EFFECT OF DIFFERENT FERTILIZER APPLICATIONS ON MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS OF SPINACH (*SPINACIA OLERACEA* L.) GROWN UNDER FIELD CONDITIONS

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Abstract. This study investigated the effects of different fertilizer applications on the morphological and physiological characteristics of spinach grown under field conditions. The treatments included a control, chemical fertilizer (CF), three levels of farmyard manure (FYM) combined with CF, and three levels of biochar (BC) also combined with CF. The experiment was conducted in a randomized block design with eight treatments, two growing periods and two sowing periods (autumn: 15 October; spring: 15 March), and three replications, resulting in a total of 48 plots. Statistically significant differences were observed among treatments for several parameters, including plant number, leaf number, leaf area (cm² plant⁻¹), SPAD value, leaf relative water content (LRWC, %), soluble solid content (SSC, °Brix), and membrane damage index (MDI, %). The CF + 30 t ha⁻¹ FYM treatment yielded the highest values for plant number (336.75 plants per 4 m²), leaf number (21.08 per plant), leaf area (48.09 cm² plant⁻¹), SPAD value (60.86), LRWC (84.42%), and SSC (9.08 °Brix), whereas the control treatment showed the lowest values across all traits. The results demonstrate that increasing FYM rates significantly improved plant performance, and BC also showed potential as an effective organic amendment.
Keywords: *biochar, chemical fertilizer, farmyard manure, physiological traits, spinach*

Introduction

Vegetable farming is increasingly recognized as a cornerstone of global nutrition, as vegetables are a rich source of essential minerals and vitamins vital for human health (Kilavuz and Erdem, 2019). Among these, spinach (*Spinacia oleracea* L.) holds a prominent place due to its short cultivation cycle and high nutritional value, making it a widely cultivated leafy vegetable globally and in Turkey (Sensoy et al., 2011; FAOSTAT, 2022). However, intensive vegetable production often relies heavily on chemical fertilizers, which, although effective in increasing yield, may degrade soil health, cause environmental pollution, and contribute to nitrate accumulation in edible tissues — a significant health concern for consumers (Abdolahi Arshad et al., 2024).

Fertilization remains a crucial determinant of crop productivity and quality, with mineral deficiencies or imbalanced fertilization strategies potentially leading to significant yield losses and compromised nutritional content. Although chemical fertilizers are commonly used to supply nutrients, recent research emphasizes the

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importance of organic and integrated fertilization strategies for sustainable crop production (Adiloglu and Eraslan, 2012). Organic fertilizers, derived from plant and animal materials, improve soil organic matter, enhance nutrient retention, and support beneficial microbial activity — factors essential for long-term soil fertility and environmental sustainability (Kacar and Katkat, 2007).

Farmyard manure (FYM), especially from sheep and cattle, is among the most widely utilized organic amendments. It is known for its capacity to improve soil structure, enhance water retention, reduce nutrient leaching, and promote microbial diversity (Schoenau, 2006; Goksu and Kuzucu, 2017). Similarly, biochar (BC)—a carbon-rich material produced via the pyrolysis of organic biomass under limited oxygen—has gained attention for its ability to improve soil physicochemical properties, increase cation exchange capacity, and mitigate the adverse effects of abiotic stressors (Blackwell et al., 2009; Lehmann and Joseph, 2009; Gunal and Erdem, 2018; Mounirou et al., 2020). Biochar's porous structure and mineral-rich profile make it particularly effective in enhancing nutrient availability and facilitating the synergistic use of chemical fertilizers.

As the global population continues to rise, so does the demand for nutrient-rich vegetables. Conventional reliance on synthetic fertilizers to meet this demand poses long-term risks to soil fertility and food quality. Consequently, integrating alternative fertilizers such as biochar and farmyard manure into existing fertilization regimes has emerged as a key strategy in sustainable agriculture. Research demonstrates that these amendments not only enhance crop yield and quality but also rehabilitate degraded soils (Luan et al., 2025; Zhang et al., 2025). For instance, Pandey et al. (2024) explored the synergistic effects of biochar and compost on cadmium-contaminated soils and found improvements in soil properties and reductions in pollutant bioavailability. Additionally, biochar is increasingly recognized for mitigating the harmful effects of salinity and heavy metals on crop performance (Soothar et al., 2021; Boostani et al., 2021; Gavili et al., 2018; Rasheed et al., 2024).

Organic amendments such as chicken manure have also been widely used to boost plant growth and nutrient uptake under challenging conditions, including heavy metal contamination (Celik and Kunene, 2021). Similarly, acidified biochar has recently been proposed as a sustainable phosphorus source capable of balancing nutrient availability and supporting robust plant growth (Sahin et al., 2024). Optimization of fertilization strategies is especially crucial for spinach production, as this leafy crop requires efficient nutrient management to avoid nitrate accumulation while ensuring high biomass output (Altuntas et al., 2018).

