Growth and Quality of Hydroponic Cultivated Spinach (*Spinacia oleracea* L.) Affected by the Light Intensity of Red and Blue LEDs

(Pertumbuhan dan Kualiti Bayam (Spinacia oleracea L.) yang Dipengaruhi oleh Keamatan Cahaya Lampu LED Biru dan Merah

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ABSTRACT

This study aimed to evaluate the effect of four light intensities (90, 140, 190 and 240 µmol $m^{-2} s^{-1}$) provided by red-blue LED light (spectrum ratio: R660/B450 = 4/1) on the growth and quality of hydroponic cultivated spinach. The results showed that when the light intensity increased, plant height, leaf number, root length, leaf width, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight were increased but specific leaf weight and shoot-to-root ratio did not increase. The highest values of growth parameters were observed under 190 µmol $m^{-2} s^{-1}$ treatment, while the lowest values were observed under 90 µmol $m^{-2} s^{-1}$ treatment. At higher light intensities, K^+ , oxalic acid and nitrate contents tended to decrease but not Ca^{2+} content. Meanwhile, the highest values of Fe^{2+} , crude fiber, soluble-solids, total polyphenol and vitamin C contents were observed under 190 µmol $m^{-2} s^{-1}$ treatment showed the lowest organic acid content. Our results indicated that among all experimental lighting treatments, 190 µmol $m^{-2} s^{-1}$ light intensity showed the best effect on the growth and quality of hydroponic cultivated spinach.

Keywords: Growth; LEDs; light intensity; quality; spinach

ABSTRAK

Kajian ini bertujuan untuk menilai kesan empat keamatan cahaya (90, 140, 190 dan 240 μ mol m⁻² s⁻¹) yang disediakan oleh lampu LED merah-biru (nisbah spektrum: R660/B450 = 4/1) terhadap pertumbuhan dan kualiti bayam hidroponik. Hasil kajian menunjukkan bahawa ketika keamatan cahaya meningkat, kepanjangan tanaman, jumlah daun, panjang akar, lebar daun, berat segar pucuk, berat kering pucuk, berat segar akar dan berat kering akar meningkat tetapi berat daun khusus dan nisbah pucuk:akar tidak meningkat. Nilai tertinggi parameter pertumbuhan diperhatikan di bawah perlakuan 190 μ mol m⁻² s⁻¹, sementara nilai terendah diperhatikan di bawah rawatan 90 μ mol m⁻² s⁻¹. Pada keamatan cahaya yang lebih tinggi, kandungan K⁺, asid oksalik dan nitrat cenderung untuk menurun tetapi tidak bagi kandungan Ca²⁺. Sementara itu, nilai tertinggi Fe²⁺, serat kasar, zat terlarut, jumlah polifenol dan vitamin C diperhatikan di bawah rawatan 190 μ mol m⁻² s⁻¹, tetapi rawatan 190 μ mol m⁻² s⁻¹ menunjukkan kandungan sid organik terendah. Hasil kajian kami menunjukkan bahawa antara semua rawatan pencahayaan, keamatan cahaya 190 μ mol m⁻² s⁻¹ menunjukkan kesan terbaik terhadap pertumbuhan dan kualiti bayam hidroponik.

Kata kunci: Bayam; keamatan cahaya; kualiti; LED; pertumbuhan

INTRODUCTION

Controlled environment agriculture is rapidly developing to address food shortages, efficiently use resources, and solve some problems related to the agricultural impacts on the environment (Kozai 2018; Miyagi et al. 2017). The ability to accurately manage physical and chemical parameters such as temperature, humidity, light, and nutrients for greenhouse plants enables year-round vegetable production, as well as creating opportunities to control the productivity and the quality of plants. In plant factories, plants with short growth cycles, relatively small height and high value are often given priority for development (Lu & Shimamura 2018). Therefore, the production of high-quality crops is an indispensable need of modern life (Graamans et al. 2018). Light-emitting diodes (LEDs) are the new fourthgeneration light source (Wang et al. 2017) with good spectral characteristics and spectral width, and can be assembled to match light quality which plants need (Goins et al. 1997). The LED has been proposed as a photosynthetic radiation source for space flight growing systems and as more efficient sources for terrestrial controlled-environment agriculture facilities (Bula et al. 1991). Moreover, LED has the advantages such as low energy consumption, small size, durability, long lifetime, cool emitting temperature and options to select specific wavelengths for target.

