A 645) + 8.02(A 663)] × V/1000 × W

Carotenoids (mg g^{-1} FW) = [7.6 (A 480) - 1.49(A 510)] × V/1000 × W

V: Volume of extract (ml), W: Fresh weight of the sample (g), and A: Absorbance at specific wave length.

Electrolyte leakage was measured as described by Lutts *et al*. (1996) using young leaf discs (2 cm diameter) of three plants for each treatment. Samples were washed with deionized water to remove surface adhered electrolytes. Leaf discs were placed in closed vials containing 10 ml of deionized water, and incubated at 25 ºC on a shaker incubator (100 rpm) for 18 h, and subsequently, electrical conductivity of the solution (EC_1) was determined.

Samples were then autoclaved at 120 ºC for 15 min, and the final electrical conductivity (EC_2) was obtained after equilibration at 25 ºC. The EL was defined as follows:

$$EL\% = EC_1/EC_2 \times 100$$

Plant height and flower diameter was measured by ruler and caliper respectively. At the end of the experiment and after removing the plant from the soil and drying in an oven at 70 °C for 24 h dry weight was determined.

In order to determine the average leaf area, 10 intact and fully matured leaves were harvested from the middle of branches, and the area of each of them was measured using leaf area meter (ADC Model: No SE 213C), and thereafter the mean leaf area was calculated. Data were statistically analyzed with MSTAT-C software, and means were compared using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Chlorophyll content

According to the analysis of variance results (Table 1) paclobutrazol, drought stress and the interaction of these factors showed significant effects on chlorophyll content of petunia ($P < 0.01$). The results showed that the highest chlorophyll content was achieved by the use of 25 and 50 mg a.i L^{-1} paclobutrazol under 90 % FC, but there was no significant difference with 90 % and 75 % FC without the use of paclobutrazol treatments. Also, the results indicated that the lowest cholorophyll content was achieved by the use of 75 mg a.i. l^{-1} paclobutrazol under 75 % field capacity (Table 4).

Table 1. Analysis of variance of the effects of drought stress and paclobutrazol on some physiological traits and flower characteristics of *Petunia × grandiflora* 'Bravo F1 Blue'.

The drought stress resulted in reduction of chlorophyll content in Kentucky bluegrass Bian and Jiang (2009), three species of turfgrass (Tatari *et al*., 2013) and frankenia (Chegah *et al*., 2013). Photosynthetic pigments determine the physiological status of the plants. Under drought stress, chlorophyll reduction is likely due to increased catabolism and the lack of required factors for its biosynthesis (Ahmadi *et al*., 2005). Abraham *et al*. (2008) have reported that application of paclobutrazol increased the chlorophyll content in *Sesamum indicum*, but drought stress caused a reduction in total chlorophyll content in this plant. It is suggested that the higher chlorophyll content in triazole treated plants may be related to the effect of triazole on cytokinin levels. Previous studies revealed that triazoles stimulate cytokinin biosynthesis which increases chloroplasts differentiation, number of chloroplasts, chlorophyll synthesis and prevent chlorophyll degradation (Fletcher *et al.,* 2000; Kalil and Rahman, 1995).

Carotenoids

The results indicated that the paclobutrazol, drought stress and the interaction of these factors had a significant effect on carotenoids content of petunia ($P < 0.01$) (Table 1). The highest amount of carotenoids was observed in control plants (irrigation at 90 % FC), and drought stress decreased the amount of carotenoids (Table 3). The highest carotenoids content was found in control plants (without the use of paclobutrazol), and use of this substance reduced the amount of carotenoids (Table 2).