Several studies have examined the role of organic inputs on spinach growth. Citak and Sonmez (2010) and Sharma et al. (2024) reported enhanced yield, improved soil fertility, and reduced dependency on synthetic fertilizers through the application of FYM and bioinoculants such as *Azotobacter*. Mufwanzala and Dikinya (2010) demonstrated that poultry manure could simultaneously serve as a nutrient source and potentially introduce salinity stress, highlighting the need for balanced organic amendments. Furthermore, combinations of poultry manure and biochar have shown promise in increasing spinach biomass and nutrient uptake (Siddiqui et al., 2021).

Recent interest in biochar and biochar-based fertilizers has grown due to their potential for improving crop performance, enriching nutrient availability, and stabilizing soil organic matter and resilience to stress factors (Keskinaslan et al., 2023; Ahmad et al., 2024; Hussain et al., 2025; Sheikh et al., 2025; Ul Haq et al., 2025; Zhang et al.,

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2025). These materials not only enhance nutrient-use efficiency but also support the antioxidant defense system in plants under stressful conditions (Rasheed et al., 2024).

Given this background, it is hypothesized that the application of FYM and BC, particularly when integrated with chemical fertilizers, will synergistically enhance the morphological and physiological traits of spinach grown under field conditions. This includes improvements in parameters such as plant number, leaf development, SPAD value, water status, and biochemical quality. Furthermore, it is expected that increasing the dose of FYM and BC will produce more pronounced effects, contributing to sustainable soil fertility and improved crop resilience across different sowing periods. The primary objective of this study is to evaluate the effects of different fertilizer combinations — including chemical fertilizer, FYM, and BC — on key growth and physiological traits of spinach cultivated in autumn and spring sowing periods in two growing seasons. The study aims to generate data that can support the development of environmentally friendly and efficient fertilization strategies to enhance spinach production and soil health under open-field conditions.

Materials and methods

Materials

The study utilized the spinach (*Spinacia oleracea* L.) cultivar ‘Matador’ (Sunagri Seeds) as the plant material. ‘Matador’ is widely recognized for its adaptability and favorable agronomic traits. It features broad, smooth, dark green oval leaves with short petioles and forms a flat rosette close to the soil surface. Noted for its rapid vegetative growth and cold resistance, this variety is among the most widely cultivated spinach types across Turkey due to its high yield potential and tolerance to varying climatic conditions.

Three types of fertilizers were used in the experiment: one chemical fertilizer (CF) and two organic fertilizers — farmyard manure (FYM) and biochar (BC). The FYM was derived from well-composted sheep manure, known for its high organic matter content and beneficial effects on soil structure and fertility. The biochar used in the study was produced from oak wood through pyrolysis under low-oxygen conditions, ensuring a carbon-rich and stable amendment with the potential to improve soil physical and chemical properties.

The chemical fertilizer (CF) was applied to deliver essential macronutrients at the following rates: 120 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, and 120 kg K_2O ha^{-1}. To achieve this, 80 kg ha^{-1} of a compound NPK fertilizer (15:15:15) was applied, supplemented by 40 kg K ha^{-1} provided as potassium sulfate and 40 kg N ha^{-1} as ammonium sulfate. This combination ensured balanced nutrient supply, supporting vigorous plant growth during the vegetative stage.

Methods

Experimental site and design

The study was conducted over three consecutive years, covering two distinct planting seasons from 2020 to 2022—autumn (15 October) and spring (15 March)—at the experimental fields of Van Yuzuncu Yil University, located in Van province, eastern Turkey. Climatic data for Van province during the study period (2020-2022) were obtained from Sadak Turhan et al. (2024). Average temperatures ranged from 1.4°C in

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December 2020 to 23.8°C in May 2021. Relative humidity fluctuated between 33.3% (June 2021) and 81.4% (February 2022). Precipitation peaked in February 2020 (79.9 mm) and March 2022 (68.1 mm), while summer months were largely dry. Wind speeds ranged from 0.9 m/s (January 2020) to 3.2 m/s (July 2021), reflecting typical regional variability in climatic conditions (Anonymous, 2022a). *Figure 1* illustrates the monthly average temperature and total precipitation in Van province during the spinach growing seasons of the experimental years.