Light regulates a variety of plant developmental pathways from germination to flowering induction and fruit development (Jiao et al. 2007). However, about 90% of the light absorbed by leaves are in the blue or red light spectrum (Terashima et al. 2009). Therefore, plant growth is significantly affected by these two light spectra (Chen et al. 2014). Previous studies have shown that the combination of blue and red LED light in the visible light spectrum is effective for photosynthesis and the normal growth of different crops (Bian et al. 2018; Viršilė et al. 2017; Wang et al. 2016). However, the light intensity appropriate for the optimal growth of each crop is an issue that needs to be clarified.

Green vegetables are an important food source for the daily intake of essential nutrients. Some vegetables are also considered as functional foods, or are used as precious herbs to promote health and prevent disease (Ülger et al. 2018). Spinach (*Spinacia oleracea* L.), an important vegetable crop, is widely produced in greenhouses and plant factories due to short-duration production cycles and faster economic return (Lu & Shimamura 2018). Also, this vegetable has high nutritional value because it contains many essential vitamins and minerals, and a rich source of omega 3 vegetable fatty acids (Ko et al. 2014). Therefore, the objective of this study was to investigate the effect of red and blue LEDs intensities on growth and quality of spinach in a hydroponic system.

MATERIALS AND METHODS

PLANT MATERIALS AND GROWTH CONDITION

This experiment was conducted in an indoor system at the Institute of Agrobiology, Vietnam National University of Agriculture. The room temperature and humidity were maintained at 27 ± 0.5 °C and $65\pm5\%$, respectively. Heat-treated F1 seeds variety PD512 of spinach (*Spinacia oleracea* L.) were provided by Phu Dien Trading & Production Co. Ltd, Vietnam. The seeds were sown in the 128-cell plug trays (Bumnong, Jeongeup, Korea) that had been filled with commercial growing substrate (Klasmann TS-2, Germany). Ten days after germination, the same size seedlings were transplanted into plastic in the circulating hydroponic system. The experiment was conducted in three hydroponic system racks with 4 rigs per rack. Each rig has 5 parallel hydroponic solution tubes and 9 plants per tube, corresponding to 45 plants per rig. Every hydroponic system rack was equipped with LEDs lighting at four light intensities of 90, 140, 190, and 240 μ mol m⁻² s⁻¹ (LEDs at the same spectral composition R660/B450 = 4/1).

Light intensity was measured by specialized equipment (UPRtek PG100N Handheld Spectral PAR meter). The LEDs were manufactured and supplied by Rang Dong Light Source & Vacuum Flask Joint Stock Company, Vietnam. The plants were grown under a 12/12 h light/dark photoperiod cycle. The modified Hoagland's nutrient solution was used in the experiment (Hoagland & Arnon 1950). The pH and EC of the nutrient solutions were maintained at 6.0-6.5 and 1,200 μ S cm⁻¹, respectively, by changing the solutions in the hydroponics containers every 7 days.

GROWTH PARAMETERS

Plant height (cm/plant) was measured from the base (close to the surface of the substrate) to the highest leaf level, and leaf number (leaves/plant) was calculated from the first true leaf. These parameters were measured every 7 days (d), and the 7 d growth rate was calculated. Root length (cm) was measured from the base root to the longest root tip at 21 d after planting. Specific leaf fresh weight was calculated as the weight of 1 dm² of leaf at 21 d after planting. Thirty plants per treatment were randomly taken for all parameters and the average value was calculated.

QUALITY PARAMETERS

Dry samples of spinach were used for mineral element determination. The contents of total Ca, K, and Fe were examined according to Sanui (1971) by the method of atomic absorption spectrophotometry at Hanoi National University of Education, Vietnam. The nitrate (NO₃) content was measured using method according to Shinn (1941) at Vietnam Academy of Science and Technology. Soluble solids content was immediately measured using a digital refractometer (model PR-201 α (Brix 0.0-60.0%), ATAGO, Co., Itd. Japan).

