Journal of Plant Physiology and Breeding

2019, 9(2): 31-46 ISSN: 2008-5168

A key role of penconazole in biomass production and responses to different soil moisture levels in *Satureja sahendica* Bornm.

Rozita Mazloumi Oskouiee[1*], Mohammad Reza Shakiba[2], Adel Dabbag Mohammadi Nassab[2] and Hassan Monirifar[3]

Received: July 22, 2019 Accepted: December 18, 2019

[1]PhD Student, Department of Ecophysiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
[2]Department of Ecophysiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
[3]Horticulture and Crops Research Department, East Azarbaijan Agricultural and Natural Resources Research and Education Center, AREEO, Tabriz, Iran.
*Corresponding author; Email: rozitamazloumi@gmail.com

Abstract

Drought stress is a significant environmental factor retarding plant growth as well as productivity. Plants adapt to environmental stress via numerous strategies such as changes in plant height, biomass, carbohydrate content and phytohomnonal levels. Therefore, a split plot experiment based on randomized complete block design with three replications was carried out to determine possible drought tolerance mechanisms in *Satureja sahendica* Bornm. induced by penconazole (PEN). The determined water (100, 60 and 30% FC) and PEN (0, 10 and 20 mg.l^{-1}) levels were applied. Result showed that drought stress significantly decreased plant height, fresh and dry weight, indoleacetic acid (IAA), gibberellic acid (GA) and induced accumulation of carbohydrates, glucose, sucrose, fructose and abscisic acid (ABA). PEN treatment even decreased further plant height, IAA, GA and increased fresh and dry weight, carbohydrate content, glucose, sucrose, fructose and ABA. PEN increased fructose and ABA up to 2.5 folds and 3.5 to 4.5 folds, respectively compared to the control, especially at the second harvest. The significant decrease in growth hormones along with significant increase of the inhibitor hormone by elevating drought stress and PEN application led to significant augmentation in (GA+IAA)/ABA ratio (~70% at 30% FC and 20 mg.l^{-1} PEN) compared to the control. Altogether, the application of 20 mg.l^{-1} PEN together with 60% FC seems an appropriate treatment for planting *Satureja sahendica* in dry regions. Furthermore, the improved fructose production and ABA level induced by PEN had outstanding role on drought tolerance of this plant.

Keywords: Biomass; Drought stress; Hormone; PEN; Plant height; *Satureja sahendia*; Soluble carbohydrate.

Citation: Mazloumi Oskouiee R, Shakiba MR, Dabbag Mohammadi Nassab A and Monirifar H, 2019. A key role of penconazole in biomass production and responses to different soil moisture levels in *Satureja sahendica* Bornm. Journal of Plant Physiology and Breeding 9(2): 31-46.

Introduction

With the increasing lack of fresh water and agricultural land, it is of vital importance to cultivate genotypes with low water consumption (Baghalian *et al.* 2011). *Satureja* (savory) is a genus of Lamiaceae, from Nepetoidae sub-family and Mentheae tribe (Cantino *et al.* 1992). In Persian, the names “Marze” and “Marze-Sahandi” are designated to the genus (*Satureja*) and the species (*S. sahendica*), respectively (Mozaffarian 2004). *Satureja sahendica* Bornm. is of about 10-30 cm height, in the form of branched, perennial and bushy aromatic herb (Ghahreman 1988). It is spread through the west and northwest of Iran, specifically Sahand mountain (Ghahreman 1988). Having a low precipitation of 240 mm, many regions of Iran are considered as arid/semi-arid. Thus water deficiency is regarded as the main stress-causing yield loss. Although drought tolerance mechanisms are not yet clarified completely, some of the stress adaptation processes are established such as: reduction of plant growth, decrease in photosynthesis, hormonal imbalance, stomatal closure and modification in the accumulation of protein and compatible solutes as well as trigger of some regulatory mechanisms (Ajithkumar and Panneerselvam 2014). Sugar accumulation and phytohormone activation are the primary plant responses against stressors (Bartoli 2013), which affect its growth and developmental processes (Jia *et al.* 2013). Glucose, sucrose and fructose are considered as common soluble sugars accumulated intracellularly (Rosa *et al.* 2009). The endogenous synthesis of gibberellic acid (GA) and indole acetic acid (IAA) is inhibited by drought stress, whilst the synthesis of abscisic acid (ABA) is promoted to economize the plant water reservoir (Taiz and Zeiger 2010). During adaptive mechanisms to suboptimal conditions, the cross-talk between sugar and hormonal signaling pathways further play a crucial role (Sairanen *et al.* 2012). Plants show complicated metabolic changes such as oxidative reactions to environmental stresses via various physiological and biochemical approaches (Kafi *et al.* 2009). Based on what mentioned, determination of optimal water supply is of potent necessity. Depending on the severity and the period of stress conditions, product reduction has been observed in many species (i.e. Kaczmarek *et al.* 2017). The application of growth regulators may have a great impact on the plant to switch towards the adaptive responses. This is an expanding enterprise in the stress affected areas. The triazoles are the largest and most important group of curative and preventative systemic fungicide to control leaf and ear diseases, in cereals, apple and other crops (Matthews 2018). More recently, it is widely considered as growth regulators, as well (Tuna 2014). The use of penconazole goes back to 1983 which was registered in the EU on a large number of crops (Matthews 2018).

Penconazole (known as PEN) with chemical formula [1-(2,4-dichloro-β-propylphenethyl)-1H-1,2,4-triazole] is a triazolic fungicide, which due to its function of signal transducers, can be used as a plant growth regulator (Fletcher *et al.* 1999; Shaki *et al.* 2018). A wide range of morphological, physiological and biochemical changes in response to PEN occurs in the plants including *Triticum aestivum* L. and *M. pulegium* L. These changes consist modification in endogenous hormonal level, stimulation of stress-protective metabolites synthesis, increase in carbohydrate and compatible solutes such as osmoprotectants (Aly and Latif 2011; Hassanpour *et al.* 2014). The mentioned properties make PEN an ideal candidate to enhance the drought resistance of medicinal plants (Hassanpour *et al.* 2012). There are very limited studies related to the triazole effect on carbohydrate content and the endogenous regulator level in medicinal plants (i.e. Hassanpour *et al.* 2013). In the present study, we evaluated drought alleviating ability of PEN, with the special focus on plant height, biomass production, carbohydrate and hormone content. The outcome was aimed to assess the possibility of improving drought tolerance of *Satureja*.

Material and Methods

This study was conducted as a split-plot experiment based on randomized complete block design with three replications at the Faculty of Agriculture, University of Tabriz, Iran. *Satureja* seeds were obtained in summer 2018 from East Azarbaijan, Agricultural and Natural Resources Research and Education Center, AREEO, Tabriz, Iran. The seeds were sown in sowing trays, in a greenhouse with a light to dark periods of 16-8 h and day to night temperatures of 25-18 °C. Seven weeks after sowing, five seedlings were transferred to an individual pot filled with pit mass-perlite (1:1). Two weeks later, the seedlings were exposed to water insufficiency. The moisture regimes were imposed on main plots as 100%, 60% and 30% field capacity (FC) and then 0, 10 and 20 mg.l^{-1} PEN was assigned as the sub-plots. The pots were weighed every other day so that the soil moisture was retained at 100%, 60% and 30% FC levels. Four months after sowing, PEN concentrations were applied to the plants. PEN treatment was re-performed 10 days after the first application at both harvests. Four weeks following PEN treatment (synchronizing the beginning of flowering), plants were collected and dried for subsequent measurements and analyses.

Plant height and shoot biomass

Plant height, aerial fresh (FW) and dry weights (DW) were measured at the beginning of flowering, the optimal time for harvesting *Satureja sahendica* to achieve the oil of better quantity and quality (Sefidkon and Akbari-Nia 2009). Height was considered from the tip of the tallest stem to the soil level (Puvanitha and Mahendran 2017). The upper part of the stem above lignified section was cut, instantly weighted (fresh weight), and dried in a drying oven at 80 °C until it reached a consistent weight (Puvanitha and Mahendran 2017).

