

## Article

# Phytotoxicity Assessment of Agro-Industrial Waste and Its Biochar: Germination Bioassay in Four Horticultural Species

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**Abstract:** This study investigated the phytotoxicity of agro-industrial wastes (almond, walnut, pistachio and peanut shells, asparagus spears, and brewer's spent grain) and their biochar through germination bioassays in several horticultural species: green pea, lettuce, radish, and arugula. Biowaste was pyrolyzed under controlled conditions to produce biochar, and both biowaste and biochar were characterized. Germination bioassay was conducted using seeds exposed to different dilutions of aqueous extract of biowaste and their biochar (0, 50, and 100%). Germination percentage, seed vigor, germination index, and root and aerial lengths were evaluated. The results showed that the phytotoxicity of the biowaste was significantly different to that of its biochar. The biochar obtained demonstrated changing effects on germination and seedling growth. In particular, biochar extracts from spent brewers grains, walnut shells, and pistachio shells showed 5–14% increases in seed vigor and root and aerial length. Furthermore, the response of different species to both agro-industrial waste and biochar revealed species-specific sensitivity. Seeds of lettuce and arugula species were more sensitive to aqueous extracts than radish and green peas. This knowledge not only elucidates the behavior of agro-industrial waste-based biochar in the early stage of plant development but also provides valuable insights regarding phytotoxicity, seed sensitivity, and the variables involved in germination.

**Keywords:** biochar; biowaste; emergence; inhibition; vegetable



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## 1. Introduction

In recent years, the need for sustainability in agricultural practices has become increasingly evident. Moreover, agricultural and industrial waste or byproducts are increasing exponentially, and the practical management and safe disposal of this biomass has become a major environmental concern around the world [1]. With concerns about environmental degradation and resource depletion, there is a growing need for innovative solutions that not only reduce waste but also increase agricultural productivity. Agro-industrial waste presents a significant opportunity in this regard [2].

In this context, pyrolysis is emerging as the most environmentally friendly way to recycle biowaste [3]. Pyrolysis has been widely used for centuries as an alternative way to handle various biowaste [4,5]. This process can reduce the weight, volume, and odor of the

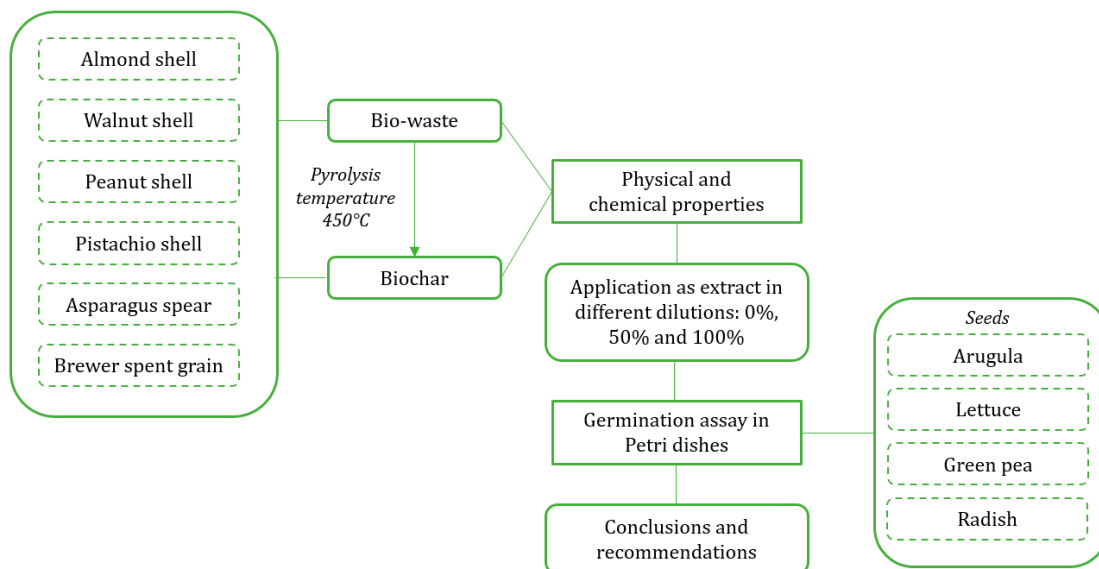
biomass and also transform the waste into a high-value-added product [3]. The products of pyrolysis are biochar, bio-oil, and syngas. All three products can be used as biofuel, but biochar can be used to improve soil quality [6], mitigate various agro-environmental stresses [7], and can also be used as a substrate alternative in soilless culture [8]. Biochar is a highly stable carbon-rich material that can remain in the soil for hundreds or thousands of years [9]. With its unique properties, it is increasingly recognized for its potential to improve soil fertility, absorb or neutralize natural toxins in decomposing organic materials, herbicides, and pesticides, sequester carbon, and mitigate greenhouse gas emissions [10]. The integration of biochar derived from agro-industrial waste into horticultural practices offers an opportunity not only to address waste management challenges but also to promote sustainable agricultural systems. However, in some cases, the waste from different food industries can be directly applied to agricultural soil as a source of labile organic N, P, and C, but these have the disadvantage of excessive fertilization and leaching [11]. Thus, converting these elements to slower cycling forms through carbonization, like biochar, can have potential benefits [3,11]. Moreover, the use of biowaste as fertilizer on soils destined for food production enriches soil fertility and structure, but also mitigates waste disposal issues, providing a sustainable solution that benefits both agricultural productivity and environmental health. However, problems can arise due to its potential to bioaccumulate, generating a risk to human health if the wastes contain any toxic compounds [12,13]. The use of biochar has raised concerns about its potential toxicity because its chemical composition depends on the biomass from which it is derived and the conditions under which it was obtained (temperature, residence time, and heating rate, among other variables). In this sense, recent studies have been carried out to explore physiological effects on biota [14]. The potential for biomass amendments to cause toxicity is a significant barrier to adoption by producers [15].

Understanding the toxic effects of contaminants is a key requirement for environmental risk assessments [13]. Physicochemical parameters can be used to characterize wastes, but they do not provide sufficient information to ensure that wastes from food production are safe for agricultural use [16]. Ecotoxicological assays play a fundamental role in this area [17]. Phytotoxicity assays consider germination and root growth of different plant species as indicators of biological responses to biosolids stabilization [13,16]. This is because seed germination is one of the most important phases in the life cycle of a plant and is highly sensitive to its surrounding environment [18]. Phytotoxicity is a way to measure the delay or inhibition of seed germination, inhibition of plant growth, or any adverse effects on plants caused by certain substances [19]. These inhibitory effects on growth may be the result of a combination of factors, such as exposure to heavy metals, ammonia, salts, and low-molecular-weight fatty acids [13]. Germination rate (GR) and root elongation (RE) assays are commonly used to evaluate the phytotoxicity of contaminants and contaminated environmental compartments [20]. Furthermore, bioassays respond not only to known compounds but also to complex mixtures of phytotoxins [19,21].

On the other hand, the effects of biochar on plant growth are highly variable and depend on the feedstock, thermochemical process conditions, application rate, soil characteristics, environmental conditions, and plant species used [22]. There is a need for more detailed studies with different types of biochar to explain the diversity of results reported in the literature, ranging from increased plant productivity to high phytotoxicity [23]. There is a scarcity of studies reporting on the influence of biochar on the early stages of plant growth, as most studies have been conducted on forest plants [23]. Some authors have investigated the effect of biochar on the germination of corn seeds [24,25] and observed that there was no effect on the early growth of corn seedlings. There were also no interactions between the type and rate of biochar and soil type. Similarly, in a study evaluating the growth of lettuce seeds using peanut shells biochar as an amendment, the authors did not observe a significant effect on vegetable yield, but a positive effect was observed, attributed to the improvement in soil parameters [26]. Abdelhafez et al. [27] evaluated walnut shell biochar produced by conventional pyrolysis, with a small water jacket connected to the

unit to capture the emitted gases. The collected liquefied smokes were tested on *Lactuca sativa* (lettuce) seed germination at different concentrations (0%–5%, *v/v*) and showed an increase in leaf chlorophyll pigments and an improvement in plant growth parameters after application at intermediate rates. Rogovska et al. [28] reported that some biochar contains phytotoxic compounds that inhibit the germination of corn, as reported in their study: hardwood, corn, and switchgrass. These authors propose leaching the biochar to eliminate possible phytotoxic compounds. On the contrary, Milon et al. [29] evaluated the maturity of compost modified with commercial biochar using vegetable seedlings through a phytotoxicity germination bioassay, where they observed that none of the extracts of the treated compost product had any phytotoxic effect on the seeds of the crops and that the treatment with higher biochar content had a higher GI compared to the other treatments, including the control.

