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INFLUENCE OF TWO TYPES OF BIOCHARS ON THE PHOTOSYNTHETIC APPARATUS OF PRICKLY-SEEDED SPINACH (*SPINACIA OLERACEA* L.)

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Aim. To compare the effect of two biochars from different raw materials and their concentrations in soil on the main plant photosynthesis processes. **Methods.** Photosynthetic activity of prickly-seeded spinach plants (*Spinacia oleracea* L.), hybrid Corvair F1, was measured under controlled conditions in a pot experiment in a growth chamber (24–26 ^{o}C, light 150 μmol photons m^{-2} s^{-1} for 16 h per day; substrate humidity 60 % of full moisture capacity) at the stage of the fourth true leaf development (BBCH 14) using a portable fluorometer (MultispeQ v1.0), recording the following parameters: quantum efficiency of photosystem II (ϕII), quantum yield of non-photochemical quenching of chlorophyll (ϕNPQ), fraction of light energy lost due to unregulated processes (ϕNO), qL – fraction of open photosystem II; Fv′/Fm′ – maximum quantum efficiency of photosystem II, ECSt – capacity of ATP synthase; gH^+ – proton conductivity; vH^+ – steady-state proton flux. The two biochars used originated from the aboveground biomass of *Miscanthus* plants (Bch1, variants D2-4) and the sewage sludge of municipal sewage treatment plants (Bch2, variants D5-7) in the amount of 1 % (D2; D5), 3 % (D3; D6), 5 % (D4; D7) from the dry mass of a heavy loamy low-humus chernozem. Control plants were grown in soil without biochar. The data were statistically processed using R and RStudio with ANOVA, Kruskal-Wallis, Tukey's HSD test and Principal Component Analysis (PCA). The measurements were conducted using ten plants per variant. **Results.** When the biochars Bch1 and Bch2 were applied, they influenced the photosynthetic properties of plants, including the chlorophyll content. Bch1 did not significantly increase the relative chlorophyll content (SPAD) in spinach leaves, while Bch2 significantly increased SPAD (by 17–19 %). The presence of biochar in the soil positively changed the temperature differential (TD) of the leaves, which indicated transpiration and marked the water supply of plants. The leaves of variants D3 (Bch1, 3 %) and D7 (Bch2, 5 %) were characterized by the most significant negative TD, the hydration of which, compared to the control, was higher by 3 and 1.7 %, respectively. The study of primary photosynthetic processes by chlorophyll fluorescence induction showed that both biochars generally had a positive effect on photosynthetic activity, particularly at 3 % addition on the photosystem II quantum efficiency (ϕII) and the maximum quantum yield in photosynthesis (Fv′/Fm′). Non-photochemical quenching without dark adaptation (NPQt) was 35–39 % lower in variants with Bch1, indicating more efficient use of light energy for photochemical processes, which may indicate that this biochar may contribute to reduced light energy dissipation and increased photosynthetic efficiency. In general, both types of biochar, reduced the loss of light energy and increased the photosynthesis efficiency by 3–7 %, thus indicating that they may be used in practice to stimulate photosynthesis and yield of *Spinacia oleracea* L. **Conclusions.** Adding both types of biochar to the typical heavy loamy low-humus chernozem in the amount of 1–5 % increased the photochemical efficiency and a 17–39 % decrease in non-photochemical quenching of chlorophyll fluorescence in spinach plants. The increase by

3–7 % in the maximum quantum yield and by 6–9 % in the quantum efficiency of photosystem II, along with lower values of ϕNPQ and NPQt compared to the control, indicate a higher efficiency of photochemical processes in plants grown in soil with added biochar. Future field studies should confirm if this increased photosynthesis is still present and leads to healthier plants and increased yield.

Key words: *Spinacia oleracea* L., photosynthesis, chlorophyll fluorescence, biochar.

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INTRODUCTION

The need to develop a more sustainable agriculture and enhanced plant productivity using limited resources, biochar has been found over the last twenty years to be a promising material (Nascimento et al, 2023; Srivastava et al, 2023). Biochar or biocarbon is a carbonaceous product formed following pyrolysis under oxygen limitation of organic materials, such as wood, agricultural waste, solid household waste and sewage (Yaashikaa et al., 2020). Its use in agroecosystems has promoted yield increase, a lower impact of harmful substances, and an increased carbon binding in soil in a number of cases (Ali et al, 2017; Hilber et al, 2017; Tang et al, 2020; Brtnický et al, 2021; Hussein et al, 2022; Wang et al, 2021; Wu et al, 2023).

Effect of biochar on the photosynthesis activity of plants has also been studied as it may have significant consequences for plant productivity and the ability of plants to adjust to environmental conditions (Lyu et al, 2016; Zemanová et al, 2017; Zhang et al, 2020; Soothar et al, 2021; Wu et al, 2023; Zhang et al, 2020). One of the critical aspects of studying the impact of biochar on plants is its ability to decrease the stress load on plants and thus increase their photosynthetic activity (Cheng-Yuan et al, 2015; Liu et al, 2022). Stress in plants is often manifested at the level of primary photosynthesis processes. It is usually accompanied by a decrease in the photosynthetic efficiency of photosystem II (ϕII), an increase in the rate of non-photochemical chlorophyll quenching (NPQ), and an impaired balance between the absorption of light energy and its use in photosynthetic reactions of the photosystems (Nath at al, 2013).

The efficiency of photosynthesis in plants is considerably dependent on the concentration of photosynthetic pigments, the efficiency of absorption and use of light energy by photosynthetic antenna complexes, and the velocity of carbon dioxide assimilation (Blankenship, 2015; Sagun et al, 2022; Simkin et al, 2022).

One of the strategies in the adaptation of plants to environmental conditions is the optimization of the content of photosynthetic pigments in leaf tissues. The application of biochar may enhance photosynthesis of plants (Jiang et al, 2022; Tang et al, 2022).

It was found that biochar considerably stimulated the photosynthesis process and the biomass accumulation in C_3 plants of , but had a limited effect on C_4 plants. In the former, biochar increased the photosynthesis rate by 27.1 % and enhanced the air permeability, transpiration rate, and relative content of chlorophyll (SPAD) (He et al, 2020).