Total soluble carbohydrate (TSC)

The samples were dried in the oven at 72 °C for 48 h. To assess total soluble carbohydrate, phenol sulfuric acid method (Dubios *et al.* 1956) was used. The absorbance at 485 nm was measured by a spectrophotometer (Perkin Elmer, Lambda 35). HPLC system (Knuer, Germany) with RI detector and Eurokat-H column was applied for separation of glucose, sucrose and fructose. The mobile phase was water containing sulfuric acid at pH= 2.5 and a flow rate of 0.7 ml.min^{-1}.

Phytohormone

Extraction, purification and determination of IAA, GA and ABA was performed according to Nefed'eva and Khraynin (2003). The amounts of ABA, IAA and GA hormones were measured using reverse-phase LC18, HPLC column. The applied wavelength was 257 nm, with methanol and acetic acid 0.2% (1:1) as the mobile phase, at the flow rate of 0.7 ml.min^{-1}. The ratio of endogenous growth hormones to ABA in the leaves were also calculated.

Statistical analysis

Analysis of variance was utilized to determine the existence of significant differences among treatments and Duncan's multiple range test ($p \leq 0.05$) was used for the comparison of means. The analyses were performed by SAS software (Release 6.12, SAS Institute Inc. Cary, NC, USA).

Result and Discussion

Plant height

Regarding the plant height, 7.38% and 14.59% reduction was observed at 60% and 30% field capacity, respectively (Table 1). The decrease in plant height has been well documented in thyme (Babaee *et al.* 2010). *Satureja hortensis*, through height reduction, decreased its transpiration rate as a mechanism of the defense procedure (Sodaii Zadeh *et al.* 2016).

The minimum plant height was obtained at 20 mg.l^{-1} PEN treatment, 7.09% lower than that of PEN-free samples (Table 2). There was no significant difference in plant height between 0 and 10 mg.l^{-1} PEN treatments (Table 2). Diminished plant height has been reported in PEN-treated *Cymbidium sinensis* (Pan and Luo 1994), *Plectranthus aromaticus* and *Plectranthus vettiveroids* (Meena Rajalekshmi *et al.* 2009) as a result of triazole effect at endogenous regulator level.

The height reduction in the second harvest was about 18.75% higher than the first harvest (Table 3). The slope of height decrease was severe with the increasing water deficiency at the first harvest. Whilst at the second harvest, a decreasing trend was observed in height, but it was slow among the soil moisture regimes (Figure 1). At the first harvest, 20 mg.l^{-1} PEN treatment reduced 9.79% plant height compared to the control. However, the height changes at 10 mg.l^{-1} PEN treatment was not significantly different from the control treatment. At the second harvest, the stems were generally short in height. Not to mention that the plant height was not affected by PEN (Figure 2). Triazole is established to show its growth arrest through inhibition of GA synthesis. Koutroubas *et al.* (2015) demonstrated that the use of paclobutrazol (PBZ), retarded plant height in sunflowers. The application of triazole also showed a direct effect as decreased tomato height (Pal *et al.* 2016), accompanied by a yield increase (Syahputra *et al.* 2016).

Shoot biomass

Higher biomass was obtained in well-watered plants, whilst 23.82% and 22.96% reduction in FW and DW, respectively, was observed at 30% FC (Table 1). However, there was no significant difference between FC of 100% and 60% treatments (Table 1). The decrease in DW has been documented in barley, wheat and kidney bean under water deficiency (Saffan 2008). DW reduction under water deficit condition might be due to several factors such as retarded cell enlargement and extended leaf aging originated from decreased turgor pressure (Shao *et al.* 2008).

FW and DW weight increased about 12.22% and 14.05%, respectively, under 20 mg.l^{-1} PEN compared to the control, while there was no significant difference between the control and 10 mg.l^{-1} concentration (Table 2). The increased biomass production in triazole treated plants has been previously observed in *M. pulegium* (Hassanpour *et al.* 2014) and *Eragrostis Tef* (Tesfahun and Menzir 2018). Triazoles, through hormonal modification (Grossman 1990), may have induced dry weight increase under drought stress. Based on our results, at the second harvest, fresh and dry yield was about twice as high as the first harvest (Table 3). The increased biomass in the second year might be due to the deeper rooting of plants (Bahreininejad *et al.* 2013).

Our results demonstrated that under drought stress, FW and DW are significantly reduced in comparison to the control. PEN treatment at both

Table 1. Comparison of means for morphological, physiological and biochemical characteristics of *Satureja sahendica* under different soil moisture levels.

Soil moisture (%)	Plant height (cm)	Fresh weight (g/pot)	Dry weight (g/pot)	TSC (mg/g dw)	Fructose (mmol/g dw)	Glucose (mmol/g dw)	Sucrose (mmol/g dw)	IAA (mmol/g dw)	GA (mmol/g dw)	ABA (mmol/g dw)	(IAA+ GA)/ ABA
FC= 100	47.01a	8.55a	1.77a	15.80c	0.00187c	0.00193b	0.02499c	0.1085a	0.9030a	0.0832b	13.69a
FC= 60	43.54b	8.26a	1.73a	21.01b	0.00233b	0.00284a	0.02738b	0.0938b	0.8637ab	0.0871b	12.77a
FC= 30	40.15c	6.51b	1.36b	41.26a	0.00414a	0.00293a	0.03166a	0.0892b	0.8405b	0.2234a	6.72b

Different letters in each column indicate significant difference at p≤ 0.05 based on Duncan's Multiple Range Test; TSC: total soluble sugars; IAA: indoleacetic acid; GA: gibberellic acid; ABA: abscisic acid.

Table 2. Comparison of means for morphological, physiological and biochemical characteristics of *Satureja sahendica* under no-PEN and PEN-treated conditions.

PEN (mg/l)	Plant height (cm)	Fresh weight (g/pot)	Dry weight (g/pot)	TSC (mg/g dw)	Fructose (mmol/g dw)	Sucrose (mmol/g dw)	Glucose (mmol/g dw)	IAA (mmol/g dw)	GA (mmol/g dw)	ABA (mmol/ g dw)	(IAA+ GA)/ ABA
PEN= 0	45.00a	7.40b	1.53b	21.49c	0.00198c	0.02501c	0.00227b	0.10324a	0.9102a	0.0689c	15.82a
PEN = 10	43.89a	7.50b	1.55b	25.08b	0.00235b	0.02747b	0.00267a	0.09783b	0.8656b	0.1325b	10.34b
PEN = 20	41.81b	8.43a	1.78a	31.49a	0.00402a	0.031564a	0.00277a	0.09040c	0.8314b	0.1923a	7.02c

Different letters in each column indicate significant difference at p≤ 0.05 based on Duncan's Multiple Range Test; PEN: penconazole; TSC: total soluble sugars; IAA: indoleacetic acid; GA: gibberellic acid; ABA: abscisic acid.

Table 3. Comparison of means for morphological, physiological and biochemical characteristics of *Satureja sahendica* at two harvest stages.

Harvest	Culm height (cm)	Fresh weight (g/pot)	Dry weight (g/pot)	TSC (mg/g dw)	Glucose (mmol/g dw)	Fructose (mmol/g dw)	Sucrose (mmol/g dw)	ABA (mmol/g dw)	(IAA+GA)/ ABA
First harvest	48.07a	5.38b	1.09b	24.24b	0.00209b	0.00262b	0.02901a	0.0904b	12.57a
Second harvest	39.06b	10.18a	2.15a	27.80a	0.00305a	0.002957a	0.02701b	0.1721a	9.55b

Different letters in each column indicate significant difference at p≤ 0.05 based on Duncan's Multiple Range Test; TSC: total soluble sugars; IAA: indoleacetic acid; GA: gibberellic acid; ABA: abscisic acid.

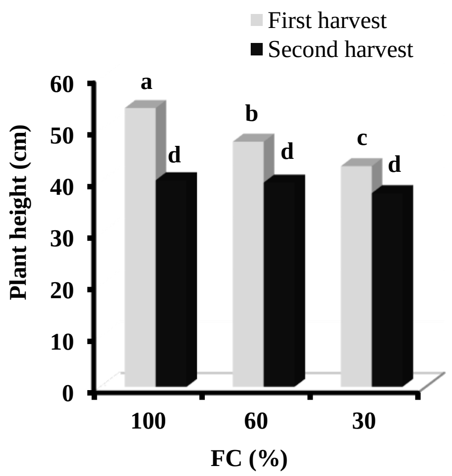

Figure 1. Plant height of *Satureja sahendica* under different soil moisture levels at two harvest stages.

