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EFFECTS OF FERTILIZER TYPES ON THE QUANTITATIVE AND QUALITATIVE ATTRIBUTES OF MUSKMELON (*CUCUMIS MELO* L. CV. AHLAM) UNDER DIFFERENT LEVELS OF DROUGHT STRESS

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Abstract

Nowadays, consuming biological fertilizers and natural compounds is considered as the major basis of sustainable agriculture in order to reduce or remove chemical substances. In this case, the effects of the Nitroxin bio-fertilizer and fertilizers (more/less common) in different drought stresses (50, 75 and 100% of field capacity), was assessed within three intervals in Khonj, Fars, Iran during two successive years. The results revealed that drought stress and fertilizer type had a significant effect on the physiological and chemical attributes of muskmelon fruit. The lowest electrolyte leakage (EL) was observed in fertilizer treatment (0 and 60 kg/h) and 100% irrigation that has shown the fertilizers reduced EL in the treated plant. Since drought stress accompanied by the fertilizers, leaf relative water content was reduced, however, the application of both chemical and biological fertilizers increased the photosynthetic pigments including chlorophyll and carotenoid. Drought stress and application of fertilizers solely, increased malondialdehyde (MDA), phenol and antioxidant enzymes activities. According to the obtained results, using chemical and biological fertilizers in the low-irrigation conditions can improve the muskmelon tolerance for overcoming the negative effects of drought stress.

Keywords: Nitroxin, leaf relative water content, Proline, antioxidant enzyme activity.

Introduction

Melons (*Cucumis melo* L.) (Ozdemir *et al.*, 2019) are one of the most important horticulture crops which are often cultivated in the tropical and temperate regions of the world (Barzegar *et al.*, 2013). Overall production of melons was estimated to be 31,166,896 tons in the world and Iran rated as the third major producer after China and Turkey, delivering nearly 145,000 tons of melons a year (FAO, 2016; Li *et al.*, 2017). Melons, especially cantaloupes are spread in a wide area with a wide range of production (Pitrat, 2018) and also melon is one of the most common and important products in Iran. Due to its long growth period and the tolerance to high temperature, melon cultivation is very common in the arid and semi-arid regions (Kusvuran *et al.*, 2011). In such areas, water shortage is the main limitation of melons production. It is obviously a significant challenge in Cantaloupes cultivation in Iran and thus, making the most out of the water resources is the most important feature to make the planter capable of planting it (Leskovar and Piccinni, 2005). Persistent droughts in Iran are noteworthy alarms in agricultural issues (Aslmarz *et al.*, 2019; Sharafi *et al.*, 2019). Drought stress occurs when the amount of the received water in a plant is less than its waste. This can be resulted due to excessive water loss, reduction in water uptake or both. Water shortage affects the growth of plants during all development stages (Alizadeh *et al.*, 2011), but the amount of damage depends on the growth stage, the time and severity of stress, and the duration of water stress. The most sensitive stages of the cantaloupe are the flowering and fruit set. Sever water deficiency significantly reduces plant growth, causing smaller fruits (Long *et al.*, 2006) and also a reduction in fruit set (Alizadeh *et al.*, 2011). Proper irrigation is essential for maximum yield and water conservation in watermelon and melon (Orta *et al.*, 2003; Wang *et al.*, 2004). Fibero *et al.* (2002) reported that severe drought stress at a long period reduced flowers, fruit set and yield in cantaloupes and melons. Moreover, Pervez *et al.* (2009) reported that the drought stress reduced number and fruit size per plant. Several researchers have shown that both regular irrigation and optimal fertilizers consumption plays an important role in improving the quality and quantity of cantaloupe (Castellanos *et al.*, 2011; Yuan *et al.*, 2013). Although nowadays, the usage of fertilizers has proved to be the fastest way to compensate for the deficiency of soil mineral elements and high yields, in many cases the usage, of these fertilizers results in environmental pollution and the degradation of physical and chemical properties of the soil and increases the cost of production (Kalhapure *et al.*, 2013). Therefore, in recent years, the integrated plant management has been introduced to maintain and increase the fertility of the soil by optimal usage of chemical, organic and biological fertilizers in appropriate proportion (Chakeral hosseini, 2006). Biological fertilizers are made up of useful microorganisms, which are produced specifically for the stabilization of nitrogen, the release of iron ions, phosphates, potassium and etc. (Wu *et al.*, 2005). These microorganisms are usually located around the root and help the plant to uptake the mineral elements (Nagananda *et al.*, 2010). Bio-fertilizers, using non-absorbent elements in the soil, help maintain the chemical balance of the soil along with increasing the optimum yield of the plant (Sharma, 2002). Nitroxin can be mentioned as one of the bio-fertilizers. Nitroxin has a set of nitrogen-fixing bacteria including Azotobacter and Azospirillum, which causes the growth and development of plant shoots and roots (Gilik *et al.*, 2001). These bacteria are capable to increase seed germination, rooting and root growth through the synthesis and secretion of growth regulators such as auxin, gibberellin and cytokinin, and thereby increase the provision of the nutrients required by the plant, such as nitrogen and phosphorus increase plant growth (Vessey, 2003). Kolhapur *et al.* (2013) reported that the combination of chemical and biological fertilizers is the best option for improving plant performance. Some researchers reported that the application of both chemical and biological fertilizers in combination form significantly increased the performance of the plant and increased the number of leaves, leaf surface, uptake of nitrogen, phosphorus and potassium in cucumber (Raeisi *et al.*, 2013; El-Sayed *et al.*, 2016), cantaloupe (Al-Fraihat, 2011) and melons (Castellanos *et al.*, 2011). Abdolnabi *et al.* (2014) reported that the usage of nitrogen fertilizers, because of its impact on growth parameters, directly affects the performance of the plants, so that the application of 125 kg of nitrogen per hectare increased the yield of the cantaloupes.

