



OPEN Evaluating the impact of different biochar types on wheat germination

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This study assessed sustainable solutions for organic waste management, focusing on biochar derived from kitchen waste. The characteristics and phytotoxicity effects of biochar produced from four different types of kitchen waste were investigated in view of potential agricultural applications. Analysis of the chemical and physical properties of the different biochar samples by X-ray fluorescence and Fourier transform infrared spectroscopy revealed a nutrient-rich composition with carbon, calcium, and potassium contents that ranged from 35 to 48%, from 1.6 to 24%, and from 1.5 to 28.5%, respectively. In phytotoxicity tests, the highest germination rate (45%) was observed with Coffee residue biochar obtained at 300 °C (application rate of 1%) and the longest shoot length (25 cm) with orange peel biochar obtained at 300 °C (application rate of 1%). Germination rate and shoot length were not significantly different between biochar-exposed soils and control soils (without biochar), indicating no toxic effect due to biochar addition. Washing biochar improved germination rates significantly (control: 98%; potato peel biochar: 92%; banana peel biochar: 83%). The longest shoot length (8.3 cm) was obtained with the washed potato peel biochar (pyrolysis temperature = 400 °C) extract. These findings suggest that biochar can be safely used as a soil amendment without harming the environment or hindering plant growth. The Tukey honestly significant difference (HSD) test results further emphasized the influence of different factors, such as pyrolysis temperature and feedstock type, in biochar applications.

Keywords Biochar, Organic waste valorization, Germination test, Soil amendment, Characterizations

Worldwide, human populations are facing challenges concerning food security due to the escalating prices of vegetables and other food items. These difficulties arise from the increasing production costs for farmers. For instance, in April 2022, the prices of fertilizers had significantly increased by up to 30% since the beginning of the year, surpassing previous peak levels. These high prices further increase production costs for farmers and exacerbate food insecurity¹. Therefore, it is crucial to find ways to reduce reliance on fertilizers, while maintaining sustainable production practices.

Biochar, a product of pyrolysis, is a carbon-rich material with various applications, especially as a soil amendment². It contains stable organic carbon compounds crucial for soil health, productivity, and climate change mitigation. One of biochar most significant benefits is its ability to reduce fertilizer requirements. Its adsorption capacity reduces nutrient leaching, increases nutrient availability and improves nutrient efficiency, allowing farmers to minimize fertilizer use and consequently to lower production costs. Sustainable biochar use could reduce global greenhouse gas emissions by up to 43%³ and enable access to carbon credits, making it more affordable for farmers. Moreover, biochar contributes to water storage by soils, enhances crop quality, meets the food safety standards, and persists in soil, gradually improving its physicochemical properties over time⁴⁻⁷.

To assess biochar effectiveness as a soil amendment, first its quality must be assessed using a germination inhibition assay, as recommended by international biochar standards. This ensures that the biochar product is free of toxic substances that might hinder seed germination and negatively affect plant growth. According to Intani et al.⁸, when studying biochar phytotoxicity, its feature variations, due to feedstock and manufacturing

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conditions, must be considered. Biochar is generally safe for agricultural use, but some types may contain hazardous chemicals from contaminants in the feedstock. Indeed, biochar toxicity can be explained by the feedstock characteristics and production temperature that influence pH levels, electrical conductivity (EC), heavy metal content, and presence of polycyclic aromatic hydrocarbons (PAHs). For instance, Ramírez-Rodríguez et al. (2007) found that pH levels > 8 reduce the nutrient bioavailability to plants⁹. Gómez (2023) showed that extreme pH conditions, as observed with biochar from olive tree pruning, can lead to phytotoxicity¹⁰. The EC, which relates to the presence of soluble salts in biochar, is another critical feature. Albuquerque et al. (2016) demonstrated that salt concentrations > 10 dS/m can adversely affect seed germination¹¹. PAHs generated during pyrolysis contaminate ecosystems, and their formation is increased at lower temperatures^{12,13}. Additionally, heavy metal concentration in biochar can increase significantly due to pyrolysis, particularly when performed at higher temperatures, posing risks to soil organisms^{14,15}. Therefore, monitoring the biochar production process is essential to minimize biochar toxicity.

Here, orange peels (OP), potato peels (PP), banana peels (BP), and coffee residue (CR) were selected as feedstock for biochar production. These materials are abundant kitchen waste products in Lebanon due to their high consumption by the population. Importantly, the phytotoxicity (wheat germination inhibition) of biochar produced with these materials has rarely, and in some cases never, been tested in the same conditions. The ultimate objective was to create a blend of these different biochar materials for soil amendment purposes. However, before blending for soil application, it was crucial to assess the toxicity of each biochar sample in order to identify potential risks associated with their use. Therefore, germination inhibition assays using wheat seeds were carried out to investigate the effects of two pyrolysis temperatures (300 °C and 400 °C) and two application rates (1% and 3%) on germination rate and shoot length. Two toxicity assessments were carried out: liquid phase tests to estimate the effects of biochar water extracts on seeds, and solid phase tests to directly assess the impact of adding each biochar type in the soil on wheat seed germination and growth. These tests are critical for assessing the potential toxicity and ensuring environmental safety. Before these tests, the biochar samples (OP, PP, BP, and CR) were comprehensively characterized (structure and composition) to understand their profiles and properties. Overall, the study aim was to investigate the effects on wheat seed germination and growth of biochar derived from OP, CR, PP and BP used as soil amendments to determine the best conditions (pyrolysis temperature and application rate) to enhance plant growth. Biochar not only reduces the need of chemical fertilizers, but also promotes sustainable agricultural and environmental practices by valorizing waste materials into effective products at low cost. By advancing knowledge on its application in farming, the aim of our research is to comprehensively assess the potential risks to the environment and agriculture associated with biochar use to ensure that these challenges are effectively managed and mitigated. Based on the obtained results, a blend of these different biochar types could be tested as a soil amendment and a follow-up study will be carried out to better understand their impact on plant growth in the presence and absence of chemical fertilizers.

Materials and methods

Biochar preparation

The various types of biomasses (BP, PP, OP and CR) were sourced from local markets and from a café in Beirut, Lebanon, during the winter of 2023. Biomass pretreatment and pyrolysis were carried out following a previously described procedure¹⁶.

Biochar characterization

Characterization of the chemical and physical properties of the different biochar samples included the determination of moisture and ash content, biochar yield, pH, and EC. X-ray fluorescence (XRF) using a PANalytical Axios mAX spectrometer (Malvern) and Fourier transform infrared (FT-IR) spectroscopy using a Nicolet NEXUS instrument also were performed. The detailed methodology is provided in Supplementary Material S1: Biochar Characterization.