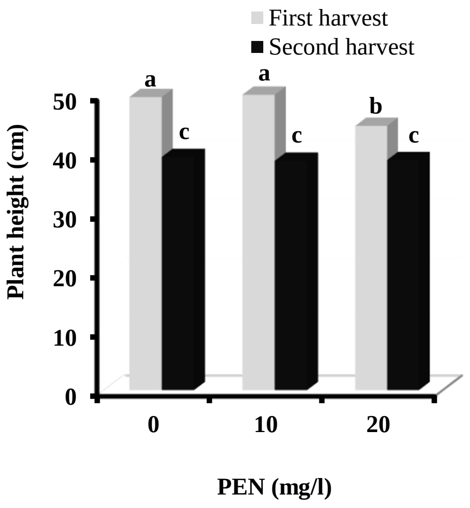

Figure 2. Plant height of *Satureja sahendica* under PEN-treated and PEN-free conditions at two harvest stages; PEN: penconazole.

harvests increased FW and DW compared to PEN-free plants. The improving effect of PEN was increasingly considerable with the growing water stress (Figures 3 and 4). Shao *et al.* (2008) reported that FW and DW reduction might further be as a result of a decrease in plant growth, as it is in agreement with the result of this study (Figures 1 and 2). Additionally, the notable decrease in *Satureja* growth under low irrigation may be attributed to unavailability of sufficient moisture in the root zone. This leads to less nutrient and water absorption and thus lower biomass production in the plant (Kamran *et al.* 2018). The increased biomass accumulation in stem improved under PEN treatments may be interpreted by its reverse relationship with suppressed height (to some threshold).

Total soluble carbohydrate (TSC)

Drought-stressed plants contained high levels of TSC than the control. Approximately, 32.98% and 161.18% increases occurred at 60% and 30% FC over well-watered plants (Table 1). The elevation of carbohydrate content has been observed in *Satureja rechingeri* (Shariat *et al.* 2016) under drought stress. According to Hennig *et al.* (2015), exacerbation of water deficiency leads to the increment of carbohydrate concentration and reduction of plant height and shoot DW.

Significant increase in TSC was determined in the plants treated by 10 and 20 mg.l^{-1} PEN (17.55% and 22.19%, respectively) as compared to control (Table 2). At the second harvest, a significant increase (14.69%) in soluble carbohydrates was achieved when compared to the first harvest (Table 3). Also, our results showed that the highest TSC was achieved at 30% FC at both harvests (Figure 5). TSC at the second harvest was 19.55, 13.44 and 19.55 percent higher than the first harvest (Figure 5).

Interaction of drought stress and PEN treatment was significant on TSC (Figure 6). Soluble carbohydrates content was highest at 30% FC with PEN consumption. The difference between 0 and 10 mg.l^{-1} PEN was not significant at 60% and 100% FC. However, soluble carbohydrates at 20 mg.l^{-1} PEN was increased by 45.19% and 47.61%, respectively, at 100% and 60% FC. Soluble carbohydrates content at 30% FC was 36.76% and 159.13% higher than those of 60% and 100% FC, respectively, by the use of 20 mg.l^{-1} PEN (Figure 6). Hassanpour *et al.* (2014) indicated that PEN treatment of drought-stressed *Mentha pulegium* L. increased sugar content as compared to the PEN free plants. It is pertinent to note that carbohydrates solute is crucial in the maintenance of mechanisms, such as cell volume compensation and cell damage reduction by free radicals (Al-Rumaih and AL-Rumaih 2007).

Sugar differentiation

Glucose, sucrose and fructose levels were significantly elevated by the increasing drought (Table 1). There was no significant difference in the glucose content between 60% and 30% FC (Table 1). Previous studies have indicated that carbohydrates (glucose, sucrose, fructose) are

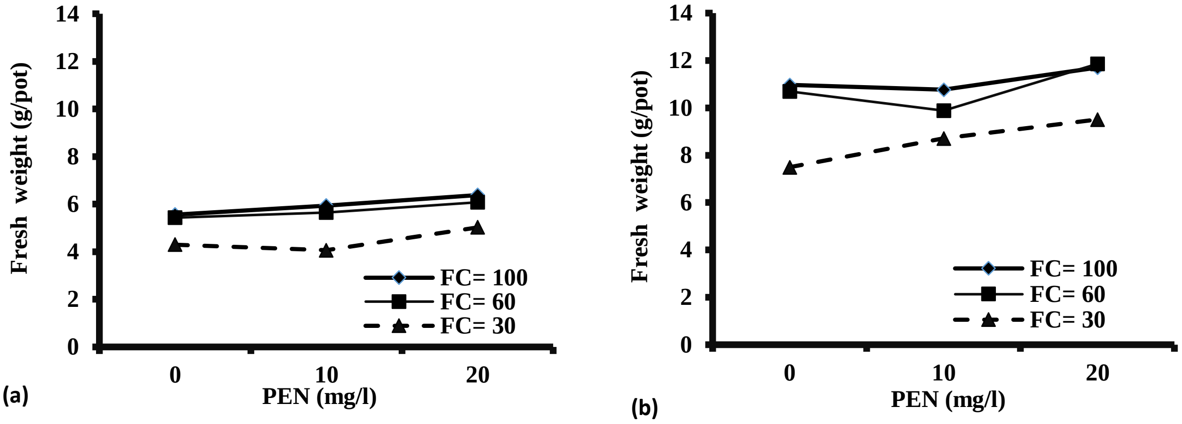

Figure 3. Effect of different soil moisture levels on fresh weight of *Satureja sahendica* under PEN-treated and PEN-free conditions at two harvest stages; a) first harvest; b) second harvest; PEN: penconazole.

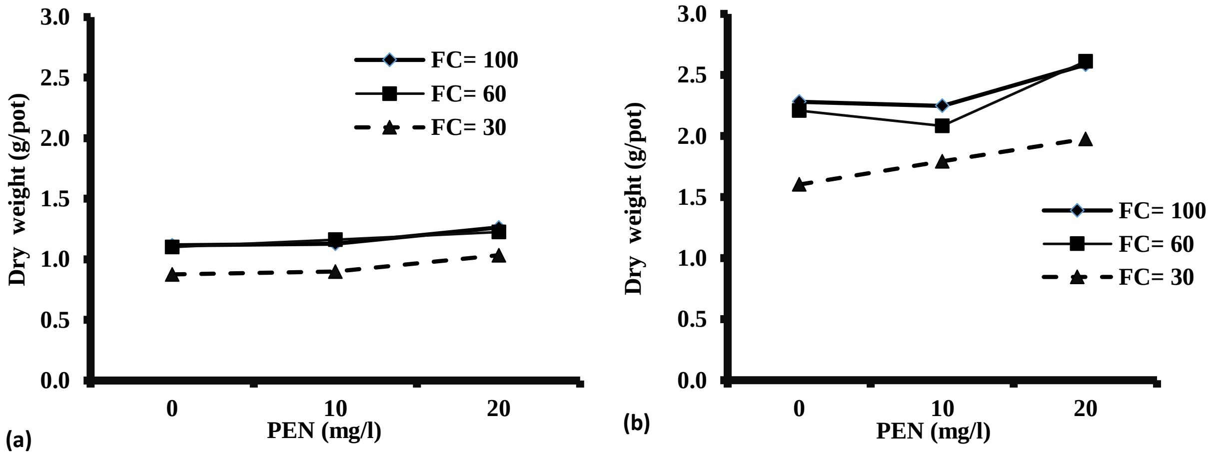

Figure 4. Effect of different soil moisture levels on dry weight of *Satureja sahendica* under PEN-treated and PEN-free conditions at two harvest stages; a) first harvest; b) second harvest; PEN: penconazole.

