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Impact of rice straw biochar in association with inorganic fertilizers and *Trichoderma harzianum* on charcoal rot *(Macrophomina phaseolina)* of maize

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Authors' Contribution | **Saeed, A., A. Akhter** and **T. Anjum** conceptualized the study, **H.A.A. Khan, W. Anwar, M.T. Abbas,** and **W. Anwar** performed data analysis.

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ABSTRACT

Phytosanitary regulations concerning pesticide residues are among the most important problems faced by today's agricultural environment. Therefore, the purpose of this study was to produce and characterize indigenously produced biochar from raw material based on rice straw. The biochar synthesized at 480°C, was used as amendment at different volumetric concentrations (3 and 6%), along with in-organic fertilizers for restricting the development of charcoal rot (*Macrophomina phaseolina*), in maize (*Zea mays* L.) The scanning electron microscopy (SEM) analysis of rice straw biochar showed micro-cracks, rough texture, and intricately formed pores, thus showcasing the complex and porous nature of the rice straw biochar resulting from the pyrolysis process. The energy dispersive X-ray spectroscopy (EDX) analysis confirmed its carbonaceous composition (approximately 54%), with the presence of functional groups like hydroxyl and carboxyl, as well as trace amounts of potassium, calcium, and silicon. Maize grown in soil amended with 6% biochar showed significant reduction in shoot and root weights (51.72 and 74.11%, respectively) when compared to 3% biochar amended soil in the presence of *M. phaseolina*. Our results revealed that 3% rice straw biochar and *Trichoderma harzianum* proved most effective for the management of charcoal rot as indicated by lowest disease incidence and disease severity (40%, each). Maize showed variable degrees of defense mechanisms activation in response to soil biochar amendments. Biochar application rate of 6% did generally not improve the maize dry biomass production and tolerance to charcoal rot. These findings will surely help us understand biochar-induced changes in physiology of maize and possible activation of defense response against *M. phaseolina*.

Keywords: Rice straw biochar, maize, bio-control agent, *T. harzianum*.

INTRODUCTION: Maize (*Zea mays*) belongs to the Poaceae family along with other domesticated cereal grain crops including wheat, rice, barley and millet providing staple food to billions of people around the globe. Maize is thought to have evolved between 55 and 70 million years ago in what is today central or South America (Ranum *et al.*, 2014). In terms of production, maize ranks third among cereal grains after wheat and rice worldwide due to the abundance of essential nutrients it provides, including macronutrients like carbs, fiber, protein, fats, vitamins like the B complex and β -carotene; and essential minerals like Mg, Zn, P and Cu etc. Maize also contains an antioxidant booster, which protects against numerous degenerative diseases (Bathla *et al.*, 2019). The protein content of maize ranges from 7 to 14% (Palacios-Rojas *et al.*, 2020), including albumin, globulin, non-nitrogen material and protamine (Kalia *et al.*, 2021). The fungal pathogen *Macrophomina phaseolina* (Tassi) Goidanich, belonging to the Botryosphaeriaceae family, is the causal agent of the disease termed "charcoal rot". *Macrophomina phaseolina*, a widespread soil-borne fungus, infects at least 500 plant species belonging to more than 100 families (Dell'Olmo *et al.*, 2022). *Macrophomina phaseolina* causes diseases including stem, root and charcoal rot, as well as seedling blight (Ghosh and Dey, 2022; Sun *et al.*, 2022). Under hot and dry conditions (Temp. 30–35 °C; humidity: 60%), soybeans and sorghum are prone to significant losses (Gupta *et al.*, 2012). There have been cases in which groundnut cultivars shown complete yield loss owing to a pre-emergence illness. During the growth of the plant, the fungus moves up from the soil and infiltrates the lower internodes, causing a reduction in strength and even fracture of the stalks (Little and Perumal, 2019). The survival of infected corn residue on the ground after harvest during winter is dependent upon the extent of temperature fluctuations (Cohen *et al.*, 2022).

Biochar's most recognizable form is charcoal, which is obtained from woody biomass (Chamorro *et al.*, 2015). The multifaceted applications of biochar extend to various industries, encompassing energy generation, flue gas purification, metallurgy, agriculture, animal husbandry, construction, and medicine. The improvement of raw biomass properties, particularly grind-ability, can be achieved through torrefaction - a process involving pyrolysis at temperatures reaching up to 300°C (Wannapeera *et al.*, 2011). Owing to its capacity for increasing cation exchange capacity (CEC) and pH stability, biochar has garnered significant interest as a potential agricultural supplement. This characteristic makes it a promising option for improving soil quality (Brassard *et al.*, 2016). The elevated pH of biochar can be attributed to varying quantities of alkaline ash within its composition, introducing elements like Ca, Mg, K oxides, hydroxides, and carbonates into the soil. Specific surface functional groups, such as alcoholic OH and COOH, combine with soil cations, thereby boosting the pH buffering capacity of soils (Han *et al.*, 2021). However, the temperature of pyrolysis and the choice of raw materials also impact the alkalinity of biochar (Khan *et al.*, 2022).

The expansive porous surface of biochar results an environment conducive to the proliferation of microorganisms and the development of plant root systems. Following the incorporation of biochar, there is the potential for favorable impacts on microbial reproduction, enzyme activity, and nutrient cycling (Gao *et al.*, 2017). Additionally, biochar contain macropores, mesopores, and micropores that offer ideal niches for diverse microorganisms, including bacteria and arbuscular mycorrhizal fungi, to flourish (Panahi *et al.*, 2020). This porous structure not only fosters a supportive habitat for microorganisms but also serves as a protective shield against potential predators (Rasool *et al.*, 2021).

Biochar holds the promise of effectively preserving beneficial microorganisms in soil (Jaiswal *et al.*, 2019), such as *Trichoderma* spp. which is renowned as a potent plant disease antagonists. Pathogen control is achieved through various mechanisms employed by antagonistic agents, including chitinase production, mycoparasitism, antibiosis, and competition for resources. Additionally, these agents foster growth and induce systemic resistance in plants (Da Silva *et al.*, 2021). Recognizing enzymes' role in decomposition and energy availability, the impact of biochar is pivotal in maintaining soil health. As a sensitive indicator of soil changes, soil enzyme activity holds great significance. Numerous studies have evidenced the direct influence of biochar amendments on various activities, encompassing enzyme activities and metabolism, driven by modifications in the physical, biological, and chemical reactions (Thies *et al.*, 2015). The suppression of diseases caused by soil-borne pathogens is closely intertwined with enzyme activities. Bezerra *et al.* (2019) showed that natural suppression of cassava black root rot has been found correlated with arylsulfatase and urease activities.

