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List of Contents

List of tables	
List of Figures	
List of abbreviations	
Résumé	
Abstract	
ملخص	
Introduction	1
Literature Review	
1.Historical of Biochar	5
2. Definition of Biochar	6
3. Production Processes	7
4. Different Types of Pyrolysis	7
4.1. Slow (or Traditional) Pyrolysis	8
4.2. Rapid Pyrolysis	8
4.3. Flash (or Ultrafast) Pyrolysis	8
5. Role of Biochar	9
6. Biochar as a Source of Nutrients	11
7. Properties of Biochar	11
7.1 Physical Characteristics	12
7.2 Chemical Characteristics	13
8. Effects of Biochar	14
8.1 Effects of Biochar on Physical and Chemical Properties of Soils	14
8.2 Effect of Biochar on Soil Nutrient Availability	15
8.3 Effect of Biochar on Soil Nutrient Retention	16
8.4. Effects of Biochar on Soil Microorganisms	17
8.5. Effect of Biochar on the Abundance of Soil Microorganisms	18

8.6. Effect of Biochar on Soil Microbial Communities	18
8.7 Effect of Biochar on the Activity of Soil Microorganisms	19
Materials and Methods	
1. Material	22
1.1.Pedological Characteristics of the Sampling Site	22
1.2. Plant Material	23
1.2.1.Selection Criteria for the Plant Species <i>Hordeum vulgare L</i>	24
1.2.2.Systematic Position	25
1.3. Chemical Product	27
2. Method	27
2.1. Conditions and Implementation of the Experiment	27
2.1.1 Conducting the Experiments	27
2.1.2. Biochar Application	27
2.1.3. Seed Planting	29
2.1.4. Extraction and Quantification of Chlorophyll Pigments	33
2.1.5. Analysis and Quantification of Metabolites	34
2.1.6. Enzymatic Parameters Assay	37
3. Statistical Analysis	40
Results and Discussion	
1. Influence of Biochar on the Synthesis of Morphophysiological, Biochemical, and	
Enzymatic Parameters in Barley Plants (Hordeum vulgare L. var. Saïda and Fouara)	42
1.1 Morphophysiological Parameters	42
1.2 Biochemical Parameters	45
1.3. Enzymatic Parameters	49
Conclusion	54
Perspective	56
Bibliographic References	58

List of Tables

9
17
20
22
24
26
26
35

List of Figures

Figure 1: Comparison Between Terra Preta and Conventional Soils	5
Figure 2: Optimizing biochar production through the pyrolysis process	7
Figure 3: Role of biochar in sustainable energy, agriculture, and environmental management	ıt
	10
Figure 4: Microscopic characterization of biochar structure	13
Figure 5: Map of the Geographical Location of Aïn Regada	23
Figure 6: Experimental Setup for Seed Germination of <i>Hordeum vulgare L.</i> var. <i>Saïda</i> , in	
Petri dishes	28
Figure 7: Experimental Setup for Seed Germination of <i>Hordeum vulgare L.</i> var. <i>Fouara</i> , i	n
Petri dishes	28
Figure 8: Experimental Setup for Seed Cultivation of <i>Hordeum vulgare L.</i> var. <i>Saïda</i> in	
plastic pots	29
Figure 9: Experimental Setup for Seed Cultivation of <i>Hordeum vulgare L.</i> var. <i>Fouara</i> in	
plastic pots	30
Figure 10: Root System of Barley Hordeum vulgare L.	30
Figure 11: Growth periods of barley (Hordeum vulgare L. var. Saïda and Fouara)	31
Figure 12: Extraction and quantification of chlorophyll pigments	34
Figure 13: Preparation of Bradford reagent	36
Figure 14: Steps of protein quantification.	36
Figure 15: Steps of Total Sugar Quantification	37
Figure 16: Preparation steps of phosphate buffer.	38
Figure 17: P reparation steps of the enzymatic extract	38

List of abbreviations

CEC	Cationic Exchange Capacity
EC	Electrical Conductivity
SOM	Soil Organic Matter
RFLP	Restriction Fragment Length Polymorphism
ITGC	The Technical Institute of Major Crops
Var	Variety
BC	Biochar
ISTA	International Seed Testing Association
VG	Germination Rate
Ngg	Number of Sprouted Seeds
GP	Germination percentage
GR	Germination Rate
IT	Tolerance Index
SIV	Seedling Vigor Index
SP	Stem Phototoxicity
RP	Root Phytotoxicity
SL	Stem Length
RL	Root Length
S.E.M	Standard Error of the Mean
BSA	Albumin From Beef Serum
BBC	Brilliant Blue of Coumassie
NaK	Phosphate Buffer
MF	Fresh Material

CAT	Catalase				
Δ DO	Difference in Optical Density Obtained After Hydrolysis of the Substrate				
Chloro	Chlorophyll				
Apx	Ascorbate-Peroxydas				
dS	DeciSiemens				
SM	Soil Moisture				
PSD	Particle Size Distribution				
OMC	Organic Matter Content				

Résumé

Cette étude s'inscrit dans une démarche d'agriculture durable visant à évaluer l'impact de l'amendement en biochar, sur deux variétés locales d'orge (*Hordeum vulgare L.*), à savoir *Saïda* et *Fouara*, aux stades de la germination et de croissance. L'expérience a été réalisée avec quatre concentrations croissantes de biochar. L'objectif de cette étude est d'évaluer l'effet de cet amendement organique sur plusieurs paramètres morpho-physiologiques (Pourcentage de germination, Vitesse de germination, longueur moyenne des tiges, longueur moyenne des racines, Seedling Vigor Index, indice de tolérance, phytotoxicité des tiges, phytotoxicité des racines), biochimiques (chlorophylles a, b, a+b; protéines totales; sucres solubles totaux) et enzymatiques (CAT, APX), afin de déterminer la dose optimale favorisant une amélioration physiologique et métabolique des espèces. Cette étude confirme que le biochar, lorsqu'il est utilisé à une dose modérée, peut agir comme un biostimulant dans le cadre d'une agriculture durable.

Mots-clés: *Hordeum vulgare L.*, biochar, paramètres morpho-physiologiques, paramètres biochimique, paramètres enzymatique.

Abstract

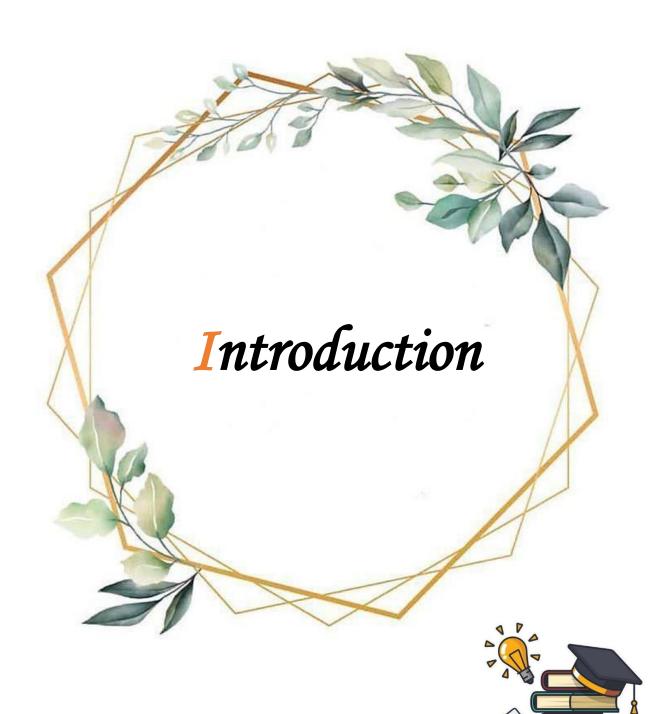
This research is part of a sustainable agriculture strategy that seeks to assess the effects of biochar, on two local barley varieties (*Hordeum vulgare L.*), specifically *Saida* and *Fouara*, during the germination and growth phases. The experiment utilized four escalating concentrations of biochar. The goal of this study is to find the best dose of this organic amendment for improving the species' physiological and metabolic health by looking at its effects on several morpho-physiological parameters (Germination percentage, germination rate, average stem length, average root length, Seedling Vigor Index, tolerance index, stem phytotoxicity, root phytotoxicity), biochemical parameters (chlorophylls a, b, a+b; total proteins; Total soluble sugars), and enzymatic parameters (CAT, APX). This study validates that biochar, employed at a moderate dosage, can function as a biostimulant within the framework of sustainable agriculture.

Keywords: *Hordeum vulgare L.*, biochar, morpho-physiological parameters, biochemical parameters, enzymatic parameters.

ملخص

تندرج هذه الدراسة في إطار الزراعة المستدامة وتحدف إلى تقييم تأثير التعديل بالبيوشار على صنفين محليين من الشعير (Hordeum vulgare L.) وهما الصنف سعيدة والصنف فوارة، خلال مرحلتي الإنبات والنمو. تم إجراء التجربة باستخدام أربع تراكيز متزايدة من البيوشار. تحدف هذه الدراسة إلى تقييم تأثير هذا المعدل العضوي على عدة معايير مورفوفسيولوجية (نسبة الإنبات، نسبة النبتات الحيوية، طول الساق، طول الجذر، مؤشر القوة الحيوية، مؤشر الطول، الكتلة الطازجة للساق والجذر)، كيميائية حيوية (الكلوروفيلات a و d و (a+b)، البروتينات الكلية؛ السكريات الكلية الذائبة) وإنزيمية (الكاتالاز، أسكوربات بيروكسيداز)، من أجل تحديد الجرعة المثلى التي تعزز التحسن الفسيولوجي والاستقلابي للنباتات. تؤكد هذه الدراسة أن البيوشار، عند استخدامه بجرعة معتدلة، يمكن أن يعمل كمنشط حيوي في إطار الزراعة المستدامة.

الكلمات المفتاحية: Hordeum vulgare L، معايير مورفوفسيولوجية، معايير بيوكيميائية، معايير انزيمية.





Introduction

The global challenge of sustaining agricultural systems also includes the problem of preserving agricultural soil. Soil improves ecosystem services and agri-food production systems critical to global and national food security (Jones et al., 2022; Smith et al., 2023). Agricultural soils are essential for offering several ecosystem services as they are dynamic living systems which contribute to the processes of biogeochemical cycling as well as the maintenance of microbial biodiversity and waterscape control (Lal et al., 2021; Martinez et al., 2024).

However, soil degradation in arid and semi-arid regions, such as Oued Zenati in Algeria, negatively impacts farm sustainability. This degradation results from a lack of organic matter, poor soil structure, and decreased water retention capacity (Benhamou et al., 2023; El Hadj et al., 2025).

Soils in these regions have silty clay loam textures, are low in organic matter, neutral or slightly acidic in pH, and have scarce water resources (Bouchenak et al., 2022). Such soils are unable to support consistent high agricultural productivity, negatively affect soil fertility, and increase the risk of abiotic stress on crops (Ait Ouarasse et al., 2024).