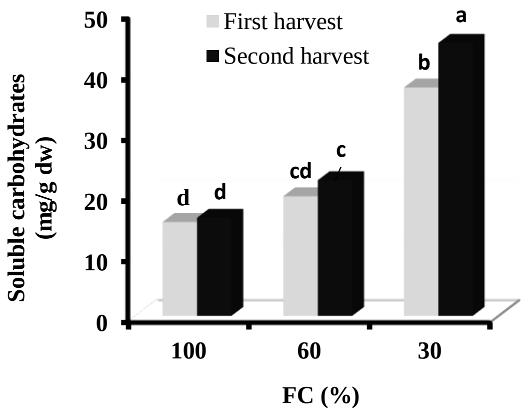

Figure 5. Soluble carbohydrates of *Satureja sahendica* under different soil moisture levels at two harvest stages.

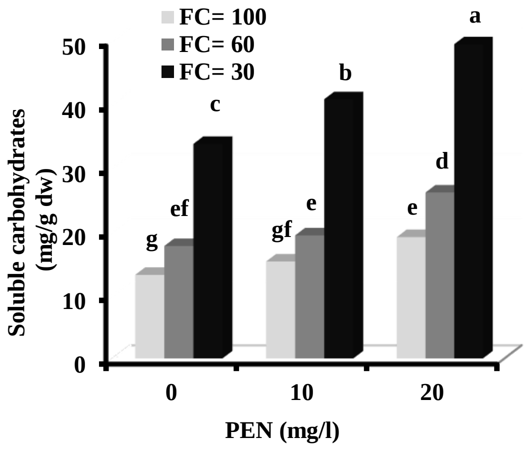

Figure 6. Soluble carbohydrates of *Satureja sahendica* at different soil moisture levels under PEN-treated and PEN-free conditions; PEN: penconazole.

fundamental compatible osmolytes (Wang 2014). In drought-stressed summer savory and basil, the water-soluble sugar content was shown to reach 4 and 8 folds of the control (Inotai 2013). It is worth to note that glucose modifies the ABA production (Cheng *et al.* 2002).

Increased glucose, sucrose and fructose was observed in PEN treated and untreated plants (Table 2). As for PEN treatments, 20 mg.l^{-1} increased over all sugar level compared to 10 mg.l^{-1} which increased glucose about 22.19%, sucrose 26.18% and fructose 103.38% over the control (Table 2).

There was an increase in glucose (14.69%), sucrose (45.88%) and fructose (12.54%) content at the second harvest over the first harvest (Table 3). Drought as well as PEN treatment increased sucrose and fructose in the *Satureja* plant at both harvest stages (Figures 7 and 8). At 30% FC, 20 mg.l^{-1} PEN augmented sucrose and fructose about 61.61% and 146% at the first harvest and about 4.84% and 128.93% at the second harvest (Figures 7 and 8). In contrast to our results, in the survey on summer savory, different increase pattern of sugar was observed in which sucrose constituted the predominant fraction (Inotai 2013). In the present study, however, fructose was strongly related with total soluble sugars under water stress conditions and was highly affected by PEN. Accumulated fructose may contribute to the storage of carbohydrates which is important in plant tolerance under abiotic stress (Guevara-Figueroa *et al.* 2015; Pardo-Rueda *et al.* 2015).

Hormones

Under drought stress, IAA content decreased in *Satureja* plants as compared to control plants (13.61% and 17.85% decrease at 60% and 30% FC, respectively) (Table 1). Correspondingly, a decreasing trend was observed in GA (4.35% and 6.92% at 60% and 30% FC, respectively) (Table 1). Decreases in IAA and GA content under water deficiency is a popular fact in plants (i.e. Wang *et al.* 2008). Water stress is cited in different references to diminish GA levels in *Lupinus albus* (Abdalla 2011) and common bean (Abass and Mohamed 2011). It is authenticated that, IAA and GA are well-known as reliable inducer for stem elongation and plant growth regulation. Recent researches emphasized their roles in response to stress conditions (Urano *et al.* 2017; Wang *et al.* 2017).

Based on our data (Table 2), PEN treatments resulted in reduction of IAA (5.24% and 12.44%) and GA (4.91% and 8.66%) contents at 10 and 20 mg.l^{-1} PEN, respectively, as compared to the the control. In the same way, PBZ treatment have decreased GA content in rice leaves in comparison to the control treatment (Syahputra *et al.* 2013). Our results showed that the plant height reduces with decreasing IAA and GA levels in PEN treated *Satureja* plants. As indicated above, the concentration of IAA (Figure 9) and GA (Figure 10) followed a significant decreasing trend in response to drought stress and PEN treatment at both harvests. This finding dicated that the restricted plant height under drought stress are due to the potent influence of IAA and GA reductions (Figures 9 and 10). Similarly, the triazole treated plants in *Mangifera indica* L. has shown a decrease in growth and DW, which was attributed to IAA and GA reduction (Sharma and Awasthi 2005).

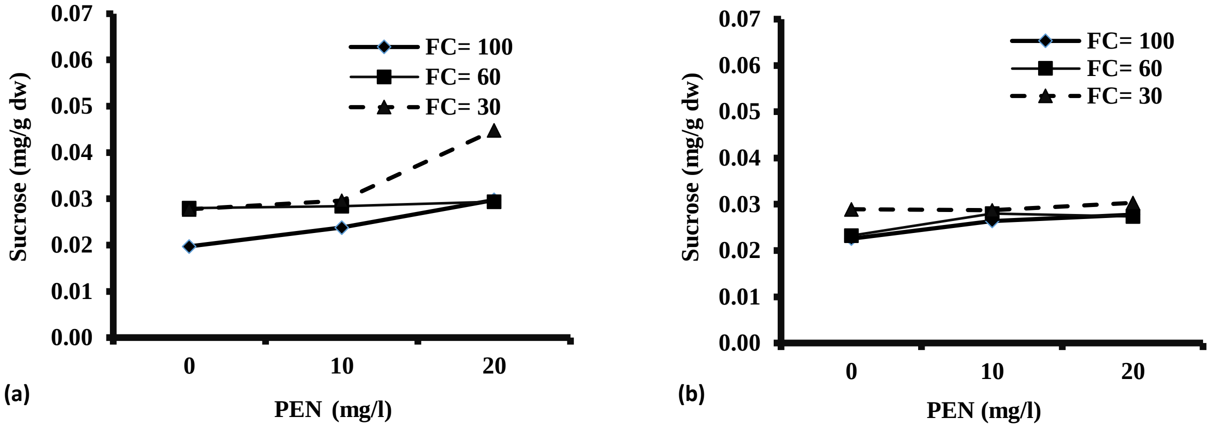

Figure 7. Effect of different soil moisture levels on sucrose content of *Satureja sahendica* under PEN-treated and PEN-free conditions at two harvest stages; a) first harvest; b) second harvest; PEN: penconazole.

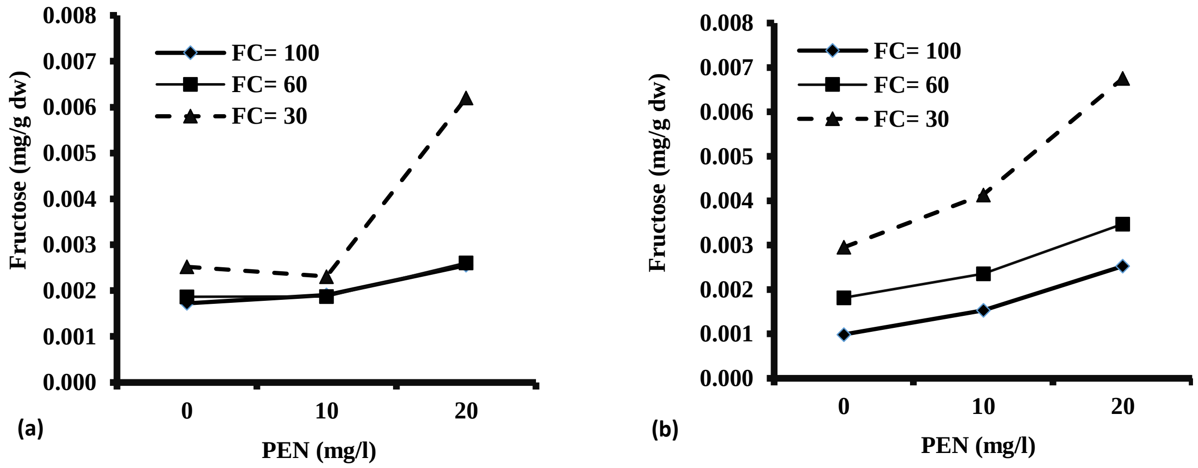

Figure 8. Effect of different soil moisture levels on fructose content of *Satureja sahendica* under PEN-treated and PEN-free conditions at two harvest stages; a) first harvest; b) second harvest; PEN: penconazole.

