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Walnut Shell Biochar Increases Seed Germination and Early Growth of Seedlings of Fodder Crops

Omer Suha Uslu ¹, Emre Babur ², Mehmet Hakkı Alma ³ and Zakaria M. Solaiman ^{4,*}

¹ Field Crops Department, Agriculture Faculty, Kahramanmaraş Sutcu Imam University, Kahramanmaraş 46050, Turkey; suhauslu@ksu.edu.tr

² Soil and Ecology Department, Faculty of Forestry, Kahramanmaraş Sutcu Imam University, Kahramanmaraş 46050, Turkey; emrebabur@ksu.edu.tr

³ Department of Forest Industry Engineering, Kahramanmaraş Sutcu Imam University, Kahramanmaraş 46050, Turkey; mhalma@yahoo.com

⁴ The UWA Institute of Agriculture and UWA School of Agriculture and Environment, University of Western Australia, Perth, WA 6009, Australia

* Correspondence: zakaria.solaiman@uwa.edu.au

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Abstract: Currently, biochars are produced from a wide range of feedstocks with a broad diversity in physicochemical characteristics. Therefore, a diverse agronomic response of crop plants to biochars application was expected. A preliminary ecotoxicological assessment is necessary before application of biochar to soil, even though biochar is a recalcitrant carbon considered as a promising soil amendment because of its ability to climate change mitigation by sequestration of carbon in the soil. Thus, a Petri dish germination test was conducted to assess the effects of six walnut shell biochar rates (i.e., 0, 10, 20, 40, 80, and 120 Mg ha⁻¹) on seed germination and early growth of seedlings of fodder crops (*Triticale* cultivar X *Triticasecale* Wittmack and *Pisum sativum* sp. arvense L. varieties Taşkent and Özkaynak). A simple Petri dish bioassay method used to determine the effect of biochar rates on seed germination. Germination rate decreased with both higher and lower rate of biochar application. Results showed that the germination rate and growth indices were dependent on plant species. The seed germination rate of all three species was stimulated at the 40 Mg ha⁻¹ rate, but Taşkent mung bean occurred at the highest rate of 120 Mg ha⁻¹. Significantly higher germination rate and growth indices observed with the 40 and 80 Mg ha⁻¹ biochar rates, respectively. Biochar application generally increased seed germination at rates ≤ 40 Mg ha⁻¹ and seedling growth indices at rates ≤ 80 Mg ha⁻¹. This rapid test can be used as the first indicator of biochar effects on seed germination rate and early growth of seedlings. Farmers could use this test before investing in biochar application.

Keywords: biochar application; plant species; Petri dishes bioassay; fertilization

1. Introduction

Nutrient and water scarcity are major stress factors affecting seed germination and plant growth [1,2]. Germination in both annual and perennial plants and critical periods for yield determination in annual crops are among the most crucial stages in the development of plants [3–6]. Thus, seed germination and seedling growth properties are essential parameters for plant establishment which can be used as early and rapid indicators of the influences of the precision application on plant yield [7].

Timely and balanced addition of nutrients like N and P from anthropogenic sources are essential to optimize plant growth in several plant species [8–11]. However, repeated use of supra-optimum rates of synthetic fertilizers is economically expensive and environmentally detrimental [12–14]. Organic fertilization and use of N-fixing species, on the other hand, are more environmentally friendly

management tools that have been extensively studied over the last 30 years [15–19]. Use of organic fertilizers and amendments such as biochar was recognized as essential to achieving agricultural sustainability in modern cropping systems because of the intensive land use and land degradation in recent years [7,20]. Biochar is a carbon-rich material produced after the pyrolysis of organic biomass [21] whose use has been globally proposed as an organic amendment to mitigate climate change by increasing carbon sequestration capacity in soils. Besides, biochar contributes to improving physicochemical and biological soil characteristics of soil health. As a result of an increase in soil health, biochar can have a positive effect on crop yields [21]. Field or pot application of varying rates of biochar has resulted in highly variable results, with an ample range of responses showing increases, decreases, or no changes in plant growth and biomass yield as a result of biochar application [22,23].

Biochars derived from different organic materials have been used to increase seed germination and improve soil fertility and other soil properties. Biochars have also been used to enhance plant productivity by minimizing the impact of abiotic stress factors such as drought through increased soil water holding capacity (WHC) and by maintaining the nutrient content through improved cation exchange capacity [15,24–26].