Despite potential advantages, the use of biochar in agroecosystems is limited by the possible presence of toxic substances (Hilber et al, 2017; Godlewska et al, 2021; Buss et al, 2022; George, 2022; Alharbi et al, 2023), the need to optimize the pyrolysis and the dosage of introduction for different soils and plant types, etc. (Mingxin and Weiping, 2022; Marazza et al, 2023; Nepal et al, 2023).

Currently, many publications have dealt with the assessment of effects of biochar on photosynthetic efficiency parameters, such as photosystem II quantum efficiency (ϕII), non-photochemical quenching(Lyu et al, 2016; Abbas et al, 2019; Zang et al, 2020; Liu et al, 2022; Hussein et al, 2022; Zhu et al, 2022; Wang et al, 2023). However, studies that simultaneously evaluate the parameters of both photosystem I and photosystem II and the capacity and conductivity of ATP synthase under the action of biochar are scanty (e.g. Yildiztugay et al, 2023; Derbali et al, 2024).

In previous studies (Kononchuk et al, 2022), we highlighted the changes in a few fluorescent parameters of photosystem II: ϕII, the maximal quantum efficiency of PSII (Fv′/Fm′), the fraction of light dedicated to nonphotochemical quenching (ϕNPQ), and the fraction of light lost via nonregulated photosynthesis processes (ϕNO) under the effect of biochar obtained from vegetative raw materials.

The aim of this work was to compare the impact of the two biochars, differing by the type of the initial raw material (biomass waste of *Miscanthus* and sewage sludge) and their amount in thesoil, on the main plant photosynthesis processes, using fluorescence and absorbance measurements for more photosynthesis parameters.

MATERIALS AND METHODS

The plant material used was prickly seeded spinach (*Spinacia oleracea* L.), hybrid Corvair F1 (Enza Zaden, the Netherlands), grown from seed, having C_3-type photosynthesis. Plants were grown in a controlled growth chamber under the following conditions: temperature 24–26 ºC; artificial light (Osram Fluora T8 36 W, Germany) (https://www.luxlight.de, 2024) 150 µmol photons m^{-2} s^{-1}; photoperiod – 16 h/day; soil humidity at 60 % of full moisture capacity. The first biochar (Bch1) was from the aboveground biomass of *Miscanthus* produced under field conditions in Chomutov on soil slightly contaminated by trace elements (TEs) (Kononchuk et al, 2022) and the second biochar (Bch2) was obtained from the sewage sludge of municipal sewage treatment plants, produced by Almeco company (Czech Republic) (Pidlisnyuk et al, 2021). The prepared soil (typical heavy loamy, low-humus chernozem) with characteristics that are presented in **Table 1**, was thoroughly mixed with a specific dose (in % of dry mass) of Bch1 (1% – variant D2, 3% – variant D3, and 5% – variant D4) and Bch2 (1% – variant D5, 3% – variant D6, and 5% – variant D7). The total volume of the pot was 1 dm³. The control plants (C) were grown in soil without biochar.

The determination of the functioning of the photosynthetic apparatus (PSA) of plants was done when the 4th true leaf unfolded (extended BBCH scale, stage 14) (Meier, 2018) using a portable fluorometer (MultispeQ v1.0, Kuhlgert et al., 2016 and https://help.photosynq.com/instruments/multispeq-v1.0.html#configuration). The measurements were conducted using ten random plants per variant.

PSA features (fluorescence- and absorbance-based photosynthesis parameters) analysed with the MultispeQ v.1.0 fluorometer (with pre-installed protocol “Photosynthesis RIDES 2.0”) (were: 1) ϕII – quantum efficiency of photosystem II; 2) NPQt, non-photochemical quenching, evaluated without the adjustment to the dark; 3) ϕNPQ, quantum yield of NPQ; 4) ϕNO, fraction of light energy, absorbed by photosystem II and lost due to unregulated processes; 5) qL, fraction of open reaction centers of photosystem II;6) Fv′/Fm′, maximum quantum efficiency of photosystem II;7) ECSt, capacity of ATP-synthase; 8) gH^+, conductivity of ATP-synthase; 9) vH^+ – steady-state proton flux (Kuhlgert et al, 2016; https://help.photosynq.com/view-and-analyze-data/references-and-parameters.html#environmental-parameters). The protocol “Photosynthesis RIDES 2.0” with connected macro we used to estimate the above-mentioned photosynthesis-related parameters photosystem II (PSII) also included state of photosystem I (PSI) measurements (PSI active centers (PSIactiv), PSI open (PSIopen)) and is described at https://photosynq.org/protocols/photosynthesis-rides-2-0.

The temperature differential (TD) and SPAD were determined by the intact methods with the mentioned device immediately before fluorescence measurement (https://help.photosynq.com). Ahead of using the device, it was calibrated using CaliQ (https://www.photosynq.com/caliq).

Statistical data processing was conducted with R (the R Project for Statistical Computing) using RStudio software (version 1.4.1103, RStudio PBC, 2021) with the “psych” and “multcomp” packages installed. Statistical data processing included descriptive statistics (mean, standard deviation), Pearson correlation analysis, analysis of variance (One-way ANOVA), and the Kruskal–Wallis test when the assumptions of normality

Table 1. **Characteristics of soil (heavy loamy low-humus chernozem) used in the pot experiment**

Agrochemical parameter	Unit	Mean ± SD	Method	Reference
pH (KCl)	–	6.66 ± 0.05	pH (KCl)	ISO 10390:2021-04
Organic matter	%	1.12 ± 0.02	Walkley-Black method	Soil organic carbon. Walkley-black method
NO_3	mg kg^{-1}	151.3 ± 4.5	Ion selective	United States Environmental Protection Agency, 2007
NH_4	mg kg^{-1}	0.18 ± 0.04	Ion selective	Fayose et al, 2020
P_2O_5	mg kg^{-1}	79.6 ± 1.0	Chirikov	United States Environmental Protection Agency, 1978
K	mg kg^{-1}	0.50 ± 0.12	Ion selective	Stevens et al, 2016

and homogeneity of variances were not met. The normality of distribution was assessed using the Shapiro-Wilk test, and the homogeneity of variances was assessed using Levene's test.