According to Li *et al.* (2018), irrigation increased the efficiency of fertilization and because of the positive correlatin between irrigation and fertilization, optimal water conditions are economically more beneficial than in the state of water shortages. In drought, nutrient uptake, especially nitrogen decreases in soil. There is an obvious need to keep the balance between the moisture in the soil and the consumption of nitrogen. Due to the close relationship between soil moisture and nutritional availability, the aim of this study was to investigate the effects of different levels of chemical and biological fertilizers for introducing the best fertilizer combination to improve the performance of muskmelon cv. Ahlam in the exposure of low and severe drought stress.

Material and Methods

In order to study the effects of the Nitroxin and fertilizers under different drought stress levels (50, 75 and 100% of field capacity), a factorial experiment with three factors (drought stress, bio, and chemical fertilizers), based on a randomized complete block design with three replications was conducted in Khonj, Fars, Iran, during two successive years (2017-2018). Drought stress was applied in three levels: 100, 75 and 50% of field capacity; the fertilizers (NPK) were applied in four levels, control (no consumption), 100% of the recommended amount, one-third less than the recommended amount, and two-third less than the recommended amount; Nitroxin (produced by MehrAsia biotechnology Company) was used in two levels of control (no consumption) and 100% application.

The amount of the application of the fertilizers was determined based on soil degradation and plant requirements. Fertilizers were applied in three parts and according to the growth stage of plant. The first part consists of 1/3(33%) of the calculated fertilizer which added to the soil along with plantation. The second part applied in thinning times and the last part added before flowering. Nitroxin was used 1 liter/hectare as rubbed into the seed. Stress treatment was done with a tanker only after the moisture level reached 50%, 75% and 100% of field capacity. The amount of the cumulative evaporation was measured daily using an evaporation pan located on the farm according to which the irrigation was done. Before the experiment, random samples were taken from the depth of 0-30 and 30-60 cm of soil and they were sent to the lab for soil analysis. After the period of treatment, the fruits were studied for their biochemical properties (plant pigments), osmotic regulator (proline), physiological traits (relative water content of the leaves, electrolyte leakage), antioxidant enzymes and phenolic compounds. Throughout the process, for achieving exact details, fruits were measured by digital scales.

Catalase

0.2 g of frozen sample was extracted in 3 ml of sodium phosphate buffer (25 mM) with a pH of 6.8. The obtained homogenates were centrifuged at 15000 rpm for 15 min at 4 °C and then the supernatant was used as an enzymatic crude extract. Catalase activity was measured by Comket and Horst method (1991): Hydrogen Peroxide decomposition was performed by reducing the absorbance at 240 nm wavelength using spectrophotometry (Jenway-6505 UV).

Peroxidase

According to Ghanati *et al.* (2002), the activity of the peroxidase enzyme was determined based on the amount of guaiacol oxidation at 480 wavelengths.

Polyphenol oxidase

The changes of polyphenol oxidase enzyme activity were measured by the Kahn method (1975) and readied based on light absorption changes at 1080 intervals at 480 nm.

Superoxide dismutase

The activity of the superoxide dismutase enzyme was measured based on the capability of the enzyme to hinder Nitro blue tetrazolium optical retrieval at 560 nm (Giannopolitis and Ries, 1977). Determination of the antioxidant content of the fruit extract is conducted based on the free radical release of DPPH by antioxidants in the absence of other free radicals in the environment, which results in a color spectrum in the environment that can be measured using the ItdT80+UV/VIS PG Instruments optical spectrometer (Miliauskas *et al.*, 2004).

