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## Article

# Evaluation of the Selenium, Iodine, and Supplementary Blue Light Influence on the Biochemical Compounds and Nutrients Content of Fenugreek (*Trigonella foenum-gracum* L.) Medicinal Plant

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**Abstract:** The development and use of selenium and iodine elements in agriculture aim to enrich agricultural food products for humans. This study was carried out as a three-factor factorial experiment in a completely randomized design (CRD). Three concentrations of sodium selenate fertilizer (0, 2, 4 mg/l), three potassium iodate (0, 2, 4 mg/l), and two supplementary radiation levels (blue light and sunlight) were used. The results showed that the highest and the lowest (3.44 & 3.12 mg/g f.w) leaf protein value was related to blue light and sunlight treatments, respectively. In the blue light treatment, the maximum and the minimum (4.77 & 3.39 mg/g f.w) leaf sugar amount was observed at 4 and 0 mg/l of the iodine, respectively. At 0 mg/l of selenium, the highest and the lowest (8.63 & 5.34 mg/g f.w) leaf vitamin C amount was recorded at the 4 and 0 mg/l of iodine, respectively. In blue light + 0 mg/l of the selenium, the highest and the lowest (2.94 & 2.10 mg/g f.w) leaf flavonoid quantity was seen at 2 and 0 mg/l of the iodine, respectively. In the blue light conditions, (5.20%) leaf nitrogen content was achieved in the selenium 4 mg/l group. In the same conditions, the maximum and the minimum (3.09 & 2.18%) leaf potassium amount was attained at selenium 2 and 4 mg/l, respectively. Under blue light conditions, the highest and the lowest (0.58 & 0.24 mg/kg dry matter) leaf selenium level was observed at 4 and 0 mg/l, respectively. In sunlight radiation levels, the maximum and the minimum (0.36 & 0.25 mg/kg dry matter) leaf selenium level were found at 4 and 0 mg/l of selenium, respectively. In the blue light conditions, the most and the lowest (1.65 & 0.72 mg/kg dry matter) seed selenium content was related to 4 and 0 mg/l of the selenium, respectively. In the blue light conditions, the highest and the lowest (8.49 & 4.25 mg/kg dry matter) leaf iodine level was recorded at 4 and 0 mg/l of iodine, respectively. In the blue light conditions, the maximum and the minimum (30.56 & 20.62 mg/kg dry matter) seed iodine value was related to 4 and 0 mg/l of the iodine, respectively. In the sunlight, the most and the lowest (24.96 & 20.29 mg/kg dry matter) seed iodine amount was achieved at 4 and 0 mg/l of the selenium, respectively.

**Keywords:** biochemical compounds; blue light; growth; immune system; nutrition

## Introduction

Selenium is an essential micronutrient element that plays a vital role in various physiological processes such as thyroid hormone metabolism, antioxidant defense system, and also improving the function of the body's immune system (Santhosh and Priyadarsini, 2014; Schomburg and Köhrle, 2008; Rayman, 2000). Iodine is also a micronutrient essential for the proper physiological functions of

humans and animals (Krzepiřko et al., 2018; Zimmermann et al., 2008). Selenium and iodine elements significantly affect the growth and quality of vegetable crops. The development and application of selenium and iodine in agriculture aims to enrich agricultural food products for humans (Wu et al., 2015; El-Ramady et al., 2015; El-Ramady et al., 2016).

Selenium feeding causes a significant increase in selenium levels in spinach (Ferrarese et al., 2012), broccoli (Sindelařova et al., 2015), cabbage (Mechora et al., 2014), radish (Schiavon et al., 2016) without any adverse effects on the biomass and quality of plants. The responses of plants to selenium are different ; for instance, selenium treatment increased the carotenoids content and reduced the chlorophyll content of the mint plant (Oraghi Ardebili et al., 2015), while the chlorophyll value of lettuce (Alsina et al., 2012) and broccoli (Ghasemi et al., 2016) was increased. Selenium has also been reported to increase total sugar in tomato fruit (Lee et al., 2007). The dry weight and iodine content of cabbage plant tissues in the hydroponic system were increased in response to the iodine increment in the nutrient solution (Gonnella et al. 2019).

Blue light can induce the expression of genes involved in the production of enzymes such as PAL (phenylalanine ammonialyase), CHS (calcene synthase), and DFR (dihydroflavono-1-4-reductase), which are critical elements in the biosynthetic pathways of anthocyanin and flavonoid compounds (Son and Oh, 2012).

In a recent research by Rui et al., the amount of carotenoid, total phenol, and total flavonoid in the edible broccoli sprouts increased significantly under the influence of selenium and LED light. Also, the survey of the thermal map and analysis diagram of the data into the main component showed that the selenium improved the nutritional value of broccoli (edible sprouts) with combined blue and red light (1R2B). Also, the highest dry weight of broccoli related to selenium treatment plus the red and blue light combination (1R2B) and the highest amount of selenium of edible sprouts was reported for the combined treatments of blue and red light (2:1 and 1:2) in combination with selenium. In there experiment, the highest amount of potassium, calcium, and zinc were related to the combination of red, blue, and green lights (1:1:1). Also, total sugar and protein content significantly increased in the blue and red light treatments (1:1, 2:1 and 1:2) in combination with selenium (Rui et al., 2020).

Blue light increased ; red lettuce leaves' total phenol levels and antioxidant capacity (Son and Oh, 2015). Total phenol and flavonoid values and antioxidant capacity of lettuce grown under higher ratios of blue light were considerably higher than other spectra (Son and Oh, 2013). Also, the comparison between blue light and other light spectra on lettuce plants shows that the amount of calcium, magnesium, manganese, and iron elements increased in the blue light conditions (Shin et al., 2013).

It has been well demonstrated that blue light receptors control the stomatal opening (Ieperen and Trouwborst, 2008). Many studies have investigated the blue light effects on stomatal conductance and photosynthetic efficiency in many various plants (Yorio et al. 2001; Hogewoning et al. 2010; Terfa et al. 2013; Wang et al. 2014; Hernández and Kubota 2015). Blue light can affect the proton pumping system, membrane permeability, and ion channel activity in plants and increase the absorption of nutrients (Kopsell et al., 2012; Kopsell and Sams, 2015; Vařtakaitė et al., 2015; Gerovac et al., 2016). Also, Blue LED light is helpful in improving nutritional quality. Thus, th, the treatment of different plants with blue light leads to more accumulation of total phenol compounds (Son and Oh, 2013; Son and Oh, 2015; Qian et al., 2016; Taulavuori et al., 2016), ascorbic acid (Xin et al., 2015), carotenoids, anthocyanin content, and leaf color (Mizuno et al., 2015). It is assumed that the photo induction of blue light receptors (cryptochromes) is directly related to the production of total phenol.

Selenium and iodine rarely occur in most T for most plants, which calls for cusses to explain the importance of enriching agricultural products (Jerře et al., 2018). In order to enhance the dietary intake of iodine and selenium, Vegetable crops biofortification is a negligible approach (Allen et al., 2006; Gonzali et al., 2017). Therefore, the purpose of this research is to investigate the effects of selenium, iodine, and blue light on growth, biomass weight, physicochemical properties, mineral elements, and the medicinal composition of trigonelline in fenugreek seeds and shoots at two growth stages, namely the 40 and 80<sup>th</sup> day after seed planting.

## Materials and Methods

The current research was conducted as a three-factor factorial experiment in a completely randomized design (CRD), including three replications in plastic pots in the educational and research greenhouses of the Zabol University in 2022. In this experiment, the first factor was three levels of sodium selenate fertilizer (S1: 0, S2: 2, and S3: 4 mg/l), and the second factor was three levels of potassium iodate (I1: 0, I2: 2, and I3: 4 mg/l) and the third factor was two additional light radiations (L1: blue light and L2: no blue light). Fenugreek plant seeds were used in this experiment, and was purchased from Pakan Bazar company (Isfahan). Seeds were treated with dishwashing liquid, they were washed three times with distilled water and then the seeds were soaked with benomyl fungicide (2 g/l water) for 20 minutes. Then, ten seeds were planted in each pot on cocopeat: perlite substrate (ratio, 1:1). After the germination phase, five seedlings were kept, and the rest was detached. Hoagland's nutrient solution supplemented with sodium selenate and potassium iodate was applied. The greenhouse is in the north-south direction, with a polycarbonate cover, cooling device, lateral ventilation, and shade. The average daily temperature was 22-28 °C, 60-70% humidity, and light intensity was 600 micromoles per square meter per second. Twenty days after planting the seeds, they seedlings' exposure to supplementary blue light began. The blue lighting system was turned on for 2 hours every morning (sunrise) until the plant samples were analyzed. Traits were measured in two growth stages, 40 and 80 days after seed sowing.

