Scientific Papers. Series B, Horticulture. Vol. LXVIII, No. 2, 2024
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

USING BIOCHAR AND ORGANIC FERTILIZER IN THE CULTIVATION OF BOK CHOY (*BRASSICA RAPA* L. SSP. *CHINENSIS* L.)

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Abstract

In period during 2022 and 2023 years a pot experiment was conducted in a greenhouse using alluvial-meadow soil. Two seeding dates were tested. Five variants with different doses of biochar and humate fertilizer (Bioforce) was developed. The test crop was leafy vegetable - Bok Choy (Brassica rapa L. ssp. chinensis L.). Results indicate that increased moisture and temperature in the vegetative house caused premature budding and flowering of the plants. In the variant with Soil + Bioforce (humic fertilizer) *showed high photosynthetic activity, while variant Biochar 112.5 g per pot + Bioforce (20%) exhibited the best biometric indicators. Plants grown in March-April were taller but had less mass compared to those from December. The mass flowering makes the method suitable for seed production but not for consumption. Sugar levels followed a similar trend in both assessments. The least amount of accumulated sugars was in variant 3 with 50% Biochar. Variant 4 consistently had the highest sugar content, ranging from 7.6 to 7.7% Brix. The low nitrate content in leaf tissue was evidenced. The nectar`s sugar content and pollen quantity were assessed to determine the honeybee potential of Bok Choy by the tested variants.*

***Key words**: Bok Choy, Brassica rapa L. ssp. chinensis L., biochar, humate fertilizer, honeybee potential.*

INTRODUCTION

In recent years, research in agriculture has been focused on developing good agricultural practices not only to achieve high yields but also to produce products that meet international quality standards and recommended levels of environmental impact. One of the primary environmental problems stemming from agricultural activities is the pollution of surface and groundwater with nitrates, as well as harmful emissions of greenhouse gases released from the soil. That is why countries developing modern agriculture are placing increasing importance on technologies for utilizing crop residues. In the context of climate change, the idea of applying biochar in agriculture has drawn considerable attention among scientific communities (Lehmann, 2007). Biochar has a wide range of applications and advantages due to its distinctive characteristics such as high cation exchange capacity, adsorption capacity, microporosity, high carbon content, and stable structure, and it is widely used in carbon sequestration, soil restoration, water and wastewater purification (Mohanty et al., 2013; Rizwan et al., 2016).

Leafy vegetables that require minimal processing are of great interest to consumers due to their easy usage. Bok choy (*Brassica rapa*) is a vegetable from the Brassicaceae family, which also includes cabbage, broccoli, cauliflower, and Brussels sprouts. It is also very closely related to turnips and rutabagas. In their results, Genesio et al. (2012) confirm the positive influence of applying biochar from rice husks on the growth and development of lettuce (*Lactuca sativa*) and Chinese cabbage (*Brassica chinensis*). The use of biochar in vegetable cultivation has been studied by many authors, and the conducted research unequivocally demonstrates that its application, in combination with balanced organic fertilization, leads to better results (Mikova et al., 2015).

Priadi and Nuro (2017) conducted experiments with Bok choy using organic and inorganic substances and found that their combination led to the highest parameters of seedling growth and leaf indices, as well as positive correlations between growth parameters.

Bok choy is still a relatively unknown vegetable crop in Bulgaria. There is scant literature data regarding its cultivation in our country, which is the subject of the present study.

The aim of the research is to evaluate the impact of combined application of carbonized plant residues (biochar) rate and humic fertilizer as soil amendments on the growth and development of Bok choy.

MATERIALS AND METHODS

During 2022 and 2023 years, a pot experiment was conducted on alluvial-meadow soil from the field of Vrazhdebna distruct - Sofia. The experiment was set up in the glazed greenhouse of the University of Forestry. The tested crop was leafy vegetable - Bok choy (*Brassica rapa L. ssp. chinensis L.*).

Five variants were developed, in three replications:

- Control - clean soil without amendments;
- Soil + Bioforce (humic fertilizer);
- Biochar 225 g per pot + Bioforce (50%) (BC 50%);
- Var. 4 Biochar 112.5 g per pot + Bioforce (20%) (BC 20%);
- Ver. 5 Biochar 67 g per pot + Bioforce (15%) (BC 15%).

The quantity of biochar (BC) applied was chosen based on literature studies (Haryanto et al., 2022), aiming to track the influence of fertilization with the humic fertilizer product name "Bioforce", and the biochar's ability to absorb and release nutrients more slowly. The application of the biofertilizer occurred through irrigation water, with the same quantity used for all variants.