Additionally, agricultural soils are increasingly exposed to various pollutants, including heavy metals, pesticides, and microplastics. The pollutants alter the chemical, biological, and physical objectives of the soil, damaging its health and productivity (Amanullaha et Khan, 2022; Zhang et al., 2023). Microplastic pollution, in particular, is increasing due to its persistence in soil, potential toxicity, and destructive impact on soil microbiology and plant growth (Xing et al., 2024).

In this context, biochar, a carbon-rich byproduct of biomass pyrolysis, has shown considerable potential to enhance several essential parameters of agricultural soils, including water retention capacity, porosity, nutrient availability, and the stimulation of microbial biomass (Liu et al., 2021; Ge et al., 2024). Additionally, biochar plays a role in the sequestration of organic carbon which helps mitigate climate change impacts (Prajapati et al., 2024). Nevertheless, its impacts can differ greatly owing to the source and chemical makeup of the biochar, application rate, and the particular soil properties of the land being treated (Chen et al., 2023).

Moreover, biochar has demonstrated as being essential to increasing the nutritional value of cereals and other food products by impacting their proteins, carbohydrates, minerals, and other



essential vitamins. These changes stem from biochar's effects on the nutrient accessibility in soil, as well as its influence on the physiological processes of plants (Hassani et al., 2024). Considering this perspective from nutrition science on biochar can help understand its contribution to agricultural productivity and food security (Lehmann et Joseph, 2015).

Barley (*Hordeum vulgare L.*), a hardy cereal widely cultivated in Algeria, and in particular in the Oued Zenati area, plays an important role in food security and animal production (Brahimi et al., 2023). However, the productivity of barley is significantly affected by soil health, often disrupted by climatic factors, contamination, and intensive farming (Hassani et al., 2024). As such, gaining adequate understanding about the physiology and biochemistry underlying barley's behavior under the amendment effects of biochar has great importance for enhancing its adaptability and productivity under this specific environmental regime.

This study aims to contribute meaningfully to the understanding of biochar-soil-plant interactions, which remain inadequately documented under Algerian agroecological conditions, by utilizing current data and recent research focused on biochar derived from local resources (Dong et al., 2024). Additionally, it seeks to offer practical recommendations for the sustainable enhancement of farming practices, addressing environmental challenges associated with soil conservation, particularly through improved water retention and increased microbial activity (Kumar et al., 2025; Liu et al., 2021). Furthermore, the socio-economic development of local farms could be bolstered through better valorization of agricultural waste into biochar, thereby fostering a circular and climate-resilient agricultural system (Ababsa et al., 2025; Brahimi et al., 2023).

This study aims to investigate the effects of biochar derived from peanut shells at various concentrations (0 %, 1 %, 2.5 %, and 5 %) on the growth and development of two barley varieties (*Hordeum vulgare L.*), *Saïda* and *Fouara*, cultivated in agricultural soil typical of the Oued Zenati region. The objective is to examine the physiological responses of barley to biochar amendments by assessing growth, photosynthetic efficiency, and morphological parameters. Additionally, by measuring the activity of specific enzymes associated with oxidative stress responses and quantifying metabolites such as proteins and sugars, this study will also illustrate the biochemical impacts of biochar on barley.

The structure of this thesis is organized as follows:

• Part I: An extensive literature review that provides the scientific foundation and context for the study.



- Part II: A detailed description of the materials and methods, including the biological material used, the experimental protocol, and the analytical techniques employed.
- Part III: Presentation and analysis of the experimental results.
- Part IV: A critical discussion of the results, leading to a general conclusion and recommendations for future research directions.





1. Historical of Biochar

The black soils of the Amazon, known as Terra preta, are among the most prized agricultural soils due to their excellent fertility, high nutrient content (C, N, P, K, and Ca), good cation exchange capacity (CEC), and their ability to retain and recycle nutrients over prolonged cultivation periods (Lima et al, 2002; Factura, 2010). Terra preta covers an area of more than 50,000 hectares. Radiocarbon dating indicates that these soils were formed between 7,000 and 500 years ago, and are pre-Columbian origin. Terra preta soils were rediscovered by the Dutch soil scientist, Wim Sombroek, in 1966 (Glaser, 2007). These soils maintain exceptionally high amounts of organic carbon, more than 70 times the nutrient content of neighboring soils (Fig. 1). One hectare deep Terra preta can contain 250 tonnes of carbon compared to 100 tonnes in unimproved soil (Lima et al, 2002; Factura, 2010). The Amazonian soil is rather low in carbon and nutrients, and it cannot be used for agricultural production even several thousand years after their abandonment (Glaser, 2007). Analyses of black soils revealed high concentrations of coal and organic matter, such as plant and animal remains. The productivity of Terra preta soils is attributed to their efficient nutrient retention and neutral pH in regions where soils are typically acidic. The current equivalent of coal used in black soils in the Amazon is biochar produced by pyrolysis (Lehmann, 2007).



Figure 1: Comparison Between Terra Preta and Conventional Soils (Lehmann, 2007).



In 2003, it was reported that the largest producer of biochar was Japan, with an annual production of 15,000 tons. In Switzerland, the composting company "La Coulette" is the leading producer of charcoal, mainly derived from forest waste. Their production allows customers to purchase biochar in small quantities, ranging from 30 to 35 liters. They also sell a compost/biochar mixture called Terra preta, in reference to the highly fertile Amazonian soil of the same name, although it is not identical, as it is a manufactured soil amendment (Sohi et al., 2010). Since January 1, 2012, a European certification for biochar has been in place, establishing guidelines to introduce controls in its manufacturing and use, and to define standard quality levels (Schmidt, 2012). Biochar has a variety of applications and benefits. Environmentally, it enables carbon sequestration and the reduction of greenhouse gases, such as CO₂ an increasingly relevant area of research due to growing environmental concerns. In terms of waste management, the pyrolysis process reduces waste volume and generates valuable by-products; currently, green waste, forest residues, and paper mill waste are being recovered. Biochar also plays a role in improving soil quality. Chemically, it contains three carbon fractions based on biodegradability (refractory, labile, and washable), as well as mineral ash. The fundamental difference between biochar and regular soil organic matter lies in biochar's high proportion of structural aromatic carbon. Additionally, biochar contributes to energy production by allowing recovery of the heat generated during the pyrolysis process (Lehmann et Joseph, 2009).

2. Definition of Biochar

The term biochar is an abbreviation of "bio-charcoal," derived from the prefix "bio" (indicating biological origin) and the English word "charcoal" (meaning charbon de bois in French) (Lehmann et al., 2009). It is a carbon-rich solid material produced by the thermal decomposition of biomass such as wood by-products, agricultural residues, green waste, or food waste under oxygen-limited or oxygen-free conditions, a process known as pyrolysis. Biochar has a porous carbon structure, many functional groups, and a large specific surface area (Lehmann et Joseph, 2015).

In recent years, biochar has attracted significant international attention as a microporous carbonaceous material derived from the thermochemical degradation of organic matter in the absence of oxygen. Its primary applications include soil amendment to improve fertility, enhancement of carbon sequestration, and promotion of water filtration through soil percolation (Lehmann et Joseph, 2009).



3. Production Processes

Various methods exist to produce biochar, but all involve heating biomass under limited or no oxygen conditions, volatilizing gases and leaving behind a carbon-rich solid residue. This fundamental process is known as thermal decomposition, most commonly achieved through pyrolysis or gasification (Vijayan et al., 2025).

• Production of Biochar by Pyrolysis Process

Pyrolysis, a term derived from the Greek pyro (fire) and lyse (decomposition), meaning 'heat decomposition'. Pyrolysis to produce biochar typically uses cellulose-rich biomass as raw material, such as wood chips and agricultural residues. During pyrolysis, carbon-rich biomass is heated in an industrial furnace at high temperatures within a low-oxygen environment. This process converts approximately 50 % of the carbon into biochar, primarily used as a soil amendment, while the remaining 50 % is transformed into bioenergy products such as synthetic gases and oils, which can be utilized for heat or energy generation (Fig. 2). Pyrolysis occurs in a closed reactor system at temperatures exceeding 300°C (Vijayan et al., 2025).

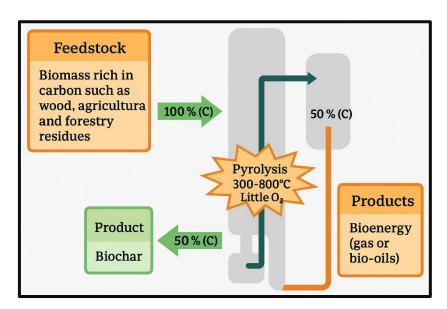


Figure 2: Optimizing biochar production through the pyrolysis process (Laurin-Lanctôt, 2015).

4. Different Types of Pyrolysis

Several types of pyrolysis exist (**Tab. 1**), each adapted to specific purposes and characterized by distinct operating conditions (**Iurchenkova et** *al.*, 2024).



4.1. Slow (or Traditional) Pyrolysis

Conditions: Moderate temperature (300–500 $^{\circ}$ C), low heating rate (0.1–1 $^{\circ}$ C/s), long residence time (from minutes to hours).

Products: Mainly biochar (charcoal), with less bio-oil and gas.

Applications: Charcoal production, soil amendment, carbon sequestration.

Benefits: Simple technology, low energy cost, solid carbon enhancement.

4.2. Rapid Pyrolysis

Conditions: High temperature (400–600°C), rapid heating rate (10–200°C/s), and very short residence time (<2 seconds).

Products: Mainly bio-oil (~60%), biochar (~20%), and gases (~20%).

Applications: Production of biofuels, chemical raw materials, and renewable energy.

Benefits: High liquid yield, energy efficiency, suitable for a wide range of biomass.

4.3. Flash (or Ultrafast) Pyrolysis

Conditions: Very high temperature (>700°C), extremely fast heating rate (>1000°C/s), ultra-short residence time (<1 second).

Products: Mainly synthetic gases and bio-oil, with little biochar.

Applications: Production of combustible gases, synthetic fuels, high-value chemicals.

Benefits: Maximizes gas production, suitable for high-energy raw materials.



Table 1: Types of pyrolysis and their characteristics (Iurchenkova et al., 2024).

Type of Pyrolysis	Temperature (°C)	Heating Rate (°C/s)	Residence Time	Dominant Products	Main Applications	Yields (%)
Slow	300-500	0,1-1	Minutes to hours	Biochar	Coal, agriculture	35-45 biochar 25-35 bio-oil 20-30 syngas
Fast	400-600	10-200	<2 seconds	Bio-oil	Biofuels, chemicals	20 biochar 60 bio-oil 20 syngas
Flash	>700	1 000	<1 Second	Gas, Bio-oil	Energy, chemicals	Biochar 10–20 % Bio-oil 10–15 % Gas (syngas) 60–75 %

5. Role of Biochar

Biochar is attracting increasing interest due to its multiple benefits in the energy, agronomic, and environmental fields. Its main roles are illustrated in the diagram below (Fig. 3), highlighting its potential as a key vector for sustainable biomass development (Mustin, 2013).



