Synergistic Effect of Pyrolyzed Bagasse and Trichoderma Viride for Sustainable Mitigation of Chili Southern Blight

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Citation | Ullah. M. Z, Rasheed. B, Akhtar. A, Akram. W, Siddique. M. A. B, Khurshid. M, Tariq. H.M. Ali. N, Raza. U, Ahmad. M, "Synergistic Effect of Pyrolyzed Bagasse and Trichoderma Viride for Sustainable Mitigation of Chili Southern Blight" pp 2197-2212, September 2025

Received | August 07, 2025 Revised | August 27, 2025 Accepted | August 29, 2025 Published | September 03, 2025.

Soil-borne diseases like southern blight severely limit chili (*Capsicum annuum* L.) production, demanding sustainable and eco-friendly management approaches. This study introduces the integration of sugarcane bagasse-derived biochar with *Trichoderma viride* as a novel strategy for enhancing chili resistance against *Sclerotium rolfsii*. Biochar was produced through pyrolysis at 450°C and characterized using SEM, EDX, and XRD, revealing porous honeycomb-like structures, high carbon content, and mineral phases such as SiO_2 and CaO. Glasshouse experiments were conducted on the chili cultivar 'Desi' using biochar at 3% & 6% (v/v) concentrations. Biochar was either applied alone or in combination with *T. viride* as well as with *S. rolfsii*. Results demonstrated that biochar treatments significantly enhanced shoot and root growth, biomass accumulation, and physiological performance under pathogen stress. Disease severity, incidence, and mortality were notably reduced, with the greatest suppression (20%) noted in chili plants treated with 6% biochar plus *T. viride*. Furthermore, higher biochar doses substantially elevated levels of defense-related compounds, including phenolics, catalase, and flavonoids, indicating induction of systemic resistance. Similarly, the combined effect of biochar and *T. viride* was also visible under in vitro assays. Overall, the integration of biochar and beneficial fungi not only improved soil health but also strengthened host defense, offering a sustainable approach to managing southern blight. These findings highlight biochar-induced resistance as a promising component of integrated disease management in chili cultivation.

Keywords: Organic Amendments, Plant Protection, Sclerotium Rolfsen, Biological Control, XRD.

Introduction:

Chili (*Capsicum annuum* L.), a member of the Solanaceae family, is one of the most economically significant vegetable and spice crops cultivated across the globe. In Pakistan, chili is primarily cultivated in Sindh, Punjab, and Baluchistan, with Sindh alone accounting for more than 82% of the country's total production [1]. Chili cultivation in Pakistan covers about 47,870 hectares, producing nearly 109,615 tons annually, with an average yield of 2,289.8 kg/ha [2]. Beyond its culinary significance, chili is also valued for its medicinal properties, being a rich source of capsaicinoids, flavonoids, and essential vitamins including A, B6, C, and K [3].

However, chili production faces a serious threat from southern blight, also known as collar rot, caused by *Sclerotium rolfsii* Sacc. (SR) a destructive soil-borne pathogen that infects more than 500 plant species [4]. The pathogen thrives under hot and humid climates, making Pakistan's chili-growing zones particularly vulnerable. Infection begins at the collar region, resulting in white mycelial growth and brown sclerotia, leading to plant wilting and substantial economic losses. Under favorable conditions, yield losses due to the disease have been reported to range between 16% and 80%[5]. Recent studies confirm that SR continues to cause significant yield losses in chili across Asia and Africa, where conducive climatic conditions promote disease outbreaks[6].

Conventional management techniques were focused on intensive utilization of chemical fungicides. The non-judicious use of these hazardous chemicals has raised concerns such as the emergence of resistant strains, environmental contamination, and residual effects[7]. Furthermore, excessive reliance on fungicides is inconsistent with international policies promoting eco-friendly crop protection strategies[8].To address these challenges, there is a pressing need to adopt integrated disease management strategies that provide sustainable and ecological alternatives. One of such integrated measures is the use of antagonistic fungi such as *Trichoderma viride*. *T. vriide* has been investigated for its ability to suppress chili's southern blight through various mechanisms, including competition, parasitism, production of secondary metabolites, and activation of host resistance[9]. *Trichoderma* spp. Improve root colonization, induce systemic resistance, and enhance antioxidant enzyme activity in chili under pathogen stress[10]. Importantly, *Trichoderma* is also compatible with organic amendments, offering additional opportunities for integrated biological disease management[11]. Similarly, soil conditioning with biochar enhances resistance against pathogens and promotes plant growth. Biochar, a carbon-rich byproduct produced through the pyrolysis of biomass such as sugarcane bagasse, improves soil physicochemical properties, enhances microbial activity, and promotes plant growth by inhibiting pathogen proliferation[12]. The porous structure of biochar not only supports the colonization of beneficial microbes but also improves the retention of essential nutrients such as N, P, K, Ca, and S. More importantly, biochar serves as an effective carrier for *Trichoderma* spp., enhancing its survival and activity in the rhizosphere[13]. Recent evidence suggested that biochar-amended soils harbor enriched microbial diversity, higher enzymatic activity, and reduced inoculum density of soil-borne pathogens[14]. Moreover, synergistic application of biochar with biocontrol agents such as *Trichoderma* has been shown to enhance nutrient uptake, suppress *S. rolfsii*, and improve growth and yield in solanaceous crops[15]. Despite these advancements, little is known about the combined application of pyrolyzed bagasse biochar and *Trichoderma viride* for managing southern blight of chili under local agro-climatic conditions.

Therefore, the objectives of the current study are (1) evaluation of the pathogenic potential of *S. rolfsii* on chili under controlled conditions, (2) assessment of individual and combined effects of *T. viride* and sugarcane bagasse biochar on chili disease suppression, (3) biochemical analysis of chili plants treated with *T. viride* and biochar both in the presence and

absence of S. rolfsii, and (4) in vitro examination of suppressive potential exerted by *T. viride* and biochar on the growth of *S. rolfsii*.

