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Effect of nanosilver and biochar application on plant physiological characteristics under water stress conditions

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Abstract – Drought negatively affects crop productivity, especially crop quality and sustainability. In recent years, the use of biochar, which plays an important role in combating drought, has come to attention. Biochar is an organic/vegetable material with high surface area, porosity, and water retention capacity. Nanoparticles accelerate soil improvement and plant growth by reducing the need for fertilizer. In light of this information, it was aimed to determine the effects of biochar and nanoparticles on plants under water stress conditions. For this purpose, biochar (B0= No biochar, B1= biochar) and nano silver (N0= no nano silver, N1= nano silver) were applied under varying levels of irrigation water (I100= Full irrigation, I75= 25% water deficit, I50= 50% water deficit), treatments on plant physical properties (leaf relative moisture content, chlorophyll content, membrane damage index) and leaf color parameters (L*, a*, b*, croma and hue) were investigated. At the end of the study, the highest irrigation water use was determined in the I100N1B0 treatment (212.4 mm) and the lowest in the I50N0B1 treatment (130.8 mm). Biochar application decreased the membrane damage index of the plant and increased the chlorophyll content, leaf relative water content, and green color intensity in the leaves of the plant. As a result, it was determined that using biochar in irrigation water deficit applications positively improved the plant's physiological characteristics. In addition, it was determined that the effects of nano silver application on plant physiology were not statistically significant.

Keywords – Water Deficit, Nano Silver, Biochar, Plant Stress, Physiological Properties

I. Introduction

The increasing population and dwindling available water resources threaten food security globally [1]. In addition, many factors, including soil erosion, low fertility, soil pollution, and salinization, are leading to land degradation and severe economic constraints [2]. Declining arable land and climate change-induced agricultural production threaten millions of people with poverty and malnutrition. Therefore, alternative and innovative practices are needed to mitigate the impacts of climate change and protect existing water resources and soil quality.

Biochar is an alternative soil amendment considered as a 'carbonized organic matter produced for use in a variety of environmental applications, including energy production, soil and water quality improvement, and climate change mitigation [3,4]. Characteristics that make biochar an alternative option for improving and enhancing soil functions include high soil cation exchange capacity, adsorption capacity, organic carbon content, water retention, and nutrient retention capacity [5]. Furthermore, biochar is an effective method to increase soil water retention in relation to field capacity, wilting point, and available water [6]. Liu et al. [7] reported that by mixing biochar obtained from straw into the soil, the water-holding capacity of the soil increased between 0.056-0.068 m3 m-3 and this was due to the high surface area of the biochar. High surface area and high porosity lead to changes in the tensile strength of the soil, which promotes plant growth [8].

Nanoparticles can be defined as nanomaterials that provide one or more nutrients to the plant and enhance plant growth and development [7,9]. Nano fertilizers are slowly soluble and highly effective fertilizers. They have the potential to be preferred more because they can be easily taken up by plants without being washed out of the soil and without binding to organic matter, clay, and lime [10]. Since nano fertilizers provide healthy growth and development of the plant, the plant gains more resistance to severe and variable atmospheric conditions and diseases. Akhoundnejad et al. [11] determined the highest plant height, stem diameter, leaf width, plant width, and root dry matter content values in onion at the dose of nanosilver nanoparticles (50 ppm).

Researchers have conducted studies on the water deficit-biochar relationship, water deficit-plant stress mechanism, and water limitation-nano silver. However, there is no study that examined the effect of biochar and nanosilver application on plant physiology together in reduced irrigation water applications. It is also possible that biochar and nanosilver application under irrigation water deficit conditions may reduce the amount of irrigation water use and plant stress. Therefore, the aim of this study was to contribute to the explanation of the relationship between biochar material with high surface area, which provides water retention, and nano silver, which will help plant nutrient uptake, and plant stress mechanisms under deficit irrigation conditions.