Part 01: Literature Review

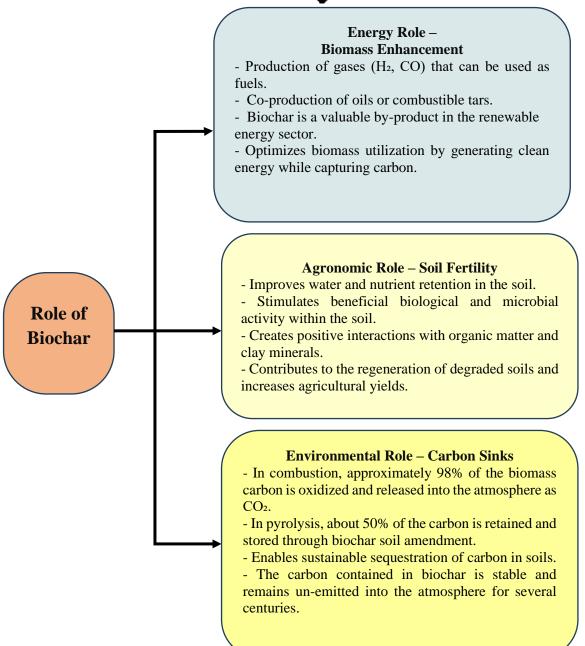


Figure 3: Role of biochar in sustainable energy, agriculture, and environmental management (Mustin, 2013).



6. Biochar as a Source of Nutrients

Biochar is a potential supplier of essential nutrients for plant development due to its diverse mineral composition. The nutrient role of biochar largely depends on the type of biomass used and the thermal conditions under which pyrolysis occurs, both of which significantly impact its mineral element content. Indeed, several studies have shown that biochar contains varying but significant concentrations of macro- and micronutrients. The **macronutrients** include (**Gao et al., 2018**):

- Potassium has been a key nutrient, typically present between 1.3% and 5.4%. The
 highest levels are often found in biochar produced from rice straw or agricultural
 waste rich in potassium.
- Phosphorus, occurring in concentrations ranging from 0.05 % to 5.9 %, in the form
 of compounds with low solubility, releasing them gradually under the influence of
 soil pH and root-plant interactions.
- Calcium and magnesium are vital elements, with calcium content ranging from 3.0% to 4.6% and magnesium ranging from 0.8% to 3.7%. These elements are crucial in counteracting acidity in the soil and in the development of plant cell structures.

Biochar can also contain **trace elements**, including zinc (up to 98 mg/kg), iron (about 500 mg/kg), and manganese, which are essential in small amounts to participate in enzymatic activities, photosynthesis, and hormonal regulation of plant growth (Ma et al., 2025).

However, the availability of the nutrients in the soil depends on several factors that include (Hossain et *al.*, 2020):

- Pyrolysis temperature, which significantly influences the solubility and chemical form of the elements.
- The alkaline pH of biochar, which enhances the mobility of certain nutrients.
- The cation exchange capacity (CEC) of biochar, which allows for increased retention of nutrients in the soil, thereby reducing leaching losses and making nutrients more available to root systems.

7. Properties of Biochar

The physical and chemical properties of biochar vary according to the sources of raw materials and production conditions (temperature, oxygen, pressure, duration, etc.), allowing biochars to be generated with specific functions such as cation exchange capacity (CEC), specific surface area, organic carbon and moisture content, pH, and particle size distribution.



As a result of these properties, biochar can affect greenhouse gas emissions such as nitrous oxide and methane (Mackenzie et al., 2013).

7.1 Physical Characteristics

Temperature is the most important factor for physical changes in biochar, followed by heating rate and pressure (Lehmann et Joseph, 2009).

• Biochar Structure

The biochar structure is amorphous, containing localized crystalline regions composed of aromatic compounds (Graber et al., 2011). The carbon skeleton is formed during the pyrolysis of organic matter. The material results in high biochar porosity, due to its sponge-like structure.

The particle size distribution of biochar is highly dependent on the raw material used. In general, wood-based biochar is coarser and retains a xylem structure, whereas the biochar obtained from finer crop residues has a more recalcitrant structure (Kumari, 2015).

• Specific Surface

The biochar density is very low, and its porous structure consists mainly of macropores and micropores (Downie et *al*, 2009). Biochar has a large specific surface area, which depends on the base material and heat treatment. Obtained by pyrolysis, it can reach areas between 20 m²/g and 3000 m²/g. The amount of water adsorbed depends directly on this surface, which allows biochar to adsorb large amounts of water. The process of water adsorption on the surface of the biochar is regulated by the functional groups (Antal et Gronli, 2003).

Porosity

The gaps in the biochar manifest themselves as macropores ($> 50 \, \mu m$), mesopores ($2-50 \, \mu m$) and micropores ($< 2 \, \mu m$), as shown in (Fig. 4). The high proportion of micropores, with a diameter of less than 3 μm , is responsible for the increase in the specific surface area, which contributes to the reduction of water mobility in the soil. The microporous structure of biochar promotes water retention and provides habitat for beneficial soil organisms (Lehmann et Joseph, 2009).



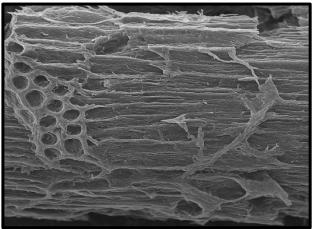


Figure 4: Microscopic characterization of biochar structure (Lehmann et Joseph, 2009).

Density

The bulk density is directly related to porosity. Indeed, the more gaps in a biochar, the lower the bulk density. The maximum actual density readings for biochar are between 2 and 2.1 g/cm³, while graphite density is 2.25 g/cm³. The actual density would increase as a function of the pyrolysis temperature. However, the average values of actual density would be between 1.5 and 1.7 g/cm³. Apparent densities are on average 0.30 to 0.43 g/cm³. This means that biochar is a very porous material with 79 to 85 % porosity (Downie et al., 2009).

7.2 Chemical Characteristics

pH

The pH of biochar is generally alkaline (pH > 7), with little variability between different biochars, typical values remaining above 7 (Lehmann et Joseph, 2009). The pH depends on the raw material used: at a pyrolysis temperature of 300°C, the pH measured for biochar from corn, groundnut, canola, and soybean is 9.4, 8, 6, 6.5, and 7.7, respectively. Increased pyrolysis temperature results in elevated levels of pH from accumulation of oxides of the alkali metals. The increase in pH imparts a positive attribute to biochar upon its incorporation into the soil (Chintala et al., 2014).

• Electrical Conductivity (EC)

The electrical conductivity and pH properties of biochar improve after thermal treatment at high temperatures (700°C), compared to biochar produced at lower temperatures (550°C) (Tu et *al.*, 2022).



• Cation Exchange Capacity (CEC)

The large specific surface area of biochar enhances its cation exchange capacity (CEC) and nutrient sorption. The CEC for biochar depends on both the type of precursor used and its granulometry. Reported values range from 29.2 to 51.1 cmol/kg when measured with ammonium acetate. This capacity tends to decrease with increasing pyrolysis temperature (Lehmann et Joseph, 2009).

However, the CEC values vary according to the origin of the material and the analytical method employed (Song et Guo, 2012).

8. Effects of Biochar

8.1 Effects of Biochar on Physical and Chemical Properties of Soils

Biochar possesses important physical and chemical properties that influence soil fertility (Sun et Lu, 2014). Furthermore, biochar can improve various physical and chemical properties of the soil. It contributes to reduced compaction, decreased bulk density, increased porosity, and better water and nutrient retention. It can also raise soil pH, improve cation exchange capacity, and increase extractable nutrient quantities (Na, K, Ca, Mg). Other benefits include increased base saturation, saturated hydraulic conductivity, total carbon and nitrogen, and reduced erosion (Zhu et al., 2025).

A key mechanism for improving yields is the increased water retention capacity of the soil due to its high porosity, allowing both water retention and infiltration. Several studies have shown that biochar can significantly increase water retention capacity and improves soil structure. It also promotes aggregate formation and stability, which contributes to better agricultural production and prevents soil degradation (Sun et Lu, 2014).

However, some effects are not always observed in the long term, as shown by variable field test results. These variations indicate that the effectiveness of biochar depends on specific application conditions. Long-term studies are needed to assess its effects on soil properties in a sustainable way (Hardy et al., 2017).

• Influencing Factors of Biochar Function

Biochar acts differently depending on several factors. The effects of biochar also vary depending on soil texture and mineralogy. These soil characteristics influence water retention capacity as well. Improved nutrient availability depends on increasing soil pH. This effect is particularly noticeable for phosphorus and potassium. However, high-temperature biochar rich in volatile matter can immobilize nitrogen. It can also reduce microbial activity, limiting plant



growth (Zhang et al., 2013).

Peake et *al.* (2014) observed effects that varied by soil type. Application rates should be adapted to the buffer capacity of the soil. 1% is recommended for coarse-textured soils. 2% is preferred for fine-textured soils.

Biochar may not affect certain crops such as corn, but it can improve subsequent crop growth. These differences are related to the depth of plant rooting. Additionally, pyrolysis temperatures also influence its effects. Interactions between soil type, plant, and dosage are crucial. It is therefore essential to understand these factors for optimal application (Peake et al., 2014).

8.2 Effect of Biochar on Soil Nutrient Availability

Nutrient availability represents the proportion of nutrients in the soil that is accessible for plant uptake and is influenced by several factors, including pH levels, moisture levels, temperature fluctuations, soil texture, and biological activity. Adding biochar to the soil has a very significant effect on nutrient availability through a number of physicochemical and biological pathways (Zhang et al., 2020).

• High pH of Acid Soils

One of the best-documented effects of biochar is its alkalizing capacity, especially in acidic soils. It can be attributed to the basic ash mineral composition (such as calcium, magnesium, potassium hydroxides or oxides, and carbonates) that results from pyrolysis. The increase in pH caused by the incorporation of biochar into the soil neutralizes acidity in the soil, stimulating root development and microbial activity improves the solubility and thus the accessibility of several of the most important nutrients, including phosphorus (P), calcium (Ca), and magnesium (Mg), that are otherwise bound in acid-insoluble form, and decreases the toxicity of certain heavy metals that can disrupt nutrient uptake (such as aluminum and iron) (Geng et al., 2022).

• Stimulation of Beneficial Microbial Activity

Biochar provides a conducive environment for microbial organisms due to its porous nature and stable properties. It offers shelter to a range of soil microorganisms, including bacteria, fungi, and actinomycetes. This structure allows for increased soil diversity and microbial balance, enhancing the activity of microorganisms involved in releasing and converting critical nutrients, such as phosphorus and nitrogen. Moreover, it promotes beneficial relationships between plants and fungi, which maximize nutrient uptake (Lehmann et Joseph, 2015).