Moreover, the addition of biochar to soil may reduce the adverse effects of climate change by increasing soil carbon stocks by improving soil physicochemical properties like pH, cation exchange capacity, soil water holding capacity, plant-nutrient retention, microbial activity, and nutrient availability [21,27–29]. Furthermore, biochar application can decrease bulk density and increase soil porosity which can significantly improve soil aeration and structure [30]. It helps to maintain plant nutrition through absorption and slow release of nutrients to plants and contributes to the reclamation of degraded soils [31]. He et al. [32] observed that biochar amendments contributed to increasing soil microbial activity and provided a suitable condition for nitrification. Biochar has also been used as a highly effective sorbent [33], that can reduce heavy metal concentrations and levels of other phytotoxic compounds that are harmful to plant development and seed germination by adsorption to their surface [34].

Studies have reported that biochar application may have both positive [24,29,35–37] and negative [38–40] effects on seed germination and seedling growth [41]. In this context, it is crucial to identify the proper biochar material for soil improvement and seed germination [17,40]. More research is needed to determine the relationship between different biochar materials and the germination process of different plant species. To this end, a simple approach involves the germination of seeds in Petri dishes in response to varying rates of biochar application. For instance, Solaiman et al. [15] utilized Petri dishes to investigate biochar effects on *Triticum aestivum* L. seed germination rate and early plant growth, and Rosende et al. [42] studied the effect of biochar applications to mine soil on germination of *Lolium perenne* L. seeds. Gascó et al. [40] conducted a Petri dish assay to determine the influence of phytotoxic compounds on germination rate for five plant species. Busch et al. [39] used a similar test to determine germination rates and plant growth indices with different hydrochars and biochars. This study reported that hydrochars severely inhibited seed germination. Hydrochar treatment caused abiotic stress by emitting phytotoxic gases and causing salt stress. In another study, the germination rate of maize was not significantly influenced by the biochar application [22].

Successful seed germination is crucial for both plant growth and development; therefore, germination rate and early growth characteristics can be an early indicator of the effects of biochar on plant productivity [7]. Such a laboratory bioassay test would be an excellent option to determine the usability of biochar in soil rehabilitation [21,43].

A seed germination test is a fast and reliable assay to determine the potential effects of biochar application on plant growth. Few studies were conducted to assess the impact of biochar on seed germination and plant growth due to the specificity of the interaction between biochars and plants. Therefore, the impact of biochar applications on seed germination should be determined before use in large-scale areas. For this, we applied a procedure used at the Oregon State University Seed Laboratory as a ‘rapid test’ for monitoring the effects of biochar on plant growth and seed germination.

This study aimed to determine the impact of biochar application rate on seed germination rate and early plant productivity.

Walnut production in the world increased from 1.80 million Mg in 2005 to 3.75 million Mg in 2016. Approximately 90% of the walnut world production is grown in eight major producing countries, including China, USA, Iran, Turkey, Mexico, Ukraine, Chile, and Uzbekistan. Turkey, with an approximate production of 15,000 Mg yr⁻¹, ranks fourth in the world for walnut production, with the province of Kahramanmaraş being the most important producer in the nation [43]. Therefore, walnut shell waste from walnut production would be a potential resource for biochar production in the province of Kahramanmaraş.

2. Materials and Methods

2.1. Biochar Production and Characterization

Biochar used in this study was produced at 550 °C from black walnut (*Juglans nigra* L.) shells. The pyrolysis duration was 120 min for walnut shells, and it was produced by using a Pyrolysis unit at Kahramanmaraş, Turkey. Biochars were sieved to pass a 2 mm screen [44] before use in soilless Petri dish bioassays. The pH and electrical conductivity (EC) of the biochar were determined in a 1:2.5 soil/water suspension with a pH/EC meter by the methods of Rhoades [45]. WHC of biochar was determined by drying the biochar for 24 h at 105 °C and then soaking it in water for 12 h [46]. WHC was calculated according to the equation: WHC = (wet mass × 100)/dry mass.

On a powdered subsample of biochar, total carbon was determined by the Walkley–Black method [47], and total nitrogen was determined by the Kjeldahl method [48]. Elemental composition of biochar (Al, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, and Zn) was measured using an ICP-OES Instrument (Agilent 5110). Before analysis, a 0.5 g sample of biochar was digested with 5 mL of HNO₃ and 4 mL of HClO₄ in a hot plate system and diluted to 25 mL with deionized water. After that, the prepared solutions were filtered (Whatman 42) and stored in the freezer until analysis [49].