Malondialdehyde and Proline content

Malondialdehyde enzyme and the osmotic adjustments of the proline were measured using Health and Packer (1968) and Bates (1973) method, respectively.

Phenolic content

Changes in phenolic content were investigated during the experiment by Swain and Hillis (1959) method.

Total chlorophyll and carotenoids

The Arnon method (1949) was used to measure total chlorophyll and carotenoids: 0.1 g of fresh leaves was mixed with 10 ml of acetone in a mortar and pestle and the extract was measured for total chlorophyll at 663 and 645 wavelengths and 470 nm for carotenoid by spectrophotometry (Jenway-6505 UV) after being centrifuged. The amount of chlorophyll and carotenoids (mg per g fresh tissue of the leaf) are measured according to the following equations:

$$\text{Chla} = \{12.25\,(A663.2) - 2.79\,(A646.8)\} \times V/1000 \times W$$
$$\text{Chlb} = \{12.21(A646.8) - 5.10\,(A663.2)\} \times V/1000 \times W$$
$$\text{ChT} = \text{chlb} + \text{Chla}$$
$$\text{Car} = [(1000(A470) - 1.8(\text{chla}) - 85.02\,(\text{chlb})\,/198]$$

A: measured absorbance V: consumed acetone
W: the weight of the sample (g)

Electrolyte leakage

Measurement of electrolyte leakage was carried out according to the method of Lutts *et al.* (1995).

Ascorbate peroxidase

The activity of the ascorbate peroxidase enzyme was also performed using the Dazy *et al.* (2008) method. After preparing the protein extracts, 2 ml phosphate buffer (0.5 M and pH 6.5), 0.2 ml of hydrogen peroxide (V/V 3%) and 0.2 ml of ascorbate 50 mm were mixed in an ice bath and immediately 0.1 ml of the enzyme extract was added; The absorbance curve was read at 265 nm.

Relative water content

In order to measure the relative water content of the leaf, the fresh weight (FW) of the isolated discs from the leaf was measured. Then, in order to determine the weight of the saturated mode (TW), the leaves were placed in distilled water for 5 hours at room temperature. In the end, to measure the dry weight (DW), the leaves were placed in the oven of 70 °C for 5 minutes. The relative water content of the leaves is calculated from the following formula (Sangeetha *et al.*, 2006):

RWC = (FW-DW/TW-DW(×100

Statistical Analysis

Data were analyzed using the analysis of variance procedure in SAS version 9.1. Means separation between treatments was performed using Duncan multiple range test.

Results and Discussion

Peroxidase Enzyme (POD)

The effects of the interaction of fertilizers and bio- The effects of the interaction of fertilizers and bio-fertilizer with drought stress on peroxidase enzyme were significant at 1% level of probability. The highest amount of peroxidase enzyme (1.8 Units/mg Pro) was obtained in 50% FC and 180 kg/ha fertilizers without bio-fertilizer application and the lowest amount (0.007 Units/mg Pro) was achieved in the 180 kg/ha chemical and bio-fertilizer application with 100% (Table 1). Also in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

Table 1: Effects of drought stress and bio-fertilizer and fertilizers on Catalase, Total chlorophyll, Superoxide dismutase, Polyphenol oxidase, Proline, Relative water content of cantaloupe.