Total organic acid, fiber and total polyphenol contents were determined at Vietnam National University of Agriculture. Total organic acid content was determined by the titration method according to Horwitz (1980). Crude fiber content was measured by digestion and gravimetric technique according to Antia et al. (2006) whereas total polyphenols content was determined by colorimetric method using folin-ciocalteu reagent according to Singleton and Rossi (1965).

Oxalic acid and Vitamin C (ascorbic acid) contents were determined at the National Institute for Food Control, Vietnam. Oxalic acid content was determined according to the method reported by Wang et al. (2014) whereas ascorbic acid content was determined according to the method reported by Gahler et al. (2003).

STATISTICAL ANALYSIS

Statistical analyses were conducted with Excel and R softwares. Data were analyzed by analysis of variance (ANOVA) and the differences between means were tested using Duncan's test (P < 0.05).

RESULTS

GROWTH PARAMETERS

In all treatments, plant height and leaf number were increased with increasing light intensity (Figures 1 & 2). The highest value of plant height was observed in 240 µmol m⁻² s⁻¹ treatment but this was similar with the 190 µmol m⁻² s⁻¹ treatment. The lowest value of plant height was observed under 90 µmol m⁻² s⁻¹ treatment. There was a statistically significant difference in plant height under different light intensities on 14 and 21 days after transplanting, but no significant difference was observed in plant height under different light intensities on 7 days after transplanting (Figure 1(A)). However, leaf number growth was slightly different from plant height growth. There was a statistically significant difference in the leaf number under different light intensities on 7, 14, and 21 days after transplanting. The highest value of leaf number was observed in 240 µmol m⁻² s⁻¹ treatment but this was similar to that in 190 μ mol m⁻² s⁻¹ treatment. The lowest value of leaf number was observed in 90 µmol m⁻² s⁻¹ treatment (Figure 1(B)).



FIGURE 1. Effect of different light intensity (LEDs at the same spectral composition R660/ B450 = 4/1) on the growth of plant height (A) and leaf number (B) of hydroponic cultivated spinach. Vertical bars represent ± SD, n = 10. DAT: days after planting





FIGURE 2. Hydroponic cultivated spinach under different light intensity (LEDs at the same spectral composition R660/B450 = 4/1) at different time intervals. I1: 90 μmol m⁻² s⁻¹, I2: 140 μmol m⁻² s⁻¹, I3: 190 μmol m⁻² s⁻¹, I4: 240 μmol m⁻² s⁻¹. DAT: days after planting

PHYSIOLOGICAL PARAMETERS

The highest value of root length was observed in 190 µmol m⁻² s⁻¹ treatment but this was similar to that in 240 µmol m⁻² s⁻¹ treatment. The lowest value of root length was observed in 90 µmol m⁻² s⁻¹ treatment. There was no statistically significant difference in leaf width between 90 and 140 µmol m⁻² s⁻¹, or between 190 and 240 µmol m⁻² s⁻¹ treatments. The highest value of specific leaf weight was observed in 190 µmol m⁻² s⁻¹ treatment but this was similar to those in 140 and 240 μ mol m⁻² s⁻¹ treatments. The lowest value of specific leaf weight was observed in 90 µmol m⁻² s⁻¹ treatment. The fresh and dry weights of shoot and root were increased with increasing light intensity from 90 to 190 µmol m⁻² s⁻¹. However, fresh and dry weights of shoot and root were decreased with increasing light intensity to 240 µmol m⁻² s⁻¹. The shoot-to-root ratio for fresh and dry weight was increased with increasing light intensity from 90 to 140 µmol m⁻² s⁻¹. However, there was no statistically significant difference between 90 and 140 µmol m⁻² s⁻¹, or between 190 and 240 µmol m⁻² s⁻¹ treatments with the shoot-to-root ratio for fresh weight, and there was no statistically significant difference among 140, 190 and 240 μ mol m⁻² s⁻¹ treatments with the shoot-to-root ratio for dry weight (Table 1).

