Front. Agr. Sci. Eng. 2025, 12(2): 391–405 https://doi.org/10.15302/J-FASE-2024598

RESEARCH ARTICLE

Evaluation of biochar as a *Trichoderma* carrier for managing *Sclerotinia sclerotiorum* in chickpea

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KEYWORDS

Bio-agent, biochar, carrier material, disease suppression, plant growth enhancement, *Sclerotinia sclerotiorum*, soil amendment, *Trichoderma*

HIGHLIGHTS

- Hardwood biochar supported the highest *Trichoderma* population and disease suppression.
- It reduced plant disease severity and increased plant root mass.
- The most effective preparation was 4% hardwood biochar graded to 100-mesh.
- This significantly suppressed disease and promoted plant growth.

Received July 8, 2024;
Accepted November 11, 2024.

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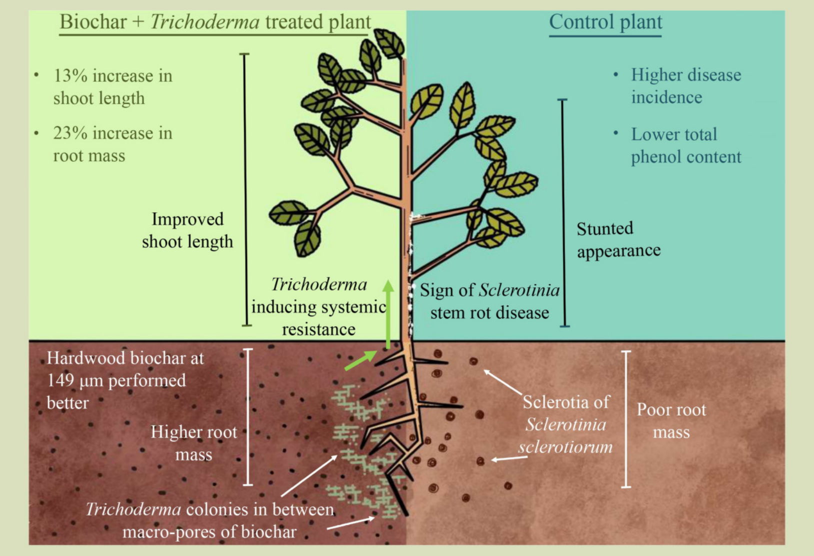
GRAPHICAL ABSTRACT



ABSTRACT

The macropores of biochar provide a suitable habitat for microbial growth, and its high carbon content serves as an energy source for beneficial microbes. This study evaluated the potential of biochar as a carrier for *Trichoderma* in managing *Sclerotinia sclerotiorum* in chickpeas. Biochar application reduced plant disease severity by 36.5% and increased plant root mass by 23.3%. For this, three types of biochar, wheat straw, organic kitchen waste, and hardwood were tested with *Trichoderma*, analyzing such as organic C, total N, P, K, Mg, and Ca; pH, and ash content. *Trichoderma* populations were monitored with biochar carrier of different mesh sizes (250, 150, 75, and 45 µm) for up to 6 weeks after inoculation. Hardwood biochar at 150 µm supported the highest *Trichoderma* population, reaching 33.5×10^5 CFU·g^{-1} after 6 weeks. Hardwood biochar also achieved the maximum disease suppression compared to other biochar types. This research highlights the dual role of biochar in enhancing plant growth and controlling disease, contributing to the standardization of biochar use in agricultural practices.

1 Introduction

The production of the annual legume, chickpea (*Cicer arietinum*) in India holds a prominent position in the global market, driven by favorable climatic and growing conditions. The ability of India to sustain and expand its chickpea production is important not only for meeting domestic demand but also for contributing to the global supply chain[1]. Chickpeas are a pivotal crop in India, contributing about 70% of the global production. The production of chickpeas in India has increased substantially, reaching 14 Mt in 2021–2022, up from 7.3 Mt in 2014–2015. This increase is attributed to improved agricultural practices and government initiatives like the National Food Security Mission[2]. Chickpeas are a vital source of protein in many countries, particularly for the vegetarian population of India and are significant in its national dietary and economic landscape. The crop is primarily grown in the states of Madhya Pradesh, Maharashtra, Rajasthan and Uttar Pradesh, with Madhya Pradesh alone accounting for 34% of the total chickpea production in India, followed by Rajasthan at 19% and Maharashtra at 16%[3]. However, *Sclerotinia sclerotiorum*, responsible for white mold disease, poses a significant threat to chickpea crops across India and in many other countries. This soilborne pathogen can cause severe yield losses, potentially exceeding 50% under favorable conditions. The disease is characterized by water-soaked lesions on stems, leaves and pods, which can result in the complete collapse of infected plants. The presence of sclerotia, hardened fungal structures, allows the pathogen to persist in the soil, making it difficult to manage[4]. The impact of *S. sclerotiorum* is particularly severe in India due to favorable environmental conditions for the pathogen, such as moderate temperatures and high humidity during the flowering and podding stages. This not only reduces crop yields but also affects the quality of the harvested grains, leading to significant economic losses for farmers. To counter this disease, the biochar could be of great value because of its alkaline properties, porous texture, good water retention capability, disease suppression and enhancement of nutrient uptake by plants[5], improving chickpea growth and productivity under field conditions. Its stable carbon structure aids in long-term carbon sequestration, contributing to climate change mitigation. Studies have demonstrated direct positive effects of biochar on plant growth and root development. Incorporating biochar into agricultural systems offers a effective and ecofriendly approach that enhances plant productivity[6]. Also, certain types of biochar can reduce the growth rate of *S. sclerotiorum*[7], thus lowering the inoculum potential in the field, and directly impacting the survival of *S. sclerotiorum* in the soil. Sclerotia are the survival structures of the pathogen, and biochar amendments can reduce their viability by creating unfavorable soil conditions or through direct antimicrobial effects. The presence of biochar in soil can indirectly be beneficial for bacteria, fungi and other soil microbes because of its macropores, which act as a habitat for the beneficial microbes to grow and multiply. Biochar can also enhance plant defense mechanisms through the induction of systemic resistance. AL-Mayahi and his colleagues found that biochar application induced systemic resistance in soybean plants against *Fusarium solani*, leading to lower disease severity[8], indicating that disease suppression by biochar addition may involve complex interactions between soil amendments, microbial communities and plant defense responses. The combination of biochar and beneficial fungi helps crops to grow well under conditions that would otherwise favor disease development[9]. Such beneficial fungi include species of *Trichoderma* that have the ability to colonize plant roots and the surrounding soil, establishing a symbiotic relationship with the host plant. They have various mechanisms that are effective against plant pathogens, including direct parasitism, competition for nutrients and space, and the production of antifungal compounds. *Trichoderma* produces various secondary metabolites, such as enzymes, antibiotics and volatile organic compounds, which have antifungal and antibacterial properties. These substances inhibit the growth and activity of soilborne pathogens. *Trichoderma* also provides induce systemic resistance in plants, activating their defense mechanisms against pathogens. Also, *Trichoderma* has been found to stimulate plant growth, enhance nutrient uptake and improve soil health, offering a sustainable and environmentally-friendly approach to disease management in agriculture[10]. Various studies have highlighted the benefits of combining biochar and *Trichoderma* spp. for disease control and soil health improvement. For example, a recent study demonstrated that the combination of biochar and *Trichoderma harzianum* significantly enhanced plant growth and provided effective biocontrol against *Ralstonia solanacearum* in eggplants[11]. This shows that biochar is a beneficial soil amendment that supports the proliferation of beneficial microbes like *Trichoderma*, which further enhance plant resistance to pathogens. Also, another study demonstrated combining biochar with *T. harzianum* enhanced phytoremediation efficiency in cadmium and arsenic-contaminated soils[12], highlighting the synergistic effects of biochar and *Trichoderma* in improving soil health and plant growth. Biochar increases enzyme activity and total microbial quality of soil, thereby enhancing plant growth[13]. This research underlines the benefits of biochar in improving soil microbial communities and nutrient availability. Despite the known benefits of biochar in enhancing soil fertility and supporting microbial growth, there is limited research on its use as a carrier for *Trichoderma* in the context of managing *S. sclerotiorum* in chickpeas, especially the characteristic comparison of different types of biochar (e.g., wheat straw, organic kitchen waste and hardwood) in supporting *Trichoderma* populations and enhancing plant growth remains underexplored. This study analyzed the properties of different types of biochar, including nutrient content (organic C, and total N, P, K, Mg, and Ca), pH, and ash content, to determine their suitability as a carrier for *Trichoderma*; properties that could enhance its survival and efficacy against *S. sclerotiorum*. Also, investigating the population dynamics of *Trichoderma* in different biochar types and size grades over a six-week period to understand the long-term viability and effectiveness of biochar-*Trichoderma* combinations. Providing insights into the dual role of biochar in enhancing plant growth and disease suppression could contribute toward the development of sustainable, environmentally-friendly agricultural practices. This study is novel in its approach to evaluating biochar as a carrier for a biological control agent, specifically in the context of managing *S. sclerotiorum* causing *Sclerotinia* stem rot in chickpea. The findings have the potential to improve disease management strategies and promote sustainable chickpea production, thereby supporting food security and agricultural sustainability at a global stage.

