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Effect of biochar amendment on physiological and biochemical properties of common bean (*Phaseolus vulgaris* L.)

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Abstract

Soil degradation, increasing pollution, and water stress are major challenges facing agriculture today, threatening soil fertility and crop productivity. In response to these issues, the adoption of sustainable and environmentally friendly solutions has become essential. Among these, the use of biochar a natural soil amendment produced through the pyrolysis of organic residues has attracted growing interest for its potential to improve soil quality and support plant growth. The objective of this study was to evaluate the effects of soil amendment with biochar on the physiological and biochemical properties of common bean *(Phaseolus vulgaris* L.*)*. Two types of biochar were tested: one derived from eggshells and the other from date seeds, both applied at different concentrations (1%, 3%, and 5%) to clay soil. The results showed that the addition of biochar significantly enhanced plant growth, with increases in root biomass, leaf area, and stem length. Biochemically, there was an increase in chlorophyll content, total soluble sugars, total proteins, polyphenols, and flavonoids. In parallel, oxidative stress markers such as proline and malondialdehyde (MDA) decreased. Antioxidant activity, assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, revealed a notable improvement in radical scavenging capacity, particularly in plants treated with 1% eggshell biochar. Date seed biochar showed marked benefits at 3% and 5% concentrations, whereas eggshell biochar had a stronger impact on photosynthesis and leaf quality. These results confirm that biochar is a promising natural amendment capable of improving soil fertility, enhancing plant stress tolerance, and contributing to sustainable agriculture through the recycling of organic waste.

Keywords: Biochar; *Phaseolus Vulgaris* L.; soil amendment; eggshells; date seeds; organic waste.

Résumé

La dégradation des sols, la pollution croissante et le stress hydrique représentent aujourd'hui des défis majeurs pour l'agriculture, compromettant la fertilité des sols et la productivité des cultures. Face à ces enjeux, le recours à des solutions durables et respectueuses de l'environnement s'impose. Parmi elles, l'utilisation du biochar, un amendement naturel issu de la pyrolyse de résidus organiques, suscite un intérêt croissant pour améliorer la qualité des sols et soutenir la croissance des plantes. L'objectif de cette étude est d'évaluer les effets de l'amendment du sol par le biochar sur les propriétés physiologiques, biochimiques de l'haricot commun *(Phaseolus vulgaris* L.*)*. Deux types de biochar ont été testés : l'un dérivé de coquilles d'œufs et l'autre de graines de dattes, appliqués à différentes concentrations (1 %, 3 % et 5 %) sur un sol argileux. Les résultats ont montré que l'ajout de biochar a significativement amélioré la croissance des plantes, avec une augmentation de la biomasse racinaire, de la surface foliaire et de la longueur des tiges. Sur le plan biochimique, on a observé une augmentation des teneurs en chlorophylle, en sucres solubles totaux, en protéines totales, en polyphénols et en flavonoïdes. Parallèlement, les marqueurs de stress oxydatif tels que la proline et le Malondialdehyde (MDA) ont diminué. L'activité antioxydante, évaluée par le test DPPH (2,2-diphényl-1-picrylhydrazyle), a révélé une amélioration notable de la capacité de piégeage des radicaux libres, en particulier chez les plantes traitées avec 1 % de biochar de coquille d'œuf. Le biochar de graines de dattes a montré des bénéfices marqués à 3 % et 5 %, tandis que celui de coquilles d'œufs a eu un impact plus fort sur la photosynthèse et la qualité des feuilles. Ces résultats confirment que le biochar constitue un amendement prometteur, capable d'améliorer la fertilité des sols, de renforcer la tolérance des plantes au stress et de favoriser une agriculture durable grâce au recyclage des déchets organiques.

Mots-clés : Biochar ; *Phaseolus Vulgaris* L. ; amendement du sol ; coquilles d'oeufs ; noyaux de dattes ; déchets organiques.

ملخص

يعَدّ تدهور التربة، وتزايد معدلات التلوث، والضغط المائي من بين أبرز التحديات التي تواجه القطاع الزراعي حالياً، مما يهدد خصوبة التربة وإنتاجية المحاصيل. وأمام هذه التحديات، تبرز الحاجة إلى اعتماد حلول مستدامة وصديقة للبيئة. ومن بين هذه الحلول، يحظى استخدام الفحم الحيوي، وهو مُحسِّن طبيعي للتربة ناتج عن التحلل الحراري للمخلفات العضوية، باهتمام متزايد لدوره في تحسين جودة التربة ودعم نمو النباتات.

تهدف هذه الدراسة إلى تقييم تأثير تعديل التربة بالفحم الحيوي على الخصائص الفسيولوجية والبيوكيميائية لنبات الفاصولياء الشائعة *(Phaseolus vulgaris* L.*)* وقد تم اختبار نوعين من الفحم الحيوي: أحدهما مشتق من قشور البيض والآخر من نوى التمر، وذلك بتراكيز مختلفة (1%، 3%، 5%) على تربة طينية.

أظهرت النتائج أن إضافة الفحم الحيوي أدت إلى تحسين ملحوظ في نمو النباتات، من خلال زيادة الكتلة الحيوية الجذرية، والمساحة الورقية، وطول الساق. وعلى المستوى البيوكيميائي، لوحظ ارتفاع في محتوى الكلوروفيل، والسكريات الذائبة الكلية، والبروتينات الكلية، البوليفينولات، الفلافونويدات، مع انخفاض في مؤشرات الإجهاد التأكسدي مثل البرولين والمالونديالديهيد (MDA). كما أظهرت القدرة المضادة للأكسدة، التي تم تقييمها باستخدام اختبار DPPH (2,2-ثنائي فينيل-1-بيكريل هيدرازيل)، تحسناً ملحوظاً في قدرة التخلص من الجذور الحرة، خاصة لدى النباتات المعالجة بـ 1% من فحم قشور البيض الحيوي. وأظهر فحم نوى التمر الحيوي فوائد واضحة عند التراكيز 3% و5%، بينما كان لفحم قشور البيض الحيوي تأثير أكبر في تعزيز عملية التمثيل الضوئي وجودة الأوراق.

تؤكد هذه النتائج أن الفحم الحيوي يُعَدّ مخصباً طبيعياً واعداً لتحسين خصوبة التربة، وتعزيز قدرة النبات على تحمل الإجهاد، ودعم الزراعة المستدامة من خلال تدوير المخلفات العضوية.

الكلمات المفتاحية

الفحم الحيوي؛ الفاصولياء الشائعة *(Phaseolus vulgaris* L.*)* تعديل التربة؛ قشور البيض؛ نوى التمر؛ النفايات العضوية

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List of abbreviations

Abbreviation	Meaning
APX	Ascorbate Peroxidase
ARA (%)	Antiradical Activity
B1	Biochar from eggshells
B2	Biochar from date seeds
BSA	Bovine Serum Albumin
Ca	Calcium
$CaCO_3$	Calcium Carbonate
CAT	Catalase
CBB	Coomassie Brilliant Blue
C	Carbon
CO	Organic Carbon
CO_3^{2-}	Carbonate Ion
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DM / dw	Dry Matter / Dry Weight
EC	Electrical Conductivity
FM / fr.wt.	Fresh Matter / Fresh Weight
G	Gram
H	Hydrogen
ID/IG	D-to-G Band Intensity Ratio (Raman Spectroscopy)
K	Potassium
MDA	Malondialdehyde
Mg	Magnesium
MO	Organic Matter
N	Nitrogen

Na	Sodium
O	Oxygen
–OH	Hydroxyl Group
OH^-	Hydroxide Ion
P	Phosphorus
PAHs	Polycyclic Aromatic Hydrocarbons
pH	Potential of Hydrogen
PO_4^{3-}	Phosphate Ion
RWC	Relative Water Content
S	Sulfur
TBA	Thiobarbituric Acid
TCA	Trichloroacetic Acid
TSS	Total Soluble Sugars
UV-Vis	Ultraviolet-Visible Spectroscopy

General Introduction

I. Introduction

The global agricultural sector faces significant challenges due to the world's growing population. The farming system must rely more on technology and chemical inputs to feed the growing population and satisfy the continuously increasing demand for food. In an effort to boost crop yields, more fertilizers and pesticides have been applied to agricultural land over time. Long-term use of pesticides and fertilizers may result in leaching losses, which lower soil fertility and pollute the ecosystem. Nutrient loss from agricultural soils can raise soil acidity and lower crop yields in addition to decreasing soil fertility and raising farming expenses. Farmers and the scientific community have turned their attention to natural leftovers and organic materials as alternatives to commercially produced goods. (**Adak *et al.*, 2024**)

One promising outcome of scientific research is biochar. Recognized for its well-established benefits, biochar is used as a soil amendment to enhance fertility and immobilize or transform heavy metals and other pollutants in agricultural soils. Often referred to as “charcoal” or “biomass-derives black charbon”, biochar also has the unique ability to sequester carbon over extended periods.

The pre-Columbian indigenous people of the Amazon region are thought to have used it for the first time between 500- and 9000-years, biochar as part of a series of soil amendments that created "terra preta," an agricultural soil with a higher pH and more nutrients leading to a more stable arrangement of soil particle than the region's current acidic and infertile soils (**Singh *et al.*, 2022**). It is simple to detect because of its dark color, high nutritional content associated with an increase in microbial population, and high aggregate stability brought on by the presence of additional carbon. The evaluated Terra Preta's soil sub-layers contained flakes of various mica species. The Terra Preta has a 25% higher variety of bacterial species than other soils, and there are many different kinds of acidobacteria.

According to its original definition, "biochar" is "charred organic matter that is applied to soil in a deliberate manner, with the intent to improve soil properties." The concept of biochar has recently been expanded to include beneficial material use (such as in construction or composite materials) that provides equal C-sinks to soil use (**Schmidt *et al.*, 2021**).