2.2. Biochar Bioassay for Fodder Seeds

The pretreatment and bioassay were conducted in an incubator of the Kahramanmaraş Sutcu Imam University, Faculty of Agriculture, Department of Field Crops Laboratory in September 2018. Three plant genotypes (one genotype of *x Triticasecale Wittmack*, Triticale and two genotypes of *Pisum sativum* sp. *arvense* L. namely Taşkent and Özkaynak) were used in this bioassay. The study was arranged as a completely randomized design (CRD) with four replications, each Petri dish being a replication. The fodder seeds used in the study were sterilized in 5% NaClO (sodium hypochlorite) solution for 5 min and then rinsed with distilled water. Different biochar doses (0, 0.5, 1.0, 2.0, 4.0, and 6.0 g per Petri dish equivalent to 0, 10, 20, 40, 80, and 120 Mg ha⁻¹ on a volume basis at 10 cm soil depth) were added to the Petri dishes on filter paper moistened with 20 mL distilled water [50]. In other words, these ratios are equal to 0%, 0.03%, 0.06%, 0.12%, 0.24%, and 0.36% of the soil mass, assuming a bulk density of 1.6 g cm⁻³ [51]. Twenty-five seeds were spread per Petri dish of 90 mm diameter, and 20 mL of distilled water was added to each Petri dish. Then, Petri dishes were closed with lids, sealed and incubated in the dark at 25 °C for 14 d, after which the germination rate and other early growth properties were evaluated. During the length of the experiment, all Petri dishes were examined every other day, and equal amounts of distilled water were added according to the need for the filter paper not to dry [52].

Germination rate, radicle length, plumula length, seedling fresh weight, dry seedling weight, and vigor index values were measured. The germination rate was calculated by dividing the amount of germinated seeds by the total number of seeds and then multiplying by 100 [53]. The radicle and plumula lengths were measured separately, and then both lengths measured as seedling length [54]. The radicle and plumula fresh weights were obtained, dried at 70 °C for 24 h and weighed to

determine seedling dry weight [54]. Vigor index was determined by multiplying the seedling length by germination rate [55,56].

2.3. Statistical Analysis

For the Petri dish bioassay, a two-way analysis of variance (ANOVA) was performed using SAS 9.3 to determine the main effects of factors (species and biochar doses) as well as their interactions. Also, ANOVA was used to find the significance of seed germination with different biochar rates. Means comparisons among treatments were made using LSD at the $p \leq 0.05$ level [57]. Correlation matrix analysis was conducted among the seed germination, seedling growth, and biochar characteristics of the laboratory bioassay.

3. Results

3.1. Biochar Properties

The main characteristics of the tested biochar are stated in Table 1. The mean pH was 8.25, which is moderately alkaline (7.9–8.4), according to the United States Department of Agriculture classification of pH ranges [58]. The mean EC was $570 \mu\text{S cm}^{-1}$, which is in the non-saline range. The tested biochar had high organic carbon, and total nitrogen concentrations and the percentage of WHC were significant.

Table 1. Basic properties of biochar derived from a walnut shell.

Properties	Mean	Properties	Mean
pH (1:2.5)	8.25	Cu (ppm)	9.56
EC (1:2.5, $\mu\text{S cm}^{-1}$)	570.00	Fe (ppm)	328.44
OC (%)	79.60	K (ppm)	6132.38
TN (%)	0.12	Mg (ppm)	596.25
WHC (%)	61.29	Mn (ppm)	77.88
Al (ppm)	327.47	Na (ppm)	420.18
B (ppm)	19.78	Ni (ppm)	7.52
Ca (ppm)	6648.85	P (ppm)	224.13
Cd (ppm)	0.02	Pb (ppm)	1.46
Co (ppm)	0.22	Zn (ppm)	11.58
Cr (ppm)	5.20		

3.2. Germination and Plant Development

In this study, the germination rate and other seedling growth characteristics were significantly different for different fodder species and treatments. The germination rate of fodders originating from Taşkent (94%) was significantly higher ($p < 0.01$) than Triticale (81%) and similar to Özkaynak (94%) species. Seed germination rate (%) ranged from 69 to 97 across different biochar doses (Table 2, Figure 1a). The maximum germination rate (97%) was found at 40 and 80 Mg ha^{-1} application for species of Taşkent origin whereas the minimum germination rate (69%) was observed for 120 Mg ha^{-1} application.

Table 2. Analysis of variance (ANOVA) of germination rate (GR), radicle length (RL), plumula length (PL), seedlings length (SL), seedling fresh weight (SFW), seedling dry weight (SDW), and vigor index (VI) values for cultivar, biochar rate, and their interactions.