2 Materials and methods

2.1 Isolation and purification of *Trichoderma*

For the isolation of *Trichoderma*, a soil sample was taken from the root zone of healthy chickpea plants from the Plant Pathology Research Field of Lovely Professional University, Punjab, India; rhizosphere-isolated *Trichoderma* spp. are likely to be more ecologically competent, meaning they can survive, proliferate and provide biocontrol more effectively when reintroduced into the same or similar soil environments[14], which is crucial for the practical application of *Trichoderma* in combination with biochar. For this, 1 g of soil was suspended in sterile water, serially diluted to 10^{-3}, spread onto rose bengal agar in 90 mm Petri dishes and incubated in an incubator at 27 °C for 96 h[15]. After the appearance of *Trichoderma*, a small section of the fungal mycelium was transferred to a Petri plate containing potato dextrose agar (PDA) and incubated to obtain a pure culture of *Trichoderma*.

2.2 Biochar preparation and testing from various sources

Three different types of biochar (wheat husk, hardwood and kitchen waste) were tested. The design used was a complete randomized design. Biochar was prepared by pyrolysis at a temperature of about 400 °C in a metal kiln. Wheat straw (WS), hardwood (HW) and organic kitchen waste (OW) are placed in the sun for 2–3 days to dry completely and then chopped into small sizes (25–50 mm) using a knife to increase surface area, facilitating uniform heating and conversion. The kiln was cleaned and dried before the process, and the bottom was filled with dry wood or firewood to facilitate burning. The kiln was filled with 6 kg of WH, making sure it was not overfilled allowing space for the air to circulate. The lid was closed leaving the air vent slightly open for controlled airflow. The dry wood at the bottom was ignited to initiate the pyrolysis of WH. The air vents were regulated for the process to proceed under limited oxygen conditions. The temperature was allowed to slowly reach 400 °C taking about 5 h to complete the pyrolysis and the kiln was cooled naturally, not opening it until it was completely cool to touch[16]. The process was repeated with OW and HW. The biochar obtained was crushed using a mortar and pestle and stored separately in a new plastic zip lock bag to prevent moisture exchange until tested for pH, electrical conductivity and organic carbon as well as various other quantitative tests such as lignin, cellulose and hemicellulose, the rest was then autoclaved to remove all contaminants for conducting microbial testing under *in-vitro* conditions[17]. The crushed biochar was graded using standard laboratory sieve at sizes, 250, 145, 75, and 45 μm. The sieved biochar was transferred to separate autoclavable bags, tightly sealed to prevent moisture, and then sterilized using an autoclave at 121 °C for 20 min for use in further experiments.

2.3 Biochar-PDA concentrations were prepared for microbial testing

For evaluating the effects of different concentrations of biochar; 1% biochar concentration was prepared by adding

0.5 g of 250 μm biochar in a 50-mL potato dextrox agar (PDA) mixed in a 100-mL Erlenmeyer flask, mix it properly to get an even consistency. 2% concentration is prepared by adding 1 g of biochar in 50 mL of PDA, 3% by adding 1.5 g of biochar in 50 mL PDA, 4% by adding 2 g of biochar in 50 mL of PDA, and lastly 5% by adding 2.5 g of biochar in 50 mL of PDA. Repeat the above procedure to obtain biochar-PDA concentrations of other grade (150, 75, and 45 μm) of biochar. The different concentration obtained was further autoclaved and chloramphenicol tincture added to prevent bacterial contamination.

2.4 Determination of nitrogen

Nitrogen was determined by the Kjeldahl method[18]. A 0.5-g sample of crushed biochar was placed in a Kjeldahl digestion flask and 10 mL of concentrated sulfuric acid and a catalyst mixture (1 g of copper sulfate and 10 g of potassium sulfate) added. The flask was gently heated at first, then the temperature was increased until the contents become clear. Distillation preceded until all ammonia was been transferred. The distillate was collected in the receiving flask and few drops of methyl red-bromocresol green mixed indicator added. The solution was then titrated with standard 0.1 mol·L^{-1} hydrochloric acid until the green color changed to a faint pink. The volume of HCl added was recorded and Nitrogen content is calculated as:

$$\text{Nitrogen} = \frac{V_{\text{HCl}} \times N_{\text{HCl}} \times 14.01}{W_{\text{sample}}} \times 100\% \qquad (1)$$

where, V_{HCl} is volume of HCl (in L), N_{HCl} is the normality of HCl (0.1 mol·L^{-1}), W_{sample} is weight of biochar sample and 14.01 is the atomic weight of nitrogen.

2.5 Determination of carbon

Carbon content in biochar was determined by the loss on ignition method that indirectly estimates carbon content by measuring the weight loss after combustion at high temperature[19]. Initially, finely crushed biochar was placed in a hot air oven to remove all moisture. Then a 1 g sample of dried biochar was placed in the crucible recording the weight of the crucible. A muffle furnace was used to heat the crucible at 375 °C for 16 h to burn off the combustible matter, leaving only the fixed carbon and ash. The difference in weight before and after ignition was then calculated, the percentage weight loss and further calcination at 550°C was performed to estimate the ash content[20], which was used to adjust the carbon estimate.