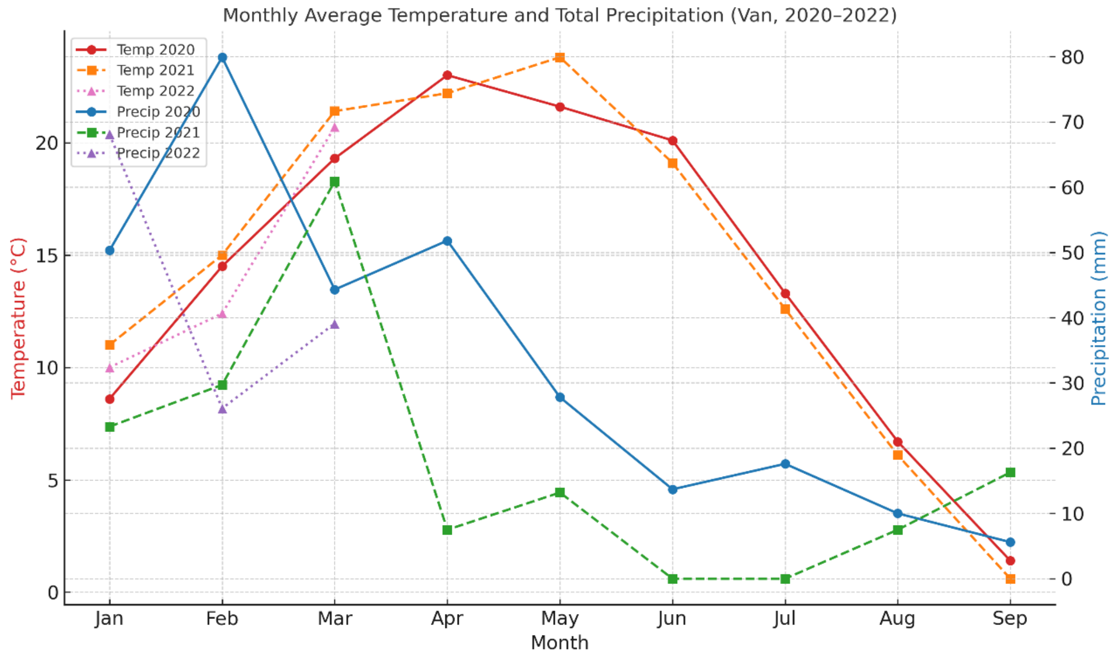

Figure 1. *Monthly average temperature and total precipitation during the spinach growing seasons in Van province (2020–2022)*

The experiment was laid out in a randomized block design with three replications. A total of 48 plots (24 per season) were established, each measuring 2 × 2 m (4 m²). The same plots were maintained over both years. The study included eight fertilizer treatments, applied in both planting periods (autumn and spring),

As follows:

1. Control (no fertilizer)
2. Chemical fertilizer (CF; 120 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, and 120 kg K_2O ha^{-1})
3. CF + 10 tons ha^{-1} farmyard manure (FYM)
4. CF + 20 tons ha^{-1} FYM
5. CF + 30 tons ha^{-1} FYM
6. CF + 1 ton ha^{-1} biochar (BC)
7. CF + 2 tons ha^{-1} BC
8. CF + 3 tons ha^{-1} BC

Fertilizers (CF, FYM, and BC) were uniformly applied to each plot approximately one week before sowing. Spinach seeds were sown at a rate of 25 kg ha^{-1} using a row-planting method, with 25 cm spacing between rows. Each plot contained eight rows. The amount of irrigation water applied was 5 mm per irrigation event on the 4 m² plots.

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In spring sowings, irrigation commenced once rainfall declined, while in autumn it continued until the onset of rainfall.

Sowing and harvest dates were as follows:

- Year 1: Autumn sowing on October 15, 2020; spring sowing on March 15, 2021. Autumn harvest on May 17, 2021; spring harvest on June 10, 2021.
- Year 2: Autumn sowing on October 15, 2021; spring sowing on March 15, 2022. Autumn harvest on May 11, 2022; spring harvest on May 28, 2022.

The study was completed over two years.

Organic fertilizer characterization

Two organic fertilizers — biochar (BC) and farmyard manure (FYM) — were analyzed prior to application. Samples (500 g) of each were tested at Martest Analysis Laboratory following the protocols described by Kacar and Kutuk (2010). The parameters analyzed included pH, electrical conductivity (EC), organic carbon content, C/N ratio, total nitrogen, water-soluble potassium oxide, and concentrations of trace elements (Cu, Fe, Zn, Mn, Ca, Mg, and Na). Phosphorus content was not examined in the fertilizer analysis.

Soil properties (2020)

The experimental field soil was alkaline (pH 8.47), moderately calcareous (8.14% lime), and low in organic matter (1.05%). Electrical conductivity was low (0.218 dS/m), indicating non-saline conditions. Macro and micronutrient levels were low: K (51.64 ppm), Mg (57.98 ppm), and Ca (337.53 ppm).

