DOI: https://doi.org/10.36719/2707-1146/32/78-85

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PACS number(s): 87.17.Ee; 73.22.-f

LIGHT SPECTRUM EFFECTS ON THE PIGMENT CONTENTS IN LEAVES OF SPROUTS OF WHEAT SEEDS TREATED WITH IRON NANOPARTICLES

Abstract

In the presented article, the effect of iron oxide (Fe_2O_3) nanoparticles on the pigment content in the leaves of wheat seedlings in different light spectra (white, red, and blue) was studied. Wheat seeds were initially kept in a suspension solution of iron oxide nanoparticles 20-40 nm in size for 3 hours and planted in pots.

The process of germination and development of seedlings took place in the dark in a phytatron for growing plants in laboratory conditions. Samples were taken from the leaves of 7-day-old seedlings. The leaves of seedlings obtained from treated and untreated seeds with iron nanoparticles were irradiated with conventional white, red and blue light for 90 minutes and then grown again in the dark for 3 days.

It can be seen from the experimental results that the pigment composition of seeds treated with iron oxide nanoparticles varies depending on the spectral composition of light. It turned out that iron oxide nanoparticles have a better effect on the synthesis of chlorophyll b (Chlb) pigment under red light, while under blue light the synthesis of both chlorophyll a (Chla) and Chlb slows down, in all cases the synthesis of carotenoids is weakened.

Keywords: *spectrum of light, wheat seedlings, chlorophyll a and b, carotenoids**,** nanoparticles*

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Dəmir nanohissəcikləri ilə örtülmüş buğda cücərtilərinin yapraqlarının piqment tərkibinə işıq spektrinin təsiri

Xülasə

Təqdim olunan məqalədə dəmir oksidi (Fe_2O_3) nanohissəciklərinin müxtəlif işıq spektrlərində (ağ, qırmızı və göy) buğda cücərtilərinin yarpaqlarında piqment tərkibinə təsiri tədqiq edilmişdir. Buğda toxumları əvvəlcə 20-40 nm ölçülü dəmir oksid nanohissəciklərinin suspansiyon məhlulunda 3 saat saxlanılıb və qablara əkilib. Bitkilərin laboratoriya şəraitində yetişdirilməsi üçün fitatronda toxumların cücərmə və inkişaf prosesi qaranlıqda baş vermişdir. Nümunələr 7 günlük cücərtilərin yarpaqlarından götürülüb. Dəmir nanohissəcikləri ilə işlənmiş toxumlardan alınan cücərtilərin yarpaqları adi ağ, qırmızı və göy işıqla 90 dəqiqə şüalanmış və sonra 3 gün müddətində yenidən qaranlıqda yetişdirilmişdir. Eksperimental nəticələrdən görünür ki, dəmir oksidi nanohissəcikləri ilə işlənmiş toxumların piqment tərkibi işığın spektral tərkibindən asılı olaraq dəyişir. Məlum olub ki, dəmir oksidi nanohissəcikləri qırmızı işıq altında xlorofil b (Chlb) piqmentinin sintezinə daha yaxşı təsir göstərir, mavi işıqda isə həm xlorofil a (Chla), həm də Chlb sintezi ləngiyir, bütün hallarda karotenoidlərin sintezi zəifləmiş olur.

