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Synergistic effect of rice straw biochar and *Trichoderma harzianum* for sustainable mitigation of chickpea collar rot

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Contribution Conceptualization of study and data collection was performed by **Raza, U.**, **M. Abdullah** and **M. A. Siddique**. **A. Akhter, W. Anwar** contributed towards data analysis and supervision of study. **N. Ali, H. Arshad, H. M. Tariq, M. Z. Ullah** aided in methodology development, performing experiments and manuscript formatting.

ABSTRACT

Chickpea is a versatile crop with respect to its adaptability to different growing regions from temperate to tropical and serve as a crucial food source for both humans and animals. Collar rot (*Sclerotium rolfsii)* is a destructive soil-borne disease that lead to significant yield losses to chickpea. The wide spread use of chemical pesticides in controlling this disease present dangerous effects to the environment and health, making it essential to provide some sustainable alternatives. The current study assessed the combined application of *Trichoderma harzianum* and rice straw biochar for mitigation of collar rot under glasshouse conditions. Two rates of biochar (3 and 6%) were used as soil amendment, with and without *T. harzianum*. *In vitro* tests indicated a high antagonistic potential of *T. harzianum* alone in inhibiting mycelial growth of *S. rolfsii* by about 60%. X-ray diffraction (XRD) spectroscopy based characterization of biochar indicated both crystalline and amorphous mineral phases such as Quartz (SiO_2), $CaCO_3$, and CaO. Scanning electron microscope and Energy Dispersive X-ray spectroscopy (EDX) demonstrated that biochar was highly porous and contain other vital elements such as P, K, O, Mg, S, Cl and Si. In pot trial, combined treatment with 6% biochar and *T. harzianum* effectively decreased the percentage severity index and disease incidence by 32 and 40%, respectively along with simultaneous stimulation of plant growth by up regulating plant defense mechanisms. The combined treatment significantly increased polyphenol oxidase (8.13 Units per g protein per min) as well as phenylalanine ammonia lyase (35 M t-cinnamic acid per mg protein) activities compared to control. These findings suggest that the synergistic effect of rice straw biochar as well as *T. harzianum* could be a sustainable and environment friendly approach for managing collar rot disease along with growth enhancement of chickpea.

Keywords: Soil-borne pathogens, biological control, induced defence, sustainable agriculture, rice straw valorisation

INTRODUCTION: Chickpea (*Cicer arietinum*) is a legume rich in carbohydrates and proteins. Chickpea belongs to the Fabaceae family that is high in the fiber content (Grasso *et al.*, 2022). It is mainly grown as a rabi-season crop in arid and semi-arid areas in rainfed and irrigated conditions. There are two primary types of chickpea, Desi and Kabuli, of which Desi has the majority of production by volume (more than 80%). Chickpea production has its roots in the south-east of the world and has spread to other semi-arid areas. Chickpeas are particularly cultivated in the Thal area of the desert in Pakistan where the environmental factors are harsh and the soil less fertile. These factors make all other crops economically unfeasible. In order to make the best use of the existing soil moisture, the farmers would generally plant chickpea as the dobari crop after rice crop harvest or during the later stages of the monsoon season since the crop has a high drought tolerance level (Soomro *et al.*, 2021).

Although Pakistan is the third-largest producer of chickpea i.e. 978,000 ha area under cultivation producing 340,000 tons annually, after India and Australia, its chickpea production is lower than its potentiality because of some limitations (Ullah *et al.*, 2020). Chickpea productivity is negatively influenced by the presence of both biotic factors (bacteria, fungi, viruses, nematodes, insects and rodents) and abiotic stresses (extreme temperatures, salinity, drought, heavy metals, and pesticide residues). Among these, the soil-borne pathogens especially *Sclerotium rolfsii* Sacc. are very detrimental (Chanu *et al.*, 2017). This pathogen invades the collar and root areas of young seedlings, resulting in the collar rot, which is indicated by yellowing and wilting of the leaves, a soft and darkening of the base of the root system, and eventually by death of the plant, usually in 25-30 days after planting (Kumar *et al.*, 2024). *Sclerotium rolfsii is a soil-born fungus with wide host range of more than 500 plant species*. The pathogen was first identified by Rolfs in 1892 in Florida and it was named by Saccardo in 1911. *S. rolfsii* thrives under warm (25–30°C) and moist conditions (Raj *et al.*, 2024). It can destroy 55-95% of chickpea seedlings under favorable conditions. One of the characteristics of the infection is the white, cottony fungal growth at the collar region, especially in high humidity. Initial infections may cause a full plant rot and collapse because of stem rot and structural loss of support (Javaid *et al.*, 2021). *S. rolfsii* can be chemically controlled using fungicides of carbamates, triazoles, phenylpyrroles and dicarboximides. Nonetheless, the exclusive use of such fungicides may deteriorate the microflora of soil and hasten the emergence of resistance to any fungicide in pathogens. This issue has led to the desire to pursue green, environmental-friendly technology (Gowdar *et al.*, 2024). Biochar is one of such alternatives, a carbon-rich biomass derivative produced by pyrolysis (Zou *et al.*, 2024). Biochar has been historically utilized in the enhancement of soil fertility and plant growth but its current stand to be noted is its use in disease suppression. Biochar is synthesized using agricultural wastes like sunflower stalks and rice straw. Biochar is marked by high surface area, cation exchange capacity, and nutrient levels (Chaturvedi *et al.*, 2021). These characteristics favour the colonization of microbes and enhance physicochemical properties of soil. Biochar could contain useful bacteria, ectomycorrhizal, and mycorrhizal fungi (Tan *et al.*, 2022). In addition to this, biochar can affect pH and nutrient availability (Naz *et al.*, 2022). Biochar has the potential to change the environment of the rhizosphere by preventing the establishment of pathogens, activating plant immunity, and alleviating pathogenic stress by changing the physiochemical conditions and pathogen toxicosis (Chi *et al.*, 2024).