The main objective of this work was to evaluate the phytotoxicity of different biowaste and biochar obtained by pyrolysis, from six agro-industrial biowastes: almond (Alm), walnut (Wal), peanut (Pea), pistachio shells (Pis), asparagus spear (Asp), and brewer spent grain (Bre), and on seed germination and seedling growth of four different species: green peas (*Pisum sativum* L.), lettuce (*Lactuca sativa* L.), radish (*Raphanus sativus* L.) and arugula (*Eruca sativa* M.), under laboratory bioassay conditions. For this, biochar and their raw materials were incorporated in different proportions in aqueous extracts (0, 50, and 100%). The aqueous extracts obtained were used for germination tests in the different types of seeds. It is noteworthy that the novelty of this work lies in its comprehensive approach, which encompasses a diverse range of biomass sources, the utilization of pyrolysis, the assessment of multiple plant species, and a focus on early-stage plant development under controlled laboratory conditions. The findings of this study could contribute valuable insights into the phytotoxicity of biochar derived from agro-industrial biowaste, with potential implications for sustainable agricultural practices. A logic diagram of the study is shown in Figure 1.



**Figure 1.** Logic diagram, a methodological scheme of the germination bioassay in horticultural species: evaluation of biowaste and biochar.

## 2. Materials and Methods

### 2.1. Biowaste Preparation and Characterization

The Alm, Wal, Pea, and Pis were provided by farms in the departments of Albardón, Calingasta, 25 de Mayo, and Pocito, respectively, in San Juan, Argentina. The biowaste Asp was provided by a farm located in Médano de Oro, Rawson, and the biowaste Bre was acquired from the brewery “Cumbre”, both located in the Province of San Juan, Argentina.

The biowaste samples were characterized as follows: pH and electrical conductivity (EC) were measured according to the methodology proposed by Belda et al. [30], using a digital pH meter (Adwa AD1000, Budapest, Hungary) and a conductivity meter (EC-214, Hanna Instruments, Rome, Italy), respectively. Organic matter (OM) content was determined according to the methodology described by Schulte and Hopkins [31]. Once the OM content was known, organic carbon (OC) was estimated using van Bemmelen factor. The Kjeldahl method was used to determine total nitrogen (TN) [32]. Proximate analysis (ash and volatile matter content (VM)) was performed according to ASTM standards [33,34], and elemental analysis was performed using an elemental analyzer (AuroEA3000, Rome, Italy). The moisture content was determined using an infrared moisture analyzer (Radwag PMR50, Radom, Poland). In addition, lignin, cellulose, and hemicellulose contents were determined [32]. All analyses were carried out in triplicate. Figure 2 shows images of the analyzed biowastes.



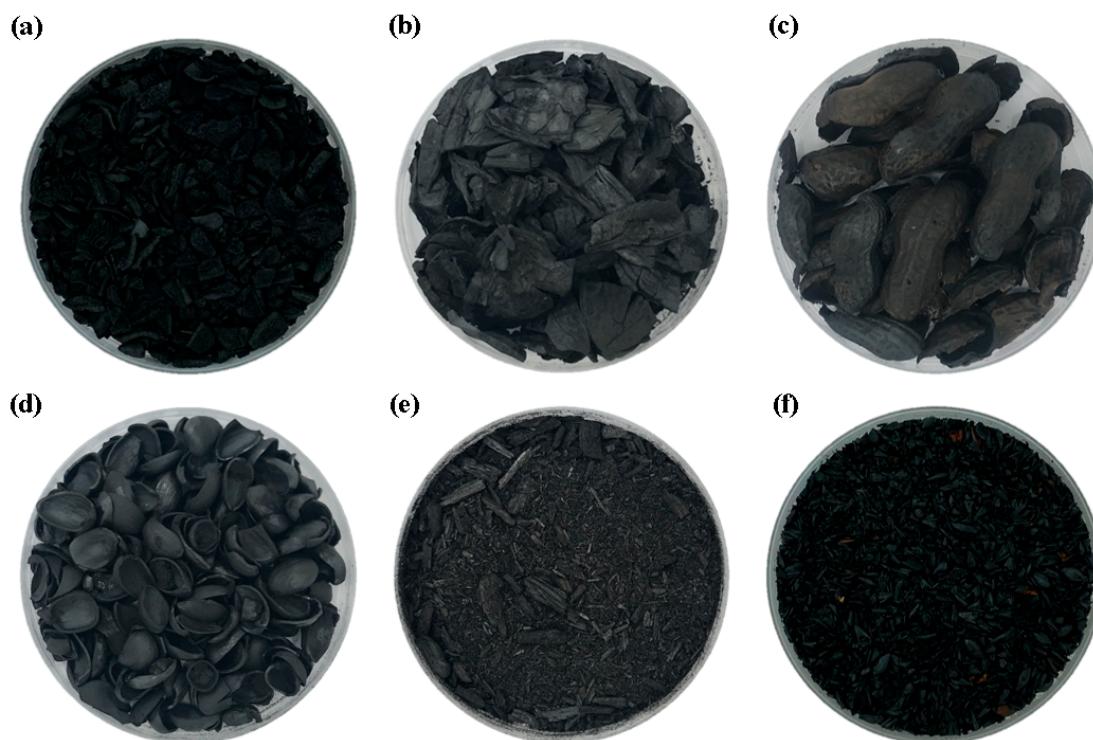
**Figure 2.** Biowastes: (a) Alm, (b) Wal, (c) Pea, (d) Pis, (e) Asp, and (f) Bre.

### 2.2. Pyrolysis Experiments for Biochar Production

The experiments were carried out in a cylindrical reactor as described by Rodriguez Ortiz et al. [35], under an inert atmosphere ( $N_2$  atmosphere) at a temperature of  $450\text{ }^\circ\text{C}$ , with a residence time of 2 h [36,37]. The reactor had a capacity of 4 kg of feedstock. To carry out the pyrolysis process, independent samples were used as received, except for Asp and Bre, which were previously dried to a moisture content of less than 10% (initial moisture: 60–80%) following the methodology described by Capossio et al. [38]. Then, the biochar was ground and sieved for analysis. The size of the samples was 1190–2380  $\mu\text{m}$ .

### 2.3. Physical and Chemical Properties of the Biochar

The pH, EC, OM, OC, TN, proximate, and elemental analysis in the biochar samples were determined following the procedures described in Section 2.1. All analyses were performed in triplicate [32]. Figure 3 shows images of the corresponding biochars obtained.



**Figure 3.** Biochars: (a) AlmB, (b) WalB, (c) PeaB, (d) PisB, (e) AspB, and (f) BreB.

#### 2.4. Germination Assay and Growth Seedling

The Alm, Wal, Pea, Pis, Asp, and Bre samples and their respective biochars were conditioned to establish the different dilution extracts: 0%, 50%, and 100%. Distilled water was used as the control solution (0%). All other solutions were prepared using distilled water, biowaste, and their biochars in the ratios shown in Table 1.

The in vitro bioassay for seeds was carried out in a Petri dish without soil, following the methodology reported by Solaiman et al. [39]. Five seeds of radish (*Raphanus sativus* L.), arugula (*Eruca sativa* M.), green peas (*Pisum sativum* L.), and lettuce (*Lactuca sativa* L.) were sown in Petri dishes on a layer of filter paper moistened with the extract of the above treatments (Table 1).