Drought stress	Biological fertilizer	Fertilizers	CAT (Units/mg Pro)	Total Chl (mg/g FW)	SOD (Units/mg Pro)	POD (Units/mg Pro)	Proline (g/gFw)	RWC (%)
50% FC	Non-usage	0 kg/h	23.57c	1.3e-h	276.8c	1.4b	195.1b	44.3l
		60 kg/h	24.63bc	1.02f-i	241.1d	0.8d	178.6c	50.2k
		120 kg/h	33.98a	0.37ij	258.3b	1.8a	214.1a	35.5m
		180 kg/h	34.57a	0.28j	375.8a	1.8a	214.3a	37.5m
	Usage	0 kg/h	24.18c	1.4efg	229.1d	1.1c	153.6d	57.5ij
		60 kg/h	11.61e	1.4e-h	224.6d	0.1f	138.7e	55.8j
		120 kg/h	26.75b	0.85g-j	286.1c	1.4b	196.8b	43.06l
		180 kg/h	26.78b	1.6ef	278.3c	1.12c	198.8b	71gh
75% FC	Non-usage	0 kg/h	10.26e	2.6d	65.2f	0.11ef	44.7fg	73.8gh
		60 kg/h	11.26e	1.9e	56.3f	0.15e	40.6g	83.6f
		120 kg/h	15.9d	0.7hij	85.6e	0.15e	49.5f	59ij
		180 kg/h	16.21d	0.55ij	89.9e	0.15e	49.6f	62.5i
	Usage	0 kg/h	11.02e	2.81d	53.3f	0.09f	34.5h	85.8e
		60 kg/h	4.7fgh	2.71d	52.1f	0.008g	30.7h	83e
		120 kg/h	12.31e	1.6ef	67.5f	0.11ef	45.2fg	71.8h
		180 kg/h	12.32e	3.2cd	65.6f	0.09f	45.7fg	126.6b
100% FC	Non-usage	0 kg/h	4.06gh	4.9b	28.2g	0.009g	17.6i	92.9e
		60 kg/h	3.7h	3.6c	28.3g	0.006g	17.8i	105.3d
		120 kg/h	6.3fg	1.3e-h	28.3g	0.012g	17.8i	74.3gh
		180 kg/h	6.5f	1.05f-i	28.3g	0.013g	17.8i	78.7fg
	Usage	0 kg/h	3.6h	5.3b	28.3g	0.007g	17.8i	121c
		60 kg/h	0.14i	5.1b	28.3g	0.0007g	17.8i	117.1c
		120 kg/h	4.3fgh	3.09cd	28.3g	0.009g	17.8i	90.2e
		180 kg/h	4.36fgh	6.1a	28.3g	0.007g	17.8i	159.5a

The averages in each column that are common in at least one element, do not reveal significant differences.

Table 2: Impact of fertilizers on Ascorbate Peroxidase, Carotenoid, Phenol, Malondialdehyde, Antioxidant, Electrolyte leakage, Catalase, Total chlorophyll, Superoxide dismutase, Polyphenol oxidase, Proline and Relative water content of cantaloupe.

Fertilizers	APX	Carotenoid	Phenol	MDA	Antioxidant	Electrolyte leakage	CAT	Total Ch	SOD	POD	Proline	RWC
0 kg/h	342.1b	4.97a	3.4c	1.1b	29.8c	26.4b	12.8b	3.1a	113.5b	0.45c	77.2b	80.8c
60 kg/h	324.3c	5.23a	3.1d	0.89c	28.1d	24.1c	9.3c	2.6b	105.1c	0.18d	70.7c	84.1b
120 kg/h	412.9a	2.59c	5b	1.5a	38.2a	38.5a	16.6a	1.3d	142.3a	0.59a	90.2a	62.d
180 kg/h	416.2a	3.72b	5.5a	1.5a	36.3b	38.1a	16.7a	2.2c	144.5a	0.55b	90.6a	90.1a

The averages in each column that are common in at least one element, do not reveal significant differences.

Table 3: Impact of bio-fertilizer on Ascorbate Peroxidase, Carotenoid, Phenol, Malondialdehyde, Antioxidant, Electrolyte leakage, Catalase, Total chlorophyll, Superoxide dismutase, Polyphenol oxidase, Proline and Relative water content of cantaloupe.

Biological fertilizer	APX	Carotenoid	Phenol	MDA	Antioxidant	Electrolyte leakage	CAT	Total Ch	SOD	POD	Proline	RWC
Non-usage	404a	3.3b	4.7a	1.4a	36.1a	35.6a	15.9a	1.6b	138a	0.55a	88a	66.5b
Usage	343b	4.9a	3.8b	1.1b	30.2b	28.1b	11.8b	2.9a	114b	0.34b	76b	92.2a

The averages in each column that are common in at least one element, do not reveal significant differences.

Table 4: Impact of drought stress on Ascorbate Peroxidase, Carotenoid, Phenol, Malondialdehyde, Antioxidant, Electrolyte leakage, Catalase, Total chlorophyll, Superoxide dismutase, Polyphenol oxidase, Proline and Relative water content of cantaloupe.

Drought stress	APX	Carotenoid	Phenol	MDA	Antioxidant	Electrolyte leakage	CAT	Total Ch	SOD	POD	Proline	RWC
50% FC	820a	0.6c	8.1a	1.9a	45a	51a	25.7a	1.1c	283a	1.2a	186a	51c
75% FC	201b	4.2b	2.6b	1.1b	36b	26b	11.7b	2.1b	66b	0.1b	42b	83b
100% FC	100c	7.6a	2.2c	0.7c	17c	18c	4.1c	3.8a	28c	0.008c	17c	104a

The averages in each column that are common in at least one element, do not reveal significant differences.