NUTRITION CONTENT AND QUALITY MINERAL ELEMENTS

The concentration of Ca²⁺ tended to increase as the light intensity increased. Ca2+ content was highest under the 240 μ mol m⁻² s⁻¹ treatment, and lowest under the 90 μ mol m⁻² s⁻¹ treatment. Ca²⁺ content under 240 µmol m⁻² s⁻¹ treatment was 50 times higher than under 90 µmol m⁻² s⁻¹ treatment, 7 times higher than under 140 µmol m⁻² s⁻¹ treatment, and 1.6 times higher than under 190 µmol m⁻² s⁻¹ treatment. In contrast to the Ca²⁺ content, the K⁺ content was decreased with increasing light intensity. The highest value of K⁺ content was observed in the 140 µmol m⁻² s⁻¹ treatment, but this was similar to that in 90 µmol m⁻² s⁻¹ treatment. Meanwhile, the lowest value of Fe²⁺ content was observed in 90 µmol m⁻² s⁻¹ treatments but this was similar to those in 140 and 240 µmol m⁻² s⁻¹ treatments. The highest value of Fe²⁺ content was observed in the 190 μ mol m⁻² s⁻¹ treatment (Table 2).

Light intensity (µmol m ⁻² s ⁻¹)	Root length (cm)	Leaf width (cm)	Specific leaf weight (g/dm ²)	Shoot FW (g/plant)	Root FW (g/plant)	Shoot DW (g/plant)	Root DW (g/plant)	Shoot-to- root ratio FW	Shoot-to- root ratio DW
90	51.32°	8.36 ^b	3.67 ^b	16.35 ^d	3.34 ^d	0.92 ^d	0.19 ^d	4.90 ^b	4.84 ^b
140	62.80 ^b	8.67 ^b	4.26ª	21.19°	4.21°	1.20°	0.24°	5.03 ^b	5.06 ^{ab}
190	72.00ª	9.75ª	4.67ª	43.74ª	6.87ª	1.64ª	0.31ª	6.37ª	5.24 ^{ab}
240	65.38 ^b	9.51ª	4.59ª	34.79 ^b	5.56 ^b	1.36 ^b	0.27 ^b	6.26ª	5.07ª

TABLE 1. Physiological parameters of hydroponic cultivated spinach under different light intensity (LEDs at the same spectral composition R660/B450 = 4/1) at 21 DAT

DAT: days after planting, FW: fresh weight, DW: dry weight. Different lower case letters in the same column indicate significant differences among treatments (P ≤ 0.05; n = 3)

TABLE 2. Mineral element content of hydroponic cultivated spinach under different light intensity (LEDs at the same spectral composition R660/B450 = 4/1) at 21 DAT

Light intensity (µmol m ⁻² s ⁻¹)	Ca (mg g-1 DW)	K (mg g ⁻¹ DW)	Fe (mg g ⁻¹ DW)
90	0.53 ^d	113.97ª	0.25 ^b
140	7.06°	118.91ª	0.23 ^b
190	18.37 ^b	100.82 ^b	0.90^{a}
240	28.62ª	86.74°	0.27 ^b

DAT: days after planting, DW: dry weight. Different lower case letters in the same column indicate significant differences among treatments ($P \le 0.05$; n = 3)

CRUDE FIBER CONTENT AND SOLUBLE-SOLIDS CONTENT

The crude fiber and soluble-solids contents were increased with increasing light intensity from 90 to 140 μ mol m⁻² s⁻¹. However, crude fiber and soluble-solids contents were decreased under 190 and 240 μ mol m⁻² s⁻¹. The highest value of the crude fiber and soluble-solids contents was observed in the 190 μ mol m⁻² s⁻¹ treatment, whereas the lowest value was observed in 90 μ mol m⁻² s⁻¹ treatment. However, there was no statistically significant difference in the soluble-solids content between 140 and 190 μ mol m⁻² s⁻¹ treatments, or between 90 and 240 μ mol m⁻² s⁻¹ treatments (Figure 3(A) and 3(E)).

ORGANIC ACID CONTENT

Although the 190 μ mol m⁻² s⁻¹ treatment showed the highest value in the two parameters discussed earlier,

it showed the lowest value of organic acid content. Compared to the three remaining intensity treatments, this lower level was statistically significant, but there was no statistically significant difference between the three remaining treatments (Figure 3(B)).

OXALIC ACID AND NITRATE CONTENTS

In contrast to the crude fiber and soluble-solids contents, oxalic acid and nitrate contents were decreased with increasing light intensity. However, the oxalic acid content was not significantly different under 90 and 140 μ mol m⁻² s⁻¹ treatments, or 190 and 240 μ mol m⁻² s⁻¹ treatments, respectively. The 190 and 240 μ mol m⁻² s⁻¹ treatments also showed no difference in nitrate content, which was statistically different from the 90 and 140 μ mol m⁻² s⁻¹ treatments (Figure 3(C) dan 3(D)).