### Measured traits

#### *Total phenol content*

The total phenolic content (TPC) was determined quantitatively using the Folin Ciocalteu reagent, with gallic acid as the standard (McDonald et al., 2001). To prepare the extract, 2 gr of the plant leaves crushed and add 5 ml of the extraction solution to it and place it at room temperature for one hour, and after centrifugation, remove the floating solution (the upper part) using Whatman filter paper, and it was used to measure the amount of phenol. Reading the amount of absorption, 100 µl of the extract was brought to a volume of 8 ml with distilled water, and then 500 µl of diluted Folin Ciocalteu (50%) was added to it; after one minute of adding Folin Ciocalteu, 1500 µl of sodium carbonate (20%) was added. The samples were held for two hours at room temperature and dark conditions, the absorbance was read at the 760 nm wavelength. To prepare the standard solution, a stock solution of gallic acid (0.1 g gallic acid with pure methanol, volume 100 ml), Folin Ciocalteu (5 ml Folin Ciocalteu with distilled water, volume 50 ml), and sodium carbonate 5.7% (1.5 g sodium carbonate in 20 ml distilled water) were prepared. Volumes of 10, 15, 20, 25, and 30 µl of gallic acid were added into small glass containers, and were added to each of them 2.5 ml of Folin Ciocalteu and 2 ml of 7.5% sodium carbonate. The absorbance of the solutions at the wavelength of 760 nm was read using a spectrophotometer (Jenway model, England). Then, the standard curve was drawn from the model absorption. The amount of total phenol was expressed using the sample and standard absorption based on mg/g of wet weight.

#### *Total flavonoids content*

Total flavonoid values were determined using the aluminum nitrate method. First, 11.5 ml of 30% ethanol and 0.7 ml of 5% sodium nitrite were added to one ml of the plant extract solution. After 5 minutes of the reaction, the solution was mixed with 0.7 ml of aluminum nitrate, and after 6 minutes, 5 ml of 5% sodium hydroxide was added to the present mixture. Ten minutes later, the absorbance of the samples was read at a wavelength of 760 nm using a spectrophotometer (Xie et al., 2015).

#### *Total sugar content*

The amount of total soluble sugar was determined by the anthrone sulfuric acid colorimetric method (Kohyama and Nishinari, 1991). First, fresh samples (0.5 g) of the plant aerial parts (leaves)

were heated in a hot water bath, including 10 ml of distilled water, for 30 minutes. Then, 0.1 ml of supernatant was mixed with 1.9 ml of distilled water, 0.5 ml of anthrone ethyl acetate and 5 ml of sulfuric acid. After shaking the solution, the soluble sugar was determined using a UV spectrophotometer at 630 nm wavelength.

#### *Total soluble protein*

Bradford analysis (1976) evaluated the total soluble protein content using the Coomassie Brilliant Blue G-250 dye. At first, 8 ml of distilled water was added to 0.5 g of fresh sample (leaves). The resulting homogenous solution was centrifuged at 3000 rpm for ten minutes at 4°C. Then, 0.2 ml of the supernatant was combined with 0.8 ml of distilled water and 5 ml of Coomassie Brilliant Blue G-250 dye (0.1 g/L). After 5 minutes, the protein content of the solution was determined using a spectrophotometer at a wavelength of 595 nm.

#### **Micronutrients**

The combustion method measured the leaf nitrogen content using an elemental analyzer (CHNS-O Elemental Analyzer model ECS4010, Italy) (Carl et al., 1997). The Spectrophotometric method measured leaf phosphorus (Rayan et al., 2001). For this purpose, one g of the dry plant material was placed in an electric furnace at a temperature of 500 °C for 4 hours. Then, 10 ml of hydrochloric acid (2 molar) was added to the samples, and the volume was brought to 1000 ml with distilled water in a flask and read at a wavelength of 420 nm. The atomic absorption method was used to measure potassium, calcium, magnesium, iron, zinc, copper, and manganese elements; therefore, 0.5 g of dry plant samples (leaves) were dissolved in 10 ml of nitric acid, and the suspension was placed at 70 °C for 24 hours until the samples were well dissolved in the acid. Finally, the solutions' absorbance values were read using an atomic absorption device model FSAA 240 (White, 1976).

#### *Selenium content in leaves and seeds*

To measure total selenium, 5 g of plant samples (dry weight) were first digested in 25 ml of a mixture of nitric acid and perchloric acid (volume ratio, 4:1) at 130 °C for one hour. After cooling, 5 ml of hydrochloric acid was added and heated at 115 °C for 20 minutes. After digesting the samples and cooling them at the laboratory temperature, the extracts were transferred to 50 ml tubes and added twice distilled water to gain a defined volume, and it was used to determine the total selenium content using an atomic emission spectrometer device (Liu and Gu, 2009).

#### *Iodine content in shoots and seeds*

The plant samples were ashed at 550 °C. Then, 20 ml of distilled water, 20 ml of one molar sulfuric acid and 10 ml of hydrogen peroxide (10 %) were added to one g of ash in a small cup. The mixture was slowly boiled and filtered using a Buchner funnel placed on a vacuum pump. The filtrated material was transferred into a small separator funnel and mixed with 3 ml of tetra-chloromethane. The purple layer of the mixture was moved in a 250 ml flask, and this process was repeated several times to ensure the extraction of all iodine. For calibration, 0.01 M of iodine solution was transferred to a 50 ml flask, and then 25 ml of this solution was kept, and the rest was diluted to draw the calibration graph. The absorbance of the samples was read at a wavelength of 450 nm (Liprot, 1971; Jackson, 1976).

#### *Vitamin C*

In determine the amount of vitamin C in leaves, a titrimetric method with iodine, potassium iodide, and potassium iodate was performed in the presence of starch reagent (Brater, 2002; Calam, 2002), the following Solution was prepared with Sodium thiosulfate (10 mmol/dm<sup>3</sup>), potassium iodide (5 mmol/dm<sup>3</sup>), and potassium iodate (1 mmol/dm<sup>3</sup>). Titration was done using sodium thiosulfate solution in an acidic environment in the presence of a starch reagent. The endpoint of the

titration was determined by discoloration (from the initial deep purple color). Vitamin C was calculated by moles of iodine titrated with sodium thiosulfate solution and reported as milligrams per 100 milliliters (Skinner, 1997). The titration process was repeated with a solution containing specific concentrations of standard vitamin C (10, 20, 40, 80, and 160 mg/100 ml), and the standard curve was drawn.

### Data analysis

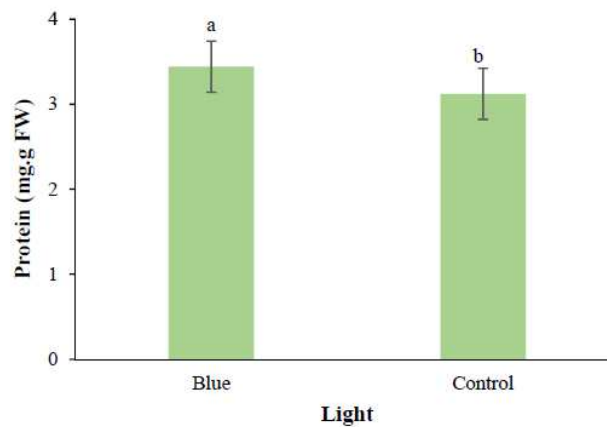
The obtained data were statistically analyzed by SAS statistical software; averages were compared with the LSD test at the 5 % level.

## Results

### Biochemical compounds

#### Protein

The highest and lowest (3.44 & 3.12 mg/g fresh weight) leaf protein value was observed on the 40th day and was subjected to supplementary blue light and sunlight treatments, respectively (Figure 5). According to the data in Table 6, in the control selenium, the highest amount of leaf protein (5.06 mg/g) on the 80<sup>th</sup> day was obtained at the 4 mg/l of iodine, and there wasn't any significant difference between the 0 and 2 mg/l of iodine levels. Also, at the selenium level of 2 mg/l, the highest quantity of leaf protein (6.17 mg/g) on the 80<sup>th</sup> day is recorded at 4 mg/l of the iodine treatment., There was no significant difference between the iodine levels of 0 and 2 mg/l. At 4 mg/l of the selenium level, there was no significant difference between the iodine levels in leaf protein content on the 80<sup>th</sup> day.