Principal Component Analysis (PCA) (Jolliffe, 2002) was used to reduce the dimensionality of the large datasets and to find and visualize interrelations among the quantitative variables. The cut-off value for statistical significance was set at 0.05.

RESULTS

In our tests biochar Bch1 in soil (1, 3, 5 %, – D2, D3, D4, respectively) did not increase the relative content of chlorophyll (SPAD) in leaves of *S. oleracea* L. but in case of Bch2 from sewage sludge an increase of 20-21 % was recorded, with little difference between the amounts applied (**Fig. 1, *a***). The SPAD index is known to correlate not only with the (spectrophotometric) chlorophyll content, but also with that of nitrogen and

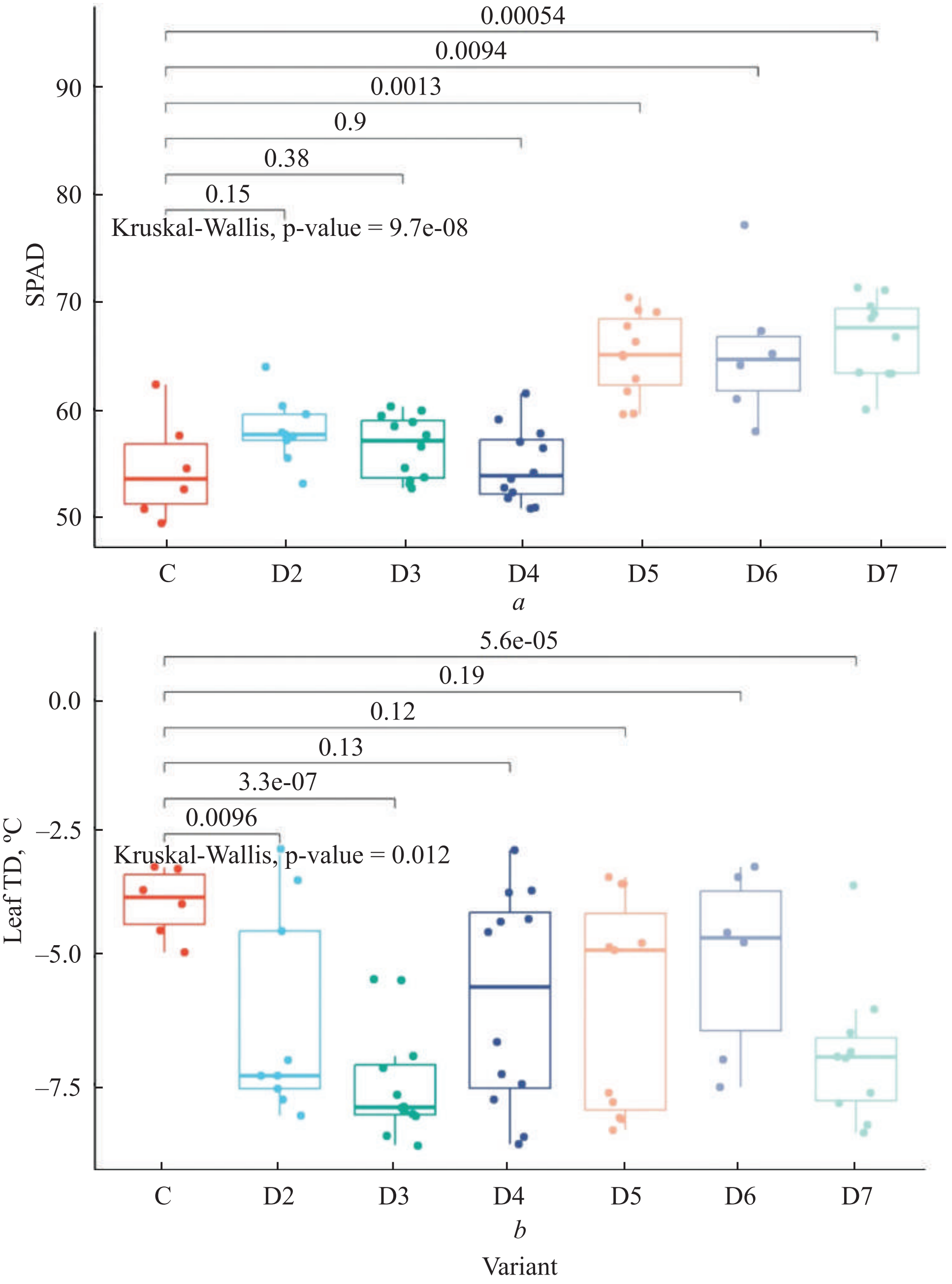

Fig. 1. The relative content of chlorophyll (SPAD) (*a*) and the temperature differential (TD) (*b*) of the leaves of prickly seeded spinach at the effect of biochars. (C) control; (D2) Bch1 (1 %); (D3) Bch1 (%); (D4) Bch1 (%); (D5) Bch2 (1 %); (D6) Bch2 (%); (D7) Bch2 (%)

Table 2. **The parameters of chlorophyll fluorescence of *S. oleracea* L. under the effect of biochars (M ± SD, n = 10)**