• Regulation of Organic Matter Mineralization

Biochar influences the activity of soil organic matter (SOM). The feature has a number of advantages: it avoids the release peaks that are not synchronized with the plant's needs, thus reducing losses through leaching or volatilization; it improves the efficiency of the application of organic fertilizers by serving as a catalyst in the nutrient releasing process; it helps to structure the soil and improves its ability to hold nutrients in the root zone. Its ability to sequester compounds retards rapid mineralization but allows a slow release of nutrients like nitrogen, phosphorus, and sulfur (Lehmann et Joseph, 2009).

8.3 Effect of Biochar on Soil Nutrient Retention

Nutrient retention refers to the ability of soil to retain valuable nutrients for plants to thrive in the zone where the roots are, that is, where the roots can easily absorb them. Biochar is significant owing to its unique physical as well as chemical attributes (Gunal, 2025).

• A Large Specific Surface Area

Biochar contains a microporous structure reminiscent of a sponge filled with many small pores. The latter plays an essential part in increasing the biochar's interaction with the nutrients in the soil. As a Consequence, the additional surface area can promote a higher capacity for holding nutrient ions, including charged ions like K⁺, Ca²⁺, and Mg²⁺. Such nutrients adhere to the surface of biochar, thus inhibiting their leaching by water immediately (El-Naggar et *al.*, 2019).

• High Cation Exchange Capacity (CEC)

Biochar surfaces incorporate active, negatively charged chemical functional groups such as carboxylic groups (-COO⁻). These functional groups draw and retain particular positive ions known as cations: K⁺ (potassium) aids plants in their growth and water control, Ca²⁺ (calcium) is used in cell structure, and Mg²⁺ (magnesium) is a structural component of chlorophyll. These ions adsorb onto the surface of the biochar for a certain period of time, and yet are still available for uptake by the roots when the plant requires them (Liang et *al.*, 2006).

Reduction of Leaching

Leaching is the process by which nutrients are carried to lower layers of soil due to rain or irrigation activities. It is most frequently observed in sandy or highly porous soils, which have limited capacity to retain water and nutrients. Biochar acts as a buffer reservoir or a barrier; it



helps keep nutrients in the area surrounding plant root systems. As a result, fewer nutrients are lost to deeper soil layers or drainage water (Dissanayake et al., 2023).

8.4. Effects of Biochar on Soil Microorganisms

Biochar influences various aspects of the soil, particularly microbial activity. **(Tab. 2)** summarizes its main effects on soil biological and physico-chemical properties, including microbial dynamics (**Huang et** *al.*, 2023).

Table 2: Effects of biochar on soil microbial communities (Huang et al.,

2023).

Aspect studied	Effects of biochar
Biological functions of soil	Biochar improves the functions performed by microorganisms (respiration, nutrient recycling, soil stability).
Physico-chemical properties of soil	It modifies the pH, apparent density and organic carbon content favorably, thus facilitating microbial development.
Microbial diversity and abundance	Biochar stimulates the diversity, activity and proliferation of microorganisms (bacteria, fungi, protozoa).
Creation of microbial habitats	The pores of biochar provide a physical shelter for microorganisms, protecting them from predators and stressful conditions.
Reduction of toxic substances	Thanks to its adsorption capacity, biochar retains harmful compounds, reducing their impact on soil microorganisms.
Water retention	Increasing the porosity and specific surface area of biochar improves the soil's ability to retain water, which is essential for microbial activity.
Availability of nutrients	Biochar increases the nutrient content (K, dissolved carbon, etc.) thus stimulating growth and functions of soil microorganisms.



8.5. Effect of Biochar on the Abundance of Soil Microorganisms

Several studies reveal the positive influence of biochar on microbial biomass growth in various soil types (Hale et al., 2015). Hale et al. (2015) demonstrated that amended soil with pine biochar produced at 600°C and inoculated with the strain Enterobacter cloacae UW5 had a bacterial population density 16% higher than the control without biochar. In addition, Ameloot et al. (2013) observed a 29% increase in carbon from microbial biomass (P < 0.05) in sandy-loamy soil amended with willow wood biochar produced at 700°C after 117 days of pot experience. In another study, a significant 62% increase in microbial biomass carbon in response to the amendment with biochar derived from Miscanthus giganteus produced at 700°C, after three months in a clay loam, was reported (Luo et al., 2013). In a study by Li et al. (2016), bacterial abundance in soil increased 161% as a result of the addition of straw and wood biochar. However, some studies have reported that the short-term application of biochar products at high temperatures (600°C) had no effect or a negative influence on microbial biomass (Dempster et al., 2012).

8.6. Effect of Biochar on Soil Microbial Communities

Soil amendment with biochar has a significant impact on the structure, composition, and diversity of soil microbial communities. This effect is primarily due to changes in soil properties such as pH, porosity, water retention capacity, and nutrient availability. These changes influence microbial competition and promote the appearance of new taxa while eliminating others, leading to an ecological reorganization towards a new functional balance (Lehmann et al., 2011; Anderson et al., 2011; Xu et al., 2018). For example, Feng et al. (2012) showed that the addition of biochar at a rate of 24 t/ha to a rice paddy soil reduces methane (CH₄) emissions not by inhibiting methanogenic archaea, but by stimulating methanotrophic bacteria (Proteobacteria) which actively oxidize methane. This demonstrates the functional modulation of biogeochemical cycles by biochar. Molecular analyses such as RFLP and pyrosequencing have revealed a differentiated taxonomic response to biochar, including the stimulation of bacterial families like Bradyrhizobiaceae, Thermomonosporaceae, and Hyphomicrobiaceae, and the inhibition of others such as Streptomycetaceae, Micromonosporaceae (Anderson et al. 2011). This suggests that biochar exerts ecological selectivity on bacterial communities. Furthermore, several studies have reported significant increases in microbial diversity indices following biochar application (Sun et al., 2013). For instance, Sun et al. (2013) and Hu et al. (2014) observed increases in diversity ranging from 12% to 37% according to the Shannon,



Simpson, and Chao indices, confirming the positive effect of biochar on microbial biodiversity. Lastly, a dose-response relationship has been documented: Increasing biochar doses lead to a proportional increase in bacterial richness, as evidenced by 16S rRNA gene sequencing (Xu et al., 2016), supporting the role of biochar as an ecological modulator of soil microbial communities.

8.7 Effect of Biochar on the Activity of Soil Microorganisms

Biochar has multiple effects on the biological functioning of soils, particularly through its interaction with microbial and enzymatic activity (**Zimmerman et** *al.*, **2011**).

The following table (Tab. 3) presents a summary of the main mechanisms by which biochar influences these processes, in relation to its physico-chemical properties and production conditions.



Table 3 : Impact of biochar on soil physicochemical properties and microbial activity (Zimmerman et *al.*, 2011).

Aspect studied	Effects of biochar
Modification of soil properties	Direct influence on microbial activity through modification of soil physico-chemical properties (pH, texture, etc.), which affects the decomposition of organic matter.
Mineralization of organic carbon	Stimulation of the mineralization of native organic C and increase in microbial respiration (CO ₂), depending on soil pH and pyrolysis temperature of biochar.
Extracellular enzymatic activity	Biochar stimulates the activity of key enzymes (alkaline phosphatase, aminopeptidase, N-acetylglucosaminidase, β-glucosidase, dehydrogenase) involved in biogeochemical cycles.
Enzyme-substrate interaction with biochar	Effects depend on physico-chemical interactions between enzymes, substrates and biochar surface (sorption/desorption, porosity, specific surface).
Effects of pyrolysis temperature	Biochar produced at high temperature (700°C) can inhibit enzymes by adsorbing substrates and enzymes, while those produced at low temperature (350°C) can stimulate them.
Protective effect on soil	Immobilization or deactivation of pathogenic enzymes, such as those of Fusarium oxysporum, suggesting a protective role for biochar on soil health.







1. Material

1.1. Pedological Characteristics of the Sampling Site

As part of the optimization of cereal crop cultivation, a pedological analysis was conducted on an agricultural plot located in Aïn Regada, in the commune of Oued Zenati (Guelma province). The geographical location of the experimental site is illustrated in (Fig. 5). This study aims to characterize the physical and chemical properties of the soil with a view to its use for barley cultivation (*Hordeum vulgare L.*). The data collected make it possible to adapt technical practices according to the local soil specificities. (Tab. 4) presents a summary of the results obtained during soil sampling and sowing preparations (Hammana et al., 2024).

Table 4: Summary of Soil Properties at the Aïn Regada Agricultural Site (Oued Zenati Commune).

Zone	Aïn Regada
Soil Type	Brown Agricultural Soil
Depth	Medium
Texture	Silty-Clay
Ph	6.74
Recommended Agricultural Use	Barley Cultivation
Sampling Date	14/02/2025
Sowing Date	27/02/2025





Figure 5: Map of the Geographical Location of Aïn Regada (Commune of Oued Zenati, Wilaya of Guelma).

1.2. Plant Material

The plant material used in this study is barley (*Hordeum vulgare L.*), a cereal species of strategic importance in semi-arid areas. Two local varieties cultivated in Algeria were selected for this trial: *Saïda* and *Fouara* (Tab. 5). These varieties are distinguished by agronomic characteristics adapted to local environmental conditions (Guetteche et al., 2023).



Table 5: Agronomic and Adaptive Characteristics of *Hordeum vulgare L.* var. *Saïda* and *Fouara*. (Benmahammed et *al.*, 2010).

Variety	Saïda	Fouara
Origin	Local (from Western Algeria)	Local (Selection from the Technical Institute of Field Crops (ITGC) in Sétif)
Adaptation Zone	Interior plains, High plateaus	High plateaus
Туре	Spring (responsive to vernalization)	Mid-winter (tolerates winter cold)
Vegetative Cycle	Semi-early	Late
Tillering	Medium	Strong
Thousand Grain Weight	High	High
Cold Resistance	Resistant	Tolerant
Drought Resistance	Resistant	Tolerant
Lodging Resistance	Yes	Yes
Disease Resistance	Sensitive to Diseases	Helminthosporiosis, Smut Tolerant to: Rhynchosporium

1.2.1 Selection Criteria for the Plant Species Hordeum vulgare L

We are interested in the study of a local cereal (*Hordeum vulgare L.*), our choice is based on a scientific approach to explore its physiological and biochemical responses to innovative organic amendments such as biochar (*Nasiri et al.*, 2023; *Bagues et al.*, 2024). This species is of major interest, as it represents the fourth most produced cereal crop worldwide, and it plays



a crucial role in human and animal nutrition, as well as in various agroecological systems (Le Guyader et al., 2025).

In Algeria, barley ranks second after wheat in terms of cultivated area, with significant importance in steppe regions due to its hardiness and low requirements (Debab et al., 2025). It is also recognized for its ability to enhance marginal soils and for its favorable interaction with organic amendments, particularly biochar, which is receiving growing interest for its effects on soil fertility, plant productivity, and the modulation of physiological processes (Abdelaal et al., 2022; Hafez et al., 2020).

Barley thus constitutes an ideal model plant to evaluate the impact of biochar on biochemical and physiological parameters, within a perspective of sustainable agriculture and improvement of soil health. In this context, the choice was made to use two local varieties, *Saïda* and *Fouara*, due to their nutritional and agronomic importance. These genotypes, widely cultivated in Algeria, are valued for their adaptation to local climatic conditions, their productivity, and their nutritional quality, making them relevant candidates for this type of assessment (Nasiri et al., 2024; Amjad et al., 2021).