**Figure 5.** Means comparison of effect of light on protein in fenugreek plant in 40 days after seed planting

**Table 6.** Means comparison of effect of selenium and iodine on flavonoid, protein, Fe, Mg, carbohydrate and vitamin c in fenugreek in 80 days after seed planting

Selenium (mg.l)	Iodine (mg.l)	Flavonoid (m.g.g FW)	Protein (m.g.g FW)	Fe (ppm)	Mg (%)	Carbohydrate (m.g.g FW)	Vitamin C (m.g.g FW)
0	0	3.45± 0.2 b	3.96± 0.2 b	132.83± 12.5 b	0.38± 0.3 a	4.21± 0.2 a	5.26± 0.2 ab
	2	5.17± 0.2 a	4.13± 0.2 b	91.20± 8.6 c	0.35± 0.2 a	5.12± 0.2 a	4.94± 0.3 b
	4	3.14± 0.1 b	5.06± 0.3 a	160.95± 14.6 a	0.38± 0.2 a	5.01± 0.2 a	5.55± 0.2 a
2	0	3.00± 0.1 b	4.93± 0.3 b	174.79± 15.0 c	0.60± 0.4 a	4.94± 0.2 b	5.54± 0.3 a
	2	5.97± 0.2 a	5.03± 0.3 b	225.79± 18.3 c	0.56± 0.3 a	5.98± 0.2 c	5.34± 0.2 a
	4	2.99± 0.1 b	6.17± 0.3 a	246.78± 18.0 a	0.47± 0.3 b	7.30± 0.3 a	5.64± 0.3 a
4	0	2.96± 0.1 b	5.32± 0.2 a	222.57± 15.5 b	0.54± 0.4 b	6.20± 0.3 b	5.10± 0.3 b
	2	5.30± 0.2 a	5.54± 0.2 a	181.87± 14.8 c	0.63± 0.5 a	7.91± 0.4 a	5.96± 0.3 b
	4	5.03± 0.2 a	5.63± 0.3 a	250.85± 19.7 a	0.64± 0.4 a	8.84± 0.4 a	6.00± 0.3 a
<b>LSD</b>		<b>0.3</b>	<b>0.35</b>	<b>9.9</b>	<b>0.06</b>	<b>0.3</b>	

Data are presented as treatment means ±SE (n=3), Differences letters indicate significantly different values at p < 0.05.

### *Total soluble sugar*

In the supplementary blue light treatment, the highest and the lowest (4.77 & 3.39 mg/g fresh weight) leaf total soluble sugar content on the 40<sup>th</sup> day was obtained at 4 and 0 mg/l of iodine, respectively. In the sunlight treatment, the highest and the lowest (4.42 & 3.52 mg/g) leaf sugar values on the 40<sup>th</sup> day are respectively related to 2 and 0 mg/l of the iodine level, and there was no significant difference between 2 and 4 mg/l of iodine levels (Table 1). In the selenium 2 mg/l treatment, the highest and the lowest (5.18 & 3.00 mg/g) leaf sugar amount on the 40<sup>th</sup> day was related to the 4 and 0 mg/l levels of iodine, respectively. Also, in the selenium treatment of 4 mg/l, the highest and the lowest (4.95 & 4.06 mg/g) leaf total sugar content on the 40<sup>th</sup> day was obtained at 2 and 0 mg/l of iodine levels, respectively (Table 5). According to Table 6, in the treatment of selenium 0 mg/l, the highest and the lowest (5.12 & 4.21 mg/g) leaf total sugar quantity on the 80<sup>th</sup> day was associated with 2 and 0 mg/l of iodine levels, respectively, and no significant difference was observed between 2 and 4 mg/l levels of iodine. Also, in the treatment of selenium 2 mg/l, the highest and the lowest (7.30 & 4.94 mg/g) leaf sugar value on the 80<sup>th</sup> day was related to 4 and 0 mg/l of iodine levels, respectively. In the 4 mg/l of selenium, the highest and the lowest (8.84 & 6.20 mg/g) total leaf sugar value on the 80<sup>th</sup> day was obtained at 4 and 0 mg/l of iodine levels, respectively.

**Table 1.** Means comparison of effect of light and iodine on Cu, selenium, carbohydrate, iodine, vitamin C and seed iodine in fenugreek in 40 days after seed planting.

Light	Iodine(mg.l)	Cu (ppm)	Selenium (ppm)	Carbohydrate (mg.gFW)	Iodine (ppm)	Vitamin C (mg.gFW)	Seed iodine (ppm)
Blue	0	59.56± 4.6 a	0.473± 0.03 a	3.39± 0.3 c	4.25± 0.3 c	5.44± 0.5 b	20.62± 1.5 c
	2	58.43± 5.3 a	0.451± 0.03 a	4.08± 0.3 b	6.08± 0.3 b	6.08± 0.5 v	25.32± 1.8 b
	4	41.48± 4.0 b	0.420± 0.03 b	4.77± 0.5 a	8.49± 0.4 a	8.48± 0.6 a	30.00± 2.5 a
Control	0	47.24± 5.1 b	0.318± 0.02 a	3.52± 0.2 b	3.75± 0.2 b	6.15± 0.4 b	20.29± 1.2 b
	2	48.75± 3.8 b	0.295± 0.02 a	4.42± 0.3 a	5.82± 0.3 a	6.37± 0.4 b	23.22± 1.7 a
	4	56.73± 4.5 a	0.323± 0.02 a	4.13± 0.3 a	5.80± 0.4 a	8.20± 0.5 a	24.96± 2.0 a
LSD		3.4	0.029	0.45	0.35	0.38	1.8

Data are presented as treatment means± SE (n=3), Differences letters indicate significantly different values at p < 0.05.

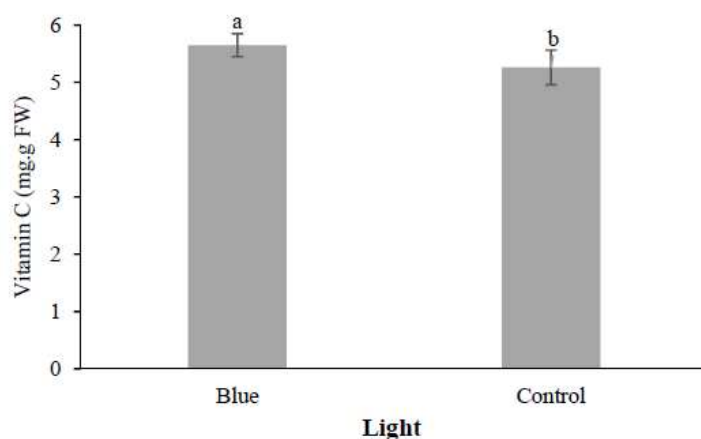
**Table 5.** Means comparison of effect of selenium and iodine on Mn, iodine, Fe, N, K, carbohydrate and vitamin C in fenugreek in 40 days after seed planting

Selenium (mg.l)	Iodine (mg.l)	Mn (ppm)	Iodine (ppm)	Fe (ppm)	N (%)	K (%)	Carbohydrate (m.g.g FW)	Vitamin C (m.g.g FW)
0	0	89.36± 5.3 b	4.55± 0.3 c	252.15± 13.3 a	4.74± 0.6 a	2.25± 0.3 b	3.32± 0.1 a	5.34± 0.3 b
	2	91.27± 5.6 b	5.69± 0.4 b	236.67± 15.5 a	4.17± 0.4 b	1.97± 0.2 c	3.73± 0.3 a	5.53± 0.3 b
	4	101.40± 6.4 a	7.40± 0.6 a	228.32± 13.7 a	3.71± 0.3 b	2.46± 0.3 a	3.46± 0.2 a	8.63± 0.4 a
2	0	61.14± 4.2 a	4.02± 0.3 c	259.51± 15.8 b	3.87± 0.2 a	2.31± 0.3 b	3.00± 0.1 b	5.05± 0.2 b
	2	63.91± 3.1 a	6.12± 0.5 b	288.58± 11.6 a	4.00± 0.3 a	2.61± 0.2 a	4.06± 0.2 c	6.16± 0.2 c
	4	66.15± 3.0 a	7.16± 0.5 a	300.72± 15.0 a	3.85± 0.2 a	2.56± 0.3 a	5.18± 0.3 a	9.00± 0.5 a
4	0	109.44± 7.9 b	3.43± 0.3 c	328.91± 13.3 b	4.54± 0.5 a	1.64± 0.2 b	4.06± 0.2 b	6.99± 0.3 b
	2	129.45± 6.1 a	6.03± 0.4 b	316.86± 19.5 b	4.79± 0.3 a	1.68± 0.2 ab	4.95± 0.3 a	6.98± 0.3 b
	4	118.05± 6.9 b	6.88± 0.5 a	366.63± 18.6 a	4.66± 0.5 a	1.82± 0.2 a	4.71± 0.3 a	7.93± 0.4 a
LSD		8.8	0.35	24.2	0.5	0.15	0.5	0.38

Data are presented as treatment means ± SE (n=3), Differences letters indicate significantly different values at p < 0.05.

