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Article

The Influence of the Spectral Composition and Light Intensity on the Morphological and Biochemical Parameters of Spinach (*Spinacia oleracea* L.) in Vertical Farming

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Abstract: The present study has been carried out to determine the effects of four different illuminators with red, far-red, blue, and white light-emitting diodes (LEDs) on the growth, morphology, pigment composition, and chlorophyll fluorescence of spinach (*Spinacia oleracea* L.) of the 'Zhirnolistny' cultivar. We investigated these variants in two photon flux densities, 400–800 nm (PFD) 120 μmol m^{-2} s^{-1} and 180 μmol m^{-2} s^{-1}. The studies were carried out in a climate chamber. Plant measurements were carried out on the 30th and 45th days of cultivation. The results showed that during the period of active growth, on the 30th day, spinach plants accumulated 2.6 and 2.4 times more fresh weight in the variant with a higher PFD (180 μmol m^{-2} s^{-1}). At the end of the growing season, only a decrease in PFD had an effect on the fresh and dry weight of plants. The highest concentration of chlorophyll on both the 30th and 45th days of vegetation was found when spinach plants were grown under red-blue (RB) LEDs in a spectrum proportion of R70:B30. It was found that the variants had a higher proportion of green radiation in the spectrum of illuminators with PFD 180 μmol m^{-2} s^{-1}, and the nitrate content in spinach was slightly lower than in other variants.

Keywords: spinach; vertical farming; light-emitting diode; spectral composition of light; productivity; photosynthesis

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1. Introduction

Spinach (*Spinacia oleracea* L.) is a popular leaf vegetable of the amaranth family (*Amaranthaceae*). It is one of the ten healthiest foods, as it contains large amounts of vitamins, minerals, and antioxidants and has anti-inflammatory, anticancer, and hypoglycemic and hypolipidemic properties [1–4]. Spinach contains plenty of vitamin K, which helps support bone health. The widespread use of spinach in dietetics is due to its low-calorie content. Despite these valuable qualities, spinach consumption is still low compared to other leafy vegetables [1]. The peculiarity of spinach cultivation is the difference in temperatures required for germination and further growth. In addition, spinach leads the list of leafy vegetables in terms of the frequency of overload with pesticides and nitrates [5,6]. Another problem is that spinach is a long-day plant; therefore, in daylight hours of 14–16 h, it develops a flowering stem, and in short daylight hours, rosettes form, and the active growth of biomass occurs. The influence of these factors usually determines the seasonal nature of spinach cultivation.

One solution to the problem of the year-round cultivation of spinach is to cultivate plants in controlled conditions of climate chambers and city farms. When growing spinach in vertical farms, it is necessary to take into account the fact that spinach is a nitrate accumulator plant, along with green crops, such as arugula and lettuce. The value of the nitrate content in spinach is inversely proportional to light intensity [7], so photosynthetic photon flux density (PPFD) plays a key role when cultivating spinach.

The costs of irradiation systems may account for a significant part of growing plants in climate chambers and city farms. A comparison of fluorescent red (R)/blue (B) = 9/5 and LED lighting (LED) R/B = 9/10, 6/5 and 9/4 with different levels of DLIs (daily light integrals) (11.5; 14.4, 17.3 and 20.2 mol m^{-2} day^{-1}) in hydroponic spinach cultivation was found that plant fresh and dry weight; photosynthesis level and energy efficiency (1.73 kWh per 100 g) were higher at DLI = 17.3 mol m^{-2} day^{-1} under LED illumination R/B = 6/5. Energy consumption under LED lighting was 38% less than under fluorescent lamps [8]. In assessing the impact the effect of red-blue (RB) LED lighting (R:B = 4:1) of different PPFD levels (90, 140, 190, and 240 µmol m^{-2} s^{-1}) on the growth and quality of hydroponically grown spinach, it was found that with increasing light intensity, plant height, the number of leaves, the length of the root system, leaf width, the weight of fresh and dry shoots, and the weight of fresh and dry roots increased, but the specific weight of the leaf and the ratio of shoots to roots did not increase. Optimal growth parameters were observed in the variant with 190 µmol m^{-2} s^{-1}. A decrease in potassium and oxalic acid was observed at higher light intensities [9]. When the effects of B, R, RB, and white (W) spectrum fluorescent lamps were studied at a PPFD of 300 µmol m^{-2} s^{-1}, it was found that blue spectrum irradiation was not suitable for growing spinach because of a sharp decline in shoot dry weight, although carotenoid content slightly increased, and R irradiation reduced nitrate content. The authors recommend combining lighting, using W-lamps for growth, and using additional lighting with B-lamps a few hours before harvesting [10]. According to the literature data, when choosing lighting for additional illumination of spinach, it has been found that the photoperiod is the most important factor, light intensity is the second most important, and light quality is the least important [11]. It was found that when the photosynthetic photon flux density is 100 µmol m^{-2} s^{-1} and the photoperiod is 13/11 h, the best light regime for obtaining plants with a larger leaf surface area was a lower light intensity with a longer photoperiod and a spectral range ratio of R:B = 4:1. To improve the quality and taste of spinach, it is necessary to increase the PPFD level and reduce the photoperiod (PPFD = 150 µmol m^{-2} s^{-1} and 9/15 h) with R:B = 4:1 spectral ranges [11].