Another biocontrol mechanism is through application of *Trichoderma harzianum,* the beneficial fungus is proven to enhance plant growth and suppress disease like collar rot. The antagonistic effect of *T. harzianum* has been through the mycoparasitism process mostly involving the production of cell wall degrading enzymes including beta-1, 3-glucanases and chitinases (Sahgal, 2021) and by the antimicrobial volatile organic compounds (Shanmugaraj *et al.*, 2023). *T. harzianum* not only reduces the proliferation of *S. rolfsii* but also improves the health and performance of plants when the pathogen becomes invasive (Chowdhury *et al.*, 2024). The excessive use of chemical pesticides has resulted in the negative environmental effects, as well as strains of pathogens developing resistant to the chemicals. Therefore, more sustainable sources of chemical protection are required. As demonstrated in the literature, biochar alone or with the help of other biological control agents is expected to be an encouraging way of managing soil-based pathogens such as *S. rolfsii*. Thus, this study was conducted to develop an environment-friendly and efficient method for managing collar rot disease in chickpea by analysing the potential of rice straw-derived biochar and *T. harzianum*, used both individually and in combination.

OBJECTIVES: The objectives of this study were to (1) evaluate the antagonistic effect of *T. harzianum* against *S. rolfsii* under laboratory conditions (2) characterize the physicochemical and structural properties of rice straw biochar using XRD, SEM, and EDX, (3) determine the effect of rice straw biochar and *T. harzianum*, alone and in combination, on collar rot incidence and severity in chickpea under glasshouse conditions (4) assess the influence of biochar and *T. harzianum* on chickpea growth and yield-related parameters (5) investigate the induction of defense-related enzymes (PAL and PPO) in chickpea plants treated with biochar and *T. harzianum.*

MATERIALS AND METHODS: Biochar preparation: Biochar was prepared by burning rice straws through the process of pyrolysis at temperature above 450°C (Biswas *et al.*, 2022). The apparatus used for making biochar was comprised of main container, a lid and a chimney (McLaughlin, 2010). The rice straws were collected from the Faculty of Agricultural Sciences, University of the Punjab were filled into the main container having small holes at the bottom for the passage of air. The rice straws were heated with limited supply of oxygen for 45 min. The final biochar product obtained was sprinkled with water and grinded into fine powder for future use (Koippully Manikandan and Nair, 2023).

Characterization of rice straw biochar: The crystalline phases and mineralogical composition of the rice straw biochar was obtained by X-ray diffraction (XRD). Samples were scanned with Cu-Kα radiation in finely powdered form (λ = 1.5406 Å) over a 2θ range of 0.3°–120°, at 40 kV and 30 mA with a step size of 0.030° (Waheed *et al.*, 2024). The patterns of diffraction were examined on the basis of Match software (CRYSTAL IMPACT), COD database. Phase identification was done through matching of experimental X-Ray peaks and JCPDS peaks (Biswas *et al.*, 2022). Surface morphology and elemental composition were analysed through SEM-EDX at 15 kV. For this, oven-dried and finely ground samples were placed on aluminium stubs with carbon tape and gold-coating through sputtering (Bhattacharyya *et al.*, 2020). SEM imaging was conducted at 500X, 1000X, 2000X and 4000X magnifications by adjusting take off angle at 34.3° (Khan *et al.*, 2024).

Culture acquisition: Pure cultures of pathogenic *S. rolfsii* as well as beneficial *T. harzianum* were obtained from the First Fungal Culture Bank of Pakistan, University of the Punjab, Lahore. These authenticated fungal strains selected for use in all experimental procedures.

Effect of biochar and *T. harzianum* on the growth of *S. rolfsii* under laboratory conditions: The *in vitro* antagonism of *T. harzianum* against *S. rolfsii* was determined with and without rice straw biochar (3 and 6 % v/v), following the dual culture technique. Seven days old colonies of both fungi were inoculated on PDA plates. On the dual culture plates, 5 mm plugs of *S. rolfsii*, and *T. harzianum* were individually placed on opposite sides of the plate, and in the biochar-amended plates, *S. rolfsii* was inoculated in the center of the plate. Plates with *S. rolfsii* only were used as a control. Incubation temperature for Petri plates was adjusted to 25°C. Inhibition of radial growth of *S. rolfsii* was measured at 3 as well as 5 days after inoculation (Attia *et al.*, 2022). Six treatments consisting of three replicates were incorporated in the *in vitro* assay. Percent inhibition was calculated by using following formula:

$$Growth\ inhibiton\ (\%) = \frac{\text{Colony diameter in control (cm)} - \text{Colony diameter in treatment (cm)}}{Colony\ diameter\ in\ control\ (cm)} x\ 100$$

Pot trial: A glasshouse experiment was conducted to ascertain the *in vivo* efficacy of rice straw biochar and *T. harzianum* combination for management of *S. rolfsii* induced infection on chickpea. The experiment was composed of twelve treatments having five replications each and six plants per pot were sustained. Pearl millet seeds were used to prepare *S. rolfsii* inoculum. For this purpose, pearl millet seeds (1 kg) were slightly boiled and packed into polythene bags. The seeds were autoclaved and inoculated with 7 day old mycelial discs of *S. rolfsii* and incubated for 10 to 15 days for colonization of fungus (Javaid *et al.*, 2023). To inoculate, 50 grams of colonized millet grains were well mixed into the potting soil and in control pots, the un-inoculated grains were used. The pots were mildly watered after inoculation and left for 7 days for the establishment of pathogen (Ali *et al.*, 2020).