### Vitamin C

According to Table 5, in the 0 mg/l of selenium treatment, the highest and the lowest (8.63 & 5.34 mg/g fresh weight) leaf total vitamin C amount on the 40<sup>th</sup> day was related to the 4 and 0 mg/l of iodine, respectively and there was no significant difference between iodine levels of 0 and 2 mg/l. Also, in the 2 mg/l of selenium treatment, the highest and the lowest (9.00 & 5.05 mg/g) leaf vitamin C value on the 40<sup>th</sup> day was achieved at 4 and 0 mg/l of the iodine, respectively. In the 4 mg/l of selenium treatment, the highest and the lowest (7.93 & 6.98 mg/g) leaf vitamin C content on the 40<sup>th</sup> day was observed at 4 and 2 mg/l of iodine, respectively, and there was no significant difference between the levels of 0 and 2 mg/l of iodine (Table 5). The results in Figure 7 show that the highest and the lowest (5.65 & 5.26 mg/g) leaf vitamin C quantity on the 80<sup>th</sup> day was recorded at the supplementary blue light and sunlight treatments, respectively. The data in Table 6 shows ed that in the selenium control level, the highest and the lowest (5.55 & 4.94 mg/g) leaf vitamin C content on the 80<sup>th</sup> day related to the iodine level of 4 and 2 mg/l, respectively. Also, in 2 mg/l of the selenium group, there was no significant difference between the iodine levels in terms of the amount of vitamin C in the leaf on the 80<sup>th</sup> day. In 4 mg/l of the selenium treatment, the highest and the lowest (6.00 & 5.10 mg/g) leaf vitamin C amount on the 80<sup>th</sup> day was seen at the iodine 4 and 2 mg/l, respectively.

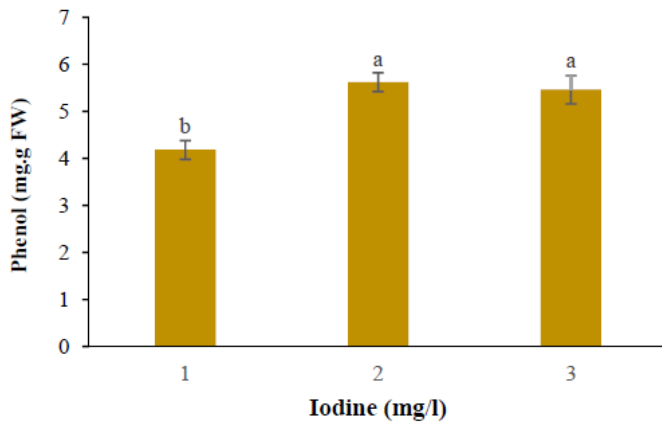


**Figure 7.** Means comparison of effect of light on vitamin C in fenugreek plant in 80 days after seed planting

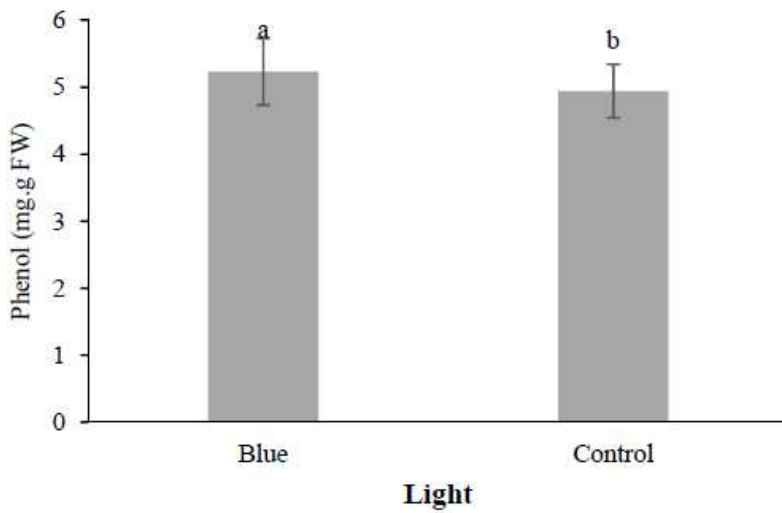
### Phenol

According to Table 7, in the blue light with control level of selenium conditions, the highest and the lowest (3.83 & 2.10 mg/g fresh weight) total leaf phenolic components value on the 40<sup>th</sup> day was achieved at 4 and 0 mg/l of iodine, respectively and no significant difference was observed between iodine levels of 0 and 2 mg/l. In the same light treatment with 2 mg/l of selenium, the highest (4.79 mg/g) leaf phenol content on the 40<sup>th</sup> day was related to the 4 mg/l of iodine, and no significant difference was observed between the 0 and 2 mg/l levels of iodine. In the blue light with 4 mg/l of selenium treatment, no statistically significant difference was observed between the levels of 2 and 4 mg/l of iodine (> 0.05). In the sunlight (control) conditions with a selenium control level, the highest (3.36 mg/g) total leaf phenol amount on the 40<sup>th</sup> day were recorded at 4 mg/l of iodine, and no significant difference was observed between 0 and 2 mg/l iodine levels. In the sunlight treatment with 4 mg/l of selenium, the highest (3.56 mg/g) total phenol quantity was observed on the 40<sup>th</sup> day, and there wasn't any significant difference between iodine levels of 0 and 2 mg/l. In the sunlight treatment with 4 mg/l of selenium, the highest (2.92 mg/g) leaf phenol value on the 40<sup>th</sup> day was observed at 4 mg/l of iodine, and no significant difference was observed between levels 2 and 4 mg/l of iodine (> 0.05) (Table 7). According to Figure 8, the highest and the lowest (5.23 & 4.94 mg/g) leaf phenol value on the 80<sup>th</sup> day was related in the blue light and sunlight groups, respectively. The Figure 4 data shows that the highest and the lowest (5.62 & 4.18 mg/g) leaf phenol amount on the 80<sup>th</sup> day was seen

at iodine 2 and 0 mg, respectively. Also, there was no significant difference between the levels of 2 and 4 mg/l ( $> 0.05$ ) (Figure 4).



**Figure 4.** Means comparison of effect of Iodine on phenol in fenugreek plant in 80 days after seed planting



**Figure 8.** Means comparison of effect of light on Phenol in fenugreek plant in 80 days after seed planting

Table 7. Means comparison of effect of selenium, iodine and light on flavonoid, P, Zn, Ca, Phenol and Mg in fenugreek in 40 days after seed planting

Light	Selenium (mg.l)	Iodine (mg.l)	Flavonoid (m.g.g FW)	P (%)	Zn (ppm)	Ca (%)	Phenol (m.g.g FW)	Mg (%)
Blue	0	0	2.10± 0.1 b	0.81± 0.07 a	39.05± 2.8 a	1.93± 0.1 a	2.10± 0.1 b	0.84± 0.1 b
	0	2	2.94± 0.2 a	0.82± 0.11 a	35.70± 1.9 a	2.35± 0.1 a	2.22± 0.1 b	0.70± 0.1 c
	0	4	2.86± 0.2 a	0.95± 0.10 a	36.13± 2.1 a	1.70± 0.2 a	3.83± 0.2 a	0.67± 0.1 c
	2	0	2.40± 0.2 c	1.28± 0.11 a	41.81± 2.0 a	3.60± 0.3 a	2.10± 0.1 b	0.98± 0.1 b
	2	2	2.93± 0.2 b	0.84± 0.08 b	33.55± 2.1 b	2.84± 0.2 b	2.21± 0.1 b	1.06± 0.1 ab
	2	4	3.93± 0.2 a	0.78± 0.09 a	39.14± 2.3 a	3.15± 0.3 a	4.79± 0.2 a	0.91± 0.1 b
	4	0	3.10± 0.2 a	1.61± 0.12 a	43.74± 2.5 a	2.82± 0.3 b	1.77± 0.1 b	1.30± 0.1 a
	4	2	2.73± 0.2 b	0.66± 0.08 b	39.68± 2.5 a	4.10± 0.2 b	2.80± 0.2 a	1.02± 0.1 b
	4	4	3.63± 0.3 a	0.63± 0.09 b	30.29± 2.5 b	2.22± 0.2 a	2.89± 0.2 a	1.29± 0.1 a
	Control	0	0	2.07± 0.1 a	1.46± 0.12 a	30.92± 3.0 a	3.04± 0.3 a	2.12± 0.1 b
0		2	2.04± 0.1 a	1.26± 0.11 a	31.02± 2.6 a	2.85± 0.3 ab	2.43± 0.2 b	1.13± 0.1 a
0		4	2.36± 0.2 a	1.44± 0.10 a	30.34± 2.0 a	3.02± 0.1 b	3.36± 0.2 a	0.88± 0.1 b
2		0	2.38± 0.2 b	0.96± 0.10 b	32.34± 2.8 b	1.65± 0.1 b	2.16± 0.1 b	0.66± 0.1 c
2		2	3.13± 0.2 a	1.54± 0.11 a	39.74± 3.5 a	2.63± 0.1 c	2.29± 0.1 b	0.70± 0.1 c
2		4	3.13± 0.2 a	1.30± 0.10 a	43.73± 4.0 a	2.91± 0.1 a	3.56± 0.3 a	0.66± 0.1 c
4		0	3.07± 0.2 a	1.10± 0.10 b	30.21± 2.1 b	2.69± 0.1 a	1.68± 0.1 b	0.66± 0.1 c
4		2	2.44± 0.2 b	1.70± 0.15 a	31.66± 2.5 b	2.59± 0.2 b	2.66± 0.2 a	0.66± 0.1 c
4		4	3.27± 0.3 a	1.71± 0.25 a	43.30± 4.1 a	3.25± 0.2 b	2.92± 0.3 a	1.11± 0.1 a
LSD				0.33	0.28	4.2	0.33	0.32

Data are presented as treatment means± SE (n=3), Differences letters indicate significantly different values at p < 0.05.