ANOVA	GR (%)	RL (cm)	PL (cm)	SL (cm)	SFW (g)	SDW (g)	VI
Cultivar	**	*	ns	ns	*	**	**
Biochar	*	*	ns	*	ns	**	**
Cultivar × Biochar	*	*	*	*	*	*	*

**, * denote statistical significance at $p \leq 0.01$ and $p \leq 0.05$, respectively; ns, not significant.

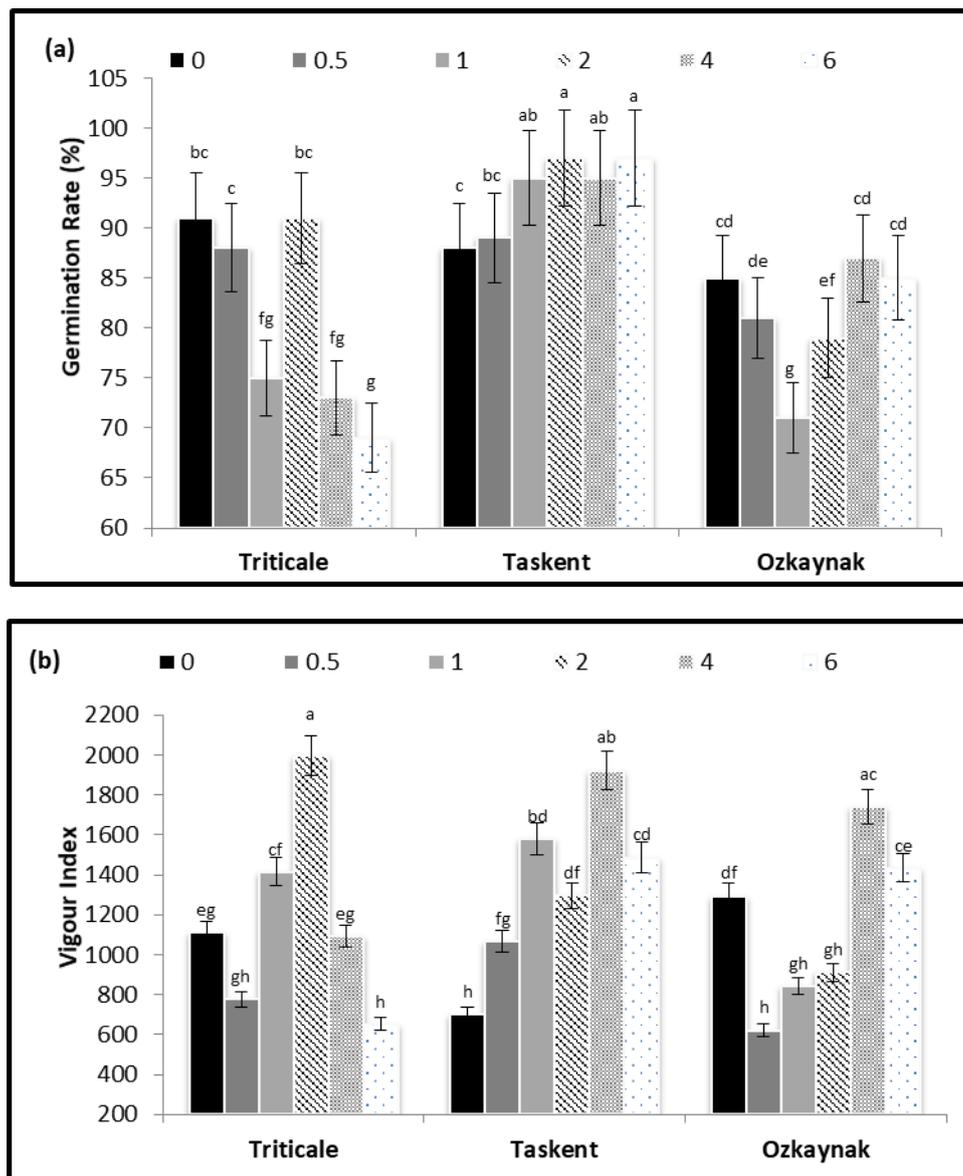


Figure 1. Effect of biochar rate on (a) germination rate (%) and (b) vigor index of Triticale, Taškent and Özkaynak. Different letters above the bars suggested that these values are significantly different at $p \leq 0.05$.

The highest values for germination rate were found at 0 and 40 Mg ha⁻¹ for Triticale (91%), 40 and 80 Mg ha⁻¹ for Taškent (97%) and 80 Mg ha⁻¹ for Özkaynak (87%). When analyzing data from all three origins together, the most suitable biochar dose was found to be 40 Mg ha⁻¹.