For the preparation of conidial suspension of *T. harzianum,* 15-day-old PDA cultures were used. The plates were filled with sterile distilled water and the spores were gently scraped (Navaneetha *et al.*, 2015). The suspension was filtered through muslin cloth followed by re-filtration with filter paper and the spore count was adjusted to 1×10^7 conidia/mL using a haemocytometer (Kumari *et al.*, 2025). The suspension was applied to pots according to the treatment plan at the rate of 15 mL per pot. The pots were kept undisturbed for an additional 7 days prior to seed sowing (Javaid *et al.*, 2021). Desi chickpea seeds were surface sterilized with 2% sodium hypochlorite solution for 3 minutes, followed by five rinses with distilled water. The seeds were air dried under laminar airflow and sown into the treated pots. After germination, thinning was done to maintain six uniform seedlings per pot (García-Latorre *et al.*, 2025). Plants were harvested at maturity. Shoot and root lengths were measured using a centimetre scale, while fresh and dry weights were recorded using a weighing balance. Measurements of the disease incidence (DI) and percent severity index (PSI) were taken at second- and fourth-week post sowing. The symptoms of collar rot were taken in terms of yellowing, wilting, and basal rotting of the stem using standardized disease rating scale.

Biochemical analysis: The activity of defence related enzymes was determined through biochemical analysis of leaves of the chickpea samples. The activity of phenylalanine ammonia-lyase (PAL) was measured according to Nagarathna *et al.* (1993) with slight modification. A 0.5g sample of fresh leaf material was ground up in 25 mM sodium borate buffer (pH 7) containing 32 mM of 2-mercaptoethanol. The extracted clear supernatant was utilized as the enzyme extract. The substrate used was L-phenylalanine and the mixture was subjected to a temperature of 32°C for an incubation time of 30 minutes. The presence of trans-cinnamic acid was quantified at 290 nm using spectrophotometer. The activity of polyphenol oxidase (PPO) was determined according to Singh and Gaur (2017). Leaf tissue (0.5g) was homogenized with 0.1M phosphate buffer (pH 6.5), and the supernatant was used as an enzyme extract. PPO activity was determined by measuring absorbance at 495nm on a spectrophotometer using catechol as a substrate.

Statistical analysis: Experimental data was analyzed by applying one-way Analysis of variance (ANOVA) using statistics software (Version 8.1). Comparisons of treatment means were done by Tukey (HSD) test at 5 % level of probability (P<0.05).

RESULTS: Characterization of rice straw biochar: The XRD profile of rice straw biochar indicated a combination of amorphous regions and crystalline mineral phases, showing its dual-phase structural nature. A prominent peak at 2θ ≈ 27.16° corresponded to quartz (SiO_2), with additional quartz-related peaks at approximately 20.2°, 26.2°, and 68°, indicating high silica content (figure 1). Broad humps between 20°–30° and 42°–46° indicated amorphous carbon (002) and turbostratic carbon (100) planes, respectively. The characteristic diffraction peaks at 2θ degree of around 39.1°, 45.1° and 50.9° were assigned to CaO, $CaCO_3$ and Ca $(OH)_2$, respectively. These peaks indicate that CaO, $CaCO_3$ and Ca $(OH)_2$ were mineral constituents in the ash form.

Scanning electron microscopy (SEM) analysis, at a magnification ranging from 500X to 4000X, showed porous and irregular surface profile (figure 2). At 500X, the appearance of roughness and open channels revealed a disintegration of the plant cell walls (figure 2A). At 1000X connected mesopores were observed, which results in increased surface area (figure 2B). While at 2000X fibrous remains and microporous regions were seen (figure 2C). At 4000X, sponge-like structures were also seen with fused spots and micropores which means there was increased adsorptive power as a result of volatilization caused by pyrolysis (figure 2D).

The energy-dispersive X-ray spectroscopy (EDS) showed that carbon (62.18%) is major component demonstrating a high carbon retention during the pyrolysis process (figure 3). The presence of oxygen (18.95%) represented surface functional groups that promoted the cation exchange capacity. The potassium (8.18%) and calcium (3.10%) contents were significant, which is an indication of enrichment of nutrients. Trace minerals such as magnesium, silicon, phosphorus, sulfur, and chlorine were also identified which help in agronomic and soil-enhancing properties of the biochar by increasing nutrient availability, microbial colonization, and stress resistance.

In Vitro assay: The untreated control recorded the maximum radial growth of the *S. rolfsii* (8.00 cm) in the *in vitro* test (figure 4). *T. harzianum* (SR+TH) inhibited the fungal growth by 60 % and mycelial growth to the length of 3.61 cm. The combination of *T. harzianum* with 6 percent rice straw biochar (SR+TH+6%RSB) recorded an inhibition rate of 56 percent followed closely by SR+TH+3%RSB, which recorded 54 percent suppression. The 3% RSB alone had the least inhibitory effect of 43%.

Impact of rice straw biochar combined with *Trichoderma harzianum* on chickpea growth under collar rot stress*:* When rice straw biochar (RSB) was amended, especially together with *T. harzianum*, there was tremendous enhancement of chickpea growth parameters under *S. rolfsii* induced stress. Pathogen inoculation caused a reduction of growth parameters but this was reversed by 6% RSB + *T. harzianum* leading. This combination had better performance than the 3% RSB treatments, which was suggestive of