Soil properties (2021—2022)

Soil nitrogen and phosphorus contents were measured and included in the study. However, phosphorus content was not analyzed in the manure used. Soil nitrogen and phosphorus contents were measured 15 days after harvest. Due to the nitrogen content of the applied fertilizers, soil nitrogen increased in the second year. The decrease in nitrogen in the control plot in the second year is likely related to spinach cultivation in that area.

The highest average phosphorus was observed in the CF + FYM3 (biochar dose 3) treatment, while the lowest was in the control.

Soil nitrogen content (%):
Year 1 (2021): Control: 0.16; CF + FYM3: 0.14
Year 2 (2022): Control: 0.11; CF + FYM3: 0.20
Soil phosphorus content (kg/da):
Year 1 (2021): Control: 14.82; CF + FYM3: 73.17
Year 2 (2022): Control: 15.23; CF + FYM3: 91.57

Biochar properties

BC had a slightly alkaline pH (8.97), EC of 0.41 dS/m, and 3.47% organic carbon content. The C/N ratio was 6.31. Nutrient content included 0.12% water-soluble K_2O and 0.55% total N. Trace element concentrations were as follows: Cu (28.96 mg/kg), Zn (15.76 mg/kg), Mn (1.44 mg/kg), Ca (407.2 mg/kg), Mg (69.68 mg/kg), Na (349.3 mg/kg), and Fe (<0.01 mg/kg).

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Farmyard manure properties

FYM was neutral in pH (7.33) with a higher EC (4.52 dS/m). It contained 7.92% organic carbon and a C/N ratio of 9.21. Nutrient levels included 1.02% water-soluble K_2O and 0.86% total N. Micronutrient contents were: Cu (29.68 mg/kg), Fe (349.6 mg/kg), Zn (26.12 mg/kg), Mn (5.53 mg/kg), Ca (2539 mg/kg), Mg (617.5 mg/kg), and Na (2343 mg/kg).

Number of plants

The number of spinach plants per plot (4 m^2) was determined by manually counting all individuals prior to harvest.

Number of leaves per plant

Fifteen spinach plants were randomly selected from each plot, and the total number of leaves per plant was counted to calculate the average leaf number per plant.

Leaf area (cm^2 $plant^{-1}$)

Leaf area was estimated by taking leaf discs using a metal ring from 15 randomly selected leaves per plot. The discs were weighed on a precision scale, and leaf area was calculated using the area-to-weight ratio method described by Celik and Kok (2011). Leaf area measurements were taken on the day the plants reached the harvest stage, after all treatments were completed. In the autumn sowing, this corresponded to approximately 230 days after sowing.

Soil plant analysis development (SPAD) value

Chlorophyll content was estimated using a Minolta SPAD-502 chlorophyll meter (Osaka, Japan). SPAD readings were taken from 15 plants in each plot. SPAD values measurements were taken on the day the plants reached the harvest stage, after all treatments were completed. In the autumn sowing, this corresponded to approximately 230 days after sowing.

Leaf relative water content (LRWC, %)

To determine LRWC, fresh weights (FW) of leaf samples were first recorded. Samples were then placed in distilled water for 4 h to obtain turgid weights (TW), followed by drying in an oven at 65°C for 48 h to determine dry weights (DW). The LRWC was calculated using the following formula (Kusvuran, 2010):

$$\text{LRWC (\%)} = [(FW - DW) / (TW - DW)] \times 100 \quad \text{(Eq.1)}$$

Soluble solid content (SSC, °Brix)

SSC of spinach leaf juice was measured using a handheld refractometer after juicing the leaves in a solid juice extractor (Kusvuran, 2010).

Membrane damage index (MDI, %)

Leaf discs (17 mm diameter) were immersed in distilled water, and electrical conductivity (EC) was measured after 5 h (Lt). The same samples were then autoclaved

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at 100°C for 10 min, and final EC was measured. MDI was calculated using the formula below (Kusvuran, 2010):

$$MDI (\%) = [(Lt - Lc) / (1 - Lc)] \times 100 \qquad \text{(Eq.2)}$$

where: Lt = EC of stressed leaf before autoclaving / EC after autoclaving; Lc = EC of control leaf before autoclaving / EC after autoclaving

Hoeing was performed for weed control, and no chemical plant protection treatments were necessary, as no significant pest or disease pressure was observed during the study period. Although yield data were collected as part of the same experimental setup, they are presented and discussed in a separate publication focusing on growth and yield parameters (Sadak Turhan et al., 2024), to allow for a more detailed treatment of the physiological and morphological responses in the current study.

Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) in IBM SPSS Statistics 21.0 software (SPSS, 2020) to evaluate the main effects and interactions between year and treatment. Mean comparisons were performed using Duncan's multiple range test at a significance level of $p < 0.05$. Results are presented as mean ± standard deviation (SD).