II. Materials and Method

Material And Experimental Plan

The study was conducted in the climate room of Van Yüzüncü Yıl University, Faculty of Agriculture. During the experiment, the average temperature of the climate laboratory was set at 25±2 °C and the humidity was set at 60±5%. Common bean (*Phaseolus vulgaris* L.) was used as plant material and nano silver was used as nanoparticle material. Soil sieved through a 4 mm sieve and mixed homogeneously was used as a growing medium. Biochar purchased from a commercial company was sieved through a 2 mm sieve to minimize wind losses and particle passage through the soil [12]. The sieved soils were filled into the pots (Figure 1a.) by mixing 1% biochar on a weight basis, totaling 4 kilograms each (Figure 1b). Four seeds were sown in each pot. Weeds seen on the soil surface in the pots were pulled out by hand and weed control was carried out after sowing the seeds.

a. b.

Fig. 1. Weighing of soils (a) and filling the pots with soils (b)

The experiment was conducted with 3 different irrigation water levels (I0: 100%; full irrigation-control; I1: 25% deficit; I2: 50% deficit), 2 different doses of nanosilver (N0: control and N1: 80 ppm,) and 2 different doses of biochar (B0: 0-control, B1: 1% w/w) in a total of 36 pots in 3 replicates.

Characteristics Of Soil And Biochar

The texture of the experimental soil was clay loam characterized by 42.2% sand, 35.4% clay, and 22.4% silt, and the volume weight of the soil was 1.27 g cm^{-3}. In the preliminary study, the pH, electrical conductivity, organic matter, and total nitrogen contents of the soil were determined as 7.88, 0.34 dS m^{-1}, 1.02 g kg^{-1}, and 0.93%, respectively. Biochar material produced from rose pruning residues was used. Biochar pH, total N, soil organic carbon, and cation exchange capacity values were determined as 8.14, 1.32%, 88.75, and 31.23 cmol kg^{-1}, respectively.

Irrigation

The amount of irrigation water to be applied to each pot was calculated based on the volume of moisture content in the pots using a portable moisture meter (HH2 Moisture Meter, WET Sensor, Delta-T Devices, Cambridge, UK). In a preliminary study, the moisture meter was calibrated to the soil used in the experiment and the specific volumetric moisture content of the medium at pot (field) capacity was determined. Irrigation was applied at a soil moisture level (I0: 100% of TK) that would reach the potting capacity (PC). Irrigation was regularly applied at I1 (75% of TK) and I2 (50% of TK), equivalent to I0 (control).

Physiological Measurements

In order to determine leaf reletive water content, fresh weights (Fw) of leaf samples were weighed and recorded. The weighed leaves were kept in pure water for four hours and their turgor weights (Tw) were weighed. Then, these leaves were kept in an oven at 65 ºC for 24-48 hours and their dry weights (Dw) were determined. Leaf relative water content was calculated according to equation (1):

$$\text{LRWC} = \frac{\text{Fw}-\text{Dw}}{\text{Tw}-\text{Dw}} \times 100$$

Chlorophyll content index (CCI) was measured using a portable SPAD-502 Chlorophyll meter (KONICA MINOLTA Japan) on 10 leaves of 10 plants in pots and the average of these measurements was recorded as SPAD value.

Membrane damage was calculated by measuring the outflow of electrolyte from leaf cells [13]. Discs (10 mm diameter) were taken from the leaves of the plants in the pots, left in 50 ml tubes in 30 ml de-ionized water for 24 hours at room temperature and then the electrical conductivity of the water in the tube was measured and the EC1 value was obtained. Then the tubes were kept in a water bath at 95-100 °C for 20 minutes, cooled to room temperature and the electrical conductivity of the water in the tube was measured again and the EC2 value was obtained. The membrane damage index (MDI) was determined by using the first and second values in the formula below.

$$\text{MDI} = \frac{EC1}{EC2} \times 100$$

Leaf color measurement; changes in leaf color were determined from different points on the upper surface of the leaves of the plants using a Minolta CR-400 (Minolta Camera Co., LTD Ramsey, NJ) color chromameter [14].