1.2.2 Systematic Position

Barley (*Hordeum vulgare L*.) is an annual herbaceous plant, primarily self-pollinating. Its genome consists of seven pairs of chromosomes (2n = 14), as presented in (**Tab. 6; Tab. 7**) (Boudersa et al., 2021). In the context of this study, two local varieties were selected: *Saïda* and *Fouara*.



Table 6: Systematic Position of Hordeum vulgare L. var. Saïda. (Bouchetat et al.,

2020).

	Position	Systematic
A Total	Kingdom:	Plant
	Division:	Tracheophyta
	Class:	Liliopsida
	Order:	Poales
	Family:	Poaceae
Salah Parangan	Subfamily:	Pooideae
	Tribe:	Triticeae
	Genus:	Hordeum
	Subgenus:	Hordeum
	Section:	Hordeum
	Species:	Hordeum vulgare L
	Variety:	Hordeum vulgare L. var. Saïda

Table 7: Systematic Position of *Hordeum vulgare L.* var. *Fouara*. (Ro et al., 2025).

	Position	Systematic
A COLOR OF THE PARTY OF THE PAR	Kingdom:	Plant
	Division:	Tracheophyta
	Class:	Liliopsida
	Order:	Poales
TO COME TO SERVICE OF THE PARTY	Family:	Poaceae
	Subfamily:	Pooideae
7/1	Tribe:	Triticeae
	Genus:	Hordeum
	Subgenus:	Hordeum
	Section:	Hordeum
	Species:	Hordeum vulgare L
	Variety:	Hordeum vulgare L. var. Fouara



1.3. Chemical Product

The biochar used in this study, synthesized by Dr. Hadia Hemmami (Department of Process Engineering and Petrochemistry, Faculty of Technology; Research Unit UDERZA, University of El Oued, 39000 El Oued), was prepared from peanut shells using a rigorous slow pyrolysis method under a nitrogen-saturated atmosphere, with gradual heating up to 600 °C and a residence time of 30 minutes at this temperature, in accordance with the technique reported by Wang et al. (2020).

The shells were previously washed, dried, ground, and then purified by Soxhlet extraction with methanol to eliminate labile compounds. After carbonization, the biochar was washed with hydrochloric acid to remove residual ash, filtered, rinsed until neutral pH, and then dried at 50 °C until constant weight. This protocol ensures a stable, purified biochar suitable for environmental applications (Wang et al., 2020).

2. Method

2.1. Conditions and Implementation of the Experiment

2.1.1 Conducting the Experiments

The experiments were conducted under controlled conditions within the experimental greenhouse of the Department of Biology at the University of 8 May 1945 of Guelma.

2.1.2. Biochar Application

2.1.2.1. Seed Germination Medium

Germination tests were conducted by applying three concentrations of biochar (Bouqbis et al., 2017), as well as a control treated only with distilled water. Aimed to evaluate the effect of biochar on the seed germination process (Ali et al., 2021).

2.1.2.2. Physical Aspect of Seed Germination

The study aims to evaluate the physiological quality of seeds as well as the actual effect of biochar on their viability and growth potential, using different germination enriched with biochar at increasing concentrations. To ensure aseptic experimental conditions, the seeds are first weighed, then disinfected by immersion in a bleach solution for 10 minutes at room temperature, before being carefully rinsed several times with distilled water. The seeds are then germinated in two types of experimental setups: Petri dishes containing moist sterile filter



paper, and plastic pots filled with soil. Irrigation is performed every other day throughout the experimental period, with 10 ml of distilled water for the controls and biochar-enriched solutions for the treated groups. Germination tests are conducted with three replicates per treatment, over a total duration of 21 days (Zhang et al., 2023).

2.1.2.3 Experimental Setup

The study was conducted on the species *Hordeum vulgare L.*, using a total of eight Petri dishes distributed across four experimental modalities, including a control. Each treatment was applied to three replicates, consisting of dishes each containing 21 seeds. The experiment was carried out over a period of 21 days, under controlled conditions established to ensure the validity of the observations (Fig. 6; Fig. 7) (Chabani et al., 2015).

Treatment	Control	Concentration Concentra		Concentration 3	
Hordeum vulgare L. var. Saïda					
Distilled Water	1450 ml				

Figure 6: Experimental Setup for Seed Germination of *Hordeum vulgare L.* var. *Saïda* in Petri dishes.

Treatment	Control	Concentration 1 Concentration 2		Concentration 3	
Hordeum vulgare L. var. Fouara					
Distilled Water	1450 ml				

Figure 7: Experimental Setup for Seed Germination of *Hordeum vulgare L.* var. *Fouara*, in Petri dishes.



2.1.3. Seed Planting

In order to evaluate the effect of biochar on seedling growth, a rigorous planting protocol was implemented. This protocol includes several successive steps ranging from seed disinfection to their cultivation in treated soil. The different stages of the process are detailed as follows (Zhang et al., 2022):

- The seeds were disinfected using a bleach solution for 10 minutes at room temperature.
- They were then carefully rinsed several times with distilled water.
- The seeds were germinated in beakers containing distilled water for 24 hours.
- After germination, they were planted in plastic pots containing soil amended with different concentrations of biochar, at a rate of 21 seeds per pot.
- The experimental setup included three replicates for each treatment (Fig. 8; Fig.9).
- Watering of the control and treated plants was carried out every other day, with 10 mL of distilled water per pot.

Treatment	Control	Treated	Treated	Treated
Hordeum vulgare L. var. Saïda				
Watering Period	21 Days	21 Days	21 Days	21 Days

Figure 8: Experimental Setup for Seed Cultivation of *Hordeum vulgare L.* var. *Saïda* in plastic pots.



Treatment	Control	Treated	Treated	Treated
Hordeum vulgare L. var. Fouara				
Watering Period	21 Days	21 Days	21 Days	21 Days

Figure 9: Experimental Setup for Seed Cultivation of *Hordeum vulgare L.* var. *Fouara* in plastic pots.

• Morphophysiological Parameters

• Germination Percentage

The germination percentage is expressed as the proportion of seeds that have developed a radicle of at least 2 mm, relative to the total number of seeds tested in a Petri dish (Fig. 10). The germination test was conducted in accordance with the guidelines of the International Seed Testing Association (ISTA, 2025).

Germination percentage = (Number of germinated seeds / Total number of seeds) \times 100

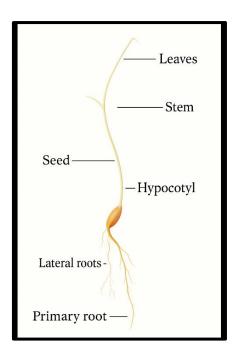


Figure 6: Root System of Barley *Hordeum vulgare L*.



Germination Rate

The germination speed index, an indicator of the rapidity of seed development, is calculated according to the method described by **Berka et** *al.* (2018). **Figure 11** illustrates the main growth periods observed in these two varieties under the controlled conditions of the experiment.

GR = (ngg on day 1) / 1 + (ngg on day 2) / 2 + ... + (ngg on day n) / n



Figure 7: Growth periods of barley (*Hordeum vulgare L.* var. *Saïda* and *Fouara*) during the experiment (photo taken as part of this study).

• Average Stem Length

Stem length was measured by carefully removing each germinated seedling from the Petri dish and measuring the length using a calibrated ruler (Betegón-Putze et *al.*, 2019).



Average Root Length

Root length is determined by carefully removing each germinated seedling from the Petri dish, then marking the ends of the root with a pencil. The distance between the marks is then measured, and the average length obtained is calculated (Betegón-Putze et al., 2019).

• Seedling Vigor Index (SVI)

This index is calculated by multiplying the germination percentage by the dry weight of the seedlings. This method allows simultaneous evaluation of the seeds' ability to germinate and the growth quality of the seedlings (Gupta et al., 2022).

$$SVI = \%$$
 of germination \times Dry Weight of Seeds (g)

• Tolerance Index

The index is calculated according to the method described by Rehman et al. (2022), which evaluates the seeds' capacity to tolerate environmental stress conditions.

• Phytotoxicity Percentage

This index is determined according to the method of Siddiqui et Abbas (2021), which allows the evaluation of the impact of phytotoxic substances on seedling growth by measuring the relative reduction in stem and root length compared to the control.

> Stem Phytotoxicity

> Root Phytotoxicity



2.1.4. Extraction and Quantification of Chlorophyll Pigments

The extraction of chlorophyll pigments was carried out according to the traditional method developed by Holden (1961); the sample processing was conducted as follows (Fig. 12):

- ➤ The fresh chopped and ground tissue (1 g) was macerated in 20 ml of 80 % (v/v) acetone, with an additional supplementation of 100 mg of calcium carbonate (CaCO₃) to neutralize any resulting acidity.
- ➤ The filtered extract obtained was stored in light-protective containers to prevent oxidation induced by light.
- Quantification was performed by spectrophotometry with measurements taken at 645 and 663 nm, after blank calibration using 80 % acetone.

The concentrations of chlorophyll a, b, and total chlorophyll (a + b) were calculated using the formulas proposed by Arnon (1949), with appropriate modifications for the solvent used:

Chlorophyll a (mg/L) = 12.7 (OD663) – 2.69 (OD645)

Chlorophyll b (mg/L) = 22.9 (OD645) - 4.86 (OD663)

Total Chlorophyll (a + b) = 8.2 (OD645) + 20.20 (OD663)



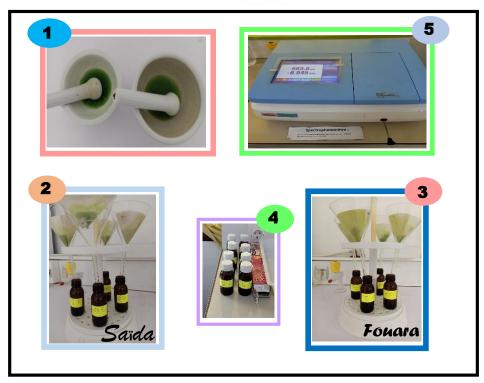


Figure 8: Extraction and quantification of chlorophyll pigments (photo taken as part of this study).

2.1.5. Analysis and Quantification of Metabolites

• Total Protein Assay

Total proteins extracted from the leaves, stems, and roots of *Hordeum vulgare L*. were quantified according to the **Bradford method** (1976), using the **Bradford** reagent composed of: 100 mg of Coomassie Brilliant Blue (G250), 50 mL of absolute ethanol (95°), 100 mL of orthophosphoric acid (85 %), all brought up to 1000 mL with distilled water. This reagent is stable for 2 to 3 weeks when stored at 4 °C (Fig. 13).

Bovine serum albumin (BSA, Merck) was used as the standard. The calibration range was prepared from a stock solution of BSA at 1 mg/mL in distilled water.



Table 8: Calibration range of a BSA solution.