Superoxide dismutase enzyme (SOD)

The effects of the tripartite interaction of fertilizers and bio-fertilizer with drought stress on superoxide dismutase enzyme were significant at a 1% level of probability. The highest amount of superoxide dismutase enzyme (375.8 Units/mg Pro) was obtained in 50% field capacity treatment and 180 kg/ ha fertilizers without bio-fertilizer application and the lowest amount (28.2 Units/mg Pro) was achieved in the 100% FC without using chemical or bio-fertilizer (Table 1). Also in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

Catalase (CAT) Enzyme

The effects of the tripartite interaction of fertilizers and bio-fertilizer with drought stress on CAT activity were significant at a 1% level of probability. The highest amount of CAT enzyme (34.57 Units/mg Pro) was obtained in 50% FC and 180 kg/ ha fertilizers without bio-fertilizer application and the lowest amount (0.007 Units/mg Pro) was achieved in the 180 kg/ha chemical and bio-fertilizer application at 100% FC (Table 1). Also in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

Total chlorophyll

The effects of the tripartite interaction effects of fertilizers with bio-fertilizer with drought stress were significant at 1% level of probability. The highest total chlorophyll (6.1 mg/g FW) was obtained in 100% FC with 180 kg ha-1 fertilizer and bio-fertilizer application and the lowest total chlorophyll content (0.28 mg/g FW) in 50% FC with 180 kg/ha fertilizers application without bio-fertilizer consumption (Table 1). Also in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

Proline

The effects of tripartite interaction of fertilizers with bio-fertilizer and drought stress were significant on the amount of proline. The highest proline content (214.3 g/gFW) was obtained in 50% FC with 180 kg/ha fertilizers without using bio-fertilizers. The lowest amount of proline (17.6 g/g FW) was in 100% FC treatment without using the chemical and bio-fertilizers (Table 1). Also, in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

Relative Water Content (RWC)

The effects of tripartite interaction of fertilizers with bio-fertilizer with drought stress were significant on leaf relative water content at a 1% level of probability. The maximum relative content water of leaf (95.5%) was obtained in the treatment of 180 kg/ha fertilizers with the application of bio-fertilizer and 100% farm capacity. The minimum relative water content (35.5%) was achieved in the treatment of 120 kg/ha fertilizers without using bio-fertilizer with 50% (Table 1). Also in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

Ascorbate Peroxidase Enzyme (APX)

Interaction effects of fertilizers and drought stress on ascorbate peroxidase enzyme were significant at 1% level of probability. The highest amount of this enzyme (909.9 μM H_2O_2 dec/min/mg Pro) was obtained in 180 kg/ha fertilizers treatment and 50% FC; the lowest amount of this enzyme (113.9 μM H_2O_2 dec/min/mg Pro) was obtained in 100% FC and 120 kg/ha chemical application (Table 5).

Table 5: The effects of fertilizers and bio-fertilizer on Ascorbate Peroxidase, Carotenoid, Phenol, Malondialdehyde, Antioxidant, Electrolyte leakage of cantaloupe.

Fertilizers	Biological fertilizer	APX (uM H_2O_2 dec./min/mg Pro)	Carotenoid (mg/gFW)	Phenol (mg/gDM)	MDA (μmol/gFW)	Antioxidant	Electrolyte leakage (%)
0 kg/h	Non-usage	376.8b	3.7c	3.9e	1.3b	31.9de	31.1e
60 kg/h		351.6c	4.7b	3.6f	1c	30.3e	28.1f
120 kg/h		442.6a	2.2d	5.5b	1.7a	42.3a	40.3b
180 kg/h		447.6a	2.9cd	6a	1.6a	39.7b	42.6a
0 kg/h	Usage	307.6d	6.3a	2.9g	0.85cd	27.7f	21.9g
60 kg/h		296.9d	5.9a	2.7h	0.78d	26f	20g
120 kg/h		383.6b	2.9cd	4.6d	1.3b	34.1c	36.6c
180 kg/h		384.8b	4.6b	4.9c	1.46b	32.9cd	33.6d

The averages in each column that are common in at least one element, do not reveal significant differences.

Interaction of fertilizer with drought stress on ascorbate peroxidase enzyme was significant at a 1% level of probability. The highest amount of this enzyme (885.6 µM H_2O_2 dec/min/mg Pro) was obtained in 50% FC without bio-fertilizer and the lowest amount of this enzyme (89.3 µM H_2O_2 dec/min/mg Pro) was obtained in the treatment of 100% FC with the bio-fertilizer application (Table 6). Also in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

Table 6: Impact of bio-fertilizer and drought stress on Ascorbate Peroxidase, Carotenoid, Phenol, Malondialdehyde, Antioxidant, Electrolyte leakage of cantaloupe.