Novelty Statement:

Southern blight of chili, caused by *Sclerotium rolfsii*, lacks sustainable and eco-friendly management options. Previous studies mainly focused on the chemical fungicides or single biocontrol agents, while the role of biochar–microbe interaction in disease suppression remains poorly understood. In particular, the synergistic potential of sugarcane bagasse biochar combined with *Trichoderma viride* has not been evaluated for chili blight management. The novelty of this study lies in testing this unexplored synergistic interaction, offering an integrated and sustainable alternative to chemical fungicides for chili cultivation.

Material and Methods:

Investigation Site:

The experiment was carried out at the Faculty of Agricultural Sciences (FAS) experimental station, University of the Punjab, Lahore. The experimental site was situated at 31°29'42"N latitude and 74°17'49"E longitude, with soil characterized as loamy to clayey in texture. Figure 1 presents a schematic flowchart outlining the methodology employed to achieve the study's objectives.

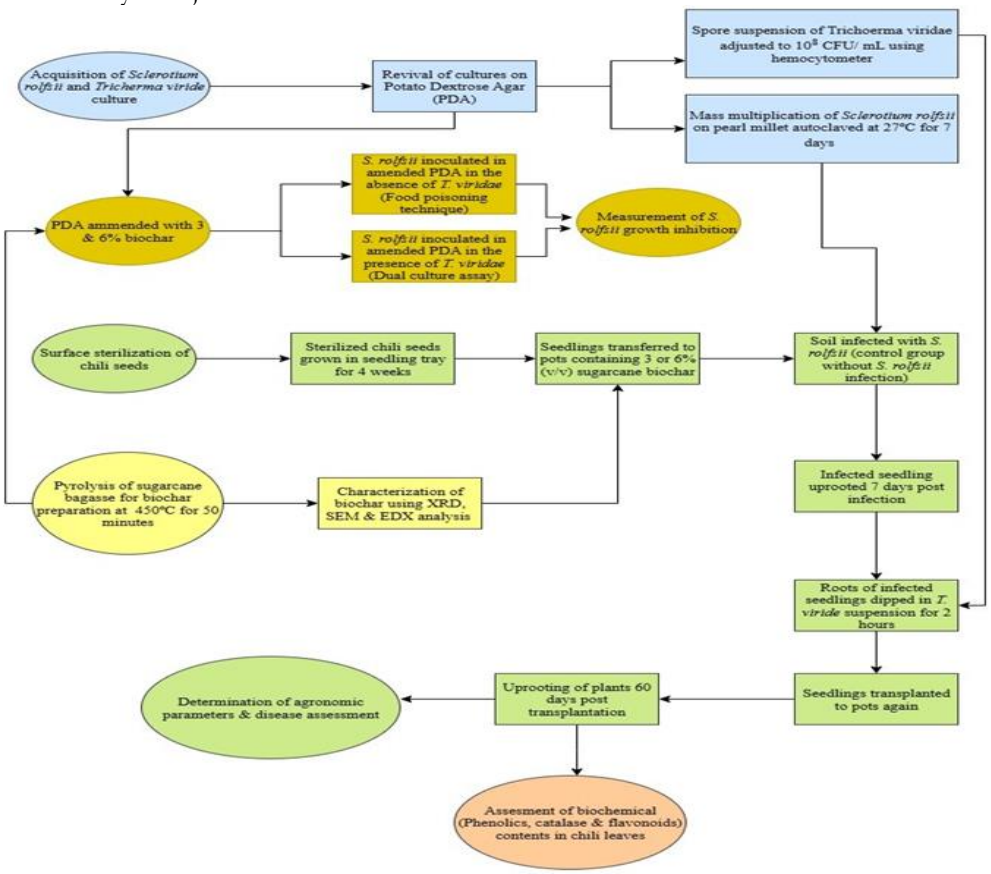


Figure 1. Flowchart of methodology.

Biochar Preparation and Characterization:

Sugarcane (*Saccharum officinarum*) bagasse was obtained from a local juice shop in Lahore and air-dried for one week. Biochar was then produced using the top-lit updraft (TLUD) kiln method [16]. TLUD setup consisted of a primary burner of 200 L capacity, an afterburner, and a chimney. The dried bagasse was pyrolyzed at 450°C for 50 minutes under restricted oxygen conditions using a portable TLUD setup. The resulting biochar was cooled, ground, and sieved through a 1 mm mesh to obtain a fine powder. The prepared biochar was then characterized using XRD, SEM, and EDX analyses.

XRD Analysis of Sugarcane Biochar.

Crystalline structure of the sugarcane biochar was analyzed using X-ray diffraction (XRD) analysis. XRD was carried out using a D8 Advance X-ray diffractometer (Bruker, Germany). Measurements were carried out using Cu Kα1 radiation (λ = 1.5406 Å), with scanning performed at a rate of 2.5° per minute over a 2θ range of 10–120° (Amin et al., 2016).

SEM and EDX Analysis of Sugarcane Biochar:

A scanning electron microscope (SEM) coupled with energy-dispersive X-ray spectroscopy (EDX) (Thermo Fisher Scientific FEI Inspect S-50) was employed to examine the biochar's morphology and elemental composition. The biochar powder was mounted on aluminum stubs using conductive carbon tape, and imaging was conducted under a vacuum pressure of 2×10^{-3} Pa. The SEM was operated at an accelerating voltage of 15 kV, and micrographs were captured at magnifications of 500×, 1000×, 2000×, 3000×, and 4000×, with each image having a live viewing time of 30 seconds[17].