Carotenoids content was increased by increasing the paclobutrazol concentration under 50 % of field capacity. Therefore, application of paclobutrazol under severe drought stress could prevent the loss of carotenoids (Table 4). Our results are in agreement with Amina and Hanan (2011), who described that water stress caused significant reduction in total chlorophyll and carotenoids contents in Sakha 8 wheat genotype, and application of paclobutrazol increased this pigments. Also, Fletcher (1985) reported that plants treated with triazole have higher amounts of chlorophyll and carotenoids. Chlorophyll and carotenoid absorb radiant energy, which is used for photosynthesis. On the other hand, carotenoids play fundamental roles in drought stress tolerance including light harvesting and protection from oxidative damage caused by stress. Therefore, increased contents of carotenoids are important for stress tolerance (Jaleel *et al.*, 2009).

Electrolyte leakage

The results indicated that the paclobutrazol and drought levels did not have any significant effects on electrolyte leakage, while interaction of factors (drought × paclobutrazol) had significant effect on this trait at 1% probability level (Table 1). The results indicated that the highest electrolyte leakage occurred by using 75 mg a.i. l^{-1} paclobutrazol under 75 % field capacity (Table 4).

In the *Bromus inermis* plant, the electrolyte leakage was increased by increase of drought stress (Tatari *et al.*, 2013). It seems that petunia in this research conditions has been able to maintain the cell membranes structure and to prevent increasing of electrolyte leakage. The amount of damage caused by water deficit in the plant depends on species, genotype, duration of exposure to stress, intensity of water deficit, plant's age and development stage and intrinsic properties (Safarnejad, 2004). Javadi (2012) reported that paclobutrazol induces more water stress tolerance in Biroty pear by affecting the vegetative, biochemical and gas exchange traits, but the use of paclobutrazol did not affect on membrane stability index in this plant.

Flowers number

According to the results, paclobutrazol, drought stress and the interaction of these factors showed significant effects on the flowers number of petunia ($P < 0.01$) (Table 1). The means comparison indicated that the highest flowers number was achieved by the 75% FC, but there was no significant difference with 50 % FC (Table 3). The lowest flowers number was found in control plants (without the use of paclobutrazol) and use of this substance increased the flowers number in petunia (Table 2). Generally, results showed that the application of paclobutrazol and drought stress increase the number of flowers in Petunia (Table 4).

According to Desclaux and Roumet (1996), drought stress seemed to trigger a signal that caused an early switch of plant development from vegetative to reproductive. Under drought stress, increased number of flowers is likely due to the reduction of nutrient uptake and vegetative growth, and change in plant growth regulators content.

Paclobutrazol reduces vegetative growth and decrease competition for photosynthetic products between vegetative and reproductive organs. Different effects of paclobutrazol on the number

Table 1. Means of squares of the studied traits of calendula in response to four high temperature length.

S.o.V	df	Mean Squares										
		Chlorophyll content	Carotenoids content	Electrolyte leakage	Number of flowers	Flower diameter	Main branch	Lateral branch	Plant height	Shoot dry weight	Root dry weight	Leaf area
Drought stress (A)	2	162.75**	9.39**	81.40 ns	50.09**	1.95**	0.02 ns	5.77 ns	1.58 ns	0.44**	0.23**	28436.19**
PBZ (B)	3	43.91**	6.98**	67.25 ns	114.8**	1.70**	0.10 ns	165.65**	107**	0.50**	0.10**	2412.6 ns
A x B	6	151.71**	12.94**	184.88**	31.85**	1.24**	0.35*	32.55**	35.64**	0.27**	0.11**	52677.54**
Error	2	3.67	0.36	43.7	12.79	0.29	0.14	3.97	1.85	0.05	0.01	10020.58
CV (%)	2	10.4	17.4	9.36	17.4	12.6	11.8	16.8	6.2	12.6	10.7	11.5

*, ** are significant at 0.05 and 0.01 of probability levels, respectively, and ns is not significant.