Parameter	C	D2	D3	D4	D5	D6	D7
Fv/Fm	0.690 ± ± 0.014	0.742 ± ± 0.004*	0.741 ± ± 0.003*	0.744 ± ± 0.005*	0.711 ± ± 0.011*	0.713 ± ± 0.010*	0.722 ± ± 0.012*
ϕNPQ	1.89e-01 ± ± 1.90e-02	1.21e-01 ± ± 6.18e-03*	1.16e-01 ± ± 7.07e-03*	1.24e-01 ± ± 9.41e-03*	1.57e-01 ± ± 1.69e-02*	1.57e-01 ± ± 1.50e-02*	1.49e-01 ± ± 1.86e-02*
ϕNO	1.67e-01 ± ± 5.36e-03	1.75e-01 ± ± 4.82e-03*	1.79e-01 ± ± 3.40e-03*	1.78e-01 ± ± 3.52e-03*	1.68e-01 ± ± 3.82e-03	1.68e-01 ± ± 4.35e-03	1.69e-01 ± ± 4.83e-03
qL	7.85e-01 ± ± 1.97e-02	8.20e-01 ± ± 3.00e-02*	8.05e-01 ± ± 2.16e-02*	8.00e-01 ± ± 2.64e-02	8.20e-01 ± ± 2.82e-02*	8.16e-01 ± ± 2.96e-02*	8.23e-01 ± ± 3.77e-02*
vH^+	2.68e-02 ± ± 7.65e-03	3.02e-02 ± ± 1.03e-02	2.55e-02 ± ± 9.37e-03	3.43e-02 ± ± 9.37e-03	1.80e-02 ± ± 1.06e-02	2.31e-02 ± ± 1.07e-02	2.31e-02 ± ± 9.33e-03
gH^+	1.25e+02 ± ± 3.31e+01	1.69e+02 ± ± 5.86e+01	1.50e+02 ± ± 5.00e+01	1.77e+02 ± ± 5.00e+01*	1.08e+02 ± ± 5.78e+01	1.80e+02 ± ± 8.89e+01	1.48e+02 ± ± 7.91e+01
ECSt	2.15e-04 ± ± 3.01e-05	1.77 e-04 ± ± 1.9e-05*	1.70 e-04 ± ± 3.6e-05*	1.96e-04 ± ± 3.4e-05	1.80e-04 ± ± 6.4e-05	1.40e-04 ± ± 5.09e-05*	1.61e-04 ± ± 2.2e-05
PSIactiv	1.17 ± 1.23	1.78 ± 0.78	1.35 ± 1.38	0.89 ± 2.81	0.82 ± 2.19	1.75 ± 0.35	1.72 ± 1.49
PSIopen	0.47 ± 0.65	0.75 ± 0.39	0.26 ± 1.17	0.86 ± 0.38	0.81 ± 1.23	1.19 ± 0.30*	1.06 ± 0.63*

Note: * – the difference is significant at $P \leq 0.05$ (compared to control) ((C) control; (D2) Bch1 (1 %); (D3) Bch1 (3 %); (D4) Bch1 (5 %); (D5) Bch2 (1 %); (D6) Bch2 (3 %); (D7) Bch2 (5 %). PSI – photosystem I; PSIactiv – photosystem I active center; PSIopen – photosystem I open center

magnesium in plant leaf tissues (Netto, 2005; França and Carvalho, 2016; Rhezali and Aissaoui, 2021). Possibly this difference between SPAD values of the two biochars is related to their different origin and production process. BCh1 originated from the aboveground waste biomass (AWB) produced under field conditions in Chomutov, whereas BCh2 was produced by the firm Almeco (Czech Republic) from municipal wastewater treatment plant sludge from Brno, Czech Republic.

The presence of biochar in the soil changed leaf TD in all experimental variants by 30–89 % compared with the control (**Fig. 1, *b***). The leaves of plants, especially in variants D3 and D7, had a statistically significant, more considerable (85–89 %).

We found a statistically significant, positive effect of both biochars studied on the quantum efficiency of photochemical transformation of energy (**Table 2, Fig. 2, *a***) and the maximal quantum yield of photosynthesis (Fv/Fm) (**Table 2**) in spinach of up to 5–9 % and 3–7,5 %, respectively.

The maximal efficiency of photosystem II and linear transportation of electrons was registered for 1 and 3 % Bch1 (**Table 2, Fig. 2*a*, *b***). There was only a slight and insignificant increase in linear transportation of electrons for 5 % Bch2 (**Table 2**, **Fig. 2, *b***).

Non-photochemical quenching of chlorophyll was influenced by the amount and type of biochar in the substrate (**Fig. 3**), values of Bch1 were up to 25 % lower than those for Bch2, with the lowest values for variants D3 and D7.

Thus, lower non-photochemical quenching of chlorophyll along with higher values of the maximum quantum yield and quantum efficiency of photosystem II in Bch1 (obtained from the aboveground biomass) demonstrated a more efficient consumption of the light energy in Bch1 than that in Bch2 from the sewage sludge.

Moreover, Bch1 apparently activated non-regulated processes at the level of photosystem II (**Table 2**), as demonstrated by parameter ϕNO that increased by 5–7 %. In general, Bch1 and Bch2 conditioned an increase in amount of open, photochemically active reaction centers of photosystem II, which could possibly explain the decrease in NPQ activity found in the experimental variants (**Table 2**). The parameter ECSt, an indicator of light-induced change in the pH of the thylakoid space (Kramer et al., 1999), differed between the variants D2–D4 and D5–D7 but did not show a dependence on the amount and type of biochar added to the soil (**Table 2**).

