# Changes in biochemical properties of tomato (cv.240) affected by combination of blue/red optical spectra and Calfomyth spray (Ca and P)

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**Abstract:** The levels of antioxidant activity and vitamins can change with varying ratios of different wavelengths. This study was conducted as a pot experiment under soilless culture conditions in order to investigate the interactive effects of light supplementation and nutrition (calfomyth solution) on some qualitative traits of tomato fruit. The research was carried out as a split-plot experiment based on a completely randomized design with three light treatments including without supplementary light (control), 60% red light + 40% blue light and 90% red light + 10% blue light. There were two nutritional treatments including no spraying with calfomyth as commercial fertilizer and foliar application with a concentration of 2 mg/L in three replicates. According to the results, the amounts of vitamin C (16.1 mg/g FW fruit), soluble solids (12.33 mg/g FW fruit), and lycopene (2.95 mg/g FW fruit) were all uppermost by the effect of a higher percentage of red light treatment. Higher percentage of the blue light resulted in the highest leaf chlorophyll content (38.4 mg/g FW leaf), but supplementary light treatments had no significant effects on the titratable acidity. Nutrition (calfomyth foliar application) showed positive impacts on all treatment traits compared to control. Beta-carotene content was affected by none of the treatments with no significant differences. According to this research, it can be expected that the use of complementary light treatments and calfomyth foliar spray may have positive effects on most of the qualitative traits in tomato fruit (cv. 240).

**Keywords:** biochemical properties, tomato, optical spectra, calfomyth, antioxidant, lycopene, phytochrome
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## 1 Introduction

Among greenhouse vegetables, tomato cultivation for fresh consumption is the most important greenhouse cropping in many European countries[1] with a per capita consumption of 40 kg/a in some countries[2]. Tomatoes are also widely used around the world due to their high levels of antioxidants, vitamins and phenolic compounds[3]. The development and production of red pigment are one of the most prominent signs of ripening in most tomato fruits. The major carotenoids accumulated in ripened red tomatoes include lycopene (90%), beta-carotene (5%-10%), lutein (1%-5%), and other carotenoids of <1%[4]. Moreover, lycopene and beta-carotene are the two main pigments responsible for coloration in tomato fruit. Carotenoids are valuable compounds in this plant that play an important role in human health[5]. Lycopene content also improves red coloration in fruits as one of the main characteristics for both the industry and consumers[6]. Thomas et al.[7] stated that both light quality and intensity play an essential role in determining the number of carotenoids synthesized in tomatoes. Evidence suggests that carotenoid biosynthesis in tomatoes is controlled by phytochromes and red light. Schopfer[8] in 1966 studied red light and reported that ascorbic acid levels increased in mustard plant as a result of red light. Also, red light could increase the amount of lycopene in tomato through phytochromes[9]. Photosynthesis can be dependent upon certain regulators and light sensors. Blue and red lights trigger different optical sensors and induce expression of genes each with a positive or negative effect on plant growth and development[10]. Therefore, it can be concluded that the presence of both wavelengths (blue and red) is essential for the plant, hence, research is now mostly focused on the achievement of an appropriate light combination[11]. Accordingly, Bukhov et al.[12] investigated chlorophyll content in barley and concluded that blue light would have a marked effect on the chlorophyll content in barley leaves and that the amount of chlorophyll will increase with rising light blue leading to improved plant growth and leaf development[13]. Calcium plays an important role in maintaining the quality of fruits and vegetables. Treatment with calcium in apple fruit, for instance, retained stiffness, increased vitamin C, and reduced carbon dioxide and ethylene[14]. The application of calcium chloride on tomatoes was found to increase the titratable acidity[15]. Foliar spray of calcium on pomegranate (cv. *Yazdi acidulous*) enhanced the solubility of soluble solids[16]. Saito and Kano[17] obtained a positive result in the amount of lycopene by the use of phosphorus in tomatoes. Increasing the amount of phosphorus in nutritional solutions for tomatoes was also reported to elevate vitamin C levels[18]. Considering the importance of blue and red wavelengths as supplementary illumination and such elements as calcium and phosphorus, as well as the vital roles of micronutrients in plant growth and development using calfomyth commercial fertilizer, this study aimed to determine the best growth conditions to produce a quality product of tomatoes.