For the experiment setup, pots with 5 kg of soil were used, to which the respective presenting of biochar p pot were added. Two sowing dates were tested on December 2 (2022), and March 10 (2023), with 10 seeds of Bok choy planted per pots. At the stage of the second true leaf, three plants were left in each container.

The biochar used for the experiment was produced from wood chips.

The humic fertilizer was applied twice with the irrigation water at both sowing dates, in the initial phase of development and at the stage of 3-4 true leaves. The dosage was 100 mL per pots diluted 1:10 according to the manufacturer's recommendations.

Maintaining an optimal watering regime close to the field capacity was ensured by frequent irrigation with a small amount of water, with the quantity of water used determined using the gravimetric method.

Phenological observations and biometric measurements were conducted during the vegetation period. The photosynthetic activity, total sugar content, nitrates, and absolute dry matter content in the experimental plants were determined. After determining the fresh weight, the leaves were placed in a dryer at 60°C, where they were dried to obtain the absolute dry weight. The leaf water content was determined using the formula described by Jin et al. (2017). The chlorophyll content was measured according to Sarah C. et al., 2013, methodology. The percentage content of sugars in the plant cell (Brix, %) was measured with a refractometer model - Digital refractometer 32145, manufactured by B & C Germany.

The biochar used to conduct the experiment was produced from wood chips. Table 1 presents the chemical analysis of biochar the data being provided by the manufacturer.

Table 1. Chemical characteristic of biochar (BC)

	pH	EC mS/m	W%	C%	N%	P%	K%	Ca%	Mg%	$CaCO_3$
BC	**10.8**	**45**	**10.3**	**61.8**	**0.39**	**0.22**	**0.85**	**2.18**	**0.23**	**5.4**

The pH reaction of biochar is strongly alkaline. It contains a large amount of carbon, which confirms the ability of BC to deposit carbon in the soil, reducing its release into the atmosphere.

Pre-measured standards were introduced into each vessel in the form of a fine powder fraction.

The humate fertilizer used for pot experiment is "Bioforce" based on lombricompost with the following commercial characteristics:

Table 2. Chemical characteristic of Bioforce

pH		Total N g/kg	P_2O_5 mg/ 100 g	K_2O mg/100 g	Humus %
H_2O	KCl				
9.0	8.4	155.5	260.0	760.3	32.3

According to data given by producer the reaction of humic fertilizer is highly alkaline, it is well stocked with nutrients.

RESULTS AND DISCUSSIONS

Evaluation of the abiotic factors during the vegetation experiment

Temperature, light, and air humidity play an important role in greenhouse production and can have a significant impact on the growth and development of plants. Light and warmth are essential vital factors determining the growth and development of vegetable crops.

Deviation from these factors can halt life processes, leading to plant death. High temperature is one of the stressful factors that significantly reduce photosynthetic activity and plant productivity (Oukarrorum et al., 2002). Agrometeorological and phenological observations are valuable sources of information for understanding the relationship between climate and plant development during the vegetation period.

During the conducted experiment (December-May), the temperature in the greenhouse was monitored.

During the vegetative growth of Bok choy at both sowing dates, entirely positive temperatures were observed. Temperatures for the first vegetation period from December 2 (2022) to February 20 (2023) ranged between 8 and 15°C, with no sharp temperature fluctuations.

For the second sowing date from March 13 (2023), to May 26 (2023) temperatures increased up to 22°C at the end of April. The optimal temperature for the development of this crop is considered to be between 13°C and 24°C. The temperatures recorded by us are suitable for the normal growth and development of the crop. Despite the suitable temperature range at both sowing dates, the plants do not form well-rounded rosettes and transition to the budding and flowering phase.

Relative humidity (RH %) is the ratio between the amount of moisture present in the air and the maximum amount of moisture that the same air can hold at the same temperature and atmospheric pressure. Relative humidity is expressed in percentages. When the air is at 100% RH, it cannot hold more water vapor. Warm air can hold more water vapor than cold air. Therefore, as the air temperature increases, Figure 1. Air temperature during the period 2022-2023 Bok choy growing season RH decreases, and as the temperature drops, RH increases. If the air becomes cold enough, moisture condenses out of the air as dew.