Statistical Analysis

The experiment was carried out as a pot study with 3 replications according to the coincidence plots factorial experiment design. SSPS (Version 23.0) package program was used for statistical analysis of the data. One-way analysis of variance (One-way ANOVA) was used to compare the findings and DUNCAN multiple comparison test was used to determine the differences between the groups [15].

III. RESULTS

During the germination and development phase of the plants, weed control and moisture monitoring were carried out regularly in the pots. When the plants reached sufficient maturity (Fig. 2), sampling was done and the experiment was terminated.

Fig. 2. General view of the plants

Irrigation Water

Different amounts of irrigation water were applied under the three irrigation regimes presented in Table 1. The irrigation interval was between 3-4 days depending on the moisture depletion in the pot. A higher amount of irrigation water was used in the full irrigation (I100) treatment compared to the other irrigation treatments.

Table 1. Amounts of irrigation water for common bean plants under treatments

Treatments	Irrigation water, mm
I100N0B0	211.1
I100N0B1	203.7
I100N1B0	212.4
I100N1B1	207.7
I75N0B0	173.1
I75N0B1	167.3
I75N1B0	174.2
I75N1B1	170.4
I50N0B0	135.2
I50N0B1	130.8
I50N1B0	136.0
I50N1B1	133.2

In this study, chlorophyll content index (CCI), leaf relative water content (LRWC), membrane damage index (MDI), and leaf color contents of plants were determined under irrigation water stress conditions of biochar amendment and nanosilver application (Tablo 2).

Table 2. Chlorophyll content index (CCI) of common beans grown under treatment, Leaf relative water content (LRWC) and membrane damage index (MDI)

Irrigation levels	Nano silver	Biochar	SPAD	LRWC, %	MDI, %
I100	N0	B0	42.73	88.38	23.04öD
		B1	43.93	91.35	22.22
	N1	B0	43.17	87.38	24.54
		B1	45.13	90.32	23.15
I75	N0	B0	39.47	75.89	32.55
		B1	39.43	77.06	31.03
	N1	B0	38.77	76.47	32.03
		B1	39.33	79.25	30.87
I50	N0	B0	32.83	62.37	43.52
		B1	34.17	67.21	41.32
	N1	B0	34.57	63.97	43.98
		B1	34.43	65.93	41.14
I100			43.74 A	89.36 A	23.24 C
I75			39.25 B	77.17 B	31.62 B
I50			34.00 C	64.87 C	42.49 A
P irrigation			0.000	0.000	0.000
P nanosilver			0.232	0.779	0.647
P biochar			0.290	0.000	0.032
P irrigation x nano silver			0.290	0.304	0.675
P irrigation x biochar			0.938	0.633	0.699
P nano silver x biochar			0.329	0.729	0.845
P irr. x nano silver x biochar			0.067	0.341	0.952

I100: Full irrigation, I75: 25% deficit irrigation, I50: 50% deficit irrigation, N0: No nano silver-control treatment, N1: Nano silver, B0: no biochars, B1: biochars.

At the end of the study, leaf colors of the plants in each pot were determined with a colorimeter, averages were taken and given in Table 3. In irrigation water levels, a*, b*, Croma and Hue values were statistically significant except for L color parameter (Table 3). In biochar applications, L*, b*, Croma and Hue values were statistically significant except a* color parameter. Nano silver application was found to be statistically insignificant in color parameters.

Table 3. Color parameters (L*, a*, b*, Croma, Hue) of common beans according to treatments