In 2020, in the phytotron of the laboratory of light culture and vertical farming of the Federal State Budgetary Educational Institution of Higher Education of St. Petersburg State Agrarian University, experiments were carried out to investigate the productivity of five varieties of spinach for cultivation in total control environment [12]. Spectral composition R/B = 5/1 with a day/night ratio = 12/12 h was considered with irradiation intensity options of 80, 110, 140, and 170 µmol m^{-2} s^{-1}. As a result of the experiment, the variety specificity of spinach plant reactions to irradiation intensity was established. The varieties 'Matador', 'Zhirnolistny', and 'Spokane' had a higher yield at 170 µmol m^{-2} s^{-1}, the varieties 'Krepysh' and 'Rembrandt' had yields at 140 µmol m^{-2} s^{-1}. The 'Zhirnolistny' variety is more valuable for cultivation in vertical farming in PPFD 170 µmol m^{-2} s^{-1} since the production of a unit of its biomass requires the least amount of energy—16.56 kW/kg with 0.04 kg/m^2 yield [12]. Avinash Agarwal et al. studied the effect of different ratios of R and B light in a spectrum of illuminators (100R:0B, 75R:25B, 50R:50B, 25R:75B, and 0R:100B) on the vegetation of spinach plants [13]. It was found that 25R:75B to 75R:25B light treatments reduced non-photochemical quenching (NPQ) and oxidative stress, while the suppression of photosynthetic activity under B-band deficiency resulted in growth arrest due to impaired C- and N-assimilation [13]. It was also found that the rate of photosynthesis tends to increase with an increasing PFD of blue light up to 100 µmol m^{-2} s^{-1}, which is associated with an increase in the nitrogen content in leaves and was not necessarily the result of an increase in dry weight per unit leaf area [14]. The treatment of light with a PFD of 120 µmol m^{-2} s^{-1} with an increase the B-component of the spectrum shows a slight increase in NPQ and, consequently, in oxidative stress, which is consistent with studies by other authors conducted on quasi-bichromatic illuminators [13]; at a PFD of 180 µmol m^{-2} s^{-1}, the increase in plant NPQ under RB illumination is less pronounced.

Most of the published research on spinach cultivation under controlled conditions focuses on assessing the impact of light intensity levels on plant growth and development [11–14]. Therefore, of particular interest is the assessment of the influence of the spectral composition of light for different levels of light intensity on the growth and development of spinach plants, as well as on the quality of the obtained product. The purpose of our study is to assess the influence of the spectral composition and intensity of lighting on the morphological and biochemical parameters of spinach under full light culture and to select optimal lighting parameters for spinach cultivation in the conditions of vertical farms.

2. Materials and Methods

2.1. Characteristics of the Spinach Variety

To grow hydroponic spinach in full light culture, we used the mid-season (28–30 days before technical ripeness) variety of spinach 'Zhirnolistny', which has medium-sized compact rosettes, which are up to 28 cm in diameter. The fresh weight of one plant reaches 20–28 g. This variety is the most attractive from an economic point of view.

2.2. Cultivation Conditions

The studies were carried out in a climate chamber developed by the Federal Scientific Research Center VIM (Moscow, Russia). Plants were grown in a hydroponic flooding setup. Mineral wool was used as a substrate. The seeds were germinated in a thermostat at a temperature of 10–12 °C and a relative humidity of 70–80%, according to the recommendations [15]. Next, one sprout at a time was planted in pots with mineral wool, which were placed on racks with hydroponics using Ebb & Flow technology. To grow spinach, eight light-insulated racks were used according to the experimental variants. The area of each rack was 0.68 m^2, and the planting density was 44 plants per 1 m^2. A microclimate was maintained in the chamber with a day/night air temperature of 18/16 °C and a relative air humidity of 75%. To prepare nutrient solutions, a complex of fertilizers for hydroponics Flora Series® (GHE, Paris, France) was used. The chemical composition of the nutrient solution was N-NO_3 15.004 mM; N-NH_4 3.0 mM; P-PO_4 2.50 mM; K 11.00 mM; Ca 4.50 mM; Mg 3.00 mM; S-SO_4 6.00 mM; Fe 40.00 µM; B 30.00 µM; Cu 1.00 µM; Zn 5.00 µM; Mn 10.00 µM; and Mo 1.00 µM. The electrical conductivity of the nutrient solution was maintained within the range of 1600–1800 $\mu S \cdot cm^{-1}$.

After reviewing the literature, some sources suggest that the optimal PFD for growing spinach is 170–190 µmol m^{-2} s^{-1}, while others claim that lower PFD levels = 100–150 µmol m^{-2} s^{-1} are more favorable in terms of growth and product safety [7,11]. To find the optimal lighting mode, we selected two PFD levels, 120 and 180 µmol m^{-2} s^{-1}, and four illumination spectra were provided by the combined LEDs of different spectral compositions (Table 1).

Table 1. Spectral characteristics of lighting options.

