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Growth and Biochemical Characters of Basil *(Occimum basilicum)* in Response to Low Irrigation and UV-B

Mohammady Wahid Ahmad *, Azadani Homayoon, Amiri Abdul Wasi

Department of Horticulture, Faculty of Agriculture, Herat University, Herat, Afghanistan

*Corresponding author email: mohammadywahid@yahoo.com

ABSTRACT

Various basil cultivars in greenhouse can be cultivated round all year with the necessary growth conditions. Environmental stresses such as low irrigation and ultraviolet radiation are among the factors affecting the plant growth and development process. In the current study, five treatments for duration of ultraviolet radiation (0, 5, 10, 15, 20 minutes per day) and four irrigation levels (100, 75, 50, 30 % of field capacity) on two green and violet type of basil based on factorial in a completely randomize design experiment was conducted. The results showed that the duration of UV irradiation, low irrigation stress and their interaction effected morphological and biochemical responses of both basil cultivars. Measured traits such as leaf thickness, secondary metabolites, percent of essential oil, sodium and potassium elements content under UV treatments, low irrigation stress, and their interactions significantly increased compared to the control, but inter-nod decreased due to ultraviolet radiation, low irrigation stress and interaction effects. The amount of soluble sugars increased due to low irrigation stress (especially relatively severe low irrigation stress, 50% FC), but decreased due to high levels of UV treatments compared to the control, according to the result, UV radiation and low irrigation stress had shown synergistically stat together. The purpose of this study was to investigate the effects of UV light duration (280–320 nm), low irrigation stress, and the combined effects of UV light duration and low irrigation stress on morphological and biochemical responses of two basil cultivars.

Keywords: Basil, Drought, Essential oil, Flavonoid, Potassium

INTRODUCTION

Various plants may experience stress during growing periods. Stress is the result of anomalous processes in physiological processes resulting from the influence of one or a combination of different biological and environmental factors. Environmental stresses are factors that limit the growth and function of plants. The damage caused by various stresses such as low irrigation, UV, salinity and temperature to plants, at a wider level is more extensive than other types of stress (Suzuki et al., 2014). The yield reduction under stress conditions has been reported from 65 to 82 percent (Kacar et al., 2009). Low irrigation, salinity, heat, UV-B radiation and flooding conditions are important stress factors that can reduce crop yields (Kacar et al., 2009). The reduction of ozone layer has increased the amount of purple Himalayan radiation and created problems for the living organisms on the earth. Human industrial activities increase atmospheric pollutants, especially halogenated compounds, which reach high levels of atmospheric stability due to the degradation of the ozone layer (Buchholz et al. 1995).

Generally, ultraviolet radiation in the wavelength range are from 180 to 400 nm, including UV-A with a wavelength of 320 to 400 nm, which affects some physiological processes but is not effective in cell killing (Kafi & Damghani, 2002). It contains a relatively stable portion of solar energy. UV-B, which has a wavelength of 280-320 nm, is the most important band with its natural filtering from ozone layer, increasing in intensity with increasing longitude. UV-C has a wavelength of 180-280 nm, which is a highly lethal cell band, but solar UV-C is absorbed in the atmosphere by ozone and oxygen and is not considered to be destructive to the environment (Kafi & Damghani, 2002). Ultraviolet radiation acts as an environmental stress on plants and reduces their physiological function changes, thereby slowing plant growth, damaging photosynthetic pigments and reducing CO2 absorption. These changes also reduce biomass productivity (Janetta & Shanthi, 2015). Damage occurs in many areas, including direct DNA damage, inactivation of proteins and enzymes, disruption of membranes and other cellular structures, and production of chemical reactants called "free radicals" (Chang. 2004). Different

plants can react differently to UV radiation. Some plants, by producing more secondary metabolites, absorb ultraviolet light and prevent its penetration into mesophilic tissues. Increase of secondary metabolites in foliar fibrosis is an appropriate mechanism to counter UV radiation in plants (Indrajith, 2009: Piri et al., 2011).

In general, environmental, adipose conditions, or both, that prevent the plant from accessing sufficient water for vital operations and its repetition leading to water loss to plant tissues are called low irrigation. For a plant physiologist, low irrigation is nothing more than a lack of rainfall. In this sense, low irrigation is the reduction of available water to the soil, which is defined as the decrease in the amount of soil water potential, but in terms of low irrigation, it is a condition that reduces the yield of a plant relative to the state of water availability (Kramer et al., 1991: Kafi et al., 2009). Low irrigation is actually a condition that reduces the water potential in the plant and reduces Turgor pressure, resulting in altered normal physiological activities in the plant. Plants encounter stress when the amount of water available in the root zone is limited or the transpiration rate is higher than the root absorption, which decreases root absorption in the first place due to water scarcity and secondly to unfavorable soil conditions such as high salinity. Also, in other adverse conditions such as high salinity, waterlogging, low soil temperature, the plant is not able to absorb normal water, during low irrigation stress and with increased reactive oxygen species and glutathione and ascorbate compounds, there are more adverse effects on the plant.