***Sclerotium rolfsii* and *Trichoderma Viride* Culture Acquisition**:

The culture of *Sclerotium rolfsii* (Acc. No. FCBP-PTF-1409) as well as *Trichoderma viride* (Acc. No. FCBP-SF-639) was procured from the First Fungal Culture Bank of Pakistan (FCBP), Department of Plant Pathology, University of the Punjab, Lahore. Fungal cultures were revived on Potato Dextrose Agar (PDA), prepared by dissolving 40 g of commercial PDA per liter of distilled water. For mass inoculum production, *S. rolfsii* was cultured on autoclaved pearl millet grains (2 kg) and incubated at 27°C for 7 days to allow sufficient multiplication of the inoculum[18]. *T. viride* was grown on PDA plates at 28°C for 5 days, after which spores were harvested by rinsing with distilled water, and the suspension was filtered through Whatman filter paper. The spore concentration was adjusted to 108 CFU per mL using a hemocytometer [19].

Planting Material and Experimental Setup:

A local chili variety (Desi) was selected to assess the effects of pyrolyzed bagasse biochar and *T. viride* on southern blight. Seeds were surface-sterilized using 2% Clorox (NaOCl) for 3 minutes and then rinsed three times with sterile distilled water. Sterilized seeds were sown in nursery trays filled with sterilized sandy loam soil and maintained in a greenhouse at 26 ± 2°C for four weeks before transplanting.

To prepare the potting mixtures, sterilized soil was mixed with compost (10% v/v). Bagasse biochar was incorporated into the soil at 3% and 6% (v/v) concentrations by thoroughly mixing with the potting mixture before filling the pots. After one month, the chili seedlings were transplanted into the pots that contained potting mixture amended with or without bagasse biochar at 3% & 6% (v/v) concentration and *S. rolfsii* inoculated pearl millet seeds. The inoculum of *S. rolfsii* was prepared by growing the pathogen on sterilized pearl millet seeds for 10–12 days at 28 ± 2°C until complete colonization. Inoculum was applied to pots by mixing the colonized millet seeds (10 g per pot) into the upper soil layer at the time of transplanting. The uninoculated control treatment consisted of millet seeds that were autoclaved but not infected. Seven days after pathogen inoculation, chili seedling roots were superficially washed with tap water, then immersed in a *T. viride* suspension (10^8 CFU/mL) for 2 hours. The *T. viride* suspension was prepared by culturing the fungus on potato dextrose broth for 7 days, followed by harvesting and adjusting spore concentration using a hemocytometer. The application was carried out as root dipping before transplanting. The treated seedlings were transplanted into pots 120 minutes after biocontrol application.

The experimental setup included chili plants with or without *S. rolfsii* (SR) inoculation. Soil was amended with two concentrations of biochar (3% and 6%) and TV, applied either individually or in combination as biocontrol agents. Each treatment was replicated five times, with each replicate represented by a single pot. The experiment followed a Completely Randomized Design (CRD) with 12 treatments and 5 replicates per treatment by using plastic

pots of 4kg per replicate. The pots were maintained in a glasshouse at 26 ± 2°C with a relative humidity of 50–70%. The experiment was continued from transplanting until harvest, covering a total duration of 60 days.

Plant Agronomic Parameters Analysis:

Chili growth parameters were assessed 60 days after transplantation. Shoot length was measured from the base of the stem to the tip using a measuring tape.

For dry weight determination, plants were oven-dried at 60°C for 7–10 days until a constant weight was achieved, and the dry weight was measured using an analytical balance. Root length was recorded using a measuring scale.

Disease Assessment:

Disease incidence, mortality rate, and severity index of chili southern blight were evaluated 60 days after transplantation (DAT). The percentage of chili mortality was calculated using the following formula[20].

$$Plant\ mortality\ (\%) = \frac{Number\ of\ dead\ plants}{Total\ observed\ plants} X100$$

The following formula was used to calculate disease incidence[21].

$$Disease\ incidence\ (\%) = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ plants\ observed} X100$$

The severity of chili southern blight caused by SR was evaluated using the disease rating scale developed by[22]. The disease rating scale ranges between 1 and 5, with 1 corresponding to immune (I), 2 for moderately resistant (MR), 3 for moderately susceptible (MS), 4 for susceptible (S), and 5 representing highly susceptible (HS) disease response, respectively. The disease severity index is calculated by using the following formula[21]:

$$Disease\ severity\ index\ (\%) = \frac{\sum severity\ ratings\ of\ all\ plants}{Total\ plants\ observed} X100$$

Biochemical Assessments:

Estimation of Total Phenolics:

Total phenolic content was estimated following the method of[23] with minor modifications. In brief, 250 mg of leaf tissue was ground in 3 mL of 80% methanol and incubated at 65°C for 15 minutes. The extract was centrifuged at 15,000 rpm, and the resulting supernatant was used for phenolic content determination. The reaction mixture consisted of 1 mL of the extract and 250 µL of Folin-Ciocalteu reagent, diluted to 5 mL with water, and incubated at room temperature for 30 minutes. Absorbance was measured at 725 nm, using catechol as the calibration standard. The phenolic content was expressed in micrograms of catechol equivalents per gram of fresh weight (µg catechol g^{-1} FW). Each treatment was analyzed in triplicate, and the entire experiment was repeated three times for consistency.

Estimation of Catalase (CAT) Activity:

Catalase activity was measured following the method of Anderson et al. (1995). Briefly, 500 mg of leaf tissue was ground in 0.05 M Tris-HCl buffer (pH 8.0) containing 0.5% v/v Triton X-100, 2% w/v polyvinylpyrrolidone, and 0.5 mM EDTA, and the mixture was centrifuged at 15,000 rpm to obtain the enzyme extract.

The reaction mixture was prepared by adding 400 µL of the extract to 5 mL of 0.1 M sodium phosphate buffer, 1.2 mL of 150 mM hydrogen peroxide, and incubating for 60 seconds in the dark. A spectrophotometer was used to determine the absorbance at 240 nm, while Beer's law was used to determine the catalase activity.