ANTIOXIDANT

In our results, polyphenol and vitamin C contents were at the highest level under 190 μ mol m⁻² s⁻¹ treatment, followed by 240, 140 and 90 μ mol m⁻² s⁻¹ treatments.

However, the polyphenol content under 140 and 240 μ mol m⁻² s⁻¹ treatments, and the vitamin C content under 90 and 140 μ mol m⁻² s⁻¹ treatments were not significantly different (Figure 4(A) and 4(B)).



FIGURE 3. Effect of different light intensity (LEDs at the same spectral composition R660/B450 = 4/1) on some nutritional parameters of hydroponic cultivated spinach at 21 DAT. Vertical bars represent \pm SD, n = 3. DAT: days after planting, FW: fresh weight Different lowercase letters in the same column indicate significant differences among treatments (P \leq 0.05; n = 3)



FIGURE 4. Effect of different light intensity (LEDs at the same spectral composition R660/B450 = 4/1) on total polyphenol content (A) and ascorbic acid (B) of hydroponic cultivated spinach at 21 DAT. Vertical bars represent \pm SD, n = 3. DAT: days after planting. FW: fresh weight. Different lowercase letters in the same column indicate significant differences among treatments (P \leq 0.05; n = 3)

DISCUSSION

Light intensity is an important factor for plant growth. Low light intensity inhibits growth and productivity of plant by affecting gas exchange (Zavala & Ravetta 2001), whereas excess light intensity has detrimental effects on the photosynthetic apparatus (Lichtenthaler et al. 2007). Plant development and physiology are strongly influenced by red and blue light (McNellis & Deng 1995). The absence of one of red or blue light wavebands creates photosynthetic inefficiencies (Hogewoning et al. 2010). The effect of light quality on plant growth rates can also be influenced by the light intensity (Johkan et al. 2012). However, optimal ratio and intensity of mixed red and blue light differs with plant species, growth periods and the targeted qualities.

In this study, growth parameters such as plant height and leaf number of spinach were increased with increasing light intensity. This result agrees with the previous study by Proietti et al. (2004), who suggested that growing spinach plants at the higher light intensity increased dry and fresh weight and leaf area of the plants. However, other growth parameters such as root length, leaf width, specific leaf weight, fresh and dry weight of shoot and root of spinach only increased with increasing light intensity from 90 to 190 μ mol m⁻² s⁻¹. On the other hand, there was a significant decrease of root length, fresh and dry weight of shoot and root of spinach when the light intensity increased to 240 μ mol m⁻² s⁻¹. This result is similar with result of Furuyama et al. (2014) who reported that total dry weight of red leaf lettuce under the 0.23 blue/red ratio increased with increasing light intensity from 100 to 200 μ mol m⁻² s⁻¹, but total dry weight was decreased with increasing light intensity to 300 μ mol m⁻² s⁻¹.

The demand of plants for the nutrient is related to its biomass production and its absorption capacity is related to its growth rate (Atkinson 1990). Calcium is an essential macronutrient needed for growth and development of plants (Thor 2019). In our study, the Ca²⁺ content tended to increase as the light intensity increased. This result agrees with the result of Grygoray et al. (2015) who also reported that the higher content of Ca²⁺ in cucumber leaves under increased light level. In addition, iron in plants is involved in the synthesis of chlorophyll, and it is essential for the maintenance of chloroplast structure and function (Rout & Sahoo 2015). On the other hand, micronutrients accumulation in plants is a possible relationship between yield and accumulation of nutrients (Rasmusson & Gengenbach 1984). Therefore, this might explain the highest value of Fe^{2+} content observed at 190 µmol m⁻² s⁻¹ treatment.

As light intensities increased, K^+ content in this study was decreased and the lowest at 240 µmol m⁻² s⁻¹ treatment. This result is consistent with the finding of a previous study that showed that low light intensity increases nutrient uptake but markedly weakened the photosynthetic capacity of plants, resulting in a significant decrease in dry matter accumulation, thus, increasing nutrient content (Kazuo & Nobutoshi 1998). In addition, Gerovac et al. (2016) reported that nutrient content was increased under low light intensity regardless of light quality. Furthermore, Zhou et al. (2019) also reported that K⁺ content in lettuce was decreased with increasing light intensities.