Numerous researchers have observed that integrating biochar into the soil shows potential in mitigating crop diseases. A study by (Akanmu *et al.*, 2020) underscores biochar's effectiveness against soil-borne pathogens. Specifically, their research found that biochar produced from poultry manure and sawdust successfully managed corn cob rot, caused by *Fusarium verticillioides*. This underscores biochar's viability for addressing soil pathogens.

Further field studies are required to determine the ideal application rate for each pathogen-plant system, as excessive concentrations of biochar may pose a threat by compromising the plants' defenses and rendering their roots vulnerable to pathogenic attack (Frenkel *et al.*,

2017). The utilization of biochar resulted in a decrease in the root colonization by arbuscular mycorrhiza fungi. This finding highlights the necessity for additional research on the advantageous and disadvantageous impacts of biochar, encompassing those associated with effects on the inhibition of plant diseases.

OBJECTIVES: The study was planned with the major objective to investigate the biochar association with in-organic fertilizers as well as *Trichoderma harzianum* in managing charcoal rot of maize.

MATERIAL AND METHODS: Biochar preparation: The process of pyrolysis was used to produce biochar from rice straw, commonly referred as rice straw biochar. The process was carried out at a temperature of 480°C for 2-3 hrs. The following procedure is outlined for the preparation of biochar (Akmal *et al.*, 2019). Rice straw was collected from the experimental fields of the University of the Punjab, Lahore. The TLUD (Top-lit Updraft) method was used with slight modifications to prepare biochar as suggested by (McLaughlin and Shields, 2010).

X-ray diffraction analysis: The level of crystallinity or amorphousness of the samples was determined using X-ray diffraction (XRD) analysis. At room temperature, the analysis was conducted within the 2 Theta (θ) regions and the angle range of 0°-90°. The XRD analysis was performed with a 40 kV and 30mA X-ray diffractometer (XRD-7000, SHIMADZU, Japan). Using Cu-K radiation in the 2(θ) range, the crystal structure of the biochar was analyzed. At room temperature, XRD patterns of the prepared material were captured on film to provide insight into the crystal structure of the biochars (Fatimah *et al.*, 2022).

SEM-EDX analysis: SEM model SSX-550 from SHIMADZU in Japan was used to assess the surface morphology of biochar samples collected and stored hermetically to prevent moisture absorption, along with selecting one small sample for SEM examination. Utilizing adhesive material, samples were adhered securely onto specimen stubs for analysis by SEM instrument assembly according to manufacturer specifications and the specimen stub containing biochar samples was safely inserted into its chamber (Zhao *et al.*, 2017). To ensure optimal electron beam interactions with the sample, the chamber was evacuated to an optimal vacuum level. Based on resolution and sample properties, an appropriate electron beam acceleration voltage was chosen. Biochar analysis was carried out using voltages between 5 kV and 20 kV (Zhao *et al.*, 2017)**.**

Preparation of soil and sowing of plants: The soil utilized in our study was sourced from the Faculty of Agricultural Sciences (FAS). Sandy loam soil was used, comprising 52.2% sand (>63 mm), 42.9% silt (>2 mm), and, 5.6% clay (<2 mm), 1.30 g/cm^3 of bulk density (Rasool *et al.*, 2021). To ensure sterility, formalin was applied to the soil (Jakovljevic, 2022). Subsequently, the treated soil served as the base for potting mixtures containing varying proportions (3% and 6% v/v) of biochar for the purpose of cultivating plants. Maize Pioneer seeds, variety 30Y87, were obtained from the Lahore, Pakistan vegetable market. The seeds were surface sterilized to assure disinfection (Davoudpour *et al.*, 2020).

Acquisition, conformation and multiplication of *M. phaseolina* culture: The *M. phaseolina* culture was obtained from the First Fungal Culture Bank of Pakistan (FCBP), University of the Punjab Lahore, Pakistan with the accession number FCBP-PTF-1156. The light microscopy analysis of *M. phaseolina* was conducted to examine the morphological features of the fungus. After microscopic confirmation, *M. phaseolina* culture was multiplied on Potato Dextrose Agar media (PDA).

Procurement and revival of *T. harzianum* as biocontrol agent: The culture of *T. harzianum* was obtained from the First Fungal Culture Bank of Pakistan (FCBP) (FCBP-SF-1277). To revive the culture of *T. harzianum*, a small amount of the preserved *T. harzianum* culture was aseptically transferred onto already prepared petri plates containing PDA growth media. The culture was then incubated under optimal conditions at 24-25°C in incubator. Regular monitoring was conducted to ensure the growth and viability of *T. harzianum* (Sreedevi *et al.*, 2011).

Experimental plan: A suitable soil type was carefully chosen for the experiment. Two different concentrations of Rice Straw Biochar, 3% and 6% (v/v), were utilized. Each treatment was replicated five times, resulting in a total of five pots per treatment. In treatments involving only soil, soil with (+MPH) or without (-MPH) *M. phaseolina,* in the presence (+BCA) or absence (-BCA) of bio control agent (*T. harzianum*) amended with 3 and 6% rice straw biochar.

Coating *T. harzianum* on maize seeds: The *T. harzianum* spore suspension was prepared by gently scraping the culture surface and suspending them in sterilized distilled water after 7 days. At a wavelength of 550nm and absorbance of 0.109, the spore count (4 × 10^6 spores/mL) of the biocontrol agent *T. harzianum* could be calculated with a spectrophotometer as suggested by (Waghunde *et al.*, 2010). Maize seeds were gently shaken in spore suspension for about one hour. The coated seeds were then sown in pots (Coninck *et al.*, 2020).

Inoculation of pathogen: The stem injection method was used to inoculate the *M. phaseolina* culture into the maize plants. Sterile syringes (05 mL) were used to inject the fungal inoculum (1×10^5 microsclerotia/mL) into the stems of maize plants (Pastrana *et al.*, 2016). Care was taken to avoid injury of the plant tissue during injection. The injected plants were kept at suitable temperature in the greenhouse for seven days to allow the pathogen to colonize and show disease symptoms.