The humidity percentage (Hm) was calculated using Eq. 1:

$$Hm (\omega) = (\text{water mass/dried peel mass}) \times 100 \quad (1)$$

Ash content was determined with Eq. 2:

$$\text{Ash content}(\%) = \frac{Ma}{M0} \times 100 \quad (2)$$

where Ma is the remaining mass after burning (g) of the sample and M0 is the initial mass before burning (g).

The biochar yield (dry basis, %) was calculated using Eq. 3:

$$\text{Biochar yield} (\%) = \frac{M2(g)}{M1(g)} \times 100 \quad (3)$$

where M2 is the weight (g) of the sample after pyrolysis and M1 is the weight (g) of the sample before pyrolysis.

Germination test in soil

Soil samples were from Anjar (33.7484682°, 35.9485286°) and Riyakk (33.870399°, 35.965320°), both located in the Beqaa Valley of Lebanon. They were analyzed at the Lebanese Agricultural Research Institute (LARI) in Tal Amara, Lebanon.

Soil samples were prepared by air-drying and sieving through a 1 mm mesh to obtain fine particles and remove rocks or large particles, ensuring sample homogeneity and consistency. Then, soil samples were blended with 1% and 3% of CR, BP, PP, and OP biochar produced at 300 °C and 400 °C (three replicates/condition). Three control samples (soil without biochar) were also prepared. Five wheat seeds (purchased locally) were added to each sample, which was then moistened with water. Samples were monitored for three weeks. The CR and OP biochar were added to the Anjar soil samples, and the BP and PP biochar to the Riyakk soil samples. The choice of soil and biochar was based on soil availability at the time of the experiment. As the primary objective was to assess the effects of each biochar on germination, using the same soil for all biochar types was not deemed necessary.

4 phytotoxicity test with biochar water extracts

As described by Gómez (2023), biochar liquid extracts were prepared by mixing 10 g of each biochar produced at 300 and 400 °C with 50 mL of sterilized distilled water. Germination rate and shoot length were measured when the control group (germinated with distilled water) reached 90% of germination¹⁰.

Statistical analysis

we conducted statistical analysis using the Tukey HSD (Honestly Significant Difference) test to assess differences between group means. The analysis was performed with SPSS software (version 23) to ensure accurate comparison and interpretation of the data.

Results and discussion

In this section, the results of the characterization (moisture and ash content, biochar yield, pH, EC, XRF, and FT-IR) of biochar samples derived from OP, PP, BP and CR are presented and discussed. This comprehensive characterizations was carried out to determine their suitability of for various applications.

Humidity, ash content, and biochar yield

The characterization of the humidity percentage and ash content of the various feedstocks (Table 1) showed that PP had the highest (34.96%) and BP the lowest humidity percentage (16.02%). Their ash content at 600 °C varied from 4.21% in CR to 9.78% in BP (and 9.24% in OP). Previous studies reported ash contents for BP of 8.50% (Anhwange et al., 2009), which is close to our results, and of 19.98%^{17,18}. The ash contents in PP (7.8%), OP (9.24%) and CR (4.21%) were within the literature range (0.9–8.6, 0.08–22.2, and 1.59–6.95%, respectively). The literature data indicate that the ash content of biochar increases with the pyrolysis temperature.

Table 2 shows the yield, pH and EC of biochar samples obtained by pyrolysis carried out at temperatures between 300 and 500 °C. Indeed, previous studies showed that the ideal temperature for biochar production is between 350 and 500 °C¹⁹.

Figure S1 (supplementary material) shows the biochar yield variations when the different feedstocks underwent pyrolysis at different temperatures. The biochar yields ranged between 51.04 and 21.24%. BP gave the highest biochar yield at all pyrolysis temperatures, whereas CR gave the lowest yield at 500 °C. Nevertheless, at 500 °C, biochar yield was similar with all four feedstock types. Abdelaal et al., (2020)²⁰ reported OP biochar yields from 52 to 32% at temperatures from 300 to 500 °C, and Najdi et al.²¹ a PP biochar yield of 41% at 300 °C. Our results are similar to these previous findings. On the other hand, Lee et al., (2022)²² reported a CR biochar yield of 62% at 300 °C, higher than in the present study. Moreover, the BP biochar yield at 300 °C was slightly lower than the one reported by Te et al., (2021)²³ at 356.1 °C. Variations in biochar yield, even when using the same feedstock and pyrolysis temperature, can be attributed to various factors, such as heating time, pyrolysis technology, and other operational parameters. In our study, differences in biochar yields may be explained by differences in feedstock varieties, heating time, heating rate, and type of pyrolysis reactor.

Physicochemical properties

XRF results

Figure 1 presents the composition of the different biochar samples obtained at pyrolysis temperatures of 300 and 400 °C. The CR biochar had the highest carbon content (~ 61 and 57% at 400 and 300 °C, respectively), followed by the OP biochar (48 and 44% at 400 and 300 °C, respectively). The BP biochar had the lowest carbon content at both temperatures (35% at 300 °C). In all samples, oxygen content was lower at 400 than 300 °C. The OP biochar

Feedstock	Humidity (%)	Ash content (%)
OP	17.88	9.24
BP	16.02	9.78
PP	34.96	7.80
CR	20.75	4.21

Table 1. Humidity and ash content of the different feedstocks. The relative error for this measurement was 0.01. *OP* range peels, *BP* banana peels, *PP* potato peels, *CR* coffee residue.

Temperature	Feedstock	Biochar yield (%)	pH	EC ($\mu\text{S}\cdot\text{cm}^{-1}$)
Pre-treatment	BP	–	6.01	7780.0
	PP	–	6.28	1580.0
	OP	–	5.21	580.00
	CR	–	5.89	900.00
300 °C	BP	51.04	8.89	880.00
	PP	43.98	7.86	4070.0
	CR	42.66	6.82	390.00
	OP	40.69	7.32	260.00
400 °C	BP	39.55	9.19	10,500
	PP	31.88	9.34	2870.0
	CR	29.28	8.56	1290.0
	OP	37.25	8.90	790.00
500 °C	BP	27.52	9.69	11,740
	PP	22.24	10.14	2980.0
	CR	21.34	9.56	1480.0
	OP	26.68	10.29	980.00

The relative error for this measurement was 0.01

Table 2. Effect of the pyrolysis temperature on biochar yield, pH and EC. *OP* range peels, *BP* banana peels, *PP* potato peels, *CR* coffee residue.