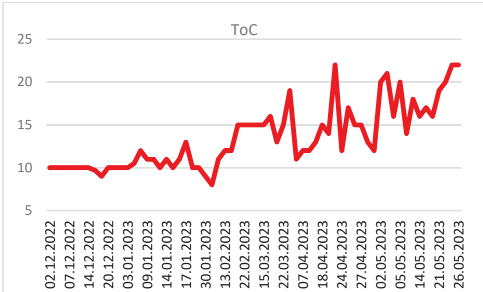

Figure 1 Temperature dynamics in greenhouse

The humidity during the crop's vegetation period is high (Figure 2), reaching up to 97%. The lowest values were recorded in early February, which resulted from the temperature rise. When values above 90% are recorded, the greenhouse is ventilated. High humidity is also associated with the plants' ability to absorb water, as the saturated air reduces transpiration in plants.

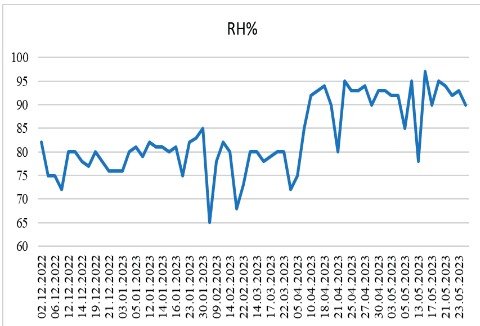

Figure 2. Relative humidity during the period 2022-2023 Bok choy growing season

The increased air humidity combined with high soil moisture likely exerts a suppressive effect on the development of the crop. Arve et al. (2011) reported that reduced ventilation, which consequently leads to increased relative humidity, affects plants in various ways and may result in plants that are less tolerant to water stress. This type of water stress leads to deformation of the stomata. To minimize the negative effects of this type of water stress,

plants respond by altering their growth pattern, producing stress proteins, enhancing regulation of antioxidants, accumulating compatible solutes, increasing the quantity of transporters involved in water and ion absorption and transportation, and by closing stomata.
The increased air humidity combined with higher temperatures is one of the probable reasons for the premature budding and flowering of Bok choy. Regardless of the temperature differences in the two development periods and at both sowing dates, mass flowering occurs between 50 and 55 days of vegetative growth.

Evaluation the impact of simultaneous application of organic fertilizers and biochar on the growth metrics and fresh yield of Bok Choy

About a week after sowing the Bok choy, the number of sprouted plants was counted in replicates.
The highest number of germinated plants were recorded in variant 3 with 50% biochar, with an average of 8.33 and 7 plants per variant, followed by variant 4 with 20% biochar. In both sowing dates, the lowest number of germinated plants was observed in the control variant, where a slower development of the plants was also observed.
Simultaneously with the recording of germinated plants, the phenological development of Bok choy was observed and described by variants.
It is noteworthy that the plants treated with biochar appear to be better developed compared to the control variant. The leaf rosette is better developed.

Table 3. Number of sprouted plants per pots during first showing date 02.12.2022

Variants	Replication			Aver. Number of germinated plants
	I	II	III	
Control	3	5	3	3.67
Bioforce	7	4	2	4.33
BC 50%	10	5	10	8.33
BC 20%	9	5	4	6.00
BC 15%	6	3	5	4.67

Table 4. Number of sprouted plants per pots during second showing date 10.03.2023

Variants	Replication			Aver. Number of germinated plants
	I	II	III	
Control	0	4	3	2.33
Bioforce	3	7	1	3.67
BC 50%	10	1	10	7.00
BC 20%	9	4	6	6.33
BC 15%	7	7	2	5.33

The data obtained upon completion of the experiment regarding the size of the leaves are presented in Tables 5 and 6.

Table 5. Size of the leaves at the first sowing date 02.12.2022

Variants	Leaves number	Leaves petiole cm	Leaves width cm	Leaves length cm
Control	6.44	2.87	2.29	3.57
Bioforce	7.44	3.27	3.48	4.94
BC 50%	9.30	2.10	3.85	4.95
BC 20%	9.70	1.96	4.17	5.01
BC 15%	9.80	2.60	3.59	4.81

The number of leaves varies between 6 and 14 in both sowing dates. Dinceva (2020) obtained the same data regarding the leaf count in a field experiment conducted at the Maritsa Vegetable Crops Research Institute. During the first sowing period, there are fewer leaves compared to the second growing period. In the first sowing date, the highest number of leaves were recorded in variant 5 with 15% biochar, and the lowest in the control variant. The plants from the first period have shorter petioles and smaller leaf rosettes compared to the second sowing date. The larger leaf mass is likely a result of higher air humidity, a statement supported by the results obtained by Arve et al. (2011). Another possible reason is the application of biochar, which during the second growth period has released a larger amount of NH_4^+. Biochar has a strong absorbing capacity and subsequently slow release of nutrients into the soil. Gomez-Eyles et al. (2013) report that the application of biochar increases the cation exchange capacity of the soil, with NH_4 ions being retained on the surface of the biochar.