Enzyme activity was expressed as µmol min^{-1} g^{-1} of protein**.**

Estimation of Flavonoids:

To determine flavonoid content, 250 mg of chili leaf tissue was soaked in 3 mL of 80% aqueous ethanol and kept in the dark at room temperature for 40 minutes. Following centrifugation, the supernatant was combined with 100 µL of 1 M sodium acetate, 4.3 mL of

80% aqueous ethanol, and 100 µL of 10% aluminum nitrate solution. After a 30-minute incubation in the dark, absorbance was measured at 495 nm. Total flavonoid content was expressed as quercetin equivalents per gram of chili leaf tissue[24].

In vitro assay of biochar and *t. Viride* against *s. Rolfsii*:

Antagonistic effect of *T. viride* alone or in synergy with two different concentrations (3 and 6%) of sugarcane biochar against SR was determined under in vitro conditions.

The effect of biochar alone on SR growth inhibition was assessed using the food poisoning technique, in which SR was inoculated at the center of PDA plates amended with biochar, and radial growth was subsequently measured.

A dual culture assay was conducted to evaluate the effect of *T. viride*, alone or combined with biochar, on SR growth. In this setup, *T. viride* and SR were inoculated at opposite ends of PDA plates amended with biochar. Each treatment was replicated three times. The inoculated plates were labeled and incubated at 25°C, and SR growth suppression was recorded at 3, 5, and 8 days after inoculation (DAI). The following formula was used to calculate the percentage growth inhibition of SR [25]:

$$Growth\ inhibition\ (\%) = \frac{C-T}{T} X100$$

Were,

C = Colony diameter in control

T = Colony diameter in treatments

Statistical Analysis:

The experimental data were analyzed by one analysis of variance (ANOVA) using Statistix software (version 8.1). Tukey HSD test was utilized to find homogeneity and to compare means at a probability of ($P < 0.05$).

Result and Discussion:

Characterization of Biochar:

XRD Analysis:

The crystalline and amorphous phases of sugarcane bagasse biochar (SBB) were analyzed using X-ray diffraction (XRD). Diffraction patterns were recorded over a 2θ range of 10–120° using Cu Kα radiation with a wavelength of 1.5406 Å. The amorphous carbon characteristic of disordered graphitic structures was indicated by a large hump between 20° and 30°. The (100) level of graphitic carbon was represented by a peak (Figure 2) that lies between 42° and 46°, indicating partial graphitization.

Sharp peaks at 28°, 29°, 43°, 68°, 73°, and 79° in the XRD pattern (Figure 2) indicated crystalline phases of ash minerals such as SiO_2, CaO, $Ca(OH)_2$, and $CaCO_3$, while a peak around 65° was likely attributed to MnO_2.

The broad baseline and diffuse peaks indicate that the biochar is predominantly amorphous with minor crystalline inclusions, a typical feature of biochar produced at moderate to high pyrolysis temperatures.

Scanning Electron Microscopy Analysis:

The surface morphology of biochar was examined using Scanning Electron Microscopy (SEM) at magnifications ranging from 500× to 4000× to characterize structural and textural features (Figure 3A–F). At 500× magnification (A), complete vascular bundles were clearly visible. At 1000× magnification (B), the biochar surface appeared rough, displaying prominent vertically aligned ridges and flake-like particles. At 2000× magnification (C), granular mineral residues, likely ash, were observed surrounding tubular apertures. At 3000× magnification (D), angular, sheet-like structures with surface fractures and fine pores were evident. At 4000× magnification (E), the biochar surface exhibited circular and oval cracks forming a honeycomb-like pattern. Clear tubular channels and honeycomb-like patterns were observed at 2000X magnification (F).

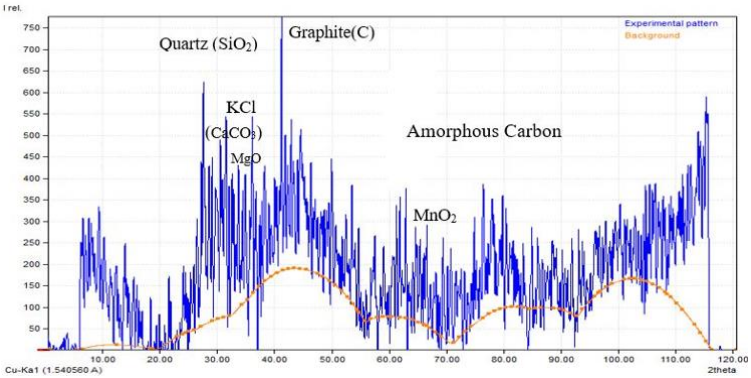


Figure 2. X-ray diffraction (XRD) spectrum of Sugarcane bagasse biochar.

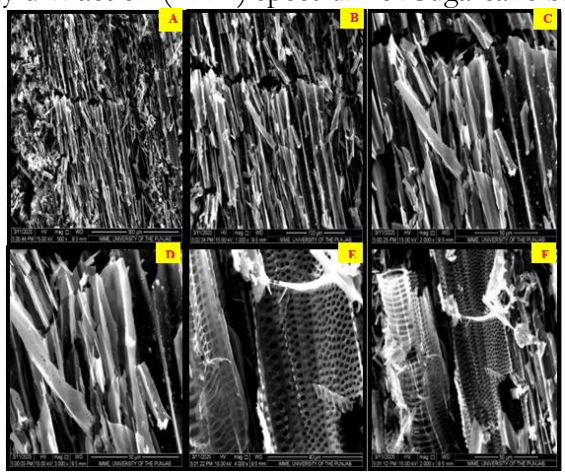
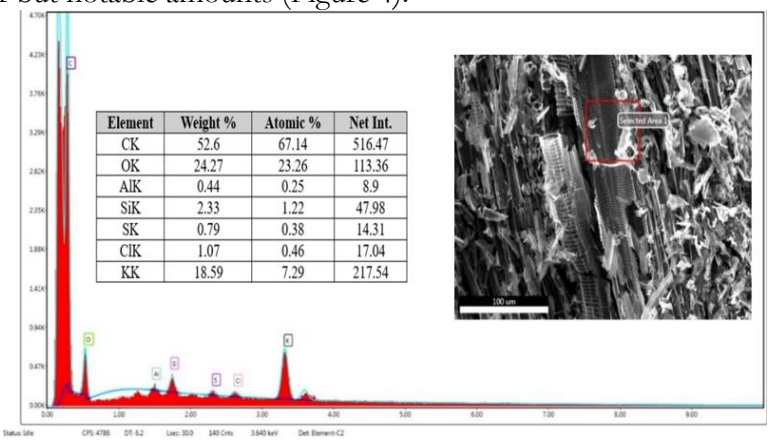