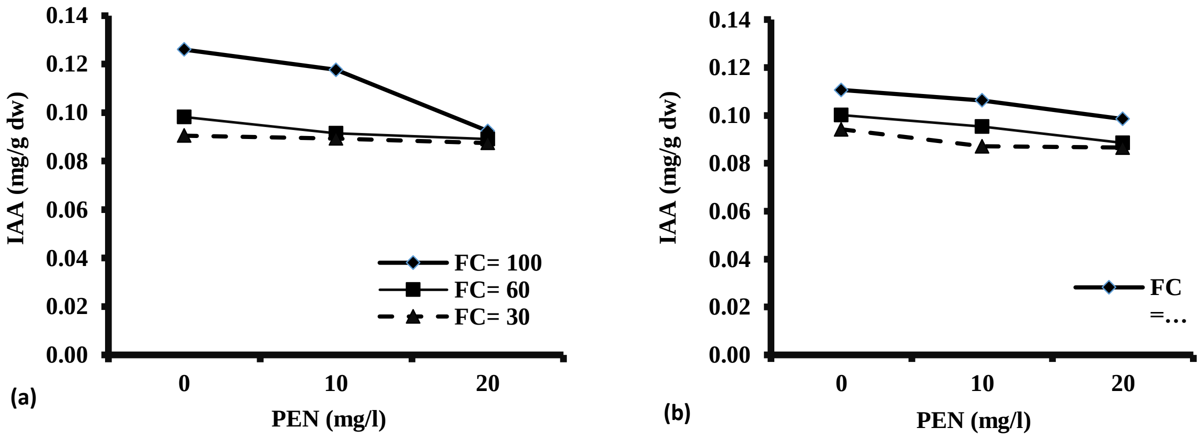

Figure 9. Effect of different soil moisture levels on indoleacetic acid (IAA) content of *Satureja sahendica* under PEN-treated and PEN-free conditions at two harvest stages; a) first harvest; b) second harvest; PEN: penconazole.

Through GA modification, plantenvironmental adjustment is improved, which plays a role inmaintaining the source-sink relationship. The latter results in sucrose increase which is observed in the present study (Figure 7).

In total, the observed reduction in plant height (Figure 2) is stemmed from inhibition of gibberellin biosynthesis by PEN (Figure 10) and prevention of kaurene to kaurenoic acid conversion in the biosynthesis pathway (Vettakkorumakankav *et al.* 1999). Triazoles further improve the auxin activity in plants by enhancing the activity of IAA-oxidase along with suppressing the transformation rate of tryptophan to IAA branches (El-Kady 2002). This was established in the results our study in which PEN decreased the level of growth regulators and ultimately was expressed as the inhibited growth.

In contrast to IAA and GA, ABA content was increased by the two factors of drought and PEN (Tables 1 and 2). At 30% FC, ABA was 168.38% higher than the control (Table1). No significant difference was determined between 60% and 100% FC (Table 1). It has been demonstrated that in the plants exposed to water deficiency, dehydrating in the plant roots leads to high ABA accumulation (Manzi *et al.* 2015). It was recorded that improving osmoregulation capacity is the result of high ABA accumulation in plants (Urano *et al.* 2009). ABA increases carbon remobilization, movement and transport of reserves during drought stress (Yang *et al.* 2003). Therefore, the ABA increment in the studied *Satureja* under PEN treatment was considered as the stimulator of plant height reduction and yield increase.

According to Table 2, ABA was significantly increased about 92.38% and 179.18% at 10 and 20 mg.l^{-1} PEN, respectively, as compared to the PEN free sample. It has been suggested that triazole induces an increase in ABA content of plants (Hassanpour *et al.* 2013).

At the second harvest, plants showed more induction effect in ABA production (90.44%) than that of the first harvest (Table 3). PEN treatment of either drought-stressed or non-stressed plants increased ABA content in the leaves when compared to the PEN free plants at both harvests (Figure 11). In comparison to PEN free plants, ABA increase at 10 and 20 mg.l^{-1} PEN was about 18.64% and 162.87% at the first harvest and 243.09% and 348.48% at the second harvest, respectively, at 30% FC (Figure11). The increase of drought tolerance due to ABA increment is a well-proven fact (Thameur *et al.* 2011).

A relative high ratio of (GA+IAA)/ABA was observed in *Satureja* leaves at 100% and 60% FC as compared to 30% (Table 1). This ratio significantly declined at 10 and 20 mg.l^{-1} PEN by 34.64% and 55.63% as compared to the control (Table 2). The (GA+IAA)/ABA ratio at the second harvest was found to be 23.97% lower than that of first harvest which confirmed the increasing accumulation of ABA as the time advances (Table 3). Variations of (GA+IAA)/ABA contents in non-stressed and stressed plants under PEN treatment showed that with the increasing water insufficiency, the ratio of growth hormones to the inhibitor hormone significantly reduced in both harvests (Figure 12). The environmental sub-optimal condition generally leads to high ABA and low IAA and GA content in the plant leaves (Verma *et al.* 2016). Our data (Figure 11, 9, 10)

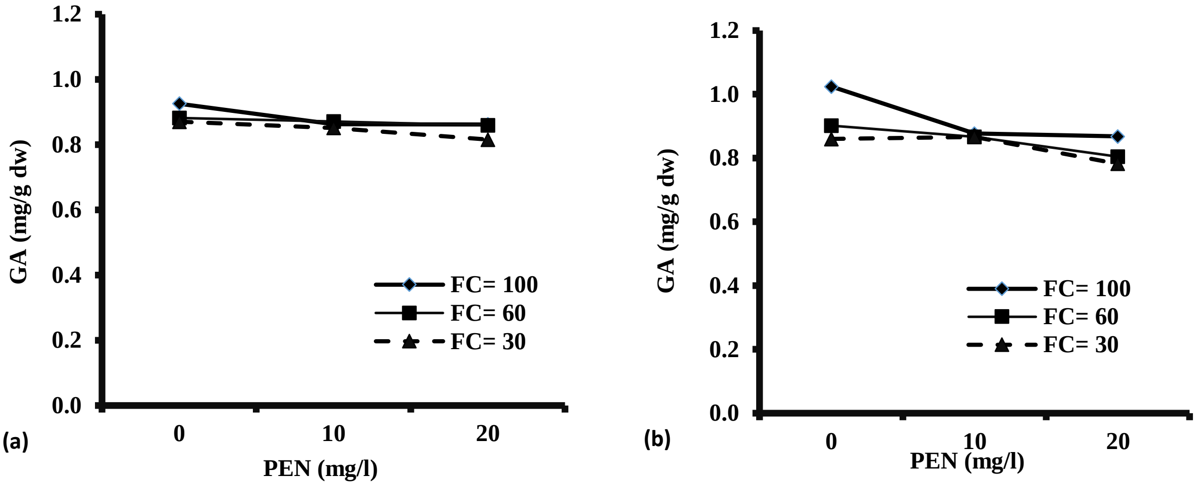

Figure 10. Effect of different soil moisture levels on gibberellic acid (GA) content of *Satureja sahendica* under PEN-treated and PEN-free conditions at two harvest stages; a) first harvest; b) second harvest; PEN: penconazole.