2.6 Determination of phosphorus

Phosphorus content in a biochar sample was determined by the molybdenum blue method, which uses colorimetric determination of phosphorus after acid digestion[21]. A 0.5-g sample of finely crushed biochar was taken and placed in a digestion flask with 10 mL of concentrated sulfuric acid and 5 mL of concentrated nitric acid. The flask was then placed on a heating mantle and heated until the solution became clear or pale yellow, indicating completed digestion. After cooling, a few drops of hydrogen peroxide was added to the flask and heated until the solution was clear. Once cooled, distilled water was added to dilute the digested solution to 100 mL, which was then filtered (Whatman No. 42). 10 mL of the filtered sample is mixed with 4 mL of ammonium molybdate solution, 2 mL of ascorbic acid solution and 1 mL of potassium antimonyl tartrate solution. This mixture was allowed to rest until a blue color developed indicating the formation of the phosphomolybdenum blue complex. A spectrophotometer was used to measure the absorbance of the blue sample at 880 nm with distilled water as a blank. A calibration curve was prepared using phosphorus standards and plot absorbance against concentration. The phosphorus content was calculated as:

$$\text{Phosphorus} = \frac{\text{Concentration from curve (mg/L)} \times \text{Total volume of digest (mL)}}{\text{Weight of biochar sample (g)}} \times 100\% \qquad (2)$$

2.7 Determination of potassium, calcium, and magnesium

Concentrations of potassium, calcium and magnesium in biochar were determined using atomic absorption spectroscopy[22]. For this, dried and finely powdered biochar was added to a digestion vessel. 10 and 5 mL of concentrated nitric acid and hydrochloric acid, respectively, were added to the sample. The digestion tube was then heated on a hot plate until the mixture turned pale yellow. After l cooling, the digestate was diluted to 100 mL with deionized water in a volumetric flask and filtered (Whatman No. 42) into a clean volumetric flask to remove any remaining undissolved materials. A series of standard solutions for K, Ca, and Mg (e.g., 0, 1, 5, 10, and 20 mg·L^{-1}). These standards were used to generate calibration curves. Absorbance of each standard was measure at the respective wavelengths for K (767 nm), Ca (423 nm), and Mg (285 nm), with deionized water used as a blank. The final concentrations as mg·kg^{-1} or % by weight were calculated as:

$$\text{Element} = \frac{\text{Concentration from ASS (mg/L)} \times \text{Final volume of digest (L)}}{\text{Weight of biochar sample (g)}} \times 100\% \tag{3}$$

2.8 Determination of cellulose

Cellulose was determined using an acid hydrolysis method[23]. Biochar was crushed and then dried to remove any moisture. A 0.5-g sample was added to an Erlenmeyer flask with and 5 mL of 72% sulfuric acid and gently mix by swirling. The flask was placed it in a water bath for 1 h at 30 °C with occasional mixing. The digested as diluted to a 4% solution with distilled water and autoclaved at 121 °C for 1 h. This was then filtered (Whatman No. 1) and the residue washed using distilled water. Using a glucose standard, 1 mL of hydrolysate, 1mL of 5% phenol, and 5 mL of concentrated sulfuric acid were combined and incubated for 30 min at 30 °C. The absorbance of the sample and the standard were measured at 490 nm. The glucose concentration was converted to cellulose as:

$$\text{Cellulose} = \frac{\text{Glucose concentration} \times 0.9 \times \text{Dilution factor}}{\text{Sample weight}} \times 100\% \tag{4}$$

where, 0.9 is the the conversion factor for glucose to cellulose.

2.9 Determination of lignin

Lignin was determined by acid hydrolysis to removing carbohydrates, followed by gravimetric analysis. This process generally yields both acid-insoluble lignin (AIL) and acid-soluble lignin (ASL). A 0.5-g sample of dried biochar sample was hydrolyzed in sulfuric acid. After cooling to room temperature, the hydrolysate was filtered (Whatman No. 42) and the residue is dried to constant weight. To calculate AIL%, the dried weight of residue was recorded before heating it in muffle furnace at 575 °C for 4 h, leaving only ash. which was weighed. Similarly, ASL% was calculated by collecting the filtrate from hydrolysis containing acid-soluble lignin and measuring its absorbance at 205 nm using a UV-Vis spectrophotometer. The calculations were as follows, with AIL% and ASL% were combined to obtain total lignin percentage[24].

$$\text{AIL} = \frac{\text{Weight of dried residue} - \text{Weight of ash}}{\text{Sample weight}} \times 100\% \tag{5}$$

$$\text{ASL} = \frac{\text{Absorbance} \times \text{Volume of filtrate} \times \text{Dilution factor}}{\text{Sample weight} \times \text{Extinction coefficient at 205 nm} \times \text{Path length}} \times 100\% \tag{6}$$

2.10 Determination of hemicellulose

Hemicellulose was determined using the acid hydrolysis method[23]. A 0.5-g sample of finely crushed and dried biochar was added to 5 mL of 72% sulfuric acid and placed in a water bath at 30 °C for 1 h with occasional mixing. The mixture was diluted to 4% with distilled water and autoclaved at 121 °C for 1 h. After cooling, the hydrolysate was filtered (Whatman No. 1) to remove the any residues. Standard solutions of xylose, mannose, arabinose and galactose were prepared at 0.1, 0.2, 0.5, 1, and 2 g·L^{-1}. The hydrolysate and standards were assayed by high-performance liquid chromatography with Animex HPX-87H ion exchange column and refractive index detector at 60°C to we determine the total hemicellulose content as:

$$\text{Hemicellulose} = \frac{\text{Sum of sugar concentration} \times \text{Dilution factor}}{\text{Sample weight}} \times 100\% \tag{7}$$

2.11 Growth of *Trichoderma*

For biochar-PDA concentration (1%, 2%, 3%, 4%, and 5%) was poured into three replicate 100 mm borosilicate glass Petri dishes and a 10-mm plug of *Trichoderma* mycelium was placed in the center of the dish. The dishes incubated at 27 °C for 48 h and the growth diameter of the fungus recorded.

2.12 Inhibition of *Sclerotinia sclerotiorum*

The inhibitory effect of biochar was tested using the soilborne pathogen *S. sclerotiorum* using three replicates of five concentrations of biochar-PDA media (1%, 2%, 3%, 4%, and 5%) and a control in Petri dishes. Seven mm plugs of *S. sclerotiorum* mycelium were placed in the center of the plates sealed with Parafilm and incubated at 25 °C of 96 h. Growth of mycelium was recored after 36, 72, and 96 h.

2.13 Estimation of leaf phenolics

Total phenolic content in leaf samples were estimated by a Folin-Ciocalteu reagent method[25]. Leaf samples were collected from the field avoid contamination by soil or debris. The leaves were dried overnight at 40 °C and dry weight recorded. Samples (1.0 g) of dried leaf were crushed in a mortar and pestle to a fine powder, then transferred to 80% ethanol in 10 mL centrifuge tubes. These were kept at room temperature for 1 h or placed it in a water bath at 27 °C then centrifuged for 10 min at 13,000 r·min^{-1}. 1 mL of supernatant was transferred to sterile borosilicate glass test tubes along with 5 mL of distilled water and 0.5 mL of 50% Folin-Ciocalteau's reagent.