Figure 3. SEM images of sugarcane bagasse biochar at varying magnifications: (A) 500×, (B) 1000×, (C) 2000×, (D) 3000×, (E) 4000×, and (F) 6000×.

Energy Dispersive X-ray Analysis:

The Energy dispersive X-ray (EDX) analysis was done with an accelerating voltage of 15 kV at a live time of 30 s. EDX analysis showed that carbon was the major element in the sample with 67.12 atomic percentage and 52.5% weight percentage. Oxygen was the most abundant element in the sample after carbon, with a weight percentage of 24.26% and an atomic percentage of 23.25%. These results align with the high organic matter content of sugarcane biochar, reflecting the pyrolytic transformation of lignocellulosic biomass. In addition to carbon and oxygen, the biochar contained several other mineral elements, including potassium (18.59%), chlorine (1.07%), sulfur (0.79%), and aluminum (0.44%), present in lower but notable amounts (Figure 4).



Element	Weight %	Atomic %	Net Int.
CK	52.6	67.14	516.47
OK	24.27	23.26	113.36
AlK	0.44	0.25	8.9
SiK	2.33	1.22	47.98
SK	0.79	0.38	14.31
ClK	1.07	0.46	17.04
KK	18.59	7.29	217.54

Figure 4. The Energy dispersive X-ray (EDX) analysis showing different elemental composition (weight% %, atomic% %, net int..) of sugarcane biochar.

Plant Agronomic Parameters:

Overall, pathogen inoculation negatively affected plant growth parameters, with shoot length significantly reduced in all treatments exposed to *S. rolfsii* (Table 1). However, the maximum plant shoot length (15 cm) was found in the combined utilization of 6% SBB biochar and *T. viride* (S+6%SBB+TV) without S. *rolfsii* treatment, which was significantly greater than all other treatments. Similarly, the maximum root length (23 cm) was observed in the absence of pathogen (-SR) in S+6%SBB+TV combination, which was significantly greater than all other treatments. Similarly, root length was notably increased in the S+6%SBB+SR+TV treatment despite *S. rolfsii* infection. In contrast, plants grown without biochar and *T. viride* showed the shortest roots (8 cm) under *S. rolfsii*-infected (+SR) conditions. These patterns suggest that *T. viride* alleviated pathogen stress through antagonism, while biochar likely improved soil structure, water retention, and nutrient availability, collectively promoting stronger root and shoot development even under infection pressure.

However, *T. viride*-inoculated plants grown in soil amended with 6% sugarcane biochar in the SR combination recorded the highest shoot dry weight (0.71 g). Among pathogen-inoculated plants, those grown in 6% SBB-amended soil exhibited a 38.23% increase in shoot dry weight compared to plants grown in 3% SBB with *T. viride*. Similarly, *T. viride*-inoculated plants growing in 6% sugarcane biochar without infection with *S. rolfsii* (-SR+TV) showed the greatest root dry weight (0.59 g). Plants in 3% SBB amended soil with TV showed increased root dry weight by 25% among pathogen-inoculated treatments as compared to plants in 6% SBB. Conversely, SR-inoculated plants in unamended soil without *T. viride* (+SR-TV) had the minimum root dry weight (0.15 g). This indicates a concentration-dependent effect of biochar, where higher levels provided better nutrient retention and supported *T. viride* colonization, leading to improved biomass accumulation. In contrast, plants under pathogen stress without amendments suffered severe reductions, highlighting the protective and growth-promoting synergy of biochar and TV.

Table 1. Effect of *Sclerotium rolfsii* (S), *Trichoderma viride* (TV), and sugarcane bagasse biochar (SBB) on shoot and root growth parameters of chili seedlings (mean ± SD). Different letters within a column indicate significant differences at $P \leq 0.05$.