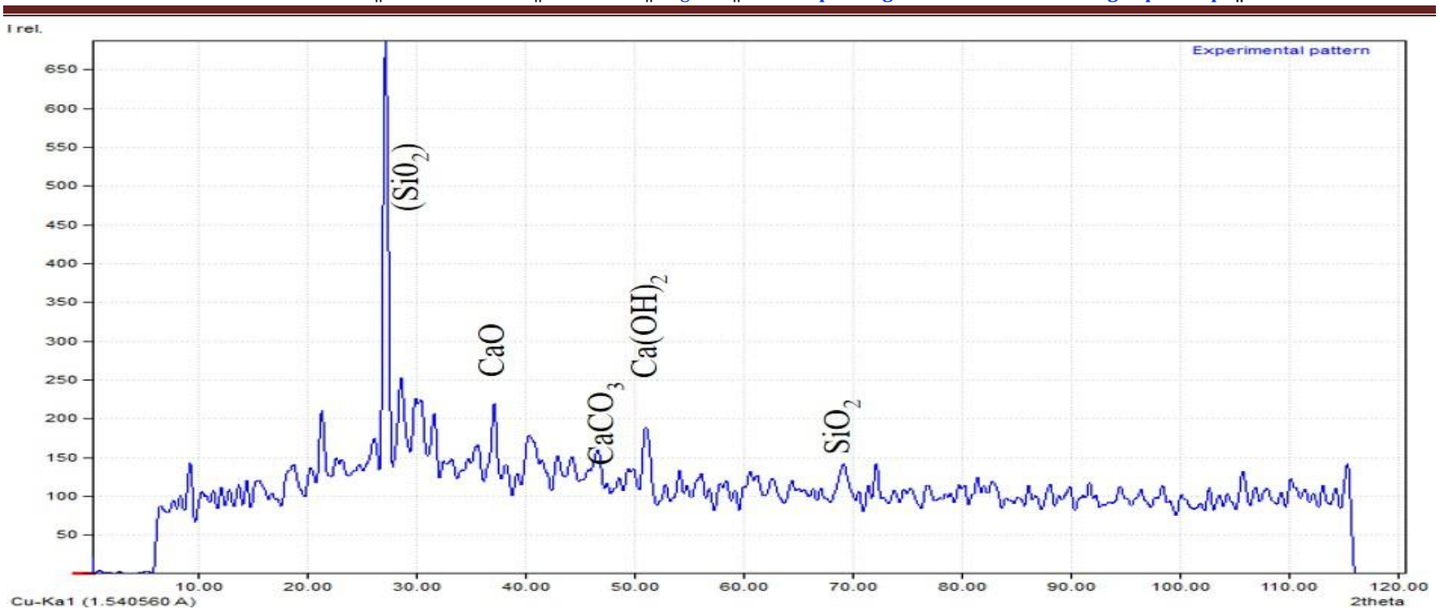


Figure 1: X-ray diffraction (XRD) analysis of rice straw biochar with clear crystalline peaks of quartz (SiO_2), CaO, $CaCO_3$, and Ca $(OH)_2$ produced by the ash.

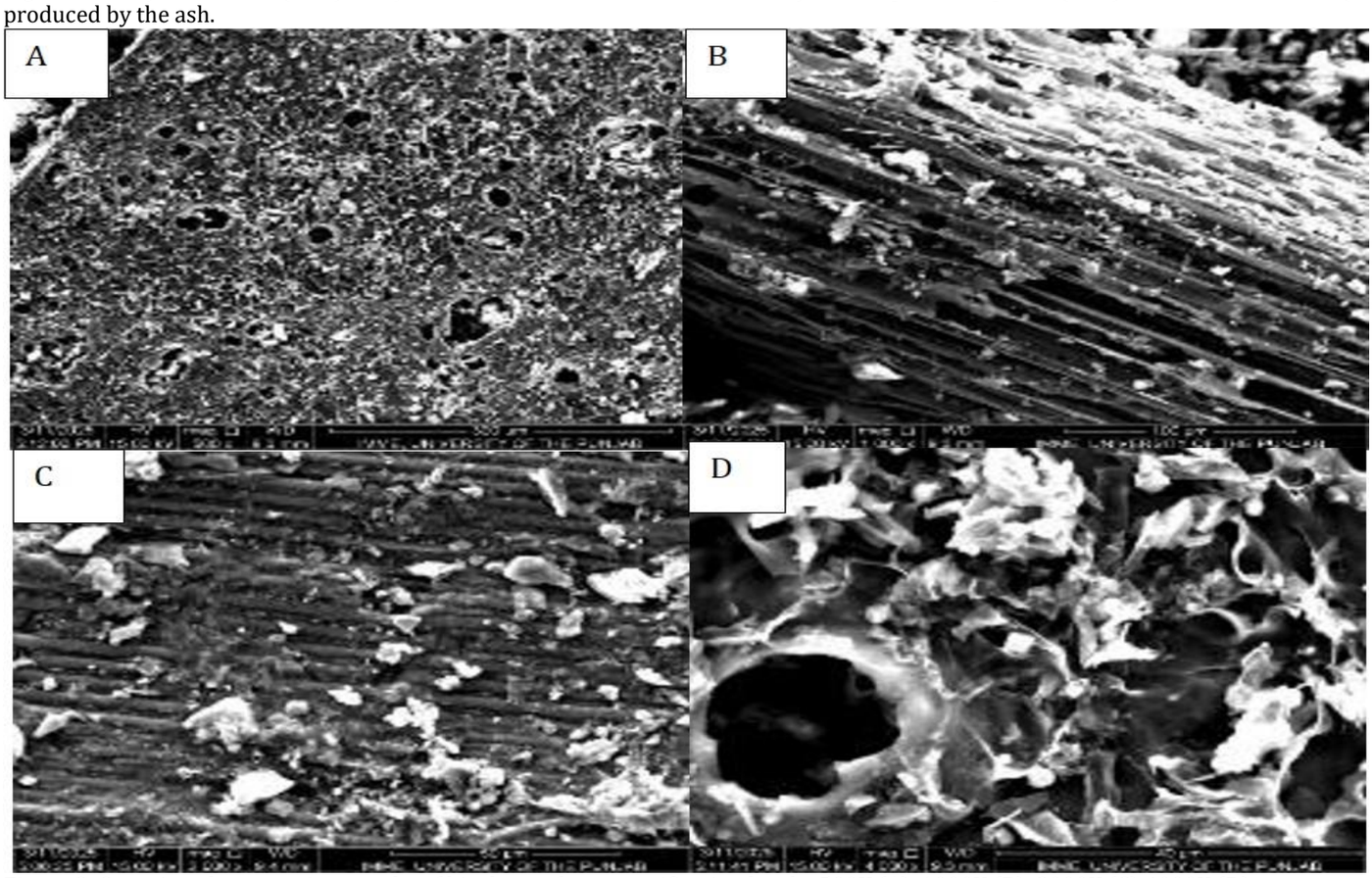
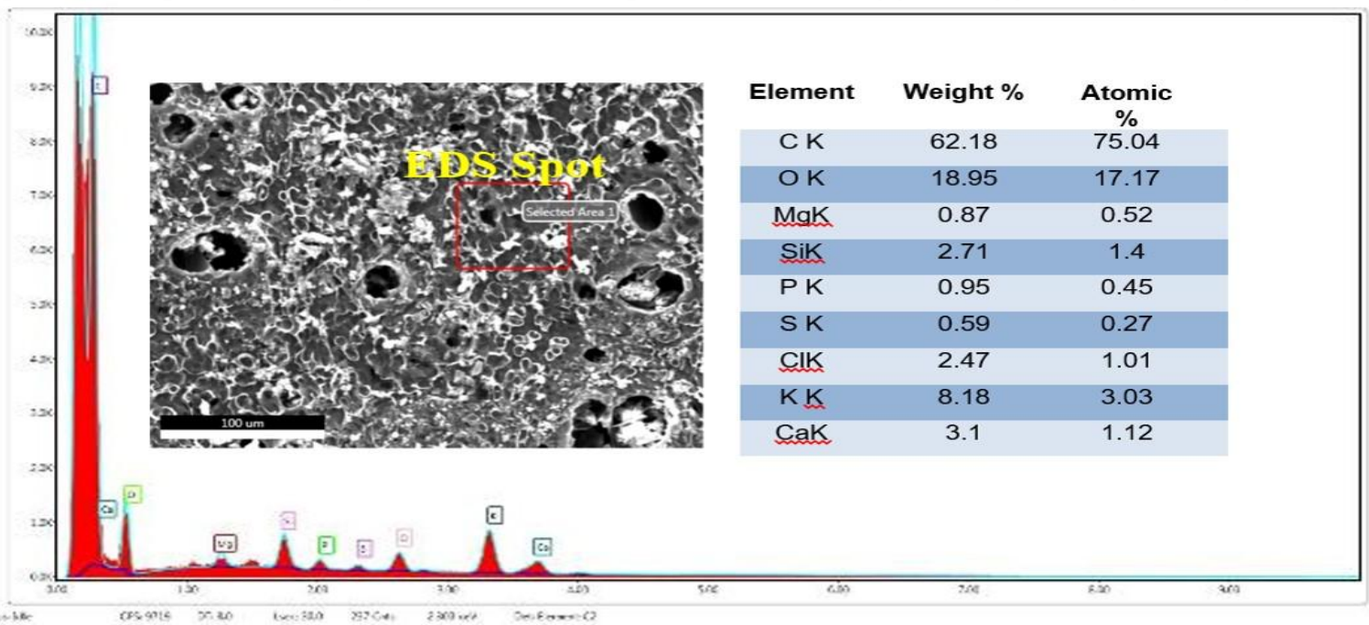