Biological fertilizer	Drought stress	APX (uM H_2O_2 dec./ min/mg Pro)	Carotenoid (mg/gFW)	Phenol (mg/g DM)	MDA (µmol/gFW)	Antioxidant	Electrolyte leakage (%)
Non-usage	50% FC	885.6a	0.52e	8.91a	2.16a	49.5a	56.1a
	75% FC	217.4c	3.43d	2.86c	1.33c	40.2b	29.3c
	100% FC	110.9e	6.13b	2.48d	0.83e	18.6d	21.1e
Usage	50% FC	755.5b	0.75e	7.15b	1.68b	41.5b	44.3b
	75% FC	184.9d	5.02c	2.3e	1.03d	33.6c	33.2d
	100% FC	89.3f	8.96a	1.9f	0.66f	15.6e	16.6f

The averages in each column that are common in at least one element, do not reveal significant differences.

Electrolyte leakage

Interaction of fertilizer with drought stress on ascorbate peroxidase enzyme was significant at a 1% level of probability. The highest amount of this enzyme (885.6 µM H_2O_2 dec/min/mg Pro) was obtained in 50% FC without bio-fertilizer and the lowest amount of this enzyme (89.3 µM H_2O_2 dec/min/mg Pro) was obtained in the treatment of 100% FC with the bio-fertilizer application (Table 6). Also in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

Table 7: The effects of fertilizers and drought stress on Ascorbate Peroxidase, Carotenoid, Phenol, Malondialdehyde, Antioxidant, Electrolyte leakage of cantaloupe.

Fertilizers	Drought stress	APX(uM H_2O_2 dec./ min/mg Pro)	Carotenoid (mg/g FW)	Phenol (mg/g DM)	MDA (µmol/gFW)	Antioxidant	Electrolyte leakage (%)
0 kg/h	50% FC	753.2b	0.76f	6.6c	1.6b	40.9cd	41.8b
60 kg/h		715.6c	0.79f	5.89d	1.34c	38.6d	37.9c
120 kg/h		903.1a	0.39f	9.39b	2.32a	52.5a	60.75a
180 kg/h		909.9a	0.56f	10.31a	1.32a	49.8b	60.22a
0 kg/h	75% FC	184.3e	5.08c	2.1h	1.1d	33.2e	21.9ef
60 kg/h		174.9e	5.35c	1.8hi	0.83de	31.3e	19.8f
120 kg/h		221.8d	2.66e	3f	1.43c	42.6c	31.8d
180 kg/h		223.5d	3.8d	3.3e	1.43c	40.6cd	31.5d
0 kg/h	100% FC	88.8g	9.08a	1.8ij	0.64ef	15.3g	15.7g
60 kg/h		82.6g	9.56a	1.6j	0.51f	14.6g	14.3g
120 kg/h		113.9f	4.7cd	2.6g	0.89d	19.7f	22.8e
180 kg/h		114.9f	6.8b	2.8f	0.89d	18.7f	22.6e

The averages in each column that are common in at least one element, do not reveal significant differences.

Antioxidant capacity

Based on the results, the interaction of the fertilizers and the drought stress on the electrolyte leakage was significant at 1% level of probability. So that the highest rate of the electrolyte leakage (60.75%) was obtained in the fertilizer treatment with 50% of field capacity. The lowest rate of the electrolyte leakage (3.14%) was achieved at the presence of the fertilizers treatment with irrigation of 100% of field capacity (Table 6). Moreover, the interaction of bio-fertilizer with drought stress on electrolyte leakage level was significant at a 1% level of probability. The highest electrolyte leakage (1.56%) was obtained in non-bio-fertilizer treatment with 50% field capacity and the lowest electrolyte leakage (6.16%) was obtained in a fertilizer application with irrigation of 100% of FC (Table 6). Also, the interaction between fertilizer and bio-fertilizer on electrolyte leakage was significant at a 1% level of probability. The highest amount of electrolyte leakage (42.6%) was found in bio-fertilizer treatment and the lowest amount of Electrolyte leakage (20%) was found in the simultaneous use of fertilizers with bio-fertilizer (Table 7). Also in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

Malondialdehyde

The effects of the interaction of the fertilizers with drought stress on the amount of malondialdehyde were significant at 1% level of probability. The highest amount of malondialdehyde (2.32 µmol/g FW) was achieved in the 180 kg/ha treatment with irrigation of 50% FC; the lowest amount of malondialdehyde (0.51 µmol/g FW) was obtained in the 60 kg/ha treatment with irrigation of 100% FC (Table 5).