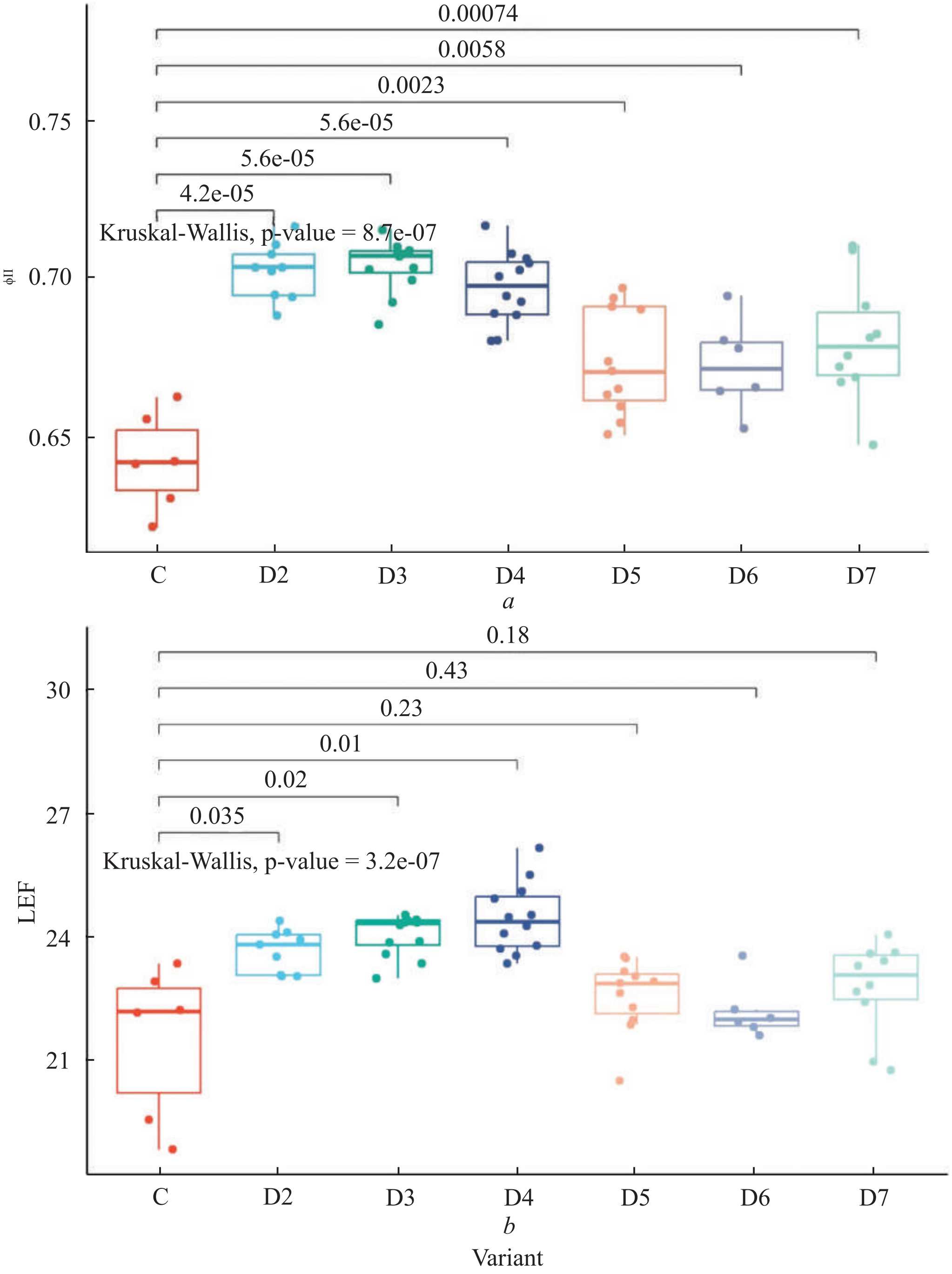

Fig. 2. The quantum efficiency of the photochemical transformation of energy (ϕII) (*a*) and linear transportation of electrons (*b*) of prickly seeded spinach at the effect of biochars. (C) control; (D2) Bch1 (1 %); (D3) Bch1 (3 %); (D4) Bch1 (5 %); (D5) Bch2 (1 %); (D6) Bch2 (3 %); (D7) Bch2 (5 %)

Moreover, Bch2 in the amounts of 3 and 5 % showed a (statistically insignificant) decrease in the flux of protons via ATP-synthase. The proton conductivity of ATP-synthase of chloroplasts did not change as compared to the control.

The fraction of PSIactiv did not change under our experimental conditions for both biochars (**Table 2**).

The share of open PSI reaction centers, which ensure the distribution of charges under stationary illumination (PSIopen), increased considerably (by two and a half times) in variants D6 and D7 with high doses of Bch2.

The visual presentation of chlorophyll fluorescence parameters in the form of a scatter plot (**Fig. 4**) dem-

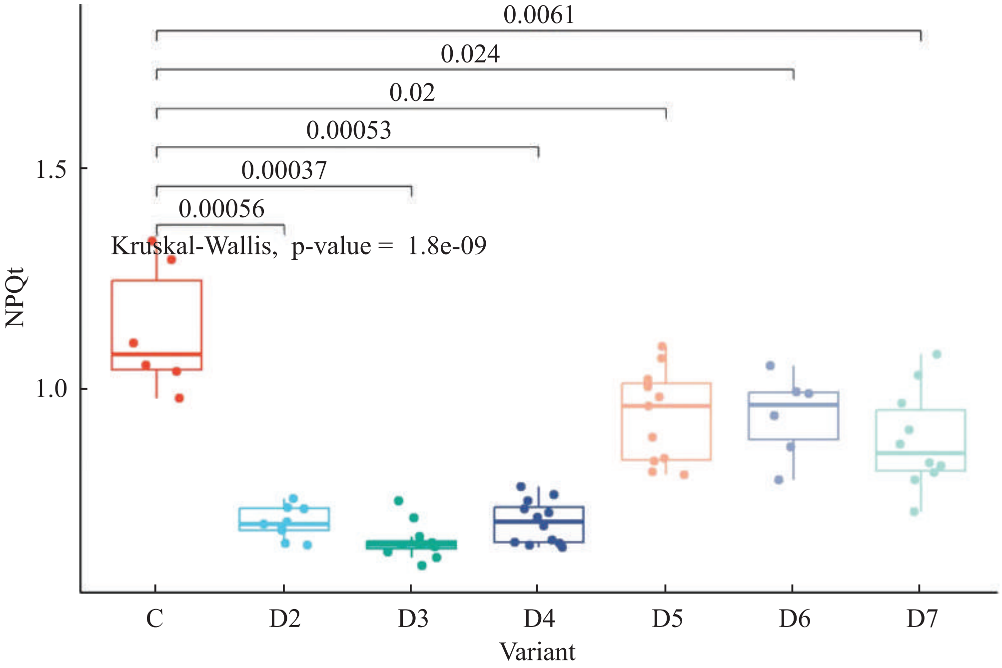

Fig. 3. Non-photochemical quenching of chlorophyll (NPQt) in prickly seeded spinach at the effect of biochars. (C) control; (D2) Bch1 (1 %); (D3) Bch1 (3 %); (D4) Bch1 (5 %); (D5) Bch2 (1 %); (D6) Bch2 (3 %); (D7) Bch2 (5 %)