Table 6. Size of the leaves at the second sowing date 10.03.2023

Variants	Leaves number	Leaves petiole cm	Leaves width cm	Leaves length cm
Control	10.25	4.44	6.16	8.95
Bioforce	10.88	5.15	5.81	9.04
BC 50%	7.29	4.80	6.04	8.39
BC 20%	9.25	5.25	5.84	9.01
BC 15%	9.71	5.63	6.37	8.50

The length of the whole plants together with the peduncles, which are shown in Figures 3 and 4, was recorded.

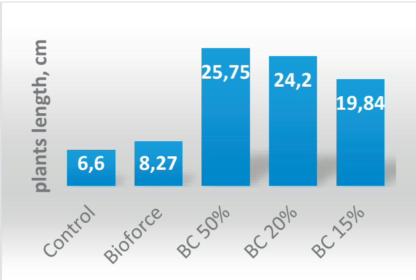

Figure 3. Plant height at harvest on the first sowing date

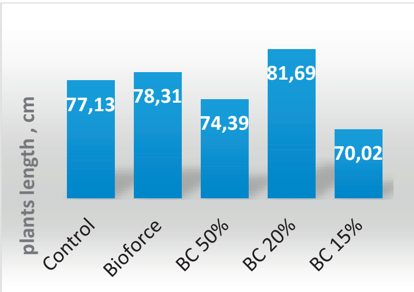

Figure 4. Plant height at harvest on the second sowing date

The height of the plants sown on December 2 (2022) ranges between 6.6 and 25.7 cm. The tallest plants were recorded in variant 3, followed by variant 2. Despite their relatively small height in the control variant, the plants from the first two variants approach the standard height of Bok choy, which ranges between 13 and 30 cm. The significant height of the plants is due to etiolation and elongation of the flowering stem.

The plants grown during the March-April period are on average about 50 cm taller than those sown in December. The strong elongation of the stems is likely due to insufficient lighting and the greater amount of infrared light. Infrared light has been proven to inhibit flowering in Bok choy. The tallest plants are in variant 4, while the shortest are in variant 5.

The number of flowering stems per variant is depicted in Figure 5.

The average number of flowering stems varies between 2.57 and 3.75. Variant 4 recorded the highest number of flowering stems, with individual plants reaching up to 6 stems, which is due to the combined effect of humic fertilizer and biochar. This variant demonstrates the highest biometric indicators, which determines the applied norm of 20% as suitable for Bok choy cultivation. The mass flowering during Bok choy cultivation in a glass greenhouse makes this method suitable for seed production but not for growing a crop suitable for consumption.

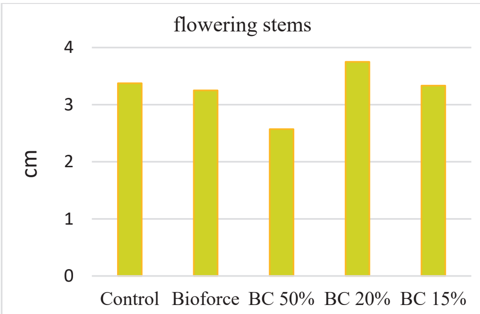

Figure 5. Average number of flowering stems

The yield of fresh weight for the first period, December to March, is lower than that recorded for the vegetation period from March to May. During the first period, it ranges between 15.2 and 17.7 g.

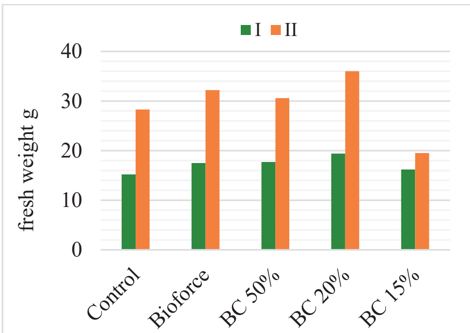

Figure 6. Fresh weight at harvest of Bok choy

It is noteworthy that the yield of fresh weight in the variant with the addition of biochar slightly exceeds that obtained from the control variant with pure soil. Although biochar itself is poor in nutrients, this is likely due to the improved water and air regime created as a result of its application, along with increased nutrient adsorption.