Biochar, as a well-known soil amendment, is a carbon-enriched material produced by pyrolysis of biomass in the absence of oxygen. Due to the specific and tunable properties of biochar, e.g., high porosity and surface area, high pH value, good stability, and high cation exchange capacity (CEC), numerous studies have recognized the great benefits of biochar for soil amendment

(**Hou *et al.,* 2020; Palansooriya *et al.,* 2020**), such as improving soil fertility, soil structure, microbial community, and carbon storage capacity (**Zhang *et al.,* 2020; Zheng *et al.,* 2019**) as well as immobilizing potentially toxic metals/metalloids (TMs) in soil (**Wang *et al.,* 2020; Xiong *et al.,* 2019; Zhao *et al.,* 2020; Zhong *et al.,* 2020**). Waste leftovers from forestry, agriculture, and animal manures are typically used to make biochar. These feedstocks are crucial because they can convert garbage into biochar, a valuable and practical product.

According to (**Hayat *et al.,* 2024**), the well-known elements found in biochar include carbon (C), oxygen (O), hydrogen (H), sulfur (S), silicon (Si), calcium (Ca), sodium (Na), nitrogen (N), phosphorus (P), potassium (K), and magnesium (Mg). Among these, C is the most abundant (about 60%), followed by Hydrogen and Oxygen. According to several researches, the content is 40%–75% carbon, 7%–20% oxygen key elements, and 7%–15% minor elements.

The literature review showed that Mg, Ca, K, and P can directly create mineral nutrients, increasing plant development characteristics, and anions such as CO_3, OH, PO_4, and SO_4 released from biochar. According to earlier studies, heavy metal removal can be significantly aided by water-based media that contain carbon and mineral biochar components (**Inyang *et al.,* 2016**). Although it also occurs in lower mineral fractions such as crop residue biochar, heavy metal sorption primarily occurs via surface complexes in media with O-rich feature groups (Alcoholic-OH, -COOH). Conversely, biochar derived from manure and sludge with elevated mineral fractions may significantly influence the sorption process through interactions between mineral fractions and heavy metals, as minerals can eliminate around 90% of heavy metals by forming precipitates. (**Shakoor *et al.,* 2019; Hayat *et al.,* 2024**).

A popular technique for producing biochar is pyrolysis, which is typically done at 300–1000°C (**Diatta *et al.,* 2020**). According to (**Li *et* You, 2022**), pyrolysis is a thermochemical process that produces value-added products like biochar (such as crop residues and municipal solid waste) in an inert atmosphere. Fast pyrolysis, flash pyrolysis, slow pyrolysis, vacuum pyrolysis, hydro-pyrolysis, and microwave pyrolysis (MWP) are the six main categories of pyrolysis technologies, which differ by their heating rate, pyrolysis temperature, residence time, reaction environments, and heating method. Microwave pyrolysis and slow pyrolysis are two of these methods that are thought to be promising and favor the production of biochar (**Li *et al.,* 2022**)

The physicochemical characteristics of biochar, such as its surface area, pH, and functional groups, as well as its abilities as a soil amendment, were significantly impacted by the pyrolysis temperature. Pyrolysis temperature affects characteristics due to the presence of volatiles at

higher temperatures. A higher pyrolysis temperature led to a drop in CEC and the concentration of surface functional groups and an increase in surface area, carbonized fractions, pH, and volatile matter (**Tomczyk *et al.,* 2020**)

However, biochar made at lower temperatures (between 250 and 400 degrees Celsius) has better yield recoveries and contains additional C=O and C-H functional groups that, following oxidation, can act as sites for nutrient exchange. Furthermore, the organic composition of biochar generated at lower pyrolysis temperatures is more varied and includes cellulose-type and aliphatic structures. These could make suitable mineralization substrates for fungi and bacteria, which are essential for aggregate formation and nutrient turnover activities

Biochar's properties are crucial for evaluating its effectiveness in removing heavy metals and determining its applications. Surface area, pore structure, pH, functional groups, elemental composition, and stability are some of the crucial properties of biochar that are covered below (**Akhil *et al.,* 2021; Yan *et al.,* 2024**)

In most cases, biochar with a large surface area and high porosity will have strong sorption capabilities. The pyrolysis process creates the porous surface of biochar when there is a rise in water loss as a result of dehydration. The International Union of Pure and Applied Chemistry states that biochar can have micro (less than 2 nm), meso (between 2 and 50 nm), and macro (more than 50 nm) holes. Regardless of the pesticide molecules' polarity or charges, biochar with smaller pores is unable to adsorb them. SEM (Scanning Electron Microscopy) can be used to characterize the pore size of biochar. While temperature has a significant impact on the synthesis of biochar, surface area is the primary factor in determining the sorption capacity of biochar. Raw materials that have been treated and those that have not may have different surface areas. The biochar made without the activation step is less porous and has a small surface area. Therefore, an activation technique is used to improve the porosity and surface area of biochar during its manufacture. The activation process may entail both chemical and physical activation. (**Yaashikaa *et al*., 2020**)

Biochar is known for its excellent stability. The stability of biochar depends on the feedstock, pyrolysis process, and pH conditions. Increasing pyrolysis temperature leads to more stable biochar.

Biochar generated at 600°C was shown to be more stable than that produced at 250°C, 350°C, and 450°C (**Yang *et al.,* 2018**). Pyrolysis with oxygen has little impact on biochar stability due

to negligible weight loss. The enhanced pyrolysis process ensures great biochar stability, making it suitable for various applications, including as an additive.

When assessing the pore characteristics of biochar, temperature is a crucial consideration. When the temperature was raised from 400 to 500 °C, the biochar's total pore volume increased from 0.5 to 0.6 cc/g, which led to pore melting and blockage (**Yakout 2017**). According to **Jia *et al.*** (**2018**), the biochar's coordinated nanoscale pores facilitate the simple entry of heavy metal ions. The pore structure of biochar is mostly determined by volatiles.

The biochar has a porous structure as volatiles are created. However, volatiles have a tendency to escape during thermochemical degradation, which causes pores and cracks to develop on the surface of biochar (**Zhang *et al.*, 2018**).

Various surface oxygen-containing functional groups define biochar. In the Raman spectra, **Chen *et al.* (2019)** found that the ratio of the D-Raman peak to the G-Raman peak (ID/IG) was 0.736 in wet sludge biochar and 0.805 in dry sludge biochar. The low ratio in wet sludge biochar was caused by an overabundance of moisture, which generally has a detrimental effect on biochar because it consumes the majority of the functional groups on the surface, lowering the Raman intensity. The pyrolysis conditions and feedstock type are the only factors that determine whether a specific functional group is present (**Zhang *et al.*, 2018**). Due to their ability to form stable complexes with heavy metals, C–H and O–H functional groups aid in increasing the adsorption of heavy metals. The quantity of functional groups in the biochar is further reduced by severe carbonization brought on by rising temperatures (**Li *et al.*, 2017**).

The quantity of surface functional groups in the biochar has a direct impact on the removal of heavy metals. Additionally, functional groups aid in learning about the characteristics of biochar. Phenolic and carboxylic groups allow for the measurement of biochar's surface charge and redox characteristics (**Wang and Liu 2017; Yuan *et al.*, 2017**).

The pH value of biochar can be used to determine whether they are basic or acidic. The pH range of biochar is typically 4.0 to 12.0. Because of an increase in volatilization, the temperature rise causes the chars' pH to move toward a highly basic pH. The biochar produced by pyrolysis at 600 °C had a basic pH of 9.0, demonstrating the alkaline nature of the biochar surface at elevated temperatures. If there is an electrostatic contact between the heavy metal and the biochar, the presence of a higher pH in the biochar increases the adsorption of heavy metals. this is due to the fact that higher pH levels generate more negative charges.

However, because the heavy metals dissociate into the solution, a high pH can also occasionally result in a decrease in the adsorption of heavy metals (**Yakout 2017; Jia *et al.,* 2018**).

Increasing pyrolysis temperature increases carbon content by releasing volatiles, but decreases hydrogen and oxygen content by breaking weak bonds in biochar. Rice straw biochar's hydrogen and oxygen content declined from 4.4 to 2.9% and 49 to 30.1%, respectively, when the pyrolysis temperature increased from 400 to 600 °C (**Yakout 2017; Jia *et al.,* 2018**). The polymerization, aromatization, and carbonization of biochar results in a high carbon content. Biochar elemental composition changes as they lose bulk. Biochar mass loss occurs at temperatures below 100 °C due to moisture content, whereas temperatures above 500 °C cause oxidation of carbon molecules and mass loss.

According to **Hammer *et al.* (2014)**, the characteristics of soil are altered by biochar. It can raise the soil's load of plant fungi and arbuscular mycorrhizal organisms. Crop yield rises as a result (**Solaiman *et al.,* 2010**). Biochar's biological characteristics allow it to function as a habitat, about microbes (**Jaafar, 2014**), earthworms can readily adapt to soil modified with biochar. The fact that earthworms favor soils treated with biochar over those that are not was further supported by **Van-Zwieten *et al.,* (2010**). Adding biochar to the soil has the potential to increase the amount of methane that plants absorb from the soil. The mechanism underlying the claim is unclear, though. Nitrogen fixation is one of the biochar's other significant biological effects on the soil. Upon being integrated into the soil, it could increase the amount of nitrogen that plants fix (**Rondon *et al.,* 2007**).

Various parameters, including pyrolysis temperature and feedstock type, can significantly impact the properties of biochar, including pH, specific surface area (SSA), porosity, chemical composition, and electrical conductivity (**Kamali *et al.,* 2021**). The properties of biochar are mostly influenced by variables like feedstocks, temperature, particle size, heating rate, etc. Instead of affecting the quality of the biochar, these factors directly affect its yield. Determining the use of biochar requires a thorough understanding of property analysis. Biochar has been produced using a variety of biomass from various sources, including solid waste, wood, plant materials, agricultural residues, etc. (**Yaashika *et al.,* 2020**)

A complex biological material that can be either organic or non-organic, biomass is derived from living or recently deceased organisms. Because they include a combination of organic and non-organic compounds and can be processed to produce energy, a variety of waste items, including animal manure, waste paper, sludge, and several industrial wastes, are also considered

biomass. Woody and non-woody biomass are the two types of biomasses. The majority of woody biomass is made up of tree and forestry wastes. Low moisture content, low ash content, high calorific value, high bulk density, and reduced porosity are the attributes of woody biomass. Animal dung, urban and industrial solid waste, and agricultural products and leftovers make up non-woody biomass. It is thought to have a higher porosity, a lower calorific value, a smaller bulk density, and a high moisture and ash content (**Tomczyk *et al.,* 2020**).