### *Flavonoids*

According to Table 7, in blue light treatment with control selenium level (0 mg/l), the highest and the lowest (2.94 & 2.10 mg/g fresh weight) leaf flavonoid value on the 40<sup>th</sup> day is related to the iodine level of 2 and 0 mg/l, respectively. Also, there was no significant difference between the levels of 2 and 4 mg/l of iodine. In the blue light conditions with 2 mg/l of selenium treatment, the highest and the lowest (3.93 & 2.40 mg/g) leaf flavonoid content on the 40<sup>th</sup> day was recorded at 4 and 0 mg/l of iodine groups, respectively. Also, in the blue light with 4 mg/l of selenium treatment, the highest and the lowest (3.36 & 2.73 mg/g) leaf flavonoid amount on the 40<sup>th</sup> day was achieved at 4 and 2 mg/l of iodine, respectively, and no significant difference was observed between 0 and 4 mg/l of iodine treatments. In the sunlight treatment with the control selenium level, there was no statistically significant difference between the iodine levels in the leaf flavonoid content on the 40<sup>th</sup> day (> 0.05). In the sunlight with 2 mg/l of selenium, no significant difference was observed between iodine levels of 2 and 4 mg/l, and in the sunlight with 4 mg/l of selenium, the highest and the lowest (3.27 & 2.44 mg/g) leaf flavonoid quantity at the 40<sup>th</sup> day was achieved at 4 and 2 mg/l of the iodine, respectively and there wasn't any significant difference between 0 and 4 mg/l of the iodine groups (Table 6). The data showed that in 0 mg/l of selenium treatment, the highest and the lowest (5.17 & 3.14 mg/g) leaf flavonoid values on the 80<sup>th</sup> day are related to 2 and 4 mg/l of iodine levels, respectively, and no significant difference was observed between iodine levels of 0 and 4 mg/l. Also, in the 2 mg/l of selenium treatment, the highest and the lowest (5.97 & 2.99 mg/g) leaf flavonoid amount on the 80<sup>th</sup> day was seen at 2 and 4 mg/l of iodine, respectively, and no significant difference was observed between 0 and 4 mg/l of iodine. In 4 mg/l of selenium treatment, no significant difference was observed in terms of leaf flavonoid content on the 80<sup>th</sup> day between 2 and 4 mg/l of iodine (Table 6).

### *Micronutrients*

#### *Leaf nitrogen (N)*

According to Table 3, in the supplementary blue light conditions, (5.20%) leaf nitrogen value on the 40<sup>th</sup> day was related to selenium 4 mg/l, and no significant difference between 0 and 2 mg/l of selenium was not observed. The Table 5 data shows that no significant difference was observed between the 2 and 4 mg levels of iodine in the control of the selenium, and the highest (4.74%) leaf nitrogen quantity on the 40<sup>th</sup> day was recorded as the control level of iodine; and in 2 and 4 mg/l of the selenium levels, no significant difference was observed between different levels of iodine. The results of leaf nitrogen content on the 80<sup>th</sup> day (Table 8) showed that in the blue light and control selenium conditions, the highest (3.19%) leaf nitrogen amount on the 80<sup>th</sup> day was achieved at the control level of the iodine. In the blue light and 2 mg/l of selenium conditions, the highest (3.64%) leaf nitrogen amount was attained at 4 mg/l of the iodine, and no significant difference was observed between the other two levels of iodine, and also in the same treatment (blue light) and 4 mg/l of selenium, no statistically significant difference was observed between the levels of iodine at the 80<sup>th</sup> day (> 5%).

**Table 3.** Means comparison of effect of light and selenium on Fe, selenium, N and k in fenugreek in in 40 days after seed planting

Light	Selenium (mg.l)	Fe (ppm)	Selenium (ppm)	N (%)	K (%)
Blue	0	255.34± 16.4 c	0.24± 0.01 c	3.53± 0.2 b	2.81± 0.1 b
	2	280.68± 17.2 b	0.52± 0.02 b	3.67± 0.4 b	3.09± 0.2 a
	4	347.06± 22.6 a	0.58± 0.02 a	5.20± 0.1 a	2.18± 0.1 c
Control	0	222.76± 10.5 c	0.25± 0.01 c	4.88± 2.4 a	1.64± 0.1 b
	2	285.19± 15.8 b	0.31± 0.02 b	4.19± 2.7 b	1.90± 0.1 a
	4	327.88± 18.5 a	0.36± 0.02 a	4.12± 2.2 b	1.25± 0.1 c
LSD		22.3	0.02	0.2	0.1

Data are presented as treatment means± SE (n=3), Differences letters indicate significantly different values at p < 0.05.

**Table 8.** Means comparison of effect of selenium, iodine and light N, Cu, Zn, Ca and Mn in fenugreek in 80 days after seed planting

Light	Selenium (mg.l)	Iodine (mg.l)	N (%)	Cu (ppm)	K (%)	Zn (ppm)	Ca (%)	Mn (ppm)
Blue	0	0	3.19± 0.1 a	66.78± 5.0 a	5.04± 0.1 a	66.24± 4.1 a	5.67± 0.3 a	32.49± 2.2 b
	0	2	2.12± 0.3 b	61.22± 4.3 ab	4.95± 0.3 a	66.92± 3.5 ab	5.69± 0.5 a	33.24± 2.1 b
	0	4	2.33± 0.2 a	57.11± 5.3 b	4.74± 0.1 a	56.68± 4.0 b	5.31± 0.4 a	37.50± 2.1 a
	2	0	2.31± 0.2 b	49.14± 3.4 b	4.66± 0.3 a	40.45± 2.9 a	5.10± 0.5 b	35.73± 2.1 a
	2	2	2.55± 0.4 b	53.67± 4.2 ab	2.88± 0.2 b	38.90± 3.0 a	6.18± 0.6 a	38.67± 2.3 a
	2	4	3.64± 0.1 a	59.29± 4.9 a	4.19± 0.1 a	40.15± 3.0 a	5.02± 0.2 b	26.13± 2.2 b
	4	0	3.54± 0.3 a	67.60± 5.4 a	3.24± 0.3 b	43.59± 3.5 a	5.65± 0.5 a	42.73± 2.6 a
	4	2	3.44± 0.2 a	60.06± 5.0 a	3.05± 0.2 b	42.91± 3.0 a	6.07± 0.2 a	45.96± 2.5 a
Control	4	4	3.11± 0.2 a	65.45± 4.5 a	4.93± 0.2 a	44.51± 3.0 a	5.55± 0.4 a	33.13± 2.3 b
	0	0	1.86± 0.3 b	50.53± 5.3 a	3.65± 0.3 a	36.54± 2.5 c	4.10± 0.3 a	27.27± 2.0 b
	0	2	3.21± 0.1 a	45.21± 4.1 a	3.18± 0.3 ab	45.24± 4.0 b	4.67± 0.5 a	34.21± 2.2 a
	0	4	3.26± 0.2 a	52.35± 4.5 a	2.95± 0.2 b	59.84± 4.5 a	4.17± 0.4 a	34.10± 2.2 a
	2	0	3.58± 0.2 a	57.77± 4.6 a	3.10± 0.3 b	62.41± 4.0 a	3.85± 0.3 b	28.09± 2.1 b
	2	2	3.42± 0.3 a	56.85± 5.0 a	2.43± 0.2 c	62.70± 4.2 a	2.84± 0.3 c	31.07± 3.0 ab

2	4	2.39± 0.1 b	57.01± 4.0 a	4.83± 0.2 a	63.22± 4.0 a	6.26± 0.4 a	33.66± 2.4 a
4	0	2.39± 0.1 a	62.16± 5.5 a	4.92± 0.1 a	66.40± 5.0 a	4.28± 0.3 a	35.37± 3.2 b
4	2	2.08± 0.1 a	54.14± 4.8 ab	3.00± 0.2 b	66.70± 4.5 a	4.33± 0.3 a	38.29± 3.0 b
4	4	2.03± 0.2 a	49.16± 3.7 b	2.87± 0.2 b	67.22± 5.1 a	3.88± 0.3 a	42.84± 3.4 a
<b>LSD</b>		<b>0.47</b>	<b>8.6</b>	<b>0.5</b>	<b>6.3</b>	<b>0.65</b>	<b>3.3</b>

Data are presented as treatment means± SE (n=3), Differences letters indicate significantly different values at p < 0.05.