Table 2. Means comparison of the main effect of paclobutrazol levels on the studied traits of *Petunia* × *grandiflora* 'Bravo F1 Blue'

PBZ (mg a.i.L^{-1})	Chlorophyll (mg/g FW)	Carotenoids (µg/g FW)	Flower diameter (cm)	Number of flower	Shoot dry weigh (g)	Root dry weight (g)	Plant height (cm)	Lateral branch
0	9.49 b	10.38 a	4.37 a	15.39 b	1.48 b	0.62 a	25.64 a	7.22 c
25	8.11 c	7.54 b	3.65 b	20.85 a	2.02 a	0.45 b	23.40 b	16.13 a
50	10.43 a	7.63 b	4.65 a	22.66 a	1.89 a	0.45 b	21.12 c	14.72 a
75	8.33 c	6.66 c	4.45 a	23.22 a	1.88 a	0.45 b	17.56 d	9.28 b

*Means followed by the same letter(s), within a column are not significantly different using Duncan's Multiple Range Test, P < 0.05.

Table 3. Means comparison of the main effect of drought stress levels on the studied characteristics of *Petunia* × *grandiflora* 'Bravo F1 Blue'.

Drought (FC)	Chlorophyll (mg/g FW)	Carotenoids (µg/g FW)	Leaf area (mm^2)	Root dry weight (g)	Shoot dry weight (g)	Flower diameter (cm)	Number of flower
90 %	9.45 a	11.04 a	910.33 a	0.63 a	1.97 a	4.71 a	18.24 b
75 %	9.31 b	6.69 b	814.42 b	0.41 b	1.89 a	3.91 b	22.16 a
50 %	8.51 c	6.43 b	876.83 ab	0.37 b	1.60 b	4.22 b	21.19 ab

*Means followed by the same letter(s), within a column are not significantly different using Duncan's Multiple Range Test, P < 0.05.

of flowers of ornamental plants have been reported in the literature. Abraham *et al*. (2008), have reported that drought stress decreased the flower number in *Sesamum indicum,* but the flower number was increased by paclobutrazol treatment. The flower number is increased in *Catharanthus* plants under paclobutrazol treatment (Jaleel *et al.,* 2006). Paclobutrazol is a triazole type plant growth retardant which blocks gibberellin biosynthesis (Barrett, 2001; Hua *et al.*, 2014; Watson and Jacobs, 2012). Blocking gibberellin synthesis leads to a reduced vegetative growth and consequently, to improved reproductive traits (Magnitskiy *et al.*, 2006; Hua *et al*., 2014). There are reports that paclobutrazol can increase total non-structural carbohydrates. Increased availability of assimilates can possibly lead to initiation of more floral buds on the branches. The proportion of photoassimilates allocation towards reproductive organs could have a direct effect on flowering (Kumar *et al*., 2012).

Flower diameter

According to the analysis of variance results, paclobutrazol, drought stress and the interaction of these factors showed significant effects on flowers diameter of petunia ($P < 0.01$) (Table 1). The means comparison indicated that the highest flower diameter (4.71 cm) belonged to control plants (irrigation at 90 % FC), and the lowest flower diameter related to 50 % and 75 % FC (Table 3).

The lowest flower diameter was achieved by using 25 mg a.i. l^{-1} paclobutrazol under 75 % field capacity (Table 4). Generally, results showed that paclobutrazol and drought stress reduce the flower diameter. Flower size responses of plants to paclobutrazol can be varied depending on species and cultivars. Gilbertsz (1992) reported that paclobutrazol reduced flower diameters in Chrysanthemum, but flower sizes in *Zinnia elegans* were not affected (Pinto *et al.*, 2005). Also, it is stated that paclobutrazol treatments resulted in significant increases in flower diameter in *Osteospermum ecklinis* (Olsen and Andersen, 1995), and had no significant effects on flower size in Hibiscus *rosa-sinensis* L. (Wang and Gregg, 1991).

Main and lateral branches

The results showed that the paclobutrazol and drought stress had no significant effects on the number of main branches in petunia, while their interaction had a significant effect on this trait ($P < 0.05$) (Table 1). Also results showed that the drought stress had no significant effects on the number of lateral branches, while paclobutrazol and interaction of factors (paclobutrazol and drought stress) had a significant effect on this trait ($P < 0.01$) (Table 1).