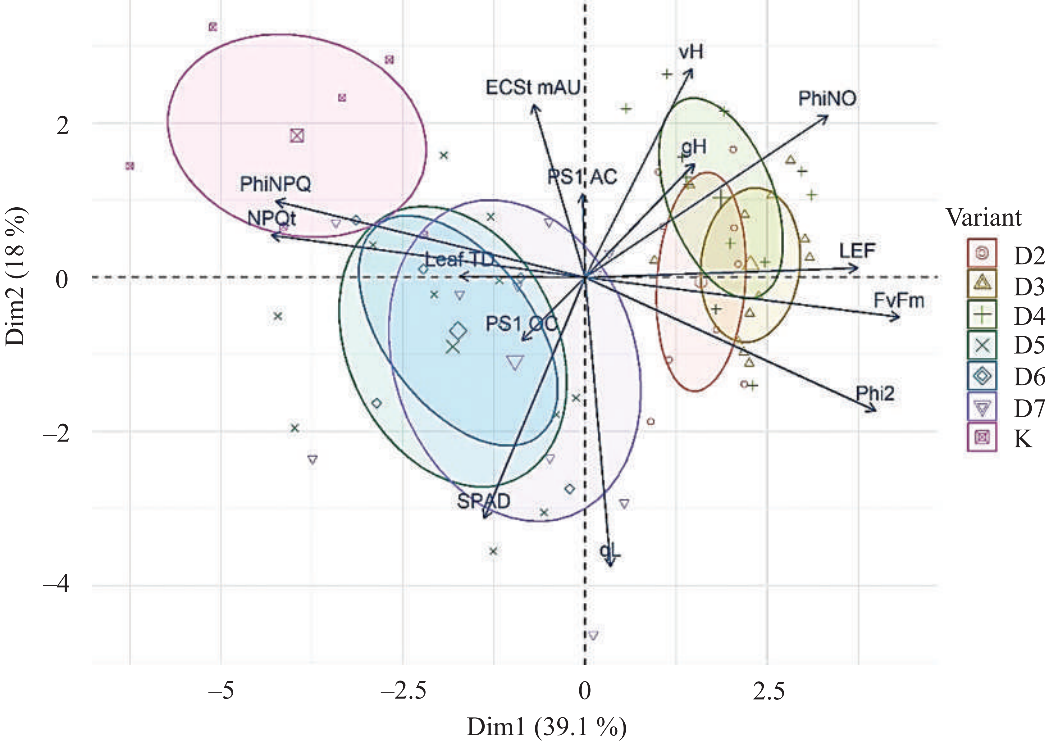

Fig. 4. The PCA of chlorophyll fluorescence parameters for *S. oleracea* L. plants, their association with the content of biochars in soil (solid circles describe the experimental variants; the abbreviations are explained in the text). Abbreviations: SPAD - relative chlorophyll; LEF – linear electron flow; Leaf.TD – leaf temperature differential; PS1.OC – photosystem I open center; PS1.AC – photosystem I active center; FvFm – efficiency of PSII in light acclimatized state; PhiNO – fraction of energy lost through non-regulated photosynthesis processes (ϕNO); NPQt – non-photochemical quenching, evaluated without the adjustment to the dark; qL – fraction of open centers of photosystem II; PhiNPQ – fraction of light involved in non-photochemical quenching(ϕNPQ); Phi2 – quantum yield of photosystem II (ϕII); vH – steady-state proton flux (vH$^+$); gH – conductivity of ATP-synthase (gH$^+$); ECSt.mAU – capacity of ATP-synthase (ECSt)

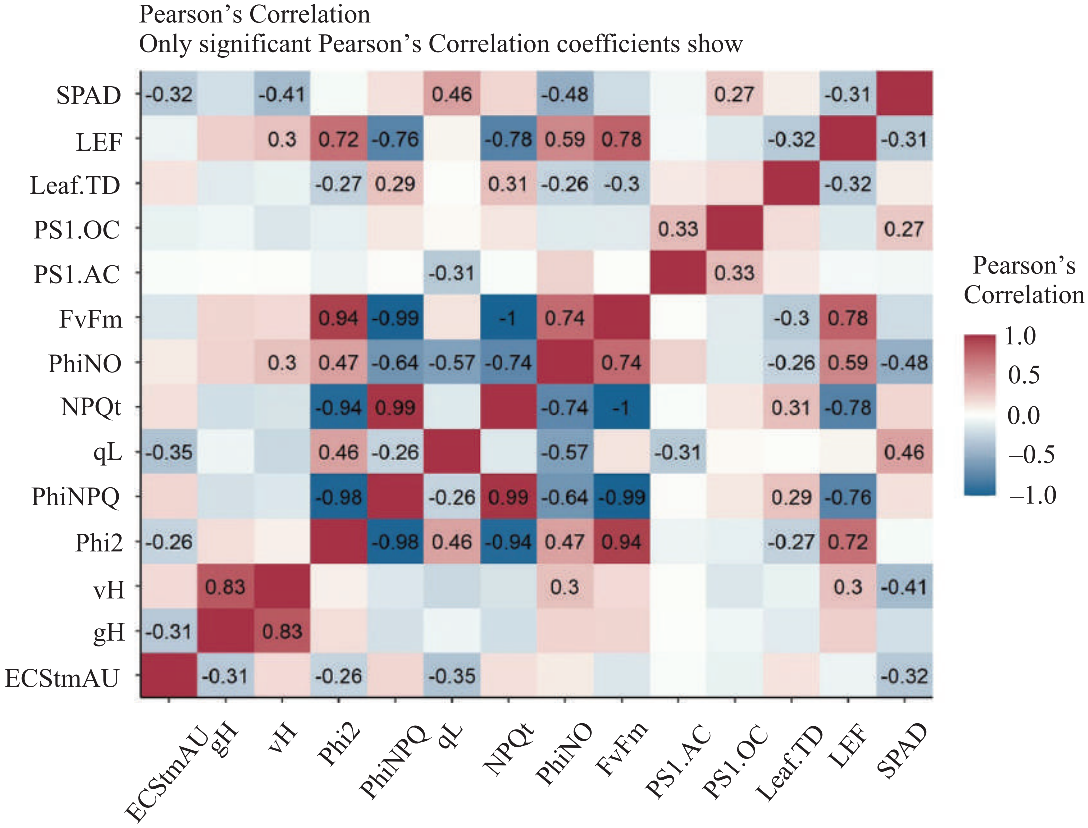

Fig. 5. Statistically significant ($p < 0.05$) correlation coefficients within the investigated parameters of the chlorophyll fluorescence induction (CFI) of prickly seeded spinach under the effect of two different biochars. Abbreviations: SPAD – relative chlorophyll; LEF – linear electron flow; Leaf.TD – leaf temperature differential; PS1.OC – photosystem I open center; PS1.AC – photosystem I active center; FvFm – efficiency of PSII in light acclimatized state; PhiNO – fraction of energy lost through non-regulated photosynthesis processes (ϕNO); NPQt – non-photochemical quenching, evaluated without the adjustment to the dark; qL – fraction of open centers of photosystem II; PhiNPQ – fraction of light involved in non-photochemical quenching(ϕNPQ); Phi2 – quantum yield of photosystem II (ϕII); vH – steady-state proton flux (vH^+); gH – conductivity of ATP-synthase (gH^+); ECSt.mAU – capacity of ATP-synthase (ECSt)

onstrates the impact of the two biochars on some physiological processes in the chloroplasts of prickly seeded spinach.