Figure 2: Scanning electron micrographs of biochar at four magnification levels: 500X (A), 1000X (B), 2000X (C) 4000X (D).



Element	Weight %	Atomic %
C K	62.18	75.04
O K	18.95	17.17
MgK	0.87	0.52
SiK	2.71	1.4
P K	0.95	0.45
S K	0.59	0.27
ClK	2.47	1.01
K K	8.18	3.03
CaK	3.1	1.12

Figure 3: Energy dispersive X-ray Spectroscopy with the description of elements present in biochar.

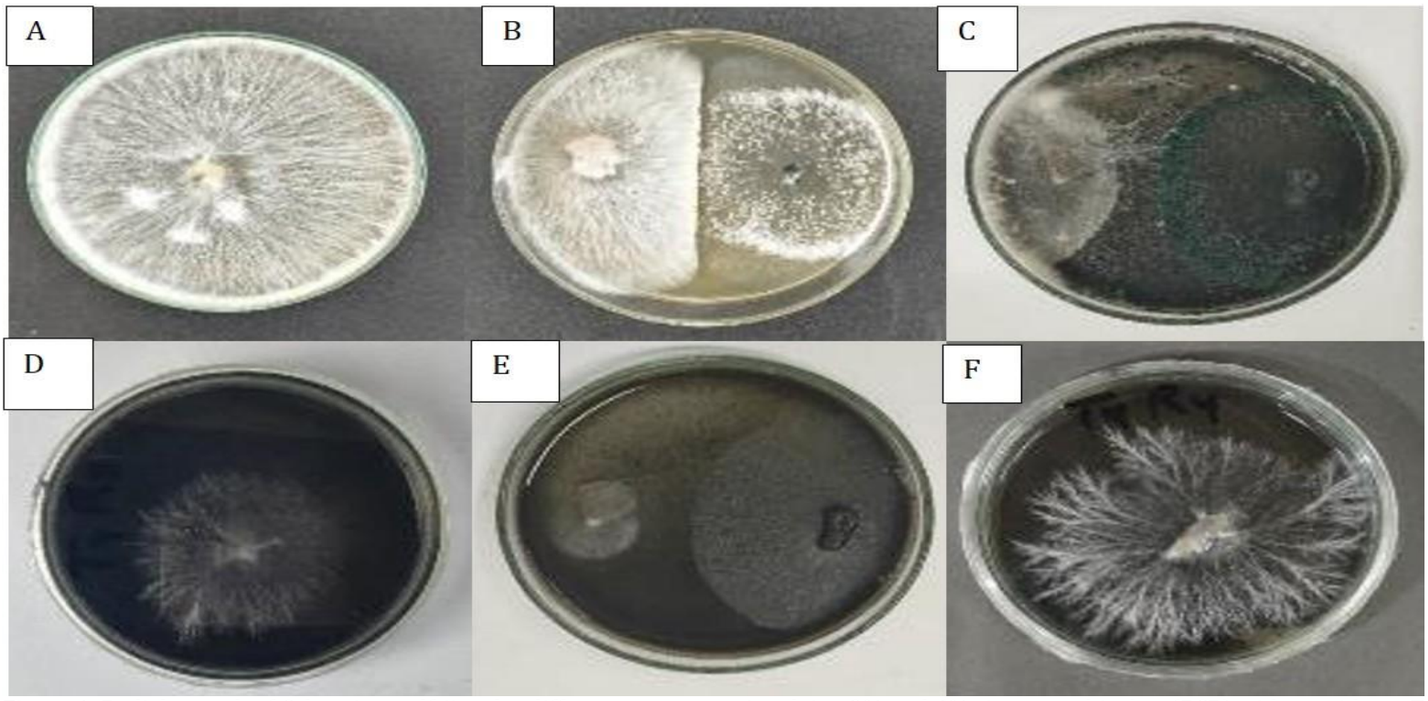


Figure 4: Radial growth of *Sclerotium rolfsii* under different treatments involving 3% and 6% rice straw biochar (RSB) with or without *T. harzianum*. *S. rolfsii* alone and with (A), + *T. harzianum* (B), + *T. harzianum* + 3% RSB (C), + 6% RSB (D), + *T. harzianum* + 6% RSB (E), and + 3% RSB (F).

a concentration dependent influence of biochar in alleviating growth suppression caused by pathogens. Similar response was exhibited by dry biomass. The maximum shoot (0.41 g) and root (0.12 g) dry weights were recorded in 6% RSB + *T. harzianum* treated plants, which was over two fold higher than that of the pathogen infected controls (figure 5, 6).

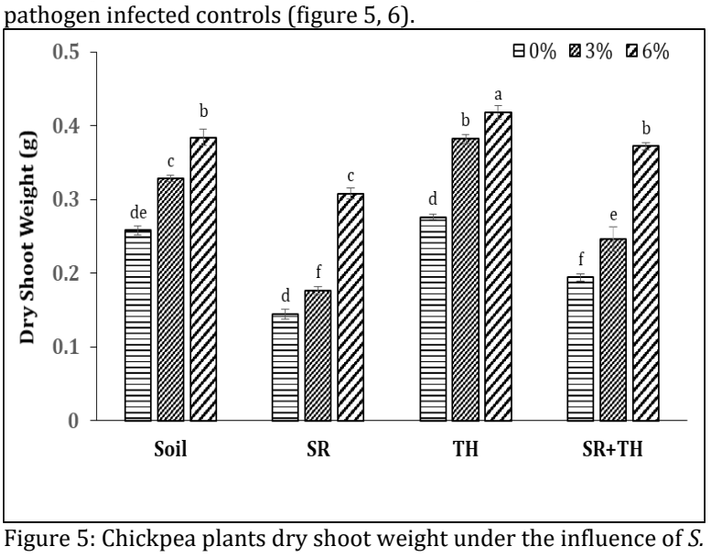


Figure 5: Chickpea plants dry shoot weight under the influence of *S. rolfsii*, *T. harzianum* (TH), and rice straw biochar (RSB).

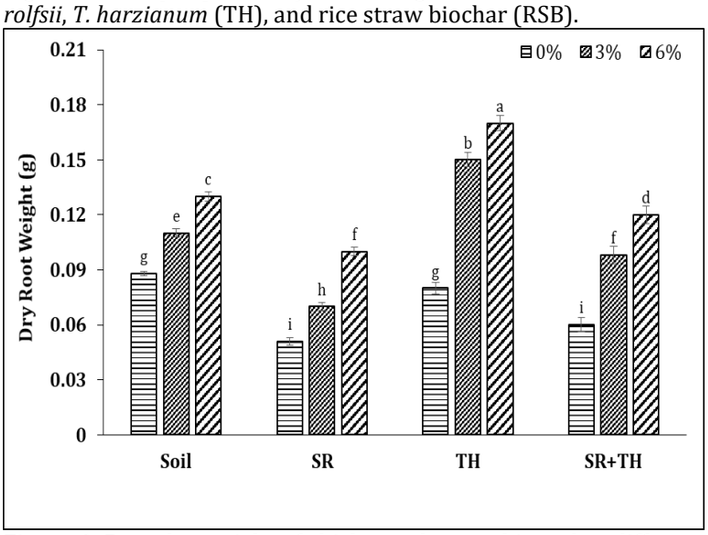


Figure 6: Root dry weight of chickpea plants subjected to different treatments involving *S. rolfsii*, *T. harzianum* (TH), and rice straw biochar (RSB).

Biochar itself also resulted in dose-dependent increases in biomass, but again with greater synergy when biochar was mixed with *T. harzianum*.

Impact of biochar and *T. harzianum* on incidence as well as severity of collar rot in chickpea: A notable reduction of collar rot incidence and severity was observed when rice straw biochar (RSB) and *T. harzianum* were applied together. The plants treated with *S. rolfsii* only exhibited maximum disease pressure (figure 7) with disease incidence of 90% and a disease severity of 78.49%.

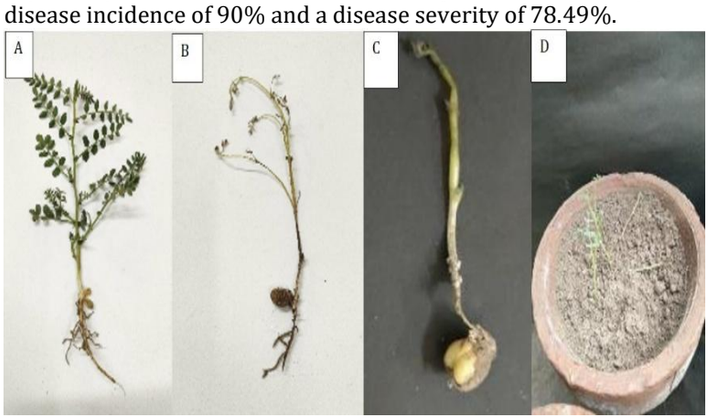


Figure 7: Healthy chickpea plant (A), Wilting symptoms due to collar rot infection in chickpea plant (B), Collar rot symptoms at seedling stage (C), Death of seedlings in the pot due to collar rot (D).