All Petri dishes were incubated at 25 °C for 72 h in the dark. Then, the germination percentage (GP; %) was calculated, and the aerial length (AL; cm) and root length (RL; cm), were measured; for this, the ImageJ software [40] was used. The mean value of root length determined for each Petri dish was used to calculate the germination index (GI) according to the following expression [7]:

$$GI = \frac{G}{G_0} \cdot \frac{L}{L_0} \cdot 100 \quad (1)$$

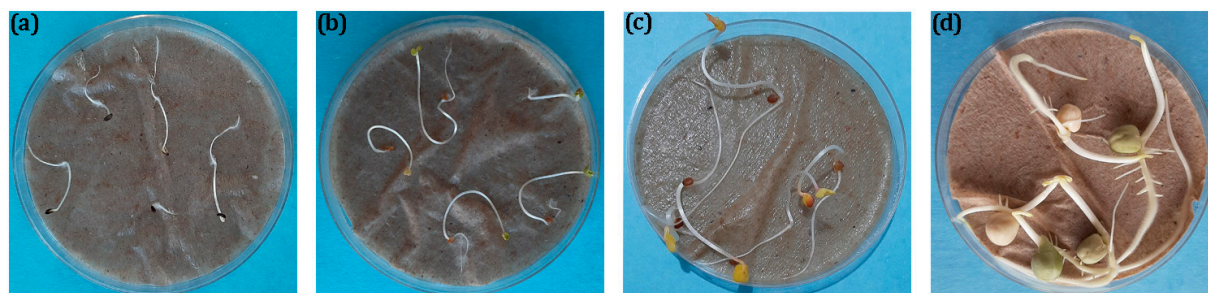
where G and L are the germination percentage and root length of the treatment and G<sub>0</sub> and L<sub>0</sub> of the control, respectively. Moreover, seed germination percentage (GP) and seed vigor (SV; %) were calculated as follows:

$$\text{Germination Percentage (GP)} = \left( \frac{\text{SNG}}{\text{SNO}} \right) \cdot 100 \quad (2)$$

$$\text{Seed Vigor (SV)} = \text{GP} \cdot \text{Seedling length} \quad (3)$$

where SNG and SNO are germinated seeds in the total and the total number of viable seeds.

According to the International Seed Testing Association (ISTA) [41], 4 days and 7 days were used as the initial and final counts for arugula and lettuce seeds, 4 and 10 days for radish seeds, and 5 and 8 days for peas, respectively (see Figure 4).



**Figure 4.** Photographs taken at the end of the Petri dish assay. Horticultural seeds emerging in control extract: (a) *Lactuca sativa*, (b) *Eruca sativa*, (c) *Raphanus sativus*, and (d) *Pisum sativum*.

**Table 1.** Description of extract preparation.

Treatment	Dilution Extract (v:v)
Control	Sterilized distilled water
Alm50	Almond shell: distilled water (1:5)
Alm100	Almond shell: distilled water (1:2.5)
AlmB50	Almond shell biochar: distilled water (1:5)
AlmB100	Almond shell biochar: distilled water (1:2.5)
Wal50	Walnut shell: distilled water (1:5)
Wal100	Walnut shell: distilled water (1:2.5)
WalB50	Walnut shell biochar: distilled water (1:5)
WalB100	Walnut shell biochar: distilled water (1:2.5)
Pea50	Peanut shell: distilled water (1:5)
Pea100	Peanut shell: distilled water (1:2.5)
PeaB50	Peanut shell biochar: distilled water (1:5)
PeaB100	Peanut shell biochar: distilled water (1:2.5)
Pis50	Pistachio shell: distilled water (1:5)
Pis100	Pistachio shell: distilled water (1:2.5)
PisB50	Pistachio shell biochar: distilled water (1:5)
PisB100	Pistachio shell biochar: distilled water (1:2.5)
Asp50	Asparagus spear: distilled water (1:5)
Asp100	Asparagus spear: distilled water (1:2.5)
AspB50	Asparagus spear biochar: distilled water (1:5)
AspB100	Asparagus spear biochar: distilled water (1:2.5)
Bre50	Brewer spent grain: distilled water (1:5)
Bre100	Brewer spent grain: distilled water (1:2.5)
BreB50	Brewer spent grain biochar: distilled water (1:5)
BreB100	Brewer spent grain biochar: distilled water (1:2.5)

### 2.5. Statistical Analyses

All analyses were conducted in triplicate and the data are reported as mean  $\pm$  standard deviation. The statistical independence of the data was tested and the normality was verified using the Kolmogorov–Smirnov test. The homoscedasticity was verified using the Levene test. All analyses were performed considering  $\alpha = 0.05$ . The characterizations of the biochar and biowaste were analyzed by one-way ANOVA and significant differences between mean values were determined by the Tukey test ( $p < 0.05$ ). Data analysis was performed using Infostat statistical software, version 1.1 (InfoStat Group, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina).

To understand the influence of the variables GP, AL, RL, GI, and SV and to describe the relationships between types of seeds (green peas, arugula, lettuce, and radish) and extract types (biowaste, biochar, and control), a multivariate statistical procedure (linear discriminant analysis, LDA) was algebraically used. Before analysis, it was ensured that the assumptions of a linear model were met. The MASS package was utilized for this analysis. Classification errors were evaluated. The statistical analysis was performed using R-Studio. Furthermore, a mixed-factorial MANOVA and Tukey test for comparison of means were

used to detect differences in GP, RL, AL, SV, and GI between the main treatments (biochar, biowaste, and control), dilution extract (0%, 50%, and 100%), and seed types. Seed type (green peas, arugula, lettuce, and radish), treatments, and dilution extract were considered as fixed factors. Multivariable contrasts were followed by univariate contrast analysis. The statistical analysis was carried out using SPSS Statistics v17.

### 3. Results and Discussion

#### 3.1. Biowaste and Biochar Characterization

The characterizations of biowaste and biochar are shown in Tables 2 and 3. The average value of biowaste exhibited higher values of OM ( $15.8 \pm 3.5$ ), OC ( $8.6 \pm 2.6$ ), moisture ( $5.7 \pm 0.9$ ), VM ( $82.1 \pm 4.2$ ), H ( $5.4 \pm 0.3$ ), and O ( $41.4 \pm 2.1$ ). The OM, OC, and TN contents are important for soil amendments, as they enhance soil quality and sustainability [42]. Alm had the highest OM and OC values, while Asp had the lowest TN and OC contents. Bre had the lowest OM content. VM varied less, with Pis having the highest and Alm the lowest (see Table 2). Ash content ranged from 0.3% to 12.1%, with Alm having the highest and Pis the lowest values. Ash contains essential nutrients for plants, while VM releases ions at high temperatures, which can benefit soil [43,44]. Biowaste shared similar elemental composition, primarily C and O, with minor proportions of H, N, and S. Similar results were reported by Rodriguez Ortiz et al. [35] and Capossio et al. [38] for almond, walnut shells, and brewer's spent grain. Complex molecules like hemicellulose, cellulose, and lignin are linked to OM and OC contents and contribute to forming humic and fulvic acids, which enhance soil fertility [45].

**Table 2.** Main properties of studied biowaste. Values are means  $\pm$  standard deviation. ANOVA. Values followed by similar letters are not significantly different between treatments (Tukey test,  $p < 0.05$ ). Letters (a, b, c, d, e) represent the levels of significance among the means of the different groups or treatments. Alm (almond shell); Wal (walnut shell); Pea (peanut shell); Pis (pistachio shell); Asp (asparagus spear); Bre (brewer spent grain); EC (electrical conductivity); OM (organic matter); OC (organic matter); TN (total nitrogen); VM (volatile matter); FC (fixed carbon); C (carbon content); H (hydrogen content); O (oxygen content).