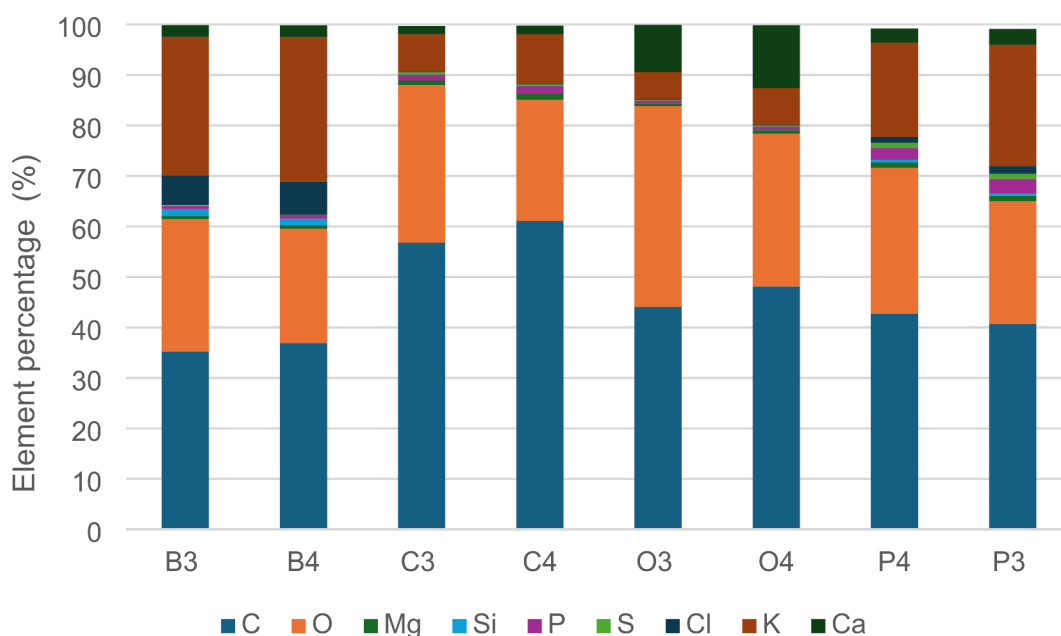


Fig. 1. Elemental composition of the biochar samples prepared using different feedstock types and two pyrolysis temperatures (300 and 400 °C). *B* banana peels, *O* orange peels, *C* coffee residue, *P* potato peels, 3: 300 °C, 4: 400 °C.

displayed the highest oxygen content at 300 °C (~39%) and the PP biochar the lowest oxygen content at 400 °C (24.27%).

The elemental composition varied significantly among biochar samples. However, the pyrolysis temperature had minimal influence on the mineral composition. The CR biochar displayed the highest phosphorus content (1.6% at 400 °C). Both CR and PP biochar types contained a notable amount of magnesium, up to 1.16%. Calcium content was relatively high in all samples, and particularly in the PP biochar (~24%). The lowest calcium concentration was detected in the BP biochar (2.29%). Similarly, potassium was detected in all samples. The highest amount was found in the BP biochar (28.5%) and the lowest in the PP biochar (1.5%). In conclusion, the presence in these biochar samples of essential elements, such as phosphorus, potassium, magnesium, and calcium, that are crucial for plant growth indicates that they are potential options for use in soil amendment applications.

It is worth noting the unhabitual presence of chlorine in the BP biochar samples. This could be due to the presence of organochlorine pesticide residues. However, the use of organochlorine pesticides has been banned in Lebanon. Therefore, more studies are needed to determine the exact source or cause of chlorine presence.

Takolpuckdee (2014)²⁴ reported very low calcium (1.246%) and higher magnesium (21.540%) contents in BP biochar, possibly due to the use of different banana varieties, methods of peel removal and pyrolysis temperatures. Previous studies found that BP pyrolyzed at 400 °C contained ~ 51% of carbon, 42.55% of potassium, and 11.53% of calcium^{23,25–27}. In another study, BP biochar produced at 450 °C had a carbon content of 69.99%. The oxygen content varied between 38% and 14% across the temperature range of 200–600 °C. The values for BP biochar in our study were lower. According to the literature, OP biochar pyrolyzed at 400 °C had a carbon content of ~ 58%. Another study showed that at the same pyrolysis temperature, the biochar derived from OP contained ~ 80.8% of carbon and 14.5% of oxygen. However, when pyrolyzed at 300 °C, OP biochar contained 80.72% of carbon and 16.16% of oxygen. All these findings indicate higher carbon and oxygen contents compared with our results^{27–29}. In their review, Abdelaal et al., (2020) reported that OP biochar produced at 400 °C had a carbon content of 47.00%²⁰. Stella et al., (2016)³⁰ found that the carbon content of OP biochar was 40.43%, and Lam et al., (2018) reported a carbon content of 42.50%³¹. These results are similar to our findings in the same pyrolysis conditions. CR biochar produced at 850 °C had the following elemental composition: 75.29% of carbon, 14.11% of oxygen, 0.85% of magnesium, 0.75% of phosphorus, 6.3% of potassium, and 2.69% of calcium^{32,33}. When PP were pyrolyzed at 350 °C, the resulting biochar contained ~ 60.8% of carbon (nearly twice the carbon content of our PP biochar) and 13.7% of oxygen³⁴. It can be concluded that both pyrolysis temperature and feedstock type significantly influence the biochar elemental composition. Specifically, an increase in pyrolysis temperature leads to an increase in the biochar carbon content. This can be attributed to the biomass thermal decomposition at higher temperatures, resulting in the breakdown of complex organic compounds and the release of volatile matter, including oxygen-rich compounds. Consequently, the carbon concentration in the biochar increases. Conversely, the oxygen content in the biochar decreases at higher pyrolysis temperatures. This is primarily due to the loss of oxygen-rich compounds during the pyrolysis process³⁵. Moreover, the mineral concentrations in biochar increase with the pyrolysis temperature. This is reflected by the high EC values observed in our biochar samples³⁶. It is important to note that variations in the biochar elemental composition can also be attributed to other factors, such as feedstock variety, pyrolysis conditions and biomass pretreatment. Different feedstock types can vary in chemical compositions, resulting in different elemental compositions of the produced biochar. Similarly, variations in pyrolysis conditions, such as heating rate and residence time, can influence the thermal decomposition extent and the resulting elemental composition. Biomass pretreatment techniques, such as washing and drying, also affect the biochar elemental composition.