The effects of the interaction of the bio-fertilizers with drought stress on the amount of malondialdehyde were significant at 1% level of probability. The highest amount of malondialdehyde (2.16 µmol/g FW) was achieved in the absence of bio-fertilizer treatment with irrigation of 50% FC; the lowest amount of malondialdehyde (0.66) was obtained in bio-fertilizer treatment with irrigation of 100% FC (Table 6).

The effects of the interaction of the bio-fertilizers with fertilizers on the amount of malondialdehyde were significant at 1% level of probability. The highest amount of malondialdehyde (1.7 μmol/g FW) was achieved in the 120 kg/ha fertilizers treatment without using bio-fertilizers and the lowest amount of malondialdehyde (0.78 μmol/g FW) was obtained in 60 kg/ha fertilizers treatment, using bio-fertilizers (Table 7). Also in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

Phenol

The interaction of chemical and bio-fertilizers was significant in the amount of phenol at a 1% level of probability.

The interaction of fertilizer and drought stress on phenol content was significant at a 1% level of probability. The highest amount of phenol (10.31 mg/g DM) was achieved in the 180 kg/ha fertilizers with irrigation of 50% FC; the lowest amount of phenol (1.6 mg/g DM) was obtained in 60 kg/ha fertilizers with irrigation of 100% FC (Table 5).

The mutual effects of fertilizers and drought stress on the amount of phenol was significant at 1% level of probability. The highest amount of phenol (8.91 mg/g DM) was achieved in the treatment of the absence of bio-fertilizers with an irrigation of 50% FC. The lowest amount of phenol (1.9 mg/g DM) was achieved in the bio-fertilizer treatment with an irrigation of 100% FC (Table 6). Also in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

Carotenoid

The interaction effects of chemical and bio-fertilizers were remarkable on the amount of carotenoid at a 5% level of probability. The highest amount of carotenoid (6.3 mg/g FW) was achieved with bio-fertilizer in the absence of fertilizer treatment. The lowest amount of carotenoid was obtained in 120 kg/ha fertilizers without using bio-fertilizer (Table 7). Moreover, the mutual effect of fertilizers with drought stress is noteworthy in a 1% level of probability. The highest amount of carotenoid (9.56 mg/g FW) was achieved in 60 kg/ha fertilizers with 100% field capacity irrigation. The lowest amount of carotenoid (0.39 mg/g FW) was obtained in 120 kg/ha fertilizers with 50% FC (Table 5). Also, the effects of the interaction of bio-fertilizer with drought stress on carotenoid were significant at a 1% level of probability. The highest carotenoid content (8.96 mg/g FW) was obtained in 100% field capacity irrigation with the bio-fertilizer application and the lowest level (0.52 mg/g FW) was obtained in 50% field capacity irrigation treatment without bio-fertilizer application (Table 6). Also in this trait, the comparison of the mean effect of fertilizers, bio-fertilizer and drought stress is shown in Tables 2, 3 and 4.

The results proved that the amount of proline in cantaloupe increased under drought stress conditions. Thus, according to the results obtained by other researchers (Tarighaleslami *et al*., 2012), it can be stated that osmolytes accumulate in the cytosol to modulate osmotic pressure. The increase of proline in the leaves of the plant reveals that proline functions as an osmotic protector during the stress period and proline accumulation is part of the physiological responses to the applied stresses. Proline is achieved under the influence of 1-proline-5-carboxylate synthase which is very telling of the proline controlling genes under drought stress (Madhava *et al*., 2006). Tarighaleslami *et al*. (2012) revealed that the application of 80 kg/ha nitrogen causes an increase in proline which is a further confirmation of this study.

Results showed that the total carotenoid and chlorophyll content decreased with increasing stress. The highest amount of the total carotenoid and chlorophyll was achieved in the application of bio-fertilizer and fertilizers and irrigation of 100% FC. Two of the most important roles of carotenoids are the protection of thylakoid membranes and the prevention of photosynthesis by chlorophylls (Zakar *et al*., 2016). One of the reasons for the reduction of chlorophyll concentration in stress conditions can be an increase in the activity of chlorophyll degrading enzymes, including chlorophyllase, which is induced under stress conditions to express genes of this enzyme (Dodd *et al*., 2005). Glutamate (a common precursor of chlorophyll and proline) is also used to produce proline under stress conditions, which may be another reason for chlorophyll depletion under stress conditions (Bybordi, 2012). Jeyaramraja *et al*. (2005) observed that mild water stress increased carotenoids, while severe water deficiency reduced carotenoids in addition to chlorophyll reduction, which was consistent with the results of this study.