Combinations	Chilli Shoot length (cm) ± SD	Chilli Root length (cm)± SD	Chilli Shoot dry weight(g)± SD	Chilli Root dry weight(g)± SD
S	7 ± 1.58^{bc}	13 ± 3.76^{ab}	0.37 ± 0.23^{d}	0.29 ± 0.02^{e}
S+SR	3.5 ± 0.29^{f}	7.7 ± 0.27^{cd}	0.2 ± 0.94^{g}	0.15 ± 0.03^{f}
S+TV	8 ± 0.30^{de}	15 ± 2.46^{ab}	0.46 ± 0.55^{c}	0.35 ± 0.04^{d}
S+SR+ TV	6.94 ± 0.37^{e}	11 ± 5.12^{ab}	0.25 ± 0.11^{fg}	0.18 ± 0.02^{f}
S+3%SBB	8.7 ± 0.91^{cd}	16 ± 6.78^{ab}	0.46 ± 0.46^{c}	0.43 ± 0.04^{c}
S+3%SBB+SR	5 ± 0.31^{f}	10.3 ± 1.99^{cd}	0.3 ± 0.09^{ef}	0.27 ± 0.03^{e}
S+3%SBB+SR+TV	8.5 ± 1.15^{cde}	14 ± 6.43^{bc}	0.34 ± 0.37^{de}	0.3 ± 0.02^{e}
S+TV+3%SBB	10.1 ± 0.43^{bc}	18 ± 1.34^{cd}	0.57 ± 0.24^{a}	0.49 ± 0.05^{b}
S+6%SBB	11.3 ± 0.39^{b}	19 ± 2.77^{ab}	0.55 ± 0.09^{b}	0.52 ± 0.05^{b}
S+6%SBB+SR	9.2 ± 0.34^{cd}	13.5 ± 2.52^{d}	0.39 ± 0.03^{d}	0.35 ± 0.03^{d}
S+6%SBB+SR+TV	11.74 ± 1.10^{b}	17 ± 4.22^{d}	0.47 ± 0.23^{c}	0.4 ± 0.03^{c}
S+6%SBB+TV	15 ± 1.06^{a}	22.5 ± 0.86^{a}	0.71 ± 0.04^{a}	0.59 ± 0.01^{a}

Disease Assessment:

To check the efficiency of sugarcane biochar at concentrations of 3% & 6%, either without or with TV combination, in inhibiting *Sclerotium rolfsii*-induced infection, a disease assessment was conducted (Table 2). Plants growing in the control (S+SR) group represented a highly sensitive disease response with the highest disease severity (75%), disease incidence (75%), and death rate (70%). Nearly 55% incidence, 48% severity, and 50% mortality rates

were found in the infected treatments containing *T. viride* alone (S+SR+TV), which led to a moderate reduction in parameters of disease, a sign of a susceptible (S) reaction. This indicates that while *T. viride* exerted antagonistic pressure on the pathogen through enzyme secretion, nutrient competition, and mycoparasitism, it alone was insufficient to fully suppress disease. Though the disease incidence and severity were reduced up to 35% and 28%, respectively, by using 3% SBB alone (S+SR+3% SBB), plants were rated as moderately sensitive (MS), and the mortality was very high at 60%. Where 3% SBB and *T. viride* were combined, disease severity (25%) and mortality (30%) were somewhat reduced, but incidence of disease remained the same (35 %), maintaining the moderately susceptible designation. A moderately susceptible response was found when 6% SBB alone produced almost similar reduction in disease severity (27%) and incidence (35%), while percentage mortality was minimized (30%). The combination of S+SR+6%SBB + TV exhibited the maximum suppression of disease, leading to moderately resistant (MR) response by minimizing incidence (15%), severity (23%), and percentage mortality (10%). This strong reduction highlights the synergistic mechanisms where *T. viride* directly inhibited *S. rolfsii* while biochar enhanced its survival, colonization, and functional activity, collectively strengthening host resistance.

Table 2. Effect of 3% and 6% sugarcane bagasse biochar, alone or with *Trichoderma viride*, on disease incidence of chili under *S. rolfsii* stress**.**

Combinations	Disease incidence (%)	Disease severity (I%)	Percent mortality	Plant Disease Response	Disease rating scale
S+SR	75%	75%	70%	Highly susceptible (HS)	5
S+SR+TV	55%	48%	50%	Susceptible(S)	4
S+SR+3%SBB	35%	28%	60%	Moderately susceptible (MS)	3
S+SR+TV+3%SBB	35%	25%	30%	Moderately susceptible (MS)	3
S+SR+6%SBB	35%	27%	30%	Moderately susceptible (MS	3
S+SR+TV+6%SBB	15%	23%	10%	Moderately resistant (MR)	2

Biochemical analysis:

The phenolic, catalase, and flavonoid concentrations were altered in chili plants by different treatments. The highest total phenol content, 3.45 mg per g of fresh tissue, was obtained from plants cultivated in soil treated with 6%SBB and with combined application of *S. rolfsii* as well as *T. viride* (S+6%SBB+SR+TV). Total phenolic content in the plants inoculated with sugarcane biochar alone was 3.31 mg g^{-1}. However, plants treated with 3% SBB and SR+TV showed phenolic content of 3.22 mg g^{-1}. The lowest phenolic content (1.99 mg/g) was recorded in plants exposed solely to pathogen stress (S+SR). Catalase activity was highest (4.12 µmol min^{-1} g^{-1}) in plants treated with the S+6%SBB+TV combination, whereas the lowest activity (2.49 µmol min^{-1} g^{-1}) was observed in plants inoculated with *S. rolfsii* alone. In pathogen-inoculated treatments, catalase activity was 14.70% higher in plants grown with 6% SBB compared to those with 3% SBB when treated with *T. viride*. Regarding flavonoids, the highest content (4.42 mg) was observed in plants treated with 6% SBB and *T. viride* in the absence of pathogen stress. Flavonoid levels increased proportionally with biochar concentration, reaching 4.09 mg in plants grown with 6% SBB alone.

S. rolfsii infection decreased flavonoid contents in all the treatments. However, the combined application of 6% sugarcane biochar with *T. viride* under pathogen stress (S+6%SBB+SR+TV) increased flavonoid content to 4.53 mg, while the lowest flavonoid level (2.13 mg) was observed in control plants.

Table 3. Impact of sugarcane biochar application alone or in synergy with *T. viride* on the biochemical content in terms of total phenolics, flavonoids, and catalase levels in chili plants infected with *S. rolfsii*.