More moisture in biomass mostly prevents biochar from forming and increases the energy required to reach the pyrolysis temperature. When compared to biomass with a high moisture content, biochar creation is economically feasible when the biomass has a low moisture content due to the remarkable reduction in heat energy and the time required for the pyrolysis process (**Yaashika *et al.,* 2020**). When compared to biochar made from solid waste and animal litter, those made from crop residue and wood biomass typically have higher SSA (specific surface area) and porosity (**Kamali *et al.,* 2021**)

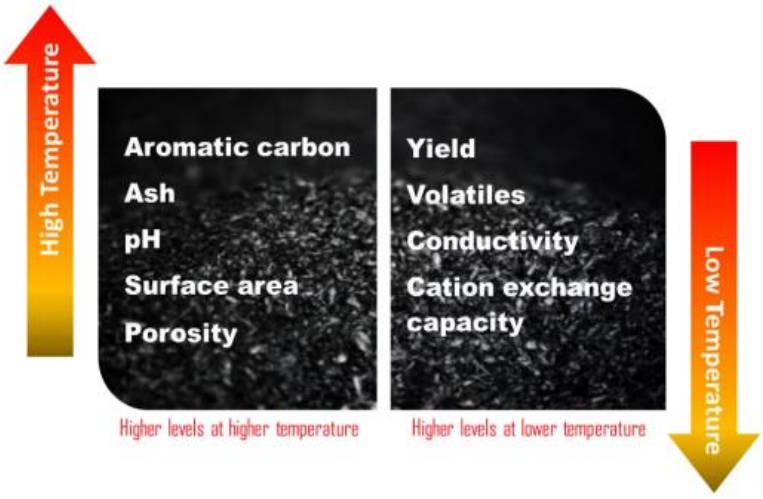

Figure 1: Evolution of physicochemical properties of biochar as a function of temperature. **Patra, B. R. (2021)**.

The pace at which heat changes during the slow pyrolysis of biomass affects the yield, volatile component release, and characteristics of biochar. Reducing the likelihood of secondary pyrolysis reactions and additional thermal cracking by keeping the pyrolysis heating rate in the lower range significantly increases the yield of biochar. The generation of volatile compounds is suppressed when the biochar's heating rate is kept below 10 °C min−1, which is followed by minor polymer structural rearrangement events that produce a stable carbon matrix.

Furthermore, through limits in heat and mass transmission, the degree of heating rate also affects the textural characteristics of biochar (**Mukherjee *et al.,* 2022**).

The extended residence time at a moderate to higher peak temperature range is crucial for slow pyrolysis efficiency, especially when the heat transfer limit at this elevated thermal condition point is large. Because of the various polymerization and secondary processes that can occur for the biomass's contents, a higher biochar production was achieved while retaining a moderate to extended residence duration (**Patra *et al.,* 2021**).

The application of biochar in agriculture has been the subject of recent research, both in large-scale field operations and in laboratory settings. Biochar is applied as a soil supplement to boost crop output and water capacity, as well as a chemical fertilizer component that encourages soil microbial activity. In acidic soils, it has been demonstrated to increase soil pH and decrease heavy metal discharge. Unlike compost, which is naturally biodegraded by microbial populations, biochar is produced by heating breaking down materials without oxygen. The benefits of compost fade quickly, whereas biochar stays in the soil for longer.

The issue of heavy metals/metalloids (HMS) and polycyclic aromatic hydrocarbons (PAHs) in soil and water has a detrimental effect on all forms of life. These contaminants result in detrimental environmental effects and inadequate farming methods. These pollutants have the potential to bioaccumulate, are persistent, toxic, and non-biodegradable. Because of its advantages, biochar is one of the best bioremediation techniques utilized to address the HMS and PAHs issue. These advantages include sustainability, cost, and carbon sequestration. Because of its physical and chemical characteristics, such as its pore structure, specific surface area, and functional groups, biochar has been used to adsorb a variety of pollutants. (**Kumar *et* Panwar, 2024**)

Biochar significantly improves dry areas with limited water supply and extremely variable water quality. The method used to prepare biochar affects the soil's capacity to hold onto water.

Following a heavy rain, biochar's wide total pore space allows it to retain water in its micropore space and facilitate water movement through larger pores from the lower soil horizon to the upper soil (0–15 cm). This improves the capacity to hold water. A study was carried out for various soil types. After applying biochar at different rates, only 25 of 60 soil types showed an improvement in water storage capacity. Applying biochar to plants under drought stress has been found to boost their photosynthetic rate.

Biochar can enhance certain enzymatic processes in soil as well as carbon, phosphorus, nitrogen, and carbon mineralization. Generally speaking, incubating with fresh pyrolyzed biochar significantly improved the behavior of many different types of enzymes. Numerous studies have shown that heavy metals negatively affect soil biological and biochemical characteristics, including soil enzyme activity. Nevertheless, it has also been asserted that by changing the soil's enzymes, adding biochar to the soil can reduce the harmful effects of heavy metals. Biochar is a significant contributor to systemic acquired resistance. The suggested rates of application for disease suppression vary depending on the type of biochar. As of yet, there is no "one concentration fits all" method for incorporating biochar into soilless systems.

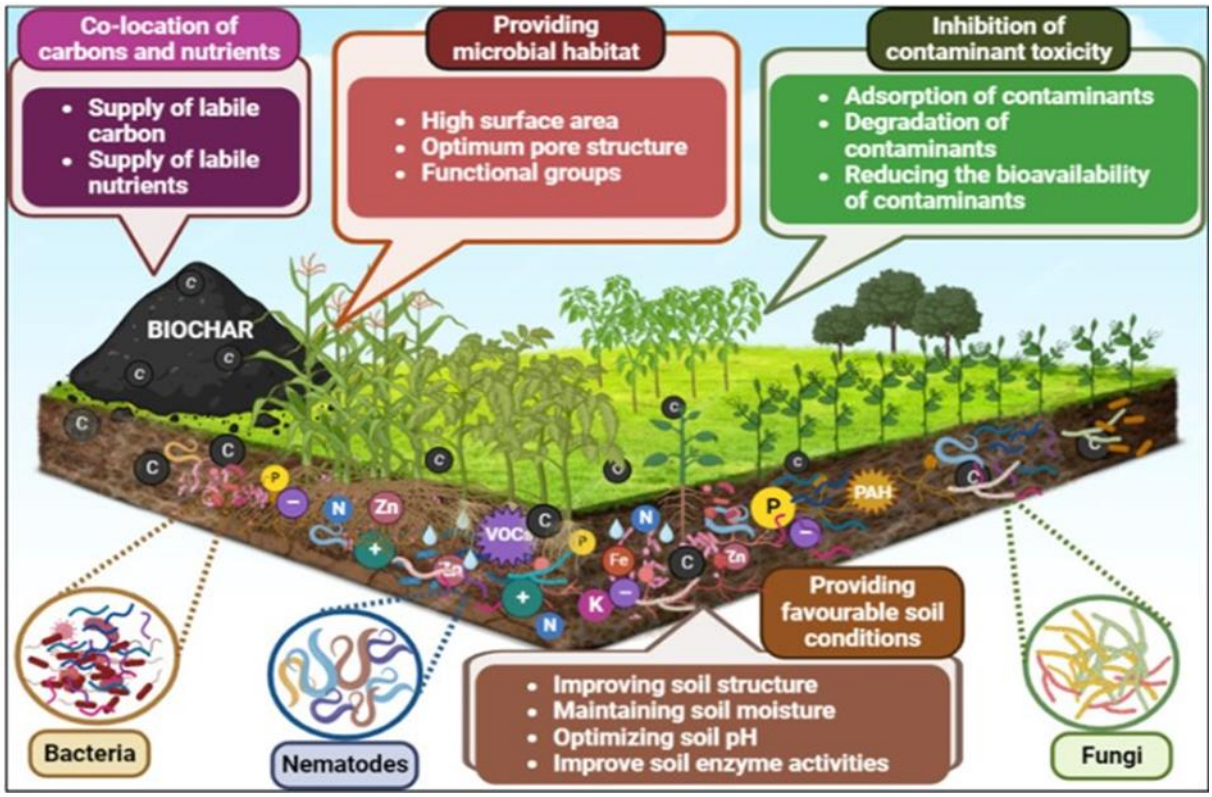

Figure 2: Biochar effects on soil biology health (**Bolan *et al.*, 2023**)

Studies repeatedly show that biochar-enriched poor soils produce larger, more robust plants that produce more and better crops. Better yet, compared to soils lacking biochar, these soils maintain their production and retain nutrients. In soils containing up to 9% biochar, plants thrive, producing more at a lower cost and with a higher yield. They may maintain this higher production for extended periods of time while using less fertilizer. Biochar greatly boosts fertilizer efficiency in soil, lowering the demand for chemicals while increasing crop yields. Maize productivity more than doubled after a Mississippi maize farmer buried 15 tons of charcoal per acre into the sandy riverbed. His use of fertilizer decreased after the first year.

Research conducted in New South Wales, Australia, found that applying 4.5 tons per acre (20 pounds per 100 square feet) to carbon-depleted soils increased the biomass of soybeans and tripled that of wheat. In 2008, Virginia Tech conducted tomato transplant experiments using less than a cup of biochar in a gallon of soil mixture and discovered a yield increase of 48% on average. Inoculating biochar with advantageous microorganisms improves crop responsiveness, boosting overall plant health and nutrient usage efficiency. Applying biochar reduces the requirement for irrigation, according to field observations (**Ekebafe *et al.*, 2013**).