Radicle lengths were significantly different among the different treatments, with the longest radicle found in Triticale species (Table 2; Figure 2a). The maximum radicle length (10.74 cm) was found for 40 Mg ha⁻¹ application for Triticale origin. Radicle lengths ranged from 1.82 cm to 10.74 cm across all biochar doses. The highest radicle length values were found in 40 Mg ha⁻¹ application (10.74 cm) for Triticale origin, 80 Mg ha⁻¹ application (8.48 cm) for Tashkent origin and 80 Mg ha⁻¹ application for Özkaynak source (6.43 cm). As a result of examining all three sources together, the highest root length was observed in 80 Mg ha⁻¹ applications (Table 2; Figure 2a).

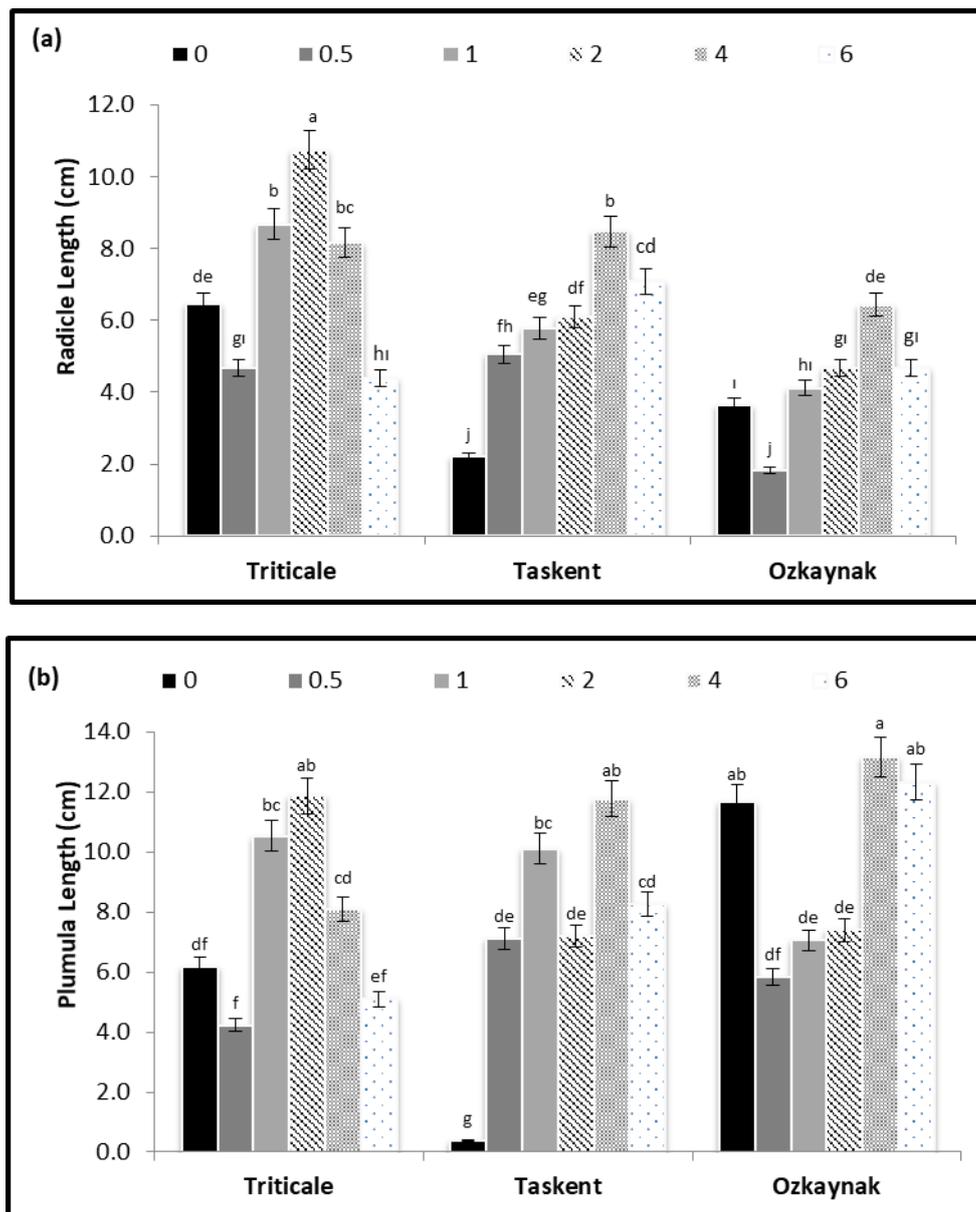


Figure 2. Effect of biochar rate on (a) radicle length, and (b) plumula length of Triticale, Taškent and Özkaynak. Different letters above the bars suggested that these values are significantly different at $p \leq 0.05$.