Results

This study evaluated the impact of different fertilizer treatments on spinach growth under field conditions by measuring several agronomic and physiological parameters. These included the number of plants, number of leaves per plant, leaf area (cm² plant^{-1}), SPAD values, leaf relative water content (RWC, %), soluble solid content (°Brix), and membrane damage index (MDI, %) (Tables 1 and 2).

Statistical analysis revealed that both fertilizer treatments and their interaction with the treatment period had significant effects on the number of plants ($P \leq 0.05$). The CF + FYM3 treatment significantly increased plant number compared to all other treatments, reaching 336.75 plants, while the control had the lowest (92.42 plants, $P \leq 0.05$). CF + FYM2 (305.33) and CF + FYM1 (254.58) also resulted in significantly higher plant numbers than the control, CF, and CF + BC1 treatments (*Table 1*). These results indicate a substantial increase in plant establishment in response to organic and chemical fertilizer combinations.

Similarly, the number of leaves per plant varied significantly among treatments and due to the treatment period × fertilizer interaction ($P \leq 0.05$). The number of leaves per plant was significantly higher in the CF + FYM3 (21.08) and CF + FYM2 (19.17) treatments compared to the control (7.33) and CF (8.75) ($P \leq 0.05$). The CF + FYM3 group was statistically different from all treatments except CF + FYM2 and CF + FYM1 (*Table 1*). This trend suggests that nutrient-rich treatments enhance vegetative growth by promoting leaf production.

Fertilizer treatments also significantly influenced leaf area ($P \leq 0.05$). CF + FYM3 resulted in the highest leaf area (48.09 cm²) and was significantly greater than all treatments except CF + FYM2 (46.30) ($P \leq 0.05$). Both were significantly higher than the control (28.08) (*Table 1*). These results reinforce the positive correlation between nutrient availability and leaf expansion.

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Overall, fertilizer applications significantly improved plant number, leaf number, and leaf area compared to the control. The highest values across all three parameters were consistently observed in the FYM treatments, particularly at the 30 tons ha^{-1} dose in combination with CF. A dose-dependent response was evident, as increasing FYM rates led to progressive improvements in these growth indicators.

Interestingly, the treatment involving 3 tons ha^{-1} of biochar combined with CF achieved results comparable to the 10 tons ha^{-1} FYM + CF treatment, particularly in terms of plant number and leaf area. Additionally, the CF-alone treatment showed similar performance to the 1 ton ha^{-1} biochar + CF treatment, suggesting that biochar can partially substitute for FYM under certain conditions. These observations align with previous studies reporting synergistic effects of organic amendments and mineral fertilizers on crop performance.

Table 1. *Effect of different fertilizer applications on morphological traits in spinach plants*

Treatments	Fall seasons' average	Spring seasons' average	Mean
Plant number (per 4 m^2)			
Control	136.50 ± 4.27 E*	48.33 ± 2.52 CD*	92.42 ± 48.39 D*
CF	171.17 ± 1.76 DE	72.17 ± 5.75 C	121.67 ± 54.36 CD
CF + BC1	240.33 ± 2.75 D	90.00 ± 3.77 C	165.17 ± 82.39 C
CF + BC2	273.50 ± 2.18 CD	114.17 ± 5.01 B	193.83 ± 87.34 C
CF + BC3	304.00 ± 3.61 C	142.17 ± 2.02 AB	223.08 ± 88.68 BC
CF + FYM1	333.17 ± 3.88 C	176.00 ± 2.65 AB	254.58 ± 86.14 B
CF + FYM2	407.00 ± 4.00 B	203.67 ± 1.26 A	305.33 ± 111.40 B
CF + FYM3	460.33 ± 3.01 A	213.17 ± 1.89 A	336.75 ± 135.40 A
Leaf number per plant			
Control	9.00 ± 0.00 BC*	5.67 ± 0.29 BC*	7.33 ± 1.83 BC*
CF	10.67 ± 0.58 BC	6.83 ± 0.29 B	8.75 ± 2.14 B
CF + BC1	12.67 ± 0.58 B	8.83 ± 0.29 B	10.75 ± 2.14 B
CF + BC2	14.17 ± 0.29 B	11.83 ± 0.29 AB	13.00 ± 1.30 AB
CF + BC3	15.83 ± 1.04 B	14.33 ± 0.76 AB	15.08 ± 1.16 AB
CF + FYM1	18.17 ± 0.29 AB	15.50 ± 0.87 A	16.83 ± 1.57 AB
CF + FYM2	21.00 ± 0.50 A	17.33 ± 0.29 A	19.17 ± 2.04 A
CF + FYM3	23.50 ± 1.00 A	18.67 ± 0.76 A	21.08 ± 2.76 A
Leaf area (cm^2 $plant^{-1}$)			
Control	33.12 ± 0.46 C*	23.05 ± 0.37 C*	28.08 ± 5.53 D*
CF	35.85 ± 1.00 C	26.43 ± 1.26 BC	31.14 ± 5.26 C
CF + BC1	40.04 ± 1.65 BC	31.73 ± 0.88 B	35.88 ± 4.70 C
CF + BC2	44.01 ± 0.72 B	34.33 ± 0.79 AB	39.17 ± 5.35 BC
CF + BC3	46.79 ± 1.01 B	36.60 ± 0.76 AB	41.70 ± 5.64 B
CF + FYM1	50.04 ± 0.85 AB	38.68 ± 1.01 AB	44.36 ± 6.28 B
CF + FYM2	52.34 ± 0.94 A	40.25 ± 0.19 A	46.30 ± 6.65 AB
CF + FYM3	54.20 ± 0.43 A	41.97 ± 0.38 A	48.09 ± 6.71 A