### *Leaf phosphorus (P)*

According to Table 7, in the supplementary blue light treatment and selenium control level, no significant difference was observed between iodine levels in terms of leaf phosphorus value on the 40<sup>th</sup> day. Also, in the same light and 2 mg/l of selenium, the highest (1.28%) leaf phosphorus content was related to the control level of the iodine. There was no significant difference between the other two levels of iodine, and also in the blue light and 4 mg/l of selenium, the highest (1.61%) phosphorus quantity was recorded at the control level of iodine. No statistical difference was seen between other iodine levels (> 5%). The results in Table 4 shown that in the supplementary blue light conditions, there was no significant difference between the levels of 0 and 2 mg/l of selenium in terms of leaf phosphorus value on the 80<sup>th</sup> day, and also in the sunlight conditions, the highest and the lowest (0.72 & 0.36%) leaf phosphorus content was related to 0 and 2 mg/l levels of selenium, respectively.

**Table 4.** Means comparison of effect of light and selenium on seed selenium, selenium, Fe, Mg and P in fenugreek in in 80 days after seed planting and seed selenium

Light	Selenium(mg.l)	Seed Selenium (ppm)	Selenium (ppm)	Fe (ppm)	Mg (%)	P (%)
Blue	0	0.72± 0.05 c	0.321± 0.02 c	153.66± 14.3 c	0.44± 0.3 b	0.75± 0.06 a
	2	1.42± 0.06 b	0.876± 0.05 b	212.16± 15.3 b	0.69± 0.5 a	0.80± 0.05a
	4	1.65± 0.10 a	0.957± 0.04 a	243.70± 22.3 a	0.74± 0.5 a	0.48± 0.05 b
Control	0	0.73± 0.05 b	0.315± 0.02 c	103.00± 14.2 c	0.30± 0.3 c	0.72± 0.03 a
	2	0.79± 0.04 b	0.680± 0.04 b	219.40± 15.0 b	0.40± 0.3 b	0.36± 0.03 c
	4	1.00± 1.00 a	0.750± 0.04 a	193.16± 17.5 a	0.47± 0.3 a	0.47± 0.04 b
LSD		0.1	0.038	9.9	0.06	0.07

Data are presented as treatment means  $\pm$ SE (n=3), Differences letters indicate significantly different values at  $p < 0.05$ .

### *Leaf potassium (K)*

According to the data in Table 3, in the supplementary blue light conditions, the highest and lowest (3.09 & 2.18%) leaf potassium value on the 40<sup>th</sup> day was related to the selenium 2 and 4 mg/l treatments, respectively. At the control selenium level, the highest and the lowest (2.46 & 1.97%) leaf potassium amount on the 40<sup>th</sup> day was recorded at iodine 4 and 2 mg/l groups, respectively. Also, at 2 mg/l of selenium treatment, no significant difference was observed between 2 and 4 mg/l of the iodine (Table 5). In the blue light with 4 mg/l of selenium treatment, the highest (4.93%) leaf potassium content on the 80<sup>th</sup> day was attained at 4 mg/l of the iodine, and there was not any significant difference between the levels of 0 and 2 mg/l of iodine. In the sunlight and 0 mg/l of selenium conditions, no significant difference was observed between 0 and 2 mg/l of the iodine treatments, and also in the sunlight with 2 mg/l of selenium, the highest and the lowest (4.83 & 2.43%) leaf potassium quantity on the 80<sup>th</sup> day was achieved at 4 and 2 mg/l of iodine, respectively. Also, in sunlight treatment with 4 mg/l of selenium, the highest (4.92%) amount was related to the control iodine level, and no significant difference was observed between levels 2 and 4 of iodine treatments (Table 8)

### *Leaf calcium (Ca)*

According to the Table 7, in the of supplementary blue light and the control level of selenium conditions, the highest (2.35%) leaf calcium value on the 40<sup>th</sup> day was related to 2 mg/l of the iodine; also no significant difference was observed between the other levels of iodine. Also, in the blue light treatment, the highest (3.60%) leaf calcium content on the 40<sup>th</sup> day was recorded at the control level of iodine, and no significant difference was observed between the levels of 2 and 4 mg/l of iodine. In the blue light treatment and 4 mg/l of the selenium, the highest (4.10%) amount of leaf calcium on the 40<sup>th</sup> day was attained at 2 mg/l of the iodine. Also, in the blue light conditions with a selenium level of 4 mg/l, the highest (3.25%) leaf calcium quantity at the 40<sup>th</sup> day was recorded at 4 mg/l of the iodine, and no significant difference was observed between 0 and 2 mg/l levels of the iodine. Also, in the sunlight treatment with 2 mg/l of the selenium, the most and the minimum (6.26 & 2.84 %) leaf calcium quantity on the 80<sup>th</sup> day was related to 4 and 2 mg/l levels of iodine, respectively. Also, in the sunlight treatment with 4 mg/l level of selenium, no significant difference was observed between iodine levels (Table 8).

### *Leaf magnesium (Mg)*

According to the Table 7, in the conditions of supplementary blue light and control level of selenium, there was no significant difference between iodine levels in terms of leaf magnesium content on the 40<sup>th</sup> day. Also, in the sunlight with 4 mg/l of selenium, the highest (1.11%) leaf magnesium amount on the 40<sup>th</sup> day was related to 4 mg/l levels of iodine. In the blue light conditions, the highest and lowest (0.74 & 0.44%) amount of leaf magnesium on the 80<sup>th</sup> day was recorded at 4 and 0 mg/l levels of the selenium, respectively, and there was not any significant difference between 4 and 2 mg/l levels of the selenium groups (> 5%). Also, in the sunlight conditions, the highest and lowest (0.47 & 0.30%) leaf magnesium amount on the 80<sup>th</sup> day was achieved at 4 and 0 mg/l of the selenium levels, respectively (Table 4). At 4 mg/l level of the selenium, the highest (0.64%) leaf magnesium quantity at the 80<sup>th</sup> day was attained at 4 mg/l level of the iodine, and no significant difference was observed between the levels of 2 and 4 mg/l of iodine (Table 6).

### *Leaf zinc (Z)*

According to the Table 7, in the conditions of supplementary blue light with the selenium level of the control, no significant difference was observed between all levels of iodine in terms of the leaf zinc amount on the 40<sup>th</sup> day. In the same light plus 2 mg/l of selenium, the highest (41.81 ppm) leaf Zn value was related to the control level of the iodine, which was not significantly different from the 4 mg/l of iodine. Moreover, in the sunlight treatment with 4 mg/l of selenium, the highest (43.30 ppm) leaf Zn amount at the 40<sup>th</sup> day was related to 4 mg/l level of the iodine. In the sunlight and the control

level of selenium, the highest (59.84 ppm) leaf Zn content on the 80<sup>th</sup> day was recorded at the 4 mg/l level of iodine, and no significant difference was observed between iodine levels in this light treatment with 2 or 4 mg/l levels of the selenium (Table 8).

#### *Leaf iron (Fe)*

In the conditions of supplementary blue light, the highest (347.06 ppm) leaf iron value on the 40<sup>th</sup> day was recorded at the iodine level of 4 mg/l. Also, in the sunlight treatment, the highest (327.88 ppm) leaf Fe content was observed at 4 mg/l of the iodine group (Table 3). In 4 mg/l of the selenium treatment, the maximum (366.63 ppm) leaf Fe amount on the 40<sup>th</sup> day was attained at the iodine level of 4 mg/l (Table 5). According to Table 4, in the blue light conditions, the highest (243.70 ppm) leaf Fe quantity on the 80<sup>th</sup> day was achieved at 4 mg/l levels of selenium. Also, in the sunlight conditions, the highest (40 219/ppm) value was related to the selenium level of 2 mg/l. In the control treatment of selenium conditions, the highest (160.95 ppm) leaf Fe content on the 80<sup>th</sup> day was recorded at the iodine level of 4 mg/l; also in the 2 mg/l selenium treatment, the highest (246.78 mg/l) value was related to iodine level of 4 mg/l (Table 6). The results of leaf iron value on the 80<sup>th</sup> day showed that in the blue light and sunlight conditions, the highest (238.29 & 200.76 ppm) leaf Fe content was observed at the iodine level of 4 mg/l (Table 2).