Plumula lengths were significantly different among the different treatments. However, plumula and seedling lengths were not statistically different between the origins ($p > 0.05$). Also, the highest values of seedling fresh weights, seedling dry weights and vigor indices were found in Taškent species. Plumula lengths ranged from 0.39 cm to 13.17 cm for all biochar doses. The highest plumula length was found in 40 Mg ha⁻¹ application (11.88 cm) for Triticale origin, 80 Mg ha⁻¹ application (11.77 cm) for Tashkent origin and 80 Mg ha⁻¹ application for Özkaynak source (13.17 cm). Across all three sources, the highest plumula length was found in 80 Mg ha⁻¹ applications (Table 2; Figure 2b).

Seedling lengths were significantly different among the different treatments. Seedling lengths ranged from 2.60 cm to 22.62 cm across all biochar doses. Seedling lengths were highest with Triticale origin for 40 Mg ha⁻¹ (22.62 cm), 80 Mg ha⁻¹ (20.25 cm) for Taškent source and 80 Mg ha⁻¹ for Özkaynak

source (19.61 cm). Across all three sources, the highest seedling length was found in 80 Mg ha⁻¹ (Table 2; Figure 3a).

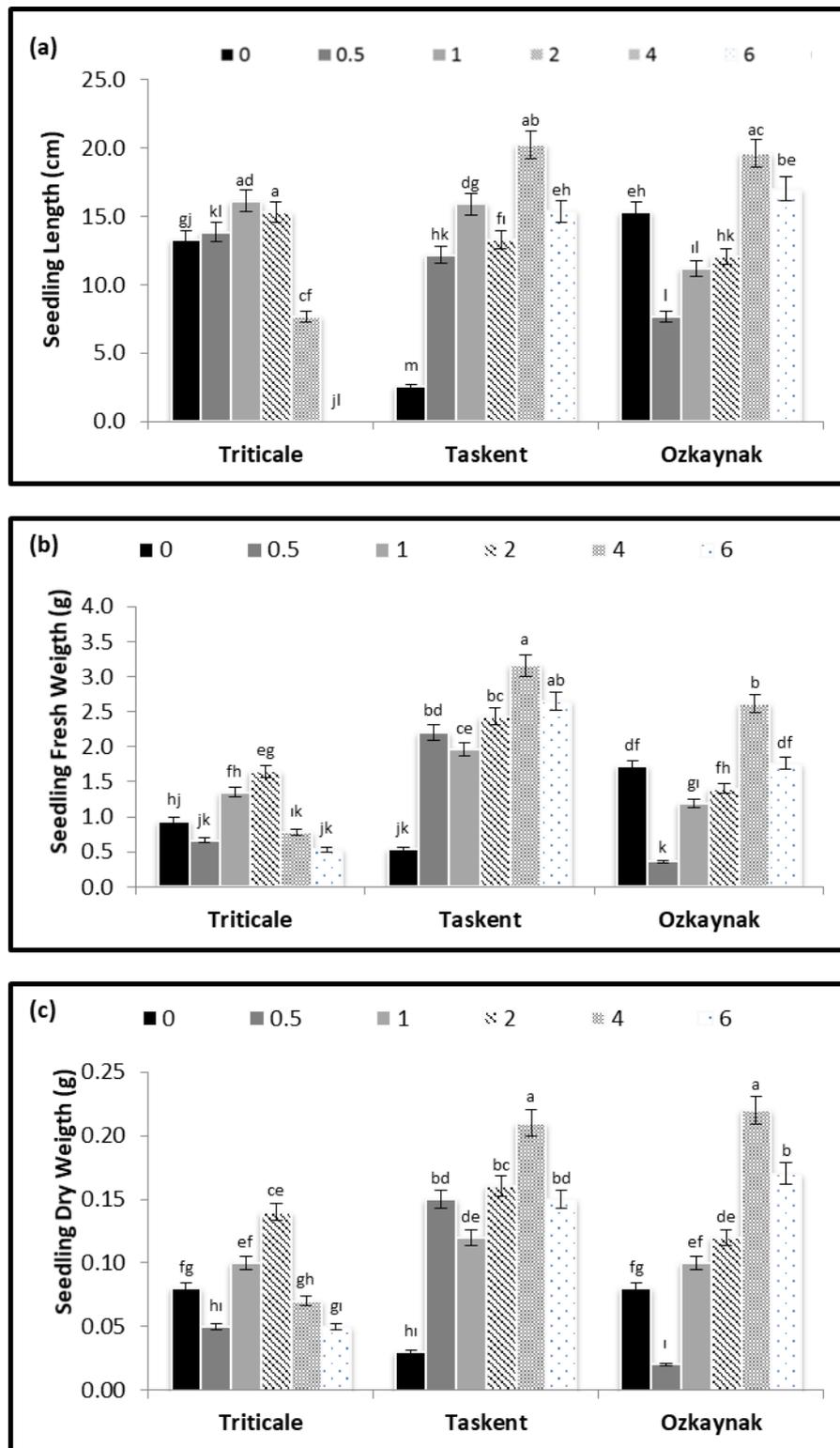


Figure 3. Effect of biochar rate on (a) seedling length, (b) seedling fresh weight, and (c) seedling dry weight of Triticale, Taskent, and Özkaynak. Different letters above the bars suggested that these values are significantly different at $p \leq 0.05$.

Seedling fresh weights were significantly different among the different treatments. Seedling fresh weights ranged from 0.36 g to 3.16 g across all biochar doses. The highest seedling fresh weights were found in 40 Mg ha⁻¹ application (1.65 g) for Triticale origin, 80 Mg ha⁻¹ application (3.16 g) for Taškent origin and 80 Mg ha⁻¹ application for Özkaynak origin (2.61 g). When analyzing all three origins together, the highest seedling fresh weight was found in 80 Mg ha⁻¹ application (Table 2; Figure 3b).

Seedling dry weights were significantly different among the different treatments. Seedling dry weights ranged from 0.02 g to 0.22 g across all biochar doses application. The highest seedling dry weights were found in 40 Mg ha⁻¹ application (0.14 g) for Triticale origin, 80 Mg ha⁻¹ application (0.21 g) for Taškent origin, and 80 Mg ha⁻¹ application for Özkaynak origin (0.22 g). When analyzing all three origins together, the highest seedling dry weight was found in 80 Mg ha⁻¹ application (Table 2; Figure 3c).