The newly-formed PCs explain about 57.1 % (the sum of PC1 – 39.1 % and PC2 – 18.0 %) of the variation in the investigated physiological parameters of photosynthesis. There are evident associations between the investigated parameters, which was additionally confirmed by a separate correlation analysis. For instance, ϕII, Fv/Fm, and LEF have a negative correlation with ϕNPQ, NPQt, and SPAD – with vH^+, gH^+ and ECSt, etc. (**Fig. 5**).

The PCA graph (**Fig. 4**) clearly demonstrate that Bch1 and Bch2, their respective variants and the control form separate groups. This distribution into groups is based on physiological indices (ϕNPQ, NPQt, ϕII, Fv/Fm, LEF), describing the primary processes of photosynthesis in *S. oleracea* L. under the impact of biochars from different raw materials.

The following statements describe the correlation between the analysed parameters: a) fluorescent parameters such as ϕII, and ϕNO were positively correlated with each other but negatively correlated with ϕNPQ; b) ϕII did not show a correlation with the SPAD, but had positively correlation with qL; c) SPAD had a positive correlation with qL and negative with such parameters as the capacity of ATP-synthase, steady-state proton flux, and ϕNO (**Fig. 4**).

DISCUSSION

It was determined that in the presence of biocarbon in the substrate, the efficiency of consumption and distribution of substances in plants improved (Hossain et al, 2020; Feng et al, 2021; Hou et al, 2022). PSA, especially the primary processes of photosynthesis, occurring at the level of photosystem II, is a rather sensitive link in the photo assimilation process of plants regarding such effects.

Fluorometric measurements reliably detecting changes in the level of primary photosynthesis processes in plants, grown in soil with or without addition of biochar have been determined before (Lyu et al, 2016; Wang et al, 2021). To determine the interrelations between the fluorescence parameters of the PSA, Principal Component Analysis (PCA) was used (Jolliffe, 2002). We also applied PCA and found compensation effects of photochemical efficiency for dissipation by regulated and non-regulated energy losses. It is characterized by the placement in different quadrants of the coordinate system of ϕII, NPQt, and ϕNO (Fig. 4) (Wang et al, 2021). The localization of the chlorophyll fluorescence parameters of the experimental variants Bch1 and Bch2 in quadrants different from the control in the two-dimensional coordinate system (Fig. 4) indicates the effect of biochar in reducing the level of regulated dissipation of light energy as heat. The phenomenon of heat dissipation occurs at the level of photosystem II (Genty et al, 1989). Non-photochemical quenching of chlorophyll fluorescence is associated with a decrease in the rate of photosystem II photochemistry. In our case, Bch1 decreased the NPQt, ϕNPQ, and increased ϕII. A similar effect of biochar was described by Tang et al. (2020). The doses of Bch1 and Bch2 did not influence this parameter; only the type of biochar was significant.

Successful regulation of the photosynthetic process generally aims at maximal values of ϕII, with the remaining aimed at a maximal ratio of ϕNPQ/ϕNO (Klughammer et al, 2008). ϕNO indicates that the absorbed light energy cannot be consumed completely through photochemical energy conversion and protective regulation mechanisms (Klughammer et al, 2008). There is the relative stability of φNO, which results from compensatory changes in φII and φNPQ (Kramer et al, 2004).

This study showed only Bch1 compared to control significant difference in ϕNO. This parameter negatively correlated with: φNPQ, qL and SPAD (Fig. 5). Both biochars increased qL, indicating that photosystem II was not inhibited, i.e., the transportation of electrons to the following stages after the restoration of plastoquinone was not blocked on this level. (Jin and Mi, 2002; Kramer et al, 2004). It was shown that both types of biochars enhanced the proportion of open PSII reaction centers and photosynthetic electron transfer rates in *Spinacia oleracea* and reduced heat dissipation, enabling full use of the light energy absorbed in leaves for photosynthesis.

The cause of the launch of the photoprotective mechanism of photosystem II is the transmembrane gradient of pH (ΔpH). It was shown that ΔpH across the thylakoid membrane of chloroplast initiates the photoprotective mechanism of photosystem II from excessive excitation (Kanazawa et al, 2017).

Neither used biochar (Bch1 and Bch2) didn’t increase ECSt; on the contrary, values decreased, demonstrating the absence of violations in the systems of the outflux of metabolites. Thus, we assume the imbalance between the presence of protons and their use for ATP synthesis was absent. Impairment of this balance usually results in the acidification of the chloroplast thylakoid lumen and, consequently, the activation of photoprotective processes (Tietz et al, 2017).

Photosystem I (PSI) is a critical component of photosynthesis in plants and plays a decisive role in the reactions of transportation of electrons during the absorption of light and transformation of energy. Active PSI centers are relevant for the efficient transformation of light energy into chemical energy and the generation of restorative energy for further metabolism processes (Kanazawa et al, 2017) The changes in the PSIactiv fraction may indicate adaptive responses of a plant or stress states, impacting the efficiency of the photosynthetic apparatus (Kanazawa et al, 2017).

We assumed that the increase in NPQ in Bch2 compared with Bch1 is caused by changes at the photosystem I level. Miyake et al (2005) showed that cyclic electron flow around PSI induced NPQ of Chl fluorescence when LEF is limited by the availability of an electron acceptor. In our opinion, it may be caused by a smaller content of chlorophyll in the leaves which characterizes (or which determines) the SPAD parameter.