In addition to reducing water potential and Turgor pressure, the stomata openings are also blocked, which results in reduced gas exchange and reduced carbon uptake and thus reduced photosynthesis (Lisar et al., 2016). Plants cope with these stresses under environmental stresses such as low irrigation, salinity, heat, and etc. Proline is one of the amino acids used to regulate the osmotic pressure of plants. Most osmotic pressure regulators include amino acids, sugars and some minerals, hormones and proteins (Assaha et al., 2016). Some researchers have proved the interaction between low irrigation stress and UV-B radiation in plants, so that these radiations can reduce the intensity of stress in plants by reducing plant water loss through reducing stomatal conductance and leaf area. The study of UV and low irrigation stress effects on lettuce showed that these two stresses sometimes have synergistic and sometimes non-synergistic effects depending on the mechanism induction of protective substances in the plant (Rajabbeigi et al., 2013). Liheng et al., (2011) examined the combined effects of UV-B radiation and low irrigation stress on susceptible and resistant wheat genotypes reported that susceptible wheat seedlings had more harmful growth effects when exposed to two stresses simultaneously, but combination of the two stresses had more positive growth effects for the resistant species. Therefore, in different studies the response of plants varied depending on the plant species.

Basil is a useful vegetable and valuable medicinal plant whose medicinal compounds are used for various medicinal and health purposes, on the other hand, it is a plant sensitive to environmental stress, especially Low Irrigation. The purpose of this research is to investigate the appropriate solution to increase the plant's resistance to adverse environmental conditions and increase the growth and medicinal compounds in the plant by combining effects of UV radiation, low irrigation, and common basil cultivars.

MATERIALS AND METHODS

This study was carried out in autumn, under greenhouse conditions on the campus of Gorgan University of Agricultural and Natural Sciences, Gorgan, Golestan province, Iran. The experiment was conducted as a factorial experiment with three factors (duration of UV irradiation, different low irrigation levels and two basil cultivars) in a completely randomized design. The duration of irradiation consisted of five levels (0, 5, 10, 15, and 20) minutes per day. Low irrigation levels were considered as four levels (100, 75, 50, and 30% of field capacity) with two basils (green and violet) cultivars. First, the green and violet basil seeds were seeded in trays separately containing two volumes of coconut fiber and one volume of perlite in a greenhouse at 25 ° C daily and 15 ° C overnight (temperature adjusted by Cold heat appliances were set up in the greenhouse) with 16 hours of light and 8 hours of darkness (shortage of light was eliminated by LED lamps on short and cloudy days) and 60% relative humidity regulated by ventilation, Cultivated.

The plants were transferred to the laboratory after reaching the three- to four-leaf stage for UV light exposure. Ultraviolet irradiation exposure for two weeks by two UV-B lamps (TL. UV-B 15W = 280-320 nm, mad in German) that mounted 60 cm high above the plants. In the six-leaf stage, plants were planted in pots containing a mixture media of two typical soil volumes, one volume of sand and one volume of soil with 0.5 volumes of fully disinfected vermicomposting. After complete plant establishment, low irrigation stress levels were applied based

on field capacity and weighting method at four levels including 100, 75, 50, and 30% of field capacity. After determining the percentage of soil moisture in crop capacity by daily weighting of the pots, the water content was added to the pots. After four weeks of low irrigation stress and flowering stage, the plants were removed and transferred to the laboratory for measurement of different traits.

To measure phenol, samples were first dried in the oven at 45 ° C and powdered by mill. Subsequently, half a gram of the sample was mixed with 5 ml of 80% methanol and kept for 5 hours on the shaker. The diluted extract was added to a solution of 20 µl with 1.16 ml of distilled water and 100 µl of Folin reagent. After 5 min, 300 microliters of 20% sodium carbonate solution was added to the solution and kept in Bon Marie for 30 min at 40 ° C. The absorbance of the samples was then read by spectrophotometer at 760 nm and the results were calculated in milligrams of gallic acid per 100 g of dry sample after obtaining the standard form. Finally, the obtained data were calculated based on the equation mg gallic acid / g dry weight. Chang (2002) method was used to measure flavonoid. The samples were first dried at 45 ° C in the oven and then powdered by mill, next, half a gram of the powdered sample was mixed with 5ml of methanol 80% and placed on shaker for 24 hours, the extract was filtered off and the extract was then filtered with filter paper. After that 0.5 ml diluted extract with 1.5 ml methanol 80%, 0.1 ml aluminum chloride 10% (10 g aluminum chloride in 100 ml distilled water), 0.1 ml potassium acetate 1 Molar (2.41 g / 100 ml distilled water), and 8.2 ml distilled water were mixed. After placing the samples in the dark for half an hour, the absorbance of the samples was measured by spectrophotometer at 415 nm. Different concentrations of Quercetin were used for standard preparation and after drawing the standard form, the amount of flavonoid was calculated according to Quercetin aquavolan in one gram of dry extract. Comazawa (2002) method was used to measure antioxidant activity. The samples were first dried at 45 ° C in the oven and then powdered with mill, next, half a gram of the powdered sample was mixed with 5 ml of 80% methanol and placed on shaker for 24 hours. The diluted extract was mixed with 1 ml of 1mM DPPH methanol solution and then the mixture was kept in the dark for 2 minutes at room temperature. Finally, the absorbance of the sample at 517 nm was read by spectrophotometer and the oxidant activity was calculated according to the relative percentage of DPPH activity and absorbance of the control sample without methanol extract using the following formula. *DPPH% = Number of sample - Number of control / Number of sample*. Sadisvam (1992) method was used to measure sugar content.