Experimental Variant	Photon Flux, µmol Photons m^{-2} s^{-1} of Spectral Ranges (nm)				
	Total PFD (400–800) (µmol m^{-2} s^{-1})	Blue (400–500) (µmol m^{-2} s^{-1})	Green (500–600) (µmol m^{-2} s^{-1})	Red (600–700) (µmol m^{-2} s^{-1})	Far-red (700–800) (µmol m^{-2} s^{-1})
Control	120	18.0	38.4	50.4	13.2
	180	27.0	57.6	75.6	19.8
V1	120	14.4	24.0	75.6	6.0
	180	21.6	36.0	113.4	9.0
V2	120	18.0	36.0	58.8	7.2
	180	27.0	54.0	88.2	10.8
V3	120	36.0	1.2	81.6	1.2
	180	54.0	1.8	122.4	1.8

Full-spectrum luminaires based on Refond RF-W40QI35DS-DF-J-Y LEDs (Shenzhen Refond Optoelectronics Co., Ltd., Shenzhen, China) were chosen as a control lighting option since their emission spectrum is as close as possible to the spectrum of sunlight. In other lighting options, lamps produced by FSBSI FSAC VIM were used; they consisted of combinations of the latest generation LED, TM LUXEON 2835 (Lumileds Holding B.V. (San Jose, CA, USA), for crop production, which included blue (445 nm), red (630 nm and 660 nm), far-red (730 nm and 660 nm), and white LEDs. We used similar spectra earlier in work devoted to the cultivation of leaf mustard, a long-day green crop [16]. The light period day/night was 12/12 h [12,15]. PFD and spectral irradiance measurements were performed using an MK350D Compact Spectrometer (UPRtek Corp., Miaoli County, Taiwan).

2.3. Determination of Biometric and Biochemical Indicators

To determine biometric parameters, 10 spinach plants of each variant were selected on the 30th and 45th days of cultivation. The following indicators were measured: fresh and dry plant weight, number of leaves, leaf surface area, and plant height. To obtain dry weight, the plants were crushed and dried in an oven at 100 °C until constant mass was obtained. The fresh and dry weight of plants was weighed on a Sartorius LA230S scale (Laboratory Scale, Jena, Germany). Leaf surface area was determined using a photoplanimeter LI-COR—LI-3100 AREA METER (LI-COR, Inc. Lincoln, NE, USA).

Sampling to determine the content of pigments was carried out from the leaves of the 3rd tier with the same uniformity of illumination in triplicate. A sample of fresh leaves weighing 0.1 g was crushed, and then pigments were extracted using 100% acetone. The optical density of the resulting solution was determined at 662 nm (chlorophyll *a*), 644 nm (chlorophyll *b*), and 440.5 nm (carotenoids) on a SPEX SSP-705 spectrophotometer (Spectroscopic Systems, Moscow, Russia). The pigment content was calculated using the Holm–Wettstein formula [17,18].

Determination of nitrates in the spinach plant was carried out from samples taken on the 45th day of cultivation to assess product safety before harvesting. Three plants were selected from each experimental variant, and the weight of each sample was 10 g. The selected samples were crushed, and the resulting sample was placed in a measuring glass and extracted with a 1% solution of potassium alum [19] and then stirred for 3 min on a magnetic stirrer. In the resulting suspension, the concentration of nitrate ions was measured using a pH meter—an Ethan ion meter (Tomanalit, Tomsk, Russia).

2.4. Fluorescence Measurement

To measure the activity of the light stage of photosynthesis on the 45th day of cultivation, a portable fluorometer, PAR-FluorPen FP 110-LM/D (Photon Systems Instruments, Drásov, Czech Republic), was used, which was designed to record active chlorophyll fluorescence and its further analysis using the PAM method or OJIP test. PAR-FluorPen FP 110-LM/D includes a detector (PIN photodiode with bandpass filters, operating optical range from 667 to 750 nm) and a blue LED emitter (a maximum of about 470 nm), an external light sensor [20].

The following parameters of chlorophyll fluorescence were determined: the rate of linear electron transport (ETR) and the non-photochemical quenching coefficient (NPQ). Active fluorescence was measured on leaves of the 3rd tier in triplicate. To measure NPQ, the leaf was dark-adapted for at least 30 min [21].

2.5. Data Analysis

All experiments were carried out threefold. All of the data were subjected to a two-way analysis of variance (ANOVA) using RStudio 1.4.1717 software. The significant differences ($p < 0.05$) in each parameter among the treatments are indicated by different letters.

3. Results and Discussion

3.1. Morphological Parameters of Spinach Plants

On the 30th day of cultivation of spinach plants, it was found that in all variants, except for the control one, the plants were more developed under lighting with an intensity of 180 μmol m^{-2} s^{-1}; the best lighting options in terms of spectral composition were white with a predominance of the red component of the spectrum (V1) and red-blue (V3). The fresh weight of these plants was 2.6 and 2.4 times higher, respectively, and they were distinguished by a greater number of leaves and a 1.9 times greater leaf surface area. The results of the obtained measurements are shown in Table 2.

Table 2. Biometric indicators of spinach plants of the 'Zhirnolistny' variety. Values represent mean SEM (n = 10). Letters indicate significant differences between experimental variants compared to the control group of plants ($p < 0.05$) according to a two-way ANOVA.