Table 4. Means comparison of the interaction effect of paclobutrazol and drought stress on physiological traits and flower traits of *Petunia × grandiflora* 'Bravo F1 Blue'.Blue'.

Drought (FC)	PBZ (mg a.i. L^{-1})	chlorophyll (mg/g FW)	carotenoids (µg/g FW)	Electrolyte leakage (%)	Flower number	Flower diameter (cm)
90 %	0	22.2 ab	4.9 ab	71.0 b	11.6 c	4.1 abc
	25	25.1 a	5.4 ab	66.2 b	19.0 ab	4.4 abc
	50	25.1 a	5.8 a	72.0 b	17.3 bc	5.1 a
	75	13.9 cd	1.5 d	69.5 b	25.0 a	5.1 a
75 %	0	23.9 ab	5.7 a	67.9 b	15.6 bc	4.9 ab
	25	15.5 c	2.0 cd	70.0 b	22.0 ab	2.5 d
	50	21.1 b	4.5 b	67.3 b	25.6 a	4.3 abc
	75	7.1 e	1.1 d	78.7 a	25.3 a	3.8 c
50 %	0	12.1 d	2.6 c	63.2 b	18.8 ab	4.0 bc
	25	11.4 d	1.4 d	73.5 b	21.5 ab	4.0 bc
	50	10.9 d	1.5 d	73.7 b	25.0 a	4.4 abc
	75	22.8 ab	4.9 ab	63.6 b	19.3 ab	4.4 abc

*Means followed by the same letter(s), within a column are not significantly different using Duncan's Multiple Range Test, $P < 0.05$.

The highest number of lateral branches were observed at 25 and 50 mg a.i. l^{-1} paclobutrazol treatments, and the lowest number of lateral branches was corresponded to the control plants (Table 2).

The highest number of lateral branches was achieved by the usage of 50 mg a.i. l^{-1} paclobutrazol under 50 % field capacity, and the usage of 25 mg a.i. l^{-1} paclobutrazol under 75 % and 90 % field capacity (Table 5).

Paclobutrazol is a triazole type plant growth retardant which blocks gibberellin biosynthesis and is effective on most plant species (Barrett, 2001; Hua *et al*., 2014; Watson and Jacobs, 2012). A reduction in the number of branches has been reported in drought stress in *Sesamum indicum* plants by Abraham *et al.* (2008), also these researchers have reported that the treatment with paclobutrazol increased number of branches in this plant.

Plant height

Results showed that the paclobutrazol and interaction effect of paclobutrazol and drought stress was significant on plant height of petunia ($P < 0.01$) (Table 1).

Paclobutrazol application at all levels studied in this experiment has significantly reduced the plant height, and higher concentrations of paclobutrazol had a greater effect on reducing plant height (Table 2). The results showed that the highest plant height was observed in control plants (irrigation at 90 % field capacity without the use of paclobutrazol), while the lowest plant height was achieved by using 75 mg a.i. l^{-1} paclobutrazol under 75 % and 50 % field capacity, and also usage of 50 mg a.i. l^{-1} paclobutrazol under 90 % field capacity (Table 5). The results indicated that paclobutrazol had a significant effect on reducing the height of petunia.

Plant size Control is one of the most important aspects of ornamental plants production. Growers can control crop height genetically, environmentally, culturally, or chemically. Paclobutrazol is an incredibly useful chemical tool. Paclobutrazol is a triazole type plant growth retardant and it is used to reduce vegetative growth of many plants such as bedding plants and indoor plants (Whipker, 2013; Denek and Keever, 1992). An inhibitor of GA biosynthesis, Paclobutrazol (pp333) has been shown to be very effective in inhibiting shoot growth in petunia (Francescangeli and Zagabria, 2008), freesia (Gianfagna and Wulster, 1986), chrysanthemum (Barrett and Bartuska, 1982), black iris (Al-Khassawneh *et al.*, 2006) and sunflower (Wample and Culver, 1983). Application of paclobutrazol improves water use efficiency by reducing vegetative growth, transpiration rate and leaf area, and consequently, increases drought tolerance (Asamoah and Atkinson, 1985).