***Açar sözlər*:** *işıq spektri, buğda cücərtiləri, xlorofil a və b, karotenoidlər, nanohissəciklər*

Introductıon

The idea of the dependence of the efficiency of the photosynthesis process on the spectral composition of light was investigated by Daubeni about 200 years ago. He found that photosynthesis does not occur at the same rate in different ranges of the light spectrum (Daubeny, 1836: 149-175). K.A.Timiryazev (1886) said that the red region of light is better absorbed by chlorophyll. Engelman first noted the presence of a second maximum in the blue-violet region of light when studying the absorption spectra of chlorophyll in his experiments (Engelman, 1883: 1-13). Currently, each range of the spectral composition of light has been studied in detail in the development, productivity, and effectiveness of the photosynthesis process of plants. It has been determined that light with a wavelength of 280-320 nm produces harmful effects on plants; 320-400 nm - plays a regulatory role, albeit in a small amount; 400-500 nm is important for the regulation of photosynthesis and photoperiodic reactions, it is absorbed by crypto chromes and phototropism; light with a wavelength of 500-600 nm has high penetration and is suitable for photosynthesis in densely leafed fields; 600-700 nm light affects photosynthesis, regulation of growth processes, photo morphogenesis, absorption of phytochrome P_{660}; the absorption of phytochrome P_{730} has a clear effect on 700-750 nm, while its share is a few percent of the total intensity; 1200-1600 nm is absorbed by intracellular water and increases the rate of thermal biochemical reactions (Kuleshova, Likhachev, Pavlova, Kuleshov, Nashchekin, Gall, 2018: 1243-1247). Thus, the specific pigments (various types of chlorophyll, carotenoids, crypto and phytochromes) that provide absorption in all ranges of visible light in plants are very well studied. The most important of these pigments are Chla and Chlb, as well as carotenoids. Depending on the type of higher plants, both the function and the amount of Chl a and Chl b pigments are different. Chl a/b ratios typically range from 3.3 to 4.2 in light-loving species under normal nutritional conditions and well adapted to light. But in shade-loving species grown in low light, this ratio can be in the order of 2.2 or even lower. Both forms of chlorophyll are involved in intense absorption of light (Endredi, 1985: 269-282). Although the dependence of the pigmentation process on the leaves of plants on the spectral composition of light has been studied in a number of studies, research in this field is still relevant. In experiments, the synthesis of pigments in the leaves of plants illuminated by light with different spectral composition during growth, maintaining their balance, and thus the dependence of photosynthesis productivity on the spectral composition of light are of important practical importance.

Kuleshova et.al investigated the synthesis of Chla and Chlb photosynthetic pigments in oat (Avena sativa) seedlings depending on the spectral content of light. It was found that the amount of Chla and also Chlb in the seedlings illuminated with red light for 9 days is higher than in other options. It is interesting that the amount of pigments reaches the maximum value in 5 days, but the following days do not change. During the experiment, the ratio of Chla/b remains unchanged, but the ratio of carotenoids to Cla and Chb decreases (Kuleshova, Likhachev, Pavlova, Kuleshov, Nashchekin, Gall, 2018: 1243-1247). The effect of nanoparticles on the pigment composition in plants has been intensively studied recently. These studies are related to the effect of nanomaterials on the photosynthesis process. It has been known that metal-based nanoparticles have a serious effect on the synthesis of pigments, especially chlorophyll and carotenoids. For example, the amount of chlorophyll in the leaves of cilantro (*Coriandrum sativum*) cultivated for 35 days in soil with ZnO added to the soil increased by 50% compared to the control variant (normal soil). The results of these experiments showed that ZnO nanoparticles added to the soil at a dose of <400 mg/kg did improves the pigments of photosynthesis (Reddy Pullagurala, Adisa, Rawat, Kalagara, Hernandez-Viezcas, Peralta-Videa, Gardea-Torresdey, 2018: 35-45). The biological activity of Fe, Cu and Mo nanoparticles was studied in the model plant *Solánum tuberósum*, and it was clear from the results that depending on the physico-chemical properties of the nanoparticles, the growth and development of the plant can be both weakened and enhanced. The effect of copper and molybdenum nanoparticles on *Solanum tuberósum* L. plants is not obvious, but iron nanoparticles at a concentration of 0.025 M have a stimulating effect on the length of shoots, roots and chlorophyll content. Fe nanoparticles at a dose of 0.0125-0.1 M reduced the content of Chla and Chlb in seedlings by 57- It has increased by more than 98% (Mushinskiy, Aminova, 2019: 12-19).

In another experiment, a very different change in photosynthetic pigment composition was observed in *Corianderum sativum L.* depending on the concentration of nickel nanoparticles. Chlorophyll a increased at 20 and 80 ppm concentrations, and decreased at 40 ppm concentration. In contrast, the content of chlorophyll b decreased at 20 and 80 ppm concentrations. The level of carotenoids was almost the same as the control group, but decreased at 40 ppm but increased at 80 ppm (Daglioglu, Acikgoz, Ozcan, Metin Kara, 2022: 22-23). When studying the effect of Al_2O_3 nanoparticles on callus development and chlorophyll content of *Ocimum basilicum* plant, it was determined that leaves, percentage of callus formation of stem segment and callus weight are more favorable than leaves as an explant source. The highest Chl-a was detected as a result of the application of Al_2O_3 nanoparticles at a dose of 75 mg/l. The lowest Chl-a was detected at a concentration of 25 mg/l (Daglioglu, Acikgoz, Ozcan, Metin Kara, 2022: 22-33). It was observed in canola plant that the amount of chlorophyll a pigment increased when applying low concentrations of Co_3O_4 nanoparticles (50 and 100 mg L^{-1}), but decreased at high concentrations. The same result was obtained for the pigment chlorophyll b, its amount was minimal at concentrations of 4000 mg L^{-1} of Co_3O_4 nanoparticles (Malihe, 2019: 29-42).