This solution was then gently mixed and kept for few minutes in the dark to react. 1 mL of 5% sodium carbonate was then added, the mixture vortexed for a few seconds and the left for 1 h in the dark. Its absorbance was measured using a UV-Vis spectrophotometer at 725 nm with ethanol as a blank. Gallic acid (GA) in 80% ethanol was used as a standard. The total phenol content as GA equivalents (μg GAE per g leaf) was calculated as:

$$\text{Total Phenol} = \frac{\text{Concentration from standard curve} \times \text{Volume of extract}}{\text{Sample weight}} \quad (8)$$

2.14 Effect of biochar on the growth of chickpea in the field

A field experiment was conducted with chickpea cv. GF 89-133. A randomized block design was used, with three replications and eight treatments including a control. The treatments included were T1 (HW biochar), T2 (OW biochar), T3 (WS biochar), T4 (HW biochar + *Trichoderma*), T5 (OW biochar + *Trichoderma*), T6 (WS biochar + *Trichoderma*), T7 (*Trichoderma*), and T8 (control). Each treatment plot was 3 m× 2 m, with three 1 m wide irrigation channels between the plots, covering a total area of about 160 m^2. Each plot consisted of 5 rows of chickpea with 40 cm of row spacing, and plant-to-plant spacing of 15–20 cm, making about 80–90 chickpea plant per plot. The biochar and *Trichoderma* treatments were applied at a rate of 5 kg per plot, 7 days before sowing the chickpea.

2.15 Determining population of *Trichoderma*

A pure culture of *Trichoderma* was initially incubated at 27 °C for 12 h to ensure active growth and used to inoculate PDA plates supplemented with one of three biochar types that had been sterilized before addition to the medium at 1% concentration each (0.5 g of biochar in 100 mL PDA). The biochar treatments were: HW biochar + *Trichoderma* (T4), OW biochar + *Trichoderma* (T5), and WS biochar + *Trichoderma* (T6). These cultures were then incubated in a at 27 °C for 1, 2, and 10 weeks after inoculation (WAI) to monitor the *Trichoderma* population over time[26]. The population of *Trichoderma* was quantified by counting colony-forming units (CFUs) on the biochar-supplemented PDA plates. This was done by serial diluting the biochar-*Trichoderma* mixtures and plating them onto fresh PDA containing biochar. CFUs were counted after incubation at 27 °C for 48 h and the sampled population calculated. The populations recored for the biochar treatments were compared to determine which biochar type was most conducive for *Trichoderma* growth. This methodology allowed for an evaluation of the synergistic effects of biochar and *Trichoderma* on microbial population stability and proliferation over extended periods.

2.16 Statistical analysis

All determinations were repeated twice. The data sets were analyzed using the IBM SPSS software 22, and by analysis of variance in the R statistical package (version 4.4.1). Means of mycelial growth for *Trichoderma* and *S. sclerotiorum*, growth inhibition, population dynamics, shoot length, disease incidence, root mass and phenol content were compared using Duncan’s new multiple range test using R package “agricole”. A *P*-value of less than 0.05 was deemed to be statistically significant.

3 Results

3.1 Biochar characteristics

The properties of the biochar materials used in this study are presented in Table 1. The lignin content was highest in HW (31.3%), followed by WS (22.6%) and OW (3.1%), which contained the lowest. Hemicellulose ranged between 22.9% and 27.4%, whereas cellulose varied from 24.4% to 34.5%, with HW having a highest amount of both. Lignin and cellulose are two important components of biomass that can significantly impact the properties and performance of biochar. HW had a higher concentration of lignin, which is a complex polymer that

Table 1 Cellulose and hemicellulose content, and C/N ratio of various organic materials used to make biochar

Type of biochar	Percentage (%)		
	Lignin	Cellulose	Hemicellulose
Hardwood	31.3	34.5	27.4
Organic waste	3.10	24.4	24.7
Wheat straw	22.6	31.1	22.9

provides rigidity and strength to plant cell walls and is typically more resistant to degradation compared to cellulose and hemicellulose. In biochar production, lignin tends to decompose at higher temperatures, which can result in the formation of more aromatic and stable carbon structures in the resulting biochar. This can lead to increased carbon sequestration potential and longer residence time in soil[27]. Cellulose content is also higher in HW biochar; this increased porosity can enhance water and nutrient retention in soil, which can benefit plant growth and productivity[28]. Overall, high lignin and cellulose content in biomass can lead to the formation of biochar with improved stability, porosity, and nutrient retention properties, which can benefit soil health and promote beneficial microorganisms as well as plant growth[29].

The properties of the biochar produced by the pyrolysis process are given in Table 2. The organic carbon content varied, the highest was in HW biochar (77.9%) and lowest in WS biochar (66.9%). WS biochar had the lowest pH, making it more conducive to fungal growth. Physicochemical changes in biochar can have an impact on microbes both directly and indirectly. In terms of microbial abundance, pH levels are crucial[30]. In contrast to weak acid conditions, slightly alkaline or neutral conditions are more favorable for fungal growth[30,31].

Trace amounts of micro- and macronutrients (C, N, P, K, Ca, and Mg) in biochar can support the growth of beneficial microorganisms, including *Trichoderma*, and can act as a slow-release fertilizer, improving soil fertility and plant health. This microbial activity is essential for nutrients cycling and can lead to improved soil fertility. The type and quantity of biochar applied to soil can have an impact on the microbial communities there. When it comes to ash content HW biochar had the lowest amount, while WS biochar had the highest. The ash content in biochar is an important factor that can influence its suitability and effectiveness in agricultural applications. Whether higher ash content is suitable depends on the specific context and intended use of the biochar.

3.2 Effect of biochar on *Trichoderma*

The three types of biochar studied as carriers for the growth of *Trichoderma* all supported *Trichoderma* growth, being it significantly higher in HW biochar, followed by OW biochar (Table 3). Also, *Trichoderma* population was consistent for all biochar sources at 1 WAI, but the population doubled by 2 WAI. HW biochar had the highest population, 33.5 × 10^5 CFU·g^{-1} *Trichoderma* by 6 WAI, closely followed by OW biochar.

3.3 Comparative analysis of *Trichoderma* and *Sclerotinia sclerotiorum* against biochar

3.3.1 Growth of *Trichoderma* with respect to different concentration and mesh size of biochar

The use of HW biochar as a growth medium for *Trichoderma*

Table 2 Properties of biochar and its composition: determination of organic C, N, P, K, Ca, Mg, and ash content from different biochar sources

Type of biochar	pH	Composition of biochar in percentage (%)							
		N	C	C/N	Ash content	P	K	Ca	Mg
Hardwood	7.80	2.37	77.9	32.9	1.38	0.22	4.20	1.89	0.57
Organic waste	8.20	0.76	71.7	94.4	6.61	0.89	1.91	7.77	1.19
Wheat straw	7.40	1.28	66.9	52.2	13.3	0.15	2.01	0.32	0.37

Table 3 Average number of fungi population at 2, 6 and 10 weeks after inoculation (WAI)