Seed vigor index showed a significant difference among the different treatments. Vigor index (VI) values ranged from 228 to 1998 across all biochar doses application. The highest vigor indexes were found in 40 Mg ha⁻¹ application (1998) for Triticale origin, 80 Mg ha⁻¹ application (1921) for Tashkent origin, and 80 Mg ha⁻¹ application (1739) for Özkaynak origin (Table 2; Figure 1b). When analyzing all three origins together, the highest seed vigor index was found in 80 Mg ha⁻¹ applications. The vigor index values correlated with seedling length and germination rate. The highest VI in the application of 40 Mg ha⁻¹ for Triticale origin seeds might be due to the accumulative effect of higher germination rate and seedling length.

In the soil-less Petri dish bioassay, biochar application rates had a significant effect on seed germination ($p < 0.05$, Table 2). Biochar application positively influenced seed germination and plant growth characteristics at the 80 Mg ha⁻¹ application; however, germination and growth decreased at higher applications ($p < 0.05$, Table 2). Consequently, the most appropriate natural soil fertilization with biochar application was found to be 80 Mg ha⁻¹ in an area of 10 cm soil depth for the species used in the experiment ($p < 0.05$), but higher biochar application rates led to negative impact ($p > 0.05$; Table 2).

Also, applying biochar to different species influenced seed germination rate and growth indexes ($p < 0.05$). For instance, Triticale and Taškent species germination rate was found highest value in 40 Mg ha⁻¹, but Özkaynak species germination rate was found highest value in 80 Mg ha⁻¹. Increased biochar application rate generally increased radicle length, plumula length, seedling fresh weight, seedling dry weight, and vigor index of the seedlings in the Petri dish bioassay, especially at the 40 Mg ha⁻¹ and 80 Mg ha⁻¹ rates of application ($p < 0.05$, Table 2).

4. Discussion

While the rapid bioassay for germination rate and seedling growth indices were used for assessing the effect of biochar on the crop productivity on agricultural soils [15], it could be used to study the soil stressors. Biochar application to soil increases some soil properties such as pH, water-holding capacity (WHC), soil organic carbon (SOC), and contributes to soil nutrient retention [59]. These positive effects can influence seed germination and plant productivity [60]. Nutrients were found in the biochar sample when applied to soils, some soil properties related to fertility improved. For example, Mendez et al. [61] found an increase in WHC after biochar application. Liang et al. [62] found that the amendment of soil with poultry litter biochar increased the resistance of soil microbial properties to drought sufficiently to increase agricultural yields. Lu et al. [63] reported that biochar increased the reaction (pH) of acidic soil. Moreover, the combination of biochars and earthworms had a positive effect on soil microbial activities and plant growth [64].