The oxygen-to-carbon (O/C) ratio of the different biochar samples obtained at two pyrolysis temperatures (300 and 400 °C) ranged from 0.68 to 0.29 (Table 3). The O/C ratio is a reliable indicator of the biochar stability³⁶. According to Spokas (2010), the O/C ratios of most biochar types are typically between 0.2 and 0.6³⁷. Our results are within this range, suggesting that our biochar samples are moderately stable. Indeed, O/C ratios close to 0.6 indicate less stable biochar samples, whereas lower O/C ratios suggest higher stability. This variability indicates the influence of other factors, in addition to the pyrolysis temperature, such as feedstock type and post-production conditioning, that need to be better investigated.

FT-IR results

The FT-IR spectrum peaks were observed within the 4000–500 cm^{-1} wavelength range, which confirmed the complex chemical nature of the biochar samples. The functional groups present in the various biochar samples are listed in Table S1 (supplementary material). Figure S2 (supplementary material) highlights the presence of differences in the analyzed spectra among the different biochar samples and pyrolysis temperatures. Specifically, the absorbance peak at 3490 cm^{-1} is indicative of OH stretching vibrations and confirmed the presence of alcoholic and phenolic functional groups associated with cellulose, hemicellulose, and lignin components in the feedstocks. The strength of this peak was more pronounced in the PP and BP biochar samples obtained at 400 °C. Additionally, the aliphatic C–H stretching vibration at 2910 cm^{-1} was more prominent in the CR and BP biochar samples obtained at 300 °C. This suggests the presence of alkanes, derived from various carbohydrate monomers. In summary, these spectral differences offer valuable insights into the variations in chemical composition resulting from differences in feedstock types and fluctuations in the pyrolysis temperature. Furthermore, an

Biochar samples	O/C ratio
B3	0.56
B4	0.46
C3	0.41
C4	0.29
O3	0.68
O4	0.47
P4	0.51
P3	0.45

Table 3. Oxygen-to-carbon (O/C) ratio of the different biochar samples obtained using different pyrolysis temperature. *B* banana peels, *O* orange peels, *C* coffee residue, *P* potato peels, 3: 300 °C, 4: 400 °C.

increase in the pyrolysis temperature to 400 °C promoted the formation and improvement of an aromatic carbon structure in biochar samples, especially in PP, OP and BP biochar. This is in line with the observations by Mokhtar et al., (2019)³⁸. Previous authors, including Sbizzaro et al., (2021) and Fawzy et al., (2021) showed that biochar with heightened aromaticity exhibits greater adsorption capacity, increased potential for carbon sequestration, and enhanced stability in soil. These features were further validated by FT-IR analysis^{5,39}. Table S2 (supplementary material) lists the main functional groups detected in the biochar samples.

Surface properties

pH and electrical conductivity

The results of the pH and EC measurements before and after pyrolysis are in (Table 2; Figs. 2 and 3).

The pH values of the biochar samples varied between 5.21 and 10.29. The pH values were lower in OP and CR. At 500 °C, the highest pH was found in the OP biochar, followed by the PP biochar, while the CR and BP biochar samples had similar pH values. Ding et al., (2014) observed that the biochar pH value increased from 6.5 to 10.8 with the increase in temperature⁴⁰. Our findings show an increase of pH values for BP biochar from 300 °C to 500 °C, consistent with the study by Tan and So (2018)⁴¹. Conversely Al-Awadhi et al., (2022) reported slightly higher pH values for CR biochar at 300–600 °C³⁶. Zhang et al., (2017)⁴² noted that higher pyrolysis temperatures increased the pH of biochar samples, rendering them more alkaline, and that this effect was influenced by the feedstock type and pyrolysis temperature and duration.

The EC values ranged between 0.26 and 11.74 ms/cm. The increase in pyrolysis temperature led to an increase in EC values. Among the feedstocks, OP had the lowest EC and BP the highest. Compared with the literature data, the EC value of the OP biochar sample at 400 °C was lower than that reported by Stella et al., (2016)³⁰ and higher than that by Sial et al., (2019)⁴³. The EC value of the CR biochar at 400 °C was close to that reported by Cervera-Mata et al., (2022)⁴⁴. The EC values of the four biochar types were significantly different, and this difference was due to the feedstock type and not the pyrolysis temperature, unlike in the study by Bartoli et al., (2022)⁴⁵ who found that both temperature and feedstock type influenced the EC. Thus, different parameters and conditions affect the biochar characteristics differently.

Their distinct chemical composition indicates that these biochar samples show promise for soil amendment, particularly in terms of potassium, phosphorus, and calcium content. This diversity makes their mixture an attractive option for soil amendment. Therefore, the toxicity of these biochar samples was tested using two methods to assess their suitability as soil amendments.

Germination test in soil

Comparison of the characteristic of the two soils used in the study highlighted their similarities and differences (Table S3 in supplementary material). Both soils were categorized as clayey soils and showed similar pH levels