Experimental Variant	PFD (μmol m^{-2} s^{-1})	Fresh Weight (g)	Dry Weight (%)	Plant Height (cm)	Number of Leaves (pcs.)	Leaf Surface Area (cm^2)
30th day of cultivation						
Control	120	5.2 ± 1.0 c	7.7 ± 1.7 b	9.6 ± 1.9 b	6.6 ± 0.5 b	93.1 ± 17.8 c
	180	4.2 ± 1.3 c	10.6 ± 3.5 a	13.3 ± 2.5 a	6.1 ± 0.8 b	96.2 ± 12.7 c
V1	120	8.0 ± 1.4 b	6.4 ± 1.0 b	9.9 ± 1.2 b	7.9 ± 1.0 a	140.2 ± 17.7 b
	180	11.1 ± 1.9 a	7.3 ± 1.0 b	15.5 ± 1.3 a	7.4 ± 0.7 a	179.3 ± 25.8 a
V2	120	6.5 ± 0.9 bc	7.0 ± 0.5 b	8.6 ± 1.2 b	6.7 ± 0.7 b	111.2 ± 13.2 bc
	180	7.7 ± 1.2 b	8.0 ± 0.6 ab	14.0 ± 1.6 a	7.0 ± 0.8 b	127.6 ± 19.9 b
V3	120	5.4 ± 0.5 c	7.8 ± 0.5 b	9.3 ± 0.9 b	7.4 ± 0.5 a	103.7 ± 7.9 c
	180	10.0 ± 2.0 ab	7.9 ± 0.4 b	12.9 ± 1.7 a	7.9 ± 0.6 a	179.6 ± 31.1 a
45th day of cultivation						
Control	120	19.7 ± 6.4 c	7.5 ± 1.0 ab	20.0 ± 1.9	9.6 ± 0.9 b	328.9 ± 79.8
	180	29.0 ± 6.0 a	7.8 ± 0.8 a	21.5 ± 2.6	10.6 ± 1.0 ab	410.1 ± 70.3
V1	120	31.71 ± 6.0 a	6.3 ± 0.3 b	20.0 ± 3.7	11.1 ± 1.7 ab	524.1 ± 112.2
	180	32.9 ± 7.6 a	7.4 ± 0.6 ab	19.2 ± 3.7	12.3 ± 1.9 a	460.5 ± 101.4
V2	120	22.2 ± 5.3 c	7.2 ± 0.6 ab	21.4 ± 3.1	10.1 ± 1.4 ab	422.8 ± 110.9
	180	29.0 ± 10.8 b	7.5 ± 0.9 ab	19.8 ± 2.7	11.9 ± 2.5 a	421.8 ± 122.0
V3	120	22.5 ± 3.3 c	6.6 ± 0.3 b	19.0 ± 1.6	10.7 ± 1.2 ab	391.5 ± 61.38
	180	33.8 ± 12.9 a	7.3 ± 1.3 ab	21.4 ± 1.3 a	12.9 ± 2.2 a	492.6 ± 174.3

On the 45th day of cultivation, the trends in spinach growth activity under lamps of different spectral compositions changed (Figure 1). The spectral composition did not have a statistically significant effect on the growth of spinach, and high light intensity had a significant effect on the fresh and dry weight of plants, as well as on the number of leaves (Table 2). The best options for fresh weight accumulation and growth of leaf surface area were V1 at 120 and 180 μmol m^{-2} s^{-1} PFD and red-blue V3 at 180 μmol m^{-2} s^{-1} PFD. In terms of yield and energy savings, lighting option V1 at 120 μmol m^{-2} s^{-1} showed good results, but the dry weight of the plants was 13.7% less; therefore, the plants contained more moisture, which reduced their nutritional value and may affect the duration of storage.

3.2. Biochemical Parameters of Spinach Plants

The data obtained during our experiments are further confirmation that the pigment composition of a plant is largely dependent on the intensity and spectral composition of light. The highest concentration of chlorophyll *a* on the 30th and 45th days of cultivation was found when spinach plants were grown under RB light (Figure 2), with a B proportion of 30%, which looks like magenta (V3). It was previously shown that the chlorophyll content has a positive relationship with the increase in the blue component of the light spectrum [22]. We also note that at higher PFD (180 μmol m^{-2} s^{-1}), total chlorophyll content was lower in all irradiance spectral compositions; hence, plants at 120 μmol m^{-2} s^{-1} were stressed due to lack of light, irrespective of irradiance spectrum. This is consistent with other studies where plants accumulated more chlorophyll at lower irradiation levels [23,24].