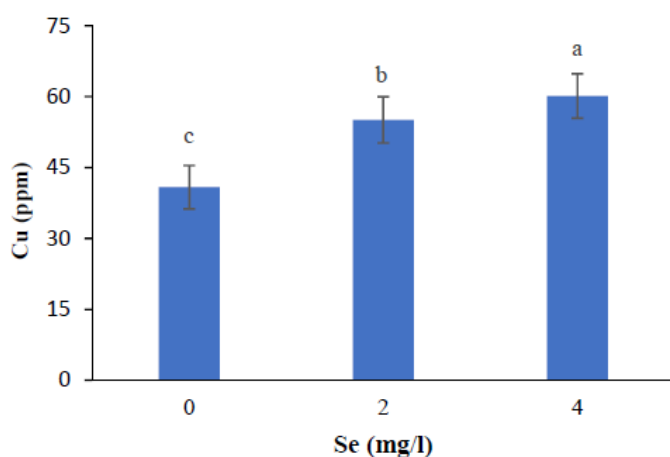
**Table 2.** Means comparison of effect of light and iodine on iodine, Fe, protein, selenium and iodine in fenugreek in in 80 days after seed planting

Light	Iodine (mg.l)	Fe (ppm)	Protein (mg.gFW)	Selenium (ppm)	Iodine (ppm)
Blue	0	203.24± 21.5 b	4.81± 0.3 b	0.758± 0.1 a	12.66± 0.7 c
	2	167.99± 19.3 c	4.96± 0.4 b	0.738± 0.1 a	14.62± 0.5 b
	4	238.29± 23.5 a	6.00± 0.4 a	0.657± 0.1 b	21.25± 0.7 a
Control	0	150.22± 20.0 c	4.64± 0.3 b	0.573± 0.1 a	13.15± 0.8 b
	2	164.58± 17.6 b	4.83± 0.3 b	0.601± 0.1 a	14.37± 1.1 b
	4	200.76± 18.2 a	5.24± 0.3 a	0.571± 0.1 a	16.84± 0.7 a
LSD		9.9	0.3	0.038	1.4

Data are presented as treatment means± SE (n=3), Differences letters indicate significantly different values at p < 0.05.

### Leaf copper (Cu)

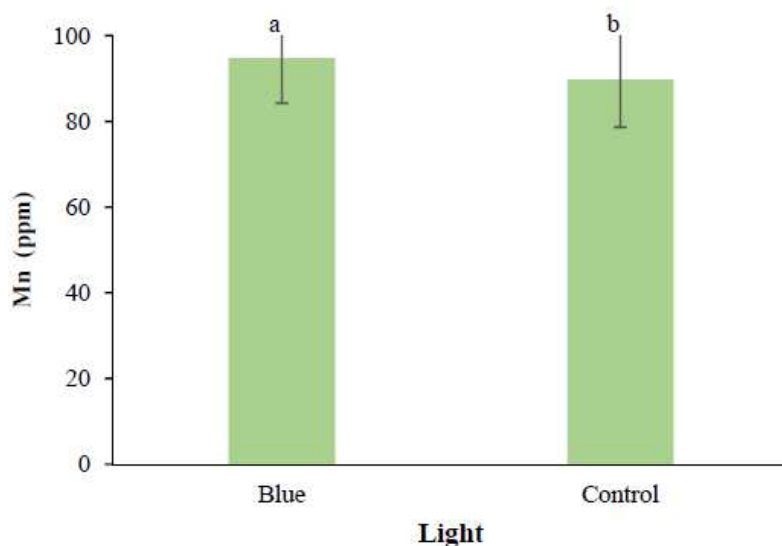
The Figure 1 data shows that the highest and lowest (60.15 & 40.85 ppm) leaf copper value on the 40<sup>th</sup> day was observed at 4 and 0 mg/l of selenium treatments, respectively. Also, according to Table 1, no significant difference was observed in the blue light treatment between the 0 and 2 mg/l levels of iodine. In the sunlight, the highest (56.73 ppm) leaf Cu quantity on the 40<sup>th</sup> day was found at 4 mg/l of the iodine. In blue light treatment with the control level of selenium, the highest (66.78 ppm) leaf Cu amount on the 80<sup>th</sup> day was related to the control level of the iodine, which is no significant difference with 2 mg/l levels of iodine (Table 8). Also, in blue light with 2 mg/l levels of selenium, the highest (59.29 ppm) leaf Cu content was recorded at the iodine level of 4 mg/l, which was not significantly different from the level of 2 mg/l (>5%). In the sunlight conditions with control level of the selenium, no significant difference was observed between different iodine levels, and in this light treatment with selenium 2 mg/l, no significant difference was observed between iodine levels. In the sunlight with a selenium level of 4 mg/l, the highest (62.16 ppm) leaf Cu value on the 80<sup>th</sup> day was found at the control level of the iodine, which was not significantly different from the iodine level of 2 mg/l. (Table 8).



**Figure 1.** Means comparison of effect of selenium on Cu in fenugreek plant 40 days after seed planting

### Leaf manganese (Mn)

The highest and the lowest (94.75 & 89.73 ppm) leaf manganese value at the 40<sup>th</sup> day was related to supplementary blue light and sunlight treatments, respectively (Figure 6). Also, in the selenium control treatment, the highest (101.40 ppm) leaf Mn amount was related to the 4 mg/l level of iodine (Table 5). No significant difference between iodine levels was observed at 2 mg/l of the selenium. In selenium 4 mg/l, the highest (129.45 ppm) leaf Mn content on the 40<sup>th</sup> day was recorded at 2 mg/l of the iodine. The leaf Mn amount data on the 80<sup>th</sup> day showed that in the supplementary blue light conditions with the control level of selenium, the highest (37.50 ppm) Mn quantity was attained at 4 mg/l of the iodine, and no significant difference was observed between the levels of 0 and 2 mg/l of iodine (Table 8). In the blue light treatment and 2 mg/l of selenium, the highest (38.67 ppm) leaf Mn content was found at 2 mg/l of iodine level, and no significant difference was observed between 0 and 2 mg/l iodine levels so the same condition was observed at the selenium level of 4 mg/l. In the sunlight conditions with the selenium control level, no statistically significant difference was observed between the iodine levels of 2 and 4 mg/l (>5%). Also, in the sunlight with 2 mg/l of the selenium, the highest (33.66 ppm) leaf Mn amount on the 80<sup>th</sup> day was achieved at 4 mg/l of the iodine, which was not significantly different from 2 mg/l. The maximum (42.84 ppm) leaf Mn quantity was related to 4 mg/l levels of iodine (Table 8).



**Figure 6.** Means comparison of effect of light on Mn in fenugreek plant in 40 days after seed planting

### *Leaf selenium (Se)*

In the supplementary blue light conditions, the highest and the lowest (0.58 & 0.24 mg/kg dry matter) leaf selenium value at the 40<sup>th</sup> day was at 4 and 0 mg/l treatments, respectively; and in sunlight conditions, the highest and the lowest (0.36 & 0.25 mg/kg d.m.) leaf Se content at the 40<sup>th</sup> day was found at the selenium 4 and 0 mg/l, respectively (Table 3). In the conditions of blue light, the highest and the lowest (0.47 & 0.42 mg/kg d.m.) leaf Se amount on the 40<sup>th</sup> day was at 0 and 4 mg/l of selenium levels, respectively. Also, no statistically significant difference was observed between the levels of 0 and 2 mg/l of selenium. In the conditions of sunlight, there was not any significant difference between different levels of selenium in terms of leaf selenium content on the 40<sup>th</sup> day (> 5%) (Table 1). The results of the selenium amount in the leaves on the 80<sup>th</sup> day showed that in the blue light conditions, the highest and the lowest (0.95 & 0.32 mg/kg d.m.) value was related to 4 and 0 mg/l concentrations of the selenium (Table 4). In the sunlight conditions, the highest and the lowest (0.75 & 0.31 mg/kg d.m.) leaf Se amount was found at 4 and 0 mg/l of the selenium, respectively. According to Table 2, in the blue light conditions, the highest and the lowest (0.75 & 0.65 mg/kg d.m.) leaf Se content was recorded at 0 and 4 mg/l of iodine treatments, respectively. Also, there was no significant difference in leaf selenium amount between the different levels of iodine on the 80<sup>th</sup> day in both blue and sunlight conditions (> 5%).

### *Seed selenium (Se)*

The Table 4 data shows that in the blue light conditions, the highest and the lowest (1.65 & 0.72 mg/kg d.m.) seed selenium amount was related to 4 and 0 mg/l levels of the selenium, respectively. On the other hand, in sunlight conditions, the highest and the lowest (1.00 & 0.73 mg/kg d.m.) seed selenium content was observed at 4 and 0 mg/l of the selenium, respectively, and there was no statistically significant difference between 0 and 2 mg/l selenium levels in terms of seed selenium (> 5%) (Table 4).