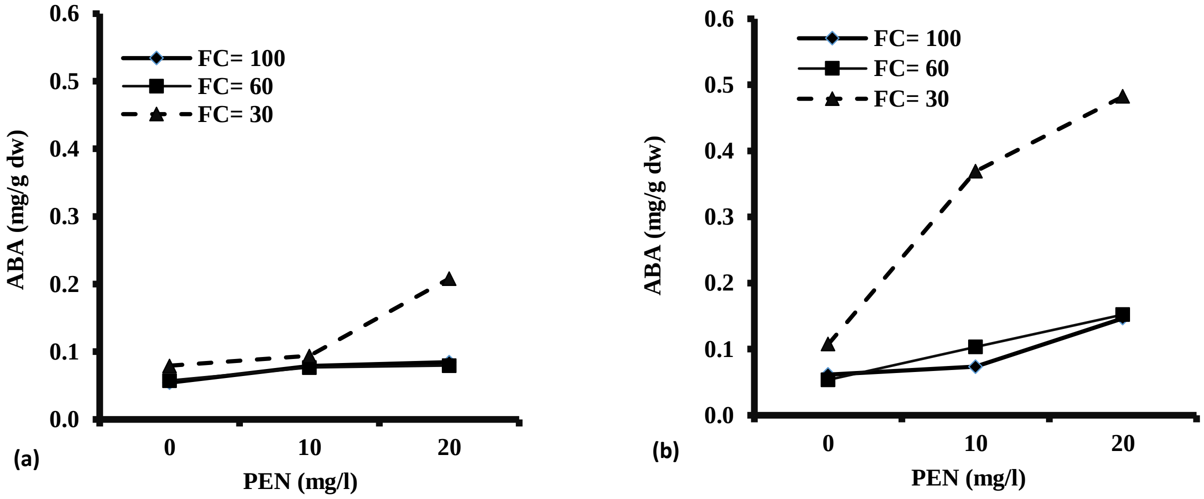

Figure 11. Effect of different soil moisture levels on abscisic acid (ABA) content of *Satureja sahendica* under PEN-treated and PEN-free conditions at two harvest stages; a) first harvest; b) second harvest; PEN: penconazole.

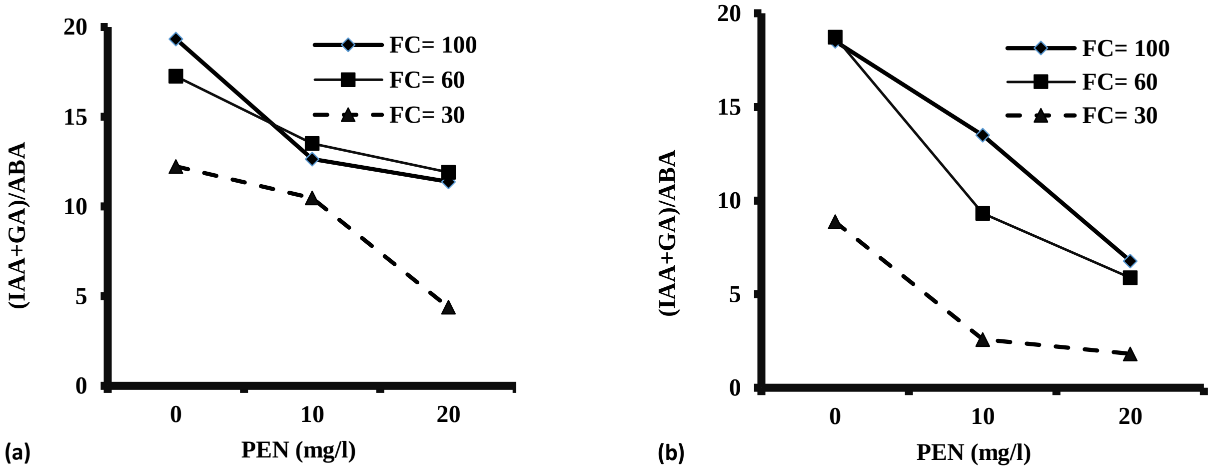

Figure 12. Effect of different soil moisture levels on (GA+IAA)/ABA ratio of *Satureja sahendica* under PEN-treated and PEN-free conditions at two harvest stages; a) first harvest; b) second harvest; PEN: penconazole; GA: gibberellic acid; IAA: indoleacetic acid; ABA: abscisic acid.

demonstrated the increase in ABA with decreasing IAA and GA which led to a higher decrease of (GA+IAA)/ABA. The reduction in the ratio of (GA+IAA)/ABA seems to be an important factor for drought tolerance induced by PEN in *Satureja* plants. Regulatory characteristics of triazoles in the plants are mediated by their capability to modify the balance of plant hormones consisting GA and ABA (Sridharan *et al.* 2015). They cause dry weight increase through modification of hormonal state under water deficiency (Grossman 1990). The growth prevention improves the stress tolerance in plants (Danquah *et al.* 2014).

Conclusion

According to the current research, drought stress reduced plant height, fresh and dry weights in *Satureja sahendica* plants. PEN application as an additive supplement augmented accumulation of TSC and additionally reduced the plant height. These changes might favor yield under drought stress. The variation caused by PEN could be attributed to IAA and GA decrease and ABA increase, especially under water deficiency conditions. Accumulation of 3.5 to 4.5 folds ABA and 2.5 folds fructose in the leaves occurred in PEN treatments. This was in contrast to the former studies in which sucrose was determined as the prominent carbohydrate fraction. ABA and fructose seem to demonstrate the major role in *Satureja* tolerance to drought stress with the passing time. In the treated plants of the second harvest, more biomass, carbohydrate and ABA was produced from shorter plants, which altogether contributed to stress tolerance compared to the first harvest. PEN treatment had an immense effect on hormonal balance via significant decrease (64%-80%) in (GA+IAA)/ABA ratio, especially at 30% FC with 20 mg.l^{-1} PEN as compared to the control. This caused a significant reduction in plant height and an increase in the yield and carbohydrate accumulation leading to ABA dominance, which has an important role in drought stress tolerance. On the other side, there was a slight significant difference in the studied features between the combined treatments of 60% and 100% FC with 0 and 10 mg.l^{-1} PEN levels. In brief, application of 20 mg.l^{-1} PEN together with 60% FC was considered as the most appropriate treatment in response to water deficiency. Hence, this treatment might be suggested for use in dry regions.

References

Abass SM and Mohamed H, 2011. Alleviation of adverse effects of drought stress on common bean (*Phaseolus vulgaris* L.) by exogenous application of hydrogen peroxide. Bangladesh Journal of Botany 41(1): 75-83.

Abdalla MM, 2011. Beneficial effects of diatomite on the growth, the biochemical contents and polymorphic DNA in *Lupinus albus* plants grown under water stress. Agriculture and Biology Journal of North America 2: 207-220.

Ajithkumar IP and Panneerselvam R, 2014. ROS scavenging system, osmotic maintenance, pigment and growth status of *Panicum sumatrense* Roth. under drought stress. Cell Biochemistry and Biophysics 68: 587-595.

Al-Rumaih MM and Al-Rumaih MM, 2007. Physiological response of two species of datura to uniconazole and salt stress. Journal of Food, Agriculture and Environment 5(3): 450-453.

Aly AA and Latif HH, 2011. Differential effects of paclobutrazol on water stress alleviation through electrolyte leakage, phytohormones, reduced glutathione and lipid peroxidation in some wheat genotypes (*Triticum aestivum* L.) grown in-vitro. Romanian Biotechnological Letters 16(6): 6710-6721.

Babaee K, Dehaghi MA, Modares Sanavi SAM and Jabbari R, 2010. Water deficit effect on morphology, proline content and thymol percentage of thyme (*Thymus vulgaris* L.). Iranian Journal of Medicinal and Aromatic Plants 26(2): 239-251 (In Persian with English abstract).

Baghalian K, Abdoshah S, Khalighi-Sigaroodi F and Paknejad F, 2011. Physiological and phytochemical response to drought stress of German chamomile (*Matricaria recutita* L.). Plant Physiology and Biochemistry 49(2): 201-207.

Bahreininejad B, Razmjoo J and Mirza M, 2013. Influence of water stress on morpho-physiological and phytochemical traits in *Thymus daenensis*. International Journal of Plant Production 7(1): 151-166.

Bartoli CG, Casalongue CA, Simontacchi M, Marquez Garcia B and Foyer CH, 2013. Interactions between hormone and redox signaling pathways in the control of growth and cross tolerance to stress. Environmental and Experimental Botany 94: 73-88.

Cantino PD, Harley RM and Wagstaff SJ, 1992. Genera of Labiatae: status and classification. In: Harley RM and Reynolds T (Eds) Advances in Labiatae Science. Pp. 511-522. Royal Botanic Gardens, Kew, UK.

Cheng W-H, Endo A, Zhou L, Penney J, Chen H-C, Arroyo A, Leon P, Nambara E, Asami T, Seo M, Koshiba T and Sheen J, 2002. A unique short-chain dehydrogenase/reductase in *Arabidopsis* glucose signaling and abscisic acid biosynthesis and functions. Plant Cell 14(11): 2723-2743.