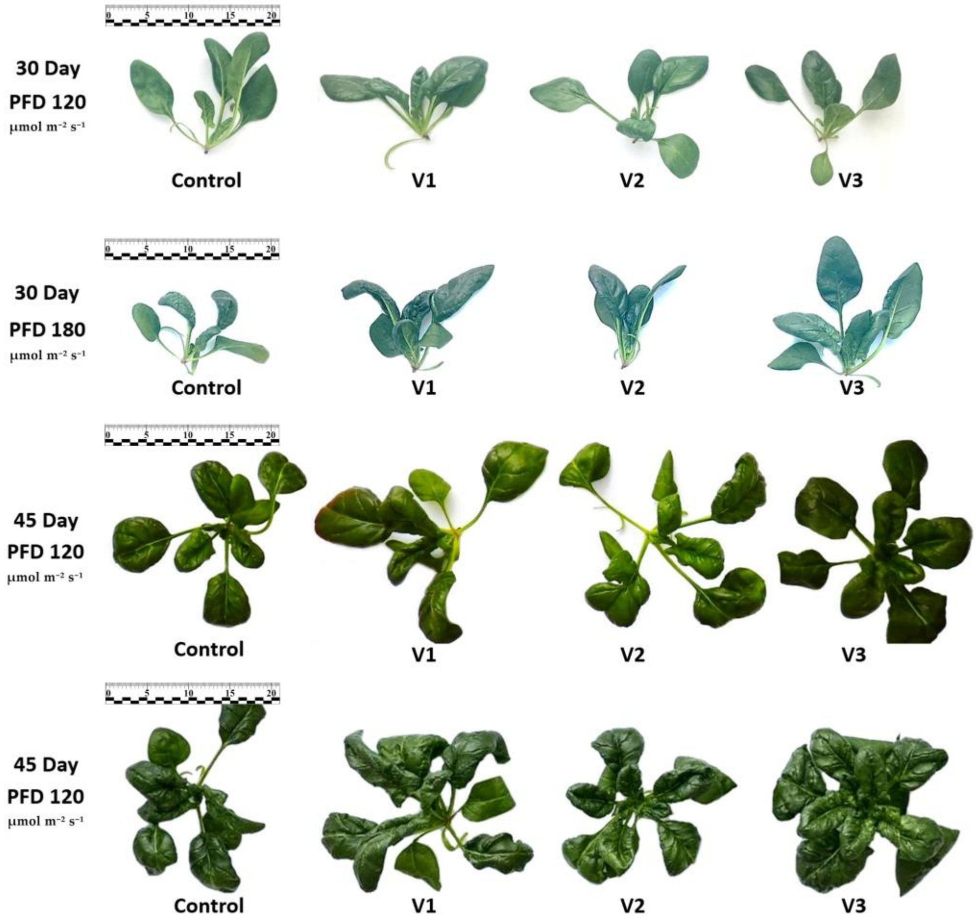
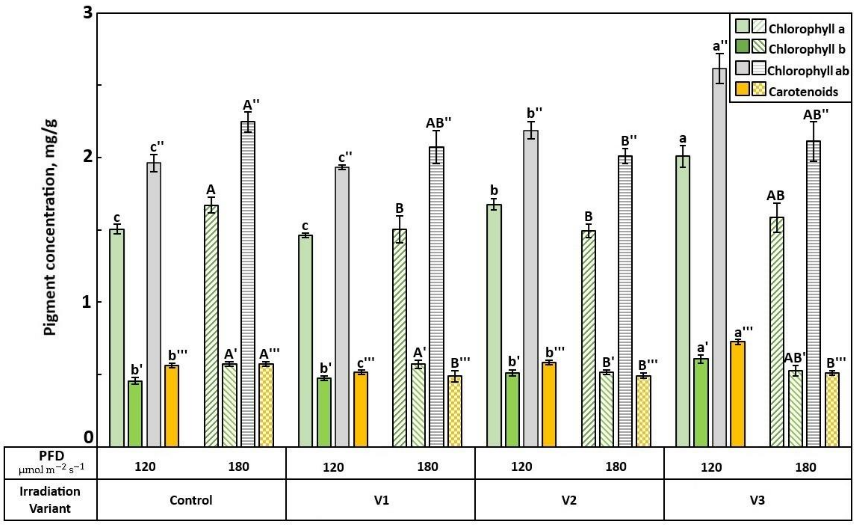


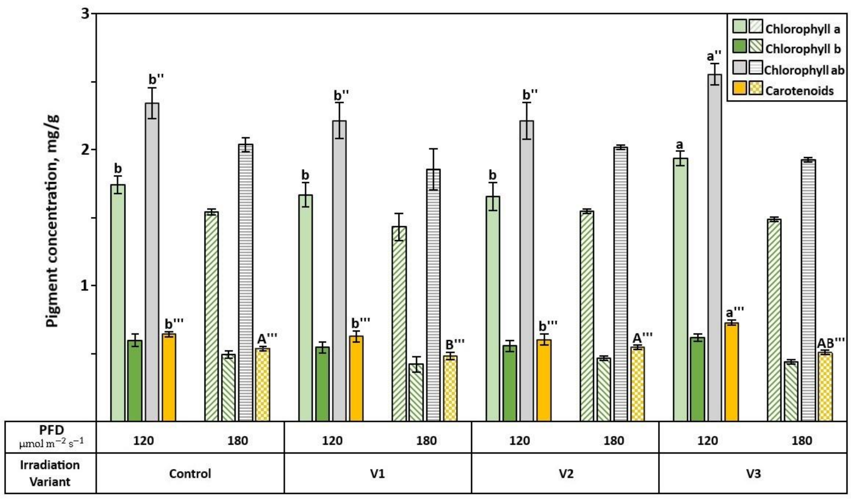
Figure 1. Appearance of spinach plants under studied spectral compositions and PFD levels.