In recent years, biochar has emerged in Algeria as a versatile amendment, both in agriculture and in civil engineering. **Mohamed *et al.* (2025)** showed that adding up to 15% biochar to local sands significantly improves shear strength, due to better particle interlocking at this optimal content. The study by **Bounouara *et al.* (2025)**, conducted in Algeria, demonstrated that combining biochar with compost and chemical fertilizer significantly enhances soil properties (organic carbon, nitrogen, pH, water retention) as well as agro-morphological characteristics of durum wheat (plant height, leaf area, water content). This treatment achieved the best yield, with 45 quintals per hectare, confirming its effectiveness in increasing wheat productivity under local conditions. Thus, the use of biochar today appears as a promising and sustainable solution in Algeria to improve soil quality, increase agricultural yields, and address challenges related to land degradation and harsh climatic conditions.

The aim of this study is to investigate the effects of biochar application on the physiological and biochemical properties of plant, especially common bean *(Phaseolus vulgaris* L.*)*

This work is divided into two parts: a general introduction and an experimental part. The introduction consists of several sections covering concepts related to biochar, including its production, characterization, properties, and the effects of its use as a soil amendment. The experimental part is composed of two parts: the first details the materials used and the methodology employed, while the second presents the results obtained, accompanied by a discussion, and concludes with a final synthesis.

Materials and Methods

II. Materials and Methods

The objective of this research is to study the effects of biochar application on the physiological and biochemical properties of the common bean *(Phaseolus vulgaris* L.*)*. The two types of biochar used for the study were prepared from eggshells and date seeds. Their preparations as well as all the other tests and experiments were conducted in the laboratory and greenhouse of the University of May 8, 1945 of Guelma, in the department of biology.

II.1 Vegetal material and soil

The soil used in this study was collected from a wheat field in Ain Arbi in the region of Guelma, Algeria.

II.1.1 Soil

Figure 3: The soil used for experiments

II.1.2 Vegetal material

The vegetal materiel consists of one variety (coco-rose) of common bean *(Phaseolus Vulgaris* L.*).* This variety was purchased from a specialized agronomic seed supplier in Guelma, with seeds imported directly from Italy.

Figure 4: the vegetal material

Common bean *(Phaseolus Vulgaris* L.*)* **classification** according to Chaux and Foury's (1994)

Kingdom: *Plantae*

Super division: *Spermatophyta*

Division: *Magnoliophy*

Class: *Magnoliopsida*

Subclass: *Rosidae*

Order: *Fabales*

Family: *Fabaceae*

Genus: *Phaseolus*

Species: *vulgaris* L.

II.1.3 Germination test

To enhance germination, the seeds were soaked in water at room temperature for one night. We utilized Petri dishes labeled with paper and tape (with the following information: plant species, variety, and replication number) that were filled with cotton balls of the same weight and covered with absorbent paper. Forty seeds vegetal material were used, with ten seeds per Petri dish and four (4) replications. Seeds were evenly distributed on top of the absorbent paper, ensuring no seeds came into contact. We sprayed 15ml of distilled water to completely saturate the papers without soaking. The boxes were placed in an incubator at 25°C for seven days and watered every two days to ensure constant humidity. Germinated seeds in Petri dishes were

counted and recorded during watering days and after seven days the germination rate percentage was calculated using the following formula according to **(**ISTA 2023 Guidelines**)**

$$\textbf{\textit{Germination rate}}\ (\%) = \frac{\textbf{\textit{Number of germinated seeds}}}{\textbf{\textit{Total number of seeds tested}}} \times \mathbf{100}$$

Figure 5: Steps of germination test

II.2 Biochar preparation

The preparation of biochar involves a pyrolysis process, which is the thermal decomposition of organic material in the absence of oxygen. In this study, we have prepared two types of biochar from eggshells and date seeds.

II.2.1 Eggshells biochar preparation

The eggshells were collected, washed with tap water, then manually crushed and sun-dried for 7H. To ensure thorough dehydration, a second drying phase was done in an incubator at 70°C for 24H. The dried material was then exposed to pyrolysis at 450°C for 2H in a muffle furnace. After cooling to an ambient temperature, the biochar was ground with a grinder and sieved to produce a fine and homogenous powder **(Akram *et al.,* 2022)**

II.2.2 Date (*Phoenix dactylefera* L.) seeds biochar preparation

Dates were collected and the seeds were extracted, washed in warm water and dried in an oven at 105°C for 24 hours.

Following the method described by **Rahmat (2021)**, the seeds were then converted into biochar by pyrolysis in a muffle furnace at 350°C during four hours.

The seeds were then grounded and sieved using a 350-micrometer sieve.

Figure 6: Eggshells and date seeds biomass after pyrolysis

II.3 Sowing of bean seeds

This experience was realized in plastic pots, which were filled with soil and biochar mixture. Three concentrations of biochar (1%, 3%, and 5%) were tested according to **Copley *et al.* (2015)** compared to a control group without biochar, with four replicates for each trial. For biochar treated groups, the biochar was incorporated into the soil before sowing and four seeds were sowed per pot after they have been soaked in water overnight to stimulate germination. The pots were irrigated two to three times per week with distilled water.

Before sowing, a field capacity test was carried out by filling each pot with soil but leaving 1/3 of the volume empty. A known volume of distilled water was poured in to saturate the soil. After 24 hours, drained excess water that poured out was collected and measured. The field capacity, which is the ideal amount to water that soil can retain, was determined by subtracting the drained water from that which was originally added.

Figure 7: Sowing bean seeds

II.4 Soil and biochar analysis

II.4.1 Measurement of pH (the electrometric method)

25 mL of distilled water was combined with a 10 g sample of soil or biochar in a 1:2.5 (w/v) ratio. After 30 minutes of shaking, the mixture was given an hour to settle. A pH meter that had been calibrated was used to measure the pH.

II.4.2 Electrical Conductivity (EC)

The electrical conductivity expressed in (µS) was measured using a conductivity meter and soil in a ratio (soil or biochar/water = 1/5) according to **(AUBERT, 1978)**

II.4.3 Soil composition

II.4.3.1 Determination of organic carbon and organic matter

The organic carbon was determined by weighting 1g of soil and invert it into a beaker, then 10 ml of potassium dichromate and 20ml of concentrated sulfuric acid have been added. After 30 min, 200 ml of distilled water and 10 ml of concentrated phosphoric acid were added before finally adding 10 to 15 drops of the colored indicator diphenylamine.

Titration was done using ammonium iron sulfate until a green color appears. A control (without the soil) was prepared using the same method. And the percentages of organic carbon and organic matter were calculated with the following formulas: **(Walkley and Black, 1934)**

$$\boldsymbol{CO\% = \frac{(n' - n)}{p} \times 1 \times \frac{0.3}{0.77}}$$

with **n'**: volume of the control

n: volume of the sample

p: weight of the soil

And $$\boldsymbol{MO\% = CO\% \times 1.72}$$

II.4.3.2 Total Limestone

Total limestone refers to the amount of limestone in the soil in all dimensions. Its concentration in the soil can be evaluated following dissolving with a moderately concentrated acid (effervescence test).

HCl attacks the soil's limestone carbon. This measurement is based on an acid-base reaction with HCl diluted by one-third. The volume of CO_2 emitted is used to calculate the amount of $CaCO_3$. **(Baize, 2000)**

In a beaker containing 10 ml of HCl diluted to 1/3 of the known weight, add 2 g of fine soil, weigh and note it (**P1**), stir, let stand, and reweigh to determine the weight (**P2**).

The limestone content is estimated with the following formula:

CaCo3% = weight of CO2 released *2.274*100/ weight of soil

Were weight of CO2 released = **P1- P2**

II.4.3.3 Texture of Soil

Based on **Dermech *et al.* (1982)** methodology which consist to make a paste by mixing a quantity of soil with water. Next, try to use this paste to make a ribbon, then transform this ribbon into ring.

Figure 8: Soil texture test steps

Identification

-No ribbon forms → Sand

-Ribbon fragments → Sandy soil

-Ribbon forms but is fragile → Loamy soil

-Ribbon forms but not the ring → Sandy loam soil

-Ribbon and ring form, but ring fragments → Heavy sandy loam soi

-Ribbon and ring form completely → Clay soil

II.4.4 Volatile matter, Ash and Moisture content determination

For the determination of moisture content, volatile matter, and ash content, the method described by **(Mahdi *et al.,* 2015)** was applied. First, the empty crucibles were weighed and their masses were recorded as **M0** using a precision scale.

Then, a known mass of soil or biochar, typically 1 g, was placed into each crucible. The crucibles containing the samples were weighed again, and this mass was recorded as **M1**. The samples were heated in a muffle furnace at different temperatures to analyze parameters:

- **105°C/24H for moisture content,**
- **350°C/3H for volatile matter, and**
- **550°C/3H½ for ash content**.

Following heating, the crucibles were removed from the furnace and allowed to cool to ambient temperature in a desiccator to avoid moisture absorption. After cooling, the crucibles were weighed again, and the resultant mass was recorded as **M2**.

Calculations were based on the loss-on-ignition method, using the formula:

$$\frac{M1 - M2}{M1 - M0} \times 100$$

with

M0: the mass of the empty crucible,

M1: the mass of the crucible plus the sample before heating,

M2: the mass after heating.

II.5 Analysis of plants

II.5.1 Morphological parameters

In order to evaluate the effects of the two types of biochar used in this study on the morphological parameters of the common bean plants, they were uprooted and the leaves, stems and roots were analyzed

II.5.1.1 Stem and root length:

After uprooting, the length of the stems and roots was measured and reported in centimeters (cm) using a graduated ruler.

II.5.1.2 Roots fresh and dry weight

After uprooting them, the roots of the plants in each group were washed and then dried between two pieces of paper towels. Afterwards, their fresh weights were directly determined using a precision scale and the values were reported. They were then placed in an incubator at 105°C for 24 hours, after which their dry weights were determined.

Figure 9: Uprooting of plants for morphological parameters determination

II.5.1.3 Leaf Area Measurement

Leaf area was estimated using a manual tracing method according to **(Breda, 1999)**. After harvesting, each leaf was placed on tracing paper, and its outline was carefully drawn to capture the exact shape and margins.