Biowaste	Alm	Wal	Pea	Pis	Asp	Bre
pH	$5.79 \pm 0.05$ <sup>ab</sup>	$6.14 \pm 0.02$ <sup>b</sup>	$6.13 \pm 0.06$ <sup>b</sup>	$5.62 \pm 0.15$ <sup>ab</sup>	$4.79 \pm 0.03$ <sup>a</sup>	$5.21 \pm 0.01$ <sup>a</sup>
EC ( $\mu\text{S cm}^{-1}$ )	$2546 \pm 100$ <sup>d</sup>	$1039 \pm 6.66$ <sup>bcd</sup>	$828 \pm 4$ <sup>ab</sup>	$335 \pm 7$ <sup>a</sup>	$2038 \pm 125$ <sup>cd</sup>	$833 \pm 0.71$ <sup>abc</sup>
OM (%)	$19.42 \pm 0.26$ <sup>a</sup>	$17.57 \pm 0.48$ <sup>b</sup>	$9.28 \pm 0.83$ <sup>d</sup>	$17.67 \pm 0.42$ <sup>b</sup>	$13.87 \pm 0.27$ <sup>c</sup>	$6.30 \pm 0.19$ <sup>b</sup>
OC (%)	$11.26 \pm 0.15$ <sup>a</sup>	$10.19 \pm 0.28$ <sup>b</sup>	$5.38 \pm 0.41$ <sup>c</sup>	$10.25 \pm 0.24$ <sup>b</sup>	$4.67 \pm 0.39$ <sup>c</sup>	$9.83 \pm 0.20$ <sup>b</sup>
TN (%)	$0.73 \pm 0.06$ <sup>a</sup>	$0.68 \pm 0.04$ <sup>a</sup>	$0.75 \pm 0.01$ <sup>a</sup>	$0.67 \pm 0.01$ <sup>a</sup>	$0.35 \pm 0.01$ <sup>a</sup>	$2.08 \pm 0.01$ <sup>a</sup>
Moisture (%)	$6.67 \pm 0.07$ <sup>c</sup>	$4.42 \pm 0.27$ <sup>a</sup>	$5.24 \pm 0.45$ <sup>ab</sup>	$4.91 \pm 0.18$ <sup>ab</sup>	$6.50 \pm 0.23$ <sup>bc</sup>	$6.46 \pm 0.17$ <sup>bc</sup>
Ash (%)	$12.13 \pm 0.99$ <sup>d</sup>	$0.69 \pm 0.15$ <sup>ab</sup>	$1.78 \pm 0.16$ <sup>abc</sup>	$0.32 \pm 0.02$ <sup>a</sup>	$5.72 \pm 0.31$ <sup>cd</sup>	$2.14 \pm 0.05$ <sup>bcd</sup>
VM (%)	$77.78 \pm 2.64$ <sup>a</sup>	$83.11 \pm 0.34$ <sup>abc</sup>	$80.65 \pm 0.20$ <sup>ab</sup>	$89.31 \pm 0.52$ <sup>c</sup>	$78.12 \pm 0.61$ <sup>a</sup>	$84.19 \pm 1.75$ <sup>bc</sup>
FC (%)	$3.41 \pm 0.25$ <sup>a</sup>	$11.78 \pm 0.60$ <sup>bc</sup>	$12.33 \pm 0.77$ <sup>c</sup>	$5.46 \pm 0.64$ <sup>a</sup>	$9.65 \pm 0.39$ <sup>abc</sup>	$7.21 \pm 0.19$ <sup>ab</sup>
C (%)	$36.79 \pm 0.77$ <sup>e</sup>	$45.65 \pm 0.28$ <sup>a</sup>	$44.76 \pm 0.40$ <sup>ab</sup>	$44.52 \pm 0.17$ <sup>bc</sup>	$41.06 \pm 0.35$ <sup>d</sup>	$43.10 \pm 0.41$ <sup>c</sup>
H (%)	$4.99 \pm 0.05$ <sup>a</sup>	$5.69 \pm 0.02$ <sup>b</sup>	$5.58 \pm 0.03$ <sup>b</sup>	$5.68 \pm 0.01$ <sup>ab</sup>	$5.27 \pm 0.04$ <sup>a</sup>	$5.50 \pm 0.01$ <sup>ab</sup>
O (%)	$37.36 \pm 0.56$ <sup>a</sup>	$42.97 \pm 0.12$ <sup>bc</sup>	$41.97 \pm 0.17$ <sup>abc</sup>	$43.73 \pm 0.04$ <sup>c</sup>	$39.54 \pm 0.28$ <sup>ab</sup>	$41.89 \pm 0.18$ <sup>ab</sup>
Other elements (%) *	$20.85 \pm 1.14$ <sup>a</sup>	$5.69 \pm 0.41$ <sup>e</sup>	$7.68 \pm 0.61$ <sup>d</sup>	$6.06 \pm 0.17$ <sup>de</sup>	$14.12 \pm 0.67$ <sup>b</sup>	$9.51 \pm 0.27$ <sup>c</sup>
Hemicellulose (%)	$9.59 \pm 0.77$ <sup>d</sup>	$55.01 \pm 1.56$ <sup>a</sup>	$49.72 \pm 1.64$ <sup>a</sup>	$10.33 \pm 1.01$ <sup>d</sup>	$16.51 \pm 0.47$ <sup>c</sup>	$29.8 \pm 0.30$ <sup>b</sup>
Cellulose (%)	$63.37 \pm 1.85$ <sup>b</sup>	$13.15 \pm 0.79$ <sup>a</sup>	$17.28 \pm 1.29$ <sup>ab</sup>	$74.56 \pm 1.71$ <sup>b</sup>	$44.29 \pm 0.69$ <sup>ab</sup>	$16.20 \pm 0.20$ <sup>a</sup>
Lignin (%)	$40.15 \pm 3.88$ <sup>ab</sup>	$45.31 \pm 1.55$ <sup>b</sup>	$25.29 \pm 1.28$ <sup>b</sup>	$27.02 \pm 0.24$ <sup>a</sup>	$26.03 \pm 1.94$ <sup>a</sup>	$31.00 \pm 3.00$ <sup>ab</sup>

(\*) Mainly N, S, and other minerals present in ashes.

When comparing the average value of the biochar with biowaste, it was found that biochar had the highest values of pH ( $9 \pm 2.5$ ), ash ( $8.2 \pm 7.1$ ), FC ( $63.7 \pm 8$ ), and C ( $73.6 \pm 7$ ), showing significant differences. The biochar of Alm, Wal, Pea, and Asp had similar pH values indicating a basic behavior, while BreB presented pH values closer to neutrality. On the other hand, PisB had acid pH values. Conversely, all biowaste had acidic pH values between 4.8 and 6.1. The change in pH of the material after pyrolysis is mainly

due to changes in the chemical composition of the material during this thermal process. Pyrolysis promotes the decomposition of organic matter, removing volatile compounds and converting carbon to a more stable structure. This usually increases the ash content, which is composed of minerals that tend to raise the pH, such as carbonates and alkali oxides [46]. In our study, this change in pH is relevant because it affects the ability of biochar to interact with soil and plants, as pH can improve the availability of certain nutrients and alter microbial activity, aspects that can influence germination and plant growth. Pyrolysis at 450 °C for 2 h: The EC varied among different extract types, not showing significant differences (see Table 3). Low EC values indicate fewer soluble salts, which makes the biochar suitable for use as an amendment in semiarid zones or for crops tolerant to salt [47]. pH and EC levels are influenced by salts and ions, which can affect plant osmotic pressure and enzyme activities [48], potentially affecting seed germination. Both high alkalinity and acidity can reduce germination rates [23]. Changes in EC may also have affected germination because the high levels of soluble salts present in most biochar (e.g., Na<sup>+</sup> and Cl<sup>-</sup>) result in higher EC levels than in most agricultural soils [49], which negatively affect nutrient availability and osmotic potential. Ash content varied significantly, from 0.3 to 12.1%. The highest ash content was found in AspB and the lowest in PisB (see Table 3). High ash content can reduce the biochar's ability to retain nutrients and increase soil salinity [50]. FC in biochar showed low variability, with values ranging from 46.9% to 69.6%. PisB had the highest FC content, while AspB had the lowest. In biowaste, FC also showed substantial variability, with values ranging from 3.4 to 12.3%. Alm, Pis, and Bre had the lowest FC values, while Asp, Wal, and Pea had the highest values (see Table 2). FC represents the stable carbon in biochar that resists decomposition, making it more durable for soil application [51]. The C content in AlmB and WalB were similar to values reported by Rodriguez Ortiz et al. [36], but differed for other elements (H, N, and O contents), possibly due to differences in pyrolysis process temperature. Komnitsas et al. [52] reported similar C and H contents, though PisB had lower N and higher O levels. Differences in biochar characteristics across studies may result from variations in pyrolysis conditions or feedstock sources [53,54]. WalB had the lowest moisture content and the highest for AspB. Pyrolysis at 450 °C for 2 h significantly reduces biowaste moisture, but the porous structure of biochar allows it to absorb moisture from the environment [55]. Despite this, the moisture content remains low, between 4% and 6%: nearly negligible. Low moisture level in biochar is associated with greater long-term stability. Other elements include mainly N, S, and trace elements: such as chlorine (Cl), iron (Fe), sodium (Na), potassium (K), or manganese (Mn), which may be present in very small amounts, depending on the origin of the sample [6,35,56]. This was observed between different biochar, with values ranging from 1.4 to 22.1% (see Table 3). After pyrolysis, micronutrient concentrations are expected to rise due to volatile loss. However, their distribution also depends on the feedstock and pyrolysis conditions (temperature, time, heating rate) [46]. Tables 2 and 3 show total micronutrient concentrations, but variations may complicate a direct link to volatile loss, likely due to feedstock heterogeneity and element redistribution.