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## 2 Materials and methods

### 2.1 Plant materials and growing conditions

In order to investigate the impacts of calcium and phosphorus together with supplementary light, the present study was carried out as a pot experiment under greenhouse conditions with soilless culture in the Faculty of Agriculture, Ferdowsi University of Mashhad (36°16′N and 59°36′E with an altitude of 985 m). A split-plot trial was run based on a completely randomized design with three treatments including natural light (control), 60% red light+40% blue light and 90% red light+10% blue light. There were two nutritional treatments viz. no foliar spray with calfomyth commercial fertilizer (a mixture of calcium and phosphorus plus other micronutrients, Table 1) and foliar application with a concentration of 2 mg/L in three replicates. The average 24 h temperature and relative humidity were 15 ℃-27 ℃ and 70%-40%, respectively. Each experimental unit consisted of 18 pots with a total of 54 pots. A tomato transplant from a hybrid variety (cv. 240) was planted in each pot in January 2016. The pots were of plastic type with a diameter of 30 cm and a height of 40 cm. The culture medium was a mixture of 40% peat moss with 40% coco peat and almost 20% perlite. The nutritional solution of Hochmuth was used for nutrition (Table 2). The percentages of nutrients in this nutrient solution was changed according to the growth stages and supplied to the plants through a pump and drip irrigation system. After 20 d of seedling transplantation, the transplants were sprayed with calfomyth once every 14 d.

**Table 1 Calfomyth nutritional ingredients**

| Composition | Percentage of constituent compounds |
|---|---|
| Total nitrogen (N) | 3% w/w equivalent to 4.2% W/V at 20 ℃ Total nitrogen (N) |
| Nitric nitrogen (N-$NO_3$) | 1.7% w/w equivalent to 2.38% W/V at 20 ℃ |
| Ureic nitrogen (N-$NH_2$) | 1.3% w/w equivalent to 1.82% W/V at 20 ℃ |
| Phosphorus pentoxide ($P_2O_5$) soluble in water | 23% w/w equivalent to 32.2% W/V at 20 ℃ |
| Calcium oxide (CaO) soluble in water | 5% w/w equivalent to 7% W/V at 20 ℃ |
| Boron (B) soluble in water | 0.1% w/w equivalent to 0.14% W/V at 20 ℃ |
| Molybdenum (Mo) soluble in water | 0.1% w/w equivalent to 0.14% W/V at 20 ℃ |

**Table 2 Nutritional solution ($10^{-6}$) used at different growth stages of the tomato plant**

| Nutrition | Transplant to first cluster | First cluster to second cluster | Second cluster to third cluster | Third cluster to fifth cluster | Fifth cluster to termination |
|---|---|---|---|---|---|
| N | 70 | 80 | 100 | 120 | 150 |
| P | 50 | 50 | 50 | 50 | 50 |
| K | 120 | 120 | 150 | 150 | 200 |
| Ca | 150 | 150 | 150 | 150 | 150 |
| Mg | 40 | 40 | 40 | 50 | 50 |
| S | 50 | 50 | 50 | 60 | 60 |
| Fe | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 |
| Cu | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Mn | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
| Zn | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| B | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |
| MO | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| EC/dS $m^{-1}$ | 0.7 | 0.9 | 1.3 | 1.5 | 1.8 |

### 2.2 Lighting treatments

Plants were illuminated by light emitting diodes (LEDs) with different percentages of red (R, 661 nm) and blue (B, 449 nm) lights. Three spectral treatments were used in this study, namely 90%R+10%B, 60%R+40%B and control. The photoperiod was 12/12h (day/night), photosynthetic photon flux density (PPFD) was 168±10 $\mu$mol/($m^2$ s). The LED lights were prototypes from General Electric Lighting Solutions (Salid, Karamax, Iran). These consisted of 0.26 m, 0.06 m, 0.05 m linear fixtures, on which were placed an array of 6 LEDs. Irradiance was measured routinely using a quantum sensor (MQ-200; Apogee Instruments, Logan, UT). Photosynthetic photon flux density intensities and light spectra were monitored using a light meter (Sekonic C-7000, Japan). The relative spectra of the light treatments are shown in Figure 1. To apply light treatments at the beginning of seedling transplant in each experimental unit, a series of LED bulbs with blue and red wavelengths were installed at a height of 30 cm above the plant surface (the distance between the bulbs and the plant was adjustable by metal clips at different growth stages). All treatments were equally illuminated from sunset for 4 h (6:00 p.m. to 10:00 p.m.) by a timer.