Table 5. Means comparison of the effect of paclobutrazol and drought stress on morphological characteristics of *Petunia × grandiflora* 'Bravo F1 Blue'.

Drought (FC)	PBZ (mg a.i. L^{-1})	Main branch	Lateral branch	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Leaf area (mm^2)
90%	0	3.1 abc	6.6 ef	30.0 a	1.5d ef	1.0 a	804.5 cd
	25	3.1 abc	18.5 a	22.2 bc	2.5 a	0.6 b	991.1 ab
	50	3.2 abc	12.6 b	16.8 d	1.6 def	0.5 bc	1055.3 a
	75	3.3 abc	10.8 bcd	20.1 c	2.1 abc	0.3 d	790.3 cd
75%	0	2.8 bc	5.8 f	24.4 b	1.4 ef	0.3 cd	914.0 abc
	25	3.1 abc	17.0 a	23.3 b	2.0 bcd	0.3 cd	802.5 cd
	50	3.1 abc	11.6 b	23.1 b	2.2 ab	0.2 d	692.6 d
	75	3.5 ab	9.6 bde	15.3 d	1.8 cde	0.5 bcd	848.5 bcd
50%	0	3.7 a	9.1 def	22.5 b	1.3 f	0.4 bcd	834.5 bcd
	25	2.7 c	12.8 b	24.6 b	1.5 ef	0.3 cd	772.3 cd
	50	3.1 abc	19.8 a	23.4 b	1.8 cde	0.2 d	900.1 abc
	75	2.8 bc	7.3 def	17.2 d	1.6 def	0.5 bcd	1000.5 ab

*Means followed by the same letter(s), within a column are not significantly different using Duncan's Multiple Range Test, P < 0.05.

Shoot dry weight

The results showed that the paclobutrazol, drought stress and the interaction effect of these factors was significant on shoot dry weight of petunia ($P < 0.01$) (Table 1). The results indicated that the highest shoot dry weight belonged to 90 % and 75 % FC, and the lowest shoot dry weight corresponded to 50 % field capacity (Table 3). The lowest shoot dry weight was found in control plants (without the use of paclobutrazol) and application of paclobutrazol increased the shoot dry weight (Table 2). According to the results, the highest shoot dry weight was achieved using 25 mg a.i. l^{-1} paclobutrazol under 90 % field capacity (Table 5). Generally, results showed that drought stress reduced shoot dry weight, and use of the paclobutrazol had a positive effect on shoot dry weight in petunia. A decrease in dry matter may be due to the considerable reduction of photosynthesis and plant growth under drought stress. Abraham et al. (2008), reported that drought stress decreased dry weight accumulation, and dry weight was increased under paclobutrazol treatment under drought stress in *Sesamum indicumand*. Bayat and Sepehri (2012) reported that the exogenous paclobutrazol application was advantageous in maize plants under drought stress conditions. Paclobutrazol treated plants with stomatal closure and osmotic adjustment allows the cell to decrease osmotic potential, and consequently, increases the gradient for water influx and maintenance of turgor. This is beneficial for maintaining physiological activity of plants under drought stress (Kramer and Boyer, 1995).

Root dry weight

The results showed that the paclobutrazol, drought stress and the interaction effect of these factors was significant on root dry weight of petunia ($P < 0.01$) (Table 1). The highest root dry weight (0.63 g) belonged to control plants (irrigation at 90 % FC) (Table 3). All treatments of paclobutrazol significantly decreased root dry weight of petunia (Table 2). The results indicated that the highest root dry weight was observed in control plants (irrigation at 90 % field capacity without using paclobutrazol), while the lowest shoot dry weight was achieved by using of 50 mg a.i. l^{-1} paclobutrazol under 75 % and 50% field capacity, and also using of 75 mg a.i. l^{-1} paclobutrazol under 90 % field capacity (Table 5). Generally, the results indicated that reduced irrigation with the use of paclobutrazol caused the root dry weight loss. Numerous examinations show that the stress resulted by water deficit would cause reduction of dry weights of root, water use efficiency, and chlorophyll content (Chegah *et al*., 2013). In grass plant, the fresh and dry weights of root were reduced due to drought stress (Mafakheri *et al*., 2011).