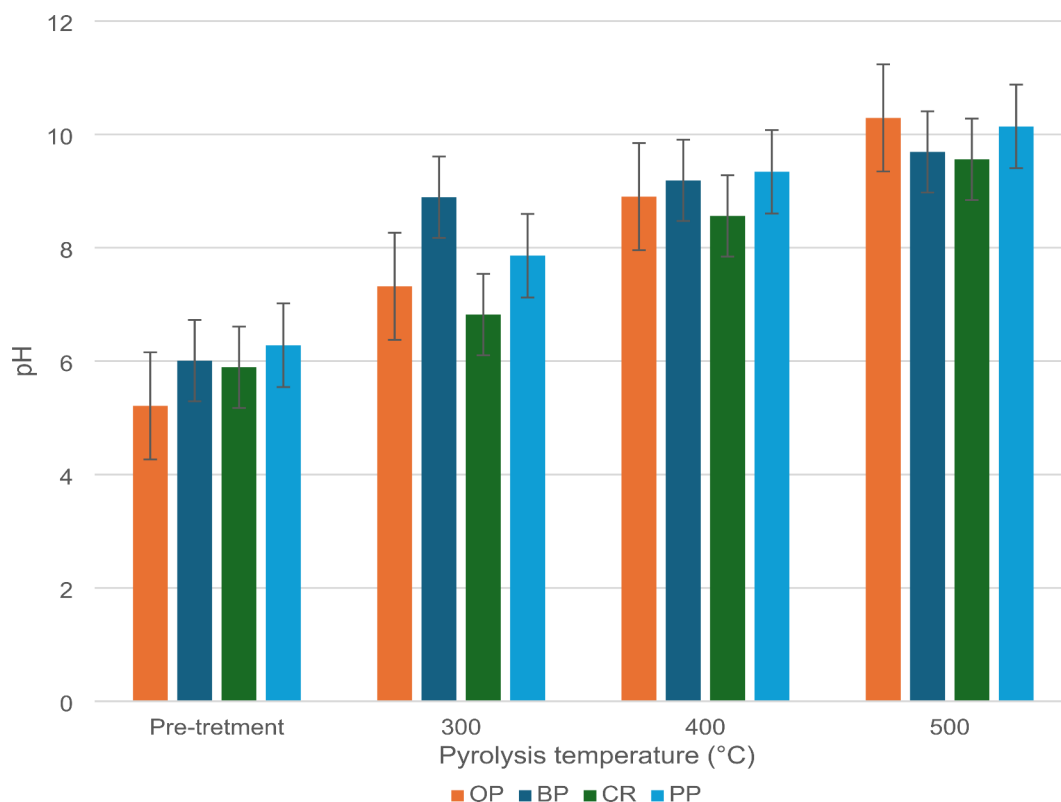


Fig. 2. pH of different feedstocks and of the biochar samples obtained by pyrolysis at the indicated temperatures. OP orange peels, BP banana peels, PP potato peels, CR coffee residue.

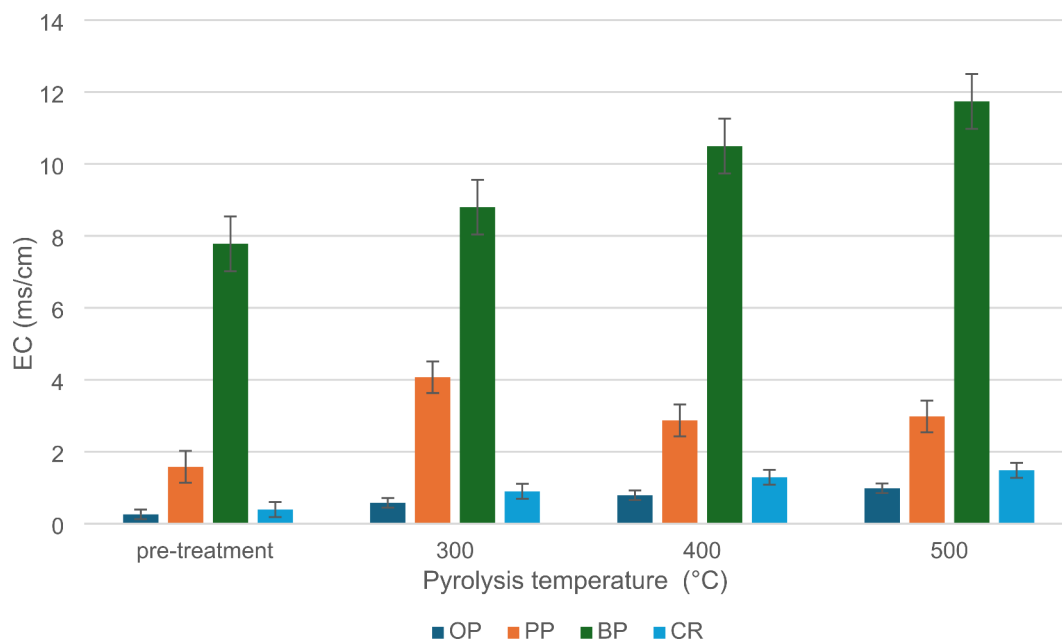


Fig. 3. Electrical conductivity (EC) of the different feedstocks (pre-treatment) and biochar samples obtained at the indicated pyrolysis temperatures. *OP* orange peels, *BP* banana peels, *PP* potato peels, *CR* coffee residue.

and EC values. However, the Riyaak soil displayed higher nitrogen and potassium contents, and the Anjar soil higher amounts of organic matter, carbonate and micronutrients, such as calcium and magnesium.

The analysis of biochar phytotoxicity (seed germination inhibition) necessitates a comprehensive consideration of the various soil features because these factors intricately shape the interactions between biochar and seeds, consequently affecting the plant growth and development. Specifically, the soil pH plays a pivotal role, influencing nutrient availability and the bioavailability of specific chemicals in the soil. This can profoundly affect seed germination and the subsequent plant growth. The organic matter content in the soil also is a critical variable because it interacts with the biochar, influencing its stability and the potential release of nutrients or chemical compounds. Moreover, the organic matter content influences the soil water-holding capacity and nutrient retention capacity, two features that directly modulate seed germination and plant growth. The soil texture and structure also are key features because they influence its physical properties, such as water retention and aeration. The soil structure affects root penetration, water movement and nutrient availability, which are crucial for plant development. Nutrient content, including macronutrients and micronutrients, influences the plant growth. Biochar adds complexity, potentially altering the nutrient availability. The water-holding capacity and drainage influence seed germination and root development. Understanding these interconnected properties is vital when studying the biochar effects on seeds. The used soils were suitable for plant growth due to their clayey texture that offers good water retention. Their similar pH and conductivity levels suggest that they are suitable for most plant species. Nutrient presence in both soils supports plant nutrition and development.

At the application rate of 1%, the highest what seed germination rate (45%) was observed with the CR biochar obtained at 300 °C. This was higher than the rates of control soil (40%) and of the soils with CR biochar at 400 °C and also with OP biochar at 300 °C and 400 °C (Fig. 4A). This is in agreement with the biochar ability to improve soil conditions and nutrient availability that support the early growth stages. The longest shoot length (25 cm) was observed in the soil with the OP biochar obtained at 300 °C (1% application rate) (Fig. 4C), suggesting a beneficial effect on the aboveground biomass. This finding underscores the biochar role in enhancing nutrient retention and promoting root growth, which are crucial for the overall plant vigor.