*Significant at $P \leq 0.05$ level; data are expressed as mean ± standard deviation. Control: no fertilizer; chemical fertilizer (CF) (120 kg N ha^{-1}: 80 kg P_2O_5 ha^{-1}: 120 kg K_2O ha^{-1}); BC1, BC2, and BC3: 1, 2, and 3 tons oak biochar ha^{-1}, respectively; FYM1, FYM2, and FYM3: 10, 20, and 30 tons of sheep manure ha^{-1}, respectively

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Table 2 presents the data on SPAD value, leaf relative water content (RWC), soluble solid content (°Brix), and membrane damage index (MDI), reflecting the physiological responses of spinach plants to the different fertilizer treatments.

Table 2. *Effect of different fertilizer applications on physiological traits in spinach plants*

Treatments	Fall seasons' average	Spring seasons' average	Mean
SPAD value			
Control	37.52 ± 1.01 C*	36.17 ± 1.05 D*	36.84 ± 1.18 D*
CF	40.70 ± 0.72 BC	38.42 ± 0.25 CD	39.56 ± 1.34 C
CF + BC1	43.22 ± 0.78 BC	41.83 ± 0.68 C	42.53 ± 1.00 C
CF + BC2	49.82 ± 0.75 B	45.43 ± 0.74 B	47.63 ± 2.49 BC
CF + BC3	52.52 ± 1.13 B	48.12 ± 0.60 B	50.32 ± 2.54 B
CF + FYM1	58.82 ± 0.94 AB	52.97 ± 0.46 B	55.89 ± 3.27 AB
CF + FYM2	61.57 ± 0.60 AB	56.37 ± 0.98 A	58.97 ± 2.94 A
CF + FYM3	63.82 ± 0.33 A	57.90 ± 0.31 A	60.86 ± 3.25 A
Leaf relative water content (%)			
Control	62.54 ± 9.57 D*	55.66 ± 3.89 D*	55.66 ± 3.89 D*
CF	76.73 ± 2.18 C	71.57 ± 0.33 C	71.57 ± 0.33 C
CF + BC1	78.48 ± 2.69 BC	76.52 ± 3.54 BC	76.52 ± 3.54 BC
CF + BC2	79.82 ± 3.09 BC	78.66 ± 3.29 B	78.66 ± 3.29 B
CF + BC3	79.61 ± 2.67 BC	79.07 ± 3.07 B	79.07 ± 3.07 B
CF + FYM1	81.63 ± 7.97 B	81.25 ± 5.75 AB	81.25 ± 5.75 AB
CF + FYM2	82.21 ± 4.15 B	82.15 ± 9.99 AB	82.15 ± 9.99 AB
CF + FYM3	93.45 ± 0.79 A	84.42 ± 1.33 A	84.42 ± 1.33 A
Soluble solid content (°Brix)			
Control	7.17 ± 0.08 ^{ns}	5.77 ± 0.19 ^{ns}	6.47 ± 0.78 ^{ns}
CF	7.97 ± 0.10	6.18 ± 0.13	7.08 ± 0.98
CF + BC1	8.37 ± 0.10	6.42 ± 0.13	7.39 ± 1.07
CF + BC2	8.75 ± 0.15	6.73 ± 0.08	7.74 ± 1.11
CF + BC3	9.10 ± 0.09	7.00 ± 0.13	8.05 ± 1.15
CF + FYM1	9.47 ± 0.13	7.58 ± 0.08	8.53 ± 1.04
CF + FYM2	9.67 ± 0.15	7.87 ± 0.03	8.77 ± 0.99
CF + FYM3	9.88 ± 0.03	8.28 ± 0.13	9.08 ± 0.88
Membrane damage index (%)			
Control	10.47 ± 0.30 ^{ns}	11.45 ± 0.39 ^{ns}	11.45 ± 0.39 ^{ns}
CF	10.08 ± 0.26	10.78 ± 0.18	10.78 ± 0.18
CF + BC1	9.45 ± 0.26	10.45 ± 0.10	10.45 ± 0.10
CF + BC2	8.62 ± 0.23	10.18 ± 0.13	10.18 ± 0.13
CF + BC3	8.15 ± 0.13	9.90 ± 0.10	9.90 ± 0.10
CF + FYM1	7.60 ± 0.05	9.45 ± 0.09	9.45 ± 0.09
CF + FYM2	7.05 ± 0.13	9.07 ± 0.13	9.07 ± 0.13
CF + FYM3	5.78 ± 0.33	8.55 ± 0.18	8.55 ± 0.18