Differences between the control and the biochar-treated soils provide key insights into the benefits of biochar application. The higher pH values in the biochar treatments compared to the control suggest that biochar may mitigate soil acidity, creating more favorable conditions for plant growth. On the other hand, biowastes offer distinct benefits in comparison to the control, particularly in terms of increasing OM and OC content, both of which are critical for soil health and fertility. While biowastes may not have the same stabilizing effects on soil structure as biochar, their higher OM content compared to the control suggests that they contribute to enhancing soil OC and nutrient cycling. Nevertheless, biowaste decomposition occurs more rapidly than biochar, meaning that its long-term benefits may be more limited. The comparison with the control underscores that biochar, despite its variability, provides a more persistent amendment to improve soil structure and stability over time, while biowastes offer more immediate OM contributions but may require more frequent application.

**Table 3.** Main properties of studied biochar. Values are means  $\pm$  standard deviation. ANOVA. Values followed by similar letters are not significantly different between treatments (Tukey test,  $p < 0.05$ ). Letters (a, b, c, d, e) represent the levels of significance among the means of the different groups or treatments. AlmB (almond shell biochar); WalB (walnut shell biochar); PeaB (peanut shell biochar); PisB (pistachio shell biochar); AspB (asparagus spear biochar); BreB (brewer's spent grain biochar); EC (electrical conductivity); OM (organic matter); OC (organic carbon); TN (total nitrogen); VM (volatile matter); FC (fixed carbon); C (carbon content); H (hydrogen content); O (oxygen content).

Biochar	AlmB	WalB	PeaB	PisB	AspB	BreB
pH	11.71 $\pm$ 0.06 <sup>d</sup>	10.86 $\pm$ 0.09 <sup>cd</sup>	10.34 $\pm$ 0.02 <sup>bcd</sup>	4.94 $\pm$ 0.29 <sup>a</sup>	9.74 $\pm$ 0.02 <sup>abc</sup>	6.41 $\pm$ 0.22 <sup>ab</sup>
EC ( $\mu\text{s}\cdot\text{cm}^{-1}$ )	4443.33 $\pm$ 20.82 <sup>cd</sup>	561.33 $\pm$ 3.06 <sup>bcd</sup>	291.0 $\pm$ 1.00 <sup>abc</sup>	175 $\pm$ 13.89 <sup>ab</sup>	6273.33 $\pm$ 262.74 <sup>d</sup>	20.65 $\pm$ 0.91 <sup>a</sup>
OM (%)	10.60 $\pm$ 0.94 <sup>bc</sup>	11.28 $\pm$ 0.83 <sup>bc</sup>	14.74 $\pm$ 0.55 <sup>c</sup>	5.65 $\pm$ 0.64 <sup>a</sup>	8.14 $\pm$ 0.68 <sup>abc</sup>	6.30 $\pm$ 0.19 <sup>ab</sup>
OC (%)	6.15 $\pm$ 0.55 <sup>b</sup>	6.54 $\pm$ 0.48 <sup>b</sup>	8.55 $\pm$ 0.32 <sup>a</sup>	3.28 $\pm$ 0.37 <sup>c</sup>	3.15 $\pm$ 2.74 <sup>a</sup>	3.66 $\pm$ 0.11 <sup>c</sup>
TN (%)	0.71 $\pm$ 0.07 <sup>ab</sup>	0.36 $\pm$ 0.02 <sup>a</sup>	1.07 $\pm$ 0.02 <sup>ab</sup>	0.35 $\pm$ 0.01 <sup>a</sup>	2.92 $\pm$ 0.02 <sup>b</sup>	4.02 $\pm$ 0.12 <sup>b</sup>
Moisture (%)	2.73 $\pm$ 0.25 <sup>cd</sup>	1.99 $\pm$ 0.21 <sup>e</sup>	4.09 $\pm$ 0.22 <sup>b</sup>	2.32 $\pm$ 0.13 <sup>de</sup>	5.15 $\pm$ 0.35 <sup>a</sup>	3.06 $\pm$ 0.11 <sup>c</sup>
Ash (%)	12.75 $\pm$ 0.29 <sup>b</sup>	2.49 $\pm$ 0.21 <sup>e</sup>	3.86 $\pm$ 0.26 <sup>d</sup>	1.31 $\pm$ 0.51 <sup>f</sup>	20.95 $\pm$ 0.33 <sup>a</sup>	8.30 $\pm$ 0.65 <sup>c</sup>
VM (%)	20.82 $\pm$ 2.33 <sup>b</sup>	28.70 $\pm$ 1.08 <sup>a</sup>	25.45 $\pm$ 0.30 <sup>a</sup>	26.71 $\pm$ 1.11 <sup>a</sup>	26.99 $\pm$ 1.17 <sup>a</sup>	20.39 $\pm$ 1.91 <sup>b</sup>
FC (%)	63.70 $\pm$ 2.46 <sup>ab</sup>	66.83 $\pm$ 0.98 <sup>abc</sup>	66.60 $\pm$ 0.38 <sup>abc</sup>	69.65 $\pm$ 0.66 <sup>c</sup>	46.90 $\pm$ 1.08 <sup>a</sup>	68.25 $\pm$ 1.44 <sup>bc</sup>
C (%)	51.24 $\pm$ 0.55 <sup>d</sup>	55.87 $\pm$ 0.12 <sup>ab</sup>	54.36 $\pm$ 0.21 <sup>bc</sup>	56.64 $\pm$ 0.2 <sup>a</sup>	44.19 $\pm$ 0.30 <sup>e</sup>	53.51 $\pm$ 0.21 <sup>c</sup>
H (%)	5.13 $\pm$ 0.03 <sup>c</sup>	5.68 $\pm$ 0.01 <sup>a</sup>	5.49 $\pm$ 0.02 <sup>ab</sup>	5.73 $\pm$ 0.03 <sup>a</sup>	4.59 $\pm$ 0.03 <sup>bc</sup>	5.33 $\pm$ 0.03 <sup>c</sup>
O (%)	31.71 $\pm$ 0.32 <sup>c</sup>	36.24 $\pm$ 0.18 <sup>a</sup>	34.67 $\pm$ 0.15 <sup>ab</sup>	36.25 $\pm$ 0.33 <sup>ab</sup>	29.09 $\pm$ 0.26 <sup>b</sup>	32.96 $\pm$ 0.43 <sup>c</sup>
Other elements (%) <sup>*</sup>	11.91 $\pm$ 0.54 <sup>b</sup>	2.20 $\pm$ 0.11 <sup>e</sup>	5.47 $\pm$ 0.38 <sup>d</sup>	1.39 $\pm$ 0.53 <sup>e</sup>	22.12 $\pm$ 0.47 <sup>a</sup>	8.20 $\pm$ 0.53 <sup>c</sup>

(\*) Mainly N, S, and other minerals present in ashes.