At the application rate of 3%, the the longest shoot length (~15 cm) was observed in the soil with the PP biochar obtained at 300° C, followed by the soil with the BP biochar obtained at 400 °C (14.85 cm), and the control soil (13 cm). Germination rate was 70% in the soils with the PP biochar obtained at 300 °C and 400 °C, and 55% in the control soil (Fig. 5B and D). These parameters were evaluated three weeks after wheat seed sowing. However, the different comparisons carried out did not find any significant difference in germination rate and shoot length ($P > 0.05$) between soil samples with and without biochar (Tables S4 and S5 in supplementary material). Nevertheless, the absence of negative effects of the biochar types on the germination rate and shoot length suggests that the different feedstock types used for biochar production, the applied doses (1% and 3%), and the pyrolysis temperatures (300 °C and 400 °C) did not have any toxic or detrimental effects on the soils. The absence of adverse effects indicates that these biochar types can be used as a soil amendment without causing harm to the environment or hindering plant growth.

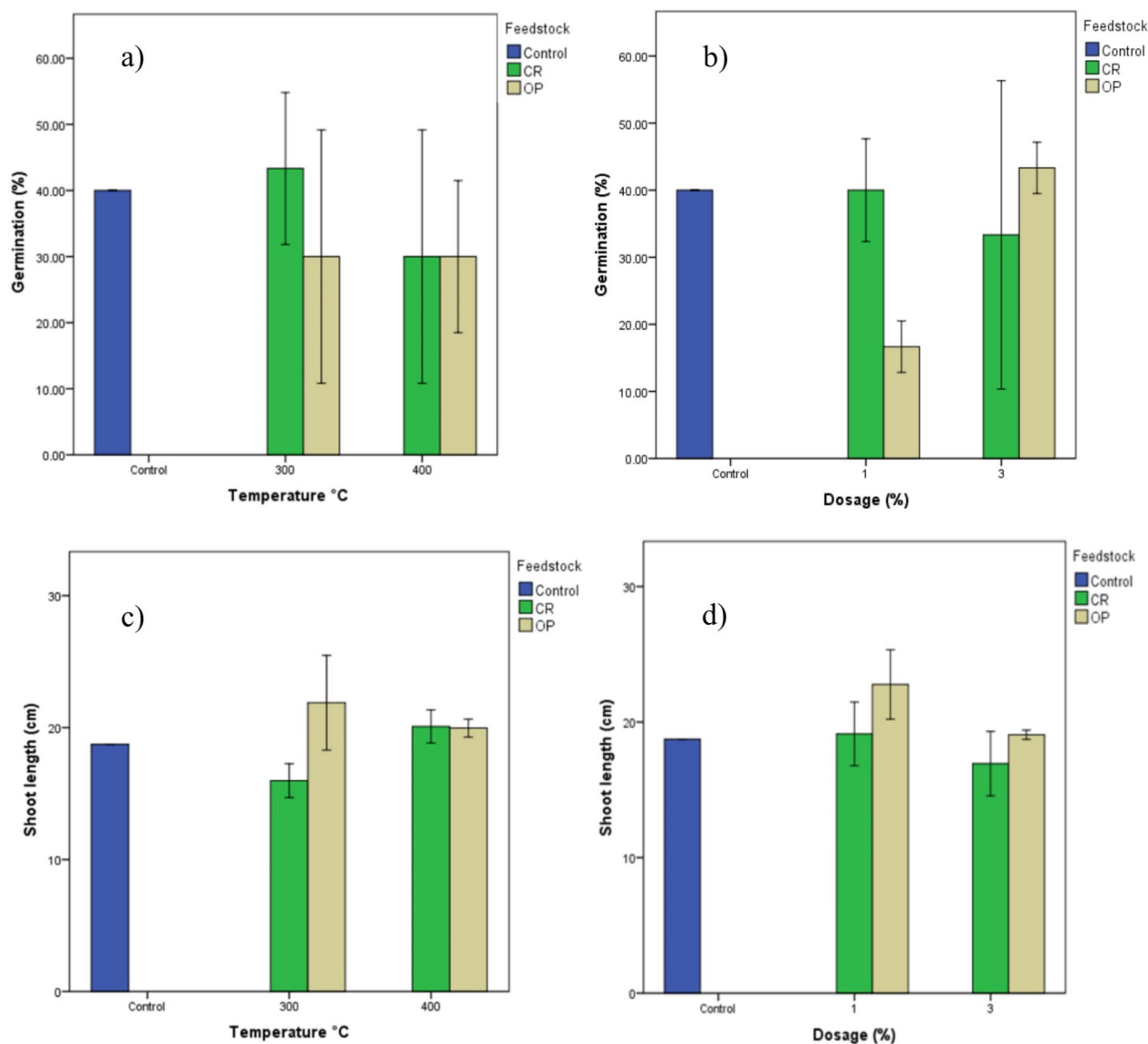


Fig. 4. Effect on wheat seed germination rate (a,b) and shoot length (c,d) in a clayey soil of CR and OP biochar samples obtained at 300 and 400 °C (a–c) and used at 1 and 3% (b–d).

Phytotoxicity test

The CR biochar obtained at 300 °C exhibited no adverse effect on wheat seed germination when incorporated directly into the soil. However, when the liquid extract of CR biochar at 300 °C was applied instead of the solid biochar, seed germination was fully inhibited. Conversely, with the liquid extract of CR biochar obtained at 400 °C, germination rate was ~69% and shoot length was 11 cm (after 10 days) (Fig. 6). These findings indicated that the phytotoxicity test using the liquid extracts was more sensitive. The toxic effect of the CR biochar extract at 300 °C was attributed to the presence of phytotoxic compounds, such as polyphenols. However, this problem was effectively addressed by increasing the pyrolysis temperature to 400 °C that helped to eliminate these harmful elements, as previously reported by Pastoriza et al.⁴⁶

When water extracts of BP and PP biochar (obtained by pyrolysis at 300 °C) were used, germination was completely inhibited, while the control group showed normal germination (data not shown). However, BP and PP biochar toxicity has been rarely described, and a previous study showed that BP biochar enhances plant growth without toxic effects²⁵. This difference was intriguing and prompted further investigations. An additional step was implemented by soaking BP and PP biochar in distilled water for two days followed by vacuum-filtering and repeated washes till the filtrate became clear, compared with its initial dark color. After biochar drying, the phytotoxicity test was repeated using the same protocol as before. The washing step, significantly improved the germination rates in biochar extract-treated soil samples. The germination rates were 98% in the control and 92% and 83% in the presence of the washed PP and BP biochar extracts, respectively. Moreover, the longest shoot length (8.3 cm) was observed in soil samples treated with PP biochar extract obtained by pyrolysis at 400 °C and the shortest shoot length with the BP biochar extracts (both temperatures) (Fig. 7).