Combinations	Phenolics (mg per g)	Flavonoids (mg)	Catalase (µmol per min per g)
S	2.11±0.18^{e}	2.13±0.28^{fg}	2.67 ± 0.26^{def}
S+SR	1.99±0.13^{f}	2.45± 0.29^{f}	2.49 ± 0.25^{ef}
S+TV	2.30±0.13^{e}	2.92± 0.30^{de}	2.82 ± 0.13^{d}
S+SR+ TV	2.55± 0.15^{cd}	3.14 ±0.17^{d}	2.75 ± 0.24^{de}
S+3%SBB	2.72±0.17^{c}	3.50±0.12^{c}	3.31 ± 0.26^{c}
S+3%SBB+SR	2.60±0.13^{cd}	3.58 ±0.17^{bc}	3.13 ± 0.29^{cd}
S+TV +3%SBB+SR	3.22±0.15^{ab}	3.83±0.28^{b}	3.41 ± 0.23^{c}
S+TV+3%SBB	2.96±0.18^{c}	3.83 ±0.22^{b}	3.56 ± 0.24^{bc}
S+6%SBB	3.31± 0.23^{ab}	4.09±0.39^{ab}	3.66 ± 0.26^{ab}
S+6%SBB+SR	3.10± 0.25^{ab}	4.28± 0.22^{ab}	3.36 ± 0.25^{b}
S+6%SBB+SR+TV	3.45± 0.22^{a}	4.53± 0.28^{a}	3.91 ± 0.38^{a}
S+6%SBB+TV	3.20±0.24^{a}	4.63± 0.39^{a}	4.12 ± 0.39^{a}

In vitro suppression of *S. rolfsii* by sugarcane bagasse biochar and *T. viride*.

Radial growth of fungi and corresponding inhibition percentages were used to compare the in vitro antifungal activity of various treatments against the test fungus (Figure 5). Although all biochar-amended treatments significantly suppressed fungal growth, the highest growth (8.4 cm) occurred in the control (SR) treatment. The lowest growth (3.6 cm) and highest inhibition (60.42%) were observed in the SR+6%SBB+TV treatment, making it the most effective among those tested. When applied alone and in combination with TV, 6% and 3% SBB treatments inhibited fungal growth by 49.19% and 42.39%, respectively. These results indicate that TV was the primary contributor to antifungal activity, which was further enhanced by the addition of SBB. The antifungal effect was concentration-dependent, with a smaller fungal diameter observed at 6% SBB (4.12 cm) compared to 3% SBB (4.84 cm), indicating that inhibition increased with higher biochar concentrations. Overall, our results indicate that the combined application of TV and SBB biochar led to the greatest inhibition of SR growth, likely due to synergistic mechanisms where TV produced antifungal enzymes and metabolites while biochar improved its colonization and suppressed pathogen growth through adsorption of toxins and modification of the soil microenvironment.

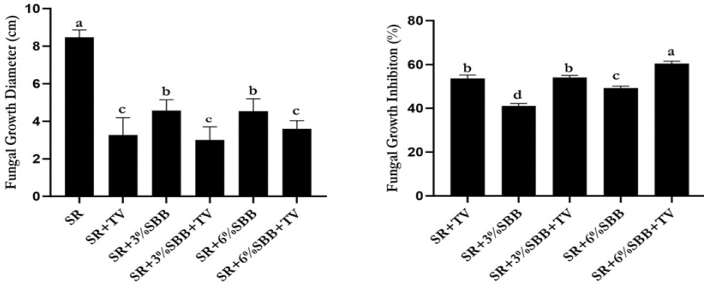


Figure 5. In vitro mycelium growth diameter and inhibbition of S. rolfsii. Treatments included S. rolfsii (SR), T. viride (TV) either alone or in combination with PDA amended with 3% sugarcane bagasse biochar (3% SBB) and 6% sugarcane bagasse biochar (6% SBB). Bars on rectangles represent standard error (SE) and lettering determined by Tuckey's HSD test (P ≤ 0.05).

Discussion:

The rising frequency of unexpected climate shifts, including fluctuations in temperature and weather patterns, has increasingly challenged the agricultural sector in recent years. As a result of the estimated 20–40% annual crop loss induced by disease and insect pests, the existing issue of food insecurity is bound to escalate. There is a growing need to explore innovative agricultural strategies to effectively address the global food crisis. Transitioning into sustainable agriculture systems involves weighing the advantages and disadvantages of different production and marketing practices, yield-enhancing strategies, and reducing the cost of crops[26].

Insights obtained by analyzing data obtained from the current study suggest that biochar has a dual role in chili cultivation, i., suppression of *S. rolfsii* and enhancement of chili growth. This dual potential was further enhanced when the biocontrol potential of *T. viride* was integrated with the enhancing characteristics of biochar. These findings can be justified with the plant growth enhancement activity of *T. viride* and the soil conditioning characteristics of biochar. Porous structure renders biochar to absorb essential nutrients such as N, P, K, and Si and enhances cation exchange capacity[27]. *T. viride* produces auxin-like compounds that promote phosphate solubilization. This process enhances root development and improves nutrient uptake in host plants[28]. The synergistic effect of biochar and *T. viride* resulted in improvement of nutrient uptake and disease suppression. Disease incidence, severity, and mortality in plants treated with biochar and *T. viride* were significantly lower, especially at 6% biochar concentration. The untreated control exhibited an 80% incidence and mortality rate, indicating a highly susceptible response. In contrast, the 6% biochar along with *T. viride* reduced disease parameters to 20–25%, reflecting a moderately resistant response. Our findings align with[29], who demonstrated that biochar amendments suppress soil-borne diseases by enhancing soil suppressiveness and activating plant defense mechanisms.

Moreover,[9] found that *T. harzianum* combined with biochar significantly reduced *Rhizoctonia solani* infection in tomato. Our findings align with[29], who demonstrated that biochar amendments suppress soil-borne diseases by enhancing soil suppressiveness and activating plant defense mechanisms. Similarly, cultivation of tomato plants compost amended with green waste biochar (GWB) at concentrations of 3 & 6% (v/v) showed a statistically significant drop in disease index. Plants in compost with 6% GWB in particular demonstrated excellent disease reduction and enhanced growth. By adding Bacillus subtilis, wilt reduction was further improved, leading to up to 80% disease control. In comparison to compost or wood biochar alone, treatments containing both GWB and B. subtilis improved the physiological characteristics of plants and reduced *Alternaria solani* [26].