Irr. L.	Nano silver	B	L*	a*	b*	C (Croma)	H (Hue)
I100	N0	B0	33.87	-12.35	15.37	19.72	129.06
		B1	32.45	-11.40	13.76	17.87	129.88
	N1	B0	33.81	-11.65	14.99	19.19	128.99
		B1	33.98	-12.01	14.91	19.15	128.99
I75	N0	B0	33.63	-11.27	13.57	17.64	130.01
		B1	33.11	-9.85	11.45	15.11	130.93
	N1	B0	34.09	-11.13	13.30	17.35	130.14
		B1	32.02	-9.85	11.15	14.88	131.51
I50	N0	B0	33.69	-11.44	13.52	17.71	130.46
		B1	33.08	-11.44	13.13	17.41	131.21
	N1	B0	33.54	-9.75	11.61	15.18	130.39
		B1	33.29	-9.88	11.12	14.88	131.82
I100			33.57	-11.93 A	14.76 A	18.98 A	129.23 B
I75			33.22	-10.52 B	12.37 B	16.25 B	130.65 A
I50			33.37	-10.55 B	12.23 B	16.17 B	131.02 A
P irrigation			0.763	0.001	0.001	0.001	0.002
P nanosilver			0.662	0.090	0.229	0.171	0.900
P biochar			0.041	0.066	0.032	0.039	0.030
P irr. x nano silver			0.498	0.058	0.174	0.123	0.619
P irr. x biochar			0.688	0.173	0.368	0.290	0.678
P nano s. x biochar			0.967	0.486	0.647	0.592	0.891
P irr. x nano s. x biochar			0.388	0.792	0.752	0.762	0.693

IV. DISCUSSION

When all treatments were considered, less water was used in biochar applications compared to the control application. When I100 treatments with full irrigation were analyzed, the I100N0B1 treatment with only biochar application consumed 3.62% less water compared to the control treatment (I100N0B0). Similarly, 3.51% and 3.33% less water was consumed in I75 and I50 restriction treatments. It is thought that biochar has a high-water retention ability due to its highly porous structure and accordingly increases the duration of the moist environment in the soil [7, 16]. When the treatments using nano silver are examined, it is seen that higher water use is realized compared to the control treatments (Table 1). It is possible that nanosilver may increase plant growth and consequently increase the water consumption of the plant.

Chlorophyll Content

Chlorophyll content is one of the pigments important for the green and healthy growth of plants. Chlorophyll, which is the basic component of chloroplast for photosynthesis of plants, has a positive relationship with photosynthesis rate. Therefore, photosynthesis rate decreases with a decrease in chlorophyll content ([17, 18].

It is known that chlorophyll content changes when irrigation water is insufficient. At the end of the study, it was determined that irrigation water treatments affected the chlorophyll content in bean leaves (Table 2). The highest chlorophyll content was determined in the full irrigation treatment and the lowest in the I50 treatment with 50% water shortage. Drought stress causes chlorophyll degradation, leading to a decrease in chlorophyll content. On the other hand, stomatal closure, which is the first response of plants exposed to drought stress, prevents CO_2 entry in order to prevent water loss by transpiration and reduces photosynthetic carbon assimilation and photorespiration. Although SPAD values were mostly higher in biochar treatments, these results were not statistically significant. Similarly, the difference between nano silver treatments was not significant.

Leaf Relative Water Content

With water deficit, the amount of water retained by the plant in the leaf cells also decreases. Due to the decrease in water, the leaf temperature of plants increases. The increase in leaf temperature significantly reduces leaf water potential, leaf relative water content (RWC) and transpiration rate. LRWC is high in the early stages of leaf development but decreases as dry matter accumulation increases [17]. LRWC, canopy temperature, stomatal resistance, osmotic potential, turgor potential, leaf water potential, transpiration rate, and leaf temperature are important traits affecting plant water relations [19, 20]. LRWC is used as a measure of leaf water content reflecting metabolic activity in tissues and for dehydration tolerance. Table 2 shows that leaf relative water content (RWC) decreased as the amount of irrigation water decreased. Irrigation water levels had a statistically significant effect on FWC content. LRWC reached the highest levels in full irrigation treatments. However, with water shortage, there was a 16% decrease in I75 and 38% decrease in I50 treatments compared to I100 and I50 treatments, respectively. In the study, biochar applications also significantly affected the LRWC content. Higher LRWC was determined in biochar treatments compared to non-biochar treatments. Biochar application increased the water storage in the root zone of the plant and accordingly, it provided water to the plant under water shortage conditions. Leaf relative water content increased by 0.76%-4.43% in the biochar treatments of full irrigation and I75 irrigation levels compared to the non-biochar treatments, while a difference of 5.71%-7.76% was observed in the I50 restriction treatment (Table 2). Thanks to the porous structure of biochar, water retention in the root zone was ensured and it was more effective under stress conditions. Nano-fertilizer (silver) applications did not show a significant change in LRWC.