Following the contour tracing, leaf area was calculated using the empirical formula and expressed in cm^2):

$$\boldsymbol{Leaf\ area = Length \times width \times 0.75}$$

II.5.1.4 Relative water content (RWC)

By following **Jones and Turner (1978)**, RWC was measured using evenly sized leaves from replicates and weighed. After recording the weight of fresh leaves (g), bean leaves were soaked in distilled water for 3 hours at room temperature in the dark and then the turgid weights were recorded. To determine dry weight, bean leaves samples were oven-dried overnight at 80°C.

$$\boldsymbol{RWC\% = \left(\frac{f.wt. - d.wt.}{t.wt. - d.wt.}\right) \times 100}$$

With **f.wt.**: fresh weight

d.wt.: dry weight

t.wt.: turgid weight

II.5.2 Biochemical parameters

Every test was conducted on bean (*Phaseolus vulgaris* L.*)* fresh leaves. Seven groups have been analyzed (control, 1%, 3%, and 5% for both date seeds and eggshells biochar) with three biological replicates for each group and so a total of 21 samples by analyses.

II.5.2.1 Total Soluble Sugars Assay

From each group about 0.5 g of fresh bean leaves were weighed. After homogenizing the samples in 80% ethanol, they were centrifuged for ten minutes at 3000 rpm and the supernatants were gathered for the analysis.

A stock solution of glucose was made by dissolving 100 mg of glucose powder in 100 ml of distilled water and we proceed to a dilution from 0-1 mg/ml from this stock solution in purpose to realize a standard curve.

Then, 1mL of either standard solution or plant extract was added to each tube.

Each tube was then filled with 2 ml of anthrone reagent (200 mg of anthrone in 100 ml of concentrated sulfuric acid).

After mixing, the tubes were heated for 10 min at 90°C in a water bath before left to cool to room temperature. And finally, the absorbance was measured using a spectrophotometer UV-Vi at 620 nm for both each sample. **According to YEMM *et* WILLIS, (1954)**

II.5.2.2 Chlorophyll Determination

The determination of chlorophyll a, chlorophyll b and total chlorophyll content was carried out according to **Arnon (1949).**

Fresh bean leaves were harvested on the same day of the analysis for each treatment corresponding to different biochar concentrations. For each sample, 0.5g of fresh leaves were weighed, cut into small pieces and ground in 20 ml of 80% acetone until a homogeneous extract was obtained. After filtration, the filtrate was collected and used for spectrophotometric analysis.

The absorbance was measured at 663 nm and 645 nm for chlorophyll a and b respectively. The pigment concentrations (expressed in mg/g fresh weight) were calculated using the following equations:

$$\textbf{Chl a } (mg/g \ \textbf{FW}) = 12.7 \times \textbf{A663} - 2.69 \times \textbf{A645}$$

$$\textbf{Chl b } (mg/g \ \textbf{FW}) = 22.9 \times \textbf{A645} - 4.68 \times \textbf{A663}$$

$$\textbf{Chl total } (mg/g \ \textbf{FW}) = 20.2 \times \textbf{A645} + 8.02 \times \textbf{A663}$$

II.5.2.3 Proline content

The proline content in leaves was determined according to **Bates *et al.*, (1973).** Fresh leaves tissues (500mg) were homogenized in 10 ml of 3% sulfosalicylic acid; the homogenate was filtered through Whatman filter paper.

In a clean glass test tube, 2 ml of the filtrate was mixed with 2 ml of glacial acetic acid and 2 ml of acidic ninhydrin reagent (prepared by dissolving 3.75g of ninhydrin in 90 ml glacial acetic acid, then adding 60 ml of 6 M orthophosphoric acid).

The reaction mixture was incubated in a water bath at 100°C for 60 minutes to allow color development after what the tubes were immediately cooled in an ice bath to stop the reaction. Then, 4ml of toluene was added to each tube, the mixture was vortexed for 1-2 minutes, this allows the separation of organic and inorganic phase.

The upper toluene phase containing the chromophore, was carefully collected and the absorbance was measured at 520nm against a toluene blank using a UV/vis spectrophotometer.

II.5.2.4 Malondialdehyde (MDA) content

0.5 g of fresh bean leaves from each group were homogenized in3 mL of 1% TCA.
This homogenate was transferred into conical centrifuge tubes and centrifuged at 2000 g for 15 minutes at 4 °C and the supernatant was collected. In clean test glass tubes 0.5 ml of the supernatant was added to 3 ml of 0. 5% TBA (prepared in 20% TCA) and left to incubate 50 min in a shaking water bath at 95 °C. In order to stop the reaction, tubes were immediately cooled in an ice bath and the mixtures underwent a second centrifugation at 10000 g for 10 min.

The optical density of the supernatants was measured at 532 nm with a UV/vis spectrophotometer and the values at 600 nm for nonspecific turbidity absorbance were subtracted.

Concentration of MDA was calculated by using the extinction coefficient of the MDA–TBA complex (=155 $mmol^{-1}$ cm^{-1}), **Cakmak and Horst (1991).**

$$\boldsymbol{MDA\ content(nmol) = \left[\frac{A_{532}-A_{600}}{1.56} \times 10^5\right] \times V/W \times 10^6}$$

II.5.2.5 Antioxidant capacity determination

In order to evaluate the antioxidant activity of bean leaves after application of both types of biochar, several tests were carried out using the same extract.

II.5.2.5.1 Extraction for antioxidant activity

According to **Abbas *et al.,* (2023)**, after homogenizing 250 mg of fresh bean leaves of each group with 3 ml of 80% methanol, each sample was incubated in a dry incubator at 65°C for 15 minutes following by a centrifugation step, the supernatant was collected for additional tests.

II.5.2.5.2 Total Phenol content

Total phenolic content of leaf extracts of plants was determined using the Folin–Ciocalteu method as described by **Li *et al.,* (2007)** with minor modifications.

This colorimetric method uses the oxidizable ability of phenolic groups to cleave the complex containing the phosphotungstic (WO_4^{-2}) phosphomolybdic (MoO_4^{-2}) in the Folin reagent under alkaline conditions to form a blue-colored complex.

200 µL of each extract or gallic acid standard (from 0 to 1g/ml) was combined with 1 ml of Folin–Ciocalteu reagent (previously diluted 10-fold with methanol). After 4 minutes of reaction, 800 µl of sodium carbonate solution (0. 75%) was added to the mixture. Both mixtures were incubated for 2 hours in the dark at room temperature. Absorbance was measured spectrophotometrically at 765 nm.

Total phenolic content was calculated using the gallic acid (0-1g/ml) calibration curve and expressed in mg of gallic acid equivalents per g of fresh weight (mg AG/g fr.wt.).

II.5.2.5.3 Flavonoid Content

According to **Bahorun *et al.,* (1996)** the flavonoids content was quantified using the aluminum chloride ($AlCl_3$) colorimetric method with quercetin (0 to 40 g/ml) as standard. In short, 1 ml

of a solution of 2% aluminum chloride (made in methanol) was added to 1 ml of each extract. The absorbance of the reaction mixture was measured at 430 nm after 10 min of incubation at room temperature.

Using the standard curve, the flavonoid content of each extract was determined and expressed as milligrams quercetin equivalent per gram of fresh weight (mg QE/g fr.wt.).

II.5.2.5.4 Determination of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity

Based on the method proposed by **Fedeli *et al.*, (2024b)**, the total antioxidant power of extracts was estimated.

1 ml of DPPH solution was added to 200 µl of extracts obtained from each sample. The DPPH solution was made by dissolving 3.9 mg of DPPH in 100 ml of 80% methanol, a control solution (200 µl of 80% methanol and 1 ml of the DPPH solution) samples were prepared.

Samples absorbance was measured at 517 nm with an UV–vis spectrophotometer after one hour of incubation in complete darkness. The percentage of antiradical activity (ARA), which was determined using the following formula, was used to express the results:

$$\boldsymbol{ARA}\ (\%) = (\mathbf{1} - \frac{\boldsymbol{sample\ absorbance}}{\boldsymbol{control\ absorbance}}) \times \mathbf{100}$$

II.5.2.6 Protein content

II.5.2.6.1 Extraction for protein and antioxidant enzymes

From each, 0.5g of fresh bean leaves were homogenized in 10 ml of cold phosphate buffer (pH 7,8; 50mM). The homogenate was then centrifuged at 6000 g for 20 minutes at 4 °C. The resultant supernatant was carefully removed from the pellet, put to appropriately labeled Eppendorf tubes, and frozen for additional analysis. **(Shahzadi *et al.*, 2024)**

II.5.2.6.2 Total protein assay

The enzymatic extract produced from fresh bean leaves was used to quantify total proteins content using the method described by **Bradford (1976).**

The Coomassie Brilliant Blue (CBB) reagent was prepared by dissolving 0.1g CBB in 50 ml of 95% ethanol. To this solution, 100ml of 85% O-phosphoric acid was added and the final volume was adjusted to 1l with distilled water.

In each tube, 50 µl of enzymatic extract was mixed with 2.5 ml of CBB reagent. The mixtures were stirred and incubated at room temperature for 5min. The absorbance was then measured at 595 nm using a UV-Visible spectrophotometer.

A standard calibration curve was established using a stock solution of BSA (0-1mg/ml). This curve was used to determine protein content in unknown samples.

II.5.2.6.3 Catalase activity assay

According to the method described by **Seckin *et al.,* (2009)**, the activity CAT was measured by producing a reaction mixture consisting of 1.0 ml of phosphate buffer (pH 7.8; 50 mM), 1.7 ml of distilled water and 0.1 ml of enzymatic extract.

This combination was incubated at room temperature for 3 min. After incubation, 0.2 ml of H_2O_2 (200mM) was added to start the reaction.

The breakdown of H_2O_2 was monitored by measuring the decrease in absorbance at 240 nm using a UV-Visible spectrophotometer. Readings were taken every 30s for a duration of 2 min.

II.5.2.6.4 APX activity assay

The ascorbate peroxidase 3 mL reaction solution was containing 2.7 ml of phosphate buffer (50 mM and 7.8 pH), 0.1 ml of ascorbic acid (7.5 mM), 0.1 ml of H_2O_2 (300 mM) and 0.1 ml of enzyme extract. The decrease in absorbance at 290 nm was followed using a UV-Visible spectrophotometer every 30s during 60s. according to **Nakano *et* Asada (1981)**.