It was previously established by way of the example of beet microgreens, which, like spinach, are short-day plants and belong to the same botanical family (*Amaranthaceae*). The concentrations of chlorophylls *a* and *b* and carotenoids were 1.2–4.3 times higher during treatment blue light 33% at PPFD 300 μmol m^{-2} s^{-1} compared to lower doses of blue light (0, 8, 16, 25%) [25]. In our experience, a similar trend can be observed at an intensity of 120 μmol m^{-2} s^{-1}. At 180 μmol m^{-2} s^{-1}, no significant differences between the content of photosynthetic pigments were observed on the 30th or 45th day of cultivation.

A previous study on the different R and B ratios of LED 83R:17B; 91R:9B; 95R:5B, and 100R:0B, which was carried out at PPFD 200 μmol m^{-2} s^{-1} and a photoperiod of 16 h, showed that the plant height and number of spinach leaves did not change significantly among the experimental variants. The fresh and dry weight of the studied plants increased in light treatment 91R:9B and 95R:5B. Chlorophyll *a* and chlorophyll *b* contents in spinach were significantly increased in 91R:9B treatment [26]. In our experiment, similar results were obtained using a light intensity of 120 μmol m^{-2} s^{-1} on the 30th and 45th days of cultivation. As it was noted above, at PFD 180 μmol m^{-2} s^{-1}, there was no significant difference in the content of photosynthetic pigments. Under an irradiation intensity of 120 μmol m^{-2} s^{-1}, carotenoid accumulation was the highest at a high proportion of blue light (V3). In a study [26], the accumulation of carotenoids in cabbage and basil also increased significantly with a high proportion of blue light. A similar effect was observed in other studies [27,28]. This confirms that a high proportion of blue light stimulates the accumulation of carotenoids in spinach, but in our study, this relationship was observed only at low PFD levels (120 μmol m^{-2} s^{-1}).



(**a**)



(**b**)

Figure 2. Concentration of photosynthetic pigments of spinach plants on the 30th (**a**) and 45th (**b**) days of cultivation. The data shown are the means, and the vertical bars indicate standard errors (n = 5). Statistically significant differences are within $p \leq 0.05$. The different letters indicate significant differences among groups. Capital letters were used in groups with plants grown at PFD 180 μmol m^{-2} s^{-1}, capital letters in groups with PFD 120 μmol m^{-2} s^{-1}, the apostrophe corresponds to chlorophylls *a* and *b* and carotenoids.

It was found [29] that spinach plants under R and red-green (RG) lighting spectrums showed signs of shading. The thickening of the leaf blade increased the content of photosynthetic pigments, and, as a consequence, resulted in a higher photosynthetic capacity compared to plants under W and RB irradiation.

In our case, spinach plants did not show signs of shading (an increase in the concentration of photosynthetic pigments) with an increase in the proportion of R and G components in the light spectrum, as shown in studies [29].

As in an early study with plants of the *Brassicaceae* family [16], nitrate content in spinach plants (Figure 3) decreases with increasing light intensity [7,9]. It is worth emphasizing that in our case, more contrasting values of nitrate concentration with an increase in illumination from 120 to 180 μmol m^{-2} s^{-1} were observed in the control and V2 variants with spectral compositions; in other variants, the difference was not significant. In the territory of the Russian Federation, the maximum permissible concentration of nitrates in leafy vegetables should not exceed 2000 mg/g [30,31]; thus, only lamps with the spectral composition control and V2 with a PFD of 180 μmol m^{-2} s^{-1} can be used for growing 'Zhirnolistny' spinach, the second of which gives greater plant productivity compared to the control.

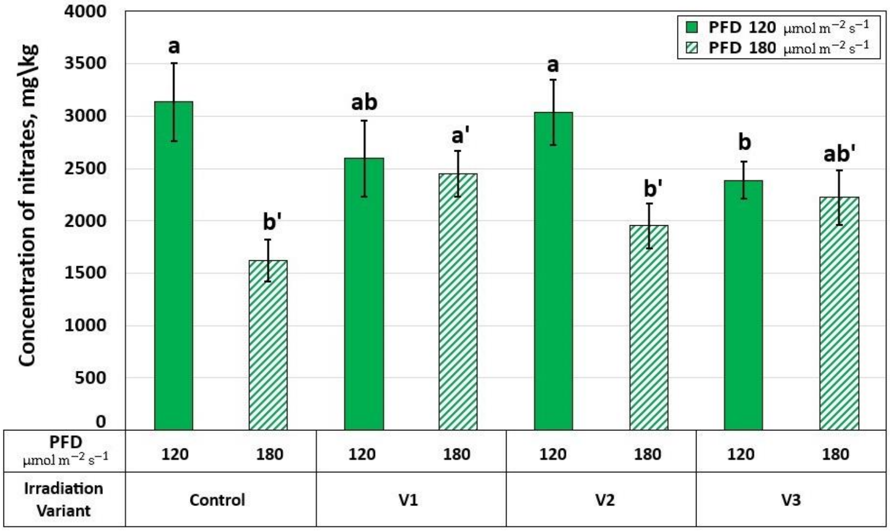


Figure 3. Nitrate concentration in 'Zhirnolistny' spinach on the 45th day of cultivation under the studied spectral compositions and PFD levels. The data shown are the means, and the vertical bars indicate standard errors (n = 5). Statistically significant differences are within $p \leq 0.05$. The different letters indicate significant differences among groups.