Chan et al. (2017) report that the application of biochar in combination with fertilization positively affects the growth and development of plants, but sometimes a negative effect is observed in variants with only biochar without added fertilizer due to reduced bioavailability, resulting from nitrogen adsorption. This is likely the reason for the lower fresh weight observed in variant 3 with the highest biochar application dose.

The fresh weight during the second vegetation period, March-May, is about 35% higher, with the highest average weight per plant observed in variant 4 with 20% biochar, corresponding to the soil volume, which is consistent with other biometric measurements obtained.

Crop yield is a result of the systemic interaction of processes occurring in plants. Assimilates produced by photosynthesis can be stored or distributed among different plant organs. Crop yield and plant development are not only a function of assimilate production and distribution but also an expression of the relationships between water and carbon, which interact with each other. This interaction is expressed through leaf temperature, stomatal conductance, relative water content in plant tissues, and leaf area.

The content of dry matter in Bok choy leaves is a direct indicator of the quality of the obtained product.

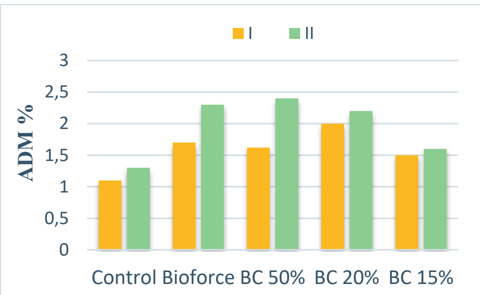

Figure 7. Absolute dry mass in 2022/2023 Bok choy plant samples

The percentage water content in plant samples is an indicator of maintaining an optimal water regime during vegetation.

Influence of combined organic fertilization and BC on some physiological indicators

Carbohydrates constitute the major portion (up to 85-90%) of the substances composing plant organisms. These are the structural, nutritional, and energetic materials of plant cells and tissues.

Measuring the content of total sugars in plant tissues (Brix %) is an important indicator because it provides us with a direct understanding of how the plant is functioning. A sugar molecule produced in the process of photosynthesis is the primary building block for everything we see growing above and below ground.

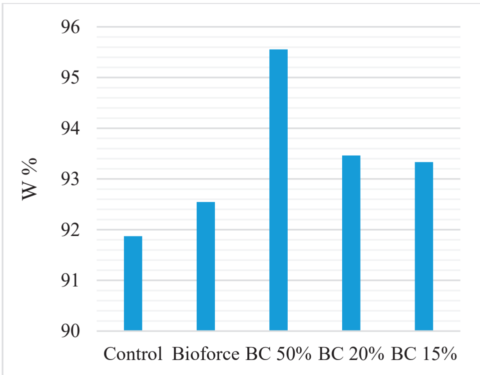

Figure 8. Moisture percentage in Bok choy plant samples 2022/2023

The percentage water content in plant samples is an indicator of maintaining an optimal water regime during vegetation.

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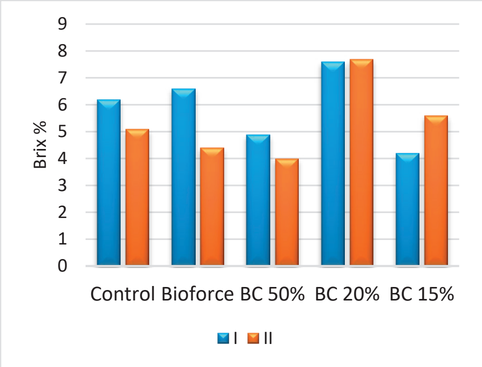

Figure 9. Percentage of total sugars in Bok choy leaf (2022/2023)

The amount of sugars follows the same trend in both observations. The least rate of accumulated sugars is found in variant 3 with 50% biochar. This is due to the higher water content in the leaf tissues observed in this variant. The highest amount of sugars is found in variant 4 for both sowing dates, ranging from 7.6 to 7.7%, which corresponds to the water content in the leaves of this variant.

Accumulation of nitrates in plants results from the uptake of nitrate ions and subsequent assimilation. Of all nitrogen sources in the soil, plants primarily uptake ammonium.

The regulation does not mention the permissible content for Bok choy, but the defined levels for spinach and lettuce are 3500 mg NO_3^-/kg.