Statistical tests:

All experimental data are presented as mean + standard deviation (SD) from five trials. Statistical analyses were performed using IBM SPSS Statistics V27 software IBM Corporation, New York, USA), with the significance level set at $p < 0.05$. The tests conducted included:

• One-way analysis of variance (ANOVA) followed by Tukey HSD (with Bon-Ferroni adjustment) and Games-Howell post-hoc tests.

• Two-way ANOVA, complemented by Tukey's post-hoc test, and Welch's ANOVA, followed by Games-Howell's post-hoc test.

• Non-parametric Kruskal-Wallis test, with post-hoc comparisons performed using Bonferroni correction.

Discussion

III. Discussion

Globally, the exponential growth of the human population has led to an increasing demand for agricultural resources, low- and middle-income countries, in particular face significant challenges in addressing food security issues. Biochar, a carbon rich material, has been advocated as a soil amendment to improve soil quality and crop productivity. However, its effects on plant growth vary depending on factors such as biochar type, application rate, plant species and soil conditions (**Kumari *et al.,* 2022**). This study aims to evaluated the physiological and biochemical effects of eggshell-and date seed- derived biochar on the common bean *(Phaseolus Vulgaris* L.*).*

IV.1. Soil and biochar physicochemical characterization

The physicochemical characterization of the soil and biochar realized in this study revealed significant variability in their properties, which may influence their agronomic effectiveness. The native soil displayed a slightly alkaline pH (7.92), moderate organic matter content (2.64%), and relatively low electrical conductivity (200 µS), in consistent with values commonly observed in clay soils of semi-arid regions **(Hassanein *et al.,* 2022)**. Eggshell-derived biochar (B1) exhibited a markedly alkaline pH (9.05), low moisture content (1.36%), and low volatile matter (2.6%), which reflects its high mineral content, particularly calcium carbonate, consistent with findings by **Wong *et al.* (2023)**, who reported that eggshell biochar significantly increased soil pH and phosphorus availability due to its high $CaCO_3$ concentration. In contrast, the date seed biochar (B2) had a lower pH (6.61) but much higher volatile matter (66.3%) and carbon-rich composition, corroborating the results of **Mahdi et al. (2015)**, who showed that date seed biochar enhances soil water retention and microbial activity due to its porous carbonaceous structure. The higher conductivity value observed in B2 (470 µS) compared to B1 (246 µS) suggests a greater ionic release, possibly linked to its elevated ash content. Overall, these results align with previous studies demonstrating that biochar properties are highly dependent on feedstock type and pyrolysis conditions, and that both eggshell and date seed biochar can serve complementary roles in improving soil fertility, through pH adjustment, nutrient retention, or organic matter enhancement.

IV.2. Morphological and physiological properties

According to **Bradbeer (1988)**, a seed is considered viable if it can germinate under optimal conditions, including when dormancy has been broken through appropriate treatments.

Conversely, a seed deemed non-viable if it fails to sprout even after dormancy-breaking measures. In our study, a germination rate of 77.5% was observed for common been (*Phaseolus vulgaris* L.) seeds, a result consistent with established standards for this species. This rate aligns with the typical germination threshold reported fer these seeds, as confirmed by Agrobio Périgord's technical guidelines on seeds germination.

Our results show an increase in root biomass (root dry weight, root fresh weight and root length) compared to the control, after the addition of both types of biochar, as well as the length of the stems, after the addition of B1, corroborate with those found by **Rondon *et al.,* (2006)** who also observed an increase in biomass after the addition of biochar to the same plant material. This increase can be explained by the improvement of soil structure by biochar, in particular by promoting water retention and nutrient availability. According to **Zulfiqar *et al.,* (2021)**, possible explanations for BC's beneficial effects on root growth include modifications to the physical characteristics of the soil, such as pH, water-holding capacity, and hormonal impacts, which speed up root development and enhance overall growth. Biochar derived from date seeds is rich in four main macronutrients which are P, K, Ca and S, **(Rahmat, 2021)**, essential for good root growth. On the other hand, eggshell biochar is rich in $CaCO_3$ and therefore mostly alkaline, which makes it possible to adjust the pH of soils, thus influencing their qualities and fertility by improving the availability of mineral elements. Calcium, the dominant element in this biochar, is required for biochemical and metabolic activities in plants, such as the synthesis of root and shoot tissue and cell wall membranes (**Ma'mor *et al.,* 2023**). The length of the bean stems after the addition of date seeds biochar shows a significant decrease compared to the control group, we hypothesize that this may be due to a shift in the growth dynamics of the plant that prioritized the development of the underground parts or may also be due to an increased allocation of resources to the roots after the biochar is positively modified soil parameters.

Bean plants' leaf area and relative water content (RWC) significantly increased following applying biochar, which is similar to the results of (**Munir *et al.,* 2020; Farhangi-Abriz *et al.,* 2018**), particularly for eggshell-derived biochar suggests enhanced physiological performance under treated conditions, the highest relative water content (RWC) at 3% and the increased leaf area especially at 5% indicate that biochar improved the plant's water retention capacity while promoting vegetative growth. These effects can be attributed to biochar's ability to enhance soil physical properties, such as porosity and water-holding capacity as while as its influence on nutrient availability, particularly calcium, which is critical for maintaining membrane stability

and cell turgor **(Yuan *et* Xu, 2011; Lehmann *et* Joseph, 2015)**. The positive correlation between RWC and leaf area supports the hypothesis that improved water status facilitates leaf expansion, as proper hydration promotes stomatal function and cell enlargement (**Farooq *et al.,* 2009; Agegnehu *et al.,* 2017**).

In contrast, date seed-derived biochar exhibited more variables effects compared to B1 which showed consistent improvements while high RWC was observed at 1% and 3% for B2, a significant decline accrued at 5% suggesting potential osmotic or structural limitations at higher concentrations. This may result from altered soil ionic balance or excessive water retention impeding aeration (**Glaser *et al.,* 2002; Sohi *et al.,* 2010**). Overall, the stimulations enhancement of physiological (RWC) and morphological (leaf area) traits the multifaceted benefits of biochar for plant performance, with optimal outcomes observed at moderate application rates.

IV.3. Biochemical properties

IV.3.1. Total soluble sugar content

The total soluble sugar content in bean leaves was clearly impacted by the application of biochar, with significant variations based on the type and concentration of biochar. The eggshell-derived biochar showed a dose-dependent response, which is in contrast to the results of **Farhangi-Abriz *et al.,* (2018)**, who did not find a significant increase in sugar content in bean leaves after applying biochar. There was a notable increase In B1 3%, though, reaching 355.264 mg/g FM, which is higher than the control. This notable increase could be the result of a microenvironment that is ideal for photosynthetic efficiency and carbohydrate accumulation at this concentration, most likely due to better potassium uptake.as well as less oxidative stress, **Spokas *et al.,* (2012)**. The date seed-derived biochar showed a more progressive and stable dose-dependent effect at higher doses, indicating a nutritional imbalance. At 5%, the sugar content decreased slightly, but it was still higher than the control. The sugar content increased moderately at 1%, then decreased slightly at 3% then increased at 5%. The consistent nutrient release and buffering properties of B2 may be an explanation of this pattern, maintaining soil conditions that favor the accumulation of sugar (**Atkinson *et al.,* 2010; Lehmann *et al.,* 2011**).

IV.3.2. Chlorophyll content

The increase in chlorophyll content observed in bean plants treated with biochar is consistent with several earlier studies that have reported beneficial effects of biochar on plant growth and

metabolism. Mechanistically, **Cheng *et al.,* (2012)** and **Enders *et al.,* (2012)** proposed that biochar enhances plant development in two ways: the direct action occurs through the supply of essential nutrients (Ca, Mg, P, K, and S), and the indirect action occurs through the modification of the soil's physical, chemical, and biological properties (in other words, improves plant physical permeability). This dual action may give rise to favorable conditions for chlorophyll biosynthesis. This is particularly evident in eggshell biochar, which demonstrated the highest chlorophyll a content (6. 7272 mg/mL at 5%) and chlorophyll b content (10. 61 mg/mL at 1%) probably due to its mineral richness and soil conditioning effects. These findings are consistent with those reported by **Farhangi-Abriz *et al.* (2018)**. Date seeds biochar on the other hand was associated with a more moderate but steady increase in chlorophyll content. The highest levels of chlorophyll a were achieved at all concentrations, the chlorophyll b at 1% and 5% treatments increased with a comparable level. To monitor the total chlorophyll content, the values were relatively high in all. These results are consistent with those recently reported by **Wang *et al.* (2014)** wherein biochar significantly increases photosynthetic rate and chlorophyll accumulation and are closely followed by reports from **Laird *et al.* (2010)** and **Beesley *et al.* (2011)** who have shown that biochar enhances the soil mineral nutrient content as a result of adsorption processes.

IV.3.3. Leaf proline content

The results reveal that leaf proline content in common bean is significantly influenced by both the type and concentration of biochar applied, the highest proline concentration was observed in the control indicating a higher level of physiological stress in the absence of amendments according to **Szabados *et al.,* (2010)**. This could be due to suboptimal soil conditions, such as poor water retention, limited nutrient availability, leading to the activation of stress-related pathways and proline biosynthesis. The addition of eggshell biochar results in a progressive decrease in proline content, this pattern signifies a decrease in stress intensity, likely resulting from improved soil conditions, and water retention, also biochar could improve nutrient availability, particularly of calcium, magnesium and phosphorus, this element known to support plant metabolism and reduce oxidative damage **(Marschner, 2012; Gill and Tuteja, 2010)**. A small amount of proline in the cytoplasm can quickly reach a high concentration and contribute more effectively to the vacuole osmotic potential balance. In a number of stressed tissues, amino acids function as a putative osmoprotective solute, reducing the osmotic potential **(Mohamedin *et al.,* 2006)**. Free proline has been suggested to have a variety of functions, including osmoprotection, protein stabilization, metal chelation, lipid peroxidation inhibition, and OH

and O_2 scavenger **Trovato *et al.,* (2008)**. These findings are consistent with those reported by **Farhangi *et al.,* (2017)** who also observed a decrease in leaf proline content in common bean leaves treated with biochar, date seeds biochar has not been able to significantly lower proline concentrations. The observed concentrations of 1% and 5% are comparable to the control while a slight decrease of 3% is noted, this suggests that this type of biochar did not improve growing conditions to the same extent as eggshell biochar, it may contain lower levels of beneficial minerals that play critical roles in stress response mechanisms.