Danquah A, de Zelicourt A, Colcombet J and Hirt H, 2014. The role of ABA and MAPK signaling pathways in plant abiotic stress responses. Biotechnology Advances 32(1): 40-52.

DuBios M, Gilles KA, Hamilton JK, Rebers PA and Smith F, 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28(3): 350-356.

El-Kady FM, 2002. Some studies on the effect of the growth regulator uniconazole on growth, some metabolic changes, level of endogenous hormones and productivity of wheat plants. MSc Thesis, Al-Azhar University, Faculty of Science, Botany Department, Egypt.

El-Maarouf-Bouteau H, Sajjad Y, Bazin J, Langlade N, Cristescu SM, Balzergue S, Baudouin E and Bailly C, 2015. Reactive oxygen species, abscisic acid and ethylene interact to regulate sunflower seed germination. Plant, Cell and Environment 38(2): 364-374.

Fletcher RA, Gilley A, Sankhla N and Davis TD, 1999. Triazoles as plant growth regulators and stress protectants. Horticultural Reviews 24: 55-138.

Ghahreman A, 1988. Flora of Iran. Research Institute of Forest and Rangeland, Tehran, Iran (In Persian).

Grossmann K, 1990. Plant growth retardants as tools in physiological research. Physiologia Plantarum 78(4): 640-648.

Guevara Figueroa T, Lopez Hernandez L, Lopez MG, Dufoo Hurtado MD, Vazquez Barrios ME, Guevara-Olvera L, Guevara Gonzalez RG, Rivera Pastrana DM, Torres-Robles H and Mercado- Silva EM, 2015. Conditioning garlic “seed” cloves at low temperature modifies plant growth, sugar, fructan content, and sucrose sucrose fructosyl transferase (1-SST) expression. Scientia Horticulturae 189: 150-158.

Hassanpour H, Khavari-Nejad RA, Niknam V, Najafi F and Razavi K, 2012. Effects of penconazole and water deficit stress on physiological and antioxidative responses in pennyroyal (*Mentha pulegium* L.). Acta Physiologiae Plantarum 34: 1537-1549.

Hassanpour H, Khavari-Nejad RA, Niknam V, Najafi F and Razavi K, 2013. Penconazole induced changes in photosynthesis, ion acquisition and protein profile of *Mentha pulegium* L. under drought stress. Physiology and Molecular Biology of Plants 19: 489-498.

Hassanpour H, Khavari-Nejad RA, Niknam V, Razavi Kh and Najafi F, 2014. Effect of penconazole and drought stress on the essential oil composition and gene expression of *Mentha pulegium* L. (Lamiaceae) at flowering stage. Acta Physiologiae Plantarum 36: 1167-1175.

Hennig A, Kleinschmit JRG, Schoneberg S, Loffler S, Janβen A and Polle A, 2015. Water consumption and biomass production of protoplast fusion lines of poplar hybrids under drought stress. Frontiers in Plant Science 6: 330. doi.org/10.3389/fpls.2015.00330.

Inotai K, 2013. The effect of water deficit on some stress parameters of *Ocimum basilicum* L. and *Satureja hortensis* L. Doctoral thesis. Doctoral School of Horticultural Sciences, Budapest, Hungary.

Jia H, Li C, Chai Y, Xing Y and Shen Y, 2013. Sucrose promotes strawberry fruit ripening by stimulation of abscisic acid biosynthesis. Pakistan Journal of Botany 45(1): 169-175.

Kaczmarek M, Fedorowicz-Stronska O, Głowacka K, Waskiewicz A and Sadowski J, 2017. $CaCl_2$ treatment improves drought stress tolerance in barley (*Hordeum vulgare* L.). Acta Physiologiae Plantarum 39: 41. https://doi.org/10.1007/s11738-016-2336-y.

Kafi M, Borzoee A, Salehi M, Kamandi A, Masoumi A and Nabati J, 2009. Physiology of environmental stresses in plants. Jehad Danshgahi Press, Mashhad, Iran, 502 pp. (In Persian).

Kamran M, Wennan S, Ahmad I, Xiangping M, Wenwen C, Xudong Z and Siwei M, Khan A, Qingfang H and Tiening L**,** 2018. Application of paclobutrazol affect maize grain yield by regulating root morphological and physiological characteristics under a semi-arid region. Scientific Reports 8: 1. doi: 10.1038/s41598-018-23166-z.

Koutroubas SD and Damlas CA, 2015. Sunflower response to repeated foliar applications of paclobutrazol. Planta Daninha 33(1): 129-135.

Man HM, Pollmann S, Weiler EW and Kirby EG, 2011. Increased glutamine in leaves of poplar transgenic with pine GS1a caused greater anthranilate synthetase α-subunit (ASA1) transcript and protein abundances: an auxin-related mechanism for enhanced growth in GS transgenics. Journal of Experimental Botany 62: 4423-4431.

Manzi M, Lado J, Jesús Rodrigo M, Zacarías L, Arbona V and Gómez-Cadenas A, 2015. Root ABA accumulation in long-term water-stressed plants is sustained by hormone transport from aerial organs. Plant and Cell Physiology 56(12): 2457-2466.

Matthews GA, 2018. A history of pesticides. CABI, UK, 310 pp.

Mozaffarian V, 2004. A Dictionary of Iranian Plant Names. Farhang Moaaser Publishing Company, Iran (In Persian).

Nefed'eva EE and Khraynin VA, 2003. The aftereffects of impulse pressure on the phytohormone content and some morphological characteristics of buckwheat plants. Russian Journal of Plant Physiology 46(2): 194-200.

Pal S, Zhao JS, Khan A, Yadav NS, Batushansky A, Barak S, Rewald B, Fait A, Lazarovitch N and Rachmilevitch S, 2016. Paclobutrazol induces tolerance in tomato to deficit irrigation through diversified effects on plant morphology, physiology and metabolism. Scientific Reports 6: 39321. doi.org/10.1038/srep39321.

Pan RC and Luo YX. 1994. Effect of PP333 on growth, development and leaf structure of *Cymbidium sinensis*. Acta Horticulturae Sinica 21(3): 269-272.

Pardo-Rueda AJ, Quintero-Ramos A, Genovese DB, Camacho-Davila A, Zepeda-Rodriguez A, Contreras-Esquivel JC and Bizarro AP, 2015. Efficient extraction of fructans from stool plant (*Dasylirion leiophyllum*) enhanced by a combination of enzymatic and sonothermal treatments. Food and Bioproducts Processing 94(4): 398-404.

Puvanitha S and Mahendran S, 2017. Effect of salinity on plant height, shoot and root dry weight of selected rice cultivars. Scholars Journal of Agriculture and Veterinary Sciences 4(4): 126-131.

Rajalekshmi KM, Abdul Jaleel C, Azooz MM and Panneerselvam R, 2009. Effect of triazole growth regulators on growth and pigment contents in *Plectranthus aromaticus* and *Plectranthus vettiveroids*. Advances in Biological Research 3(3-4): 117-122.

Rosa M, Prado C, Podazza G, Interdonato R, González JA, Hilal M and Prado FE, 2009. Soluble sugars--metabolism, sensing and abiotic stress: a complex network in the life of plants. Plant Signaling and Behavior 4(5): 388-393.

Saffan ES, 2008. Effect of salinity and osmotic stresses on some economic plants. Research Journal of Agriculture and Biological Sciences 4: 159-166.

Sairanen I, Novak O, Pincik A, Ikeda Y, Jones B, Sandberg G and Ljung K, 2012. Soluble carbohydrates regulate auxin biosynthesis via PIF proteins in *Arabidopsis*. The Plant Cell 24: 4907-4916.

Sefidkon F and Akbari-nia A, 2009. Essential oil content and composition of *Satureja sahendica* Bornm. at different stages of plant growth. Journal of Essential Oil Research 21(2): 112-114.

Shaki F, Ebrahimzadeh Maboud H and Niknam V, 2018. Penconazole alleviates salt-induced damage in safflower (*Carthamus tinctorius* L.) plants. Journal of Plant Interactions 13(1): 420-427.