To measure the soluble sugars, 40 mg of the herbal sample was diluted in porcelain oven with 5 ml of ethanol 80% and placed in test tubes and kept for 10 min at 70 ° C in the Bon Mary bathroom. It was then centrifuged at 1000g for 10 minutes. The obtained alcoholic extract containing the soluble sugars was transferred to another tube and repeated four more times on the residue. Next, the alcoholic extract was concentrated by heat until it reached a volume of about one fifth; the sieve extract was mixed with chloroform at a ratio of 1: 5 and centrifuged at 1000 g for 15 min. Finally, the upper phase was separated and used to measure different types of soluble sugars. To measure total sugar, 200 µL of concentrated extract was mixed with 3 ml of antron reagent and incubated at 100 ° C for 20 minutes in a Bain-marie bathroom and after cooling the extract, the absorption rate of each sample was read by spectrophotometer at 620 nm. Finally, taking into account the standard form of total sugar content for each sample was calculated. Gafari (2004) method used to measure the potassium and sodium content as following, first one gram of the powdered plant samples was placed in Chinese bushes and Burned in an electric oven for 12 hours at 550 ° C to turned into ashes. Then distilled water plus 5 ml of chloric acid-2 normal was added to each sample and incubated in Bain-marie for half an hour at 100 ° C. Then, the contents of each simple were transferred to a 50 ml balloon, and then got reached to 50 ml by distillate water after clearing each sample; it was transferred to a plastic lid and used for measuring potassium and sodium.

To measure potassium, each sample was diluted 1: 1 with distilled water and the number of absorption was read by Flame Photometer. Then the standards were prepared with concentrations of 0, 10, 20, 30, 40, 50 mg/l potassium chloride in concentrated standard solution, 5000 mg/l potassium chloride and a control, after reading the standard sample numbers by Flame photometer was standard form and potassium content of each sample was calculated based on mg / g standard form, the standard form was plotted and the potassium content of each sample was calculated based on the standard form in mg / g. The same procedure was used for the measurement of sodium, and in the final step each sample was diluted with1: 2 distilled water and then read by a Flame Photometer. Sodium chloride was used to prepare the sodium standard at concentrations of 0, 10, 20, 30, 40, 50 mg/ L and then calculated by using the standard form of sodium. Percentage of essential oil was obtained by distillation using Clevenger apparatus. First, the plant sample was weighed and then each plant sample was placed in a Clevenger

apparatus for 4 hours to extract the essential oil by distillation. It was calculated based on the initial weight of the plant sample, as follows. *Essential oil percent = weigh of Essential oil / weigh of sample*100*

Finally, data analysis was done using SAS 9.1 software and comparisons were made using Duncan's test at the level of 0.05%. Figures and tables were drawn using Excel software.

RESULTS

Based on the results of variance analysis, there was a significant difference between UV irrigation treatments, low irrigation stress and their interactions on most of the basil traits measured based on Duncan test at level of P≤ 0.05 and P≤ 0.01.

Table (1): Source of variation

Source of variable	DF	Sugar content	antioxidant	flavonoid content	phenol content	sodium	potassium	Essential oil
Cultivars (a)	1	5.33^{ns}	1122.50**	889.47**	126.50**	51.36**	382.36**	3.020**
Ultraviolet (b)	4	327.36**	243.87**	66.78**	40.18**	74.91**	43.10**	0.046*
Low irrigation stress (c)	3	11482.16**	1984.56**	327.08**	46.71**	170.10**	201.30**	0.205**
a*b	4	24.41^{ns}	41.41^{ns}	5.62*	4.82**	1.38ns	20.61*	0.124**
a*c	3	22.71^{ns}	58.92^{ns}	42.66**	9.43**	1.32ns	210.19**	0.076**
b*c	12	167.50**	207.16**	13.20**	6.11**	14.89**	24.02**	0.051**
a*b*c	12	43.20*	9.75^{ns}	4.97**	3.91**	0.74ns	19.81**	0.034**
Error	80	19.21	26.02	1.60	0.94	4.61	4.65	0.012
C.V		16.78	8.74	7.35	4.11	5.16	6.40	17.52

Table (1) Continue

Source of variable	Df	Root thickness	Stem thickness	Inter-nod
(a) Cultivars	1	0.008 $^{n.s}$	3.04**	4732.005**
(b) Ultraviolet	4	0.037 $^{n.s}$	0.06*	223.02**
Low irrigation stress (c)	3	12.24**	3.74**	622.90**
a*b	4	0.90**	0.13**	73.07*
a*c	3	0.34*	0.39**	295.91*
b*c	12	0.29*	0.03 $^{n.s}$	49.07*
a*b*c	12	0.30**	0.09**	49.51*
Error	80	0.12	0.02	22.55
C.V		8.74	5.40	8.39

Inter-node

According to the results of variance analysis, there is a significant difference between the applications of UV radiation; low irrigation stress, cultivars and interaction effects on inter- nodes (Table 1). The results in Average Comparison of cultivars showed that inter- of nodes in violet cultivar was about 19.97% more than green cultivar. There was still a significant difference between all levels of UV irradiation compared to the control, and the 20-min irradiation alone reduced approximately 11.88% of inter- nodes compared to the control. This

difference is also significant between different levels of low irrigation, as severe low irrigation levels of 30% field capacity reduced about 16.93% of the inter- nodes compared to the control, which had a greater effect on reducing the length of the nodes and overall height of the plant than UV (Table 2). Also, the result of interaction effect showed the highest inter-node length related to the combined treatment of (UV 0, 100% FC, purple cultivar) with 79.39 mm, and the lowest inter-node length related to the combined treatment of (UV 20 min / day, 50% FC, and green cultivar) with 43.18 mm (Table 3).