Application of *T. harzianum* decreased disease incidence to 70% while disease severity to 60.2%. Combining *T. harzianum* with 3% RSB resulted in the reduction of disease incidence to 60% and the reduction of severity to 43.5%, which represents a moderately resistant effect. Use of 6% RSB alone resulted in further reductions, with disease incidence declining to 50% and severity to 38%. Simultaneous application of 6% RSB along with *T. harzianum* yielded the most effective control as the disease incidence as well as severity decreased to 40% and 32.1%, respectively. This indicates the considerable protective efficacy of the combination treatment.

Defence enzyme response to Biochar and *T. harzianum* in chickpea: There was a significant up-regulation of phenylalanine ammonia lyase (PAL) activity in all *S. rolfsii* inoculated treatments relative to untreated treatment. Combined application of 6% rice straw biochar and *T. harzianum* induced greater levels of PAL activity (35.24 Mole t-cinnamic acid per mg protein) among amended plants compared to activity in plants amended with 3% biochar (32.52 Mole t-cinnamic acid per mg protein). This indicates that combined amendments provoked pathogen-induced defense response. The level of PAL in plants treated with 6% biochar and *S. rolfsii* was 7.14% more than that of 3% biochar (figure 8).

The activity of the polyphenol oxidase (PPO) was in the same trend. Moderate activity was recorded in individual treatments with *T. harzianum* or biochar. Higher PPO activity was measured in the treatment that consisted of 6% biochar, *T. harzianum*, and *S. rolfsii* (8.12 Units per g protein per min), as compared to (6.71 Units per g

protein per min) in 3% biochar combination (figure 9). These findings show that integration of *T. harzianum* and biochar, especially in high concentrations, is very effective in promoting the biochemical defence mechanism of chickpea against collar rot.

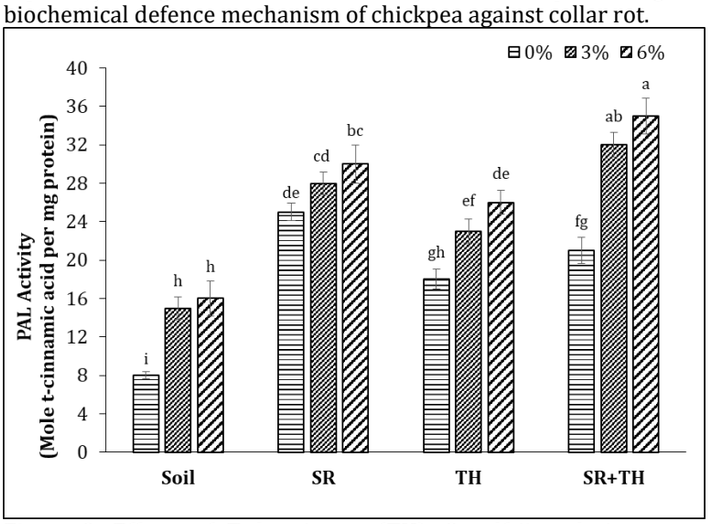


Figure 8: Effects of *T. harzianum* (TH), *S. rolfsii*, and rice straw biochar (RSB) on the activity of phenylalanine ammonia lyase (PAL) in chickpea plants.

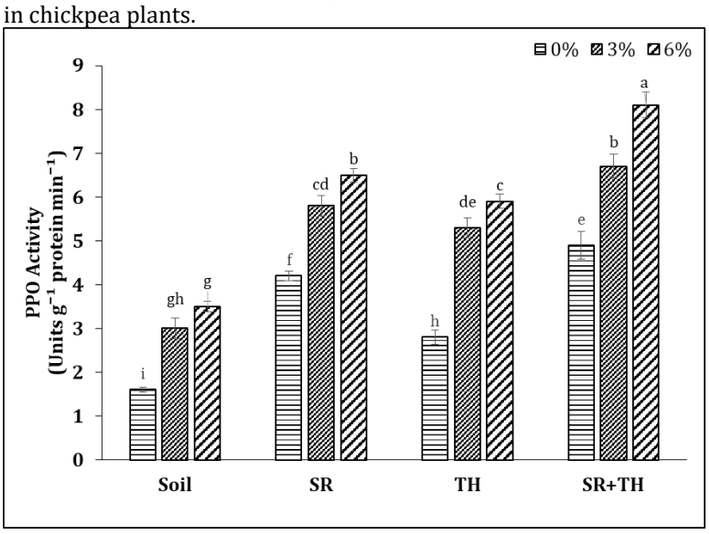


Figure 9: Effects of *T. harzianum* (TH), *S. rolfsii*, and rice straw biochar (RSB) on the activity of the enzyme polyphenol oxidase (PPO) in chickpea plants.

DISCUSSION: Long term and heavy application of chemical pesticides in controlling plant diseases has remained a subject of great concern because of its negative impacts on the environment and diseases to human. As a result, there is an increasing concern in finding alternative environment friendly mitigation techniques, which can control plant disease without introducing damaging side-affects. Integrated disease environments that combine various biological and organic practices are increasingly becoming a long term answer to sustainability (Jaiswal *et al.*, 2022). Of these, biochar has attracted much interest because of its multiple functionalities of enhancing soil characteristics, which manipulates microbial communities and induces plant defence responses (Poveda *et al.*, 2021).