IV.3.4. Malondialdehyde (MDA) levels

Similar to the results reported by **Raziye Kul *et al.,* (2021)** malondialdehyde (MDA) levels in groups treated with biochar of bean (*Phaseolus vulgaris* L.*)* leaves were considerably lowered in comparison to the control group, suggesting a reduction in oxidative stress. The effect of eggshell-derived biochar was the most noticeable, MDA levels were considerably reduced at all tested concentrations, but especially at 5% in comparison to the control. This decrease is explained by the biochar's high mineral content, particularly calcium, which is essential for maintaining cell membrane stability and decreasing lipid peroxidation **(Cakmak, 2005)**. Previous research has demonstrated that biochar improves a number of soil properties, such as pH, nutrient availability, water retention, and beneficial microbial activity, so that plants are better able to resist abiotic stress **(Farhangi-Abriz *et al.,* 2017; El-Naggar *et al.,* 2019)**. However, date seeds derived biochar demonstrates a significant decrease in MDA only at 3% at 1% and 5% concentrations, the effect was less apparent. The distinction could result from variations in the two types of biochar's chemical composition and structural characteristics, which affect how well they can regulate the plant's antioxidant response.

IV.3.5. The antioxidant activity (total phenols content, total flavonoid content, DPPH radical scavenging).

Phenolics, which have at least one phenol unit with one or more hydroxyl substituents, are a type of secondary metabolites in plants **(Yang *et al.,* 2018)**, which play multiple essential roles in plant physiology and have potential healthy properties on human organism, mainly as antioxidants, anti-allergic, anti-inflammatory, anticancer, antihypertensive, and antimicrobial agents **(Daglia, 2011)**. Our results show an increase in the total polyphenol content depending on the concentration but also on the type of biochar. Eggshells biochar at 1% recorded the highest value, which indicates a higher efficacy at low doses, which is quite the opposite of date seeds biochar where the peak concentration of polyphenols is obtained at 5%. The reason

behind the varying plant responses to different biochar concentrations lies primarily in the alteration of soil pH **(Wong *et al.,* 2023)**. As for flavonoids, date seeds biochar seems to have a more significant effect on this parameter, probably because of its richness in carbons, mineral elements and its ability to improve water retention on the ground **(Kavvadias *et al.*, 2024; Rahmat, 2021)**. **Phares *et al.,* (2020)** also found an increase in the content of polyphenols and flavonoids in another plant of the same family (Fabaceae) as the bean, cowpea (*Vigna unguiculata*), exponentially to the concentration of biochar and claims that this increase would be due to an improvement in soil fertility following the addition of biochar. According to **Nasiri *et al.,* (2024)**, biochar effectively raises the levels of secondary metabolites in soil by introducing a substantial amount of organic molecules and the effectiveness of the plant's antioxidant system in kidney beans. Antioxidant activity in this study was measured by the DPPH method and all biochar-treated groups recorded a higher percentage of activity than the control. These results demonstrate that biochar, after increasing the content of phenolic compounds in bean leaves, certainly effectively influenced its antioxidant capacity. **Zulfiqar *et al.,* (2021)** also found in Alpinia zerumbet that, by raising phenolic acid concentrations, biochar is helpful for boosting plant antioxidant capacity.

IV.3.6. Total protein content

In contrast to the findings of **Farhangi-Abriz *et al.,* (2018)**, who reported no significant increase in protein content in leaf protein content after biochar application, our study reveals distinct dose dependent effects for different biochar types. Eggshell derived biochar consistently enhanced total soluble protein levels at both 1% and 5% application rates. Whereas date seeds biochar showed a more variable response, increasing protein content at 1% but decreasing it below control protein levels at 5%. The beneficial effects of B1 likely stem from its ability to improve soil nitrogen availability through enhanced nutrient cycling **(Lehmann et Joseph, 2015)** and its calcium carbonate content that promotes microbial activity and pH regulation tested concentrations with a maximum value of 1% and 5%, while dated seed biochar has a less uniform effect only the 1% treatment increased protein content, a decrease, with 5% of B2 below control levels, was observed ,there are several of interconnected mechanisms that contribute to the observed increase in soluble proteins, especially with B1. It is commonly known that biochar increases the soil's availability of nutrients, especially nitrogen, which is necessary for the synthesis of proteins and amino acids. Biochar which is typically rich in calcium carbonate and other essential minerals, enhances nutrient cycling, pH regulation **(Uzoma *et al.,* 2011; Liu *et al.,* 2018)**. These factors collectively stimulate nitrogen assimilation

pathways leading to greater protein accumulation. The negative impact observed with higher B2 concentrations may results from ionic unbalances or disruption of critical root-soil-microbe interactions, highlighting the importance of biochar source and application rate in protein biosynthesis.

IV.3.7 Antioxidant enzymes (CAT, APX)

Catalases, ascorbate peroxidases, glutathione/thioredoxin peroxidases, glutathione sulfo-transferases, and some peroxiredoxins are among the H_2O_2-metabolizing enzymes found in plants. Nevertheless, CAT and APX are the most prominently different enzymes since the former is mostly found in peroxisomes and catalyzes a dismutation reaction without the need for a reductant **(Sofo *et al.,* 2015)**.

Our results found in this study about CAT and APX, can be explained by the fact that the addition of biochar to reduce the availability of free radicals, so the plant could therefore reduce the synthesis of certain antioxidants in order to adjust their quantity to its needs. According to **Farhangi-Abriz *et* Turabian (2016**), biochar decreased the amount of O_2^- and H_2O_2 as ROSs in bean leaves and roots, which decreased some antioxidant activities. They also report that to minimize oxidative damage and preserve cellular metabolic processes under stress, a balance between ROS creation and breakdown is necessary. The amount of ROS in plant tissues may be regulated by the antioxidant system. On the contrary, increases in APX activity were found in all groups compared to the control except the eggshell biochar at 3%. Plants can still activate the synthesis of other enzymes more efficient than catalase at low concentrations of H_2O_2 such as APX, which would explain our results obtained in relation to the activity of APX in bean leaves after the addition of both types of biochar. With ascorbate acting as a specialized electron donor, APX specifically has a greater affinity for H_2O_2 **(Sofo *et al.,* 2015)**.

Conclusion and perspectives

Conclusion and perspectives

This study demonstrated the significant potential of biochar as a sustainable soil amendment to improve the physiological and biochemical performance of common bean (*Phaseolus vulgaris* L.). Two types of biochar, derived from eggshells and date seeds through pyrolysis, were applied at different concentrations to evaluate their effects on plant growth, nutrient status, and stress response. The results clearly show that both biochar positively influenced various morphological parameters, such as root and shoot development, and improved biochemical indicators, including chlorophyll content, soluble sugars, proteins, and antioxidant activity.

Notably, the date seeds biochar exhibited a stronger effect on root biomass and antioxidant capacity, particularly at 3% and 5% concentrations, while eggshell biochar was more effective in enhancing photosynthetic pigments and leaf water status. Additionally, the reduction in stress markers such as proline and malondialdehyde confirms the biochar's role in enhancing plant tolerance to environmental stress.

These findings highlight the agronomic value of biochar, not only for improving plant productivity and soil quality but also for promoting circular economy principles through the valorization of organic waste. This work supports the broader application of biochar in sustainable agriculture and opens the door to future research on optimizing its use across different crops and soil types.

With this in mind, several perspectives deserve to be explored to strengthen and expand the applications of this technology.

- It would first be relevant to test the effectiveness of biochar as a crop adaptation strategy to abiotic stresses, particularly in drought or salinity conditions, in order to assess its potential to improve plant resilience in a context of climate change.
- In addition, a better understanding of the effect of biochar on soil diversity and microbial activity would help to elucidate its role in stimulating soil-plant interactions, particularly in legume root systems.
- Finally, the study of the link between the physicochemical properties of the different biochar (pH, conductivity, cation exchange capacity, mineral richness) and the biochemical responses of plants could pave the way for the optimization of pyrolysis parameters and the raw materials used, with a view to producing "tailor-made" biochar for specific agronomic needs