The analysis of scientific literature in this field shows that depending on the type, dose, exposure time and type of plants, nanoparticles have both positive and negative effects on the amount of photosynthetic pigments in white light. Experiments on the effect of nanoparticles on the synthesis of pigments depending on the spectral composition of light are very few and such experiments are needed.

Materials and methods.

2.1 Material. The object of the study was seedlings and plants of the soft wheat variety (*Triticum aestivum L*) Mirbashir-128. The Mirbashir-128 wheat variety was obtained from the intraspecific hybridization of the Bezostaya-1 variety with the "S-271" variety of Pakistani origin of the Azerbaijan Scientific Research Institute of Agriculture. The variety is resistant to dormancy and has a height of 95-100 cm. The variety has medium maturity and ripens at the same time as the Bezosta-1 variety. It is resistant to wilting. The bush is compact, the sprouts and the plant are dark green. The spike is of medium length and medium density. Spike scales are semicircular, the spike lets are short, weakly spreading and rough.

The average productivity of the variety is 63.4 s/ha under irrigation. Potential productivity is 60-70 s/ha. The grain is large and the weight of 1000 grains is 37-47 g. The amount of protein in the grain is 14.7-15.3%, the amount of gluten is 28-30%. It is weakly and moderately susceptible to fungal diseases. Drought resistance is average. The optimal sowing period in the boiling conditions is from September 20 to October 10, and in the irrigation conditions from October 20 to November

10. 80-100 kg of phosphorus, 40-60 kg of potassium, 80-100 kg of nitrogen fertilizers are applied per hectare.

2.2. Nanoparticles. The nanoparticles Fe_2O_3 in powder form were purchased from Sky Spring Nanomaterials, Inc. (USA). The characteristics of the particles were as follows. Average particle size: 18 nm, purity: 99.9% and surface area > 80 (m^2/g) as reported by the commercial agent. Before planting, wheat seeds were kept in distilled water for 3 hours in the control variant, and in the suspension solution of iron nanoparticles in the test variant. For this purpose, 1 mg of nanoparticles was added to 50 ml of distilled water and processed in ultrasonicator for 10 minutes.

Experiments. Swollen wheat seeds were planted in soil in vegetation pots, watered every 3 days and germinated in dark conditions. The experiments were carried out on 7-day-old seedlings. On the 8th day of the experiment, the amount of chlorophyll a and b, carotenoids was determined by taking a leaf sample to determine the pigment content of each variant. After taking the sample, the seedlings were illuminated in normal white light, blue and red light for 90 minutes. Then the vegetation pots were kept in the dark again. Blue light was obtained with C3C-22 and red light with KC-13 glass filters. The emission spectra of these filters are given in figure 1.

Each variant of plants grown in the dark was illuminated with white, red and blue light for 3 days, once every 90 minutes, and leaf samples were taken to determine the pigment content. After lighting, the amount of chlorophyll a and b, carotenoids was determined by taking leaf samples from each variant. The amount of chlorophylls and carotenoids was determined by a standard spectrometric method. For this, 0.1 g of leaves are crushed in a mortar and 10 ml of 95% acetone is added and mixed. After the extract was centrifuged at 5000 cycles for 5 minutes, the amount of pigments was measured at wavelengths of 440, 645, 663 nm using SPECORD 250 plus spectrophotometer.