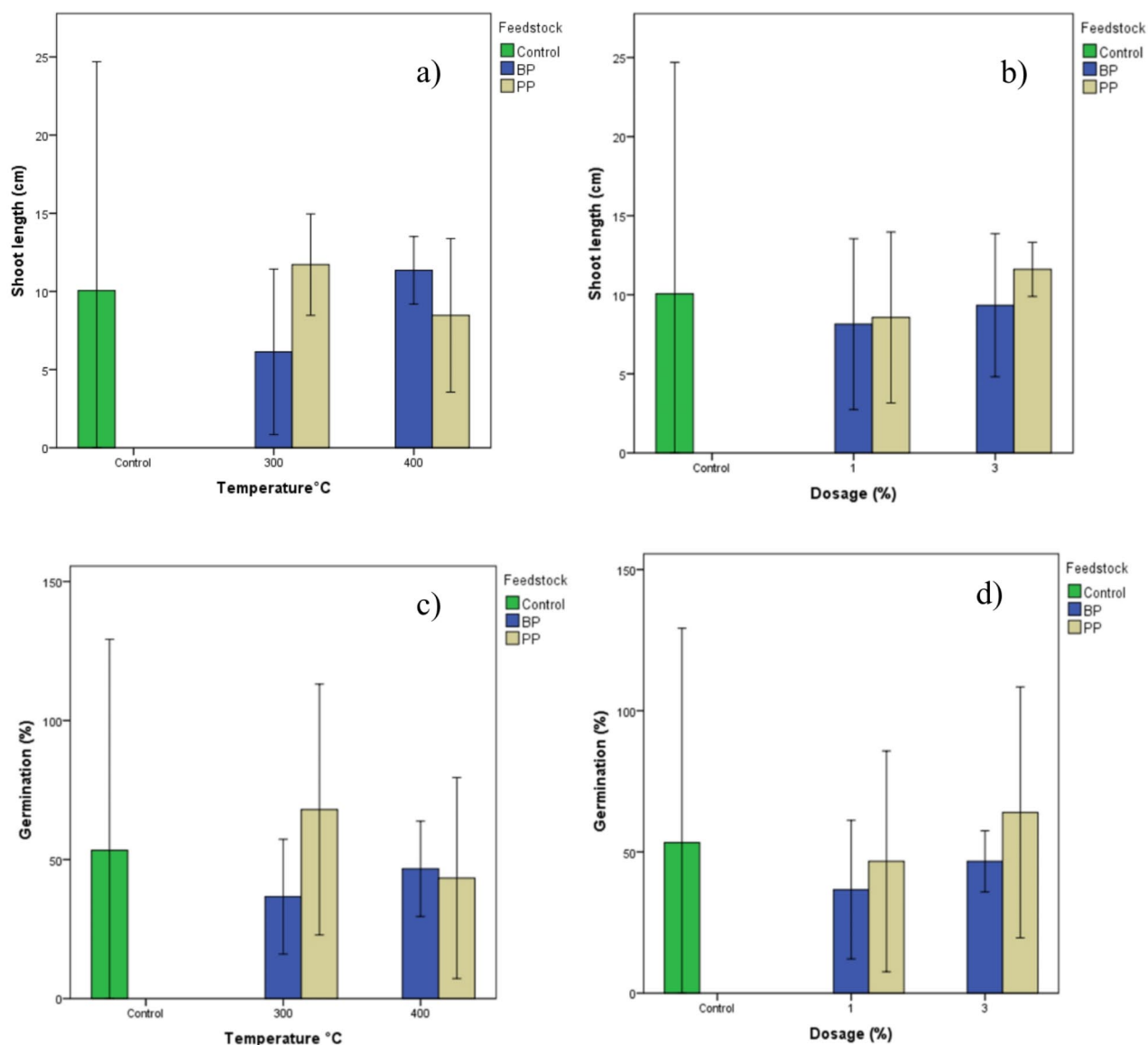


Fig. 5. Effect on wheat shoot length (a,b) and wheat seed germination (c,d) in a clayey soil of BP and PP biochar samples obtained at 300 and 400 °C (a–c) and used at 1 and 3% (b–d).

Although the PP and BP biochar extracts had high pH levels, which have been associated with phytotoxicity in other studies, phytotoxicity was more likely due to PAHs that were eliminated during the washing step. These findings suggest that biochar washing is a successful strategy to overcome biochar toxicity. Additionally, when biochar is incorporated into the soil, its phytotoxicity is reduced because irrigation acts as a natural washing step. Various studies, including the work by Intani et al., (2019)⁸, emphasized the importance of biochar washing as an essential and cost-effective approach to reduce/eliminate toxic compounds.

Tables S6–S10 (supplementary material) list the results of the different comparisons carried out using the Turkey HSD test in order to determine (i) the influence of the pyrolysis temperature on CR, OP, BP, and PP biochar effect on wheat seed germination and shoot length in soil; and (ii) the effect of the different feedstock types and the pyrolysis temperature on germination and shoot length in the phytotoxicity test with biochar extracts.

It is important to note that the obtained results are specific to the experimental conditions used in this study. Different experimental conditions, such as pyrolysis temperature, biochar amount, biochar type, seed type, and soil conditions, can affect germination rates. Even when the same biochar is added at the same concentration, different results can be observed for different crops (Table 4), as reported by Oliveira Da Mota et al.⁴⁷. This highlights the necessity of performing phytotoxicity tests before applying any produced biochar, even if positive effects were previously reported.