Root and stem thickness

Results of stem and root thickness analysis indicated that there was a significant difference between the two cultivars in stem thickness, but no significant difference in root thickness between the two cultivars. Ultraviolet radiation levels alone had a significant effect on shoot thickness and increased shoot thickness, Maximum effect was in 5 and 20 min irradiation about 3.56% compared to control. But irradiation levels alone did not have a significant effect on changes in root thickness, whereas low irrigation stress levels alone had a significant effect on shoot and root thickness reduction, which reduced shoot and root thickness by about 25.79% and 30.53% respectively (Table 2), Interaction effect of cultivar, UV, and low irrigation stress on shoot and root thickness was also significant. The highest stem thickness was related to (UV 20 min/day, 100% FC, and Violet cultivar) with 3.913 mm and the lowest related to the (UV 0, 30% FC, and Green cultivar) with 5.266 mm. Also, the highest root thickness was obtained in combined treatment (UV 20 minutes per day, 30% FC and purple cultivar) whit 5.266 mm and the lowest at the combined treatment (UV0, 30% FC, and green cultivar) with 2.866 mm was obtained (Table 3). This result indicates that UV irradiation levels prevented further reduction of stem and root thickness due to severe low irrigation stress.

Total phenol content

There was a statistically significant difference in total phenol content between all variables (Table 1). Phenol content in violet cultivar at 24.67 mg /g was about 8.30% higher than in green cultivar at 22.62 mg /g. Also, different levels of UV alone increased phenol content in both basil cultivars compared to control. The 20-min irradiation level increased the phenol content by about 11.80% compared to the control. Different levels of low irrigation alone increased the amount of phenol in the plant, which is about 10.96% compared to control (Table 2). Interaction effects result showed the highest total phenol content obtained at combined treatment of (5, 15, 20 min/day UV, 6% FC, and purple cultivar) and the lowest in (UV 0, 100% FC, and green cultivar) obtained (Table 3). The results of interactions showed that total phenol content in the combined treatments was higher than the UV and low irrigation treatment alone that is shows the synergistic state.

Total flavonoid content

There was also a statistically significant difference in flavonoid content between all variables. The flavonoid content of violet cultivar at 19.94 mg /g is about 27.33% higher than green cultivar at 14.49 mg /g. There was no significant difference in levels of 0, 5 and levels of 15, 20 minutes UV treatment in flavonoid content, Ultraviolet irradiation at level of 20 min /day with 19.18 mg /g increased flavonoid by about 19.29% compared to the control with 15.48 mg /g. And low irrigation stress at 30% of field capacity with 21.38 mg /g, increased flavonoid content at about 35.36% compared to control with 13.82 mg /g, Indicating more low irrigation effects than ultraviolet radiation on flavonoid accumulation in basil (Table 2). Also, according to the results of interaction effects, maximum flavonoid in combined treatment of UV 5, 15, and 20min /day, 30% FC, and violet cultivar) with an average of 27.32 mg/g obtained, the lowest was obtained in the combination of (UV 0, 100% FC, and Green cultivar) at 7.514 mg /g dry weight. Increase of flavonoid content in the co-treatment was about (65.32%) compared to the control purple and green cultivars, which had more effects than low irrigation and UV treatments alone (Table 3).

Antioxidant activity (DPPH)

There was a significant difference in the antioxidant activity of different treatments, as shown in the table of variance analysis there are significant difference between the levels of low irrigation, UV and their interactions,

but there was no significant difference between the interaction effects of cultivar, low irrigation, and UV. There were significant differences in antioxidant activity (DPPH) between the two cultivars of basil, which was 45.25% violet cultivar and 39.13%in green cultivar. The 20 min level of UV irradiation increased the antioxidant activity by 18.45%, while the increase at high low irrigation stress (30% field capacity) alone was 32.98%, indicating a greater effect of Low irrigation stress due to ultraviolet radiation on antioxidant activity in both basil cultivars (table 2). According to the results of the interaction between UV and low irrigation stress, the highest antioxidant activity was in combined treatment (UV20 min /day, 30% FC), and the lowest was related to (UV 0, 100% FC). The antioxidant activity of the combined UV and low irrigation treatments was higher than the individual UV and low irrigation treatments alone (Figure 1).