Treatments	Population (× 10^5 CFU·g^{-1})		
	1 WAI	2 WAI	6 WAI
HW biochar + *Trichoderma*	5.87 ± 0.06^{c}	12.1 ± 0.37^{c}	33.5 ± 0.06^{d}
OW biochar + *Trichoderma*	5.62 ± 0.02^{bc}	11.9 ± 0.33^{bc}	31.6 ± 0.10^{c}
WS biochar + *Trichoderma*	5.57 ± 0.32^{ab}	11.2 ± 0.25^{b}	30.0 ± 0.13^{b}
Trichoderma	4.92 ± 0.15^{a}	10.7 ± 0.22^{a}	22.6 ± 0.13^{a}

Note: Means followed by the same letter within columns are not significantly different according to Duncan's test ($P < 0.05$); ± represents SD.

was suitable across various concentrations at a 150-µm mesh size. As shown in Fig. 2, the addition of biochar at 1%, 2%, 3%, 4%, and 5% provided significant growth of *Trichoderma* across all the tested mesh sizes. The best results were with 250 and 150 µm, as evident in Fig. 1(a) (I and II). In contrast, the 45 µm gave the least growth, as evident in Fig. 1(a) (IV). Importantly, 1% biochar supported the highest *Trichoderma* growth with the 150 µm mesh size, whereas 5% biochar supported minimal or no growth across all mesh sizes (Fig. 1(a)). This indicates that as the concentration of biochar increases, the growth of *Trichoderma* decreased across the various mesh sizes (Fig. 2). This finding highlights the importance of optimizing both biochar concentration and mesh size to maximize the growth and efficacy of *Trichoderma*.

3.3.2 Growth of *Sclerotinia sclerotiorum* with respect to different biochar concentrations and mesh sizes

The data presented in Table 4 indicates that varying concentrations of HW biochar, particularly at 150 µm, effectively inhibited the growth of *S. sclerotiorum*. Specifically, 4% and 5% biochar gave the greatest *S. sclerotiorum* suppression, as evident in Figs. 3 and 4. It is important to note that the choice of feedstock significantly influences the properties of biochar, potentially more so than the pyrolysis temperature[32]. Hence in this study, the use of 150 µm HW biochar at the 4% and 5% had a clear negative effect on the growth of *S. sclerotiorum*[33].

3.4 Comparative analysis

The results indicate that HW biochar, when applied at specific concentrations, had a dual effect; it inhibits the growth of the *S. sclerotiorum* while simultaneously promoting the growth of *Trichoderma*. This finding was particularly evident for 4% HW biochar at 150 µm size providing the most effective inhibition for *S. sclerotiorum* and promotion of *Trichoderma*, as evident in Fig. 5. These results indicate that HW biochar has the potential to be a useful material for integrated disease management, specifically for controlling *Sclerotinia* stem rot in chickpeas. The ability of HW biochar to simultaneously suppress pathogen and support bioagent growth highlights its potential as a dual-purpose soil amendment.

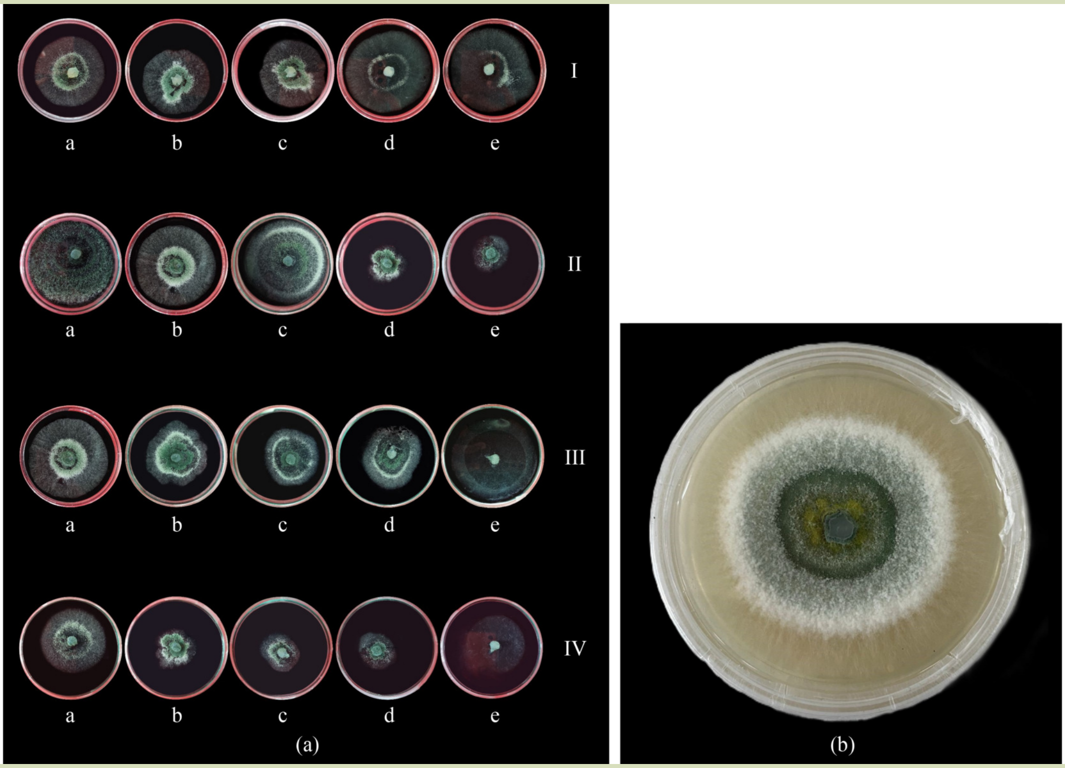


Fig. 1 (a) Growth of *Trichoderma* in combination with biochar in different size grades. I, 250 µm; II, 150 µm; III, 75 µm; and IV, 45 µm size; a–e are the 1%, 2%, 3%, 4%, and 5% biochar in PDA media, respectively. (b) Control growth of *Trichoderma* after 48 h of incubation.

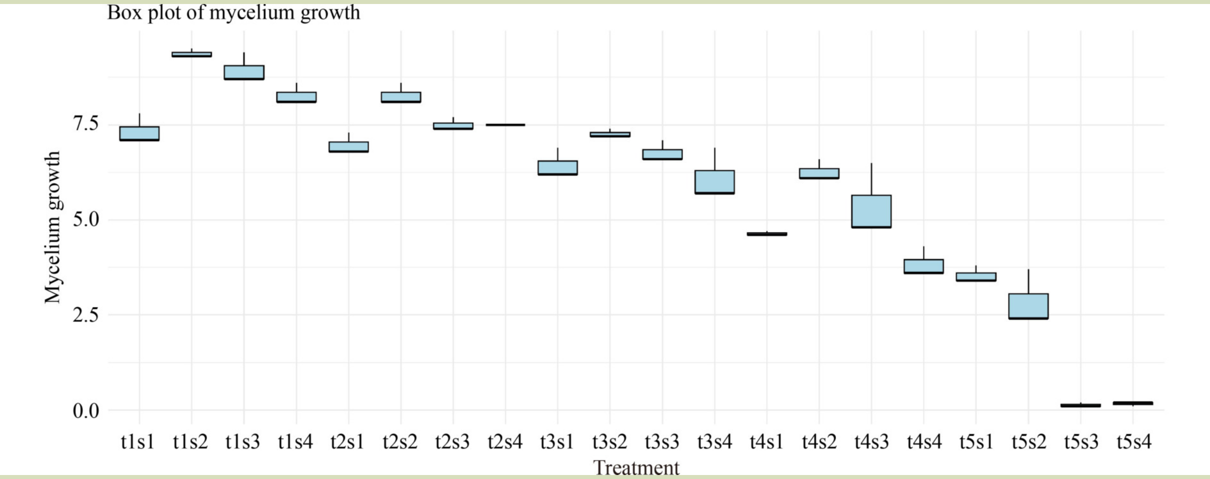


Fig. 2 Growth of *Trichoderma* with different biochar proportions and size grades. t1–t5 are biochar at 1%, 2%, 3%, 4%, and 5%, respectively; and s1–s4 are for mesh sizes of 250, 150, 75, and 45 µm, respectively.