Numerous studies have shown that light and nitrogen-containing fertilizers were the two main factors that affected nitrate levels in plants, including the intensity and quality of light (Bian et al. 2015). The nitrate content in our study was decreased with increasing light intensity. This result agrees with previous studies which reported that the greatest amount of nitrate was found in leaves grown at low light intensity, while the least nitrate was found in leaves grown at high light (Dapoigny et al. 2000; Gaudreau et al. 1995; Lillo 1994; Proietti et al. 2013). In addition, it is now known that oxalic acid can be degraded by oxalate oxidase, whose activity is enhanced by high light (Loewus 1999). Therefore, our result showed that the oxalic acid content was decreased with increasing light intensity. On the other hand, many reports have shown that higher light intensity could lead to higher contents of soluble sugar (Gruda 2005; Scaife & Schloemer 1994). Soluble-solids content in our study was increased with increasing light intensity from 90 to 140 µmol m⁻² s⁻¹ but was decreased with increasing light intensity up to 240 µmol m⁻² s⁻¹.

Light quality enhances the amounts of health-related phytochemicals such as vitamin C (ascorbic acid) through the stimulation of secondary metabolites (Dorais et al. 2008; Poiroux-Gonord et al. 2010; Rosales et al. 2011). Moreover, ascorbic acid biosynthesis and accumulation in plants was highly dependent on light quality and intensity (Bian et al. 2015). Many reports have shown that higher light intensity could achieve higher contents of ascorbic acid (Gruda 2005; Proietti et al. 2004; Scaife & Schloemer 1994). In this study, ascorbic acid was increased with increasing light intensity from 90 to 190 μ mol m⁻² s⁻¹. However, ascorbic acid was decreased with increasing light intensity to 240 µmol m⁻² s⁻¹. This result agrees with the previous study by Fu et al. (2017) who suggested that vitamin C concentration was parabolically correlated with light intensity (first increasing, then decreasing), and

was linearly negatively associated with nitrogen supply. Vitamin C concentration was increased with increasing light intensity from 60 to 140 μ mol·m⁻²·s⁻¹ but was reduced with increasing light intensity to 220 μ mol·m⁻²·s⁻¹.

Meanwhile, other secondary metabolites in plants which are phenolic compounds were thought to act as direct or indirect antioxidants by enhancing the efficiency and production of other antioxidant compounds (Kumar & Goel 2019). So far, several studies have focused on evaluating the effect of light quality on the concentration of phenolic compounds in plants, such as phenolic acid and flavonoids on tomato and lettuce plants, respectively (Rajashekar et al. 2009; Son & Oh 2013). However, studies on the impact of light intensity on this group of substances has been quite limited. In our study, total polyphenol content was increased with increasing light intensity from 90 to 190 µmol m⁻² s⁻¹ but it was decreased with increasing light intensity to 240 µmol m⁻² s⁻¹.

The results in our study indicate that the light intensity higher than 190 μ mol m⁻² s⁻¹ did not increase the concentration of antioxidants. However, reasonable additional LEDs could be used to improve the yield and nutritional quality of leafy vegetables grown under artificial lighting. Each different crop has a different optimal light intensity threshold at which the plant grows and accumulates the best nutrients. Among these intensity ranges, light intensity of 190 μ mol·m⁻²·s⁻¹ proved to be the most effective to enhance the productivity and quality of hydroponic spinach.

CONCLUSION

Hydroponic spinach grew better as the light intensity increased, while the amount of substances that were not beneficial to human health, such as K⁺, oxalic acid, and nitrate, tended to decrease. In contrast, Ca²⁺ content increased at higher light intensity. However, Fe²⁺, crude fiber, soluble-solids, total polyphenol and vitamin C content reached their highest values under 190 µmol m⁻² s⁻¹ treatment, but the same treatment gave the lowest organic acid content. In this study, the treatment that was most suitable for indoors hydroponic spinach is a combination of red and blue LEDs (R660/B450 = 4/1) with an intensity of 190 µmol m⁻² s⁻¹.

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