Inorganic fertilizer application: The NPK fertilizers were selected based on the desired nutrient composition. For this experiment, the nutrient content equivalent to 20 t/ha compost was used (0.12g N, 0.06g P_2O_5, and 0.24g K_2O). The mineral nutrient NPK was used in the form of urea for nitrogen, orthophosphate for P_2O_5, and murate of potash for K_2O. Commercial NPK fertilizers with the appropriate nutrient ratios were sourced. The NPK fertilizers were carefully measured and weighed according to the desired nutrient content (Pandit et al., 2019). To make stock solution for NPK application, 7.2g N, 3.6g P_2O_5 and 14.4g K_2O were dissolved in 500 mL of water. After 20 days of the first treatments, NPK fertilizers were put directly into the soil at a rate of 8.3 mL/pot. The calculated amount of NPK fertilizers for each nutrient (N, P_2O_5, and K_2O) was spread evenly over the soil area in each pot (Pandit et al., 2019).

Disease incidence and severity assessment: To determine the disease incidence, infected plants were counted, and the percentage of disease incidence was calculated using the formula below (Akhter et al., 2016).

$$\text{Disease Incidence} = \frac{\text{No. of symptomatic plants}}{\text{Total number of plants}} \times 100$$

Percentage severity index was calculated by the following formula as described by Rasool *et al.* (2021)

$$\text{Percentage Severity index} = \frac{\text{sum of all disease rating}}{\text{maximum number of rating} \times \text{total number of observation}} \times 100$$

Growth Parameters assessment: Maize crop was harvested after 90 days of implementing the pot treatments. The plants from each treatment were carefully uprooted from the pots, taking care not to damage the roots. The harvested plants were gently cleaned to remove any adhering soil particles. The above-ground plant parts, including the stems and leaves were separated from the roots and agronomic parameters were measured. All the harvested plants were placed in respective polythene bags that were properly labeled with treatment numbers.

Total Chlorophyll: To assess photosynthetic pigment concentrations (chlorophyll), freshly harvested leaves were finely ground in a pestle and mortar using 80% acetone. The resulting mixture was filtered and then subjected to centrifugation at 10,000 g for 5 minutes to quantify the levels of chlorophyll a and chlorophyll b. The absorbance of the filtered solution was subsequently measured at 645 and 663 nm using a spectrophotometer (Badawy *et al.*, 2021).

Chlorophyll A content = 12.7 × OD (663) – 2.69 × OD (645)
Chlorophyll B content = 22.9 × OD (645) - 4.68 × OD (663)
Total chlorophyll (A+B) content =20.2 × OD (645) + 8.02 × OD (663)

***In-vitro* analysis:** The impact of rice straw biochar (RSB) on the inhibition of *M. phaseolina* was examined *in vitro* on Potato Dextrose Agar. Prior to incorporation into the PDA medium, the biochar underwent sieving. The growth medium was supplemented with RSB before autoclaving. Subsequently, the PDA mixture was poured into 90mm petri plates and allowed to solidify at ambient temperature. Following this, agar plugs with a diameter of 6mm, extracted from a 7-day-old *M. phaseolina* culture, were aseptically collected using a cork borer and positioned at the center of the plates. To assess the antifungal capacity of *T. harzianum*, a dual culture method was employed. PDA plates were inoculated with *T. harzianum* along the periphery of the petri plates (Chowdappa *et al.*, 2013). The inoculated petri plates were placed in an incubator for 3 to 4 days at 25°C (Riaz *et al.*, 2021).

Statistical analysis: To determine homogeneity, the Tukey's HSD All-Pairwise Comparisons Test was conducted, and means were analyzed at a significance level of P≤0.05. Statistix 8.1 was employed for data analysis. The results were evaluated using a three-way

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analysis of variance (ANOVA). The *M. phesolina*, *T. hazianum*, and soil composition were the three main variables in the analysis.

RESULTS: X-ray Diffraction (XRD) Analysis: The crystalline or amorphous nature of the materials was determined using X-ray diffraction (XRD) analysis. The XRD graph of rice straw biochar (RSB) is shown in figure 1.

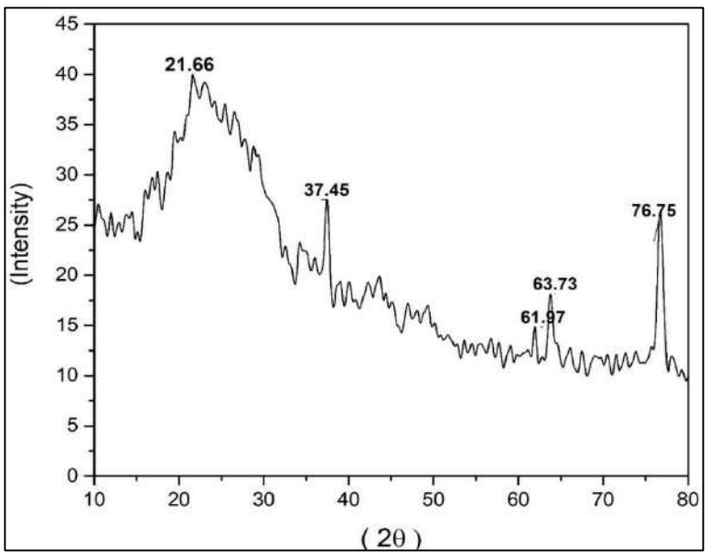

Figure 1: The X-ray diffactogram of Rice Straw Biochar

The biochar was analyzed within the 2 Theta (θ) regions, at an angle of 0° to 90° and constant room temperature (25°C). The RSB exhibited a prominent peak within the range of 15° to 40° (2θ), indicating the presence of the turbostratic graphite plane (002). Thus, the biochar derived from rice straw comprises an amorphous carbon structure. The XRD analysis revealed the presence of quartz in the biochar, as peaks were at 2θ values of 21.8° and 37.0°. The 2θ values of 61° and 63° correspond to the diffraction angles of the (10) and (11) crystallographic planes of graphite, respectively. While the 2θ of 76° corresponded to the presence of amorphous carbon.

Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX): The SEM analysis revealed a very porous structure of the RSB, with interconnecting gaps and channels throughout the surface (figure 2).

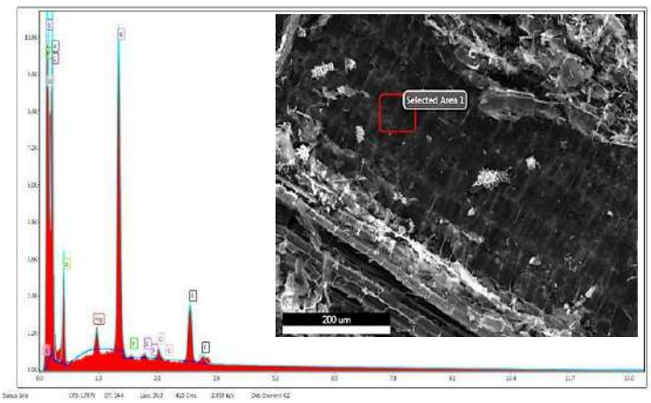

Figure 2: The EDX elemental analysis of rice straw biochar.

Several irregularities, cracks, and fissures can be observed, which are indicative of the structural alterations that took place during the pyrolysis process. The rough texture observed on the surface can be attributed to the presence of residual ash and the inherent structure of rice straw. The EDX analysis provided confirmation of the carbonaceous composition of the biochar, exhibiting a carbon content of approximately 54%. The presence of functional groups, such as hydroxyl and carboxyl, was indicated by the detection of oxygen and hydrogen. Furthermore, minute quantities of potassium, calcium, and silicon were detected. When observed under 250x magnification rice straw biochar revealed a rough and textured surface with visible cracks and uneven patterns pores. At 500x, more intricate details become visible, such as micro-cracks, canals, and a complex arrangement of interconnected pores. Successive magnifications of 1000x, 2000x, and 3000x revealed prominent fissures, a roughened texture, and intricately formed pores (figure 3).

Morphological identification of *M. phaseolina*: The colony color on both sides (upper and lower) was white to grey, eventually turning black with time (table 1). The hyphae were septate and 2-4µm wide, sub-hyaline to dark brown in color. Microsclerotia were black, spherical to oblong or irregular microsclerotia ranging from 90-180µm (av. 128µm) to 50-100µm (av. 81µm) formed in culture after 4-5 days of incubation (figure 4).

Morphological Features	Description
Colony appearance	Initially white to grey
Colony color	Turns black with age
Hyphae	Septate, 2-4µm wide, sub-hyaline to dark brown
Microsclerotia color	Black
Microsclerotia shape	Spherical to oblong or irregular
Microsclerotia size range	90-180µm (average 128µm) to 50-100µm (average 81µm)

Table 1: The morphological characteristics of *M. pahseolina.*

Growth parameters: The agronomic parameters of maize were significantly influenced by the presence of *M. phaseolina, T. harzianum*, and the composition of soil biochar. The interaction between soil-biochar composition, *T. harzianum*, and *M. phaseolina* had a significant impact on plant biomass production (table 2). The inoculation of the pathogen to the plants resulted in a generally suppressive impact on various parameters related to plant growth.

Maize shoot length: Among the biochar amended treatments, the maximum shoot length (73 and 70.2cm) was recorded in 3% rice straw biochar amended treatment (S+3% RSB) in the presence (+BCA) and absence (-BCA) of biocontrol agent without pathogen stress (-MPH), respectively (Figure 5). While the minimum shoot length of 46.4cm was observed in 6% RSB amended treatment (S+6%RSB+MPH-BCA), received pathogen but not BCA. On the other hand, the lowest (52cm) shoot length was recorded in un-amended soil control in the presence of pathogen (+MPH-BCA) (figure 5).

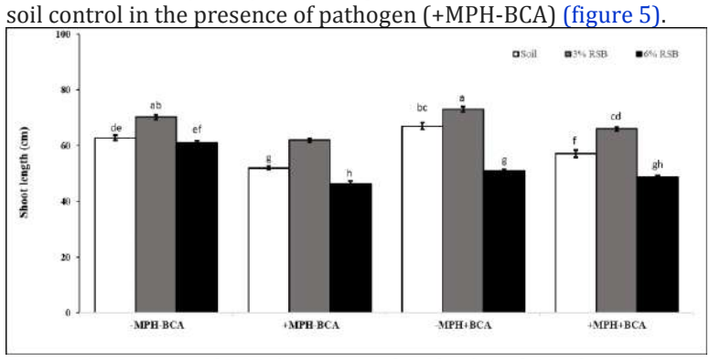

Figure 5: **:** Impact of RSB (rice straw biochar) and *M. phaseolina* on shoot length of maize in various soil substrate compositions: soil, soil with RSB (3 and 6%). These compositions were assessed both in the presence (+MPH+BCA) and absence (-MPH-BCA) of the pathogen and biocontrol agent. The presented values are expressed as the mean ± standard error (Da Silva *et al.*)

Root length: Without disease stress (-MPH), the 3% RSB amended potting mixture (S+3% RSB), with (+BCA) as well as without (-BCA) biocontrol agent, had the maximum root lengths (25 cm and 23.6 cm), respectively (figure 6). Under pathogen stress, the 6% rice straw biochar-amended treatment (S+6%RSB+MPH-BCA) had a minimum root length of 16.8 cm. Moreover, un-amended soil control in the presence of the pathogen but without the biocontrol agent (+MPH-BCA), also among the lowest (17.4cm).

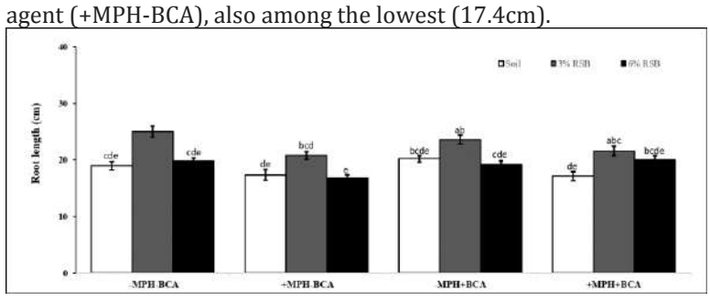

Figure 6: Impact of RSB and M. phaseolina on root length of maize in various soil substrate compositions: soil, soil with RSB (3 and 6%). These compositions were assessed both in the presence (+MPH+BCA) and absence (-MPH-BCA) of the pathogen and biocontrol agent. The presented values are expressed as the mean ± standard error (Da Silva *et al.*).