Table 4 Effect of biochar on *Sclerotinia sclerotiorum*

Biochar proportion (%)	Growth at three times (cm)			
	36 h	72 h	96 h	Average
1% (0.5 g biochar in 50 mL PDA)	1.8	3.4	6.7	3.9
2% (1 g biochar in 50 mL PDA)	2.0	3.8	9.0	4.9
3% (1.5 g biochar in 50 mL PDA)	3.7	4.7	7.0	5.1
4% (2 g biochar in 50 mL PDA)	1.9	2.2	2.5	2.2
5% (2.5 g biochar in 50 mL PDA)	1.6	2.0	2.4	2.0
Control	1.7	2.8	5.9	3.5

Note: This table shows the growth of *S. sclerotiorum* at different concentrations of HW biochar in potato dextrose agar (PDA).

3.5 Field analysis

3.5.1 Shoot length and root mass

The results shown in Table 5 reveal that the application of HW biochar (T1) and the combination of HW biochar with *Trichoderma* (T4) significantly improved chickpea shoot length and root dry mass of compared to the control. The root dry mass was highest in T1 (4.82 g) and T4 (4.63 g), and similar trends were observed for shoot length (Table 5). These findings support the hypothesis that biochar, particularly HW biochar, and its combination with *Trichoderma*, can enhance plant growth, including shoot length and root mass. The increased root dry mass observed in T1 and T4 is consistent with the reported effects of *Trichoderma* on plant growth promotion and root architecture[13,34]. *Trichoderma* species are recognized for their ability to stimulate biomass production and lateral root growth through auxin-dependent mechanisms[35]. This process involves the modulation of auxin response pathways, which are crucial for root formation[14].

3.5.2 Total phenol

Phenolic compounds in plants serve as strong indicators of plant defense response to stress, including pathogen infection[36]. These compounds are measured by quantifying their concentration in plant tissues and comparing them to a reference sample, such as in ethanol. This comparison helps to assess whether the plant is increasing or decreasing phenol production in response to the stress factor under study[37]. In this study, the phenol content was quantified from the leaves of randomly tagged plants across different treatments. The combination treatments of biochar and *Trichoderma* (T1, T2, T3, T5, and T7) had higher phenol content, with T4 (HW biochar + *Trichoderma*) having the highest concentration of 480 $\mu g \cdot g^{-1}$ GAE, compared to the control, which showed an

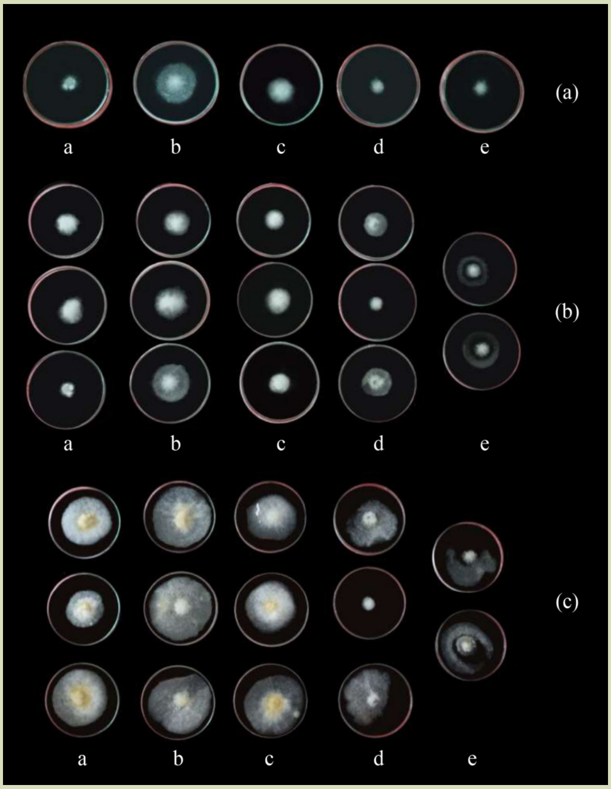


Fig. 3 Growth of *Sclerotinia sclerotiorum* at different concentrations on 150 µm mesh hardwood biochar. (a–c) The growth of *S. sclerotiorum* after 36, 72, and 96 h of incubation, respectively; a–e are 1%, 2%, 3%, 4%, and 5% biochar in PDA media, respectively.

average of 408 µg·g^{-1} GAE (Table 5). This increase in phenol concentration indicates that the biochar and *Trichoderma* combination not only inhibits pathogen development but also enhances plant defense mechanisms, allowing it to better tolerate stress conditions[38]. Plant phenolic compounds act as signaling molecules and possess antimicrobial properties[39]. In this study, the total phenolic concentration was significantly higher in plants treated with a combination of *Trichoderma* and HW biochar compared to all other treatments.

3.5.3 Diisease incidence

The combination of *Trichoderma* with biochar significantly suppressed disease incidence in field under all treatments (Table 5). Of the tested combinations, HW biochar with *Trichoderma* provided the greatest disease suppression, with the lowest disease incidence of 44.8% (Table 5). Also, plants treated with this combination also had the highest phenol content compared to other treatments. This elevated phenol content suggests that the HW biochar and *Trichoderma* combination may induce biochemical changes within the plant, which in turn could enhance its defense mechanisms and significantly limit the development of *S. sclerotiorum*.

4 Discussion

This study explored the potential of biochar, particularly HW biochar, as a carrier material for the biocontrol agent *Trichoderma* in managing *S. sclerotiorum* in chickpea crops.