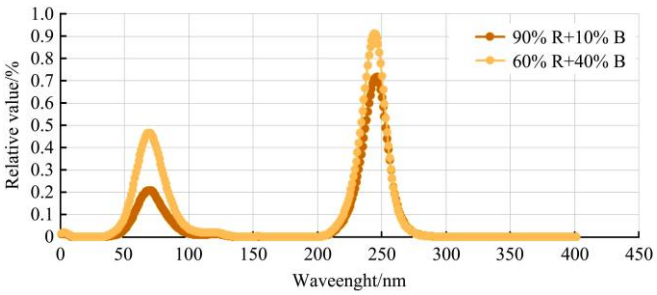


Figure 1 Relative spectral photon flux of the light sources red and blue (RB) utilized

### 2.3 Examined traits

#### 2.3.1 Total leaf chlorophyll content

Dissolve 200 mg of fresh leaf from fully developed leaves and shed in a mortar, then crush it to crush well. 10 mL of 99% methanol was added to the samples, then centrifuged at 6000 r/min for 10 min. The extract of the supernatant was transferred to a glass balloon. The sample was poured into a balloon in a cuvette spectrophotometer and read separately at the wavelengths of 663 nm for chlorophyll a and 653 nm for chlorophyll b and 470 nm for carotenoids by a spectrophotometer. The following levels of chlorophyll a, b, total chlorophyll and carotenoid were obtained in milligrams per gram of fresh sample weight[19].

$$\text{Chl a} = 15.65A_{666} - 7.340A_{653} \quad (1)$$

$$\text{Chl b} = 27.05A_{653} - 11.21A_{666} \quad (2)$$

$$\text{Chl T} = \text{Chl a} + \text{Chl b} \quad (3)$$

#### 2.3.2 Vitamin C content in fresh tomato fruits

Jacob's method was used to determine vitamin C content. In this method, mix 10 mL of tomato juice with 20 mL of distilled water and add 2 mL of 1% starch solution. The solution was poured into the burette and placed under the burette of a small human, then opened the burette valve gently to enter the solution, during which time the potassium iodide drop was added until to see the first color change in the solution inside the human, then close the burette and read the number on the burette and obtain the following vitamin C content of the fruit in mg/L[20].

$$\text{Vitamin C} = \frac{\text{Volume of potassium iodide solution} \times 0.88}{\text{Sample size (10)}} \quad (4)$$

2.3.3 Titratable acidity based on the used volume of caustic soda

10 g sample (tomato pulp) was mixed with 90 mL distilled water and titrated with 0.1 N NaOH and phenolphthalein (as an indicator) to reach pH 8.1. The amount of total acidity was calculated according to the percentage of citric acid using the following equation[20]:

$$\text{Titratable acidity} = \frac{\text{mL consumable NaOH} \times 0.1 \times 0.064}{\text{Sample volume}} \times 100 \quad (5)$$

2.3.4 Percentage of soluble solids (BRIX) by the refractometer

Desktop Refractometer Model MG55320 was used to determine the percentage of soluble solids. By placing a drop of fruit juice on the refractometer, the Brix number was read from the calibrated lens.

2.3.5 Beta-carotene content

To determine the amount of beta-carotene, one gram of dried tomato tissue was milled using a mill machine. Then 16 mL of acetone-hexane solution (hexane to acetone 6: 4 ratio) was added to the milled tomato tissue and centrifuged for 10 min at 5000 r/min. Then, the absorbance of the solution was measured using a spectrophotometer at wavelengths of 663 nm, 645 nm, 505 nm and 453 nm and the amount of beta-carotene was calculated in mg per 100 g using the following equation[21].

$$\text{Beta-carotene} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453} \quad (6)$$

2.3.6 Lycopene content

For lycopene measurement, all operations are similar to the beta-carotene measurement method, but the following formula is used for the calculation[21]:

$$\text{Lycopene} = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453} \quad (7)$$

**2.4 Statistical analysis**

The data were subjected to two-way analysis of variance (ANOVA) and the LSD test was used as a post-test. $p \leq 0.01$ was considered not significant. Charts were drawn using Excel 2013 software.