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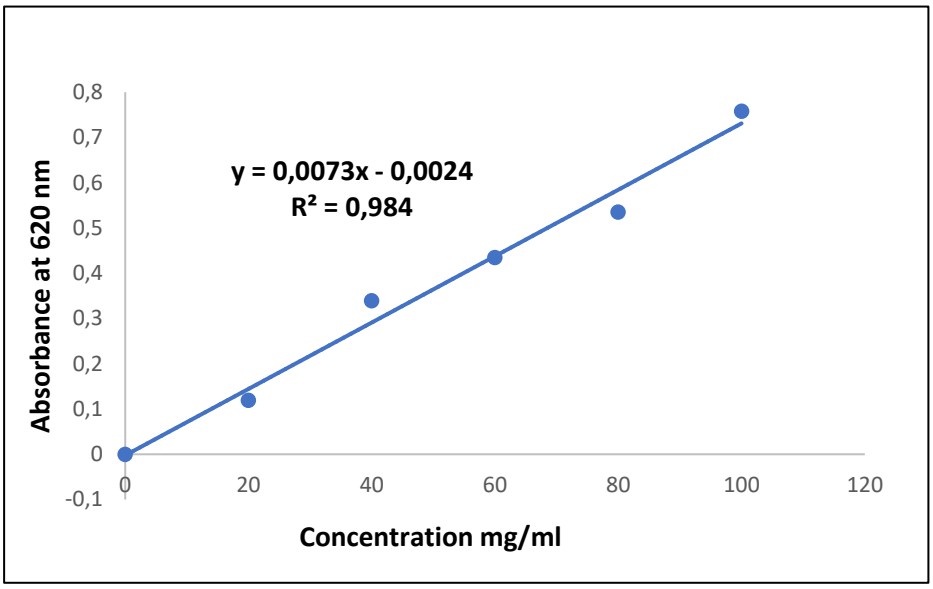
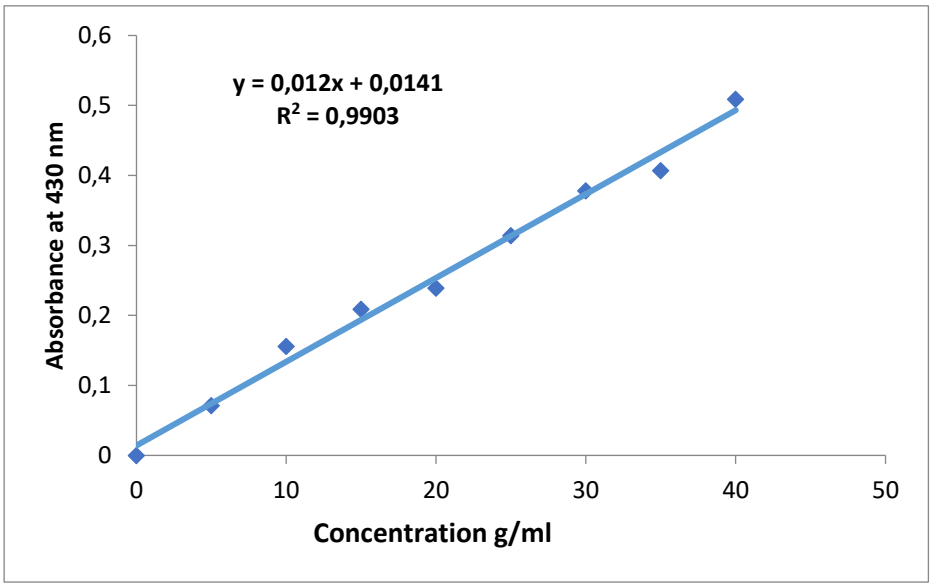
Annexes

Calibration Curve for Total Soluble Sugars Determination



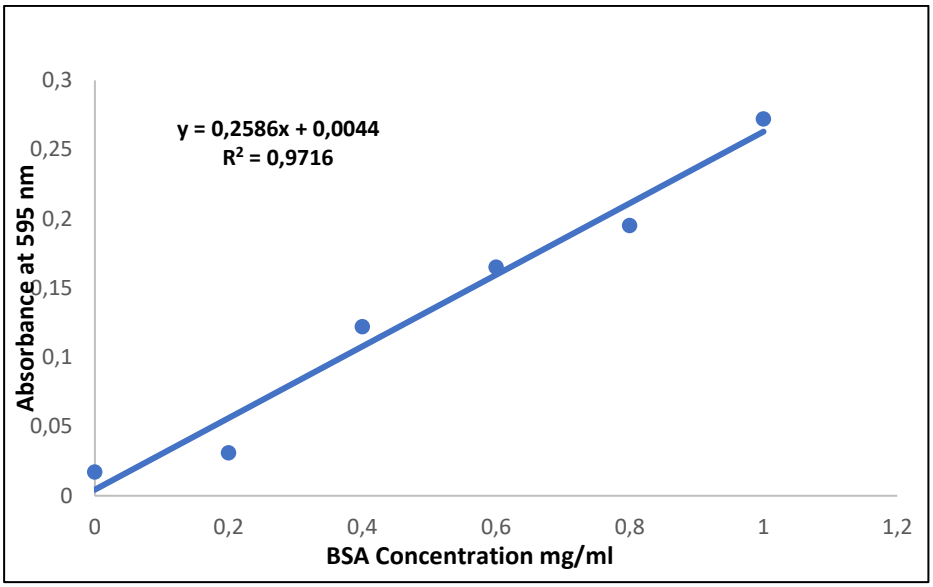
Annex 1: Calibration Curve for Total Soluble Sugar Determination Using Glucose Standard

Calibration Curve for Flavonoid Content Determination



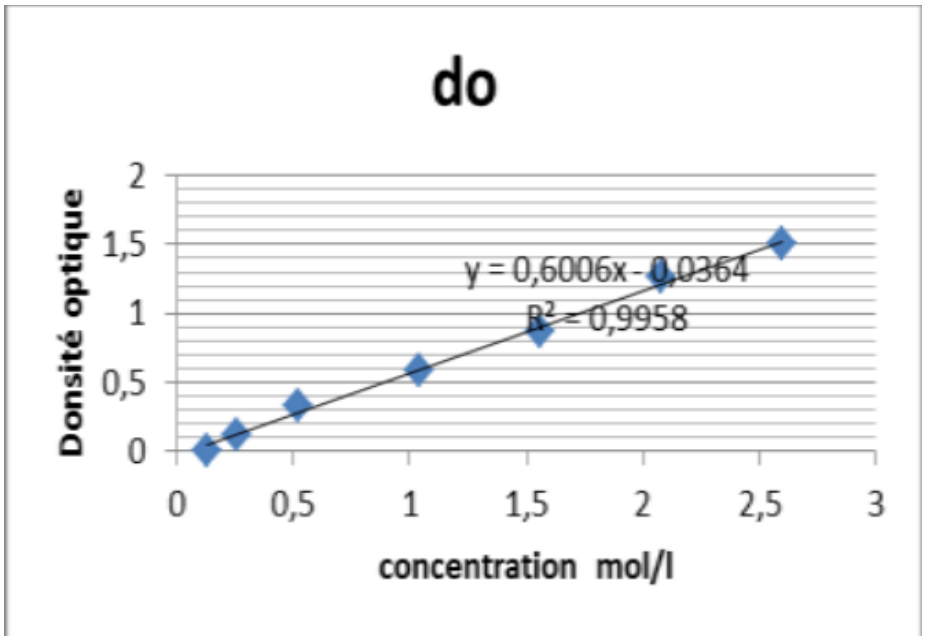
Annex 2: Calibration Curve for Flavonoid Content Using Quercetin Standard

Calibration Curve for Protein Quantification



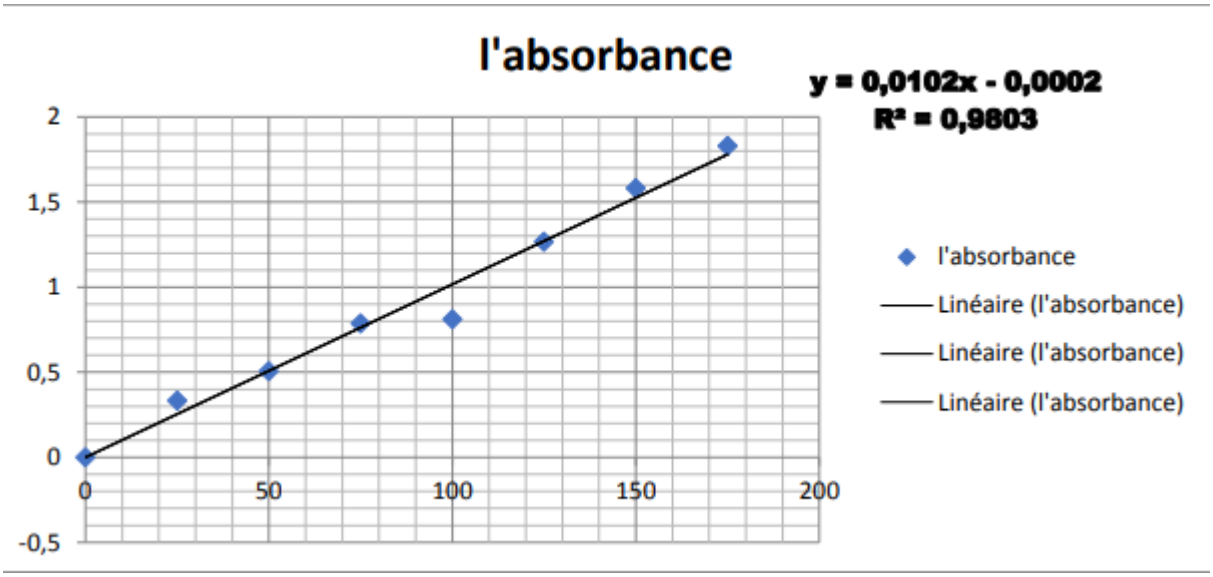
Annex 3: Calibration Curve for Protein Quantification Using Bovine Serum Albumin (BSA)

1.5. Calibration Curve for Leaf Proline content Determination



Annex 5: Calibration Curve for Proline Quantification Using L-Proline Standard (khouildat et Benzahi, 2014)

Calibration Curve for Total Polyphenol Content Determination



Annex 6: Calibration Curve for Total Polyphenol Content (Amara et al., 2022)

Table: pH classification (Baize, 1988)

pH values	Interpretations
Below 3.5	Hyper acidic
Between 3.5 and 5	Very acidic
Between 5 and 6.5	Acidic
Between 6.5 and 7.5	Neutral
Between 7.5 and 8.7	Basic/alkaline
Above 8.7	Very basic/alkaline

Table: CaCO3 interpretation (Baize, 1988)

CaCO3 (%)	Interpretation
Below 1%	Non-calcareous
1 to 5%	Slightly calcareous

5 to 25%	Moderately calcareous
25 to 50%	Strongly calcareous
50 to 80%	Very strongly calcareous
Above 80%	Excessively calcareous

Table: soil quality classes according Durand J.H scale (1983)

Class	**CE (µS)**	**Quality**
Class I	0 to 500	Non saline
Class II	500 to 1000	slightly saline
Class III	1000 to 2000	Saline
Class IV	2000 to 4000	Very saline
Class V	Above 4000	Extremely saline

Table: interpretation of organic matter

MO (%) values	**interpretations**
Below 1	Very poor
Between 1 and 2	Poor
Between 2 and 4	Moderate
Above 4	Rich