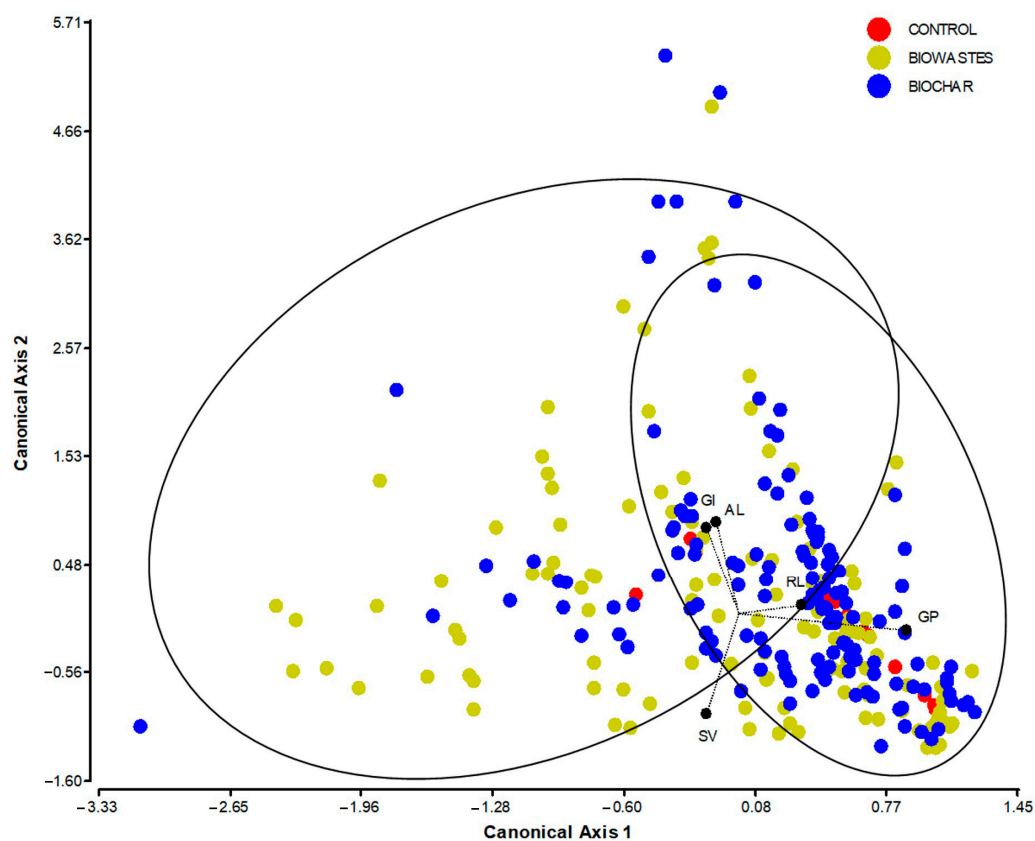
### 3.2. Germination Bioassay

Figure 5 shows how different types of biochar, biowaste, and control treatments influence the evaluated variables (SV, GI, GP, RL, and AL) through linear discriminant analysis (LDA). The analysis demonstrated that the GP variable contributed the most to differentiating between the biochar and biowaste groups. Canonical axis 1 explained 86.3% of the variation between groups, while axis 2 explained 13.7%. The positioning of biochar and biowaste treatments on opposite ends of axis 1 further confirmed that GP was crucial in distinguishing between the two groups. The total error rate of classification was 35%. These results suggest that the type of extract used significantly impacted plant growth and development, likely due to differences in the chemical composition and physical structure of biochar [57,58]. These findings align with earlier research showing that biochar improves soil structure and nutrient availability, boosting early plant growth [59,60]. For instance, Shamim et al. [61] examined biochar from three tree species and found that while germination rates remained unchanged, seed vigor increased in paddy crops (*Oryza sativa*) with two of the biochar types. Martínez-Gómez et al. [62] studied the effect of grape pomace biochar on nematode activity and found that biochar produced at 350 °C reduced nematode populations, while biochar at 750 °C showed some toxicity to tomato seed germination.

Seed response to the different biowaste and biochar extracts varied significantly by species (Figure 6). Canonical axis 1 explained 77.73% of the variation between groups, and axis 2 explained 21.87%. This figure shows how radish (*Raphanus sativus* L.) and green pea (*Pisum sativum* L.) seeds were positioned on opposite sides, influenced by differences in RL and SV. The total classification error rate was 29%, with pea seeds being the least sensitive to treatments, while radish and lettuce (*Lactuca sativa* L.) seeds showed more inhibition in germination (Tables S1–S4, Supplementary Materials).

Gezahegn et al. [63] also reported the sensitivity of these species. For green peas, no significant differences were found between extracts for any variables evaluated (Table S1, Supplementary Materials). Lettuce treated with walnut shell biochar showed the highest average values of AL, GP, and SV. Pistachio shell biochar generated the highest GI and RL values. Asparagus spear biowaste, however, had the most negative effect on lettuce seed performance (Table S4, Supplementary Material). For arugula, Bre biochar led to the highest values of AL and RL, while walnut shell biochar gave the highest SV and GP values (Table S2, Supplementary Materials). However, walnut shell biowaste had the greatest positive impact, on lettuce. The lowest average values for these variables were reached with

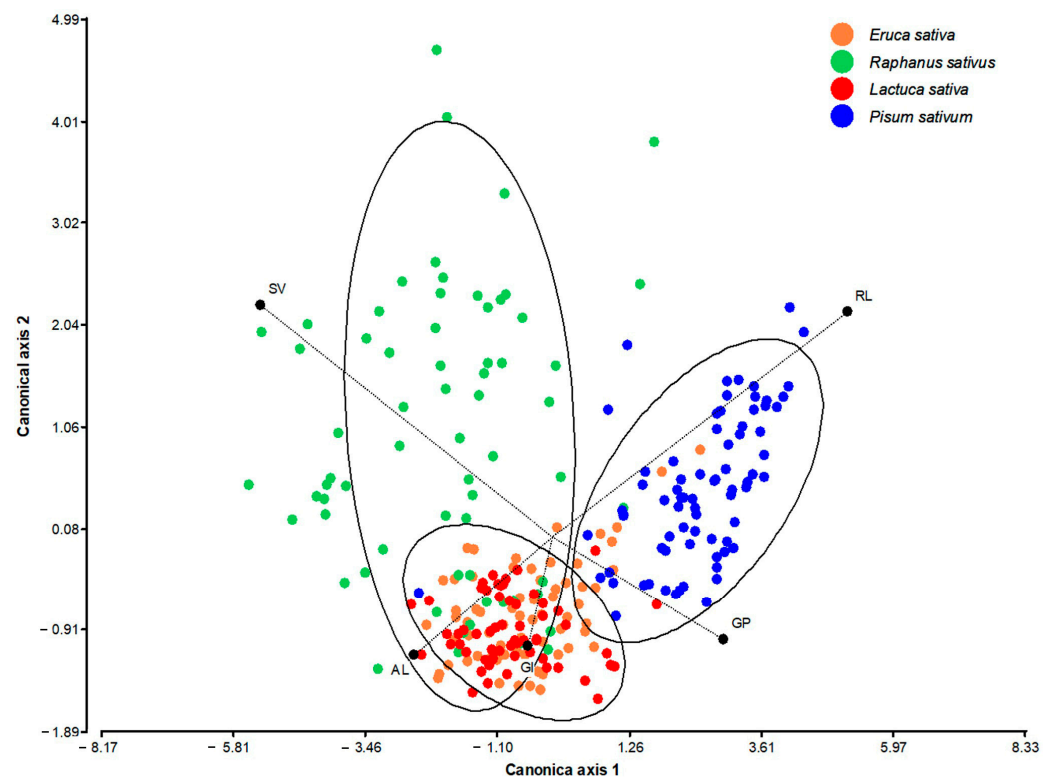
the addition of asparagus spear biowaste. For radish, peanut shell biochar generated the highest average values for the GI, RL, and GP variables. Walnut shell biomass generated the highest values for the AL and SV variables. The lowest values in the variables GI, AL, GP, and SV were reached with the addition of almond shell biochar, while for RL, with the addition of asparagus spear biochar. The negative effects of asparagus spear biochar and biowaste may be related to its low pH, high conductivity, and low OC and TN (see Table 2). These characteristics certainly influenced the effects on seed germination for all the species evaluated. Although some biowaste increased certain seed parameters, its high organic load and low stability make it less suitable without prior treatment, such as pyrolysis (see Table 2). These findings emphasize the importance of using multiple seed species and germination variables to avoid biased results based on seed sensitivity. Likewise, the variables analyzed responded differently to the treatments applied. The most commonly used variable, GP, showed high values in most of the phytotoxicity analyses. However, the rest of the variables decreased significantly. This behavior highlights the need to use more than one variable in this type of test.



**Figure 5.** Relationships between biomass and biochar types and control and their influence of variables (GI, GP, SV, AL, and RL) with discriminatory potential through a multivariate statistical procedure, such as linear discriminant analysis (LDA).