Shoot weight*:* The 6% rice straw biochar-amended treatment (S+6%RSB+MPH-BCA) had a minimum shoot weight of 23.2 g in the presence of pathogen, while the un-amended soil control (+MPH-BCA) had a minimum shoot weight of 21.4 g. When the 3% biochar treatment was compared to the 6% RSB-comprising treatment in the absence (-MPH) of pathogen and biocontrol agent, a 16.06% increase in shoot weight was observed in the 3% RSB amended soil treatment (figure 7).

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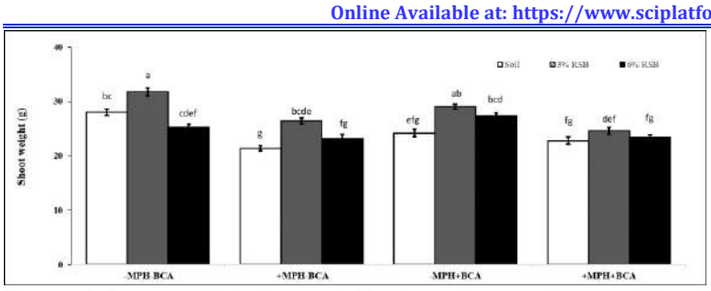

Figure 7: Impact of biochar and *M. phaseolina* on shoot weight of maize in various soil substrate compositions: soil, soil with RSB (3 and 6%), these compositions were assessed both in the presence (+MPH+BCA) and absence (-MPH-BCA) of the pathogen and biocontrol agent. The presented values are expressed as the mean ± standard error (Da Silva *et al.*, 2021).

The S+3% RSB treatment had the maximum shoot weight (31.8 g) both without the biocontrol agent and pathogen stress.

Root weight: The 3% rice straw biochar amended treatment (S+3% RSB) had the maximum root weight (2.7 g) in the absence (-BCA) of the biocontrol agent and pathogen stress. The 6% rice straw biochar-amended treatment (S+6%RSB+MPH-BCA) had a minimum shoot weight of 1.24 g in the presence of the pathogen, while the un-amended soil control (+MPH-BCA) had a minimum shoot weight of 1.44 g. The root weight of 6% rice straw biochar amendment treatment (S+6%RSB+MPH-BCA), when subjected to pathogen stress, exhibited a percentage decrease of 16.1% compared to the control soil without any amendment (figure 8).

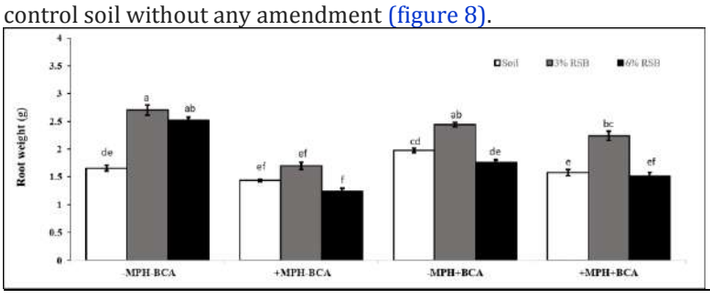

Figure 8: Impact of RSB and *M. phaseolina* on root weight of maize in various soil substrate compositions: soil, soil with RSB (3 and 6%). These compositions were assessed both in the presence (+MPH+BCA) and absence (-MPH-BCA) of the pathogen and biocontrol agent. The presented values are expressed as the mean ± standard error .

Plant dry biomass and total chlorophyll contents: Shoot dry weight: When the maize plants were exposed to charcoal rot stress, their shoot dry weight significantly decreased. However, when the soil was amended with 3% rice straw biochar (3% RSB), there was an increase of 17.2% in the shoot dry weight as compared to the 6% RSB amended treatment without (-BCA-MPH) the bio-control agent and pathogen (table 3). Furthermore, when inoculated with both *M. phaseolina* and the biocontrol agent (+BCA+MPH), the maximum shoot dry weight of 6.78±0.15 g was observed in the 3% biochar-amended treatment (S+3% RSB+BCA+MPH). In contrast, the un-amended soil control under pathogen stress had the minimum shoot dry weight of 5.52±0.15g.

Root dry weight: The *M. phaseolina* inoculation reduced root dry weights of maize in all treatments, both with (-BCA) or without (+BCA) bio-control agent (*T. harzianum*). The maximum root dry weight (1.98±0.16 and 2.1±0.12g) was noted in 3%+RSB amended soil with biocontrol agent (+BCA) that was either infected (+MPH) or un-inoculated (-MPH) with the pathogen, respectively. While under *M. pahseolina* stress, the lowest (1.38±0.08 g) root dry weight was recorded in the un-amended soil treatment (table 3).

Chlorophyll content: With the interacting effect of P x S x BCA, plant chlorophyll concentrations varied significantly ($P < 0.05$) (table 3). The biochar-containing soil substrates significantly increased the amount of chlorophyll in the maize (Table 3). The plants cultivated in 3% RSB and 6% RSB, either un-inoculated (-MPH) or inoculated (+MPH), had the highest chlorophyll levels (0.51 ± 0.007 & 0.4 ± 0.009, respectively). The soil-only potting medium inoculated with *M. phaseolina* had the lowest chlorophyll contents (0.36 ± 0.05), which was 41.67% lower than the Soil + 3%RSB treatment.

Treatment	Shoot Length	Root Length	Shoot Weight	Root Weight	Shoot Dry Weight	Root Dry Weight	Total Chlorophyll Content
S	***	***	***	***	***	***	**
P	***	***	***	***	***	***	**
BCA	Ns	Ns	*	Ns	**	Ns	***
S×P	Ns	Ns	Ns	**	***	Ns	Ns
S×BCA	Ns	Ns	***	***	*	**	***
BCA×P	Ns	Ns	*	***	***	Ns	***
S×BCA×P	*	*	*	***	Ns	Ns	***

Table 2: Tble 2: The three way analysis of variance (ANOVA) based statistical analysis to analyze the significance of the relationship between *M. phaseolina, T. harzianum*, and soil-biochar composition on maize development and physiological parameters.