## 3 Results and discussion

### 3.1 Effects of light spectra and nutrition on Total leaf chlorophyll content

Total leaf chlorophyll content alone (Table 3) was not significantly affected by simple light supplementation and nutrition, but the interaction of these two factors was significant at 5% probability level. The highest chlorophyll content (38.4 mg/g LW) belonged to treatments sprayed by calfomyth. Moreover, there were no significant differences between the various irradiated wavelengths (Figure 2). In other words, by spraying elements such as calcium and phosphorus, we were able to increase the amount of chlorophyll in tomato leaves. As can be seen in Figure 2, the light treatments did not have a significant effect, but still, the light treatment, which had a higher percentage of blue light, showed a higher chlorophyll number, which confirms the positive effect of the blue spectrum on photosynthesis. According to reports, blue light renders chlorophyll biosynthesis and opening of stomata[22] and increases the amount of chlorophyll as well as the rate of photosynthesis up to around 30% depending on the plant type[23]. Yanagi et al.[24] also found out the elevated amount of chlorophyll resulting from the positive effect of blue light on the activity of the cryptochrome system. Similarly, Wu et al.[25] reported that the blue light spectrum raised the chlorophyll content in the pea plant. The results of other studies are consistent with the results of this research. According to the report of Ajdanian et al.[26], the more the percentage of the blue light, the more the amount of chlorophyll was considerably increased based on 1% LSD. The highest amount of chlorophyll a, b and total was observed under 60R:40B treatment with values of 9.4, 5.68, and 15.09 mg/g FW leaf, respectively[26], but no remarkable differences were observed in chlorophyll contents of two lettuce cultivars grown under red plus blue LEDs and white plus red LEDs in some research[37].

**Table 3 Results of analysis of variance for the data**

| Source | *Df* | Total chlorophy ll | Vitamin C | Titratable acidity | Soluble solids content | Lycopene | Beta carotene |
|---|---|---|---|---|---|---|---|
| Block | 6 | $8.82^{ns}$ | $0.004^{ns}$ | $1168.5^{ns}$ | $1.68^{ns}$ | $0.11^{ns}$ | $0.18^{ns}$ |
| Light supplementary | 2 | $4.22^{ns}$ | $0.37^{*}$ | $1758.5^{ns}$ | $49.68^{**}$ | $2.04^{*}$ | $0.06^{ns}$ |
| Nutrition | 1 | $1.55^{ns}$ | $0.11^{*}$ | $15488^{*}$ | $14.22^{*}$ | $2.17^{*}$ | $0.02^{ns}$ |
| Light supplementary × Nutrition | 2 | $65.22^{*}$ | $0.03^{*}$ | $1383.5^{ns}$ | $9.37^{*}$ | $1.83^{*}$ | $0.01^{ns}$ |
| Error | 6 | 8.72 | 0.007 | 988.5 | 0.8 | 0.1 | 0.02 |

Note: *: Significant at $p \leq 0.05$. **: Significant at $p \leq 0.01$. ns: Insignificant.

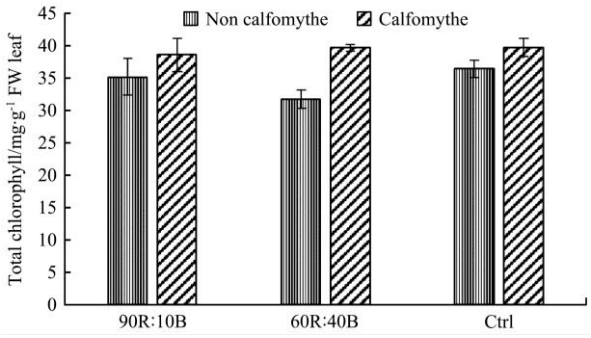


Figure 2 Comparison of supplementary light and nutrition impacts on the total chlorophyll content of the leaves ($p \leq 0.05$)

### 3.2 Effects of light spectra and nutrition on Vitamin C

Vitamin C levels in the tomato fruit (ANOVA, Table 3) showed significant fluctuations at 5% probability level as a result of separate impacts of supplementary light and nutrition. The interaction effect of light supplementation and nutrition significantly influenced the vitamin C levels of tomato fruit at a probability of 5%. According to Figure 3, the amount of vitamin C was uppermost (1.16 mg/g LW) in the treatment sprayed with calfomyth and lightings of 90% R+10% B. The tomato fruits in the control light with no foliar spraying contained the lowest amount (0.49 mg/g LW) of vitamin C. Therefore, increasing the percentage of red light with a small amount of blue light can increase the amount of vitamin C in tomatoes, which proves the effect of red light on vitamin C levels. Ascorbic acid (vitamin C) as the most abundant antioxidant in plants is one of the most important causes of redox (oxidation and reduction) in cells[27]. Previous research demonstrated that vitamin C levels increased by red light due to phytochemicals in the mustard plant[8], which is in line with our observation. Elevations in vitamin C levels with calcium treatment were observed in apple and kiwi fruits[28], tomatoes[15], and strawberries[29]. Calcium treatments with