Assays using biowaste showed different results compared to biochar and control treatments. Overall, biochar and control treatments performed similarly for most variables. However, the GP variable showed significant differences between biochar and control treatments (MANOVA,  $F = 23.59$ ,  $p < 0.0001$ ). Biochar treatments had the highest values in variables such as AL with  $4.47 \pm 2.37$  and GI with  $110.08 \pm 80.4$ . In contrast, the control treatments showed the highest values for GP ( $93.33 \pm 12.52$ ), RL ( $4.48 \pm 2.98$ ), and SV ( $840.27 \pm 516.69$ ). Biowaste treatments had the lowest average values for all variables, indicating they are not as effective in promoting plant growth compared to biochar or controls. Germination performance varied based on the type and dilution of biowaste and

biochar extracts (MANOVA,  $F = 5.18$ ,  $p < 0.0001$ ). In this study, data from Table 4 suggested that GP, RL, AL, and GI were affected by seed type, types of extracts, extract dilutions, and their interactions. SV was only significantly influenced by the type of extracts (MANOVA,  $F = 14.18$ ,  $p < 0.0001$ ) and interactions (MANOVA,  $F = 4.27$ ,  $p < 0.0001$  and MANOVA,  $F = 5.07$ ,  $p < 0.0001$  for seed type  $\times$  type of extracts and type of extracts  $\times$  dilution extracts, respectively). The data showed that seed type significantly influenced (MANOVA,  $F = 5.68$ ,  $p < 0.001$ ) the efficiency of the type of extract in modifying GP. The data suggested that GP, RL, AL, GI, and SV decreased in response to biowaste addition, and this effect varied in response to seed type, type of extract, dilution extract, and their interactions (see Table 4). The MANOVA results indicated that biochar should be specifically designed before large-scale field applications to maximize its efficiency in germination and improve crop productivity.



**Figure 6.** Relationships between types of seeds and their influence on variables with discriminatory potential through a multivariate statistical procedure, such as linear discriminant analysis (LDA). GI (germination index), GP (germination percentage), SV (seed vigor), AL (aerial length) and RL (root length).

The low values in the biowaste treatments suggest a less favorable effect on plant growth and development compared to biochar and controls. This could be due to the higher organic load and lower stability of the biowaste compared to biochar, which negatively affects nutrient availability and soil aeration [64]. Control groups played a critical role in distinguishing the inherent effects of biochar and biowaste treatments. Although the control group performed comparably in certain variables such as RL and SV, biochar consistently outperformed the control in GI and overall plant growth metrics. This suggests that the contribution of biochar goes beyond providing basic growing conditions, actively enhancing soil structure, water retention, and nutrient availability [6,7]. The porous structure of biochar increases soil aeration and improves water-holding capacity, essential for plant development, particularly under stress conditions like drought [8]. Its ability to retain nutrients while minimizing leaching could explain the consistent improvements observed in GI and other growth variables [23]. This capacity to act as both a physical

and chemical amendment contrasts with the control soil, which, while offering a natural environment for seeds, lacks the enhanced retention and buffering properties that biochar provides [8]. Moreover, the role of biochar in carbon sequestration and its potential to reduce greenhouse gas emissions further highlight its importance in sustainable agriculture [27]. These properties are not present in untreated soils, emphasizing the added environmental benefits of biochar beyond just promoting plant growth. On the other hand, the poor performance of biowaste treatments, in comparison to both control and biochar, underscores the importance of biochar's stability. Unlike biowaste, which may introduce excess organic matter, leading to potential toxicity or nutrient imbalances, biochar offers a more stable and controlled method of improving soil health without the risk of organic overload [12,13].

**Table 4.** Statistical results of the factorial MANOVA on GP, RL, AL, GI, and SV variables in germination bioassay.

Source of Variation	GP (Germination Percentage)		RL (Root Length)		AL (Aerial Length)		GI (Germination Index)		SV (Seed Vigor)	
	F	<i>p</i> -Value	F	<i>p</i> -Value	F	<i>p</i> -Value	F	<i>p</i> -Value	F	<i>p</i> -Value
Seed type	5.68	***	121.160	***	323.801	***	2.385	***	158.655	NS
Types of extracts	23.59	***	8.291	***	18.700	***	6.785	***	14.184	***
Dilution extracts	80.08	***	9.873	***	7.750	***	1.697	***	17.936	NS
Seed type × Types of extracts	9.22	***	3.107	***	7.595	***	1.889	***	4.273	***
Seed type × Dilution extracts	6.89	***	4.853	***	6.052	***	0.908	***	5.582	NS
Types of extracts × Dilution extracts	10.29	***	2.762	***	8.508	***	2.085	***	5.071	***
Seed type × Types of extracts × Dilution extracts	5.18	***	1.588	***	4.016	***	0.978	***	2.307	NS

\*\*\*  $p < 0.001$ , NS indicates  $p > 0.05$ .

Additionally, biochar applied at a rate of 50% enhanced germination variables more than biochar applied at 100% (Tables S5–S9, Supplementary Materials). For the different dilution extracts, pea seeds germinated in all extracts and dilutions, while radish and lettuce seeds did not germinate under certain conditions. This suggests phytotoxicity in specific extracts (see Table S3, Supplementary Materials). For lettuce, seeds did not germinate for extracts Bre50, Bre100, Asp50, and Asp100. A GI value of less than 60% indicates phytotoxicity. Therefore, the unsuitable extracts for green peas were Asp50 and Asp100; for arugula, Alm50 and Alm100, Asp50 and Asp100, and AspB and PeaB at 100%. Additionally, radish seeds showed low GI values (<60%) with Alm and Asp biowaste extracts and their biochar. The treatments that caused phytotoxicity in lettuce included Alm (50 and 100), Bre (50 and 100), Asp (50 and 100), and PeaB at 100%. In general, it was observed that Asp and Alm inhibited seed germination and root elongation. This biowaste had a high EC and content of other elements that could be the cause of their phytotoxicity (see Tables 2 and 3). Similarly, its biochar and PeaB, according to the results, are not suggested for use in sensitive seeds due to high EC, OM, O, ash content, and other elements.

Seed germination is a crucial stage for crop production. Some biochar may contain phytotoxic substances such as dioxins, furans, polycyclic aromatic hydrocarbons, phenolic compounds, and heavy metals that can be harmful to crops, soil, and even humans [59]. Moreover, recent studies by Enaime et al. [65] evaluated the phytotoxicity of raw and treated olive oil mill effluents, obtaining extracts at different concentrations in maize and tomato seeds. Concerning seeds, it was observed that tomato was more sensible than maize, and phytotoxicity was related to monomeric phenols and other toxic compounds. The pyrolysis thermal treatment contributed to alleviating the phytotoxicity of the biowaste

studied; this result is consistent with the findings reported by Cui et al. [66]. However, various technologies are currently used to remove phytotoxicity from biochar, including biological and oxidative aging, as well as thermal post-treatment [67]. Efficiency, cost, and sustainability vary for each technology, so further evaluation is suggested.

He et al. [68] reported that the application of almond shell biochar (ASB) significantly enhanced crop growth by improving the physicochemical and biological properties of saline alkaline soils. Sanchez et al. [7] showed that the addition of almond shell biochar (ASB) at low rates to saline soils reduced pH and EC. Increasing the amendment rate led to an increase in moisture, OM, and OC and a decrease in cation exchange capacity, which had a positive effect on soil quality. In addition, treatment with 5% ASB produced better results in total plant length, fresh and dry weight, leaf area, and leaf chlorophyll content of arugula plants. Sanchez et al. [6] reported the positive effect of pistachio shell biochar (PSB) addition at different mass ratios that was analyzed considering the physicochemical properties of the mixtures and the agronomic parameters of green pepper plants and fruits under greenhouse conditions. Alkharabsheh et al. [69] and Xu et al. [70] conducted comprehensive reviews on biochar, exploring its potential for carbon sequestration, its role in enhancing soil fertility, and its influence on the productivity and quality of crops. In addition, the carbon-to-nitrogen ratio (C/N) is a key factor affecting the overall turnover rates of soil OM. One critical point is that biochar with a high C/N ratio can result in nitrogen immobilization in the soil, where microbes consume available nitrogen to break down the high-carbon material. This process can temporarily reduce the nitrogen available for plants, affecting crop productivity in the short term. However, over time, biochar can enhance soil OC levels and improve overall soil fertility [71]. Biochar application involves both carbon mineralization and nitrogen availability in the soil; thus, these impacts are strongly influenced by the presence of plant-growth-promoting bacteria and the nitrogen content of the soil [72]. According to the papers consulted and the research work of the working group, the incorporation of biochar in field applications can, in the medium and long term, have an impact on optimizing soil nutrient dynamics, improving soil fertility, increasing nutrient retention, and reducing leaching; increasing water retention capacity, especially in sandy soils or under drought conditions; carbon sequestration, contributing to climate change mitigation; and reducing greenhouse gases (e.g., methane and nitrous oxide emitted by the soil).