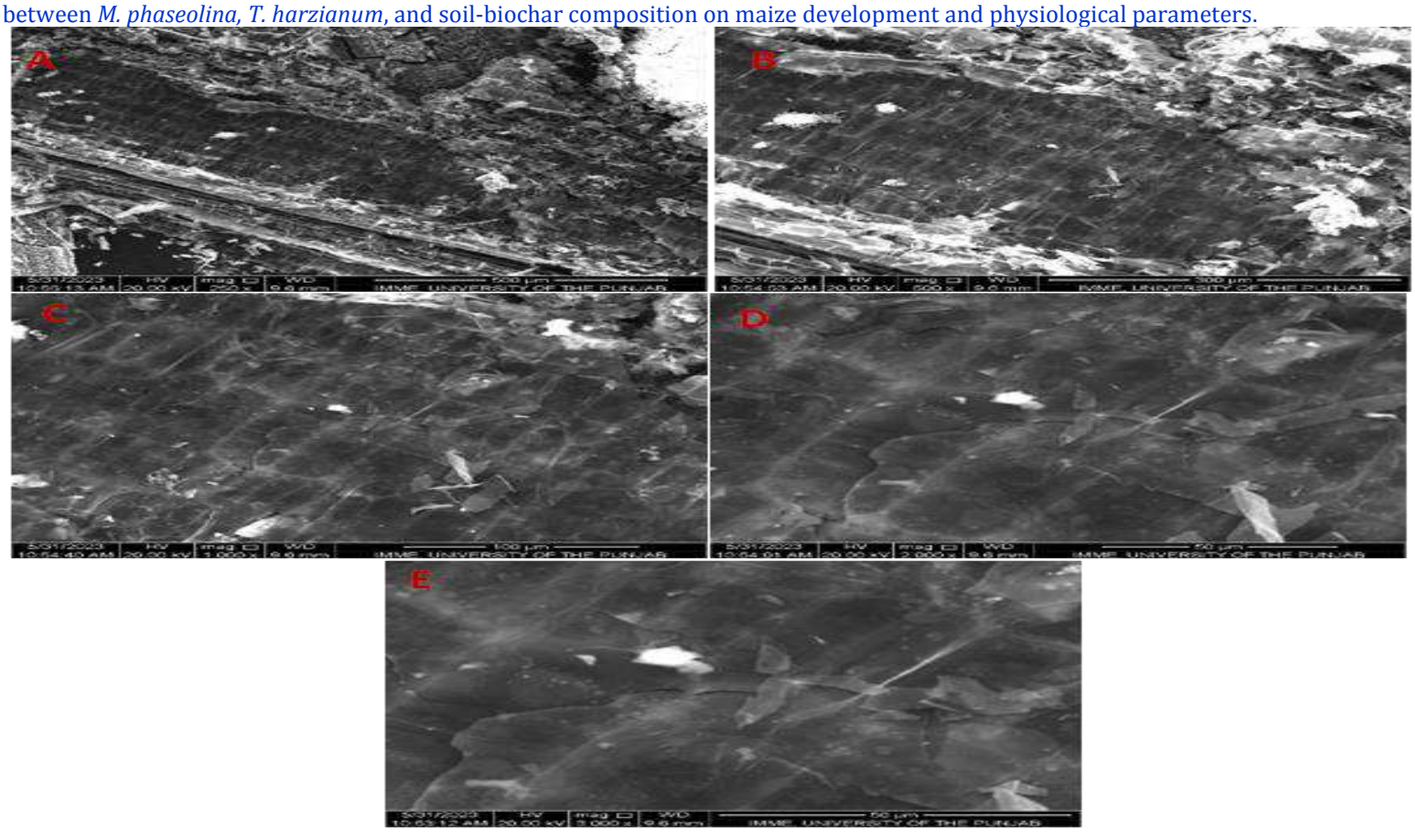

Figure 3: (A,B,C,D, E) SEM representing the structure, channels, and pore size of rice straw biochar synthesized at 480 °C.

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Treatment	Dry Root Weight	Dry Shoot Weight	Total chlorophyll content (µg/ml)
Soil	1.66±0.18^{a}	6.84±0.11^{a}	0.36^{b}
Soil + MP	1.38±0.08^{ab}	5.52±0.15^{b}	0.30^{d}
Soil + BCA	1.84±0.11^{abc}	6.76±0.18^{c}	0.35^{c}
Soil + MP + BCA	1.52±0.13^{bcd}	5.84±0.11^{cd}	0.39^{b}
Soil + 3%RSB	2.1±0.12^{bcd}	7.74±0.17^{cd}	0.51^{a}
Soil + 3%RSB + MP	1.76±0.09^{bcd}	6.5±0.1^{cd}	0.42^{b}
Soil + 3%RSB + BCA	1.98±0.16^{bcd}	7.38±0.08^{cd}	0.38^{b}
Soil + 3%RSB + MP + BCA	1.72±0.08^{cd}	6.78±0.15^{de}	0.38^{b}
Soil + 6%RSB	1.76±0.17^{cde}	6.68±0.22^{e}	0.38^{b}
Soil + 6%RSB + MP	1.56±0.05^{de}	6.04±0.09^{f}	0.39^{b}
Soil + 6%RSB + BCA	1.78±0.13^{de}	6.66±0.09^{f}	0.40^{b}
Soil + 6%RSB + MP + BCA	1.62±0.08d^{e}	6.34±0.11^{g}	0.37^{bc}

Table 3: Effect of rice straw biochar on maize plant dry biomass (root and shoot) and total chlorophyll content. All values are represented ±SD three-way ANOVA at P≤0.05.

Treatments	Disease incidence Percentage (%)	Severity index Percentage (%)	Response of Disease
Soil + MP	100	84^{a}	Highly susceptible
Soil + MP + BCA	80	76^{b}	Highly susceptible
Soil + 3%RSB + MP	60	68^{c}	Moderately susceptible
Soil + 3%RSB + MP + BCA	40	40^{f}	Susceptible
Soil + 6%RSB + MP	80	65^{d}	Highly susceptible
Soil + 6%RSB + MP + BCA	60	60^{e}	Highly susceptible

Table 4: Estimation of disease incidence, percent severity index (PSI), and disease response parameters for charcoal rot in maize in different soil-biochar compositions.