Shao H-B, Chu L-Y, Abdul Jaleel C and Zhao CX, 2008. Water-deficit stress-induced anatomical changes in higher plants. Comptes Rendus Biologies 331(3): 215-225.

Shariat A, Karimzadeh G, Assareh MH and Zandi_Esfahan E, 2016. Drought stress in Iranian endemic savory (*Satureja rechingeri*): in vivo and in vitro studies. Journal of Plant Physiology and Breeding 6(1): 1-12.

Sharma D and Awasthi MD, 2005. Uptake of soil applied paclobutrazol in mango (*Mangifera indica* L.) and its persistence in fruit and soil. Chemosphere 60(2): 164-169.

Sodaei Zadeh H, Shamsaie M, Tajamoliyan M, Mirmohammady Meybody AM and Hakim Zadeh MA, 2016. The effects of water stress on some morphological and physiological characteristics of *Satureja hortensis*. Journal of Plant Process and Function 5(15): 1-11 (In Persian with English abstract).

Sridharan R, Raja S and Sakthivel P, 2015. Effect of triazole compounds on induced changes in growth biomass and biochemical content of white radish (*Raphanus sativus* L.). Journal of Plant Stress Physiology 1(1): 43-48.

Syahputra BSA, Sinniah UR, Syed Rastan SO and Ismail MR, 2013. Changes in gibberellic acid (GA3) content in *Oryza sativa* due to paclobutrazol treatment. Journal Food Pharmaceutical Sciences 1: 14-17.

Syahputra, BSA, Sinniah UR, Ismail MR and Swamy MK, 2016. Optimization of paclobutrazol concentration and application time for increased lodging resistance and yield in field-grown rice. The Philippine Agricultural Scientist 99(3): 221-228.

Taiz L and Zeiger E, 2010. Plant Physiology. 5th edition. Sinauer Associates Inc., Sunderland, UK.

Tesfahun W and Menzir A, 2018. Effect of rates and time of paclobutrazol application on growth, lodging, yield and yield components of Tef [*Eragrostis Tef* (Zucc.) Trotter] in Ada Woreda, East Shewa, Ethiopia. Journal of Biology, Agriculture and Healthcare 8(3): 104-117.

Thameur A, Ferchichi A and López-Carbonell M, 2011. Quantification of free and conjugated abscisic acid in five genotypes of barley (*Hordeum vulgare* L.) under water stress conditions. South African Journal of Botany 77(1): 222-228.

Tuna AL, 2014. Influence of foliarly applied different triazole compounds on growth, nutrition, and antioxidant enzyme activities in tomato (*Solanum lycopersicum* L.) under salt stress. Australian Journal of Crop Science 8(1): 71-79.

Urano K, Maruyama K, Jikumaru Y, Kamiya Y, Yamaguchi-Shinozaki K and Shinozaki K, 2017. Analysis of plant hormone profiles in response to moderate dehydration stress. The Plant Journal 90(1): 17-36.

Urano K, Maruyama K, Ogata Y, Morishita Y, Takeda M, Sakurai N, Suzuki H, Saito K, Shibata D, Kobayashi M, Yamaguchi-Shinozaki K and Shinozaki K, 2009. Characterization of the ABA- regulated global responses to dehydration in *Arabidopsis* by metabolomics. The Plant Journal 57(6): 1065-1078.

Verma, V, Ravindran P and Kumar PP, 2016. Plant hormone-mediated regulation of stress responses. BMC Plant Biology 16: 86. doi.org/10.1186/s12870-016-0771-y.

Vettakkorumakankav NN, Falk D, Saxena P and Fletcher RA, 1999. A crucial role for gibberellins in stress protection of plants. Plant Cell Physiology 40(5): 542-548.

Wang L-F, 2014. Physiological and molecular responses to drought stress in rubber tree (*Hevea brasiliensis* Muell. Arg.). Plant Physiology and Biochemistry 83: 243-249.

Wang B, Wei H, Xue Z and Zhang WH, 2017. Gibberellins regulate iron deficiency-response by influencing iron transport and translocation in rice seedlings (*Oryza sativa*). Annals of Botany 119(6): 945-956.

Wang C, Yang A, Yin H and Zhang J, 2008. Influence of water stress on endogenous hormone contents and cell damage of maize seedlings. Journal of Integrative Plant Biology 50(4): 427-434.

Yang JC, Zhang JH, Wang ZQ, Zhu QS and Liu LJ, 2003. Involvement of abscisic acid and cytokinins in the senescence and remobilization of carbon reserves in wheat subjected to water stress during grain filling. Plant, Cell and Environment 26(10): 1621-1631.

نقش کلیدی پنکونازول در تولید بیوماس در سطوح رطوبتی متفاوت خاک در گیاه مرزه سهندی (*Satureja sahendica* Bornm.)

رزیتا مظلومی اسکویی[1*]، محمد رضا شکیبا[2]، عادل دباغ محمدی نسب[2] و حسن منیری‌فر[3]

۱- دانشجوی دکتری فیزیولوژی گیاهان زراعی، گروه اکوفیزیولوژی گیاهی، دانشکده کشاورزی، دانشگاه تبریز، تبریز.
۲- گروه اکوفیزیولوژی گیاهی، دانشکده کشاورزی، دانشگاه تبریز، تبریز.
۳- بخش تحقیقات نهال و بذر، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی آذربایجان شرقی، تبریز.
*مسئول مکاتبه؛ Email: rozitamazloumi@gmail.com

چکیده

تنش خشکی به عنوان یک عامل محیطی مهم، محدود کننده رشد و عملکرد گیاهان به شمار می‌آید. گیاهان از طریق راهبردهای متعدد مانند تغییر در ارتفاع گیاه، بیوماس، محتوای کربوهیدرات و سطوح هورمونی به تنش‌های محیطی سازگار می‌شوند. بنابراین، یک طرح اسپلیت پلات در قالب بلوک‌های کامل تصادفی با سه تکرار برای تعیین سازوکارهای تحمل به خشکی القاء شده به وسیله پنکونازول در گیاه مرزه سهندی به اجرا درآمد. تیمار سطوح آبیاری (نرمال، ۶۰٪ = FC و۳۰٪ = FC) و پنکونازول (شاهد، ۱۰ و۲۰ میلی گرم در لیتر) اعمال شد. نتایج نشان داد که تنش خشکی منجر به کاهش معنی‌دار ارتفاع گیاه، عملکرد تر و خشک، محتوای اسید ایندول استیک (IAA) و اسید ژیبرلیک (GA) و القای افزایش قابل توجه کربوهیدرات‌های محلول کل (TSC)، گلوکز، ساکارز، فروکتوز و اسید آبسیزیک (ABA) می‌گردد. تیمار پنکونازول منجر به کاهش ارتفاع بوته، IAA، GA و افزایش عملکرد تر و خشک، TSC، گلوکز، ساکارز، فروکتوز و ABA شد. تیمار پنکونازول سطح فروکتوز و ABA را تقریبا ۲/۵ و ۴/۵-۳/۵ برابر بیشتر از شاهد، به ویژه در برداشت دوم، افزایش داد. کاهش معنی‌دار هورمون‌های رشد به موازات افزایش چشمگیر هورمون بازدارنده با افزایش تنش خشکی و تیمار پنکونازول منجر به کاهش معنی‌دار در نسبت GA+IAA)/ABA) شد (حدود ۷۰ درصد در سطح آبیاری ۳۰ درصد و غلظت ۲۰ میلی گرم در لیتر پنکونازول در مقایسه با شاهد). در مجموع به نظر می‌رسد که کاربرد ۲۰ میلی‌گرم در لیتر پنکونازول همراه با رژیم رطوبتی برابر با ۶۰ درصد ظرفیت مزرعه‌ای، می‌تواند تیمار مناسبی برای زراعت مرزه سهندی در مناطق خشک باشد. علاوه بر این، افزایش تولید فروکتوز و سطح ABA القاء شده به وسیله پنکونازول نقش برجسته‌ای در تحمل به خشکی این گیاه دارد.

واژه‌های کلیدی: ارتفاع گیاه؛ بیوماس؛ پنکونازول؛ تنش خشکی؛ کربوهیدرات‌های محلول؛ مرزه سهندی؛ هورمون.