Tubes	0	01	02	03	04	05
BSA albumin solution (μl)	00	20	40	60	80	100
Distilled water (μl)	100	80	60	40	20	00
BBC (ml)	4	4	4	4	4	4

An aliquot fraction of 100 μ L from each point of the standard curve as well as protein extracts from the samples was mixed with 4 mL of Bradford reagent (Fig. 14).

- Optical densities were measured using a spectrophotometer (JENWAY 63000) at a wavelength of 595 nm.
- The total protein concentration was then determined by interpolation on the standard calibration curve obtained from bovine serum albumin (BSA).

$$Y = 0.01113 X + 0.000190 R = 100.00\%$$

 $\mathbf{Y} = \text{Optical Density (OD)}.$

X = Total Protein Concentration.



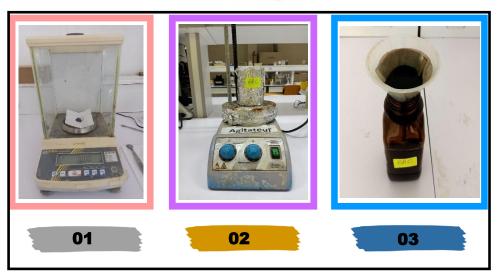


Figure 9: Preparation of Bradford reagent (photo taken as part of this study).

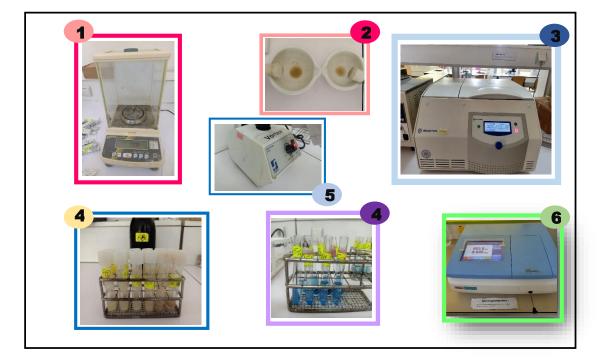


Figure 10: Steps of protein quantification (photo taken as part of this study).

01. Weigh 500 mg of plant material. **02.** Grind with distilled water using a mortar. **03.** Allow the homogenate to precipitate for 30 minutes. **04.** Prepare the reaction mixture (100 μ L homogenate + 4 mL Bradford reagent) in tubes protected from light. **05.** Shake the reaction mixture using a vortex. **06.** The reading is taken at a wavelength of 595 nm using a spectrophotometer.



• Total Sugar Assay

Total soluble sugars are quantified according to the colorimetric method of Rosales et al. (2023) based on the anthrone reaction in a sulfuric acid medium. The anthrone reagent is prepared by dissolving 150 mg of anthrone in a mixture of 75 mL of concentrated sulfuric acid (96 %) and 25 mL of distilled water. The standard solution is a glucose solution at $50 \,\mu\text{g/mL}$.

For the extraction, 100 mg of fresh plant material are incubated in 3 mL of 80 % ethanol at room temperature for 48 hours, then heated in a water bath at 70 °C for 30 minutes. After filtration, 2 mL of the extract are taken for quantification.

The quantification is performed on $100 \,\mu\text{L}$ of extract, incubated with the anthrone reagent. Absorbance is measured at 585 nm using a JENWAY 63000 spectrophotometer. An illustration of the test is presented in (Fig. 15). The sugar concentration is determined from the calibration curve.

$$Y = 0.000487 X - 0.06814 R2 = 99.98 %$$

 $\mathbf{Y} = \text{Optical Density (OD)}.$

X = Total Sugar Concentration.

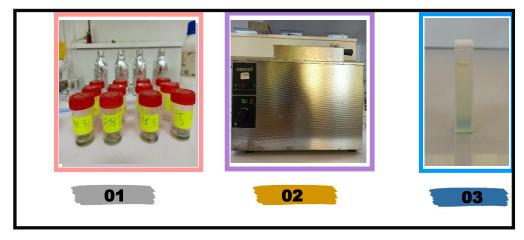


Figure 11: Steps of Total Sugar Quantification (photo taken as part of this study).

2.1.6. Enzymatic Parameters Assay

• Preparation of the Enzymatic Extract

The method used to obtain the plant enzymatic extract is that described by Holden (1975).

After 14 days of treatment, 500 mg of fresh plant material were ground in a mortar at cold temperature using a phosphate buffer (50 mM NaK, pH 7.2), at a ratio of 1 mL of



buffer per 1 g of fresh material (Fig. 16).

The obtained homogenate is then filtered through an appropriate cloth, followed by cold centrifugation at 12,000 g for 20 minutes using a centrifuge (Sigma 3-16K). The supernatant collected constitutes the enzymatic extract used for subsequent assays. (Fig. 17).

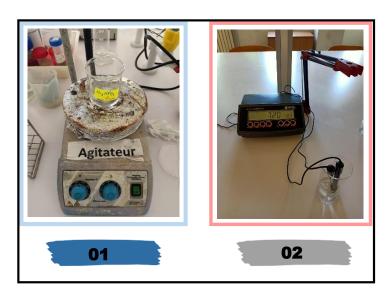


Figure 12: Preparation steps of phosphate buffer (50 mM NaK, pH = 7.2) (photo taken within the framework of this study).

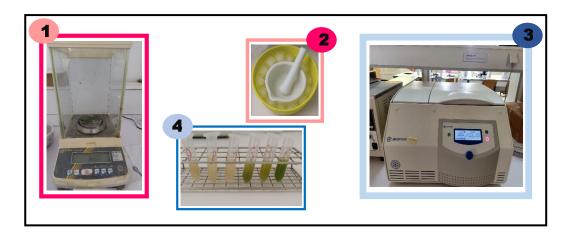


Figure 13: Preparation steps of the enzymatic extract (photo taken within the framework of this study).



• Determination of Catalase (CAT) Activity

Catalase (CAT) activity was determined by spectrophotometric method according to Cakmak et Horst, (1991).

The degradation of hydrogen peroxide (H_2O_2) was monitored by the decrease in absorbance at 240 nm over 3 minutes using a spectrophotometer (JENWAY 63000), employing a molar extinction coefficient $\epsilon = 39,400~\text{M}^{-1}\cdot\text{cm}^{-1}$. The final reaction mixture (3 mL) consisted of: 100 μ L of crude enzyme extract, 50 μ L of 0.3 % (v/v) H_2O_2 , and 2850 μ L of 50 mM phosphate buffer, pH 7.2.

$$Act = \frac{\Delta DO \times 10}{\epsilon \times L \times 0.05 \times mg \text{ of proteins}}$$

Calibration was performed without enzyme extract. The enzymatic reaction was initiated by the addition of H₂O₂. Catalase activity is expressed in nmol of H₂O₂ degraded per minute per mg of protein.

 Δ **D0**: Difference in optical density obtained after substrate hydrolysis

NB: All enzymatic activity measurement results are expressed relative to the amount of fresh material.

• Determination of Ascorbate Peroxidase (APX) Activity

APX activity is measured spectrophotometrically according to the protocol established by Nakano et Asada, (1981).

The oxidation of ascorbate is monitored at 290 nm for 1 minute using a spectrophotometer (JENWAY 63000), employing a molar extinction coefficient $\varepsilon = 2,800~\text{M}^{-1}\cdot\text{cm}^{-1}$. The reaction mixture (3 mL) consists of: 100 μ L of enzyme extract, 50 μ L of 0.3 % H₂O₂, and 2850 μ L of NaK phosphate buffer containing 0.5 mM ascorbate (pH 7.2).

The blank is prepared without enzyme extract. Enzyme activity is expressed in nmol of ascorbate oxidized per minute per mg of protein, according to Sbartai, (2008).

$$Act = \frac{\Delta A \times V_t}{\epsilon \times \Delta t \times L \times Ve \times P}$$



Act: Enzymatic activity expressed in nmol·min⁻¹·mg⁻¹ of protein.

 ΔA : Average absorbance difference.

Vt: Total volume of the reaction mixture in mL.

Ve: Volume of the enzymatic extract in mL.

P: Protein content in mg.

T : Reading time in minutes.

L : Cuvette path length in cm.

ε: Molar linear extinction coefficient.

3. Statistical Analysis

The statistical analysis conducted in our study was performed using Minitab Statistical Software 22 (version 22). For each assay, three replicates were analyzed (n = 3). The results correspond to the means of these measurements ± the standard error. To test the significance of differences between means for each dependent variable, univariate analysis of variance (ANOVA) was used, followed by the Tukey post-hoc test, which allowed us to determine significant differences between the means of homogeneous groups.





1. Influence of Biochar on the Synthesis of Morphophysiological, Biochemical, and Enzymatic Parameters in Barley Plants (*Hordeum vulgare L.* var. *Saïda* and *Fouara*)

1.1 Morphophysiological Parameters

• Effect of Biochar on the Germination Percentage

Figure 19 illustrates the effect of biochar (BC) on the germination percentage of barley seeds (*Hordeum vulgare L.* var. *Saïda*). A one-way ANOVA followed by Tukey's test revealed a highly significant increase in germination percentage in seeds treated with 1 % and 2.5 % BC compared to the control (p < 0.01). In contrast, the germination percentage in the 5 % BC treatment showed a non-significant decrease (p > 0.05) relative to the control.

Figure 20 illustrates the effect of biochar (BC) on the germination percentage of barley seeds (*Hordeum vulgare L.* var. *Fouara*). A one-way ANOVA followed by Tukey's test revealed a very highly significant increase in germination percentage in seeds treated with 2.5 % BC compared to the control ($p \le 0.001$). A significant increase was also observed in the 1 % BC group (p < 0.05), while the 5 % BC treatment resulted in a non-significant decrease relative to the control (p > 0.05).

• Effect of Biochar on the Germination Rate

Figure 21 illustrates the effect of biochar (BC) on the germination rate of barley seeds (*Hordeum vulgare L.* var. *Saïda*). A one-way ANOVA followed by Tukey's test revealed a very highly significant increase in germination rate in seeds treated with 2.5 % BC compared to the control ($p \le 0.001$). A significant increase was also observed in the 1 % BC group (p < 0.05), while the 5 % BC treatment resulted in a non-significant decrease relative to the control (p > 0.05).



Figure 22 illustrates the effect of biochar (BC) on the germination rate of barley seeds (*Hordeum vulgare L.* var. *Fouara*). A one-way ANOVA followed by Tukey's test revealed a highly significant increase in germination rate in seeds treated with 2.5 % BC compared to the control (p < 0.01). A non-significant increase was also observed in the 1 % BC group (p > 0.05), whereas the 5 % BC treatment resulted in a non-significant decrease relative to the control (p > 0.05).

• Effect of Biochar on Stem Length

Figure 23 illustrates the effect of biochar (BC) on the stem length of barley (*Hordeum vulgare L.* var. *Saïda*). A one-way ANOVA followed by Tukey's test revealed a very highly significant increase in stem length in the groups treated with 1 % and 2.5 % BC compared to the control (p < 0.001). Conversely, a very highly significant decrease in stem length was observed in the 5 % BC group compared to the control (p < 0.001).