In our study, the biochar pH was moderately alkaline. Not only high alkalinity but also strong acidity inhibits seed germination [65]. Chidumayo [66] noticed that biochar application to soil increased its pH, exchangeable P and K, and seed germination. Olszyk et al. [17] (2018) reported biochar application generally altered soil pH and EC. Biochar application can improve soil fertility and nutrient retention by increasing soil pH, soil organic carbon, Ca, K, Mn, soil exchangeable-P, and other nutrients [59,60]. Also, biochar application had direct effects on soil properties, and its remediation such as Cely et al. [67] have been presented. Organic soil fertilizer application positively

affects not only soil fertility but also plant biomass productivity. These effects depend on the quality of organic fertilizer.

The effect of biochar on the germination of plant species needs to be investigated before sowing [15,68]. In this study, the impact of biochar rate on seed germination and seedling growth indices varied among three agricultural plants (x *Triticosecale Wittmack*, *Pisum sativum sp. arvense* L. Taşkent and Özkaynak).

The results showed that the germination rate decreased with both higher and lower rates of biochar application. The optimum germination rate was found with 40 Mg ha⁻¹ biochar application because the germination rate was not decreased compared to the control. Also, the results illustrated that the plant species significantly affected germination rate and growth indices. The seed germination rate of all three species was stimulated at the 40 Mg ha⁻¹ application rate, but Taşkent mung bean occurred at the highest rate also in 120 Mg ha⁻¹. Solaiman et al. [15] found that seed germination rates significantly influenced by biochar application similarly to our study. The germination rates generally increased at the lower rates (10–50 Mg ha⁻¹) and decreased at higher rates of application (>50 Mg ha⁻¹) ($p < 0.05$, Table 2). Rondon et al. [69] stated that plant production and N-fixation of common bean (*Phaseolus vulgaris* L.) yield increased with biochar application at 30 and 60 g kg⁻¹ soil. However, there was a decrease in crop production as the biochar rate increased. In our study, we noticed that biochar application improved plant growth to be related to increased nutrient availability from biochar higher pH value.

Germination rate and seedling growth indexes such as plant height, root length, biomass accumulation, and Dickson quality index of okra were significantly influenced by biochar treatments [70]. Improving soil porosity and water holding capacity with biochar application contributed to increased root growth and high concentrations of nutrient uptake by the plant. It also helped to increase aboveground plant biomass [71,72].

The results of ANOVA test (Table 2) showed that germination rate and growth properties were influenced primarily by plant species. The germination rate was slightly higher in *Pisum sativum sp. arvense* L. Taşkent species. Olszyk et al. [17] reported that biochar application significantly influenced the germination rate of carrot, cucumber, and tomato species. As opposed to this, Gascó et al. [40] noticed that biochar application leads to decreased germination rate and early seedling growth.

Seedling growth indexes such as germination rate, radicle length, plumula length, seedling fresh weight, dry seedling weight, and vigor index values were slightly increased by biochar application in the present study and also reported elsewhere [17]. However, Gascó et al. [40] reported that different biochars have no effects on the seedling dry weight. Olszyk et al. [17] defined the impact of biochar on plant germination. It was determined that dry seedling weights of different plants significantly increased. Meng et al. [73] found no influence on germination rate but significant effect on older seedling fresh weight.

Consequently, the biochar rates generally increased seed germination at rates of application <40 Mg ha⁻¹ and a higher rate of biochar application inhibited seed germination under the bioassay conditions. This study recommended that the soil-less Petri dish bioassay would use for ecotoxicological test for biochar because it is rapid, simple, and inexpensive as mentioned in another study [15]. Moreover, there was a noticeable germination rate difference among plant species which also differed with different rates of biochar application. The information obtained from this study will increase our understanding of biochar applications on seed germination rate and seedling growth for their potential usage. Based on the obtained results of the present experiment, we suggest that the use of the soil-less Petri dish bioassay to determine possible effects of biochar on seed germination and early growth of fodder crops.

5. Conclusions

In conclusion, the rapid test investigated in this study demonstrated that biochar could increase seed germination and seedling growth with appropriate application rates. Taşkent species showed significant effects on germination as well as vigor index. Among the studied biochar doses, the use

of 40 Mg ha⁻¹ could be a better option to achieve enhanced germination rates and seedling growth indices. Agricultural areas are vast; preliminary laboratory studies may help before irreversible biochar application to the farm field. Therefore, the maximum crop yield can be obtained with efficient use of biochar and fertilizer with an economic return. Also, biochar application can provide a simple or immediate improvement to poor soil conditions such as pH, EC, WHC, and some other properties. Besides, it contributes to carbon sequestration in soil which helps to reduce global warming [74].

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