It can be noted that in the variants with a greater proportion of the G light in the spectrum (control and V2) at a PFD level of 180 μmol m^{-2} s^{-1}, the nitrate content in spinach is slightly lower than in other variants.

Recent studies show that green light has positive functions that help reduce nitrate content in plants since it is assumed that green light is involved in the regulation of nitrogen metabolism and increases plant resistance to abiotic stress [32].

The possibility of changing the spectral composition of lighting options V1 and V3 in favor of the green and red range a few days before harvest to reduce the concentration of nitrates is considered a perspective [14,33].

Light intensity has a profound effect on the accumulation of nitrates by plants, as it induces the activity of nitrate metabolic enzymes. For example, the concentration of nitrates in Brassica microgreens decreased by 37.7–84.5% with increasing light intensity from 110 to 545 μmol m^{-2} s^{-1} [34].

According to the standards of the European Union [35] established by the Council Regulation of the European Economic Community 1881/2006 of December 19, 2006, the maximum permissible nitrate content in spinach is 3500 mg/kg. That is, all nitrate values obtained using the emitters under study do not exceed the EU MAC, which means they can be used to produce products for the EU.

3.3. Chlorophyll Fluorescence Parameters

On the 45th day of spinach cultivation, the influence of the spectral composition and light intensity on the magnitude of the photosynthetic electron flow through photosystem II (ETR), and non-photochemical fluorescence quenching (NPQ) was studied (Figure 4). It is known that at low irradiation intensity, the most effective from the point of view of the photosynthetic activity of light is irradiation with light in the red range of the spectrum [36], while growing under monochrome light is not effective [37]. For this reason, we expected that with a total PFD RED of 120 μmol m^{-2} s^{-1}, the highest rate between photosystems would be in variants V3 (81.6 μmol m^{-2} s^{-1} RED) and V1 (75.6 μmol m^{-2} s^{-1} RED);

however, only variant V1 had the highest ETR at low irradiation intensity. Variant V3, where the light spectrum is red-blue, showed the lowest rate of electron flow through photosystem II. This is due to the low penetration of both R and B light into spinach leaves, as well as the absence of G light in the spectrum [38]. RB light had a significant effect on plant photomorphogenesis at an intensity of 120 µmol m^{-2} s^{-1}. Judging by the slope of the non-photochemical fluorescence quenching curve of NPQ spinach grown under RB light (V3) with an intensity of 120 µmol m^{-2} s^{-1}, after a bright flash, photosynthesis saturation occurred faster, and more energy dissipated as heat.