Figure 24 illustrates the effect of biochar (BC) on the stem length of barley (*Hordeum vulgare L.* var. *Fouara*). A one-way ANOVA followed by Tukey's test revealed a very highly significant increase in stem length in the groups treated with 1 % and 2.5 % BC compared to the control (p < 0.001). Conversely, a very highly significant decrease in stem length was observed in the 5 % BC group compared to the control (p < 0.001).

• Effect of Biochar on Root Length

Figure 25 illustrates the effect of biochar (BC) on root length of barley (*Hordeum vulgare L.* var. *Saïda*). A one-way ANOVA followed by Tukey's test revealed a very highly significant increase in root length in the groups treated with 1 % and 2.5 % BC compared to the control (p < 0.001). Conversely, a very highly significant decrease in root length was observed in the 5 % BC group compared to the control (p < 0.001).

Figure 26 illustrates the effect of biochar (BC) on root length of barley (*Hordeum vulgare L.* var. *Fouara*). A one-way ANOVA followed by Tukey's test revealed a very highly significant increase in root length in the groups treated with 1 % and 2.5 % BC compared to the control (p < 0.001). Conversely, a very highly significant decrease in root length was observed in the 5 % BC group compared to the control (p < 0.001).



• Effect of Biochar on the Seedling Vigor Index (SVI)

Figure 27 illustrates the effect of biochar (BC) on the Seedling Vigor Index (SVI) of barley (*Hordeum vulgare L.* var. *Saïda*). A one-way ANOVA followed by Tukey's test revealed a highly significant increase in SVI in seedlings treated with 1 % BC compared to the control (p < 0.01), and a very highly significant increase in the 2.5 % BC group compared to the control (p < 0.001). In contrast, a non-significant decrease was observed in the 5 % BC group (p > 0.05).

Figure 28 illustrates the effect of biochar (BC) on the Seedling Vigor Index (SVI) of barley (*Hordeum vulgare L.* var. *Fouara*). A one-way ANOVA followed by Tukey's test revealed a non-significant increase in SVI in seedlings treated with 1 % BC compared to the control (p > 0.05), and a significant increase in the 2.5 % BC group compared to the control (p < 0.05). In contrast, a non-significant decrease was observed in the 5 % BC group (p > 0.05).

• Effect of biochar on the tolerance index

Figure 29 illustrates the effect of biochar (BC) on the tolerance index of barley (*Hordeum vulgare L.* var. *Saïda*). A one-way ANOVA followed by Tukey's test revealed a very highly significant increase (p < 0.001) in the tolerance index of seedlings treated with 1 %, 2.5 %, and 5 % BC compared to the control.

Figure 30 illustrates the effect of biochar (BC) on the tolerance index of barley (*Hordeum vulgare L.* var. *Fouara*). A one-way ANOVA followed by Tukey's test revealed a very highly significant increase (p < 0.001) in the tolerance index of seedlings treated with 1 %, 2.5 %, and 5 % BC compared to the control.

• Effect of Biochar on Stem Phytotoxicity

Figure 31 illustrates the effect of biochar (BC) on stem phytotoxicity in barley (*Hordeum vulgare L.* var. *Saïda*). A one-way ANOVA followed by Tukey's test revealed a non-significant increase in stem phytotoxicity in seedlings treated with 1 % BC compared to the control (p > 0.05). A significant increase was observed in the 2.5 % BC group (p < 0.05), while a very highly significant increase was recorded in the 5 % BC group compared to the control ($p \le 0.001$).



Figure 32 illustrates the effect of biochar (BC) on stem phytotoxicity in barley (*Hordeum vulgare L.* var. *Fouara*). A one-way ANOVA followed by Tukey's test revealed a non-significant increase in stem phytotoxicity in seedlings treated with 1 % BC compared to the control (p > 0.05). A non-significant increase was observed in the 2.5 % BC group (p > 0.05), while a very highly significant increase was recorded in the 5 % BC group compared to the control ($p \le 0.001$).

• Effect of Biochar on Root Phytotoxicity

Figure 33 illustrates the effect of biochar (BC) on root phytotoxicity in barley (*Hordeum vulgare L.* var. *Saïda*). A one-way ANOVA followed by Tukey's test revealed a non-significant increase in root phytotoxicity in seedlings treated with 1 % BC compared to the control (p > 0.05). A highly significant increase was observed in the 2.5 % BC group ($p \le 0.01$), while a very highly significant increase was recorded in the 5 % BC group compared to the control ($p \le 0.001$).

Figure 34 illustrates the effect of biochar (BC) on root phytotoxicity in barley (*Hordeum vulgare L.* var. *Fouara*). A one-way ANOVA followed by Tukey's test revealed a non-significant increase in root phytotoxicity in seedlings treated with 1 % BC compared to the control (p > 0.05). A significant increase was observed in the 2.5 % BC group (p < 0.05), while a very highly significant increase was recorded in the 5 % BC group compared to the control ($p \le 0.001$).

1.2 Biochemical Parameters

- Effect of Biochar on the Synthesis of Chlorophyll Pigments
- Chlorophyll Synthesis in the Leaves of Barley (*Hordeum vulgare L.* var. *Saïda*)

Figure 35(A) illustrates the effect of biochar (BC) on chlorophyll \boldsymbol{a} content in the leaves of barley (*Hordeum vulgare L.* var. *Saïda*). A one-way ANOVA followed by Tukey's post hoc test revealed a statistically non-significant increase in chlorophyll \boldsymbol{a} content in plants treated with 1% BC (p > 0.05), compared to the control. A non-significant decrease (p > 0.05) was observed in the 5 % BC treatment. In contrast, a very highly significant increase (p < 0.001) was recorded in plants treated with 2.5 % BC.

Figure 35(**B**) illustrates the effect of biochar (BC) on chlorophyll \boldsymbol{b} content in the leaves of barley (*Hordeum vulgare L.* var. *Saïda*). A one-way ANOVA followed by Tukey's post hoc test revealed a statistically non-significant increase in chlorophyll \boldsymbol{b} content in plants treated with 1 % BC (p > 0.05), compared to the control. A non-significant decrease (p > 0.05) was observed



in the 5 % BC treatment. In contrast, a very highly significant increase ($p \le 0.001$) was recorded in plants treated with 2.5 % BC.

Figure 35(C) illustrates the effect of biochar (BC) on total chlorophyll content (a+b) in the leaves of barley (*Hordeum vulgare L.* var. *Saïda*). According to the results of a one-way ANOVA followed by Tukey's post hoc test, a very highly significant increase (p < 0.001) in total chlorophyll content was observed in plants treated with 1 % and 2.5 % BC compared to the control. Conversely, a significant decrease (p < 0.05) was recorded in the 5 % BC group relative to the control.

- Chlorophyll Synthesis in the Leaves of Barley (*Hordeum vulgare L.* var. *Fouara*)

Figure 36(A) presents the effect of biochar (BC) on chlorophyll \boldsymbol{a} content in the leaves of barley (*Hordeum vulgare L.* var. *Fouara*). A one-way ANOVA followed by Tukey's post hoc test revealed a statistically non-significant increase (p > 0.05) in chlorophyll \boldsymbol{a} content in plants treated with 1 % BC compared to the control. A non-significant decrease (p > 0.05) was observed in the 5 % BC treatment. In contrast, a very highly significant increase (p < 0.001) was recorded in plants treated with 2.5 % BC.

Figure 36(**B**) illustrates the effect of biochar (BC) on chlorophyll **b** content in the leaves of barley (*Hordeum vulgare L.* var. *Fouara*). A one-way ANOVA followed by Tukey's post hoc test revealed a statistically non-significant increase (p > 0.05) in chlorophyll **b** content in plants treated with 1 % BC compared to the control. A non-significant decrease (p > 0.05) was observed in the 5 % BC treatment. In contrast, a highly significant increase (p < 0.001) was recorded in plants treated with 2.5 % BC.

Figure 36(C) illustrates the effect of biochar (BC) on total chlorophyll (a+b) content in the leaves of barley (*Hordeum vulgare L.* var. *Fouara*). According to the results of a one-way ANOVA followed by Tukey's post hoc test, a significant increase (p < 0.05) in total chlorophyll content was observed in plants treated with 1 % BC compared to the control. A very highly significant increase (p < 0.001) was recorded in plants treated with 2.5 % BC. Conversely, a very highly significant decrease (p < 0.001) was observed in the 5 % BC group relative to the control.



- Effect of Biochar on the Synthesis of Total proteins
- Total Protein Synthesis in the Leaves, Stems and Roots of Barley (*Hordeum vulgare L. var. Saïda*)

Figure 37 presents the effect of biochar (BC) on total protein content in the leaves, stems, and roots of barley (*Hordeum vulgare L.* var. *Saïda*). Statistical analyses were performed using one-way ANOVA followed by Tukey's post hoc test.

In the leaves, a significant decrease in total protein concentration was observed in plants treated with 5 % biochar (BC) compared to the control (p < 0.05). In contrast, protein levels in plants treated with 1 % and 2.5 % BC showed non-significant increases compared to the control (p > 0.05).

In the stems, biochar (BC) treatments at 1 % and 2.5 % led to a statistically very highly significant increase in total protein content compared to the control ($p \le 0.001$). In contrast, a highly significant decrease (p < 0.01) was observed in the 5 % BC treatment. Among all treatments.

In the roots, a non-significant decrease in total protein concentration was observed in plants treated with 5 % biochar (BC), while a non-significant increase was noted at 1 %, and a significant increase was recorded at 2.5 %, compared to the control.

- Total Protein Synthesis in the Leaves, Stems and Roots of Barley (*Hordeum vulgare L.* var. *Fouara*)

Figure 38 presents the effect of biochar (BC) on total protein content in the leaves, stems, and roots of barley (*Hordeum vulgare L.* var. *Fouara*). The data were analyzed using one-way ANOVA followed by Tukey's post hoc test.

At the foliar level, a significant increase in total protein concentration was observed in plants treated with 2.5 % BC (p < 0.05), while the 1 % BC treatment resulted in a non-significant increase (p > 0.05) and the 5 % BC treatment led to a non-significant decrease (p > 0.05), compared to the control.

At the stem level, total protein concentrations increased very highly significantly with the 1 % and 2.5 % BC treatments (p < 0.001), whereas a very highly significant decrease was observed at 5 % BC (p \leq 0.001).



At the root level, a significant increase in protein content was observed in the 2.5 % BC treatment compared to the control (p < 0.05). A non-significant increase was also recorded at 1 % BC (p > 0.05), while the 5 % BC treatment resulted in a non-significant decrease (p > 0.05).

- Effect of Biochar on the Synthesis of Total Soluble Sugars
- Total Soluble Sugars Synthesis in the Leaves, Stems and Roots of Barley (*Hordeum vulgare L.* var. *Saïda*)

Figure 39 illustrates the effect of biochar (BC) on the concentration of total soluble sugars in the leaves, stems, and roots of barley (*Hordeum vulgare L.* var. *Saïda*). Statistical differences were assessed using one-way ANOVA followed by Tukey's post hoc test.