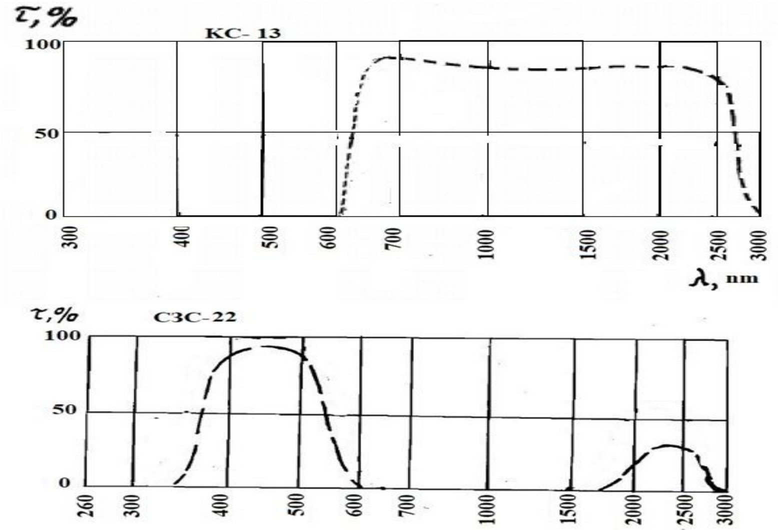

Figure 1. The emission spectra of glass filters

The amount of pigments was determined according to the following formulas:

Chl a (mg/g-fresh weight) = 9,784xA_{663} – 0,990xA_{645}

Chl b (mg/g) = 21,426xA_{645} – 4,650xA_{663}

Chl a +Chl b (mg/g) = 5,134xA_{663} + 20,436xA_{645}

Carotenoids (mg/g) = 4,695·A_{440} – 0,268·(a + b)

A = absorbency at corresponding wave length, values 9.784, 0.990, 21.426, 4.650 and 0.288 is the molar absorptivity coefficient according to Holm (1954) and Wetsttein (1957) for acetone (absorption of 1 cm). After calculating the concentrations, the amounts of pigment per g of fresh matter were calculated applying the formula:

$$C = \frac{\text{c1Vr}}{m}$$

C = content of pigment (mg/g) of fresh matter; C1 = the concentration of pigment calculated by the previous formula (mg/l); v = the starting volume of extract (ml); r = dilution; m = the weighed fresh plant (g). Figure 2 shows the results of experiments showing the effect of Fe_2O_3 nanoparticles on the synthesis of Chl a pigment in wheat sprouts. As we mentioned, wheat seeds were inflated in dispersion solution of iron nanoparticles and planted in vegetation pots. Plants germinated in the dark were kept in the dark again after being illuminated with white, blue and red light for 90 minutes on the 8th day. After one, two and three days, the seedlings were illuminated again and the amount of chlorophyll a was determined. It was clear from the results that the effect of iron nanoparticles in seedlings irradiated with white light is not different compared to the control. In blue and red light, the amount of chlorophyll a is slightly less than in the control.

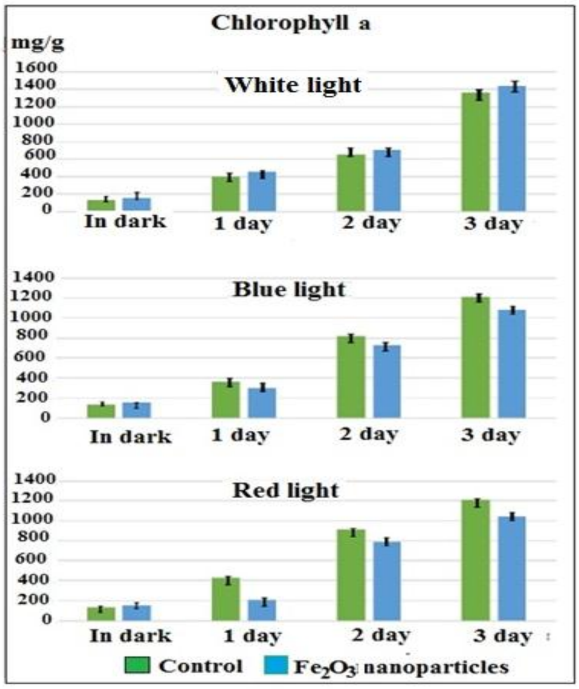

Figure 2. Effect of iron nanoparticles on the synthesis of chlorophyll a pigment in different light spectra