Biochar and *T. viride* not only reduced disease incidence but also improved overall plant growth parameters. The combined treatment of biochar and TV resulted in the highest shoot and root length, and maximum shoot and root dry weight. Even under pathogen stress, plants grown in biochar performed significantly better. These results align with the findings of[30] that biochar improves soil structure, increases water retention, and enhances nutrient availability. Similarly, [31] recorded that *Trichoderma* species promote root development through auxin-like activity and improve nutrient uptake. Therefore, the improved growth performance observed in this study can be attributed to the combined effects of enhanced soil physical properties and microbial-induced growth stimulation.

The X-ray diffraction (XRD) analysis revealed the SBB had a predominantly amorphous carbon structure with a broad hump between 20° and 30° (2θ), which is a property of disordered carbonaceous materials[32]. Described that biochar produced at moderate pyrolysis temperatures typically exhibits poor crystallinity and contains disorganized graphitic layers. The additional crystalline peaks at 43° and other angles indicated the presence of graphite microcrystals, silica (SiO_2), and calcium compounds (CaO, $CaCO_3$), along with trace

minerals (such as MnO_2). This chemical composition is similar to the residual ash content obtained from biomass. Such mineral residues enhance the stability of biochar, as reported by[33].

Scanning electron microscopy (SEM) further indicated that the sugarcane-derived biochar had a layered structure. There were vascular bundles and honeycomb-like pores visible in the structure. These characters indicate that the biochar can serve as a physical habitat for microbial colonization, increasing aeration and water retention in soil. EDX revealed carbon as the major element in addition to a high concentration of potassium and medium levels of silicon, Sulphur, and chlorine. A similar distribution of elements has been found by[34], who associated the K and Si content of biochar with enhanced plant nutrition and suppression of pathogens. The dual culture assays showed an obvious inhibition of radial growth of SR by both 3% and 6% SBB, with greater inhibition at 6%. This concentration-dependent inhibition corroborates the earlier finding by[35], who reported that biochar at higher doses interferes with pathogen development by pH changes, leaching of antimicrobial compounds, and producing non-conducive conditions for fungal growth. Importantly, the synergistic interaction was evident with the combination of SBB with *Trichoderma viride* showing the greatest inhibition (56.42%). *Trichoderma* spp. are recognized for their antagonistic activity through mechanisms of mycoparasitism, antibiosis, and competition for nutrients[36]. Such porosity of biochar probably increased the survival and colonization potential of *T. viride* as reported by[13]. Biochemical analyses on chili plants showed increases in phenolic content, catalase activity, and flavonoid level in the biochar and *T. viride*, especially under pathogen stress. The maximum amounts of phenol (3.44 mg/g), catalase (3.90 umol min-1 g-1), and flavonoids (4.52 mg) were found in SBB+TV treated plants in the presence of *S. rolfsii*. The treatment was suggested to activate the resistance and oxidative stress management pathways. Phenolics and flavonoids have antimicrobial and antioxidant properties. According to[37], phenolics and flavonoids are important for the inhibition of fungal invasion and strengthening of plant cell walls. Catalase is an essential antioxidant enzyme. Catalase breaks down reactive oxygen species produced as a result of pathogen attack. Similar biochemical responses were observed in plants sprayed with biocontrol agents along with organic amendments[38].

Our study reveals the mechanism behind resistance activation in chili as a result of synergy between biochar and *T. viride*. Our findings suggest waste materials can be converted into a carbon-rich soil amendment. Organic amendments increase the permeability of the soil structure by increasing the carbon sequestration in soil. Soil conditioning with organic materials encourages deep root development, thus enhancing nutrient uptake. Moreover, biochar application stimulates the defense system of plants and suppresses SR grown both in vivo and in vitro. Thus, biochar conditioning of soil along with application of *T. viride* is an integrated approach for *S. rolfsii*-induced disease mitigation in chili.

Conclusion:

The combined application of sugarcane bagasse biochar and *Trichoderma viride* proved effective in suppressing southern blight of chili while simultaneously promoting plant growth and enhancing defense-related biochemical responses. These findings suggest that integrating biochar with microbial biocontrol agents offers a sustainable and environmentally friendly alternative to chemical fungicides for collar rot management in chili. However, further investigations are recommended to assess the performance of biochar produced from diverse biomass sources, its long-term impact on soil microbial communities, and its effectiveness across different agro-climatic conditions and pathosystems. Such studies will help in optimizing biochar-based strategies for broader application in integrated disease management.

Acknowledgement: The authors would like to thank the University of the Punjab, Lahore, for facilitating the study.

Author's Contribution:

Muhammad Zia Ullah (MZU), Beenish Rasheed (BR), Muhammad, Abu Bakar Siddique (MABS), Hafiz Muhammad Tariq (HMT), Nasir Ali (NA), Muhammad Ahmad (MA) and Umari Raza (UR) proposed the methodology and carried out the experiments, whereas Adnan Akhter (AA), Muhammad Khurshid (MK) and Waheed Akram (WA) designed the study. Muhammad Zia Ullah (MZU), Beenish Rasheed (BR), Muhammad Ahmad (MA), and Umari Raza (UR) gathered and examined data. Adnan Akhter (AA), Muhammad Khurshid (MK), Hafiz Muhammad Tariq (HMT), Nasir Ali (NA), Muhammad Abu Bakar Siddique (MABS), and Waheed Akram (WA) made contributions to the draft of the work, insightful, commentary, and crucial edits for the finished version. The combined efforts of all the authors produced a thorough and significant study result.

Conflict of interest:

There is no conflict of interest in this paper.

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