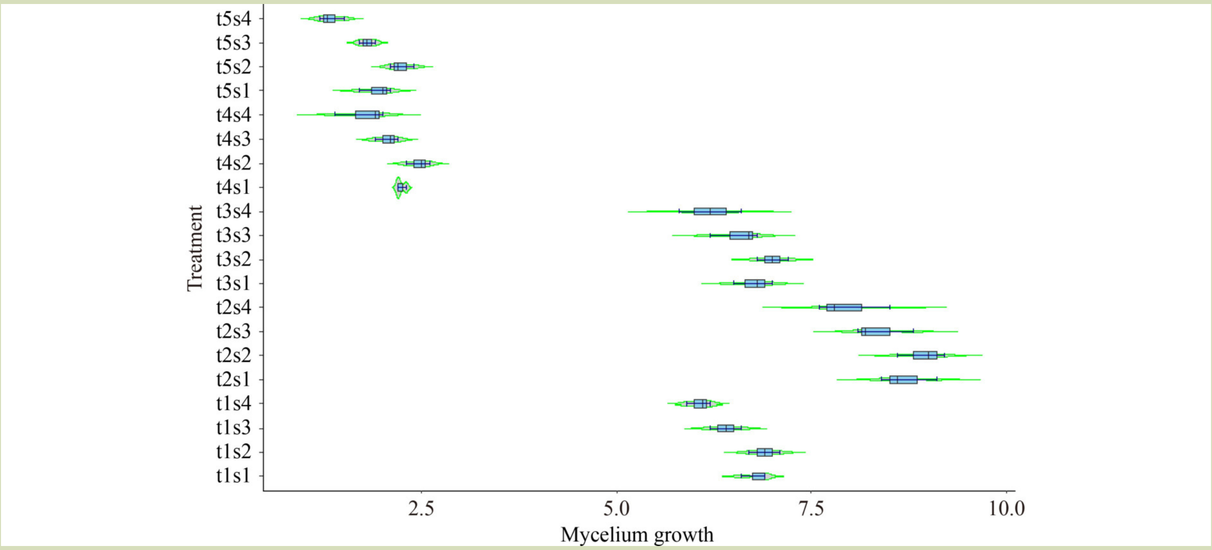


Fig. 4 Growth of *Sclerotinia sclerotiorum* with different size grades of hardwood biochar. t1–t5 are biochar at 1%, 2%, 3%, 4%, and 5%, respectively; and s1–s4 are for mesh sizes of 250, 140, 75, and 45 µm, respectively.

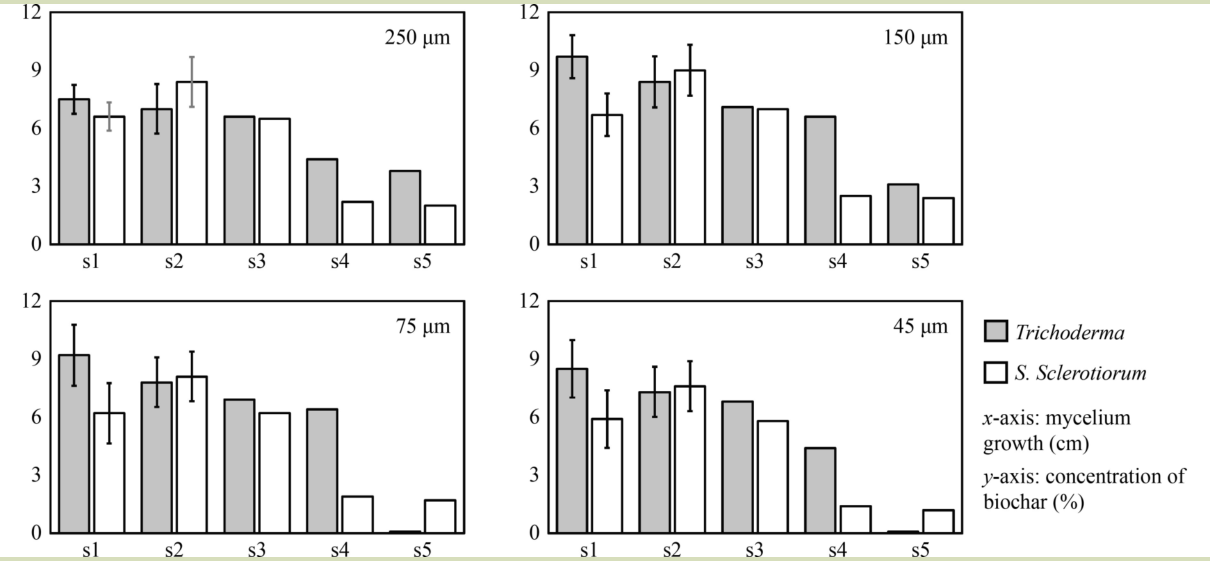


Fig. 5 Comparative growth of both *Trichoderma* and *Sclerotinia sclerotiorum* with five concentrations of hardwood biochar. s1–s5 is hardwood biochar at 1%, 2%, 3%, 4%, and 5%, respectively.

Table 5 Shoot length, root mass, disease incidence and total phenol content of chickpea grown in the field

Treatments	Shoot length per plant (cm)				Average DI (%)	Average root mass per plant (g)	Phenol (μg·g^{-1} GAE)
	30 days	60 days	90 days	Average			
T1 (HW biochar)	12.2	26.4	45.5	28.2 ± 16.9	45.5 ± 4.74^{ab}	4.7 ± 0.08^{e}	0.47 ± 0.01^{b}
T2 (OW biochar)	12.4	25.5	44.7	26.2 ± 15.7	52.2 ± 3.41^{bc}	4.2 ± 0.07^{b}	0.45 ± 0.01^{ab}
T3 (WH biochar)	12.5	24.9	42.5	26.2 ± 15.1	48.1 ± 3.07^{ab}	4.5 ± 0.07^{cd}	0.45 ± 0.01^{ab}
T4 (HW + *Trichoderma*)	12.5	25.3	45.6	28.1 ± 16.7	44.8 ± 4.42^{a}	4.6 ± 0.06^{de}	0.48 ± 0.01^{b}
T5 (OW + *Trichoderma*)	11.8	25.7	44.6	26.4 ± 16.7	57.6 ± 4.34^{cd}	4.3 ± 0.04^{b}	0.45 ± 0.01^{ab}
T6 (WH + *Trichoderma*)	12.1	26.1	44.9	26.7 ± 16.5	51.0 ± 2.33^{abc}	4.4 ± 0.09^{bc}	0.45 ± 0.02^{ab}
T7 (*Trichoderma*)	11.8	25.5	45.2	26.2 ± 14.7	52.3 ± 4.45^{bc}	4.2 ± 0.24^{b}	0.43 ± 0.01^{ab}
Control	11.1	22.1	40.8	24.7 ± 15.0	61.2 ± 2.91^{d}	3.7 ± 0.30^{a}	0.40 ± 0.06^{a}

Note: Means followed by the same letter within columns are not significantly different according to Duncan's test ($P < 0.05$); ± represents SD.

The results demonstrated significant disease suppression and enhanced plant growth, with HW biochar in combination with *Trichoderma* providing the most promising outcomes. The implications of these findings are significant for sustainable agriculture, particularly for chickpea production. The combination of HW biochar and *Trichoderma* resulted in a notable reduction in the incidence of *S. sclerotiorum*, with disease incidence dropping to 44.8%. This suppression is likely due to the synergistic effects of *Trichoderma* and biochar, where biochar provides a conducive habitat for *Trichoderma* to grow and effectively colonize the soil and plant roots. Similarly, macropores in biochar are also connected to the microbial population. Generally, fungi survive in these macropores and most microbial populations, such as bacteria, fungus, actinomycetes and algae ranging from 0.5 to 5 μm in size[40]. It was already known that the macropores of biochar serve as excellent homes for a variety of microorganisms by protecting them from predators and water deficit, supplying the fungus with carbon for energy, and providing mineral nutrients[41]. Depending on the molecules remaining after pyrolysis, biochar can serve as a substrate for microbes[9,42]. Ash contains essential minerals, including P, K, Ca, and Mg, which can benefit plant growth[43]. Higher ash content in biochar can increase the availability of these nutrients in the soil, acting as a slow-release fertilizer[44]. However, in some cases, particularly in soils that are already neutral or alkaline, high ash content can lead to excessive alkalinity. This can limit the availability of certain micronutrients, such as Fe and Mn, which are more available in slightly acidic soils[43]. Depending on the source material, higher ash content could also mean higher levels of unwanted substances, including heavy metals, which could be detrimental to soil health and plant growth if not properly managed[45].