Figure 3 shows the results of experiments showing the effect of Fe_2O_3 nanoparticles on the synthesis of Chl b pigment in wheat sprouts. In the experiments, the amount of chlorophyll b was determined with the same procedure. The amount of chlorophyll b was also determined after one, two and three days after the seedlings were re-illuminated. It was clear from the results that iron nanoparticles significantly accelerated the synthesis of chlorophyll b in seedlings kept in the dark. This increase was also observed in seedlings irradiated with white light. However, iron nanoparticles reduced the synthesis of chlorophyll b in seedlings exposed to blue and red light. This reduction was greater at red light. Figure 4 shows the effect of Fe2O3 nanoparticles on the amount of Chl (a+b) in wheat sprouts. As a result of the effect of iron nanoparticle, depending on the spectral composition of the light, there is a trend of increase and decrease. Figure 5 shows the effects of Fe2O3 nanoparticles on the amount of carotenoids in wheat sprouts. It was clear from the results of the experiments that iron nanoparticles reduce the amount of carotenoids in sprouts illuminated with white light and blue, red light. The decrease is mostly observed in red light.

Discussion. The discovery of photosynthesis in the late 18th century was an important event in plant physiology. From the very first days of the discovery of photosynthesis, scientists began to be interested in the question of how this process depends on the spectral composition of light. Detailed and molecular-level studies have shown that the photosynthetic effect of light coincides with the light absorption spectrum of the chlorophyll molecule. This discovery proved that chlorophyll is the key molecule in the photosynthesis process. Different types of chlorophyll are now known to science, but the most important for higher plants are Chl a and b.

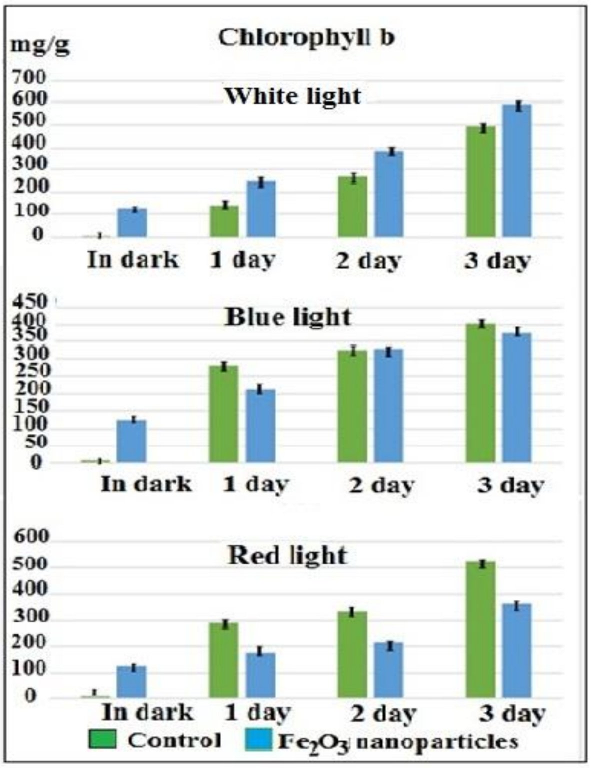

Figure 3. Effect of iron nanoparticles on the synthesis of chlorophyll b pigment in different light spectra

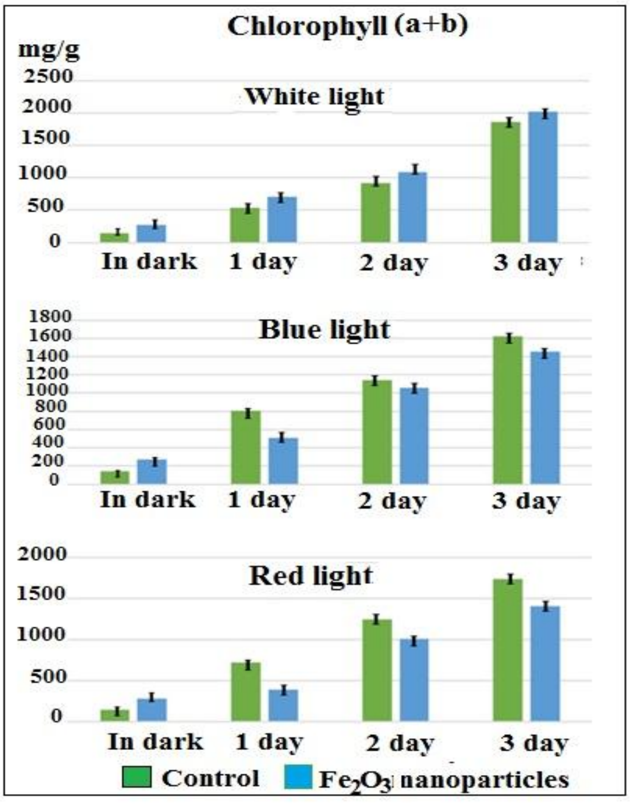

Figure 4. Effect of iron nanoparticles on the synthesis of chlorophyll (a+b) pigments in different light spectra