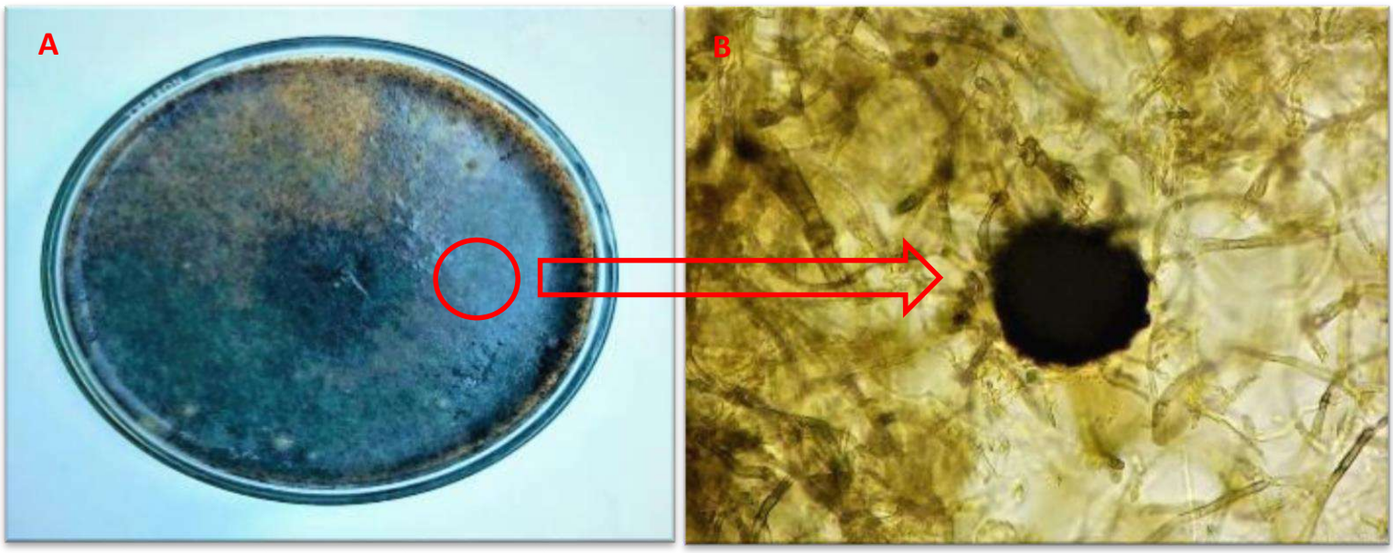

Figure 4: The *M. phaseolina* colony morphology on PDA microsclerotia on PDA.

Treatment	Colony diameter (cm)	%age Inhibition
Control	4.3±0.05^{a}	---
3%RSB + MP	3.56±0.07^{b}	17.12^{c}
BCA + MP	2.07±0.03^{c}	51.7^{b}
3% RSB + MP + BCA	1.69±0.05^{d}	60.69^{a}

Table 5: Mycelial growth inhibition (%) of *M. phaseolina* (Burrell *et al.*) in control (un-amended), 3% RSB and *T. harzianum* (BCA) amended PDA. The provided outcomes represent mean values ± standard error. Distinct letters in superscript indicate significant differences at (P ≤ 0.05).

Disease assessment: The maize plants exhibited varying levels of susceptibility to *M. phaseolina* inoculation, which were influenced by the composition of the soil (table 4). The plants that were planted in the only soil (S + MP) had a very vulnerable reaction to charcoal rot, as evidenced by the maximum PDI (100%) and disease incidence (84%). The addition of biochar to the soil substrate resulted in a suppressive impact on pathogen growth. An evident reduction in PSI and DI was found in the plants cultivated in soil containing biochar. The treatment that included 3% biochar (S+3%RSB+ MP + BCA) exhibited the most effective plant defense against the occurrence of charcoal rot, as evidenced by the lowest PDI (40%) and DS (40%).

***In vitro* influence of rice straw biochar and *T. harzianum* on *M. phaseolina* mycelium growth and development:** The colony diameter in the control treatment was 4.3 cm. Treatment with 3% biochar (3%RSB+MP) resulted in a 3.56 cm colony diameter, resulting in a significant 17.12% growth suppression. *T. harzianum* had a more noticeable suppressing effect on *M. phaseolina*, with a colony diameter of 2.07 cm (table 6). The combination treatment containing both biochar and BCA reduced the colony diameter to 1.69 cm, and an inhibition of 60.69% in comparison with control (table 5).

DISCUSSION: Farmers and government organizations need integrative measures to limit agriculture's excessive use of chemical inputs to achieve global sustainability goals. Biochar formulations can manage plant diseases, improve soil, and sequester carbon (Rasool *et al.*, 2021). Stakeholders may capitalize on the increased demand for sustainable products that utilize green waste with eco-friendly choices such as biochar production (Lehmann and Joseph, 2015). The significance of this transition is highlighted by the negative effects induced by phytopathogens, which result in reduced agricultural yields and degraded product quality, resulting in significant economic losses for agricultural entrepreneurs (Savary *et al.*, 2019). Agrochemical overuse worsens environmental and health issues and promotes disease resistance (Schmitz *et al.*, 2014). Thus, various *M. phaseolina* isolates with improved fungicide resistance have underlined the urgent need for sustainable disease control strategies in coordination with sustainable amendments (Kaur, 2014). Historically, biochar application has been used to improve soil quality; however, in the last two decades, researchers have become increasingly interested in its potential as a carbon sequestration method and as an organic plant protection agent (Lehmann and Joseph, 2015). Biochar application can alter how plants respond to disease stress as well as crop yield (Graber *et al.*, 2010). To the best of our understanding, this research represents the inaugural investigation into the role of biochar, both with and without a bio-control agent, on the development of a root rot pathogen known as *M. phaseolina* in maize crops.