In the leaves, the 2.5 % biochar (BC) treatment led to a statistically very highly significant increase in total soluble sugar concentration compared to the control (p < 0.001, Tukey's test). A non-significant increase was observed at 1 % BC (p > 0.05), while a non-significant decrease was recorded at 5 % BC (p > 0.05).

In the stems, the 2.5 % biochar (BC) treatment resulted in a statistically significant increase in total soluble sugar concentration compared to the control (p < 0.05, Tukey's test). A non-significant increase was observed at 1 % BC (p > 0.05), while a non-significant decrease was recorded at 5 % BC (p > 0.05).

In the roots, the 2.5 % and 1 % biochar (BC) treatments resulted in a very highly significant increase in total soluble sugar concentration compared to the control (p < 0.001, Tukey's test). In contrast, the 5 % BC treatment led to a non-significant decrease (p > 0.05).

- Total Soluble Sugars Synthesis in the Leaves, Stems and Roots of Barley (*Hordeum vulgare L.* var. *Fouara*)

Figure 40 illustrates the effect of biochar (BC) on the concentration of total soluble sugars in the leaves, stems, and roots of barley (*Hordeum vulgare L.* var. *Fouara*). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

In the leaves, the application of 2.5 % biochar (BC) led to a very highly significant increase in total soluble sugar concentration compared to the control (p < 0.001, Tukey's test). A highly significant increase was also observed at 1 % BC (p < 0.01), while a non-significant decrease was recorded at 5 % BC (p > 0.05).

In the stems, a very highly significant increase in total soluble sugar concentration was observed in the 1 % and 2.5 % biochar (BC) treatments compared to the control ($p \le 0.001$,



Tukey's test). Conversely, a very highly significant decrease was recorded at 5 % BC (p < 0.001).

In the roots, the 2.5 % biochar (BC) treatment resulted in a highly significant increase in total soluble sugar concentration compared to the control (p < 0.01, Tukey's test). A non-significant increase was observed at 1 % BC (p > 0.05), while a non-significant decrease was recorded at 5 % BC (p > 0.05).

1.3. Enzymatic Parameters

- Effect of Biochar on Catalase (CAT) Activity
- Catalase (CAT) Activity in the Leaves, Stems and Roots of Barley (Hordeum vulgare L. var. Saïda)

Figure 41 presents the effect of biochar (BC) on catalase activity in the leaves, stems, and roots of barley (*Hordeum vulgare L.* var. *Saïda*). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

In the leaves, catalase activity showed a significant increase in plants treated with 2.5 % biochar (BC) compared to the control (p < 0.05, Tukey's test). In contrast, a non-significant increase was observed in the 1 % BC treatment (p > 0.05), while the 5 % BC treatment resulted in a non-significant decrease (p > 0.05).

In the stems, a very highly significant increase in catalase activity was observed in both the 2.5 % and 1 % biochar (BC) treatments compared to the control (p < 0.001, Tukey's test). In contrast, the 5 % BC treatment resulted in a very highly significant decrease (p < 0.001).

In the roots, a very highly significant increase in catalase activity was observed in both the 2.5 % and 1 % biochar (BC) treatments compared to the control (p < 0.001, Tukey's test). In contrast, the 5 % BC treatment resulted in a very highly significant decrease (p < 0.001).

- Catalase (CAT) Activity in the Leaves, Stems and Roots of Barley (Hordeum vulgare L. var. Fouara)

Figure 42 illustrates the effect of biochar (BC) on catalase activity in the leaves, stems, and roots of barley (*Hordeum vulgare L.* var. *Fouara*). Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test.

At the foliar level, a highly significant increase in catalase activity was observed in plants treated with 2.5 % biochar (BC) compared to the control ($p \le 0.01$, Tukey's test). The 1 % BC



treatment resulted in a non-significant increase (p > 0.05), while the 5 % BC treatment led to a non-significant decrease (p > 0.05).

At the stem level, a very highly significant increase in catalase activity was observed in both the 2.5 % and 1 % biochar (BC) treatments compared to the control (p < 0.001, Tukey's test). In contrast, the 5 % BC treatment resulted in a very highly significant decrease (p < 0.001).

At the root level, a very highly significant increase in catalase activity was observed in both the 2.5 % and 1 % biochar (BC) treatments compared to the control (p < 0.001, Tukey's test). In contrast, the 5 % BC treatment resulted in a very highly significant decrease (p < 0.001).

- Effect of Biochar on Ascorbate Peroxidase (APX) Activity
- Ascorbate Peroxidase (APX) Activity in the Leaves, Stems and Roots of Barley (Hordeum vulgare L. var. Saïda)

Figure 43 presents the effect of biochar (BC) on ascorbate peroxidase (APX) activity in the leaves, stems, and roots of barley (*Hordeum vulgare L.* var. *Saïda*). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

In the leaves, APX activity increased very highly significantly in the 1 % and 2.5 % biochar (BC) treatments compared to the control (p < 0.001, Tukey's test). In contrast, a significant decrease was observed in the 5 % BC treatment compared to the control (p < 0.05).

In the stems, a very highly significant increase in APX activity was observed at 2.5 % biochar (BC) (p < 0.001) compared to the control. A significant increase was also noted at 1 % BC (p < 0.05), while a very highly significant decrease was recorded at 5 % BC (p < 0.001), according to Tukey's test.

In the roots, a very highly significant increase in APX activity was observed at 2.5 % biochar (BC) (p < 0.001) treatments compared to the control. A significant increase was also noted at 1 % BC (p < 0.05), while a very highly significant decrease was recorded at 5 % BC (p < 0.001), according to Tukey's test.

- Ascorbate Peroxidase (APX) Activity in the Leaves, Stems and Roots of Barley (Hordeum vulgare L. var. Fouara)

Figure 44 shows the effect of biochar (BC) on ascorbate peroxidase (APX) activity in the leaves, stems, and roots of barley (*Hordeum vulgare L.* var. *Fouara*). Statistical significance was assessed using one-way ANOVA followed by Tukey's post hoc test.



At the foliar level, APX activity increased very highly significantly in the 1 % and 2.5 % biochar (BC) treatments compared to the control (p < 0.001). In contrast, a very highly significant decrease was observed in the 5 % BC treatment (p < 0.001).

At the stem level, APX activity increased very highly significantly in the 1 % and 2.5 % biochar (BC) treatments compared to the control (p < 0.001). In contrast, a very highly significant decrease was observed in the 5 % BC treatment (p < 0.001).

At the root level, APX activity increased very highly significantly at 2.5 % and 1 % biochar (BC) (p < 0.001) treatments compared to the control, while a non-significant decrease was observed at 5 % BC (p > 0.05).







Conclusion

Soil degradation in arid and semi-arid regions, including the Oued Zenati area in Algeria, significantly jeopardises agricultural sustainability. This phenomenon primarily results from insufficient organic matter, inadequate water retention capacity, and modified soil structure, which impairs fertility and restricts crop productivity. In light of these challenges, the application of biochar, an organic amendment produced through the pyrolysis of biomass, has attracted growing interest for its potential impact on soil restoration and enhancement of agricultural yields. The precise effects of biochar on local crops, especially barley, are inadequately recorded in the Algerian agro-ecological framework.

This study sought to determine how biochar made from peanut shells affected the physiological performance and growth of two local barley varieties, *Saïda* and *Fouara*. The effects of applying varying concentrations of biochar on physiological parameters like germination, root and shoot growth, and biochemical processes involving protein and sugar synthesis, as well as the activity of antioxidant enzymes (CAT and APX), were the main focus of this investigation. In order to provide solutions for improving agricultural productivity and soil management in degraded areas, it was intended to further our understanding of the physiological mechanisms of plants under the influence of this organic amendment.

The findings demonstrated that the growth and physiological performance of the two barley varieties under study were significantly impacted by the application of biochar made from peanut shells. In fact, this concentration activated antioxidant enzymes like catalase and ascorbate peroxidase, which are indicators of resistance to oxidative stress, and enhanced germination, vegetative growth, and the biosynthesis of proteins and soluble sugars. This implies that biochar may be extremely important for managing soil and raising agricultural output, especially in areas where soil degradation is a problem.

Higher biochar concentrations demonstrated growth-inhibiting effects, particularly at the root level, indicating toxicity linked to overly high dosages. These findings emphasise how crucial it is to apply biochar at precise dosages in order to optimise its advantages and reduce any negative effects on crops.

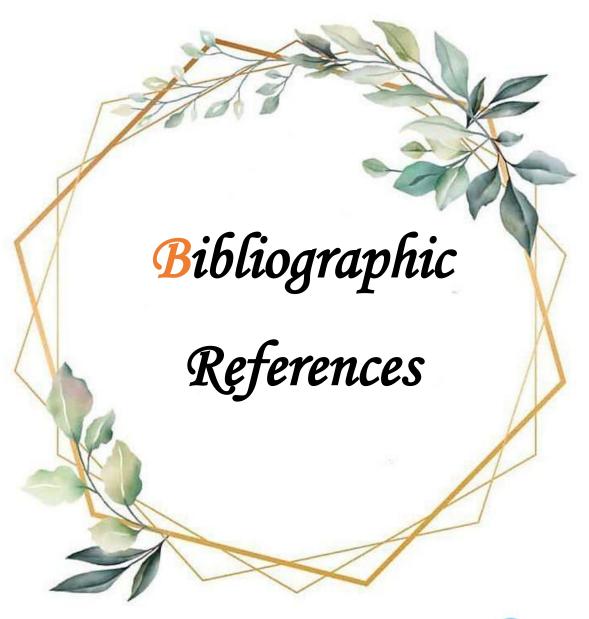






Perspectives

- 1. To identify the most efficient and locally appropriate sources, it would be pertinent to compare the effects of biochar made from various raw materials, such as date pits or wheat residues.
- 2. More research on how biochar affects the rhizosphere and soil microbial communities may shed light on how it promotes microbial biodiversity, which is critical to soil productivity and health.
- 3. Research is essential to evaluate soil quality following biochar treatment, especially with regard to soil structure, porosity, water retention, and cation exchange capacity (CEC).
- 4. Other oxidative stress biomarkers like malondialdehyde (MDA), glutathione S-transferase (GST), and glutathione (GSH) could be used to get a more thorough assessment of biochar's effects on plant health. These biomarkers would show signs of oxidative stress-induced cellular damage and the plants' capacity to withstand these effects.
- 5. To gain a better understanding of how biochar affects plant cell structure, a thorough histological examination of plant tissues (roots, shoots, and leaves) is necessary.
- 6. It would be relevant to examine how biochar affects plants' antioxidant quality, specifically by assessing the concentrations of bioactive compounds and overall antioxidant capacities. Furthermore, to evaluate the nutritional enhancement of treated crops, the effect of biochar on the concentration of vital minerals like calcium, magnesium, potassium, and iron should be investigated.









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