Table (2): Compression average of measuring parameters

Treatments	Potassium mg/g	Sodium mg/g	Sugar content mg/g	Anti-oxidant %	Flavonoid mg/g	Phenol mg/g	Essential oil %
Cultivars							
violet	31.29 b	40.92 b	26.32 a	45.25 a	19.94 a	24.67 a	0.438 a
green	35.49 a	42.23 a	25.90 b	39.13 b	14.49 b	22.62 b	0.827 a
Ultraviolet							
0 min/day	32.12 d	40.12 c	30.28 a	38.22 c	15.48 c	21.81 d	0.553 b
5 min/day	32.87 cd	40.76bc	29.008 a	40.74 bc	15.80 c	22.88 c	0.703 a
10 min/day	33.49 bc	40.96bc	26.42 b	42.03 b	16.92 b	23.97 b	0.623 ab
15 min/day	34.69 ab	41.41 b	21.15 c	43.09 b	18.69 a	24.84 a	0.651 a
20 min/day	35.41 a	44.63 a	23.33 c	46.87 a	19.18 a	24.73 a	0.634 ab
Low irrigation stress							
100 FC	31.06 d	39.33 c	12.03 c	33.62 c	13.82 d	22.33 d	0.529 c
75 FC	32.55 c	40.71 b	13.87 c	36.92 b	15.49 c	22.92 c	0.575 c
50 FC	37.12 a	44.91 a	24.21 b	48.02 a	18.17 b	24.25 b	0.757 a
30 FC	34.10 b	41.36 b	54.33 a	50.17 a	21.38 a	25.08 a	0.670 b

Table (2) Continue

Treatments	Root thickness (cm)	Stem thickness (mm)	Inter-nod (mm)
Cultivars			
violet	4.045 a	2.869 a	62.88a
green	4.028 a	2.550 b	50.32 b
Ultraviolet			
0	4.025 a	2.652 c	60.08 a
5 min/day	4.043 a	2.745 a	56.58 bc
10 min/day	4.008 a	2.738 ab	59.14 bc
15 min/day	4.004 a	2.657 bc	54.13 dc
20 min/day	4.102 a	2.755 a	53.07 d
Low irrigation stress			
100 FC	4.601 a	3/14 a	61.65 a
75 FC	4.450 a	2.82 b	58.83 b
50 FC	3.891 b	2.52 c	54.81 c
30 FC	3.196 c	2.33 d	51.21 d

Percentage of essential oil

Based on the results of variance analysis there is a significant difference between cultivar, UV and low irrigation stress in essential oil percentage based on Duncan test (Table 1). The percentage of essential oil in green cultivar was 47.03% more than violet cultivar, which indicates that there is more essential oil in green than purple cultivar. Ultraviolet radiation also had an effect on the essential oil content, with a significant difference between the levels of 5 and 15 minutes UV compared to the control. Low irrigation stress increased essential oil percentage in both basil cultivars by alone and had more effect on increasing of oil percentage than UV irradiation. The highest effect was at 50% of field capacity, with the difference of essential oil increase at 50% of field capacity being about 30.11% compared to control. Although there was a significant difference in the percentage of essential oil in 30% of field capacity compared to the control, it showed a decrease in essential oil percentage compared to 50% of field capacity, indicating a decrease in essential oil percentage due to severe low irrigation stress and increased percentage of essential oil is in moderate low irrigation stress in basil. Also, based on the results of triple interaction effects of cultivar, UV and low irrigation stress, the highest essential oil percentage was obtained combined treatment of (green cultivar, UV 5 min/day, and 50% FC), and the lowest essential oil in combined treatment of (UV5 Min /day, 30% FC and purple cultivar) obtained (Table 3).

Potassium and sodium content

There is a significant difference between the amount of potassium in the different levels of UV, different levels of low irrigation, cultivars, and their interaction (table 1). There was a significant difference between the amount of sodium in different levels of UV, low irrigation, cultivars and interaction effects of UV and low irrigation stress, but there was no significant difference between the interaction effects of UV and cultivars, cultivar and low irrigation stress and triple interaction effects based on Duncan test. According the compression of mean square there was no significant difference between the levels of 0 and 5 minutes' radiation per day in terms of potassium and sodium. But there was a significant difference between the control treatment and the levels of 10, 15, and 20 min of irradiation, with the highest effect being 20 min radiation per day, the 20-min irradiation with 35.41 and 44.63 mg /g dry weight, respectively, increased the percentage of potassium and sodium by 9.29% and 10.10% compared to the control with 0.5 and 2.5 mg / g dry weight, respectively. There was a significant difference across all low irrigation levels, with the highest level of potassium and sodium devoted to the 50% field capacity. The 50% level of field capacity increased by 31.06% and 39.33% potassium and sodium, respectively, compared to the control (table 2). According to interaction effect of variable the highest amount of potassium was obtained in combined treatment of (UV 20 min /day, 50% FC, and green cultivar) with 41.24 mg /g dry weight. the lowest amount of potassium in combined treatment of (UV 0, 100%FC, and green cultivar) with 25.18 mg/g dry weigh obtained (table 3). Also the highest level of sodium was obtained in the combined treatment of (UV 20 min/day, 50%FC) with 48.19mg/g dry weigh (Figure 1).

Total sugar content

There was no significant difference between two basil cultivars in terms of total sugar content, but was significant difference between the levels of UV irradiation low irrigation stress and their interaction effects. High levels of UV irradiation reduced total sugar content compared to the control, but no significant difference was observed at irradiation of 5min/day than control. Levels of 15 and 20 minutes' irradiation per day with 21.15 and 23.33 mg /g dry weight, respectively, resulted in decreasing 26.55% of total sugar compared to the control. High levels of low irrigation stress caused a significant increase in total sugar content; level of 30% field capacity with 54.33 mg/g got increasing about 77% of total sugar content compared to the control with 12.03 mg/g. According to the results of the interaction effects, the highest total sugar content was obtained in combined treatment of (UV 5 min/day, 30% FC and purple cultivar) with 67.13 mg/g dry weight, and the lowest was obtained in the combination treatment of (UV 20 min / day), 100% FC and green cultivar) at 7.73 mg /g dry weight (Table 3). This result indicates an increase in the sugar content during severe low irrigation stresses.