However, its large-scale application may have an impact on some factors that pose some challenges and future research: Its effect is variable, depending on the type of soil on which it is applied, the crop to be grown, and the climatic conditions of the region, leading to periodic soil + biochar studies to adjust the dose and form of application, as well as the addition of fertilizers. It is also necessary to consider whether the added biochar contains traces of heavy metals or organic substances toxic to crops, which could lead to their accumulation.

In other words, although the benefits of applying biochar to the soil are very encouraging in terms of productivity and environmental sustainability, it is necessary to evaluate the raw material to be used to obtain biochar, as well as the properties of the biochar obtained, to adapt its implementation to each agricultural context (soil–crop–climate–work). It is, therefore, essential to develop pilot-scale trials before field application, as well as continuous crop monitoring to certify the efficient and sustainable use of biochar in the field.

Biochar is a stable carbon material produced by heating biomass at high temperatures through pyrolysis, a process that occurs in the absence of, or with limited, oxygen. This process can release undesirable emissions such as particulate matter (PM), volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), carbon monoxide (CO), and nitrogen oxides (NO<sub>x</sub>), especially if the process is not well controlled. In addition to biochar, the products gas and bio-oil, when utilized in conjunction with an appropriate separation system, have the potential to serve as an energy vector [73]. In this context, emission control strategies, changes in reactor design, use of catalysts, and integration of

real-time monitoring systems have been developed to mitigate air pollution from pyrolysis processes [74–77].

In terms of the economic feasibility of biochar production from different biomasses, building a pyrolysis plant requires a significant investment in infrastructure, but then brings savings in terms of environmental management and the type of substrate obtained, as it has numerous advantages over other fertilizers and substrates added to the soil, as already mentioned in the previous section. The design of the plant should also take into account the different biomasses used in agricultural, forestry, and urban waste, as they have different initial compositions and, therefore, produce biochars of different quality and characteristics. With regard to the location of these plants, it makes more sense to locate them in areas close to the sources of biowaste due to transport costs.

Biochar adoption in farming faces several challenges, primarily related to economic considerations and scalability. The initial costs of production and application can be significant, particularly for small-scale farmers, and the economic viability often hinges on the availability of affordable feedstock. Additionally, scaling up production requires advanced technology and infrastructure, which may not be accessible in all regions. However, the long-term benefits of biochar, such as improved soil health, reduced fertilizer needs, and potential participation in carbon credit markets, present significant economic opportunities. To facilitate practical adoption, farmers should start small by testing biochar on specific plots, utilize locally available biomass, and seek guidance from agricultural experts. With proper support and education, biochar can become an effective tool for enhancing sustainability and resilience in agriculture [6].

In conclusion, the great potential of biochar production was deeply discussed here. However, it faces technical, economic, and regulatory challenges. To improve its performance, and reduce production costs and hazardous emissions, it is essential to optimize pyrolysis processes, develop markets for its commercialization, and promote policies that encourage its production and use for environmental protection and sustainable management of biowaste. In addition, the integrated use of byproducts (such as bio-oil and syngas) can improve the economics and sustainability of the process.

Overall, the findings of this work emphasize the promise of converting waste to biochar in fostering sustainable agricultural systems, transforming waste into a valuable resource for enhancing soil fertility, carbon sequestration, and ecosystem resilience.

The results obtained shed light on the potential of agro-industrial wastes and their biochars as an effective soil amendment. The use of these biowastes and their biochars provides not only a means of waste management but also an opportunity to enhance agricultural sustainability. The reuse of biowaste that would otherwise contribute to environmental pollution reduces the environmental footprint associated with waste disposal while simultaneously improving soil health and productivity. Furthermore, the diverse responses of horticultural species to both agro-industrial waste and biochar underscore the importance of considering plant diversity in sustainable agricultural practices. This nuanced understanding enables biochar applications to be tailored to specific crops, optimizing benefits while minimizing potential risks. Overall, the findings of this work emphasize the promise of converting waste to biochar in fostering sustainable agricultural systems, transforming waste into a valuable resource for enhancing soil fertility, carbon sequestration, and ecosystem resilience.

#### 4. Conclusions

This work explored the uses of regional biowaste and their biochar as substrates through phytotoxicity assays on horticultural seeds. The results obtained suggest that these materials have the potential to improve conditions in the germination stage.

Furthermore, the detailed characterization of the source materials revealed that the specific physicochemical properties of the biowaste and their biochar significantly influence the phytotoxicity of the extracts obtained. This deeper understanding of the components of the samples is crucial for their effective application in agriculture. It was noteworthy

that the biowaste and biochar from asparagus spears and almond shells, under the studied conditions, showed high phytotoxicity for the tested seeds. In general, the biochar extracts performed better in the germination tests than the control and the biowaste. However, biochar from brewer spent grain, walnut shells, and pistachio shells are recommended for agricultural use as an amendment or substrate, because they have suitable properties. Overall, for all seeds studied growing in the mentioned extracts, RL and AL variables showed increases ranging from 6% to 14% compared to the control treatments without biochar. Additionally, SV showed increases of approximately 5% to 10% compared to the control in the PeaB and WalB extracts. However, no improvement was observed in the GP values. On the other hand, among the seeds evaluated, it was observed that some species showed greater sensitivity to some aqueous extracts, due to inhibitory effects on germination and/or root elongation. This group of seeds was lettuce and arugula, followed by radish and, finally, pea.

In summary, this study provides strong evidence that regional biowaste and its biochar can be used as valuable resources in sustainable agriculture. By incorporating these materials into agricultural practices, one can not only improve crop yields but also reduce dependence on chemical fertilizers while addressing the growing problem of organic waste. This approach presents a promising path towards building more sustainable and environmentally friendly agricultural systems in the regions studied.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14112573/s1>, Tables of descriptive statistics of variables studied for each seed. Table S1: Descriptive statistics of variables studied in green pea seeds; Table S2: Descriptive statistics of variables studied in arugula seeds; Table S3: Descriptive statistics of variables studied in radish seeds; Table S4: Descriptive statistics of variables studied in lettuce seeds. Annexed tables of the MANOVA analysis. Table S5: Germination index; Table S6: Root length; Table S7: Aerial length; Table S8: Germination percentage; Table S9: Seed vigor.

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## Abbreviation

Alm	Almond shell
Wal	Walnut shell
Pea	Peanut shell
Pis	Pistachio shell
Asp	Asparagus spear
Bre	Brewer spent grain

AlmB	Almond shell biochar
WalB	Walnut shell biochar
PeaB	Peanut shell biochar
PisB	Pistachio shell biochar
AspB	Asparagus spear biochar
BreB	Brewer spent grain biochar
EC	Electrical conductivity ( $\mu\text{S cm}^{-1}$ )
OM	Organic matter (%)
OC	Organic carbon (%)
FC	Fixed carbon content (%)
VM	Volatile matter (%)
C	Carbon content (%)
H	Hydrogen content (%)
O	Oxygen content (%)
TN	Total nitrogen (%)
GP	Germination percentage (%)
SNG	Germinated seed in the total ( $n^\circ$ )
SN0	Total number of viable seeds ( $n^\circ$ )
GI	Germination index (%)
G	Germination percentage of treatment (%)
G0	Germination percentage of control (%)
L	Root length of the treatment (cm)
L0	Root length of the control (cm)
SV	Seed vigor (%)
AL	Aerial length (cm)
RL	Root length (cm)

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