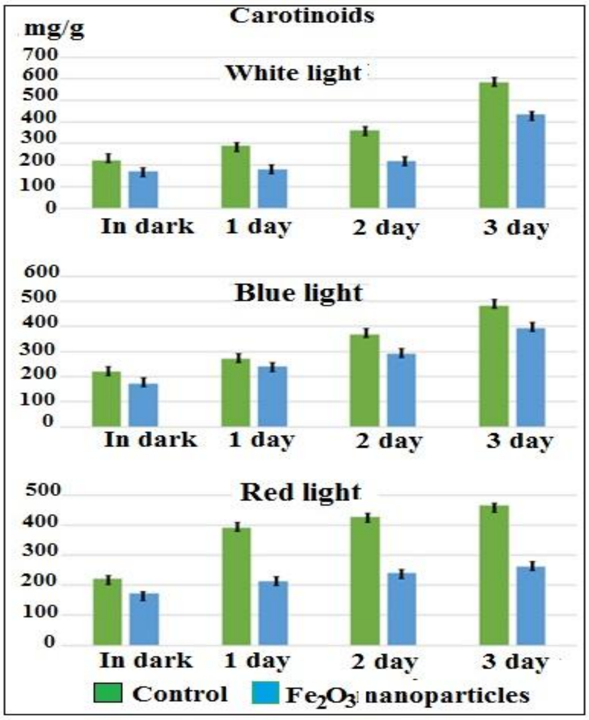

Figure 5. Effect of iron nanoparticles on the synthesis of carotinoids in different light spectra

Chla, as the main pigment of photosynthesis, absorbs light and emits high-energy electrons to the P680 and P700 reaction centers of the photosynthesis system. Chlb, in the role of auxiliary pigment, transfers the absorbed energy to Chla pigment. Chlorophyll content, significantly increased in red light relative to the control. The opposite trend was observed in blue light in the early phase of leaf senescence. At later stages, physiological indexes were gradually higher than that of control, resulting in a delay in leaf senescence. Compared to the control, red and blue light both significantly increased the chlorophyll a/b ratio (Wang, 2016: 82-90). It was clear from the results of the experiments conducted with the lettuce plant that the combined effect of red and blue light is more effective than the monochromatic effect of these rays. Thus, the amount of chlorophyll pigment and the rate of photosynthesis are the highest in lettuce leaves grown in combined blue and red light compared to monochromatic light (Wang, 2016: 250-260). Naznin showed that the amount of chlorophyll and carotenoids decreased in lettuce grown under red light only (Naznin, 2019: 93-103).

The analysis of scientific literature in this field shows that depending on the type, dose, exposure time and type of plants, nanoparticles have both positive and negative effects on the amount of photosynthetic pigments in white light. Experiments on the effect of nanoparticles on the synthesis of pigments depending on the spectral composition of light are very few and such experiments are needed. Substantial studies have shown that nanoparticles are less toxic than their bulk counterparts. Therefore, it was observed that, for example, in plants with low concentrations of ZnO nanoparticles, higher photosynthetic pigments were formed (Jahani, Khavari-nejad, Mahmoodzadeh, Saadatmand, 2019: 29-42). Stimulation of photosynthetic pigments was also observed in experiments with copper and molybdenum nanoparticles (Miri, 2017: 1297-1303). In our experiments, it was observed that the amount of chlorophyll and carotenoids changes depending on the spectral composition of light in wheat plants whose seeds were stored in iron nanoparticles.

Conclusion

Depending on the spectral composition of light, red and blue light, it was observed that iron nanoparticle did not significantly affect the synthesis of Chla in white light, but it was relatively delayed in red and blue light. It is interesting that Chlb increases in white light due to the effect of iron nanoparticle, decreases in red light, and does not change significantly in blue light. And carotenoids are reduced in all cases due to the effect of iron nanoparticle.

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Received: 30.01.2023 Accepted: 29.04.2023