The highest electrolyte leakage was obtained in the fertilizers with 50% field capacity and the lowest electrolyte leakage was obtained in the fertilizers treatment with 100% FC. Application of chemical and bio-fertilizer in 100% field capacity reduced the electrolyte leakage; however, the application of drought stress in 50% field capacity was not capable of reducing electrolyte leakage. Electrolyte leakage increases with drought stress. As the water content of the plant organs drops under drought stress, the amount of damage to the membrane increases, and leads to the increased permeability and electrolyte leakage of the cell and its death (Apel and Hirt, 2004). Irrigation stress, by inducing oxidative stress and generating oxygen free radicals, peroxidates cell membrane fatty acids and increases membrane permeability and ion leakage (Guo *et al*., 2006). According to the results, the consumption of fertilizers with 50% FC without using any bio-fertilizers causes a reduction in RWC.

Drought stress reduced the relative water content of watermelon leaves by 20 to 25% (Ferus *et al*., 2011), which was consistent with the results that the minimum leaf water content was obtained with deficit irrigation stress. The leaf relative water content is measured by the status of the water of the plant and reflects the metabolic activities of the tissues and is further, an indicator of deficit irrigation in the plant (Siddique *et al*., 2000). Plants that are affected by the drought stress reduce their intercellular spaces and the amount of water in their parts by increasing the osmotic content within the tissues for a more intense uptake from the soil which by itself decreases the relative water content in the face of drought stress (Nayyar and Gupta). According to the achieved results in drought stress (50% FC), malondialdehyde content increased using chemical and bio-fertilizer. Lipid peroxidation causes adverse environmental impacts. The increase in the content of lipid peroxidation reveals that drought stress causes lipid peroxidation in the membrane by creating free oxygen radicals (Mirzaee *et al.*, 2013).

In the present study, under drought stress (50% FC), phenol content increased both in the face of lack of bio-fertilizer and consumption of chemical fertilizer. Moreover, by increasing the stress, the antioxidant capacity increased. These results are in agreement with Peterlunger *et al.* (2000). The capability of the plant to face the drought stress occurs by altering chemical constituents and producing a diverse range of secondary metabolites. The plants apply an antioxidant system to alleviate stress conditions to reduce cellular membrane damage caused by free radicals by increasing the accumulation of phenolic compounds (Terzi *et al.*, 2010). By accumulating free radicals in stress conditions, the plant becomes damaged and releases compounds that are often secondary metabolites for their life. These compounds can reduce the damage caused by free radicals. In different plants, due to the different resistance to drought stress, the amount of accumulated phenolic compounds varies greatly to decrease the free radicals (Gharibi *et al.*, 2015).

The results showed that at 50% FC along with using fertilizers without any bio-fertilizers, the antioxidant enzymes, POD, APX, SOD, and CAT increased. To tackle the undesired effects of oxidative stresses in unhealthy conditions, the plants develop an antioxidant defense mechanism including the antioxidant enzymes such as POD, SOD, APX and CAT. The levels of antioxidant enzymes under different biological stresses in the tolerant species are higher than those of susceptible species (Wang *et al.*, 2009). Under different stress conditions, the increase in the activity of the POD enzyme incurs protection from oxidative damages, being corked and cross-linking of the cell walls in unhealthy conditions (Moussa and Abdel-Aziz, 2008). Turkan *et al.* (2005) reported that within the drought stress, the number of antioxidant enzymes increased, which is in agreement with the results of this study. Mirzaee *et al.* (2013) reported increased activity of antioxidant enzymes in roots and shoots of rapeseeds. Increasing the antioxidant enzymes activity results in more protection of the plant under stress conditions. Catalase and SOD convert toxic O2 radicals into H2O2. The antioxidant enzymes decompose hydrogen peroxide into water and oxygen and reduce its harmful effects (Ozkur *et al.*, 2009).

Conclusion

According to the results, a decrease in the photosynthetic pigments and the relative leaf water content and an increase in proline and antioxidant enzymes' activity indicate the effects of drought stress and the production of free oxygen radicals which leads to further oxidative damage. Generally, it is safe to claim that for a better function in drought stress, the consumption of chemical and bio-fertilizers can prove to be efficient. For the generalization of the influence of bio-fertilizers on other plants, different amounts and methods are to be tested. Although fertilizers have a significant role in cultivation, what matters more is the management of the mixture of bio and fertilizers to reach a sustainable agriculture industry.

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