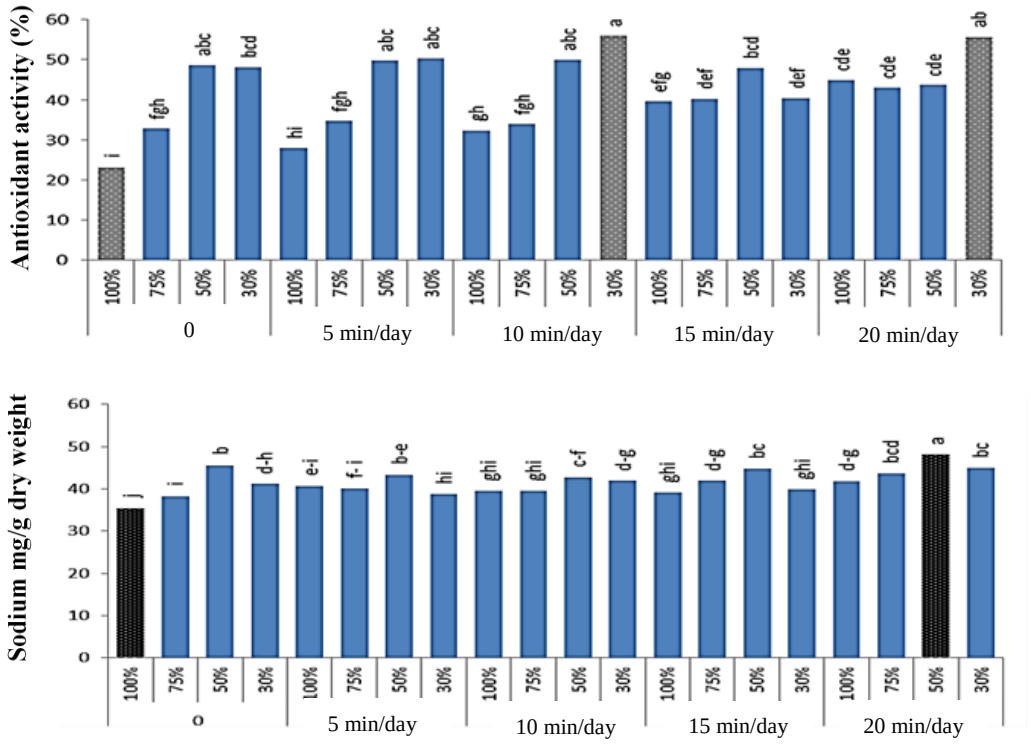

Figure (1). Combined effect of UV (min/day) and low irrigation stress (field capacity) on amount of antioxidant (%) and sodium (mg/g dry weight)

Table (3): Triple combined effect of cultivars, UV, and low irrigation stress on measuring parameters (compression average)