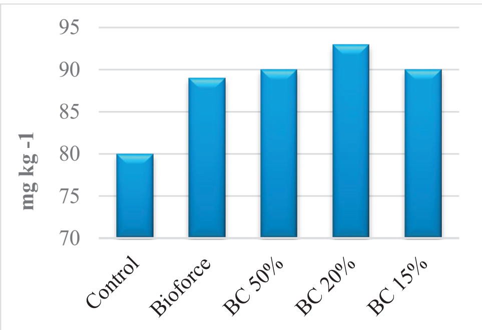

Figure 10. Nitrate content of Bok choy leaf (2022/2023)

The gradual increase in nitrates in the variants with added biochar is likely due to biochar's ability to absorb nutrients and release them over a longer period. The nitrate values range from 80 to 93 mg/kg, with the highest value recorded in variant 4, which also has the best-developed leaf mass.

Despite the presence of nitrates in fresh Bok choy leaves, the concentration does not exceed the regulated limit in Regulation 1881/2006, which classifies the product as suitable for consumption.

Photosynthesis is a unique physiological process inherent to photoautotrophic green plants.

Chlorophylls, the primary colour pigments in green vegetables, have two main types: chlorophyll "a" and chlorophyll "b". Chlorophyll "a" typically occurs in concentrations 2-3 times higher than chlorophyll "b" in agricultural products (Kirca et al., 2006). Carotenoids in plant tissues have two main functions: they participate in the absorption and transfer of radiant energy to chlorophyll "a" and protect chlorophyll molecules.

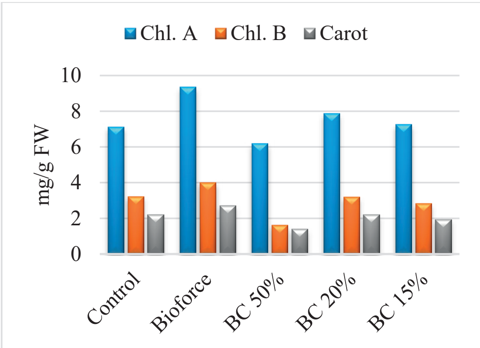

Figure 11. Content of plastid pigments in leaves of Bok choy

With the highest photosynthetic activity, variant 2 with the addition of humic fertilizer Bioforce. The absence of biochar accelerates the absorption of nutrients. Among the variants with added biochar, variant 4 with 20% of the norm again shows the best indicators. The ratio of 1:2 between chlorophyll a and chlorophyll b is preserved in all variants. The content of carotenoids ranges between 1.42 and 2.74 mg/g FW. The absence of elevated levels of carotenoids indicates the absence of stress in the cultivated plants.

CONCLUSIONS

The soil and climatic conditions inside the greenhouse are favourable for growing Bok choy, and the growth period progresses under favourable conditions.

The increased humidity combined with higher temperatures is one of the probable reasons for premature budding and flowering of Bok choy. Regardless of the temperature differences in the two development periods and at both dates, mass flowering occurs between the 50th and 55th day of vegetative growth.

The highest number of germinated plants is recorded in variant 3 with 50% biochar, averaging 8.33 and 7 plants per variant, followed by variant 4 with 20% biochar. In both seeding dates, the least germinated plants are observed in the control variant, where a slower development of the plants is also observed.

Plants grown during the March-April period are on average about 50 cm taller than those seeded in December. The strong elongation of the stems is likely due to insufficient light and a greater amount of infrared light. Infrared light has been proven to inhibit flowering in Bok choy. The tallest plants are in variant 4, while the shortest are in variant 5.

The mass flowering during the cultivation of Bok choy in a greenhouse makes this method suitable for seed production but not for growing a crop suitable for consumption.

The yield of fresh biomass for the first period from December to March is lower than that reported for the vegetation period from March to May. The fresh biomass during the second vegetation period from March to May is about 35% higher, with the highest average mass per plant observed in variant 4 with 20% biochar relative to soil volume, which corresponds to other biometric measurements obtained.

There is a gradual increase in nitrates in the variants with added biochar (BV), which is likely due to the ability of biochar to absorb nutrients and release them over a longer period. The nitrate values range between 80 to 93 mg/kg-1, with the highest value reported in variant 4, which also exhibits the best-developed leaf mass. Despite the presence of nitrates in fresh bok choy leaves, the concentration does not exceed the levels regulated by Regulation No. 1881/2006, which designates the production as suitable for consumption.

Variant 2, with the addition of Bioforce humic fertilizer, exhibits the highest photosynthetic activity. The absence of biochar results in faster nutrient absorption. Among the variants with added biochar, variant 4 with 20% of the recommended amount consistently shows the best performance.

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