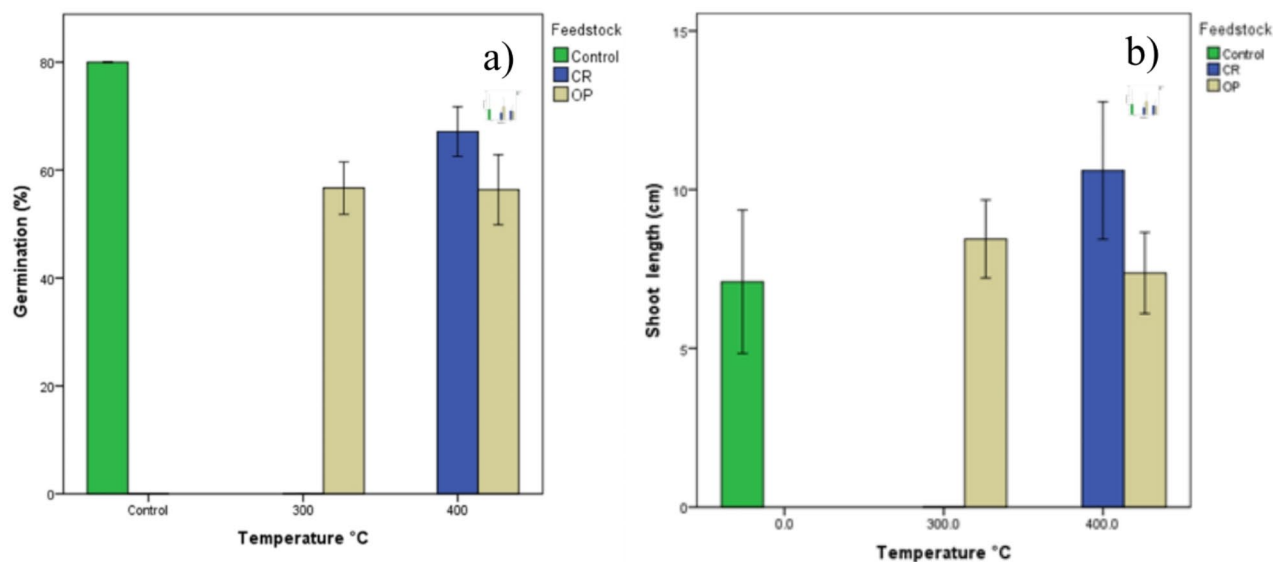


Fig. 6. Effect of CR and OP biochar extracts on wheat seed germination rate (a) and shoot length (b) in function of the pyrolysis temperature (300 and 400 °C).

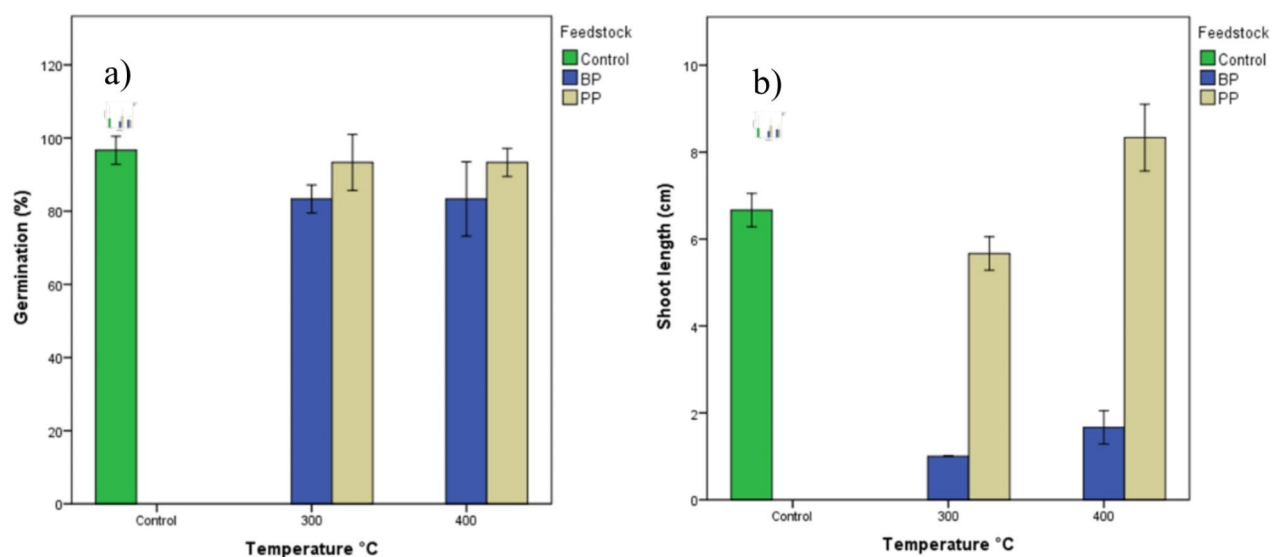


Fig. 7. Effect of washed BP and PP biochar extracts (obtained by pyrolysis at 300 °C and 400 °C) on wheat seed germination rate (a) and shoot length (b).

Conclusion

This study evaluated the phytotoxicity and effectiveness of biochar produced from kitchen waste (BP, PP, OP and CR) as a soil amendment with the aim of reducing the dependence on expensive fertilizers and supporting sustainable agriculture practices. Our analysis revealed that in the obtained biochar samples, carbon content ranged from 48 to 35%, calcium from 24 to 1.6%, and potassium from 28.5 to 1.5%, and the O/C ratio from 0.68 to 0.29, indicating moderate stability and suitability for agricultural use. The highest germination rate (45%) was observed with the CR biochar obtained by pyrolysis at 300 °C and used at the concentration of 1% and the longest shoot length (25 cm) with the OP biochar obtained by pyrolysis at 300 °C and used at the concentration of 1%. Moreover, the phytotoxicity tests demonstrated that addition of a biochar washing step significantly improved germination rates (PP biochar: 92%, BP biochar: 83%) and shoot length by reducing the toxic compounds in some biochar types.

The encouraging results of the germination and phytotoxicity tests suggest that BP, CR, PP and OP biochar can enhance plant growth and are generally non-toxic for agricultural use. However, the germination rate variability underscores the importance of considering factors, such as pyrolysis temperature, biochar application rates, seed types, and soil conditions, and to always carry out analyses to identify other toxicity-causing factors, such as

Biochar	Application rate (%)	Germination rate (%)	Reference
Rice husk	1	91	48
Wheat Chaff	1	95	48
Wheat Chaff	10	81	48
Coconut shells	5	80	49
Coconut shells	5	40	49
Coconut shells	10	80	49
Coconut shells	10	67	49
Orange peels	1	100	50
Lantana	1.80	95.23	51
coffee residue	1.0	93.3	52
Coffee residue	10	76	52
Banana peels	2.4	66	53
Banana peels	2.4	76	53
Viticulture waste	5	72	47
Viticulture waste	5	4	47
Orange peels	1	18	This study
Orange peels	3	45	This study
Coffee residue	1	40	This study
Coffee residue	3	36	This study
Banana peels	1	45	This study
Banana peels	3	50	This study
Potato peels	1	50	This study
Potato peels	3	60	This study

Table 4. Germination rates of various seeds after addition of different biochar types (and at different application rates).

heavy metals, PAHs and pH levels. Moreover, phytotoxicity tests must be performed before biochar application to ensure its positive impact on crop growth. More studies should be carried out to precisely understand the impact on plant growth of biochar used as a soil amendment.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Author contributions

All co-authors contributed equally to the work.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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