cultivars	UV (min/day)	Low irrigation (FC)	Flavonoid (mg/g)	Phenol (mg/g)	Sugar content (mg/g)	Potassium (mg/g)	Root thickness (cm)	Stem thickness (mm)	Inter- not (mm)
violet	0	100	11.44 opq	21.28 klm	16.92 l-o	26.69 lmn	4.166 d-i	3.136 c-f	79.39 a
		75	16.15 j-m	21.77 jkl	16.95 l-o	30.85 g-k	4.000 f-k	3.166 cde	76.90 ab
		50	20.75 def	24.88 b-g	20.67 j-m	36.43 def	4.266 d-i	2.533 k-p	59.72 e-j
		30	23.27 bc	24.40 c-h	63.98 ab	31.39 ghi	3.366 k-o	2.266 pq	57.33 f-l
	5	100	12.74 nop	23.92 d-h	12.34 n-q	29.59 i-m	4.133 e-i	3.460 b	72.41 abc
		75	15.30 lm	23.53 f-i	14.55 l-q	32.99 f-i	4.400 b-h	3.173 cde	68.27 b-e
		50	17.63 h-k	24.22 c-h	24.26 ijk	32.46 ghi	4.266 c-i	2.450 l-q	57.09 f-l
		30	27.01 a	26.32 ab	67.13 a	30.89 g-k	3.333 k-o	2.226 q	46.99 m-q
	10	100	17.36 h-k	25.23 bcd	10.98 opq	37.76 a-d	4.200 d-i	3.213 bcd	71.94 abc
		75	17.06 h-l	25.80 abc	14.39 l-q	31.21 g-j	4.933 abc	3.160 cde	70.06 bcd
		50	18.54 ghi	25.35 a-d	34.65 gh	31.74 ghi	3.700 h-m	2.786 g-k	72.44 abc
		30	24.09 b	23.64 e-i	50.04 de	26.14 mn	2.833 n-o	2.406 m-q	53.66 h-p
	15	100	18.68 ghi	24.18 d-h	9.320 pq	32.52 ghi	4.666 a-f	3.243 bc	63.89 c-f
		75	20.50 efg	25.14 b-e	10.88 opq	33.40 fgh	3.933 g-l	2.933 e-i	60.85 e-i
		50	21.38 cde	25.15 b-e	18.51 k-n	38.38 a-d	3.733 h-m	2.613 j-n	56.50 f-m
		30	27.77 a	26.92 a	43.17 ef	27.51 k-n	3.400 j-n	2.470 l-q	52.57 i-q
	20	100	18.80 fgh	25.21 b-e	9.190 pq	33.91 efg	5.266 a	3.913 a	62.23 d-h
		75	20.37 efg	25.10 b-f	12.88 n-q	32.24 ghi	4.833 a-d	3.030 c-g	63.30 d-g
		50	22.70 bcd	25.09 b-f	20.88 jkl	32.68 ghi	4.066 f-j	2.733 h-l	55.46 f-n
		30	27.201 a	26.29 ab	54.76 cd	29.67 i-l	3.666 i-m	2.470 l-q	55.30 f-o
green	0	100	7.514 r	16.89 p	13.51 n-q	25.81 n	4.900 abc	2.870 f-j	58.32 f-k
		75	10.73 pq	18.84 no	14.65 l-q	30.34 h-k	4.566 b-g	2.565 k-o	49.06 k-q
		50	15.83klm	22.34 ijk	30.25 hi	38.27 a-d	4.066 f-j	2.435 m-q	52.98 i-q
		30	18.13 hij	24.04 d-h	65.31 ab	37.18 cde	2.866 n-o	2.245 pq	46.99 m-q
	5	100	10.40 q	17.64 op	13.61m-q	27.44 k-n	4.350 c-i	2.960 d-h	52.62 i-q
		75	12.17 opq	20.06 mn	13.82 l-q	31.95 ghi	4.900 abc	2.660 j-m	49.44 k-q
		50	14.46 mn	23.47 ghi	27.62 hij	38.64 a-d	4.000 f-k	2.595 j-n	54.15 g-p
		30	16.73 i-l	23.85 d-i	58.69 bc	38.69 a-d	3.233 m-o	2.440 m-q	50.36 j-q
	10	100	12.57 nop	20.47 lm	15.46 l-p	27.74 j-n	5.066 ab	3.125 c-f	51.76 i-q
		75	11.95 opq	22.28 ijk	16.69 l-o	32.37 ghi	4.200 d-i	2.645 j-n	54.12 g-p
		50	17.08 h-l	23.80 d-i	31.58 gh	40.20 abc	3.833 h-m	2.300 opq	49.55 k-q
		30	16.70 i-l	25.17 d-e	37.52 fg	40.80 ab	3.300 l-o	2.275 pq	49.58 k-q
	15	100	13.09 no	24.04 d-h	11.14 opq	31.60 ghi	4.566 b-g	2.865 f-j	55.28 f-o
		75	14.46 mn	23.48 ghi	12.62 n-q	32.97 f-i	4.566 b-g	2.415 m-q	45.32 pq
		50	17.27 h-l	24.73 b-h	17.18 k-o	41.14 a	3.900 g-m	2.360 n-q	46.40 n-q
		30	16.39 j-m	25.09 b-f	49.42 de	39.98 abc	3.266 l-o	2.360 n-q	52.25 i-q
	20	100	15.65 klm	24.47 c-h	7.836 q	37.55 bcd	4.766 a-e	2.675 i-m	48.68 l-q
		75	16.21 j-m	23.19 hij	11.29 opq	37.20 cde	4.166 d-i	2.525 k-p	49.97 k-q
		50	16.02 klm	23.44 ghi	16.48 l-o	41.24 a	3.350 k-o	2.460 l-q	43.18 q
		30	16.47 j-m	25.09 b-f	53.33 cd	38.74 a-d	2.700 o	2.235 q	45.78 opq

DISCUSSION

The results showed that under UV irradiation shoot thickness increased and no significant difference in root thickness was observed, but under low irrigation stress shoot and root thickness was significantly decreased, in interaction treatments, the thickness reduction was further reduced by low irrigation stress, which showed a synergistic state. In the study of low irrigation stress effects on green basil, it was found that control plant had the highest thickness without stress and decreased stem and root thickness under stress, which could be due to reduced growth of vegetative organs under stress conditions (Sirousmehr et al., 2014). Study of UV rays on the chili peppers; found that ultraviolet radiation had no effect on root thickness but increased stem thickness due to the persistence of ethylene by ultraviolet radiation, which decreases the longitudinal growth and increases the transverse growth; it is consistent with the results obtained in this study (Hossini et al., 2011). Increased stem thickness by UV radiation in green basil was also reported (Ciurli et al., 2017). Based on the results, the amount