*Significant at $P \leq 0.05$ level; ^{ns}: not significant, data are expressed as mean ± standard deviation. Control: no fertilizer; chemical fertilizer (CF) (120 kg N ha^{-1}: 80 kg P_2O_5 ha^{-1}: 120 kg K_2O ha^{-1}); BC1, BC2, and BC3: 1, 2, and 3 tons oak biochar ha^{-1}, respectively; FYM1, FYM2, and FYM3: 10, 20, and 30 tons of sheep manure ha^{-1}, respectively

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Statistical analysis revealed that both fertilizer treatments and the interaction between treatment period and fertilizer type significantly affected the SPAD value ($P \leq 0.05$). SPAD values were significantly higher in CF + FYM3 (60.86) and CF + FYM2 (58.97) compared to all other treatments. The control had the lowest value (36.84) ($P \leq 0.05$). (*Table 2*). These findings suggest that the enhanced nutrient availability in the FYM + CF treatments promoted chlorophyll synthesis and improved leaf greenness.

Similarly, leaf relative water content was significantly influenced by fertilizer treatments and their interaction with treatment periods ($P \leq 0.05$). CF + FYM3 (84.42%) had significantly higher relative water content than all treatments, while the control had the lowest (55.66%) ($P \leq 0.05$) (*Table 2*). The increased RWC in the fertilized plots reflects better water retention and osmotic balance, indicating improved plant water status under nutrient-enriched conditions.

Although no significant differences were found among treatments for soluble solids (°Brix) or membrane damage index (%), the highest °Brix value (9.08) was measured in the 30 tons ha^{-1} FYM + CF treatment, suggesting enhanced sugar accumulation under improved nutrient conditions. Conversely, the lowest mean °Brix (6.47) was recorded in the control group. Likewise, the lowest MDI (8.55%), indicating reduced membrane damage and better cellular stability, was observed in the 30 tons ha^{-1} FYM + CF treatment, while the control group exhibited the highest mean MDI (11.45%) (*Table 2*).

In general, SPAD value, leaf relative water content, and soluble solid content showed substantial improvements in response to fertilizer applications compared to the control. These parameters consistently increased with the application rate of FYM, with the 30 tons ha^{-1} FYM + CF treatment yielding the highest values. The control treatment recorded the lowest values across all measured physiological parameters. Interestingly, the 3 tons ha^{-1} biochar + CF treatment yielded values comparable to the 10 tons ha^{-1} FYM + CF application, while the CF-alone treatment produced results similar to the 1 ton ha^{-1} biochar + CF treatment. These findings are consistent with previous studies demonstrating the beneficial effects of integrating organic amendments such as FYM and biochar with chemical fertilizers on plant physiological health and performance (*Table 2*).

Discussion

The results of this study, which examined the effects of different fertilizer applications on spinach under field conditions, are consistent with previous research, confirming the positive role of fertilization in enhancing spinach growth parameters. Various metrics such as plant number, leaf number, leaf area, SPAD value, and relative water content were all influenced by the treatments applied, echoing similar findings from earlier studies.

The plant number in this study varied between 39.66 and 478.67 per plot, with the lowest value recorded in the control treatment and the highest in the treatment combining 30 tons ha^{-1} of farmyard manure with chemical fertilizer. Fertilization improved germination rates and seedling survival by enhancing soil fertility and plant vigor. This aligns with studies like Xu and Mou (2016), who observed an increase in plant number with vermicompost applications, and Roy et al. (2009), who found that combining cow manure with chemical fertilizers led to higher plant numbers. Similarly, Jakhro et al. (2017) reported that combining farmyard manure with inorganic fertilizers enhanced plant number, a finding that supports the notion of organic and inorganic fertilizers' synergistic effects on spinach growth.

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Regarding leaf number, values ranged from 5.33 to 25.00 per plant. The highest leaf count was observed in the treatment with 30 tons ha^{-1} of farmyard manure combined with chemical fertilizer, which aligns with Kovacs et al. (2016), who reported increased leaf numbers with higher doses of horse manure. Similarly, Citak and Sonmez (2010) demonstrated that farmyard manure and chemical fertilizers combined yielded the highest leaf numbers compared to other treatments.