Biochar and compost are high-carbon compounds and are widely acknowledged to enhance soil attributes and structure. One of the most essential measures of soil quality is its porosity. Images of biochar made from rice straw obtained with a scanning electron

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microscope at a magnification of 3000x revealed distinct tabular channels and a porous structure. Aslam *et al.* (2014) came to the conclusion that biochar enhances soil qualities by making it more porous, less dense, having high infiltration rates, and having a granular structure. Soil organic carbon, microbial diversity, and biochar's adaptability are all factors that may contribute to enhanced soil characteristics.

Soil erosion and subsequent decreased agricultural yields are direct results of intensive farming practices. Poor soil structure increases the risk of disease due to reduced aeration and damp soil (Otten and Gilligan, 2006). Uzoma *et al.* (2011) and Zahra *et al.* (2021) reported that biochar made with manure as the biomass source has high ash concentrations. Kim *et al.* (2012) showed that biochar with higher ash levels aids in the incorporation of soil mineral components. We found that the SEM-EDX analyses of rice straw biochar had healthy concentration of mineral nutrients. Moreover, XRD analysis of rice straw biochar validated the heterogeneity of biochar material by showing that it contains a wide range of mineral compositions (Shaaban *et al.*, 2013). This biochar, when added to soil, improves soil stability and aggregation, which in turn increases water retention, nutrient availability, root penetration, and crop development and yield (Burrell *et al.*, 2016).

It is widely acknowledged that bio-control agents like *Trichoderma* spp., *Penicillium axalicum, Bacillus subtilis* and some others cause the plants to be resistant to foliar pathogen, such as *B. cinerea,* C. *acutatum*, and *R. solanacearum* (De Cal *et al.*, 2008; Attia *et al.*, 2020). Rasool *et al.* (2021) recently investigated how biochar, in combination with plant growth promoting rhizobacteria (PGPR), affect the rigourness of the disease, as well as the physiological responses of tomatoes to early blight.

Elad et al. (2011) indicated resistance response to *Botrytis cinerea* at higher biochar application rate, and Zwart and Kim (2012) found that biochar made from pine (*Pinus spp*.) origin reduced *Phytophthora spp*. induced stem lesions in ornamental trees such as *Acer rubrum* and *Quercusrubra*. While lower concentrations of biochar had no effect on foliar pathogens responsible for powdery mildew and grey mold on strawberry (Meller Harel *et al.*, 2012). However, Atucha and Litus (2015) found that pinewood biochar was beneficial in combating replant disease in peach rootstock when applied at substantially greater rates of 10 and 20% (v/v). As a result, biochar type and concentration used in the growing media are crucial for preventing disease and encouraging plant development.

In this study, plants were examined visually for disease incidence and severity index. Our results demonstrated a significant influence of 3%RSB on charcoal rot suppression in maize. Disease control with biochar is mostly determined by the type of biomass used and the pyrolysis conditions (Akhter *et al.*, 2016). Rasool *et al.* (2021) and Araujo *et al.* (2019) reported that alone and combined application of biochar and compost suppressed *M. phaseolina*. Soils that are rich in nutrients alters plant tolerance and resistance to pathogen invasion, penetration, reproduction, and development .

There have been conflicting findings about the impact of biochar on chlorophyll levels, as evidenced by Akhter *et al.* (2016), which observed a reduction in chlorophyll content in tomato plants cultivated in biochar. In addition to enhancing the solubilization of nutrients such P and K, the co-application of *T. harzianum* and biochar resulted in elevated and persisted chlorophyll concentrations, even in the presence of *M. phaseolina*. Similarly, Rasool *et al.* (2021) found that the synergistic effect of PGPR and biochar increased wheat chlorophyll content significantly. The variations in the response of PGPR when exposed to various biochars at varying concentrations in the soil substrate might be linked to modifications that result from biochar in the signaling mechanisms between plants and microorganisms (Rondon *et al.*, 2006). In line with our findings, Chan *et al.* (2007) reported that biochar and mineral fertilizers improved nitrogen uptake. Kaplan *et al.* (2016) found that nitrogen availability improved maize plant development and dry matter output. Biochar, coupled with NPK fertilizers, increased plant biomass and fertilizer effectiveness (Alburquerque *et al.*, 2013).

Agegnehu *et al.* (2016) suggested that biochar have the potential to supply easily accessible and transferable phosphorus by either making the natural phosphorus accessible or directly dispersing it. Phosphorus deficiency in calcareous soils is caused by phosphorus precipitating as $CaHPO_4$ (di-calcium phosphate) (Abbass *et al.*, 2022). Higher P concentrations in plants as well as soil confirmed the favorable impacts of organic inputs on plants due to appropriate phosphorus availability (Nigussie *et al.*, 2012). Similarly, Steiner *et al.* (2008) showed that the addition of compost and biochar improved overall growth of plants, resulting in greater K absorption. Direct and indirect rising potassium levels in plants and soil may be caused by increased N and P availability.

Macrophomina pahseolina can survive in the soil as mycelia on host debris (Dorozhkin and Ivaniuk, 1979), while chlamydospores can survive without host debris (Basu, 1971). We also discovered that *T. harzianum* inhibited *M. pahseolina* mycelial growth more effectively, which could be related to the formation of extracellular chemicals produced in response to fungal infections. On *et al.* (2015) reported on the antifungal activity of crude extracts of *B. subtilis* cultures against *A.* solani. Organic amendments and *T. harzianum* have a direct antifungal impact, which provides another potential for decreasing the quantity of *M. phaseolina* overwintering inoculum in soil and plant waste. However, future experiments will determine whether or not this assumption can be trusted.

CONCLUSION AND FUTURE PROSPECTS: Maize response to *M. phaseolina* depended on biochar concentration while resistance against charcoal rot was stronger at lower biochar amendment rates i.e. 3%. Rice straw biochar was found to be efficient in inducing resistance against *M. phasolina* in maize plants at 3% amended treatment alone, as well as in synergism with *T. harzianum*. However, further investigations are needed for the concentration level of biochar to be employed as a soil supplement needs. Furthermore, research should be focused on evaluating the processes underlying biochar-induced resistance in maize plants against *M. phaseolina* as well as other economically important pathosystems. To address the potential risks connected with biochar application on plant health, the scientific community must focus on understanding the biochemical reactions activated by biochar-borne compounds. Further investigation into the efficacy of various biochar types in combating several diseases affecting a certain crop is needed since this would enhance the economic significance of biochar.

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