of secondary metabolites, including phenol, flavonoid, antioxidant, were increased significantly by UV, low irrigation stress and their interaction respectively, increasing of secondary metabolites was due to the interaction of the treatments more than either alone, indicating a synergistic state. These compounds either inhibit UV penetration into sensitive tissues and prevent damage, or play an antioxidant role against free radicals induced by UV stress in the plant and alleviate oxidative stress; also the increase in flavonoids content can be due to the concentration or the high activity of phenol alanine ammonia lyase enzyme under UV stress (Guo & wang, 2008). Increased phenolic compounds and antioxidant system activity by UV-B and UV-C treatments can be recognized as biological markers of UV radiation intensity in the plant (Khurami et al., 2014). The greater leaf area will cause, the greater amount of phenol content to trace radicals due to more UV absorption, this cause may be due the leaf morphological structure (Lee et al, 2009). Studies show that ultraviolet rays stimulate cynamic acid production by stimulating the activity of phenolalanine ammonia lyase enzymes, and thereby activate the biosynthetic pathway of flavonoids; Studies have also shown that the amount of chalcone synthase enzyme activity that plays a key role in the biosynthesis of flavonoids is increased by UV (Liu et al., 2012). One of the effective non-enzymatic protective mechanisms stimulated by UV and low irrigation stress is found to be the synthesis of flavonoids and phenolic compounds, which in addition to its proper antioxidant role, plays an important role as suitable UV absorbing compounds (Jaakola et al., 2013) Increased levels of flavonoids under UV, low irrigation stress and their interaction in lettuce plant reported (Rajabbeigi et al., 2013). As indicated in the results, Purple Basil cultivar had greater capacity for free radical scavenging (DPPH) than Green cultivar, indicating more antioxidant property of Purple cultivar than Green cultivar. Increased capacity to capture free radicals (DPPH) under UV has been studied in various studies (Shah abdaghlo et al., 2016). The results show a decrease in the content of soluble sugars due to UV and an increase in soluble sugars due to low irrigation stress. Interaction treatments also reduced the soluble sugars content compared to the low irrigation treatment alone, indicating relative adaptation of the plant to low irrigation stress due to ultraviolet radiation pretreatment. The decrease in soluble sugars in the UV treatment indicates a decrease in photosynthesis due to peroxidation of the thylakoid membrane or UV absorption by the photosystem 2, degradation of D1, D2 proteins and degradation of the Rubisco enzyme (Embasht & Agrawal, 1998). It has also been shown that reducing the biosynthesis of sugars at the oxidative stress induced by these rays causes the degradation of carbohydrates (Salama et al., 2011). Increasing the amount of soluble sugars due to low irrigation can be a response to changes in relative water content and leaf water potential and is one of the mechanisms of response plants to low irrigation stress, un necessary of photosynthetic materials due to reduced growth under low irrigation stress and synthesis of soluble sugars from non-photosynthetic pathways also causes the accumulation of sugars (Ehdaei et al, 2006). Increased soluble sugars content in leaf of bean, castor and thyme under low irrigation stress have also been reported (Brojerdinia et al., 2015: Karimi et al., 2012: Bestgani et al., 2017: El-Esawi and alayafi., 2019). As shown in the results, the amount of sodium and potassium increased due to ultraviolet radiation and low irrigation treatments compared to the control. Increasing in the concentration of potassium and sodium in the UV application may be due to changes in the metabolism processes of the elements or an increase in the concentration due to a decrease in the amount of dry matter under UV radiation (Correia et al., 2012). However, as a result of low irrigation treatments, the amount of these two elements was initially increased and then decreased, with the 50% capacity being the highest concentration of these two elements, but at a 30% level again the downward trend was due to a decrease in soil uptake in the severe low irrigation conditions. Under low irrigation stress, potassium plays a role in regulating stomach activity and helps plants adapt to water scarcity. Potassium also strengthens the antioxidant system in plants, thereby protecting them from oxidative stress caused by various environmental stresses (Hasanuzzaman et al., 2018).

The results also showed that the green cultivar had more sodium and potassium under moderate stress conditions, which indicates the greater need of the plant to accumulate these elements to cope with the stress conditions and indicates greater susceptibility of the green cultivar. Increased levels of potassium and sodium under low irrigation stress reported (Tadayyon et al., 2018). An increase in sodium content under low irrigation stress in coriander plant was also reported, which is in agreement with the results of this study (Ahmadian & Noorzad, 2013). The results also showed that UV levels and relatively severe low irrigation stress increased the percentage of essential oil in the basil. It has been said that the increase in essential oil percentage in relatively severe stresses may be due to the decrease in flowering shoot yield. In the Medicinal Plants Interaction of Essential Oil Percentage and Flowering Branch Yield are considered as Two Components of Essential Oil Yield

(Abaszadeh, 2017). The duration of UV irradiation significantly increased the essential oil of peppermint plant, which is agreement whit this study (khany, 2014).

CONCLUSION

Based on the results of growth parameters measurements, it was found that basil plant does not tolerate severe stress levels up to 30% of field capacity due to drastic reduction of growth factors, elemental uptake, and reduced plant efficiency for accumulation of effective growth materials. The effects of severe stresses were somewhat reduced by the use of UV radiation as pretreatment. But in the irradiation duration treatments, the plant had different reactions that require further investigation of the irradiation duration in the plant.

It was also found that the duration of irradiation and low irrigation stress accumulate secondary metabolites such as phenol and flavonoid, antioxidant activity, increase in essential oil percentage and accumulation of compatible solvent such as proline, and also elements involved in stomata activities such as sodium and potassium. It is considered as an effective plant mechanism in response to stress.

Ultraviolet radiation at level of 20 min/day with low irrigation stress at level of 30% field capacity had more effect on secondary metabolites accumulation and protective compounds, but level of 5 min/day with 50% field capacity had more effect on essential oil percentage. In some measured parameters, purple basil was less sensitive than green basil in reducing some measured traits such as photosynthetic pigment and etc. Also, the concentration of secondary metabolites and compatible solvents was higher in violet cultivar, but in the total sodium and potassium and essential oil percentages in green cultivar were higher than purple one.

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