Membrane Damage Index

At the end of the study, irrigation water amounts and biochar application were found to be significant for membrane damage index, while nano silver applications were not statistically significant (Table 2). The lowest membrane damage was determined in I100 treatments where full irrigation water was applied, while the highest membrane damage was determined in I50 treatments where 50% water deficit was applied. Cell growth and cell division in plants are affected by water deficit. In particular, the decrease in turgor in plants under drought conditions suppresses cell expansion and growth [18, 20]. Drought stress causes cellular dehydration, which leads to osmotic stress resulting in a decrease in cytosolic and vacuolar volume and the removal of water from the cytoplasm to the extracellular space [21]. A marked decrease in cell volume and subsequent cell shrinkage becomes apparent as an immediate symptom caused by dehydration [22]. Membrane damage indices were also determined to be statistically significant as the plant root zone was moist in biochar applications (Table 2). Although biochar material varies according to the raw material and pyrolysis process, it has the ability to absorb and retain moisture due to its high porosity. This feature can increase soil water holding capacity [16, 23]. However, due to its high surface area, it can retain water not only inside its pores but also between particles [24]. It is suggested that the ability of biochar to adsorb and retain water is due to its pore size, which can range from 0.1 to 10 µm [7].

Color Parameters

The color of plant leaves in each pot was evaluated according to the International Commission on Illumination (CIE) system and expressed as L*, a*, b*. L* coordinate indicates lightness - darkness and takes values in the range of 0 - 100 (black - white, respectively). a* gives green - red, b* blue - yellow coordinates. a* Green - red coordinate takes positive values for reddish colors and negative values for greenish ones, while b* takes positive values for yellowish colors and negative values for bluish ones [25]. Chroma (C*) indicates the degree from gray to pure chromatic color (color intensity), while Hue (h) indicates the color perceived visually. Accordingly, an increase in chroma value means an increase in color intensity (brightness), whereas a decrease in Hue means more red coloration [26, 27, 28]. The results of biochar and nano silver application on the color parameters of plant leaves under different irrigation water levels in the experiment are given in Table 3.

Since the negative a* value (-) in full irrigation conditions indicates that the green color ratio is high, it is seen that the a* value increases positively and the green color decreases under water restriction conditions. Although there was a difference in the irrigation levels x nano silver interaction, it was not statistically significant.

Croma value was also higher in full irrigation treatment (I100). Since an increase in Croma value indicates an increase in color intensity (brightness), it may have caused Croma values to be determined high in the full satisfaction of plant water needs. The decrease in Croma value under water stress conditions supports this situation. Contrary to expectations, Croma values generally decreased in nano silver applications. However, this decrease was not statistically significant.

V. CONCLUSION

At the end of the study, leaf relative water content and chlorophyll value decreased and membrane damage index increased with the decrease in irrigation water. Biochar application increased leaf relative moisture content and decreased membrane damage index. Nano fertilizer application did not show a significant change in the physiological properties of the plant. When the color parameters of the plant leaves were examined, the color intensity (brightness) and the amount of green color of the plant leaves decreased with the decrease in irrigation water. Higher color intensity and amount of green color were measured in biochar treatments compared to non-biochar treatments. There was no significant change in nano silver application. As a result, it was determined that the use of biochar in irrigation water restriction applications provided water retention in the root zone of the plant and accordingly, the plant gained tolerance against stress in physiological properties. Under irrigation water stress conditions, it was observed that soil application of nano silver application had little effect on water retention and plant stress reduction. It was suggested that the amount of nano silver should be increased and foliar application studies should be increased in the future.

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