Leaf area values, ranging from 21.89 to 58.90 cm^2 per plant, were also significantly influenced by fertilizer treatments. The highest leaf area was found in the treatment with 30 tons ha^{-1} of farmyard manure plus chemical fertilizer, mirroring findings from studies like Shaheen et al. (2017) and Altuntas et al. (2018), who observed similar results with organic fertilizers, especially farmyard manure. Toksoy (2019) further corroborated these results, suggesting that biochar could serve as a viable alternative to organic fertilizers in spinach cultivation.

Chlorophyll content, measured by SPAD values, ranged from 35.63 to 66.30 SPAD units, with the highest chlorophyll content observed in the 30 tons ha^{-1} farmyard manure plus chemical fertilizer treatment. This is consistent with Kumarpandit et al. (2017) and Montemurro et al. (2015), who found increased SPAD values with organic fertilizer applications, particularly farmyard manure. Similarly, studies like Aisha et al. (2013) and Jafarpour and Rahimzadeh (2015) highlighted significant improvements in chlorophyll content due to biofertilizers and organic amendments.

The leaf relative water content ranged from 50.00% to 94.34%, with the highest values achieved by the 30 tons ha^{-1} farmyard manure plus chemical fertilizer treatment. This is in line with findings from Dichio and Montanaro (2005) and Ashraf and Iram (2005), who reported that organic fertilizers could significantly enhance leaf water retention, particularly in spinach under varying water availability conditions.

Further, the membrane damage index ranged between 5.33% and 10.73%, with the lowest values observed in the biochar and farmyard manure treatments. This reduction in membrane damage due to organic fertilizers correlates with studies by Basdic and Kabay (2022), Kaya and Dasgan (2013), and Furkan (2019), who emphasized the protective role of organic fertilizers under stress conditions. Our study showed that organic treatments improved stress tolerance, as evidenced by lower membrane damage compared to the control.

While yield is an essential indicator for assessing treatment efficiency and agricultural sustainability, the current study was designed to focus on plant morphological and physiological traits under different fertilization regimes. The yield outcomes from this experiment have been previously published in a separate manuscript (Sadak Turhan et al., 2024), which complements the findings presented here.

Overall, the fertilizer treatments applied in this study resulted in significant improvements in all growth parameters, consistently outperforming the control group. These findings support the growing body of evidence that organic and integrated fertilization strategies are beneficial for spinach cultivation, contributing to enhanced growth, yield, and stress resilience. The observed improvements are particularly notable in the combination of farmyard manure and chemical fertilizers, which consistently produced the highest values for key growth parameters.

Conclusion

In vegetable production, proper and balanced fertilization is crucial for optimal growth and yield. The absence of essential nutrients or the use of incorrect fertilization

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practices can significantly impact both the quantity and quality of crops. While chemical fertilizers are commonly used in vegetable cultivation worldwide, promoting the use of organic fertilizers is key to improving the sustainability of agricultural practices and maintaining ecological balance. This study aimed to assess the effects of biochar as an organic fertilizer supplement to conventional chemical fertilizers on spinach growth and development under different planting periods.

The results indicate that organic fertilizers had a significant positive impact on spinach growth. Specifically, an increase was observed in plant number, leaf number, leaf area, SPAD value, leaf relative water content, and soluble solid content. The combination of farmyard manure at 30 tons ha^{-1} and chemical fertilizer produced the highest values for these parameters. The other fertilization treatments, ranked by effectiveness, were farmyard manure at 20 tons ha^{-1} + chemical fertilizer, farmyard manure at 10 tons ha^{-1} + chemical fertilizer, and biochar applications. Additionally, the lowest membrane damage index was recorded with the farmyard manure at 30 tons ha^{-1} + chemical fertilizer treatment.

Overall, farmyard manure applications resulted in superior growth and development compared to other fertilization methods. This study highlights the potential of organic fertilizers, particularly farmyard manure, when used in conjunction with chemical fertilizers, to enhance spinach cultivation, improving both yield and quality. Moreover, these findings lay the groundwork for future research on sustainable fertilization strategies and provide valuable insights for upcoming studies.

This study provides detailed insights into how different fertilizer treatments influence key morphological and physiological traits of spinach. Although yield data were not discussed herein, they are available in a related publication (Sadak Turhan et al., 2024), which together with the present work offers a comprehensive assessment of the agronomic performance of the tested treatments.

In conclusion, our results are consistent with existing literature, demonstrating the beneficial effects of organic fertilizers like farmyard manure and biochar, when combined with chemical fertilizers, on spinach growth. These findings highlight the importance of integrated fertilization strategies in enhancing vegetable production while ensuring sustainability and improving crop quality.

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