Moreover, the application of biochar enhanced relative leaf chlorophyll content, which facilitated the synthesis of enzymes and electron transporters involved in photosynthetic carbon assimilation, thereby improving photosynthetic function in leaves, as was also found byWang et al, 2021and Hou et al, 2021. Consequently, the light energy absorbed by the leaf was more effectively utilized in photochemical processes, resulting in an increase in φII and a decrease in φNPQ. In summary, these findings confirm the potential of biochar to improve chlorophyll fluorescence traits. Further investigation is warranted to elucidate the internal mechanisms by which biochar enhances chlorophyll fluorescence characteristics.

CONCLUSIONS

Two different biochars, one from above-ground biomass of *Miscanthus* (Bch1) and one from sewage sludge (Bch2), when introduced into pots with chernozem soil, increased the maximum quantum efficiency of PSII by 3–7 % and decreased the level of regulated dissipation of light energy in the form of heat by 17–39 %. An increase in quantum efficiency was determined in PSII with both types of biochar, by 5–9 %; the biochar obtained from the sewage sludge caused a higher, by 17–19 %, relative chlorophyll content (SPAD) in the parenchyma of leaves.

Based on our analysis of the photosynthesis apparatus (PSA) features (including chlorophyll fluorescence induction parameters) analysed via fluorescence and absorbance measurements with the MultispeQ v.1.0 fluorometer we determined that the efficiency of photochemical processes in *S. olerace*a L. plants growing in the soil with the addition of Bch1 and Bch2 was higher by 9.3 and 5.1 % respectively than in the control plants. Addition of biochar could be one of the strategies for improving the functional PSA state of spinach plants and increase yield in the end. Further (field) studies should confirm our findings.

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Вплив двох видів біочару на фотосинтетичний апарат рослин шпинату городнього (*Spinacia oleracea* L.)

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Мета. Порівняти вплив двох видів різносировинних біочарів та їхніх концентрацій у ґрунті на основні процеси фотосинтезу рослин. **Методи.** Фотосинтетичну активність рослин шпинату городнього (*Spinacia oleracea* L.) гібриду Corvair F1 вимірювали у контрольованих вегетаційних умовах, у посудинах в камері для вирощування (t = 24–26 °C, рівень фотосинтетично активної радіації 150 мкмоль фотонів $м^{-2}$ $с^{-1}$ 16 год на добу; вологість субстрату – 60% повної вологоємності) на стадії розвитку четвертого справжнього листка (BBCH 14) за допомогою портативного флюориметра MultispeQ v1.0 (https://www.photosynq.com), фіксуючи такі параметри як квантова ефективність фотосистеми II (ϕII), квантовий вихід нефотохімічного гасіння хлорофілу (ϕNPQ), частку світлової енергії, що втрачається через нерегульовані процеси (ϕNO), qL – частка відкритих реакційних центрів ФСІІ; Fv′/Fm′ – максимальна квантова ефективність ФСІІ, ECSt – ємність АТФ-синтази; gH^+ – провідність АТФ-синтази; vH^+ – стаціонарний потік протонів. Два використані біочари походили з надземної біомаси рослин *Miscanthus* (Bch1, варіанти D2–4) та з осаду стічних вод муніципальних очисних споруд (Bch2, варіанти D5–7) у кількості 1 % (D2; D5), 3 % (D3; D6), 5 % (D4; D7) від сухої маси чорнозему типового важкосуглинкового слабогумусованого. Контрольні рослини (К) вирощували у ґрунті без внесення біочару. Отримані дані були піддані статистичній обробці в середовищі R та RStudio з використанням ANOVA, методу Крускал-Волліс, HSD тесту Тьюкі та методу головних компонент (МГК, Principal Component Ana-lysis). Вибірка у межах окремого варіанту досліду складала 10 рослин. **Результати**. За умов внесення біочарів Bch1 і Bch2 було виявлено, що вони впливають на фотосинтетичні властивості рослин, зокрема, на вміст хлорофілів. Біочар Bch1 не призводив до значного зростання відносного вмісту хлорофілу (SPAD) в листках шпинату, натомість, Bch2 суттєво (на 17–19 %) збільшував SPAD. Присутність біочару в ґрунті позитивно змінювало температурний диференціал (TD) листків, який є індикатором транспірації і вказує на водозабезпечення рослин. Найбільш значимим від’ємним TD характеризувались листки варіантів D3 (Bch1,

3 %) і D7 (Bch2, 5 %), оводнення яких, у порівнянні з контролем, було вищим на 3 та 1,7 % відповідно. Дослідження первинних процесів фотосинтезу за індукцією флуоресценції хлорофілу виявило, що обидва біочари загалом мають позитивний вплив на фотосинтетичну активність, зокрема на квантову ефективність фотосистеми II (ϕII) та максимальний квантовий вихід фотосинтезу (Fv′/Fm′) при додаванні 3 %. Нефотохімічне гасіння без темнової адаптації (NPQt) було нижчим у варіантах з біочаром Bch1 на 35–39 %, що свідчить про ефективніше використання світлової енергії для фотохімічних процесів і вказує на те, що біочар може сприяти зменшенню дисипації світлової енергії та підвищенню фотосинтетичної ефективності. Загалом, обидва види біочарів зменшили втрати світлової енергії та підвищили ефективність фотосинтезу на 3–7 %, що вказує на можливість практичного застосування цих добрив як засобу стимуляції фотосинтезу і урожаю *Spinacia oleracea* L. **Висновки.** Внесення в чорнозем типовий важкосуглинковий слабогумусовий біочарів обох видів у кількості 1–5 % обумовило підвищення фотохімічної ефективності та зниження на 17–39 % рівня нефотохімічного гасіння флюорисценції хлорофілу в рослинах шпинату. Зростання, на 3–7 % максимального квантового виходу та на 6–9 % квантової ефективності фотосистеми II разом із меншими значеннями ϕNPQ та NPQt, порівняно з контролем, свідчать про вищу ефективність фотохімічних процесів у рослин, що росли в ґрунті з доданням біочарів. Потрібно провести подальші польові дослідження, аби підтвердити продовження тривалості підвищеної ефективності фотосинтезу і зрештою отримання здоровіших рослин та вищого врожаю.

Ключові слова: *Spinacia oleracea* L., фотосинтез, флюоресценція хлорофілу, біочар.

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