### *Leaf iodine (I)*

According to the data in Table 1, in the supplementary blue light conditions, the highest and the lowest (8.49 & 4.25 mg/kg dry matter) amount of leaf iodine on the 40<sup>th</sup> day was related to 4 and 0 mg/l of iodine treatments, respectively. In the sunlight (control) conditions, there was no significant difference between different levels of iodine in terms of iodine value on the 40<sup>th</sup> day (>5%). The results have shown that in the selenium control level (0 mg/l), the highest and the lowest (7.40 & 4.55 mg/kg d.m.) leaf iodine value on the 40<sup>th</sup> day was recorded at 4 and 0 mg/l of the iodine levels, respectively. Also, at 2 mg/l of the selenium group, the highest and the lowest (7.16 & 4.25 mg/l d.m.) leaf iodine

content on the 40<sup>th</sup> day was found at 4 and 0 mg/l of iodine, respectively (Table 5). At 4 mg/l of the selenium, the highest and the lowest (6.88 & 4.25 mg/l d.m.) leaf iodine quantity on the 40<sup>th</sup> day was achieved at 4 and 0 mg/l of the iodine, respectively. In the blue light conditions, the highest and the lowest (21.25 & 12.66 mg/l d.m.) leaf iodine value on the 80<sup>th</sup> day was attained at 4 and 0 mg/l of iodine treatments, respectively. In the sunlight conditions, the highest and the lowest (16.84 & 13.15 mg/l d.m.) leaf iodine content on the 80<sup>th</sup> day was related to 4 and 0 mg/l of the iodine, respectively, and also, no significant difference was observed between 0 and 2 mg/l of iodine levels (Table 2).

### *Seed iodine (I)*

In the supplementary blue light conditions, the highest and the lowest (30.56 & 20.62 mg/kg d.m.) amount of seed iodine was related to 4 and 0 mg/l of the iodine, respectively. Conversely, in the sunlight conditions, the highest and the lowest (24.96 & 20.29 mg/kg d.m.) seed iodine content was recorded at 4 and 0 mg/l of selenium, respectively. Also, there was no significant difference between 2 and 4 mg/l of iodine treatments (> 5%) (Table 1).

## **Discussions**

### *Biochemical compounds*

The highest amount of vitamin C on the 80<sup>th</sup> day was related to the supplementary blue light treatment. In the control selenium level, the highest leaf vitamin C content on the 80<sup>th</sup> day was recorded at 4 mg/l of the iodine. The blue LED light is helpful in improving the nutritional quality of vegetable crops. Among its effects, we can mention increased ascorbic acid levels in edible products (Xin et al., 2015). The highest amount of leaf phenol on the 80<sup>th</sup> day was related to 2 mg/l of the iodine. Studies have shown that blue light is a valuable tool in improving the nutritional quality of edible plants, which can be pointed out by increasing phenolic compounds (Son and Oh, 2015; Taulavuori et al., 2016). It has also been reported that blue light alone causes a 69% increase in total phenol content in Chinese cabbage sprouts compared to darkness conditions (Qian et al., 2016). Therefore, it is assumed that the photo-induction of blue light receptors (cryptochromes) is directly related to the production of phenol. In addition, increasing the ratio of blue light in combination with far-red compared to far-red alone or the low ratio of blue light in the treatment has led to more accumulation of phenol in lettuce (Son and Oh, 2013). Research found that blue light increased the amount of total phenol in red leaf lettuce (Son and Oh, 2015). The total phenol and flavonoid value of lettuce grown under higher ratios of blue light was significantly higher than other light spectra (Son and Oh, 2013). Blue light can activate the expression of genes involved in the production of enzymes such as PAL (phenylalanine ammonialyase), CHS (calcene synthase), and DFR (dihydroflavono-l-4-reductase), which are key elements in the biosynthetic pathways of flavonoid compounds (Son and Oh, 2012). In a research by Rui et al., selenium treatment with combined blue & red light (1R:2B) improved the nutritional value of broccoli (edible sprouts), and also the content of total sugar and protein increased in the various blue and red light treatments (1:1 & 2:1 and 1:2) in combination with selenium significantly (Rui et al., 2020). Selenium has been reported to increase the total sugar in tomato fruit (Lee et al., 2007). Blue light facilitates protein biosynthesis in plants and increases plant photosynthetic capacity (Li and Pan, 1995).

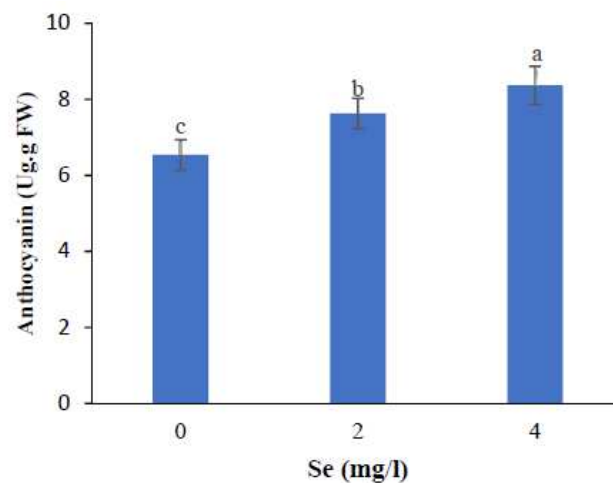
### *Micronutrients*

An experiment found that the amount of calcium, magnesium, manganese, and iron in lettuce plants increased under blue light conditions compared to other spectrums (Shin et al., 2013). Blue light can affect the proton pumping system, membrane permeability, and ion channel activity in plants and increase the absorption of nutrients (Kopsell et al., 2012). Reports have indicated that feeding the plants with selenium causes a significant increase in the selenium level of spinach (Ferrarese et al., 2012), broccoli (Sindelařova et al., 2015), cabbage (Mechora et al., 2014) and radish (Schiavon et al., 2016) without adverse effects on the biomass and quality of plants. In another research, by Rui et al. stated that selenium treatment with combined blue and red light 1R2B (1:2)

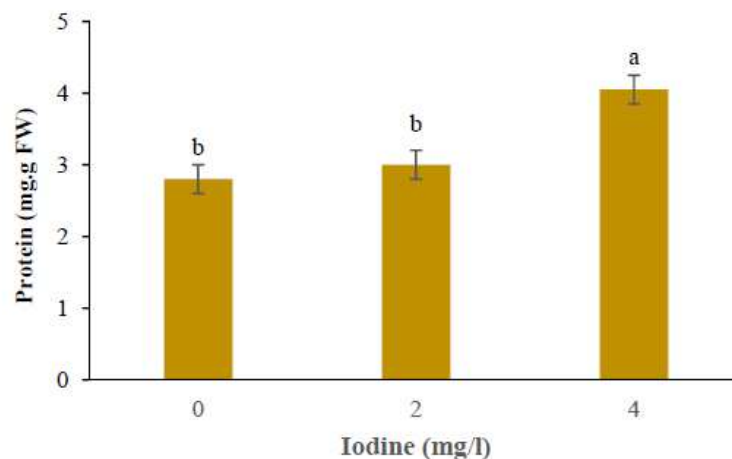
improved the nutritional value of broccoli (edible sprouts), and the highest selenium content in the edible sprouts was obtained from combined blue and red light (2:1 and 1:1) plus selenium treatment (Rui et al., 2020). Also, a research shows that the treatment of lettuce plants with blue-red light (10:90) increased the amount of selenium in the leaves (Brazaitytė et al., 2021). The results of the experimental research showed that when Se and I treatments were used separately or simultaneously, the content of these two elements increased in lettuce leaves without any negative interaction (Puccinelli et al., 2021). In a hydroponic culture of cabbage, it was observed that adding iodine to the nutrient solution increases the iodine content of the plant tissues, which is essential for human food (Gonnella et al., 2019). In general, it can be said that due to the application of supplementary blue light, the stomata of the plant are more affected, and the transpiration of the plant increases, and consequently it causes an increase in the suction flow in the xylem of the plant, which increases the flow rate of water and nutrients to inside the plant and as a result, the absorption of elements also increases.

## Conclusions

The highest amount of vitamin C on the 80<sup>th</sup> day was related to the supplementary blue light treatment. In the blue light conditions, the highest leaf protein content on the 40<sup>th</sup> day was observed at 4 mg/l of the selenium. In the blue light conditions, the highest leaf nitrogen quantity on the 40<sup>th</sup> day was recorded at 4 mg/l of selenium. In the blue light conditions, the highest leaf selenium value on the 40<sup>th</sup> day was found at 4 mg/l of the selenium. In the blue light conditions, the highest amount of seed selenium was attained at 4 mg/l of the selenium group. In the conditions of blue light, the highest leaf iodine content on the 40<sup>th</sup> day was observed at 4 mg/l of the iodine. In the blue light conditions, the highest amount of seed iodine was achieved at 4 mg/l of the iodine.



**Figure 2.** Means comparison of effect of selenium on anthocyanin in fenugreek 80 days after seed planting



**Figure 3.** Means comparison of effect of Iodine on protein in fenugreek plant in 40 days after seed planting

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