The presence of soil borne pathogens causes significant economic losses in the whole world, which tend to worsen the quality and quantity of agriculture products (Anand and Rajeshkumar, 2022). The pathogens have the ability to persist in the soils for years through the production of resistant propagules, and hence it is difficult to control such pathogens. The attempts to control them conventionally are usually inefficient or detrimental to the environment, and researchers examine the use of sustainable substitutes. Biochar made of a wide range of plant residues through pyrolysis has become a promising resource to help reject soil borne diseases alongside enhancing the fertility of the soil (Alkharabsheh *et al.*, 2021). It has been revealed in several studies that biochar improves soil aeration, porosity as well as microbial activity all of which inhibits the settlement and growth of pathogens. Rice straw biochar treatments considerably enhanced the growth of chickpea and mitigated symptoms of collar rot infection by *S. rolfsii*. Such results are similar to the findings of Ma *et al.* (2021) and Ullah *et al.* (2024) who noted that the amendment of biochar promotes the development of the root system and resistance to soil borne pathogens due to the improvement of soil physical properties.

The concentration of the biochar is very important in determining its effectiveness. In the current experiment, the increased level of biochar (6%) was superior to the lower amount (3%), causing more disease suppression and increased plant growth. Similar results were observed in the study conducted by Reyes-Cabrera *et al.* (2017) with soybean, in which the mean plant height and root length were significantly elevated at higher concentrations of biochar as compared to lower concentration. The findings of Pradhan *et al.* (2022) and Joseph (2015) confirm that such effects can be achieved through the use of higher doses of biochar that promote water retention, the availability of nutrients, and soil structure overall contributing to a healthy plant growth and plant resistance to pathogens. Likewise, Mukherjee and Lal (2014) reported enhanced legume yield and improved soil fertility when biochar was applied at 5–10%, whereas lower doses (1–2%) had minimal effects. Besides, Graber *et al.* (2010) have reported that disease risk is minimized under higher biochar concentrations, either through direct inhibition of pathogen growth or propagation of beneficial rhizosphere microorganisms.

In vitro experiments in our study revealed that *T. harzianum* had significant inhibitory effect on the mycelial growth of *S. rolfsii*. Similar antagonistic activity has been reported on *T. harzianum* against *S. rolfsii* where it is attributed to mechanisms including myco-parasitism, nutrient deprivation, and production of hydrolytic enzymes like glucanases and chitinases (Kumar *et al.*, 2024). Furthermore, Wang *et al.* (2024) reported up to 78.5% growth inhibition of *S. rolfsii* under *in vitro* conditions by the use of *T. harzianum*, which supports our findings.

Interestingly, a combination of biochar with *T. harzianum* showed the best prospect in the study in which it exhibited greater control of collar rot, and better plant growth than their isolated applications. These synergetic effects have also been emphasized in previously observed studies, biochar integration with *Trichoderma* spp. increased diseases suppression and plant vitality in a variety of crops, such as maize and sunflower (Kumari *et al.*, 2025). This synergistic effect can be explained by the fact that biochar is able to create a porous microenvironment and a source of carbon allowing the colonization and growth of beneficial microorganisms, which increases their antagonistic action against soil borne disease-causing organisms. The positive effects of this comprehensive approach were further supported by the activity of the defence related enzymes. According to our findings, chickpea plants treated with biochar + *T. harzianum* had a higher level of phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) that are indicators of an enhanced plant defence mechanism. PAL is a major enzyme which is involved in phenylpropanoid pathway that results in lignin, flavonoid and phenolic compound production which have a significant effect in plant resistance. PPO mediates oxidation of phenolics to quinones that are toxic against invading pathogen and also helps fortify the cell walls (Constabel and Barbehenn, 2008). Previous studies suggest that biochar has the ability to trigger either systemic resistance (ISR) or systemic acquired resistance (SAR) in plants particularly when used in combination with beneficial microbes (Elad *et al.*, 2011). Tao *et al.* (2025) revealed that *Bacillus subtilis* and biochar mixture led to higher microbial activity and fungal disease suppression of chrysanthemum due to improvements in microbial antagonism facilitated by the porous nature of biochar. These results agree with ours as in our study biochar and *T. harzianum* treatments increased PAL and PPO activity which subsequently decreased the incidence of the disease. The elevated activity of defense enzymes in legumes under biochar and *Trichoderma* inoculation was also observed by Rasool *et al.* (2021), Javaid *et al.* (2021) and Singh *et al.* (2013). The other beneficial effect of biochar is enhancing the availability of nutrients. These findings have been supported by previous studies which indicate that when soils are amended with biochar, they have increased plant available macro- and micro-nutrients such as P, Zn, Fe, K, and S that promote better nutrient uptake and growth (Pradhan *et al.*, 2022). The increased nutrient availability was probably synergistic with better soil structure and caused a resistance increase that may have made chickpea plants more resistant to *S. rolfsii* infection. Overall, our results will show that the combination of rice straw biochar and *T. harzianum* is a long lasting and environmentally safe approach to control the collar rot disease in chickpea.

This synergistic combination not only reduces the growth of the pathogen, but it also enhances soil status, nutrient cycling, and plant defence measure, and this makes it a good alternative to fungicides. The extensive use of such strategies related to a bio-based management can lead to the sustainable agriculture process as they reduce the usage of hazardous pesticides and ensure high production of crops.

CONCLUSIONS: This study highlights the potential of integrating rice straw biochar with *T. harzianum* for sustainable management of chickpea collar rot. The combined treatment significantly reduced disease incidence and severity, improved plant growth, and enhanced defence enzyme activities, indicating both direct suppression of *S. rolfsii* and induction of host resistance. Using rice residues to produce biochar not only helps manage agricultural waste but also improves soil quality and supports environmental sustainability. Although the results under controlled conditions were promising, testing at the field level is still needed to ensure the approach works across different environments. Future studies should focus on the long-term effect on soil health and crop yields, and on finding the best ways to combine biochar with biological control agents for maximum benefit.

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