molecular charge bind to membranes and stabilize them, thereby, preventing free radicals and reactive oxygen species from binding to membranes. Calcium is also effective in protecting the health of biomembranes actually playing the role of antioxidants (e.g. vitamin C) to prevent vitamin C degradation. Calcium also retards the oxidation of ascorbic acid by improving the activity of ascorbate peroxidase enzyme[30]. Concerning the use of phosphorus, Saito and Kano[17] stated that increased levels of phosphorus could also increase vitamin C contents. The results of this research agree with Nirupama et al.[15], who noticed increased titratable acidity as a result of calcium treatment on tomatoes.

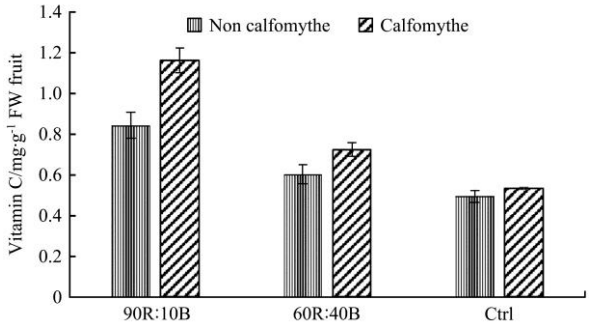


Figure 3 Comparison of supplementary light and nutrition impacts on the amount of vitamin C ($p \leq 0.05$)

### 3.3 Effects of light spectra and nutrition on titratable acidity

The titratable acidity had a significant difference at 5% probability by the use of calfomyth foliar spray, but supplementary light had no effect on this trait (Table 3). The sprayed treatment displayed an increasing trend of 85% compared to control. Different percentages of light failed to increase titration acidity, so their effect was neglected. But elements such as calcium and phosphorus had a significant effect on the solution of calfomyth. The highest levels of acidity in plants sprayed with cauliflower at 130 were shown to be 71.33 in control treatments. Any treatment that retards crop metabolism and ripening can reduce the rate of titratable acidity changes. Since calcium has been shown to delay ripening and to reduce both ethylene production and respiratory rate, it can diminish the rate of acidity changes[30]. Tsantili et al.[31] showed that apple and lemon fruits treated with calcium had lower respiratory rates, so low amounts of organic acids were consumed in the respiratory route, with reduced respiration probably resulting in high titratable acidity.

### 3.4 Effects of light spectra and nutrition on soluble solids (BRIX)

The amount of soluble solids (BRIX) was significantly influenced by the use of supplementary light (at 1% probability) and nutrition with calfomyth solution (at a 5% probability level) (Table 3). The interaction of these two factors was also significant at 5% probability level. BRIX was maximum (12.33%) by using 90% red+10% blue light treatment (Figure 4). Lo et al.[32] detected that red light treatment led to the increased content of sugar in tomato fruits. According to Vassey[33], red light regulates sucrose synthesis through the action of sucrose phosphate synthase (SPS) as one of the key enzymes in the synthesis of sugars, which conforms to the results of the present study. Also, calcium application was reported to raise soluble solids compared to control treatments[16]. The high contents of soluble solids in calcium-treated fruits were attributed to the likely formation of a thin layer on the outer surface of fruit's peel that can reduce respiration rate and prevent the breakdown of carbohydrates giving rise to retained soluble solids in fruits[30]. In addition, the rising level of soluble solids during the growth period could have resulted from an increase in the enzyme invertase, which transforms sucrose[30].

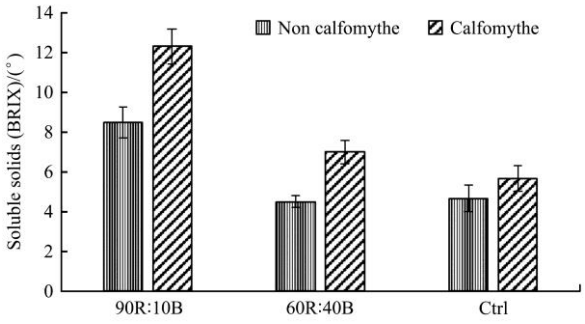


Figure 4 Comparison of supplementary light and nutrition impacts on the amount of soluble solids (BRIX) ($p \leq 0.05$)