The increase in *Trichoderma* population in the different biochar treatments followed a clear trend. Initially, the population was stable to 1 WAI for all biochar sources, but a significant increase was observed by 2 WAI, with populations doubling at this stage. Notably, HW biochar with *Trichoderma* gave the greatest increase, indicating its superior capacity for supporting *Trichoderma* colonization[46]. Biochar has been recommended as a carrier for beneficial microbes in a soil ecosystems[47] and *Trichoderma* can use biochar as a habitat for development and a source of nutrition, so it can be used as a suitable carrier[48]. Also, biochar may serve as a source of organic C and energy for fungi. However, this is greatly influenced by the type of biochar as well as the quantity of organic molecules present[49].

The increased phenol content observed in chickpeas treated with this combination supports the hypothesis that biochar, in conjunction with *Trichoderma*, induces systemic resistance in plants as reported by Ahmad et al.[11]. Phenols are crucial compounds in plant defense, acting as antimicrobial agents and signaling molecules that activate various defense pathways[39]. The findings of the present study are consistent with previous research, which has shown that biochar can enhance soil health and promote plant growth by improving soil structure, nutrient retention and microbial activity[50,51]. However, this present study adds a new dimension by demonstrating the specific benefits of biochar in managing plant diseases when used as a carrier for biocontrol agents. The improved growth and root mass in chickpea plants treated with HW biochar and *Trichoderma* further underscore the dual benefits of this approach, which not only controls disease but also promotes overall plant health. Another new aspect of this work was to determine how efficiently concentrations and size grades of biochar could support this dual functionality, from promoting the growth of bioagent to suppression of plant pathogen, thus we found that 4% HW biochar of 150 μm effectively inhibits the growth of the pathogen *S. sclerotiorum* while greatly promoting the growth of the beneficial *Trichoderma*.

Previous studies have primarily focused on the use of biochar for soil improvement and carbon sequestration, with limited emphasis on its role in disease management. However, recent research has begun to explore the potential of biochar in enhancing the efficacy of biocontrol agents. For example, a recent study demonstrated that biochar can suppress several soilborne pathogens by altering soil microbial communities and enhancing beneficial microbes, including *Bacillus* and *Pseudomonas*[52]. The present study builds on this by providing empirical evidence that HW biochar at specific concentrations and size grades can significantly enhance *Trichoderma* growth and activity, leading to effective suppression of *S. sclerotiorum* under field conditions. This research also aligns with the findings of multiple studies[13,53,54], which report that biochar application can inhibit pathogen growth while promoting the activity of beneficial soil microbes. One of these studies found that green waste biochar, particularly at 6% concentration, significantly reduced disease severity, especially when combined with *B. subtilis*, which enhanced disease suppression by up to 80%. That research highlights the synergistic potential of biochar and *B. subtilis* in managing foliar pathogens and improving plant physiology[53]. The increased phenol content observed in the present study indicates that the biochar-*Trichoderma* combination triggers plant defense mechanisms, a hypothesis supported by previous studies on the role of phenolic compounds in plant defense[48].

The practical implications of the present research are significant for chickpea production, particularly in regions prone to *Sclerotinia* stem rot. The use of HW biochar in combination with *Trichoderma* offers a sustainable and effective strategy for managing this disease while also promoting plant growth. The dual benefits of disease suppression and enhanced plant biomass make this approach particularly attractive for farmers seeking to reduce reliance on chemical fungicides and improve crop yields. The findings suggest that the application of 4% HW biochar of 150 μm size is optimal for disease suppression and plant growth promotion. This information can guide farmers in the practical application of biochar and *Trichoderma* in chickpea fields, potentially leading to more resilient crops and higher yields.

While the results of the present study are promising, there are several limitations that should be acknowledged. First, the study focused on a specific combination of biochar and *Trichoderma* in a controlled field setting. The effectiveness of this approach may vary under different environmental conditions, soil types and crop varieties. Future research should investigate the long-term effects of biochar and *Trichoderma* combinations on soil health, microbial diversity and plant disease resistance across a range of agroecological zones. Another limitation is the potential variability in biochar quality, which is influenced by feedstock type, pyrolysis conditions and post-production handling. Further research is needed to standardize biochar production methods to ensure consistent quality and efficacy. Additionally, exploring the effects of different biochar types, concentrations, and application methods on a wider range of crops and pathogens would provide a more comprehensive understanding of the potential of biochar in sustainable agriculture.

The present study provides valuable insights into the role of biochar as a carrier for biocontrol agents in disease management. The findings solidify that HW biochar in combination with *Trichoderma* is a promising strategy for managing *S. sclerotiorum* in chickpea production, offering both disease suppression and growth promotion benefits. Further research is needed to optimize this approach and explore its broader applications in agriculture.

5 Conclusions

This study successfully demonstrated that HW biochar can significantly enhance the growth of *Trichoderma* while simultaneously inhibiting the pathogen *S. sclerotiorum* in chickpea production. The most effective combination was found to be 4% HW biochar of 150 μm, which not only reduced disease incidence but also promoted plant growth and increased phenol content, suggesting enhanced plant defense mechanisms. These findings indicate that HW biochar has considerable potential as a dual-purpose soil amendment, providing both disease suppression and microbial growth promotion in an integrated disease management strategy. However, for agricultural practices, it is recommended that HW biochar be applied at optimal concentrations and size grade to maximize its efficacy in disease control and plant growth promotion. Specifically, 4% HW biochar should be considered as a standard for managing *Sclerotinia* stem rot in chickpeas, with potential adaptability to other crops.

Despite the promising results, this research also highlights areas needing further research. The long-term effects of biochar on soil health, nutrient cycling and microbial dynamics require more in-depth study. Additionally, the variability in biochar properties due to different feedstocks and pyrolysis conditions require the development of standardized production protocols. Future research should also investigate the interactions between biochar and other biocontrol agents or organic amendments across diverse agricultural environments. Addressing these gaps will be crucial for advancing the use of biochar in sustainable agriculture and optimizing its benefits for plant disease management.

Acknowledgements

We are thankful to everyone who has helped us over the course of our research journey. A great help came from Ms. Suman (laboratory officer in-charge) for providing us with all the necessary equipment for the conduction of experiments; a warm appreciation to all our fellow colleagues for helping us out with the field work and data collections; and last but not least, we are very thankful to the Department of Plant Pathology, School of Agriculture, Lovely Professional University for providing us the platform to work on and encouraging us to promote our research ideas onto a global avenue and Researchers from The Ecole Nationale d' Agriculture de Meknès, Morocco for their contribution to this work.

Compliance with ethics guidelines

Prashant Paveen, Vipul Kumar, Prahlad Masurkar, Devendra Kumar, Amine Assouguem, Chandra Mohan Mehta, and Rachid Lahlali declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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