The reduction of root dry weight of marigold and impatiens has been reported as a result of using growth retardant (Latimer, 1991). In another report, paclobutrazol retarded shoot growth, enhanced lateral root growth, produced darker green leaves, and resulted in a more extensive root system (Lin and Zhang, 1999). The inhibition effects of growth retardants depend on the type, method of use and dose of these compounds (Latimer, 1991). Effect of Paclobutrazol on root dry weight depends on the used concentration, so that at low concentrations paclobutrazol increased the root dry weight, and this trait is reduced at high concentrations of paclobutrazol (Williamson *et al.*, 1986; Wieland and Wample, 1985). Paclobutrazol reduced root dry weight in Raritan strawberries (Stang and Weis, 1984), while this trait increased by usage of paclobutrazol in Belrubi strawberry (Ramina *et al.,* 1985).

Leaf area

The results showed that the drought stress and interaction effect of paclobutrazol and drought stress was significant on leaf area of petunia ($P < 0.05$), while main effect of paclobutrazol was not significant on this trait (Table 1). The mean comparison results showed that the highest leaf area (910.33 mm^2) occurred by irrigation at 90 % FC (Table 3). Highest leaf area was achieved by the use of 50 mg a.i. l^{-1} paclobutrazol under irrigation at 90 % field capacity (Table 5). Generally, the results indicate that drought stress reduced the leaf area in the petunia.

Drought is a major abiotic stress that limits ornamental plant production. The common adverse effect of drought stress on crops is the reduction of leaf area.

The reduction of leaf area of marigold, zinnia (Latimer, 1991) and geranium (Cox, 1991) has been reported as a result of the use of paclobutrazol.

It is suggested that the reducing effect of growth retardant on leaf area is associated with the prevention of the synthesis of GA, the enhancement of ABA and the prevention of cell elongation (Gopi *et al*., 2005; Ahmad Nazarudin *et al.*, 2007). Previous studies reveal that responses of plant species and cultivars to paclobutrazol are quite different. The difference in response of plants to paclobutrazol may be due to differences in the type of plant, method of use and the dose of this substance (Gianfagna and Wulster, 1986; Francescangeli and Zagabria, 2008).

Barrett and Bartuska (1982) suggested that foliar application of paclobutrazol must travel through the phloem in leaf tissue before reaching xylem tissue in the stem, but is more readily transported through the xylem than through the phloem. Although foliar sprays as a method for use of regulator, has practical advantages over irrigation in the commercial scale but, the translocation route of paclobutrazol is xylem (Rademacher, 2000), hence, application of paclobutrazol as irrigation is more efficient (Francescangeli and Zagabria, 2008).

Cramer and Bridgen (1998) reported that the paclobutrazol sprays or drenches at any concentration or application number were ineffective for controlling plant height in *Mussaenda* 'Queen Sirikit'. Latimer (1991) also stated that paclobutrazol had not a significant effect on reducing the plant height in marigold.

CONCLUSION

According to the results, paclobutrazol increased the number of flowers, aerial part dry weight and number of lateral branches, and decreased the plant height, root dry weight and amount of carotenoids in petunia. Also, the results showed that drought stress increased the number of flowers per plant, and reduced the content of chlorophyll, carotenoids, flower diameter, shoot and root dry weight and leaf area of petunia. With an increase in the concentration of paclobutrazol under 50 % field capacity, carotenoids and chlorophyll content increased. Application of paclobutrazol under severe drought stress could prevent chlorophyll and carotenoidsto be declined, and use of 75 mg a.i.l^{-1} paclobutrazol had the greatest effect on height controlling of petunia plant under drought stress. Generally, application of paclobutrazol is partially effective in overcoming adverse effects of drought stress.

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