### 3.5 Effects of light spectra and nutrition on Lycopene

The use of supplementary lights had significant effects on the amount of lycopene in fresh tomato fruit at a 5% probability level. Also, the use of calfomyth solution and the interaction of these two factors were significant at 5% probability level (Table 3). According to the comparison of mean data (Figure 5), the highest amount (2.95 mg/g LW) of lycopene weight was recorded in treatment with 90% red+10% blue lights, which also received calfomyth spray. Lycopene content was lowermost (0.65 mg/g LW) in the control fruits not sprayed by calfomyth. As highlighted by various studies, the biosynthesis of carotenoids is a complex process regulated by hormones and illumination mechanisms[9]. In this regard, researchers found that the two key enzymes, viz. 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and phytonethenatease (PSY) regulate this pathway. Tomato fruit contains two genes encoding for the PSY protein, namely PSY1 expressed in ripened fruits, and PSY2 expressed in green tissues playing no role in ripening[34]. Phytochromes have been reported to increase the accumulation of carotenoids by controlling the activity of the PSY enzyme mediated by red light[9]. Lois et al.[35] concluded that increased expression of DXS and PSY1 genes at the time of fruit ripening correlated with the amount of lycopene accumulation in tomatoes. Phytochromes were also found to play a major role in the deposit of lycopene in tomato fruit[9]. Levels of lycopene, beta-carotene, lutein and phytoene are almost doubled owing to enhanced expression of PSY. Additionally, exposure of the plant to red light could significantly increase the activity of phytonutrientase (PSY) as a key enzyme in the carotenoid biosynthesis pathway[36]. All of the above findings on the influence of red light on improved levels of lycopene accord with our results. However, red light had no significant effect on the amount of beta-carotene probably because the fruits in this study were all harvested and analyzed at the full ripening stage. The spray of calfomyth solution also amplified the lycopene content in our tomatoes, similar to the use of calcium to raise lycopene levels[17,18]. The color of tomato fruit was also improved along with an elevated amount of lycopene by an increase in the amount of phosphorus[17]. Lycopene and carotenoids are in the form of 40 carbons and are synthesized from acetyl coenzyme A. In a few steps, the substance is converted to a compound of six carbons called malonic acid, which is the precursor to terpenes, and this synthesis is known as the Malone path because of its name. With the addition of phosphorus to the five-carbon molecular structure called isoprene tonil pyrophosphate (IPP), the bond is formed by the bonding of its four molecules, the 20-carbon granyl granyl

diphosphate (GGDP)[17]. Therefore, the presence of phosphorus in the path of lycopene synthesis is an essential element that we increased the amount of phosphorus available to the plant in this experiment along with calfomyth foliar application and therefore increased the amount of lycopene in tomato fruit.

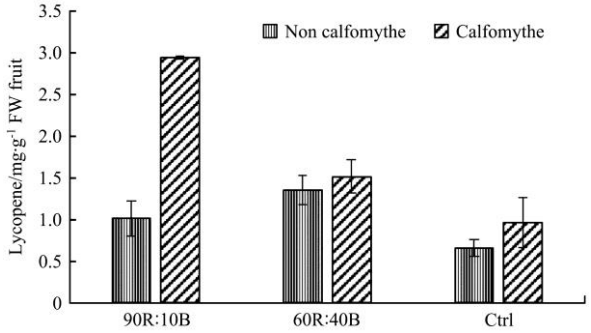


Figure 5 Comparison of the supplementary light and nutrition impacts on the amount of lycopene ($p \leq 0.05$)

### 3.6 Effects of light spectra and nutrition on Beta-Carotene

As shown in Table 3, the use of supplementary light and nutrition had no significant impacts on the beta-carotene content of tomato fruit. One of the most important reasons for the lack of effect of light and nutrition on the beta-carotene content of tomatoes in this experiment is that it is probably related to the fact that the fruits in this study were all harvested and analyzed in full maturity.

## 4 Conclusions

In this study, some biochemical traits that affect the quality of tomato fruit were investigated by LED lamps and nutrition based on phosphorus and calcium elements. The results indicated the positive effects of supplementary LEDs on the traits affecting tomato fruit quality. As expected, nutrient supplementation as foliar spray could positively influence the examined traits. The overall outcomes of this research signify that many traits irradiated by LED light (combined red and blue) and calfomyth foliar application were superior to those at natural conditions. It can, therefore, be recommended that the use of such lights with calfomyth spray as a commercial fertilizer could be feasible for better and ideal production under controlled (greenhouse) conditions.

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