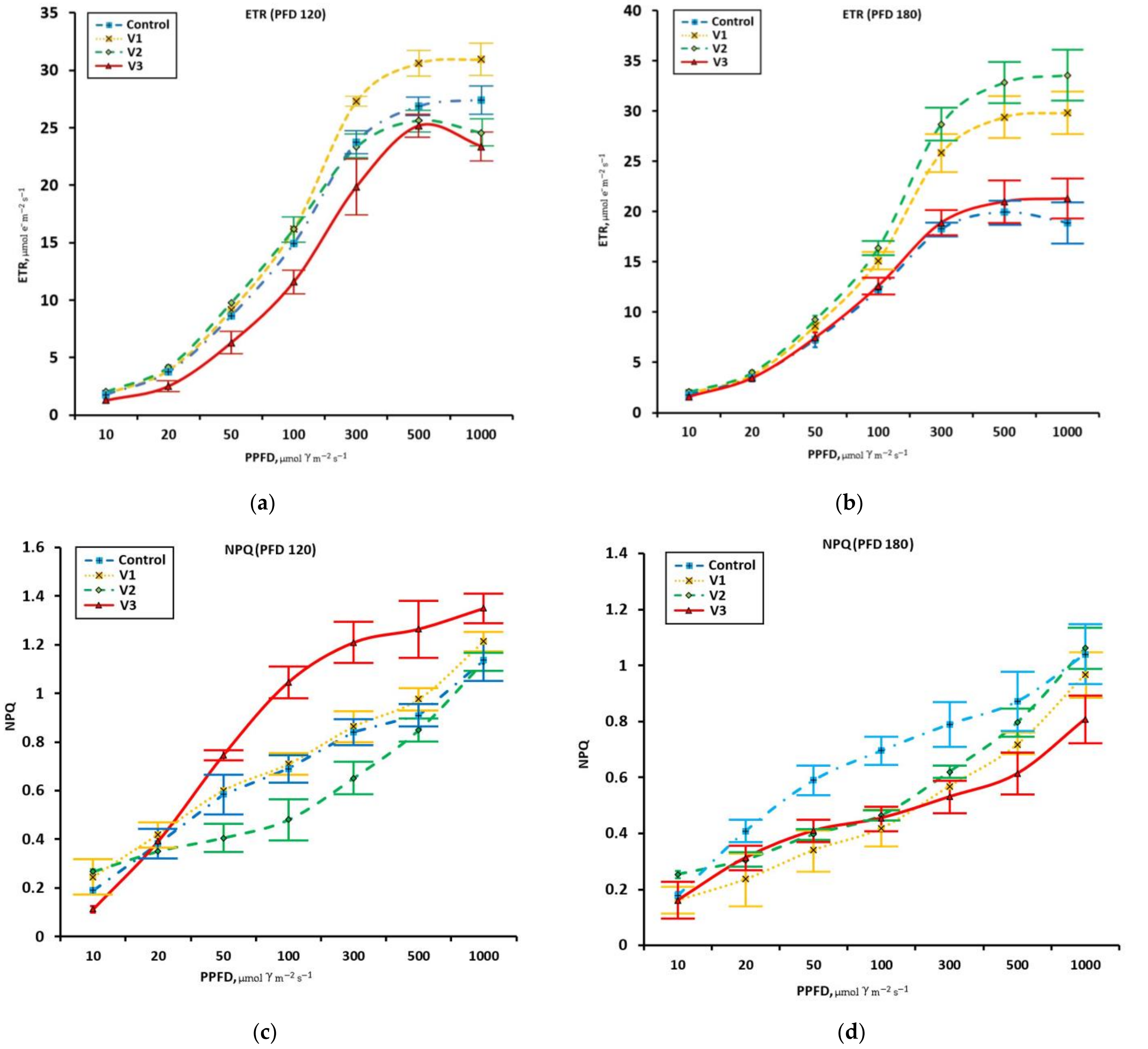


Figure 4. Dependences of the rate of electron transport (ETR) (**a**,**b**) and non-photochemical quenching (NPQ) of chlorophyll fluorescence (**c**,**d**) on the PAR of spinach plants. Values represent mean SEM (n = 6).

At an intensity of 180 µmol m^{-2} s^{-1}, the highest ETR was detected in options V1 and V2, which is due to the high proportion of both R and G light in the irradiation spectrum, while no significant differences were found between lighting options. G light has a beneficial effect on photosynthesis processes in spinach, allowing light to be more evenly distributed throughout the entire thickness of the leaf between the chloroplasts [36,39].

The spectral composition of illumination, in general, did not cause significant changes in spinach plants; at the same time, there was a tendency toward a decrease in ETR in plants with increasing actinic light intensity cultivated under RB light conditions (variant V3). This effect was observed in plants grown at intensity levels of 120 and 180 μmol m^{-2} s^{-1}.

The results obtained suggest that it is the low ETR value and high NPQ that may be the cause of the stress state of spinach plants grown under red-blue light, but this did not affect the final productivity of the plants.

Light intensity directly affects plant growth parameters (Figure 1), thereby determining their productivity. But the light spectrum also plays an important role, so in option 1, an increase in PFD from 120 to 180 μmol m^{-2} s^{-1} did not lead to a significant increase in the fresh weight of plants, and this can be associated with the high ETR value of this option, even at low light levels. With this spectrum option, additional photosynthetic photons are not used effectively to gain biomass; growth may be delayed due to the excess light intensity [40]. Accordingly, this spectrum option is more effective for photosynthesis and, as a consequence, the productivity of spinach plants at low PFD values, but the plant dry weight was 13.7% less. At high PFD values, the sensitivity of spinach plants to the spectral composition is less pronounced; this can also be associated with the light saturation of photosynthesis [41]. At the same time, energy costs increase to maintain a high PFD without a proportional yield response. For other green crops, increasing the photoperiod may also increase productivity [42], but for spinach plants, this is not permissible due to the start of the flowering phase, and the selection of light quality remains preferable to increase productivity. However, the mechanisms of action of different parts of the spectrum on the productivity and quality of spinach at different light levels are largely unknown, which merits further study of the influence of lighting conditions.

4. Conclusions

In the middle of the active growth period on day 30, spinach plants accumulated 2.6- and 2.4-fold more crude mass under V1 and V3 illumination at an intensity of 180 μmol m^{-2} s^{-1} and differed by 1.9-fold in the leaf surface area. At the end of cultivation, before harvesting spinach on the 45th day, only high light intensity had a significant effect on the fresh and dry weight and number of plant leaves. Reduced photosynthetic efficiency, as indicated by lower ETR values, was observed in the control and V3 plants, but this did not have a significant effect on plant productivity. The best options in terms of fresh weight accumulation and increasing leaf surface area were V2 (the total proportion of R and G in the spectrum was maximum) and red-blue V3, with a PFD of 180 μmol m^{-2} s^{-1}. In terms of yield and energy savings, lighting option V1 at 120 μmol m^{-2} s^{-1} showed good results, but the plant dry weight was 13.7% less. A high light intensity is often expensive and difficult to maintain, and V1 treatment will be preferable. In these lighting options, spinach plants comply with EU standards for nitrate content. The results of this study may provide spinach growers with a better understanding of optimal lighting conditions to improve spinach productivity.

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